Sample treatments prior to capillary electrophoresis-mass spectrometry

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Summary

Sample preparation is a crucial part of chemical analysis and in most cases can become the bottleneck of the whole analytical process. Its adequacy is a key factor in determining the success of the analysis and, therefore, careful selection and optimization of the parameters controlling sample treatment should be carried out. This work revises the different strategies that have been developed for sample preparation prior to capillary electrophoresis-mass spectrometry (CE-MS). Namely, the present work presents an exhaustive and critical revision of the different samples treatments used together with on-line CE-MS including works published from January 2000 to July 2006.

Keywords: Capillary electrophoresis/ Mass spectrometry/ CE-MS/ Sample pretreatment/ Couplings/ Hyphenated techniques/ Review

Contents

1 Introduction.

It is generally assumed that in order to provide an adequate chemical analysis any analytical method must include the following steps: sampling (sample must be representative of the object under investigation), sample preservation (sample should be kept stable until the analysis in completed), sample preparation, sample analysis *per se* and data treatment. Often, one of the the bottlenecks of this analytical process is sample preparation since it is usally a time-consuming and laborious step. The purpose of any sample preparation is the clean-up of the sample and/or the extraction, enrichment or preconcentration of the analytes, improving in this way the quality of the analytical results obtained. However, it has to be considered that any sample treatment will depend on both the sample nature and the following analytical technique that is going to be employed, requiring an almost case-by-case development. Therefore, no universal sample preparation is available.

The choice and optimization of a suitable sample pretreatment is not easy, especially with highly complex sample matrices like biological fluids (plasma, serum, whole blood, urine, etc.) or other natural matrices including e.g., foods, plant extracts or environmental samples. Ideally, sample preparation should be as simple as possible, not only because it will reduce the time required, but also because the greater the number of steps, the higher the probability of introducing errors. If possible, sample preparation should be carried out without loss of the analytes (or with the minimum loss) while eliminating as many interferences as possible from the matrix. Finally, it should also include, when necessary, a suitable dilution or concentration of the analytes in order to obtain an adequate concentration for the subsequent analysis. Sometimes, it may also include the transformation of the analytes into different chemical forms that can make

easier e.g., their separation or detection.

At the present time, developments in sample pretreatment strategies involve the use of new extraction materials, the use of automated protocols and/or its integration into miniaturized formats such as microchips or micrototal analysis sytems (μ-TAS) that should allow a rapid and sensitive analysis of the target analytes, especially in complex samples [1]. This research area has provided interesting and promising results and it will surely be one of the working areas in the future Analytical Chemistry.

Nowadays, the inherent advantages of the use of capillary electrophoresis (CE) as separation technique are well known and can be summarized in high separation efficiency, low analysis time, high resolution power and low consume of samples and reagents. It is at the moment one of the premier analytical separation techniques for the analysis of biological compounds such as peptides, proteins and polynucleotides and has been applied with success to a great variety of analytes [2-6]. Its different separation modes (CZE, MEKC, ITP, etc.) have allowed facing the problem of the separation of either neutral or charged analytes based on different physico-chemical properties (charge/mass ratio, molecular weight, polarity or isoelectric point). Besides, the different detectors available (UV-Vis, laser induced fluorescence (LIF), mass spectrometry (MS), electrochemical, etc.) have also broadened its use and applications, although UV is the most widely used detector in CE equipments so far.

Nevertheless, the small capillaries used in CE separations accommodate only small volumes of sample, which require either the use of a suitable on-line or off-line preconcentration procedure or the use of a more sensitive detector like LIF or MS. In this regard, MS gives information on the molecular weight of the analytes and enables the separation of co-migrating molecules increasing selectivity and specificity acting as a second dimension. Furthermore, it also compensates the migration time variation that

normally takes place in CE and provides unequivocal structural information via fragmentation patterns that can be obtained for instance via MS^n procedures. Therefore, the on-line coupling of CE with MS gives rise to an impressive analytical tool that combines the high resolution power and separation speed of CE with the high sensitivity and selectivity of the mass spectrometer [4, 7, 8]. However, in order to take advantage of the many possibilities derived from using CE-MS, it is of extreme importance a suitable selection of CE separation parameters (buffer composition, pH, preconcentration procedures), ionization technique (usually electrospray, ESI), and ESI and MS working parameters.

Thus, when developing a suitable CE-MS procedure several aspects have to be taken into account. Only highly volatile buffers can be used and they are tipically an aqueous or hydroorganic solution containing e.g., acetic acid, formic acid, ammonium hydroxide at low concentrations. The use of non volatile components like cyclodextrins (CDs), inorganic salts (e.g., containing sodium, phosphate, etc) or surfactants (as SDS) are precluded since they are strong inhibitors of ESI efficiency, increase the noise and reduce the sensitivity of the system. Different strategies have been proposed to overcome this limitation including the partiall filling technique [9].

An additional consideration prior to use CE-MS is the development of suitable sample pretreatment procedures [10]. As an example, direct injection of samples with a high protein content results in short capillary longevity (proteins precipitate and can irreversibly adsorb onto the silanol groups of the internal capillary wall) [11]. Furthermore, despite the selectivity of the mass spectrometer, highly complex samples may also induce some ionization suppression or even a complete loss of the MS signal. The objective of this work is, therefore, to provide an overview of the various samples preparation protocols that have recently been proposed prior to on-line CE-MS covering

relevant publications from January-2000 till July-2006.

2. Sample treatments.

In the time covered by the present review, different and interesting sample treatments prior to CE-MS have been proposed, which are sumarized in Table 1. As will be next discussed, in some occasions a single or simple treatment procedure was not enough to ensure the correct analysis of the sample, requiring the use of several consecutive sample treatments. In other cases, a single extraction or preconcentration procedure was enough to reduce the sample complexity or to improve the LODs achieved by CE-MS.

2.1 Liquid-liquid extraction (LLE).

This classical sample treatment allows the extraction of both trace analytes or macrocomponents. The selectivity and efficiency of the extraction process in LLE depends mainly on the election of the immiscible solvents, but other factors may also affect the distribution of the solute into both phases like the pH, the addition of a complexation agent, the addition of salts (salting out effect), etc. Although the use of LLE alone provides goods results in terms of extraction efficiency and clean-up of the samples, it is often carried out in combination with other preconcentration procedures as it will clearly be seen in the subsequent examples and sections.

Rudaz et al [12] developed a CE-MS stereoselective analysis of tramadol and its main phase I metabolite in plasma after LLE with hexane-ethyl acetate (80:20, v/v); samples were evaporated and redissolved in 0.01 M HCl. The best enantioseparation

was achieved using a coated polyvinyl alcohol (PVA) capillary and a 40 mM ammonium acetate buffer at pH 4.0 with 2.5 mg/ml sulfobutyl ether β-CD as the chiral selector. To avoid the entrance of the CDs in the MS and, as a result, the loss of the MS signal, the partial filling technique was applied.

Strickmann et al [13] developed an on-line capillary electrochromatography (CEC)-ESI-MS method for the determination of etodolac and metabolites in urine. CEC, although difficult to perform, is together with CZE the preferred CE mode for on-line coupling with MS, because of the highly volatile buffers frequently used. The drug and metabolites in urine could be analyzed by CEC-ESI-MS after LLE extraction using an equal volume of ethyl-acetate and then evaporated and redissolved into the separation buffer.

Wey et al [14] have developed a CE-ESI-MS method for the analysis and confirmation testing of morphine and related opioids in human urine by using a BGE containing 25 mM ammonium acetate at pH 9. High analyte concentrations (2-5 μg/ml) could be monitored in plain and diluted urine samples without further treatment directly by CE-MS. However, for the recognition of lower concentrations LLE at alkaline pH and solid phase extraction (SPE) were used and compared. Concerning the LLE procedure, a mixture of dichloromethane and dichloroethylene was used as extraction solvent. Mean recovery values ranged between 76 and 86% except for the metabolites nordihydromorphine and normorphine which were 14 and 25% respectively. However, the SPE procedure using a mixed mode polymer phase (namely, Bond Elut and Vac-Elut) provided higher recoveries, between 83 and 96% for all the compounds. For this particular application, SPE was shown to be more time consuming than LLE since an additional evaporation step was required to eliminate water from the eluate.

