Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com

Research Article

MASS SPECTRAL FRAGMENTATION OF CATHINONES BY HIGH-RESOLUTION TOFMS USING A SOFT IONIZATION SOURCE

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Received on: 03/10/12 Revised on: 10/11/12 Accepted on: 15/11/12

ABSTRACT

A set of 25 cathinones has been analyzed by GC high-resolution time-of-flight mass spectrometry (TOFMS) using a soft ionization source, referred in here as the microplasma photoionization (MPPI) source. This plasma-based, wavelength-selectable ionization source enables softer (i.e., ~ 8 to 12 eV) ionization of the test cathinones relative to electron ionization at 70 eV. Plasma gases such as Xe, Kr, and Ar, which generate distinct vacuum ultraviolet (VUV) resonance lines (i.e., Xe @ 8.44/9.57 eV, Kr @ 10.03/10.64 eV, and Ar @ 11.62/11.83 eV), have been evaluated for the identification of underivatized and derivatized cathinones. Derivatization of the test compounds with trifluoroacetic anhydride and α-methoxy-α-(trifluoromethyl)- phenylacetyl pyrazole was evaluated because the MPPI mass spectra of the underivatized cathinones yield primarily immonium ions and are identical to those obtained by electron ionization, thus leading to inconclusive MS identification of the test compounds. The MPPI mass spectra of TFA-derivatized cathinones yield both benzoyl and immonium ions, and certain TFA-derivatized cathinones yield molecular ions especially when using Xe as plasma gas, thus making possible their identification by MS.

Keywords: GC high-resolution TOFMS, soft-ionization source, cathinones, designer drugs

INTRODUCTION

Cathinones are an emerging group of designer drugs that contain derivatives of cathinone (β-ketoamphetamine), β-ketoanalogs of methylenedioxyamphetamines and pyrrolidinophenones[1]. S (-)-Cathinone is a natural compound found in young leaves of Khat bush (*Catha edulis*) and exhibits stimulant activity similar to amphetamine[2]. Synthetic cathinones, which have appeared in the European recreational drug market since mid-2000s, include ring-substituted cathinone derivatives like methcathinone, mephedrone, methedrone, metylone, and 3,4-methylenedioxypyrovalerone [3,4,5].

3-Bromo- and 3-fluoromethcathinone are two new designer drugs sold via the internet as "bath salts" or "plant feeders"[6,7]. Other designer drugs belong to phenylethylamines (i.e., amphetamines), tryptamines, and piperazines such as N-benzylpiperazine, 1-(3,4-methylenedioxybenzyl)-piperazine, and 1-(3-trifluromethyl)-phenylpiperazine[8].

Quantitative analysis of cathinones by GC-electron ionization (EI)/MS is widely used in forensic laboratories, and it is known that the EI spectra of cathinones lack molecular ions and exhibit mainly immonium ions $(C_nH_{2n+2}N)^+$ at m/z 44, 58, 72, 86, and 100, depending on the compound, thus making their identification extremely difficult in the absence of authentic standards (see Figure 1). The EI spectra of cathinones with common ring substituents are discussed elsewhere [9]. It has been reported that the dissociation of the carbon-carbon bond between the aromatic ring and the amine group takes place even at 12eV, so the immonium ion is always the dominant fragment ion in EI[9]. Alternative ionization techniques such as chemical ionization[9] with methane as reagent gas and liquid chromatography with electrospray ionization (ESI) coupled to MS[9] have been reported. The characteristic ionization processes under ESI include: loss of water, loss of methyl or ethyl groups attached to the nitrogen atom in straight chain derivatives, and loss of neutral fragment by dissociation of the carbon-nitrogen bond, which is dominant in cathinones with tertiary amine functionality [9].

Knowledge of the molecular mass of designer drugs is very important in their identification, and it is the first step in the screening procedure for finding active components of "legal highs"[9]. Once the mass-to-charge (m/z) ratio of the molecular ion is known, such information is used to classify the compound into one of the four categories of designer drugs given above. For example, if the molecular ion is an odd mass ion, then the compound might be a phenylethylamine or a cathinone, according to the nitrogen rule in MS, whereas an even mass ion might classify it as a tryptamine or a piperazine because of the even number of nitrogen atoms in these compounds [9]. Following this, a chemical formula can be derived for the molecular ion from the experimental m/z and the isotope distribution. Use of GC interfaced to a high-resolution TOF mass spectrometer equipped with a soft ionization source provides a powerful analytical tool for this purpose, because we have demonstrated for a set of nine stimulants that by inducing photoionization using VUV photons from a Xe plasma, not only is the fragmentation of molecular ion reduced, but selectivity can be achieved by not ionizing certain compounds that have ionization energies above the photon energies generated by the plasma gas [10].

The ionization source used in this study incorporates a microplasma discharge as the source of resonance radiation in the VUV[11]. The specific emission wavelengths produced by the discharge, which are usually comprised of multiple resonance lines, are selected by choosing the plasma gas. Radiation with wavelengths in the range of 104-150 nm, corresponding to photon energies between ~ 8 and 12 eV, can enable the soft ionization of analytes with less fragmentation than EI, and increased selectivity can be achieved by proper selection of the plasma gas. Different plasma gases generate distinct VUV resonance lines : Ar at 104.82 nm (11.83 eV) and 106.67 nm (11.62 eV); Kr at 116.49 nm (10.64 eV) and 123.58 nm (10.03 eV) and Xe at 129.56 nm (9.57 eV) and 149.96 nm (8.44 eV). By utilizing a windowless design, this soft ionization source can be operated with the full range of rare gas mixtures, including He and Ne, with emission lines below the ~105-115 nm cut-off of VUV transmissible

materials such as LiF and MgF_2. The only requirement for photoionization is that the photon energy be greater than the ionization potential of the target compound. Therefore, the photoionization conditions can be chosen such that the solvent or the matrix components are not ionized under the conditions selected for ionization of the target compound.

This study was undertaken to investigate the soft ionization (~8 eV -12eV) of a set of 25 cathinones in a high-resolution (~ 10,000) time-of-flight (TOF) mass spectrometer equipped with this microplasma photoionization (MPPI) source, and interfaced to a gas chromatograph. Table 1 lists the 25 test cathinones and their chemical structures are shown in Figure 2.

