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RESEARCH PAPER

Development of a GC-APCI-QTOFMS library for new psychoactive substances and comparison to a commercial ESI library

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Abstract Gas chromatography coupled to atmospheric pressure chemical ionization quadrupole time-of-flight mass spectrometry (GC-APCI-QTOFMS) was evaluated for the identification of new psychoactive substances (NPS). An in-house high mass resolution GC-APCI-QTOFMS test library was developed for 29 nitrogen-containing drugs belonging mostly to synthetic stimulants. The library was based on 12 intra-day measurements of each compound at three different collision energies, 10, 20 and 40 eV. The in-house library mass spectra were compared to mass spectra from a commercial library constructed by liquid chromatography-electrospray ionization (LC-ESI) QTOFMS. The reversed library search scores between the in-house GC-APCI library and the commercial LC-ESI library were compared once a week during a 5-week period by using data measured by GC-APCI-QTOFMS. The protonated molecule was found for all drugs in the full scan mode, and the drugs were successfully identified by both libraries in the targeted MS/MS mode. The GC-APCI library score averaged over all collision energies was as high as 94.4/100 with a high repeatability, while the LC-ESI library score was also high (89.7/100) with a repeatability only slightly worse. These results highlight the merits of GC-APCI-QTOFMS in the analysis of NPS even in situations where the reference standards are not immediately available, taking advantage of the accurate mass measurement of the protonated molecule and product ions, and comparison to existing soft-ionization mass spectral libraries.

Keywords New psychoactive substances · Mass spectral library · Gas chromatography · Atmospheric pressure chemical ionization · Electrospray ionization · Time-of-flight mass spectrometry

Introduction

New psychoactive substances (NPS) pose a challenge for forensic analysts due to the multitude of emerging substances and the continuous changes at the drug scene. New kind of demands are made on drug screening and quantification procedures as the reference standards for NPS and their metabolites are usually not immediately available [1].

Conventional gas chromatography-mass spectrometry (GC-MS) relies on electron ionization (EI), while fragmentation takes place in the ion source. This technique allows reproducible search against very large spectral libraries. However, the libraries produced with quadrupole mass analysers are based on unit resolution and the spectra show extensive fragmentation without necessarily possessing the ions of molecular species. During recent years, liquid chromatography-mass spectrometry (LC-MS) has been increasingly used for drug analysis, particularly taking advantage of high-resolution mass spectrometry (HRMS) analysers [2–4]. An important benefit of LC-MS over EI-GC-MS is the capability to include a wider range of various drugs in the method without derivatization. Another benefit of LC-MS is related to the soft ionization mechanisms, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), producing less fragmentation in the ion source and revealing more frequently the molecular species [5].

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The presence of the molecular species, the protonated molecule in case of nitrogen-containing NPS, is crucial for structural elucidation. In HRMS, the protonated molecule can be directly used to estimate the molecular formula of a compound or a fragment and thus drastically reduce the number of false positive structures. Secondly, it allows facile retrospective data analysis without another measurement by searching the stored data for potential newly discovered NPS. Thirdly, the presence of the protonated molecule may help reaching lower limits of detection (LOD). During the last decade, the merits of the APCI ion source have been recognized even within GC analysis. Applications of GC-APCI-HRMS include metabolic profiling [6] and the analysis of androgenic anabolic steroids (AAS) [7], food packaging contaminants [8] and organic pollutants in groundwater samples [9]. Despite the increasing number of GC-APCI applications, a major limitation of the technique is the lack of such comprehensive spectral libraries that are now available for EI or ESI sources [5, 9]. Consequently, in each of the applications cited above, confirmations were carried out by measuring reference standard spectra.

In our previous communication, we introduced a new platform for the simultaneous identification and quantification of NPS in blood matrix, without the necessity of using authentic reference standards [10]. The instrumentation consisted of GC coupled to nitrogen chemiluminescence detection (NCD) and APCI-quadrupole time-of-flight mass spectrometry (QTOFMS). The GC flow was divided in appropriate proportions between QTOFMS and NCD, for accurate mass-based identification and for single-calibrant quantification, respectively. The present study elaborates the qualitative analysis segment further by validating an in-house GC-APCI-QTOFMS test library of 29 different drugs belonging mostly to the category of synthetic stimulants. Based on the hypothesis that existing commercial LC-ESI-QTOFMS libraries could be used in connection with GC-APCI-QTOFMS, the spectra produced by the latter technique were searched against both libraries and the scores compared.