2.2 Solid-phase extraction (SPE).

Sample preparation using SPE was firstly introduced in the mid-1970s, replacing LLE due to its simplicity, selectivity and the better LODs that it provides. Since then, SPE has gained a wide acceptance due to the ease of automation, high analyte recovery, extraction reproducibility, ability to increase selectively analyte concentration and commercial availability of many SPE devices and sorbents, including the use of molecular imprinted polymers (MIPs) [15, 16].

Concerning the use of SPE it is probably the most widely used sample pretreatment procedure prior to CE-MS. Recently, Hernández-Borges et al [17] have determined five triazolopyrimidine sulfoanilide herbicides (cloransulam-methyl, diclosulam, florasulam, flumetsulam and metosulam) in soy milk by SPE-CZE-MS using C_{18} cartridges. For this purpose, CE-UV and CE-MS instruments were used. To increase the sensitivity of the method, normal stacking mode (NSM) was also used for on-line preconcentration of the SPE extract, providing LODs down to 74 µg/L. Mean recovery percentages ranged between 40 and 94% with good separations when working with aqueous solutions and SPE-NSM-CE-UV as shown in Figure 1A. However, the use of SPE combined with NSM-CZE-UV for analysis of the mentioned pesticides in soy milk did not provide suitable results because of the high number of interferences from the sample matrix (see Figure 1B). In order to overcome this limitation CE-MS was used. Thus, the main ESI-MS parameters (nebulizer pressure, dry gas flow rate, dry gas temperature and sheath-liquid composition) were optimized by means of a central composite design. Optimum separation buffer was composed of 24 mM formic acid and 16 mM ammonium carbonate at pH 6.4, while the sheath-liquid was composed of acetonitrile:water 82.5:17.5 (v/v) with 2% of TEA at 0.35 mL/h flow rate. The

combined use of SPE-NSM-CE-MS allowed the detection of these pesticides in soy milk as can be seen in Figure 1C.

Peterson et al [18] developed a specific CE-ESI-TOF-MS method for the determination of serotonin (5HT) and its precursos tryptophan (Trp) and 5-hydroxytryptophan (5HTP) in human platelet rich plasma. Analytes were removed from the plasma and preconcentrated by SPE using Oasis MCX columns with mean recoveries between 71.6 and 95.3%. Submicromolar LODs were obtained for standard mixtures of all the compounds except for 5HTP which had LODs in the low micromolar range. When the method was applied to the analysis of plasma extracts from healthy volunteers as well as from pathological samples the levels of both 5HT and Trp were determined while 5HTP was not found present in any of the samples. In a previous work of the same group [19] also a CE-ESI-TOF-MS method was used for the determination of catecholamines (dopamine, norepinephrine and epinephrine) and their O-methoxylated metabolites (3-methoxytyramine, normetanephrine, metanephrine) in urine. In this case the capillary was coated with polyvinyl alcohol and the injection of the samples was carried out electrokinetically. Catecholamines and metanephrines were removed from the urine samples and preconcentrated by SPE using cation-exchange sorbents (Oasis MCX) with mean recovery values over 80% for all the analytes, except for epinephrine (75%).

Vuorensola et al [20] have also analyzed eight catecholamines in aqueous and alcoholic (ethanol, methanol and 1-propanol) non-aqueous solutions by CE-MS but in this case using sheathless nanospray coupling. A comparison was made between different separation electrolytes for the separation of these compounds. Although non-aqueous media (in methanol) was more efficient than water, both methods were applied to the analysis of urine samples extracted with Oasis HLB cartridges using a previously

developed protocol [21]. The sensitivity of the non-aqueous nanospray method (0.48-1.30 μM) was only slightly better than that of a previous aqueous method using coaxial sheath-liquid coupling [22].

SPE procedures are often used after the LLE or solid-liquid extraction of the analytes assisted or not by ultrasounds, microwaves, etc. Rodríguez et al [23] have used C_8 cartridges for the extraction of pesticides thiabendazole and procymidone from fruits (apples, grapes, oranges, pears, strawberries) and vegetables (tomatoes) after a suitable sonication of the homogenized samples with methanol:water 1:1 for 15 min. Separation was achieved using a buffer of formic acid-ammonium formate at pH 3.5 with 2% of methanol (the sheath-liquid was the same as the separation buffer). LOQs of the SPE-CE-MS procedure (using also a stacking technique) ranged between 0.005 and 0.05 mg/kg, with mean recovery values of 64 and 75% for thiabendazole and procymidone, respectively.

Recently, Juan-García et al [24] have also extracted six pesticides (thiabendazole, pyrifenox, pirimicarb, pyrimethanil, procymidone and dinosed) from peaches and nectarines with a mixture water:acetone 1:1 (v/v) prior to their SPE extraction with C_{18} cartridges before their CE-MS or CE-MS/MS determination. In this case, a buffer consisting of 0.3 M ammonium acetate-acetic acid pH 4 in 10% methanol (the sheath-liquid had the same composition) was used. Recovery percentages ranged between 58 and 99% with relative standard deviation values (RSD %) between 9 and 19%. Under optimized CE-MS/MS conditions the minimum detectable levels of the six pesticides in spiked samples were between 0.01 and 0.05 mg/kg.

Sentellas et al [25] described the optimization of a clean-up and preconcentration procedure for the determination of fifteen heterocyclic amines in human urine samples. In this work, Oasis MCX and LiChrolut TSC cartridges were studied by using UV

detection. Peak intensities obtained after clean up for both sorbents were similar for most of the amines; however, Oasis MCX cartridges were selected because they provided slightly better recoveries for some of the amines. When urine samples were analyzed, interferences preventing the analytes identification were observed with both cartridges, that is why a LLE procedure using dichloromethane was used. The optimized clean-up procedure together with a previously published field-amplified sample injection (FASI)-CE-MS method [26] was used for the quantification of heterocyclic amines in hydrolyzed spiked human urine, obtaining LOD down to 0.3 ng/mL.

As it has been previously indicated, BGE solutions as well as sheath-liquid compositions should be volatile enough in CE-MS. In spite of this limitation, several works have appeared in which CDs are used as components of the separation electrolyte to analyze SPE extracts by CE-MS [27-29]. For instance, Servais et al [29] have used nonaquous CE (NACE)-ESI-MS with heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin (HDAS-β-CD) in the BGE for the enantioselective determination of low concentrations of salbutamol in SPE extracts from human urine. The selected separation electrolyte consisted of 10 mM ammonium formate and 15 mM HDAS-β-CD in methanol acidified with 0.75 M formic acid. This approach was applied to the quantitative determination of salbutamol enantiomers in human urine after SPE using Isolute HCX-3 cartridges. The SPE-NACE-MS allowed the determination of both compounds at concentrations ranging from 8 to 14 ng/mL.

2.3 Solid-liquid extraction.

Extraction from solid matrices has to be carried out after an adequate

homogenization or trituration of the sample, which can be enhanced (as well as the extraction efficiency) by lyophilization or by the use of liquid-nitrogen. The extraction of organic compounds, for example, involve the desorption of the analytes from the sample matrix and their later dissolution into the solvent, which is controlled by the solubility, mass transfer and matrix effects. Extraction can be improved by either assisting the process with ultrasounds, microwaves, etc. Sonication, for example, helps in the homogenization of the sample and, consequently, it can be used for the rapid an easy extraction of analytes from solid samples. Thus, Groom et al [27] have analyzed nitroaromatic and cyclic nitramine contaminants originated from military explosives and propellants (TNT, TNB, RDX, HMX, CL-20) in soil and marine sediments using sonication with acetonitrile together with sulfobutylether-β-cyclodextrin (SB-β-CD) assisted CE-ESI-MS. In this work, it was also stated that the presence of highly charged SB-β-CDs may affect the identification of target explosive analytes. Optimum BGE consisted of 10 mM SB-β-CD and 10 mM ammonium acetate at pH 6.9 using acetonitrile alone as sheath-liquid.

Feng et al [30] have analyzed several alkaloids (aconitine, hypaconitine, mesaconitine, brucine, strychnine, icajine, atropine, novacine) and their hydrolysis products in Chinese medicine preparations (Maqianzi, the seed of *Strychnos pierrian*, and Wutou, aconite root of *Radix aconiti praeparata*) by CE-ESI-MS. Pulverized samples were immersed in methanol overnight and afterwards ultrasonicated for 30 min prior to their CE-MS determination. Goodwin et al. [31] were able to separate and determine herbicides glyphosate and glufosinate and their derivatives (aminomethylphosphonic acid and methylphosphinicpropionic acid) in wheat samples using CE-ESI-MS with a sheathless interface. In this case wheat samples were extracted in a mixture of water-acetone 1:1 with magnetic stirring for 1 h. The separation buffer

was 1 mM ammonium acetate/acetic acid at pH 6.3 in a mixture methanol:water (50:50, v/v). The best reproducibility in terms of migration times and peak areas was obtained using a capillary coated with linear polyacrilamide. The extract was directly injected in the CE system and the final LOD was 1 μM in water and 2.5 μM in the wheat water-acetone extract.

