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ORIGINAL PAPER

Chemical profile of meta-chlorophenylpiperazine (*m*-CPP) in ecstasy tablets by easy ambient sonic-spray ionization, X-ray fluorescence, ion mobility mass spectrometry and NMR

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Abstract Meta-chlorophenylpiperazine (*m*-CPP) is a new illicit drug that has been sold as ecstasy tablets. Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) and X-ray fluorescence spectrometry (XRF) are shown to provide relatively simple and selective screening tools to distinguish *m*-CPP tablets from tablets containing amphetamines (mainly 3,4-methylenedioxymethamphetamine (MDMA)). EASI-MS detects the active ingredients in their protonated forms: $[m\text{-CPP+H}]^+$ of *m/z* 197, $[\text{MDMA+H}]^+$ of *m/z* 194, and $[\text{2MDMA+HCl+H}]^+$ of *m/z* 423 and other ions from excipients directly on the tablet surface, providing distinct chemical fingerprints. XRF identifies Cl, K, Ca, Fe, and Cu as inorganic ingredients present in the *m*-CPP tablets. In contrast, higher Cl concentrations and a more diverse set of elements (P, Cl, Ca, Fe, Cu, Zn, Pt, V, Hf, Ti, Pt, and Zr) were found in MDMA tablets. Principal component analysis applied to XRF data arranged samples in three groups: *m*-CPP tablets (four samples), MDMA tablets (twenty three samples), and tablets with no active ingredients (three samples). The EASI-MS and XRF techniques were also evaluated to quantify *m*-CPP in ecstasy tablets, with concentrations ranging from 4 to 40 mg of *m*-CPP per tablets. The *m*-CPP could only be differentiated from its isomers (*o*-CPP and for the three isomers *p*-CPP) by traveling wave ion mobility mass spectrometry and NMR measurements.

Keywords Drug monitoring/drug screening · Ambient mass spectrometry/EASI-MS · Forensics/toxicology · Metals/heavy metals · X-ray spectroscopy (XPS | XRF | EDX) · NMR/ESR

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Introduction

Ecstasy is a well-known illicit drug with variable composition, but it usually contains 3,4-methylenedioxymethamphetamine (MDMA) as the main active ingredient. Recently, a new

molecule has been detected in street drugs sold as ecstasy tablets all over the world [1]. This new drug has been identified as meta-chlorophenylpiperazine (*m*-CPP), which is a piperazine-based 5-HT receptor agonist. Additionally, this molecule interacts with several serotonin receptors, as well as adrenergic and dopaminergic receptors [2]. *m*-CPP is being introduced as an alternative to MDMA due to similar effects on the serotonergic system as those caused by the most common ecstasy drugs. In the streets, *m*-CPP tablets are often called "rainbows", "harlequins", or "confetti" [3]. *m*-CPP does not present any licensed medicinal application and has been included on lists of controlled psychotropic substances due mainly to its toxicity and recent appearance in the ecstasy market.

In forensic investigations, *m*-CPP has been detected in tablets displaying no logos, normally with a yellow color and round shape. *m*-CPP tablets displaying off-white colors with striking multicolored flecks have also been encountered [3]. In Brazil, seizures of *m*-CPP tablets started in 2006, and recently, several colors, shapes, sizes, and logos have been found, which makes unrealistic any attempt to distinguish *m*-CPP and MDMA tablets based on visual examination. Figure 1 presents some typical *m*-CPP and MDMA tablets apprehended by the Brazilian police in the ecstasy market. Therefore, *m*-CPP has acquired the same illegal status as typical active ingredients of ecstasy (MDMA), methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA).

Forensic laboratories analyze ecstasy tablets mainly using ecstasy testing kits, which are often based on the Marquis or nitroprusside tests. These tests are based on the development of specific colors such as dark blue or black when MDMA is present. For *m*-CPP, no color change is displayed, leading to false-negative results [4]. Other techniques have to be employed to confirm kit results, such as thin-layer chromatography (TLC) [3], gas chromatography using nitrogen-phosphorous detection [3], gas chromatography coupled with mass spectrometry [5], high-performance liquid chromatography (HPLC) with ultraviolet detection [6] and liquid chromatography coupled to mass spectrometry (LCMS) [7]. These instrumental techniques naturally require skilled operators and are time-consuming. Hence, the development of powerful, secure, fast, and efficient methods to identify the presence of this drug is in priority for forensic analysis.

Recently, a new class of ambient ionization techniques for mass spectrometry [8–19] has been developed. These techniques allow desorption, ionization, and mass spectrometry (MS) characterization of analytes directly in their natural surfaces and matrices in open atmosphere with no or little sample preparation [20], being an attractive tool for direct forensic characterization of illegal drugs. Among these techniques, easy ambient sonic-spray ionization (EASI) is simple, non-destructive, and easily implemented [21]. An EASI source can be constructed and installed in a few minutes from simple MS laboratory parts (Fig. 2) and can also be operated with self-pumping provided by the Venturi effect [22]. EASI relies on the force of a high velocity stream of N_2 (or even air) to accomplish analyte desorption and ionization by sonic spray ionization [23]. EASI has already been successfully tested in various forensic applications such as aging of ink writing [24], authenticity of perfumes [25], and identification of counterfeit banknotes [26]. EASI has also been coupled to TLC [27] to identify seven common ecstasy drugs: MDMA, MDEA, MDA, metamphetamine, amphetamine, caffeine, and lidocaine.

Trace metal impurities can also be present in illicit *m*-CPP tablets from different sources such as leaching metal

Fig. 1 *m*-CPP (*first column on the left*) and MDMA tablets (*right*) seized by the Brazilian police

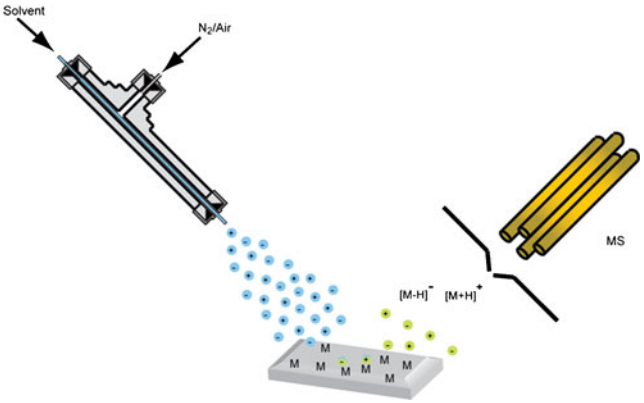

Fig. 2 Schematic of an EASI-MS system in operation on a surface solid. Sonic spray produces a bipolar stream of very minute charged droplets (*blue* spray) that bombard the solid surface causing desorption and ionization of the analyte molecules that rest on the target spot (*green* dots). Analytes are ionized, often as $[M+H]^+$ or $[M-H]^-$, or both. EASI is assisted only by compressed nitrogen or air, and causes no oxidation, electrical discharge, or heating interferences

from the reaction vessels, residues of catalysts and reducing agents, dyes, or contamination in adulterants or diluents. These trace metals also function as important chemical markers and may serve to distinguish between MDMA and *m*-CCP containing tablets. X-ray fluorescence (XRF) can provide a fast and suitable technique to characterize these elements in MDMA and *m*-CCP street tablets due to its multielemental capability, good sensitivity, high precision, short analytical time, and non-destructive nature. XRF has been applied to a great variety of samples for element determination [28, 29]. In this work, easy ambient sonic-spray ionization mass spectrometry (EASI-MS) and XRF (allied to chemometric analysis) have been applied to the direct analysis of street *m*-CPP and ecstasy tablets. Also, the abilities of EASI-MS and XRF techniques in *m*-CPP quantitation were evaluated in ecstasy tablets. The linearity via correlation coefficients (R^2, n=9) and the limits of detection (LOD) were determined, and the *m*-CPP concentrations in ecstasy tablets were calculated.