Materials and methods

Reference standards 7-Aminonitrazepam, bupropion, cyclizine, dextromethorphan, ephedrine, hydroxybupropion, methadone, 3,4-methylonedioxymethamphetamine, pseudoephedrine and tapentadol were obtained from various pharmaceutical and reference standard manufacturing companies and were of pharmaceutical purity. All other test substances were seized material received from drug enforcement authorities for testing and found to be >95% pure [11].

Sample preparation Each reference standard (1–3 mg) was dissolved in methanol to obtain a stock solution of 1 mg/mL. The stock solution was further diluted into a 5-μg/mL working solution mixture containing 2–6 reference standards.

Three post-mortem case urine samples were chosen to demonstrate the method's applicability, including a mephedrone (0.65 μg/mL), an MDMA (2 μg/mL) and a *meta*-chlorophenylpiperazine (mCPP) finding, all additionally confirmed by EI-GC-MS. A urine sample (1 mL) was transferred into a 6-mL centrifuge tube, 50 μL of internal standard (20 μg/mL dibenzepin-D3 in methanol) and 300 μL of 1 M Tris buffer (pH 11) was mixed with the sample, and the mixture was extracted in a vortex mixer with 500 μL of butyl acetate for 2 min. After centrifugation, an aliquot (150 μL) of the organic phase was transferred into a conical autosampler vial for analysis by GC-APCI-QTOFMS.

Instrumentation A 7890B Series GC System equipped with a 7693 Automatic Liquid Sampler and a split/splitless injector was coupled through a two-way splitter with makeup gas (He) to an APCI 6540 UHD Accurate-Mass QTOF mass analyser and a 255 Nitrogen Chemiluminescence Detector (all Agilent Technologies, Santa Clara, CA).

Gas chromatography

The injector liner was a Single taper Ultra Inert liner with glass wool (Agilent 5190-2293). The analytical column was a DB-5MS (30 m × 0.25 mm id with 0.1 μm film) capillary column (Agilent Technologies). After the analytical column, the GC flow was divided between the NCD and the APCI ion source through a two-way splitter, using 0.55 m × 0.18 mm and 2 m × 0.18 mm uncoated deactivated fused-silica post-columns to obtain a 10:1 flow ratio, respectively. The splitter pressure was 15.8 psi and the flow ratio was calculated using the Effluent Splitter Calculator (with Makeup) (Agilent Technologies). NCD was not used in the current study.

The GC was operated in the pulsed splitless injection mode with an equilibration time of 0.5 min and 50 mL/min purge flow to split vent at 0.75 min. A pulse pressure of 50 psi for 0.75 min was applied prior to using initial head pressure of 24.9 psi. The injector port temperature was 250 °C and the transfer line temperature 320 °C. The injection volume was 1.5 μL. The oven temperature was initially held at 100 °C for 0.75 min and then increased by 30 °C per min to 320 °C, which was held for 6 min. Helium was used as carrier gas at 1 mL/min in the constant flow mode [10].

Mass spectrometry

The method was modified from the previous study [10]. QTOFMS was operated in the APCI-positive ionization mode, drying gas (nitrogen) flow at 5.0 L/min and gas temperature at 365 °C with a constant pressure of 0.6 psi. The

current of the corona discharge needle was 1000 nA and capillary voltage 1000 V. The fragmentor voltage was 140 V and skimmer voltage 65 V.

QTOFMS data were recorded over the *m/z* range of 50–500 with an acquisition rate of 5 spectra/s. MS/MS spectra of all compounds were collected in the targeted MS/MS mode using collision energies of 10, 20 and 40 eV in separate measurements. The precursor ion width was ~1.3 *m/z*. Initial external mass calibration was carried out using the ESI source with an ESI tuning mix (Agilent Technologies). The mass spectrometer was operated in 2 GHz, Extended Dynamic Range mode.

All the MS and MS/MS data were collected with the MassHunter Data Acquisition B.04.00 software (Agilent Technologies), and MassHunter Qualitative Analysis B.04.00 software (Agilent Technologies) was used for initial data processing and compound identification.

Spectral libraries

The MassHunter Personal Compound Database and Library Manager B.07.00 software (Agilent Technologies) were used to create a custom database and spectral library from GC-APCI-QTOFMS acquisition data. The commercial LC-ESI-QTOFMS spectral library was a MassHunter METLIN PCDL B.04.00 (Agilent Technologies).

