Research Article

Drug Testing and Analysis

Received: 11 February 2010 Revised: 23 April 2010 Accepted: 26 April 2010 Published online in Wiley Interscience: 27 May 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.134

# Differentiation of structural isomers in a target drug database by LC/Q-TOFMS using fragmentation prediction

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**Isomers cannot be differentiated from each other solely based on accurate mass measurement of the compound. A liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOFMS) method was used to systematically fragment a large group of different isomers. Two software programs were used to characterize *in silico* mass fragmentation of compounds in order to identify characteristic fragments. The software programs employed were ACD/MS Fragmenter (ACD Labs Toronto, Canada), which uses general fragmentation rules to generate fragments based on the structure of a compound, and SmartFormula3D (Bruker Daltonics), which assigns fragments from a mass spectra and calculates the molecular formulae for the ions using accurate mass data. From an in-house toxicology database of 874 drug substances, 48 isomer groups comprising 111 compounds, for which a reference standard was available, were found. The product ion spectra were processed with the two software programs and 1 – 3 fragments were identified for each compound. In 82% of the cases, the fragment could be identified with both software programs. Only 10 isomer pairs could not be differentiated from each other based on their fragments. These compounds were either diastereomers or position isomers undergoing identical fragmentation. Accurate mass data could be utilized with both software programs for structural elucidation of the fragments. Mean mass accuracy and isotopic pattern match values (SigmaFit; Bruker Daltonics Bremen, Germany) were 0.9 mDa and 24.6 mSigma, respectively. The study introduces a practical approach for preliminary compound identification in a large target database by LC/Q-TOFMS without necessarily possessing reference standards.** 

**Keywords:** structural isomers; drug; liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOFMS); mass fragmentation *in silico*; accurate mass

## Introduction

Analytical techniques exploiting accurate mass measurement have become common in the pharmaceutical industry and drug metabolism studies,[1] as well as in analytical toxicology[2] and doping analysis[3] using large target databases. Current liquid chromatograpy/time-of-flight mass spectrometry (LC/TOFMS) instruments are fast, sensitive, and cost-effective in routine laboratory analysis.[4] They provide mass accuracy comparable to more expensive instruments together with moderately high mass resolution, which facilitates the determination of the elemental composition of small molecules.

An analytical challenge with accurate mass-based identification is the differentiation of isomers from each other, as these compounds cannot be differentiated solely based on accurate mass data, although in most cases they can be separated by means of LC. Further structural information can be produced with MS techniques by fragmenting the molecule and identifying the compound characteristic fragments.[5] Several large libraries of electron ionization (EI) reference mass spectra are available for use with gas chromatography-mass spectrometry (GC-MS),[6–8] which makes tentative identification of library compounds fast and convenient. Interpretation of the mass spectra acquired from electrospray ionization (ESI) LC/MS is more challenging, since less fragmentation occurs, and thus less structural information is achieved compared to EI with GC/MS.[9] ESI-MS fragment spectra tend to vary in ion intensities with different instruments,[5,9] and although reference mass spectral libraries for ESI-MS exist, it is not straightforward to exploit the data between different mass analyzers and laboratories without careful standardization of the conditions for compound identification.[10,11]

Both commercially available and in-house built software has been developed to predict *in silico* mass spectral fragmentation in MS analyses. In some programs, such as ACD/MS Fragmenter[12,13] or Mass Frontier,[14,15] the fragment prediction is mainly based on general rules of fragmentation reactions. Non-commercial software that simulates fragmentation and forms a reconstructed mass spectrum based on fragmentation rules includes MASSIS[16] and MASSIMO.[17] The following two software programs for fragment prediction do not rely on the general rules of mass fragmentation, but take into account optimal bond energies in order to predict the most stable fragments and estimate by a validated algorithm the probability of the predicted fragment. Fragment iDentificator (FiD)[18] uses scoring functions to rank competing fragmentation pathways of a molecule that can explain the mass peaks observed in the product ion (MS/MS) spectrum. The algorithm calculates the dissociation energies of the cleaved bonds and estimates the energetic favorability of the alternative fragments. Another recently published algorithm, Density Functional Theory (DFT),[19] calculates the thermodynamically most stable position for the

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protonation in a molecule. This information can be utilized in the prediction of the cleavage site of the molecule.

Mass fragmentation prediction with ACD/MS Fragmenter was successfully used in our previous study[20] for quetiapine metabolism and differentiation of the structurally isomeric metabolites. In that study, in-source collision-induced dissociation (ISCID) with LC/TOFMS was used to produce the fragments, and structural elucidation of the metabolites was done without reference standards. In ISCID analysis, sample background or other co-eluting analytes can interfere with the identification of compound characteristic ions. In the present study, a hybrid quadrupole TOFMS instrument (Q-TOFMS) is used for systematic, reference-standard-based analysis of a large number of different isomeric drugs with the purpose of producing compound characteristic fragments and differentiating the isomers from each other. Two software programs are used to specify mass fragmentation of the compounds *in silico*: one predicting the possible fragments based on the molecular structure of the compound (ACD/MS Fragmenter), and another assigning fragments from mass spectra acquired by MS/MS analysis and calculating the molecular formulae for the ions based on accurate mass measurement (SmartFormula3D).

## Experimental Section

### Materials

All solvents and reagents were of analytical grade from Merck (Darmstadt, Germany), except the HPLC-grade methanol, which was purchased from Rathburn (Walkerburn, UK). Water was purified with a Millipore DirectQ-3 instrument (Bedford, MA, USA). The selected 111 standards were from several different suppliers.

### Sample preparation

Isomeric compounds were searched from an LC/TOFMS in-house toxicology database of 874 substances, for which 462 reference standards were at hand, and 111 compounds were found from these standards. The compounds constituted 48 isomer groups with 2–4 compounds each, with *m/z* ranging from 150.1277 to 387.1559. Sixteen reference standard mixtures were prepared, containing 6–7 of the selected compounds of 1 µg/mL in 0.1% formic acid and methanol (9 : 1). Compounds in the same mixture were known to separate chromatographically.

### Liquid chromatography/quadrupole time-of-flight mass spectrometry

The liquid chromatograph was an Agilent 1200 series instrument (Waldbronn, Germany) including a vacuum degasser, autosampler, binary pump, and column oven. Chromatographic separation was performed in gradient mode at 40 °C with Phenomenex Luna PFP(2) 100 × 2 mm (3 µm) column and a PFP 4 × 2.0 mm pre-column (Torrance, CA, USA). Mobile phase components were 2 mM ammonium acetate in 0.1% formic acid and methanol and the flow rate was 0.3 mL/min. The proportion of methanol was increased from 10% to 40% over 5 min, to 75% at 13.50 min, to 80% at 16 min and held at 80% for 4 min. The post-time was 8 min, comprising a total run time of 28 min per sample, and the injection volume was 10 µL.

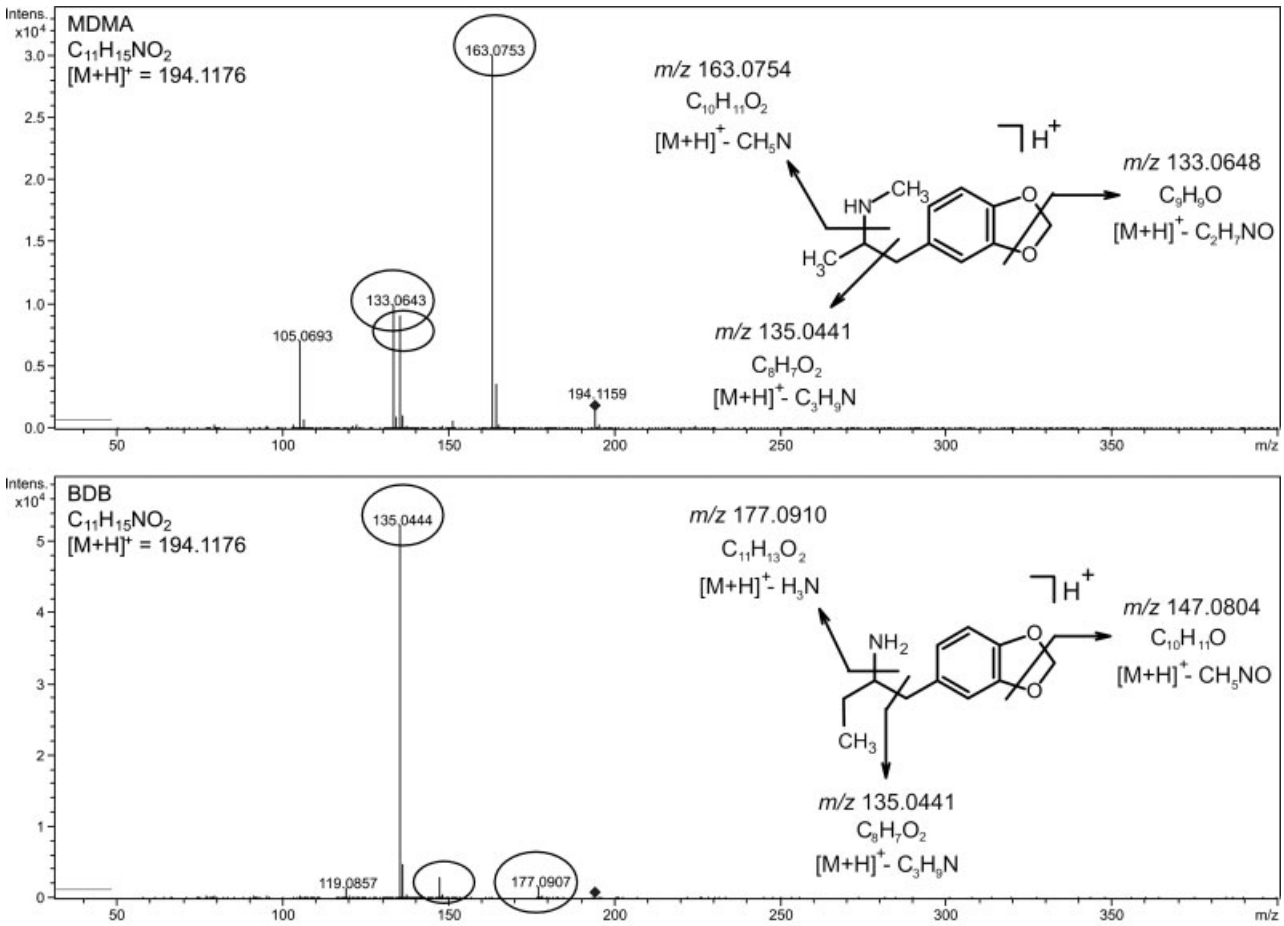
The mass analyzer was a Bruker Daltonics micrOTOF-Q mass spectrometer (Bremen, Germany) with an orthogonal electrospray ionization source and a six-port divert valve. The instrument was operated in positive ion mode with *m/z* range of 50–800. The nominal resolution of the instrument was 10 000 (FWHM). The nebulizer gas pressure was 1.6 bar and the drying gas flow 8.0 L/min. The drying temperature was 200 °C. The capillary voltage of the ion source was set at 4500 V and the end plate offset at −500 V. The quadrupole collision energy in MS mode was 6.0 eV and the collision cell radio-frequency 100.0 Vpp. The quadrupole transfer time was 60.0 µs and pre-pulse storage time 8.0 µs. The spectra rolling average was set at 2 and spectra time 0.6 s. Instrument calibration was performed externally with sodium formate solution, consisting of 10 mM sodium hydroxide in isopropanol and 0.2% formic acid (1 : 1, v/v). Ten sodium formate cluster ions, $(Na(NaCOOH)_{1-10})$ *m/z* values between 90.9766 and 702.8635, were selected for calibrating the instrument. Post-run internal mass scale calibration of individual samples was performed by injecting the calibrant at the beginning and at the end of each sample run. The calibrator ions in the post-run internal mass scale calibration were the same, excluding the ion *m/z* 702.8635, as used in the instrument calibration.