Suomi et al [32] determined five neutral irioid glycosides (cyclopentanol monoterpene derivatives) in plant samples by micellar electrokinetic chromatography (MEKC) using SDS as surfactant. The separation system was coupled via a coaxial sheath-liquid flow ESI interface to a MS using the partial filling (PF) technique to avoid the entrance of the micelles in the MS. The separation, which was optimized by MEKC-UV, was achieved by using a BGE consisting of 100 mM SDS in 20 mM ammonium acetate at pH 9.5 (Figure 2). The compounds were detected as lithium adducts by the addition of 1.0 mM lithium acetate to the sheath-liquid (water-methanol 50:50, v/v). The extraction of the samples was carried out with boiling water for 60 min of the crushed dry leave samples (after 40 min at room temperature to wet the leaves throughly), and after evaporation to dryness, the extract was redissolvend in Milli-Q water and injected in the CE system. Catalpol, verbenalin, loganin and possibly 10-cinnamoyl catalpol were found in the examination of seven plants species in the genera *Plantago, Veronica, Melampyrum, Succisa* and *Valeriana*. LODs for the iridoid glycosides ranged were 25 mg/L except for catalpol which was 50 mg/L. In a second work by the same group, Suomi et al [33] separated a higher number of irioid glycosides (eleven) in several plants belonging also to the genera *Plantago, Veronica, Melampyrum, Succisa* and *Valeriana* by PF-MEKC-ESI-MS. In this case, extraction of the dry leave samples was also carried out with boiling water for 60 min.

Recently, Arráez-Román et al [34] have tested different liquid-phase extraction

procedures to establish which could provide the highest content of polyphenols and bitter acids from hop characterized by CE-MS (optimum BGE was 80 mM ammonium acetate at pH 10.5). For this purpose hop pellets were powdered and extracted with different solvents like hexane, methanol, methanol:water, etc. by shaking. Among them, the extraction with hexane to remove lipids, carotenoids and chlorophylls and later with methanol (to extract sugars, organic acids and phenolic compounds) allowed the detection of the highest number of compounds.

Juan-García et al [35] determined five quinolone residues (danofloxacin, enrofloxacin, flumequine, ofloxacin and pipemidic acid) in chicken and fish by CE-MS by solvent extraction of the minced muscle tissues. A sodium phosphate buffer at pH 7.0 was added to the spiked samples which were later extracted with dichloromethane (rotary shaking). The organic layers were then extracted with 0.5 M NaOH. This aqueous phase was adjusted to pH 7 and extracted with hexane to eliminate the fat and it was then passed through a C_{18} cartridge following a suitable SPE protocol. Mean recovery values of the whole procedure ranged between 45 and 99% for chicken samples and between 52 and 90% for fish samples. The proposed method is sufficiently sensitive to analyse these quinolone in both samples because the LOQs achieved (50 ng/g) were below the maximum residue limits (100-200 ng/g) established by the EU.

2.4 Solid-phase microextraction (SPME).

SPME was firstly developed by Pawliszyn and co-workers in 1989 and became commercially avaible in 1993 [36]. Since its development, SPME has been increasingly used since its setup is small and convenient, it can be used to extract analytes from very small samples, it provides a rapid extraction and transfer to analytical instrument and

can be easily combined with other extration and/or analytical procedures improving in a large extent the sensitivity and selectivity of the whole method.

The on-line coupling of SPME with CE has been described in several occasions [37, 38], however, the use of such coupling is still a non-resolved topic because of the very small injection volumes required in CE. As a result, SPME-CE analyses are typically carried out in an off-line mode, by manually desorbing the analytes in an appropriate organic solvent, and later introducing it into the CE system. Rodríguez et al. [39] carried out the analysis of a group of pesticides (ioxynil, *o*-phenylphenol, haloxyfop, acifluorfen, picloram) in fruit samples by using SPME prior to CE-MS. In that work, the buffer used consisted of 32 mM $HCOONH_4$/HCOOH at pH 3.1 while the sheath-flow was made of 32 mM separation buffer with a 20 % of methanol with 14 μL/min flow. After testing different SPME fibers, the use of CW-TPR allowed the extraction of these pesticides from water and fruit samples down to 0.02-5 mg/kg (LOQ).

Hernández-Borges et al. [40] tested different SPME fibers and CE-MS for the extraction and quantitative determination of a group of pesticides (pyrimethanil, pyrifenox, cyprodinil, cyromazine and pirimicarb) in orange and grape juices. The buffer used consisted of a volatile aqueous solution containing 0.3 M ammonium acetate/acetic acid at pH 4 while the sheath-liquid was made of a mixture isopropanol:water (65:35, v/v) at 0.22 ml/h flow. In this case, SPME parameters (e.g. extraction time, sodium chloride percentage, pH and desorption time) were optimized by means of a chemometrical approach. The best results were achieved by direct immersion of a PDMS-DVB fiber which allowed achieving LODs of these pesticides at concentrations down to 15 ng/mL in water samples and down to 40 ng/mL in fruit juices.

Other SPME modifications like stir-bar sorptive extraction (SBSE) [41] or fiber-in-tube SPME [42] have not yet been combined with CE-MS.

2.5 Pressurized liquid extraction (PLE).

Pressurized liquid extraction (PLE), also called accelerated solvent extraction, is a sample preparation technique in which a solvent at elevated temperature and pressure is used as extractant. By adequately chosing the solvent, its temperature and pressure it is possible to control, among other factors, the dielectric constant of the extractant and with that the polarity of the compounds that can be obtained. Moreover, PLE works in an automatic way, it requires small amounts of solvents and low extraction times. Therefore, PLE can provide fast extractions and purifications allowing testing a high number of extraction conditions under controlled conditions.

The possibilities of the combined use of PLE and CE-MS were recently demonstrated by Herrero et al [43-45]. PLE-CE-MS was applied to the extraction and characterization of the main antioxidants (i.e., polyphenols) from rosemary [43] and the extraction and characterization of phycobiliproteins from the microalga *Spirulina platensis* [44, 45]. In this latter case, a thorough optimization of both the PLE extraction conditions (including sonication of the sample prior to PLE) and CE-MS conditions had to be carried out, demonstrating that PLE-CE-MS can be a fast, automatic and highly informative method for natural products investigations [46].

2.6 Other procedures.

Apart from the previosly described sample treatment procedures, Table 1 also

shows different and interesting alternatives for this purpose. Thus, introduced in the mid-nineteenth century, soxhlet extraction has been one of the extraction methods more used until the development of modern extraction techniques. The need of cooled, condensed solvents for the extraction makes this technique a slow alternative (up to 24-48 hours of extraction) with a very high consumption of organic solvents that have to be evaporated, although with very high recoveries and also with multiple sample extraction possibilities. Concerning its combination prior to CE-MS several approaches have appeared [47-49]. Very recently, Edwards et al [48] have used soxhlet extraction in combination with LLE and SPE for the characterization by CE-ESI-MS of secondary metabolites (flavonoids) from the antihyperglycaemic plant *Genista tenera*. In this case, air-dried and powdered plants were extracted in a soxhlet apparatus with ethanol. After filtration and evaporation the residue was redissolved in water and extracted successively with diethyl ether, ethyl acetate and butanol. After another evaporation and redissolution of part of the extract a SPE procedure with C_{18} was carried out. Optimum buffer was composed of water:2-propanol (95:5, v/v) containing 10 mM ammonium carbonate at pH 9.25. The CE-MS study of the extract allowed the identification of five flavonoid aglycones, five flavonoid-monoglycosides, two flavonoid-diglycosides, one flavonoid-triglycoside, thee monoacetyl-flavonoids, one diacetyl-flavonoid and one acetyl-flavonoid-glycoside. Wahby et al [49] have also used soxhlet extraction for the extraction of atropine (tropane alkaloid) and choline (quaternary base) in hairy root cultures of *Cannabis sativa L.* Hairy root cultures were rinsed with tap and distilled water, frozen in liquid nitrogen and lyophilized. The dry material was ground to a fine powder and extracted in a soxhlet apparatus with 70% of aqueous methanol for 16 h. After cooling, the extracts were filtered and concentrated. Both compounds could be determined in the samples with LODs of 18 mg/L for choline and 320 µg/L for atropine

using a BGE of 20 mM ammonium acetate at pH 8.5 and a sheath-liquid composed of 50:50, v/v 2-propanol:water with 0.5% (v/v) formic acid 0.18 mL/h.