MATERIALS AND METHODS

Chemicals

Cathinones were obtained either from Ceriliant (Round Rock, TX, USA), as solutions at concentrations of 1 mg/mL in methanol, or Cayman Chemical (Ann Arbor, MI, USA) as solids, and were stored at 4ºC. 3,4-Methylenedioxy-methamphetamine (MDMA) at 1 mg/mL in methanol was obtained from Cerriliant. Individual stock solutions of the compounds obtained from Cayman Chemical were prepared in ethyl acetate. Serial dilutions of all test compounds at 100 ng/µL, 50 ng/µL, 20 ng/µL, 10 ng/µL, and 5.0 ng/µL, were prepared in ethyl acetate. Spectroscopic-grade ethyl acetate and methylene chloride were purchased from J.T. Baker (Phillipsburg, NJ, USA) and EMD (Gibbstown, NJ, USA), respectively. Research Plus Grade Xe and Ultra High Purity Kr were purchased from Scott Specialty Gases (Plumsteadville, PA, USA) and Ultra High Purity Ar was purchased from Airgas USA, LLC (Long Beach, CA, USA).

Derivatization with TFAA

Trifluoroacetic anhydride (TFAA), Reagent Plus >99% was purchased from Sigma Aldrich (St. Louis, MO, USA). A 100 µL aliquot of TFAA/ethyl acetate (5:1, v/v) was added to each vial containing either residue from a single test compound or a composite solution, and the vial was capped, its contents were mixed with a vortex mixer, and then heated at 80 ºC for 10 min on a hotplate stirrer (Optichem digital hotplate stirrer, ChemGlass, Vineland, NJ, USA). After cooling to room temperature, the excess TFA/ethyl acetate was removed by evaporation to dryness under a gentle stream of nitrogen and the residue was redisolved in 50 µL ethyl acetate.

Derivatization with MTPA-Pyrazole

α-Methoxy-α-(trifluoromethyl) phenylacetyl pyrazole (MTPA-pyrazole) was obtained from Wako Chemicals (Richmond, VA, USA). A 100 µL aliquot of stock solution in ethyl acetate at 12.3 mg/mL was added to the vial containing the test compounds that needed to be derivatized and the vial was capped, its contents were mixed with a vortex mixer, and then heated at 80 ºC for 10 min on a hotplate stirrer. After cooling to room temperature, any excess reagent was removed by evaporation to dryness under a gentle stream of nitrogen, and the residue was redisolved in 100 µL ethyl acetate.

GC- MPPI high-resolution MS analysis

The exact mass measurements were conducted with a research TOF mass spectrometer that was equipped with the MPPI source, operated in positive mode, and was interfaced to an Agilent 7890 GC (Agilent Technologies, Santa Clara, CA, USA). The TOF mass spectrometer was a modified Agilent 6220 Accurate-Mass TOF LC/MS system with a flight path of 2 m and a 4 GHz data acquisition system. Spectral data were acquired at a rate of 5scans/sec and the mass range for data acquisition was 42 to 600 u. The mass axis was calibrated daily with perfluorotributylamine (PFTBA), which was delivered to the MPPI source via a calibration valve, using Ar plasma (ionization energy of PFTBA is 11.3-11.7 eV [12]). The resolution of the TOF mass spectrometer was approximately 10,000 (FWHM) at m/z 271.9867. Samples were introduced via a 30 m x 0.25 mm id x 0.25 µm film thickness HP-5MS capillary column from Agilent Technologies. The oven temperature was programmed from 50ºC to 245ºC at 35ºC/min and then to a final temperature of 300ºC at 15 ºC/min, where it was held for 3 min. Helium was used as carrier gas at a flowrate of 1.2 mL/min. The injector temperature was 250ºC, the source temperature was 175ºC, and the GC-MS transfer line temperature was 300ºC. The injector, fitted with a double tapered liner, was set in splitless mode for 2 min after the injection (purge flow was 50 mL/min). Data processing was performed using the MassHunter Qualitative Analysis software (Agilent Technologies, version B.05.00) and possible chemical formulas were obtained using the Qual Formula Calculator algorithm incorporated in the MassHunter software.

Soft ionization source

The MPPI source schematic is shown elsewhere [10]. Vacuum ultraviolet (VUV) light is produced by resonant microwave devices designed to ignite and sustain a plasma discharge at reduced pressures [11]. These miniaturized flow resonance lamps typically operate on a few watts of power near 2.5 GHz at flowrates below 10 mL/min. The resulting plasma emits at a specific wavelength determined by the gas composition and pressure. The gaseous analyte flows through a channel in the plenum and is exposed to VUV light through an orifice. The microplasmas are offset from the sample ionization chamber, allowing metastable atoms to be dispersed and thus reducing their interaction with the analyte. Electrostatic deflectors situated between the outputs of the plasma devices and entrances to the ionization chamber prevent plasma ions and electrons from entering the ionization zone and interacting with the analyte. The sample ions are extracted and formed into a beam using a custom ion source based on the design of the Agilent Technologies EI source.

Table 1. GC retention times, chemical formulas and *m/z* for the underivatized and TFA-derivatized test cathinones

	Underiv	Underiv	Underiv	TFA-deriv	TFA-deriv	TFA-deriv
Compound name	**RT (min)**	**Formula**	**Calc. *m/z***	**RT (min)**	**Formula**	**Calc. *m/z***
Cathinone	a	$C_9H_{11}NO$	149.0835	4.51	$C_{11}H_{10}F_3NO_2$	245.0658
3-Fluoromethcathinone	4.1	$C_{10}H_{12}FNO$	181.0897	4.56	$C_{12}H_{11}F_4NO_2$	277.072
4-Fluoromethcathinone	4.21	$C_{10}H_{12}FNO$	181.0897	4.56	$C_{12}H_{11}F_4NO_2$	277.072
Methcathinone	4.28	$C_{10}H_{13}NO$	163.0992	4.67	$C_{12}H_{12}F_3NO_2$	259.0815
2-Fluoroethcathinone	4.3	$C_{11}H_{14}FNO$	195.1054	4.93	$C_{13}H_{13}F_4NO_2$	291.0877
N,N-dimethylcathinone	4.36	$C_{11}H_{15}NO$	177.1149	b	b	b
2-Methylmethcathinone	4.44	$C_{11}H_{15}NO$	177.1148	4.94	$C_{13}H_{14}F_3NO_2$	273.0971
Ethcathinone	4.45	$C_{11}H_{15}NO$	177.1148	4.98	$C_{13}H_{14}F_3NO_2$	273.0971
Buphedrone	4.55	$C_{11}H_{15}NO$	177.1148	4.88	$C_{13}H_{14}F_3NO_2$	273.0971
2-Methylethcathinone	4.65	$C_{12}H_{17}NO$	191.1305	5.23	$C_{14}H_{16}F_3NO_2$	287.1128
3-Methylethcathinone	4.8	$C_{12}H_{17}NO$	191.1305	5.23	$C_{14}H_{16}F_3NO_2$	287.1128
Diethylpropion	4.92	$C_{13}H_{19}NO$	205.1461	b	b	b
Mephedrone	4.75	$C_{11}H_{15}NO$	177.1148	5.08	$C_{13}H_{14}F_3NO_2$	273.0971
Pentedrone	4.85	$C_{12}H_{17}NO$	191.1305	5.14	$C_{14}H_{16}F_3NO_2$	287.1128
2-Ethylethcathinone	4.94	$C_{13}H_{19}NO$	205.1461	5.51	$C_{15}H_{18}F_3NO_2$	301.1284
4-Methylethcathinone	4.97	$C_{12}H_{17}NO$	191.1305	5.38	$C_{14}H_{16}F_3NO_2$	287.1128
3-Methoxymethcathinone	5.25	$C_{11}H_{15}NO_2$	193.1097	5.42	$C_{13}H_{14}F_3NO_3$	289.092
3,4-Dimethylethcathinone	5.37	$C_{13}H_{19}NO$	205.1461	5.72	$C_{15}H_{18}F_3NO_2$	301.1284
Methedrone	5.46	$C_{11}H_{15}NO_2$	193.1097	5.67	$C_{13}H_{14}F_3NO_3$	289.092
2,3-Methylenedioxy-methcathinone	5.49	$C_{11}H_{13}NO_3$	207.089	5.85	$C_{13}H_{12}F_3NO_4$	303.0713
Methylone	5.75	$C_{11}H_{13}NO_3$	207.089	6.01	$C_{13}H_{12}F_3NO_4$	303.0713
Butylone	6.05	$C_{12}H_{15}NO_3$	221.1046	6.21	$C_{14}H_{14}F_3NO_4$	317.0869
Ethylone	5.92	$C_{12}H_{15}NO_3$	221.1046	6.29	$C_{14}H_{14}F_3NO_4$	317.0869
Eutylone	6.07	$C_{13}H_{17}NO_3$	235.1203	6.4	$C_{15}H_{16}F_3NO_4$	331.1026
Pentylone	6.21	$C_{13}H_{17}NO_3$	235.1203	6.45	$C_{15}H_{16}F_3NO_4$	331.1026
a not able to analyze w/o deriv						
b does not derivatize with TFAA						