Selection of the drugs for the in-house test library was based on the common availability of NPS in the authors' laboratory and in the commercial library. Methanolic solutions of 5 μg/mL for each compound were injected to ensure sufficient abundance of protonated precursor ions in the targeted MS/MS mode. The in-house GC-APCI-QTOFMS library was created from averaged relative abundances of 12 intra-day measurements. Noise removal criteria for the library spectra were set as follows: (1) infrequent peaks occurring in less than nine measurements were removed, (2) 20 most abundant peaks were selected, (3) peaks with a relative abundance less than 5% were removed. If there were less than five qualifying peaks, the threshold for qualifying a relative abundance was decreased to 2%, (4) mass error less than 1.0 mDa was required for a peak to qualify and (5) fragment ions peaks must originate from the molecular formula of their respective precursor ions. Accurate masses of qualified peaks were then converted into theoretical (exact) *m/z* values.

Comparison of libraries

The in-house GC-APCI-QTOFMS library was constructed 7 weeks prior to commencing the library comparison studies (Table 2). The following search query parameters were applied: the reverse-search scoring method, ≤1 mDa mass error for product ions, and 20 largest peaks with relative intensity higher than 2% from base peak were scored.

Results and discussion

Table 1 compares the averaged library search scores between the in-house GC-APCI-QTOFMS test library and the commercial LC-ESI-QTOFMS library for 29 drugs measured by GC-APCI-QTOFMS during a 5-week period. The in-house GC-APCI library was based on averaged relative abundances from 12 intra-day measurements for each compound, using the same QTOFMS series mass analyser as used for producing the commercial LC-ESI library. In both libraries, the mass spectra of compounds were measured at three different collision energies, 10, 20 and 40 eV. The protonated molecules were found for all drugs in the full scan mode and they were successfully identified by both libraries in the targeted MS/MS mode. The GC-APCI library score averaged over all collision energies was as high as 94.4/100 with a mean repeatability of 1.5%, while the LC-ESI library score was also high (89.7/100) with a mean repeatability of 2.9%. All 29 drugs were identified as the first candidate when searched against the commercial LC-ESI library, unless a positional isomer or stereoisomer was present in the library. Consequently, 4-fluoromethcathinone was sometimes identified as 2-fluoromethcathinone and ephedrine could not be distinguished from pseudoephedrine using MS/MS spectra alone. For most compounds, the score values between ESI and APCI libraries were similar, as illustrated with the methylone spectra (Table 1 and Fig. 1a).

Table 2 qualitatively describes selected three differences between the two libraries. By comparing these descriptions to Table 1 library scores, we came into conclusions that differences between the GC-APCI and LC-ESI spectra arise predominantly from two different mechanisms: (1) *Low abundance of targeted $[M + H]^+$ ions reaching the quadrupole collision cell*. Drugs containing a primary or secondary amine group are known to be difficult to analyse without derivatization due to their polar nature and interaction with the GC column [12], which was compensated here by injecting relatively high drug concentrations (5 μg/mL). For instance, the $[M + H]^+$ yield for mCPP was low, and consequently, the number of peaks in the MS/MS spectrum was low in GC-ACPI (Fig. 1b). Those spectra with a low number of peaks gave a high score while the discriminating power was fairly low. Using a newly changed liner, the number of qualifying detected peaks increased from 2 to 5 (figure not shown); thus, proper maintenance of the instrument is crucial to ensure sufficient ionization efficiency. (2) *Different external conditions*. For instance, hydroxybupropion was fragmented more in GC-APCI than in LC-ESI, resulting in more low-mass fragments (Fig. 1c). Some compounds may be inherently more labile or their ionization efficiency is more susceptible to external factors, such as humidity or contamination of the ion source. Also, we do not know whether there were any differences in adduct formation or stereoisomeric forms of

Table 1 Comparison of averaged library search scores between in-house GC-APCI-QTOFMS library and commercial LC-ESI-QTOFMS library for 29 drugs measured by GC-APCI-QTOFMS during a 5-week period