A crucial aspect of *m*-CPP analysis is isomerism, since two other positional isomers of *m*-CPP could be present: 1-(2-chlorophenylpiperazine) or *o*-CPP and 1-(4-chlorophenylpiperazine) or *p*-CPP (Fig. 3). Contrary to *m*-CPP, *o*-CPP and *p*-CPP have not been included on lists of controlled psychotropic substances. *p*-CPP, for instance, has been reported in France and Bulgaria in 2007 [3], so the confirmation of the presence of *m*-CPP isomer is essential. Here, the differentiation of *m*-CPP from its isomers was done using traveling wave ion mobility mass spectrometry [30] and nuclear magnetic resonance spectrometry (NMR) [3, 30, 31].

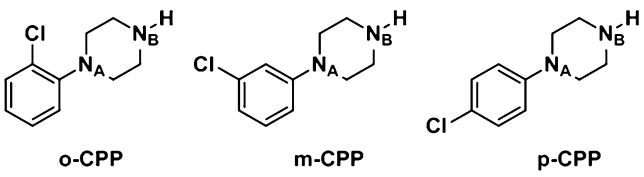

Fig. 3 Isomeric structures of *o*-CPP, *m*-CPP, and *p*-CPP

Methods and materials

HPLC- and PA-grade methanol and formic acid were purchased from Burdick and Jackson (Muskegon, MI, USA). Thirty tablets apprehended as ecstasy were provided by the Rio de Janeiro State Civil and Brazilian Federal Police. Methanol-D was purchased from Sigma-Aldrich Chemicals, USA. *o*-CPP, *m*-CPP, *p*-CPP, and lactose standard samples were purchased from Alfa Aesar Company (USA).

To evaluate the ability of EASI-MS and XRF techniques in *m*-CPP quantification in ecstasy tablets, nine *m*-CPP tablets were prepared as mixtures containing *m*-CPP+ lactose excipient with total tablet mass of 300 mg, with *m*-CPP concentrations ranging from 3 to 80 mg m-CPP $tablet^{-1}$. The correlation coefficients (R^2, n=9) and the LOD were determined, and *m*-CPP was quantified in ecstasy tablets.

EASI-MS

Experiments were performed on a single quadrupole mass spectrometer (LCMS-2010EV-, Shimadzu Corp., Japan) equipped with a home-made EASI source. Acidified methanol (0.1% in volume of formic acid) at a flow rate of 20 μL min^{-1} and compressed N_2 at a pressure of 100 psi were used to form the sonic spray. The tip-to-tablets distance was ca. 2 mm, and the capillary-tablet-entrance angle was 45°. Each tablet was directly analyzed by EASI-MS, without any sample preparation. Spectra were collected from the surface of each tablet for about 10 s.

To confirm the structure of compounds found in the *m*-CPP and ecstasy tablets, electrospray ionization tandem mass spectrometry (ESI(+)-MS/MS) was applied using a Micromass (Manchester, UK), a hybrid instrument (quadrupole/time-of-flight mass spectrometer (QTof)) with ESI-QTof configuration having 5,000 mass resolution and less than 20 ppm mass accuracy in the time-of-flight (Tof) mass analyzer. The following operating conditions were used: a 3-kV capillary voltage, 8 V cone voltage, and desolvation gas temperature of 100 °C. ESI-MS/MS were collected after 4–40 eV collision-induced dissociations (CID) of selected ions with argon. Selection was performed by Q1,

using a unitary *m*/*z* window, and collisions were performed in the rf-only hexapole collision cell, followed by mass analysis of product ions by the high-resolution orthogonal reflectron Tof analyzer.

X-ray fluorescence

The energy dispersive X-ray fluorescence (ED-XRF) experiments were performed using an X-ray spectrometer Shimadzu®, model EDX 700 (Kyoto, Japan). The measurements were performed under air, with a beam collimation of 3 mm, 25% of detector dead time, with the current automatically adjusted during spectrum acquisition to keep the detector dead time of 25%. The Shimadzu EDX 700 also presents the following characteristics: (1) an Rh X-ray generator, with tube voltage ranging from 5 to 50 kV, and tube current from 1 to 1,000 μA; (2) a semiconductor detector, Si(Li), with detection area of 10 mm^2 and resolution of <155 eV.

For spectral acquisition, tablets were crushed using a mortar and placed into XRF cells on Mylar™ film (3 μm thickness). The measurement time was 250 s. In all cases, the spectra were recorded from 0 to 40 keV, with an energy step of 0.02 keV, resulting in 2,048 points for each spectrum.

Chemometric analysis

For XRF, the spectral data were mean-centered. Then, to classify the tablets after XRF fingerprinting, principal component analysis (PCA) was applied to the XRF data using the software Pirouette v. 3.11. The entire spectra were used in PCA analysis (0 to 40 keV), resulting in 2,048 points (variables) and 90 samples (30 in triplicate).

Traveling wave ion mobility mass spectrometry

Traveling wave ion mobility mass spectrometry separates ions based on their distinct mobilities in a drift gas, typically nitrogen, air, or helium, under the influence of a weak electric field gradient, where ion mobility (K) is defined as:

$$K = n/E \cong L^2/V\,x\,t_d \quad (1)$$

ν is the average velocity of an ion moving through the drift cell, E is the electric field gradient applied along the drift cell, L is the length of the drift cell, V is the total voltage drop applied along the drift cell, and t_d is the drift time of the ion (i.e., the time taken for an ion to drift the length of the cell). The factors determining ion mobility under low field conditions ($<2\times10^{-17}$ V cm^{-2}) are defined by the simplified Mason–Schamp equation [32]:

$$K = \frac{3}{16} \times \frac{q}{N} \times \left(\frac{1}{\mu} \times \frac{2\pi}{kT}\right)^{\frac{1}{2}} \times \frac{1}{\Omega} \quad (2)$$

where q is the charge of an ion, N is the number gas density of the drift gas, k is Boltzmann's constant, μ is the reduced mass of the drift gas–ion pair, T is the temperature of the drift cell, and Ω is the collision cross-section of the drift gas/analyte ion pair.

