Universidad de Córdoba

Departamento de Química Analítica

NUEVAS APLICACIONES DE DETECTORES ANALÍTICOS NO CONVENCIONALES BASADOS EN PROCESOS DE IONIZACIÓN

NEW APPLICATIONS OF NON-CONVENTIONAL ANALYTICAL DETECTORS BASED ON IONIZATION PROCESSES

Tesis Doctoral

Isabel Márquez Sillero

Córdoba, Septiembre 2013

TITULO: *NUEVAS APLICACIONES DE DETECTORES ANALÍTICOS NO CONVENCIONALES BASADOS EN PROCESOS DE IONIZACIÓN*

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NUEVAS APLICACIONES DE DETECTORES ANALÍTICOS NO CONVENCIONALES BASADOS EN PROCESOS DE IONIZACIÓN

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Trabajo presentado para aspirar al

Grado de Doctor en Ciencias

LA DOCTORANDA,

Fdo. **Isabel Márquez Sillero**

Licenciada en Ciencias Químicas

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CERTIFICAN: Que la Tesis Doctoral "NUEVAS APLICACIONES DE DETECTORES ANALÍTICOS NO CONVENCIONALES BASADOS EN PROCESOS DE IONIZACIÓN" ha sido desarrollada en los laboratorios del Departamento de Química Analítica de la Universidad de Córdoba y de la empresa G.A.S. en Dortmund, y que, a nuestro juicio, reúne todos los requisitos exigidos a este tipo de trabajo.

Y para que conste y surta los efectos pertinentes, expide el presente certificado en la ciudad de Córdoba, 20 de Junio de 2013.

Miguel Valcárcel Cases **Mª Soledad Cárdenas Aranzana**

Miguel Valcárcel Cases, Full Professor of Analytical Chemistry of the University of Córdoba, and

María Soledad Cárdenas Aranzana, Full Professor of Analytical Chemistry of the University of Córdoba,

IN QUALITY OF:

Supervisors of the Doctoral Thesis of ISABEL MÁRQUEZ SILLERO, entitled "New applications of non-conventional analytical detectors based on ionization processes",

CERTIFIES THAT:

1) The experimental work of the PhD thesis has been developed in the laboratories of the Department of Analytical Chemistry of the University of Córdoba and in the company G.A.S. of Dortmund (Germany).

2) According to our judgment the thesis meets all the requirements of this type of scientific work.

3) ISABEL MÁRQUEZ is the first author of all the scientific papers presented in the thesis. According to the University rules and the internal agreements in our research group, the first author of a paper is the full responsible for the implementation of the experimental work and also to produce the first draft of the paper. In addition, she has also been actively participated in the meetings with the supervisors each month to check and discuss the progress of the doctoral work.

Córdoba, July 12th 2013.

Miguel Valcárcel Cases **Mª Soledad Cárdenas Aranzana**

TÍTULO DE LA TESIS: NUEVAS APLICACIONES DE DETECTORES ANALÍTICOS NO CONVENCIONALES BASADOS EN PROCESOS DE IONIZACIÓN

DOCTORANDO: Isabel Márquez Sillero

INFORME RAZONADO DEL LOS DIRECTORES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda Isabel Márquez Sillero cursó brillantemente los estudios del Máster en Química Fina Avanzada, obteniendo excelentes calificaciones en las asignaturas del mismo. El trabajo fin de Máster se publicó en la revista Journal of Chromatography A, situada en el primer cuartil del área de conocimiento.

La temática de la Tesis se encuadra en una línea de investigación puntera, como es el desarrollo de nuevos sistemas analíticos de vanguardia que permitan obtener información de manera rápida, fácilmente interpretable por el cliente y que pueda ser empleada para la toma de decisiones. En este sentido, el empleo de detectores de respuesta universal es crucial. En esta Tesis Doctoral se ha profundizado en el conocimiento y aplicación de detectores basados en procesos de ionización y de tipo evaporativo, cuya aplicabilidad es bastante limitada en el contexto de los sistemas de vanguardia. Asimismo, se han empleado medios de extracción no convencionales como los nanotubos de carbono y los líquidos iónicos para aumentar la eficiencia y selectividad de los procesos.

La realización de la investigación recogida en la Memoria que se presenta, ha permitido a la doctoranda adquirir además una sólida formación analítica, adiestrándose en el manejo de técnicas de microextracción, de separación cromatográficas, así como detectores nos convencionales de tipo evaporativo, de descarga corona y de movilidad iónica, bajo distintas configuraciones instrumentales. Además, ha desarrollado interfases específicas para el acoplamiento de técnicas de microextracción con la espectrometría de movilidad iónica. Por otra parte, conviene resaltar también la variedad de muestras y familias de analitos que han sido objeto de esta Tesis Doctoral lo que ha supuesto un aprendizaje muy importate para la doctoranda con vistas a su futuro laboral en el ámbito de la investigación o la empresa, completando así su formación integral en el ámbito analítico. Todo ello ha dado lugar la

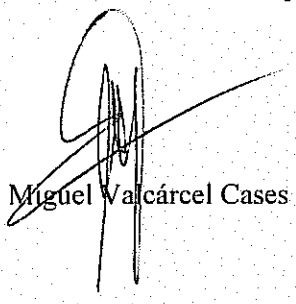
publicación de 7 artículos científicos, 5 de ellos ya publicados en revistas del primer cuartil del área de Química Analítica, y dos artículos de revisión ya publicados. También han sido fruto de esta Tesis Doctoral siete comunicaciones, 4 orales y 3 flash (oral+póster) a Congresos nacionales.

La estancia realizada en el grupo de investigación de La empresa GAS (Dortmund, Alemania) durante el desarrollo de la Tesis Doctoral ha completado de forma satisfactoria dicha formación, profundizando en el conocimiento de la espectrometría de movilidad iónica, ámbito de trabajo de esta empresa, spin-off de la Universidad de Dortmund.

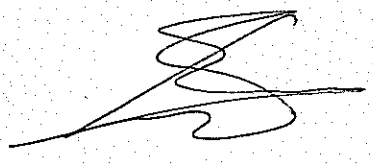
Por todo ello, consideramos que la investigación desarrollada y recogida en esta Memoria, reúne todos los requisitos necesarios en cuanto a originalidad, innovación y calidad, y autorizamos la presentación de la Tesis Doctoral de Doña Isabel Márquez Sillero.

Córdoba, 11 de julio de 2013

Firma del/de los director/es



Fdo.: Miguel Valcárcel Cases



Fdo.: Mª Soledad Cárdenas Aranzana

Mediante la defensa de esta Memoria de Tesis Doctoral se pretende optar a la obtención de la Mención de “Doctorado Internacional” habida cuenta de que la doctoranda reúne los requisitos para tal mención:

1. Cuenta con los informes favorables de dos doctores pertenecientes a instituciones de Enseñanza Superior de países distintos a España.

2. Uno de los miembros del tribunal que ha de evaluar la Tesis pertenece a un centro de Enseñanza Superior de otro país distinto a España.

3. Parte de la defensa de la Tesis Doctoral se realizará en una lengua distinta de las lenguas oficiales en España.

4. La doctoranda ha realizado una estancia de tres meses en la empresa G.A.S. Gesellschaft für Analytische Sensorsysteme mbH en Dortmund (Alemania), que han contribuido a su formación y permitido desarrollar parte del trabajo experimental de esta Memoria.

Índice

Objeto / Aim

La tendencia hacia la simplificación, automatización y miniaturización de los procesos de medida engloba también a la información que se genera en los laboratorios. En este sentido, es deseable el empleo de procesos de medida que ofrezcan información analítica simple, rápida, fiable y fácilmente comprensible para el cliente de manera que se agilice la toma de decisiones. En los últimos años, se ha puesto de manifiesto el potencial que los detectores no convencionales tienen en este contexto, debido fundamentalmente a la universalidad de su respuesta. Sin embargo, existen problemas asociados a la baja sensibilidad y selectividad de este tipo de detectores, los cuales pueden solucionarse mediante el diseño de interfases adecuadas que permitan su acoplamiento con módulos de extracción para la separación y preconcentración de los analitos de interés. Por otra parte, se está potenciando la incorporación de nuevos materiales en una amplia variedad de procesos de medida con el fin de mejorar sus propiedades analíticas. En este contexto, los nanotubos de carbono se han revelado como una herramienta eficaz para el desarrollo de procesos de extracción y los líquidos iónicos se pueden considerar como una alternativa a los disolventes orgánicos convencionales.

Teniendo en cuenta lo anteriormente expuesto, el objetivo principal de la Tesis Doctoral que se presenta en esta Memoria es evaluar de forma sistemática el potencial del uso de detectores no convencionales con el empleo de nuevos medios de extracción que supongan una mejora en las propiedades analíticas de los procesos de medida. Con este objetivo global, los objetivos específicos de la investigación a desarrollar son los siguientes:

- Diseñar y validar nuevas estrategias analíticas de vanguardia para la determinación rápida, simple y fiable de parámetros de interés en el ámbito alimentario. Se emplearán dos detectores no convencionales de tipo evaporativo y de respuesta cuasi-universal como el detector de dispersión de luz (ELSD) y el detector de transferencia de carga-corona (C-CAD) en cromatografía líquida.

- Incrementar la selectividad de las determinaciones mediante el empleo de nanopartículas de carbono como sorbentes en sistemas de extracción eficientes.

- Evaluar el potencial de la espectrometría de movilidad iónica para obtener información cualitativa y cuantitativa sobre compuestos volátiles presentes en muestras de interés alimentario. En este sentido será crucial el disponer de una interfase adecuada para llevar a cabo el acoplamiento entre el detector y la técnica de microextracción.

- Demostrar la aplicabilidad del triple acoplamiento espacio de cabeza - cromatografía multicapilar - espectrometría de movilidad iónica para analizar directamente el perfil de volátiles de una muestra con vistas a obtener información analítica sobre la presencia de compuestos que puedan afectar a la calidad del alimento.

- Aplicar los sistemas diseñados para la determinación de analitos en muestras de interés en diversas áreas como cosmética, alimentación o medio ambiente.

The current trend to simplify, automate and miniaturize analytical measurement processes has also reached laboratory-produced information. It is therefore desirable to use measurement procedures capable of providing accurate, easily understood analytical information in a simple, expeditious manner with a view to facilitating decision-making processes. In recent years, unconventional detectors have proved highly promising for this purpose by virtue of their providing universal responses. However, their low sensitivity and selectivity must be overcome by using effective interfaces to extraction equipment in order to separate and preconcentrate the target analytes. Also, new materials are being increasingly used in a wide variety of measurement processes to improve their analytical performance. In this context, carbon nanotubes have proved a useful tool for developing efficient extraction procedures and ionic liquids an effective alternative to conventional organic solvents.

Based on the foregoing, the primary aim of this doctoral work was to assess in a systematic manner the potential of unconventional detectors used in combination with the new extraction media for improving the analytical performance of measurement processes. The specific objectives derived from this general objective are as follows:

- *To design and validate new vanguard analytical strategies for the simple, accurate, rapid determination of nutritional parameters by using two universal-response detectors: an evaporative light scattering detector (ELSD) and a corona-charged aerosol detector (C-CAD).*

- *To improve the selectivity of the ensuing determinations by using carbon nanoparticles as sorbents for efficient extraction.*
- *To assess the potential of ion mobility spectrometry for extracting qualitative and quantitative information about volatile compounds present in nutritional samples by using a suitable interface to couple the detector and microextractor.*
- *To validate the use of the headspace–multicapillary chromatography–ion mobility spectrometry combination for the direct determination of volatile profiles with a view to extracting analytically useful information about the presence of substances potentially affecting food quality.*
- *To use the resulting systems for the determination of analytes in cosmetic, food and environmental samples.*

Introducción

Panorámica general de los detectores no convencionales basados en la nebulización, evaporación e ionización de la muestra

1. SISTEMAS ANALÍTICOS DE VANGUARDIA

Una de las tendencias de la Química Analítica es el desarrollo de procesos de medida que sean simples y rápidos y que a su vez, garanticen la fiabilidad y la calidad en los resultados obtenidos. Por ello, es necesario un cambio en el planteamiento de estas metodologías, de manera que se orienten a alcanzar, en la mayor medida posible estos requerimientos. En este contexto, surgen las estrategias analíticas de vanguardia actuales que se caracterizan por ofrecer una respuesta rápida, fiable y fácil de interpretar. Los sistemas analíticos basados en estas estrategias permiten procesar un elevado número de muestras con vistas a seleccionar sólo aquellas que contengan información relevante relacionada con el problema a resolver. Esta respuesta puede estar basada en índices globales o respuestas binarias tipo si/no. En algunas ocasiones, los sistemas analíticos de vanguardia permiten obtener información aún más detallada sobre los compuestos de interés sin perder las características de rapidez, sencillez y bajo coste. También, la necesidad de realizar análisis *in-situ*, requiere de dispositivos simples, robustos, respuesta rápida y facilidad en el transporte [1].

Los sistemas de vanguardia emplean detectores poco sofisticados, de uso sencillo y de bajo coste de adquisición y mantenimiento. Estos detectores pueden ser convencionales (espectrofotómetro, fluorímetro e infrarrojo) o no convencionales como el detector evaporativo de dispersión de luz (*evaporative light scattering detector*, ELSD), detector de transferencia de carga-corona (*corona-charged aerosol detector*, C-CAD) y el espectrómetro de movilidad iónica (*ion mobility spectrometer*, IMS). En la Figura 1 se comparan de forma esquemática estos tres detectores no convencionales teniendo en cuenta diferentes procesos de análisis.

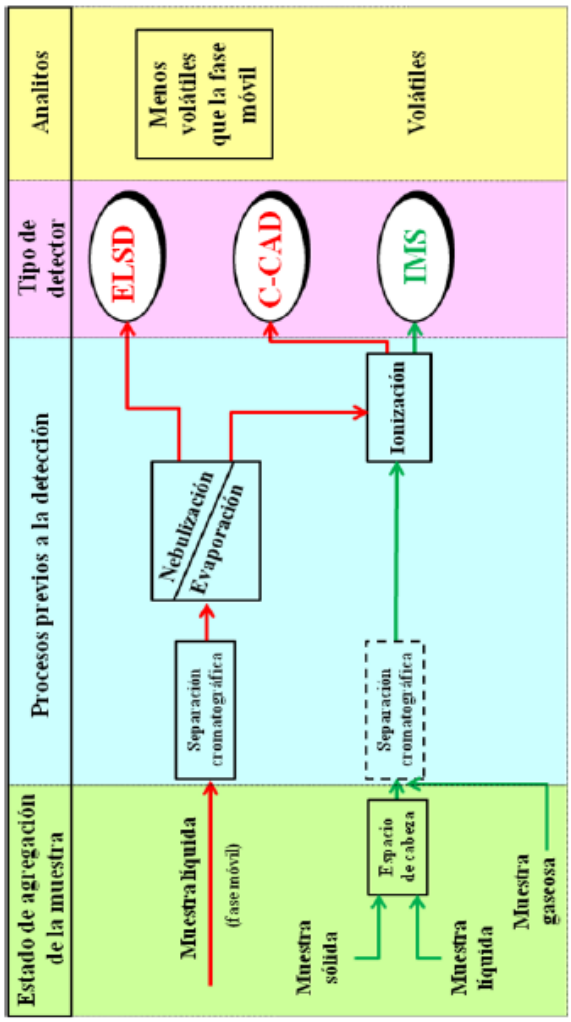


Figura 1. Visión conjunta de los detectores de tipo evaporativo y el espectrómetro de movilidad iónica en el proceso analítico.

El ELSD y el C-CAD se usan de manera exclusiva para muestras líquidas (o su extracto) de manera que la fase móvil la transporta a través del sistema cromatográfico. En cambio, el IMS está especialmente diseñado para el análisis de muestras gaseosas aunque también es compatible con la fracción volátil de muestras líquidas o sólidas previo paso de los analitos desde estas al espacio de cabeza. Para los tres detectores es posible introducir una separación cromatográfica de los analitos antes de su detección. Esta es prácticamente ineludible en los detectores evaporativos. El detector evaporativo de dispersión de luz y el de transferencia de carga tienen en común la nebulización y evaporación de la fase móvil en la que se encuentran los analitos. Este último y el espectrómetro de movilidad iónica comparten la ionización de los compuestos de interés presentes en la muestra antes de la detección de los mismos. El IMS se usa para la determinación de analitos volátiles, mientras que en los detectores de tipo evaporativo la volatilidad de los analitos tiene que ser inferior a la de la fase móvil que se use para llevar a cabo su separación cromatográfica. A continuación, se describe brevemente su fundamento, se muestra una comparación con detectores convencionales, se da a conocer los sistemas miniaturizados y portátiles y los acoplamientos aplicados a las diferentes áreas de análisis.

2. DETECTORES DE TIPO EVAPORATIVO

Los detectores de tipo evaporativo son una alternativa a los detectores no convencionales pues generan una respuesta cuasi-universal para una amplia variedad de compuestos o sus familias independientemente de sus propiedades físico-químicas. El detector de transferencia de carga-corona y el detector evaporativo de dispersión de luz se han desarrollado recientemente para su aplicación en cromatografía líquida de alta resolución (*high performance liquid chromatography*, HPLC) y cromatografía de fluidos supercríticos (*supercritical fluid chromatography,* SFC). En ambos detectores la fase móvil

se nebuliza, produciendo partículas de analito que son ópticamente detectadas en el caso del ELSD o por transferencia de carga para el C-CAD. Ambos se caracterizan fundamentalmente por ser robustos, sensibles, muy reproducibles, simples, de fácil uso y poseer un campo de aplicación bastante amplio.

2.1. Detector evaporativo de dispersión de luz (ELSD)

El ELSD se presentó por primera vez en 1966 en unos laboratorios australianos de Union Carbide. Pero no fue hasta 1991 cuando se empezó a utilizar de forma más continua en el ámbito de la Química Analítica, fundamentalmente como detector en cromatografía de líquidos. El funcionamiento del detector evaporativo de dispersión de luz consta de tres etapas (Figura 2): nebulización, evaporación y detección [2].

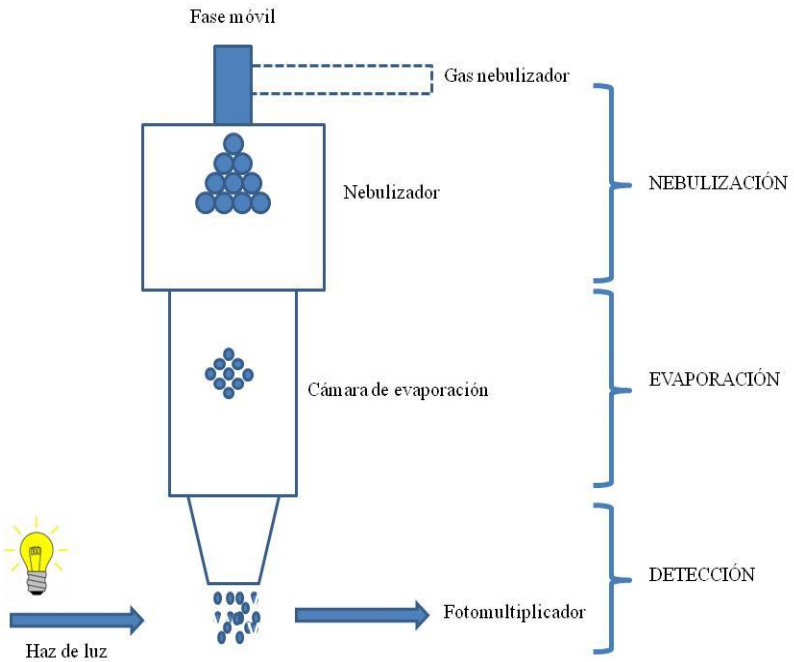


Figura 2. Esquema del funcionamiento del detector evaporativo de dispersión de luz.

El efluente procedente de la columna cromatográfica entra al nebulizador y por efecto Venturi se forma el aerosol. Para ello, el nebulizador necesita un caudal alto de gas (aire o cualquier gas inerte como nitrógeno, argón, helio o dióxido de carbono) para formar las gotas de tamaño uniforme. La homogeneidad y reproducibilidad en el tamaño de las gotas depende de la relación entre el diámetro de la boquilla de entrada y de los caudales de la fase móvil y el gas nebulizador. La distribución del diámetro de las gotas es un parámetro importante que afecta a la detección, y por tanto a la sensibilidad y precisión del método [3]. En la etapa de evaporación, el aerosol es arrastrado por la corriente de gas a una cámara de evaporación, donde el tamaño de las gotas se reduce como consecuencia de la eliminación de la fase móvil. La temperatura de la cámara de evaporación se elige en función de la composición de la fase móvil y la volatilidad de los analitos. Esta temperatura debe ser capaz de eliminar por completo la fase móvil sin producir pérdidas de analito. La temperatura de evaporación se encuentra entre 30-100 ºC. Después de la evaporación, el aerosol formado idealmente por partículas sólidas de analito entra en una célula óptica, a través de la que pasa un haz de luz. La luz dispersada se mide en un fotodiodo o fotomultiplicador, generando la señal analítica que es proporcional a la cantidad de analito que ha entrado en la célula óptica.

El ELSD se ha usado para la determinación de una amplia variedad de compuestos tanto en matrices sintéticas como naturales. Sus principales campos de aplicación son: farmacia, alimentación y bebidas y muestras biológicas, entre otros. Se usa como detector cromatográfico debido a su respuesta global y por su compatibilidad con los componentes de la fase móvil. Existen varios intentos de adaptación del ELSD a la cromatografía capilar y microcapilar. Una de las ventajas de usar el ELSD con la cromatografía capilar es la linealidad de la respuesta, debido a la uniformidad en el tamaño de la

gota como resultado de la disminución del caudal. De todos modos, la miniaturización del nebulizador es fundamental para asegurar la compatibilidad entre el microflujo del efluente procedente de la columna y el proceso de nebulización. De las aproximaciones desarrolladas, la primera de ellas, creada por Demirbüker, emplea caudales entre 5-100 μL^{-1}, aunque su principal limitación es que requiere de un estricto control de la geometría del nebulizador para asegurar la nebulización de la fase móvil [4]. En la segunda alternativa, se introduce un capilar de sílice fundida en la boquilla de entrada del nebulizador [5].

2.2. Detector de transferencia de carga-corona (C-CAD)

El detector de transferencia de carga-corona se introdujo comercialmente en 2004 por ESA Biosciences (Chelmsford, MA) [6], aunque esta tecnología fue desarrollada en 1970 [7,8]. Se basa en la combinación de la cromatografía líquida con la creación de un aerosol. La Figura 3 ilustra el funcionamiento del detector. En primer lugar, el eluyente procedente de la columna cromatográfica se nebuliza con una corriente de nitrógeno (o aire) de manera que se generan pequeñas gotas que facilitan la evaporación posterior de la fase móvil para formar entonces partículas del analito en cuestión. Paralelamente, una segunda corriente de nitrógeno se carga positivamente a su paso por un sistema de descarga corona de platino. Esta carga se transfiere a continuación a la corriente opuesta de partículas de analito que llega a un colector, donde un medidor de electrones genera una señal cuya intensidad es directamente proporcional a la cantidad de analito presente. Su uso está menos extendido que el ELSD y se emplea exclusivamente como detector en cromatografía líquida y de fluidos supercríticos. Sin embargo, los nuevos diseños de este detector están ampliando su campo de aplicación. Así el detector Corona® Ultra™, permite su acoplamiento a cromatografía líquida de ultra alta resolución (*ultra performance liquid chromatography*, UPLC)

manteniendo la velocidad y la resolución de esta modalidad cromatográfica [9,10]. Permite alcanzar una mayor sensibilidad pudiendo detectar cantidades de analito situadas en el nivel de nanogramo y picogramo. Este nuevo diseño se puede apilar en el equipo de UPLC y así ahorrar espacio y minimiza el volumen muerto. Una nueva versión de este detector permite que la temperatura del nebulizador pueda controlarse entre 5-35 ºC, lo que ayuda a proporcionar una respuesta más uniforme. El detector ofrece la posibilidad de trabajar con diferentes disolventes orgánicos, tales como acetona para HPLC de fase reversa y alquil cetonas o tolueno para fase normal. Estos disolventes absorben radiación en la mayor parte del espectro ultravioleta (*ultra-violet*, UV) y no son adecuados para muchas aplicaciones de HPLC-UV.

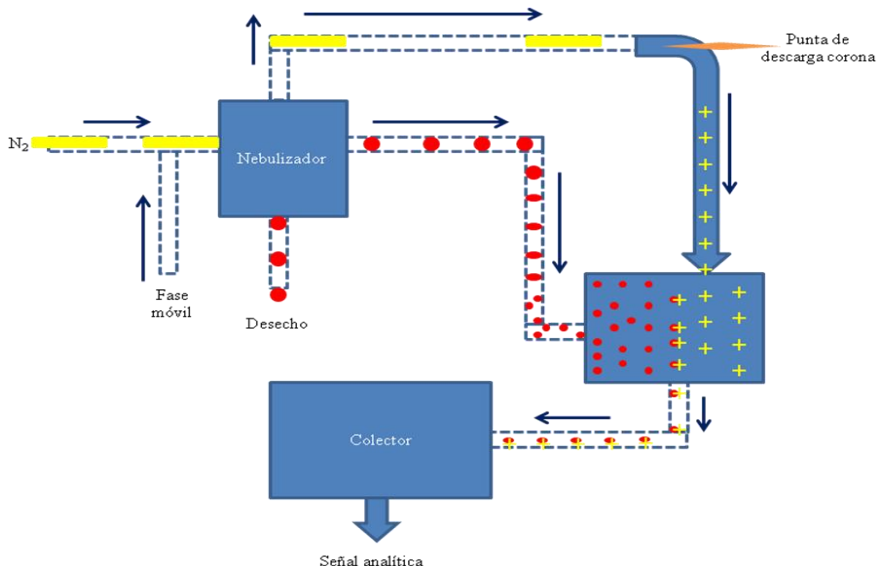


Figura 3. Esquema del funcionamiento del detector de transferencia de carga-corona.

2.3. Comparación con otros detectores

C-CAD y ELSD se consideran universales pero bajo esta categoría se pueden incluir también otros como el espectrómetro de masas (*mass spectrometer*, MS), índice de refracción (*refraction index*, RI),

quimioluminiscente y ultravioleta a baja longitud de onda [11-13]. Aunque el detector RI se usa con frecuencia, es el más limitado en cuanto a sensibilidad se refiere y no es compatible con el gradiente de elución en cromatografía. En cambio, el detector ultravioleta ofrece una mayor sensibilidad a baja longitud de onda, que es a su vez la región de menor selectividad. Sin embargo, la detección está limitada sólo a compuestos con grupos cromóforos como aminoácidos, carbohidratos, lípidos, polímeros, etc. Además, tiene facilidad de uso pero la respuesta depende de la absortividad molar, lo cual puede variar en órdenes de magnitud entre estructuras análogas. En el espectrómetro de masas, al igual que en los dos detectores anteriores, la respuesta depende de la estructura química del analito, pero ofrece información estructural de los compuestos. Sin embargo, como la respuesta depende del proceso de ionización, el análisis cuantitativo es menos robusto. Otras limitaciones a destacar son su elevado coste de adquisición y mantenimiento, lo que restringe su uso en laboratorios de rutina y necesita de personal cualificado para interpretar correctamente los espectros obtenidos [14]. En la Tabla 1 se resume de forma esquemática las características de estos detectores.

Tabla 1. Comparación de las características analíticas de los detectores "universales".

	C-CAD	ELSD	UV	RI	MS
Sensibilidad	++	++	+++	+	++++
Reproducibilidad	++++	++	++++	+++	+++
Compatibilidad cromatográfica	++++	++++	++	+	+++
Aplicabilidad	+++	+++	+	+	+++
Facilidad de uso	++++	++++	+++	+++	+

El C-CAD y ELSD están ganando terreno en los últimos años puesto que presentan ventajas sobre los detectores anteriormente comentados. Ambos permiten detectar compuestos de baja volatilidad y su respuesta no depende de las propiedades químicas del analito (relación m/z, potencial de oxidación, emisión a una longitud de onda específica, etc), aunque el detector de transferencia de carga lo hace de manera más homogénea. Las principales características de los detectores evaporativos son su alta sensibilidad (5-200 ng) y precisión (mejor del 2% expresada como desviación estándar relativa). Su uso es simple, no necesita de personal cualificado, y los únicos parámetros a controlar son la presión del gas de nebulización, la temperatura y la atenuación de la señal [15]. Sin embargo, presentan el inconveniente de que la respuesta depende de la composición de la fase móvil, obteniéndose mayor respuesta en aquellas fases móviles con un alto contenido de disolventes orgánicos. Este problema es más marcado en el C-CAD y para evitarlo se puede usar una compensación de gradiente inverso [16]. Otra carencia es que no ofrece información espectral, así que no es posible identificar inequívocamente al analito como en los detectores de MS y UV-diodos en fila. A pesar de estas limitaciones, sus ventajas respecto a otros detectores han hecho que su campo de aplicación se incremente.

Recientemente, en la bibliografía han comparado estos detectores para la determinación de diferentes compuestos en una amplia variedad de muestras usando diferentes columnas cromatográficas. Hay casos en los que la sensibilidad del C-CAD es mayor dependiendo de si se usa en cromatografía líquida de interacción hidrofílica o en fase reversa [17,18], mientras que para el ELSD, esta diferencia no es notable. Generalmente, el detector de transferencia de carga-corona es más sensible y preciso que el de dispersión de luz [13,19]. Se ha comprobado que cuando se trabaja en microcromatografía a altas temperaturas la sensibilidad mejora en ambos detectores [20]. A bajas

concentraciones, el C-CAD es mejor que el ELSD, pero esta diferencia no es tan significativa a altas concentraciones (≈10 mg L^{-1}) [21]. Por otro lado, hay casos en los que el ELSD presenta una mayor precisión y un intervalo dinámico más amplio que el C-CAD.

Aunque actualmente los ámbitos biológico, clínico y farmacéutico son los principales campos de aplicación del C-CAD y ELSD, cada vez es mayor la presencia de estos detectores en el análisis medioambiental y de alimentos.

3. ESPECTROMETRÍA DE MOVILIDAD IÓNICA

La espectrometría de movilidad iónica (*ion mobility spectrometry*, IMS) emergió en 1970 como una técnica analítica avanzada para la determinación de compuestos orgánicos volátiles o semivolátiles basada en la separación de sus iones gaseosos bajo la influencia de un campo eléctrico a presión atmosférica y en un tiempo de milisegundos. Sin embargo, hasta 1990 no se introdujeron comercialmente los instrumentos de IMS. Esta técnica es similar a la espectrometría de masas-tiempo de vuelo con la diferencia de que la separación se produce a presión atmosférica, lo que la hace más simple en cuanto a instrumentación se refiere y permite separar los iones por forma y tamaño. La IMS se caracteriza por su alta sensibilidad, robustez, rapidez en la respuesta, bajo coste de adquisición y portabilidad. Se han diseñado y desarrollado numerosos espectrómetros de movilidad iónica para ser utilizados en el campo militar, forense, alimentario y farmacéutico, entre otros.

3.1. Fundamento

La IMS se fundamenta en la diferente movilidad que muestran los iones gaseosos bajo el efecto de un campo eléctrico constante. Un espectrómetro de movilidad iónica está formado por una cámara de ionización, un obturador de iones, una cámara de separación y una unidad de detección.

Estos componentes describen las etapas que constituyen el funcionamiento de un espectrómetro de movilidad iónica. La Figura 4 muestra un esquema general de un espectrómetro de movilidad iónica y las principales etapas se comentan en detalle a continuación.

3.1.1. Introducción de muestra e ionización

En primer lugar, la muestra gaseosa se introduce mediante un gas portador en la cámara de ionización, donde se forman los iones cuya carga depende de la fuente de ionización empleada. El objetivo de los sistemas de introducción de muestra es transportar los analitos hasta la cámara de ionización, volatilizándolos en caso de que sea necesario. La configuración de este elemento depende de la naturaleza de la muestra, del tipo de ionización que se vaya a emplear y de los requisitos del análisis [22]. El más común es la vaporización o desorción térmica [23-25], aunque también se pueden emplear membranas [26,27], spray o electrospray (muestra líquida) [28,29], microextracción en fase sólida (muestra líquida) [30,31] y vaporización por ablación láser (muestra sólida) [32,33].

La ionización ocurre a presión atmosférica y existe una amplia variedad de fuentes de ionización, aunque las más comunes son las fuentes radiactivas de ionización química [23,25]. Una alternativa a los materiales radiactivos son las fuentes de ionización de descarga corona (*corona discharge*, CD) [34,35]. La ionización también se puede llevar a cabo a través de la fotoionización (por láser [36] o lámpara ultravioleta [37]), ionización en electrospray [29], ionización en superficie [38] e ionización en microplasma [39].

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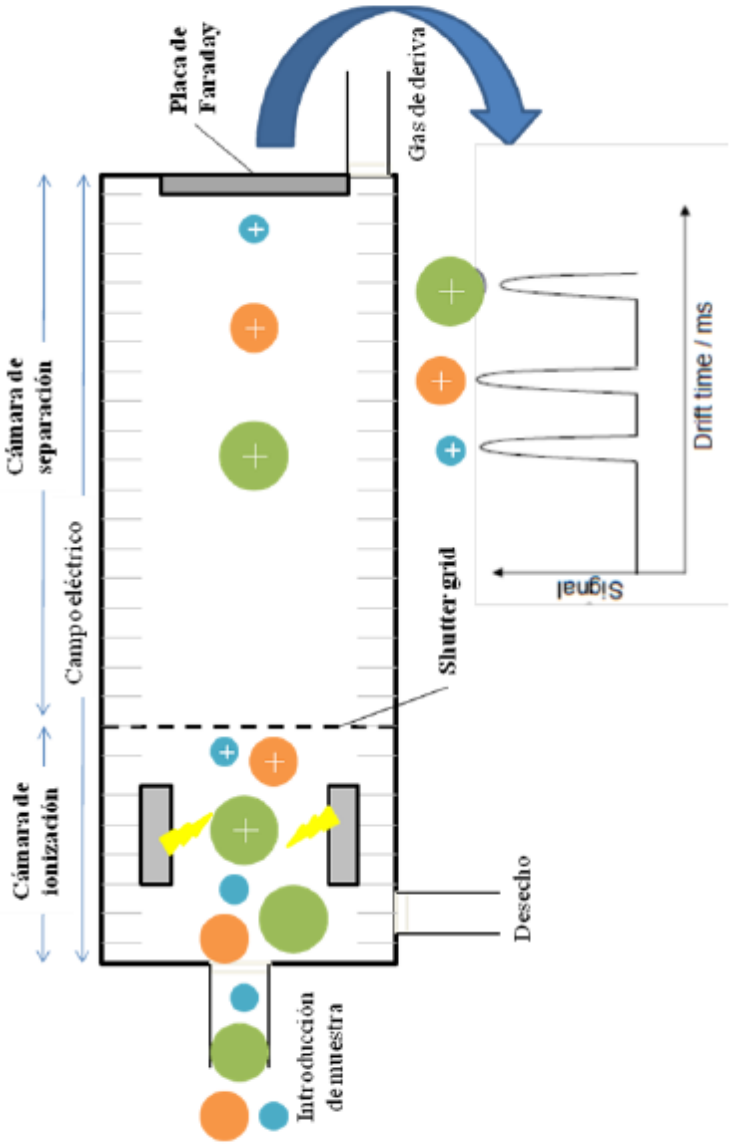

Figura 4. Esquema general del funcionamiento de un espectrómetro de movilidad iónica convencional.

Las fuentes radiactivas no precisan de una fuente de alimentación externa, no tienen componentes portátiles pero necesitan de un control de posibles fugas radiactivas. La fuente radiactiva más usada es ^{63}Ni, aunque también se han utilizado ^{3}H y ^{241}Am. Dentro de las fuentes no radiactivas, las basadas en descarga corona producen una corriente de iones hasta 100 veces mayor que la generada por la fuente radiactiva ^{63}Ni, lo que da lugar a límites de detección menores. Como inconveniente se puede destacar que necesita una fuente de alimentación externa y de un mantenimiento. Tanto la ionización radiactiva como la de descarga corona generan iones positivos y negativos. La fuente de ionización basada en la fotoionización incluye: lámparas UV, láser, MALDI (*matrix-assisted laser desorption ionization*) y SELDI (*surface enchanced laser desorption ionization*). Los principales inconvenientes de estas fuentes de ionización por UV son el coste de las lámparas y la necesidad de reemplazarlas periódicamente una vez que finaliza su vida útil. Su principal ventaja es la selectividad en la respuesta, bien por la selección de una longitud de onda o por una energía de ionización apropiada. La ionización por electrospray se usa para muestras líquidas y para evitar la saturación del detector es necesario incluir un tiempo de limpieza para eliminar restos de matriz de la aguja. La ionización en superficie emplea un cristal de molibdeno dopado con iridio calentado como máximo a 500 ºC. En este tipo de ionización, la respuesta depende de la estructura del analito y ante el depósito de determinados compuestos, la superficie cambia y no permite la ionización. Por último, la fuente de ionización en microplasma consiste en un vaso capilar que proporciona un caudal de gas He o N_2 y filamentos de plata a su alrededor para formar los electrodos donde tienen lugar microdescargas. Una alternativa a las fuentes radiactivas o de fotoionización es la fuente de ionización por llama que además permite ampliar el campo de detección de compuestos pero, debido a la complejidad de su incorporación al tubo de deriva, aún no se ha comercializado.

3.1.2. Separación de iones

Los iones procedentes de la cámara de ionización se transfieren a la cámara de separación a través de un obturador de iones o "*shutter grid*". El obturador de iones permite el paso de los iones a un intervalo de tiempo fijo. Su apertura está sincronizada con el inicio del proceso de adquisición de datos y es la base para medir los tiempos de desplazamiento de los diferentes iones, desde el *shutter grid* hasta el detector. El dispositivo consiste en filamentos o cables delgados conductores colocados próximos entre ellos, de forma paralela y coplanar y bajo tensión, sobre un soporte no conductor. Cuando está abierto, presentan el mismo potencial y cambia a diferentes potenciales cuando está cerrado. La rendija de entrada suele abrirse durante 200-300 μs cada 20-30 ms aproximadamente. El obturador de iones es un factor limitante en la forma y resolución de los picos pues tiempos de apertura cortos limitan la sensibilidad de IMS, pero si aumentan, puede haber problemas de ensanchamiento de los picos.

La separación de los iones tiene lugar en la cámara de separación o tubo de deriva. Para que tenga lugar la separación, los iones se someten a un campo eléctrico débil constante a través de un gas de deriva que fluye en dirección opuesta al movimiento de los iones hacia el detector y del gas portador de muestra. El gas de deriva no debe contener moléculas con las que los iones puedan formar *cluster* o reaccionar con ellos. Los iones colisionan con las moléculas del gas de deriva, lo que causa una reducción en la velocidad relativa de los iones, en función del tamaño y la forma de los mismos. Los primeros tubos de deriva, que aparecieron en 1970 [40,41], consisten en anillos metálicos (acero inoxidable, aluminio o cobre) y anillos aislantes (zafiro, cristal, cerámica o teflón) apilados. Entre los anillos se producen diferencias de potencial y se establece un campo eléctrico constante, lineal y uniforme. Los campos eléctricos pueden variar entre 200 y 400 Vcm^{-1},

en tubos de deriva entre 5 y 20 cm y una fuente de alto voltaje desde ± 1 a 8 kV. Cuanto más largo sea el tubo de deriva, más tiempo tardan los iones en llegar al detector, por lo que tienen más tiempo para separarse. Con ello se incrementa la resolución pero disminuye la intensidad de la señal. Los diseños y materiales que se emplean para la construcción del tubo de deriva influyen en la resolución, en la relación señal/ruido y en la durabilidad.

Los gases usados suelen ser nitrógeno, aire sintético, helio, argón, dióxido de carbono o hexafluoruro de azufre. Normalmente, el caudal del gas de muestra es de 50-200 mL min^{-1} y el del gas de deriva hasta 700 mL min^{-1} y ambos fluyen en sentido opuesto de manera que el gas de muestra permite llevar la muestra en forma gaseosa hasta la cámara de ionización y el gas de deriva impide que los iones sigan reaccionando en el tubo de deriva.

3.1.3. Detección de iones y adquisición de la señal

Al final de la cámara de separación se encuentra el detector. El más usado es una placa de Faraday, que consiste en un electrodo circular que se coloca al final del tubo de deriva y recibe el impacto de los iones a detectar. Los iones se neutralizan por transferencia de electrones y la señal se amplifica a potenciales de 1 a 10 V. Es un detector muy simple y de baja sensibilidad.

Este detector genera señales analógicas que posteriormente se digitalizan y se almacena en forma de espectros. Existen varias formas de visualizar los datos en un gráfico XY, donde el tiempo de deriva de las especies iónicas se representa en el eje X y la intensidad de la señal en el eje Y. El espectro de movilidad se obtiene promediando un número determinado de espectros para eliminar ruido. Otro modo es representando cada espectro de movilidad en función del tiempo de análisis (eje Z), creando un gráfico tridimensional. La Figura 5 muestra diferentes gráficas obtenidas para una

muestra de vino con un espectrómetro de movilidad iónica con una fuente de ionización radiactiva de ^{3}H.

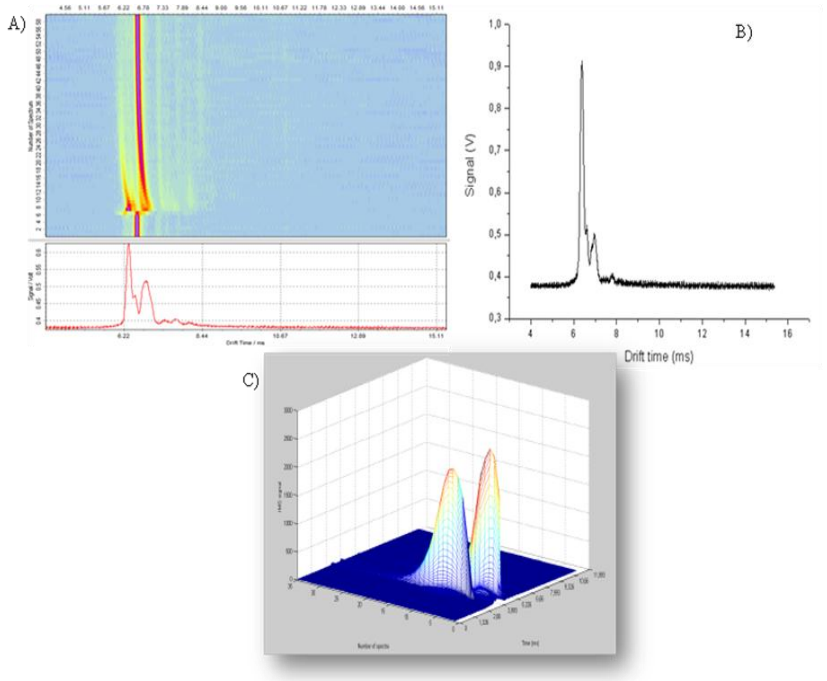

Figura 5. Posibilidades de visualización de la señal obtenida en un espectrómetro de movilidad iónica. A) Mapa topográfico; B) Gráfico bidimensional; c) Gráfico tridimensional.

3.2. Tendencias de la espectrometría de movilidad iónica

Las tendencias de esta técnica se han centrado fundamentalmente en dos aspectos:

- Miniaturización
- Hibridación

En este último caso se contempla tanto el acoplamiento en serie con otros detectores, básicamente la espectrometría de masas por la compatibilidad entre ambas, como con sistemas automáticos de introducción de muestra para ampliar la aplicabilidad de estos detectores.

3.2.1. Detectores IMS miniaturizados

Existen diferentes sistemas miniaturizados en IMS que se caracterizan por su sensibilidad (pueden detectar compuestos a concentraciones inferiores a ng L^{-1}), su velocidad de respuesta (en pocos ms), lo que permite obtener la señal en tiempo real y su robustez [42]. Sin embargo, presentan como inconvenientes la limitada selectividad debido a la baja resolución y el uso restringido de fuentes radiactivas. Además, la principal limitación está relacionada con la pérdida de resolución es la reducción de las dimensiones de los diferentes elementos del sistema. El tubo de deriva debe ser pequeño, lo que limita la migración de iones [43]. Otro parámetro crítico a tener en cuenta es la miniaturización de la rendija de entrada que se utiliza para controlar el pulso de iones que pasa de la fuente de ionización al tubo de deriva. El tiempo de apertura de la rendija y su diseño afecta también a la resolución final de las medidas y a la relación señal-ruido. Cuando el tiempo de apertura de la rendija es corto, se produce una pérdida de la señal a causa de la pequeña cantidad de iones que llegan al tubo de deriva. Sin embargo, cuando el tiempo de deriva es mayor, la señal aumenta pero los picos se ensanchan y hay una pérdida de resolución en el IMS [44]. La posibilidad de controlar este parámetro es un aspecto deseable en la miniaturización de IMS con el fin de ajustar el tiempo de apertura para obtener la máxima resolución. En la última década, varios grupos de investigación han realizado grandes avances en el diseño de tubos de deriva de IMS, con unas nuevas configuraciones que permiten simplificar la construcción de tubos de deriva, posibilitando una producción en serie de bajo coste, así como introducir mejoras sustanciales en la sensibilidad y resolución. Una alternativa a los tubos de deriva de anillos apilados son los tubos de deriva basados en diseños de tubos de vidrio y tubos de deriva de resistencia. Zimmermann propuso un nuevo concepto de IMS miniaturizado con dimensiones de chip basado en diseños anteriores [45,46]. Las fuentes de ionización usadas en IMS miniaturizados están limitadas a la de radiación UV

[43] y la descarga corona [47], probablemente por restricciones de seguridad existentes en la mayoría de los países a las fuentes radiactivas. Los IMS miniaturizados se han usado para la determinación de compuestos orgánicos volátiles teniendo como finalidad su futura aplicación en el área de seguridad [48] y médica [49].

A pesar de estas limitaciones, la miniaturización de estos detectores facilita su portabilidad y esto ha dado lugar a la comercialización de diversos IMS portátiles. La mayoría de estos dispositivos son sistemas cerrados con circuitos de gas (nitrógeno o aire sintético) que contienen niveles de humedad mayores que los sistemas abiertos. En los sistemas abiertos, el uso de un gas inerte como gas de entrada y de deriva mejora la sensibilidad y la resolución de los espectros IMS, evitando los problemas relacionados con la humedad de un circuito de gas interno [50]. Al principio, la mayoría de los instrumentos IMS portátiles se usaron para la detección de explosivos y agentes de guerra química [51,52]. El primer uso visible fuera del campo militar es el uso en la detección de explosivos en equipaje de pasajeros en la aviación comercial. Por otra parte, la mayoría de los instrumentos portátiles son adecuados para llevar a cabo la determinación de analitos específicos como la cuantificación de azufre en gas natural [53], benceno, tolueno, etilbenceno y xileno en aguas [54], sibutramina en suplementos dietéticos [55], nicotina en aire [56], presencia de heroína en cocaína [57] o compuestos orgánicos volátiles [58].

3.2.2. Hibridación instrumental

La IMS pueden acoplarse a la espectrometría de masas denominándose espectrometría de masas de movilidad iónica (IM-MS) o espectrometría de movilidad iónica- espectrometría de masas de trampa de iones (IMS-Ion Trap-MS), dependiendo del tipo de detector de masas hibridado. Igualmente, puede

acoplarse con técnicas de separación cromatográfica, de líquidos o de gases empleando columnas convencionales o multicapilares.

La movilidad iónica acoplada a la espectrometría de masas es una técnica que separa simultáneamente iones gaseosos en base a su masa, forma y tamaño. Los avances producidos en MS se reflejan también en IM-MS, lo que permite su aplicación al estudio estructural de biomoléculas. Aunque es una técnica de baja resolución, puede proporcionar información de gran interés sobre la estructura microscópica y población de las especies. Dadas estas ventajas, IM-MS se está convirtiendo en una técnica fiable y versátil para la biología estructural [59].

Desde 1970, IMS ha sido considerado como un detector potencial para GC. Sus ventajas frente a la espectrometría de masas es que no necesita un sistema de vacío, es simple, portátil, reduce espacio, peso y costes de adquisición y mantenimiento. El espectrómetro de movilidad iónica permite la separación de compuestos en función de su masa, tamaño y forma, en cambio el espectrómetro de masas lleva a cabo la separación en base a la relación masa/carga de los iones generados. Conlleva, en la mayoría de los casos la fragmentación de la molécula de analito generando un espectro que permite la identificación inequívoca del compuesto. En la Tabla 2, se comparan esquemáticamente las características de ambos espectrómetros.

Tabla 2. Comparación de las características de los espectrómetros de movilidad iónica y masas.

	IMS	MS
Resolución	Baja	Alta
Respuesta	Masa, tamaño y forma	Masa y carga
Vacío	No	Sí
Portabilidad	Alta	Baja
Facilidad de uso	Simple	Complejo
Coste	Bajo	Elevado
Espacio/Peso	Bajo	Alto

Por otro lado, la presencia de iones interferentes presentes en la muestra es un problema a la hora de extraer la información que procede de los compuestos de interés. No obstante, una pre-separación cromatográfica minimiza el número de componentes en la etapa de ionización, simplificando la respuesta [60]. Recientemente, se ha empleado para el análisis de muestras en matrices complejas en la estación espacial internacional [61]. Otra alternativa de miniaturización del sistema consiste en sustituir la columna convencional de cromatografía de gases por una columna multicapilar. En este caso, se emplea una columna con una longitud entre 4 y 250 mm y un diámetro entre 2 y 5 mm rellena de cientos de capilares con un diámetro de 20 a 80 µm. Sus ventajas son sus reducidas dimensiones, alta capacidad y respuesta rápida, aunque tienen baja disponibilidad y un alto coste de adquisición [62]. La IMS se puede acoplar a otras técnicas tales como UPLC [63], cromatografía de fluidos supercríticos [64] o TOF [65]. El acoplamiento con estas técnicas se emplea mayoritariamente en el área de metabolómica.

En cuanto al acoplamiento con sistemas automáticos de introducción de muestra actualmente, se dispone de un IMS acoplado a un automuestreador de espacio de cabeza lo que permite ampliar su uso para la determinación de

compuestos en matrices complejas de diferente naturaleza y estado de agregación. Este acoplamiento es muy simple y está disponible comercialmente. Además, se puede incluir el uso de una columna semicapilar o una columna multicapilar de gases para realizar una separación cromatográfica previa a la detección de los analitos.

* * * * *

En esta Memoria de Tesis Doctoral se han desarrollado nuevas aplicaciones de estos detectores no convencionales, de manera que se pueda extender su campo de aplicación al análisis de muestras de alimentos o productos de cuidado personal, para determinar tanto macrocompuestos como compuestos minoritarios. Los procesos de medida desarrollados se han dividido en dos bloques. En el primero de ellos se recogen aquellos que emplean detectores de tipo evaporativo para la determinación de parabenos en productos cosméticos, vitaminas hidrosolubles en leche y suplementos vitamínicos y azúcares en distintos alimentos y productos empleados en repostería. En este último caso, se ha comparado la respuesta del C-CAD y ELSD para el problema analítico planteado. El segundo bloque se centra en el empleo de la espectrometría de movilidad iónica para la determinación de contaminantes en vinos y presencia de productos de degradación de ácidos grasos omega-3 en aceites y leches enriquecidos. Por último, se mostrará un resumen de los resultados obtenidos, así como las conclusiones más relevantes de esta Tesis Doctoral.

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Herramientas analíticas

El trabajo experimental desarrollado en esta Memoria se ha llevado a cabo gracias al uso de una serie de herramientas analíticas, entre las que se incluyen estándares y reactivos, muestras, aparatos e instrumentación de diferente naturaleza. En esta sección de la Memoria de Tesis Doctoral se enumeran dichas herramientas y se describen aquellas que se han considerado más relevantes y novedosas.

1. ESTÁNDARES Y REACTIVOS

1.1. Analitos (patrones)

Los analitos utilizados para llevar a cabo la investigación fueron de pureza analítica o superior. Los compuestos objeto de estudio en la presente Memoria se enumeran a continuación agrupados por familias:

- Parabenos: Metilparabeno, etilparabeno, propilparabeno y butilparabeno se usan comúnmente como conservantes y se suelen encontrar en combinaciones de dos o más en alimentos, productos cosméticos y de farmacia. Recientes investigaciones afirman que el uso de parabenos provoca riesgos potenciales en la salud debido a su actividad estrogéna y disruptora en el sistema endocrino. Además, se les relaciona con el desarrollo de cáncer de mama. La concentración máxima total permitida de parabenos por la legislación europea es de 0.4% en peso.

- Vitaminas hidrosolubles: De las siete vitaminas seleccionadas, seis de ellas pertenecen al grupo de vitaminas B: vitamina B_1 o tiamina, vitamina B_3 o ácido nicotínico, vitamina B_5 o ácido pantoténico, vitamina B_6 o piridoxina, vitamina B_7 o biotina y vitamina B_9 o ácido fólico. La otra vitamina empleada fue la vitamina C o ácido ascórbico. Todas ellas pertenecen al grupo de vitaminas hidrosolubles, las cuales

son coenzimas o precursores de coenzimas necesarias para muchas reacciones químicas del metabolismo.

- Azúcares: De este grupo de analitos se han determinado fructosa, glucosa, sacarosa, maltosa, lactosa y maltotriosa. Los azúcares se clasifican según el número de unidades de los que están formados: monosacáridos formados por una sola unidad (fructosa y glucosa), disacáridos formados por dos unidades iguales o diferentes (sacarosa, maltosa y lactosa) y trisacáridos formados por tres unidades (maltotriosa). Los nombrados anteriormente se encuentran entre los mayoritarios en la naturaleza.

- 2,4,6-Tricloroanisol: Es el principal haloanisol responsable del efecto "sabor a corcho" en vinos. Un vino se considera defectuoso cuando la concentración de este analito se encuentra entre 10-40 ng L^{-1} aunque se puede percibir en concentraciones inferiores a 10 ng L^{-1}. Su presencia en vinos provoca grandes pérdidas en la industria vinícola.

- Productos de degradación de los ácidos grasos poliinsataurados omega-3: En los últimos años, se están incorporando los ácidos de cadena larga omega-3 a numerosos alimentos. Son susceptibles de sufrir una oxidación lipídica, lo que afecta negativamente a las propiedades sensoriales (olor y sabor) de los mismos. Los factores que producen la oxidación lipídica son principalmente temperatura, oxígeno y luz. Hexanal, 2-butanona, acetona y disulfuro de dimetilo son algunos de los productos de degradación que se crean en los alimentos enriquecidos con omega-3 como consecuencia de su degradación.

1.2. Disolventes orgánicos

A lo largo de esta Memoria se han utilizado varios disolventes orgánicos, adquiridos con una pureza analítica o superior en Panreac (Barcelona, España), los cuales se enumeran a continuación:

- Metanol.
- Acetonitrilo.
- Acetona.
- Etanol.

Se han empleado con diferentes finalidades, entre las que cabe destacar las siguientes:

- Preparación de disoluciones estándar de analitos.
- En procesos de extracción en fase sólida.
- Preparación de fases móviles en cromatografía de líquidos.

1.3. Otros reactivos

- Ácido clorhídrico.
- Ácido acético.
- Acetato amónico.
- Carbonato de sodio.
- Nitrógeno BIP, de pureza 5.0.
- Nitrógeno Premier.
- LiChrolut EN (40-120 µm).

1.4. Nuevos medios de extracción

En los métodos desarrollados y descritos en esta Memoria se hace uso de nuevos materiales extractantes que se utilizaron en fase sólida

empaquetando el sorbente en cartuchos de SPE comerciales y en unidades de microextracción en gota.