The combination of automated sample preparation in CE is especially useful for the analysis of complex samples [50] since it can improve the selectivity and sensitivity of the determination as well as to decrease the time involved in the sample treatment [50, 51]. One of the main lines of research in this area is the combination of flow injection systems with CE and, in a less extent, with CE-MS. Thus, Santos et al [52] reported a new method for the separation and detection of 9 biogenic amines by the used of a flow manifold coupled to a CE-ESI-MS for the automatic filtration of the samples and their insertion into the CE vials. The on-line filtration was carried out using a flow injection system coupled to the CE instrument. The BGE was composed of 25 mM citric acid at pH 2.0. Two injection modes (hydrodynamic and electrokinetic) were tested. Although electrokinetic injection provided better sensitivity, it was also found to give worse precision and linear range and, therefore, hydrodynamic injection was selected. The method allowed the detection of amines between 0.018 and 0.09 μg/mL. The method was applied to the determination of biogenic amines in red and white wines with mean recovery values around 100%.

The use of microwave radiation for sample pretreatment has attracted growing interest in the past few years and has yield a numerous amount of publications [53-56]. Microwave radiation provides a homegeneous and instant heating of the sample yielding into very quick and effective extraction/digestion and thus strongly decreasing sample pretreatment times. Van Lierde et al [57] used microwave-assisted acid digestion of porcine and human skin to extract chromium species from these samples. The mechanism of chromium transport through the skin and the relationship between chromium allergy and chromium species (in vitro permeation experiments) was studied.

For this purpose, CE-was used with inductively coupled plasma-mass spectrometry (ICP-MS) using a BGE composed of 50 mM phosphate buffer (pH 2.5). For the digestion of the samples, skin membranes were dried at 30ºC for 24 hours, after that HNO_3 and H_2O_2 were added. Digestion was carried out at different microwave intensities for a total of 25 min. The LODs of the method ranged between 6 and 12 µg of Cr per liter.

3 Microfluidic devices.

Clearly, development and/or use of microchip-CE are not objectives of this paper, however, microchip-CE devices deserve a special attention because they can automate sample preparation and, furthermore, they can integrate this step together with the chemical analysis under a single format that may allow a ultrarapid and sensitive analysis of the target analytes [58]. However, at the moment most of the applied aproaches suffer from several limitations regarding their fabrication, manipulation or the LODs that can be achieved. This can explain the very low number of publications found showing the on-line coupling of microchip-CE with MS.

A recent application of a microbead-packed polydimethylsiloxane (PDMS) microchip with an integrated electrospray emitter for sample pretreatment prior to sheathless ESI-TOF-MS was presented by Lindberg et al [59]. This system was applied for the desalting and enrichment of six neuropeptides from a physiological solution. Figure 3 shows a schematic picture of the PDMS microchip design used in that work. Electrical contact for the sheathless ESI was achieved by coating the integrated emitter with a conductive graphite powder after applying a thin layer of PDMS as glue. Both the coating and the bond of the PDMS structures were found to have a very good

durability (a continuous spray was obtained over 800 h). Another PDMS microfluidic system was previously developed by the same group [60] and applied to the analysis of peptides but in this case only sample injection, separation and ESI emitter structures were integrated in a single platform. As found in the literature, PDMS microchips with an integrated ESI-emitter have been fabricated using different principles [61-66]. As an example, Dahlin et al [62] presented a PDMS-based microchip for in-line SPE-CE with an integrated electrospray emitter tip coupled to a TOF-MS. The chip was fabricated in such a way that mixed PDMS was cast over steel wires in a mold. The removed wires defined 50 μm cylindrical channels where fused silica capillaries were inserted. The microchip was fabricated in a two-level cross design. In one of these channels hypercross-linked polystyrene beads acted as SPE sorbent for desalting. In this work, six-peptide mixtures at different concentrations were dissolved in physiological salt solutions and injected, desalted, separated and sprayed into the MS for the analysis. LODs were in the femtomole levels.

Microfluidic devices have also been used for in-line digestion of proteins [67, 68]. Wang et al [67] have proposed the use of a microfluidic device with a CE channel connected to a MS (via an ESI interface) which contains a digestion bed on a monolithic substrate to carry out the in-line protein digestion. The application of this device for the rapid digestion, separation and identification of proteins was demonstrated for melittin, cytochrome c and bovine serum albumin. The rate and efficiency of the digestion was related to the flow rate of the substrate solutions through the reactor. For cytochrome c and bovine serum albumin the digestion time was 3-6 min at room temperature, while for melittin was 5 s. Microdevices provide a convenient platform for automated sample processing in proteomic applications.

4 Use of stacking techniques in CE-MS.

Although strictly speaking the use of stacking techniques (except on-line SPE) cannot be considered part of the sample treatment, we would like to include a brief comment here about the use of these techniques together with CE-MS. On-line preconcentration strategies based on sample stacking [69-71], sweeping [72] and/or solid-phase extraction (SPE) [73-75] have shown their usefulness for improving the limits of detection (LOD) achieved by CE. Concerning the use of these techniques together with CE-MS they are not easy to apply and, in some cases, their use is limited. For instance, many of these preconcentration strategies involve the use of CDs, surfactants, and other non-volatile compounds that are precluded in CE-MS or can affect the stability of the electrical circuit in CE-ESI-MS. For example, the use of stacking with matrix removal (SWMR) is not possible in CE-MS since there is not outlet vial necessary in this case to reverse the polarity and to eliminate the sample matrix. In Table 1 it can be observed that these techniques are not widely applied in CE-MS.

The use of the electrokinetic injection in the mode called field-enhancement sample injection (FESI) also called field-amplified sample injection (FASI) or field-amplified sample stacking (FASS) is one of the most commonly stacking techniques in CE-MS, because, despite the presence of the siphoning effect that can take place, the sensitivity improvement can be high, although in this case only one type of charged analyte (cations or anions) can be introduced into the capillary [25, 26, 76-79].

Hernández-Borges et al [17] have used normal stacking mode (NSM) for the preconcentration of pesticides after their SPE extraction from soy milk samples. This technique is easy to apply because only a low conductivity matrix is required (which

can be achieved by the use of organic solvent) since focusing takes places due to the abrupt change in the local electric field between the sample matrix and the BGE. In this case, the stacking was achieved by injecting a high amount of the sample (up to 100 s at 20 psi) dissolved in pure acetonitrile. This specific type of stacking is often called *acetonitrile stacking* because of the good sensitivity improvement that the use of acetonitrile alone in the sample matrix has provided, which has also been observed by different authors [80-84].

5 Conclusions and future outlook.

Some current trends in the todays' sample pretreatment area are expected to continue in the future as important research areas within this attractive field of Analytical Chemistry. This is the case for the search of new extraction materials including the development of molecular imprinted polymers (MIPs) to adsorb specific analytes mimicking for instance immunorecognition. These new extraction materials can play a definitive role in the development of completely automated analytical processes able to provide information on analyte composition and concentration without the intervention of the operator. In this regard, the integration of sample preparation devices into miniaturized formats (e.g., microchips, μ-TAS) seem to be a very atractive way to achieve this goal while increasing even more the throughput and analysis speed of these methods. These future procedures combined with on-line stacking techniques and CE-MS can give rise to an ever more impressive and powerful analytical system.

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Figure captions

Figure 1.- NSM-CE-UV electropherogram of: A) a standard solution containing ca. 1 mg/L of each pesticide and; B) a SPE extract from soy milk sample containing 200 µg/L of each pesticide. Injection: 60 s at 0.5 psi. Running electrolyte: 24 mM formic acid, 16 mM ammonium carbonate at pH 6.4; Total length: 57 cm (50 cm effective length); Voltage: +23 kV; Temperature: 22ºC. (1) Metosulam; (2) Cloransulam-methyl; (3) Diclosulam; (4) Florasulam; (5) Flumetsulam. C) Extracted ion electropherograms of a soy milk sample containing 200 µg/L of each pesticide analyzed under SPE-NSM-CE-ESI-MS optimized conditions. Redrawn from [17] with permission.