Mass fragmentation was performed in AutoMS(n) mode. When the intensity of the peak crosses the threshold level, the instrument measures every other spectrum in MS/MS mode and the alternate spectrum in MS mode. If several ions overlap, the instrument changes the ion for fragmentation after five spectra (3 s). The collision energy varies depending on the mass of the ion: light molecules are fragmented with less collision energy than heavier ones. Three different AutoMS(n) methods were created: general, high-collision energy and low-collision energy. In the general method, the collision energy for ions between 100 and 600 *m/z* varied linearly from 17 to 48 eV; in high-collision energy, from 22 to 56 eV; and in low-collision energy, from 12 to 36 eV. The absolute intensity threshold level in AutoMS(n) analysis was set at 30 000 cnts. All 16 mixtures were analyzed by the three methods to find out the optimal fragmentation energy for each compound.

### Software

DataAnalysis 4.0 software by Bruker Daltonics (Bremen, Germany) was used for post-run internal mass spectrum calibration and further processing of the data acquired in the analyses. An automatic compound finding function of DataAnalysis, AutoMS(n), was used for fast identification of the compounds in the total ion current (TIC) chromatograms. Parameters for AutoMS(n) were determined: the intensity threshold was set at 2500 cnts and the maximum number of compounds to be identified was 250.

A mass spectra processing tool of DataAnalysis, SmartFormula3D, was used for calculating molecular formulae for possible fragments and precursor ions based on their accurate masses and isotope distribution matches, mSigma values. The elements included in the calculations were C, H, N, O, Cl and S. SigmaFit algorithm provides a numerical comparison of theoretical and measured isotopic patterns and can be utilized as an identification tool in addition to accurate mass determination. The calculation of SigmaFit value includes generation of the theoretical isotope pattern for the assumed protonated molecule,[21] and calculation of a match factor based on the deviations of the signal intensities.[22] The lower the mSigma value, the better the isotopic match. SmartFormula3D includes an algorithm that estimates whether a formula for a product ion is a subset of a formula for the precursor ion. It




| Precursor MDMA | m/z calculated | error [mDa] | mSigma |
|---|---|---|---|
| C 11 H 16 N 1 O 2 | 194.117555 | -0.56 | 13.7 |

| Fragment | Intensity | m/z measured | m/z calculated | error [mDa] | mSigma | parity | Loss |
|---|---|---|---|---|---|---|---|
| C 10 H 11 O 2 | 29919 | 163.075328 | 163.075356 | 0 | 4.6 | even | C 1 H 5 N 1 |
| C 9 H 11 O 2 | 557 | 151.07462 | 151.075356 | 0.7 | 13.4 | even | C 2 H 5 N 1 |
| C 9 H 9 O 1 | 9785 | 133.064292 | 133.064791 | 0.5 | 5.4 | even | C 2 H 7 N 1 O 1 |
| C 8 H 9 | 7018 | 105.069337 | 105.069877 | 0.5 | 2 | even | C 3 H 7 N 1 O 2 |

| Precursor BDB | m/z calculated | error [mDa] | mSigma |
|---|---|---|---|
| C 11 H 16 N 1 O 2 | 194.117555 | -0.78 | 11.4 |

| Fragment | Intensity | m/z measured | m/z calculated | error [mDa] | mSigma | parity | Loss |
|---|---|---|---|---|---|---|---|
| C 11 H 13 O 2 | 23838 | 177.090918 | 177.091006 | 0.1 | 6.8 | even | H 3 N 1 |
| C 10 H 11 O 1 | 4941 | 147.081061 | 147.080441 | -0.6 | 1.6 | even | C 1 H 5 N 1 O 1 |
| C 8 H 7 O 2 | 46629 | 135.043977 | 135.044056 | 0.1 | 0.7 | even | C 3 H 9 N 1 |
| C 9 H 11 | 536 | 119.085435 | 119.085527 | 0.1 | 25.4 | even | C 2 H 5 N 1 O 2 |

**Figure 1.** Mass spectra and fragmentation schemes of MDMA and BDB ($[M+H]^+ = 194.1176$, $C_{11}H_{15}NO_2$). Results of SmartFormula3D identification are presented in tables automatically formed by the software. The identified characteristic fragments, corresponding to theoretical masses of *m/z* 163.0754, 135.0441 and 133.0648 for MDMA; and *m/z* 177.0910, 147.0804 and 135.0441 for BDB, are circled in the spectra. Possible structures for fragments provided by ACD/MS Fragmenter and SmartFormula3D are represented with arrows.

calculates a formula for the neutral or radical loss and determines if it fits with the observed mass difference for precursor and product ions. Product ions that cannot be related to the precursor ion are omitted; conversely, precursor ions that cannot be composed of any of the product ions are excluded. The precursor and product ion spectra of each compound were processed with the SmartFormula3D program. The mass tolerance for the precursor ion was set at 4 mDa and the isotopic pattern match value at 50 mSigma, and for product ions, 5 mDa and 100 mSigma, respectively. Electron configuration was set even for precursor ions and both even and odd for product ions. SmartFormula3D gives the sum formula, mass error, isotopic pattern match and electron configuration of the precursor and product ions in a chart (Figure 1), which can automatically be transferred to a spreadsheet.

ACD/MS Fragmenter 11.01 from Advanced Chemistry Development (Toronto, Canada) is a rule-based fragmentation prediction software. The program generates a fragmentation scheme for the drawn molecular structure using fragmentation rules of mass spectrometry known in the literature, as well as the selected ionization mode and the number of fragmentation steps. ACD/MS Fragmenter predicts both even and odd electron fragments, and forms a tree-model of all the possible fragments. The software provides information about the routes of fragmentation and all possible structures for a specific mass as well as the exact masses of the fragments. Experimental spectra of each compound were compared to the predicted fragment schemes, and the detected fragments were selected from the tree. The program parameters used in this study were API positive mode ionization, and the number of fragmentation steps was five. The fragmentation reactions were selected to include hetero and homolytic cleavage, neutral losses and hydrogen rearrangements. Other parameters of ACD/MS Fragmenter were left at their default values.

## Results and Discussion

The results of SmartFormula3D and ACD/MS Fragmenter for each compound were compared and compound characteristic fragments were identified based on the information achieved from the programs. The most abundant and isomer specific fragment ions in a mass spectrum were selected for each parent compound. Table 1 shows all of the 111 compounds studied, belonging to 48 isomer groups, and the fragmentation data. For each compound, 1–3 fragments were identified, adding up to 305 fragments. In 80% of the cases the total number of identified fragments was three. For six compounds, only one fragment could be identified, due to poor fragmentation (e.g. ropivacaine and metolazone) or because neither of the programs predicted the observed fragments (e.g. chlorcyclizine). Ten isomer pairs could not be differentiated from each other based on fragmentation; however, eight of these pairs could be differentiated with proper chromatographic separation. The compounds, which had similar fragmentation, were either diastereomers (e.g. ephedrine and pseudoephedrine, $[M+H]^+ = 166.1226$; Table 1, isomer group 2), or position isomers (e.g. 2C-T-4 and 2C-T-7, $[M+H]^+ = 256.1366$; Table 1, isomer group 21), where the position of the fragmenting side chain or substituent did not affect the fragments formed in the MSn analysis. The differences in spectra intensities were not used as an identification parameter in this research, because neither of the software predicted the ion abundances. Two isomer pairs, protriptyline and nortriptyline ($[M+H]^+ = 264.1747$; Table 1, isomer group 23), as well as *cis*-3-methylfentanyl and *trans*-3-methylfentanyl ($[M+H]^+ = 351.2431$; Table 1, isomer group 46), could be differentiated from each other neither by chromatography nor by their fragmentation.