1.4.1. Nanotubos de carbono de pared múltiple (MWCNTs)

Se adquirieron en Sigma-Aldrich (Madrid-España). Estos nanotubos de carbono tienen una longitud que varía entre 0.5-200 µm y un diámetro interno entre 5 y 10 nm. En su síntesis se obtiene un producto con una pureza superior al 95%. Los nanotubos de carbono de pared múltiple se eligieron como material sorbente para la preconcentración y separación de parabenos en productos cosméticos por las interacciones π-π que establecen con los anillos aromáticos de los analitos de interés. Para esta aplicación, se empaquetaron 20 mg de este material en cartuchos cormeciales de 3 mL. Antes de cada muestra, se acondicionaron con 5 mL de metanol y equilibraron con 5 mL de agua Milli-Q. El material pudo reusarse 200 veces sin que se apreciara pérdida en su capacidad sorbente (Capítulo 2).

1.4.2. Líquidos iónicos (ILs)

En dos aplicaciones propuestas en esta Memoria (Capítulos 6 y 7) se empleó el líquido iónico 1-hexil-3-metilimidazolio bis(trifluorometilsulfonil)-imida [Hmim] [NTf_2] (Merck, Darmstadt, Alemania) como extractante en la microextracción en gota empleada previa a la introducción en el sistema de detección. Se usó este líquido iónico por su alta densidad lo que permitió una mayor estabilidad de la gota, facilitando así el empleo de mayores volúmenes que los que se pueden utilizar con disolventes convencionales. Además, la baja presión de vapor que presentan los líquidos iónicos minimiza la evaporación de la gota, lo que da lugar a medidas más reproducibles. El volumen de gota de líquido iónico necesario para llevar a cabo la extracción fue de 2 µL en ambas aplicaciones.

1.5. Materiales de laboratorio

- Microjeringas de vidrio de 50 µL y de 10 µL, ésta última con punta biselada.
- Material de vidrio de diferente volumen como probetas, matraces aforados, tubos de ensayo, vasos de precipitado y botes de vidrio ámbar.
- Viales de vidrio de 5, 10 y 20 mL.
- Micropipetas de hasta 200, 1000 y 5000 µL.
- Tapones magnéticos con septum de silicona.
- Cartuchos comerciales de extracción en fase sólida de 3 mL de capacidad.
- Fritas de polipropileno de 20 µm de tamaño de poro.
- Filtros desechables de Nylon de 0.45 µm de tamaño de poro y con un diámetro interno de 13 y 25 mm.
- Agitador magnético.
- Mortero.
- Varilla de vidrio.
- Espátula.
- Cúter.
- Parafina.
- Guantes de látex y máscara de protección para el rostro.

- Contenedores de plástico de diferentes tamaños para la correcta gestión de residuos de laboratorio.

2. MUESTRAS

En el trabajo presentado en la Memoria se han analizado diferentes tipos de muestras:

- Productos cosméticos: Se analizaron diferentes tipos de productos cosméticos (crema para después del sol, de manos, hidratante, antiarrugas, champú, maquillaje) mediante el método propuesto en el Capítulo 2. Las muestras se adquirieron en centros comerciales y se almacenaron a temperatura ambiente hasta su análisis.

- Agua: El método descrito en el Capítulo 6 se aplicó a la determinación de 2,4,6-TCA en muestras de agua embotellada. Todas las muestras se conservaron en su envase original y se adquirieron en centros comerciales.

- Vino: En los Capítulos 6, 7 y 8 se emplearon diferentes tipos de vino (de mesa, dulce, blanco, tinto y espumoso). Se mantuvieron en su envase original y se analizaron dentro de las 48 h posteriores a su compra en centros comerciales de Córdoba. Se adquirieron estas muestras envasadas en diferentes materiales (vidrio, tetra brick y bag in box) y tapones (corcho, goma y rosca) para estudiar su efecto en la presencia del analito de interés.

- Tapones de corcho: Se analizaron con el método propuesto en el Capítulo 8. Los corchos procedían de botellas de vino comerciales y de una bodega. El corcho se troceó en pequeños trozos cuyo tamaño aproximado fue de 1 mm x 2 mm x 3 mm que se almacenaron a 4 ºC

hasta su análisis para prevenir posibles pérdidas de compuestos volátiles.

- Aceite de linaza: Esta muestra se adquirió en comercios de Alemania y de Córdoba. Se utilizó en la investigación recogida en el Capítulo 9 en la que se tuvo en cuenta diferentes condiciones de almacenamiento (temperatura, oxígeno y luz) a lo largo de 36 días.

- Suplementos dietéticos: Se conocen también como suplementos nutricionales o alimenticios, se consumen por vía oral y contienen un "ingrediente alimenticio" destinado a complementar la alimentación, en este caso, las vitaminas. Se adquirieron en farmacias de Córdoba y en diferentes presentaciones, como pastillas, cápsulas o cápsulas suaves de gelatina. Como se explica en el Capítulo 3, el único tratamiento previo de la muestra fue la dilución en agua Milli-Q.

- Productos lácteos: Se analizaron una serie de productos lácteos entre los que se encuentran leche, leche enriquecida con omega-3 natural o con diferentes aromas, leche en polvo, batidos de diferentes sabores y leche enriquecida con vitaminas. Todas las muestras se adquirieron en comercios de Córdoba. Dependiendo del objeto de estudio, las muestras se conservaron en diferentes condiciones de almacenamiento de temperatura, oxígeno y luz. Los Capítulos 3, 4 y 9 recogen los estudios que se realizaron con estas muestras.

- Productos de repostería: Se analizaron una gran variedad de productos de repostería (salsas, siropes, gelatinas, mieles, coberturas y mermeladas) para conocer su contenido en azúcares (Capítulo 4). Fueron suministradas por una repostería de Montalbán (Córdoba) y se conservaron en su envase original y a 4 ºC hasta su análisis.

3. APARATOS Y PEQUEÑA INSTRUMENTACIÓN

Durante el desarrollo del trabajo experimental realizado en esta Tesis Doctoral, se emplearon los siguientes aparatos e instrumentos comunes en el laboratorio analítico:

- Interfase multicanal HP 35900C (Agilent, Palo Alto, CA), conversor A/D, para el registro de los datos obtenidos con los detectores de tipo evaporativo.

- Baño de ultrasonidos (Branson 3510E, Connecticut, EEUU), para la desgasificación de las diferentes fases móviles empleadas y homogeneización de muestras.

- Bomba cuaternaria de alta presión Hewlett-Packard 1050 Series (Agilent Technologies, Madrid, España).

- Bomba de HPLC Jasco 1585 (Jasco Analítica España, Madrid, España).

- Estación de vacío VacElut (Scharlab, Madrid, España), constituida por una cámara con paredes de vidrio con una tapa de polietileno de 20 posiciones, dotada de una gradilla para situar los tubos en los que se recoge el eluido de cada cartucho. Este dispositivo tiene un sistema de control de presión mediante el cual se monitoriza el grado de vacío existente en la cámara de extracción.

- Centrífuga Centronic BL-II (J.P. Selecta, Barcelona, España), con una velocidad máxima de 13500 rpm.

- Generador de nitrógeno Mistral 4 (Clan Tecnológica, Sevilla, España) necesario para el adecuado funcionamiento de los detectores de tipo evaporativo.

- Cámara de secado con convención natural Binder ED53, la cual puede alcanzar una temperatura máxima de 300 ºC.

- Horno para columnas cromatográficas LKB Bromma 2155 (Uppsala, Suiza) con una temperatura máxima de 90 ºC.

- Agitador magnético (Velp Científica, Milán, Italia) para la agitación de muestras.

- Medidor de flujo Alltech Digital Flow Check HR™ (Chromatographie Service GmbH) para controlar el caudal de entrada y salida al espectrómetro de movilidad iónica.

- pHmetro Crison micropH 2000 (Crison, Barcelona, España).

- Balanza analítica OHAUS Explorer (OHAUS, Nänikon, Suiza) que puede realizar medidas de masas desde 0.01 a 110 mg, con un error de 0.0001 g.

4. INSTRUMENTACIÓN

En el desarrollo de esta Tesis Doctoral se ha hecho uso de diferentes equipos instrumentales. Se han utilizado sistemas cromatográficos acoplados a detectores de tipo evaporativo como son el detector de transferencia de carga-corona (C-CAD) y el detector evaporativo de dispersión de luz (ELSD). El primero ha permitido la determinación de parabenos en productos cosméticos y de vitaminas en leche y suplementos dietéticos. En una última etapa del trabajo experimental, se ha realizado una comparación entre ambos detectores tomando como problema analítico modelo la determinación de azúcares en diferentes tipos de productos de repostería y productos lácteos (Bloque I).

Además, se han empleado detectores de espectrometría de movilidad iónica (IMS) fabricados por la empresa Gesellschaft für Analytische Sensorsysteme (G.A.S. mbH, Dortmund, Alemania) para el análisis de 2,4,6-tricloroanisol (2,4,6-TCA) en muestras líquidas (agua y vino) y sólidas (tapones de corcho) así como para obtener el perfil volátil de productos alimenticios ricos en ácidos grasos poliinsaturados omega-3. Para llevar a cabo este trabajo experimental, se han utilizado diferentes acoplamientos y sistemas de introducción de muestra (Bloque II).

En este apartado se describen de manera detallada los sistemas de detección, sistemas cromatográficos e interfase usados en el trabajo experimental desarrollado.

4.1. Sistemas de detección

Los sistemas de detección empleados a lo largo de esta Tesis Doctoral se caracterizan por ser detectores no convencionales. Las características más relevantes de los tres empleados en esta Memoria se describen a continuación:

4.1.1. Detector de transferencia de carga-corona (C-CAD)

El detector C-CAD (*Corona-Charged Aerosol Detector*) de la empresa ESA, Inc. (Chelmsford, Estados Unidos) se empleó en los Capítulos 2, 3 y 4 de la Memoria. Su peso aproximado es de 10 kg y sus dimensiones de 52.5 cm x 23 cm x 28.6 cm. Su funcionamiento comprende tres etapas: nebulización, evaporación y detección. El efluente procedente del sistema cromatográfico se nebuliza en forma de aerosol con nitrógeno. En la etapa de evaporación se elimina la fase móvil, generando partículas de analito. El modelo empleado en esta Memoria trabaja a una temperatura constante de 60 ºC. La corriente de partículas de analito incide con otra corriente de nitrógeno cargada positivamente como resultado de su paso por un sistema de descarga corona de

platino. Esta carga se transfiere a las partículas de analito que se recogen en un colector donde un electrómetro genera la señal analítica. La presión del gas recomendada es 2.4 bares y un caudal aproximado de 4 mL min^{-1}. En la Figura 1 se muestra una fotografía del equipo C-CAD que se utilizado.

Figura 1. Detector de transferencia de carga-corona.

4.1.2. Detector evaporativo de dispersión de luz (ELSD)

Del inglés "*Evaporative Light Scattering Detector*", el ELSD es un detector que al igual que el anterior, permite la detección de compuestos que son menos volátiles que la fase móvil utilizada para su separación por cromatografía líquida (Figura 2). Sus dimensiones son 45 cm x 25 cm x 45.5 cm y un peso aproximado de 16.5 kg y se adquirió en la casa comercial ESA, Inc. (Chelmsford, Estados Unidos). El funcionamiento es equivalente al C-CAD, incluyendo las tres etapas ya mencionadas. El modelo empleado en esta Tesis Doctoral permite controlar las temperaturas de la cámara de nebulización y evaporación. Esto es una ventaja ya que permite adaptar la detección a la volatilidad de los compuestos de interés y/o la fase móvil empleada para la separación de los mismos. Los valores máximos de trabajo son 70 ºC y 150 ºC, respectivamente. Como gas nebulizador se puede usar nitrógeno o helio a una

presión de 0.5-3.9 bares y un caudal entre 1 y 4 mL min^{-1}. El ELSD está provisto de una célula de dispersión de luz donde la luz proveniente de un foco emisor se dispersa al incidir sobre las partículas de analito. Un fotomultiplicador recibe la luz dispersada. En el Capítulo 4 de esta Memoria se describe el método analítico propuesto para la determinación de azúcares en diversos productos de repostería y productos lácteos usando el ELSD como sistema de detección.

Figura 2. Detector evaporativo de dispersión de luz.

4.1.3. Detector de espectrometría de movilidad iónica (IMS)

A continuación se describen los aspectos técnicos de los equipos IMS usados en los diferentes trabajos experimentales de esta Memoria. Ambos fueron diseñados y fabricados por la empresa Gesellschaft für Analytische Sensorsysteme mbH (G.A.S, Dortmund, Alemania).

- IMS-portátil

El equipo de movilidad iónica tiene un tamaño de 449 mm x 375 mm x 177 mm y un peso de 6.5 kg. El dispositivo está equipado con una fuente de ionización de Tritio con una actividad de 300 MBq, válida para la formación

tanto de iones positivos como negativos. El tubo de deriva tiene una longitud de 90 mm, en el cual se crea un campo eléctrico de 333 V cm^{-1} a un voltaje constante de 2 kV. El equipo opera a presión y temperatura ambiente. Para la adquisición de los datos se usó el software GASpector de G.A.S. Este detector se empleó para la determinación de 2,4,6-tricloroanisol en muestras de agua y vino (Capítulos 6 y 7). En la figura 3 se muestra una fotografía del equipo usado en esta Tesis Doctoral.

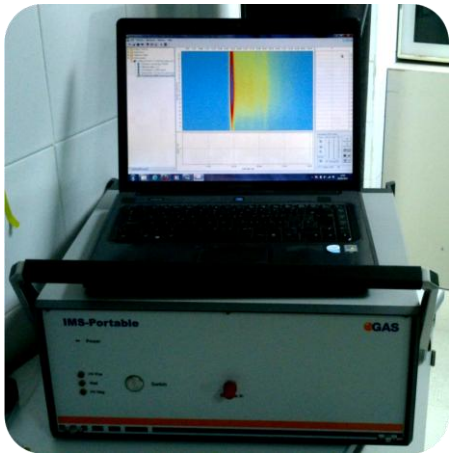

Figura 3. Vista del espectrómetro de movilidad iónica portátil.

- FlavourSpec®

Este equipo comercial se basa en el triple acoplamiento espacio de cabeza - separación cromatográfica - espectrometría de movilidad iónica. Su diseño es especialmente adecuado para el análisis directo de la fracción volátil de muestras líquidas o sólidas. La Figura 4 muestra una fotografía de este equipo. Hasta ahora, su mayor campo de aplicación está en el análisis de alimentos. El módulo de espacio de cabeza (CTC-PAL, CTC Analytics AG, Zwingen, Suiza) consta de una bandeja de muestras con capacidad para 32 viales, un módulo de agitación y calentamiento y un brazo mecánico con doble función: (i) introducir los viales en el módulo de incubación; y (ii) inyectar el espacio de cabeza en el inyector del equipo de movilidad iónica. La separación

cromatográfica se llevo a cabo en una columna multicapilar OV-1701. El detector dispone de una fuente de ionización Tritio (6.5 KeV). El tubo de deriva empleado tiene una longitud de 60 mm y está sometido a un campo eléctrico de 350 V cm^{-1}. El equipo trabaja a presión atmosférica y permite ajustar la temperatura de la columna multicapilar, del tubo de deriva y del inyector. Los datos se adquirieron a través de un ordenador integrado en el equipo y procesados usando el Software LAV de G.A.S. Este detector se usó para la determinación de 2,4,6-TCA tanto en muestras líquidas (vino, leche y aceite) como sólidas (tapones de corcho), (Capítulos 8 y 9).

Figura 4. Imagen fotográfica del equipo comercial FlavourSpec®.

4.2. Sistemas cromatográficos

Las separaciones cromatográficas se han llevado a cabo utilizando dos tipos de columnas dependiendo del detector empleado para cada problema analítico: convencionales y multicapilares. La Figura 5 muestra una fotografía en la que se pueden ver estos dos tipos de columnas:

4.2.1. Columnas cromatográficas HPLC

Para la separación cromatográfica de las distintas familias de analitos se emplearon dos tipos de fase estacionaria.

(i) Lichrosorb RP-C18 para la determinación de parabenos y vitaminas (Capítulos 2 y 3)

(ii) Kromasil amino para la determinación de azúcares (Capítulo 4)

Las dimensiones de las columnas fueron 250 mm x 4.6 mm y 5 µm de tamaño de partícula y fueron suministradas por la casa comercial Análisis Vínicos (Tomelloso, Madrid).

4.2.2. Columnas multicapilares (MCCs)

En esta Memoria se ha empleado la columna multicapilar OV-1701MCC, (14% - cianopropilfenilo, 86% - dimetilpolisiloxano) suministrada por la casa comercial Multichrom, Ltd. (Novosibirsk, Rusia) y se ha acoplado a dos detectores de movilidad iónica, de forma externa (Capítulo 7) o bien, integrada en el equipo (Capítulos 8 y 9). Estas columnas multicapilares (Fig. 5) se caracterizan por presentar una longitud inferior a la de las columnas capilares convencionales (aprox. 20 cm) y un volumen de 0.45 mL. Consisten en el empaquetamiento de un elevado número de capilares (aprox. 1200) con un diámetro interno de 40 µm y un grosor de 0.2 µm. El diámetro total de la columna es de 3 mm. Al dividirse el flujo a la entrada en los capilares, se consigue aumentar el volumen de muestra a inyectar, permitiendo trabajar con caudales de hasta 400 mL min^{-1}. El número de platos teóricos aumenta frente a las columnas capilares y mejora la resolución incluso en condiciones isotérmicas.

Figura 5. Foto comparativa de las dimensiones de una columna multicapilar y de una convencional.

4.3. Interfase

En los Capítulos 6 y 7 se ha llevado a cabo el acoplamiento de la microextracción en gota de líquido iónico con el detector IMS. Para ello, se ha hecho uso de una interfase que permite la introducción directa del líquido iónico recogido en la jeringa utilizada en la microextracción. El líquido iónico queda retenido en una pequeña cantidad de lana de vidrio situada en el interior de la interfase mientras que los analitos extraídos se transfieren al detector IMS mediante una corriente de nitrógeno y de un sistema de calentamiento. Dicha interfase que se muestra en la Figura 6, se fabricó haciendo uso de elementos disponibles comercialmente. Consta de un tubo de acero inoxidable de 3 cm de longitud y 3 mm de diámetro interno relleno de lana de vidrio. En la parte superior está unido a una conexión en forma de T y ésta a su vez conectada en su parte superior a dos unidades Swagelok 1/8-in., una de ellas provista de un septum de polidimetilsiloxano y otra que permite la entrada de nitrógeno. El tubo de acero se conecta en la parte inferior con otra unidad de acero de igual diámetro que permite su unión con una Swagelok 1/8-in. para el acoplamiento al detector o a la columna multicapilar. Todos los componentes de la interfase se adquirieron en la casa comercial Supelco (Madrid, España).

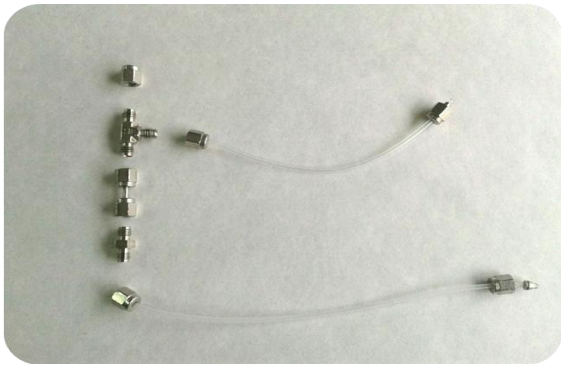

Figura 6. Fotografía descriptiva de todas las unidades que forman la interfase.

Bloque I

Nuevas aplicaciones de los detectores evaporativos

Capítulo 1

Evaporative-based detectors as global response instruments

ENCYCLOPEDIA OF ANALYTICAL CHEMISTRY

Evaporative-based detectors as global response instruments

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ABSTRACT

Evaporative-based detectors are a new generation of instruments which provide a universal response for a wide variety of compounds or their families whose determination by other conventional detection systems is limited by different reasons. Both evaporative light scattering detector (ELSD) and corona-charged aerosol detector (C-CAD) have been recognized as global response detectors in liquid and supercritical chromatographies. Their advantage over optical or electrochemical detectors is that they do not require from the analytes to present special physicochemical properties. The evaporative-based detectors nebulized the mobile-phase/liquid stream effluent, producing analyte particles, that are optically detected through the scattered light in the case of ELSD or by charge transfer in the case of the C-CAD. The foundation of the detection and the main applications of these detectors will be deeply commented in this article. Moreover, a comparison with conventional detectors (ultraviolet/visible (UV/Visible), refractive index (RI), and mass spectrometers (MSs)) is included. In the light of the bibliography available on this topic, future applications of and perspectives on the use of these detectors can also be anticipated.

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1. Introduction

The evolution of analytical chemistry has been marked by three specific trends: automation, miniaturization and simplification. Automation is a reality in most of the analytical laboratories as it affects all the steps of the (bio)chemical measurement process, although to a different extend [1]. Indeed, it is almost complete in the instrumentation and data acquisition and processing while the preliminary operations remain as a bottleneck because of their peculiar characteristics. Miniaturization is also present in instrumentation via the availability of portable instruments that permits in situ measurements. This tendency has also been materialized in sample treatment through the proposal of miniaturized devices, which allows sample processing using minimal volumes/amounts of solvents, sorbents and reagents. The commercial availability of these elements is the key factor for the implementation of microextraction techniques in routine laboratories.

The combination of these two trends has undoubtedly facilitated the simplification of the (bio)chemical measurement processes. The automation permits apparatus and instruments to work unattended, the introduction of new materials more efficient than the conventional ones, and reduces the steps required in new (bio)chemical measurement processes. However, simplification also affects to the information generated by the laboratory by two main reasons: the increased number of samples that the laboratory has to analyze in a working day and the type of information demanded by the clients. Indeed, it is well recognized nowadays that the quality of the results is directly related to the usefulness of the information they provide for timely, well-founded decision-making. In order to satisfy the clients' information demands and increase the productivity-related analytical properties of the (bio)chemical measurement processes, a renewal of routine analytical procedures is mandatory, as they should be faster and simpler than the conventional ones

[2]. Moreover, the information delivered should also be simpler, less expensive and easier to understand. Vanguard-rearguard analytical strategies and total indices are key elements in this evolution, which lead to the use of universal response instruments for obtaining high-quality analytical information [3,4].

1.1. Vanguard-rearguard analytical strategies

The combination of analytical systems that deliver information in a simple and rapid manner (vanguard systems) with conventional methods (rearguard systems) is a perfect strategy to maximize the efficiency of a routine laboratory. The operational principle of this combination, proposed by our research group in 2005, is schematically represented in Figure 1 [3].

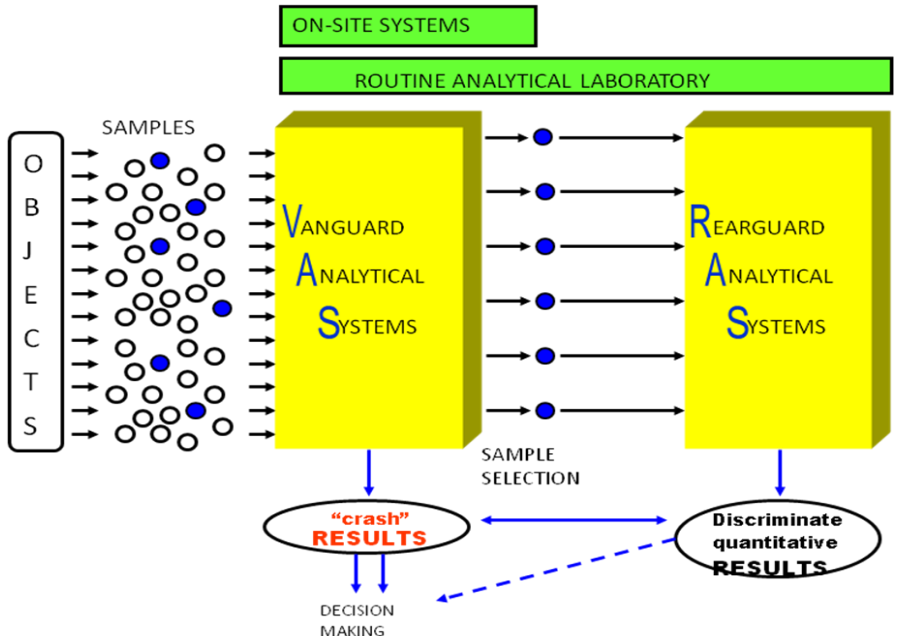

Figure 1. Schematic representation of the vanguard-rearguard analytical strategies. (Reproduced from Ref. 3. © Elsevier, 2005.)

As it can be seen, all the samples representative of the object/system under study, are systematically processed in the in the vanguard analytical system. After being processed, the samples are classified according to the client or to legal requirements (e.g. toxicity, contamination). The crash information obtained at this stage permits the rapid decision making. Only those samples providing a positive response in the vanguard system are further processed in the rearguard one. The information obtained in this conventional procedure is not only more accurate and precise but also time-consuming and expensive.

The vanguard analytical systems are simple in nature and, in this way, they offer a bypass to the preliminary operations of the (bio)chemical measurement processes, thus minimizing their negative impact on the results. The information expected from a vanguard system is mainly qualitative, and therefore, rapid response analytical tools are extensively used in this context. Such is the case with portable instruments, sensors, screening methods and universal response detectors. The qualitative response has specific connotations that have been deeply discussed elsewhere [5]. The most simple binary response answers the question: is the analyte present in the sample? This implies the unequivocal attribution of the signal to the target compound. However, in most of the cases, it is necessary for the analyte to be present above a given concentration to consider the sample positive. This quantitative connotation should be taken into consideration when the instrumental signal, generated in response to a given concentration or amount of analytes(s), has to be converted into a binary yes/no response. Two references are usually identified in this context: the cut-off concentration or decision limit and the threshold concentration or alarm limit. The former is related to the concentration of the analyte(s) fixed by the organism or laboratory to warrant the reliability of the information, thus reducing the false negatives. The latter

is established by the client or legislation in order to determine if the sample present or not in a given attribute.

The vanguard analytical systems should be design to provide a high level of productivity-related properties (speed, cost and risk) by maintaining an appropriate level of reliability of the results. In this context, the rearguard system plays a triple role: to confirm the crash information provided by the vanguard system; to systematically control the results despite the response (positive, negative or inconclusive) generated according to a previously established quality plan; and to expand the information of the vanguard system by transforming the qualitative response in quantitative data and the total index into a discriminate information for each analyte.

1.2. Universal response instruments

The development of rapid response analytical systems can be improved by using detectors that provide a uniform response for a family of analytes, regardless of their physicochemical properties. It was the pharmaceutical industry that first looked for such detectives whose response avoids the need for individual calibration standards while permitting the detection and quantification of newly synthesized or unknown compounds. The majority of the (bio)chemical measurement processes developed in this context involve liquid chromatographic separations of the target analytes either from other components of the sample matrix or among the family constituents, and compatibility with a great variety of mobile phases was also required.

Few detectors have been included under the category of "universal": low wavelength UV, chemiluminiscent nitrogen, RI, and mass spectrometry. In contrast to more specific detectors, such as UV or fluorescence ones, they present favorable characteristics to be used as generic detectors. They have

been used as directly coupled with liquid chromatographs rather than stand alone detectors in fast response analytical methods (mainly sample screening systems). The limitations of these instruments, such as lack of robustness in quantitative analysis of MSs, incompatibility with gradient elution of RI, the limitation in organic components of mobile phases of low UV and chemiluminiscent nitrogen detectors, and the dependence on the molecular structure of the analytes of the former - opens this field for the development of new detectors with more universal response. Such is the case of the evaporative-based instruments: ELSD and C-CAD. In these instruments, the mobile phase is nebulized and the solutes are evaporated. Next, the light scattering due to the charge associated with aerosol particles is measured. They are considered to be robust detectors and can be used to detect major and minor components as long as they are less volatile than the mobile phase used for the chromatographic separation; otherwise, the compounds are lost during the evaporation step.

ELSD and C-CAD are the subject matter of this article, and their main characteristics and applications are deeply studied in the following sections. Moreover, a systematic comparison among the universal detectors reported up to date included in a separate section in order to highlight their strengths and weaknesses. Finally, the future uses of ELSD and C-CAD are envisaged.

2. Operating principle of evaporative-based detectors

2.1. Evaporative light scattering detector

The ELSD functioning involves three consecutive steps (Fig. 2): i) nebulization, ii) evaporation, and iii) detection [6].

In the *nebulization step*, the effluent from a chromatographic column enters a Venturi-type nebulizer, where it is transformed into an aerosol. These

nebulizers need a high flow of carrier gas (air or inert gas, such as nitrogen, carbon dioxide, argon, or helium) over the liquid surface producing a high amount of small droplets with remarkably uniform size. The formation of uniform, reproducible, and stable aerosols depends on the relation of the nozzle diameter and the flow rates of mobile phase and nebulizing gas. For a constant diameter of the nozzle, stable aerosols are formed only for a limited range of flow rates, and further, the flow rate of the nebulizing gas must be adjusted in relation to the flow rate of the mobile phase. Distribution and mean values of droplets diameter are considered to be very critical parameters, which strongly influence the analytical characteristics (detectability, sensitivity, and repeatability) of the ELSD methods [7].

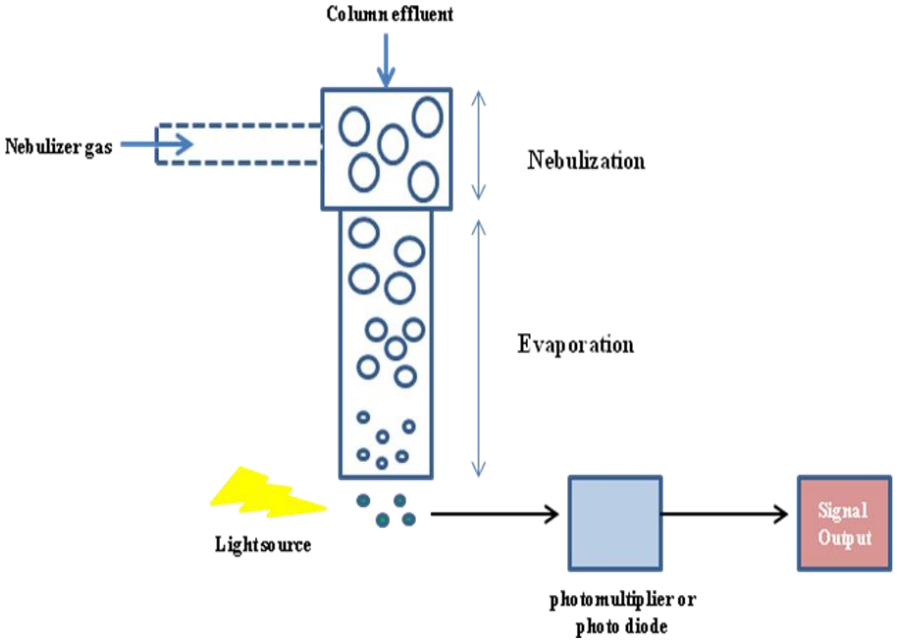

Figure 2. Scheme of the evaporative light scattering detector functioning.

ELSDs are classified into two types according to their structure after the nebulization unit: in type A or type B [8]. In ELSD of type A, the entire aerosol enters the heated evaporation tube (drift tube), where the evaporation process begins. However, in the ELSD of type B, the aerosol enters a glass chamber or a focusing cone (nebulization chamber), before the evaporation step. In this element, the droplets of high size are condensed on the walls of the chamber and wasted. The proportion of the wasted aerosol depends on mobile phase volatility and varies from >90% (aqueous mobile phases) to <10% (highly volatile organic mobile phases). Each type presents its own benefits, and thus, the appropriate choice depends on the nature of the analyte and the composition of the mobile phase. ELSD of type B requires lower evaporation temperature than type A, and thus, it is more sensitive for volatile, semi-volatile or thermosensitive analytes. On the other hand, for nonvolatile analytes, ELSD of type A appears to be more sensitive since the entire quantity of analyte reaches the optical cell.

In the *evaporation step*, the aerosol is driven by the gas stream to the evaporation chamber, where the size of the aerosol droplets is reduced of the evaporation of the mobile phase, which is performed in a heated drift tube. The completeness of the mobile-phase evaporation and the extent of analytes losses are mainly determined by the evaporation temperature, which should be selected in accordance to the mobile-phase and analytes volatilities, the mobile-phase flow rate, and the ELSD type (A or B). The evaporation temperature is usually set between 30 ºC and 100 ºC.

After the evaporation process, the aerosol, ideally composed by solid particles of analyte, enters the optical cell and passes through a light beam for *analytes detection*. The scattered light is measured by a photomultiplier or a photodiode, providing the output signal. Light scattering processes are

classified into two types: elastic scattering, in which the scattered radiation is of the same frequency as the incident radiation; and inelastic scattering, in which the scattered radiation is of a different frequency. In ELSDs, inelastic scattering is considered to be negligible and it is not further examined. Elastic scattering is classified into three types: Rayleigh-Debye, Mie, and refraction-reflection. Refraction-reflection mechanism, which has its origin in the induced secondary emission of particles in the path of the incident beam, has also been reported as a potential mechanism of scattering in the ELSD optical cell [9]. The type of interaction between the light and the particles depends on the size, shape, and surface properties of the particles and the wavelength (λ) of the incident light. Rayleigh-Debye scattering occurs with the smallest particles ($D/\lambda < 0.1$), Mie scattering becomes the predominant mechanism for $0.1 < D/\lambda < 1$, and the refraction-reflection mechanism occurs in case that the particle size is greater than the wavelength ($D/\lambda > 1$). In the case of $\lambda = 0.35$ μm (the wavelength with the maximum emission of tungsten lamp), Mie and refraction-reflection processes prevail, since analyte particles with radius smaller than 100 nm are usually not detectable. Furthermore, refraction is relatively more important compared to reflection in the refraction-reflection domain. In most cases, more than one scattering mechanisms occur in the ELSD optical cell, because of (i) variations of the aerosol droplet diameter (which depends on the nebulization and evaporation processes), (ii) the polychromatism of the light source, and (iii) dependence of the mean droplet diameter on the sample concentration.

2.2. Corona charged aerosol detector

The functioning of C-CAD is schematically depicted in Fig. 3. Briefly, the efluent of the chromatographic system is first nebulized with a nitrogen (or air) stream and then the droplets formed are dried to remove the mobile phase, generating analyte particles, in a way similar to ELSD. Then, this stream of

analyte particles is merged with a secondary positively charged nitrogen stream as a result of its prior passage through a high-voltage platinum corona wire. This charge is then transferred to the opposing stream of analyte particles and further to a collector, where an electrometer generates a signal whose intensity is directly proportional to the quantity of analyte present.

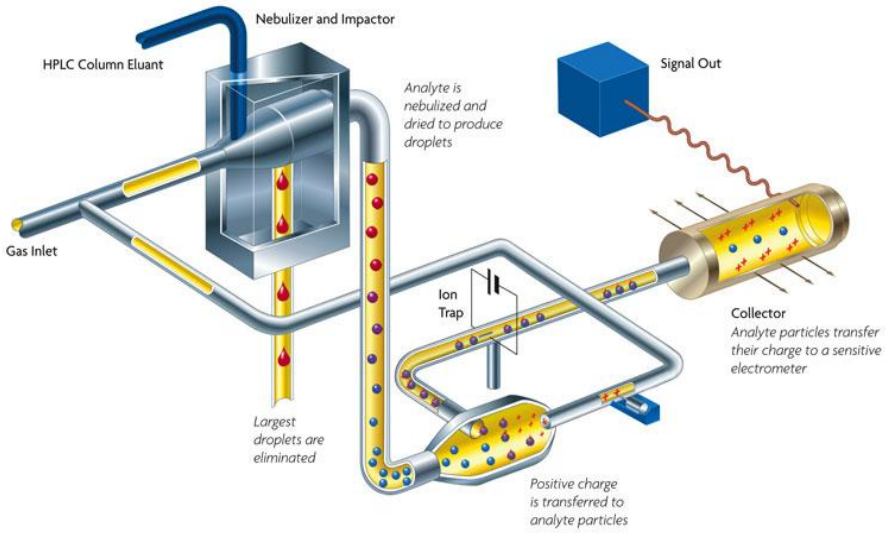

Figure 3. Scheme of the corona-charged aerosol detector functioning.

Since its commercialization, it has been exclusively used as detector in liquid and supercritical fluid chromatographies, although it has been less used than ELSD. However, a new version of the C-CAD, the Corona® ultra™ detector, permits its coupling with ultraperformance liquid chromatography (UPLC) as it maintains the speed and resolution of UPLC for the analysis of any nonvolatile or semivolatile analyte, with or without a chromophore. In addition, the Corona® ultra™ detector has also the flexibility to operate with standard liquid chromatography with no further modifications. The sensitivity achieved is in the range nanogram to picogram level.

3. The response of evaporative-based detectors

ELSD and C-CAD are considered quasi universal instruments as they can provide a response for any analyte with the only requirement of being less volatile than the mobile phase. Several criteria can be used to predict the compound volatility such as the molecular weight, the melting/boiling points, and the vapor pressure. Among them, vapor pressure is the best indicator as the other parameters can be affected by other properties of the analytes, such as hydrogen bound formation. Considering the vapor pressure as reference, it can be concluded that the lower the vapor pressure, the better response to the detector. However, there are several factors that can affect the analytical signal provided by the ELSD and C-CAD.

The temperature inside the evaporation and/or nebulization chambers is crucial to operate at the optimum sensitivity level. In case of low temperature, excessive background noise and baseline instability is obtained because of a poor evaporation of the constituents of the mobile phase. Apart from the analytes losses, high evaporation temperature causes rigorous solvent evaporation, which destroys uniformity of particle size, and favors the formation of liquid rather than solid particles. Both effects result in a decrease of the sensitivity. It should also be mentioned that not all the instruments permits these temperatures to be changed by the operator.

The sensitivity of both detectors is negatively influenced by the generation of solid particles after the nebulization/evaporation processes as they increase the background noise. These particles can appear as consequence of using poor purity solvents in the mobile phase, which present nonvolatile impurities. The column bleeding can also decrease the sensitivity of the detector in a very similar way. The use of polymeric columns instead of silica-

based ones is a good alternative to prevent potential particulate matter from reaching the detector.

Owing to the operational functioning of evaporative-based detectors, the use of volatile mobile phases is mandatory. This property conditions not only the organic solvents that can be used (acetonitrile and methanol as recommended ones) but also the buffer additives that can be used, which must be also of volatile nature. Formic acid, acetic acid, trifouroacetic acid, and ammonium acetate are usually employed when the addition of a modifier to the mobile phase is needed to achieve/improve the chromatographic separation of the analytes.

The viscosity of the mobile phase also affects the response of the evaporative-based detectors. The viscosity increased while increasing the percentage of water in the mobile phase composition. Thus, highly organic mobile phases facilitate the nebulization process and therefore increase the detector response [10]. It also permits the use of lower temperatures inside the detector, and thus, more volatile analytes can be detected.

The clear influence of the mobile-phase composition on the evaporative-based detectors recommends chromatographic separation to be carried out under isocratic conditions to overcome the 'gradient effect'. Two alternatives have been proposed in the literature to mitigate this limitation, which is more marked for the C-CAD. The first one involves the use of a gradient compensation approach [11,12]. The instrumental configuration requires the use of a second pump that delivers a post-column inverse gradient. Both flows are merged before their introduction into the C-CAD detector in order to obtain a constant composition. The second alternative is based on the construction of three dimensional models. The one proposed by Mathews et al.

[13] introduces a nonvolatile, nonretained compound at regular intervals during the gradient and the variations in the signals for this compound are attributed to the changes in the mobile-phase composition. ELSD was the detection system evaluated in this approach. Hutchinson et al. [14] correlate in their three dimensional model the C-CAD response, the analyte concentration, and the mobile phase composition and evaluated using analytes with different psycochemical properties to assess the uniformity of the detector response.

The relationship between the concentration of the analyte(s) and the instrumental signal provided by the detector is an important issue. Experimental evidence suggest that the response of evaporative-based detectors is non linear; constituting a considerable limitation or drawback in quantitative studies. In the case of ELSD, the area of the chromatographic peak (A) can be correlated with the analyte mass (m) according to the exponential relationship:

$$A = a\, m^{b}$$

where a and b are coefficients whose value depend on the ELSD instrumentation as well as the nebulization and evaporation processes (flow rates of the nebulization gas and mobile phase, composition of the mobile phase, evaporation temperature, etc.). A linear calibration curve can be constructed using double logarithmic coordinates:

$$\log A = b \log m + \log a$$

Concerning the C-CAD response, a second-order relationship between concentration and response is usually observed and thus, a quadratic equation will fit to experimental data [15].

Despite these considerations, it should be pointed out that over narrow concentration intervals, linear calibration equations can be used with acceptable correlation coefficients. This fact simplifies data handling and analytes quantification, which also helps to extend their use in routine laboratories.

4. Analytical applications of evaporative light scattering detector

ELSD was first reported by the Australian laboratories of Union Carbide in 1966. During the past 25 years, ELSD has moved into the mainstream of detection choices for liquid chromatographic separation. The inherent advantage of ELSD to detect any analyte - regardless of the optical (UV absorptivity), electrochemical, or other properties - is the main reason for expanded applicability of ELSD. In fact, ELSD is considered to be a quasi-universal, rather than a fully universal, detector since, as it was previously pointed out, analytes with higher volatility than the mobile phase cannot be detected. This section describes the main applications of ELSD in chromatographic techniques and also as stand-alone detector in vanguard strategies.

4.1. Evaporative light scattering detector as detector in chromatographic systems

ELSD has been typically used as a chromatographic detector. This is due to (i) its low selectivity, which requires separation prior to detection; (ii) the global nature of its response; and (iii) compatibility with the usual components of the mobile phase.

ELSD has been effectively used for the determination of a wide variety of compounds in various synthetic or natural matrices. Figure 4 shows the main application areas of ELSD: pharmaceuticals, food and beverages, biological samples, among others.

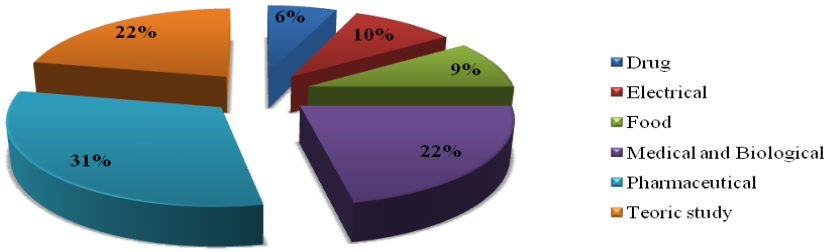

Figure 4. Main application fields of evaporative light scattering detector.

High-performance liquid chromatography/evaporative light scattering detector (HPLC-ELSD) methods have been developed and validated for the determination of isepamicin, gentamicin, neomycin, kanamycin, tobramycin and amikacin in raw materials; various formulation types (injection, cream, ointment, powder, aerosol); and biological fluids [16-20].

Ginsenosides are triterpenic saponins are considered to be the main bioactive constituents of the herbal medicine ‘ginseng’. They derived from the roots and rhizomes of different *Panax* species (Araliaceae), with antistress, antihyperglycemic, and potential antitumor properties [21,22]. The main problems encountered in performing HPLC/UV analysis of ginseng are high level of baseline noise and poor sensitivity due to the weak UV absorption. This feature also limits the choice of solvents and mobile-phase modifiers for improved separation. The developed ELSD method showed a lower detection

limit in comparison with UV detection [23]. Steroids were one of the first classes which were investigated for ELSD applicability. In 1984, HPLC/ELSD determination of 17 steroids on normal- and reversed-phase (RP) analytical columns was reported. Stable baselines were obtained under gradient elution, whit a detection limit of ca. 0.5 μg mL^{-1} [24]. During the past years, polyethers and their monoalkyl and arylalkyl derivates have gained more and more interest in various scientific fields, especially in pharmaceutical technology. In particular, poly(ethylene glycol) (PEG) derivatives are extensively used as essential additives in nonionic surfactants and wetting agents in laundry and industrial cleaners, solubilizers in enhanced oil recovery, ingredients in the cosmetic and food industries, emulsifiers in pharmaceutical preparations, and solubility enhancers in biochemical membrane technology. Numerous studies have been published on the analysis of polyesthers by HPLC/ELSD. Excellent results were obtained for a wide variety of polyether derivatives including PEGs, polypropylene glycol (PPGs), polybutilene glycol (PBGs) and PPG amides, and for alkylethoxylates, PPGs and PEG-PPG copolimers [25,26].

The potential of ELSD for the determination of carbohydrates, as well as lipids, in various matrices has been extensively investigated. Indeed, it is currently considered to be one of the prevailing detection techniques in HPLC methods. HPLC/ELSD methods have been reported for numerous oligosaccharides (glucose, fructose, maltose, saccharose, raffinose, ribose, sylose, arabinose sorbose, sorbose, mannose, galactose, erythritol, sylitol, mannitol, sorbitol), chitin, dextrans, maltodextrins, and cyclodextrins in various foods, drinks, and plant tissues, and tobacco [27,28]. Detection limits are in the microgram per milliliter range. Besides HPLC, mono-, di- and trisaccharides were determined by supercritical fluid chromatography/evaporative light scattering detector (SFC/ELSD) [29].

The lipids (polar and nonpolar) can be determined with HPLC/ELSD. This technique was proposed for the identification and quantification of 12 lipid classes (paraffin, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols, and fatty acids amides) used as lubricants in food packaging materials [30].

Owing to its poor selectivity, the direct application of ELSD to complex matrices (such as the biological samples) is very limited. Efficient sample clean-up, elimination of matrix interferences, and analytes enrichment are required prior to HPLC/ELSD determination. Several HPLC/ELSD methods for natural products and biological samples have been reported, including determination of antibiotics, carbohydrates and lipids in plasma, urine, and tissues [31-33].

Specific chromatographic applications of ELSD include the chiral separation of polar compounds of pharmaceutical interest (drugs and underivatized amino acids) by hydrophilic interaction chromatography [34]. Recently, the ELSD has also been used in a new analytical method for the quantification of lactose and lactulose in milk [35]. Lactulose can be derived from lactose by either alkaline isomerization or enzymatic transgalactosylation of fructose. An HPLC column with an amino-bonded polymeric matrix yielded better results compared to amino-bonded silica-phase resin or cation-exchange resin. The new method has detection limits for lactose and lactulose of 3.8 and 2.5 mg L^{-1}, respectively. Also, a liquid chromatographic method was developed to determine the modification degree of polyethylene glycolated proteins. This method effectively separates free PEG from other species in conjugation mixtures on a C4 RP column [25]. Pellicciari et al. have reported the first series of conformationally constrained analogs of homotaurine. The

partial constriction of the skeleton was realized through the insertion of a cyclopropyl ring, between the α-, β- and β,γ-positions, thus affording, respectively, *trans*- and *cis*-2-aminomethylcyclopropane-1-sulfonic acids and *trans*- and *cis*-(2-aminocyclopropyl)methanesulfonic acids. The resolution of all four racemic mixtures was accomplished using HPLC system carrying the polysaccharide-based Chiralpak® IB® column as the chiral stationary phase. The coupling with the ELSD has been particularly valuable during this chromatographic study [36].

There have been several attempts at adapting ELSD to microcapillary and capillary liquid chromatography; the most salient of which have been reviewed by Gaudin et al. [37] who also assessed their own configuration. One of the main advantages of using ELSD with microcapillary and capillary liquid chromatography is the linearity of the response, which can be ascribed to an increased uniformity in droplet size distribution as the likely result of the reduced flow rate [10].

In all cases, miniaturizing the nebulizer is the key to ensuring compatibility between the effluent microflow from the column and the nebulization process. Miniaturization can be accomplished in two ways. The first is based on a device created by Demirbüker et al. [38]. Their miniaturized detector can operate at mobile-phase flow rates over the range 5-100 μL min^{-1} and has provided detection limits below 8 ng for triacyl glicerols present in cohune oil [39]. This configuration was used by other authors to construct a laboratory-made capillary nebulizer for accommodation in a comercial detector. The main shortcoming of these modified detectors is that they require strict control of the nebulizer geometry in order to ensure efficient nebulization of the column effluent. Occasionally, an additional external gas heating device is needed [40]. The other way of ensuring compatibility is a simplified variant

of the previous modification involving the insertion of a fused silica capillary into the standard nebulizer nozzle [41]. This has been used to determine lipids and underivatized amino acids at the low nanogram level.

4.2. Evaporative light scattering detector in vanguard/rearguard analytical strategies

The ELSD has been combined with other detection systems to supplement the information provided by UV/VIS, MS, among others [42]. Several applications have been proposed in the form of stand-alone screening systems or vanguard/rearguard analytical strategies for clinical, toxicological, and food analyses. For example, ELSD has been used for the direct screening of human serum and urine in clinical/toxicological analyses for the determination of paracetamol [43]. In this case, the paracetamol was retained on a LiChrolut column, which was inserted in a continuous flow injection system. The limit of detection (LOD) was 0.3 μg mL^{-1}, and the precision of the method 1%. ELSD has been used in environmental and industrial process control for the sequential determination of nonionic surfactants and fats in degreasing bath samples [44]. ELSD in conjunction with liquid chromatography permits the direct screening of biological fluids for bile acids with LOD of 0.4 μg mL^{-1} and a the linear range between 1-100 μg mL^{-1} [45,46].

The analytical information requested by customers of routine analytical laboratories should be simple, fast, cheap, and easy to understand. Some analytical problems require no discriminate information to be solved; rather, general information, usually in the form of total indices. A total index is a measurand that describes a group of (bio)chemical nature/structure and/or similar operational behavior [4]. The potential group of compounds can range form a few to millions. These compounds can be of the same chemical

structure or different. ELSD has also been used to determine an individual analyte or an analyte family discriminating between the target compounds and their potential interferents. Fractionation allows the separation of a sample into different groups or families of compounds of similar nature, structure, or operational properties. The following are some examples of the combined use of fractionation and ELSD:

- Wan et al. have studied the effects of cholalic acid from artificial bezoar. The content of cholalic acid was detected by HPLC/ELSD. The content of cholalic acid in the hydrolyzed sample was higher than that in the nonhydrolyzed sample in the same batch. Through hydrolysis, the binded cholalic in the artificial bezoar was transformed into free cholalic acid so that the content of cholalic was increased. Therefore, it is more reasonable to control the quality of artificial bezoar by taking the contents of total cholalic acid and free cholalic acid as indexes [47].
- The phospholipid and sphingolipid of milk is of considerable interest regarding their nutritional and functional properties. The determination of these compounds of the milk fat globule membrane was carried out using HPLC/ELSD [48].
- An autosampler has been designed for the sequential determination of the lactose, fat, and protein contents in milk. It relies on the coprecipitation of proteins and fats, and the determination of sugars in the filtrate. With the use of selective solvents, fats and proteins are sequentially redissolved in a selective manner and driven to the detector [49].
- The sequential determination of total sugar, class IV caramel, and caffeine in soft drinks can be implemented by using a multiparametric analyzer based on the on-line coupling of a miniaturized solid phase extraction (SPE) unit with serially arranged detectors (viz. UV/VIS

detection for caramel and ELSD for sugars and caffeine) [50]. The preconcentration of the caffeine on the SPE column permits its detection despite its lower level in comparison with the other two components.

- ELSD has also been proposed as global detector for the continuous fractionation/quantification of the three main polyphenol families present in red and rosé wines. The compounds are retained on an RP-C_{18} minicolumn and selective solvents are used to sequentially elute the three fractions [51].
- The degree of exhaustion of alkaline degreasing baths involves the determination of the surfactant and/or grease contents. These two parameters can be at line monitored by using an autoanalyzer, which includes an on-line separation of the two fractions and the sequential monitoring in an ELSD [52].

These studies show the usefulness of ELSD for the development of vanguard analytical strategies and its contribution in the type of information requested by the clients in routine laboratories.

5. Analytical applications of corona-charged aerosol detector

The C-CAD was first commercialized in 2004 by ESA Biosciences. Its main application field is the pharmaceutical industry, in which its applicability to a wide range of analytes together with the sensitivity and ease of use are the most valuable characteristics.

Corona charged aerosol detection has been used in packed column supercritical fluid chromatography (pSFC) for the determination of pharmaceutically related compounds [53]. pSFC is the technique of choice in combinatorial chemistry, impurity profiling, and quality analysis on account of

its high efficiency and rapidity [54-57]. pSFC has been successfully coupled to a wide variety of universal response detectors, UV being the most popular. The C-CAD can be easily coupled to pSFC by directly connecting the outlet of the backpressure regulator to the inlet of the detector. This alternative is preferred to the preback pressure regulator to avoid the split of mobile phase, thus preserving the high sensitivity and wide dynamic range of the instrument. The authors used a mobile phase compensation to reduce the effect of its composition in the sensitivity. Especially remarkable is the influence of the CO_2/organic modifier ration on the C-CAD response as it directly affects the generation of the aerosol and thus the response. The authors found that the nebulization efficiency was higher at a lower percentage of the organic modifier, thus at a high CO_2 flow rate. Concerning the analytical figures of merit, the sensitivity was between 0.6 to 2.3 mg L^{-1}. Those values were similar to those provided by the UV detector for the same analyte, with the advantage that C-CAD produces a response even for compounds with no chromophores. The precision was ca 10%.

The C-CAD detector has been used in combination with different liquid chromatographic modalities to determine a wide variety of analytes. Kou et al. developed a size exclusion chromatographic method with C-CAD for the determination of PEG polymer. The objective of the method was to detect purities, impurities, and polydispersity of PEG reagents [58]. The authors compared the performance of the detector with that of ELSD and RI for the target issues. C-CAD was more sensitive than RI and showed a wider linear range than ELSD, which allows better estimation of the purity and polydispersity.

Reversed-phase liquid chromatography coupled to C-CAD has been proposed for the determination of parabens in cosmetics [59]. In order to

improve the sensitivity and selectivity of the method, the analytes were isolated and preconcentrated form the sample matrix using multiwalled carbon nanotubes packed in conventional solid-phase extraction cartridges. The chromatographic conditions were optimized for the detection and separation of the analytes using isocratic conditions to avoid the need for mobile-phase gradient compensation. Under these conditions, it was found that not all the parabens studied, namely methyl, ethyl propyl and butyl, showed the same response in the C-CAD as the response increased when increasing the length of the alkyl chain of the compound was increased. It is in accordance with the fact that the response of this detector depends on the particle size. The method was compared with others reported in the literature for the same compounds, C-CAD being less sensitive but simpler and faster.

Pharmaceutical quality control of L-aspartic acid and L-alanine has also been carried out using ion-pair liquid chromatography and C-CAD [60]. Amino acids have been used in a wide variety of fields, such as cosmetics, agrifood, medicine and nutrition, the last two requiring a proper and rigorous quality control of the final product. The main limitation of the methods proposed up to date is the detector used (UV/VIS), as most of the amino acids do not present a chromophore in their structures. In this context, the evaporative-base detectors have raised as an alternative of choice because of their universal response, which overcome that shortcoming. The work reported by Holzgrabe at al. used a C-CAD detector to detect amino acids (asparagine and alanine) as well as process related impurities. The authors considered the method advantageous in contrast to the thin layer chromatography test or amino acid analyzer methods. Indeed, it is possible to separate the major organic acids (fumaric, maleic, malic, citric and succinic acids) and amino acids (glutamine and glycine) known to occur during the synthesis of both amino acids at the 0.006-0.03% level (referred to a 10 g L^{-1} reference

concentration). Perfluoroheptanoic acid was used as a typical ion pairing reagent for the separation of amino acids in RP mode. The results obtained with C-CAD were compared with those provided by ELSD. The ELSD was between 3.6 and 42 times less sensitive than C-CAD, being the nonvolatile amino acids those which present the greatest difference in sensitivity.

6. Comparison of universal detectors

ELSD and C-CAD are considered to be universal. Both detectors are mass dependent, and the response generated does not depend on the spectral or physicochemical properties of the analyte. The main positive characteristics of C-CAD system are universal detection of nonvolatile analyte, a response independent of chemical properties, a broad dynamic response range with high sensitivity from low nanogram to high microgram amounts of analytes, good precision for a diverse range of analytes, and simple and reliable operation, requiring only setting of few controllable parameters, such as the gas input pressure, the temperature or the temperature range, and signal output range [61]. But C-CAD system has the same limitation as ELSD; namely, the response is affected by mobile-phase composition, with higher responses observed at higher organic contents. This problem has been resolved by using inverse gradient compensation; it has been proposed for high pressure liquid chromatography and supercritical fluid chromatography [11]. Another drawback of this type of detector is that no spectral information is acquired, so it is not possible to identify a certain peak or perform peak purity analysis as in UV-diode array detectors or MS detectors. However, ELSD and C-CAD offer advantages over other types of detection: RI, low-wavelength UV and MS [62-64]. The characteristics of these detectors are schematically compared in Table 1.

