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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

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Product ion spectra obtained with liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) were applied to the identification of seized drug samples from atmospheric pressure matrix-assisted laser desorption/ionization product ion spectra (AP-MALDI-MS/MS spectra). Data acquisition was performed in the information-dependent acquisition (IDA) mode, and the substance identification was based on a spectral library previously created with LC-ESI/MS/MS using protonated molecules as precursor ions. A total of 39 seized drug samples were analyzed with both AP-MALDI and LC-ESI techniques using the same triple-quadrupole instrument (AB Sciex 4000QTRAP). The study shows that ESI-MS/MS spectra can be directly utilized in AP-MALDI-MS/MS measurements as the average fit and purity score percentages with AP-MALDI were 90% and 85%, respectively, being similar to or even better than those obtained with the reference LC/ESI-MS/MS method. This fact enables the possibility to use large ESI spectral libraries, not only to ESI analyses but also to analyses with other ionization techniques which produce protonated molecules as the base peak. The data obtained shows that spectral library search works also for analytical techniques which produce multi-component mass spectra, such as AP-MALDI, unless isobaric compounds are encountered. The spectral library search was successfully applied to rapid identification of confiscated drugs by AP-MALDI-IDA-MS/MS.

Keywords: electrospray ionization; atmospheric pressure matrix-assisted laser desorption/ionization; tandem mass spectrometry; library search; drug

Introduction

In forensic sciences, gas chromatography-mass spectrometry (GC-MS) has for 40 years been the dominant technique for general unknown screening. Electron ionization (EI) produces highly reproducible, information-rich mass spectra that can be used for the identification of drugs against large commercial libraries. With the development of the atmospheric pressure ionization (API) interfaces, such as electrospray ionization (ESI),[1,2] the hyphenated technique of liquid chromatography-mass spectrometry (LC-MS) is gaining ground in drug identification. The advantages of LC-MS over GC-MS include suitability for thermally labile, non-volatile, and polar compounds, without need for derivatization. This is important since new drugs and metabolites tend to be more polar and less fitting for GC analysis than the old ones. In recent years, quite a few mass spectral libraries have been produced by collision induced dissociation (CID) in the source area (in-source CID)[3,4] or in the collision cell (MS/MS) of a triple quadrupole,[5–14] a hybrid ion trap mass spectrometer[14–16] or a quadrupole-TOF instrument[14,17–19] following ESI.

Two interesting points should be addressed regarding to the use of MS/MS libraries. First, transferring MS/MS libraries between two instruments is easy since only two variables, the collision energy and collision gas density, have a significant effect on the appearance of mass spectrum.[8] Weinmann *et al*. showed that an MS/MS library generated with four different collision energies can be shared between the same manufacturer's different mass spectrometers.[5] Gergov *et al*. showed that two libraries, created independently using similar instruments, were fully compatible.[8] In a study by Jansen *et al*.[10] the mass spectra obtained using a linear-ion-trap tandem mass spectrometer operated in the enhanced product ion scan (EPI) mode were compared with those obtained in the triple quadrupole product ion scan mode on the same as well as on two other instruments from different manufacturers. They concluded that, although the inter-instrument differences in ion relative intensity were significant, the mass spectra looked almost similar. Second, the ion source used has a negligible effect on the mass spectra. Kienhuis *et al*.[6] compared product ion spectra generated by different triple quadrupole and hybrid ion trap instruments connected with GC or LC and concluded that especially the product ion spectra between the triple quadrupole MS systems compared very well. This was true also for the in-source CID libraries.[20]

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Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS)[21] is a soft ionization method that has mainly been used for large biomolecules. In MALDI-MS, the sample is co-crystallized with a UV-absorbing matrix compound,[22,23] such as 2,5-dihydroxycinnamic acid or α-cyano-4-hydroxycinnamic acid (α-CHCA), and irradiated with a short pulse from a UV laser resulting in the desorption and ionization of the analytes as well as the matrix compound. The matrix compound gives high background noise, complicating the use of MALDI-MS with small molecules (MW < 800).