From the 305 identified fragments, ACD/MS Fragmenter predicted 89% and SmartFormula3D 93%, while in 82% of cases the identified fragment was predicted by both programs. Only 7% of the fragments were identified solely by ACD/MS Fragmenter and 11% by SmartFormula3D. Of the identified fragments, 89% were formed by even electron neutral losses and 11% by odd electron radical losses. The structure of the identified fragment could not be determined based on SmartFormula3D results alone, because the program does not give structural information, only the sum formula. The validity of the fragment identification based on SmartFormula3D evaluation was ensured with mass accuracy and isotopic pattern match. The reason why ACD/MS Fragmenter and SmartFormula3D did not predict the same fragments in all cases remained unclear. The aim of this study was not to identify all fragments formed in the analysis, but to find the characteristic fragments in order to differentiate isomers from each other.

Both programs exploit accurate mass data in their prediction, which was the key feature in the identification of the compound characteristic fragments. The mean mass accuracy was 0.9 mDa and the mean SigmaFit value 24.6, as calculated from the absolute values of the precursor and fragment ions. Several research articles about the relationship between mass accuracy and ion abundance with Q-TOFMS instrument have been published.[23,24] Both mass accuracy and isotopic pattern match values are dependent on the ion abundance and show reduced match values when ion abundance is very low ($<1000$) or high ($>1 \times 10^6$). The same feature was seen in the present study and was taken into account when identifying precursor ions and fragments with SmartFormula3D. In some occasions the identification parameters had to be extended as high as 250 mSigma (ketobemidone, hydrocodone and milnacipran) to enable the identification of the parent compound or an obvious fragment structure predicted by the ACD/MS Fragmenter. A poor SigmaFit value of fragments did not always arise from high or low ion abundance. An extensively fragmenting molecule can have fragments differing only 2 Da from each other, resulting in the overlap of the isotopes, which may cause errors in isotopic pattern match measurement. That is why a good mass accuracy could be achieved, although the SigmaFit value did not fulfill the identification criteria (Table 1), and thus SigmaFit values higher than 200 were left out of the calculations.

Differentiation of isomers is presented here in detail by three examples of different isomer groups: MDMA and BDB; histapyrrodine, imipramine and nortrimipramine; and cocaine and scopolamine (Figures 1–3).

Methylenedioxymethamphetamine (MDMA, Ecstacy) and 1,3-benzodioxolylbutanamine (BDB), sharing a molecular formula of $C_{11}H_{15}NO_2$ and $[M+H]^+$ 194.1176, are structurally very similar compounds (Figure 1, isomer group 8 in Table 1). The only difference in their structure is the position of one methyl group. MDMA and BDB are chromatographically well-separated (Rt 7.18 min and 8.38 min, respectively), and their individual mass spectra are visually dissimilar. Both molecules undergo fragmentation of the amine group, which leads to fragments *m/z* 163.0754 for MDMA ($[M+H]^+$ - $CH_5N$) and *m/z* 177.0910 for BDB ($[M+H]^+$ - $H_3N$). The fragments *m/z* 133.0648 for MDMA and *m/z* 147.0804 for BDB are formed as a summation of the cleavage of amino group and the breakage of the methylenedioxy ring. MDMA and BDB share one common fragment, *m/z* 135.0441, which forms in the cleavage of the aliphatic side chain ($[M+H]^+$ - $C_3H_9N$). SmartFormula3D did not identify the ion *m/z* 135.0441 with the selected software parameters to be cleaved from MDMA, although the mass accuracy and isotopic pattern match were within the identification criteria (Figure 1, Table 1) for that ion. The fragmentation reaction, from which the fragment *m/z* 135.0441 would be formed, is in congruence with the reactions of other compounds with similar structure, e.g. BDB, MDDMA, MDEA and MBDB, for which SmartFormula3D identified the fragment *m/z* 135.0441 correctly. All other fragments were predicted by both software programs and they were even electron neutral losses.

**Table 1.** Molecular formula, mass accuracy and isotopic pattern match for observed fragments of 111 compounds belonging to 48 isomer groups, listed by increasing molecular mass of precursor ions

| | Mass $[M+H]^+$ | Rt min | Molecular formula | Compound | Frag. 1 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 2 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 3 *m/z* | Error mDa | mSigma | Neutral or radical loss |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 150.1277 | 6.12 | $C_{10}H_{15}N$ | Methamphetamine | 119.0855 | −0.1 | 2.7 | $CH_5N$ | 91.0542 | −0.1 | 1.0 | $C_3H_9N$ | | | | |
| | 150.1277 | 7.27 | $C_{10}H_{15}N$ | Phentermine | 133.1012 | 0.7 | 5.2 | $H_3N$ | 91.0542 | 0.0 | 2.4 | $C_3H_9N$ | | | | |
| 2 | 166.1226 | 3.54 | $C_{10}H_{15}NO$ | Ephedrine[a] | 148.1121 | −0.4 | 1.0 | $H_2O$ | 133.0886 | 1.1 | 4.3 | $CH_5O$ | 117.0699 | 0.7 | 7.1 | $CH_7NO$ |
| | 166.1226 | 3.97 | $C_{10}H_{15}NO$ | Pseudoephedrine[a] | 148.1121 | −0.6 | 1.3 | $H_2O$ | 133.0886 | 0.7 | 3.4 | $CH_5O$ | 117.0699 | 0.3 | 16.4 | $CH_7NO$ |
| | 166.1226 | 7.08 | $C_{10}H_{15}NO$ | PMA | 149.0961 | −0.4 | 8.5 | $H_3N$ | 121.0648 | 0.4 | 9.6 | $C_2H_7N$ | | | | |
| 3 | 178.1226 | 5.72 | $C_{11}H_{15}NO$ | Ethylcathinone | 160.1121 | −0.7 | 6.9 | $H_2O$ | 132.0808 | 0.6 | 7.7 | $C_2H_6O$ | | | | |
| | 178.1226 | 6.08 | $C_{11}H_{15}NO$ | Phenmetrazine | 160.1121 | −0.2 | 1.9 | $H_2O$ | 134.0964 | 0.7 | 6.9 | $C_2H_4O$ | 117.0699 | 0.0 | 96.3 | $C_2H_7NO$ |
| | 178.1226 | 7.66 | $C_{11}H_{15}NO$ | 4-MMC | 160.1121 | 0.1 | 4.6 | $H_2O$ | 145.0886 | −0.5 | 18.6 | $CH_5O$ | 119.0855 | 0.5 | 1.3 | $C_2H_5NO$ |
| 4 | 180.1019 | 6.66 | $C_{10}H_{13}NO_2$ | MDA | 163.0754 | 0.1 | 7.1 | $H_3N$ | 135.0441 | 0.8 | 3.5 | $C_2H_7N$ | 133.0648 | 0.6 | 13.1 | $CH_5NO$ |
| | 180.1019 | 12.08 | $C_{10}H_{13}NO_2$ | Phenacetin | 152.0706 | 0.0 | 1.9 | $C_2H_4$ | 138.0913 | 0.7 | 6.4 | $C_2H_2O$ | 110.0600 | 0.1 | 2.6 | $C_4H_6O$ |
| 5 | 180.1383 | 4.36 | $C_{11}H_{17}NO$ | Methylephedrine | 162.1277 | −0.3 | 5.4 | $H_2O$ | 147.1043 | −0.8 | 0.8 | $CH_5O$ | 117.0699 | 0.1 | 9.1 | $C_2H_9NO$ |
| | 180.1383 | 7.59 | $C_{11}H_{17}NO$ | PMMA[b] | 149.0961 | −0.4 | 4.2 | $CH_5N$ | 121.0648 | 0.4 | 5.5 | $C_3H_9N$ | | | | |
| | 180.1383 | 8.40 | $C_{11}H_{17}NO$ | Methoxyphenamine[b] | 149.0961 | −0.8 | 1.5 | $CH_5N$ | 121.0648 | −0.2 | 2.8 | $C_3H_9N$ | | | | |
| | 180.1383 | 9.84 | $C_{11}H_{17}NO$ | Mexiletine | 163.1117 | −0.6 | 31.1 | $H_3N$ | 121.0648 | 0.9 | 93.6 | $C_3H_9N$ | 105.0699 | 0.9 | 30.0 | $C_3H_9NO$ |
| 6 | 181.0720 | 5.63 | $C_7H_8N_4O_2$ | Theobromine | 163.0614 | 1.0 | 7.2 | $H_2O$ | 138.0662 | 0.1 | 10.5 | CHNO | | | | |
| | 181.0720 | 7.51 | $C_7H_8N_4O_2$ | Theophylline | 124.0505 | 0.3 | 4.9 | $C_2H_3NO$ | | | | | | | | |
| 7 | 182.1176 | 1.90 | $C_{10}H_{15}NO_2$ | Etilefrine | 164.1070 | 0.0 | 6.1 | $H_2O$ | 135.0679 | 0.2 | >200 | $C_2H_7O$ | | | | |
| | 182.1176 | 1.97 | $C_{10}H_{15}NO_2$ | HHMA | 151.0754 | −0.5 | 0.2 | $CH_5N$ | 133.0648 | 0.7 | 5.5 | $CH_7NO$ | 123.0441 | 0.2 | 3.5 | $C_3H_9N$ |
| | 182.1176 | 3.61 | $C_{10}H_{15}NO_2$ | HMA | 165.0910 | 0.2 | 4.6 | $H_3N$ | 137.0597 | 0.9 | 7.0 | $C_2H_7N$ | 133.0648 | 0.4 | 3.8 | $CH_7NO$ |
| | 182.1176 | 7.84 | $C_{10}H_{15}NO_2$ | 2-CH | 165.0910 | 0.0 | 2.0 | $H_3N$ | 150.0675 | −0.9 | 5.6 | $CH_6N$ | 105.0699 | 0.7 | 5.1 | $C_2H_7NO_2$ |
| 8 | 194.1176 | 7.18 | $C_{11}H_{15}NO_2$ | MDMA | 163.0754 | 0.0 | 4.6 | $CH_5N$ | 135.0441 | 0.4 | 8.5 | $C_3H_9N$ | 133.0648 | 0.5 | 5.4 | $C_2H_7NO$ |
| | 194.1176 | 8.38 | $C_{11}H_{15}NO_2$ | BDB | 177.0910 | 0.1 | 6.8 | $H_3N$ | 147.0804 | −0.6 | 1.6 | $CH_5NO$ | 135.0441 | 0.1 | 0.7 | $C_3H_9N$ |
| 9 | 196.1332 | 4.01 | $C_{11}H_{17}NO_2$ | HMMA | 165.0910 | 0.1 | 1.4 | $CH_5N$ | 137.0597 | 0.8 | 1.0 | $C_3H_9N$ | 133.0648 | 0.8 | 6.7 | $C_2H_9NO$ |
| | 196.1332 | 7.04 | $C_{11}H_{17}NO_2$ | 3,4-DMA[b] | 179.1067 | 0.1 | 4.1 | $H_3N$ | 164.0862 | 0.6 | 1.0 | $CH_6N$ | 151.0754 | −0.6 | 1.0 | $C_2H_7N$ |
| | 196.1332 | 8.89 | $C_{11}H_{17}NO_2$ | 2,5-DMA[b] | 179.1067 | 0.0 | 4.1 | $H_3N$ | 164.0862 | −0.1 | 3.3 | $CH_6N$ | 151.0754 | −0.8 | 0.3 | $C_2H_7N$ |
| 10 | 205.1335 | 5.34 | $C_{12}H_{16}N_2O$ | Psilocin | 160.0757 | 0.2 | 1.8 | $C_2H_7N$ | 132.0808 | 0.8 | 15.6 | $C_3H_7NO$ | | | | |
| | 205.1335 | 8.71 | $C_{12}H_{16}N_2O$ | 5-MeO-AMT | 188.1070 | 0.7 | 3.1 | $H_2N$ | 173.0835 | 0.3 | 6.6 | $CH_6N$ | 147.0679 | −0.2 | 5.4 | $C_3H_8N$ |
| 11 | 208.1332 | 7.36 | $C_{12}H_{17}NO_2$ | MDDMA[b] | 163.0754 | −0.3 | 2.0 | $C_2H_7N$ | 135.0441 | 0.5 | 3.1 | $C_4H_{11}N$ | 133.0648 | 0.5 | 3.9 | $C_3H_9NO$ |
| | 208.1332 | 8.01 | $C_{12}H_{17}NO_2$ | MDEA[b] | 163.0754 | −0.4 | 2.4 | $C_2H_7N$ | 135.0441 | 0.1 | 5.4 | $C_4H_{11}N$ | 133.0648 | 0.1 | 6.1 | $C_3H_9NO$ |
| | 208.1332 | 8.64 | $C_{12}H_{17}NO_2$ | MBDB | 177.0910 | 0.2 | 9.1 | $CH_5N$ | 147.0804 | −0.2 | 7.0 | $C_2H_7NO$ | 135.0441 | −0.2 | 4.1 | $C_4H_{11}N$ |
| 12 | 210.1489 | 10.80 | $C_{12}H_{19}NO_2$ | DOM | 193.1223 | 0.1 | 6.5 | $H_3N$ | 178.0988 | −0.1 | 2.5 | $CH_6N$ | 165.0910 | −0.2 | 3.9 | $C_2H_7N$ |
| | 210.1489 | 11.55 | $C_{12}H_{19}NO_2$ | 2-CE | 193.1223 | −0.1 | 2.4 | $H_3N$ | 178.0988 | −0.2 | 3.6 | $CH_6N$ | 135.0804 | 0.4 | 15.8 | $C_3H_9NO$ |
| 13 | 226.1438 | 4.17 | $C_{12}H_{19}NO_3$ | Terbutaline | 152.0706 | −0.4 | 2.9 | $C_4H_{10}O$ | 135.0441 | −1.7 | 1.5 | $C_4H_{13}NO$ | 125.0597 | 0.6 | 8.8 | $C_5H_{11}NO$ |
| | 226.1438 | 7.75 | $C_{12}H_{19}NO_3$ | 3,4,5-TMA | 209.1172 | 0.7 | 1.5 | $H_3N$ | 194.0937 | 0.5 | 1.8 | $CH_6N$ | 181.0859 | 0.7 | 4.0 | $C_2H_7N$ |