Table 2. Fragment ions in the Kr-MPPI spectra of selected cathinones derivatized with MTPA-pyrazol [a]

Compound	MTPA-derivative	Calc *m/z*	Fragment ions
Cathinone	$C_{19}H_{18}F_3NO_3$	367.1233	176.0739 (100%) 260.0945 (40%) 132.0819 (5%)
Methcathinone	$C_{20}H_{20}F_3NO_3$	379.1390	274.1003 (100%) 189.0538 (20%) 379.1255 (5%)
Methedrone	$C_{21}H_{22}F_3NO_4$	409.1495	274.1064 (100%) 135.0478 (35%) 409.1425 (15%)
Pentedrone	$C_{22}H_{24}F_3NO_3$	407.1703	302.1368 (100%) 407.1543 (5%)
Pentylone	$C_{23}H_{24}F_3NO_5$	451.1601	302.1359 (100%) 451.1576 (10%)

[a] The chemical structure of the MTPA-derivative is similar to the TFA-derivative, except that the $COCF_3$ group is replaced by $COC(CF_3)(OCH_3)(C_6H_5)$

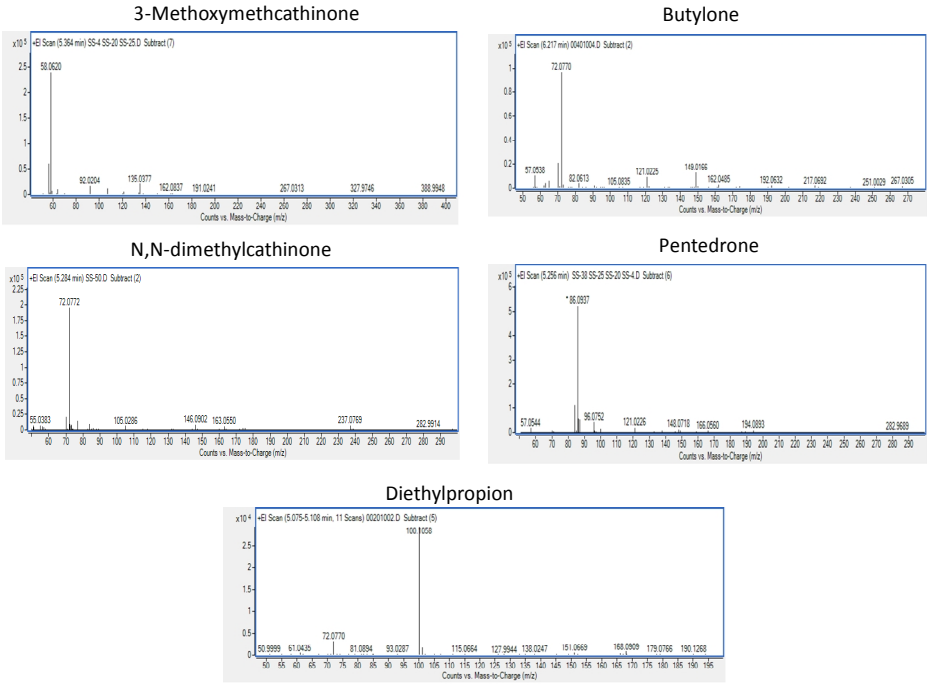
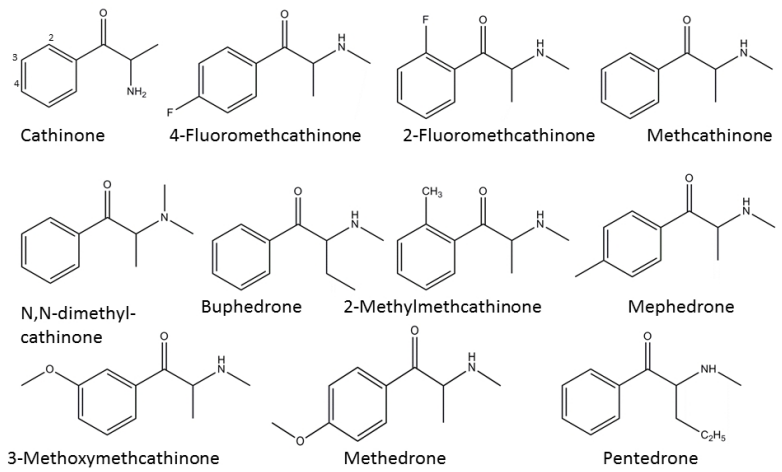
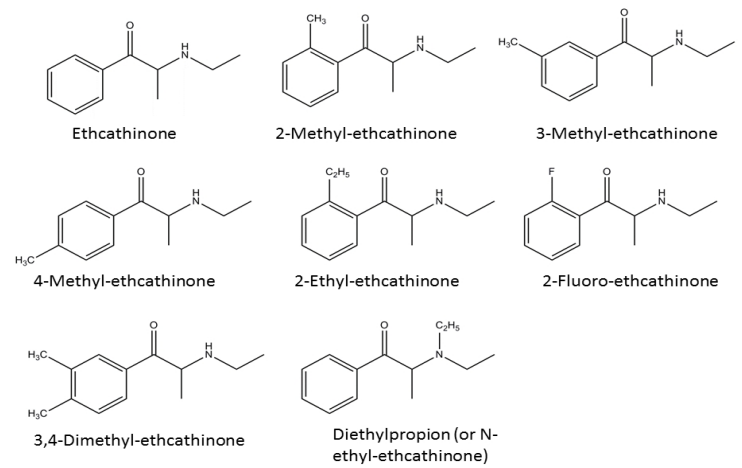