Atmospheric pressure (AP)-MALDI-MS differs from conventional vacuum MALDI-MS mainly in that the laser desorption/ionization step is conducted under AP, as is the case in the ESI technique. AP-MALDI provides easier interfacing to existing API instruments and easier sample transfer than vacuum MALDI instruments. Salo *et al.* used ultrathin-layer chromatography plates and high performance thin-layer chromatography plates coupled to AP-MALDI-MS for the detection small drug molecules directly from the plates.[24,25] Pihlainen *et al.* used both DIOS-MS and AP-MALDI-MS with an ion trap for the analysis of seized drugs.[26] Wu *et al.* studied the coupling of the single-drop microextraction technique with AP-MALDI-MS for example in the analysis of drugs in urine,[27] and nortriptyline and quinine in urine and plasma.[28,29] Shrestha *et al.* used AP-MALDI-MS in the analysis of drugs and metabolites.[30]

The potential of AP-MALDI-MS in forensic sciences has not yet been generally discovered. This may be partly due to the fact that routine analysis is very much based on target libraries that do not currently exist for AP-MALDI-MS. In the present study, we explored the AP-MALDI-MS/MS technique with mass spectral library search for the analysis of drugs in seized material. The samples were analyzed using the information depended acquisition (IDA) mode that excludes the matrix background peaks. The analytes were fragmented in the linear collision cell using a collision energy spread (CES) feature which allows the collection of data of three different collision energies (CEs) in one EPI spectrum to obtain greater spectral information, and identification of the analytes was performed with the use of an existing mass spectral library obtained by LC/ESI-MS/MS.[9,11] The results obtained with the AP-MALDI-MS/MS were compared with those obtained with LC/ESI-MS/MS.

Materials and methods

Chemicals and reagents

Methanol was purchased from J.T. Baker (Mallinckrodt Baker, Deventer, the Netherlands), acetonitrile from Rathburn (Rathburn Chemicals Ltd, Walkerburn, Scotland), ammonium acetate from Merck (Merck KGaA, Darmstadt, Germany), and α-cyano-4-hydroxycinnamic acid (α-CHCA) from Sigma (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Water was Direct-Q3-purified (Millipore, Bedford, USA). Seized material was supplied by the Crime Laboratory of the National Bureau of Investigation and the Finnish Customs Laboratory.

Samples and solutions

Stock solutions of the seized samples were prepared by dissolving the samples with methanol-water (50/50 *v/v*) to a concentration of 1 mg/ml (corresponding to 2.4–7.3 mM of main components). For LC-ESI/MS/MS, the stock solutions were further diluted with 10 mM ammonium acetate buffer (pH 3.2) to a concentration of 0.01 mg/ml (corresponding to 24–73 μM of main components). A saturated matrix solution was prepared by dissolving 25 mg of α-CHCA to 2 ml of acetonitrile-water (70/30, *v/v*).

Liquid chromatography-mass spectrometry

The LC used was an Agilent 1100 Series LC system consisting of a G1329A autosampler, a G1312A binary pump, and a G1316A column oven (Agilent Technologies, Waldbronn, Germany), and equipped with a Gemini C18 column (100 mm × 2.0 mm, particle size 3 μm) from Phenomenex (Torrance, CA, USA) and a Gemini C18 guard column (4.0 mm × 2.0 mm, particle size 3 μm). The column oven temperature was set to 35 °C. The injection volume was 10 μl and the flow rate was 200 μl/min. The mobile phase composed of (A) 10 mM ammonium acetate buffer (pH 3.2) and (B) acetonitrile/formic acid (100/0.1, *v/v*). The elution conditions applied were as follows: 0–10 min, linear gradient from 5% to 95% B; 10–12 min, 95% B; 12–14 min, linear gradient from 95% to 5% B, followed by a 12 min re-equilibrium period at the initial condition (5% B).

ESI-IDA-MS/MS analyses were performed with an AB Sciex 4000 QTRAP (AB Sciex, Concord, Canada) controlled by the Analyst 1.5.1 software. The instrument was operated in the positive ion mode using the following conditions: ionization voltage 5000 V, curtain gas 20, collision gas high, temperature 400 °C, gas1 40, gas2 70, and declustering potential 30 V. The IDA acquisition method consisted of an enhanced MS (EMS) survey scan followed by three enhanced product ion (EPI) scans for the three most abundant ions in the survey scan. The collision energy for EMS scans was set to 10 eV. The dependent scan was an EPI scan, which was carried out at three different CEs: 20, 35 and 50 eV. The resulting EPI spectra were then searched against the mass spectral library. For EPI scans, the collision gas density wase $3.8x10^{-5}$ Torr. In all experiments, the 'dynamic fill time' was used, and the scan range and scan rate were *m/z* 50–500 and 4000 u/s, respectively.