**Table 1.** (*Continued*)

| | Mass $[M+H]^+$ | Rt min | Molecular formula | Compound | Frag. 1 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 2 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 3 *m/z* | Error mDa | mSigma | Neutral or radical loss |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 236.1645 | 6.62 | $C_{14}H_{21}NO_2$ | *O*-desmethylnortramadol | 218.1539 | 0.1 | 15.4 | $H_2O$ | 187.1117 | 0.6 | 32.0 | $CH_7NO$ | | | | |
| | 236.1645 | 9.16 | $C_{14}H_{21}NO_2$ | Dinortramadol | 218.1539 | 0.0 | 1.9 | $H_2O$ | 189.1274 | 0.0 | 9.2 | $CH_5NO$ | 121.0648 | 0.4 | 1.4 | $C_6H_{13}NO$ |
| 15 | 237.1598 | 4.43 | $C_{13}H_{20}N_2O_2$ | Procaine | 164.0706 | 0.1 | 3.0 | $C_4H_{11}N$ | 120.0444 | 0.2 | 4.7 | $C_6H_{15}NO$ | 100.1121 | 0.3 | 1.2 | $C_7H_7NO_2$ |
| | 237.1598 | 4.81 | $C_{13}H_{20}N_2O_2$ | Dropropizine | 175.1230 | 0.6 | 4.4 | $C_2H_6O_2$ | 160.0995 | 0.2 | 16.6 | $C_3H_9O_2$ | 132.0808 | 0.2 | 25.2 | $C_4H_{11}NO_2$ |
| 16 | 245.2012 | 10.90 | $C_{16}H_{24}N_2$ | DPT | 144.0808 | −1.1 | 7.2 | $C_6H_{15}N$ | 114.1277 | −0.4 | 3.3 | $C_9H_9N$ | | | | |
| | 245.2012 | 13.24 | $C_{16}H_{24}N_2$ | Xylometazoline | 230.1778 | 0.4 | 13.9 | $CH_3$ | 175.1481 | 0.6 | 15.8 | $C_3H_6N_2$ | 161.1325 | 0.6 | 1.8 | $C_4H_8N_2$ |
| 17 | 247.1805 | 7.77 | $C_{15}H_{22}N_2O$ | Mepivacaine | 98.0964 | −0.1 | 1.8 | $C_9H_{11}NO$ | | | | | | | | |
| | 247.1805 | 9.39 | $C_{15}H_{22}N_2O$ | 5-MeO-MIPT | 174.0913 | −0.9 | 4.9 | $C_4H_{11}N$ | 159.0679 | −0.1 | 3.6 | $C_5H_{14}N$ | 86.0964 | 0.8 | 2.4 | $C_{10}H_{11}NO$ |
| | 247.1805 | 10.51 | $C_{15}H_{22}N_2O$ | Milnacipran | 230.1539 | −0.9 | 7.5 | $H_3N$ | 129.0699 | 0.3 | >200 | $C_5H_{14}N_2O$ | 100.0757 | −0.1 | 2.0 | $C_{10}H_{13}N$ |
| 18 | 248.1645 | 7.56 | $C_{15}H_{21}NO_2$ | Ketobemidone | 230.1539 | −0.6 | 9.5 | $H_2O$ | 201.1148 | −0.2 | 52.8 | $C_2H_7O$ | 190.1226 | 0.3 | >200 | $C_3H_6O$ |
| | 248.1645 | 9.67 | $C_{15}H_{21}NO_2$ | Pethidine | 220.1332 | −1.0 | 7.4 | $C_2H_4$ | 202.1226 | 0.4 | 4.3 | $C_2H_6O$ | 174.1277 | 0.0 | 20.9 | $C_3H_6O_2$ |
| 19 | 250.1802 | 6.72 | $C_{15}H_{23}NO_2$ | *O*-desmethyltramadol | 232.1696 | 1.4 | 93.5 | $H_2O$ | 187.1117 | 1.7 | 54.1 | $C_2H_9NO$ | 58.0651 | 1.5 | 20.9 | $C_{12}H_{16}O_2$ |
| | 250.1802 | 9.38 | $C_{15}H_{23}NO_2$ | Nortramadol | 232.1696 | 3.6 | 19.6 | $H_2O$ | 201.1274 | 1.0 | 36.2 | $CH_7NO$ | 189.1274 | 1.4 | 5.6 | $C_2H_7NO$ |
| | 250.1802 | 12.23 | $C_{15}H_{23}NO_2$ | Alprenolol | 208.1332 | 0.5 | 5.6 | $C_3H_6$ | 173.0961 | 0.3 | 6.1 | $C_3H_{11}NO$ | 116.1070 | 0.5 | 38.3 | $C_9H_{10}O$ |
| 20 | 253.1366 | 12.73 | $C_{15}H_{12}N_2O_2$ | Oxcarbazepine | 236.0706 | 0.0 | 10.9 | $H_3N$ | 208.0757 | −0.2 | 157.1 | $CH_3NO$ | 180.0808 | 0.6 | 183.0 | $C_2H_3NO_2$ |
| | 253.1366 | 13.38 | $C_{15}H_{12}N_2O_2$ | Phenytoin | 182.0964 | −0.1 | 4.2 | $C_2HNO_2$ | | | | | | | | |
| 21 | 256.1366 | 12.32 | $C_{13}H_{21}NO_2S$ | 2C-T-4[b] | 239.1100 | −0.7 | 8.5 | $H_3N$ | 224.0866 | −0.3 | 7.5 | $CH_6N$ | 197.0631 | −0.8 | 9.5 | $C_3H_9N$ |
| | 256.1366 | 12.82 | $C_{13}H_{21}NO_2S$ | 2C-T-7[b] | 239.1100 | −0.2 | 9.8 | $H_3N$ | 224.0866 | 0.3 | 7.4 | $CH_6N$ | 197.0631 | 0.3 | 10.9 | $C_3H_9N$ |
| 22 | 256.1696 | 11.86 | $C_{17}H_{21}NO$ | Diphenhydramine | 167.0855 | −1.7 | 6.9 | $C_4H_{11}NO$ | 152.0621 | −0.5 | 0.7 | $C_5H_{14}NO$ | | | | |
| | 256.1696 | 12.94 | $C_{17}H_{21}NO$ | Nororphenadrine | 181.1012 | −0.4 | 8.6 | $C_3H_9NO$ | 166.0777 | −0.1 | 11.8 | $C_4H_{12}NO$ | 153.0699 | −0.2 | 2.4 | $C_5H_{13}NO$ |
| 23 | 264.1747 | 14.11 | $C_{19}H_{21}N$ | Protriptyline[b] | 233.1325 | −0.7 | 10.3 | $CH_5N$ | 191.0855 | −0.6 | 14.5 | $C_4H_{11}N$ | 155.0855 | −0.5 | 2.2 | $C_7H_{11}N$ |
| | 264.1747 | 14.24 | $C_{19}H_{21}N$ | Nortriptyline[b] | 233.1325 | −0.7 | 7.3 | $CH_5N$ | 191.0855 | −0.2 | 74.2 | $C_4H_{11}N$ | 155.0855 | −0.2 | 10.7 | $C_7H_{11}N$ |
| 24 | 264.1958 | 8.26 | $C_{16}H_{25}NO_2$ | *O*-desmethylvenlafaxine | 246.1852 | 0.1 | 4.5 | $H_2O$ | 201.1274 | 0.9 | 1.8 | $C_2H_9NO$ | 133.0648 | 0.8 | 4.4 | $C_7H_{17}NO$ |
| | 264.1958 | 9.10 | $C_{16}H_{25}NO_2$ | Tramadol | 246.1852 | 0.8 | 43.2 | $H_2O$ | 201.1274 | 0.0 | 7.5 | $C_2H_9NO$ | 58.0651 | 2.1 | 6.7 | $C_{13}H_{18}O_2$ |
| | 264.1958 | 10.59 | $C_{16}H_{25}NO_2$ | Norvenlafaxine | 246.1852 | −0.4 | 21.8 | $H_2O$ | 215.1430 | −0.2 | 6.4 | $CH_7NO$ | 121.0648 | 0.0 | 9.7 | $C_8H_{17}NO$ |
| 25 | 266.1652 | 9.13 | $C_{17}H_{19}N_3$ | Mirtazapine | 235.1230 | −0.3 | 37.8 | $CH_5N$ | 209.1073 | −0.3 | 12.1 | $C_3H_7N$ | 195.0917 | −1.4 | 1.4 | $C_4H_9N$ |
| | 266.1652 | 11.36 | $C_{17}H_{19}N_3$ | Antazoline | 196.1121 | −0.4 | 4.5 | $C_3H_6N_2$ | 175.1104 | 0.7 | 3.0 | $C_7H_7$ | 91.0542 | −0.3 | 1.8 | $C_{10}H_{13}N_3$ |
| 26 | 267.1703 | 4.12 | $C_{14}H_{22}N_2O_3$ | Atenolol | 190.0863 | 0.5 | 3.5 | $C_3H_{11}NO$ | 178.0863 | 0.7 | 191.9 | $C_4H_{11}NO$ | 145.0648 | 0.0 | 8.5 | $C_4H_{14}N_2O_2$ |
| | 267.1703 | 5.65 | $C_{14}H_{22}N_2O_3$ | Practolol | 225.1234 | 0.2 | 2.6 | $C_3H_6$ | 190.0863 | 0.1 | 2.0 | $C_3H_{11}NO$ | 178.0863 | 0.1 | 4.7 | $C_4H_{11}NO$ |
| 27 | 267.1856 | 12.25 | $C_{18}H_{22}N_2$ | Cyclizine | 167.0855 | −1.0 | 2.0 | $C_5H_{12}N_2$ | 152.0621 | −0.5 | 1.3 | $C_6H_{15}N_2$ | | | | |
| | 267.1856 | 14.00 | $C_{18}H_{22}N_2$ | Desipramine | 236.1434 | −0.3 | 4.8 | $CH_5N$ | 208.1121 | 0.0 | 7.5 | $C_3H_9N$ | 72.0808 | 1.8 | 0.6 | $C_{14}H_{13}N$ |
| 28 | 268.1696 | 10.13 | $C_{18}H_{21}NO$ | Pipradrol | 250.1590 | −1.4 | 8.5 | $H_2O$ | 172.1121 | 0.1 | 41.1 | $C_6H_8O$ | 167.0855 | 0.1 | 3.9 | $C_5H_{11}NO$ |
| | 268.1696 | 10.88 | $C_{18}H_{21}NO$ | Azacyclonol | 250.1590 | −0.2 | 0.4 | $H_2O$ | 167.0855 | 0.5 | 1.0 | $C_5H_{11}NO$ | 143.0855 | 0 | 21.8 | $C_7H_{11}NO$ |