Table 1. Comparison of the analytical performance of universal response detectors.

	C-CAD	ELSD	UV	RI	MS
Sensitivity	5-25 ng	50-200 ng	High pg	Low µg	pg-ng
Reproducibility	Better than 2%	Poor	Better than 2%	Good	Good
Type of response	Independent on chemical structure	Independent on chemical structure	Dependent on chemical structure	Dependent on chemical structure	Dependent on chemical structure
Chromatographic compatibility	High	High	Medium	Low	High
Applicability	Broad	Broad	Limited	Limited	Broad
Ease of use	Simple	Complex	Easy	Fair	Complex

For example, the RI detector, while widely used, has significant limitations in sensitivity and is not compatible with gradient elution. Low-wavelength UV provides higher sensitivity and improved gradient compatibility. However, it is unable to detect compounds that lack a sufficient UV chromophore such as many underivatized amino acids, carbohydrates, lipids, polymers, surfactants, drug substances, and natural products. In addition, the magnitude of the response depends on the molar absorptivity, which can vary by orders of magnitude even among analogous structures. Mass spectrometry is considered to be a specific and universal detection method, which is capable of providing structural information about the compound under study. However, as the response depends on the ionization process, quantitative analysis using MS coupled with HPLC is currently less robust, and the high price of the instruments limits its use for routine analysis [65]. Moreover, it requires skilled analysts in order to correctly interpret the analytical response obtained (viz. mass spectra).

Several articles recently published specifically compare the analytical performance of ELSD and C-CAD for the determination of different compounds in a variety of samples. The chromatographic and detector conditions are showed in Table 2. It should also be mentioned that in most of these articles, MS is also included as this detector shares with the ELSD and C-CAD the mobile-phase removal before analytes detection.

Mitchell et al. [15] compared the sensitivity of ELSD and C-CAD in the RP and the hydrophilic interaction liquid interface chromatography (HILIC) modes for very polar compounds. For the ELSD, the total flow from the column is introduced into the drift tube, while the C-CAD has an internal split that directs a significant portion of the nebulized spray to the waste. As a general conclusion, the use of HILIC provided better sensitivity for both

Table 2. Comparison of the chromatographic and evaporative-based detector conditions for the determination of different families of compounds.

Compounds	Chromatographic conditions	Detector conditions		Ref.
		ELSD	C-CAD	
Polar compounds (saccharides, amino-acids, nucleosides/nucleotides)	Mobile phases: 5% acetonitrile-95% buffer (RP) and 90% acetonitrile-10% buffer (HILIC) Flow: 1 mL min^{-1} Columntemperature: 35ºC Injection volume: 5 µL	Gas flow: 1.6 L min^{-1} Drift tube temperature: 60ºC	Range: 100 pA Filter: None	[15]
Gabapentin	Column temperature: 25 ºC Injection volume: 5 µL Flow: 0.2-1.1 mL min^{-1}	Drift tube temperature: 45-85 ºC Nitrogen flow: 1-2.3 L min^{-1}	Gas pressure: 35 psi	[66]
Lipids (Squalene, cholesterol, ceramide IIIB)	Mobile phases: Squalene: 100% propan-1-ol, 30 µL min^{-1} Cholesterol: 100% butan-1-ol, 15 µL min^{-1} Ceramide IIIB: 100% butan-1-ol, 60 µL min^{-1} Injection volume: 0.2 µL Temperature: 100, 125, 150ºC	Air pressure: 1 bar Drift tube temperature: 50 ºC Nebulizer: 40 ºC	Air pressure: 35 psi Filter: Medium	[67]
Polyketide	Mobile phase: 100% acetonitrile, 1 mL min^{-1}	Nitrogen pressure: 3 bar Drift tube temperature: 55 ºC Sample volume: 20 µL	Gas pressure: 35 psi Range: 100 pA Sample volume: 10 µL	[64]
Poly(ethylene glycol)	Mobile phase: CO_2 with methanol-water (9/1, in v/v) Column temperature: 50 ºC Flow (CO_2): 2 mL min^{-1}	Drift tube temperature: 60 ºC Flow: 0.8 L min^{-1}	Gas pressure: 35 psi Gas flow: 1.53 L min^{-1} Drift tube temperature: 30ºC	[68]
Saikosaponins	Injection volume: 20 µL Column temperature: 27.5 ºC	Nitrogen pressure: 3 bar Drift tube temperature: 50 ºC	Gas pressure: 35 psi	[69]

detectors, thanks to the higher percentage of polar organic solvent in the mobile phase, which clearly favors the evaporation step, generating larger particles. The C-CAD was ~ 10 times more sensitive in HILIC than in RP chromatography for the 12 compounds selected. This difference was less pronounced for ELSD. No information about the LODs is given, so a direct comparison between ELSD and C-CAD cannot be carried out. The authors conclude that ELSD and C-CAD do not provide a universal response for all the analytes, although these differences are minimal when the same operational mode is used. In the case of gabapentin, a highly polar antiepileptic drug, the authors calculated the LODs and LOQs obtained with RP and HILIC modes using ELSD and C-CAD [66]. They concluded that although in RP, C-CAD was much more sensitive than ELSD, they perform almost equal in HILIC, considering the linear range, sensitivity, precision, and accuracy. The baseline stability was better for ELSD with specific stationary phases.

Hazotte et al. [67] show the feasibility of using the microchromatography at high temperatures with evaporative-based detectors for the determination of lipids. Irrespective of the detector used, temperature improves the response intensity if peak heights are considered. For the complex lipid samples, C-CAD provides better performance than ELSD at low concentration, the LODs being similar to those provided by MS. From this study, it can be said that the C-CAD is the only detector with a universal response as it is capable of detecting all the lipids with a linearity of two orders of magnitude. It also surpasses the MS in its ease of use. Similar conclusions were reached by Pistorino and Pfeifer [64] when they compared the potential of ELSD and C-CAD for polyketide analysis. The ELSD was the less sensitive (1 mg L^{-1}), while the LOD obtained with C-CAD (0.012 mg L^{-1}) was even

lower than that reached with mass spectrometry. On the other hand, ELSD surpassed the other detectors in precision and dynamic calibration range.

ELSD and C-CAD have been quantitatively compared using supercritical fluid chromatography and a certified reference material of PEG [68]. The final interest was to use the C-CAD for the molecular mass determination of this synthetic polymer. All the analytical figures of merit were better for C-CAD than for ELSD, although at higher concentration (e.g. 10 mg mL^{-1}) no significant differences were found with the intensities of ELSD and C-CAD. In addition, the intensities of the chromatographic peaks decrease with an increase in the degree of polymerization in both cases, but the decrements of intensities using C-CAD were smaller than those using ELSD. Compared to ELSD, the quantitativeness of C-CAD is so good that the molecular mass distribution of certified reference material (CRM) PEG 1000 can be closely determined without any calibration of detections.

7. Conclusions

The evaporative-based detectors are universal response instruments, very versatile for the determination of a variety of families of compounds. The foundation of the response, independent from the majority of physicochemical properties of the analytes (volatility excepted), is behind this versatility. Moreover, they fully compatible with the most used mobile phases in liquid and supercritical fluid chromatographies. Although the majority of the applications have been proposed in the chromatographic context, their usefulness in the development of fast-response analytical systems has also been pointed out. This is especially important for the ELSD, while C-CAD still remains unexploited in this field. The influence of the mobile-phase composition, more marked for C-CAD than for ELSD, can be the reason for this limitation, despite its better sensitivity. Future work will be aimed at

introducing the alternatives currently available to avoid this negative effect in its chromatographic uses to develop vanguard systems which used C-CAD. Moreover, the demonstrated applicability of ELSD in this context should be expanded with new configurations and analytical problems.

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Capítulo 2

Determination of parabens in cosmetic products using multi-walled carbon nanotubes as solid phase extraction sorbent and corona-charged aerosol detection system

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Determination of parabens in cosmetic products using multi-walled carbon nanotubes as solid phase extraction sorbent and corona-charged aerosol detection system

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ABSTRACT

The potential of carbon nanotubes for the solid phase extraction of parabens in cosmetic products and the detection using a corona-charged aerosol detector (C-CAD) is presented in this work. The analytical procedure is based on a conventional solid phase extraction step for which 20 mg of multi-walled carbon nanotubes were packed in a 3-mL commercial SPE cartridge. Methylparaben, ethylparaben, propylparaben and butylparaben were thus isolated and preconcentrated from the pre-treated samples and subsequently separated on a RP-C_{18} column using acetonitrile:water, 50:50 (v/v) as mobile phase. The analytical signals for the individual parabens were obtained using C-CAD. The experimental variables affecting the extraction procedure and the instrumental detection have been deeply studied. Limits of detection were in the range of 0.5–2.1 mg L^{-1}, while the linear range was extended up to 400 mg L^{-1}. The average precision of the method varied between 3.3–3.8% (repeatability) and 4.3–7.6% (reproducibility). Finally, the optimized procedure was applied to the determination of the target preservatives in a variety of cosmetic products with satisfactory results.

1. Introduction

Parabens are alkyl esters commonly used as preservatives to prevent foods, cosmetics and pharmaceuticals from microbial and fungal attack. These compounds, including methylparaben (MP) methylparaben (EP), propylparaben (PP) and butylparaben (BP) are often found in combinations of two or more substances in almost all types of cosmetics (e.g. facial make-ups, deodorants, gels, creams or skin lotions) [1,2]. Recent investigations state that the super-scale use of these preservatives in cosmetics can result in potential health risks due to their estrogenic activity, modulating and disrupting the endocrine system. Moreover, their potential contribution to the incidence of breast cancer has been highlighted recently [3–6]. The European Union permits the use of parabens with a maximum concentration for each compound of 0.4% (w/w) and a total maximum concentration of 0.8% (w/w), expressed as p-hydroxybenzoic acid (EU cosmetics directive 76/768/EEC) [7]. Therefore, the simultaneous determination of the most commonly employed parabens in cosmetic products is desirable. Taking the sample complexity into account, highly selective methods are required for this purpose.

Various analytical techniques have been used for the determination of parabens in different matrices, including gas chromatography [8–10], high performance liquid chromatography (HPLC) [11–15], capillary zone electrophoresis [16,17] micellar electrokinetic chromatography (MEKC) [18–20] or ultra performance liquid chromatography [21]. HPLC techniques are more commonly used for parabens determination since gas chromatographic separation requires a prior derivatization of the compounds. In any case, sample treatment steps are mandatory before analyte injection into the chromatographic system [22]. Different sample preparation methods have been proposed for determining parabens in cosmetic samples. Solid phase extraction (SPE) [19,23,24] and solid phase microextraction [25–28] are the commonest

alternatives of choice for this determination due to their simplicity and effectiveness of extraction. Besides, these techniques allow high selectivity and sensitivity, reducing or eliminating the volume of organic solvent employed.

The application of carbon nanotubes in the analytical field as potential SPE sorbents is gaining importance in the recent years because of their special chemical and physical properties. These nanometric materials have a high hydrophobic surface area and exhibit strong interaction capabilities for various compounds. The described adsorption mechanisms involve the establishment of weak interactions, more precisely, π–π stacking, Van der Waals forces, other hydrophobic interactions and electrostatic forces [29]. This fact facilitates the adsorption of analytes in a selective and reproducible manner. As a result, multi-walled carbon nanotubes (MWCNTs) have been used as sorbent material for the determination of a variety of organic compounds in different samples [30–33]. However, the extraction performance of MWCNTs for parabens has not been previously utilized.

Corona-charged aerosol detector (C-CAD) has been recently introduced as a new universal detection system for HPLC [34]. Its performance can be compared with that of others detectors of universal response such as the evaporative light scattering detector (ELSD) [35], refractive index (RI) [36], low-wavelength ultra-violet (UV) [37], and mass-spectrometer [38]. The operating principle of C-CAD is based on the detection of charged particles with a selected range of mobility instead of measuring individual gas-phase ions with different m/z ratio. Consequently, the signal obtained depends primarily on the particle size while, in comparison to UV or RI detectors, individual spectroscopic analyte properties negligibly affect the instrumental response obtained. As well as the ELSD, the response is not

dependent on the analyte structure, being only limited by the fact that they can detect compounds provided that they have lower volatility than the mobile phase. C-CAD is characterized by a robust, sensitive, reproducible response and can be afforded at adequate acquisition and maintenance expenses. In addition, it boasts a wide dynamic range, ease of use and has been previously employed for the determination of non- or semi-volatile compounds encountered in pharmaceutical, food, consumer product, industrial chemical and life science applications [39–41].

The research study presented in this work is focused on the development of a new application of MWCNTs as sorbent material for parabens extraction, preconcentration and sample clean-up step. Moreover, for the first time the evaluation of the applicability of the C-CAD in this context with a systematic study of the instrumental variables affecting the analytical response has been accomplished. Finally, the developed and optimized procedure for parabens has been applied to the analysis of a series of cosmetic products, demonstrating the feasibility of the novel system for a wide range of matrix complexities.

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade or better. Acetonitrile (Scharlab, Barcelona, Spain) and Milli-Q ultrapure water (Millipore, Madrid, Spain), were employed as components of the chromatographic mobile phase. Methanol and acetone (Panreac, Barcelona, Spain) were used in the solid phase extraction procedure. Hydrochloric acid, purchased from Panreac, was employed to adjust the pH of samples and aqueous standards.

The analytes (methylparaben, ethylparaben, propylparaben and butylparaben) were obtained from Sigma–Aldrich (Madrid, Spain). Stock standard solutions of each analyte were prepared in methanol at a concentration of 5 g L^{-1} and stored in glass-stoppered bottles in the dark at 4 ^{o}C. Working solutions containing all the analytes were prepared by dilution of the stocks in mobile phase or ultrapure water depending on the purpose.

Multi-walled carbon nanotubes, purity 95%, were purchased from Sigma–Aldrich and used as sorbent materials for the preconcentration of the selected analytes. The external and internal diameters were in the range 20–30 and 5–10 nm, respectively, whereas the length varied between 0.5 and 200 μm.

Samples of different types of cosmetic products were purchased from local commercial stores and kept at room temperature until their analysis.

2.2. Apparatus

Analytes detection was carried out using a C-CAD, purchased from ESA Biosciences (Chelmsford, MA, USA). A Mistral 4 nitrogen generator was obtained from Clan Tecnológica (Seville, Spain). Signals were acquired using a HPChem Station software interfaced via an HP 35900C A/D converter, both from Agilent Technologies (Madrid, Spain). The detector settings were kept constant in all experiments using a gas pressure of 37 psi.

The chromatographic separation of the analytes was performed using a Hewlett Packard 1050 high pressure pump (Agilent Technologies) for the mobile phase delivery and a high pressure injection valve (Rheodyne 7725, Cotati, CA, USA) fitted with a 20-μL stainless steel sample loop. Chromatographic separation was carried out at room temperature on a RP-C_{18} column (250 mm × 4.6 mm) obtained from Análisis Vínicos (Tomelloso,

Spain). Besides, an ultrasonic bath (J.P. Selecta, Barcelona, Spain) was employed for the mobile phase degasification. The mobile phase composition was acetonitrile:water 50:50 (v/v) and the flow rate was fixed at 0.5 mL min^{-1}.

Solid phase extractions were performed using a Vac Elut-20 sample-processing station (Scharlab), equipped with a vacuum-control valve and PTFE cartridge adapters (Varian, Barcelona, Spain). Three milliliters commercial SPE cartridges were packed with an appropriate amount of sorbent using frits to avoid losses of material.

A high speed centrifuge with a microprocessor control (J.P. Selecta) was used in the sample treatment step.

2.3. Procedure

The experimental setup employed is given in Fig. 1. An accurately weighed amount of sample (1.0 g) was properly diluted in ultrapure water (between 1:20 and 1:100, v/v); then, the sample solution was treated with ultrasounds for 30 min and was centrifugated for 15 min. The pH of the supernatant was adjusted to pH 3 using HCl 0.1 M and 1 mL of this liquid phase was subjected to the SPE procedure. This sample treatment facilitates the subsequent extraction process.

The solid phase extractions were performed in laboratory-packed cartridges containing 20 mg of MWCNTs. The cartridges were preconditioned with 5 mL of methanol and equilibrated with 5 mL of Milli-Q water prior to each extraction procedure. Then, 1 mL of the pre-treated sample, obtained as foregoing explained, was passed through the sorbent at a flow rate of 1 mL min^{-1}. Subsequently, a washing step was conducted using 4 mL of Milli-Q water at pH 3 and the cartridges were dried for 2 min. Finally, the analytes

were eluted with 2 mL of acetone at a flow rate of 1 mL min^{-1}. The extracts were evaporated to dryness under a nitrogen stream at room temperature and redissolved in 500 µL of the mobile phase before their injection in the chromatographic system. The peak area was used as the analytical signal. The cartridge can be reused for ca. 200 extractions by including a washing step with water between samples analysis.

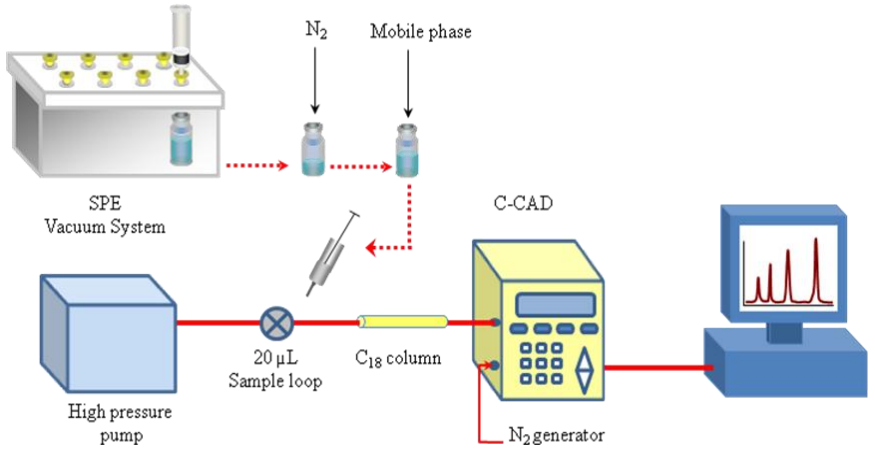


Figure 1. Schematic description of the analytical procedure developed for parabens determination in cosmetic products: HPP, high pressure pump; IV, high pressure injection valve.

3. Results and discussion

3.1. Chromatographic separation and detection

The optimization of the chromatographic separation of the target analytes was influenced by the detection system. The response of the C-CAD depends on the amount of organic solvent in the mobile phase as it influences the transport efficiency of the nebulizer and the generated signal. A high organic content in the mobile phase leads to an increase in the transport efficiency of the nebulizer, which results in a greater number of particles reaching the detector chamber and a higher signal. An empirical solution to this problem is the so-called mobile phase gradient compensation [42,43].

Therefore, it would be desirable to establish chromatographic conditions that avoid the variability in the solvent composition without the need of a gradient compensation. Taking this fact into account, the chromatographic separation of parabens was optimized using an isocratic composition of the mobile phase to generate stable baselines, working with fixed mixtures of solvents and constant flow rates during each run. From the different mixtures evaluated, acetonitrile:water (50:50, v/v) was chosen for the analytes separation since it offered the best resolution of the peaks and a stable baseline. The flow rate was maintained at 0.5 mL min^{-1}. Under these chromatographic conditions each run needs about 30 min to be completed. The retention times of the parabens assayed are listed in Table 1. The analytes selected are efficiently separated in 25 min. However, 5 min were added to the run in order to prepare the system for the following injection. It should be mentioned that this time can be shorten for standards to 15 min without affecting peak resolution by reducing the water content in the mobile phase to 25%. Nevertheless, the analysis of samples with a high content of hydrophilic components (i.e. sugars) leads to interferences for the quantification of MP. A typical chromatogram obtained for an aqueous standard containing the four compounds at a concentration of 200 mg L^{-1} is shown in Fig. 2.

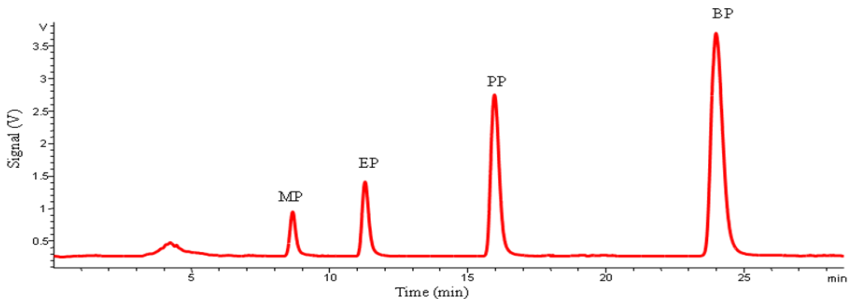
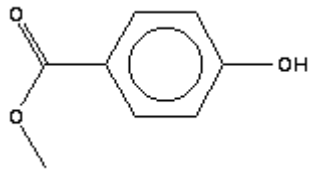


Figure 2. Chromatogram of a standard solution of parabens at a concentration of 200 mg L^{-1} working at 50 pA.

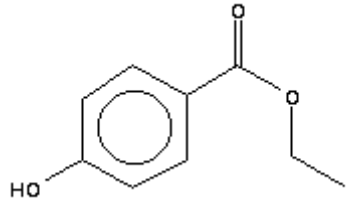
The principal variables affecting the C-CAD response were the evaporative chamber temperature and the nebulizing gas pressure. Other instrumental parameters to consider were the attenuation of the signal and the final filter applied. The C-CAD employed works at a fixed evaporative chamber temperature of 60 ºC in order to evaporate the mobile phase in the column effluent. In comparison with the new generation of C-CAD detectors, the system used in this research does not allow this temperature to be changed. Nevertheless, the results obtained under this working temperature were adequate for the present application. An important instrumental parameter in this type of detectors is the nebulizer gas pressure, which affects the uniformity and size of the droplets formed. Nitrogen is the nebulizer gas recommended for a proper functioning of the detector and the optimum gas pressure is in the range of 35–40 psi. Therefore, an intermediate pressure of 37 psi, provided by a nitrogen gas generator (purity 99.99%), was employed. A critical parameter to consider was the attenuation rate of the signal, which is inversely related to the sensitivity of the detection. The attenuation range of the detector varies from 1 to 500 pA. This parameter was changed taking into account the different concentration levels of the parabens assayed. The highest responses correspond to 1 pA (lower attenuation) while for higher concentrations it was set at 200 pA in order to obtain an adequate instrumental response. The lower attenuation (1 pA) was selected for the first analysis of diluted commercial samples with unknown concentrations in order to detect the presence of analytes using the highest gain of the detector. When some of the analytes' signals were saturated, the attenuation was increased as required. Finally, the detector allows establishing a filter for the application of a smooth of the signal. A medium filter was selected for the present application.

3.2. Optimization of the solid phase extraction procedure

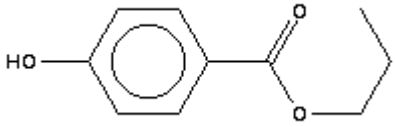
According to the literature, MWCNTs have obvious advantages as SPE sorbents for the isolation and preconcentration of organic compounds. The interaction of carbon nanotubes with the aromatic ring of organic substances through π-π interactions has been previously described [29]. Taking into account the chemical structure of the target analytes (see Fig. 3), MWCNTs were selected as sorbent material for the preconcentration and separation of parabens in cosmetic products. No references dealing with the application of MWCNTs as sorbent in this context have been reported up to date. Therefore, a systematic study of the variables potentially affecting the sorption process was deeply conducted.



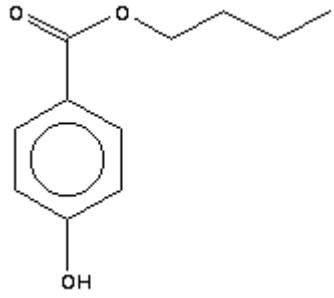
Methylparaben (MP)



Ethylparaben (EP)



Propylparaben (PP)



Butylparaben (BP)

Figure 3. Molecular structures of the paraben assayed.

The experimental variables evaluated were the following: sample pH, eluent and clean-up reagents, sample volume and dilution, and amount of sorbent. For this study, an aqueous standard solution with the parabens most commonly employed in cosmetics (MP, EP and PP) at a final concentration of 0.5 µg mL^{-1} was used. After each extraction, the extracts were evaporated to dryness under a nitrogen stream and reconstituted in 500 µL of the mobile phase acetonitrile:water (50:50, v/v) for chromatographic analysis.

3.2.1. Sample pH

According to their chemical structure, the charge of the parabens studied can be affected by the pH of the sample (pKa values varying from 8.27 and 8.45 for MP and BP, respectively). Therefore, their retention on the sorbent could be influenced by this variable. The sample pH was investigated in the range 2–10 by adding appropriate volumes of HCl 0.1M or NaOH 0.1M to the aqueous standards. According the results, the signals remained almost constant from 2 to a pH value of 3. Then, a considerable decrease of the extraction happened when the pH varied between 3 and 7, leading to a less marked reduction of the signal at higher pH. Therefore, pH 3 was selected as the optimum value of this variable. It should be noted that these results are not concordant with the expected behavior when the analytes pKa values are taken into account, which would lead one to anticipate only a lower interaction of the analytes with the sorbent due to their ionization when their pKa is reached. A possible interpretation of the data obtained would be that the MWCNTs employed have some type of acid impurities that are in their neutral form at the lower pH values assayed, leading to the maximum hydrophobic interactions with the analytes. This fact is more relevant than the influence of the pKa of the selected parabens, which can be detected in a lower extent in a basic media.

3.2.2. Eluent and clean-up reagents

Different solvents (acetonitrile, methanol and acetone) were evaluated for their use as eluents of the retained analytes. The worst result was that obtained with acetonitrile, whereas acetone provided quantitative elution of the three parabens assayed and therefore, it was chosen as optimum. Finally, the eluent volume was studied and it was found that no carry over between samples was obtained for 2 mL of acetone. For the clean-up step to be implemented between samples, 4 mL of distilled water were found to be enough for an adequate interference removal and cartridge conditioning.

3.2.3. Sample volume and amount of sorbent

The volume of sample that can be subjected to the solid phase extraction without reaching the breakthrough value is directly related to the sorbent capacity and, consequently, to the quantity of MWCNTs used for the extraction. Moreover, the sorbent amount affects the quantity of analyte that can be retained and the volume of eluent required. The studies conducted demonstrated that 20 mg of MWCNTs allowed a quantitative retention of all the parabens at the concentrations evaluated (50 mg L^{-1}) when 1 mL of the standard solution was passed through the cartridge. When the extraction was applied to cosmetic samples, the chromatographic signals of MP and EP were overlapped by those coming from matrix compounds. In order to reduce this negative effect, the sample was diluted in ultrapure water. A dilution factor of 1:10 (v/v) was enough to minimize this effect. However, higher dilution factors were applied as required in order to adequate the initial concentration of the parabens in the sample to the dynamic calibration range of the method.

The maintenance of the sorbent capacity was checked by the regular extraction of a control standard solution with all the analytes at 50 mg L^{-1},

which also permits to control the reusability of the cartridge. The sorbent was replaced when a variation higher than 10% was obtained for the peak areas.

By including the clean-up step between samples, the cartridge can be reused for ca. 200 extractions with negligible variations on the sorbent capacity.

3.3 Figures of merit

The figures of merit of the method are summarized in Table 1. The calibration graphs were constructed for six aqueous standards containing the analytes used in the optimization step plus BP since some of the samples to be analyzed contained this compound as indicated in the label. The standards were subjected to the whole procedure under the optimized conditions previously described. The lowest detectable concentrations were obtained using the S/N = 3 ratio. According to the results, the sensitivity of the method increases with the alkyl chain of the analyte, this property being better for BP. This is in accordance with the fact that the response in C-CAD depends on the particle size.

Table 1. Figures of merit the proposed method.

Analyte	Interval ($mg\ L^{-1}$)	R	LOD[a] ($mg\ L^{-1}$)	LOQ[b] ($mg\ L^{-1}$)	t_r[c] (min)
Methylparaben	1 – 400	0.996	2.1	5.3	8.6
Ethylparaben	1 – 400	0.990	1.5	4.6	11.3
Propylparaben	0.5 – 400	0.998	0.7	3.0	15.9
Butylparaben	0.5 – 400	0.997	0.5	2.0	24.0

[a] LOD, limit of detection.
[b] LOQ, limit of quantification.
[c] t_r, chromatographic retention time.

The precision (repeatability and reproducibility) of the method (Table 2), expressed as relative standard deviation, was evaluated for five and three replicates of a working aqueous standard prepared at a low concentration of the calibration curve (15 mg L^{-1}). The values calculated for intraday experiments were lower than 4% in all cases, whereas the interday reproducibility ranged between 3.3% for BP and 7.6% for EP.

Table 2. Intra- and interday precisions of the proposed method.

Analyte	Intraday precision (RSD%, n=5)	Interday precision (RSD%, n=3)
Methylparaben	3.8	7.0
Ethylparaben	3.7	7.6
Propylparaben	3.6	6.3
Butylparaben	3.3	4.3

3.4. Analysis of samples

A series of samples of cosmetic products of different natures were obtained from local stores and analyzed using the proposed method following the procedure described in Section 2.3. A previous dilution of the samples in ultrapure water was necessary prior to their solid phase extraction. An exemplary chromatogram obtained for the sample "moisturizing cream 1" is depicted in Fig. 4. Since the concentration of the parabens was not specified in the products labels, a standard dilution of 1:10 (v/v) was initially applied to all the samples. A higher dilution ratio was used (up to 1:100, v/v) depending on the analytes concentration and the signals obtained.

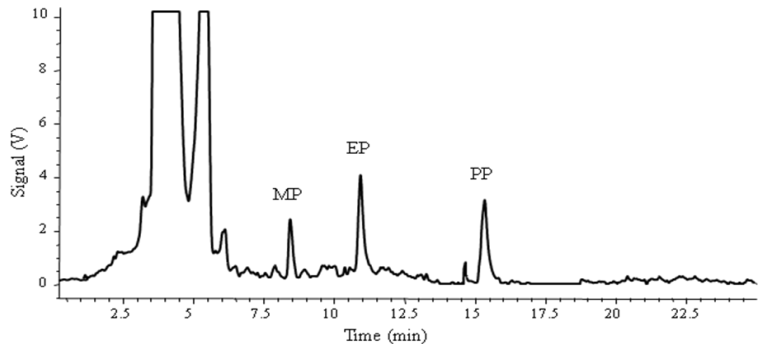


Figure 4. Exemplary chromatogram obtained for a sample of moisturizing cream with a dilution rate of 1:100 (v/v) working at 50 pA.

The maximum dilution rate that was finally applied and the concentration levels found for the parabens are shown in Table 3.

Table 3. Determination of parabens in cosmetic products.

Samples	Dilution	Concentration (mg L^{-1}) ± SD[a]			
		Methylparaben	Ethylparaben	Propylparaben	Butylparaben
Moisturizing cream 1	1: 100	1490 ± 45	1150 ± 40	590 ± 20	-
Moisturizing cream 2	1:60	504 ± 19	-	348 ± 15	-
Moisturizing cream 3	1:25	858 ± 29	208 ± 6	125 ± 4	270 ± 9
Antiwrinkle cream	1:60	1476 ± 45	-	616 ± 20	-
Make-up	1:50	1109 ± 35	-	-	-
Lotion	1:50	615 ± 21	320 ± 11[b]	290 ± 10	-
Hair gel for ends	1:50	1210 ± 38	310 ± 10	125 ± 4	75 ± 3
Hands cream	1:20	190 ± 6	154 ± 5	46 ±2	30 ± 1
After sun	1:20	308 ± 12	-	112 ± 4	-

[a] SD, standard deviation
[b], not labeled but found
–, not labeled and non-detected

The values found ranged from 30 mg L^{-1} for BP to 1490 mg L^{-1} for MP. On the other hand, MP and PP were detected in almost all the samples since they constitute the most commonly used preservatives due to their antimicrobial activity and solubility in water. The analytes were detected in all cases when their presence in the products was labeled. However, EP was found in the lotion sample despite not being specified in the product label.

The carryover was evaluated by analyzing a blank of water after the extraction of standard and samples, leading to no signal in the detector. A washing step was conducted after each extraction in order to assure the SPE cartridge remains clean.

Finally, the recoveries for each analyte from a series of selected samples were evaluated under the optimum extraction conditions. Different types of cosmetic products (make-up, antiwrinkle cream, moisturizing cream and hair gel) were selected for this study in order to spread the range of matrices evaluated. The samples, previously analyzed, were spiked with 300 mg L^{-1} of each analyte and subjected to the whole procedure described in Section 2.3. The final concentrations were obtained using the calibration curves and the relative percentages of recuperation were calculated as the ratio between the concentration of the analyte found after the spiking process and the initial concentration of each analyte in the sample. The results obtained are shown in Table 4. The high recovery values obtained ensure the accuracy of the concentrations found in the non-spiked commercial samples.

Table 4. Recoveries of parabens from different types of cosmetic products (n = 3). 300 mg L^{-1} of each analyte were spiked to the samples.

Sample	Methylparaben		Ethylparaben		Propylparaben		Butylparaben	
	Concentration found ± SD*	Recovery (%)	Concentration found ± SD*	Recovery (%)	Concentration found ± SD*	Recovery (%)	Concentration found ± SD*	Recovery (%)
Make- up	1292 ± 50	92 ± 4	244 ± 8	82 ± 3	301 ± 13	100 ± 1	308 ± 15	103 ± 2
Antiwrinkle cream	1563 ± 50	88 ± 1	283 ± 11	94 ± 4	870 ± 30	95 ± 1	288 ± 10	96 ± 2
Moisturizing cream 3	1041 ± 35	90 ± 1	475 ± 23	94 ± 5	418 ± 20	99 ± 2	564 ± 20	99 ± 2
Hair gel for ends	1282 ± 35	85 ± 2	561 ± 26	92 ± 3	428 ± 20	101 ± 3	389 ± 15	104 ± 3

* Concentration in mg L^{-1}
SD, standard deviation

3.5. Comparison with other methods

The present method can be compared with other alternatives described for parabens determination in cosmetic products. Table 5 summarizes the main analytical information of the comparable methods reported for this type of samples. It can be derived from the data that the proposed system presents similar recovery and repeatability values compared with the majority of the other approaches. On the other hand, the limits of detection achieved are higher. Nevertheless, it should be highlighted that the present method has been optimized for the concentration level at which the target analytes are expected to be found in cosmetic products and, therefore, a better sensitivity is not required. In contrast, the higher selectivity achieved with this alternative, making use of an uncomplicated sample treatment, is of special interest due to the complexity of the matrices. Compared with some of the methods [8,12], the costs of acquisition and maintenance of the system presented in this article are lower. Besides, the complexity of the analytical systems employed [13,19] and sample pretreatment requiring several steps [15,19] of other methods described, can be compared with the ease of operation and functioning of C-CAD and the SPE performance. In addition, a wider range of cosmetic

samples with different complexity has been evaluated in the present in comparison with other applications [12,13,15].

Table 5. Comparison of the proposed method with other developed alternatives for the determination of parabens in cosmetics.

Method	Cosmetic product	LOD	RSD (%)	Recovery (%)	Reference
SDME[a]-GC-MS	Mouthwash solution, gels	0.001-0.015 μg L^{-1}	< 12.1	92-105	[8]
SFE[b]/HPLC-MS	Lanoline cream, skin milk	4.7–19.3 μg L^{-1}	< 18.6	-	[12]
HPLC/CL[c]	Wash-off cosmetics	1.9–5.3 μg L^{-1}	< 3.1	93-106	[13]
HPLC/UV	Foam shampoo	0.02–0.05 mg L^{-1}	< 3.2	98-105	[15]
FIA[d]-SPE-MEKC	Gel, lotions, water/oil-based creams	0.07–0.1 mg L^{-1}	< 2.3	93-102	[19]
SPE-HPLC-C-CAD	Creams, shampoo, make-up, lotions	0.5–2.1 mg L^{-1}	< 3.8	90-104	Proposed method

[a] SDME: single drop microextraction.
[b] SFE: supercritical fluid extraction.
[c] CL: chemiluminescence.
[d] FIA: flow injection analysis.

4. Conclusions

The experimental work reported in this article has been aimed at the evaluation of the potential of two relatively new analytical tools, carbon nanotubes and C-CAD, in the field of cosmetics. The results obtained, in terms of sensitivity, precision and recovery values have demonstrated its applicability for the determination of parabens in such matrices. Concerning the SPE procedure, it is demonstrated for the first time that MWCNTs can be used as effective SPE material for the extraction of parabens. The π-π

interaction established between the analyte and the carbon nanotubes surface simplified the isolation step due to the low retention of potential interferents, leading to a high selectivity and ruggedness of the extraction with clean extracts without chromatographic interferences. Moreover, the low amount of stationary phase and the reusability of the cartridge is a great advantage as it notably reduces the cost of the analysis. As far as the C-CAD is concerned, we have demonstrated its capability for the determination of parabens with very good analytical features without the need of a compensation gradient. The universal response of the detector allowed the determination of the target compounds, providing signals that are dependent on the analytes' particle size. Therefore, it could be stressed that the system described in this work meets the analytical requirements with a simple design that does not necessitate trained personnel, employing instrumentation with an easy functioning and a low cost of acquisition and maintenance. This makes the proposed method a valid alternative for the determination of parabens in cosmetic products.

Acknowledgement

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Capítulo 3

Determination of water-soluble vitamins in infant milk and dietary supplement using a liquid chromatography on-line coupled to a corona-charged aerosol detector

Determination of water-soluble vitamins in infant milk and dietary supplement using a liquid chromatography on-line coupled to a corona-charged aerosol detector

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ABSTRACT

A simple and rapid method for the simultaneous determination of seven water-soluble vitamins (thiamine, folic acid, nicotinic acid, ascorbic acid, pantothenic acid, pyridoxine and biotin) was developed by high performance liquid chromatographic separation and, corona-charged aerosol detection. The water-soluble vitamins were separated on a Lichrosorb RP-C_{18} column under isocratic conditions with a mobile phase consisting of 0.05M ammonium acetate:methanol 90:10 (v/v) at the flow rate 0.5 mL min^{-1}. The vitamins were extracted from the infant milk (liquid and powder format) using a precipitation step with 2.5M acetic acid remaining the analyte in the supernatant. As far as dietary supplements are concerned, only a dilution with distilled water was required. The detection limits ranged from 0.17 to 0.62 mg L^{-1} for dietary supplements and 1.7 to 6.5 mg L^{-1} for milk samples. The precision of the method was evaluated in terms of relative standard deviation (%, RSD) under repeatability and reproducibility conditions, being the average values for each parameter 2.6 and 2.7 for dietary supplements and 4.3 and 4.6 for milk samples. The optimized method was applied to different infant milk samples and dietary supplements. The results of the analysis were in good agreement with the declared values.

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1. Introduction

Vitamins are organic compounds designated as nutrients because they cannot be synthesized by the body [1]. Vitamins can be divided into two groups, the water-soluble and the fat-soluble ones. The water-soluble vitamins include the B-group vitamins (thiamine, folic acid, nicotinic acid, pantothenic acid, riboflavin, cyanocobalamin, pyridoxine and biotin) and vitamin C (ascorbic acid). In developed countries, food fortification has proven to be an effective and low-cost way to increase the micronutrient supply. Measurements of vitamin levels in food and other samples have long been of interest to the health and nutritional fields and to the food industry.

The official methods for water-soluble vitamins analysis are often based on tedious, and sometimes not completely specific, microbiological assays [2,3]. Vitamins have been determined individually in food samples and pharmaceutical formulations using various analytical techniques such as spectrophotometric, spectrofluorimetric, and electrochemical, as well as separation techniques, e.g. capillary zone electrophoresis and high-performance liquid chromatography (HPLC). C.J. Blake reviewed the analytical procedures proposed for the determination of water-soluble vitamins in foods and dietary supplements [4]. Liquid chromatography allows the simultaneous separation of combinations of water-soluble vitamins in a rapid, sensitive and accurate manner. In general, these methods have the advantages of solvent economy, easy coupling with other techniques, and small amounts of sample required [5,6]. The chromatographic separation of water-soluble vitamins in food can be carried out using neither normal phase [7] or reversed-phase [8] modes and ultraviolet detection (UV) with single wavelength [9-11] or photodiode array [12,13].

The corona-charged aerosol detector (C-CAD) is a new type of detector introduced for liquid chromatography that has recently become applied in a wide variety of analysis [14-17]. In the C-CAD, the effluent from the column is nebulized and dried in a stream of nitrogen, which acts also as an ionizing gas. Aerosol particles formed become charged in a corona discharge source and are then detected by a sensitive detector (electrometer). The C-CAD measures a physical property of the analyte and responds to almost all non-volatile compounds, regardless of their nature and spectral or physicochemical properties [18]. In comparison with this universal detector, UV detection provides higher sensitivity and improved gradient compatibility but the detection is limited to chromophores, and the response magnitude depends upon molar absortivity, which can vary by orders of magnitude even among analogous structures.

The main objective of the present work was to develop a simple HPLC method with C-CAD for the chromatographic separation and quantification of seven water-soluble vitamins: thiamine, folic acid, nicotinic acid, ascorbic acid, pantothenic acid, pyridoxine and biotin. The C-CAD is proposed as an alternative to the conventional detectors used up to now taking advantage of its universal response, as it only requires the analyte to be less volatile than the mobile phase. The results achieved were excellent and the method was applied to the analysis of infant milk and dietary supplements samples.

2. Materials and methods

2.1. Reagents and samples

All reagents were of analytical grade or better. Ammonium acetate (98%) (Sigma-Aldrich, Madrid, Spain) and methanol (Panreac, Barcelona, Spain), were employed as components of the chromatographic mobile phase.

Acetic acid from Panreac, was used in the extraction step of vitamins from the infant milk samples.

The analytes (thiamine, folic acid, nicotinic acid, ascorbic acid, pantothenic acid, pyridoxine and biotin) were obtained from Sigma–Aldrich. The structures of the seven vitamins used in this study are provided in Fig. 1. Stock standard solutions of each analyte were prepared in Milli-Q ultrapure water (Millipore, Madrid, Spain) at a concentration of 2 g L^{-1} and stored in glass-stoppered bottles in the dark at 4ºC. The solutions were manually agitated to ensure the solubility of the analytes. Working solutions containing all the analytes were prepared by dilution of the stocks in ultrapure water. Stock standard solutions were prepared every day to prevent potential degradation of the vitamins.

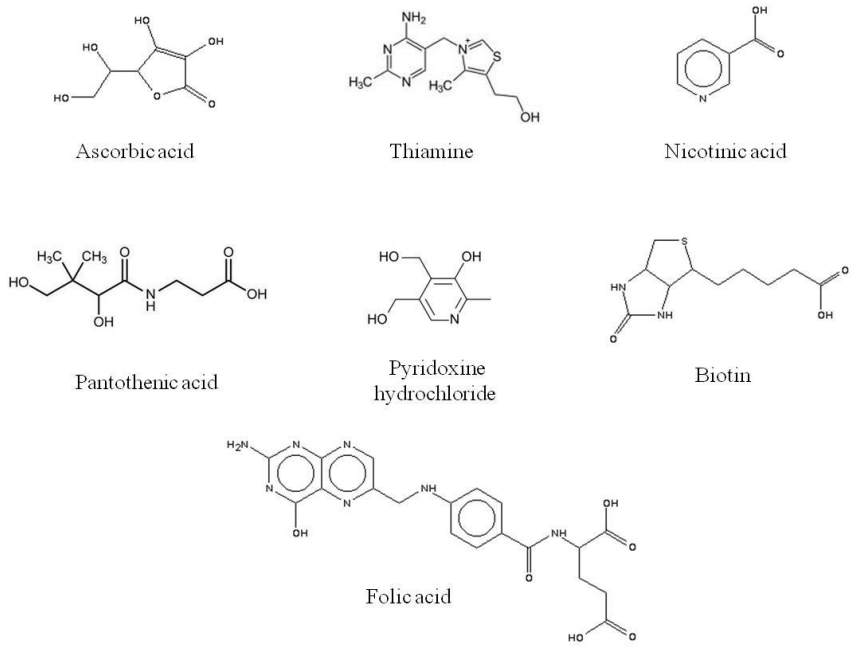

Figure 1. Molecular structures of the different vitamins studied.

Samples of different dietary supplements and infant milk products were purchased from local commercial stores and kept at room temperature until their analysis.

2.2. Chromatographic conditions

The chromatographic separation of the analytes was performed using a micro-HPLC pump Jasco 1585 (Jasco Analítica Spain, Madrid, Spain) for the mobile phase delivery and a high pressure manual injection valve (Rheodyne 7725, Cotati, CA, USA) fitted with a 20 µL stainless steel sample loop. Chromatographic separation was carried out at room temperature on a Lichrosorb RP-C_{18} column (5 µm, 250 mm × 4.6 mm) obtained from Análisis Vínicos. Besides, an ultrasonic bath (J.P. Selecta, Barcelona, Spain) was employed for the mobile phase degasification. The mobile phase composition was 0.05M ammonium acetate:methanol 90:10 (v/v) and the flow rate was fixed at 0.5 mL min^{-1}.

Analytes detection was carried out using a C-CAD, purchased from ESA Biosciences (Chelmsford, MA, USA). A Mistral 4 nitrogen generator was obtained from Clan Tecnológica (Seville, Spain). Signals were acquired using a Biocrom XP software interfaced via an A/D converter, both from Análisis Vínicos (Tomelloso, Spain). The detector settings were kept constant in all experiments using a gas pressure of 37 psi. The peak area was used as the analytical signal.

2.3. Sample preparation

The experimental treatment employed was different for each type of sample. An accurately weighed amount (5 – 250 mg) of dietary supplement samples, (pills and capsules), was properly crushed or unwrapped and diluted in ultrapure water (25 mL). Then, the sample solution was treated with

ultrasounds (ca. 1 min) to ensure the sample homogenization. The sample was directly injected in the liquid chromatograph. Preparation of infant powder milk samples consisted of weighing an appropriate amount (typically 4 g) and further dissolution of the powder in 25 mL of heated (40-45ºC) ultrapure water. Further, 10 mL of 2.5 M acetic acid was added for protein precipitation. The mixture was centrifuged for 15 min at 4000 rpm using a high speed centrifuge with a microprocessor control (J.P. Selecta). The supernatant was directly used for the determination of the hydrosoluble vitamins.

3. Results and discussion

Generally, the analysis of water soluble vitamins in food is very difficult due to the complexity of the matrices, which are as different as biological fluids or foodstuff [8,12,19]. In this work, we have evaluated the potential of high-performance liquid chromatography coupled with a corona-charged aerosol detector (HPLC-C-CAD) for the simultaneous analysis of water soluble vitamins dietary supplements and infant milk.

3.1. Chromatographic separation and detection

Water-soluble vitamins are commonly separated by reversed-phase liquid chromatography using a C_{18} column at room temperature. The response of the C-CAD depends on the composition of the mobile phase, increasing the baseline when increasing the percentage of organic solvent in its composition. An empirical solution to this problem, the so-named mobile-phase compensation, was proposed by Górecki et al. [20]. The composition of the mobile phase reaching the detector is kept constant by mixing the effluent from the column with a second mobile phase stream of exactly reverse composition, delivered by an additional HPLC pump. In this paper, we used a constant mobile phase composition and flow rate during each run.

Mobile phases composed by solvents (Milli-Q water, methanol, acetonitrile, phosphoric acid, trifluoroacetic acid and, ammonium acetate) in different proportions were evaluated in the optimization of the mobile phase for the separation of the target analytes using C-CAD. From this study, 0.05M ammonium acetate: methanol (90:10, v/v) was chosen for the vitamins separation since it offered the best resolution of the peaks and a stable baseline. The flow rate was maintained at 0.5 mL min^{-1}. The retention times of the vitamins assayed are listed in Table 1. The chromatographic profile obtained for an aqueous standard containing seven compounds at a concentration of 50 mg L^{-1} using this isocratic elution program is shown in Fig. 2 (A).

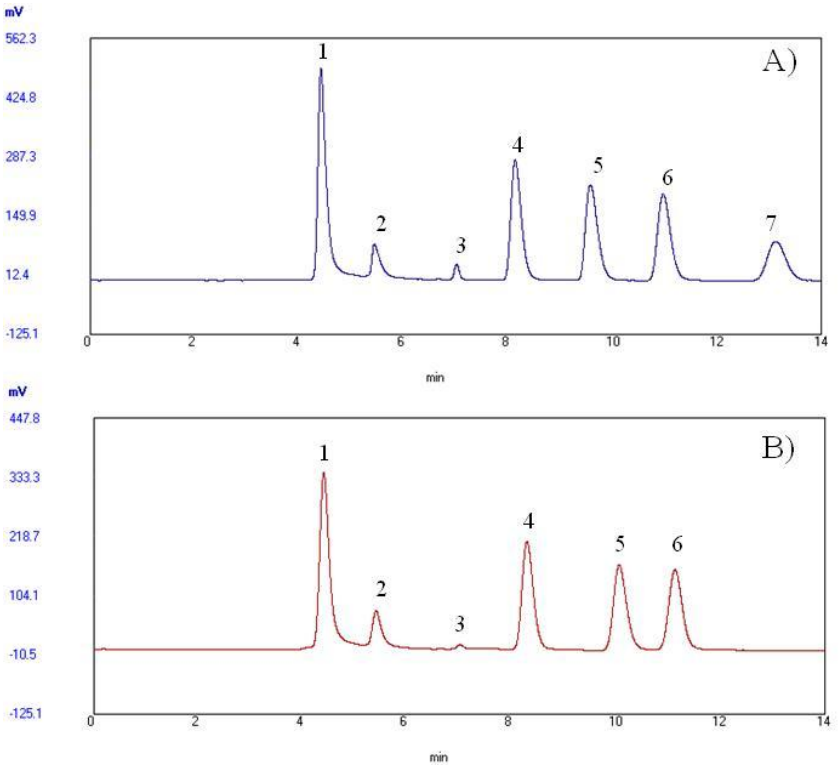

Figure 2. C-CAD chromatograms obtained for (A) a standard solution of vitamins at a concentration of 50 mg L^{-1} of each working at 50 pA; (B) a dietary supplement sample (complex 1) with a dilution rate of 1:25 (v/v) working at 5 pA. Chromatographic peaks: (1) ascorbic acid; (2) biotine; (3) nicotinic acid; (4) panthotenic acid; (5) thiamine; (6) pyridoxine hydrochloride; (7) folic acid.

The variables affecting the C-CAD signal were: evaporative chamber temperature, the nebulizing gas pressure, filter and attenuation of the signal. The optimum values were chosen in terms of peak shape, column efficiency, chromatographic analysis time, selectivity, and resolution.

The evaporative chamber temperature employed to evaporate the mobile phase is fixed at 60ºC. The system used in this research does not allow this temperature to be changed. The nebulizer gas pressure is an important parameter which affects the size and the uniformity of the droplets formed in the nebulization chamber. Generally, the nebulizer gas employed in the detector is nitrogen and the optimum range of pressure is 35-40 psi. Therefore, an intermediate pressure of 37 psi, provided by a nitrogen gas generator (purity 99.99%), was employed. Different filters (none, low, medium and high) were evaluated with the injection of aqueous standards containing the analytes studied at concentration of 50 mg L^{-1}. The filter is an electronic device that removes a portion of the noise in the signal output. A filter provides a smoother baseline making it easier for peak detection and quantification. A higher filter was selected for the present application allowing an adequate smooth of the signal. In the same way, the attenuation range of the detector was studied in the range from 1 to 500 pA at different concentration levels. The lower attenuation (1 pA) corresponds with the highest response of the detector. For dietary supplement samples it was necessary that the dilution of the sample due to the high concentration of the analytes. In this case, 5 pA was the attenuation selected to avoid the saturation of the signal. For infant milk samples, a higher attenuation, 50 pA was necessary in order to reduce the width and intensity of the peak due to the presence of remaining sample matrix component at the beginning of the chromatogram.

3.2. Optimization of the extraction procedure for infant milk samples

The complexity of the milk samples hinders their direct analysis Therefore, a sample pre-treatment step was required in order to eliminate for the protein and fat contents while avoiding the coprecipitation of the vitamins. The experimental variables evaluated were the following: milk sample pre-treatment, sample volume, stirring rate and time agitation. For this study, commercial milk samples enriched with vitamins at a known concentration given in the label were used and the pretreated sample was analyzed by HPLC-C-CAD using the previously optimized instrumental conditions.

Several treatments have been proposed for the determination of vitamins in different food sample (biscuits, cereals, baby foods), which involve the use of sulfuric acid [21] or hydrochloric acid followed by enzymatic digestion with taka-diastase [11]. For infant milk samples, proteins are precipitated using trifluoro- or trichloro- acetic acids [12]. However, these reagents are incompatible with the C-CAD, taking into account the instability of baseline and the high signal obtained. Therefore, acetic acid was chosen as precipitating reagent of the proteins contained in the infant milk samples. After mixing the sample with the acid, a centrifugation step was needed for phase separation to allow the determination of the vitamins in the supernatant. The clearance of the liquid phase depended on the concentration of acetic acid added.

Preliminary experiments demonstrated that the cleanest extracts were obtained for a concentration of 2.5 M acetic acid, the final concentration depending on the volumes of sample and acid used. Therefore, the next variable studied was the sample/acetic acid volume ratio. In addition to the qualitative evaluation of the precipitation step (viz. clearance of the

supernatant) the dilution factor obtained after precipitation should also allows the detection of the vitamins in the liquid phase.

This ratio was evaluated from 1:0.1 to 1:1 by maintaining constant the sample volume (25 mL). It was found that the addition of 10 mL of acetic acid 2.5 M provided the best results in terms of proteins precipitation and method sensitivity. This effect can be observed in the chromatograms depicted in Fig. 3.

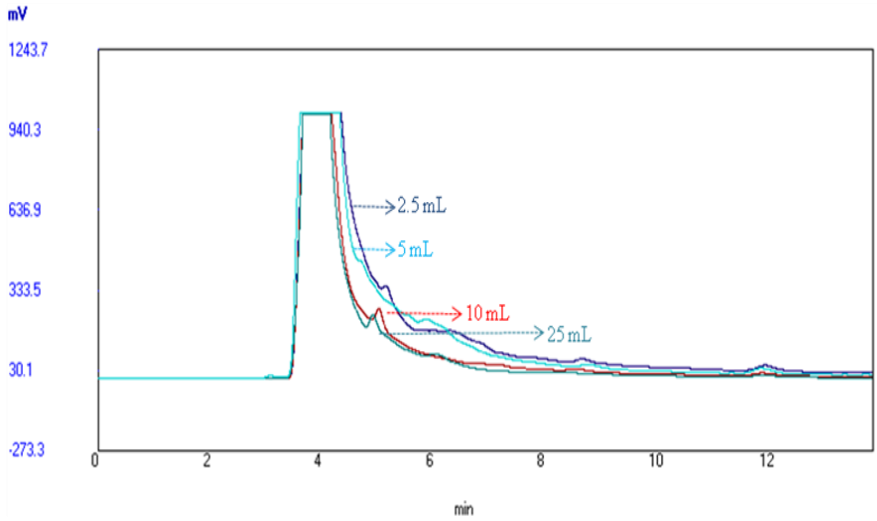

Figure 3. Chromatograms obtained for 25 mL infant milk sample employing different volumes (2.5, 5, 10 and 25 mL) of acetic acid in the precipitation step.

Next, the variables related to the centrifugation step, time and speed, were evaluated. In this step, the increase of the temperature is also a key factor as the variable can degradate the vitamins. The centrifugation speed was evaluated between 1000 and 6000 rpm with the time varying between 5 and 20 min. The chromatographic analysis of the extracts pointed out that 4000 rpm for 15 min provided acceptable phase separation with negligible influence on the sensitivity.