Figure 2.- (a) An on-line PF-MEKC-ESI-MS electropherogram of the mass spectrometric data. The capillary was 80 cm long and applied voltage was +15 kV (current 10 µA). Sample was injected at 50 mbar pressure for 10 s. The electrolyte solution contained 20 mM ammonium acetate at pH 9.2 and a solution of 100 mM SDS was injected for 200 s at 50 mbar pressure. The sheath-liquid contained 1 mM lithium acetate dissolved in water-methanol (50:50 v/v) and it was pumped to the electrospray interface at 180 µL/min. Mass area of 100-600 m/z was scanned. (b) A PF-MEKC-UV electropherogram of the sample of (a). Compounds were detected at 20 cm. Peak assignments: (1) catalpol; (2) ketologanin; (3) verbenalin; (4) loganin; (7) 10-cinnamoyl catalpol. Reprinted from [32] with permission.

Figure 3.- Schematic picture of the PDMS microchip design. A) Shows the microchip as mounted on the holder in front of the MS. Graphite coated emitter was placed between two brass plates (B) to which the high voltage was applied. Holder (C) was

mounted on an *xyz*-adjustable table for easy alignment of the microchip in front of the TOF-MS orifice. Reprinted from [59] with permission.

Figure

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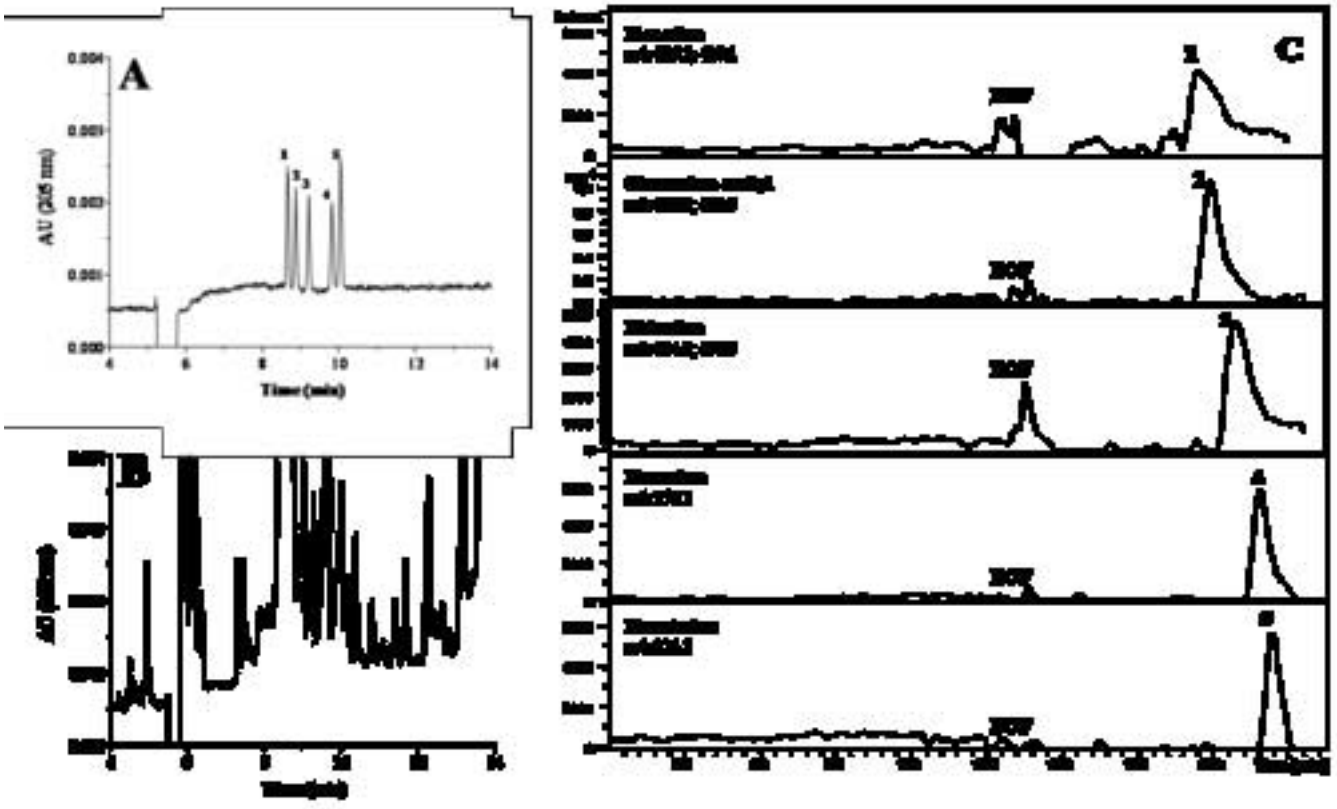

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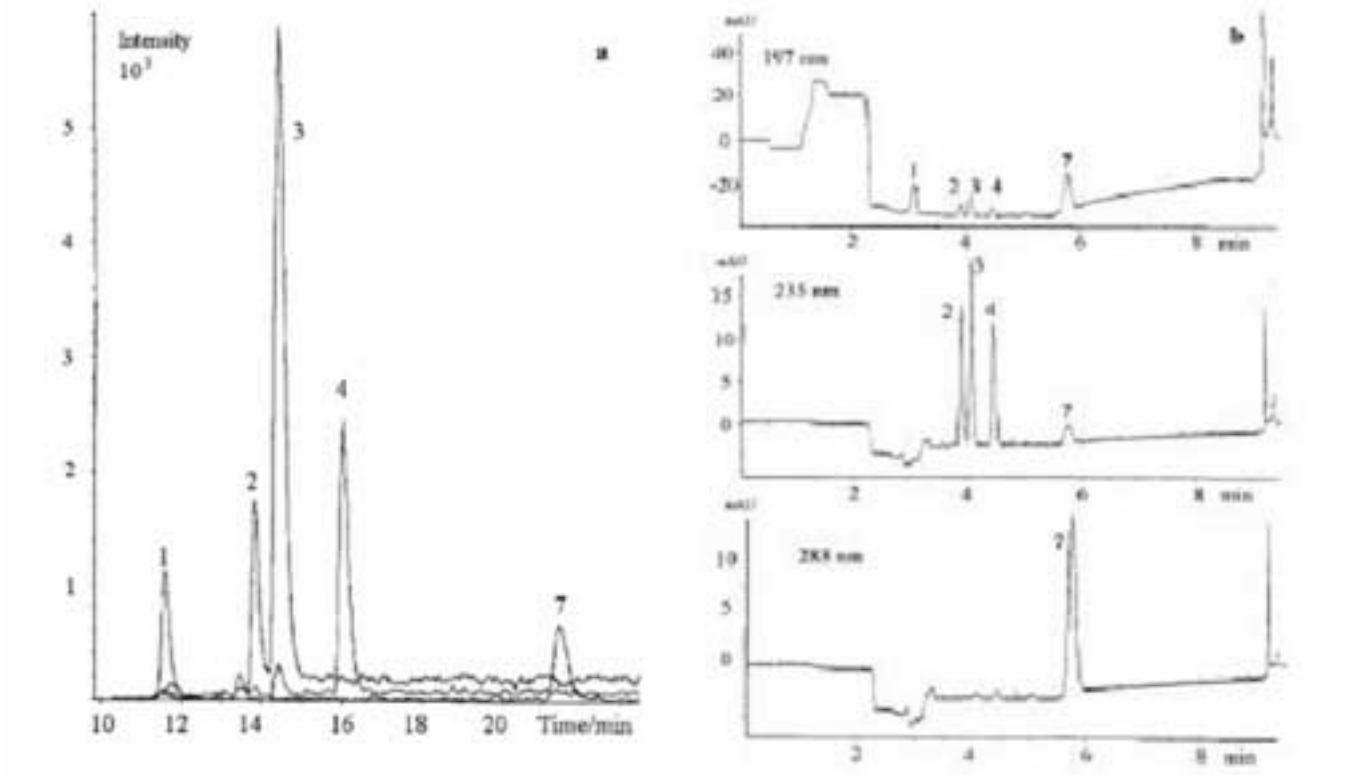