Figure 1. EI spectra of five cathinones





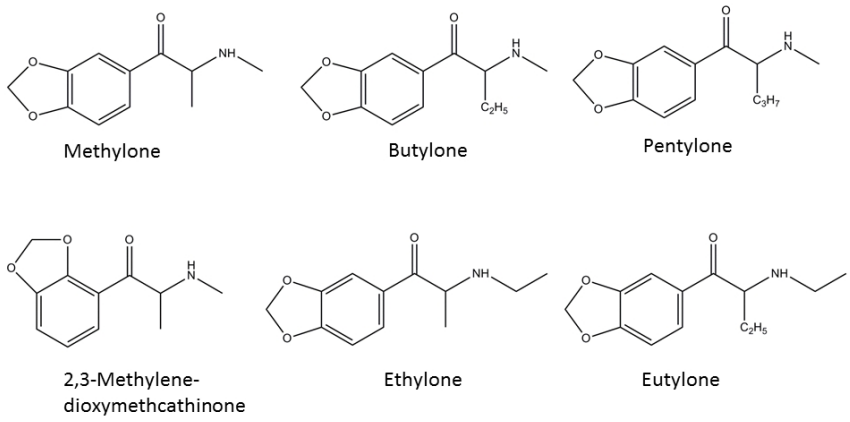


Figure 2. Chemical structures of the test compounds

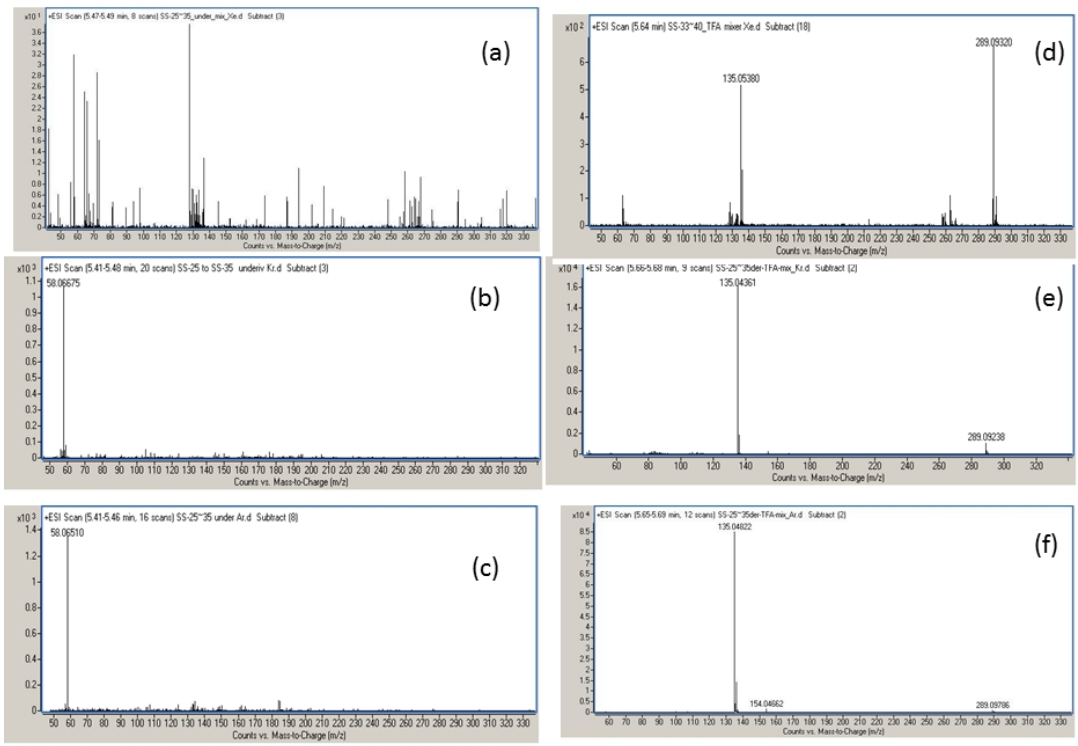


Figure 3. Xe-, Kr- and Ar-MPPI mass spectra of the underivatized and the TFA-derivatized methedrone. (a) Xe/underivatized, (b) Kr/underivatized, (c) Ar/underivatized, (d) Xe/TFA-derivatized, (e) Kr/TFA-derivatized, (f) Ar/TFA-derivatized.