3.3. Figures of merit

The calibration curves were obtained by plotting concentration (mg L^{-1}) against peak area. For each vitamin, seven aqueous standards were prepared in the concentration range 0.5 to 200 mg L^{-1}, each one analyzed by duplicate. The results obtained are given in Table 1. Regarding the limit of detection (LOD) and the limit of quantification (LOQ), they were calculated as the concentration providing a signal to noise ratio of 3 and 10, respectively. LODs varied between 0.17 (for ascorbic acid) to 0.62 mg L^{-1} (for nicotinic acid) for dietary supplements and 1.7 (pantothenic acid) to 6.5 mg L^{-1} (for nicotinic acid) for milk samples, respectively. The values obtained for each vitamin are shown in the Table 1. The linearity was maintained up to 100 mg L^{-1} for dietary supplements and 200 mg L^{-1} for infant milk samples.

Table 1. Analytical features of the proposed method for the determination of water-soluble vitamins in aqueous standards and infant milk samples.

Analyte	Dietary supplement			Infant milk		
	LOD[a] (mg L^{-1})	LOQ[b] (mg L^{-1})	Slope	LOD[a] (mg L^{-1})	LOQ[b] (mg L^{-1})	Slope
Ascorbic acid	0.17	0.6	60.541	-	-	-
Thiamine	0.26	0.8	12.382	2.2	7.0	14.637
Nicotinic acid	0.62	2.0	31.288	6.5	22.0	37.677
Pantothenic acid	0.21	0.7	53.988	1.7	5.7	53.906
Pyridoxine hydrochloride	0.33	1.0	27.204	3.0	10.0	27.278
Biotin	0.45	1.4	11.698	4.6	15.2	12.168
Folic acid	0.54	1.8	23.486	5.8	19.0	22.773

[a] Limit of detection.
[b] Limit of quantification.

In order to evaluate the potential matrix effect on the quantitative determination of vitamins in infant milk samples, an in-matrix calibration graph was constructed for each analyte within the linear range calculated with

aqueous standards. The vitamins were added to non-enriched whole milk before the precipitation step. The slopes for in-matrix and ex-matrix calibration lines obtained for each analyte are compared in Table 1. As it can be seen, no interference from the endogenous matrix components or the sample pretreatment was detected, taking into account the close values obtained in all cases. Moreover, the other analytical features are comparable for all the vitamins.

Table 2. Precision of the method calculated under repeatability and reproducibility conditions.

Vitamins	Repeatability (RSD %, n=5)	Reproducibility between samples (RSD %, n=3)	Reproducibility between days (RSD %, n=3)
Dietary supplement samples			
Ascorbic acid	1.8	2.1	1.9
Thiamine	2.1	2.2	2.2
Nicotinic acid	2.3	2.0	2.1
Pantothenic acid	3.2	3.6	3.4
Pyridoxine hydrochloride	2.6	2.9	2.7
Biotin	3.5	3.9	3.7
Folic acid	2.4	2.8	2.7
Infant milk samples			
Thiamine	4.9	8.7	5.9
Nicotinic acid	3.2	4.0	3.5
Pantothenic acid	5.7	6.0	4.9
Pyridoxine hydrochloride	2.5	2.8	2.7
Biotin	5.2	4.8	4.3
Folic acid	4.3	4.0	3.9

RSD, relative standard deviation.

The precision of the method was calculated by measuring repeatability and reproducibility for each vitamin in the samples. The inter- and intra-day experiments were evaluated for five and three replicates of the pre-treated

samples. The precision was expressed as relative standard deviation (RSD). The values calculated are summarized in Table 2 and varied from 1.8 to 5.7% (repeatability) and 1.9 to 8.7% (reproducibility), indicating acceptable precision for the analysis.

3.4. Analysis of samples

3.4.1. Dietary supplement samples

The method was applied to the determination of vitamins in dietary supplements. The commercial product was provided in tablets (pills and capsules) and they crushed or unwrapped, dissolved and diluted in Milli-Q water. A typical chromatogram obtained is shown in Figure 2 (B). The concentrations found for each vitamin in the analyzed samples are given in Table 3. The good correlation with the labeled values demonstrated the applicability of the proposed method for the analysis of vitamins in these samples.

3.4.2. Infant milk samples under liquid and powder formats

The proposed method was also tested of the determination of the target analyte in milk samples. For this purpose, four different commercial infant formula samples were analyzed. The powder format samples were prepared following the commercial instructions (Section *2.3. Sample preparation*). Table 3 shows the results obtained of the method for determination of water-soluble vitamins in powder and liquid infant milk samples. The values obtained by the present method agreed with the values labeled for infant milk samples. A typical chromatogram of infant milk sample is shown in Fig. 4.

Samples	Concentration (mg L^{-1}) ± SD[a]													
	Ascorbic acid		Thiamine		Nicotinic acid		Pantothenic acid		Pyridoxine hydrochloride		Biotin		Folic acid	
	Concentration found (n=3)	Labeled value	Concentration found (n=3)	Labeled value	Concentration found (n=3)	Labeled value	Concentration found (n=3)	Labeled value	Concentration found (n=3)	Labeled value	Concentration found (n=3)	Labeled value	Concentration found (n=3)	Labeled value
Complex 1	289±3	300	28±2	30	10±1	15	89±5	95	40±2	45	42±3	45	-	-
Complex 2	-	-	-	-	-	-	-	-	-	-	194±6	200	-	-
Complex 3	-	-	-	-	-	-	-	-	-	-	-	-	16±3	20
Liquid infant milk	n.d.	-	7±1	10	8±1	10	9±1	10	12±1	15	n.d.	3	-	-
Powder infant milk 1	n.d.	-	143±2	145	11±1	10	17±2	20	71±3	75	n.d.	2	8±3	10
Powder infant milk 2	n.d.	-	20±2	25	157±3	150	117±2	120	39±1	40	n.d.	1	7±1	5
Powder infant milk 3	n.d.	-	12±1	10	94±2	90	54±2	50	8±1	10	n.d.	0.8	6±3	5

SD[a], standard deviation
- not labeled
n.d. not detected

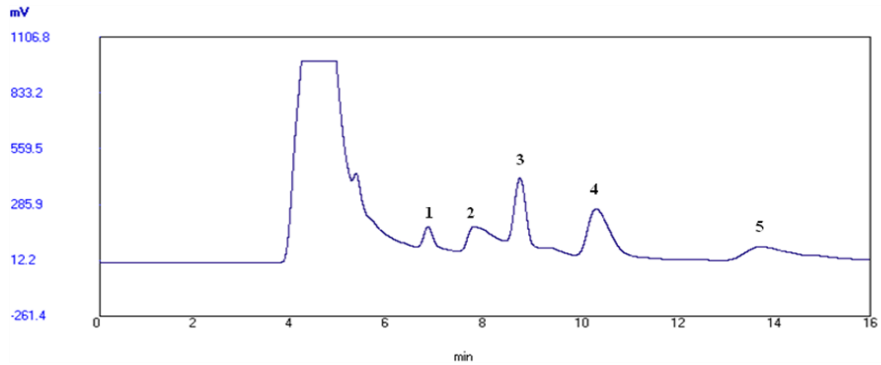

Figure 4. Chromatogram obtained after the analysis of an infant milk sample (power infant milk 1) using the proposed method (detector attenuation 50 pA). (1) nicotinic acid; (2) panthotenic acid; (3) thiamine; (4) pyridoxine hydrochloride; (5) folic acid.

4. Conclusions

In the developed method, seven water-soluble vitamins from the B-complex (thiamine, folic acid, nicotinic acid, pantothenic acid, pyridoxine and biotin) and vitamin C (ascorbic acid) are separated, identified, and quantitatively determined by HPLC-C-CAD coupling. The universal corona-charged aerosol detector is characterized by a robust and reproducible response, easy functioning, low cost of acquisition and maintenance. Moreover, the mobile phase consisting of methanol and acetate ammonium is fully compatible with C-CAD. The response of the detector allowed the determination of the compounds providing signals that are dependent on the analytes' particle size. Therefore, it can detect any compound less volatile than the mobile phase without the need of a chromophore moiety in its structure. This is an advantage over ultraviolet detection as it does not allow the use of a single wavelength for the detection of all vitamins [9]. The HPLC-UV-MS hyphenation provides higher sensitivity and measures several vitamins simultaneously [11]. However, the cost of the instrument is much higher than that of the C-CAD. The sensitivity of the proposed method is adequate for the analytical problem afforded. Also, the separation was completed within 15

min, under isocratic mobile phase conditions with acceptable precision. The procedure was applied to the determination of the vitamins in dietary supplement and infant milk samples with very good results. The simplicity of the procedure would make it highly desirable for quality control of products in the food and pharmaceutical industries.

Acknowledgements

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Capítulo 4

Comparison of two evaporative universal detectors for the determination of sugars in food samples by liquid chromatography

Comparison of two evaporative universal detectors for the determination of sugars in food samples by liquid chromatography

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ABSTRACT

The corona-charged aerosol detector (C-CAD) was developed to improve the sensitivity, reproducibility and quantification of the evaporative light scattering detector (ELSD). In this article the performance of both detectors for the detection and quantification of sugars in food samples is systematically compared. Fructose, glucose, sucrose, maltose, lactose and maltotriose were separated by liquid chromatography on a NH_2-column using a isocratic composition acetonitrile:water, 70:30 (v/v) as mobile phase. The analytical signals for the individual sugars were obtained using the C-CAD and ELSD detectors. The C-CAD was able to detect the target analytes in more dilute samples as regards ELSD, which results in a better sensitivity. The intra-day and inter-day variations were lower 3.2% and 3.9% for ELSD and 5.4% and 6.6% for C-CAD, respectively. The detection limits were between 10.2 mg L^{-1} and 17.4 mg L^{-1} for ELSD in the linear range 25–3000 mg L^{-1}. The C-CAD provided LODs between 1.2-7.6 mg L^{-1} within a narrower dynamic range (5-1000 mg L^{-1}). The optimized methods were applied to the analysis of different sauces, confectionary products and dairy products samples. The sample pre-treatment is very simple, only a dilution with Milli-Q water being necessary, except for the dairy products, which requires a precipitation of proteins with acetic acid. The results obtained for both detectors were very similar, being the selection of the most appropriate one based on the analytical quality level required for the determination.

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1. Introduction

Corona-charged aerosol detector (C-CAD) and evaporative light scattering detector (ELSD) are a new generation of detectors for high pressure liquid chromatography (HPLC) [1,2]. ELSD and C-CAD are considered quasi-universal instruments as they can provide a response for any analyte with the only requirement of being less volatile than the mobile phase. These detectors functioning involve three consecutive steps: nebulization, evaporation and detection. The effluent from the HPLC column is nebulized and dried in a gaseous stream, which acts also as an ionizing gas in the case of C-CAD. Aerosol particles formed by non-volatile analytes become charged in a corona discharge source and are detected by a sensitive electrometer (C-CAD) or the aerosol enters the cell and passes through a light, being the scattered light measured by a photomultiplier or photodiode (ELSD). Their main characteristics are the universal detection of non-volatile analytes, regardless their chemical although it should be noticed that the response is affected by mobile phase composition. This problem has been resolved by using inverse gradient compensation [3]. However, C-CAD and ELSD offer advantages other conventional universal detectors such as refractive index (RI) [4], low-wavelength ultra-violet (UV) [5] and mass-spectrometer (MS) [6]. The RI detector, while widely used, has significant limitations in sensitivity and it is not compatible with gradient elution. Low-wavelength UV provides higher sensitivity and improved gradient compatibility, but the detection is limited to chromophores, and the magnitude of the response depends upon molar absorptivity, which can vary by orders of magnitude even among analogous structures. Although UV detection remains the primary technique for many HPLC analyses, it is unable to detect compounds that lack a sufficient UV chromophore such as many underivatized amino acids, carbohydrates, lipids, polymers, surfactants, drug substances and natural products. The MS is widely used to provide structural information although it is expensive in both

acquisition and maintenance. Moreover, it requires skilled analysts in order to correctly interpret the analytical response obtained (viz. mass spectra).

Several articles recently published specifically compare the analytical performance of C-CAD and ELSD in the pharmaceutical and clinical areas. Jia et al. compared the sensitivity of both detectors with reverse phase and hydrophilic interaction liquid interface chromatography (HILIC) for the determination of gabapentin [7]. Also, Hazotte et al. show the feasibility of HILIC for the determination of lipids [8]. In both cases the authors conclude that C-CAD was much more sensitive than ELSD. Similar conclusions were obtained when it compared the potential of ELSD and C-CAD for polyketide [6] and saikosaponins [9] analysis. For the poly(ethylene glycol) analysis using supercritical fluid of chromatography at lower concentration (10 mg mL^{-1}) negligible differences were found between C-CAD and ELSD [10].

These detectors have been used for the analysis of samples with a high content of hydrophilic components (i.e. sugars). Terol et al. used high temperature liquid chromatography with ELSD for the determination of carbohydrates in milk and varieties of orange [11]. Other authors used ELSD for the qualitative analysis of sugars in fruits such as melon [12] or sweet orange peels [13]. Also, C-CAD has been widely employed for the determination of these analytes in the analysis of beverages [14,15]. Owing to the favourable response of these detectors for carbohydrates, some investigations about the use of different organic solvents as components of the mobile phases for the C-CAD detection of these analytes have been addressed [16,17].

The aim of this research is to compare the sensitivity, linearity and precision of these two detectors C-CAD and ELSD, for the determination of

sugars in different types of food sample namely, sauce, syrup, covering, jelly, jam, honey, milk and milkshake. The analytical feature for both detectors has been obtained and all the food samples have been systematically analyzed under the optimum conditions in order to evaluate the suitability of the detectors for the proposed analytical problem.

2. Materials and methods

2.1. Reagents and samples

All reagents were of analytical grade or better. Acetonitrile (Scharlab, Barcelona, Spain) and Milli-Q ultrapure water (Millipore, Madrid, Spain), were employed as components of the chromatographic mobile phase. Acetic acid from Panreac (Barcelona, Spain), was used in the treatment of milk samples.

The analytes (fructose, glucose, maltose, lactose and maltotriose) were obtained from Sigma–Aldrich (Madrid, Spain). Stock standard solutions of each analyte were prepared in Milli-Q ultrapure water at a concentration of 4 g L^{-1} and stored at 4 ºC. Working solutions containing all the analytes were prepared by dilution of the stocks in ultrapure water.

Sauces, confectionary products and dairy products were purchased from local commercial stores and kept at room temperature until their analysis.

2.2. Apparatus

The chromatographic analysis was performed using a micro-HPLC pump Jasco 1585 (Jasco Analítica Spain, Madrid, Spain) for the mobile phase delivery and a high pressure manual injection valve (Rheodyne 7725, Cotati, CA, USA) fitted with a 20 µL stainless steel sample loop. Chromatographic separation was carried out at room temperature on a NH_2-Kromasil column (5

µm, 250 mm x 4.6 mm) obtained from Análisis Vínicos (Tomelloso, Spain). The mobile phase composition was acetonitrile:water 70:30 (v/v) and the flow rate was fixed at 1 mL min^{-1}.

The corona-charged aerosol detector (C-CAD) was acquired to ESA Bioscience (Chelmsford, MA, USA). The instrumental parameters were set as follows: evaporative chamber temperature 60 ºC, attenuation range 20 pA (maximum interval 1-500 pA, the lower attenuation provided the highest signal) and medium signal filtering.

The evaporative light scattering detector (ELSD) was also purchased from ESA. In this case, the temperature of the nebulization and evaporation can be modified by the operator. A value of 45 ºC and 55 ºC were selected as optimum for this application. The signal attenuation was 9 (maximum interval 1-9).

In both cases, nitrogen obtained using a Mistral 4 nitrogen generator (Clan Tecnológica, Seville, Spain) was selected as nebulizing gas. A pressure of 37 psi (gas purity ≈ 99.99%) was also fixed for all analyses.

Signals were acquired using a HP 35900C A/D converter, both from Agilent Technologies (Madrid, Spain).

The chromatographic data analysis was carried out using a Biocrom XP software (Análisis Vínicos).

A high speed centrifuge with a microprocessor control (J.P. Selecta) was used in the sample treatment step of dairy products.

2.3. Sample preparation

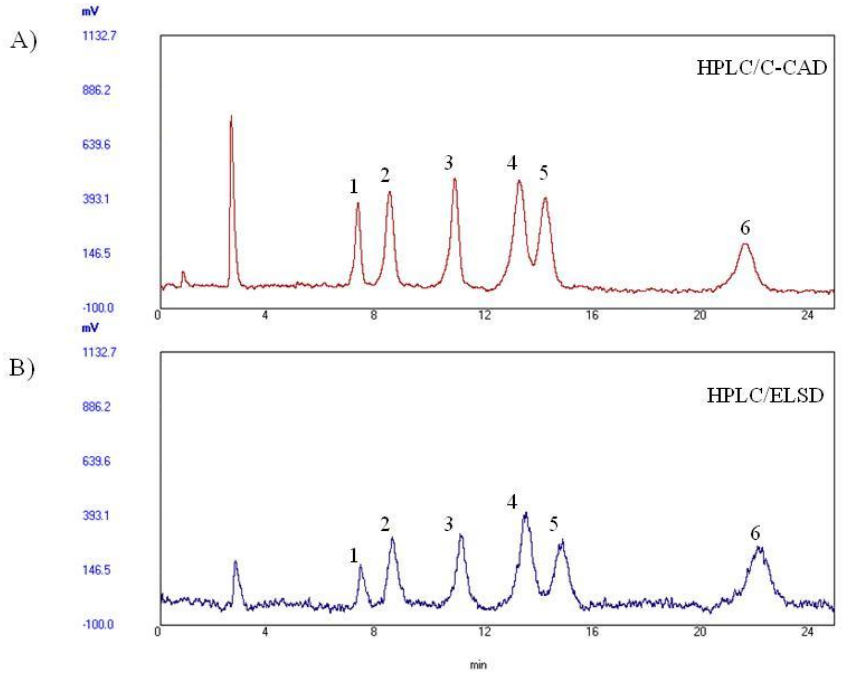
The procedure followed for the different samples included in this article was as follows. An accurately weighed amount of sample (1.0 g) was dissolved/diluted in 40 mL ultrapure Milli-Q water. Then, a further dilution (between 1:2 and 1:25, v/v) was carried out in order to adequate concentration of the compounds in the sample to the linear range of the detector. Finally, the diluted sample was filtered through a 0.45 µm disposable Nylon filter. Dairy products needed an additional step to remove the protein fraction. For this purpose, 10 mL of an aqueous solution of acetic acid 2.5 M was added to 10 mL of sample. The mixture was centrifuged at 4000 rpm for 15 min. The supernatant was injected in the liquid chromatograph for analytes separation and quantification. Peak area was used as the analytical signal in both detectors.

3. Results and discussion

3.1. Optimization of the chromatographic separation conditions

The influence of the instrumental parameters on the chromatographic separation and evaporative detectors response was evaluated by the direct injection of aqueous standards containing the analytes prepared at a concentration of 100 mg L^{-1}. When a variable is optimized, its optimum value is fixed for further studies. The optimization of the chromatographic separation of the target analytes was influenced by the detection system as in the evaporative detectors, the response depends on the amount of organic solvent present in the mobile phase. For this reason, different mixtures of acetonitrile:water were studied to obtain an adequate analytes separation with the lowest background noise. The background noise values (intensity) obtained for the different mixtures of mobile phase (v/v) were 0.65 for 80:20, 0.92 for 70:30 and 1.16 for 60:40. From the different ratios evaluated, acetonitrile:water 70:30 (v/v) in isocratic composition was chosen as it

provided the best resolution of the peaks and a stable baseline. The flow rate was maintained at 1 mL min^{-1}. Figure 1 shows typical chromatograms obtained for the chromatographic separation of standards containing the carbohydrates at concentration between 125 and 500 mg L^{-1} with C-CAD and ELSD, being the compounds separated within 25 min in both cases. As it can be seen, the



chromatographic peaks were higher and narrower with the C-CAD for the same concentration of analyte.

Figure 1. Typical chromatograms obtained for the separation of the six carbohydrates selected using (A) C-CAD and (B) ELSD detectors. Instrumental conditions using the mobile phase acetonitrile:water 70:30 (v/v) and a flow of 1 mL min^{-1}: 20 pA and medium filter for the C-CAD and a value 9 of attenuation and high filter. Analytes: (1) fructose (125 mg L^{-1}); (2) glucose (250 mg L^{-1}); (3) sucrose (250 mg L^{-1}); (4) maltose (500 mg L^{-1}); (5) lactose (500 mg L^{-1}) and (6) maltotriose (500 mg L^{-1}).

3.2. Optimization of the detection conditions

The main instrumental variables affecting to the evaporative detectors are: nebulization and evaporation chamber temperature, nebulizing gas pressure, signal attenuation and filter. The optimized variables and their initial

and optimum values are summarized in Table 1. Their influence on the detectors response is separately commented in the following sections.

Table 1. Optimized instrumental variables of the ELSD and C-CAD methods.

Variable	Initial value	Interval	Optimum value	
			C-CAD	ELSD
Attenuation	1	1 - 9	-	9
Output range (pA)	50	1 - 500	20	-
Nebulization temperature (ºC)	35	35 - 55	-	45
Evaporization temperature (ºC)	45	45 - 65	-	55
Filter	Low	None – high	Medium	High

3.2.1. Corona - charged aerosol detector

The configuration of the detector used in this article does not allow the modification of the temperature nebulization and evaporation steps, therefore, all the experiments were carried out at 60 ºC. Concerning the nebulizing gas, nitrogen was used and it was continuously provided via a N_2 generator. The working pressure was 37 psi, with a gas purity of 99.99%. The sensitivity of the detector can be modulated by changing the signal attenuation. This parameter (output) can be set within the interval 1-500 pA, being inversely related to the sensitivity (e.g. 1 pA is the minimum attenuation, thus the maximum sensitivity). In this application, it was fixed at 20 pA as it provided an adequate response for the selected concentration. However, it can be adapted to the concentration of the analyte in the sample to avoid the saturation of the signal. Also, this parameter affects to the baseline, as at low values, the noise in the baseline is higher. The last instrumental parameter studied was the filter, which is used to smooth the signal. This detector used three qualitative values: high, medium and low. In this application the medium

filter was selected. In the case of working at the maximum sensitivity a higher filter will be necessary to decrease the noise of the baseline.

3.2.2. Evaporative light scattering detector

The principal variables affecting the ELSD response were the nebulization and evaporation temperatures and, as it was the case with the C-CAD, attenuation of the signal, nitrogen pressure and filter. The nebulization and evaporation temperatures depend on the composition of mobile phase. In the instrument`s manual, a nebulizer temperature setting between 30 ºC and 35 ºC is generally given as optimal; and when organic solvents such as acetonitrile (boiling point < 80 ºC) are used the optimal temperature has to be raised up to 40 ºC. Both temperatures were studied simultaneously in the range 35-55 ºC for nebulization and 45-65 ºC for evaporation, maintaining the mandatory difference of 10 ºC between both values. At higher temperature, the baseline was instable and the area peak values lower. The best results were obtained with a nebulization temperature of 45 ºC and an evaporation temperature of 55 ºC which were fixed for further experiments. In this detector, the attenuation of the signal can be modulated between 1 and 9, the highest response corresponding to 9; this value was set to obtain the higher sensitivity although it negatively affects to the background noise, increasing the baseline instability. The nitrogen pressure provided by the nitrogen generator was of 36 psi. For this evaporative detector a high filter was selected as optimum value among the three options available (high, medium and low).

3.2. Figures of merit for the determination of sugars using ELSD and C-CAD

The relationship between the concentration of the analyte(s) and the instrumental signal provided by the detector is an important issue. Experimental evidence suggests that the response of evaporative-based

detectors is nonlinear, constituting a considerable limitation or drawback in quantitative studies. The ELSD response follows the relationship:

$$A=a\ m^{b}$$

where A is the response and m is the mass injected on-colum. Tha a and b are coefficients whose value depend on the ELSD instrumentation. A linear calibration curve can be constructed using log-log coordinates [18]:

$$\log A = b \log m + \log a$$

Concerning the C-CAD response, a second-order relationship between concentration and response is usually observed and thus, a quadratic equation will fit to experimental data [19].

To evaluate linearity of these detectors, seven calibration standards ranging from 5 to 3000 mg L^{-1} were initially analyzed. The concentrations were plotted against their corresponding peak areas. As expected a non-linear relationship was obtained for both detectors. However, the linearity was achieved following a double logarithmic representation. The characteristic parameters for both detectors are given in Tables 2 and 3. Although a second-order equation was expected for C-CAD, the values obtained for coefficient to suggest that, within the concentration range assayed, a quasi-linear response was obtained following the double logarithm approach. The different response of both detectors affects to the sensitivity and linear ranges. Also, the highest sensitivity of the C-CAD can be easily inferred from the data shown in Fig. S1 (electronic supplementary material) were the peak areas obtained for the same concentration interval is given for both detectors. The higher response results in signal saturation at a lower concentration. As a consequence, a narrower linear interval was obtained for some analytes.

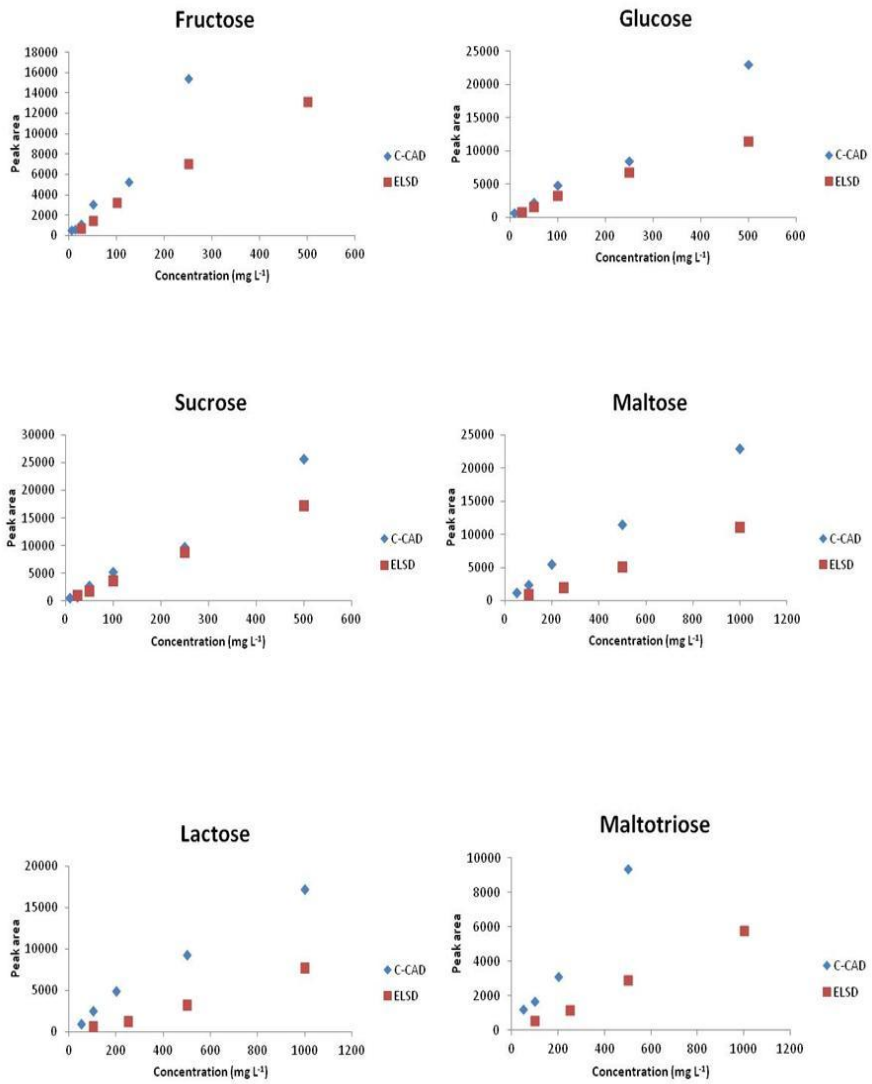


Figure S1. Comparison of the peak area obtained of the six analyte using both evaporative detectors within the common linear range. For details, see the text.

Table 2. Figures of merit of the ELSD for the identification and quantification of sugars.

Analyte	Linear range (mg L^{-1})	Regression equation log-log scale	R	LOD[a] (mg L^{-1})	Repeatability (n=5, %)	Reproducibility (n=9, %)
Fructose	25-100	log y= 0.9841 log x + 1.5099	0.9884	10.2	2.6	1.9
Glucose	25-2000	log y= 0.8642 log x + 1.7485	0.9990	12.1	2.3	2.2
Sucrose	25-2000	log y= 0.9551 log x + 1.6555	0.9999	11.3	3.1	2.8
Maltose	50-2000	log y= 1.0014 log x + 1.3076	0.9995	16.3	2.1	2.7
Lactose	50-3000	log y= 1.0396 log x + 1.0474	0.9993	16.7	3.2	3.3
Maltotriose	50-3000	log y= 1.0771 log x + 0.9044	0.9958	17.4	2.8	3.9

[a] Limit of detection.
y, peak area
x, concentration (mg L^{-1})

Table 3. Figures of merit of the C-CAD for the identification and quantification of sugars.

Analyte	Linear range (mg L^{-1})	Regression equation log-log scale	R	LOD[a] (mg L^{-1})	Repeatability (n=5, %)	Reproducibility (n=9, %)
Fructose	5-250	log y= 0.9764 log x + 1.7820	0.9919	1.2	1.8	2.5
Glucose	10-500	log y= 0.8897 log x + 1.8721	0.9835	3.8	2.8	3.4
Sucrose	10-500	log y= 0.9810 log x + 1.6948	0.9822	3.0	2.4	4.7
Maltose	25-1000	log y= 0.9935 log x + 1.3970	0.9986	6.5	4.9	6.6
Lactose	25-1000	log y= 0.9413 log x + 1.4517	0.9943	6.8	5.4	5.3
Maltotriose	25-1000	log y= 0.9442 log x + 1.4035	0.9943	7.6	4.6	3.4

[a] Limit of detection.
y, peak area
x, concentration (mg L^{-1})

The limits of detection (LOD) and limit of quantification (LOQ) values were estimated using serially diluted standards and were determined as the lowest amount injected that provided peaks with signal-to-noise ratio of 3 or 10, respectively. The results from this study are listed in Table 2 for ELSD and Table 3 for C-CAD. In all cases, the limits of detection for C-CAD were lower than that for ELSD. It also results in a better detection limits (between 8.5 and 2.3 times lower).

The precision of the method, expressed as relative standard deviation, was evaluated for five (repeatability) and nine (three replicates, 3 days, reproducibility) replicates of a working aqueous standard prepared at a concentration of 200 mg L^{-1}. The results from the precision study are shown in

Tables 2 and 3. The percent of RSD ranged from 1.8 (for fructose) to 6.6% (for maltose) for the C-CAD method, and from 1.9 (for fructose) to 3.9% (for maltotriose) for the ELSD method. Although the aerosol formation mechanism is the same for both detectors, C-CAD results were less precise for some analyte. The differences in precision between the detectors arise from the differences in their detection foundation.

Although both methods could be used to quantify the six sugars of interest in this study, the C-CAD method was more sensitive and equally precise in some cases compared to ELSD method. The response of both methods is non-linear; however, by plotting the calibration functions on a logarithmic scale, linearity was achieved with regression coefficients higher than 0.9822.

3.3. Samples

Finally, the efficiency of both detectors was compared by analyzing a series of samples of different nature following the previously optimized conditions. They have been divided into sauces, confectionary products and dairy products samples. The main results and conclusions are presented below.

A variety of sauces (ketchup, mayonnaise, mustard and yogurt) and confectionary products of different nature (syrup, covering, jelly, jam, honey) were obtained from local stores and analyzed using the proposed method following the optimal instrumental conditions described in the Section 2.3. for each detector. All the samples were initially diluted 1:40 (w/v) in ultrapure water and, in some cases, higher dilutions were used depending on the analytes concentration. The dilution that was finally applied and the concentration levels found for the sugars in the non-diluted samples are shown in Table 4 for both detectors.

Table 4. Determination of sugars using C-CAD and ELSD.

Samples	Label contained	Concentration found ($g\ L^{-1}$) ± SD											
		Fructose		Glucose		Sucrose		Maltose		Lactose		Maltotriose	
		C-CAD	ELSD	C-CAD	ELSD	C-CAD	ELSD	C-CAD	ELSD	C-CAD	ELSD	C-CAD	ELSD
Sauce													
Ketchup	Sugar	0.89± 0.02	0.86± 0.02	0.91± 0.03	0.93± 0.02	1.89± 0.08	1.85± 0.06	-	-	-	-	-	-
Mayonnaise	Sugar	-	-	-	-	1.77± 0.08	1.93± 0.06	-	-	-	-	-	-
Mustard	Sugar	0.41± 0.01	0.43± 0.01	0.48± 0.02	0.53± 0.01	3.4± 0.2	3.4± 0.1	-	-	-	-	-	-
Yogurt	Sugar, mustard, milk, yogurt	0.24± 0.01	0.26± 0.01	0.40± 0.01	0.44± 0.01	13.1± 0.6	13.2± 0.4	3.5± 0.2	3.8± 0.1	-	-	-	-
Syrup													
Chocolate I	Glucose-fructose syrup, sugar	0.09± 0.01	0.13± 0.01	0.59± 0.02	0.70± 0.02	3.3± 0.1	3.2± 0.1	0.55± 0.04	0.71± 0.02	-	-	0.19± 0.01	0.19± 0.01
Chocolate II	Glucose-fructose syrup, sugar	0.47± 0.02	0.40± 0.01	5.9± 0.2	5.7± 0.1	5.3± 0.2	5.3± 0.2	5.9± 0.4	5.9± 0.2	-	-	0.49± 0.02	0.47± 0.02
Caramel	Glucose-fructose syrup, sugar, burnt sugar	0.08± 0.01	0.07± 0.01	0.26± 0.01	0.30± 0.01	3.9± 0.2	3.8± 0.01	0.75± 0.05	0.94± 0.03	-	-	-	-
Mint	Glucose syrup, sugar	18.7± 0.4	19.4± 0.5	65±2	66±2	26±1	26±1	-	-	-	-	-	-

Covering													
Caramel I	Glucose-fructose syrup, sugar	17.5± 0.4	17.9± 0.5	37± 1	37± 1	7.3± 0.3	7.4± 0.2	20± 1	19.9± 0.5	-	-	8.1± 0.4	8.2± 0.3
Caramel II	Glucose-fructose syrup, sugar	8.3± 0.2	8.1± 0.2	19.4± 0.9	19.3± 0.4	0.67± 0.03	0.64± 0.02	13.3± 0.9	13.2± 0.4	-	-	14.5± 0.7	14.4± 0.6
Apricot	Glucose-fructose syrup, sugar	0.43± 0.02	0.64± 0.02	1.47± 0.04	1.65± 0.04	1.75± 0.08	1.97± 0.06	0.37± 0.02	0.44± 0.02	-	-	-	-
Strawberry	Sugar, fructose, inverted sugar	0.77± 0.02	0.81± 0.02	1.79± 0.06	1.83± 0.05	0.28± 0.01	0.38± 0.01	1.7± 0.1	1.56± 0.04	-	-	0.49± 0.02	0.46± 0.02
Banana	Sugar	2.28± 0.06	2.23± 0.06	4.9± 0.2	4.9± 0.1	1.46± 0.07	1.53± 0.05	0.08± 0.01	0.12± 0.01	-	-	-	-
Jelly													
White	Glucose syrup, sugar	0.87± 0.03	0.69± 0.02	2.15± 0.07	2.35± 0.05	0.93± 0.04	0.91± 0.03	1.7± 0.2	1.80± 0.05	-	-	0.58± 0.03	0.62± 0.02
Strawberry	Glucose-fructose syrup, sugar	1.96± 0.05	1.93± 0.05	7.0± 0.2	7.1± 0.2	0.96± 0.05	1.10± 0.03	5.1± 0.3	5.2± 0.1	-	-	1.75± 0.08	1.89± 0.07
Jam													
Raspberry	Glusose-fructose syrup, sugar	0.28± 0.01	0.34± 0.01	1.52± 0.05	1.64± 0.04	0.10± 0.01	0.13± 0.01	1.54± 0.10	1.39± 0.04	-	-	-	-
Lemon	Glucose syrup, sugar	0.19± 0.01	0.23± 0.01	0.63± 0.02	0.72± 0.02	0.61± 0.03	0.53± 0.02	0.51± 0.03	0.47± 0.02	-	-	-	-

Honey													
Natural		3.64± 0.09	3.7± 0.1	3.6± 0.2	3.5± 0.1	-	-	-	-	-	-	-	-
Processed		49± 1	47± 1	55± 2	55± 1	40± 2	40± 1	-	-	-	-	-	-
Dairy products													
Whole milk	Carbohydrate	-	-	2.03± 0.07	2.22± 0.06	-	-	-	-	35± 2	35± 2	-	-
Whole milk without lactose	Carbohydrate, lactose <0.01g	-	-	74± 4	70± 2	-	-	-	-	-	-	-	-
Cacao semi-skimmed milkshake	Sugar, milk without lactose < 0.01g	23.4± 0.6	22.1± 0.6	17.3± 0.6	15.5± 0.4	0.36± 0.02	0.41± 0.01	1.34± 0.09	1.45± 0.04	1.45± 0.08	1.37± 0.05	-	-
Vanilla semi-skimmedmilk shake	Sugar	0.14± 0.01	0.13± 0.01	1.04± 0.04	1.07± 0.03	13.2± 0.6	12.8± 0.4	-	-	9.4± 0.5	9.2± 0.3	-	-
Strawberry semi-skimmedmilk shake	Sugar	0.26± 0.01	0.33± 0.01	18.9± 0.6	18.6± 0.5	41± 2	43± 2	-	-	8.5± 0.5	8.5± 0.3	-	-

SD, standard deviation.

In addition, the qualitative information as regards the sugar content indicated in the label for each product is also given. In the case of the sauces, sucrose was detected in all samples. The maximum value found was 13.2 g L^{-1} for yogurt sauce. This sample contains milk and yogurt in a percentage lower than 1% but the lactose was not detected probably due to the initial dilution needed for compatibility with the chromatographic system. Confectionary products like syrups, coverings, jellies and jams contain glucose syrup or glucose-fructose syrup in their composition. Glucose syrup is extensively used to prevent crystallization of sugar. It acts as a good preservative and prevents spoilage of the product without unduly increasing its sweetness. Therefore, it provides high viscosity and facilitates browning reactions at the products. When a confectionary product contains glucose-fructose syrup in the label, it indicates that the concentration of glucose is higher than the fructose. The mint syrup presents more concentration of sugars than the other syrups. On the other hand, the caramel coverings are the samples with higher concentration of sugars in this group. Honey samples of natural and processed origin were analyzed. The sugar content in the natural honey was lower than that found in the processed one which, in addition, presents similar concentration level of fructose and glucose. The presence of maltotriose in some of the samples is associated to the simultaneous presence of glucose and maltose. According to the literature, it can be ascribed to the addition of glucose syrup. Its concentration is lower than that of glucose and maltose, in a given sample. As it can be observed, the concentrations found were not significantly different for both detectors. Exemplary chromatograms obtained for a ketchup, mint syrup, strawberry covering and natural honey samples using C-CAD and ELSD are depicted in Figure 2.

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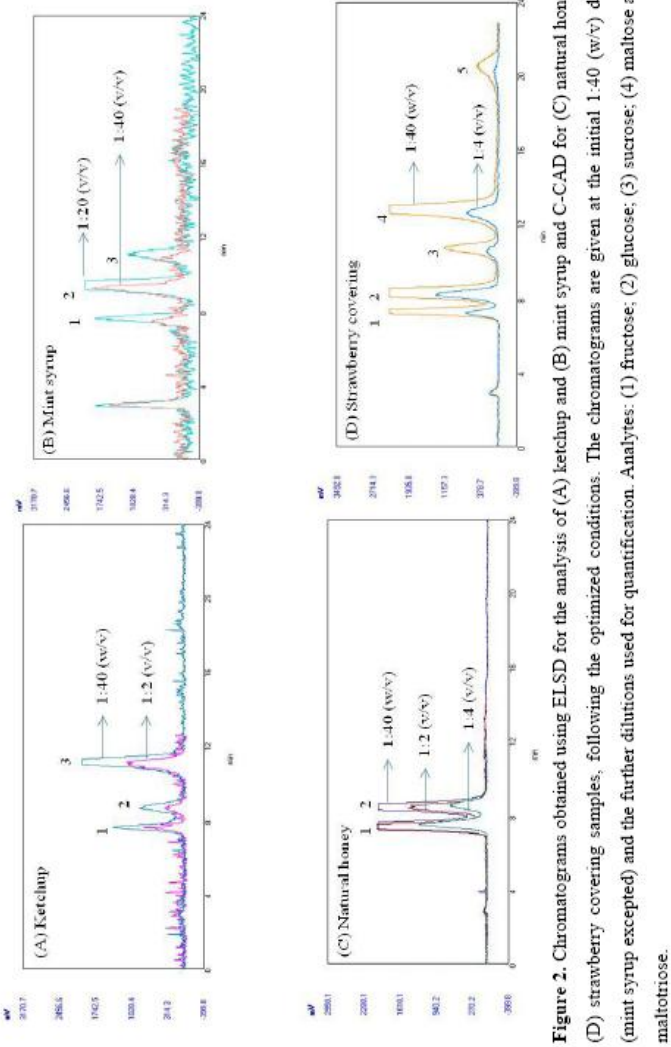

Figure 2. Chromatograms obtained using ELSD for the analysis of (A) ketchup and (B) mint syrup and C-CAD for (C) natural honey and (D) strawberry covering samples, following the optimized conditions. The chromatograms are given at the initial 1:40 (w/v) dilution (mint syrup excepted) and the further dilutions used for quantification. Analytes: (1) fructose; (2) glucose; (3) sucrose; (4) maltose and (5) maltotriose.

The proposed method was also tested for the determination of the target analytes in dairy products. Five different samples (whole milk, milk without lactose and cacao, strawberry and vanilla semi-skimmed milkshakes) were analyzed. Table 3 shows the results obtained for the C-CAD and ELSD. Glucose was detected in whole milk with and without lactose although the concentration in the lactose-free was higher. Only milkshakes presented others sugars different of the glucose and lactose contained in the milk samples. Three milkshake were analyzed, the strawberry milkshake contains higher concentration of sucrose and glucose and, the cacao milkshake presents higher concentration of fructose. The maltotriose was not detected in the dairy products analyzed. In the case of whole milk without lactose and cacao semi-skimmed milkshake, the label indicates that the concentration of lactose is lower of 0.1 g L^{-1}. The sensitivity of the proposed method allows the determination of the label concentration, but the dilution necessary in the pre-treatment of sample hinders the quantification, being the final concentration of the lactose lower than 0.001 g L^{-1}.

4. Conclusions

Two types of evaporative detectors coupled to HPLC, corona-charged aerosol detector (C-CAD) and evaporative light scattering detector (ELSD), have been used for the determination of six different carbohydrates (fructose, glucose, sucrose, maltose, lactose and maltotriose) in real food samples. Results from the comparison studies have been evaluated in terms of sensitivity, linearity and precision. The sensitivity obtained by the C-CAD was superior to the ELSD, being the limits of detection for the C-CAD lower than 7.6 mg L^{-1} (maltotriose); while the lowest limit of detection for the ELSD was 10.2 mg L^{-1} (fructose). The response of both methods is non-linear; however by plotting the calibration functions on a logarithmic scale, linearity was achieved for both detectors. For all carbohydrates, the regression coefficients

obtained with the ELSD were higher than the values obtained with the C-CAD. The results from the precision demonstrated that C-CAD is less precise for fructose, maltose, lactose and maltotriose. The repeatability (n=5) and reproducibility (n=9) variations were below 4% for ELSD and 6% and 7% for C-CAD, respectively. C-CAD presents a better signal-to-noise ratio for the mobile-phase used, acetonitrile:water 70:30 (v/v). The proposed methods were employed for the detection and quantification of sugars in a wide variety of confectionary products (sauces, syrups, coverings, jams, jellies and honeys) and dairy products (milk and milkshakes). The concentrations for each compound in the analyzed samples are similar for both detectors. The sample pre-treatment employed was only a dilution in an adequate volume of Milli-Q water although, proteins precipitation with acetic acid was necessary for dairy products. The cost and maintenance features are higher for the C-CAD than the ELSD. Taking into account the simplicity of the procedure required for both detectors and the information of the Tables 2 and 3, it can be concluded that although the detection limits are lower for the C-CAD, ELSD is more appropriate to carry out the determination of sugars of the proposed samples, considering the high concentration of the carbohydrates in the samples, the absence of interferences and its lower acquisition and maintenance costs.

Acknowledgements

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Bloque II

Nuevas aplicaciones de la espectrometría de movilidad iónica

Capítulo 5

Ion-mobility spectrometry for environmental analysis

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Ion-mobility spectrometry for environmental analysis

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ABSTRACT

Advances and changes in practical aspects of ion-mobility spectrometry (IMS) have led to its widespread use for applications of environmental concern due to its unique characteristics, which include portability, ruggedness, relatively low acquisition costs and speed of analysis. However, limitations regarding the complexity of environmental samples and strict requirements on limits of detection have to be overcome.

This article critically reviews existing environmental applications using IMS for the determination of different families of compounds. We also consider the analytical tools developed to solve the limitations regarding selectivity and sensitivity, including those approaches that have led to advances in the instrumentation of IMS and its combination with other techniques for extraction and pre-concentration of analytes, pre-separation of analytes and its coupling to other detection systems.

Finally, we discuss current trends that facilitate the deployment of IMS for on-site or in-field analysis.

1. Introduction

Environmental analysis is continually expanding for routine and in-situ determinations of recognized contaminants in different matrices. For these applications, legislation gets stricter and lower thresholds are imposed. The generation of quality results in a rapid, reliable way that facilitates timely decision making is desirable, and, in this sense, the development of sensitive, selective detection methods that contribute to this purpose is necessary [1]. Ion-mobility spectrometers are among the analytical tools that have promoted the achievement of this goal by virtue of ease of use, analytical flexibility and short times of analysis. This type of device has favorable technical parameters compared to other analytical techniques with respect to size, weight and power consumption. Besides their fast and accurate measuring performance at a relatively low expenditure, the lack of vacuum requirements and portability make their required measuring conditions suitable for increasing their use for on-site and in-field analysis. These characteristics, added to recent developments in the technique, are leading to continually increasing applications in environmental locations, where on-site measurements are of great relevance to assess quickly the concentration and the spatial distribution of chemicals, while avoiding the sources of errors caused by collection, transport and storage of samples [2].

Ion-mobility spectrometry (IMS) emerged in the 1970s as a modern analytical technique for the determination of volatile and semi-volatile organic compounds based on the separation of their gaseous ions in a weak electric field at ambient pressure. However, it was not until the 1990s, after advances in technology and design that IMS instruments were introduced commercially [3]. Since then, numerous IMS analyzers have been designed and developed, especially for explosives, drugs (particularly illicit drugs) and detection of chemical-warfare agents (CWAs). Nowadays, a significant number of hand-

held devices are used in airports worldwide and the technique has even reached the International Space Station to monitor air quality there [4]. A wide variety of fields (e.g., toxicology, pharmaceuticals, medical diagnostics, food, process and industrial control, and, especially in recent years, proteomics) have taken advantage of IMS. In addition, the applicability of IMS to environmental venues has been of great significance due to the characteristics cited [5].

Fig. 1 depicts the relative contribution of IMS to different areas of study according to the *ISI Web of Knowledge* database (May 2010). This graphic highlights the contribution of IMS to environmental applications.

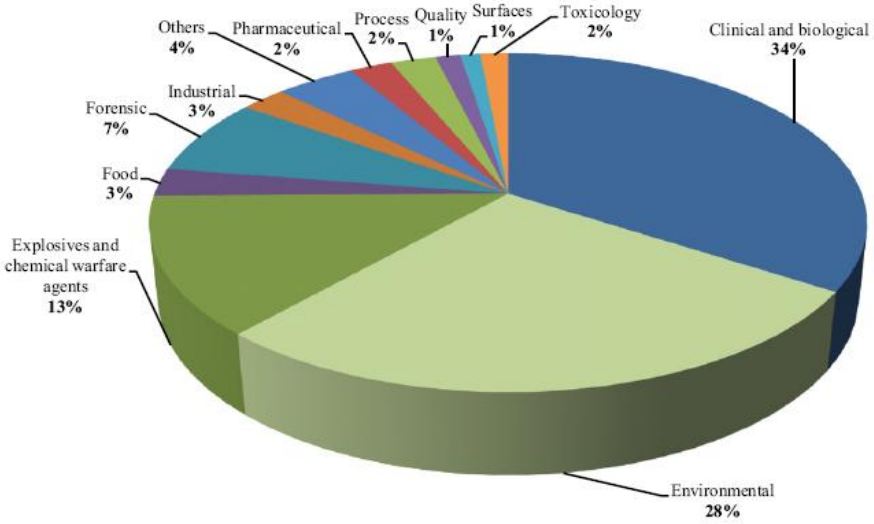

Figure 1. Contribution of ion-mobility spectrometry (IMS) to different areas of study. The percentages were calculated over 721 references (ISI Web of Knowledge, May 2010).

Nevertheless, limitations of IMS related to the nature of environmental samples have had to be overcome and some others must be solved if use of IMS for environmental monitoring is to become widespread. The complexity of matrices, the influence of humidity, the need to form gaseous ions, and the

low limits of detection (LODs) required are key issues to be considered, if current and stricter quality thresholds are to be met.

Improvements in engineering and technology, the combination of IMS with extraction and pre-concentration techniques, and its coupling to other detection system, have enhanced analytical capabilities for application in environmental venues. Effective ionization sources [e.g., electrospray ionization (ESI)] have been developed to make the technique more compatible with analysis of liquid samples. Conventional IMS has led to field-asymmetric wavelength IMS (FAIMS), which has been increasingly employed for environmental purposes [6]. In addition, new interfaces for sample introduction and the coupling of IMS to extraction techniques [e.g., solid-phase microextraction (SPME) and liquid-phase microextraction (LPME)] are worth noting [7]. Problems of selectivity associated with moisture and other interferences can be avoided by coupling IMS to other techniques {e.g., gas chromatography (GC) [8] or mass spectrometry (MS) [9]}.

In this article, we review contributions of IMS in the environmental field taking into consideration its fundamentals and how they influence the type of analysis that can be performed regarding the nature of compounds to determine and samples to analyze. We also highlight limitations of IMS in terms of selectivity and sensitivity for environmental analysis and consider the ways in which they can be solved with existing methods and future developments.

2. Fundamentals of IMS

We present some basic considerations of IMS in this section. An in-depth description of the working principles of IMS is outside the scope of this

article, and more comprehensive, detailed information can be found elsewhere in the literature [4–11].

2.1. General scheme

Traditional IMS is normally performed in a drift tube with a linear voltage gradient containing a purified gas atmosphere, often air or nitrogen (drift gas) under ambient pressure. Conventionally, drift tubes comprise circular metal rings separated by insulators and attached to a voltage divider. In an alternative design, planar drift tubes are employed and drift rings are replaced by drift plates separated by insulating rails. The drift gas is introduced at the end of the separation chamber, and can have the same direction of the carrier gas in unidirectional flow systems, or, most commonly, an opposite direction (counter-flow systems). This gas serves to maintain cleanliness in the drift tube, suppressing ion molecule reactions. Also, it provides a source of neutral molecules for ion-neutral collisions, and supplies molecules to form reactant ions [4].

Fig. 2 shows a conventional device and its main components, which may include an ionization chamber (also termed a reaction chamber), a shutter grid, a separation chamber (or a drift region) and a detection unit. These components are related to the four main steps involved in IMS functioning, namely: sample introduction, molecule ionization, and ion separation and detection.

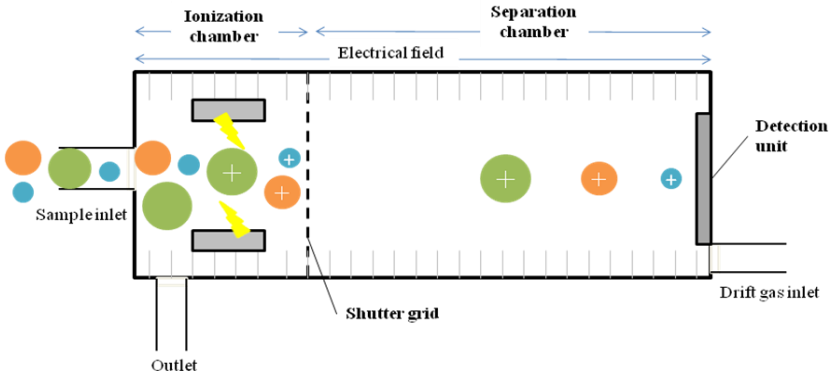

Figure 2. A conventional ion-mobility spectrometry (IMS device.)

2.2. Sample introduction and ionization

Initially, ions need to be formed from neutral sample molecules, for which the sample is introduced in a carrier gas into the ionization chamber where different ions form, depending on the ionization source employed.

The most commonly employed are radioactive sources for chemical ionization and, among them, ^{63}Ni sources are notable [10]. These sources are based on the emission of β particles that collide with the molecules of the supporting atmosphere, leading to reactant ions that are used in the chemical ionization of the sample via proton transfer and charge-exchange reactions.

Fig. 3 shows an example of mobility spectra for monomethylhydrazine and hydrazine using the ^{63}Ni ion source. Negative ions can be also formed as a consequence of direct reactions with emitted electrons or by charge-transfer reactions that include dissociative or associative electron attachments. Tritium (β-emitter source) and americium (α emitter) have also been used in IMS sources although to a lesser extent [3]. Details of ion and molecule reactions are available [11].

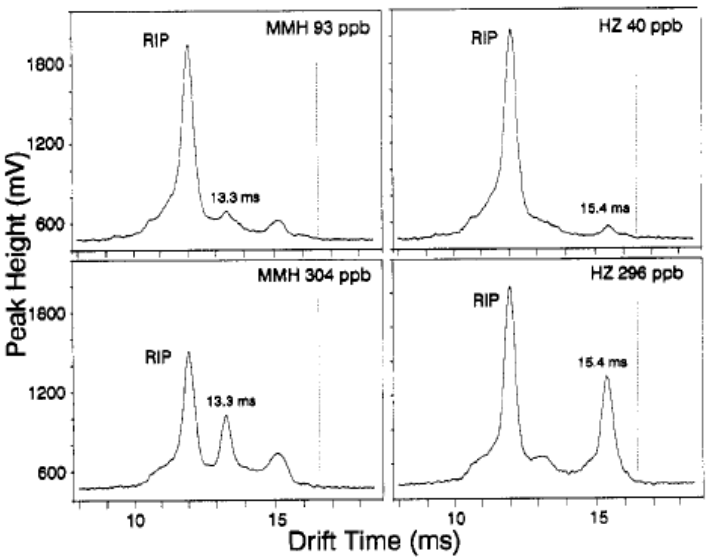

Figure 3. Ion- mobility spectra of monomethylhydrazine and hydrazine at different concentration levels using ^{63}Ni-IMS with 5-nonanone reagent gas chemistry. The reactant-ion peak (RIP) is principally $(5\text{-Non})_2\ H^+$. (Reprinted with the permission from [34]. ©1993, Elservier Science BV).

Alternatives to radioactive sources have been developed in order to avoid the drawbacks and the requirements associated with radioactive materials. Corona-discharge (CD) sources may resemble the ion chemistry found in radioactive sources although they combine mixed mechanisms of ionization that lead to responses more complicated than other methods. In addition, photoionization has frequently been employed for the direct ionization of samples without reactant ions being formed. Ultraviolet (UV) lamps have been applied in various instances, leading to comparatively simple responses and large ranges of quantitative responses. Laser sources are particularly relevant for solid samples, since they permit simultaneous desorption or vaporization and ionization of the components. In surface ionization, molecules are brought into contact with a solid and molecules undergo dissociation by loss of hydrogen atoms or alkyl groups and electron transfer. Another ionization mode to consider is ESI, which is of special

interest in environmental analysis due to the possibility of directly introducing liquid samples.

