Research Article

Rapid Communications in Mass Spectrometry

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# The analysis of amphetamine-like cathinone derivatives using positive electrospray ionization with in-source collision-induced dissociation

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**RATIONALE:** Amphetamine-like cathinone derivatives have become popular as recreational drugs over the past several years but their identification for forensic purposes is made difficult as they undergo extensive fragmentation under commonly used electron ionization (EI) conditions to afford ambiguous mass spectra. To overcome this, the feasibility of using positive electrospray ionization (ESI) with in-source collision-induced dissociation (CID) to produce distinguishable product ion mass spectra was examined.
**METHODS:** A set of six homologous cathinone derivatives was analyzed using an LTQ/Orbitrap™ high-resolution mass spectrometer to establish if there are any commonalities or uniqueness in their mass spectra. These compounds and a number of other cathinone derivatives were also analyzed on a single quadrupole mass spectrometer to establish the feasibility of using in-source CID for their identification in forensic drug samples.
**RESULTS:** The ESI product ion mass spectra of the $[M+H]^+$ ions of six model compounds were found to be readily interpretable and product ion formation pathways are presented. The use of such mass spectral data in the analysis of forensic drug samples facilitated the discrimination of closely related cathinone derivatives that were difficult to distinguish using conventional gas chromatography/electron ionization mass spectrometry. A product ion mass spectral library of 22 commonly encountered cathinone derivatives was also developed.
**CONCLUSIONS:** It has been shown that the product ion ESI mass spectra of cathinone derivatives are readily interpretable and are useful for the identification of this drug group in forensic samples. Copyright © 2012 John Wiley & Sons, Ltd.

The use of amphetamine-like cathinone derivatives as recreational drugs has increased considerably over the past several years.[1,2] Modification of the parent structure may potentially result in the production of thousands of derivatives and the rapid evolution of this drug class has posed analytical difficulties for forensic drug chemistry laboratories as reference standards are rarely available and interpretation of mass spectral data has to be relied upon for initial identification.

The electron ionization (EI) mass spectra of cathinone derivatives are predictable but suffer from the problem that the base peak is normally the ambiguous low mass iminium ion (**A**, Fig. 1). The acylium (**B**) and arylium (**C**) ions (identified by a mass difference of 28 *m/z* units: loss of CO) are also present but at low intensities. The molecular ions tend to be of very low abundance or even absent. One technique that has been used to calculate the molecular weight from an EI mass spectrum is to sum the masses of the **A** and **B** fragment ions but this has proven to be unreliable on several occasions due to co-eluting compounds. Chemical ionization (CI) may be used to obtain molecular weight information but fragmentation is limited and this technique is not normally used for routine forensic drug analysis.

Liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) has been used for the quantification of cathinones but generally LC/MS methods are not used for routine screening where unknowns may be encountered.[3,4] An LC/MS/MS method has been reported for the identification of seven methcathinone derivatives but again this is of limited value to laboratories where new cathinone derivatives are likely to be encountered.[5] In the electrospray ionization (ESI) mass spectra of cathinone derivatives, in addition to the $[M+H]^+$ ion, a $[M+H-18]^+$ ion of variable intensity, resulting from loss of water, is frequently found. However, it has also been observed that by using suitable dissociation techniques, cathinone derivatives possess rich fragmentation chemistries.[6] Product ion ESI mass spectra

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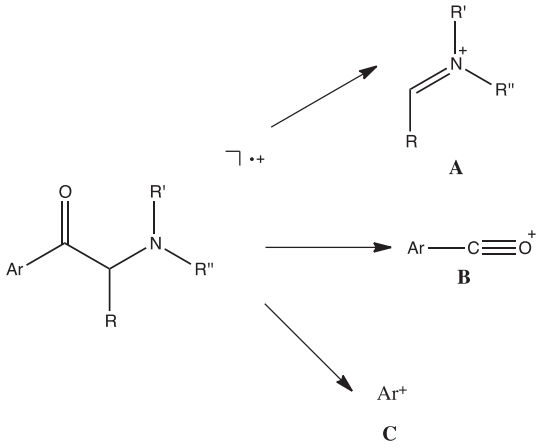
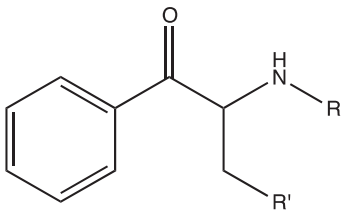



**Figure 1.** Electron ionization (EI) fragmentation for cathinone derivatives.




1. R = Me, R' = H
2. R = R' = Me
3. R = Me, R' = Et
4. R = Et, R' = H
5. R = Et, R' = Me
6. R = R' = Et

**Figure 2.** Structures of cathinone derivatives **1–6**.

from in-source collision-induced dissociation (CID) have previously been evaluated for the development of spectral libraries and for mass spectrometer tuning[7,8] and this prompted us to evaluate the potential usefulness of this technique for the identification of cathinone derivatives.

To establish if product ion ESI mass spectra could be used to discriminate closely related cathinone derivatives and to elucidate product ion formation pathways, a set of six homologous compounds, namely the *N*-methyl- and *N*-ethyl-α-methyl, ethyl and propyl cathinone derivatives (compounds **1–6**, Fig. 2), was analyzed by MS/MS and high-resolution mass spectrometry, using an LTQ/Orbitrap™ mass spectrometer system. A simpler single quadrupole ESI-MS instrument (Agilent 1100 mass-selective detector, MSD) with in-source CID[7] was then evaluated for its potential usefulness in the analysis of cathinone derivatives encountered in forensic drug samples.