Ion mobility mass spectrometry measurements were carried out on a Waters Synapt HDMS (Waters Co., Manchester, UK) instrument, by infusing separately *o*-CPP, *m*-CPP, and *p*-CPP standard solutions (1 mmol L^{-1}) prepared in acidic methanol (0.01 *v*/*v*%). Mixtures of *o*-CPP/*m*-CPP/*p*-CPP isomers (1:1:1 *v*%) were also analyzed. Samples were analyzed at a 5 μL min^{-1} flow rate into the electrospray ionization source of instrument, with capillary and cone voltages fixed at 3.0 kV and 40 V, respectively. The ion $[CPP+H]^+$ of *m*/*z* 197 transmitted through the quadrupole are accumulated at the Trap cell, periodically injected at the IM cell and mobility separated, after which they are transferred to the Oa-TOF analyzer by the transfer cell while maintaining mobility separation. A detailed description of the Synapt HDMS has been given elsewhere [33, 34].

Here, three drift gases were used (He at 4.0, N_2 at 3.0, and CO_2 at 1.5 mbar) to study the effect of their composition on drift time and resolution. The travelling wave velocity was set to 200 to 303 ms^{-1} with a pulse height changes from 5 to 30 V. This change depends directly on the type of drift gas used. Finally, after the optimization of experimental procedure, four ecstasy samples identified as CPP were investigated. The isomer present in CPP tablets was elucidated.

NMR

The ^1H NMR spectra were obtained with a Varian Inova 500 MHz spectrometer, operating at 300.07 and 499.88 MHz for ^1H [35]. Spectra were acquired for *o*-CPP, *m*-CPP, and *p*-CPP standards with ca. 30 mg mL^{-1} solutions with a probe temperature of 298 K. Deutered methanol (CD_3OD) was used as solvent.

Results

EASI-MS

A set of 30 ecstasy tablets selected by the Rio de Janeiro Civil Police was analyzed by EASI-MS. Figure 4 shows a typical EASI(+)-MS for the group of four samples, identified as *m*-CPP tablets and for the group of 23 samples, identified as an MDMA tablet, whereas the insert

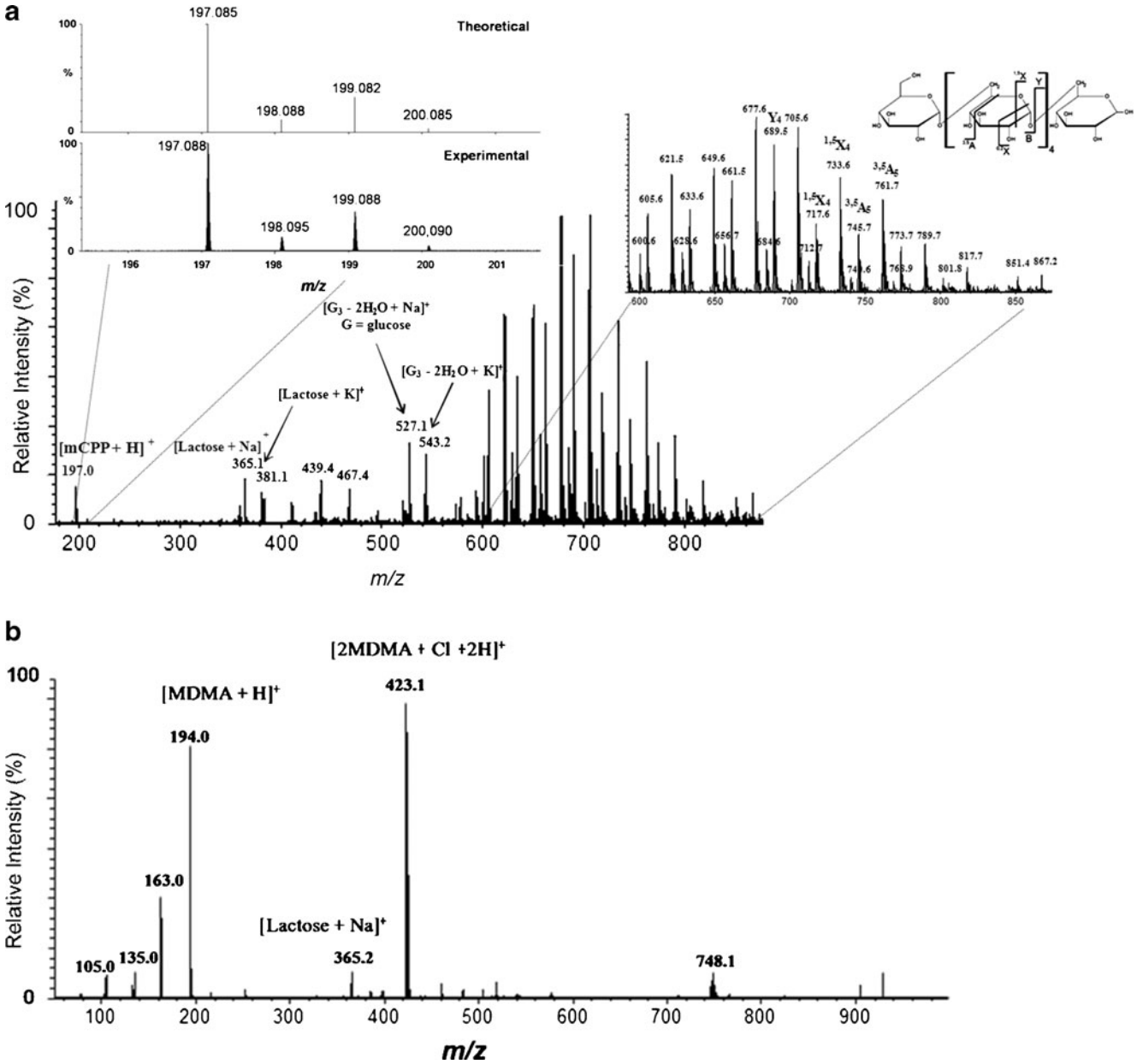

Fig. 4 EASI(+)-MS of typical **a** *m*-CPP and **b** MDMA tablets

shows data for the ion of *m/z* 197 with higher resolution and accuracy obtained via ESI-MS. Typical and contrasting chemical profiles are observed for *m*-CCP and MDMA tablets, which provide unequivocal differentiation. In the EASI(+)-MS of *m*-CPP tablets (Fig. 4a), the active *m*-CCP ingredient is identified as its protonated molecule $[M+H]^+$ of *m/z* 197. Additionally, the experimental isotopologue pattern of *m*-CPP is characteristic due to the chlorine atom (the relative intensity of the ion of *m/z* 199 is 1/3 of that of *m/z* 197). This experimental ratio matches the theoretical pattern (insets in Fig. 4a), corroborating the structural identification of *m*-CPP. A large number of abundant ions from excipients are also detected, such as $[lactose+Na]^+$ of *m/z* 365 and $[lactose+K]^+$ of *m/z* 381, $[glucose+Na]^+$ and $[glucose+K]^+$ ions of *m/z* 527 and 543 and several oligosaccharide ions of *m/z* values in the 600–900 range, detected as Na^+ (*m/z* 605, 633, 661, 689, 717, 745, 773, and 801) and K^+ (*m/z* 621, 649, 677, 705, 733, 761, 789, 817) adducts. The A, B, X, and Y fragments identified in Fig. 4a (insert) correspond to typical glycosidic cleavages [36, 37]. These sugar excipients provide the *m*-CCP tablets a "soft texture" (like "chewing gum"), which differs from the "hard texture" typical of MDMA tablets.