2.3. Separation and detection of ions

Ions derived from the sample molecules are transferred to the separation chamber via an electronic shutter grid, which is biased to annihilate the ions at the end of the ionization chamber and is periodically opened by trigger pulses to allow ions to pass through towards the drift region. Once in the separation chamber, product ions suffer a number of collisions with the supporting gas molecules and attain a rather constant velocity (drift velocity, v_d) that is proportional to the electric field strength (E) by a constant that corresponds to the mobility of the ion (K) [2]:

$$K = \frac{v_d}{E} \tag{1}$$

Ions of different mobilities experience a separation process in the drift region and reach the detection unit at different drift times (t_d), which, in a drift tube with a length ‘‘L’’, are related to the ion mobility according to the following equation:

$$K = \frac{L/t_d}{E} \tag{2}$$

Mobility values depend on environmental conditions and are commonly normalized to standard temperature (T_0 = 273 K) and pressure (P_0 = 760 mmHg) to give reduced mobilities (K_0) according to the following equation:

$$K_0 = (273/T)(P/260)K \quad (3)$$

where T is the absolute temperature of the drift gas and P is the atmospheric pressure at the time of the measurement.

The mobility and associated drift time are characteristic of the ion, and depend on the ion charge, mass and structural parameters (e.g., size and shape) that affect their collision cross-section [4]. Under fixed ionization and separation conditions, ion mobilities are characteristic properties of analytes.

Apart from traditional IMS devices, based on the time-of-flight approach previously described, other methods of ion separation based upon field-dependent mobilities have been developed in the past decade, leading to microfabricated differential mobility spectrometry (DMS) or FAIMS [6]. In these systems, although the sample is ionized using the same methods as those described above, ions are separated in a strong electric field with high-frequency asymmetric waveforms in a constant gas flow. Positive and negative ions are driven simultaneously to the separation zone and are moved between two parallel plates of electrodes. Depending on the strength of the compensation voltage applied, only certain ions can pass through the electrodes to reach the detection unit. The compensation voltage is swept over a range of voltages for ion selection and display. This approach has considerable importance in environmental analysis.

The detection unit normally comprises a Faraday plate that is preceded by an aperture grid to prevent distortion or fronting peaks, thereby preserving resolution and sensitivity. Collisions and annihilation of the ions in the plate lead to an ion current that is amplified and delivers a time-dependent signal,

which appears in the IMS spectrum as a peak with a defined voltage at a drift time.

Fig. 4 shows a typical IMS signal in positive mode, where available reactant ions led to a signal that corresponded to the reactant-ion peak (RIP), whose intensity decreased with analyte concentration. Neutral molecules led to specific ions that have characteristic drift times, allowing their selective identification.

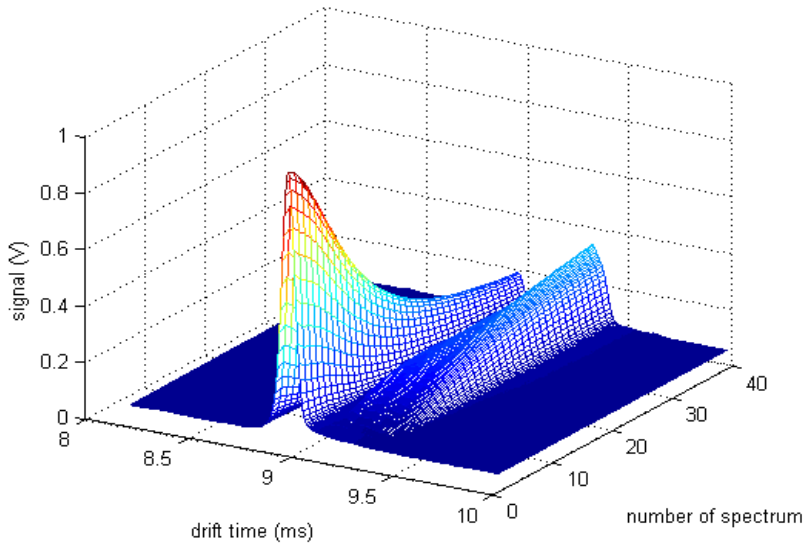

Figure 4. Typical IMS spectra working with a radioactive ionization source in positive mode. Acetone is detected using a 3H-IMS device with nitrogen as sample and drift gas. Peak corresponding to the analyte increases as the reactant-ion peak (RIP) decreases.

3. Environmental applications

IMS boasts a number of characteristics that make the technique feasible for on-site, real-time applications, since it provides a good balance between usable information and ruggedness, reliability and portability. IMS instruments combine low technical costs with high speed data acquisition. IMS devices can operate over a full range of environmental conditions, being more

easily adapted to real-time monitoring and field applications than many other laboratory instruments [5]. Table 1 sets out the main aspects of comparison between MS detectors, commonly used in environmental analysis, and IMS.

Table 1. Critical comparison between the main characteristics of ion-mobility spectrometry (IMS) and those of mass spectrometry (MS).

	Aspect Compared	IMS	MS
Advantages of IMS	Cost	Low	High
	Portability	High	Medium
	Ease of use	Easy	Complex
	Maintenance	Low	High
	Speed	High	Medium
Disadvantages of IMS	Sensitivity	Medium	High
	Selectivity	Low	High
	Sample introduction	Medium	Medium
	Applicability	Limited	Broad

Table 2 summarizes existing applications of IMS in environmental analysis. In the following sections, we analyze the data presented in Table 2, taking into consideration the type of analyte, the matrix and the tools developed to overcome the limitations of IMS with respect to selectivity and sensitivity.

3.1. Target pollutants

IMS instruments can determine almost any substance that is introduced into the ionization chamber provided that molecular properties are favorable to the ionization source. Analytes with proton affinities higher than that of water (691 kJ/mol) can be detected in positive mode of devices with a radioactive source. In negative-ion reactions, electron affinity is the favorable molecular property, so substances, such as aromatic hydrocarbons, carboxylic acids, nitro-alkanes, or nitro-aromatic compounds and halocarbons, exhibit

Table 2. Determination of different families of chemical compounds with ion-mobility spectrometry (IMS) in the environment.

Compound	Matrix	Sample preparation	Pre-separation	IMS mode	LOD	Ref.
Organic						
Perfluorocarbons	Air	PDMS membrane	None	^{63}Ni-IMS	340-780 ng/L	[12]
Dimethylsulfate	Air	None	Tenax	^{63}Ni-IMS	3 µg/L	[13]
Diisopropyl-, diethyl-, dimethyl-methylphosphonate	Soil	SPME	None	^{63}Ni-IMS	6.3-7.6 mg/kg	[14]
Chemical warfare agents	Soil	TD	None	^{63}Ni-IMS	<15 pg	[15]
2-furfural, 5-methyl-2-furfural	Synthetic water	HS	None	CD-IMS	5.3 -6.7 µg/L	[16]
Trinitrotoluene, 1,3,5-trinitro-1,3,5-triazine	Real explosives contaminated water	SBSE	None	^{63}Ni-IMS	0.1 -1.5 µg/L	[17]
Methyl tert-butyl ether (MTBE)	Water Air	MEU	MCC	^{63}Ni-IMS	50 µg/L	[18]
MTBE	Water	MEU	None	^{63}Ni-IMS CD-IMS	0.1 mg/L	[19]

MTBE	Water Gasoline	HS-SPME	None	CD-IMS	0.7 -4.9 µg/L	[20]
MTBE	Water	None	MCC	UV-IMS	20 µg/L	[21]
PAHs	Soil (PVC model matrices, RM, soils)	None	None	Laser-IMS	5-120 mg/kg	[22]
BTEX	Ground water	HS-SPME	MCC	UV-IMS	Upper µg/L range	[7]
Malathion, ethion, dichlorovos	Air	None	None	^{63}Ni-IMS	$2.1\ 10^{-10}$-$9.4\ 10^{-10}$g	[23]
Sulfotel, propoxur, nicotine	Liquid matrices	TD	None	ImCell™-Am-IMS	6.4-32.4 mg/L	[24]
Atrazine, ametryn	Well water Soil	HS-SPME	None	CD-IMS	10 -15 µg/L 23-37 ng/g	[25]
Triazines	Water	Electrospray needle and nebulizer	None	ESI-FAIMS-MS	0.1 -2 µg/L	[26]
Trihalomethanes	Tap, swimming-pool, river, drinking water	IL-SDME	Semi-capillary column	^{3}H-IMS	0.1-0.9 µg/L	[27]
Haloacetic acids	Tap water	None	None	ESI-FAIMS-MS	0.5 -4 µg/L	[28]
Chlorocarbons	Water	None	GC	^{63}Ni-DMS	20-80 pg	[29]

Trichloroethylene, tetrachloroethylene	Soil	None	SS	^{63}Ni-IMS	< 1 µg/mg	[30]
Chlorophenols	-	None	LC	ESI-IMS	0.135-2.23 mg/L	[31]
1,2,4 - Trichlorobenzene	Water	SPME	GC	FAIMS	< 1 µg/L	[32]
Trichloroethene	Synthetic air	None	MCC	UV-IMS	< 25 µg/L	[33]
Hydrazine, monomethylhydrazine, ammonia	Air	Methylsilicone membrane	None	^{63}Ni-IMS	6 µg/L	[34]
Isocyanates	Synthetic air	None	None	IMCell-IMS	-	[35]
Terpenes	Air	None	MCC	UV-IMS	5-20 mg/L	[36]
1-propanol, ethanol	Synthetic air	HS	None	UV-IMS	1-3 mg/L	[38]
Aniline	Water	Heated injector	None	^{63}Ni-IMS	0.5 mg/L	[39]
n-alkanethiols	Air	None	GC	^{63}Ni-DMS	-	[40]

Inorganic compounds						
Nitrate, nitrite	Drinking, river, creek water	None	None	ESI-IMS	10-40 µg/L	[41]
Aluminum sulfate, lanthanum chloride, strontium chloride, uranyl acetate, uranyl nitrate, zinc sulfate	Water	None	None	ESI-IMS	0.16-13 mg/L	[42]
Ammoniacal nitrogen	Ground, tap, river water	HS	None	CD-IMS	9.2 µg/L	[43]
Ambient ammonia	Air	DMS membrane	None	^{63}Ni-ASIMS	0.1 µg/L	[44]

favorable response with negative-ion chemistry. Radioactive sources are not sensitive to some analytes, including small hydrocarbons, which might be seen with other ionization sources. CD-IMS enables the detection of compounds that are difficult to determine with other sources (e.g., n-alkanesor saturated hydrocarbons). Photoionization is possible with a large number of substrates and is particularly effective for the determination of aromatic and unsaturated compounds, since the ionization energy of these substances is mostly below 10 eV. It is possible to enhance the selectivity of IMS measurements by choosing the right lamp with different distribution of intensity according to the wavelength with different photoionization energies. Besides, substances with low volatility can be analyzed with laser desorption/ionization. Surface ionization was developed for compounds with low-ionization energies, and ESI sources allow the determination of high-molecular-weight and non-volatile compounds. Almost any pollutant can therefore potentially be determined using IMS, selection of the ionization source being the key aspect to take into consideration.

3.1.1. Organic compounds

So far, most IMS detection has been devoted to determination of explosives and CWAs. This detection has, for the most part, been limited to gas-phase samples using ^{63}Ni or corona-ionization sources [12,13]. Once these substances reach an environmental compartment, their determination can be considered an environmental application [14–17]. For verification of their presence, it is necessary to detect these compounds or their degradation products on surfaces on which they have settled or condensed. ESI has been used for the detection of CWAs in water, but the ability of IMS to separate and to detect CWAs and their degradation products deposited on surfaces has not been investigated.

One of the substances intensively evaluated with IMS is methyl tert-butyl ether (MTBE), interest being motivated by its widespread use as octane-enhanced replacement for lead in gasoline and the subsequent contamination of environmental matrices. Mainly, this determination has been carried out with ^{63}Ni-IMS [18] in positive mode due to its high proton affinity, although CD-IMS [19,20] and UV-IMS [21] have also been used.

Aromatic compounds [e.g., polycyclic aromatic hydrocarbons (PAHs)], which can be found in a number of environmental matrices as a consequence of combustion of hydrocarbon fuels, have been determined using laser desorption-ionization (LDI) with IMS [22]. BTEX (benzene, toluene, ethylbenzene and xylene isomers) have also received much attention for IMS detection, UV sources being the most commonly employed with this purpose. Ionization in positive mode has been used since BTEX chemistries favor the formation of positive ions [7]. BTEX signals have been also evaluated in other studies not as the target pollutants but as possible interfering substances in the determination of other compounds.

A variety of pesticides has been measured with IMS leading to very low LODs. The major group of pesticides determined in this context comprises organophosphorus compounds (OPCs). They have high proton affinities and therefore readily produce positive ions in the reaction region of the IMS instrument, even in the presence of several other constituents present in ambient air. Positive ion-mobility spectra of different OPCs (e.g., malathion, ethion and dichlorvos) have been studied in air at ambient pressure using an IMS device with a ^{63}Ni-ionization source [23].

The determination and the identification of sultotep, propoxur and nicotine within the radioactive source of a cell was also possible. This study

used an IMS model that contains two different sensors: an open-type ionization cell (ImCell) and a commercial semiconductor cell (SCCell) [24].

Also, triazines and their degradation products (e.g., ametryn) can easily be detected with a CD-ionization source in positive mode [25]. These pesticides, together with other related substances, have also been analyzed with ESI-FAIMS-MS [26].

Much work has been done for the detection of halogenated pollutants. Some of these organohalogens are classified as disinfection by products, and, among others, include trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane and bromoform) [27] and haloacetic acids (monobromo-, monochloro-, dibromo-, dichloro- and trichloro-acetic acid) [28]. Direct ionization of these substances in IMS instruments with a radioactive ionization source in negative mode leads to the formation of the free halide, with a characteristic drift time that facilitates its quantification.

Also, a ^{63}Ni ionization source can be used for the sensitive detection of traces of chlorocarbons [29,30]. Chlorophenols were selected as analytes of interest to explore the performance of LC-ESI-IMS, due to their industrial and environmental importance and the variety of structural shapes of more than a dozen congeners and positional isomers [31].

In other cases, CD ionization has been used for determining chlorobenzenes in water. Chlorobenzenes can be detected in IMS employing UV-IMS [7] and FAIMS operating with a UV-ionization source [32].

The response of UV-IMS and the advantages of the combination with multicapillary columns (MCC) have been used for detection of trans-1,2-dichloroethene, trichloroethene and tetrachloroethene [33].

Other industrial compounds (e.g., hydrazines in air) have been detected with radioactive sources [34]. Their strong proton affinities facilitated their ionization and allowed low LODs.

Isocyanates, very toxic pollutants, including toluene, 2,4-diisocyanate [35], can be detected at sensitivity of low parts per billion in a short period of time.

UV-IMS is also optimally capable of detection of trace substances {e.g., terpenes in ambient air (e.g., indoor air quality control, process control, odor detection) [36]} and different terpene isomers have been studied with CD ionization and photoionization [37].

IMS is also particularly sensitive to alcohols. In most cases reported, the instruments used for this detection were equipped with a UV-ionization source [38].

Besides, the usefulness of IMS in the environmental field was demonstrated for the determination of other environmental contaminants {e.g., aniline [39], nalkanethiols [40], metalloorganic compounds (Be and Cu-diketonates in air) or carboxylates, which have been determined mainly with radioactive ionization sources}.

3.1.2. Inorganic compounds

IMS is considered particularly sensitive for organic compounds, but it has also been used for the determination of inorganic substances. Fertilizers

(e.g., nitrate and nitrite) in water samples have been determined with ESI-IMS [41], which was also employed of detection of inorganic cations in aqueous solutions (e.g., aluminum sulfate, lanthanum chloride, strontium chloride, uranyl acetate, uranyl nitrate, zinc sulfate in water) [42].

Also, IMS is one technique for determination of ammoniacal nitrogen. The direct quantitative analysis of this compound in various water samples was studied with CD and ^{63}Ni ionization sources [43]. ^{63}Ni-IMS was also used for the determination of ambient ammonia in air [44]. In addition, negative IMS can be employed for selected inorganic pollutant gases and gas mixtures in air with radioactive ionization sources.

3.2. Matrices analyzed

Many references in the literature on these pollutants are devoted to the study or the evaluation of the ionization process and the mobility of the ions formed [45,46]. However, the challenge arises when the study is applied to real environmental samples analyses. Due to the nature of IMS measurements, ions are mainly ionized once they are in their gaseous form, so air or gaseous samples can be easily handled in IMS, whereas liquid or solid samples require previous vaporization or additional sample preparation. Different approaches have been used to deal with the issues related to the introduction of these types of samples as described in [47]. In this section, we summarize the existing applications for each type of sample, and Table 2 gives examples.

3.2.1. Gaseous

IMS is ideal for the determination of organic vapors in air. Despite being the least problematic for sample introduction, IMS has few applications devoted to real environmental samples. Generally, the preparation of standards, and contaminant interferents make calibration difficult for gaseous

samples. Much work has focused on the analysis of ambient air for determination of explosives or monitoring toxic substances (e.g., n-alkanethiols, diborane and hydrazines). Other compounds (e.g., alcohols and terpenes) have been studied in gaseous samples.

An interesting application in real environmental samples was developed by Larry et al., who collected air samples in the vicinity of known NH_3 emitters and found high NH_3 concentrations and a direct relationship between winds emanating from the sites where fertilizer-manufacturing facilities were located.

3.2.2. Liquid

The principal type of sample analyzed with IMS for environmental applications is water. Matrices comprising typical natural water samples are complex, and the analyte concentrations can be rather low, necessitating sample clean-up and pre-concentration prior to final analysis. Due to these issues, less complex samples (e.g., synthetic water) can initially be employed to evaluate developed systems. The potential of ESI-IMS for the analysis of water samples with different complexity was demonstrated for nitrate-nitrite in environmental water samples (tap, river and creek water). Pesticides (e.g., atrazine and ametryn) have been determined in well-water samples. Inorganic salts have been detected in river water and ammoniacal nitrogen has been used to obtain information about water quality in wastewater processing, and river, tap, ground and drinking waters. Disinfection by-products, including chlorinated and brominated substances in tap water and other types of waters, can be also determined using IMS. Groundwater samples from industrial areas have been also analyzed for determination of chlorobenzenes.

Applications to analyze gasoline samples have been described [20]. MTBE has been determined in this type of matrix by employing a headspace (HS) technique that avoids the problems related to the nature of the sample.

3.2.3. Solid

The application of IMS does not extend to the analysis of solid matrices for environmental analysis, due to the complexity of the sample and the need for previous extraction or a special technique. Some investigations have been devoted to the analysis of contaminated soils for the detection of CWAs (precursor and degradation products), and herbicides (ametryn and atrazine) using SPME. Direct analysis of the samples was achieved for determination of CWAs with thermal desorption and for detection of trichloroethylene and tetrachloroethylene. In the latter application, a small sub-surface IMS instrument was constructed and tested with several environmental contaminants to determine its potential for monitoring gaseous volatile organic compounds.

A practical approach for this type of analysis is laser-based IMS, whose usefulness was demonstrated in the detection of PAHs and petroleum products directly from soil matrices, relying on the strong UV-absorption properties of the analytes and weak absorption of the matrix materials to make sample preparation or extraction of analytes unnecessary.

3.3. Sample preparation for environmental analysis using IMS

Despite the good sensitivity and the selectivity that the characteristic drift times confer to the measurements, IMS applications in environmental sites is limited by these two analytical properties, because of the low LODs required in environmental analysis and the complexity of the samples, where the presence of known interferences (e.g., moisture, ammonia, matrix

components, other target substances) and unknown interferences is of relevance. In the following sections, we describe the approaches developed to overcome these limitations.

These analytical properties (sensitivity and selectivity) are not independent and several treatments can improve both simultaneously (e.g., the combination of the techniques of extraction and pre-concentration). However, we consider the different ways of improvement separately.

3.3.1. Improvement in selectivity

Ionization, ion separation and interpretation of IMS spectra are simple for single substances or for analytes whose ionization is more favorable than that of the components of the matrix. However, the introduction of analytes in complex samples (e.g., environmental), or the determination of mixtures of various target compounds leads to complicated IMS signals. This difficulty may arise from multiple and competitive ionization interactions as a consequence of the limited reactant ions in chemical-based sources or limited energy available in the ionization source. How ionization of a substance can be suppressed in the presence of other compounds has been described (e.g., interferences of the sample or another target analytes that have preferable ionization in the source used). This is so in reducing the signal of benzene in the presence of toluene with photoionization as a consequence of the lower ionization energy required for toluene (8.83 eV for toluene versus 9.24 eV for benzene). In radioactive sources particularly, ionization of substances with low proton affinity is reduced when compounds with higher proton affinities are present in the sample. In the same way, in negative polarity, ionization of substances with higher electron affinities can hinder the ionization of analytes with lower electron affinities. Total absence of the analyte peak or reduction of its intensity or even a loss of peak resolution is therefore likely to happen in

environmental analysis. This effect depends on the concentration of the interferences.

In other circumstances, it is possible that a compound (interference or other target analyte) has the same drift time and can be falsely identified as the target analyte, leading to a false-positive response. Indeed, poor selectivity is the major issue in IMS, limiting its applicability in environmental analysis. For example, aliphatic chlorinated hydrocarbons in radioactive sources working in the negative polarity form free chlorine and the signals appear at the same drift time as the analytes.

An important interference to consider in environmental analyses is moisture, which causes the major problem using IMS under ambient atmospheric conditions due to cluster reactions between ions and water molecules, leading to significant changes of the spectra. Besides, traces of ammonia in the supporting atmosphere can gain charge at the expense of reactant ions, with a subsequent different reaction chemistry and appearance of the analyte peak at a different drift time.

As a consequence, improvement of selectivity in IMS through correction of matrix effects and problems associated with mixtures of analytes is required to obtain reliable IMS measurements for environmental analysis. The main approaches employed to overcome these problems are summarized, as follows.

3.3.1.1. Change of ion chemistry

An initial solution is to change the chemistry of the IMS system by introducing controlled, low levels of substances termed dopants or reagent gases (normally, methanol, acetone or ammonia) with an ionization potential

slightly below that of the target analyte. In this way, the response of interfering substances with ionization capability would be excluded.

3.3.1.2. Selective extraction

Analytes are separated from the matrix and then made to travel to the detector. The commonest approach involves use of membrane inlets or membrane-extraction units, which are particularly valuable to prevent moisture and ammonia from ambient air while analytes dissolve and permeate through them [34]. The problem associated with their use is the probable reduction of the concentration of analytes entering the IMS system. For example, dimethylsilicone membrane has been used for the determination of MTBE. This membrane was also efficient for the removal of negative effects of interfering substances (e.g., BTEX and tert-butyl-alcohol up to 600 µg/L). Also, the use of extraction techniques before IMS detection provides selectivity to the measurement by extracting the target analyte while minimizing background interferences. SPME and LPME have been employed in this way. In the case of SPME, selection of the fiber coating is the key aspect to consider. Polydimethylsiloxane (PDMS) fibers can be used to extract non-polar substances, whereas polyacrylate (PA) fibers are suitable for extraction of polar compounds. For example, a dodecylsulfate-doped polypyrrole fiber provides selective extraction of pesticides and MTBE from different samples. The selection of the proper extractant in LPME also allows selective extraction of the proper analyte. An ionic liquid has been used to extract selectively trihalomethanes from water using the ionic liquid-based single-drop microextraction technique (IL-SDME).

3.3.1.3. Pre-separation

The pre-separation of the constituents of the matrix and target analytes making the different elements traveling to the detector periodically is another possibility employed to enhance selectivity. This has been achieved using a short column packed with a simple adsorption material and a pyrolyzer to serve as a pre-separation step. But the most common approach is pre-separation of the constituents of the matrix using a previous chromatographic separation technique. LC and GC allow the possibility of making the analysis of liquid samples compatible with IMS analysis; at the same time, they add another dimension and improve IMS measurements by providing orthogonal information additional to retention times and simplify product-ion formation. Fig. 5 shows an example of multidimensional separation data analysis when a series of analytes are pre-separated before IMS detection. The effect of interfering substances from the matrix can be eliminated and the intended analytes in mixtures can be separated efficiently, traveling to the ionization source in succession and avoiding competition for charge.

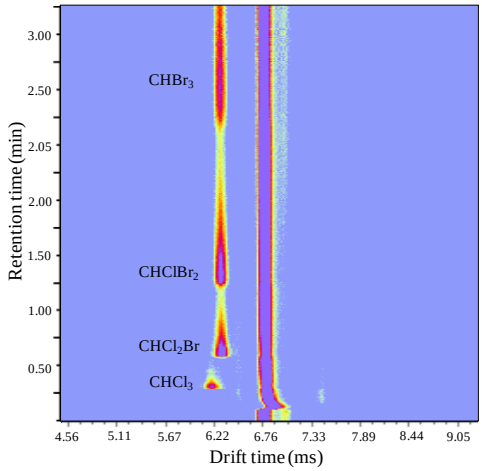

Figure 5. Multi-dimensional data analysis of four analytes (chloroform, bromodichloromethane, dibromodichloromethane and bromoform). The compounds are pre-separated in HP-5 semi-capillary column before their detection with a ^{3}H-IMS device. Drift time (X axis) and retention time (Y axis) in the column provide qualitative information about the analytes. (Reprinted with the permission from [27]. ©2009, Elsevier Science BV).

LC combined with IMS does not extend to environmental applications due to the liquid nature of the eluents from the column. An example is the separation of more than 14 chlorophenols employing LC-ESI-IMS. In the case of GC, capillary columns with different polarities have been used for environmental purposes. Usually, a counter-flow system is employed for this coupling (see Fundamentals of IMS, Section 2, above), in which the eluent from the column is directly introduced at a low flow rate (~100 mL/min) into the ionization chamber from the side of the analyzer and near the shutter grid in the drift-gas direction. Sometimes, the injector in GC has been used to inject the sample although the column is not used.

An attractive alternative to conventional capillary columns to improve selectivity is the MCC, which comprises a single bundle of hundreds of capillary columns that permit a considerable efficiency in separation at high flow rates and optimal sample capacity for IMS [48]. Moreover, their small size and the possibility of working at ambient temperature and isothermal separation make MCCs attractive for portable IMS analyzers. These columns have been used in the environmental field [33]. Semi-capillary columns can also be employed as an intermediate solution that allows working at intermediate flow rates with simple connections. An HP-5 semi-capillary column has been used for trihalometanes separation.

3.3.1.4. Coupled detection systems

Selectivity of the determinations can be enormously enhanced in coupled systems such as those with MS, where the IMS device acts as a pre-filter before the mass spectrometer and the latter provides a definitive identification of ions in a mobility spectrum [49]. In this sense, the technique ESI-FAIMS-MS allows the direct determination of 11 pesticides or 5 haloacetic acids without pre-fractionation.

3.3.2. Improvement of sensitivity

Although sometimes the direct use of IMS is perfectly sufficient to allow high sensitivity, there are some environmental applications in which LOD values must be lowered in order to meet the legislative requirements. This can be achieved by selecting the ideal ionization source for the target analyte, using a pre-concentration technique or removing the interfering substances that compete for charge, as described above.

3.3.2.1. Ionization source

The selection of the proper ionization source according to the nature of sample and analyte must be done taking into account the different aspects considered above. Although it depends on the application, in general, it can be said that CD sources usually lead to better sensitivity, a higher signal-to-noise ratio and a wider working range compared to ^{63}Ni-IMS, since they allow higher total ion current by about an order of magnitude. CD-IMS permits the most sensitive detection of halogenated compounds, whereas both types of source are generally more sensitive than photoionization-based devices. Other sources (e.g., ESI or laser based) would increase sensitivity for the analysis of liquid or solid samples.

3.3.2.2 Pre-concentration

The employment of extraction techniques before IMS detection not only removes interfering substances, as previously pointed out, but also reduces the final volume of extraction, leading to an improvement in sensitivity. LODs in the range 0.5 µg/L-0.98 mg/L have been obtained for CWAs, herbicides and other environmental contaminants using SPME. Also, stir-bar sorptive extraction (SBSE) interfaced to IMS exhibits excellent sensitivity with LODs in the range 100 ng/L–1.5 µg/L for explosives, whereas LODs in the range 0.1–1.5 µg/L are achieved with IL-SDME for

trihalomethanes, where analytes are extracted into a 2 µL-drop of the extractant.

The improvement of sensitivity using different approaches can be exemplified with MTBE; a direct on-line procedure based on the use of a membrane inlet and ^{63}Ni or CD sources led to LODs of 100 µg/L. This value can be improved to 10 µg/L when the inlet membrane acts as an enriching zone and the flow through the extraction unit is interrupted. We have shown that ^{63}Ni and CD sources permit more sensitive detection of MTBE compared to photoionization detectors. Using the combination MCC-^{63}Ni-IMS, LODs of 50 µg/L in water samples can be obtained. The sensitivity was lower using MCC-UV-IMS, with which the lower concentration detectable was 20 µg/L in water. Concentrations higher than 30 µg/L can be detected using water-adsorbing polymers. The lower LODs in water have been achieved with dynamic HS-IMS and the pre-concentration of the analyte on Tenax tubes. The combination with extraction techniques can also enhance the sensitivity, and, when CD-IMS is combined with HS or SPME, concentrations of 0.7 µg/L can be determined.

For pesticides (e.g., atrazine and ametryn), CD-IMS leads to LODs of 23–37 ng/g (soil) and 10–15 µg/L (water). In a more recent study, ESI-FAIMS-MS allowed the LOD to be decreased to 0.1 µg/L in water for application in the environment because this method permits ion sorting and discrimination against the considerable ‘‘chemical noise’’ and non-specific cluster and fragment ions, which are typically generated in ESI.

4. Miniaturized IMS for field analysis

Among the characteristics of IMS systems that make them especially suitable for miniaturization, the following can be cited:

(1) sensitivity, which permits detection at the low ng/L level;

(2) speed of response (in a very few seconds), which permits real-time monitoring; and,

(3) ruggedness, low power consumption and lower costs of consumables.

However, in miniaturized instruments, two main shortcomings can also be identified:

(1) limited selectivity, probably as the result of poor resolution and cross-product generation in the analysis of mixtures; and,

(2) limited use of radioactive sources in portable instruments, probably as a result of existing safety regulations in most countries.

Several approaches have been proposed in the literature for the design of miniaturized IMS systems. This reduction in dimensions is complex for time-of-flight IMS as a result of the complexity of the design. Moreover, several disadvantages (e.g., peak broadening due to non-homogeneity of the electric field, space-charge effects and diffusion) are more significant at small dimensions. This leads to a decrease in resolution from 25 in conventional instruments to 10–15 in miniaturized time-of-flight IMS [4]. As far as ionization sources are concerned, UV-laser radiation [50] and CD ionization [51] have been successfully used in miniaturized IMS instruments (drift-tube diameters 1.7 mm and 2.5 mm, respectively).

The first miniaturized spectrometers used a FAIMS and they involved rectangular drift tubes and a planar electrode configuration [52]. The planar device provided good separation power but suffered from high power consumption and hindered the development of handheld instruments.

Zimmermann et al. proposed a new concept of miniaturized IMS with chip dimensions based on the aspiration-condenser-type IMS previously developed by Puumalainen [53] and Paakkanen et al. [54]. The ion focusing aspiration condenser IMS is planar and fully integrated in a glass unit. In order to overcome the limitations of previous spectrometers in terms of resolution, it uses effective ion focusing before separation. For this aim, they used two parallel gas streams (sample gas and drift gas) flowing under laminar conditions. The ions inside the sample stream were focused by means of geometric constraints before entering the separation region. The presence of a transverse electric field forced ions out of the sample gas into the drift gas towards the bottom electrode. Ion species with different mobilities were separated into individual ion beams. By increasing the deflection voltage, the ions were focused to the detector electrode, which resulted in a more selective ion separation. Negative and positive ions could be detected by the simple change in polarity. A major advantage of having separate gas streams is that humidity does not affect the peak position in the spectrum. This miniaturized IMS has been successfully used for the determination of hazardous compounds, trace gases and CWAs.

5. Final remarks

IMS is a detection technique that allows qualitative and quantitative information associated with the analysis of chemicals with a very low response time (~5 s) and at ambient conditions. Its advantages are promising in environmental applications and the possibility of miniaturizing IMS adds to its suitability as a field-deployable, hand-held device to permit simple, rapid determinations on site. Although, in comparison with other techniques, the applications of IMS are still scare, the bibliography reveals that it can be used to detect and to identify a wide series of pollutants with varied chemistries and structures. Nevertheless, significant problems associated with IMS for

detection in the environment include the nature of samples, insufficient sensitivity for specific applications, ionization selectivity, and the potential of confusing results from complex mixtures.

The introduction of the sample is a first handicap when non-gaseous samples are to be analyzed, and, in most cases, additional techniques for extraction or preconcentration are needed (e.g., membranes, SPME, HS, thermal desorption). Due to the occurrence of ion-molecule reactions and relatively poor resolution of the species formed, IMS is not generally used to identify unknown compounds, but it is increasingly being applied to cases where the processes involved are known.

These inconveniences can be avoided by pre-separating the constituents of the sample, although the development of new modalities of IMS and its coupling with other techniques (e.g., MS), together with miniaturization of the devices, are among the current trends in IMS analysis.

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Capítulo 6

Determination of 2,4,6-trichloroanisole in water and wine samples by ionic liquid-based single-drop microextraction and ion mobility spectrometry

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Determination of 2,4,6-trichloroanisole in water and wine samples by ionic liquid-based single-drop microextraction and ion mobility spectrometry

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ABSTRACT

This article presents for the first time the joint use of ionic liquid-based single drop microextraction (IL-SDME) and ion mobility spectrometry (IMS) for the determination of 2,4,6-trichloroanisol (2,4,6-TCA) in water and wine samples. An imidazolium-based IL was used as extractant taking into account both, its affinity for the analyte and its negligible response in the detector. Water samples were directly analyzed by IL-SDME while wines required a previous solid phase extraction step to remove the interference of ethanol in the ion mobility spectra. All the parameters affecting the extraction were optimized in order to achieve the highest sensitivity, taking into account the lower concentrations reported for this compound in the matrices selected. Moreover, the use of ion mobility working under the negative ionization mode confers an adequate selectivity. The limits of detection and quantification were 0.2 and 0.66 ng L^{-1}, respectively. The precision of the method calculated at 10 ng L^{-1} was 1.4% (repeatability, n = 5) and 2.2% (reproducibility, n = 5, 3 days). The analysis of water and wine samples reported the presence of 2,4,6-TCA in wine samples commercialized in bottles with cork stoppers.

1. Introduction

2,4,6-Trichloroanisole (2,4,6-TCA) is considered to be one of the most frequent causes of musty odors of wines [1]. This potent odorant can also produce sensorial alterations in other products such as coffee or raisins, and its incidence in water has also been recently reported [2–4]. The main sources of these off-flavours in both matrices are the chlorine-based disinfectants added to water distribution systems and the bleaching solutions used during production of cork stoppers, washing of oak barrels, etc. It is known that consumers will reject wines containing concentrations of 2,4,6-TCA over 10 ng L^{-1} [5]. Although in water, this haloanisole is able to confer a musty taste and odour at low concentration, being its perception threshold lower than 4 ng L^{-1} [6]. Fortunately, the perception limits for other haloanisoles are significantly higher [7].

The majority of existing analytical methods developed for the determination of 2,4,6-TCA use gas chromatography (GC) coupled with electron-capture detector (ECD) [8], flame ionization detection [9] or mass spectrometry (MS) [10], although atomic emission detection and olfactometry has also been recently used. As regards sample treatment, solid-phase extraction (SPE) [11], solid-phase microextraction (SPME) [12–16] and pervaporation [17,18] are the alternatives of choice for 2,4,6-TCA isolation and preconcentration.

Solvent microextraction or single-drop microextraction (SDME), was first introduced by Jeannot and Cantwell in 1996 [19]. In this technique, the acceptor phase is a drop of water-immiscible solvent suspended either in the needle tip of a microsyringe or a small PTFE tubing, which can be in direct contact with the sample or its headspace. After a certain extraction time, the solvent is withdrawn into the microsyringe and injected into the detection

system. As it is the case with SPME, in SDME the preconcentration and extraction steps occur simultaneously, providing fast and inexpensive analyses using very simple equipment [20]. Caraseket et al. have proposed this miniaturized extraction technique for the determination of 2,4,6-TCA and 2,4,6-tribromoanisole in wine samples by HS-SDME, with analytes separation and detection by GC–ECD [21].

From the above, it follows that the determination 2,4,6-TCA requires the implementation of sample treatment for analyte isolation and preconcentration combined with a sensitive and selective instrumental technique. In this work, the use of ionic liquid based-SDME (IL-SDME) coupled to ion mobility spectrometry (IMS) is proposed for the first time for the direct extraction of TCA from waters and, after a SPE procedure, for the analysis of wine samples. The advantages of using ILs in sorptive microextraction techniques have been recently reviewed by our research group [22]. IMS is an analytical technique that characterizes molecules by the gas phase mobility of their ions formed at ambient pressure in a weak electric field [23]. IMS, working under the negative ionization mode, can be used in the determination of 2,4,6-TCA due to its instrumental simplicity, portability, sensitivity, fast response and relatively low cost. The extraction process allows reaching the low detection limits required for this application at the same time it makes compatible the direct expeditious detection by IMS.

2. Materials and methods

2.1. Reagents and samples

All reagents were of analytical grade or better. The ionic liquid 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide [Hmim][NTf_2] was supplied by Merck (Darmstadt, Germany). Methanol and sodium chloride from Panreac (Barcelona, Spain), sodium hydrogen carbonate ($NaHCO_3$) from

Merck, as well as Milli-Q ultrapure water (Millipore, Madrid, Spain), were used in the SPE procedure.

Stock standard solutions of 100 mg L^{-1} of 2,4,6-trichloroanisole in methanol were obtained from Sigma–Aldrich. Working solutions were prepared at the appropriate concentration by dilution of the standards in methanol and stored in a refrigerated environment.

LiChrolut EN (40–120 µm), obtained from 500 mg commercial SPE cartridges (Merck) was used as sorbent material.

Drinking water samples and wine samples were purchased from local commercial stores. All samples were kept in their original bottles and analyzed within 2 days from purchasing.

2.2. Apparatus

Headspace IL-SDME was implemented by means of a 10-µL microsyringe with bevel tip (Agilent, Palo Alto, CA, USA), which was used to expose 2 µL of the ionic liquid ([Hmim][NTf_2]) in the headspace of a 10 mL extraction vial (Supelco, Madrid, Spain) sealed with stainless steel caps fitted with PTFE/Silicone septa from Análisis Vínicos (Tomelloso, Spain). A Velp Cientifica magnetic stirrer (Milan, Italy) was also employed for the continuous agitation of the sample. Experiments were carried out using a portable Ion Mobility Spectrometer produced by Gesellschaft für Analytische Sensorsysteme (G.A.S. mbH, Dortmund, Germany). The device is equipped with a Tritium ionization source (St. Petersburg, Russia) with an activity of 300 MBq. A sample inlet lets a continuous stream of nitrogen 5.0 (Carburos Metálicos, Sevilla, Spain) at 50 mL min^{-1} pass through the ionization chamber where ions are formed and focused to a shutter grid made of stainless steel.

The shutter opening time was set at 100 µs, allowing the ions to pass at short pulses towards the separation chamber where parallel drift rings were connected by a series of resistors to create an electric field of 333 V cm^{-1}, a fixed voltage of 2 kV and grid pulse 100 ms. A flow of nitrogen at 175 mL min^{-1} in the opposite direction of the ions drifting was employed as drift gas in order to prevent non-ionized impurities from entering the separation chamber. The separated ions reach a Faraday plate and the signal is acquired by a computer. The device can be operated in negative or in positive mode, but all spectra were recorded in the negative ion mode. Data were acquired and analyzed using GASpector software (Version v3.99.035 DSP).

A stainless-steel injection unit designed and described in a previous work [24] was used for the coupling of the SDME extraction procedure and the IMS detector.

SPE was performed using a VacElut-20 sample-processing station (Scharlab), equipped with a vacuum-control valve and PTFE cartridge adapters (Varian, Barcelona, Spain). 3-mL commercial SPE cartridges were packed with 50 mg of Lichrolut EN using PTFE frits to avoid losses of material.

2.3. Safety considerations

Despite their initial consideration as green solvents, the toxicity of ionic liquids has received broad attention [25], more studies should be developed in order to clearly establish their effects on the environment and living being. The ionic liquid employed in this study, [Hmim][NTf_2], is labeled as irritant material and therefore it was handled using protective gloves. The wastes were stored in an appropriate container for their later management.

2.4. Procedure

The extraction procedure of 2,4,6-TCA in the different samples was as follows: 8 mL of the water sample or standard solution prepared in Milli-Q water containing 350 g L^{-1} of sodium chloride and a 1 cm PTFE magnetic bar were placed in a 10-mL glass vial which was tightly sealed with a silicone septum. The bevel tip microsyringe was filled with 2 µL of [Hmim][NTf_2] and was inserted in the vial through the septum until its needle tip was located in a fixed position about 1 cm above the surface of the solution. The plunger was depressed and a microdrop of ionic liquid was exposed on the headspace above the volume sample at 30 ºC for 30 min while the sample was agitated at 1100 rpm. After the extraction, the IL enriched with the analyte was retracted and directly introduced into the injection unit maintained at 30 ºC, being the IL retained in the glass wool bed while the 2,4,6-TCA was transferred to the IMS for direct fast response. In the present method, peak area was used as analytical signal. The injection unit was substituted by a clean one every five injections (10 µL of ionic liquid injected). The removable element can be easily cleaned by washing the tube with methanol and using a new glass wool piece. The cleaned injection unit required only 30 min of equilibration in order to achieve the optimum working conditions.

Wine samples were processed as follows: Aliquots of 2 mL were passed through a 50 mg LiChrolut EN SPE cartridge at a flow rate of 1 mL min^{-1}. Then 4 mL of a 1% $NaHCO_3$ aqueous solution were flowed through the sorbent to remove potential interferents. Then, the 2,4,6-TCA was eluted by means of 2 mL of methanol. The methanolic extract was transferred to a 10 mL glass vial, diluted up to 8 mL with Milli-Q water and analyzed as described for the water samples. To prevent the cross-contamination between samples, the SPE cartridges were not reused.

3. Results and discussion

SDME has been successfully employed to face a wide variety of analytical problems. However, the properties of the organic solvents commonly used, such as low viscosity and high tendency to evaporation, results in drop instability and poor precision levels. The use of ILs as alternative to these conventional solvents increases the drop stability, thanks to their higher viscosity, which also facilitates the formation of larger-volume drops. Moreover, the lower vapor pressure of ILs minimizes the drop evaporation, which results in better reproducibility of the measurements [22,26–30].

3.1. Optimization of the injection conditions

The IL employed for this application must boast the following characteristics: (i) high affinity for 2,4,6-TCA; (ii) low vapor pressure, low melting point and high hydrophobicity in order to form stable drops in the SDME procedure; (iii) adequate viscosity so that it allows the carrier gas flow well through the injection unit at the same time it is not easily removed from the glass wool and; (iv) leads to no interfering signal in the IMS spectra. Imidazolium based ILs have been previously proved to have high affinity to compounds with benzene rings, so the extraction of 2,4,6-TCA was expected. Moreover, their physical properties make these solvents suitable for their use as extractants and all of them show an adequate viscosity for their introduction in the injection unit.

The ILs studied for the extraction of the analyte were 1-octyl-3-methylimidazoliumhexafluorophosphate [Omim-PF_6], 1-hexyl-3-methyl-imidazoliumhexafluorophosphate [Hmim-PF_6] and 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide [Hmim][NTf_2]. The three ILs led to high extraction efficiencies. However, the limiting factor was

the interfering signals appearing in the IMS. Since [Hmim][NTf_2] was the IL that led to cleaner IMS spectra, it was selected for subsequent experiments for the extraction of 2,4,6-TCA.

The extractant volume was limited by the capacity of the injection unit and the viscosity of the ionic liquid. Studies using volumes of IL from 0.5 to 2 µL were accomplished. Results show that an increase in peak area occurs when the acceptor volume is also increased up to 2 µL. Therefore, the volume of IL selected was 2 µL for all the subsequent experiments.

The temperature in the injection unit was a parameter of study. When the temperature of desorption is higher than 30 ºC some disturbances appeared, which could be the result of the presence of impurities in the extractant that reach the detector after their volatilization at higher temperatures. Therefore, the injection temperature was fixed at 30 ºC.

3.2. Conditions of the headspace IL-SDME procedure

The headspace IL-SDME was optimized using standard solution containing the analyte at a concentration of 100 ng mL^{-1}. Initial experimental conditions were as follows: Aliquots of 8 mL of aqueous standards were placed in a 10-mL vial containing 350 g L^{-1} of NaCl and the solution was magnetically stirred at 500 rpm during 20 min, being the temperature fixed at 60 ºC. The optimized variables and their optimum values are summarized in Table 1. When a variable is optimized, its optimum value is fixed for further studies.

Table 1. Optimized variables of the headspace IL-SDME procedure.

Variable	Initial value	Interval	Optimum value
Temperature (ºC)	60	30-70	30
NaCl concentration (g L^{-1})	350	0-350	350
Stirring rate (rpm)	500	0-1100	1100
Extraction time (min)	20	10-40	30
Sample volume (mL)	8	5-9	8

3.2.1. Extraction temperature and time

Temperature might have an important influence on the relative recovery of the analytical technique, affecting the extraction of the analyte, the viscosity of the ionic liquid and, therefore, the mass-transfer process. Its effect on the extraction of 2,4,6-TCA was investigated from 30 to 70 ºC. As it is shown in Fig. 1A, analyte extraction decreases with increase of temperature. In principle, higher temperatures would favour the release of the analyte from the sample to the headspace of the vial. However, taking into account that the sorption process in the microdrop is exothermic, an increase in the temperature effectively reduces the partition coefficient of the analyte between the gaseous phase and the IL drop. In addition, working at high temperatures increases the concentration of water molecules in the headspace, which could hinder the analytes sorption in the micro-drop. Therefore, the working temperature selected for further studies was 30 ºC.

The extraction time was evaluated between 10 and 45 min. Generally, long extraction times lead to higher extraction efficiencies, as the amount of analytes that can be transferred to the microdrop is enhanced. There is an increase in the signal until 20 min, being constant over this value. Therefore 30 min was selected as optimum.

3.2.2. Stirring rate and ionic strength

Agitation of the sample is assumed to reduce the time required to establish the partition equilibrium between the aqueous and the gaseous phases since the transfer coefficients of the analytes in the aqueous phase are enhanced. Besides, stirring the sample induces convection in the headspace, facilitating also the mass transference towards the micro-drop. The effect of this variable on the extraction was studied by varying the stirring rate from 0 rpm to 1100 rpm using a magnetic stirrer. The maximum speed of the agitator offered the best results. Therefore, 1100 rpm was the rate used for subsequent experiments.

Ionic strength can affect the affinity of the analyte for the extraction phase since less water molecules are available for the solubilization of the analyte, which facilitates its transference towards the headspace. The salting-out effect was examined by monitoring the variation of peak areas with a salt concentration ranging from 0 g L^{-1} to 350 g L^{-1}, the maximum solubility of NaCl in water at the working temperature. According to Fig. 1B, the relative peak area of the analyte increases with NaCl concentration, achieving the best results at 350 g L^{-1}. This value was considered as optimum for further experiments.

3.2.3. Sample performance

The volume ratio of the sample-to-headspace phase also affects the extraction. Following the classical equations, an exhaustive extraction would require among other requisite very small volume of headspace [31]. Working with water as sample matrix, higher volume ratios will lead to higher sensitivity since the headspace decreases with the increase of sample volume, which enhances the concentration of 2,4,6-TCA in the headspace. Therefore, its influence was studied using a 10 mL glass vial, the sample volume being

increased from 5 mL to 9 mL (Fig. 1C). Peak areas of the 2,4,6-TCA increased significantly with water sample volume, reaching a maximum at 8 mL, whereas with 9 mL the signal decreases because the headspace is not sufficient to produce the extraction of the analyte in the drop. Thus, a sample volume of 8 mL of water sample was adopted to establish the analytical features of the method.

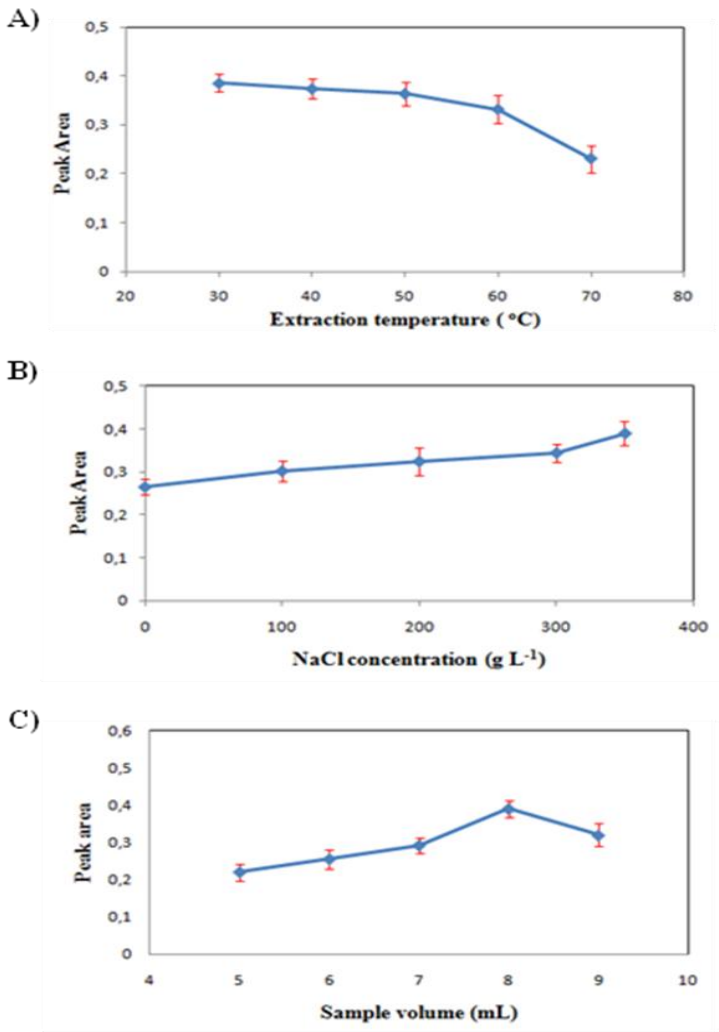


Figure 1. Optimized variables of the headspace IL-SDME procedure: (A) extraction temperature; (B) NaCl concentration; (C) sample volume. Each data was calculated by triplicate.

3.3. Analytical perfomance

The calibration graph was constructed from seven working standards prepared in Milli-Q water containing 2,4,6-TCA at different concentrations in the range from 0.1 to 100 ng L^{-1} ($y = 0.1893x^{0.1642}$, where y is the peak area and x the concentration of 2,4,6-TCA expressed in ng L^{-1}). The standards were subjected to the whole extraction procedure described in Section 2.4, under the extraction optimized conditions. The measured signals were related to the concentration of the analyte in the samples by a curve relation that could derive from the type of ionization in the IMS detector, which leads to the saturation of the IMS signal as a consequence of the limited reactants formed in the ionization source. For the calculation of the detection and quantification limits, a linear relationship was fitted to the lower portion of the calibration curve in the range 1–10 ng L^{-1}.

The limit of detection (LOD), calculated as three times the standard deviation of the blank signal divided by the slope of the calibration curve, was 0.2 ng L^{-1}. The limit of quantification (LOQ), established for ten times the standard deviation of the blank signal divided by the slope of the calibration curve, was 0.66 ng L^{-1}.The repeatability was evaluated over five replicates of a working standard at 10 ng L^{-1}, resulting to be 1.4% (as relative standard deviation, RSD). Moreover, the reproducibility of the method was evaluated under inter-day conditions for 3 consecutive days, the value being 2.2%.

3.4. Water samples

The proposed headspace IL-SDME–IMS method was applied to the determination of 2,4,6-TCA in six different water samples. The direct analysis of the samples revealed the absence of the target analyte. In order to validate the analytical methodology, a recovery study at two different concentrations (5 and 100 ng L^{-1}) was carried out over the six water samples. Then, the

concentrations were obtained using the calibration curves and the relative percentage of recuperation was calculated. The results obtained are summarized in Table 2.

Table 2. Recovery of 2,4,6-TCA from the six water samples analyzed following the proposed method (n=3).

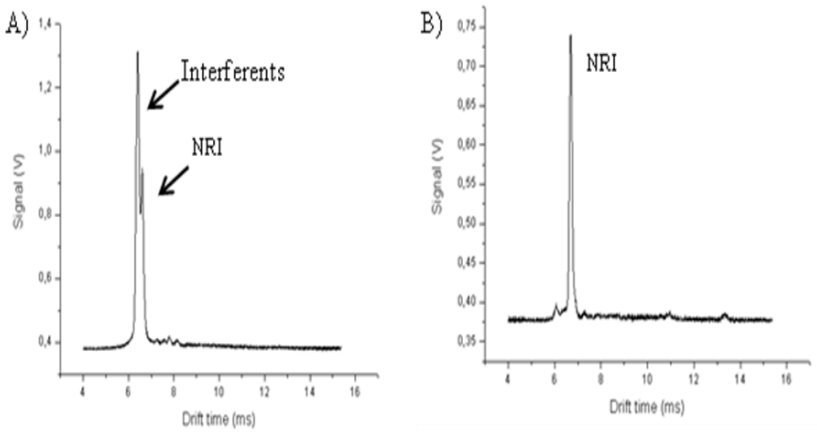
Samples	% Recovery ± SD[a] (5 ng L^{-1})	% Recovery ± SD[a] (100 ng L^{-1})
Water 1	99 ± 2	100 ± 1
Water 2	98 ± 2	99 ± 1
Water 3	96 ± 2	98 ± 1
Water 4	98 ± 2	98 ± 1
Water 5	97 ± 2	94 ± 1
Water 6	98 ± 2	98 ± 1

[a] SD, standard deviation

3.5. Wine samples

Wine samples were analyzed following the previously described procedure. However, the interference of some compounds in the wine matrix, (mainly the ethanol) was observed in the ion mobility spectra. The signal overlapped with that corresponding to the 2,4,6-TCA hindering its determination. Therefore, an SPE step was implemented prior to the IL-SDME in order to eliminate these interferents. LiChrolut EN was selected as sorbent material and a white wine sample bottled in a glass container and threat stopper was analyzed. Initially, 2 mL of wine sample were passed through a 50 mg cartridge and the sorbent was dried using air. Then, 4 mL of water were added to remove potential interferents and after a dying step, 2 mL of organic solvent were passed through the sorbent. The organic phase was placed in the 10 mL glass vial, diluted up to 8 mL with water and subjected to the optimized IL-SDME–IMS procedure. It was observed that, although the interferent peak was markedly reduced, it still appears in the spectra. Therefore, a washing-up step was included prior to elution using different percentages of sodium

bicarbonate ($NaHCO_3$), phosphate acid and ammonium acetate in water. Phosphate acid and ammonium acetate lead to the co-elution of the target analyte and thus, interfering signals in the IMS. Therefore, an aqueous solution of $NaHCO_3$ (1%, v/v) was used before the elution step. Fig. 2 compares the



spectra obtained without (A) and with (B) the optimized SPE procedure.

Figure 2. Ion mobility spectra of the white wine sample (glass bottle with a thread stopper) obtained following the IL-SDME-IMS without SPE (A) and with SPE (B). NRI: negative reactant ions.

Once the interference from the matrix sample was eliminated, the efficiency of the SPE procedure for the quantitative isolation of 2,4,6-TCA was evaluated. The sorbent amount affects the quantity of analyte that can be retained and the volume of eluent required. For this study, 2 mL of blank white wine spiked with 50 ng L^{-1} of the target compound were passed through the cartridges packed with the different amounts of sorbent (between 10 and 500 mg). The studies conducted demonstrated that 50 mg were enough for quantitative retention of the analyte at the concentration evaluated.

Different solvents (acetonitrile, acetone, hexane, methanol, ethyl acetate, propanol and mixtures of them) were evaluated for their use as eluents taking also into account that the extract would be subjected to a subsequent SDME procedure. Hexane and ethyl acetate caused the dissolution of the IL-drop.