Atmospheric pressure matrix assisted laser desorption/ionization-mass spectrometry

An AP-MALDI PDF + 713 ion source (MassTech Inc., Columbia, MD, USA) with an Nd:YAG laser (λ = 355 nm) was combined with the 4000 QTRAP mass analyzer. Target 6 software (MassTech Inc., Columbia, MD, USA) was used for operating the AP-MALDI ion source. The 'Pulsed dynamic focusing' (the time to collect/concentrate ions prior to extraction into the MS) was set to 20 μs. Stock solutions (1 μl) were applied on the target plate using the dried droplet method,[31] in which the solution is applied on the plate and the solvent allowed to dry resulting in a dried sample spot on the plate surface. Samples were moved with spiral motion (velocity 10 mm/min, maximum range 2.25 mm, spacing 0.5 mm, motion steps 0.5 mm, and spiral direction outward) during the measurement. The IDA acquisition method consisted of an EMS survey scan followed by five EPI scans for each of the three most abundant ions in the survey scan. The MS voltages were as follows: ionization voltage 4500 V, curtain gas 10, collision gas high, temperature 25 °C, gas1 and gas2 0, and declustering potential 10 V. The collision energies for EMS and EPI scans were 10 eV and 35 ± 15 eV (20, 35 and 50 eV), respectively. In all experiments the 'fixed fill time' of 150 ms was used, Q0 trapping was on, the scan range was *m/z* 50-500, and the scan rate for EMS and EPI experiments were 4000 and 1000 u/s, respectively.

Results and discussion

Method optimization

In AP-MALDI-MS/MS experiments, the parameters of the laser were optimized to give a stable total ion chromatogram (TIC) with a maximum intensity during the measurement. From the tested values of 10, 20, 50, 75, 100, 150, and 200 Hz, the repetition value of 75 Hz was found to be optimal. The laser energy was adjusted with sample 21 (Table 1) just above the ionization threshold value, typically around 10% of the maximum value, providing an estimated pulse energy of approximately 250 μJ. The high volatility of the amphetamines is a known problem during sample preparation.[32,33] To prevent the evaporation of amphetamine from the samples during AP-MALDI-MS/MS measurements, the 'Interface Heater temperature' was set to 25 °C. Thermal degradation of samples stored on a target plate within a heated AP-MALDI ion source has been noted before.[34] High ion source temperatures also promote fragmentation of the protonated molecule.[26] Although the use of a low ion source temperature has been reported to clog the MS orifice,[30] no clogging of the transfer line was observed in our experiments.

The Analyst software allowed the operator to use a set of *m/z* values either to be included or excluded during measurement. In AP-MALDI-MS/MS the matrix peaks could be eliminated from MS/MS measurements with the use of an exclude list, taking into account that the matrix peaks did not coincide with possible analyte peaks. However, an exclude list was not used in this study because identification of each compound was confirmed by MS/MS measurements regardless whether the *m/z* value of the protonated compound coincided with *m/z* values from the matrix. The use of an include list (containing the *m/z* values of protonated molecules) was found to be necessary in some instances, especially with samples containing amphetamine. For example, in sample 28 (Table 1), containing cocaine and amphetamine, the amphetamine clearly detected in the EMS scan was not detected in the IDA survey if not present in the include list. The same phenomenon was observed with all samples containing amphetamine only (samples 11, 20–25, and 27). The reason for this behavior remained unclear. The use of the include list also resulted in less data, simplifying data processing. For fast compound recognition, the mass spectra were recorded with one collision energy but the potential spread for collision energy (from 20 to 50 V) was used in order to obtain richer mass spectra and confirm the identification.[35]

The actual LC-ESI/MS/MS method was not optimized in this study because it was a routine analytical method in the laboratory and has been previously optimized and validated for the analysis. The LC-ESI/MS/MS data was interpreted by viewing the total ion chromatogram (TIC) as well as the base peak chromatogram (BPC) for peaks and then using the 'IDA explorer' tool in the software to obtain the mass spectra. The use of an exclusion list was not necessary in the LC-ESI/MS/MS runs. However, the use of the include list was necessary with the same samples containing amphetamine as with the AP-MALDI-MS/MS method.