Figure 4b shows a typical EASI(+)-MS for the MDMA tablets. MDMA is detected mainly as $[MDMA+H]^+$ of *m/z* 194, as well as $[2MDMA+HCl+H]^+$ of *m/z* 423/425.

Fragment ions characteristic of MDMA (*m/z* 163, 135, and 105) are also observed [12]. Lactose is again detected as $[lactose+Na]^+$ of *m/z* 365. For some MDMA tablets, caffeine was also detected as $[M+H]^+$ of *m/z* 195.

The structures of the key ions of *m/z* 194 and 423 were confirmed via ESI-MS/MS (Fig. 5). The $[MDMA+H]^+$ ion of *m/z* 194 mainly formed the fragments of *m/z* 163, 135, 133, and 105 (Fig. 5a). The ion of *m/z* 423 formed $[MDMA+H]^+$ of *m/z* 194 as the main fragment (Fig. 5b). This ion has apparently not been previously reported and should be also monitored as an indicative of MDMA hydrochloride in ecstasy tablets.

To confirm *m*-CPP detection, ESI(+)-MS/MS was performed for the ion of *m/z* 197 (Fig. 6a). The CID of $[m\text{-CPP}+H]^+$ of *m/z* 197, as rationalized in its spectrum, agrees well with its structure and connectivity. The CID of *m/z* 197 involved initially cleavages of the piperazine ring with the loss of NC_2H_5 likely as a neutral cyclic molecule, thus producing the ion of *m/z* 154. Subsequently, the loss of $Cl^{\cdot}$ (*m/z* 119) and $H^{\cdot}$ (*m/z* 118) or direct neutral losses of HCl (*m/z* 118) followed by HCN (or HNC) loss (*m/z* 91) leading finally to the tropylium ion (or its isomeric benzoyl cation). Additionally, the accurate *m/z* value (197.086) for the ion of *m/z* 197 was also in agreement with the calculated *m/z* of 197.088 (inset in Fig. 4a). Accurate mass measurement can be used therefore for a more secure identification of *m*-CCP.

Figure 6b also presents the ESI(+)MS/MS for protonated lactose of *m/z* 365, with fragment ions of *m/z* 347, 305, 203, and 185 being observed. Nyadong et al. [38] distinguished lactose from sucrose using the diagnostic ions of *m/z* 347 and *m/z* 305 for lactose formed by the losses of water and formaldehyde, respectively. Therefore, the disaccharide is attributed to lactose. Figure 6c shows the ESI(+)-MS/MS of *m/z* 527 $[G_3-2\ H_2O+Na^+]$, where G= glucose. Dissociation occurs as expected via glucose elimination as a neutral molecule.

In the set of the 30 ecstasy samples analyzed, four presented *m*-CPP, and its confirmation as isomers *o*-CPP and *p*-CPP was done via ion mobility mass spectrometry and NMR measurements (see below). For the remaining 26 samples, three show negative results for MDMA. Two of these samples present lidocaine ($[M+H]^+$ of *m/z* 235 and $[2M+H]^+$ of *m/z* 469) as active ingredient. The third sample presents another amphetamine derivative, $[M+H]^+$ of *m/z* 254.

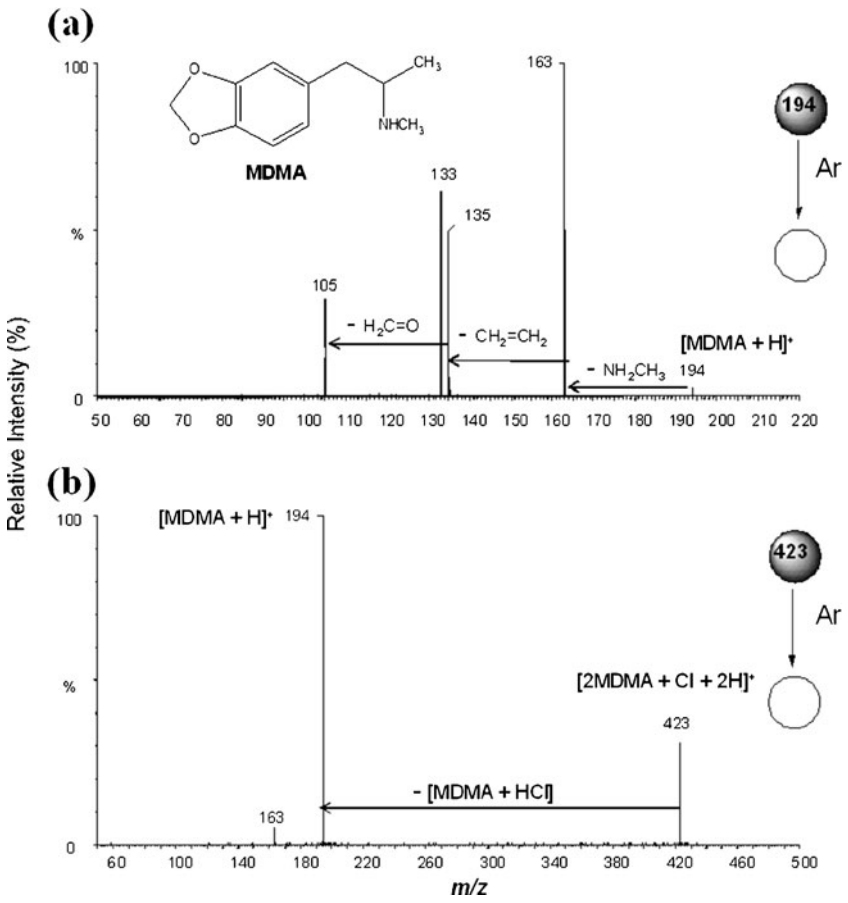

Fig. 5 ESI(+)-MS/MS for **a** $[MDMA+H]^+$ of *m/z* 194 and **b** $[2MDMA+Cl+2\ H]^+$ of *m/z* 423 detected in ecstasy tablets