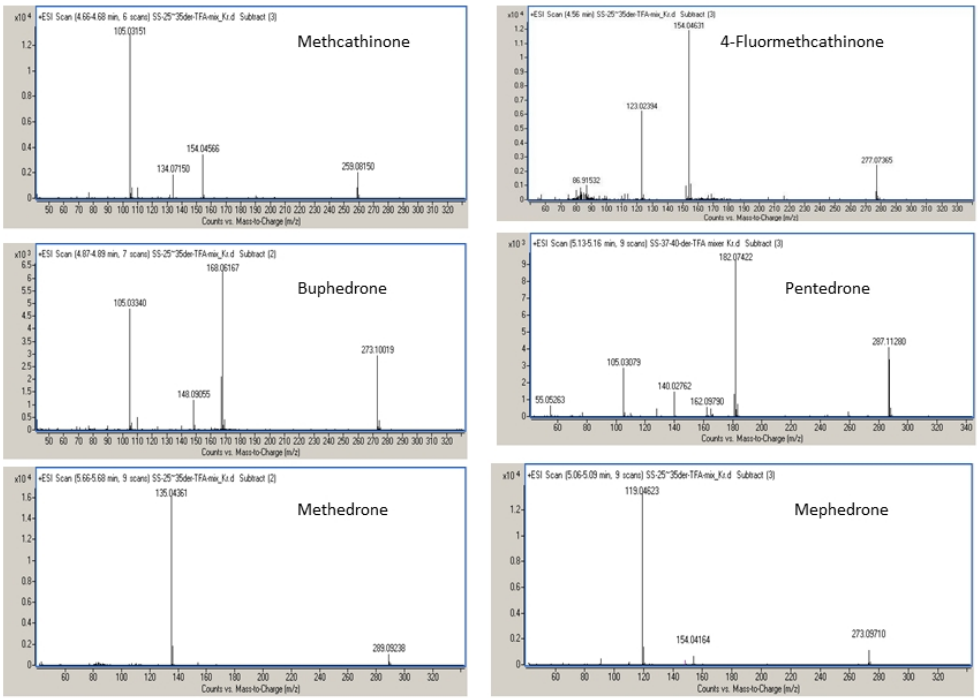


Figure 4. Kr-MPPI mass spectra of selected methcathinones derivatized with TFAA

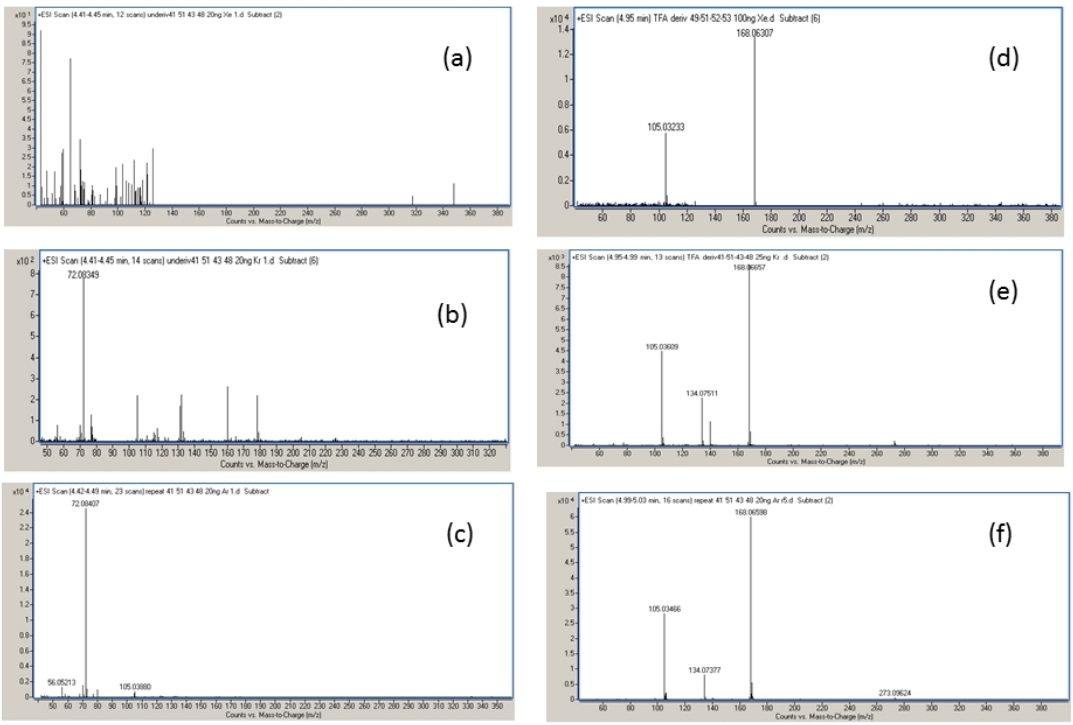
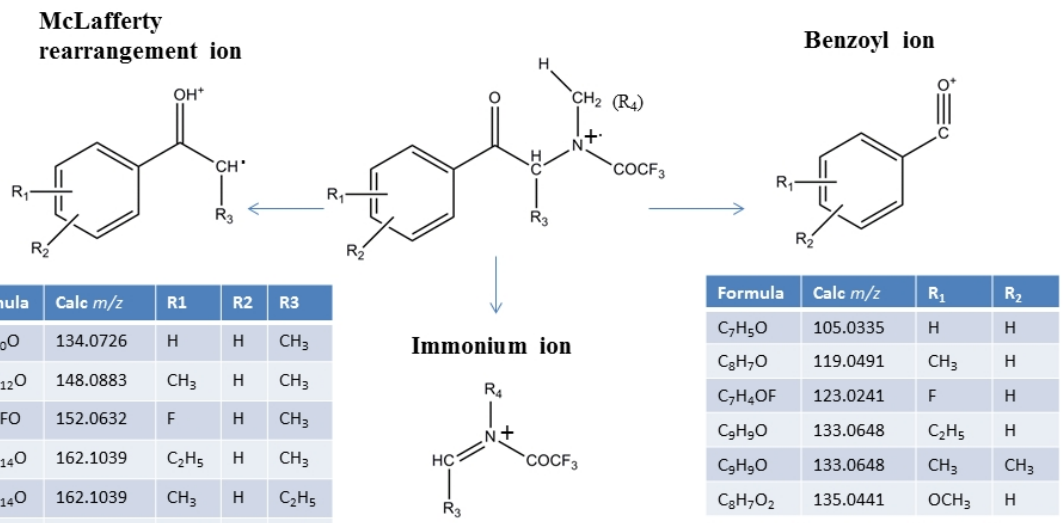


Figure 5. Xe-, Kr- and Ar-MPPI mass spectra of the underivatized and the TFA-derivatized ethcathinone. (a) Xe/underivatized, (b) Kr/underivatized, (c) Ar/underivatized, (d) Xe/TFA-derivatized, (e) Kr/TFA-derivatized, (f) Ar/TFA-derivatized.



Formula	Calc *m/z*	R1	R2	R3
$C_9H_{10}O$	134.0726	H	H	CH_3
$C_{10}H_{12}O$	148.0883	CH_3	H	CH_3
C_9H_9FO	152.0632	F	H	CH_3
$C_{11}H_{14}O$	162.1039	C_2H_5	H	CH_3
$C_{11}H_{14}O$	162.1039	CH_3	H	C_2H_5
$C_{11}H_{14}O$	162.1039	H	H	C_3H_7
$C_{10}H_{10}O_2$	164.0832	OCH_3	H	CH_3

Formula	Calc *m/z*	R_1	R_2
C_7H_5O	105.0335	H	H
C_8H_7O	119.0491	CH_3	H
C_7H_4OF	123.0241	F	H
C_9H_9O	133.0648	C_2H_5	H
C_9H_9O	133.0648	CH_3	CH_3
$C_8H_7O_2$	135.0441	OCH_3	H

Formula	Calc *m/z*	R_3	R_4
$C_5H_7NOF_3$	154.0540	CH_3	CH_3
$C_6H_9NOF_3$	168.0631	C_2H_5	CH_3
$C_6H_9NOF_3$	168.0631	CH_3	C_2H_5
$C_7H_{11}NOF_3$	182.0787	C_3H_7	CH_3
$C_7H_{11}NOF_3$	182.0787	C_2H_5	C_2H_5

Figure 6. MPPI fragmentation of the TFA-derivatized cathinones and the accurate m/z data for the benzoyl ions, immonium ions, and the McLafferty rearrangement ions of different cathinones.