Drug	Collision energy (eV)					
	10		20		40	
	Average score (CV%)					
	ESI	APCI	ESI	APCI	ESI	APCI
7-Aminonitrazepam	100[a] (0)	100[a] (0)	82.9 (3.7)	96.1 (2.5)	53.5 (8.1)	93.4 (12.4)
Buphedrone	96.4 (1.9)	99.3 (0.3)	95.1 (1.4)	99.7 (0.1)	92.6 (2.4)	98.8 (1.5)
Bupropion	82.6 (7.6)	94.6 (4.8)	84.3 (8.5)	93.6 (6.5)	89.8 (1.1)	97.7 (2.4)
3,4-Dimethylmethcathinone (3,4-DMMC)	95.9 (0.7)	97.9 (0.6)	95.9 (0.5)	99.1 (0.2)	91.9 (3.7)	99.1 (0.2)
meta-Chlorophenylpiperazine (mCPP)	86.7[a] (6.3)	95.6[a] (2.0)	79.6 (2.6)	97.1[a] (0.8)	75.2 (8.8)	100[b] (0)
Cyclizine	89.4 (0)	92.3 (0)	90.0 (1.2)	96.5 (1.2)	84.0 (4.1)	89.2 (4.2)
Desoxypipradrol (2-DPMP)	92.6 (0.4)	96.3 (0.2)	88.0 (2.1)	96.4 (1.5)	86.5 (8.3)	93.7 (5.6)
Dextromethorphan	100[b] (0)	63.8 (4.6)	80.8 (1.6)	96.2 (0.5)	55.3 (3.6)	73.6 (3.4)
Dibutylone	93.8 (0.9)	99.7 (0.4)	93.8 (0.5)	99.8 (0.1)	88.4 (3.4)	95.7 (0.8)
Ephedrine	88.4 (1.4)	97.9 (1.0)	91.4 (2.1)	99.1 (0.5)	74.2 (8.8)	98.0 (2.4)
Ethylone (bk-MDEA)	94.7 (0.9)	99.4 (1.0)	94.6 (0.6)	99.9 (0)	80.3 (6.2)	96.2 (5.6)
2-Fluoromethamphetamine	97.7 (0.3)	99.4 (0.2)	100[b] (0)	100[b] (0)	97.3 (1.7)	100[a] (0.1)
3-Fluoromethamphetamine	93.8 (1.1)	98.9 (0.7)	100[b] (0)	100[b] (0)	88.3 (1.7)	99.8[a] (0.4)
4-Fluoromethamphetamine	99.0 (0.4)	99.6 (0.1)	97.8[a] (1.2)	97.5[a] (1.7)	97.4 (0.9)	96.5 (1.5)
4-Fluoromethcathinone	91.6 (1.0)	93.6 (0.9)	93.6 (1.5)	97.6 (2.2)	67.4 (16.9)	87.6 (11.2)
Hydroxybupropion	33.9[a] (8.5)	97.0 (1.8)	39.1 (29.3)	95.8 (3.2)	58.2 (3.2)	98.0 (2.5)
MDAI	98.5 (0.3)	99.5 (0.1)	98.5 (0.4)	99.6 (0.2)	88.5 (3.1)	97.1 (0.2)
Mephedrone (4-MMC)	88.8 (0.3)	99.1 (0.2)	90.5 (0.4)	99.6 (0.2)	89.1 (1.9)	96.0 (1.3)
Methadone	80.3 (9.6)	92.8 (5.2)	71.6 (6.9)	86.5 (6.6)	75.9 (6.1)	92.4 (4.9)
5-Methoxy-DALT	90.4 (5.3)	91.3 (4.9)	95.6 (0.9)	98.8 (0.4)	72.9 (1.4)	92.3 (0.8)
3,4-Methylenedioxymethamphetamine (MDMA)	97.0 (0.6)	93.8 (0)	98.1 (0.5)	99.8 (0.1)	93.1 (1.2)	99.8 (0.1)
Methylone (bk-MDMA)	94.5 (0.7)	96.9 (0.6)	96.8 (2.0)	98.9 (0.3)	92.9 (3.4)	98.8 (0.7)
α-Methyltryptamine (AMT)	99.9[a] (0)	100[b] (0)	66.9 (6.7)	99.3 (0.2)	84.4 (0.8)	100[b] (0)
Naphyrone	83.2 (2.1)	89.9 (1.9)	81.3 (2.9)	92.1 (1.1)	86.9 (4.0)	93.3 (4.3)
Pentedrone	95.6 (0.9)	97.5 (0.5)	91.6 (0.7)	99.1 (0.1)	87.2 (2.4)	98.2 (1.0)
Pseudoephedrine	94.0 (2.4)	99.9 (0.1)	93.9 (0.6)	99.8 (0.1)	79.1 (9.7)	99.7 (0.4)
α-Pyrrolidinobutiophenone (α-PBP)	95.8 (0.7)	98.6 (0.3)	94.9 (0.4)	98.3 (0.3)	92.8 (0.7)	99.5 (0.2)
α-Pyrrolidinopropiophenone (α-PPP)	97.0 (0.4)	98.2 (0.3)	89.8 (1.0)	98.5 (0.4)	[c]	[c]
Tapentadol	93.1 (0.4)	98.9 (0.2)	88.9 (0.3)	98.8 (0.2)	94.6 (0.4)	99.5 (0.1)