From the rest of solvents, methanol was finally selected as eluent because it did not interfere in the detection system. A volume of 2 mL was used to maintain constant the analyte concentration and the volume used in the IL-SDME–IMS procedure.

Once optimized, the whole method was applied to the determination of 2,4,6-TCA in ten wine samples: four red wines, four white wines and two sparkling wines. The samples were differenced by their container (glass, bag in box or tetra brick) or their stoppers (cork, rubber or thread). All the samples were analyzed following the proposed SPE–IL-SPME–IMS procedure. Only the samples with cork stoppers provided a positive response for 2,4,6-TCA being the results summarized in Table 3. The concentrations are referred to the non diluted wine sample. The ion mobility spectra are shown in Fig. 3. In addition, a recovery test was carried out at 5 and 50 ng L^{-1}concentration in the non diluted samples, in the three different types of wine matrices analyzed in this article, being the results listed in Table 4. In Fig. 4, it is showed the spectra ion mobility for every concentration of 2,4,6-TCA in a white wine sample.

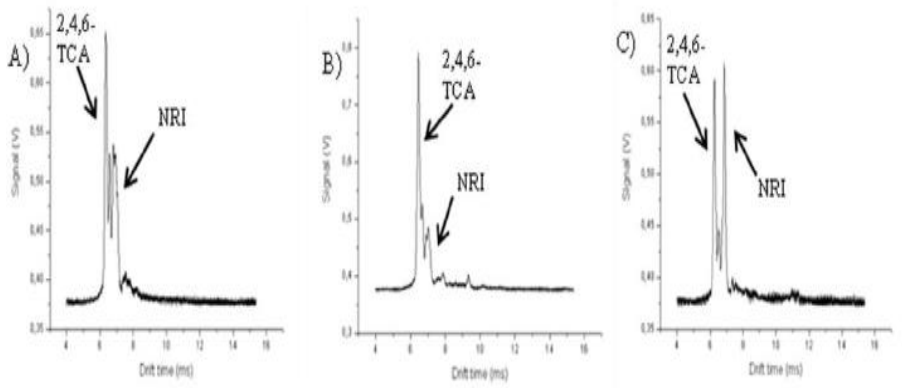


Figura 3. Ion mobility spectra of a white, sparkling and red wine sample, respectively where 2,4,6-TCA was detected. NRI: negative reactant ions.

Table 3. Analysis of wine sample with different stoppers and containers by the proposed method.

	Sample	Container	Stopper	Concentration found (ng L^{-1}) ± SD[a]
Red wine	Sample 1	Glass	Cork	124±5
	Sample 2	Glass	Cork	98±4
	Sample 3	Glass	Cork	57±4
	Sample 4	Tetra brick		n.d.
White wine	Sample 1	Glass	Thread	n.d.
	Sample 2	Glass	Cork	29±1
	Sample 3	Glass	Cork	89±2
	Sample 4	Bag in box		n.d.
Sparkling wine	Sample 1	Glass	Rubber	n.d.
	Sample 2	Glass	Cork	168±6

[a] SD, standard deviation of the measurements (n=3) n.d.= non detected

Table 4. Recovery percentages of 2,4,6-TCA after the analysis of commercial wine samples using the proposed method.

Sample	Container/Stopper	% Recovery ± SD[a] (5 ng L^{-1})	% Recovery ± SD[a] (50 ng L^{-1})
White wine	Glass/Thread	104 ± 4	95 ± 3
Sparkling wine	Glass/Rubber	97 ± 4	96 ± 3
Red wine	Tetra brick	101 ± 4	97 ± 3

[a] SD= standard deviation (n=3).

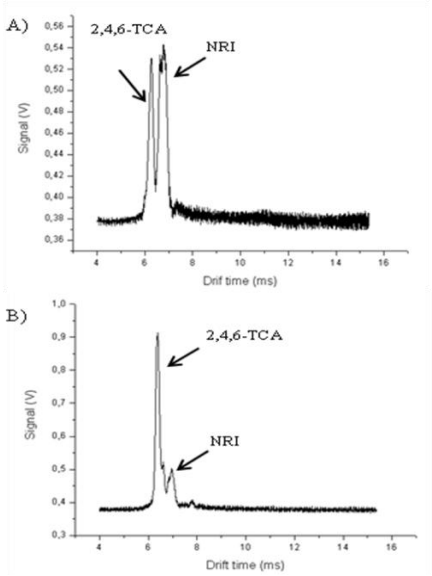


Figure 4. Ion mobility spectra of blank white wine sample spiked with (A) 5 ng L^{-1} and (B) 50 ng L^{-1} of 2,4,6-TCA. NRI: negative reactant ions.

4. Conclusions

The results obtained demonstrated the potential of the combined use of IL-SDME and IMS for the determination of 2,4,6-TCA in water and wine samples. The coupling between both techniques has been achieved by using an interface previously developed by our research group which prevents the ionic liquid from entering the spectrometer. The existence of a removable piece

simplifies the interface cleaning and allows its reusability. The use of the ionic liquid 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide [Hmim][NTf_2] has the advantage of both, analyte extraction and instrumental compatibility as no signal coming from degradation products or impurities were observed in the ion mobility spectrometer. Moreover, the instrumental coupling provides an adequate selectivity in waters, being necessary a SPE step for wine samples to avoid the interference of ethanol. The most common technique for analyzing 2,4,6-TCA is gas chromatography coupled with mass spectrometry (GC–MS), but the haloanisoles need to be derivatised prior to their chromatographic analysis due to their high polarity, which can cause broad and tailed peaks. The ion mobility spectrometry is an alternative analytical technique highly sensitive, comparatively inexpensive, simple, portable and, allow fast responses since spectra are available in the milliseconds range. The proposed system can be also compared with other analytical methodologies described for the determination of 2,4,6-TCA in wine samples in terms of sensitivity precision and extraction time (Table 5).

Table 5. Comparison of the proposed method with other methods developed for the determination of 2,4,6-TCA in wine samples.

Method[a]	LOD ($ng\ L^{-1}$)	RSD (%)	Extraction time (min)	Ref.
HS-SPME-GC-ECD	0.3	10	70	[8]
HS-SPME-GC-MS	0.2	10.1	70	[8]
SPE-LVI-GC-MS	0.2	< 6	10	[11]
SBSE-GC-MS-MS	0.03	13.3	60	[15]
HS-SDME-IMS	0.1	< 3	30	This work

[a] ECD: electron capture detector; LVI: large volume injection; SBSE: stir bar sorptive extraction. For the other acronyms, see the text.

In this sense, the use of SPE–HS-SDME–IMS provides a limit of detection of 0.2 ng L^{-1}, which is lower than those obtained with other methodologies, although the SBSE–GC–MS–MS showed the lowest LOD using 5 mL of sample with a higher extraction time. Concerning the precision, the proposed approach was the best option in terms of repeatability (1.4%, n = 5) and reproducibility (2.2%, n = 5, 3 days). Finally, the application of the proposed method to the analysis of a variety of water and wine samples pointed out that only those wine samples bottled with cork stoppers gave positive response for 2,4,6-TCA, which corroborates that the source of contamination of the wine samples with this compound is the material used for package sealing.

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Capítulo 7

Direct determination of 2,4,6-trichloroanisole in wines by single-drop ionic liquid microextraction coupled with multicapillary column separation and ion mobility spectrometry detection

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Direct determination of 2,4,6-trichloroanisole in wines by single-drop ionic liquid microextraction coupled with multicapillary column separation and ion mobility spectrometry detection

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ABSTRACT

This article evaluates the capability of single drop ionic liquid microextraction coupled with multicapillary column (MCC) and ion mobility spectrometry (IMS) for the determination of 2,4,6-trichloroanisole (2,4,6-TCA) in wines. The proposed methodology permits the direct analysis of the samples without any additional treatment other than dilution. This is achieved thanks to the selectivity provided by the ionic liquid selected as extractant, 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide, as well as the response of the analyte in the IMS working in negative ionization mode. Moreover, the multicapillary column avoids the interference of ethanol in the ion mobility spectra. The analysis of the sample takes ca. 35 min to be completed. The limit of detection was low as 0.01 ng L^{-1} using 2 mL of wine sample. Different calibration curves were constructed using aqueous standards, red and white wines, being the signals comparable, with a RSD similar to the method variability. Finally, a set of samples of different nature and packed in different containers were analysed. It was found than those with cork stoppers presented the highest concentration of 2,4,6-TCA.

1. Introduction

The wine industry considers aroma to be of great importance for product quality and consumer acceptance. The presence of haloanisoles is a great enological problem because of their extraordinary low sensory threshold and their determination along with their halophenols precursors is of great interest to the wine industry [1]. They are originated from a defensive reaction of some microorganisms through the biomethylation of their corresponding halophenols, which are present in wine due to the use of polluted materials in cellars. The main compound responsible for this defect is 2,4,6-trichloroanisole (2,4,6-TCA). The concentration considered as a defect in wine ranges from 10 to 40 ng L^{-1} [2], although the perception threshold for 2,4,6-TCA lies within 10 ng L^{-1} [3]. Therefore, the analytical methods for the determination of 2,4,6-TCA should include efficient preconcentration techniques to reach the low levels at which this compound can be present in the wine samples.

A variety of methods have been applied for the quantitative determination of 2,4,6-TCA, in wine samples: classical techniques, such as liquid–liquid extraction (LLE) [4], supercritical fluid extraction (SFE) [5], pressurized liquid extraction (PLE) [6], purge and trap (PT) [7], solid-phase extraction (SPE) [8], pervaporation (PV) [9], stir bar sorptive extraction (SBSE) [10] and, even to a greater extent, solid-phase microextraction (SPME) [11,12] and, more recently, single drop microextraction (SDME) [13] and, dispersive liquid–liquid microextraction (DLLME) [14].

The latest studies have been focused on the development of new extraction methods based on SPME because they are simpler than the traditional extraction techniques, eliminate the use of solvents, and reduce the sample preparation time. SPME methods have also been used for the analysis

of various components, off-flavours and contaminants in wine samples [15]. SDME has become a feasible alternative for sample pretreatment.

Gas chromatography (GC) is generally used for determining haloanisoles [16] in wines and in cork stoppers. In the specific case of chloroanisoles, a previous derivatization step is always recommended before GC to improve sensitivity and reduce peak tailing. Ion mobility spectrometry (IMS) is an analytical technique that characterizes molecules by their gas phase mobility [17]. This technique is suitable for determination of 2,4,6-TCA due to its sensitivity by the presence of chlorine atoms in the target analyte [18]. Ion mobility spectrometers are highly sensitive, comparatively inexpensive and allow fast responses since spectra are available in the milliseconds range [19]. These advantages make IMS devices suitable for the detection of volatiles substances in different fields. IMS suffers a limited selectivity, which has been previously overcome by separation of the analytes using conventional gas capillary chromatographic columns [20,21] or multicapillary columns [22,23].

Multicapillary columns (MCCs) have relatively small dimensions (50–300 mm in length and approximately 2–3 mm in external diameter) [24], which makes them a suitable pre-separation tool for portable purposes. Furthermore, these columns can work under isothermal separations conditions, avoiding the use of an oven and reducing the dimensions of the whole equipment required to perform the field measurements. In combination of an ion mobility spectrometer, the MCC is operated with relatively high flow rates because this leads directly to an increase in the sensitivity and selectivity in IMS [25].

The main goal of this article is to propose a simple, cheap, fast and sensitive method for the determination of 2,4,6-TCA in different wine samples by IMS using the combination of ionic liquid-single drop microextraction (IL-SDME) with a multicapillary column, to improve both the sensitivity and selectivity of the measurements. Thus, the method makes use of the good affinity of the ionic liquid 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide to the haloanisole which facilitate its preconcentration by means of headspace-single drop microextraction (HS-SDME). On the other hand the MCC reduces the interference of the ethanol present in wine samples on the ion mobility spectra.

2. Materials and methods

2.1. Reagents and samples

All reagents were of analytical grade or better. The ionic liquid 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide [Hmim][NTf_2] was supplied by Merck (Darmstadt, Germany). Methanol and sodium chloride from Panreac (Barcelona, Spain) as well as Milli-Q ultrapure water (Millipore, Madrid, Spain), were used in the extraction step.

Stock standard solutions of 100 mg L^{-1} of 2,4,6-trichloroanisole in methanol were obtained from Sigma–Aldrich. Working solutions were prepared at the appropriate concentration by dilution of the standard in methanol and stored in a refrigerated environment.

Wine samples were purchased from local markets in Córdoba. All samples were kept in their original containers and analysed within 2 days from purchasing.

2.2. Description of the IMS

2,4,6-Trichloroanisole was determined using a portable Ion Mobility Spectrometer fabricated by Gesellschaft für Analytische Sensorsysteme (G.A.S. mbH, Dortmund, Germany). The main components of the ion mobility spectrometer are an ionization chamber, a shutter-grid, a separation chamber, an ion collector and voltage generators. All parts of the IMS that are in contact with the analytes are constructed from inert material. The device is equipped with a Tritium ionization source (St. Petersburg, Russia) with an activity of 300 MBq. A sample inlet lets a continuous stream of nitrogen 5.0 (Carburos Metálicos, Sevilla, Spain) at 50 mL min^{-1} pass through the ionization chamber where ions are formed and focused to a shutter grid made of stainless steel. The shutter opening time was set at 100 µs, allowing the ions to pass at short pulses towards the separation chamber where parallels drift rings were connected by a series of resistors to create an electric field of 333 V cm^{-1}, a fixed voltage of 2 kV and a grid pulse of 100 ms. A high voltage supply is connected to the drift rings placed at equal distance to create the electric field in the drift tube. Isolators made from PEEK and resistors are placed between the drift rings. The drift tube has an inner diameter of 21 mm and 60 mm in length. A flow of nitrogen at 175 mL min^{-1} in the opposite direction of the ions drifting was employed as drift gas in order to prevent non-ionized impurities from entering the separation chamber. A gas outlet line makes the gaseous streams exit the instrument. All spectra were recorded in the negative ion mode. Data were acquired and analysed using GASpector software (Version v3.99.035 DSP).

2.3. Apparatus

HS-SDME was implemented by means of a 10-µL microsyringe with bevel tip (Agilent, Palo Alto, CA, USA), which was used to expose 2 µL of the ionic liquid ([Hmim][NTf_2]) in the headspace of the 10 mL extraction vial

(Supelco, Madrid, Spain) sealed with stainless steel capsules with PTFE/Silicone septa from Análisis Vínicos (Tomelloso, Spain). A Velp Cientifica magnetic stirrer (Milan, Italy) was also employed for the continuous agitation of the sample and was located in an oven to reach the required temperature for the extractions.

A multicapillary column OV-1701MCC, 14% – cyanopropylphenyl, 86% – dimethylpolysiloxane (Multichrom, Ltd., Novosibirsk, Russia) with a length of 20 cm, a volume of 0.45 mL, ca. 1200 capillaries with an inner diameter of 40 µm and a film thickness of 0.2 µm was also used. The total column diameter is 3 mm, which provides the column with a high sample capacity, allowing us to operate with a nitrogen carrier gas flow at its optimal value (50 mL min^{-1}). The column was hold at 30 ºC during the experiments and was connected to the IMS by means of a conventional stainless steel connector.

A stainless-steel injection unit designed in a previous work [26] was used for the coupling of the SDME extraction procedure and the IMS detector and to prevent the ionic liquid from reaching the IMS. No modification of the IMS inlet was necessary. The injection unit consisted of a 3-mm I.D., stainless steel (SS) tubing of 3 cm in length packed with cleaned glass wool (70 mg). It was provided in its extremes with 1/8-in. swagelok connectors to allow its easy removal for clean-up purposes. This tube was connected upstream with a union tee provided with a swagelok nut fitted with a poly-(dimethylsiloxane) septum, and through which a carrier gas can enter the injector. Downstream, a 1/16-in. tube (5 cm) allowed the connection of the unit to the IMS for direct analysis. The carrier gas, whose flow could be controlled by means of a millimeter valve, was connected to the injection unit using a SS 1/8-in. connector.

2.4. Analytical procedure

The configuration of the system constructed for the determination of the 2,4,6-TCA is depicted in Fig. 1. The extraction procedure was as follows: aliquots of 2 mL of the wine sample or standard solution containing 350 g L^{-1} sodium chloride and a 1 cm PTFE magnetic bar were placed in the 10 mL vial. Then, 6 mL of distilled water were added and the vial was tightly sealed with a silicone septum. The bevel tip syringe was filled with 2 µL of [Hmim][NTf_2], and further inserted in the vial through the septum until its needle tip was located about 1 cm above the surface of the stirred solution. The plunger was depressed and a microdrop of the acceptor phase was exposed on the headspace above the aqueous solution at 30 ºC for 30 min. Then, the ionic liquid was retracted and the syringe was moved to the injection unit maintained at 30 ºC in order to achieve complete volatilization of the target analyte. Once injected, the ionic liquid was retained in the glass wool while the carrier gas transferred the volatilized analyte into the multicapillary column also kept at 30 ºC. Finally, the analyte reached the IMS for detection and quantification.

The interchangeable part of the injection unit was substituted by a clean one every five injections (i.e. 10 µL of ionic liquid having been injected). Meanwhile, the replaced element can be easily cleaned by washing the tube with methanol and using a new glass wool piece. This removable characteristic of the proposed interface allows one to make a considerable number of measurements within a working day.

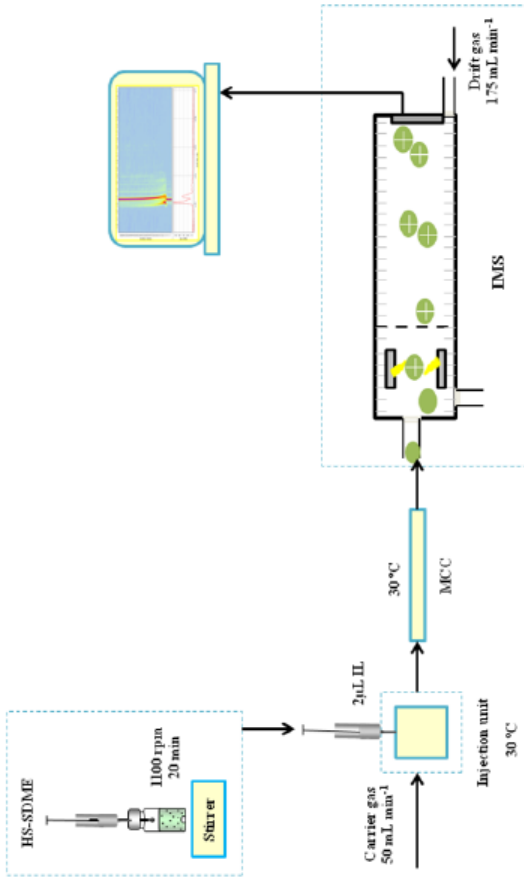

Figure 1. Schematic diagram of the configuration designed for the determination of the 2,4,6-TCA.

3. Results and discussion

3.1. Influence of ethanol on the extraction and detection

The concentration of ethanol is a crucial parameter in the determination of the 2,4,6-TCA because its percentage in wines is ca. 12%. In our procedure, the ethanol is a main interferent in the measurement of the 2,4,6-TCA by ion mobility spectrometry. To minimize the interference of ethanol, sample dilution [27] or calibration functions by matrix have been proposed. In this paper, we have take advantage of the compatibility between MCC and IMS to eliminate the negative influence of ethanol in the ion mobility spectra.

In addition, ethanol can also affect to the stability of the ionic liquid drop during the extraction. Therefore, a preliminary study was carried out in order to evaluate the compatibility of the proposed extraction procedure with the sample composition. The initial conditions were set as follows: 8 mL of an aqueous standard of 2,4,6-TCA at a concentration of 50 ng L^{-1}, ethanol 20% (v/v); extraction conditions: temperature 60 ºC; NaCl concentration 350 g L^{-1}; stirring rate 500 rpm; 20 min; instrumental conditions as indicated in experimental section. The percentage of ethanol in the aqueous standards was studied between 2 and 20% (v/v). It was observed that percentage over 5% dissolved the drop. Fig. 2 compares the ion mobility spectra obtained for 2,4,6-TCA with (5%) and without ethanol. As it can be seen, the signals were comparable which demonstrated the capability of the MCC to eliminate the spectral interference of ethanol. Under the conditions, the proposed coupling is fully compatible with the sample-analyte binomial selected being the sample dilution the sole requirement in order to adjust the evaluate content to 5%. Next, all the variables affecting the HS-IL-SDME were optimized to obtain the best analytical features for the determination of 2,4,6-TCA in wine samples.

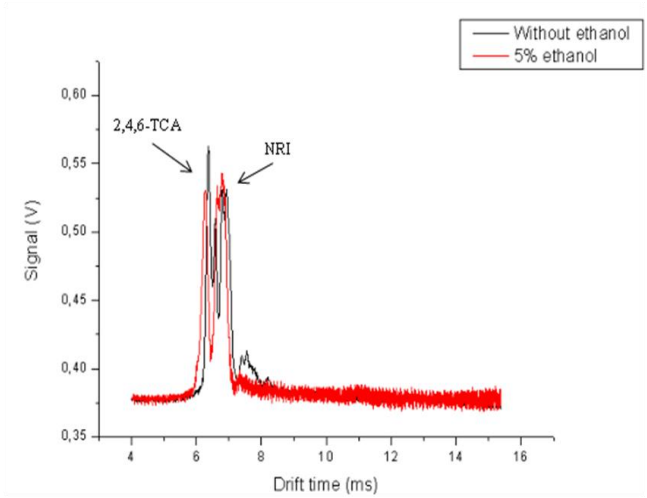

Figure 2. Ion mobility spectra of an aqueous standard of 2,4,6-TCA prepared at a concentration of 50 ng L^{-1} without ethanol and with 5% (v/v) of ethanol. NRI: negative reactant ions.

3.2. Optimization of the HS-IL-SDME parameters

The optimization of the extraction procedure involves several factors, including the type of ionic liquid and its volume; temperature, addition of salt, sample volume, stirring rate and extraction time. The study and optimization of these variables were performed by modifying one variable at a time while keeping the others unchanged. The HS-IL-SDME was optimized using standard solutions containing the target analyte at a concentration of 100 µg L^{-1}. The initial value for all the variables at the beginning of the optimization is indicated in Table 1.

Table 1. Optimization of the main variables affecting the headspace IL-SDME procedure.

Variable	Initial value	Interval	Optimum value
Temperature (ºC)	60	30-70	30
NaCl concentration (g L^{-1})	350	0-350	350
Sample volume (mL)	8	5-9	8
Stirring rate (rpm)	500	0-1100	1100
Extraction time (min)	20	10-40	30

3.2.1. IL, drop volume and desorption temperature

In recent years, the ionic liquids (ILs) have been introduced as novel extractants in miniaturized liquid extraction techniques. They have been especially useful in SDME. Although organic solvents have been commonly used as extractants in this technique, ILs have been proposed as an alternative because they offer high viscosity (which facilitates the formation of a larger-volume drop), low vapor pressure and good thermal stability, avoiding evaporation and irreproducible losses of the solvent in HS-SDME. Moreover, some of them are immiscible with water, leading to longer extraction times [28]. These properties make ILs, ideal extractants in the SDME procedure.

The ILs studied for the extraction of 2,4,6-TCA were 1-octyl-3-methylimidazoliumhexafluorophosphate [Omim-PF_6], 1-hexyl-3-methyl-imidazoliumhexafluorophosphate [Hmim-PF_6] and 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide [Hmim][NTf_2]. They were selected taking into account their affinity towards organic compounds. In addition, parameters such as hydrophobicity and viscosity were considered with the aim of avoiding the introduction in the detection system and the removal of the IL from the interface. However, the limiting factor was the interfering signals appearing in the IMS. Since [Hmim][NTf_2] was the IL that led to cleaner IMS spectra, its optimum volume was studied. The extractant volume was limited by the capacity of the injection unit and the viscosity of the ionic liquid. Studies using volumes of IL from 0.5 to 2 µL were accomplished. Results show that an increase in peak area occurs when the acceptor volume is also increased up to 2 µL. Therefore, the volume of IL selected was 2 µL for all the subsequent experiments.

The temperature in the injection unit was a parameter of study. When the desorption temperature was higher than 30 ºC some disturbances appeared,

which could be the result of the volatilization of impurities in the IL that generate an interfering signal in the IMS. Therefore, the injection temperature was fixed at 30 ºC.

3.2.2. Extraction temperature

Temperature also plays an important role in extraction processes, affecting the extraction of the analyte, the viscosity of the ionic liquid and, therefore, the mass-transfer process. Its effect on the extraction of 2,4,6-TCA was investigated from 30 to 70 ºC. As it is shown in Fig. 3A, analyte extraction decreases with increase of temperature. This could be attributed to the reduction of the partition coefficients of the analytes between the IL drop and the gaseous phase at high temperatures as the sorption process in the microdrop is exothermic. In addition, working at high temperatures increases the concentration of water molecules in the headspace, which could hinder the

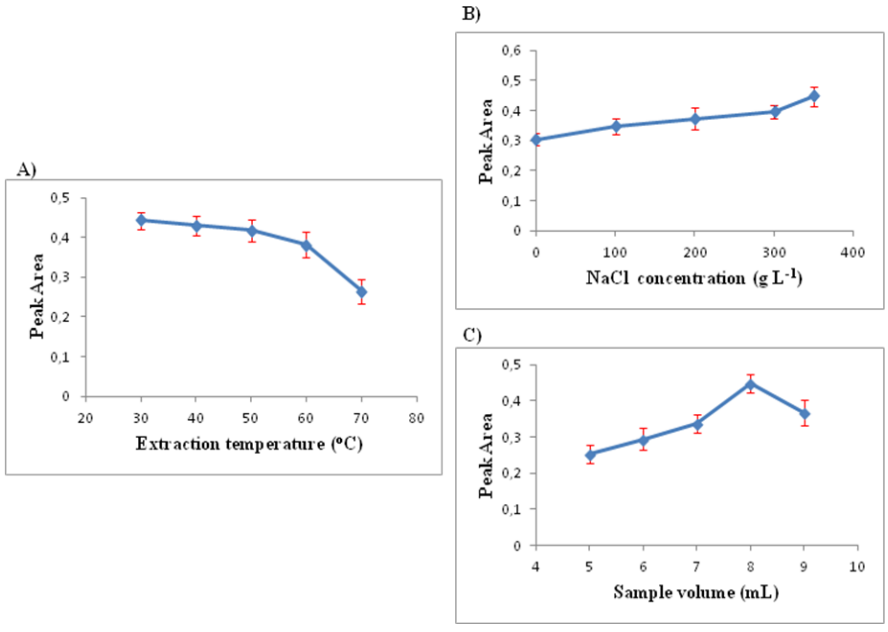

Figure 3. Influence of selected variables on the HS-IL-SDME procedure: (A) Extraction temperature; (B) NaCl concentration; (C) Sample volume.

analytes sorption in the micro-drop. Therefore, the working temperature selected for further studies was 30 ºC.

3.2.3. Addition of salt

The presence of salt in the sample/standard can affect the affinity of the analyte for the extractant since less water molecules are available for the solubilization of the analyte, which facilitates its transference towards the headspace. The salting-out effect was examined by monitoring the variation of peak areas with a salt concentration ranging from 0 g L^{-1} to 350 g L^{-1}, the maximum solubility of NaCl in water at the working temperature. According to Fig. 3B, the relative peak area of the analyte increases with NaCl concentration, achieving the best results at 350 g L^{-1}. This value was considered as optimum for further experiments.

3.2.4. Sample/headspace volumes

In HS-SDME, the minimization of the headspace volume would lead to higher sensitivities. These related variables were jointly studied by using 10 mL glass vials. The volume of sample was studied between 5 and 9 mL (Fig. 3C). Peak areas for 2,4,6-TCA increased significantly with increasing aqueous standard volume, reaching a maximum at 8 mL, whereas with 9 mL the signal decreases because there is not enough headspace to extract the analyte in the drop. Thus, a volume of 8 mL was adopted as optimum.

3.2.5. Stirring rate and extraction time

Agitation of the sample is assumed to reduce the time required to establish the partition equilibrium between the aqueous and the gaseous phases since the transfer coefficients of the analytes in the aqueous phase are enhanced. Besides, stirring the sample induces convection in the headspace, facilitating also the mass transference towards the micro-drop. The effect of

this variable on the extraction was studied by varying the stirring rate from 0 rpm to 1100 rpm using a magnetic stirrer. The maximum speed of the agitator offered the best results. Therefore, 1100 rpm was used to evaluate the influence of the extraction time on the 2,4,6-TCA signal.

The extraction time was evaluated between 10 and 45 min. Generally, longer extraction times lead to higher extraction efficiencies, as the amount of analytes that can be transferred to the microdrop is enhanced. In this case, an increase in the peak area of 2,4,6- TCA was observed up to 20 min, reaching a steady state over this value. Therefore, 30 min was chosen as optimum to minimize the variability.

3.3. Analytical performance

The calibration graph for 2,4,6-TCA was constructed using six working aqueous standards with 5% of ethanol prepared in Milli-Q ultrapure water and spiked with the analyte at concentrations between 0.01 and 500 ng L^{-1}. The aqueous standards were extracted as described above under the HS-IL-SDME optimized conditions and the 2 µL of the IL used for the extraction were then injected in the interface allowing the analyte to be transferred to the MCC-IMS coupling. In order to evaluate the absence of matrix effect, calibration curves for the 2,4,6-TCA in Milli-Q water, white and red wine were calculated. The calibration graphs were $y = 0.4804x^{0.1359}$ for the standard, with ethanol, $y = 0.4516x^{0.1336}$ for the red wine and, $y = 0.4548x^{0.1452}$ for the white wine. For comparative purposes, the variations on the peak area at a lower and higher concentration were calculated, being 4 and 6% for 500 ng L^{-1} and 0.1 ng L^{-1}, respectively. These values were acceptable taking into account the method variability. Moreover, a t-paired test was also applied to the data, which corroborates the statistic equivalence at a confidence level of 95%. This study demonstrated the absence of matrix effect and also the negligible

influence of the ethanol present in the samples under the optimized conditions of the HS-IL-SDME procedure.

The limit of detection (LOD) was calculated according to the IUPAC definition as the minimum concentration of 2,4,6-TCA that provides an analytical signal (peak area) statistically distinguishable from the blank. It resulted to be 0.01 ng L^{-1}. The limit of quantification (LOQ) was set as the minimum concentration of the linear range (0.05–25 ng L^{-1}) of the dynamic calibration interval, being 0.05 ng L^{-1}. The precision of the method was evaluated studying reproducibility and repeatability for the compound at 1 ng L^{-1} concentration level. For repeatability, five extractions were performed on the same day under optimum conditions. Reproducibility was assessed by performing extractions on three different days. The RSDs for both parameters were below 6%.

3.4. Application of the method to wine samples

Once the proposed method was optimized and evaluated, it was applied to determine the 2,4,6-TCA content in wines. Numerous samples of wine (red, table, white, sherry and sparkling) with different types of containers and stoppers were included in this study. The ethanol content of the investigated wines varied between 11.5 and 15% (v/v). Each determination was made in triplicate. The results obtained with their standard deviation are shown in Table 2. All the wines bottled in glass containers and cork stopper presented contamination with 2,4,6-TCA, independently of the type of wine. Normally, the wines in tetra brick and bag in box had the lowest concentration of 2,4,6-TCA. Among the studied wines, the sample 2 of sparkling wine presented the highest concentration of analyte.

Table 2. Results of the analysis of commercial wine samples by the proposed method (n=3).

	Sample	Container	Stopper	Concentration found (ng L^{-1}) ± SD[a]
Red wine	Sample 1	Glass	Cork	120±5
	Sample 2	Glass	Cork	94±4
	Sample 3	Glass	Cork	53±3
	Sample 4	Glass	Cork	84±3
	Sample 5	Glass	Cork	116±5
	Sample 6	Glass	Cork	19±1
	Sample 7	Tetra brick	Cork	n.d.
Table wine	Sample 1	Glass	Thread	n.d.
	Sample 2	Glass	Cork	102±3
White wine	Sample 1	Glass	Rubber	n.d.
	Sample 2	Glass	Cork	29±1
	Sample 3	Glass	Cork	44±2
	Sample 4	Tetra brick		n.d.
	Sample 5	Bag in box		n.d.
	Sample 6	Bag in box		23±1
	Sample 7	Bag in box		n.d
	Sample 8	Bag in box		n.d.
Sweet wine	Sample 1	Glass	Cork	89±3
	Sample 2	Tetra brick		9±1
Sparkling wine	Sample 1	Glass	Rubber	6±1
	Sample 2	Glass	Cork	150±6

[a] SD= standard deviation (n=3).
n.d.=non-detected.

By way of sample, Fig. 4 shows the topographic plot obtained after the extraction and instrumental determination of 2,4,6-TCA in red wine sample 5.

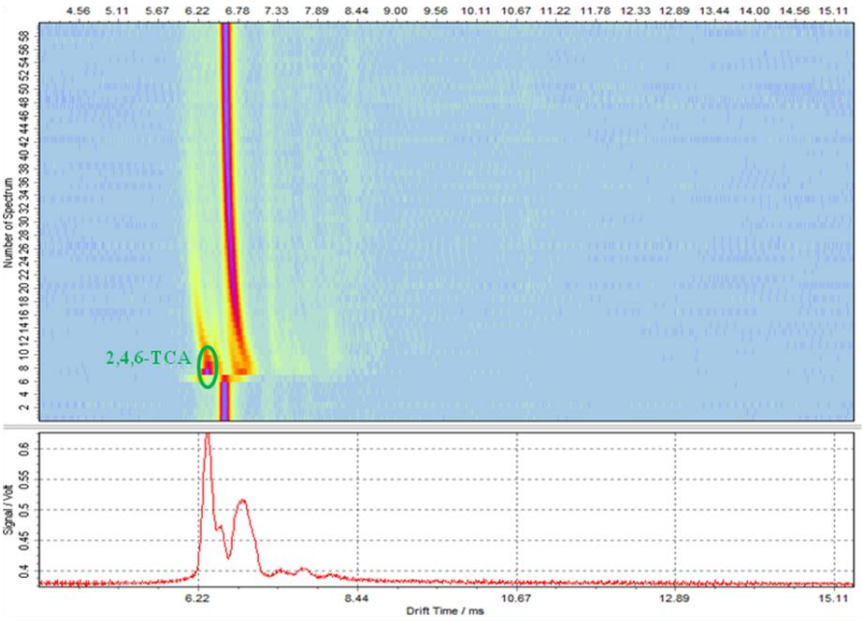

Figure 4. Topographic plot using a multicapillary column.

4. Conclusions

In this work a reliable, almost automatic method for determination of 2,4,6 TCA in different types of wines bottled in a variety of containers with a variety of stoppers has been developed, optimized, validated and applied to the analysis of commercial wine samples. The positive figures of merit of the proposed methodology are based on the different roles played by each of the modules of the instrumental hyphenation designed and used: headspace-single drop extraction-multicapillary column-ion mobility detection. The suitability of the ion mobility spectrometry working in the negative ionization mode to detect chlorine atoms allows reaching high levels of sensitivity. Moreover, the use of single drop microextraction contributes to achieve high preconcentration levels. On the other hand, the affinity of the imidazolium group towards the target analyte increases the selectivity while the

.multicapillary column located prior to the spectrometer reduces the interference from the matrix components, mainly ethanol. The configuration results in a clear simplification of the whole analytical process. The interface prevents the ionic liquid from entering the spectrometer, making compatible the extraction and detection. The characteristics of the method are compared with those provided by other methodologies for the determination of the same analyte in wines as it can be seen in Table 3. The method is the most sensitive, precise and rapid as regards the previously reported alternatives.

Table 3. Comparison of the proposed method with other methods developed for the determination of 2,4,6-TCA in wine samples.

Method[a]	LOD (ng L^{-1})	RSD (%)	Extraction time (min)	Reference
PT-GC-AED	5	< 13	---	[7]
SPE-LVI-GC-MS	0.2	< 6	10	[8]
SBSE-GC-MS-MS	0.03	13.3	60	[10]
SPME-GC-MS-MS	2.50	5.2	60	[11]
HS-SPME-GC-ECD	0.4	< 8	60	[12]
HS-SDME-GC-ECD	8.10	12.4	25	[13]
DLLME-GC-ECD	2.3	< 10	---	[14]
HS-SDME-MCC-IMS	0.01	< 6	30	This work

[a] AED: atomic emission detection; LVI: large volume injection; ECD: electron capture detector; for the other acronyms, see the text.

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Capítulo 8

Headspace-multicapillary column-ion mobility spectrometry for the direct analysis of 2,4,6-trichloroanisole in wine and cork samples

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Headspace-multicapillary column-ion mobility spectrometry for the direct analysis of 2,4,6-trichloroanisole in wine and cork samples

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ABSTRACT

Headspace–multicapillary column–ion mobility spectrometry coupling has been evaluated for the direct analysis of wine and cork stopper samples for the determination of 2,4,6-trichloroanisole (2,4,6-TCA). The instrumental configuration permits the sample to be introduced in headspace vials which are placed into the autosampler oven in order to facilitate the transference of the volatile compounds from the sample to its headspace. Further, an aliquot of 200 μL of the homogenized gaseous phase is injected into the multicapillary column in order to separate the target compounds from potential interferents. The detection of 2,4,6-TCA was carried out in an ion mobility spectrometer with a radioactive source and working under negative mode. All the system was computer controlled, including data acquisition and treatment. The limits of detection achieved were 0.012 ng L^{-1} for wine and 0.28 ng g^{-1} for the cork stopper. The procedure was applied to the analysis of commercial wine samples in different packages and 2,4,6-TCA was found in all of those closed with a cork stopper. The excellent recovery values obtained testify for the goodness of the method as no interference from the sample matrix exits.

1. Introduction

Cork has been used as closure for wine bottles since the 17th century because of its unique physical properties (flexibility, lightness, hydrophobicity, gas impermeability, chemical inertness and resistance to extreme heat). However during these more than 300 years, the incidence of moldy and musty off-flavors in wines sealed with cork stoppers has increased significantly. 2,4,6-Trichloroanisole (2,4,6-TCA) was first reported to be the primary compound responsible for cork taint in 1981 [1]. This compound can migrate from the cork stopper to the wine, changing its organoleptic properties and undermining its quality. In 1996, a study set up by the European Cork Confederation, investigated the causes and origins of taints in cork. It was found that 2,4,6-TCA was responsible for the cork taint in at least 80% of the cases when it was detected in bottled wines. Taking into account the estimated incidence of cork taint in wine bottles, which ranges from 0.5 to 7%, this implies large economic losses [2,3]. Several microorganisms such as moulds, yeasts or bacteria are involved in the formation of 2,4,6-TCA. There are also many other causes that explain the presence of 2,4,6-TCA in wine such as the use of fungicides, biocides, herbicides and wood preservatives [4]. Olfactory thresholds for 2,4,6-TCA vary from 4 ng L^{-1} to 10 ng L^{-1} in white wine and to up 40 ng L^{-1} in red wine; so, even at such low concentrations, its presence becomes a problem in wine quality [5]. It is therefore necessary to develop analytical methods with enough sensitivity to determine this compound in naturally tained cork and in wine samples.

Transmission of 2,4,6-TCA from cork stoppers to wine will depend on several factors, including the location of 2,4,6-TCA (on the surface of or within the stopper); the rates at which 2,4,6-TCA can migrate through the cork matrix and the volume of wine in contact with the closure, among others [6].

Several approaches have been proposed for the quantitative determination of haloanisoles in wines and cork stoppers. Traditionally, 2,4,6-TCA determination has relied on chromatographic techniques such as gas chromatography–mass spectrometry [7,8]. However, due to the low concentration level of 2,4,6-TCA, a preconcentration step is usually required. Pressurized fluid extraction [9], dispersive liquid–liquid microextraction [10], stir bar sorptive extraction [11], solid-phase extraction with different sorbents [12], and solid-phase microextraction [13], have been reported to solve this analytical problem. Our research group has proposed the determination of 2,4,6-TCA in water [14] and wine [15] samples by ionic liquid-based single-drop microextraction and ion mobility spectrometry (IMS). The limits of detection achieved were on the low nanogram per liter level, depending on the instrumental configuration used.

Ion mobility spectrometry is a modern analytical technique for the determination of volatile and semi-volatile organic compounds based on the separation of their gaseous ions in a weak electric field at ambient pressure. Ion mobility spectrometers generate selective and sensitive results in short analysis times [16]. Several IMS analyzers have been designed and developed, especially for explosives, drugs (particularly illicit drugs) and detection of chemical warfare agents [17]. Nowadays, a wide variety of fields, including environmental [18], pharmaceutical [19], food [20], medical diagnostics [21], process and industrial control [22], and proteomic [23], among others, have taken the advantages of IMS as detector. For some applications, the use of a multicapillary column (MCC) coupled to IMS would help to improve the selectivity of the analysis. Such is the case of the determination of trans-1,2-dichlorethene, trichloroethene and tetrachloroethene [24].

The main objective of the present study is the direct determination of 2,4,6-TCA in wine and cork stopper samples using the combination headspace–multicapillary column (HS–MCC) for improving IMS analysis. The sample is directly added to a headspace vial and it is processed without any additional treatment, improving the previous method developed in our research group [15]. The instrumental configuration allows a fast, sensitive and enhanced selective detection of the volatile organic compound detection in the headspace of solid (cork) and liquid (wine) samples.

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade or better. Methanol and ethanol from Panreac (Barcelona, Spain) as well as Milli-Q ultrapure water (Millipore, Madrid, Spain), were used in the optimization and calibration steps.

A stock standard solution of 100 mg L^{-1} of 2,4,6-trichloroanisole in methanol was obtained from Sigma–Aldrich (Madrid, Spain). Working solutions were prepared at the appropriate concentration by dilution of the stock in Milli-Q water and stored in a refrigerated environment.

Wine samples commercialized in different containers with cork, thread and rubber stoppers were purchased from local markets in Cordoba. All samples were kept in their original containers and analyzed within 2 days from purchasing. The cork stoppers were also analyzed following the proposed method. They were removed from the bottles, cut in small pieces (ca. 1 mm × 2 mm × 3 mm) and stored at 4 ºC until analysis to prevent potential losses of the volatile analyte. In addition, unused corks were obtained from a wine cellar. The cork stoppers were analyzed and their 2,4,6-TCA content was lower than the established value in the UNE 56930:2005 [25].

2.2. Apparatus

The standards and samples (wine and cork stopper) were placed in 20 (liquid samples) or 10 mL (solid samples) vials (Supelco, Madrid, Spain) sealed with stainless steel magnetic caps fitted with PTFE/Silicone septa from Análisis Vínicos (Tomelloso, Spain).

Analyses were performed on a FlavourSpec® fabricated by Gesellschaft für Analytische Sensorsysteme (G.A.S. mbH, Dortmund, Germany) which consists of three main elements: an automatic headspace injector; a multicapillary column; and an ion mobility spectrometer. The injection was carried out using a headspace autosampler fitted with a 1 mL gastight HS-syringe including a robotic arm and an oven for sample heating/headspace generation. The syringe was kept at 80 ºC. The multicapillary column (OV-1701MCC, 14% – cyanopropylphenyl, 86% – dimethylpolysiloxane) (Multichrom, Ltd., Novosibirsk, Russia) has a length of 20 cm, a volume of 0.45 mL, ca. 1200 capillaries with an inner diameter of 40 µm and a film thickness of 0.2 µm. The total column diameter is 3 mm, which provides the column with a high sample capacity. It was placed in a chromatographic oven which permits the temperature to be maintained at 60 ºC during separation. The detector was equipped with a Tritium ionization source (St. Petersburg, Russia) with an activity of 300 MBq. A sample inlet lets a continuous stream of nitrogen 5.0 (Carburos Metálicos, Sevilla, Spain) at 30 mL min^{-1} to pass through the ionization chamber where ions are formed and focused to a shutter grid. The ions pass at short pulses toward the separation chamber where parallel drift rings were connected by a series of resistors to create an electric field of 333 V cm^{-1}, a fixed voltage of 2 kV. The separated ions reach a Faraday plate and the signal is acquired by a computer.

A dedicated data acquisition and analysis software LAV version 1.5.17. from G.A.S. was also used. All spectra were recorded in the negative ion mode and the peak area was used as analytical signal.

2.3. Analytical procedure

Two different analytical procedures were designed for liquid (wines) or solid (cork stopper) samples. For the analysis of wine samples, an aliquot of 8 mL of the aqueous standard or the wine, containing the 2,4,6-TCA at concentrations within the linear range, was placed in a 20 mL vial. The sample was stirred in the oven of the autosampler at 500 rpm during 15 min at a temperature of 40 ºC. Then, 200 µL of sample headspace was automatically injected by the heated syringe (80 ºC) into the IMS equipment. After injection, the carrier gas (N_2, 30 mL min^{-1}) transferred the injected sample to the MCC for timely separation. Then, the analyte separated, from potential interferents, was driven into the ionization chamber of the ion mobility spectrometer prior to detection.

For the analysis of solid samples, 500 mg of cork cut in small pieces was placed in a 10 mL headspace glass vial and tightly sealed with the magnetic cap. Then, the previously described procedure was followed, but the oven temperature of the autosampler oven was 50 ºC.

3. Results and discussion

3.1. Optimization of the instrumental parameters

First of all, the operating conditions of the HS–MCC–IMS were optimized with a standard solution containing the analyte at a concentration of 50 ng L^{-1} and all the analyses were made by triplicate. The following variables were considered in this procedure, namely; drift gas and carrier gas flow rates, injection volume, grid pulse and system temperature. The influence of the drift

gas and carrier gas flow rates was examined over the range of 100–500 mL min^{-1} and 30–140 mL min^{-1}, respectively. A high drift gas flow rate (500 mL min^{-1}) was used to avoid contamination of the system with residual traces from previous runs and to reduce the flush time as a result. Increasing the carrier gas flow rate decreased the maximum peak intensity, probably due to dilution of the sample in the ionization chamber, 30 mL min^{-1} being chosen as more appropriate. The grid pulse has a direct influence on the shape, resolution and intensity of the peaks as this variable governs the time during which ion gates are open and allow ions to enter the drift region. A grid pulse width of 1000 µs resulted in higher signal intensity than obtained at 200 µs, at the expense of a lower resolution. Therefore, a pulse width of 500 µs was selected as optimal.

The study of the injected headspace volume was limited by the volume of the syringe (1 mL). This variable was studied in the interval 0.1–1 mL and the results obtained are shown in Fig. 1. As it can be seen, a maximum was obtained for an injected volume of 200 µL. In order to discriminate whether it can be an effect of the limited reactant ions formed or a negative influence of the volume of the plug introduced into the MCC, the same experiment was repeated at lower concentration, 5 ng L^{-1}. As it can be seen in Fig. 1, the same tendency was observed, so the decrease cannot be attributed to a potential saturation of the IMS. In order to obtain the highest sensitivity, a volume of 200 µL was selected for the subsequent experiments.

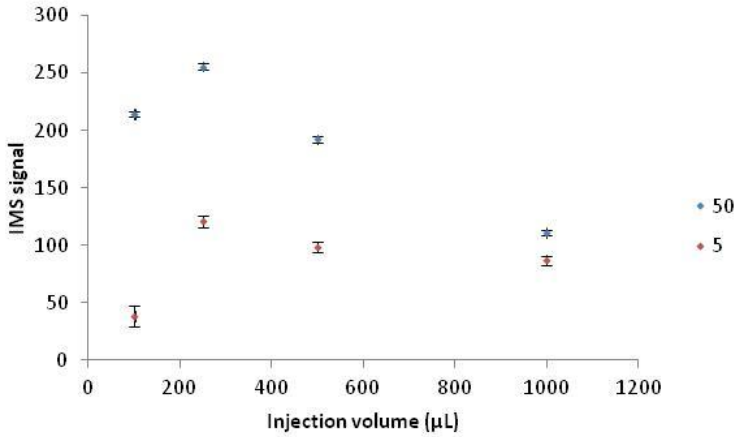

Figure 1. Influence of the injected headspace volume in the peak of 2,4,6-TCA in the HS-MCC-IMS. For details, see the text.

3.2. Optimization of the extraction conditions of 2,4,6-TCA from wine samples

Once the instrumental variables were optimized, the parameters affecting the extraction of 2,4,6-TCA from wine samples were studied. The optimization of the extraction procedure involves several factors, including sample volume, stirring rate, heating time and temperature and the concentration of ethanol taking into account its variable concentration in the wine samples. The extraction was optimized using standard solution containing the analyte at a concentration of 50 ng L^{-1}. Initial experimental conditions were as follows: aliquots of 10 mL of an aqueous standard containing ethanol 15% (v/v) were magnetically stirred at 500 rpm during 10 min at 50 ºC in the oven of the headspace autosampler. The study and optimization of these variables was performed by modifying one variable at a time while keeping the others at the more appropriate established value.

3.2.1 Extraction temperature and percentage of ethanol

Both variables can affect to the release of the 2,4,6-TCA to the headspace. The role of temperature in most of the extraction techniques is well established. On the other hand, the extraction of 2,4,6-TCA from the wine sample to the headspace can also be affected by the presence of ethanol. Therefore, in order to investigate the influence of both variables in the HS–MCC–IMS procedure, the influence of the percentage of ethanol was evaluated at two temperatures: 40 and 60 ºC. The results obtained are shown in Fig. 2A. As it can be seen, at 40 ºC, the signal increased with the percentage of ethanol up to 10%, remaining almost constant over this value. In contrast, a marked decrease in the 2,4,6-TCA signal was obtained with increasing percentages of ethanol when the temperature was set at 60 ºC. Taking into account the composition of the samples to be analyzed following this procedure, a percentage of 15% of ethanol (v/v) in the aqueous standards and an extraction temperature of 40 ºC were selected for further optimization.

3.2.2. Stirring rate and extraction time

Agitation of the sample is assumed to reduce the time required to establish the partition equilibrium between the aqueous and the gaseous phases. The effect of this variable on the extraction was studied by varying the stirring rate from 250 rpm to 750 rpm. An increase in the signal up to 500 rpm, was observed being constant over this value. However, an increase in the background noise was also observed at higher stirring rates. Therefore, 500 rpm was chosen to evaluate the extraction time between 5 and 30 min (Fig. 2B). In general, it can be said that longer extraction times lead to higher extraction efficiency. In this case, an increase in the peak area for 2,4,6-TCA was observed up to 15 min, decreasing over this value. For higher extraction time values the area peak decreased. Thus, an extraction time of 15 min was considered as more appropriate for further experiments.

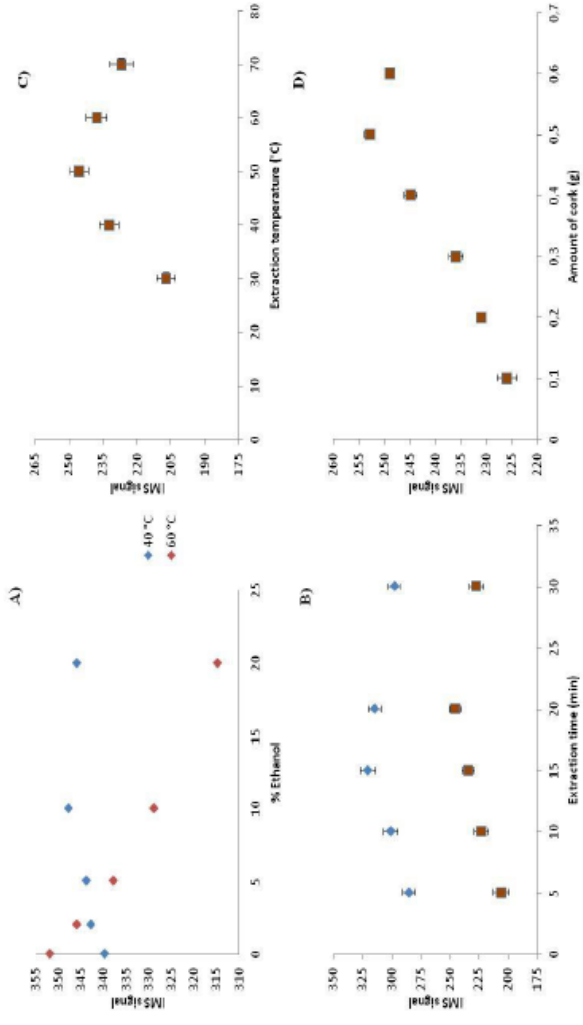

Figure 2. Effect of the percentage ethanol (A); extraction time (B); extraction temperature (C); and amount of cork (D) on the 2,4,6-TCA detection by HS-MCC-IMS in liquid and solid samples. For details, see the text.

3.2.3. Sample/headspace volumes ratio

The sample to headspace volumes ratio affects the sensitivity of the method since the headspace decreases with the increase of sample volume, thus increasing the concentration of the analyte in the headspace. Therefore, its influence was studied using 20 mL glass vials, the sample volume being increased from 2 mL to 10 mL. Peak areas of the 2,4,6-TCA increased with increased sample volume, reaching a maximum at 8 mL, whereas with 10 mL, the signal shows a slightly decrease because the headspace is not sufficient to produce the extraction of the analyte.

3.3. Optimization of the extraction process of 2,4,6-TCA from cork stoppers

Taking into account that the presence of 2,4,6-TCA in the wine samples is a direct consequence of its migration from the cork stoppers, this sample was also considered in this work. All the cork stoppers used in this optimization steps were blank samples with a certificate of analysis. The analysis of cork stoppers for haloanisoles usually involves a liquid extraction of the analytes they are grounded using a freezer mill to minimize the potential losses of volatile compound [9,10]. In order to facilitate the direct analysis of the cork sample in the headspace module, the cork stoppers were cut following two different approaches. In the first case, the stoppers were cut into slices of 3 mm in thickness (ca. 5 slices per stopper). Four slices selected from the section in contact with the wine were placed in 10 mL vials and spiked with 200 µL of a standard solution of 2,4,6-TCA prepared at a concentration of 50 ng mL^{-1} in methanol. The vial was hermetically sealed and stored at 4 ºC until analysis. In the second situation, a similar procedure was followed but in this case each slice was cut into small pieces (ca. 1 mm × 2 mm × 3 mm). In both cases, the vials were maintained at 4 ºC during 48 h to facilitate potential interaction with the sample matrix while preventing losses by volatilization of the analyte. The

samples were analyzed using the previously optimized instrumental parameters and a headspace oven temperature of 50 ºC. The signals obtained for both approaches were compared (Fig. 3). As it can be seen, the second approach was more efficient in releasing the 2,4,6-TCA to the headspace of the vial as the analytical signal was higher. Therefore, it was selected to study the temperature of the oven. This variable was evaluated in the interval 30–70 ºC (Fig. 2C). The analytical signal increased with the temperature up to 50 ºC, remaining almost constant over this value. However, it was also observed that the background noise also increased at higher temperatures and therefore 50 ºC was selected to study the influence of the extraction time. This variable was evaluated in the interval 0–30 min, a maximum being obtained for 20 min (Fig. 2B). The amount of cork stopper added to the vial was studied between 0.1 and 0.6 g (Fig. 2D). The IMS signals were very low for the lowest amount of sample. The best response was obtained for 0.5 g of stopper which corresponds to a sample to headspace ratio of 1:1 and therefore, it was selected as more appropriate sample amount.

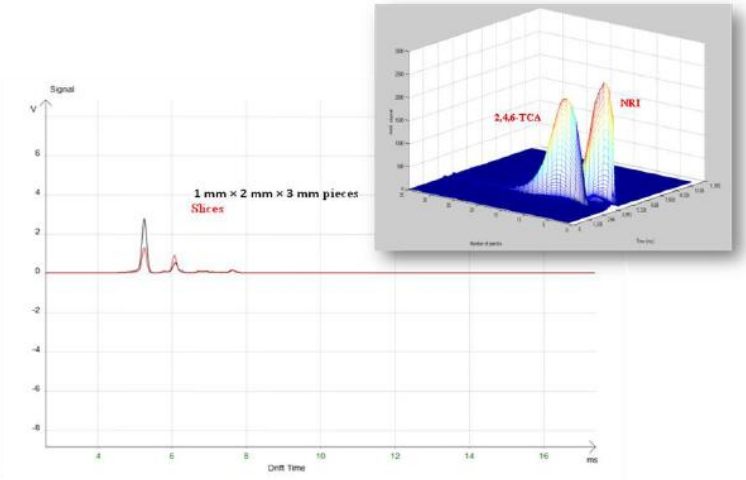

Figure 3. Comparison of the ion mobility spectra obtained of the cork stopper sample cut in slices and small pieces following the HS-MCC-IMS.