## EXPERIMENTAL

### Reagents and chemicals

LCMS grade water, acetonitrile and formic acid were obtained from Fisher Scientific (Dublin, Ireland). All reagents used for the syntheses of the cathinone derivatives were obtained from Sigma Aldrich (Arklow, Ireland).

### Syntheses of cathinone derivatives

The cathinone derivatives **1–6** were synthesized as previously described.[9] This involved bromination of the appropriate ketone followed by the reaction of the resulting α-bromo ketone with a solution of methylamine, ethylamine or propylamine in tetrahydrofuran as required. All the other cathinone derivatives were synthesized in a similar way using pyrrolidine for MDPV, MDPBP, β-naphyrone and α-PVP, dimethylamine for *N*,*N*-dimethylcathinone and 4-*N*,*N*-trmethylcathinone, and benzylamine (in the presence of triethylamine) for benzedrone. All the compounds were converted into their hydrochloride salts using a solution of hydrogen chloride in diethyl ether.

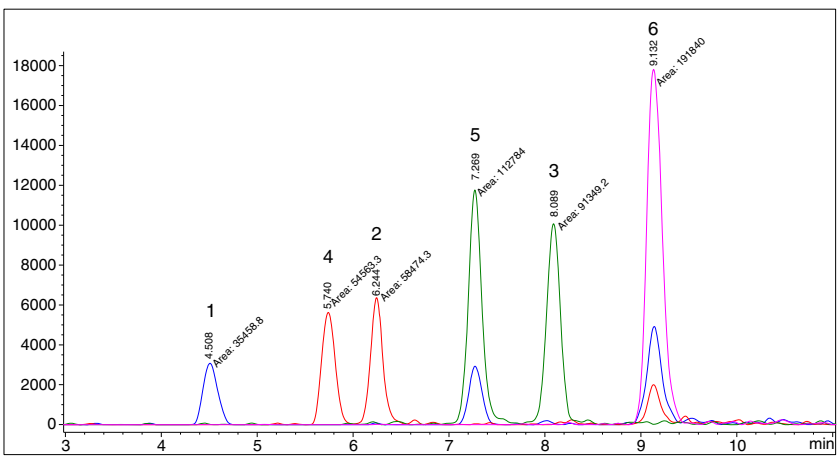



**Figure 3.** LC separation for cathinone derivatives **1–6** (EICs for $[M+H-H_2O]^+$ ions).

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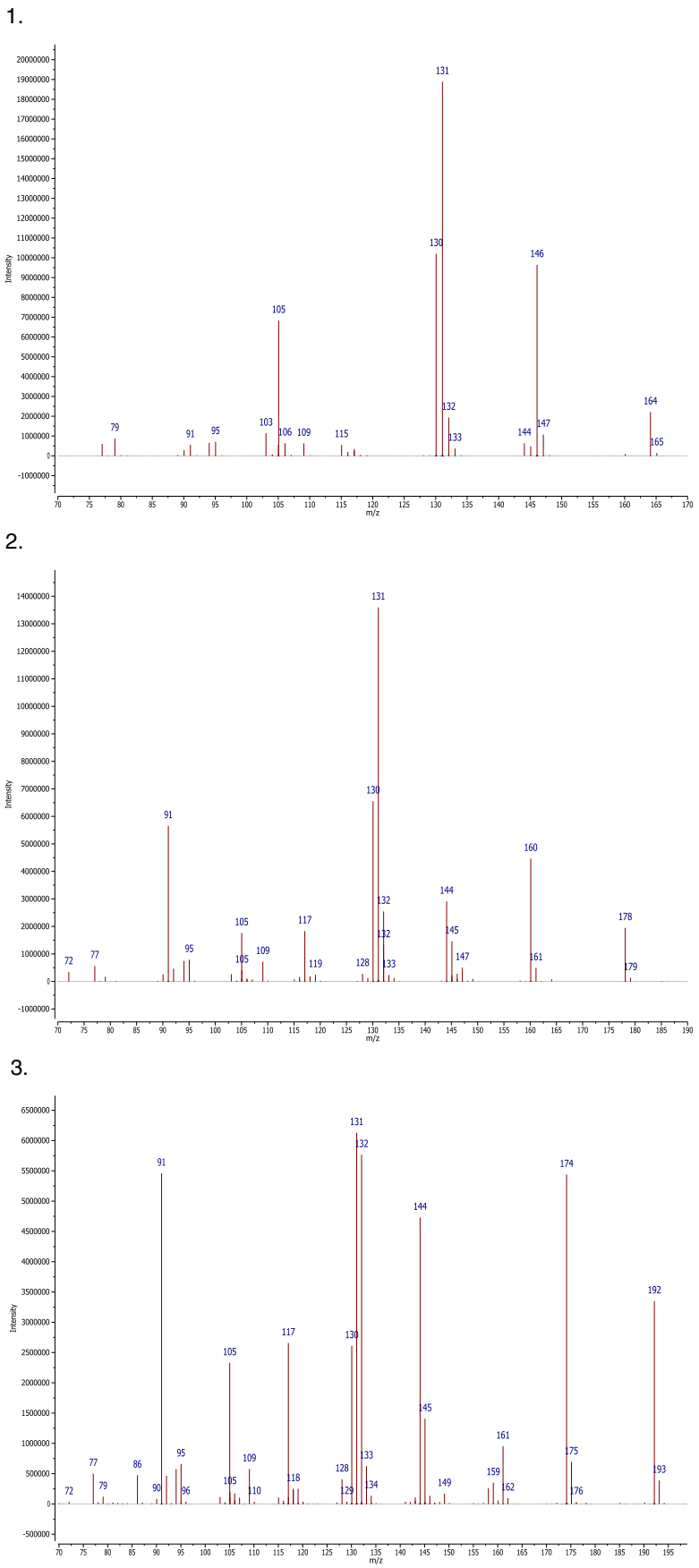
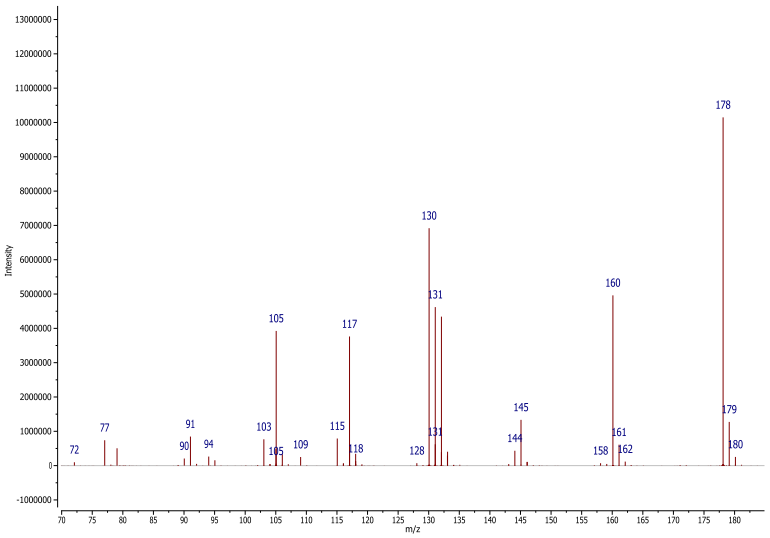