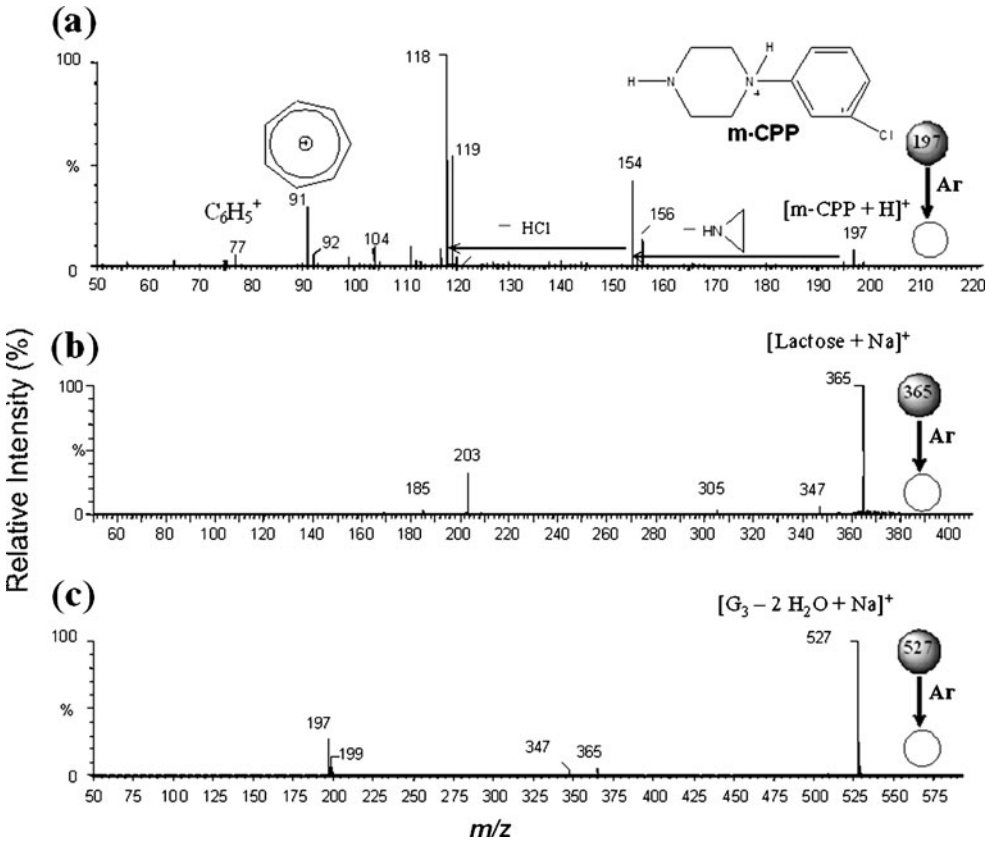

Fig. 6 ESI(+)-MS/MS for **a** $[m\text{-CPP+H}]^+$ of *m/z* 197 and for the ions of **b** *m/z* 365 and **c** *m/z* 527 detected in the *m*-CPP tablets

X-ray fluorescence

Figure 7a shows the combined ED-XRF spectra for the four *m*-CPP tablets. All spectra show the presence of Kα characteristic lines for Cl (2.60 keV), K (3.30 keV), Ca (3.62 keV), Fe (6.40 keV) and Cu (8.02 keV), the Cl line being the most prominent, due to the presence of Cl in the structure of *m*-CPP.

Figure 7b shows an expansion of the Cl, K, and Ca lines in the 2–4-keV region of the ED-XRF spectra. The abundance of the Cl line is shown to be directly related to the amount of *m*-CPP, as detected by EASI-MS.

The region of a high-intensity line is related to the Compton (Rh Kα (19.20 keV), Rh Kβ (21.56 keV) lines) and Rayleigh (Rh Kα (20.16 keV) and Rh Kβ (22.74 keV) lines) effects. These Compton (incoherent scatter) and Rayleigh (coherent scatter) effects contribute significantly to perform quantitative organic ED-XRF analysis, being associated with light elements (C, H, O, among others) that are not visualized in ED-XRF spectra. This scattering region can provide powerful information for qualitative or even quantitative determinations of light elements when treated with chemometric analysis [39–42].

Figure 8a shows the combined ED-XRF spectra for the ecstasy tablets. When compared with ED-XRF spectra of *m*-CPP tablets (Fig. 7), they are found to be very distinct. Kα lines for Cl (2.60 keV), Ca (3.62 keV), Fe (6.40 keV) and Cu (8.02 keV) are present for all ecstasy samples, as well as for the ED-XRF spectra of *m*-CPP tablets. The intensities of Kα lines for Cl in MDMA tablets are, however, much higher, which agrees with EASI-MS that detected $[2\text{MDMA+Cl+2 H}]^+$ as a major ion (Fig. 4b). This result indicates that the MDMA concentration is higher than *m*-CPP concentration in ecstasy tablets, where it is often found as its MDMA.HCl salt [43]. Muratsu et al. [44] also used X-ray fluorescence to determine trace metals in a variety of controlled substances, being able to discriminate the tablets based on the amount of MDMA. HCl present in the tablet.

Other Kα lines, present only in few samples, correspond to P (1.98 keV), Ti (4.48 keV), Zn (8.64 keV) and Pt (9.42 keV). The Fe Kβ line (7.06 keV) is verified for some samples with high Fe concentration.

Figure 8b shows the ED-XRF spectra of some ecstasy tablets with new inorganic profiles. Two ecstasy samples (top spectrum, **A**) displays high concentrations of Ca (Kα and Kβ lines at 3.62 and 4.00 keV, respectively), with intensities higher than those corresponding to the Compton and Rayleigh effects. These samples do not present MDMA as active ingredient, but lidocaine is detected instead. For two ecstasy samples (middle spectra, **B**), very low concentrations of inorganic elements were observed,

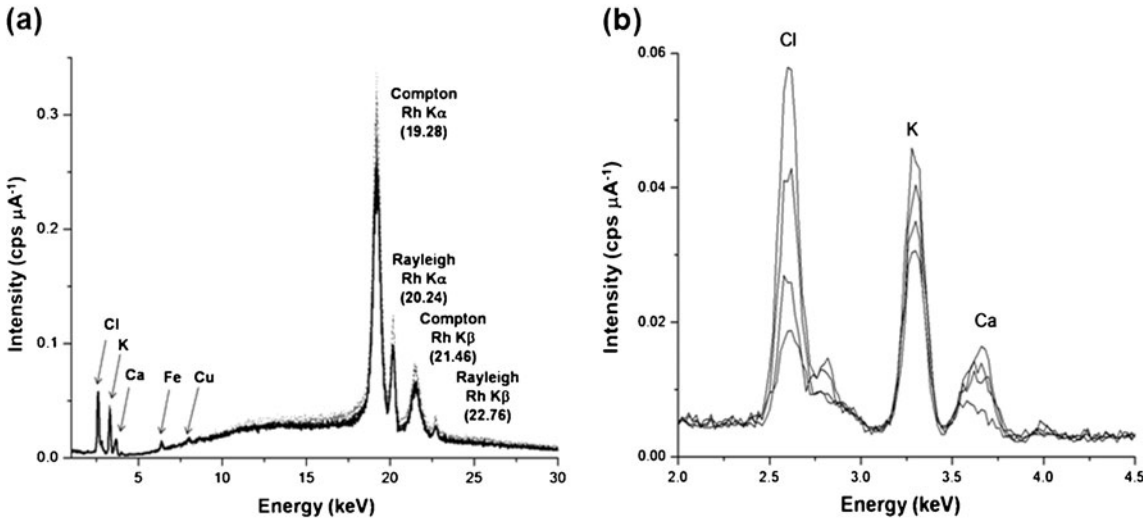

Fig. 7 **a** ED-XRF spectra of four *m*-CPP tablets and **b** an expanded view in the 2–4.5-keV region

probably due to distinct MDMA purification processes. For a single sample, new elements were observed such as V (5.00 (Kα) and 5.50 keV (Kβ)), Hf (7.90 (Lα) and 9.02 keV (Lβ)), and Zr (15.76 (Kα) and 17.68 keV (Kβ)), (bottom spectrum, **C**). The presence of these elements can exclusively be related to excipients, since they are not used as catalysts for MDMA synthesis.