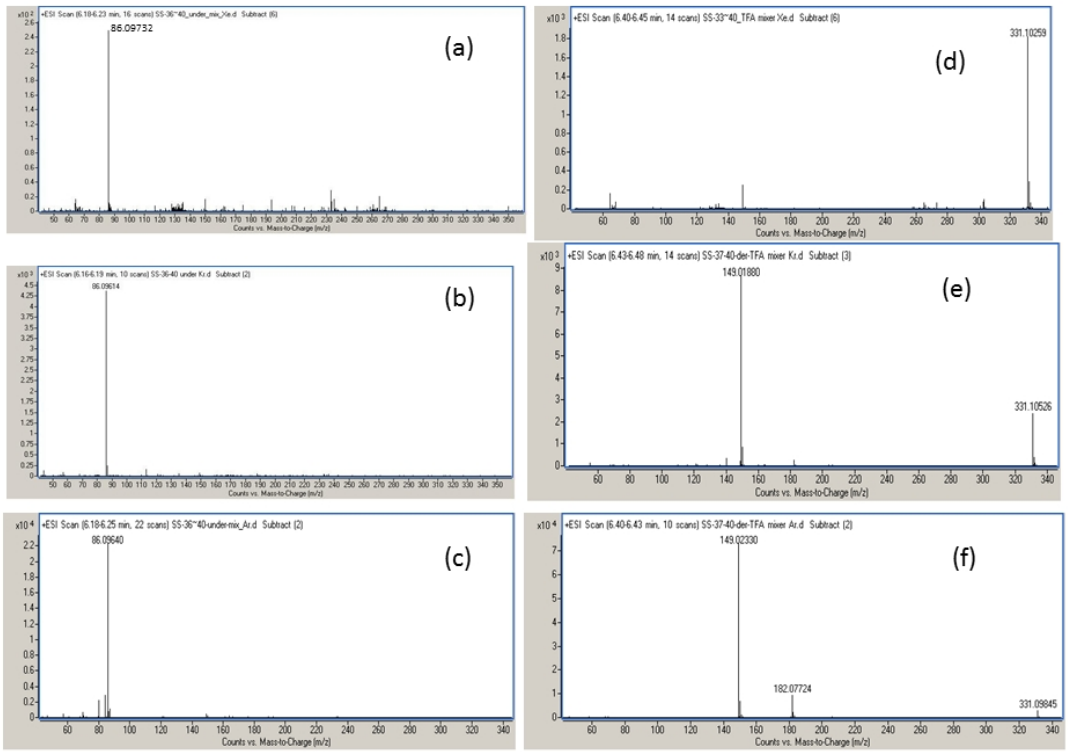


Figure 7. Xe-, Kr- and Ar-MPPI mass spectra of the underivatized and the TFA-derivatized pentylone. (a) Xe/underivatized, (b) Kr/underivatized, (c) Ar/underivatized, (d) Xe/TFA-derivatized, (e) Kr/TFA-derivatized, (f) Ar/TFA-derivatized.

RESULTS AND DISCUSSION

Mass spectral fragmentation of test compounds in the MPPI source

Figure 3 shows the Xe-, Kr-, and Ar-MPPI spectra of the underivatized (spectra a-c, respectively) and the TFA-derivatized methedrone (spectra d-f, respectively). The Xe-MPPI spectrum (Figure 3, a) of the underivatized compound is very weak, most likely because methedrone is not ionized as its ionization energy is higher than the resonance line of Xe at 8.44 eV. Kr-MPPI (Figure 3, b) and Ar-MPPI spectra (Figure 3, c) of the underivatized methedrone yield a fragment ion at *m/z* 58.0651-*m/z* 58.06675 (calc. *m/z* 58.0651), which is an immonium ion formed by an α-cleavage reaction of the carbon-carbon bond between the aromatic ring and the amine, whereas the MPPI spectra of the TFA-derivatized methedrone (Xe, Kr , and Ar in Figure 3,d-f, respectively) yield a methoxybenzoyl ion ($C_8H_7O_2^+$) at *m/z* 135.0441. In addition, the presence of a molecular ion at *m/z* 289.0924 or 289.0932 (calc *m/z* 289.0920) in the MPPI spectra d-e in Figure 3, with its intensity decreasing as the photon energy increases, facilitates the identification of this compound in the category of cathinones because: an odd molecular ion at *m/z* 289.0920 for the TFA-derivative; an immonium ion at *m/z* 58.0651 in the underivatized compound spectrum, and a methoxybenzoyl ion at *m/z* 135.0441 in the TFA-derivative. The exact assignment of the methoxy group onto the phenyl ring is not possible by MS.

When using low-resolution EI and derivatization with TFAA, methoxycathinone cannot be distinguished from the designer drug MDMA, because the methoxybenzoyl ion($C_8H_7O_2^+$) of methoxycathinone has the same mass-to-charge ratio as the methylenedioxybenzyl ion ($C_8H_7O_2^+$) in MDMA [13]. However, with the MPPI source the two compounds can be distinguished, because methoxycathinone yields an abundant methoxybenzoyl ion ($C_8H_7O_2^+$) at *m/z* 135.0441, whereas MDMA yields an abundant ion at *m/z* 162.0691. The latter ion, which is a methylenedioxyphenylpropene ion ($C_{10}H_{10}O_2^+$), has been verified in our laboratory using a deuterated analog of MDMA that contains 5 deuterium atoms (one at Cβ, one at Cα, and three at the carbon atom attached to nitrogen atom). Perfluoroacylation weakens the bond between the nitrogen atom and the α-carbon of MDMA allowing the formation of the $C_{10}H_{10}O_2$ ion [13].

Figure 4 shows the Kr-MPPI spectra of the TFA-derivatives of methcathinone, 4-fluoromethcathinone, buphedrone, pentedrone, methedrone (4-methoxymethcathinone), and mephedrone (4-methyl-methcathinone). As expected, the Kr-MPPI spectra show distinguishable molecular ions at *m/z* 259.0815, *m/z* 277.0736, *m/z* 273.1002, *m/z* 287.1128, *m/z* 289.0924, and *m/z* 273.0971, respectively. Fragment ions formed by the α-cleavage reaction of the Cα-Cβ bond between the aromatic ring and the amine group correspond to the benzoyl ion ($C_7H_5O^+$) at *m/z* 105.0335, fluorobenzoyl ion ($C_7H_4OF^+$) at *m/z* 123.0241, methylbenzoyl ion ($C_8H_7O^+$) at *m/z* 119.0491 and methoxybenzoyl ion ($C_8H_7O_2$+) at *m/z* 135.0441. TFA-derivatized immonium ions such as $C_5H_7NOF_3$ at *m/z* 154.0540, $C_6H_9NOF_3$ at *m/z* 168.0631, $C_7H_{11}NOF_3$ at *m/z* 182.0787 are also present. For most of the measurements the mass accuracy is within 0.003 u, despite the fact that mass calibration was done with Ar plasma and not Kr plasma. Work in progress in our laboratory is focusing on finding alternative calibration compounds to use with Xe- and Kr-MPPI to improve mass accuracy.