**Figure 4.** Product ion electrospray ionization mass spectra for protonated cathinone derivatives **1–6**.

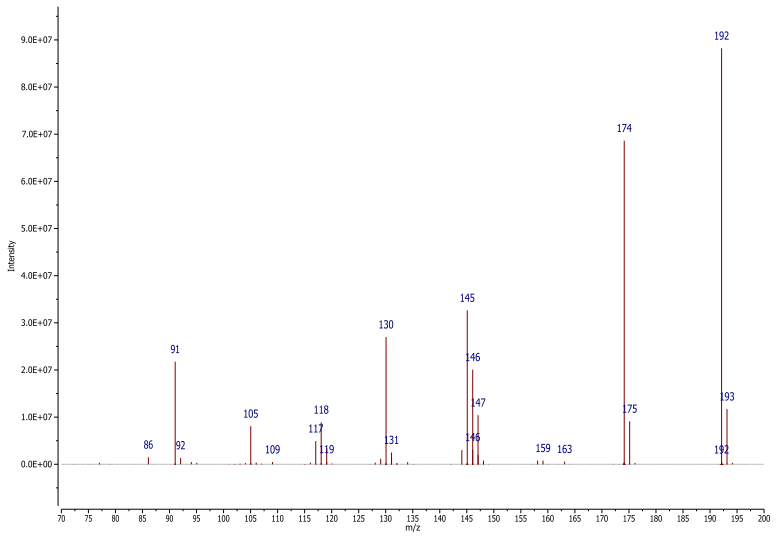


4.




5.




6.