**Table 1.** (*Continued*)

| | Mass $[M+H]^+$ | Rt min | Molecular formula | Compound | Frag. 1 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 2 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 3 *m/z* | Error mDa | mSigma | Neutral or radical loss |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 275.2118 | 9.75 | $C_{17}H_{26}N_2O$ | Ropivacaine | 126.1277 | −0.7 | 2.1 | $C_9H_{11}NO$ | | | | | | | | |
| | 275.2118 | 10.71 | $C_{17}H_{26}N_2O$ | 5-MeO-DIPT | 174.0913 | −0.8 | 3.8 | $C_6H_{15}N$ | 159.0679 | −0.1 | 1.8 | $C_7H_{18}N$ | 114.1277 | 0.1 | 1.9 | $C_{10}H_{11}NO$ |
| 30 | 278.1903 | 11.84 | $C_{20}H_{23}N$ | EDDP | 249.1512 | −0.7 | 6.6 | $C_2H_5$ | 234.1277 | −0.3 | 11.2 | $C_3H_8$ | 186.1277 | 0.5 | 17.1 | $C_7H_8$ |
| | 278.1903 | 13.67 | $C_{20}H_{23}N$ | Maprotiline | 250.1590 | −1.2 | 5.0 | $C_2H_4$ | 219.1168 | −0.2 | 8.4 | $C_3H_9N$ | 191.0855 | 0.2 | 176.6 | $C_5H_{13}N$ |
| | 278.1903 | 14.20 | $C_{20}H_{23}N$ | Amitriptyline | 233.1325 | −1.0 | 7.0 | $C_2H_7N$ | 205.1012 | −0.1 | 82.4 | $C_4H_{11}N$ | 155.0855 | −0.2 | 7.9 | $C_8H_{13}N$ |
| 31 | 280.1696 | 10.75 | $C_{19}H_{21}NO$ | *E*-10-hydroxynortriptyline[a] | 262.1590 | −0.6 | 1.0 | $H_2O$ | 231.1168 | −0.9 | 8.9 | $CH_7NO$ | 216.0934 | −0.5 | 13.1 | $C_2H_9NO$ |
| | 280.1696 | 11.97 | $C_{19}H_{21}NO$ | *Z*-10-hydroxynortriptyline[a] | 262.1590 | −0.9 | 8.0 | $H_2O$ | 231.1168 | −0.7 | 11.5 | $CH_7NO$ | 216.0934 | −0.6 | 16.5 | $C_2H_9NO$ |
| | 280.1696 | 12.85 | $C_{19}H_{21}NO$ | Doxepin | 235.1117 | −0.5 | 8.9 | $C_2H_7N$ | 217.1012 | −0.3 | 41.9 | $C_2H_9NO$ | 107.0491 | −0.3 | 1.9 | $C_{12}H_{15}N$ |
| 32 | 281.2012 | 12.81 | $C_{19}H_{24}N_2$ | Histapyrrodine | 210.1277 | −0.3 | 1.0 | $C_4H_9N$ | 132.0808 | 1.0 | 18.4 | $C_{10}H_{15}N$ | 98.0964 | −0.3 | 1.3 | $C_{13}H_{13}N$ |
| | 281.2012 | 13.99 | $C_{19}H_{24}N_2$ | Imipramine | 236.1434 | −0.4 | 1.5 | $C_2H_7N$ | 208.1121 | −0.2 | 4.0 | $C_4H_{11}N$ | 86.0964 | 0.8 | 1.0 | $C_{14}H_{13}N$ |
| | 281.2012 | 14.48 | $C_{19}H_{24}N_2$ | Nortrimipramine | 250.1590 | −0.2 | 3.4 | $CH_5N$ | 208.1121 | −0.4 | 0.7 | $C_4H_{11}N$ | 196.1121 | 0.4 | 4.9 | $C_5H_{11}N$ |
| 33 | 285.1420 | 13.20 | $C_{17}H_{20}N_2S$ | Promethazine | 240.0841 | −1.0 | 2.8 | $C_2H_7N$ | 198.0372 | −0.8 | 5.6 | $C_5H_{13}N$ | 86.0964 | 0.7 | 1.2 | $C_{12}H_9NS$ |
| | 285.1420 | 13.82 | $C_{17}H_{20}N_2S$ | Promazine | 240.0841 | −0.2 | 6.7 | $C_2H_7N$ | 212.0528 | −0.1 | 3.5 | $C_4H_{11}N$ | 86.0964 | 0.8 | 1.9 | $C_{12}H_9NS$ |
| 34 | 286.1438 | 3.02 | $C_{17}H_{19}NO_3$ | Hydromorphone | 227.0703 | 0.9 | 183.0 | $C_3H_9N$ | 185.0597 | −0.1 | 10.2 | $C_5H_{11}NO$ | | | | |
| | 286.1438 | 1.97 | $C_{17}H_{19}NO_3$ | Morphine | 229.0859 | 0.0 | 6.2 | $C_3H_7N$ | 211.0754 | 0.1 | 25.9 | $C_3H_9NO$ | 201.0910 | 0.7 | 11.8 | $C_4H_7NO$ |
| | 286.1438 | 4.67 | $C_{17}H_{19}NO_3$ | Norcodeine | 268.1332 | −0.8 | 31.5 | $H_2O$ | 225.0910 | 0.4 | 70.7 | $C_2H_7NO$ | 215.1067 | 1.5 | 49.4 | $C_3H_5NO$ |
| 35 | 287.0582 | 13.34 | $C_{15}H_{11}N_2O_2Cl$ | Demoxepam | 269.0476 | −0.9 | >200 | $H_2O$ | 241.0527 | 0.0 | >200 | $CH_2O_2$ | 179.9847 | −0.5 | 15.6 | $C_7H_9N$ |
| | 287.0582 | 14.66 | $C_{15}H_{11}N_2O_2Cl$ | Oxazepam | 269.0476 | −0.4 | 5.2 | $H_2O$ | 241.0527 | −0.5 | 32.8 | $CH_2O_3$ | 231.0684 | −0.1 | 26.1 | $C_2O_2$ |
| 36 | 294.1852 | 10.78 | $C_{20}H_{23}NO$ | *E*-10-hydroxyamitriptyline[a] | 276.1747 | −1.6 | 1.6 | $H_2O$ | 231.1168 | −1.1 | 21.1 | $C_2H_9NO$ | 216.0934 | −0.7 | 11.6 | $C_3H_{12}NO$ |
| | 294.1852 | 11.98 | $C_{20}H_{23}NO$ | *Z*-10-hydroxyamitriptyline[a] | 276.1747 | −1.4 | 1.2 | $H_2O$ | 231.1168 | −1.0 | 48.0 | $C_2H_9NO$ | 216.0934 | −0.5 | 14.7 | $C_3H_{12}NO$ |
| 37 | 300.1594 | 5.19 | $C_{18}H_{21}NO_3$ | Codeine | 243.1016 | −0.3 | 2.8 | $C_3H_7N$ | 225.0910 | 0.4 | 33.1 | $C_3H_9NO$ | 215.1067 | 0.2 | 5.0 | $C_4H_7NO$ |
| | 300.1594 | 6.68 | $C_{18}H_{21}NO_3$ | Hydrocodone | 243.1016 | −0.7 | 5.5 | $C_3H_7N$ | 241.0859 | 0.9 | >200 | $C_3H_9N$ | 213.0910 | −0.1 | 77.5 | $C_4H_9NO$ |