All spectra were measured by GC-APCI-QTOFMS at 5 μg/mL once a week during five consecutive weeks; search query was carried out against both LC-ESI and GC-APCI library with scores scaled from 0 (no match) to 100 (identical match). Averaged scores were obtained using the reverse scoring method

[a] MS/MS spectrum contained only two peaks

[b] MS/MS spectrum contained only one peak

[c] Spectrum was not available in commercial LC-ESI library and hence was not measured by GC-APCI

hydroxybupropion between the GC-APCI and LC-ESI measurements. Eventually, the criteria 2 and 3 in Table 2 are less critical for identification than the criterion 1. Therefore, using accurate mass values as an identification criterion in HRMS measurements is more reliable and consistent than using ion ratios. Such sophisticated search algorithms with less weight on ion ratios have already been described [13].

Our tentative results suggest that the present method is feasible as applied to biological samples. As an example, the following results were obtained by using optimal collision energies according to Table 2 with three different post-mortem urine samples, containing mephedrone (10 eV), MDMA (10 eV) and mCPP (20 eV). With mephedrone, the MS/MS library scores deviated from those obtained with averaged pure standards (Table 1) only 1.8% using the LC-ESI library and 0.9% using the GC-APCI library. Similarly with MDMA, the scores deviated only 2.9 and 1.9% and with mCPP 3.5 and 2.8%, respectively.

Li et al. [5] have reasonably stated that extensive fragmentation in GC-EI leads to the less specific ion species and loss of sensitivity. This argument is supported by recent studies in which the performance of GC-APCI and GC-EI were compared. GC-APCI was superior in finding a higher number of compounds in water samples [9] and in avocado fruit [14]. In addition, up to tenfold improvement in LOD was reported when 16 different androgenic anabolic steroids were measured [7]. A notable advantage of GC-APCI-HRMS is the soft ionization together with accurate mass measurement that

Table 2 Qualitative interpretation of differences between LC-ESI-QTOFMS and GC-APCI-QTOFMS spectra for selected drugs at optimal collision energy

Drug	Collision energy (eV)	Criterion 1[a]	Criterion 2[b]	Criterion 3[c]
7-Aminonitrazepam	20	Pass	Pass	Pass
Buphedrone	10	Pass	Pass	Pass
Bupropion	10	Pass	Fail	Pass
3,4-Dimethylmethcathinone (3,4-DMMC)	10	Pass	Pass	Pass
meta-Chlorophenylpiperazine (mCPP)	20	Pass	Pass	Pass
Cyclizine	40	Pass	Fail	Pass
Desoxypipradrol (2-DPMP)	20	Pass	Pass	Pass
Dextromethorphan	20	Pass	Pass	Fail
Dibutylone	10	Pass	Fail	Pass
Ephedrine	20	Pass	Pass	Pass
Ethylone (bk-(MDEA)	10	Pass	Fail	Pass
2-Fluoromethamphetamine	10	Pass	Pass	Pass
3-Fluoromethamphetamine	10	Pass	Pass	Pass
4-Fluoromethamphetamine	10	Pass	Pass	Pass
4-Fluoromethcathinone	10	Pass	Pass	Pass
Hydroxybupropion	20	Pass	Fail	Fail
MDAI	10	Pass	Pass	Pass
Mephedrone (4-MMC)	10	Pass	Pass	Pass
Methadone	10	Pass	Pass	Pass
5-Methoxy-DALT	40	Pass	Fail	Pass
3,4-Methylenedioxymethamphetamine (MDMA)	10	Pass	Fail	Pass
Methylone (bk-MDMA)	10	Pass	Pass	Pass
α-Methyltryptamine (AMT)	20	Pass	Fail	Pass
Naphyrone	20	Pass	Fail	Pass
Pentedrone	10	Pass	Pass	Pass
Pseudoepherine	20	Pass	Pass	Pass
α-Pyrrolidinobutiophenone (α-PBP)	20	Pass	Fail	Pass
α-Pyrrolidinopropiophenone (α-PPP)	20	Pass	Pass	Pass
Tapentadol	20	Pass	Pass	Pass

Optimal collision energy implies presence of precursor ion and at least 3 fragment ions; fragmented spectrum was preferred over precursor ion- only spectrum

[a] Criterion 1: All or five most intensive peaks in APCI can be found in ESI

[b] Criterion 2: Three most intensive peaks are in the same order with both APCI and ESI

[c] Criterion 3: APCI does not contain any unique peaks over ESI

allows identification without a reference spectrum, as exploited by Portolés et al. in the analysis of water samples [9].