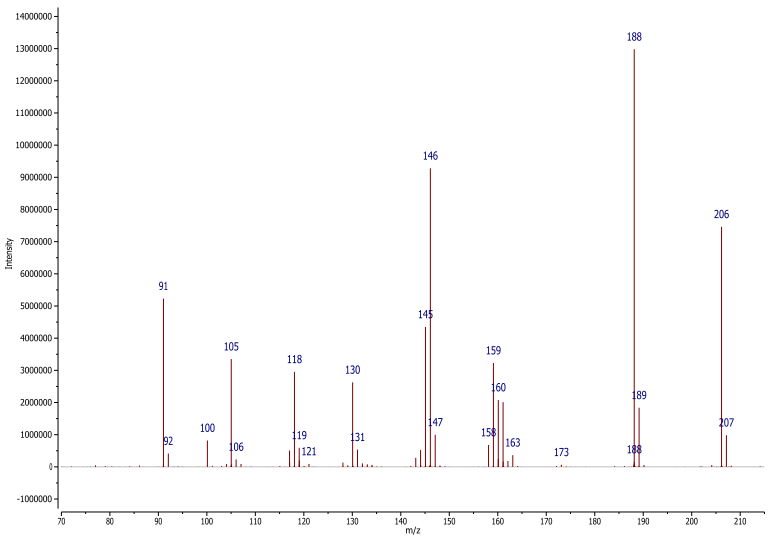



**Figure 4.** *(continued)*

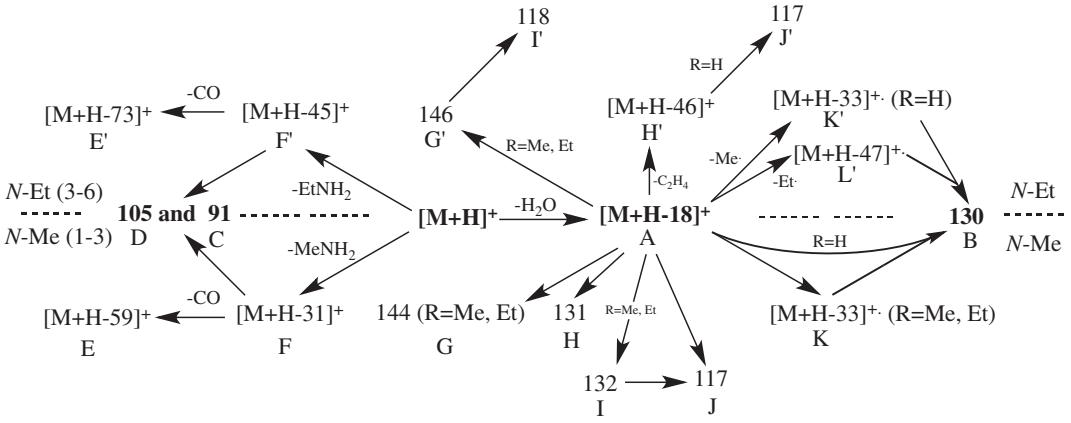



**Figure 5.** Product ion formation pathways for cathinone derivatives **1–6**.

**Table 1.** Accurate mass measurements for ions in cathinone derivatives **1–3**

| | | | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|---|---|
| Ion | Formula | Theor. mass (*m/z*) | Measured mass (*m/z*) | Mass error (ppm) | Measured mass (*m/z*) | Mass error (ppm) | Measured mass (*m/z*) | Mass error (ppm) |
| [M + H] | R = H, $C_{10}H_{14}NO$ | 164.1070 | 164.1067 | –1.8 | – | – | – | – |
| | R = Me, $C_{11}H_{16}NO$ | 178.1226 | – | – | 178.1225 | –0.6 | – | – |
| | R = Et, $C_{12}H_{18}NO$ | 192.1383 | – | – | – | – | 192.1381 | –1.0 |
| **A** | R = H, $C_{10}H_{12}N$ | 146.0964 | 146.0961 | –2.1 | – | – | – | – |
| | R = Me, $C_{11}H_{14}N$ | 160.1121 | – | – | 160.1119 | –1.2 | – | – |
| | R = Et, $C_{12}H_{16}N$ | 174.1277 | – | – | – | – | 174.1275 | –1.1 |
| **B** | $C_9H_8N$ | 130.0651 | 130.0653 | 1.5 | 130.0654 | 2.3 | 130.0653 | 1.5 |
| **C** | $C_7H_7$ | 91.0542 | 91.0541 | –1.1 | 91.0541 | –1.1 | 91.0542 | 0.0 |
| **D** | $C_7H_5O$ | 105.0335 | 105.0335 | 0.0 | 105.0334 | –1.0 | 105.0334 | –1.0 |
| **E** | R = H, $C_8H_9$ | 105.0699 | 105.0697 | –1.9 | – | – | – | – |
| | R = Me, $C_9H_{11}$ | 119.0855 | – | – | 119.0854 | –0.8 | – | – |
| | R = Et, $C_{10}H_{13}$ | 133.1012 | – | – | – | – | 133.1007 | 3.8 |
| **F** | R = H, $C_9H_9O$ | 133.0648 | 133.0652 | 3.1 | – | – | – | – |
| | R = Me, $C_{10}H_{11}O$ | 147.0804 | – | – | 147.0801 | –2.0 | – | – |
| | R = Et, $C_{11}H_{13}O$ | 161.0961 | – | – | – | – | 161.0959 | –1.2 |
| **G** | $C_{10}H_{10}N$ | 144.0808 | – | – | 144.0806 | –1.4 | 144.0806 | –1.4 |
| **H** | $C_9H_9N$ | 131.0730 | 131.0730 | 0.0 | 131.0724 | –4.6 | 131.0729 | –0.8 |
| **I** | $C_9H_{10}N$ | 132.0808 | – | – | 132.0802 | –4.6 | 132.0802 | –4.5 |
| **J** | $C_8H_7N$ | 117.0573 | 117.0571 | –1.7 | 117.0572 | –0.9 | 117.0572 | –0.9 |
| **K** | R = Me, $C_{10}H_{11}N$ | 145.0886 | – | – | 145.0882 | –2.8 | – | – |
| | R = Et, $C_{11}H_{13}N$ | 159.1043 | – | | – | – | 159.1040 | –1.9 |