| 38 | 301.0738 | 14.89 | $C_{16}H_{13}N_2O_2Cl$ | Clobazam | 259.0633 | −1.3 | 6.7 | $C_2H_2O$ | 224.0944 | 0.0 | 5.8 | $C_2H_2ClO$ | | | | |
| | 301.0738 | 15.38 | $C_{16}H_{13}N_2O_2Cl$ | Temazepam | 255.0684 | −1.1 | 14.3 | $CH_2O_2$ | 228.0575 | −0.2 | 26.9 | $C_2H_3NO_2$ | | | | |
| 39 | 301.1466 | 14.13 | $C_{18}H_{21}N_2Cl$ | Chlorcyclizine | 201.0466 | −0.2 | 10.8 | $C_5H_{12}N_2$ | | | | | | | | |
| | 301.1466 | 15.53 | $C_{18}H_{21}N_2Cl$ | Norclomipramine | 270.1044 | −1.2 | 1.9 | $CH_5N$ | 242.0731 | −1.1 | 13.1 | $C_3H_9N$ | 72.0808 | 1.5 | 2.1 | $C_{14}H_{12}NCl$ |
| 40 | 302.1387 | 2.49 | $C_{17}H_{19}NO_4$ | Oxymorphone | 284.1281 | −1.1 | 5.6 | $H_2O$ | 242.1176 | 0.0 | 1.7 | $C_2H_4O_2$ | 227.0941 | 1.3 | 18.2 | $C_3H_7O_2$ |
| | 302.1387 | 6.25 | $C_{17}H_{19}NO_4$ | Noroxycodone | 284.1281 | 0.0 | 3.2 | $H_2O$ | 227.0941 | −0.1 | 70.3 | $C_3H_7O_2$ | 187.0754 | 0.4 | 46.6 | $C_5H_9NO_2$ |
| 41 | 302.1751 | 5.08 | $C_{18}H_{23}NO_3$ | Dihydrocodeine | 245.1172 | 0.0 | 3.6 | $C_3H_7N$ | 227.1067 | 0.0 | 17.3 | $C_3H_9NO$ | 201.0910 | 0.2 | 15.9 | $C_5H_{11}NO$ |
| | 302.1751 | 10.44 | $C_{18}H_{23}NO_3$ | Isoxsuprine | 284.1645 | −1.8 | 1.8 | $H_2O$ | 150.0913 | −0.7 | 34.8 | $C_9H_{12}O_2$ | 135.0804 | 0.6 | 3.8 | $C_9H_{13}NO_2$ |
| 42 | 304.1543 | 6.00 | $C_{17}H_{21}NO_4$ | Scopolamine | 156.1019 | −0.3 | 9.1 | $C_9H_8O_2$ | 138.0913 | −0.1 | 4.6 | $C_9H_{10}O_3$ | 121.0648 | 0.4 | 14.3 | $C_9H_{13}NO_3$ |
| | 304.1543 | 9.58 | $C_{17}H_{21}NO_4$ | Cocaine | 182.1176 | −0.5 | 2.3 | $C_7H_6O_2$ | 150.0913 | −0.5 | 11.1 | $C_8H_{10}O_3$ | 105.0335 | −0.6 | 9.0 | $C_{10}H_{17}NO_3$ |

**Table 1.** (*Continued*)

| | Mass $[M+H]^+$ | Rt min | Molecular formula | Compound | Frag. 1 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 2 *m/z* | Error mDa | mSigma | Neutral or radical loss | Frag. 3 *m/z* | Error mDa | mSigma | Neutral or radical loss |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 | 314.1751 | 7.33 | $C_{19}H_{23}NO_3$ | Ethylmorphine | 257.1172 | 0.0 | 4.4 | $C_3H_7N$ | 239.1067 | 0.9 | 21.1 | $C_3H_9NO$ | 229.1223 | 2.3 | 4.2 | $C_4H_7NO$ |
| | 314.1751 | 12.57 | $C_{19}H_{23}NO_3$ | Reboxetine | 176.1070 | 0.8 | 3.7 | $C_8H_{10}O_2$ | 158.0964 | 0.7 | 11.1 | $C_8H_{12}O_3$ | 91.0542 | 0.1 | 4.3 | $C_{12}H_{17}NO_3$ |
| 44 | 325.1911 | 9.38 | $C_{20}H_{24}N_2O_2$ | Quinidine[a] | 307.1805 | −1.3 | 6.7 | $H_2O$ | 253.1335 | −0.1 | 16.8 | $C_4H_8O$ | 160.0757 | −0.1 | 37.8 | $C_{10}H_{15}NO$ |
| | 325.1911 | 9.72 | $C_{20}H_{24}N_2O_2$ | Quinine[a] | 307.1805 | −1.2 | 3.5 | $H_2O$ | 253.1335 | −0.4 | 17.6 | $C_4H_8O$ | 160.0757 | −0.1 | 41.4 | $C_{10}H_{15}NO$ |
| 45 | 328.1543 | 5.56 | $C_{19}H_{21}NO_4$ | Naloxone | 310.1438 | 0.8 | 7.9 | $H_2O$ | 268.1332 | 1.2 | 167.9 | $C_2H_4O_2$ | 253.1097 | 0.1 | 33.3 | $C_3H_7O_2$ |
| | 328.1543 | 6.75 | $C_{19}H_{21}NO_4$ | 6-MAM | 268.1332 | −0.4 | 41.3 | $C_2H_4O_2$ | 211.0754 | 0.3 | 6.0 | $C_5H_{11}NO_2$ | 193.0648 | −1.3 | 25.9 | $C_5H_{13}NO_3$ |
| 46 | 351.2431 | 12.30 | $C_{23}H_{30}N_2O$ | *cis*-3-methylfentanyl[a] | 230.1539 | −0.4 | 5.3 | $C_8H_{11}N$ | 202.1590 | −1.3 | 10.3 | $C_9H_{11}NO$ | 134.0964 | 0.4 | 0.3 | $C_{14}H_{19}NO$ |
| | 351.2431 | 12.30 | $C_{23}H_{30}N_2O$ | *trans*-3-methylfentanyl[a] | 230.1539 | −0.1 | 8.4 | $C_8H_{11}N$ | 202.1590 | −2.2 | 33.4 | $C_9H_{11}NO$ | 134.0964 | 0.7 | 2.1 | $C_{14}H_{19}NO$ |
| 47 | 366.0674 | 12.93 | $C_{16}H_{16}N_3O_3SCl$ | Metolazone | 258.9939 | −1.5 | 20.3 | $C_7H_9N$ | | | | | | | | |
| | 366.0674 | 13.73 | $C_{16}H_{16}N_3O_3SCl$ | Indapamide | 132.0808 | 0.2 | 6.4 | $C_7H_7N_2O_3ClS$ | 117.0573 | 0.0 | 174.6 | $C_8H_{10}N_2O_3ClS$ | 91.0542 | 0 | 6.4 | $C_9H_{10}N_3O_3ClS$ |
| 48 | 387.1559 | 12.47 | $C_{21}H_{26}N_2OS_2$ | Thioridazine-5-sulfoxide | 262.0355 | −0.5 | 8.4 | $C_8H_{15}N$ | 258.0406 | −0.7 | 34.7 | $C_7H_{15}NO$ | 126.1277 | 0.2 | 2.5 | $C_{13}H_{11}NOS_2$ |
| | 387.1559 | 12.73 | $C_{21}H_{26}N_2OS_2$ | Mesoridazine | 372.1325 | −2.2 | 4.9 | $CH_3$ | 274.0355 | −1.0 | 9.8 | $C_7H_{15}N$ | 126.1277 | 0.1 | 5.6 | $C_{13}H_{11}NOS_2$ |