Recently, Koper et al. also analyzed trace metals in MDMA tablets using inductively coupled plasma (ICP) optical emission spectrometry and ICP-mass spectrometry (ICP-MS) [43]. Although ICP-MS offers rapid and sensitive multi-element analysis, sample preparation procedures are inevitable, and additional problems can exist: elements such as Zr, Ta, Ti, U, and Th are chemically inert, resulting in incomplete dissolution, influencing stabilities and concentrations in final solutions. Since inert elements (Zr, Ti, and Hf; Fig. 8b) are not easily soluble, the ED-XRF technique seems more efficient for indentifying this class of elements. Other alternative techniques that do not require complicated sample-preparation procedures, also avoiding dissolution/digestion as one of the most time-consuming sample preparation procedures prior to analysis, are associated with the use of mass spectrometric methods such as laser ablation inductively coupled plasma mass spectrometry, glow discharge mass spectrometry, and secondary ion (neutral) mass spectrometry that provide a direct analysis of solid materials. In these cases, the analyte ions are generated through evaporation (or

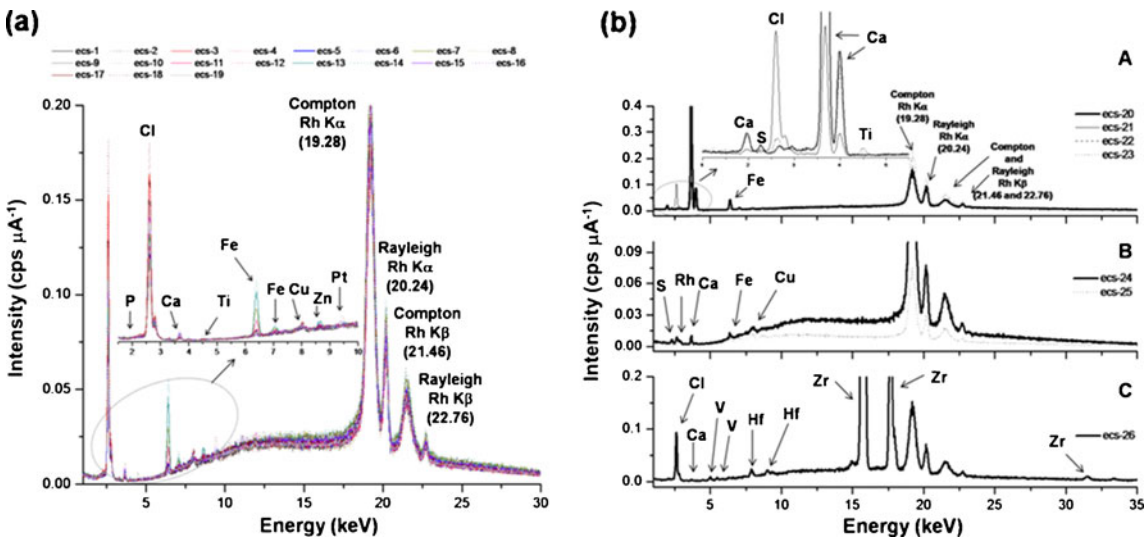

Fig. 8 ED-XRF spectra of ecstasy tablets with **a** similar and **b** distinct (*A*–*C*) inorganic profiles

ablation), atomization and ionization processes, and then subjected to MS analyses [45].

Generally, three variations are used for the synthesis of the active substance (MDMA) via reductive amination of 3,4-methylenedioxyphenyl-2-propanone (piperonylmethyl-ketone, MD-P2P, or PMK) with methylamine: (a) H_2 at elevated pressure (3–4 bar) and platinum (Pt); (b) $NaBH_4$ at lower temperatures (cold method); and (c) aluminum/mercury amalgam reduction ($HgCl_2$+Al) [46]. Note that ED-XRF indicates Pt as an ecstasy tablet ingredient. Other elements such as B, Na, and Al are light elements and cannot be easily detected via XRF. Therefore, the detection of Pt in one ecstasy sample indicates that route (a) was used. For other samples, probably route (b) was used.

Chemometry

Figure 9 shows PCA scores plot for ED-XRF data, where the three first PCs account for ca. 85% of total variance. Again, the *m*-CPP tablets are grouped closely. The ecstasy tablets are, however, highly dispersed due to variable inorganic profiles. Five groups are formed: *m*-CPP, similar, A, B, and **C.** With exception of *m*-CPP and A groups, others contain MDMA as active ingredient.

m-CPP quantitation

The abilities of EASI(+)-MS and XRF in quantifying *m*-CPP contents in ecstasy tablets were also evaluated. Admixtures of *m*-CPP and lactose excipient were prepared at nine different concentrations of 3, 10, 20, 30, 40, 50, 60, 70, and 80 mg *m*-CPP per tablet. Figure 10a shows the EASI(+)-MS for a mixture of *m*-CPP/lactose at 3 mg *m*-CPP tablet^{-1}. To construct the analytical curve, the absolute intensity (cps) of the ion *m/z* 197 as a function of

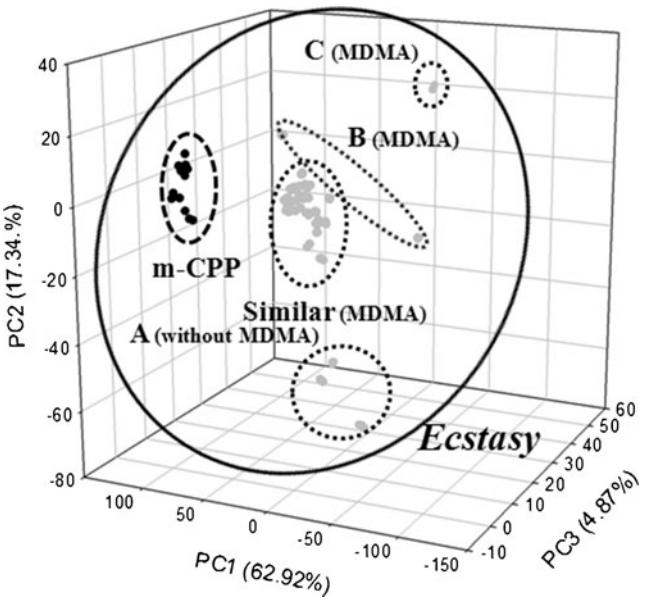

Fig. 9 PCA scores plot for ED-XRF data. *Dark circles* correspond to *m*-CPP tablets whereas *gray spots* correspond to the ecstasy tablets