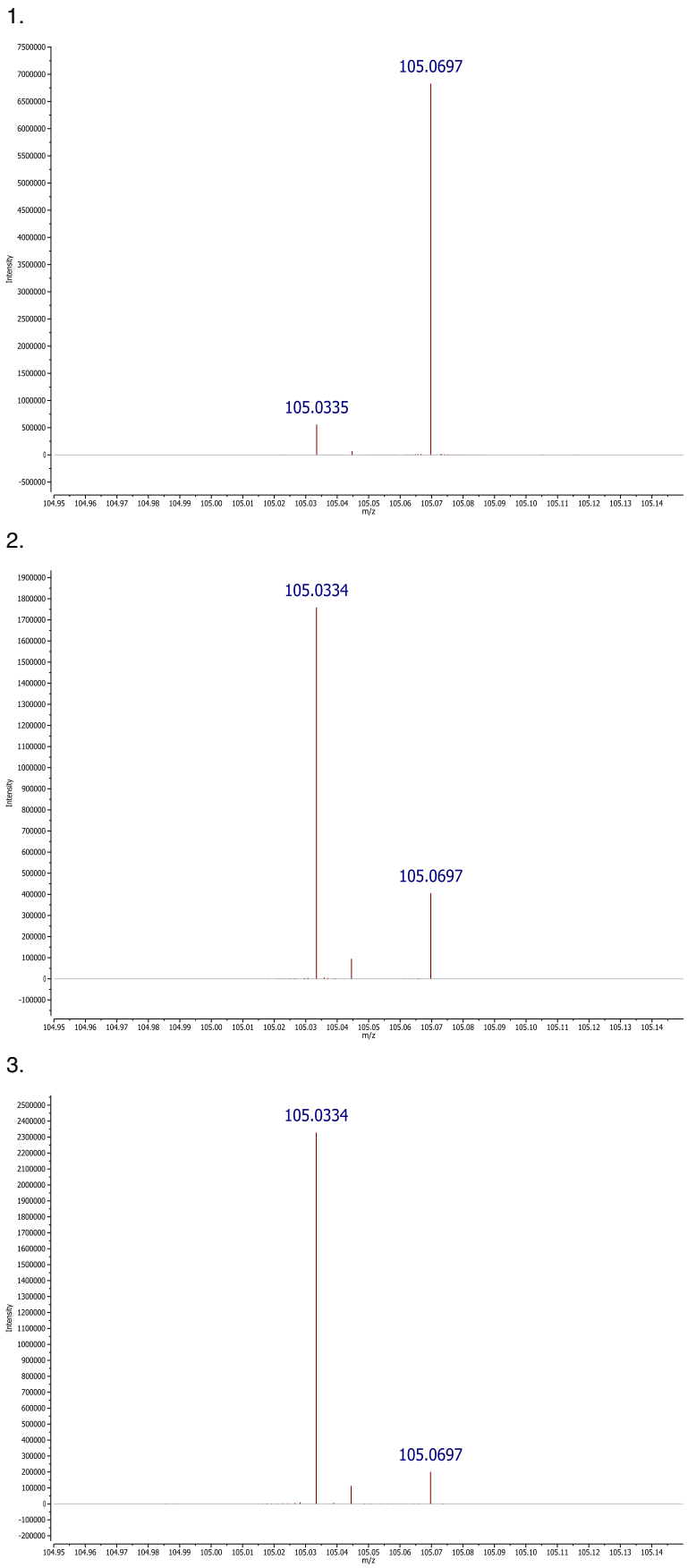



**Figure 6.** Accurate masses and abundances for *m/z* 105 product ion in compounds **1–3**.

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**Table 2.** Accurate mass measurements for ions in compounds **4–6**

| | | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|
| Ion | Formula | Theor. mass (*m/z*) | Measured mass (*m/z*) | Mass error (ppm) | Measured mass (*m/z*) | Mass error (ppm) | Measured mass (*m/z*) | Mass error (ppm) |
| [M + H] | R = H, $C_{11}H_{16}NO$ | 178.1226 | 178.1224 | –1.1 | – | – | – | – |
| | R = Me, $C_{12}H_{18}NO$ | 192.1383 | – | – | 192.1377 | –3.1 | – | – |
| | R = Et, $C_{13}H_{20}NO$ | 206.1539 | – | – | – | – | 206.1539 | 0.0 |
| **A** | R = H, $C_{11}H_{14}N$ | 160.1121 | 160.1118 | –1.9 | – | – | – | – |
| | R = Me, $C_{12}H_{16}N$ | 174.1277 | – | – | 174.1272 | –2.9 | – | – |
| | R = Et, $C_{13}H_{18}N$ | 188.1434 | – | – | – | – | 188.1433 | –0.2 |
| **B** | $C_9H_8N$ | 130.0651 | 130.0651 | 0.0 | 130.0647 | –3.1 | 130.0649 | –1.9 |
| **C** | $C_7H_7$ | 91.0542 | 91.0540 | –2.2 | 91.0538 | –4.4 | 91.0538 | –4.2 |
| **D** | $C_7H_5O$ | 105.0335 | 105.0334 | –1.0 | 105.0331 | –3.8 | 105.0331 | –3.7 |
| **E′** | R = H, $C_8H_9$ | 105.0699 | 105.0696 | –2.9 | – | – | – | – |
| | R = Me, $C_9H_{11}$ | 119.0855 | – | – | 119.0852 | –2.7 | – | – |
| | R = Et, $C_{10}H_{13}$ | 133.1012 | – | – | – | – | 133.1007 | –3.7 |
| **F′** | R = H, $C_9H_9O$ | 133.0648 | 133.0642 | –4.5 | – | – | – | – |
| | R = Me, $C_{10}H_{11}O$ | 147.0804 | – | – | 147.0798 | –4.1 | – | – |
| | R = Et, $C_{11}H_{13}O$ | 161.0961 | – | – | – | – | 161.0959 | –0.9 |
| **G′** | $C_{10}H_{12}N$ | 146.0964 | – | – | 146.0962* | –1.8 | 146.0961 | –2.1 |
| **H′** | R = H, $C_9H_{10}N$ | 132.0808 | 132.0802 | –4.5 | – | – | – | – |
| | R = Me, $C_{10}H_{12}N$ | 146.0964 | – | – | 146.0962* | –1.8 | – | – |
| | R = Et, $C_{11}H_{14}N$ | 160.1121 | – | – | – | – | 160.1117 | –2.4 |
| **I′** | $C_8H_8N$ | 118.0651 | – | – | 118.0647 | –3.4 | 118.0646 | –4.4 |
| **J′** | R = H, $C_8H_7N$ | 117.0573 | 117.0571 | –1.7 | – | – | – | – |
| **K′** | R = H, $C_{10}H_{11}N$ | 145.0886 | 145.0883 | –2.1 | – | – | – | – |
| **L′** | R = H, $C_9H_9N$ | 131.0730 | 131.0725 | –3.8 | – | – | – | – |
| | R = Me, $C_{10}H_{11}N$ | 145.0886 | – | – | 145.0882 | –2.8 | – | – |
| | R = Et, $C_{11}H_{13}N$ | 159.1043 | – | – | – | – | 159.1038 | –2.7 |