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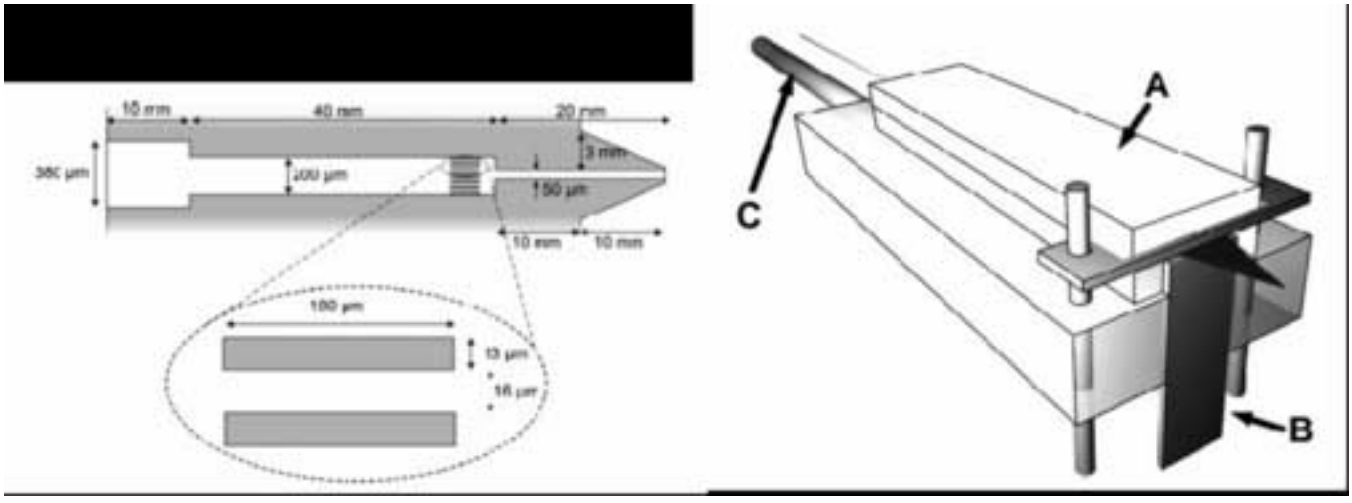

Tables

Table 1.- Some examples of sample treatments, analytes and matrices studied by CE-MS.

Analyte	Matrix	Treatment	Interface	Analyzer	Buffer	Observations	References
Hops acids, oxidized derivatives and iso-α-acids	Beer, hops pellets	Extraction (acetone/water) for hops pellets; SPE for beer	ESI (sheath-liquid: 2-propanol:water 50:50 v/v, 0.1% TEA, 3 µL/min)	IT	160 mM $(NH_4)_2CO_3$-NH_4OH pH 9	-	[85]
Glycoproteins	Bovine proteins	Microcon filtration, enzymatic deglycosilation	ESI (sheath-liquid: 1% HOAc in 2-propanol:water 1:1, 4 µL/min)	IT, TOF	Several buffers	Coated capillary	[86]
Peptides	Horse cytocrome c and myoglobin	Enzymatic digestion, SPE	ESI (sheath-liquid: 0.1% HCOOH in 50% MeOH, 3 µL/min)	IT	20/40/40 ACN/100 mM $HCOONH_4$ pH 3/water v/v/v	FASI. LOD: 10^{-9} M	[79]
Carbohydrates	Urine	-	ESI (sheathless)	QTOF	50 mM NH_4Ac-32% NH_3 pH 11 and 12	-	[87]
Glycopeptides	Plasma	Lyophilization, digestion	ESI (sheath-liquid: 2-propanol-water 1:1 v/v, 0.4% HCOOH, 2.5 µL/min)	QIT	50 mM $HCOONH_4$ pH 2.7; 50 mM triethylammonium acetate pH 5.0; 50 mM $HCOONH_4$ pH 8.0	MS^n, off line MALDI-TOF-MS, coated capillaries	[88]
Tobacco-N-nitrosamines	Rabbits' serum	SPE	ESI (sheath-liquid: MeOH-water 50:50 v/v, 0.5% formic acid, 10 µL/min)	IT	75 mM ammonium formate (pH 2.5) or citrate (pH 2.4)	-	[89]
Cytokinins	Coconut water	SPE	ESI (sheath-liquid: 0.3% formic acid in 50% v/v MeOH:water, 4 µL/min)	IT	25 mM amonium formate/formic acid (pH 3.4), 3% v/v ACN	MS^2, stacking, LOD: 0.05-0.18 µM	[84]
Phenolic compounds	Virgin olive oil	SPE	ESI (sheath-liquid: 2-propanol:water 60:40 v/v, 0.1% v/v TEA)	IT	60 mM NH_4OAc pH 9.5 with 5% 2-propanol	Standards obtained by semipreparative HPLC	[90]
Neuropeptides	Physiological salt solution	Microbead-packed PDMS microchip	ESI (sheathless)	TOF	25:75 v/v ACN:10 mM acetic acid	Microchip; LOD: 20 fmol	[59]
Chromium species	Porcine and human skin	Microwave assisted digestion	ICP	SF	50 mM phosphate buffer pH 2.5	LOD: 6-12 µg/L	[57]
^{10}B-BPA (boronophenylalanine)	Cell culture	Trypsin digestion, freeze-thawing cycles-ultrasonication, ulrafiltration	ESI (sheath-liquid: 5 mM NH_4Ac in 50 % v/v MeOH- water, 10 µL/min)	IT	0.5 M HCOOH	LOD: 3 µM; use of HR-ICP-MS	[91]
Flavonoids	Plant *(Genista tenera)*	Soxhlet, LLE, SPE	ESI (sheath-liquid:	IT	Water:2-propanol 95:5, v/v,	MS^2	[48]

			IPA:water 50:50 v/v, 0.5 µL/min)		10 mM ammonium carbonate (pH 9.25)		
Choline, atropine	Hairy root cultures of *Cannabis sativa L.*	Soxhlet	ESI (sheath-liquid: 50:50 v/v 2-propanol:water, 0.5% v/v formic acid 0.18 mL/h)	IT	20 mM NH_4OAc, pH 8.5	LOD: 18 mg/L (choline), 320 µg/L (atropine)	[49]
Polyphenols, bitter acids and oxidation products	Hops	Extraction with different solvents	ESI (sheath-liquid: 60:40 v/v, 2-propanol:water, 0.1% TEA, 0.28 mL/h)	IT	80 mM NH_4OAc/NH_4OH, pH 10.5	Caracterization of the methanolic extract of hops	[34]
Quinolone residues	Chicken, fish	Solvent extraction, SPE	ESI (sheath-liquid: 60 mM $(NH_4)_2CO_3$, pH 9.2, 10 µL/min)	QIT	60 mM $(NH_4)_2CO_3$, pH 9.2	MS^n, LOD: 20 ng/g	[35]
Glycopeptides	Urine	Gel filtration chromatography, anion exchange chromatography	ESI (sheathless)	QTOF	0.1 M HCOOH in MeOH:water 6:4, v/v	LOD: 0.05-0.25 mg/mL	[92]
Isoquinoline alkaloids	Herb(*Fumaria officinalis*) and phytopharmaceuticals	Soxhlet, LLE, SPE, ultrasounds	ESI (sheath-liquid: isopropanol-water 1:1 v/v, 3µL/min.)	IT	ACN-MeOH 9:1 v/v, 60 mM NH_4OAc and 2.2 M HOAc	MS^2	[47]
Antioxidants	*Rosmarinus officinalis L.*	PLE	ESI (sheath-liquid: 2-propanol-water 60:40 v/v, 0.1% v/v TEA; 0.24 mL/h)	IT	40 mM NH_4OAc/NH_4OH, pH 9	-	[43]
Nitroaromatic and cyclic nitramines	Soil and marine sediment	ACN sonication	ESI (sheath-liquid: 100% ACN, 6 µL/min)	QIT	10 mM SB-β-CD-10 mM NH_4OAc (pH 6.9)	LOD: 0.025-0.5 mg/L	[27]
Caffeine and metabolites	Urine	SPE	ESI (sheath-liquid: MeOH-water-HCOOH 79.7:19.8:0.5 v/v/v, 0.5 mL/min)	Q	50 mM $(NH_4)_2CO_3$, pH 11.0	-	[93]
Pesticide residues	Peaches and nectarines	Solvent extraction, SPE	ESI (sheath-liquid: 0,3 M NH_4OAc-HOAc, pH 4, in 10% MeOH, 5 µL/min)	QIT	0.3 M NH_4OAc-HOAc, pH 4, in 10% MeOH	MS^3, LOQ: 0.001-0.2 mg/kg	[24]
Salbutamol enantiomers	Human urine	SPE	ESI (sheath-liquid: ACN-water 75/25 v/v, 0.1% HCOOH, 2.5 µl/ min)	IT	10 mM $HCOONH_4$ and 15 mM HDAS-β-CD in MeOH, 0.75 M HCOOH	LOQ: 18-20 ng/mL	[29]
Methylenedioxy-derivates of amphetamine	Urine	SPE	ESI (sheath-liquid: ACN-water-HOAc 50:49.5:0.5)	IT	50 mM NH_4OAc/ HOAc, pH 4.5	LOD: 0.31-4.29 ng/mL	[94]
Pesticides	Soy milk	SPE	ESI (sheath-liquid: ACN/water 82.5:17.5 v/v, 2% TEA, 0.35 mL/h)	IT	24 mM HCOOH and 16 mM $(NH_4)_2CO_3$, pH 6.4	LOD: 74-150 µg/L	[17]
Phosphorylated and acidic	Escherichia coli DH5-α	Cell lisation	ESI (sheathless)	QIT	80% v/v 20 mM NH_4OAc	MS^2	[95]