Comparison of spectral identification from seized samples

Table 1 shows the spectral library search results for 39 seized samples analyzed with AP-MALDI-MS/MS and LC-ESI/MS/MS. The spectral library search was performed against the same ESI-MS/MS spectral library of drugs that was based on the work by Mueller *et al.*[9] and Dresen *et al.*[11] and created using AB Sciex QTRAP instruments. The success of identification was measured by calculation of fit (Fit), reverse fir (RevFit), and purity fit (Purity) values by the instrument software. The Fit value gives information about similarity of the signals in the reference mass spectrum with those in the unknown mass spectrum. The RevFit reflects the similarity of the signals in an unknown mass spectrum with those in a reference mass spectrum. Finally, the Purity value is a combination of Fit and RevFit values, thus giving an estimation of how clean the measured mass spectrum is when compared to the reference mass spectrum (i.e. it estimates whether the measured mass spectrum contains exactly the same ions as the reference mass spectrum). The average Fit value for compounds detected with both methods was 90% and 88% for AP-MALDI-MS/MS and LC-ESI/MS/MS, respectively (Table 1). The average Purity value for compounds detected with both methods was 85% and 81% for AP-MALDI-MS/MS and LC-ESI/MS/MS, respectively. The Fit and Purity values obtained are consistent with the LC-ESI/MS/MS results reported by Gergov *et al.*,[8] Mueller *et al.*,[9] and Dresen *et al.*,[11] and most of them are clearly above the satisfactory confidence level of 70% proposed by Gergov *et al.*[8] It should be noted that the Fit and Purity values were higher with the AP-MALDI-MS/MS method when the sample contained a single analyte, whereas these values were higher with the LC-ESI/MS/MS method when the samples contained several analytes. This is understandable because chromatographic separation can produce mass spectra with less interference from other compounds, whereas in the AP-MALDI-MS/MS method all compounds are ionized at the same time. These results show that a mass spectral library created with different mass spectrometers (here from the same manufacturer) and with different ion sources can be readily used in the identification of unknowns. Most of the correctly identified compounds, except for the tryptamines, were the first ones on the hit list. Only negligible differences were noticed in the mass spectra obtained with AP-MALDI-MS/MS and LC-ESI/MS/MS (Figure 1), and the fragmentation patterns were similar to those of the reference library. The matrix background arising from α-CHCA did not interfere with the findings because the identification of compounds detected was confirmed with MS/MS measurements. In most cases, the AP-MALDI-MS/MS and LC-ESI/MS/MS findings were similar, but in some cases LC-ESI/MS/MS outperformed AP-MALDI-MS/MS. For instance in sample 28, only the cocaine was detected with AP-MALDI-MS/MS, whereas with LC-ESI/MS/MS also amphetamine, lidocaine, ecgonine methyl ester, and benzoylecgonine were detected. Thus, there might be some ion suppression from the matrix or other compounds. Another reason could be poor shot-to-shot and sample-to-sample reproducibility with crystalline matrices.[36] For this application the investigation of other matrices could be beneficial. Seized drug samples can contain also other ingredients, such as sugars which can be easily analyzed with AP-MALDI,[37] but in this application they did not seem to interfere with the analysis.

Conclusions

Our study shows that product ion mass spectra produced with ES ionization can be directly used as a spectral library for AP-MALDI-MS/MS measurements using a linear ion trap/triple quadrupole instrument. The quality parameters (Fit and Purity) obtained in the identification of AP-MALDI MS/MS spectra,

Table 1. Library search results for 39 seized drug samples by AP-MALDI-MS/MS and LC-ESI/MS/MS with corresponding Fit, RevFit, and Purity percentages.