*Ions G′ and H′ have the same formula

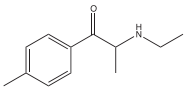
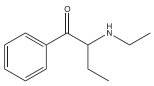
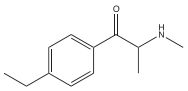
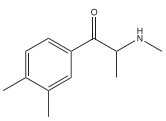
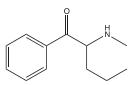
**Table 3.** Quality matches, using a PBM search, for cathinone derivatives **1–6**

| | | Cathinone derivative | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** |
| Library quality match | *1* | **90** | – | – | – | – | – |
| | *2* | – | **91** | – | – | – | – |
| | *3* | – | – | **91** | – | – | – |
| | *4* | – | 25 | – | **91** | – | – |
| | *5* | – | – | 2 | 38 | **91** | – |
| | *6* | – | – | – | – | 9 | **90** |



**Table 4.** Quality matches, using a PBM search, for 4-MEC in a case sample

| Cathinone derivative | Structure | Quality match (120 V) | Quality match (70 V) |
|---|---|---|---|
| 2-(Ethylamino)-1-(4-methylphenyl)propan-1-one (4-MEC) |  | 91 | 83 |
| 2-(Ethylamino)-1-(4-methylphenyl)butan-1-one (NEB) |  | 37 | 78 |
| 1-(4-Ethylphenyl)-2-(methylamino)propan-1-one (4-EMC) |  | 28 | 64 |
| 1-(3,4-Dimethylphenyl)-2-(methylamino)propan-1-one (3,4-DMMC) |  | 9 | 64 |
| 2-(Methylamino)-1-phenylpentan-1-one (Pentedrone) |  | 9 | 64 |

### High-resolution mass spectra and MS/MS studies

High-resolution mass spectral and MS/MS analyses were performed on an LTQ/Orbitrap™ Discovery mass spectrometer (Thermo Scientific, Bremen, Germany). This hybrid system consists of a linear ion trap (LTQ™) coupled to an Orbitrap™ Fourier transform mass spectrometer for accurate mass measurements. Samples of compounds **1–6**, dissolved in acetonitrile/water (1:1) containing 0.1% formic acid, were infused at a rate of 5 μL/min. Full scan high-resolution (30 000) spectra (*m/z* 50–200/210/250) were acquired in positive electrospray ionization (ESI) mode. The measured masses were within ± 5 ppm of the theoretical masses. The following conditions were used: drying gas (nitrogen) flow rate, 10 L/min; capillary temperature, 310 °C; spray voltage, 4 V; capillary voltage, 22 V, tube lens voltage, 77 V. A normalized collision energy™ (NCE) of 45% (of a maximum of 5 eV) was used for CID.[10,11]

### Liquid chromatography/mass spectrometry with in-source CID

LC/MS was performed on Agilent 1100 LC system (Böblingen, Germany) using an Allure PFP Propyl column (5 μm, 50 × 2.1 mm; Restek, Bellefonte, PA, USA): eluent A – acetonitrile containing 0.1% formic acid, eluent B – water containing 0.1% formic acid. The LC system was coupled to a Hewlett Packard/Agilent 1100 MSD (Agilent Technologies, Santa Clara, CA, USA) using the following conditions: positive ESI mode, capillary voltage 3000 V, drying gas ($N_2$) flow rate 12 L/min at 350 °C, nebulizer gas ($N_2$) pressure 60 psig, scan range *m/z* 50–250. Samples for LC/MS analysis were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 5 μg/mL (2 μL injected). The mobile phase was 12% A (0–2 min) followed by a linear gradient up to 50% A at 10 min and then up to 80% A at 15 min at a flow rate of 800 μL/min and column temperature of 35 °C. The fragmentor voltage for in-source CID was set at 120 V. Library searches were performed using MSD ChemStation™ software (version G1701D, Agilent Technologies) with a probability-based matching (PBM) algorithm (U + A, 2; Tilting, on; flag threshold, 3%; cross-correlation sort, off).

## RESULTS AND DISCUSSION

The majority of the cathinone derivatives encountered in our laboratory during the course of routine forensic drug work are *N*-methyl or *N*-ethyl derivatives with the α alkyl chain varying from 1 to 3 carbons in length. Thus, the compounds shown in Fig. 2 were synthesized as representative models to establish if such closely related compounds could be readily discriminated.[12,13] All six compounds were separable under the LC conditions used (Fig. 3). The product ion mass spectra for compounds **1–6** are shown in Fig. 4. The formation pathways (Fig. 5) were determined by MS/MS with accurate mass measurement on an LTQ™ linear ion trap coupled to an Orbitrap™ Fourier transform mass spectrometer with a normalized collision energy (NCE) of 45%. For the *N*-methyl derivatives (compounds **1–3**), loss of water from the