3.4. Analytical performance

The calibration graph for the determination of 2,4,6-TCA in wines was constructed by using seven aqueous standards with 15% (v/v) of ethanol containing the analyte at concentrations between 0.01 and 100 ng L^{-1}. The standards were subjected to the whole analytical procedure described in Section 2.3. Each point of the calibration curve was obtained from the analysis of three replicates. Regarding to the limit of detection (LOD) and the limit of quantification (LOQ), they were calculated as three times and ten times the standard deviation of the blank signal divided by the slope of the lower linear range of the calibration curve in the range 1–50 ng L^{-1}, respectively. The LOD was 0.012 ng L^{-1} while the LOQ was 0.04 ng L^{-1}. Concerning the precision it was calculated at two concentration levels under repeatability (intraday precision) and reproducibility (between days) conditions. The results, expressed as relative standard deviation resulted to be 3.4% (5 ng L^{-1}) and 2% (50 ng L^{-1}) (repeatability, n = 5) and 6.8% (5 ng L^{-1}) and 4% (50 ng L^{-1}) (reproducibility, 3 days, n = 15).

To construct the calibration graph for 2,4,6-TCA in cork samples, variable volumes of a standard solution of the analyte were added to a blank cork sample prepared as previously described to obtain a final concentration within the ng g^{-1} range. The LOD and LOQ values were calculated using the same criteria adopted for wine samples, and resulted to be 0.28 ng g^{-1} and 0.92 ng g^{-1}, respectively. The repeatability and reproducibility values were calculated spiking the cork stoppers with the 2,4,6-TCA at two concentration levels using 5 and 15 replicates respectively. The results obtained expressed as relative standard deviation were 5.3% (2 ng g^{-1}) and 5% (20 ng g^{-1}) (repeatability, n = 5) and 7.2% (2 ng g^{-1}) and 7% (20 ng g^{-1}) (reproducibility, 3 days, n = 15).

3.4.1. Analysis of wine samples

The proposed method was successfully applied to the analysis commercial wines. Red, white, table and sparkling wines were purchased in local markets in a variety of packages (glass, tetra brick and bag in box) with different stoppers (cork, rubber and thread). The results obtained are listed in Table 1. As it can be seen, 2,4,6-TCA was detected and quantified in all the samples bottled in glass containers and with cork stoppers. In addition, one sample with rubber stopper provided a positive response for 2,4,6-TCA. Since no reference materials were available to validate method, the analysis of enriched wine samples was used for validation purposes. Thus, one red and two white blank wine samples were selected to evaluate the potential interference of sample matrix in 2,4,6-TCA detection and quantification. The analyte was spiked at two concentration levels (5 and 50 ng L^{-1}) and the samples were analyzed by triplicate. The recoveries were calculated as the percentage ratio between the concentrations of analyte found, using the calibration curve, and the concentration added to each wine. The recovery results obtained were between 86 and 98% and with relative standard deviations, of ca. 8% (Table 2).

Table 1. Analysis of wine and cork samples by HS-MCC-IMS coupling.

	Sample	Container	Stopper	Concentration found ± SD
Wine				
Red wine	Sample 1	Glass	Cork	79 ± 2
	Sample 2	Glass	Cork	39 ± 1
	Sample 3	Glass	Cork	21 ± 1
	Sample 4	Glass	Cork	60 ± 1
	Sample 5	Glass	Cork	71 ± 2
	Sample 6	Glass	Cork	25 ± 0.4
	Sample 7	Tetra brick		n.d
	Sample 8	Glass	Cork	129 ± 4[a]
White wine	Sample 1	Glass	Rubber	n.d.
	Sample 2	Glass	Cork	44 ± 2
	Sample 3	Glass	Cork	59 ± 2
	Sample 4	Tetra brick		n.d.
	Sample 5	Bag in box		n.d.
	Sample 6	Bag in box		23 ± 1
	Sample 7	Bag in box		n.d.
	Sample 8	Bag in box		n.d.
Table wine	Sample 1	Glass	Thread	n.d.
	Sample 2	Glass	Cork	99 ± 4[a]
Sparkling wine	Sample 1	Glass	Rubber	15 ± 1
	Sample 2	Glass	Cork	117 ± 6[a]
Cork stopper				
Natural cork	Sample 1			n.d.
	Sample 2			n.d.
Commercial cork	Red wine (Sample 4)			2 ± 0.6
	Sample 1 White wine (Sample 3)			n.d.
				3 ± 0.2
	Sparkling wine (Sample 2)			12 ± 0.7

Concentration found in wine, ng L^{-1}; concentration found in cork stopper, ng g^{-1}; SD, standard deviation; n.d., not detected.

[a] Diluted sample (1:2, v/v)

3.4.2. Analysis of cork samples

Two natural cork stoppers and four cork stoppers of bottled wine samples in which 2,4,6-TCA was detected were analyzed. The results obtained are shown in Table 1. As expected, natural cork stoppers did not provide any signal for the analyte while in the stoppers of the wine samples, 2,4,6-TCA was quantified at concentrations between 2 and 12 ng g^{-1}. Next, a recovery study was carried out by spiking the analyte at two levels (2 and 20 ng g^{-1}). The recovery values are listed in Table 2 and the average value was 94% with a good standard deviation 6%, which demonstrated the applicability of the proposed approach to the identification and quantification of 2,4,6-TCA in cork stopper samples.

Table 2. Recovery of 2,4,6-TCA from wine and cork stopper samples.

Wine	Concentration added 50 ng L^{-1}	Concentration added 5 ng L^{-1}
Red wine (Sample 7)	91 ± 3	86 ± 8
White wine (Sample 4)	96 ± 1	94 ± 5
White wine (Sample 8)	98 ± 3	96 ± 4
Cork stopper	**Concentration added 20 ng g^{-1}**	**Concentration added 2 ng g^{-1}**
Natural cork (Sample 1)	94 ± 4	91 ± 6

Values are expressed as percentage ± standard deviation

4. Conclusions

The proposed HS–MCC–IMS method allows the determination of 2,4,6-TCA in wines and cork stoppers without any sample treatment other than a simple cutting of the stoppers. The procedure is fully automated as all the modules (headspace, multicapillary column and ion mobility spectrometer) are hyphenated in a unique device. All the conditions can be easily programmed and the signal processing is also carried out using dedicated software. The optimized procedure is very similar for both matrices and the limits of

detection are in the very low nanogram per liter (or gram) level, which allows the detection of the analyte at concentrations below the perception threshold. No interference from other wine matrix components was detected, being the negligible interference of ethanol the most remarkable. The instrumental configuration used in this article has clearly simplified the analytical procedure needed for the determination of 2,4,6-TCA in wine/cork samples using IMS. In previous articles published by our research group, the use of SDME with ionic liquids was necessary for the extraction and preconcentration of 2,4,6-TCA from wine samples. The presence of interferences required a solid phase extraction with LiChrolut [14] or the coupling with a multicapillary column [15] for the separation of the volatile interferences. Moreover, the direct application to solid samples is also remarkable. Also, gas chromatographic–mass spectrometric procedures reported up to date for the determination of 2,4,6-trichloroanisole, require preconcentration from the sample and further separation from other volatile interferences. González-Peñas et al. used a fiber of polydimethylsiloxane–divinylbenzene for the extraction of the analyte because it is simpler than the traditional extraction techniques, eliminating the use of solvents, and reducing the sample preparation time [13]. In other cases, it is necessary the derivatization of the 2,4,6-TCA [20]. Compared to these methodologies, the proposed HS–MCC–IMS approach is more selective on account of the negative ionization mode selected and the multicapillary column, simpler as no sample treatment is required, fully automated as once the sample is added to the headspace vial, the instrument can work unattended. Moreover, the acquisition cost and maintenance of the HS–MCC–IMS coupling is lower than GC–MS approach.

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Capítulo 9

On-line headspace-multicapillary column-ion mobility spectrometry hyphenation as a tool for efficient determination of off flavours in foods

On-line headspace-multicapillary column-ion mobility spectrometry hyphenation as tool for efficient determination of off flavours in foods

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ABSTRACT

In this work, an ion mobility spectrometer (IMS) with a tritium ionization source on-line coupled to a headspace (HS) autosampler and a multicapillary column (MCC) was evaluated for the monitoring of the degradation products of long chain omega-3 acids in milk with different flavours (cacao, fruits, cereals and nuts) and linseed oil samples. In this combination, the multicapillary column is used as an interface between the HS and the IMS, providing the individual separation of the volatile compounds. The evolution of volatile profile (from 0 to 36 days) under different storage conditions (temperature, oxygen and light) demonstrates the capability of the HS-MCC-IMS coupling for the estimation of the degradation of the samples. The proposed method permits the detection of hexanal, 2-butanone, acetone and dimethyl disulfide as degradation products of the omega-3 acids. The limits of detection were in the interval 0.3 μg L^{-1} (for hexanal in milk) to 3.0 μg L^{-1} (for dimethyl disulfide in linseed oil) while the limits of quantification varied between 1.1 μg L^{-1} (for hexanal in milk) and 9.6 μg L^{-1} (for dimethyl disulfide in linseed oil). The precision of the method was evaluated as relative standard deviation and, the values were lower than 8% in all cases. After the degradation study, it can be concluded that the stability of the milk samples during storage is more affected by the light while temperature was more critical for oil samples.

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1. Introduction

During the last 10 years, there has been an increasing industrial interest in incorporating long chain omega-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in foods and dietary supplements. This is owing to the fact that there is a growing body of evidence that these omega-3 have a number of health beneficial effects [1,2]. A major challenge in relation to the use of omega-3 acids in food applications is their susceptibility to lipid oxidation, which will give rise to the formation of undesirable fishy off-flavours and odors that make the food unpalatable. The factors affecting lipid oxidation of omega-3 enriched foods are mainly oxygen, temperature, light and metal ions present as contaminants (Fe, Cu). The off-flavours formed from omega-3 oxidation are particularly unpleasant. Furthermore, the human sensory apparatus have a low threshold for volatile off-flavours resulting from oxidation of omega-3 acids [3]. The lipid oxidation events may take place in all the different foods enriched with omega-3 such as mayonnaise, yogurts, spreads, oil, milk, seafood, etc. It is well documented that the presence of volatile ketones, alcohols and aldehydes, produced by the decomposition of peroxides are responsible for sensory degradation, mainly in oil fish [4].

Many recent studies have investigated the oxidative and sensory stability of omega-3 fatty acids enriched products [5-11]. M. Gomes Reis et al. present a rapid characterization of milk fat without the need for fat extraction and use direct transesterification by thermochemolysis-gas chromatography/ mass spectrometry (MS) [12]. On the other hand, electrospray ionization tandem MS has been used to identify and quantify triacylglycerols in camel milk [13]. Chromatography methods have been developed for the determination of PUFAs in fish oil dietary supplements and seafood [14-15].

Ion mobility spectrometry (IMS) is an analytical technique with a wide applicability [16-27]. This technique is characterized by the simplicity of its analytical information, high sensitivity, comparatively inexpensive and allows fast responses since spectra are available in the milliseconds range. The functional principle of IMS has been previously described [16,17]. Generally, the IMS can be used in different fields such as detection of explosives and chemical warfare agents, illegal drugs [18,19], control food [20], environmental contaminants [21] or volatile compounds in human exhaled air [22] and others [23]. However, for the analysis of individual substances in complex samples, IMS selectivity is very limited and a pre-separation of the analytes is required. Recently, a multicapillary column (MCC) has been coupled to IMS, allowing the detection of volatile organic compound in complex matrices. This coupling combines the high selectivity of chromatographic separation with the good sensitivity of IMS. A MCC has small dimensions (50-300 mm in length, 2-3 mm in external diameter, 1000 capillaries 20/100 μm in inner diameter), can work under isothermal conditions and can also eliminate the negative effect of the water vapor molecules in the IMS signal [24-27].

In this research, we evaluate the potential of the triple hyphenation headspace, multicapillary column and ion mobility spectrometry as an alternative analytical technique to obtain a chemical profile of volatiles with a view to knowing the lipid degradation produced in food samples enriched with long chain omega-3 polyunsaturated acids. Also, the identification and quantification of the main secondary products generated by the oxidation is achieved. This research will contribute to expand the applicability of IMS in the food analysis, mainly focused on the quality control.

2. Experimental

2.1. Reagents and standards

All reagents were of analytical grade or better. The analytes, 2-butanone, acetone and dimethyl disulfide, were obtained from Sigma-Aldrich (Munich, Germany) and hexanal was purchased from Fluka (Munich, Germany). Methanol from Sigma-Aldrich and Milli-Q ultrapure water (Millipore, Darmstadt, Germany) were also employed in the development of the analytical method.

Stock standard solutions of each analyte were prepared in methanol, at a concentration of 10 mg L^{-1} and stored in dark at 4 ºC until analysis.

Working standard solutions used for calibration were obtained by dilution of the stocks in Milli-Q water. In addition, working standard solutions prepared in linseed oil and milk samples were used in the recovery study.

2.2. Apparatus

Analyses were performed on a FlavourSpec® fabricated by Gesellschaft für Analytische Sensorsysteme (G.A.S. mbH, Dortmund, Germany). The instrument was equipped with a heated splitless injector, which enabled direct sampling of the headspace and was coupled to an automatic sampler unit (CTC-PAL, CTC Analystics AG, Zwingen, Switzerland). The sample headspace was injected with a 2.5 mL gastight HS-syringe. The analytes were separated on a multicapillary column OV-1701MCC, 14% - cyanopropylphenyl, 86% - dimethylpolysiloxane (Multichrom, Ltd., Novosibirsk, Russia). It has a length of 20 cm, a volume of 0.45 mL, ca. 1200 capillaries with an inner diameter of 40 µm and a film thickness of 0.2 µm. The total column diameter is 3 mm, which provides the column with a high sample capacity. It was placed in a chromatographic oven in order to maintain

the temperature constant at 40 ºC during analytes separation. The detector was equipped with a Tritium ionization source with an activity of 300 MBq. A sample inlet lets a continuous stream of nitrogen 5.0 (Air Liquide, Düsseldorf, Germany) at 30 mL min^{-1} to pass through the ionization chamber where ions are formed and focused to a shutter grid. A flow of nitrogen at 500 mL min-1 in the opposite direction of the ions drifting was employed as drift gas in order to prevent non-ionized impurities from entering the separation chamber.The ions pass at short pulses towards the separation chamber where parallel drift rings were connected by a series of resistors to create an electric field of 333 V/cm, a fixed voltage of 2 kV. The separated ions reach a Faraday plate and the signal is acquired by a computer. The instrument was operated at ambient pressure.

A dedicated data acquisition and analysis software LAV version 1.5.17. developed by G.A.S. was used. All spectra were recorded in the positive ion mode. Peak area and separation time were used as analytical signals.

2.3. Samples

Milk, flavoured milk (cacao, fruits, cereals and nuts) enriched with omega-3 acids and linseed oil samples were purchased at local supermarkets. The samples were kept in 20 mL transparent glass vials with a headspace of ca. 15 mL. Then, the vials were sealed with PTFE/silicone septa and stored during 36 days under different conditions of temperature, oxygen and light (Table 1). Control samples were stored at 4 ºC in the dark without headspace until analysis.

The samples were analyzed after 1, 4, 8, 15, 22, 29 and 36 days of storage to study the evolution of the volatile profile of each sample.

Table 1. Storage conditions used during 36 days for every type of sample.

Storage conditions	Temperature (ºC)	Oxygen	Light
Control sample	4		
1	4	✓	
2	23	✓	
3	23	✓	✓
4	40	✓	

2.4. Procedure

For analysis, a volume of 5 mL of sample was added to a 20 mL glass vial, hermetically sealed with PTFE/silicone septum and placed in the autosampler. After 15 min of incubation at 40 ºC, 100 µL of the headspace was automatically injected by means of a 2.5 mL syringe (80 ºC) into the injector (80 ºC) of the IMS. After injection, the carrier gas passing through the injector transferred the sample to the MCC (40 ºC) for timely separation. Then, the analytes were eluted and driven into the ionization chamber for ionization prior to detection by the ion mobility spectrometer (60 ºC). The degradation products were identified by comparison of the retention times with those provided by the standards obtained under the same instrumental conditions and quantified by the peak area.

3. Results and discussion

IMS is an analytical technique capable to separately detect gaseous compounds in a mixture of analytes. The separation is based on the specific

drift times, as the ionized compounds need to pass a fixed distance (drift tube) under a defined electric field. Compared to other techniques e.g. TOF-MS, ions travel at atmospheric pressure versus a flow of inert gas. The drift time of each substance is determined by its ion's mass and geometric structure, as slowing collisions with the drift gas molecules are more frequent for sterically demanding structures [16]. IMS is a vanguard analytical technique that provides analytical information in a simple, rapid and, inexpensive manner. The IMS can be used to obtain a chemical profile of volatiles with a view to characterizing and classifying samples [28]. In this paper, we evaluate the potential of the technique to detect degradation products of omega-3 acids in diary products and linseed oil.

IMS is clearly improved if it is hyphenated with a headspace module for automatic sample conditioning and a multicapillary column to separate analytes before the detection step.

3.1. Selection of the HS-MCC-IMS conditions

The gaseous injection volume of 100 µL was chosen and the injector temperature was set at 80 ºC. The flow rates of the drift and carrier gas were fixed at 500 and 30 mL min^{-1}, respectively. An incubation temperature of 40 ºC during 15 min was enough to release the volatile fraction from the sample to the headspace of the vial. The chromatographic separation was carried out at 40 ºC while the separation of the ions in the drift tube occurred at 60 ºC.

By way of example, Fig. 1 shows the topographic plot for the identification of the IMS signals obtained for the volatile compounds (hexanal, 2-butanone, acetone and dimethyl disulfide). Each analyte was individually injected into the spectrometer working under the optimal instrumental

conditions. The aldehydes and ketones present the typical monomer-dimer ion behaviour in the IMS signal.

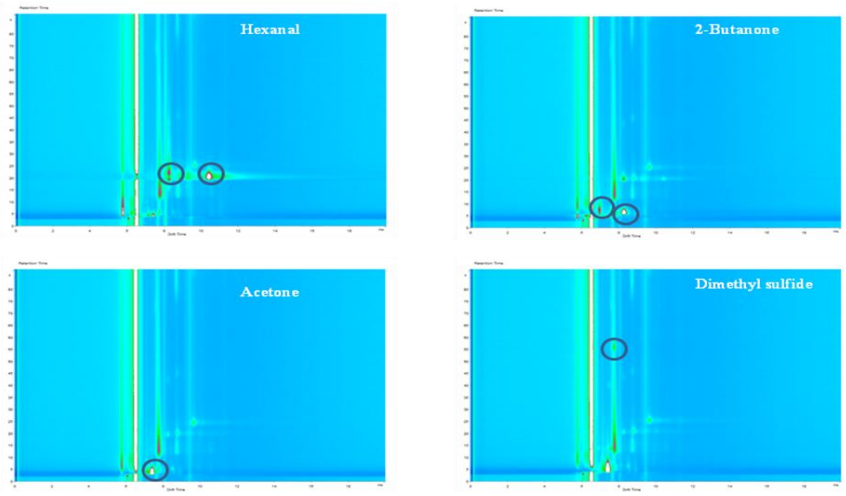

Figure 1. Topographic plot of the identification of the IMS signal with the volatile compounds studied.

3.2. Analytical features of the individual analytes

It is stated in the literature that hexanal, dimethyl disulfide, 2-butanone and acetone are the most representative compounds of the volatile fraction in milk and oil samples [29]. Therefore, in this study they were selected as targets to evaluate the inference of the storage conditions on the quality of both commodities. Prior to the analysis of commercial milk and oil samples, the method was analytically characterized in terms of sensitivity, linearity and precision. For this purpose, nine aqueous standards containing the target compounds were prepared at concentrations between 0.3 and 200 µg L^{-1} and processed following the optimized method. Each pair of data of the calibration curve were obtained as the average value of three replicates.

The linear range obtained for each analyte is listed in Table 2. Concerning the limit of detection (LOD) and limit of quantification (LOQ),

they were calculated as three and ten times the standard deviation of the blank signal divided by the slope of the linear range (between LOQ-50 µg L^{-1}). As it can be seen in Table 2, LODs were in the interval 0.3 µg L^{-1} (for hexanal in milk) to 3.0 µg L^{-1} (for dimethyl disulfide in linseed oil) while the LOQs varied between 1.1 µg L^{-1} (for hexanal in milk) and 9.6 µg L^{-1} (for dimethyl disulfide in linseed oil). The precision of the method was evaluated under repeatability (intraday precision) and reproducibility (interday precision) conditions at a concentration of 25 µg L^{-1} for each compound. The results, expressed as relative standard deviation ranged from 2.4 to 6.7% (repeatability, n=5) and from 2.7 to 7.2% (reproducibility, three days, n=15) in both samples. The values were lower than 7% in all cases.

Table 2. Figures of merit of the proposed method.

	Milk			Linseed oil		
	Hexanal	2-Butanone	Dimethyl sulfide	Hexanal	2-Butanone	Dimethyl sulfide
LOD[a] (µg L^{-1})	0.3	0.7	2.1	0.5	1.5	3.0
LOQ[b] (µg L^{-1})	1.1	2.4	7.0	1.8	4.7	9.6
Intraday precision (RSD[c]%, n=5)	2.7	2.8	6.7	2.4	3.1	5.9
Interday precision (RSD[c]%, n=15)	2.9	3.4	7.0	2.7	3.1	6.5

[a] LOD, limit of detection
[b] LOQ, limit of quantification
[c] RSD, relative standard deviation

In order to evaluate the usefulness of the proposed method, different commercial flavoured milk samples (cacao, fruits, cereals and nuts), omega-3 enriched milk and linseed oil samples were analyzed following the optimized procedure. The dimethyl disulfide was not detected in the samples analysed and the acetone was detected but not quantified because its concentration was

too high and a saturated signal was obtained as a result. In coherence with this data, no reference to acetone has been included in Table 2. The results obtained for hexanal and 2-butanone in the sample analyzed are shown in Table 3.

Table 3. Determination of volatile compounds in samples.

Samples		Concentration found (µg L^{-1}) ± SD[a] (%)	
		Hexanal	2-butanone
Milk with omega-3	Without aroma 1	3.4 ± 0.1	11.2 ± 0.5
	Without aroma 2	2.0 ± 0.1	9.0 ± 0.3
	Without aroma 3	2.0 ± 0.3	13.4 ± 0.4
	Cacao	6.0 ± 0.5	5.7 ± 0.1
	Fruits	9.0 ± 0.2	2.7 ± 0.2
	Cereals	n.d.	3.1 ± 0.3
	Nuts	n.d.	4.2 ± 1.0
Linseed oil	Sample 1	10.3 ± 0.4	25.0 ± 1.0
	Sample 2	14.0 ± 0.5	31.3 ± 1.0

[a] SD, standard deviation
n.d. not detected

Finally, the recoveries for each analyte were evaluated using the optimized HS-MCC-IMS method by spiking the samples listed in Table 3 with 10 µg L^{-1} of each analyte. The final concentrations were obtained using the calibration curves and the relative percentages of recuperation (initial + spiked) were calculated as the ratio between the concentration of each analyte in the sample and the concentration found in the spiked samples. As it can be seen in Table 4, the recovery values varied between 82 and 100%.

3.3. Evaluation of the storage conditions on the volatiles profile of the samples

The main aim of the proposed method was to evaluate the presence of degradation products of the long chain omega-3 acids in dairy products and edible oil samples. In this context, the potential influence of the storage

conditions on the modification of the volatile fraction would be interesting as these variations can also negatively affects to the sensory characteristics of the

Table 4. Recoveries of degradation products from different samples (n=3). 10 $\mu g\ L^{-1}$ of each analyte were spiked to the samples.

		Hexanal		2-butanone		Dimethyl sulfide	
		Found ± SD[a]	Recovery (%)	Found ± SD[a]	Recovery (%)	Found ± SD[a]	Recovery (%)
Milk with omega-3	Without aroma 1	11.8 ± 0.4	88 ± 2	20.1 ± 1.0	95 ± 3	8.9 ± 0.3	89 ± 6
	Without aroma 2	10.8 ± 0.3	90 ±3	18.2 ± 1.0	96 ± 2	9.2 ± 0.4	92 ± 4
	Without aroma 3	10.2 ± 0.5	85 ±2	23.2 ± 2.1	99 ± 3	9.1 ± 0.4	90 ± 5
	Cacao	13.1 ± 0.5	82 ± 1	15.2 ± 0.3	97 ± 3	9.3 ± 0.5	93 ± 7
	Fruits	17.9 ± 1.0	94 ± 4	12.7 ± 0.5	100 ± 4	9.1 ± 0.2	91 ± 5
	Cereals	9.4 ± 0.3	94 ± 6	13.1 ± 0.4	100 ± 4	9.4 ± 0.5	94 ± 9
	Nuts	9.2 ± 0.2	92 ± 1	13.9 ± 0.4	98 ± 5	8.5 ± 0.2	85 ± 6
Linseed oil	Sample 1	18.7 ± 1.0	92 ± 1	33.6 ± 2.3	96 ± 3	9.1 ± 0.5	91 ± 6
	Sample 2	22.8 ± 1.0	95 ± 1	39.6 ± 2.2	96 ± 3	9.5 ± 0.5	95 ± 7

[a] SD, standard deviation

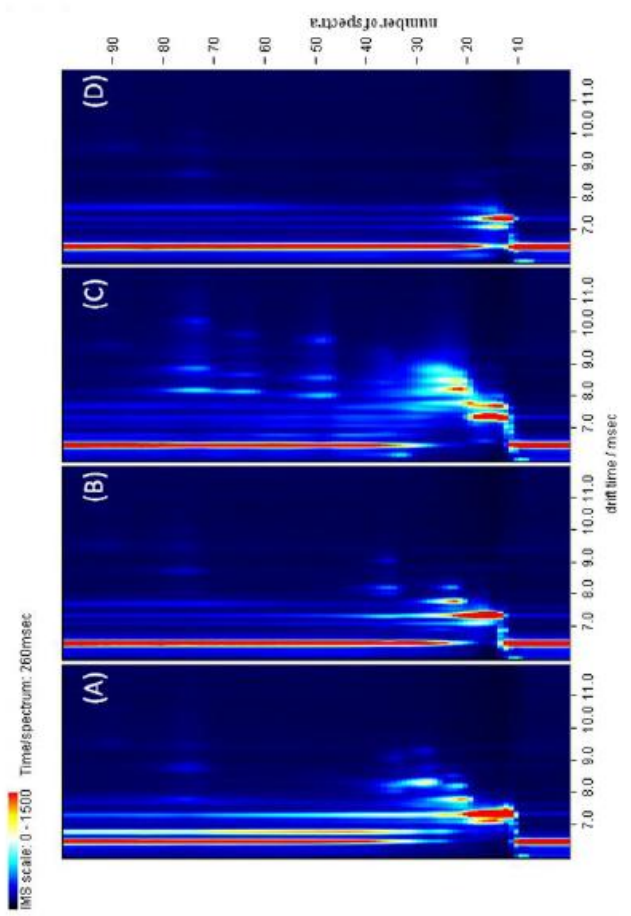

Figure 2. Topographic plot of HS-MCC-IMS spectra. (A) cacao milk with omega-3, (B) milk with omega-3, (C) linseed oil and (D) milk.

foodstuff thus influencing the quality perception of the consumers. In this study, raw milk, raw milk enriched with omega-3, cacao milk with omega-3 and linseed oil were selected as representative samples. There different environmental storage conditions were included in the study: oxygen, temperature and light. They were studied either individually or simultaneously for a total period of 36 days. Table 1 summarized the parameters take into account in this study. The headspace of each vial was analyzed after 1, 4, 8, 15, 22, 29 and 36 days and, in order to evaluate the reproducibility of the conditions, three samples were prepared and analyzed with the same frequency. Six analytical signals (drift times 7.325, 7.688, 8.812, 8.825, 9.171 and 10.754 ms) were considered as reference for the variation of the volatile profile of the sample.

Fig. 2 shows a typical spectra collected at 150 kHz for a cacao milk with omega-3 (Fig. 2A), milk enriched with omega-3 (Fig. 2B), linseed oil (Fig. 2C) and a whole milk (Fig. 2D). Each sample was analyzed directly after purchasing. This was defined as reference for the later data interpretation. From this figure, it can be already observed the different profile of the volatile fraction of the samples, which is indicative of the capability of the instrumental configuration to differentiate among them.

The evolution of the volatile profile of the samples as consequence of the generation of different degradation products of the omega-3 acids during the study is depicted in Fig. 3. Negligible modification of the volatile profile was observed for the raw milk sample, therefore, no interference from other matrix components exists and the variation on the volatile profile can be exclusively attributed to the degradation of the omega-3 compounds.

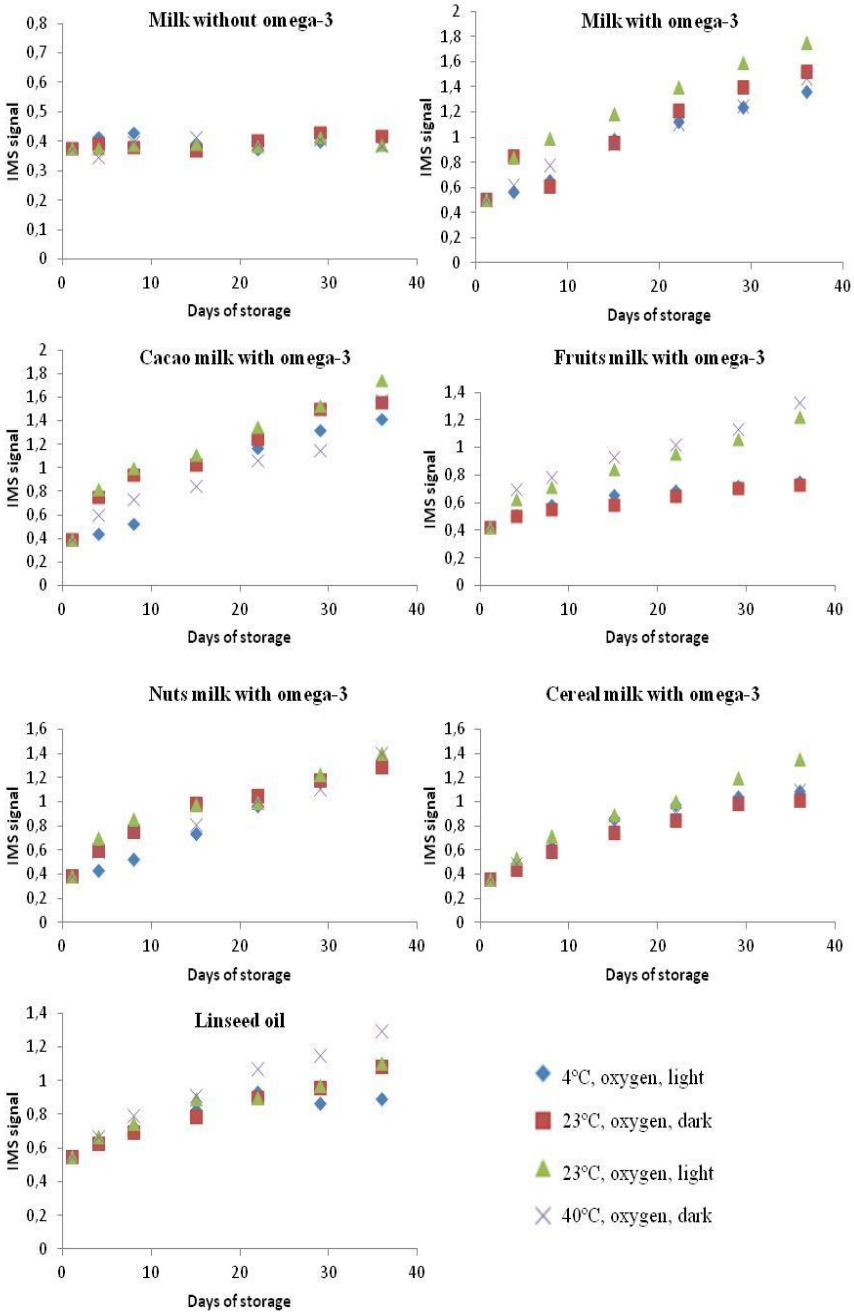

Figure 3. Evolution of the IMS signal for the samples stored under different conditions and time, using the proposed vanguard technique.

By way of example, Fig. 4 shows the chromatograms corresponding to a cacao milk sample. The comparison of the chromatograms demonstrated the increasing prensence of the hexanal during 36 days under conditions (23 ºC, oxygen and light). During this study, it was observed that the milk samples were affected by the presence of the fungi of different families depending of the storage conditions regardless the absence/presence of light. However, taking in consideration the reproducibility of the data, it has no influence on the volatile composition of the samples. From the comparison of the samples analyzed, it can be concluded that the temperature is the most critical variable for the oil sample while the presence of light is more important for the milk samples as the degradation is higher under this conditions with a lower influence of the temperature. For those milk samples with a lower omega-3 content, temperature is also an important variable (fruits milk and cereals milk with omega-3) being the degradation tendency rather similar. The nut milk sample showed stability similar to that of the cacao milk. Fig. 5 represents the influence of condition 3 (23 ºC, oxygen and light) for all the milk samples included in this study which remarks the results commented before.

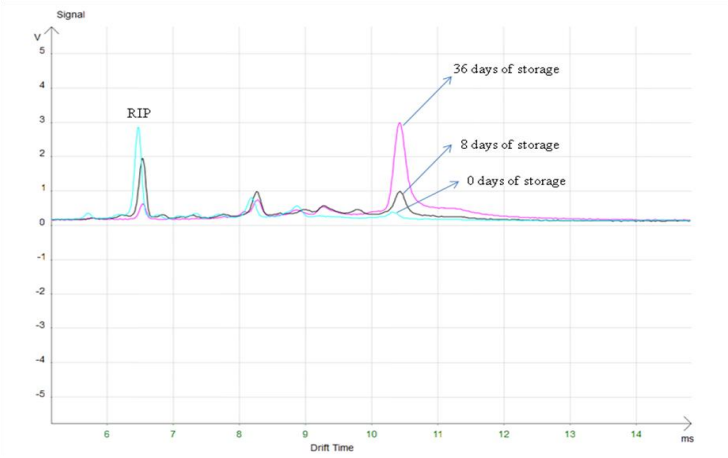

Figure 4. Comparison of the hexanal amount with the chromatograms obtained in the analysis of cacao milk sample during 36 days in the storage conditions of 23 ºC, oxygen and light. RIP: reactant ion peak.

Table 5. Recoveries of degradation products from different samples stored during 20 days (n=3). 10 µg L^{-1} of each analyte were spiked to the samples at the beginning of the study.

		Hexanal			2-butanone			Dimethyl sulfide		
		Found[1] ± SD[a]	Found[2] ± SD[a]	Recovery (%)	Found[1] ± SD[a]	Found[2]± SD[a]	Recovery (%)	Found[1] ± SD[a]	Found[2]± SD[a]	Recovery (%)
Milk with omega-3	Without aroma 1	9.5 ± 0.5	17.3 ± 2.3	89 ± 1	17.3 ± 1.2	26.8 ± 2.3	97 ± 2	n.d.	8.7 ± 0.2	87 ± 4
	Without aroma 2	5.6 ± 0.3	14.8 ± 1.0	95 ± 2	15.0 ± 1.0	24.9 ± 2.2	99 ± 2	n.d	9.0 ± 0.3	90 ± 3
	Without aroma 3	5.6 ± 0.4	15.0 ± 1.0	96 ± 1	17.2 ± 1.3	26.7 ± 2.1	97 ± 1	n.d.	9.3 ± 0.3	93 ± 6
	Cacao	20.5 ± 1.0	29.3 ± 2.0	96 ± 1	19.3 ± 1.0	28.3 ± 3.0	95 ± 2	2.6 ± 0.3	10.5 ± 0.4	83 ± 2
	Fruits	20.1 ± 1.0	29.5 ± 2.0	98 ± 2	14.0 ± 0.3	23.0 ± 2.4	93 ± 4	n.d.	9.6 ± 0.2	96 ± 3
	Cereals	3.4 ± 0.2	12.8 ± 1.4	96 ± 3	10.6 ± 0.4	19.3 ± 2.0	94 ± 3	n.d.	9.1 ± 0.5	91 ± 4
	Nuts	4.2 ± 0.2	13.5 ± 1.0	95 ± 1	18.4 ± 1.0	27.7 ± 2.2	96 ± 1	n.d.	8.9 ± 0.2	89 ± 3
Linseed oil	Sample 1	17.0 ± 1.0	25.2 ± 1.3	93 ± 2	33.8 ± 3.1	41.4 ± 3.3	94 ± 3	n.d.	9.2 ± 0.5	92 ± 2
	Sample 2	22.8 ± 1.0	29.3 ± 2.0	89 ± 1	41.3 ± 3.0	48.7 ± 4.2	95 ± 1	n.d.	8.8 ± 0.5	88 ± 7

[a]SD, standard deviation

[1] Concentration of the analyte in samples after 20 days of storage in the condition 3

[2] Concentration of the analyte in spiked samples after 20 days of storage in the condition 3

n.d. not detected

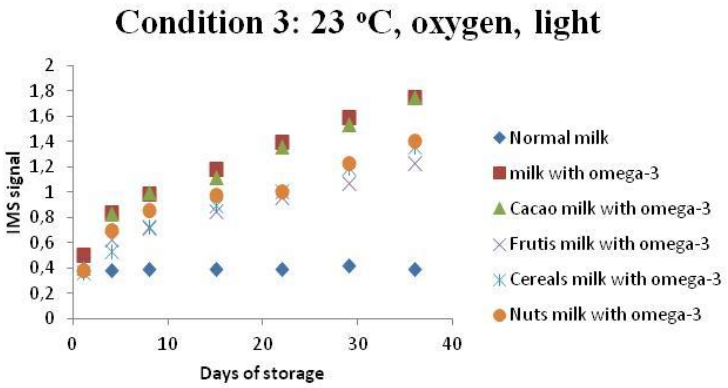

Figure 5. Evolution of the IMS signals for all milk samples in the storage condition 3.

Also, a recovery study was carried out taking in account the degradation occurred during storage. The recoveries were calculated for samples stored under condition 3 (oxygen, room temperature and light) during 20 days. In this case, the relative percentages of recuperation were calculated as the ratio between the concentration of the analyte found in the spiked sample after 20 days and the concentration of each analyte after 20 days of storage in samples without spiking. The results obtained are shown in Table 5. It can be seen that they are coherent with thoses obtained for non-degraded samples, which corroborates the usefulness of the proposed method.

4. Conclusions

In this work, we have evaluated the performance of a commercial available hyphenation HS-MCC-IMS to the study the degradation of omega-3 acids in milk and oil samples during 36 days. Using a multicapillary column coupled to IMS combines the high selectivity of chromatographic separation with the good sensitivity of IMS. As a result, a HS-MCC-IMS system provides an excellent analytical tool, even for measurements in complex samples like milk and oil. The application of the proposed method to the analysis of milk

enriched or not with omega-3 (cacao, fruits, cereals and nuts) and linseed oil samples stored under different conditions (temperature, oxygen and light) have demonstrated the degradation produced in the samples. It can be concluded that from the three variables studied, the temperature and the light are the most influential in the linseed oil and milk samples, respectively. The volatile profile obtained using positive polarity has allowed the identification of hexanal, 2-butanone, acetone and dimethyl disulfide in degradated samples. In addition, the increasing of the concentration in the degradation products has been detected and quantified. This method can be easily applied to monitor the formation of volatiles compounds as consequence of the omega-3 acids' degradation without pre-treatment of the samples.

The limits of dectection were in the interval 0.3 µg/L (for hexanal in milk) to 3.0 µg/L (for dimethyl disulfide in linseed oil) while the limits of quantification varied between 1.1 µg/L (for hexanal in milk) and 9.6 µg/L (for dimethyl disulfide in linseed oil). The precision of the method was evaluated as relative standard deviation and, the values were lower at 8% in all cases. The method is simple, fast, ease in the data interpretation and with a low cost of acquisition and maintenance. In summary, HS-MCC-IMS provides a vanguard methodology for the monitoring of degradation products in enriched omega-3 food samples.

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Resultados y discusión

En este apartado de la Memoria de Tesis Doctoral se presenta un resumen de los resultados más relevantes obtenidos dentro de los objetivos marcados en esta Memoria. Los trabajos experimentales se han agrupado en tres bloques en función del detector no convencional y el tratamiento previo de muestra empleado. En el primer bloque, se comenta la simplificación del tratamiento de muestra gracias a la mejora en la configuración instrumental propuesta. En el segundo bloque se da a conocer nuevas aplicaciones de los detectores no convencionales empleados en el área alimentaria para la determinación de macro y micro compuestos mediante el análisis directo de la muestra. En el tercer bloque se comenta el uso de las nanopartículas de carbono en el tratamiento de muestra.

1. Simplificación del tratamiento de muestra mediante la mejora de la configuración instrumental para la determinación de contaminantes a niveles traza

En esta Memoria de Tesis Doctoral se ha empleado la espectrometría de movilidad iónica para la determinación de 2,4,6-tricloroanisol (2,4,6-TCA) en distintas matrices: agua, vino y corcho. Este haloanisol se considera una de las principales causas del "sabor a corcho" en los vinos y también en otros productos como café o agua. Este compuesto migra del corcho al vino, afectando a sus propiedades sensoriales y disminuyendo su calidad, lo que produce grandes pérdidas económicas en la industria del vino. Las principales fuentes de contaminación son los desinfectantes clorados añadidos a los sistemas de blanqueamiento durante la producción de tapones de corcho, lavado de barricas, etc. Se ha comprobado que el umbral de sensibilidad de los consumidores a este producto se sitúa en el nivel de 10 ng L^{-1}. Por este motivo, es necesario disponer de métodos analíticos lo bastante sensibles para la determinación de este contaminante en muestras de corcho y vino principalmente. En esta Memoria se presentan tres alternativas basadas en el

uso de la espectrometría de movilidad iónica. Para ello se ha empleado dos instrumentos diferentes que comparten el uso de una fuente de ionización radiactiva de ^{3}H, ya comentada en la introducción de esta Memoria. Esta fuente genera tanto iones positivos como negativos, siendo este último el más adecuado para la determinación de 2,4,6-TCA dada la presencia de átomos cloro en su estructura. Como se ha puesto de manifiesto en los capítulos 6, 7 y 8, las metodologías propuestas han ido evolucionando en simplicidad, cumpliendo así con una de las tres tendencias básicas de la Química Analítica. A continuación se resumirán los aspectos más relevantes de cada una de ellas, finalizando con una comparación en función de las propiedades básicas y complementarias.

Para llevar a cabo la extracción del analito de la muestra líquida, se hizo uso de la microextracción en gota de líquido iónico (*single drop microextraction*, SDME). El líquido iónico además de extraer los analitos permite su preconcentración sin necesidad de usar disolventes orgánicos. Por otro lado, hay que tener en cuenta que el sistema de introducción de muestra en IMS es un cuello de botella para la aplicación de esta técnica de vanguardia sobre todo para muestras líquidas y sólidas. Para resolver esta limitación, se usó una interfase diseñada anteriormente en el grupo de investigación con elementos de bajo coste y de fácil ensamblaje. En la interfase se retiene el líquido iónico empleado en la extracción en una porción de lana de vidrio que se encuentra en su interior y el analito se transfiere cuantitativamente al espectrómetro de movilidad iónica. Es necesario realizar una limpieza periódica (cada cinco inyecciones) de la lana de vidrio para evitar la acumulación de líquido iónico que podría ocasionalmente entrar en el espectrómetro de movilidad iónica interfiriendo en la señal del analito.

Se ensayaron diferentes líquidos iónicos para la extracción del 2,4,6-TCA: 1-octil-3-metilimidazoliohexafluorofosfato [Omim-PF_6], 1-hexil-2-metil-imidazoliohexafluorofosfato [Hmim-PF_6] y 1-hexil-3-metilimidazolio bis(trifluorometilsulfonil)imida [Hmim][NTf_2]. Se eligieron líquidos iónicos con grupos imidazolio porque es conocida su alta afinidad por compuestos con anillos aromáticos, como es el caso del 2,4,6-TCA, y sus propiedades físicas los hacen ideales para su uso como extractantes. Aunque todos ellos presentaron alta eficiencia en la extracción, tan sólo el [Hmim][NTf_2] cumplió con las siguientes características:

- Alta afinidad por el 2,4,6-TCA
- Baja presión de vapor, bajo punto de fusión y alta hidrofobicidad para formar gotas estables en la SDME
- Adecuada viscosidad que permita pasar el gas portador y al mismo tiempo no se elimine con facilidad de la lana de vidrio
- Espectro limpio en el IMS

La cantidad total de líquido iónico está limitada por su viscosidad y las dimensiones de la interfase, seleccionándose 2 µL como volumen óptimo. Para llevar a cabo la optimización de la etapa de extracción se empleó un estándar de 100 ng mL^{-1} de 2,4,6-TCA. Las condiciones experimentales iniciales fueron 8 mL de estándar acuoso contenidos en un vial de 10 mL que contenían 350 g L^{-1} de NaCl para aumentar la fuerza iónica y así favorecer el paso de los analitos al espacio de cabeza. La disolución se agitó a 500 rpm durante 20 min y la temperatura de extracción se fijó a 60 ºC. La Figura 1 muestra de forma gráfica las condiciones finales de extracción.

En principio, el paso del analito de la muestra al espacio de cabeza debe estar favorecido con el aumento de la temperatura. Sin embargo, el proceso de adsorción en la microgota es exotérmico, un aumento de la

temperatura reduce el coeficiente de partición del analito entre la fase gaseosa y la gota de líquido iónico. Además, a altas temperaturas la concentración de moléculas de agua en el espacio de cabeza podría dificultar la adsorción de los analitos en la microgota. El paso de los analitos de interés al espacio de cabeza también se favorecen con la velocidad de agitación y la fuerza iónica de la muestra, por ello se trabajó a la máxima velocidad del agitador y a la máxima solubilidad de NaCl. La agitación también contribuye a reducir el tiempo necesario para establecer el equilibrio de partición entre la fase acuosa y gaseosa. Cuanto mayor sea el tiempo de extracción mayor es la cantidad de analito transferida, hasta que se alcanza el equilibrio. Otro parámetro importante en el proceso de extracción es la relación entre el volumen de muestra y el volumen de espacio de cabeza del vial. Por tanto, la concentración de 2,4,6-TCA se favorece en el espacio de cabeza cuanto mayor sea el volumen de muestra. El volumen máximo de muestra que pudo emplearse sin que se afectara la estabilidad de la gota de extractante fue de 8 mL.

Figura 1. Diagrama esquemático de la etapa de microextracción en gota de líquido iónico para la determinación de 2,4,6-TCA.

Una vez optimizadas las variables del proceso de microextracción, el método descrito se caracterizó en términos de sensibilidad y precisión obteniéndose un límite de detección menor que los alcanzados con otras metodologías ya descritas en bibliografía, aunque el método SBSE-GC-MS-

MS propuesto por L. Maggi llega a límites de detección inferiores pero el tiempo de análisis es mayor. La precisión del método se evaluó en términos de repetitividad y reproducibilidad, llevando a cabo 5 repeticiones del procedimiento en el mismo día y 3 repeticiones en tres días consecutivos. Los valores obtenidos en términos de desviación estándar relativa (RSD, %) fueron de 1.4% y 2.2%, respectivamente que como se muestra en la Tabla 1, es el método con mejor precisión.

El método propuesto se aplicó a la determinación de 2,4,6-TCA en seis muestras de agua embotellada. El análisis directo de las muestras reveló la ausencia del analito. Para validar la metodología analítica, se realizó un estudio de recuperación a dos concentraciones diferentes (5 y 100 ng L^{-1}). El intervalo de valores de recuperación estuvo entre 96 y 99% para bajas concentraciones, mientras que para altas concentraciones varió entre 94 y 100%.

El procedimiento descrito se aplicó también a diferentes muestras de vino, pero en este caso no se pudo realizar el análisis directo de la muestra debido a interferencias de la matriz, principalmente el etanol, en el espectro de movilidad iónica. La señal del etanol solapa con la del 2,4,6-TCA impidiendo su determinación. Por tanto, se implementó una etapa de extracción en fase sólida usando LiChrolut EN como material sorbente previa a la microextracción en gota. En la Figura 2 se muestra una comparación de los espectros obtenidos sin y con etapa de extracción en fase sólida, cuyas condiciones están descritas en el Capítulo 6.

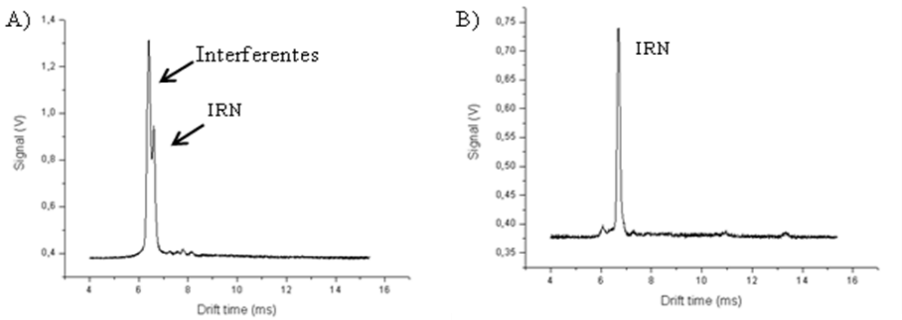

Figura 2. Espectros de un vino blanco (botella de vidrio y tapón de rosca) obtenidos A) sin SPE y B) con SPE. IRN: iones reactantes negativos.

Una vez optimizado el método para su aplicación a muestras de vino, se analizaron diez muestras diferentes (cuatro de vino tinto, cuatro de vino blanco y dos de vino espumoso). Considerando que las principales fuentes de contaminación del vino con 2,4,6-TCA se encuentran en la etapa de producción de tapones de corcho, limpieza de barricas y envasado del vino, entre las muestras analizadas había variedad en cuanto a tapones (corcho, goma o rosca) y tipo de envase (vidrio, *bag in box* o tetra brik). Sólo en las muestras con tapón de corcho y envase de vidrio se detectó el 2,4,6-TCA. Para cada tipo de vino se llevó a cabo un estudio de recuperación a dos niveles de concentración (5-50 ng L^{-1}), oscilando los porcentajes entre 97-104% y 95-97%, respectivamente.

Como ya se comentó a lo largo de esta Memoria, la IMS presenta baja resolución debido a las reacciones ion-molécula que ocurren en la cámara de ionización a presión atmosférica. Para evitar esta problemática y eliminar la etapa de tratamiento de muestra de la metodología propuesta anteriormente, en el Capítulo 7 se acopló al IMS una columna multicapilar (MCC) capaz de separar el 2,4,6-TCA de los interferentes presentes en la muestra en condiciones isotérmicas. Este acoplamiento permite realizar el análisis directo de la muestra, eliminando el uso de disolventes y reduciendo el tiempo de análisis.

Las columnas multicapilares ofrecen mayor sensibilidad que las columnas cromatográficas habituales debido al elevado número de capilares individuales que las componen. Además, la selectividad de la IMS aumenta enormemente debido a la elución secuencial de los analitos de la columna multicapilar. Pueden trabajar en condiciones isotérmicas de separación a temperatura ambiente o bien hasta una temperatura de 200 ºC. En combinación con el espectrómetro de movilidad iónica, los caudales de gas portador y gas de deriva (nitrógeno) tienen que ser más altos.

La configuración del sistema fue similar al anterior con la única diferencia de que entre la inyección en la interfase y la entrada al espectrómetro se situó la columna multicapilar. Ésta se introdujo en el interior de un horno cromatográfico para mantener constante la temperatura de separación (30 ºC). El resto de parámetros, tanto de la microextracción en gota de líquido iónico como de la detección, se mantuvieron en los valores óptimos establecidos en el procedimiento anterior.

Antes de caracterizar el método en términos de sensibilidad y precisión, se evaluó la ausencia de efecto matriz realizando un calibrado para 2,4,6-TCA en agua Milli-Q, vino blanco y vino tinto en el intervalo de 0.01-500 ng L^{-1}. Mediante la prueba t por parejas, se demostró la ausencia de efecto matriz y la escasa influencia del etanol presente en las muestras. El límite de detección fue de 0.01 ng L^{-1}. La precisión del método se evaluó en términos de repetitividad y de reproducibilidad para una concentración 1 ng L^{-1} de 2,4,6-TCA; para ambos parámetros se obtuvo un valor de RSD inferior al 6%.

Una vez optimizado y caracterizado, el método propuesto se aplicó a la determinación de 2,4,6-TCA en una amplia variedad de muestras de vino (de mesa, tinto, blanco, dulce y espumoso) teniendo en cuenta la variedad en el

tipo de envase y el tapón. El contenido alcohólico entre los vinos analizados varió entre 11.5 y 15%. Independientemente del tipo de vino, las muestras envasadas en vidrio y tapón de corcho presentaron contaminación con 2,4,6-TCA. En esta ocasión hay que destacar la presencia de este contaminante en un vino envasado en *bag in box* y en un espumoso con tapón de goma, aunque su concentración fue muy baja.

La nueva configuración permite el análisis directo de las muestras reduciendo el tiempo de análisis y mejorando la sensibilidad, selectividad y precisión de la determinación de 2,4,6-TCA.

Finalmente, mediante el empleo de un equipo comercial FlavourSpec® se consiguió la completa automatización y simplificación del método propuesto. El equipo lo constituyen tres elementos: un inyector automático de espacio de cabeza, una columna multicapilar y un espectrómetro de movilidad iónica. Además, integra un brazo automuestreador, que permite el calentamiento y agitación del vial que contiene la muestra (líquida o sólida) para transferir el analito al espacio de cabeza y la inyección posterior de un volumen exactamente medido de la fase gaseosa en el espectrómetro de movilidad iónica que, al igual que en el método anterior, está acoplado a una columna multicapilar. Es importante destacar que este equipo permite controlar de forma automática los caudales de gas de entrada y de deriva y las temperaturas de la jeringa, columna multicapilar y tubo de deriva. El inyector automático está provisto de una jeringa, que puede calentarse hasta una temperatura de 80 ºC para evitar pérdidas por condensación del analito en la transferencia desde el vial hasta el inyector del equipo. Además, el empleo de un automuestreador, evita las irreproducibilidades debidas a la manipulación de las muestras por parte del operador. En este caso, los viales sellados que contienen la muestra se depositan en una bandeja y a partir de este momento

todo el proceso está automatizado. Tal vez los aspectos negativos que presenta este equipo frente al espectrómetro de movilidad iónica usado anteriormente son su menor versatilidad y mayor coste de adquisición y mantenimiento.

En primer lugar, se optimizaron todas las variables implicadas en el proceso, se caracterizó en términos de propiedades analíticas y finalmente se aplicó a la determinación de 2,4,6-TCA en vinos y tapones de corcho. Entre todas las variables estudiadas, la temperatura de extracción junto con el porcentaje de etanol y la disposición de la muestra de corcho fueron las más importantes. El porcentaje de etanol y la temperatura de extracción afectan conjuntamente a la transferencia del analito al espacio de cabeza. La interferencia del etanol se evaluó a dos temperaturas 40 y 60 ºC, los resultados obtenidos se muestran la Figura 3.