metabolites in prokaryotes					pH 9.5 and 20% v/v 2-propanol		
Selenium	Nuts (*Bertholletia excelsa*)	Defatted nuts hydrolization	ICP	Q	Ammonium pH 9.25 with 2% v/v OFM anion-BT	Study of the association of selenium to proteins; electrokinetic injections	[96]
Polypeptides and proteins	Urine	SPE, lyophilization	ESI (sheath-liquid: 30% MeOH, 0.5% HCOOH)	TOF	30% MeOH, 0.5% HCOOH, 69.5% water	Identification of protein pattern in Type 1 diabetics	[97]
Neuropeptides	-	SPE	ESI (sheathless)	TOF	25:75 ACN:10 mM HOAc	PDMS microchip, LOD: 0.1 µg/ml	[62]
Polypeptides	Human urine	Ultrafiltration, SPE, lyophilization	ESI (sheath-liquid: 30% v/v isopropanol, 0.4% v/v HCOOH, 2 µL/min)	TOF	20 % ACN, 0.25 M HCOOH, 79.5% water	Indentification of polypeptides and patterns of polypeptides specific for prostate cancer	[98]
Lipophiclic peptaibol alamethicin	Culture broth of *Trichoderma viride*	Preparative HPLC	ESI (sheath-liquid: 2-propanol:water 1:1 v/v; 1% HCOOH; 4 µL/min)	IT, TOF	NACE: 12.5 mM $HCOONH_4$ in MeOH (pH_{app}= 7.4) Aqueous: 25 mM borate pH 11.0	MS^n	[99]
Peptide mixture	-	Digestion	ESI (sheath-liquid: MeOH-water 50:50 v/v; 0.05 v/v HCOOH, 4 µL/min)	IT	0.9 M HCOOH, pH 2	Peptide modeling; characterization of enzyme cleavage patterns. MS^2	[100]
Amino acids	Orange juice	Derivatization with FITC and DNS	ESI (sheath-liquid: MeOH-water 50:50 v/v with 25% 100 mM NH_4Ac pH 6 5 mM β-CD; 3.5 µL/min)	IT	100 mM NH_4Ac pH 6 5 mM β-CD	Capillary coating,	[101]
γ-glutamyl-S-ethenyl-cysteine (GEC)	*Vicia narboneusis L.* seeds	Solvent extraction stirring or ultrasounds	ESI (sheath-liquid: MeOH-water 50:50 v/v 0.1% v/v HOAc, 3 µL/min)	IT	20 mM NH_4HCO_3 pH 7	LOD: 0.021 mg/mL	[102]

Proteins	*Spirulina platensis* microalga	Sonication, PLE, ultrafiltration, precipitation-dialysis-freeze drying	ESI (sheath-liquid: water-2-propanol 75:25 v/v, 0.5 % v/v HOAc, 6 µL/min)	IT	40 mM ammonium hydrogen carbonate pH 7.8 in water-ACN-2-propanol 45:50:5 % v/v/v	PLE optimization	[45]
Proteins	*Spirulina platensis* microalga	Sonication, PLE, freeze drying	ESI (sheath-liquid: water-2-propanol 75:25 v/v, 0.5 % v/v HOAc, 6 µL/min)	IT, TOF	40 mM ammonium hydrogen carbonate pH 7.8 in water:ACN 2-propanol 45:50:5 % v/v/v	-	[44]
Anthocyanins	Wine and wine musts	SPE	ESI (sheath-liquid: MeOH:water 80:20, 0.25% v/v HOAc)	IT	200 mM monochloroacetate-ammonium, pH 2 or 200 mM borate-ammnonium, pH 9	Acidic and basic BGE. LOD: 0.8-1.5 mg/L (acidic); 4-10 mg/L (basic)	[103]
Antidepressants	Water	SPE	ESI (sheath-liquid: 5 mM $HCOONH_4$ in 8:2 isopropanol/water, 1 µL/min)	QTOF	1.5 M HCOOH, 50 mM $HCOONH_4$ in ACN/water 85:15	LOD: 22-280 µg/L	[104]
Basic proteins	Chicken and turkey egg white, wine, minced meat	Lyophilization (white egg); meat (homogenizaiton and buffer extraction)	ESI (sheath-liquid: MeOH:water 50:50 v/v, 0.05 % v/v HOAc 4 µL/min)	IT	75 mM NHOAc/ HOAc, pH 5.5	Polymer capillary coating; LOD: 2.9 fmol; addulteration detection	[105]
Manganese	Liver	Homogenization, liquid nitrogen, extraction in Tris-HCl	ICP	Q	10 mM Tris-HCl pH 7.4	Speciation study; LOD: 1.1 µg Mn/L	[106]
Glycoproteins	Plasma	Affinity chromatography, lyophilization	ESI (sheath-liquid: 2-propanol/1 M HOAc 1:1 v/v, 3 µL/min)	QIT	1 mM HOAc, 4 M urea	Characterization of glyco isoforms, coated capillaries	[107]
Oxycodone phase I and II metabolites	Urine	SPE	ESI (sheath-liquid: water-methanol 1:1 v/v, 1% formic acid, 5.0 µL/min)	IT	20 mM ammonium acetate pH 9	MS^n, computer simulation of fragmentation	[108]
Benzodiazepines	Urine	SPE	ESI (sheath-liquid: MeOH-water 80:20 v/v, 2 µL/min)	IT	100 mM formic acid, 1 mM TEA	MS^2, use of dynamically coated capillaries (CEofix); LOD: 50-100 ppb	[109]
Heterocyclic amines	Urine	LLE-SPE	ESI (sheath-liquid: MeOH-20 mM HCOOH 75:25, 5 µL/min)	IT	16 mM HCOOH/$HCOONH_4$ 40 mM pH 4.5, 60% MeOH	LOD: 0.3-45 ng/mL	[25]