**Table 5.** PBM quality matches for in-source product ion mass spectral library used for routine screening

| | Compound | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Butylone | 91 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **2** | Methylone | – | 91 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **3** | MDPBP | – | – | 90 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **4** | MDPV | – | – | – | 90 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **5** | Methedrone | – | – | – | – | 91 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **6** | Flephedrone (4 isomer) | – | – | – | – | – | 90 | – | – | 90 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **7** | 4-Methylethcathinone | – | – | – | – | – | – | 91 | – | – | 34 | – | – | – | – | – | – | 9 | – | – | 2 | 56 | – |
| **8** | Mephedrone | – | 32 | – | – | – | – | – | 90 | – | – | 32 | 12 | – | – | – | – | – | – | – | – | – | – |
| **9** | Flephedrone (3 isomer) | – | – | – | – | – | 74 | – | – | 91 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **10** | 4-Ethylmethcathinone | 4 | – | – | – | – | – | 56 | – | – | 91 | – | – | – | – | – | – | 9 | – | – | 42 | 42 | – |
| **11** | Ethcathinone | – | 4 | – | – | – | – | – | – | – | – | 91 | 45 | – | – | – | – | – | – | – | – | – | – |
| **12** | Buphedrone | – | 4 | – | – | – | – | – | 2 | – | – | 36 | 91 | – | – | – | – | – | – | – | – | – | – |
| **13** | Pentylone | – | – | – | – | – | – | – | – | – | – | – | – | 90 | – | – | – | – | – | – | – | – | – |
| **14** | *N,N*-Dimethylcathinone | – | – | – | – | – | – | – | – | – | – | – | – | – | 90 | – | – | – | – | – | – | – | – |
| **15** | Methcathinone | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 90 | – | – | – | – | – | – | – |
| **16** | β-Naphyrone | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 90 | – | – | – | – | – | – |
| **17** | Pentedrone | 9 | – | – | – | – | – | 28 | – | – | 9 | – | – | – | – | – | – | 90 | – | – | 9 | 9 | – |
| **18** | Benzedrone | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 91 | – | – | – | – |
| **19** | A-PVP | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 90 | – | – | – |
| **20** | 3,4-Dimethylmethcathinone | 2 | – | – | – | – | – | 22 | – | – | 38 | – | – | – | – | – | – | 25 | – | – | 90 | 4 | – |
| **21** | *N*-Ethylbuphedrone | – | – | – | – | – | – | 36 | – | – | 9 | – | – | – | – | – | – | 12 | – | – | 2 | 91 | – |
| **22** | 4-*N,N*-Trimethylcathinone | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 91 |

$[M+H]^+$ ion, as previously reported, was observed.[6] The base peak, m/z 131 (ion **H**), in the three compounds arises from subsequent loss of the α alkyl chain (Fig. 5 and Table 1).

The *m/z* 130 ion (ion **B**, Fig. 5), prominent in the spectra of cathinone derivatives **1–3**, had the formula $C_9H_8N$, and is formed by the loss of methane from the $[M+H-H_2O]^+$ ion in **1** whereas in compounds **2** and **3** it arises from initial loss of a methyl radical, followed by loss of a further methyl or ethyl radical, respectively. The precursor ions were identified as *m/z* 146 (**1**), 145 (**2**) and 159 (**3**). The *m/z* 144 ion (ion **G**, $C_{10}H_{10}N$) in the mass spectra of **2** and **3** was found to arise from the $[M+H-H_2O]^+$ ion by loss of methane and ethane, respectively. Interestingly, the *m/z* 105 ion is predominately $C_8H_9$ (ion E, Fig. 5, precursor ion *m/z* 133) in **1** and $C_7H_5O$ ($[PhCO]^+$, ion **D**, precursor ions *m/z* 147 and 161, respectively) in compounds **2** and **3** (Fig. 6). The $[M+H-33]^+$ ion (*m/z* 131, 145 and 159, respectively) in the three compounds was not due to the loss of $CH_5N$ (methylamine and hydrogen) from the $[M+H]^+$ ion, as had been previously reported, but resulted from the loss of water and a methyl radical.[5] For the three *N*-ethyl derivatives (compounds **4–6**), loss of ethene from the $[M+H-H_2O]^+$ ion produces *m/z* 132, 146 and 160, respectively, while the *m/z* 131, 145 or 159 ions were formed by loss of an ethyl radical (Fig. 5 and Table 2). The $C_9H_8N$ ion (*m/z* 130) was again observed in the three spectra with the precursor ions being identified as *m/z* 131 and 145 (**4**), *m/z* 145 (**5**), and *m/z* 159 (**6**).

Having established that product ion mass spectra offer useful structural information and are readily interpreted, a simpler single quadrupole electrospray mass spectrometer (Agilent 1100 MSD) with in-source CID was then evaluated. A fragmentor voltage of 120 V was found to produce mass spectra similar to those obtained with a 45% NCE on the LTQ/Orbitrap™. The recommended working range for the fragmentor voltage is between 30 and 150 V and it was found that working above 120 V could result in complete loss of the $[M+H]^+$ ion with *N*-methyl-α-methyl cathinone derivatives such as methcathinone. A library containing the in-source product ion mass spectra of compounds **1–6** was created using Chemstation™ software (version G1701D, Agilent Technologies) and, from comparison of the library matches using the software's probability-based match (PBM) algorithm, excellent discrimination of the compounds was observed (Table 3).[14,15] To further investigate the feasibility of utilizing in-source product ion mass spectra to identify cathinone derivatives, an Agilent Chemstation™ library of five isobaric compounds ($C_{12}H_{17}NO$, M = 191, Table 4), was created. All these compounds produced an $[M+H]^+$ ion and an $[M+H-H_2O]^+$ ion (10–20% abundance) when the fragmentor voltage was set at 70 V on the Agilent 1100 MSD. Increasing the in-source fragmentation voltage to 120 V resulted in significant and reproducible product ion formation, and much improved discrimination, greatly facilitating the identification of 4-methylethcathinone (4-MEC) in a 'bath salts' product encountered during routine forensic analysis (Table 4).

In another instance, the use of in-source product ion mass spectra facilitated the identification of a change of the active ingredient from ethcathinone (compound **4**) to buphedrone (compound **2**) in a 'bath salts' product. The compounds were difficult to separate by GC and their EI mass spectra were found to be very similar with a low mass *m/z* 44 ion being the major distinguishing factor.[16] However, the two compounds were readily resolved by LC (Fig. 3). Their in-source product ion mass spectra were found to be quite different and, overall, LC/MS was found to be superior to GC/MS in distinguishing the two compounds. Significant differences in the mass spectra included (i) a more dominant *m/z* 91 ion in buphedrone (**2**), (ii) relatively more intense *m/z* 105 and 117 ions in ethcathinone (**4**), (iii) the relative intensities of the *m/z* 130/131/132 cluster, and (iv) the ratio of the $[M+H]^+/([M+H-H_2O]^+)$ ions. The relatively higher intensity of the *m/z* 105 ion in ethcathinone (**4**) may be explained by the fact that it is comprised of both $[C_6H_5O]^+$ and $[C_9H_8]^+$ ions. A library of 22 cathinone derivatives commonly encountered during the course of our forensic investigations was constructed and, apart from 3- and 4-flephedrone, excellent discrimination is observed with a range of derivatives including ring-substituted compounds (Table 5).