Abbreviations: PMA = paramethoxyamphetamine, 4-MMC = 4-methylmethcathinone, MDA = 3,4-methylenedioxyamphetamine, PMMA = paramethoxymethamphetamine, HHMA = 3,4-dihydroxymethamphetamine, HMA = 4-hydroxy-3-methoxyamphetamine, 2C-H = 2,5-dimethoxyphenethylamine, MDMA = methylenedioxymethamphetamine, BDB = 1,3-benzodioxolylbutanamine, HMMA = 4-hydroxy-3-methoxymethamphetamine, 3,4-DMA = 3,4-dimethoxyamphetamine, 2,5-DMA = 2,5-dimethoxyamphetamine, 5-MeO-AMT = 5-methoxy-$\alpha$-methyltryptamine), MDDMA = 3,4-methylenedioxy-N,N-dimethylamphetamine, MDEA = 3,4-methylenedioxy-N-ethylamphetamine, MBDB = N-methyl-1,3-benzodioxolylbutanamine, DOM = 4-methyl-2,5-dimethoxyamphetamine, 2C-E = 2,5-dimethoxy-4-ethylphenethylamine, 3,4,5-TMA = 3,4,5-trimethoxyamphetamine, DPT = N,N-dibutyltryptamine, 5-MeO-MIPT = N-isopropyl-5-methoxy-N-methyltryptamine, 2C-T-4 = 4-isopropylthio-2,5-dimethoxyphenethylamine, 2C-T-7 = 4-propylthio-2,5-dimethoxyphenethylamine, 5-MeO-DIPT = N,N-diisopropyl-5-methoxytryptamine, EDDP = methadone metabolite, 6-MAM = 6-monoasethylmorphin.
[a] = diastereomers.
[b] = position isomers.

Histapyrrodine, imipramine and nortrimipramine share a molecular formula of $C_{19}H_{24}N_2$ and $[M+H]^+$ 281.2012 (Figure 2, isomer group 32 in Table 1). Imipramine and nortrimipramine are structural isomers with the same tricyclic molecule skeleton. Histapyrrodine is structurally different from these two compounds. All three compounds were chromatographically separated, and the retention times were 12.81 min for histapyrrodine, 13.99 min for imipramine, and 14.48 min for nortrimipramine. The mass spectrum of histapyrrodine was obviously different from the spectra of imipramine and nortrimipramine, which for their part were visually quite similar. The fragment *m/z* 210.1277 of histapyrrodine is formed by the pyrrolidine ring fragmentation ($[M+H]^+$ - $C_4H_9N$), the fragment *m/z* 132.0808 is formed after the benzene ring cleavage from the former ($[M+H]^+$ - $C_{10}H_{15}N$), and the fragment *m/z* 98.0964 is a methylaminobenzene residue ($C_6H_{12}N$). All the fragments detected for histapyrrodine were compound characteristic, so its differentiation from the other two structural isomers was undisputable. Imipramine and nortrimipramine could be differentiated from each other based on the fragment ions *m/z* 236.1434 of imipramine and *m/z* 250.1590 of nortrimipramine, which are formed in the cleavage of the amino group. The fragment ions *m/z* 208.1125, *m/z* 196.1121 and *m/z* 86.0964 are detected for both compounds; however, the ion *m/z* 196.1121 of imipramine is very low in intensity. The three fragment ions identified for imipramine and nortrimipramine were all built up in the fragmentation of the alkyl side chain. The identified fragments of histapyrrodine, imipramine and nortrimipramine were even electron neutral losses and were predicted by both ACD/MS Fragmenter and SmartFormula3D, except fragment *m/z* 250.1590 of nortrimipramine, which was only predicted by ACD/MS Fragmenter. The fragmentation reaction, which builds up the fragment *m/z* 250.1590, is logical and consistent with the known reactions for amines. The mass accuracy and isotopic pattern match for the ion were −0.2 mDa and 3.4 mSigma, respectively, and thus fulfill the identification criteria (Table 1).

Cocaine and scopolamine, which share molecular formula $C_{17}H_{21}NO_4$ and $[M+H]^+$ 304.1543, are plant alkaloids that include a tropane ring in their structure[18] (Figure 3, isomer group 42 in Table 1). The retention times were 9.58 min for cocaine and 6.00 min for scopolamine. The MSn spectra of cocaine and scopolamine are compound characteristic and can easily be differentiated from each other. The three fragments identified for cocaine and scopolamine are formed in the fragmentation of the ester bonds, or the carbon atom next to the ester bond. The fragments identified for cocaine were *m/z* 182.1176, *m/z* 150.0913 and *m/z* 105.0335, which were formed by fragmentation of $C_7H_6O_2$, $C_8H_{10}O_3$ and $C_{10}H_{17}NO_3$, respectively. The characteristic fragments of scopolamine were *m/z* 156.1019, *m/z* 138.0913 and *m/z* 121.0648. These fragments were formed in cleavage of $C_9H_8O_2$, $C_9H_{10}NO_3$ and $C_9H_{13}NO_3$, respectively. The fragments identified for cocaine and scopolamine were even electron neutral losses, predicted and identified by both ACD/MS Fragmenter and SmartFormula3D.

The number of fragments per compound predicted by ACD/MS Fragmenter ranged from 34 to 232, and SmartFormula3D suggested 1–4 different formulae for the precursor ions and 2–15 possible formulae as product ions relatable to the precursor ion, respectively. The long list of possible fragments of ACD/MS Fragmenter, with many potentially false positive predictions, might be difficult to use on its own for structural elucidation without comparison with experimental data. Also the fact that the software did not predict the same fragments in all cases shows some lack of robustness. That is why care should be taken when interpreting mass spectral data with these software programs. However, with accurate mass data and good chromatographic separation, the reliability of the software is superior compared to nominal mass data.

The programs used in this study give neither exact knowledge about the charge distribution and the location of the radical site nor approximations of the probability and abundance of the predicted fragment. These features have given rise to criticism,[18,19] and consequently software has been developed that take into account the thermodynamic and stability aspects as well as the probability rates of the predicted fragments. Identification and structural elucidation of all detected fragments might be crucial when studying drug metabolism where the structure of the metabolite is unknown and needs to be identified based on its fragments. In the present study, an adequate approach was to identify compound characteristic fragments in order to differentiate the structural isomers.

The present study was carried out using pure standards, and the compounds in the same mixture were known to be chromatographically separated. This arrangement does not quite correspond to authentic samples, where isomers with retention times close to each other can co-elute. Such a pair is for example etilefrine and HHMA, with a retention time difference of only 0.07 min (Table 1; isomer group 7). In this case, the MS/MS spectrum would be a combination of both of these compounds. However, etilefrine and HHMA have compound characteristic fragments, and hence, if fragment ions of HHMA are seen with the fragment ions of etilefrine in the same spectrum, it can be concluded that both compounds are in the sample.

The structural elucidation with tandem mass spectrometry has been used, for example, in drug metabolism research,[26] analysis of impurities in pharmaceuticals[27] and technical chemicals[28] as well as in detection of environmental toxins.[29] Several studies concerning differentiation of structural isomers with MS/MS techniques have been published, but these works deal with the differentiation of two or three compounds. For instance, studies have been published about differentiation of MDEA and MBDB; methamphetamine and phentermine;[30] hydromorphone, morphine and norcodeine;[31] clobazam and temazepam;[32] as well as metolazone and indapamide.[33] Tramadol and *O*-desmethyl venlafaxin sharing a molecular formula of $C_{16}H_{25}NO_2$ and $[M+H]^+$ 264.1958, have been reported to undergo similar fragmentation,[34] yielding only a single fragment of *m/z* 58. In our study, tramadol and *O*-desmethyl venlafaxine were differentiated based on *O*-desmethyl venlafaxine's characteristic fragment, *m/z* 133.0648. Similar results have been reported about isomers that could not be distinguished based on their fragments, including the compounds studied in the present work (MDEA and MDDMA,[35] and ephedrine and pseudoephedrine[36]).