It has been previously pointed out that a more widespread use of GC-APCI is restricted by the lack of spectral libraries. Moreover, it has been even argued that such libraries would be less reproducible since the ionization in APCI is more affected than in ESI by external conditions such as humidity, temperature, flow conditions, source geometry and cone voltage [5]. Our study supports the feasibility of GC-APCI drug libraries, as the coefficient of variation of both ESI and APCI library score values during a 5-week period was low and always less than 17% for all drugs except for hydroxybupropion (Table 1). Interestingly, Wachsmuth et al. [15] stated that continuous infusion of water into the APCI source resulted in even softer ionization. In their setting, water infusion reduced in-source fragmentation and enhanced the abundance of protonated molecule, resulting in more reproducible peak areas and increased sensitivity. Obviously, the formation of radical cations is also diminished by water infusion, which will be the topic of a future study.

The main variables affecting the MS/MS spectra are gas density and collision energy which are similar regardless of instrument type, hyphenation or ionization source [16]. Kienhuis et al. [17] demonstrated the principle of universal applicability of MS/MS spectra by acquiring data from

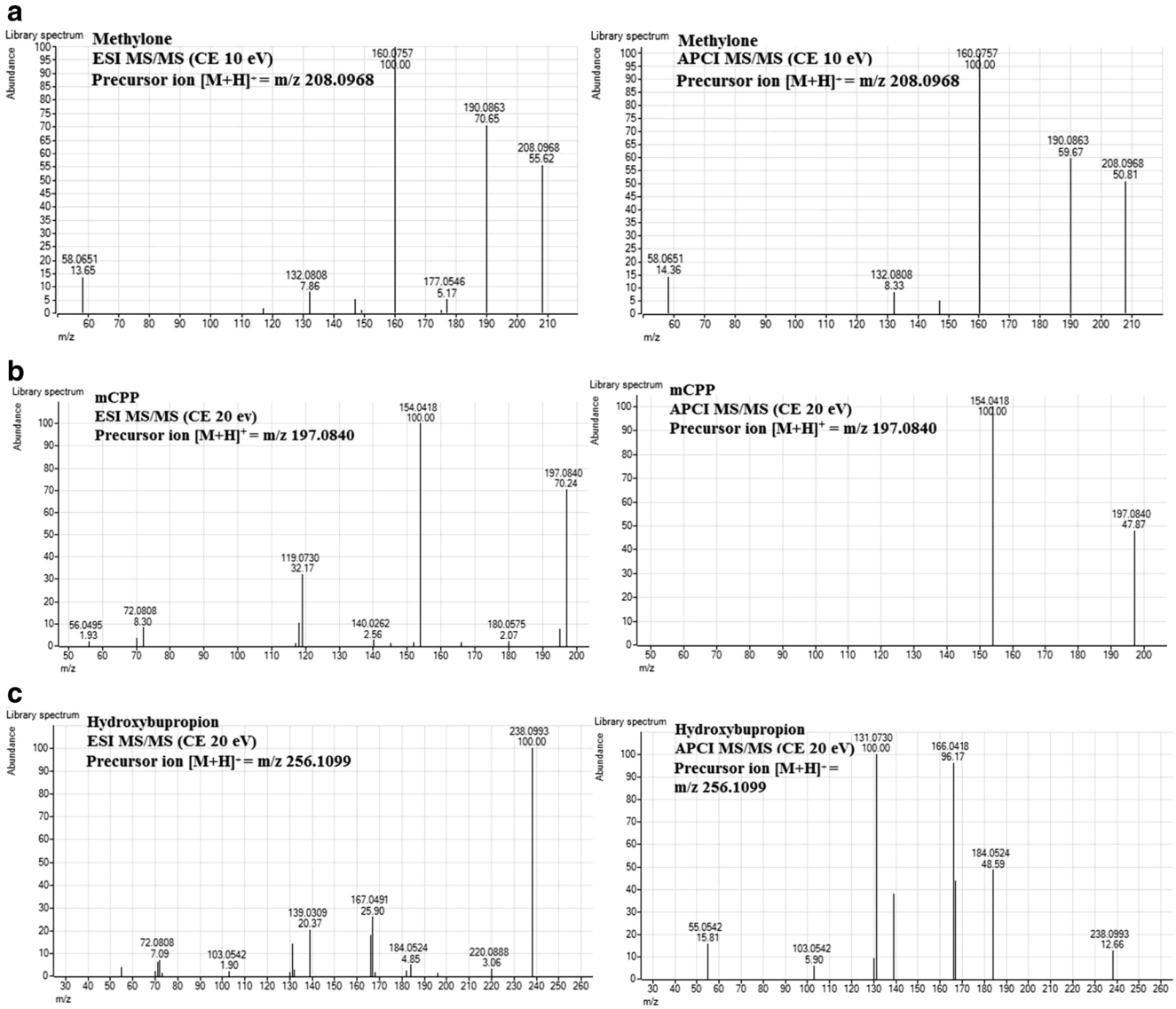

Fig. 1 LC-ESI-QTOFMS and GC-APCI-QTOFMS spectra of methylone at CE 10 eV (**a**), mCPP at 20 eV (**b**) and hydroxybupropion at 20 eV(**c**) showing absolute library score differences of 2.4/100, 17.5/100 and 56.7/100, respectively (see Table 1). Exact mass of the ions (*m/z*) and relative abundance are displayed on top of each peak; the threshold for relative abundance values in GC-APCI library is 5%

different soft ionization techniques, GC-chemical ionization (CI)-MS/MS, LC-CI-MS/MS, LC-APCI-MS/MS and LC-ESI-MS/MS, and analysing the data using an LC-CI-MS/MS library. In the present study, we have confirmed that the APCI spectra of basic drugs bear significant resemblance to their ESI spectra. These findings are supported