Overall, it was found that product ion mass spectra produced by in-source CID following ESI have potential for use in the routine forensic analysis of cathinone derivatives but one challenge to be overcome is to change the analysts' 'mind-set' away from the traditional use of the widely used and accepted electron ionization that normally accompanies GC. The reproducibility of in-source CID across instruments may be a limiting factor and some optimization will be needed for different instruments. However, in the context of its use in accredited forensic laboratories, such optimization would be a normal practice, as all new methods have to be fully validated before use. It has been also suggested that the reproducibility problem may be overcome by the use of library search algorithms that are weighted to the *m/z* values of the ions and not to absolute abundances.[7,17]

## CONCLUSIONS

It has been shown that the in-source product ion mass spectra of cathinone derivatives are readily interpretable and useful for the identification of this type of drug. Electrospray ionization mass spectrometry is widely used for molecular weight determination in the course of forensic drug analysis but it has been demonstrated here that, with suitable dissociation conditions, its usefulness may be extended to produce more informative mass spectra facilitating the discrimination of closely related cathinone derivatives. Although the technique may require some adaptation for different instrument platforms, this drawback is outweighed by the fact that it provides both molecular weight and structural information not obtainable when cathinone derivatives are analyzed by the more conventional GC/EIMS methodology. A generalized product ion formation scheme for the cathinone derivatives has been also formulated.

## REFERENCES

[1] J. P. Kelly. Cathinone derivatives: A review of their chemistry, pharmacology and toxicology. *Drug Test. Anal.* **2011**, *3*, 439.
[2] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Synthetic cathinones. Available: http://www.emcdda.europa.eu/publications/drugprofiles/synthetic-cathinones (accessed August 14, 2012).

[3] L. K. Sorensen. Determination of cathinones and related ephedrines in forensic whole-blood samples by liquid-chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. B* **2011**, *879*, 727.
[4] C. Bell, C. George, A. T. Kicman, A. Traynor. Development of a rapid LC-MS/MS method for direct urinalysis of designer drugs. *Drug Tes. Anal.* **2011**, *3*, 496.
[5] P. Jankovics, A. Váradi, L. Tölgyesi, S. Lohner, J. Németh-Palotás, H. Kőszegi-Szalai. Identification and characterization of the new designer drug 4'-methylethcathinone (4-MEC) and elaboration of a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method for seven different methcathinone analogs. *Forensic Sci. Int.* **2011**, *210*, 213.
[6] D. Zuba. Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods. *Trends Anal. Chem. (TrAC)* **2012**, *32*, 15.
[7] A. W. T. Bristow, W. F. Nichols, K. S. Webb, B. Conway. Evaluation of protocols for reproducible electrospray in-source collisionally induced dissociation on various liquid chromatography/mass spectrometry instruments and the development of spectral libraries. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 2374.
[8] W. Weinmann, M. Stoertzel, S. Vogt, M. Svoboda, A. Schreiber. Tuning compounds for electrospray ionization/in-source collision-induced dissociation and mass spectra library searching. *J. Mass Spectrom.* **2001**, *36*, 2013.
[9] P. Kavanagh, J. O'Brien, J. Fox, C. O'Donnell, R. Christie, J. D. Power, S. D. McDermott. The analysis of substituted cathinones. Part 3. Synthesis and characterisation of 2,3-methylenedioxy substituted cathinones. *Forensic Sci. Int.* **2012**, *216*, 19.
[10] Wiley-Blackwell Data Collections. Electrospray ionization: acquisition conditions. P. Rösner, F. Westphal. Available: www.wiley-vch.de/stmdata/pdf/DD_Electrospray_Ionization.pdf (accessed August 14, 2012).
[11] Normalized Collision Energy™ Technology. Thermo Scientific. Available: www.thermo.com/eThermo/CMA/PDFs/Articles/articlesFile_21418.pdf (accessed August 14, 2012).
[12] Research on Head Shop drugs in Dublin: Part 1. Available: http://drugs.ie/features/feature/headshop_drugs_research (accessed August 14, 2012).
[13] Research on Head Shop drugs in Dublin: Part 2. Available: http://www.drugs.ie/features/feature/research_on_head_shop_drugs_in_dublin_part_2 (accessed August 14, 2012).
[14] Creating and using a performance-based API-LC/MSD mass spectral library with NIST MS search software. Agilent Technologies. Available: www.chem.agilent.com/Library/applications/5988-5445EN.pdf (accessed August 14, 2012).
[15] H. J. Hübschmann. *Handbook of GC/MS: Fundamentals and Applications*. Wiley-VCH Verlag, Weinheim, Germany, **2008**, p. 328. ISBN 3-527-30170-4.
[16] K. Zaitsu, M. Katagi, H. T. Kamata, A. Miki, H. Tsuchihashi. Discrimination and identification of regioisomeric β-keto analogues of 3,4-methylenedioxyamphetamines by gas chromatography-mass spectrometry. *Forensic Toxicol.* **2008**, *26*, 45.
[17] P. Marquet, N. Venisse, É. Lacassie, G. Lachâtre. In-source CID mass spectral libraries for the 'general unknown' screening of drugs and toxicants. *Analusis* **2000**, *28*, 925.