Imazamox pesticide	Water	SPE	ESI (sheath-liquid: MeOH $HCOONH_4$ (5 mM) 50:50 v/v, pH 3.7, 4 µL/min)	IT	10 mM $HCOONH_4$ in 0.01% MeOH-water, pH 7.0	LOD: 20 ng/L	[110]
Glycosaminoglycan oligosaccharides	Human embryonic kidney 293 cells	Dialysis, lyophilization, digestion	ESI (sheathless)	QTOF	50 mM NH_4OAc pH 12.0 in water:MeOH 40:60 v/v	MS^2	[111]
Serotonin, tryptophan and 5-hydroxytryptophan	Plasma	SPE	ESI (sheath-liquid: MeOH/water 60:40 v/v, 0.2% HCOOH, 2 µL/min)	TOF	1.5% HCOOH (pH 2.07)	LOD: 0.13-3.23 µM	[18]
Pesticides	Fruit juices	SPME	ESI (sheath-liquid: isopropanol-water 65% v/v, 0.22 mL/h)	IT	0.3 M HOAc, pH 4	Chemometric optimization. LOD 40-150 µg/L	[40]
9 biogenic amines	Red and white wines	FI system	ESI (sheath-liquid: 70:30 v/v MeOH/water, 1.0% HCOOH, 4 µL/min)	Q	25 mM citric acid pH 2	LOD 0.018-0.09 µg/mL	[52]
Pesticides	Water, grape, apple, orange, tomato	SPME	ESI (sheath-liquid: 32 mM $HCOONH_4$-HCOOH pH 3.1 + 20% MeOH, 14 µL/min)	Q	32 mM $HCOONH_4$-HCOOH pH 3.1	LOQ: 0.02-5 mg/Kg	[39]
Peptides and proteins	Urine	SPE, lyophilization	ESI (sheath-liquid: 30% v/v MeOH, 0.5% v/v HCOOH, 5 µl/min)	TOF	30% v/v MeOH, 0.5% v/v HCOOH, pH 2.4	-	[112]
Dopamine and methoxycatecholamines	Urine	Enzymatic hydrolysis, cation exchange extraction, SPE	ESI (sheath-liquid: MeOH-water 80:20 v/v, 0.5 % v/v HOAc, 6 µl/min)	IT	50 mM NH_4OAc HOAc, pH 4	Comparison CE-UV, CE-MS with LC-EC; LOD: 0.7-1.4 µM	[113]
Alkaloids	Herbs (*Strychnos pieman, Radix aconiti praeparata*)	Ultrasonication	ESI (sheath-liquid: water-MeOH 1:9 v/v, 0.5% HOAc, 3 µL/min)	QIT	NH_4OAc, HOAc, MeOH	-	[30]
Pesticides	Wheat	Extraction with water-acetone	ESI (sheathless)	IT	1 mM NH_4Ac/HOAc (pH 6.3) in MeOH:water 50:50 v/v	Coated capillary; LOD: 2.5 µM	[31]
Heterocyclic aromatic amines	Urine	LLE, SPE	ESI (sheath-liquid: MeOH:20 mM HCOOH 75:25, v/v, 3 µL/min	IT	16 mM $HCOOH/HCOONH_4$, pH 4.5, 60% MeOH	FASI. LOD: 0.8-21 ng/g	[26]
Enantiomeric drugs	Plasma	LLE	ESI (sheath-liquid: ACN/5 mM NH_4OAc/HCOOH 75:25:0.1 (v/v), 2 µL/min)	TOF	25% MeOH, 75% 5 mM NH_4OAc (pH 6), 1.0% HOAc, 0.3% HS-β-CD	Electrokinetic injection; LOQ: 10 ng/mL	[114]
Polypeptides	Dialysis fluids, urine,	Anion exhange	ESI (sheath-liquid: 30%	TOF	30% MeOH and 0.5%	Polipeptide pattern	[115]

	serum	chromatography, lyophilization	MeOH, 0.5% HCOOH, 69.5% water, pH 2.3-2.5, 10 µl/min)		HCOOH, 69.5% water, pH 2.3-2.5	stablishment	
Substituted methoxy phenols and aromatic acids	Biomass burning aerosol	Filter extract (water)	ESI (sheath-liquid: water 50% 2-propanol 50% v/v, 3 µl/min)	IT	20 mM NH_4OAc-10% MeOH pH 9.1; 1 M NH_4OH pH 11	LOD: 0.1-1.0 µM	[116]
Glycoalkaloids and relative aglycones	Potatoes	Extraction with MeOH	ESI (sheath-liquid: MeOH-water 1:1 v/v, 1% HOAc, 2.5 µL/min)	IT	90:10 v/v MeCN-MeOH containing 50 mM NH_4OAc 1.2 M HOAc	MS^2; LOD: 10-50 µg/L	[117]
Irioid glycosides	Plants	Water extraction	ESI (sheath-liquid: 1.0 mM lithium acetate in water-MeOH 50:50 v/v, 200 µL/h)	IT	100 mM SDS in 20 mM ammonium acetate, pH 9.5	LOD: 15-50 mg/L; calculation of water-micelle partition coefficients	[33]
Procymidone and thiabenzadole	Fruits, vegetables	Sonication, SPE	ESI (sheath-liquid: 20 mM HCOOH -12 mM $HCOONH_4$ pH 3.5 with 2% MeOH, 13 µl/min)	Q	20 mM HCOOH -12 mM $HCOONH_4$ pH 3.5 with 2% MeOH	LOQ: 0.005-0.05 mg/kg	[23]
Oxycodone and major metabolites	Urine	SPE, LLE	ESI(sheath-liquid: water-methanol 50:50 v/v, 1% formic acid, 5 µL/min	IT	25 mM ammonium acetate pH 9	MS^n, hydrodinamic injection (LOD:10-300 ng/ml) and FASS (LOD: 1-50 ng/ml)	[77]
Catecholamines	Urine	SPE	ESI (sheathless)	QQQ	Various BGE containing NH_4OAc, water, MeOH, ethanol, HOAc, propanol	LOD: 0.48-1.30 µM	[20]
Catecholamines and metanephrines	Urine	SPE	ESI (sheath-liquid: 75:25:0.1 MeOH/water/HOAc v/v, 1.5 µl/min)	TOF	1% HOAc (pH 2.8)	Electrokinetic injection	[19]
Furosemide	Urine	SPE	ESI (sheath-liquid: MeOH-water-ammonia 50:49:1 v/v, 5 µL/min)	IT	20 mM NH_4OAc pH 9 with TEA	MS^2	[118]
Opioids	Urine	SPE, LLE	ESI (sheath-liquids: MeOH-water 60:40 v/v, 1% HOAc or 1% HCOOH, 3µl/min or 5 µl/min)	IT	25 mM NH_4OAc pH 9	Use of FASS	[76]
Drugs	River water	LLE, SPE	ESI (sheath-liquid: 2-propanol-water 80:20 v/v, 0.1% v/v HOAc or 0.1% v/v TEA; 4 µl/min)	Q	20 mM NH_4OAc pH 5.1	LOD: 18-134 µg/L	[119]

Steroidal alkaloids	Leaves and seeds (*Solanum sodomaeum*), berries (Solanum *elaeagnifolium*)	Extraction with ethanol and HCl	ESI (sheath-liquid: isopropanol-water 50:50 v/v, 0.5% HCOOH; 3 µl/min)	Q	25 mM NH_4OAc and 1 M HOAc in MeOH-ACN 20:80 v/v	LOD: 0.05 µg/mL	[120]
Irioid glycosides	Plants	Water extraction	ESI (sheath-liquid: 1.0 lithium acetate in water-MeOH 50:50 v/v, 200 µL/h)	IT	100 mM SDS in 20 mM NH_4OAc, pH 9.5	LOD: 25-50 mg/L	[32]
Morphine and related opiods	Urine	LLE, SPE	ESI (sheath-liquid: MeOH:water 60:40 v/v, 1% HOAc, 3 µl/min)	IT	25 mM ammonium acetate and NH_3 (pH 9)	MS^3, LOD 100-200 ng/mL	[14]
Gonadotropin-releasing hormone	Serum, urine	Inmunoaffinity CE	ESI (sheath-liquid: 20 mM HOAc in 50% MeOH, 0.5 ml/min)	Q	60 mm NH_4HCO_3 pH 8.0, 1% v/v ACN	-	[121]
Tramadol and its main phase I metabolite	Plasma	LLE	ESI (sheath-liquid: Isopropanol-water 1:1 v/v, 0.5% formic acid, 3 µl/min)	Q	40 mM ammonium acetate buffer, pH 4.0, sulfobutyl ether β-CD (2.5 mg/ml)	Partial filling	[12]
Etodolac and its urinary phase I metabolites	Urine	LLE	ESI (sheath-liquid: ACN-10 mM ammonium formate pH 3.0 1:1 v/v, 3 µl/min)	IT	ACN-10 mM ammonium formate pH 3.0 1:1 v/v	CEC (C_{18} capillaries); electrokinetic injection	[13]
Nitrocatechol-type glucuronides	Urine	SPE	ESI (sheath-liquid: ACN-20mM ammonium acetate 1:1 v/v, 5 µl/min)	QQQ	20 mM ammonium acetate, pH 6.84	Stacking, LOD 7 ng/mL	[83]
Codeine, dihydrocodeine and their glucuronides	Urine	SPE	ESI (sheath-liquid: MeOH-water-acetic acid 69:39:1 v/v/v, 3 µL/min)	IT	25 mM NH_4OAc pH 9.	MS^2; LOD: 100-200 ng/mL	[122]
Amphetamine and designer drugs	Urine	LLE	ESI (sheath-liquid: MeOH:water:HOAc 60/39/1 v/v/v, 3 µL/min)	IT	20 mM NH_4OAc, 20 mM HOAc pH 4.6	MS^2	[123]
Proteins	-	In-line digestion microchip	ESI (sheathless)	Q	10 mM $(NH_4)HCO_3$, 100 mM HCOOH	-	[67]