Sample	AP-MALDI-MS/MS				LC-ESI/MS/MS			
	Compounds detected	Fit	RevFit	Purity	Compounds detected	Fit	RevFit	Purity
1	Dipropyltryptamine (DPT)	97.2	96.1	94.8	DPT	88.9	95.1	86.8
2	Dextromethorphan (DM)	90.5	88.3	87.4	DM	90.9	73.0	71.6
3	5-methoxy-α-methyltryptamine (5-MeO-AMT)	64.6	76.4	52.1	5-MeO-AMT	84.8	79.5	77.2
4	DPT	97.1	98.5	96.3	DPT	87.5	91.4	81.6
5	5-methoxy-diisopropyltryptamine (5-MeO-DiPT)	96.3	98.3	96.0	5-MeO-DiPT	83.1	83.4	79.7
6	α-Methyltryptamine (AMT)	84.5	84.4	78.3	AMT	82.6	87.2	80.1
7	2,5-dimethoxy-4-iodophenethylamine (2C-I)	94.4	97.6	95.9	2C-I	94.7	97.4	93.4
8	Morphine	86.0	86.8	83.9	Morphine	93.2	91.7	87.3
	Codeine	85.0	84.0	79.6	Codeine	88.3	93.0	84.7
	Papaverine	88.8	88.1	87.7	Papaverine	77.8	61.3	60.6
	Noscapine	78.1	78.3	76.1	Noscapine	74.8	73.9	72.9
9	para-Methoxyphenylpiperazine (MeOPP)	87.2	85.3	83.0	MeOPP	85.2	66.3	61.6
10	meta-Chlorophenylpiperazine (mCPP)	90.5	91.9	89.2	mCPP	97.6	96.5	95.5
11	Amphetamine (23 w-%)[a]	93.9	94.1	93.9	Amphetamine	88.1	84.7	84.6
12	Cocaine	83.7	83.8	83.6	Cocaine[b]	52.8	38.6	24.0
					Ecgoninemethylester	74.7	35.8	34.4
					Benzoylecgonine	88.2	87.3	87.1
13	6-Monoacetylmorphine (6-MAM)	85.2	80.0	76.7	6-MAM	91.1	91.9	84.8
	Heroin	83.6	79.6	78.9	Heroin	89.1	83.4	81.9
	Papaverine	82.0	66.3	63.9	Papaverine	82.0	84.3	81.5
14	3,4-Methylenedioxymethamphetamine (MDMA)	87.7	84.7	84.2	MDMA	92.4	88.7	88.0
15	MDMA	90.6	90.0	89.4	MDMA	90.5	89.0	88.3
16	5-Methoxytryptamine (5-MeOT)	80.5	80.9	67.4	5-MeOT	88.9	86.5	83.4
17	2,5-dimethoxyphenethylamine (2C-H)	94.1	90.9	88.3	2C-H	91.8	92.4	87.7
18	5-methoxy-dimethyltryptamine (5-MeO-DMT)	89.6	79.7	73.0	5-MeO-DMT	88.4	79.1	74.1
19	Methamphetamine (13 w-%) [a]	95.5	77.6	77.1	Methamphetamine	87.0	59.9	59.6
20	Amphetamine (56 w-%) [a]	97.6	91.9	91.7	Amphetamine	95.1	86.8	86.5
21	Amphetamine (34 w-%) [a]	99.8	100.0	99.8	Amphetamine	79.2	66.9	66.8
22	Amphetamine (19 w-%) [a]	99.8	100.0	99.8	Amphetamine	96.2	94.3	94.3
23	Amphetamine (39 w-%) [a]	99.8	97.8	97.6	Amphetamine	96.2	96.3	96.2
24	Amphetamine (25 w-%) [a]	99.8	100.0	99.8	Amphetamine	99.8	91.9	91.7
25	Amphetamine (30 w-%) [a]	99.8	96.8	96.6	Amphetamine	96.7	95.7	95.6
26	6-MAM	81.1	84.8	80.3	6-MAM	48.7	37.7	35.7
	Heroin	80.6	83.2	80.6	Heroin	96.5	96.4	95.4
					Amphetamine	93.6	87.1	87.1
					Chloroquine[b]	59.0	30.0	26.8
27	Amphetamine	99.6	99.9	99.6	Amphetamine	98.4	8.6	78.6
28	Cocaine	85.2	85.2	85.0	Cocaine	90.9	90.9	90.4
					Amphetamine	98.7	72.9	72.9
					Lidocaine	81.0	79.2	78.7
					Benzoylecgonine	90.1	81.5	81.0
					Ecgonine methyl ester	63.9	18.5	18.0
29	5-MeO-DMT	87.6	83.8	78.8	5-MeO-DMT	78.2	79.0	73.7
30	3-Methylfentanyl	94.5	71.0	70.5	3-Methylfentanyl	98.7	91.1	90.4
31	6-MAM	79.9	76.6	73.1	6-MAM	89.7	83.9	81.4
	Heroin	85.0	75.7	75.2	Heroin	92.9	92.9	92.0
32	Methylenedioxypyrovalerone (MDPV)	94.9	93.1	92.6	MDPV	84.0	92.5	80.4
33	MDPV	95.6	93.7	92.9	MDPV	84.4	92.8	79.6
34	MDPV	96.3	95.0	94.6	MDPV	82.2	73.9	68.8
35	DPT	97.1	97.4	95.4	DPT	94.1	92.0	90.0
36	5-MeO-DiPT	92.7	92.9	89.8	5-MeO-DiPT	94.5	95.7	93.7
37	4-Hydroxy-di-isopropyl-tryptamine (4-HO-DiPT)	97.4	94.1	93.2	4-HO-DiPT	99.4	97.7	97.6
38	5-MeOT	79.5	83.9	67.1	5-MeOT	88.0	93.2	84.0
39	2C-H	94.4	95.9	93.1	2C-H	77.6	84.4	77.6
Average	all analytes	90.1	88.1	85.3	all analytes	86.7	79.5	77.7