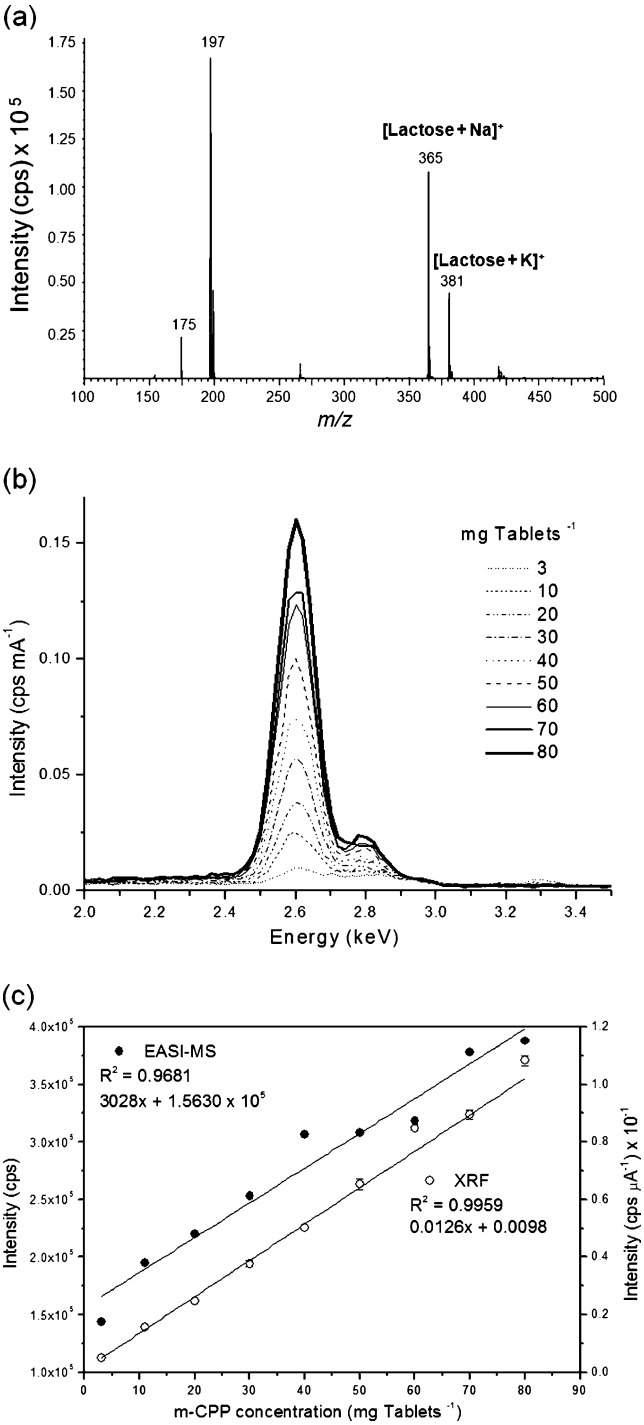

Fig. 10 **a** EASI(+)-MS for a mixture of *m*-CPP with lactose excipient at 3 mg *m*-CPP tablets^{-1}; **b** XRF spectra for *m*-CPP/lactose mixtures at 3–80 mg *m*-CPP tablets^{-1}; **c** analytical curves for EASI(+)-MS and XRF quantifications of *m*-CPP in tablets where *m*-CPP levels lower that 3 mg tablet^{-1} can be reached

amount of *m*-CPP (milligrams per tablet) was considered. Similarly to EASI-MS, the intensities of the Kα lines for Cl (2.60 keV) were used to build the analytical curve using the XRF technique (Fig. 10b).

Figure 10c shows two linear analytical curves obtained for EASI-MS and XRF measurements indicating that indeed reasonably accurate quantifications of *m*-CPP in tablets can be performed by either direct EASI(+)-MS or

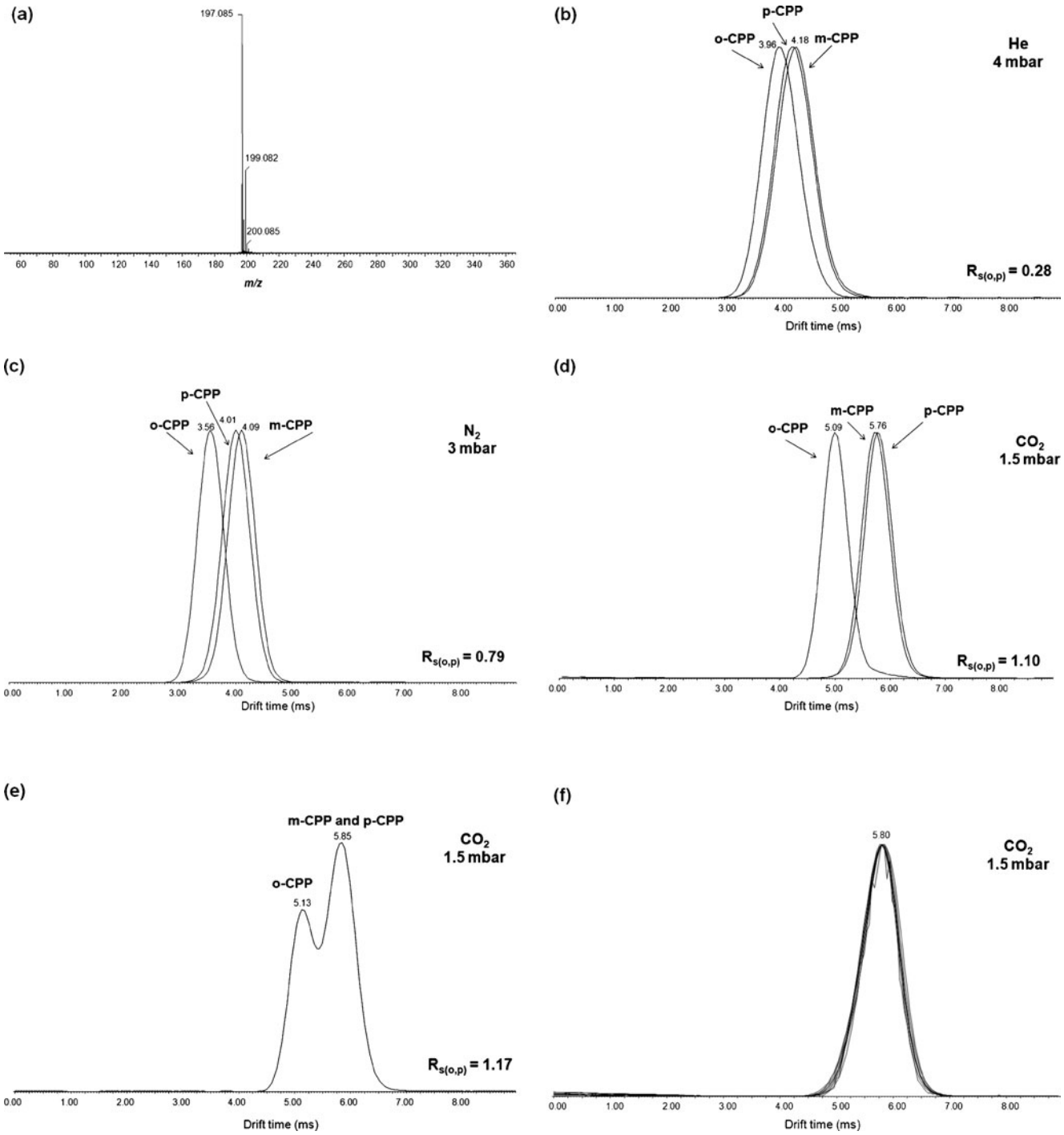

Fig. 11 **a** ESI(+)-TWIM-MS data for the $[M+H]^+$ ion of *m/z* 197; overlaid mobility chromatograms acquired in **b** He, **c** N_2, and **d** CO_2 drift gases for ions of *o*-CPP, *m*-CPP, and *p*-CPP. All isomers were infused separately in ESI(+) source; mobility chromatograms acquired in **e** CO_2 drift gas for a mixture containing the three isomers; and **f** overlaid mobility chromatograms for four ecstasy samples containing probably only *m*-CPP