by previous studies in which an ESI library was used together with other soft ionization sources. For instance, Östman et al. [18] used ESI-derived MS/MS library spectra in the analysis of 39 seized drug samples with atmospheric pressure matrix-assisted laser desorption/ionization. Similarly, Gwak and Almirall [19] applied ESI-derived spectra to detect 35 different NPS by ion mobility spectrometry coupled with QTOFMS in the direct analysis in real time (DART) mode. Signal intensity and degree of fragmentation of the measured compounds prior to reaching the collision cell can affect the quality of MS/MS spectra. Therefore, a major cause of variation in MS/MS spectra using different ion sources is attributed to thermal degradation in GC or the variable tendency to form different charge states or adducts in ESI. In the latter case, the reproducibility of spectral matching can be increased by working up a good quality consensus spectrum in which infrequently appearing peaks have been removed [20].

Conclusions

The present study is a step forward within our concept of simultaneous identification and quantification of NPS by the GC-NCD-APCI-QTOFMS platform, without the necessity of

using authentic reference standards. Identification of nitrogen containing NPS by this method provides advantages over conventional GC-EI-MS, including molecular formula based identification and retrospective analysis. As there are no GC-APCI spectrum libraries currently available for drug analysis, we studied the compatibility of a commercial LC-ESI library with the data produced by GC-APCI. The MS/MS spectra were highly similar between the commercial LC-ESI library and our in-house GC-APCI test library, suggesting universal application of soft ionization MS/MS spectra for basic drugs. However, the accurate mass of the protonated molecule was a more reliable indicator of compound identity than the reverse library search score that is dependent on ion ratios. Consequently, polar drugs are likely to benefit from derivatization, which is commonly used to improve their GC analysis and ionization efficiency.