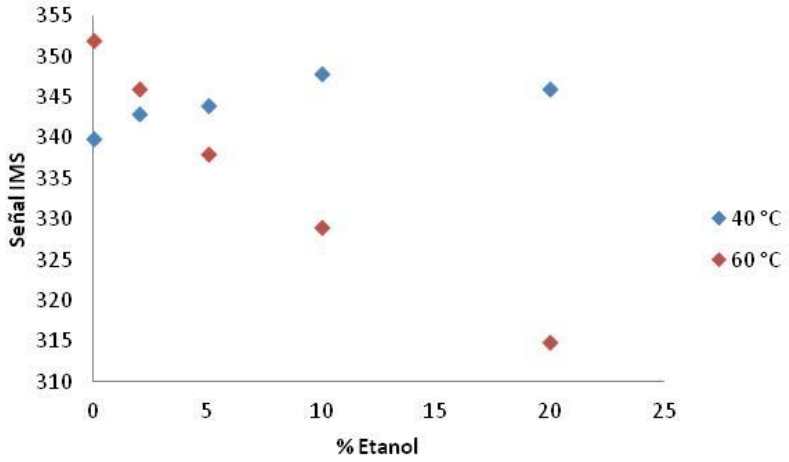

Figura 3. Efecto del porcentaje de etanol en la detección de 2,4,6-TCA por HS-MCC-IMS para muestras líquidas y sólidas.

Para una temperatura de 40 ºC, la señal aumenta cuando el contenido de etanol es superior al 10% en cambio para 60 ºC no se produce este aumento de señal con el aumento de etanol. Teniendo en cuenta que los vinos

analizados tienen un porcentaje de etanol alrededor de 15% (v/v), se seleccionó 40 ºC como temperatura óptima de extracción.

Por otro lado, se observó que el tamaño de la muestra de corcho influía de manera considerable en la migración del 2,4,6-TCA de la muestra sólida al espacio de cabeza. En estudios previos descritos en bibliografía, algunos autores usan una extracción líquido de los analitos de la muestra previamente troceada en un molinillo a baja temperatura para evitar pérdidas del analito. En nuestro caso, para facilitar el análisis directo, los tapones de corcho se cortaron de dos maneras diferentes: en láminas y en trozos de pequeño tamaño (1 mm x 2 mm x 3 mm). La mayor transferencia de 2,4,6-TCA al espacio de cabeza se obtuvo para el corcho troceado.

Al igual que en los dos casos anteriores, el método propuesto se caracterizó en términos de sensibilidad y precisión, siendo el límite de detección alcanzado de 0.012 ng L^{-1}. En términos de desviación estándar relativa la precisión fue inferior al 4%. A modo de resumen global de este apartado del Capítulo de Resultados y Discusión, en la Tabla 1 se comparan las características de los métodos propuestos con otras metodologías ya descritas en bibliografía.

Tabla 1. Comparación de los tres métodos propuestos con otros descritos en la bibliografía para la determinación de 2,4,6-TCA en muestras de vino.

Método[a]	Volumen de muestra (mL)	LOD[b] (ng L^{-1})	RSD[c] (%)	Tiempo (min)	Referencia
PT-GC-AED	500	5	< 13	-	[1]
HS-SPME-GC-ECD	20	0.3	10	70	[2]
HS-SPME-GC-MS	10	0.4	< 8	60	[3]
SPE-LVI-GC-MS	50	0.2	< 6	10	[4]
SBSE-GC-MS-MS	5	0.03	13.3	60	[5]
SPME-GC-MS-MS	4	2.5	5.2	60	[6]
HS-SDME-GC-ECD	20	8.1	12.4	25	[7]
DLLME-GC-ECD	5	2.3	< 10	-	[8]
SPE-HS-SDME-IMS	**8**	**0.1**	**< 3**	**40**	**Capítulo 6**
HS-SDME-MCC-IMS	**2**	**0.01**	**< 6**	**35**	**Capítulo 7**
HS-MCC-IMS	**8**	**0.012**	**< 4**	**20**	**Capítulo 8**

[a] PT: purga y trampa; AED: detección de emisión atómica; ECD: detector de captura electrónica; LVI: inyección de gran volumen; SBSE: extracción soportada en barrita agitadora; DLLME: microextracción dispersiva líquido-líquido.
[b] Límite de detección
[c] RSD, desviación estándar relativa

Se analizaron un total de 22 muestras de diferentes vinos y 5 tapones de corcho (3 de ellos correspondientes a vinos analizados y los otros procedentes de una bodega con certificado de análisis). Como ya se concluyó anteriormente todas las muestras de vino con tapones de corcho presentaron 2,4,6-TCA. Además los tapones de corcho comerciales presentaron también este contaminante. Ante la ausencia de un material de referencia para validar este método se realizó un estudio de recuperación a dos concentraciones diferentes (5 y 50 ng L^{-1}) tanto en vino como en corcho. Los valores de recuperación variaron entre 86 y 98% con una desviación estándar relativa máxima del 8%.

La configuración instrumental simplifica claramente el procedimiento analítico necesario para la determinación de 2,4,6-TCA en muestras de vino y corcho sin necesidad de tratamientos previos de muestra y de manera totalmente automatizada.

A modo de resumen de este bloque, la Figura 4 recoge la contribución de cada uno de los módulos que pueden integrar un instrumento de espectrometría de movilidad iónica resaltando sus aportaciones fundamentales en relación con la mejora de las propiedades analíticas. La triple hibridación HS-MCC-IMS permite la simplificación y automatización completa del proceso analítico, enfocado a la determinación de contaminantes volátiles en muestras líquidas o sólidas. Por su parte, los dos primeros módulos permiten aumentar la selectividad de la medida mediante movilidad iónica. El primero de ellos en base a la temperatura seleccionada y el segundo en base a la distinta interacción que puedan establecer los compuestos en la fase estacionaria. En cuanto al incremento de sensibilidad, se produce en su mayor parte en las etapas de separación y detección de los analitos.

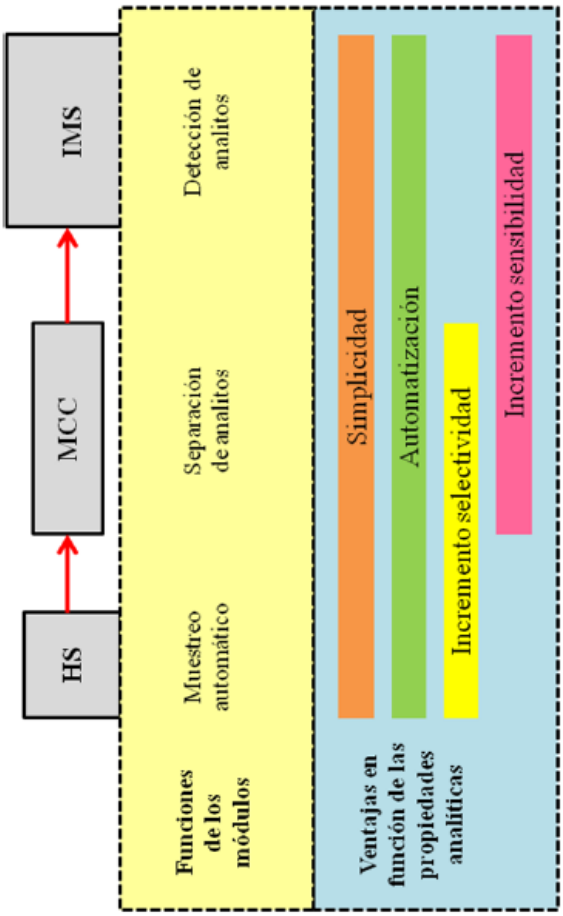

Figura 4. Esquema de las funciones y ventajas de cada uno de los módulos del método propuesto.

2. Análisis directo empleando detectores no convencionales para la determinación de macro- y micro-compuestos

2.1. Análisis directo mediante espectrometría de movilidad iónica

El desarrollo de técnicas sensibles y selectivas con detección a niveles traza e identificación inequívoca de analitos es una línea de investigación muy relevante en Química Analítica. No obstante, y atendiendo a una de las tendencias comentadas en la introducción de esta Memoria, la simplificación, no solo de los procesos de medida sino también de la información que de ellos se deriva nos llevó a evaluar la capacidad de la espectrometría de movilidad iónica para extraer información del perfil global de volátiles de una muestra dada. Para ello, se seleccionó como problema analítico modelo la monitorización de la degradación de alimentos enriquecidos con ácidos poliinsaturados omega-3. Esta investigación se llevo a cabo en la empresa alemana G.A.S., que ha diseñado y suministrado los espectrómetros de movilidad iónica empleados en esta Tesis Doctoral. Para llevar a cabo este estudio, se utilizó el equipo HS-MCC-IMS, el cual permite el análisis directo de la fracción volátil de las muestras con una gran selectividad gracias al empleo de la columna multicapilar previo al análisis de movilidad iónica.

Durante los últimos diez años, se está produciendo un considerable aumento en la incorporación de ácidos grasos poliinsaturados omega-3 a una gran variedad de productos alimenticios. Estos compuestos son susceptibles de sufrir una oxidación lipídica que da lugar a sabores y olores indeseables en el alimento que los contiene. Los factores que producen la oxidación lipídica son principalmente la presencia de oxígeno, luz y temperatura. Aunque es muy amplia la variedad de productos enriquecidos con ácidos omega-3, las muestras seleccionadas fueron leche enriquecida con omega-3 (de diferentes aromas y sabores) y aceite de linaza rico en estos ácidos grasos. La leche enriquecida está ampliando su campo comercial en España en los últimos años

mientras que el aceite de linaza es un producto muy usado por deportistas en otros países como Alemania. Para inducir la oxidación lipídica en las muestras, se añadieron 5 mL de cada una en un vial y se sometieron a diferentes condiciones de almacenamiento durante 36 días. Estas condiciones fueron: 4 ºC, 23 ºC y 40 ºC alternadas con la presencia o ausencia de luz y oxígeno. Como referencia, se tomo el espectro de movilidad iónica de la muestra recién abierto el envase. A lo largo de los 36 días de almacenamiento se realizaron análisis de las diferentes muestras por triplicado. Se seleccionaron diferentes zonas de intensidad y se comprobó la influencia de las condiciones de almacenamiento en el perfil volátil de las muestras. La Figura 5 muestra la evolución de una de las zonas estudiadas (hexanal) para una muestra de leche enriquecida con omega-3 en las diferentes condiciones de almacenamiento a lo largo de los 36 días de estudio.

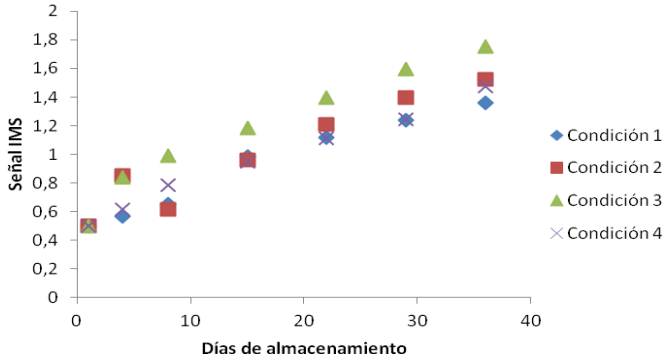

Figura 5. Evolución de la señal IMS correspondiente al hexanal para una muestra de leche enriquecida con ácidos omega-3 almacenada en diferentes condiciones de almacenamiento y tiempo. Condición 1: 4ºC y oxígeno; condición 2: 23 ºC y oxígeno; condición 3: 23 ºC, oxígeno y luz; condición 4: 40 ºC y oxígeno.

Durante este estudio se apreció el crecimiento de hongos en las muestras, aunque no se pudo establecer ningún patrón de comportamiento. En cualquier caso, la reproducibilidad de las medidas descarta su influencia significativa en el proceso de degradación de los ácidos poliinsaturados

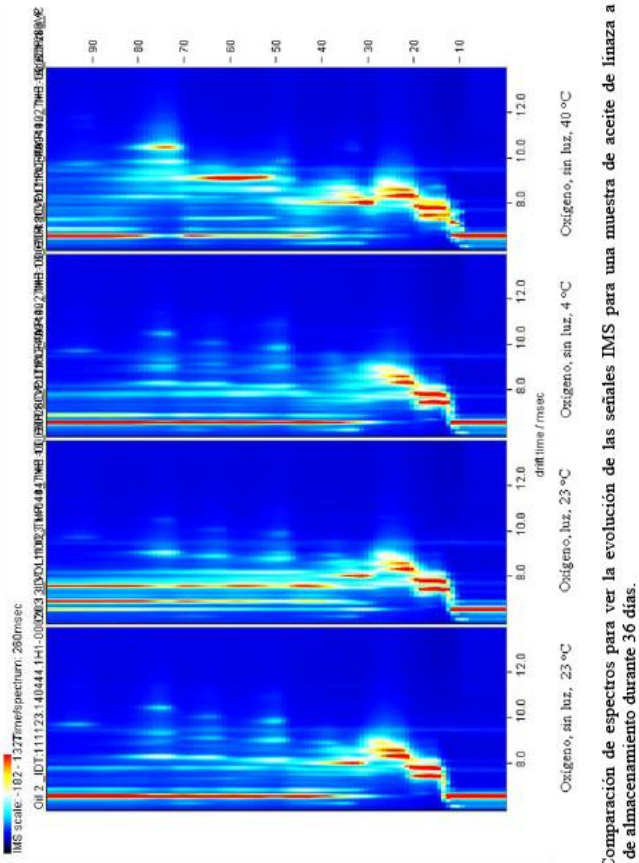

Figura 6. Comparación de espectros para ver la evolución de las señales IMS para una muestra de aceite de linaza a las diferentes condiciones de almacenamiento durante 36 días.

omega-3. Se puede confirmar que la degradación en las muestras de aceite se ve más afectada por la temperatura. La Figura 6 muestra a través de diferentes espectros este hecho. En cambio, las muestras de leche son más sensibles a la luz que las de aceite aunque estén bajo las mismas condiciones de almacenamiento. Se observó que para algunas muestras de leche con bajo contenido en ácidos grasos como es el caso de la leche con cereales o fruta, la temperatura también es una variable de peso. La leche con nueces o con cacao presenta un comportamiento similar. La Figura 7 muestra de manera gráfica la degradación de las muestras de leche durante los 36 días de almacenamiento.

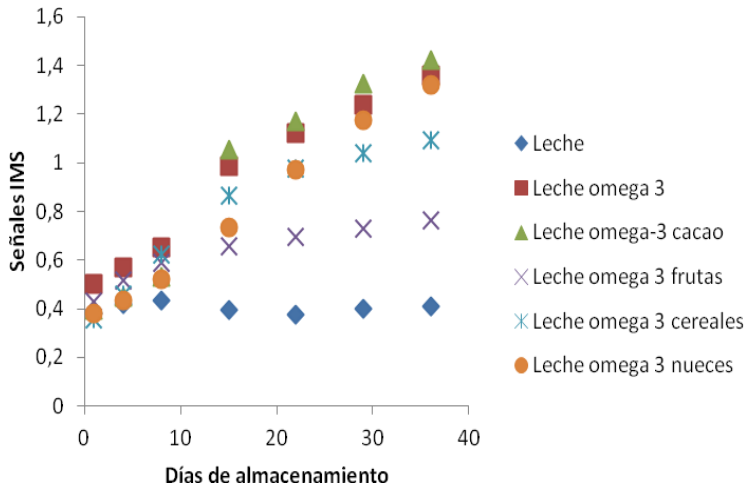

Figura 7. Evolución de las señales IMS para todas las muestras de leche en las condiciones de almacenamiento de 4 ºC, oxígeno y sin luz.

Está documentado que la presencia de acetonas, alcoholes y aldehídos producidos por la descomposición de peróxidos son responsables de la degradación sensorial en alimentos. Por este motivo, se procedió a la identificación de algunos productos de degradación: acetona, 2-butanona, hexanal y dimetil disulfuro. El método se caracterizó en términos de sensibilidad y precisión. Los límites de detección variaron entre 0.3 µg L^{-1} en leche para hexanal y 9.6 µg L^{-1} en aceite de linaza para dimetil disulfuro. La

precisión (expresada como desviación estándar relativa) fue mejor del 7% para ambas matrices.

También se llevó a cabo un estudio de recuperación en todas las muestras a una concentración de 10 µg L^{-1} y los valores variaron entre el 82 y el 100%. También se llevó a cabo un estudio de recuperación pero teniendo en cuenta la degradación de las muestras. Los valores obtenidos fueron similares a los obtenidos en muestras sin degradar.

Hay que destacar que la acetona pudo detectarse pero no cuantificarse en ninguna de las muestras debido a que su concentración excedía del intervalo lineal estudiado. El disulfuro de dimetilo no se detectó en ninguna muestra, a excepción de una muestra de cacao.

A la vista de los resultados obtenidos, se puede concluir que se ha demostrado la utilidad de la espectrometría de movilidad iónica para el análisis global de productos de degradación volátiles en muestras de diferente tipo, que nos permite conocer de forma rápida y fiable el grado de calidad de dichas muestras.

2.2. Análisis directo empleando detectores de tipo evaporativo

Durante el desarrollo de esta Tesis Doctoral, además del espectrómetro de movilidad iónica también se han empleado otros detectores no convencionales como son el detector de transferencia de carga-corona (C-CAD) y el detector evaporativo de dispersión de luz (ELSD). Ambos detectores permiten la determinación de compuestos no volátiles en muestras líquidas de manera simple, robusta y bajo coste. Se caracterizan por generar una respuesta independiente de las propiedades fisicoquímicas de los analitos de interés, por ello se conocen también como detectores "cuasi-universales".

El Capítulo 3 de esta Memoria se dedica al desarrollo de un método para la determinación de siete vitaminas hidrosolubles en leches infantiles y suplementos dietéticos. Las vitaminas son unos compuestos esenciales ya que no pueden ser sintetizadas por nuestro organismo. Por tanto, la fortificación de los alimentos es una manera efectiva y de bajo coste de aumentar estos micronutrientes. Por ello, tiene un gran interés su determinación en el área nutricional, en el campo de la salud y en la industria alimentaria y otros tipos de muestras.

Los métodos oficiales para la determinación de vitaminas hidrosolubles son tediosos y a veces no son específicos para estos compuestos. Por ello, se decidió desarrollar un método alternativo a los ya existentes que permita su separación cromatográfica y cuantificación de manera simple y rápida haciendo uso del C-CAD. Como se ha comentado anteriormente, en este detector, la fase móvil procedente de la columna se nebuliza y las partículas de analito se cargan positivamente a través de una corriente de nitrógeno previamente cargada por un sistema de descarga corona. Las partículas cargadas llegan a un electrómetro que emite una señal cuya intensidad es proporcionar a la concentración de analito.

En este tipo de detector, la composición de la fase móvil influye en la respuesta ya que un porcentaje alto de disolventes orgánicos producen un aumento de la línea de base. En este caso, se utilizó como fase móvil 0.05M acetato amónico:metanol (90:10, v/v) que proporcionó una línea de base estable y una buena resolución de los picos. Además de la composición de la fase móvil, en la respuesta del detector también afectan otras variables como la temperatura de evaporación, la presión de nitrógeno, así como el filtro y la atenuación de la señal. En el detector utilizado en esta Memoria, la temperatura de evaporación no puede modificarse y la presión de gas es la

establecida por un generador de nitrógeno. El filtro es un dispositivo electrónico que elimina una porción de ruido de la señal, es decir, suaviza la señal de la línea de base facilitando la detección y cuantificación de los analitos de interés. Se optimizó para tres valores de filtro: alto, medio y bajo, proporcionando el valor medio los mejores resultados. Al igual que se optimizó el filtro, la atenuación de la señal también se estudió en un intervalo de 1 a 500 pA. El valor de 1 pA corresponde con una mayor respuesta del detector. El valor seleccionado depende de la respuesta que tenga el detector hacia cada uno de los analitos para evitar la saturación de la señal.

El método se aplicó a muestras de leche infantiles en diferentes formatos y a suplementos dietéticos. La concentración de los analitos es diferente para cada tipo de muestra, encontrándose a nivel de macro-compuestos en los suplementos dietéticos y a niveles de micro-compuestos en las muestras de leche. Por tanto, se tuvo que establecer un valor de atenuación diferente para cada tipo de muestra, 5 pA para los suplementos dietéticos y 50 pA para el caso de los productos lácteos. La Figura 8 muestra el cromatograma de un estándar que contiene las siete vitaminas hidrosolubles a una concentración de 50 mg L^{-1}.

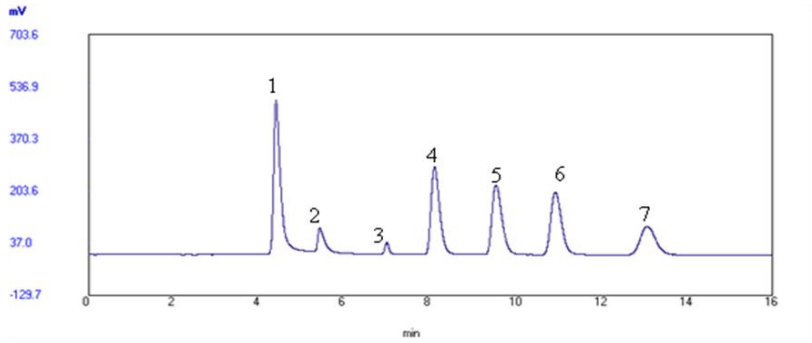

Figura 8. Cromatograma correspondiente al análisis de un estándar que contiene las siete vitaminas: (1) ácido ascórbico; (2) biotina; (3) ácido nicotínico; (4) ácido pantoténico; (5) tiamina; (6) piridoxina; (7) ácido fólico.

Antes del análisis de las muestras, se llevó a cabo la caracterización del método. Los límites de detección variaron entre 0.17 y 0.62 mg L^{-1} para suplementos dietéticos y entre 1.7 y 6.5 mg L^{-1} para las muestras de leche. La precisión se estudió en términos de repetitividad con 5 réplicas siendo menor al 6% y de reproducibilidad, en tres días consecutivos obteniéndose un valor inferior al 9%. Se evaluó el efecto matriz para la muestra de leche y se compararon las pendientes de las curvas de calibrado realizadas en ausencia y en presencia de matriz. Como se puede observar en la Tabla 2, no se detectaron interferentes por parte de la matriz de la muestra ni por la etapa de pretratamiento.

Tabla 2. Comparación de los valores de pendiente obtenidos para las curvas de calibrado sin y en presencia de la matriz de leche.

Vitaminas	Pendiente	
	Estándar acuoso	Leche
Ácido ascórbico	60.541	-
Tiamina	12.382	14.637
Ácido nicotínico	31.288	37.677
Ácido pantoténico	53.988	53.906
Piridoxina	27.204	27.278
Biotina	11.698	12.168
Ácido fólico	23.486	22.773

La complejidad de las muestras de leche dificulta su análisis directo, por ello fue necesaria una etapa de pretratamiento para eliminar las proteínas y el contenido graso. La eliminación de parte de estos interferentes, pues no fue total, se llevó a cabo centrifugando la muestra con 2.5M de ácido acético. El extracto final se analizó para determinar las vitaminas presentes en la muestra. Al no conseguir la eliminación total de los interferentes, el ácido ascórbico no se pudo determinar pues la señal correspondiente a la matriz de la muestra solapaba con la del analito. Para las demás vitaminas presentes en las muestras, el valor determinado se corresponde con el valor de la etiqueta. En la

Figura 9 se muestra la disminución de interferencia al tratar la muestra de leche con ácido acético.

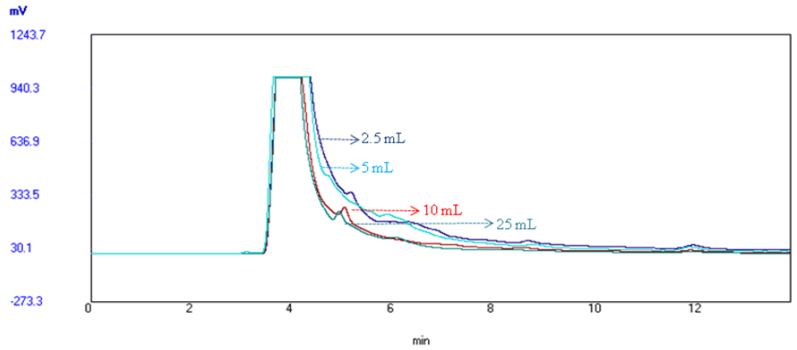

Figura 9. Cromatogramas obtenidos para 25 mL de leche empleando diferentes volúmenes de ácido acético 2.5 M en la etapa de tratamiento de muestra.

En cambio para los suplementos dietéticos tan sólo fue necesaria la disolución de la muestra en un volumen adecuado de agua. La buena correlación de los resultados obtenidos con los etiquetados, demuestra la aplicabilidad del método propuesto para el análisis de vitaminas hidrosolubles en este tipo de muestras.

Los métodos existentes son más sensibles que el método propuesto pero el coste de la instrumentación y su mantenimiento es mayor. La sensibilidad del C-CAD es adecuada para el problema analítico planteado y además se trata de un detector robusto, con una respuesta reproducible y fácil de usar.

El detector de transferencia de carga-corona se comercializó después del detector de dispersión de luz, con el objetivo de mejorar la sensibilidad, reproducibilidad y cuantificación. El detector de dispersión de luz funciona de forma similar al C-CAD, el efluente procedente de la columna se nebuliza por efecto Venturi y se forma un aerosol que pasa a una cámara de evaporación

con una temperatura más elevada que permite eliminar de forma completa la fase móvil de las partículas de analito. Sobre estas partículas incide un haz de luz y la dispersión se mide en un fotomultiplicador. En este caso, lo que no existe es el proceso de ionización que es la base de la detección del C-CAD. El Capítulo 4 de esta Memoria se centró en realizar una comparación de ambos detectores en términos de sensibilidad, linealidad y precisión. Para llevar a cabo este estudio comparativo se usaron azúcares como analitos de interés por la respuesta que muestra el detector hacia ellos.

Para ambos detectores, la fase móvil usada para la separación cromatográfica de los analitos fue acetonitrilo:agua (70:30, v/v). En el caso del ELSD, a diferencia del C-CAD, se puede controlar tanto la temperatura de nebulización como la temperatura de evaporación. Ambas temperaturas se estudiaron simultáneamente en un intervalo de 35-55 ºC para la temperatura de nebulización y 45-65 ºC para la temperatura de evaporación, manteniendo un intervalo de 10 ºC entre ambas temperaturas. Los valores óptimos fueron 45 y 55 ºC. Ambos detectores permiten atenuar la señal para evitar la saturación de los cromatogramas. El parámetro que controla este efecto es distinto para los dos detectores. La atenuación en el ELSD varía entre 1 y 9, en este caso, el detector es más sensible a 9. ELSD mostró una menor sensibilidad hacia los analitos por lo que se eligió el máximo filtro y la máxima sensibilidad para llevar a cabo la determinación. En el caso del C-CAD, se usó una atenuación de 20 pA (intervalo del detector 1-500 pA) y un filtro medio. La Figura 10 muestra los cromatogramas correspondientes a cada uno de los detectores.

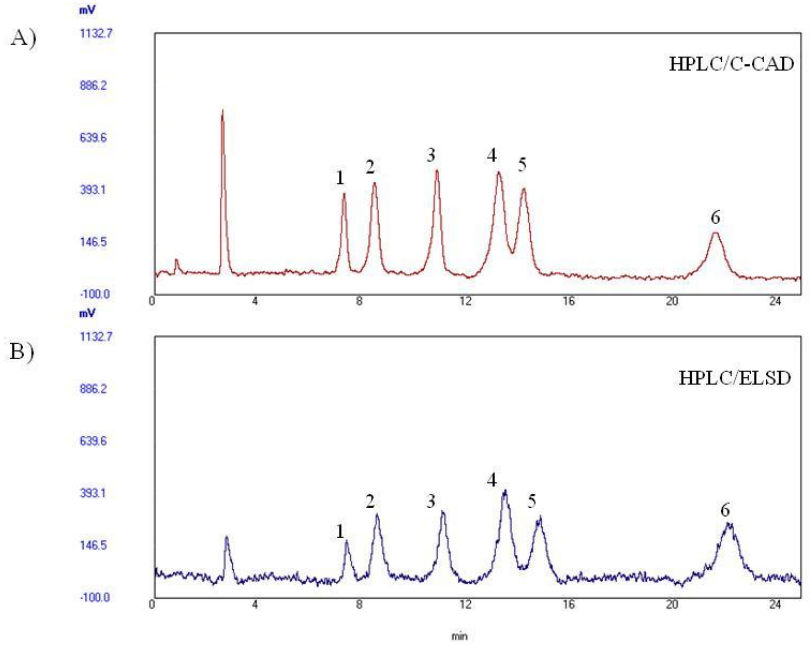

Figura 10. Cromatogramas obtenidos para la separación de los seis azúcares usando C-CAD y ELSD. (1) fructosa (125 mg L^{-1}); (2) glucosa (250 mg L^{-1}); (3) sacarosa (250 mg L^{-1}); (4) maltosa (500 mg L^{-1}); (5) lactosa (500 mg L^{-1}); (6) maltotriosa (500 mg L^{-1}).

Para evaluar la linealidad de ambos detectores, se analizaron estándares en un intervalo de concentración de 5 a 3000 mg L^{-1}. Como la respuesta de estos detectores no es lineal sino exponencial, para poder establecer los intervalos de linealidad se representó el logaritmo de la señal frente al logaritmo de la concentración. Para todos los analitos el C-CAD mostró una mayor sensibilidad, aunque el intervalo lineal de estudio fue menor que para el ELSD. Los límites de detección para el C-CAD variaron entre 1.2 y 7.6 mg L^{-1}, lo que se corresponde con una señal 8.5 y 2.3 veces mayor que la obtenida para el ELSD. En cambio, la precisión que se estudió para un estándar preparado a una concentración de 200 mg L^{-1}, fue similar para los dos detectores variando en un intervalo de 1.9-3.9% de desviación estándar relativa para el ELSD y entre 1.8-6.6% para el C-CAD.

Finalmente, ambos detectores se emplearon para el análisis de fructosa, glucosa, sacarosa, maltosa, lactosa y maltotriosa en una amplia variedad de muestras como salsas, productos lácteos y productos de repostería que se obtuvieron de locales comerciales de la zona. Tanto para las salsas como para los productos de repostería se diluyó 1 g exactamente pesado en 40 mL de agua antes de su análisis, aunque para algunas muestras esta dilución fue mayor para evitar la saturación de la señal para algunos de los analitos. Siropes, gelatinas, mermeladas y coberturas contienen jarabe de glucosa-fructosa en su composición para evitar la cristalización del azúcar y aumentar la viscosidad. Mediante la cuantificación de los analitos en las muestras se pudo comprobar que cuando en la etiqueta aparece jarabe de glucosa-fructosa, la glucosa se encuentra en mayor concentración que la fructosa, y viceversa. En todos los casos, todas las muestras que contenían maltotriosa también tenían en su composición maltosa. Para las muestras de miel, se observó que en la muestra de origen natural el contenido de azúcar era menor que en la muestra procesada. De entre los productos lácteos analizados, se incluyeron muestras comercializadas con y sin lactosa y en la que el contenido de este disacárido es inferior al 1%. Dado la dilución a la que se trabajó, no fue posible cuantificar este analito aunque si se puede decir que su concentración se sitúa por debajo del límite del método para ambos detectores. Los valores de concentraciones obtenidos en el análisis de las muestras fueron similares para ELSD y C-CAD.

Teniendo en cuenta los resultados obtenidos se puede concluir que ambos detectores son adecuados para la determinación de azúcares en este tipo de muestras. Aunque el C-CAD presente mejor sensibilidad para estos compuestos, el ELSD presenta un menor coste y para la concentración de azúcares presente en las muestras analizadas su sensibilidad es suficiente.

3. Determinación de conservantes usando nanopartículas de carbono como sorbente

Aunque el análisis directo de la muestra es la opción ideal en cualquier proceso de medida, esto es en la mayoría de los casos inviable, bien porque la concentración de analito sea muy baja, por los potenciales interferentes de la matriz de la muestra o incluso por la incompatibilidad del medio en el que se encuentran los analitos con la instrumentación analítica empleada. En este contexto, los materiales nanoestructurados, en general y las nanopartículas de carbono en particular han encontrado un amplio campo de aplicación en la simplificación de las etapas previas del proceso de medida. En esta Memoria se ha evaluado la capacidad de los nanotubos de carbono multicapa (*multi-walled carbon nanotubes*, MWCNTs) en extracción en fase sólida. En el Capítulo 2, se llevó a cabo la separación de cuatro parabenos (metil-, etil-, propil- y butil-parabeno) en productos cosméticos y de cuidado personal. Los parabenos son comúnmente usados como conservantes pero recientemente se ha demostrado que pueden ser un riesgo para la salud por su actividad estrógenica, además se les relaciona con el desarrollo del cáncer de mama. La interacción de los nanotubos de carbono con los anillos aromáticos de sustancias orgánicas a través de enlaces π-π ya ha sido descrito en bibliografía [9]. Puesto que los parabenos presentan aromaticidad en su estructura química, los nanotubos de carbono se seleccionaron como material sorbente para la preconcentración y extracción de estos analitos. Este material permitió la obtención de extractos limpios sin interferentes en la separación cromatográfica, permitiendo una alta selectividad y robustez en la etapa de extracción. Tan sólo se necesitaron 20 mg de MWCNTs y un mismo cartucho se pudo reutilizar hasta 200 veces sin que se detectara pérdidas de eficiencia en el proceso de extracción, lo que abarata el coste del análisis.

Puesto que no había referencias previas a la aplicación de los nanotubos de carbono como material sorbente en este contexto, se llevó a cabo un estudio de las variables que potencialmente podrían afectar al proceso de adsorción. Las variables estudiadas fueron las siguientes: pH de la muestra, eluyente, disolventes para la etapa de limpieza, volumen de muestra y dilución y la cantidad de sorbente. En la Figura 11 se muestra una imagen que muestra cada una de las etapas y los valores óptimos utilizados para llevar a cabo el proceso de extracción de los analitos.

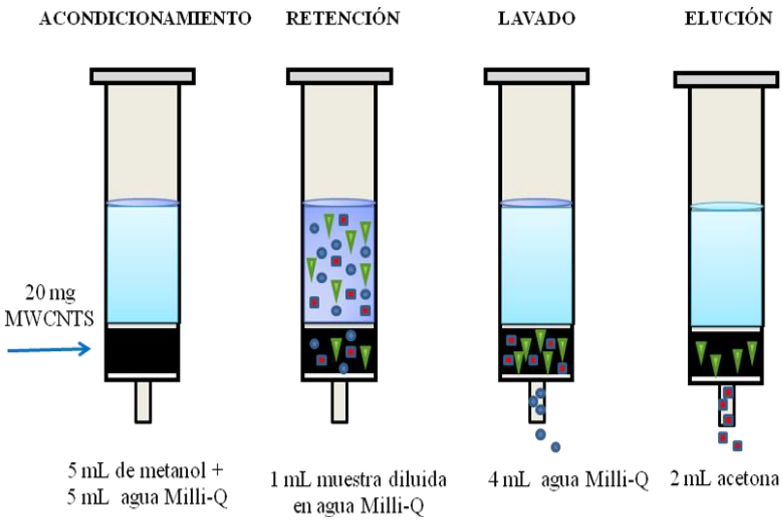

Figura 11. Esquema del proceso de extracción en fase sólida para la separación de parabenos.

De las variables experimentales estudiadas hay que destacar que los resultados que se obtuvieron para el valor de pH no fueron concordantes con el comportamiento esperado teniendo en cuenta el valor de pKa de los analitos (8.27-8.45). En la Figura 12 se muestran los valores obtenidos para esta variable. Una posible interpretación de los resultados podría ser que los MWCNTs tengan impurezas ácidas que a valores de pH bajos se encuentren

en su forma neutra, permitiendo el máximo número de interacciones con los analitos mediante la formación de puentes de hidrógeno.

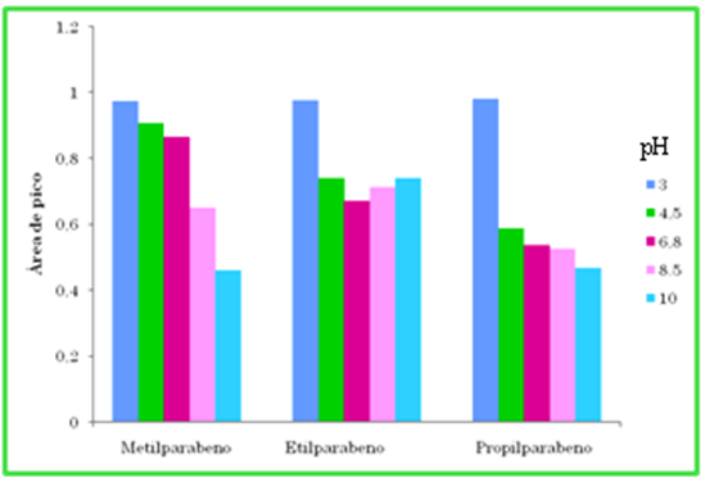

Figura 12. Comparación del comportamiento de los nanotubos de carbono a diferentes valores de pH en el proceso de extracción en fase sólida.

La detección se llevó a cabo con el detector no convencional de transferencia de carga-corona. Para evitar incompatibilidades del extracto en acetona con la fase móvil usada (acetonitrilo:agua 50:50, v/v), el extracto se evaporó con nitrógeno a temperatura ambiente y los analitos se redisolvieron en 500 µL de la fase móvil antes de su inyección en el sistema cromatográfico. La Figura 13 muestra un cromatograma correspondiente a una crema hidratante.

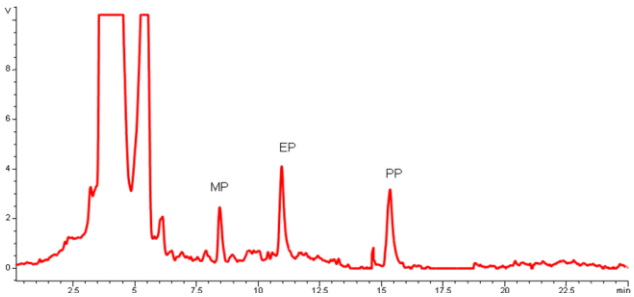

Figura 13. Cromatograma obtenido para una muestra de crema hidratante con una dilución 1:100 (v/v). MP: metilparabeno; EP: etilparabeno; PP: propilparabeno.

Si se compara con otros métodos (Tabla 3), el método propuesto presenta valores de recuperación y precisión similares a los métodos existentes. Sin embargo, la sensibilidad es más baja. Aunque si tenemos en cuenta la concentración en la que los analitos se encuentran en las muestras (mg L^{-1}) no es necesario una alta sensibilidad.

Tabla 3. Comparación del método propuesto con otros métodos alternativos para la determinación de parabenos en productos cosméticos.

Método[a]	LOD[b]	RSD[c] (%)	Recuperación (%)	Referencia
SDME-GC-MS	0.001-0.015 µgL^{-1}	<12.1	92-105	[10]
SFE/HPLC-MS	4.7-19.3 µg L-1	<18.6	-	[11]
HPLC-CL	1.9-5.3 µg L^{-1}	< 3.1	93-106	[12]
HPLC-UV	0.02-0.05 mg L^{-1}	< 3.2	98-105	[13]
FIA-SPE-MEKC	0.07-0.1 mg L^{-1}	< 2.3	93-102	[14]
SPE-HPLC-C-CAD	**0.5-2.1 mg L^{-1}**	**< 3.8**	**90-104**	**Capítulo 2**

[a] SFE: extracción de fluídos supercríticos; CL: quimioluminiscencia; FIA: análisis de inyección de flujo; MEKC:cromatografía electrocinética micelar
[b] LOD, límite de detección
[c] RSD, desviación estándar relativa

Cabe destacar que en el momento de publicación de este artículo, era la primera vez que se evaluaba el detector C-CAD con este fin. El diseño descrito en este trabajo se caracteriza principalmente por su simplicidad, la cual hace que sea una alternativa válida a los métodos existentes para la determinación de parabenos en productos cosméticos.

Con esto se cierra el apartado de Resultados y Discusión donde se ha abordado de manera comparativa el trabajo experimental desarrollado en esta Memoria de Tesis Doctoral.

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Conclusiones

Conclusions

A lo largo de la realización de esta Tesis Doctoral, se ha profundizado en el desarrollo de nuevas metodologías analíticas mediante el uso de detectores no convencionales, en concreto detectores de tipo evaporativo y espectrómetros de movilidad iónica. Asimismo, se ha avanzado en el empleo de nuevos medios de extracción. Todo ello ha resultado en el diseño, propuesta y evaluación de herramientas que permiten la simplificación y automatización de las metodologías desarrolladas que son a su vez más rápidas y económicas que las propuestas hasta la fecha. Las principales conclusiones derivadas de los resultados obtenidos en el trabajo de investigación presentado en esta Memoria se resumen a continuación.

- Se han desarrollado métodos cromatográficos empleando detectores de respuesta cuasi-universal, como el de transferencia de carga-corona y el evaporativo de dispersión de luz, para el análisis cuantitativo de varias familias de compuestos en una amplia variedad de muestras. Así se ha propuesto:

  - Un método analítico simple para la determinación de parabenos en productos cosméticos usando C-CAD como detector. Se evaluó el uso de los nanotubos de carbono como material sorbente en extracción en fase sólida como tratamiento previo de la muestra.

  - La determinación selectiva de vitaminas hidrosolubles usando C-CAD. La cuantificación se llevó a cabo a niveles traza para muestras de leche y a niveles de macro-compuestos en suplementos dietéticos.

  - Comparación de los dos detectores de tipo evaporativo, C-CAD y ELSD, a través de la determinación de azúcares en salsas,

productos lácteos y de repostería. Los métodos desarrollados se caracterizan por ser fiables, simples y robustos. El análisis de las muestras es directo, exceptuando una etapa de desproteinización en los productos lácteos.

- Se han desarrollado métodos analíticos empleando la espectrometría de movilidad iónica para realizar tanto análisis cualitativo como cuantitativo de uno o varios analitos presentes en una muestra. En este sentido:

  - Se ha demostrado la utilidad del acoplamiento de un espectrómetro de movilidad iónica con la técnica de microextracción en gota de líquido iónico para la determinación de 2,4,6-TCA. Para efectuar el acoplamiento, se ha empelado una interfase que permite el análisis de las muestras líquidas seleccionadas, agua y vino, las cuales no pueden inyectarse directamente en este detector.

  - Se ha incluido una columna multicapilar en el acoplamiento empleado previamente para aumentar la sensibilidad y selectividad de la determinación de 2,4,6-TCA en muestras de vino.

  - Se ha empleado un equipo comercial que acopla en línea un módulo espacio de cabeza, una columna multicapilar y un espectrómetro de movilidad iónica permitiendo el análisis directo, rápido y automatizado de 2,4,6-TCA procedente tanto de muestras líquidas (vino) como sólidas (tapones de corcho).

  - Se ha desarrollado un método analítico con el fin de clasificar muestras de leche enriquecidas con ácidos omega-3 y aceite de linaza

de acuerdo a los productos de degradación generados en diferentes condiciones de almacenamiento usando el perfil completo de volátiles derivado del análisis por espectrometría de movilidad iónica de las muestras. Por último, se pudo llevar a cabo la cuantificación de algunos de estos productos de degradación.

Estas aplicaciones han permitido incrementar la aplicabilidad de estos detectores no convencionales tanto a nuevas familias de compuestos como a muestras.

De los resultados derivados de esta Tesis Doctoral se puede concluir que los detectores no convencionales podrían ser implantados en laboratorios de rutina pues generan información fiable de manera simple, rápida y de bajo coste.

Autoevaluación de la Tesis Doctoral

Una vez finalizada la elaboración final de la Memoria de esta Tesis Doctoral puede resultar enriquecedor llevar a cabo un análisis crítico global de los avances obtenidos y evaluar tanto las mejoras de las metodologías propuestas como las posibles deficiencias y limitaciones de la investigación desarrollada.

En primer lugar, una de las aportaciones de la presente Tesis Doctoral ha sido el desarrollo de métodos cromatográficos para la determinación de parabenos, vitaminas y azúcares usando detectores de respuesta cuasi-universal. El escaso tratamiento de muestra y la simplicidad de uso de los detectores suponen un grado de simplificación notable frente a los métodos ya existentes. Además, el empleo de nanotubos de carbono como

medio de extracción en fase sólida ha permitido llevar a cabo el tratamiento de la muestra en una única etapa. Es cierto que los límites de detección alcanzados no son muy bajos pero si suficientes para llevar a cabo la determinación en las muestras analizadas de acuerdo con los límites impuestos por la legislación y el cliente. Esta limitación impide abordar la determinación de componentes minoritarios tanto en las muestras analizadas como en otras matrices. Para ello sería necesario evaluar el empleo de técnicas de microextracción que proporcionen elevados factores de preconcentración con gran selectividad para poder abordar estos problemas analíticos con los detectores de respuesta universal. En este contexto, sería interesante llevar a cabo la comparación entre el detector ELSD y C-CAD en base a la respuesta que proporcionan para componentes minoritarios, para poder evaluar su funcionamiento a esos niveles de concentración.

En segundo lugar, cabe destacar además, que se ha demostrado la aplicabilidad de diferentes espectrómetros de movilidad iónica para su uso en metodologías analíticas de vanguardia en el campo de la alimentación en muestras con diferente estado de agregación. En esta Tesis Doctoral se ha mejorado una de las propiedades básicas de la Química Analítica: la selectividad. Esto se ha podido conseguir gracias al empleo de una columna multicapilar previa a la introducción de los analitos al IMS. Se ha ampliado también la aplicabilidad de los líquidos iónicos, demostrándose su capacidad como medio de extracción en la etapa de microextracción en gota, permitiendo el análisis de muestras líquidas con IMS. También se ha empleado la IMS para estudiar el perfil volátil de muestras complejas. Los resultados obtenidos han respondido de forma apropiada a los objetivos marcados en los trabajos presentados. En cuanto a los trabajos experimentales dedicados a la determinación de 2,4,6-tricloroanisol, sería adecuado ampliar el estudio a otros haloanisoles problemáticos y también conocer las necesidades del sector

vinícola para que la metodología pueda ser implantada en sus laboratorios. Por otro lado, con el fin de asegurar la calidad de los productos enriquecidos, sería necesario ampliar el número y variedad de muestras para entrenar el sistema. Asimismo, sería interesante evaluar la aplicabilidad del acoplamiento HS-MCC-IMS para el análisis de muestras de interés clínico o ambiental para el análisis global de contaminantes volátiles.

With this doctoral work, we have expanded the development of new analytical methodologies by using unconventional detectors (specifically, evaporative detectors and ion mobility spectrometers). Also, we have investigated the use of new extraction media. The main outcome of this research is the design, development and assessment of tools for facilitating the simplification and automation of alternative methodologies that are more expeditious and economical than existing choices.

The main conclusions drawn from the results of the research work described in this Report are as follows:

- We have developed new chromatographic methods by using quasi-universal detectors such as corona charged aerosol detectors (C-CAD) and evaporative light scattering detectors (ELSD) for the quantitative analysis of various compound families in a different types of samples. Specifically, we have

  - developed a simple analytical method for determining parabens in cosmetic products with a C-CAD by using carbon nanotubes as sorbent for the solid-phase extraction of samples prior to their processing.

  - devised a selective determination method for water-soluble vitamins at trace levels in milk samples and macro levels in dietary supplements by using a C-CAD.

  - compared the performance of two types of evaporative detector (C-CAD and ELSD) in the determination of sugars in sauces, and in dairy and confectionary products. The ensuing methods are

simple, accurate and robust. Samples require no pretreatment other than deproteination of dairy products.

- We have developed effective analytical methods using ion mobility spectrometry (IMS) for qualitative and quantitative analyses of one or more analytes in the same sample. Thus:

  – We have demonstrated the usefulness of IMS in combination with ionic liquid drop microextraction for the determination of 2,4,6-tricloroanisole (2,4,6-TCA). The two techniques were coupled via an interface that affords the analysis of selected liquid, water and wine samples which cannot be directly injected into an ion mobility spectrometer.

  – We have incorporated a multi-capillary column in the previous arrangement to increase sensitivity and selectivity in the determination of 2,4,6-TCA in wine samples.

  – We have used a commercial ion mobility spectrometer on-line coupled to a headspace module and a multi-capillary column for the rapid, automated, direct determination of 2,4,6-TCA in both liquid (wine) and solid samples (cork stoppers).

  – We have developed an effective analytical method for classifying milk samples enriched with omega-3 acids and linseed oils in terms of the degradation products formed under different storage conditions as determined from the whole volatile profile derived from the ion mobility spectra for the samples. In addition, some degradation products were successfully identified.

The previous developments expand the scope of the unconventional detectors used here to new compound families and sample types.

Based on the results of this doctoral work, unconventional detectors produce accurate information in a simple, rapid, economical manner and can thus be useful in routine analytical laboratories.

Self-assessment of this Doctoral Work

This Section presents a critical overall analysis of the outcome of this doctoral work as regards the advantages of the new methodologies, and the potential deficiencies and limitations of our research work.

The most immediate contribution of this work is the development of new chromatographic methods for determining parabens, vitamins and sugars by using quasi-universal detectors. The need for little sample treatment and the operational simplicity of the detectors constitute two remarkable advantages over existing choices. In addition, using carbon nanotubes as a solid phase extraction sorbent material allows samples to be treated in a single step.

Although the resulting detection limits are not especially low, they are quite acceptable for analysing the sample types addressed in this work in accordance with existing regulations and the client's needs. The inability to quantify minor compounds in the sample types studied here and in other matrices might be overcome by using microextraction techniques affording high preconcentration factors and a high selectivity in order to circumvent this analytical shortcoming of universal detectors. For example, it would be interesting to compare the response of ELSD and C-CAD to minor

components with a view to assessing their performance at low analyte concentration levels.

We have also demonstrated the usefulness of different ion mobility spectrometers for vanguard analytical methodologies applied to food samples in various states of aggregation.

In this work, we have improved selectivity, one of the basic properties of Analytical Chemistry, by using a multi-capillary column prior to introduction of the analytes into the ion mobility spectrometer.

Also, we have expanded the scope of ionic liquids by using them as extraction media in the single-drop microextraction step to enable the analysis of liquid samples by ion mobility spectrometry (IMS).

In addition, we have successfully used IMS to examine the volatile profile of complex samples.

The outcome of this research therefore fulfils the initial objectives.

The experimental work conducted here on the determination of 2,4,6-tricloroanisole could be extended to other troublesome haloanisoles and suited to winemakers' needs as regards laboratory analyses.

Also, quality assurance for the enriched products could be improved by using a greater number and variety of samples to train the analytical system.

Finally, it would be interesting to assess the usefulness of the HS-MCC–IMS combination with samples of clinical or environmental interest with a view to the overall analysis of volatile contaminants.

Producción científica derivada de la Tesis Doctoral

ARTÍCULOS CIENTÍFICOS

1. *Determination of parabens in cosmetic products using multi-walled carbon nanotubes as solid phase extraction sorbent and corona-charged aerosol detection system.*

- I. Márquez-Sillero, E. Aguilera-Herrador, S. Cárdenas, M. Valcárcel.
- Publicado en la revista **Journal of Chromatography A** (1217, 2010, 1).
- Índice de Impacto: 4.194 (séptima posición de las revistas en el área de Química Analítica según el *Journal Citation Report* de 2010).

2. *Determination of 2,4,6-tricholoroanisole in water and wine samples by ionic liquid-based single-drop microextraction and ion mobility spectrometry.*

- I. Márquez-Sillero, E. Aguilera-Herrador, S. Cárdenas, M. Valcárcel.
- Publicado en la revista **Analytica Chimica Acta** (702, 2011, 199).
- Índice de Impacto: 4.555 (quinta posición de las revistas en el área de Química Analítica según el *Journal Citation Report* de 2011).

3. *Ion-mobility spectrometry for environmental analysis.*

- I. Márquez-Sillero, E. Aguilera-Herrador, S. Cárdenas, M. Valcárcel.
- Publicado en la revista **Trends in Analytical Chemistry** (30, 2011, 677).
- Índice de Impacto: 6.273 (segunda posición de las revistas en el área de Química Analítica según el *Journal Citation Report* de 2011).

4. *Direct determination of 2,4,6-trichloroanisole in wines by single-drop ionic liquid microextraction coupled with multicapillary column separation and ion mobility spectrometry detection.*

- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel.
- Publicado en la revista **Journal of Chromatography A** (1218, 2011, 7574).
- Índice de Impacto: 4.531 (sexta posición de las revistas en el área de Química Analítica según el *Journal Citation Report* de 2011).

5. *Evaporative-based detectors as global response instruments.*
- I. Márquez-Sillero, S. Cárdenas.
- Publicado en la revista **Enciclopedia of Analytical Chemistry 2012**
- DOI: 10.1002/9780470027318.a9238.

6. *Headspace-multicapillary column-ion mobility spectrometry for the direct analysis of 2,4,6-trichloroanisole in wine and cork samples.*
- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel.
- Publicado en la revista **Journal of Chromatography A** (1265, 2012, 149).
- Índice de Impacto: 4.531 (sexta posición de las revistas en el área de Química Analítica según el *Journal Citation Report* de 2011).

7. *Determination of water-soluble vitamins in infant milk and dietary supplement using a liquid chromatography on-line coupled to a corona-charged aerosol detector.*
- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel.
- Aceptado en la revista **Journal of Chromatography A** (2013).
- Índice de Impacto: 4.531 (sexta posición de las revistas en el área de Química Analítica según el *Journal Citation Report* de 2011).

8. *On-line headspace-multicapillary column-ion mobility spectrometry hyphenation as tool efficient determination of off flavours in foods.*
- I. Márquez-Sillero, S. Cárdenas, S. Sielemann, M. Valcárcel.
- Enviado a la revista **Journal of Chromatography A** para su publicación.

9. *Comparison of two evaporative universal detectors for the determination of sugars in food samples by liquid chromatography.*
- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel.
- Enviado a la revista **Microchemical Journal** para su publicación.

COMUNICACIONES A CONGRESOS

1. I Congreso Científico de Investigadores en Formación (Córdoba 2009)

- Empleo combinado de detectores de respuesta universal y nanopartículas de carbono para la determinación selectiva de parámetros de control de calidad de alimentos.
- I. Márquez-Sillero, E. Aguilera-Herrador, S. Cárdenas, M. Valcárcel.
- Comunicación oral.

2. XII Reunión del grupo regional andaluz de la sociedad española de química analítica, GRASEQA (Córdoba 2010)

-Determinación de parabenos en productos cosméticos usando nanotubos de carbono de pared múltiple como sorbente en extracción en fase sólida y el detector de aerosol cargado corona como sistema de detección.
- I. Márquez-Sillero, E. Aguilera-Herrador, S. Cárdenas, M. Valcárcel.
- Comunicación en póster y presentación oral-flash del mismo.

3. Jornadas doctorales andaluzas (Almería 2010)

- Mejora de las propiedades analíticas mediante la combinación de sistemas no convencionales de medida con nuevos sorbentes y medios de extracción.
- I. Márquez-Sillero, E. Aguilera-Herrador, S. Cárdenas, M. Valcárcel.
- Comunicación en póster y presentación oral del mismo.

4. I Congreso Científico de Investigadores en Formación en Agroalimentación del eidA3 y II Congreso Científico de Investigadores en Formación (Córdoba 2012)

- Mejora en la determinación selectiva de 2,4,6-tricloroanisol en muestras líquidas y sólidas mediante el uso de la espectrometría de movilidad iónica.
- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel

- Comunicación oral.

5. XII Reunión del grupo regional andaluz de la sociedad española de química analítica, GRASEQA (Málaga 2012)

-Determinación de 2,4,6-tricloroanisol en muestras de agua y vino combinando la microextracción en gota de líquido iónico y la espectrometría de movilidad iónica.

- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel
- Comunicación en póster y presentación oral-flash del mismo.

6. XXIII Reunión nacional- VII Congresso ibérico de espectroscopía (Córdoba 2012)

- Detección de productos de degradación producidos en la oxidación lipídica de ácidos grasos poliinsaturados omega-3 por espectrometría de movilidad iónica.
- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel
- Comunicación oral.

7. II Congreso Científico de Investigadores en Formación en Agroalimentación del eidA3 (Córdoba 2013)

- Detectores de tipo evaporativo para la cuantificación de vitaminas y azúcares en diferentes muestras de alimentos.
- I. Márquez-Sillero, S. Cárdenas, M. Valcárcel
- Comunicación oral.