To date, software for *in silico* fragment prediction has mostly been used for structural elucidation of drug metabolites[15,37,38] where the compound structures are unknown or just approximated and the identification and structural determination of all observed fragments in the mass spectra is crucial. There have been no publications on differentiation of structural isomers by MS/MS spectra and identification of the characteristic fragments with fragment prediction software, except our previous study of quetiapine.[20] The advantage of this method – combining accurate mass and fragment prediction in order to elucidate compound structure – over identification by spectral library comparison lies in the fragment structure determination it enables.

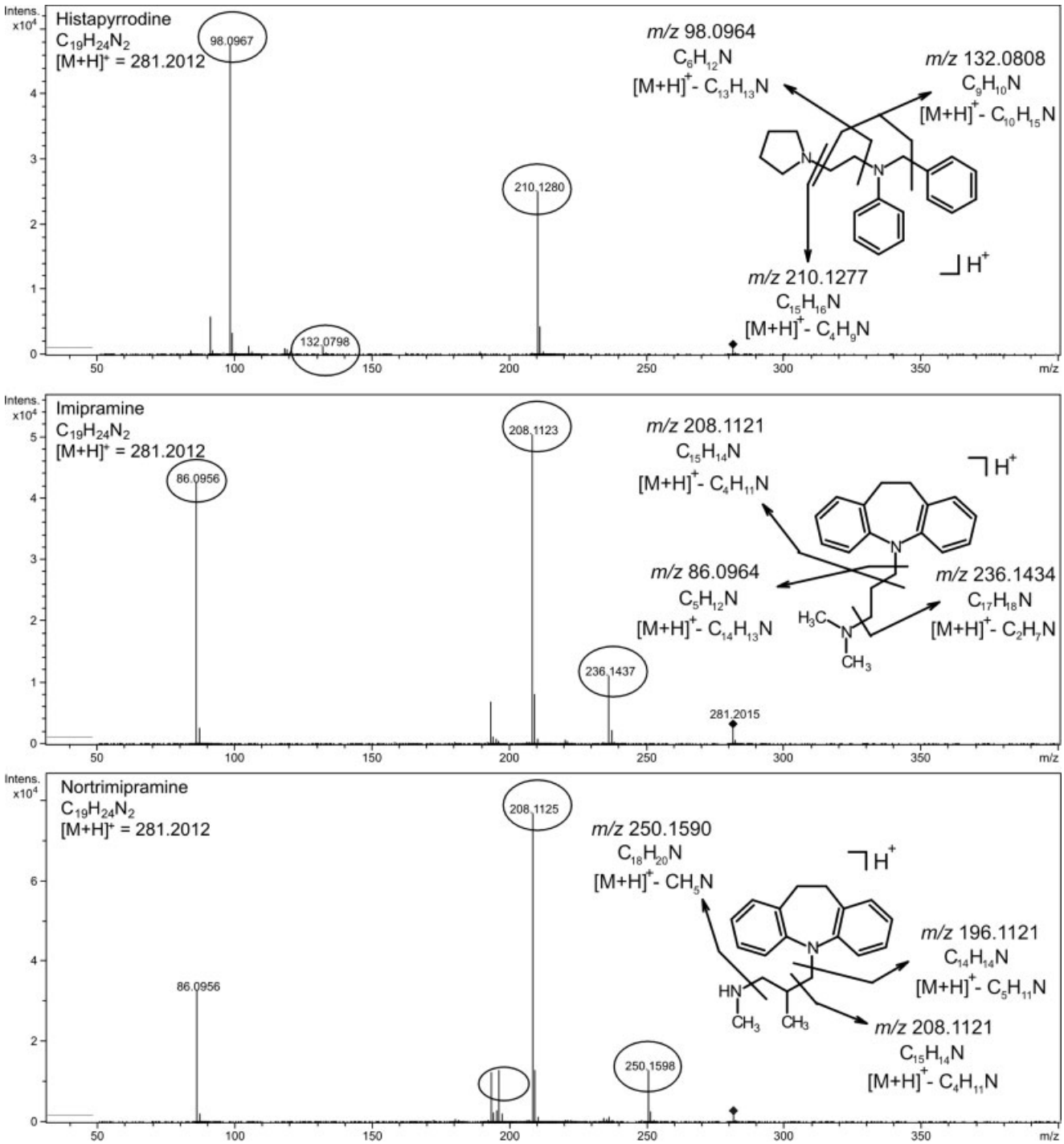



**Figure 2.** Mass spectra and fragmentation schemes of histapyrrodine, imipramine and nortrimipramine ($[M+H]^+ = 281.2012$, $C_{19}H_{24}N_2$).

## Conclusion

Poor accessibility of reference standards has hindered substance identification within drug screening, especially for new drugs, designer drugs, and metabolites. Formula-based identification against a target database of exact monoisotopic masses is a partial remedy, but even this approach fails with isomeric compounds. The aim of the present study was to differentiate all isomers found in a comprehensive target database, based on LC/Q-TOFMS product ion spectra of the reference standards available, and to identify the compound characteristic fragments. The results from 48 isomer groups demonstrated an indisputable advantage of the predictive software in assigning relevant mass fragments to structural isomers and in defining the molecular formulae of the fragments. The two software programs proved to be valuable for interpretation of experimental accurate mass data. However, one should be aware of the differences in the performance of each software program and the possibility of false positive predictions. The use of fragmentation prediction allows a target database to be built up that contains the exact monoisotopic masses of both precursor and the most

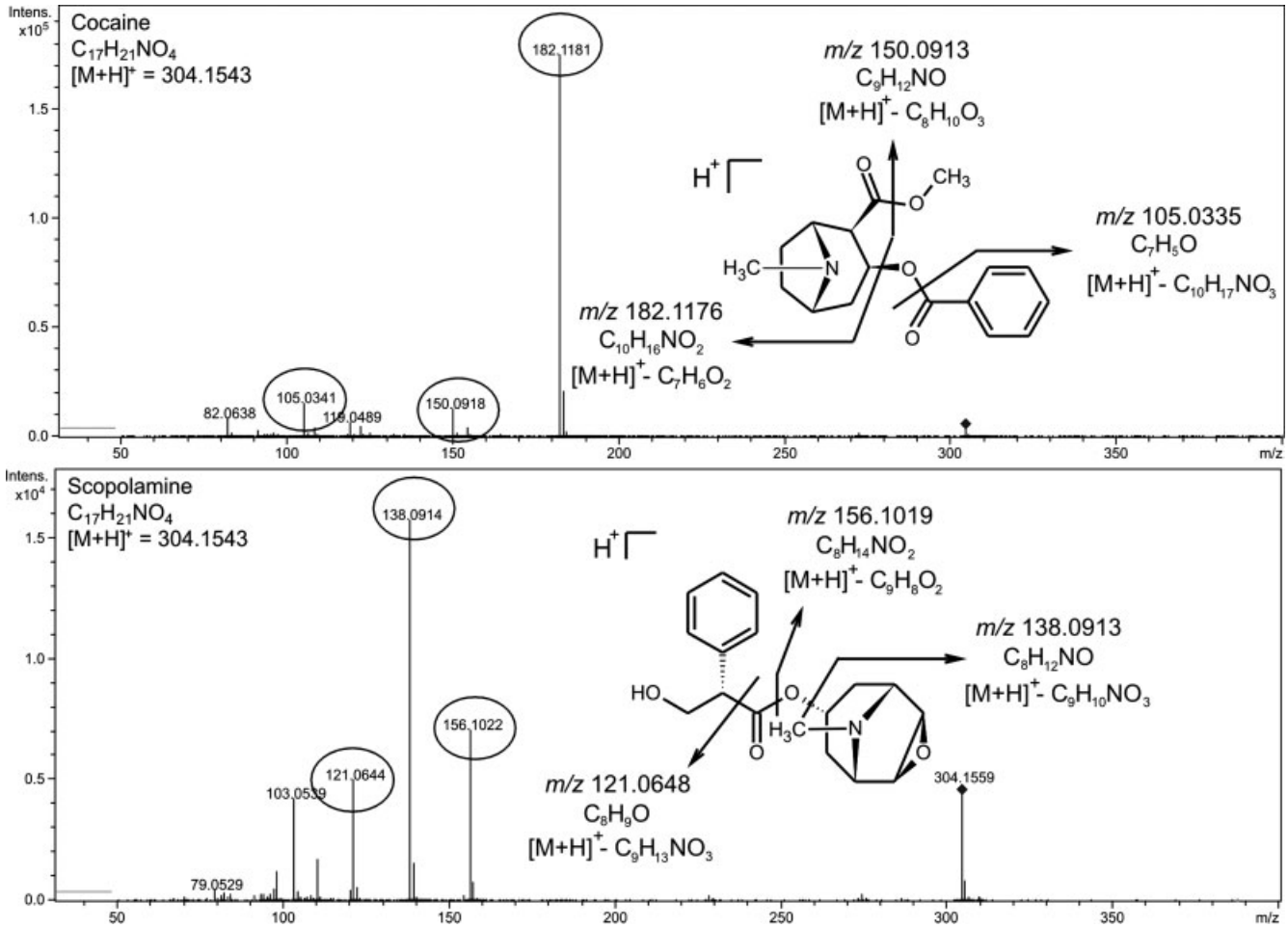



**Figure 3.** Mass spectra and fragmentation schemes of cocaine and scopolamine ($[M+H]^+ = 304.1543$, $C_{17}H_{21}NO_4$).

characteristic fragment ions, even for those compounds for which a reference standard cannot be readily obtained. Compound characterization in a biological sample can be carried out using these two fragment prediction software programs, as accurate mass data enables the elucidation of fragment structures. This, in turn, makes a rapid tentative identification of a range of compounds feasible in pharmaceutical, toxicology, and forensic contexts.

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