Figure 5 shows the MPPI spectra of the underivatized (spectra a-c) and the TFA-derivatized ethcathinone (spectra d-f). The fragmentation pathway of ethcathinones is very similar to that of cathinones and is outlined in Figure 6. As expected, the underivatized ethcathinone exhibits an immonium ion at *m/z* 72.0808, and the TFA-derivatized ethcathinone forms a benzoyl ion at *m/z* 105.0335 and an immonium ion at *m/z* 168.0631. In contrast to methcathinones, which give strong molecular ions especially with Xe-MPPI, ethcathinones barely exhibit any molecular ions. Nonetheless, once the immonium ion ($C_4H_{10}N^+$) at *m/z* 72.0808 is identified in the MPPI spectrum of the underivatized compound, there is a high probability that such compound is an amphetamine- or cathinone-type designer drug, in which the ethyl group is attached either at the amine group or the Cα. Following derivatization with TFAA, the fragment ions of the TFA-derivatized compound will facilitate the assignment of the R groups on the phenyl ring, the Cα, and the amine group. Figure 6 indicates the various combinations of R_1, R_2, R_3 and R_4 groups for the two major fragment ions in the MPPI spectra of methcathinones and ethcathinones.

In addition to the benzoyl and the immonium ions found in the MPPI spectra of methcathinones and ethcathinones, there are less abundant fragment ions at *m/z* 134.0726, *m/z* 148.0883, *m/z* 152.0632, *m/z* 162.1039, and *m/z* 164.0832 that are formed via a McLafferty rearrangement (see Figure 6). In this process, a hydrogen radical is transferred to the oxygen atom in the carbonyl group, and the new radical site can initiate an α-cleavage reaction leading to the formation of odd-electron ions such as $C_9H_{10}O^{+\cdot}$, $C_{10}H_{12}O^{+\cdot}$, $C_9H_9FO^{+\cdot}$, $C_{11}H_{14}O^{+\cdot}$, and $C_{10}H_{10}O_2^{+\cdot}$, respectively (see Figure 6).

Methylenedioxycathinones (i.e., methylone, butylone, pentylone, 2,3-methylenedioxy-methcathinone, ethylone, and eutylone) exhibit similar fragmentation in the MPPI source as the other cathinones. Specifically, these underivatized compounds yield immonium ions at *m/z* 58.0651(from methylone and 2,3-methylenedi oxymethcathin -one), *m/z* 72.0808 (from butylone and ethylone), or *m/z* 86.0964 (from pentylone and euthylone) and the TFA-derivatized methylenedioxy-cathinones yield the methylenedioxybenzoyl ion at *m/z* 149.0233 and the corresponding TFA-derivatized immonium ions (i.e., $C_5H_7NOF_3$ at *m/z* 154.0540, $C_6H_9NOF_3$ at *m/z* 168.0631, $C_7H_{11}NOF_3$ at *m/z* 182.0787). The MPPI spectra of the underivatized and the TFA-derivatized pentylone are shown in Figure 7 (spectra a-c for the underivatized compound and spectra d-f for the TFA-derivative).

Because positional isomers like 2-, 3-, 4-fluorocathinones or fluoroethcathinones, or 2-, 3-, 4-methylcathinones or methylethcathinones, cannot be identified from the MS data alone, use retention time information to distinguish between such positional isomers is needed, and thus there is still a need for reference compounds in order to positively identify cathinones. In our study we worked only with 2-methyl and 4-methylmethcathinone, but Power et al. reported that all three isomers of methcathinones can be separated by GC [14] and that either NMR or IR can easily differentiate between the positional isomers. In the case of functional isomers such as methyl-methcathinone, ethcathinone and buphedrone, the fragment ions in the MPPI spectra of the TFA derivatized compound often help elucidate the chemical structure. For example, both ethcathinone and buphedrone yield a benzoyl

ion at *m/z* 105.0335, because there are no alkyl groups on the phenyl ring for either compound, whereas methyl methcathinone yields a methylbenzoyl ion at *m/z* 119.0491, due to the methyl group on the phenyl ring of methylmethcathinone. Once methylmethcathinone is identified from the group of three TFA-derivatized compounds with molecular formula $C_{13}H_{14}F_3NO_2$, then ethcathinone and buphedrone are distinguished from the fragment ions generated by McLafferty rearrangement (i.e., ion at *m/z* 134.0726 should belong to ethcathinone and ion at *m/z* 148.0883 to buphedrone). Both spectra should exhibit immonium ions at *m/z* 168.0631. N,N-dimethylcathinone is also a functional isomer but it does not derivatize with TFAA, so this cathinone will not exhibit a benzoyl, and can be identified only from the presence of immonium ion at m/z 58.0651 and retention time match with the authentic standard. Similar examples include pentedrone/ methylethcathinone, 3,4-dimethylethcathinone/2-ethylcathinone,butylone/ethylone, and pentylone/eutylone.

The mass spectral data for the underivatized and the TFA-derivatized cathinones is summarized below:

- All underivatized compounds were ionized with Kr and Ar plasma and their MPPI spectra are similar to the EI spectra suggesting that their ionization potentials are probably in the range of 9-10 eV, and the slight excess energy causes the fragmentation of the molecular ion leading to the formation of the immonium ion. Only N,N-dimethylcathinone, diethylpropion, pentedrone, and pentylone were ionized with Xe probably because their ionization energies are below 8.44 eV, whereas the remainder of compounds have ionization energies above 8.44 eV. No such data could be found in the literature to substantiate this finding.
- The TFA-derivatives of the test cathinones and methylenedioxy-cathinones exhibit molecular ions, whose relative intensity increases, as the photon energy decreases.
- The degree of fragmentation of the TFA-derivatives of the test cathinones can be controlled with different plasma gases. Two major fragment ions are formed in the Kr and Ar spectra of cathinones : the benzoyl ions and the immonium ions. Fragment ions resulting from McLafferty rearrangement have also been found.
- The methylenedioxybenzoyl ion at *m/z* 149.0233 was found in the Kr and Ar spectra of 3,4-methylene dioxycathinones and 2,3-methylenedioxy-cathinone. In addition, fragment ions corresponding to the TFA-derivatized immonium ions at *m/z* 154.0540, *m/z* 168.0631, and *m/z* 182.0787 were found in the MPPI spectra of TFA-derivatized methylenedioxycathinones.
- N,N-dimethylcathinone and diethylpropion (also known as N-ethyl-ethcathinone or N,N-diethylcathinone) cannot be derivatized with TFAA and therefore are difficult to identify. One literature report [15] even indicates that N, N-dimethylcathinone was incorrectly identified as ethcathinone, but subsequent derivatization with penta fluoropropionic anhydride confirmed the presence of a secondary amine. At the present time they can be tentatively identified from the presence of a fragment ion at *m/z* 72.0808 or *m/z* 100.1121, respectively, and by matching their GC retention times to those listed in Table 1.