XRF analysis. From these curves, the *m*-CPP concentrations in four ecstasy samples were calculated (see Fig. 7), these values ranging from 4 to 40 mg tablets^{-1}. Two samples presented amounts of *m*-CPP lower than is normally reported (22 to 80 mg *m*-CPP tablet^{-1}) [47, 48]. The LOD values calculated from EASI-MS and XRF data were of 7.43 ng and 1.80 mg *m*-CPP tablet^{-1}, respectively.

Traveling wave ion mobility mass spectrometry

Firstly, the effect of drift gas on drift time and resolution for the ions $[M+H]^+$ of *m/z* 197 from the CPP isomers (*o*-CPP, *m*-CPP, and *p*-CPP) was studied. Figure 11a shows a typical ESI(+)-MS for the ion $[M+H]^+$ of *m/z* 197 corresponding to CPP isomers. Figure 11b–d shows the drift plots for the *o*-CPP, *m*-CPP, and *p*-CPP ions in He, N_2, and CO_2 at pressures of 4, 3, and 1.5 mbar, respectively. Among the drift gases under study, only CO_2 was able to provide clear separation of the *o*-CPP ion from its isomers *m*-CPP and *p*-CPP at an optimum pressure of 1.5 mbar ($R_{s(o,p)}$=1.10 where *o* and *p* correspond to isomers *o*-CPP and *p*-CPP). Probably, the higher molar mass and polarizability volume of CO_2 is the determinant factor in the separation of *o*-CPP from its isomers [44, 45]. For the three gases used, however, the *m*-CPP and *p*-CPP isomers displayed nearly the same drift time. The separation efficiency of CO_2 was verified when *o*-CPP/*m*-CPP/*p*-CPP mixtures (1:1:1 *v*%) were analyzed, and a reproductive result was obtained (Fig. 11e). Again, only two isomeric analytes were resolved.

Four ecstasy samples containing CPP as active ingredient were also analyzed by ESI(+)-TWIM-MS (Fig. 11f). A unique drift time peak at 5.80 ms was observed, suggesting the presence of only *m*-CCP, or its mixture with the *p*-CCP isomer. But most likely, only the *m*-CPP is present, since the synthetic route should form both *o*-CPP and *p*-CPP. For example, the synthesis of *o*-chloroaniline or *o*-dichlorobenzene often present as impurities *p*-chloroaniline and *p*-dichlorobenzene, respectively. Additionally, the *o*-CPP and *p*-CPP isomers have shown no or low pharmacological activities [48].

^{1}H NMR

Figure 12 shows ^{1}H NMR spectra of *o*-CPP, *m*-CPP and *p*-CPP and for an ecstasy sample identified as CPP tablet. The main differences observed are related to shifts (δ) of hydrogens in phenyl ring (δ, 7.7–6.5 ppm): *o*-CPP, δ (ppm) 7.43 (d,1), 7.33 (t,2), 7.21 (d,3), and 7.12 (t, 4); *m*-CPP, δ (ppm) 7.26 (t, 1), 7.05 (s, 2), 6.96 (d, 3), and 6.92 (d, 4) [49]; and *p*-CPP, δ (ppm) 7.26 (d, 1) and 7.00 (d, 2). For the piperazine ring, chemical shifts in 3.30 and 2.00 ppm regions are observed for the three isomers.

Four ecstasy samples were identified as CPP tablets by EASI(+)-MS (ion of *m/z* 197, Figs. 4a and 11a). For these samples, NMR results confirm uniquely the presence of the *m*-CPP isomer (see Fig. 7d). Others signals observed in region from 5.5 to 3 ppm (Fig. 12d) are related to lactose and starch excipients [9].

Conclusions

EASI-MS and ED-XRF data provide relatively simple and fast screening tools to characterize and classify *m*-CPP and MDMA tablets in forensic investigations. These fast techniques with no sample preparation can also provide information of the excipients used in the formulations and synthetic process. PCA applied to XRF data placed samples into three groups: *m*-CPP tablets (four samples), MDMA tablets (23 samples), and tablets with no active ingredients (three samples). Also, the EASI-MS and XRF techniques are shown to provide reliable quantification of *m*-CPP in ecstasy tablets with quite linear responses (R^2=0.9681 and 0.9959, respectively). The *m*-CPP concentration in four real samples ranges from 4 to 40 mg *m*-CPP tablet^{-1}. The *m*-CPP can be differentiated from its isomers (*o*-CPP and *p*-CPP) by traveling wave ion mobility mass spectrometry (drift time=

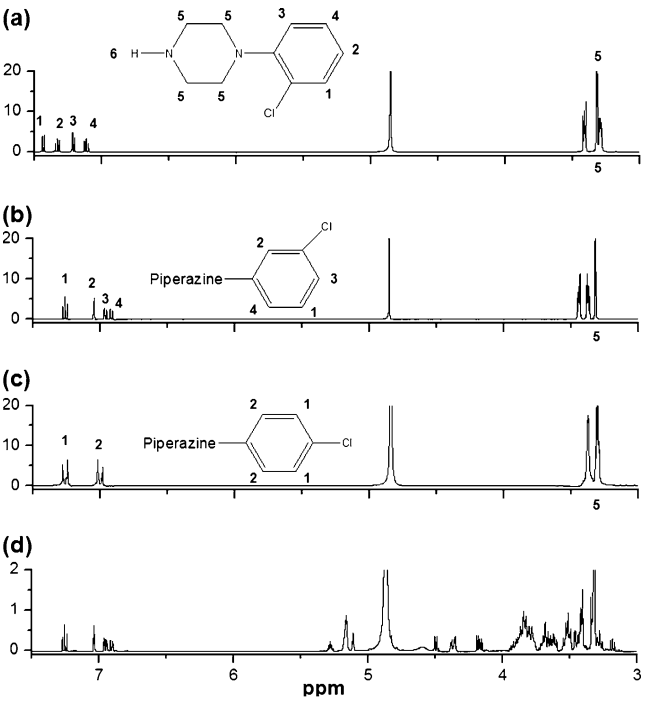

Fig. 12 ^{1}H NMR spectra of *o*-CPP **(a)**, *m*-CPP **(b)**, *p*-CPP **(c)** and of an ecstasy sample identified as *m*-CPP tablet **(d)**, with their respective signal assignments

5.77–5.80 ms) and NMR (δ (ppm) 7.26 (t, 1), 7.05 (s, 2), 6.96 (d, 3), and 6.92 (d, 4)) measurements.

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