Compliance with ethical standards This article does not contain any studies with living human participants or animals performed by any of the authors. The analysis of drugs from the autopsy specimens was performed according to the request of judicial authorities. The studies have been approved by the appropriate ethics committee and have been performed in accordance with the ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. UNODC (2013) The challenge of new psychoactive substances. [Cited 25 Sep 2016] Available from: https://www.unodc.org/documents/scientific/NPS_Report.pdf.
2. Sundström M, Pelander A, Angerer V, Hutter M, Kneisel S, Ojanperä I. A high-sensitivity ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine. Anal Bioanal Chem. 2013;405(26):8463–74.
3. Chindarkar NS, Wakefield MR, Stone JA, Fitzgerald RL. Liquid chromatography high-resolution TOF analysis: investigation of MS^E for broad-spectrum drug screening. Clin Chem. 2014;60(8):1115–25.
4. Kinyua J, Negreira N, Ibáñez M, Bijlsma L, Hernández F, Covaci A, et al. A data-independent acquisition workflow for qualitative screening of new psychoactive substances in biological samples. Anal Bioanal Chem. 2015;407(29):8773–85.
5. Li D-X, Gan L, Bronja A, Schmitz OJ. Gas chromatography coupled to atmospheric pressure ionization mass spectrometry (GC-API-MS): review. Anal Chim Acta. 2015;891:43–61.
6. Carrasco-Pancorbo A, Nevedomskaya E, Arthen-Engeland T, Zey T, Zurek G, Baessmann C, et al. Gas chromatography/atmospheric pressure chemical ionization-time of flight mass spectrometry: analytical validation and applicability to metabolic profiling. Anal Chem. 2009;81(24):10071–9.
7. Raro M, Portolés T, Pitarch E, Sancho JV, Hernández F, Garrostas L, et al. Potential of atmospheric pressure chemical ionization source in gas chromatography tandem mass spectrometry for the screening of urinary exogenous androgenic anabolic steroids. Anal Chim Acta. 2016;906:128–38.
8. Cherta L, Portolés T, Pitarch E, Beltran J, López FJ, Calatayud C, et al. Analytical strategy based on the combination of gas chromatography coupled to time-of-flight and hybrid quadrupole time-of-flight mass analyzers for non-target analysis in food packaging. Food Chem. 2015;188:301–8.
9. Portolés T, Mol JG, Sancho JV, Hernández F. Use of electron ionization and atmospheric pressure chemical ionization in gas chromatography coupled to time-of-flight mass spectrometry for screening and identification of organic pollutants in waters. J Chromatogr A. 2015;1339:145–53.
10. Ojanperä I, Mesihää S, Rasanen I, Pelander A, Ketola RA. Simultaneous identification and quantification of new psychoactive substances in blood by GC-APCI-QTOFMS coupled to nitrogen chemiluminescence detection without authentic reference standards. Anal Bioanal Chem. 2016;408(13):3395–400.
11. Rasanen I, Kyber M, Szilvay I, Rintatalo J, Ojanperä I. Straightforward single-calibrant quantification of seized designer drugs by liquid chromatography-chemiluminescence nitrogen detection. Forensic Sci Int. 2014;237:119–25.
12. Kataoka H. Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis. J Chromatogr A. 1996;733(1-2):19–34.
13. Oberacher H, Pavlic M, Libiseller K, Schubert B, Sulyok M, Schuhmacher R, et al. On the inter-laboratory transferability of a tandem mass spectral reference library: 2. Optimization and characterization of the search algorithm. J Mass Spectrom. 2009;44(4):494–502.
14. Hurtado-Fernández E, Pacchiarotta T, Longueira-Suárez E, Mayboroda OA, Fernández-Gutiérrez A, Carrasco-Pancorbo A. Evaluation of gas chromatography-atmospheric pressure chemical ionization-mass spectrometry as an alternative to gas chromatography-electron ionization-mass spectrometry: avocado fruit as example. J Chromatogr A. 2013;1313:228–44.
15. Wachsmuth CJ, Dettmer K, Lang SA, Mycielska ME, Oefner PJ. Continuous water infusion enhances atmospheric pressure chemical ionization of methyl chloroformate derivatives in gas chromatography coupled to time-of-flight mass spectrometry-based metabolomics. Anal Chem. 2014;86(18):9186–95.
16. Gergov M, Weinmann W, Meriluoto J, Uusitalo J, Ojanperä I. Comparison of product ion spectra obtained by liquid chromatography/triple-quadrupole mass spectrometry for library search. Rapid Commun Mass Spectrom. 2004;18(10):1039–46.
17. Kienhuis PG, Geerdink RB. A mass spectral library based on chemical ionization and collision-induced dissociation. J Chromatogr A. 2002;974(1-2):161–8.
18. Östman P, Ketola RA, Ojanperä I. Application of electrospray ionization product ion spectra for identification with atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry—a case study with seized drugs. Drug Test Anal. 2013;5(2):68–73.
19. Gwak S, Almirall JR. Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). Drug Test Anal. 2015;7(10):884–93.
20. Yang X, Neta P, Stein SE. Quality control for building libraries from electrospray ionization tandem mass spectra. Anal Chem. 2014;86(13):6393–400.