Separation of stereoisomers

Derivatization with (+) alpha-methoxy-alpha-(trifluoromethyl)-phenylacetyl-pyrazol (MTPA-pyrazol) reported by Matsushita et al. [16], which was evaluated only to a limited extent for a few compounds, allowed the separation of stereoisomers but did not provide additional information that could be used in structure elucidation. Specifically, the bulky alpha-methoxy-alpha-(trifluoromethyl)-phenylacetyl that replaces the hydrogen atom in the primary or the secondary amine group of cathinone and methcathinone, respectively, leads primarily to the formation of the derivatized immonium ion via elimination of the benzoyl radical, with the exception of cathinone (see Table 2). Nonetheless, if detection of stereoisomers is needed, such chiral reagent would certainly be useful for this purpose.

CONCLUSION

The MPPI spectra of underivatized cathinones are identical to those generated by EI, thus leading to inconclusive MS identification of such compounds, but the MPPI spectra of TFA-derivatized cathinones exhibit both benzoyl ions and immonium ions making possible their identification by MS. Furthermore, it has been demonstrated that by using Xe as the plasma gas, we were able to obtain molecular ions for all TFA-derivatized methcathinones using a softer ionization process. This makes the identification of such designer drugs possible. This is not always the case when dealing with functional isomers like MDMA and methoxymethcathinone, because in the absence of a soft ionization source, methoxycathinone cannot be distinguished from MDMA, as the methoxybenzoyl ion ($C_8H_7O_2^+$) has the same mass-to-charge ratio as the methylenedioxybenzyl ion ($C_8H_7O_2^+$). However, with the MPPI source the two compounds can be distinguished because methoxycathinones form the methoxy benzoyl ion ($C_8H_7O_2^+$) at *m/z* 135.0441 whereas MDMA forms the ion $C_{10}H_{10}O_2^+$ at *m/z* 162.0691. Positional isomers like 2-, 3-, 4-fluoromethcathinones or fluoroethcathinones, and 2-, 3-, 4-methylmethcathinones or methyl ethcathinones, cannot be identified from the MS data alone, use of retention time matching with authentic standards is needed to positively identify such cathinones. Finally, N,N-dialkyl substituted cathinones form only immonium ions in MPPI even under the softest ionization conditions with Xe , thus require additional confirmatory analysis for unambiguous identification.

REFERENCES

1. Westphal F, Junge T, Girreser U, Greibl W, Doering C. Mass, NMR, and IR Spectroscopic characterization of pentedrone and pentylone and identification of their isocathinone by-products. Forensic Science Intern. 2012, 271:157-167.
2. Paul BD, Cole KA. Cathinone (Khat) and Methcathinone (CAT) in Urine Specimens: a Gas Chromatographic- Mass Spectrometric Detection Procedure. J. Anal. Toxicol. 2001, 25: 525-530.
3. http://www.emcdda.europe.eu/publications/drug-profiles/synthetic-cathinones.
4. Brandt SD, Freeman S, Sumnall HR, Measham F, Cole J. Analysis of NRG "legal highs" in the UK: Identification and formation of novel cathinones. Drug Test. Analysis 2011, 3: 569-575.
5. Westphal F, Junge T, Klein B, Fritschi G, Girreser U. Spectroscopic characterization of 3,4-methylenedioxypyrrolidinobutyrophenone: A new designer drug with α-pyrrolidinophenone structure. Forensic Sci. Intern. 2011, 209: 126-132.
6. Meyer MR, Vollmar C, Schwaninger AE, Wolf E, Maurer HH. New cathinone-derived designer drugs 3-bromomethcathinone and 3-fluoromethcathinone: studies on their metabolism in rat urine and human liver microsomes using GC-MS and LC-high-resolution MS and their detectability in urine. J. Mass Spectrom. 2012, 47:253-262.

7. Archer, RP. Fluoromethcathinone, a new substance of abuse. Forensic Sci. Intern. 2009, 185: 10-20.
8. Abdel-Hay KM, Awad T, DeRuiter J, Clark CR. Differentiation of methylenedioxy-benzylpiperazines (MDBP) by GC-IRD and GC-MS. Forensic Sci. Intern. 2010, 195: 78-85.
9. Zuba D. Identification of cathinones and other active components of "legal highs" by mass spectrometric methods. Trends in Anal. Chem. 2012, 32: 15-30.
10. Lopez-Avila V, Cooley J, Urdahl R, Thevis M. Determination of stimulants using gas chromatography/high-resolution time-of-flight mass spectrometry and a soft ionization source. Rapid Communic. Mass Spectrom. 2012, 26:2714-2724.
11. Xue J, Urdahl RS, Cooley JE. Plasma resonances in a microwave-driven microdischarge. Applied Physics Letters. 2012, 100: 064102-064105.
12. NIST Chemistry WebBook, NIST Standard Reference Database Number 69, Available: http://www.workbook.nist.gov/chemistry.
13. Awad T, Clark CR, DeRuiter J. Chromatographic and Mass Spectral Studies on Methoxymethcathinones Related to 3,4-Methylenedioxymethamphetamine. J. Chrom. Sci. 2006, 44:155-161.
14. Power JD, McGlynn P, Clarke K, McDermott SD, Kavanagh P, O'Brian J. The analysis of substituted cathinones. Part 1: Chemical analysis of 2-, 3- and 4-methylmethcathionone. Forensic Sci. Intern. 2011, 212: 6-12.
15. Camillieri A, Johnston MR, Brennan M, Davis S, Caldicott DGE. Chemical analysis of four capsules containing the controlled substance analogues 4-methylmethcathinone, 2-fluoromethamphetamine, α-phthalimidopropiophenone and N-ethylcathinone. Forensic Sci. Intern. 2010, 197:59-66.
16. Matsushita T, Takatsu M, Yoshida Y, Moriyasu M. On-Column Chiral Derivatization Reagent for GC Separation of Optical Isomeric Amphetamine and Methamphetamine. Bunseki Kagaku 2007, 56:1089-1095.

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Viorica Lopez-Avila, William Gao, and Randall Urdahl. Mass spectral fragmentation of Cathinones by high-resolution tofms using a soft ionization source. *J Pharm Sci Innov*. 2012; 1(6): 44-53.