(Continues)

Table 1. *(Continued)*

Sample	AP-MALDI-MS/MS				LC-ESI/MS/MS			
	Compounds detected	Fit	RevFit	Purity	Compounds detected	Fit	RevFit	Purity
	analytes detected with both methods	90.1	88.1	85.3	analytes detected with both methods	87.7	82.7	80.7
	single analyte in samples	92.7	91.0	87.9	single analyte in samples	89.9	84.3	83.0
	multiple analytes in samples	83.4	80.7	78.4	multiple analytes in samples	84.6	81.8	79.1

a: Quantification was previously carried out by LC equipped with chemiluminescence nitrogen detection (LC-CLND); b: the signal was saturated.

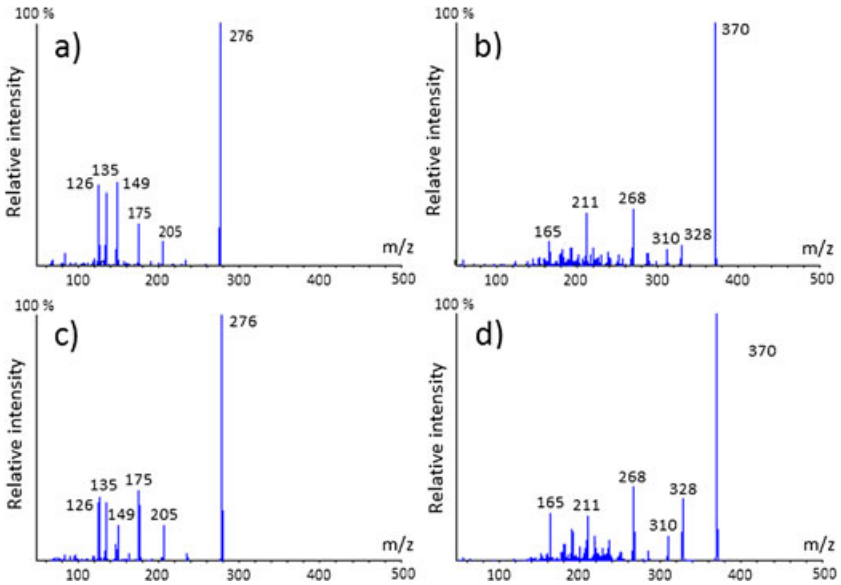

Figure 1. AP-MALDI-MS/MS mass spectra of (a) methylenedioxypyrovalerone (MDPV) (Table 1, sample 32) and (b) heroin (Table 1, sample 13) and LC-ESI/MS/MS mass spectra of (c) MDPV and (d) heroin, respectively, measured from seized samples.

measured with IDA, were as good as or even better than those obtained in LC-ESI/MS/MS measurements for drugs from seized material. In a larger context this means that universal product ion mass spectral libraries can be created, regardless of which instrumentation or ionization techniques have been utilized, provided that the precursor ion is the same, for example a protonated molecule.

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