Fragmentation Pathways and Structural Characterization of Synthetic Cathinones Using Electrospray Ionization and High Resolution Mass Spectrometry

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ABSTRACT

Designer drugs continue to present a number of challenges to the forensic toxicology community. Synthetic cathinones are a growing class of psychostimulants that can be chemically characterized as beta-keto amphetamines. Although derived from cathinone (*Catha edulis*), these synthetic drugs are substituted at the phenyl ring, amino group or propanone terminus. The functional substituents greatly influence fragmentation pathways and ion formation in both electron impact (EI) and electrospray ionization (ESI). Many of the cathinones, particularly the tertiary amines (pyrrolidine derivatives), undergo extensive fragmentation in EI, yielding poorly specific mass spectra with a limited number of diagnostic ions for selected ion monitoring. LC-MS is advantageous because of its ability to optimize ionization conditions to yield highly specific fragmentation ions through ESI. High resolution mass spectrometry is a powerful tool for structural elucidation. The fragmentation pathways and structural characterization of synthetic cathinones were identified using LC-Q/TOF-MS.

To understand the influence that functional substituents have on the fragmentation pathway, the target compounds included a variety of secondary and tertiary amines, methylenedioxy derivatives, benzylic, and amino substituents. The twenty-two synthetic cathinones in this study were methcathinone, 3-FMC, 4-FMC, methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-MEC, MDPBP, pentedrone, pentylone, 3,4-DMMC, alpha-PVP, 4-EMC, MPBP, MDPV, pyrovalerone, and naphyrone.

INTRODUCTION

The ability to reliably identify and quantify drugs in toxicology samples relies heavily on the instrumentation techniques. In gas chromatography-mass spectrometry selected ion monitoring (SIM) and in liquid chromatography multiple reaction monitoring (MRM) allow for the collection of predetermined diagnostic ions for individual drugs. The ion transitions selected should be specific to the drug of interest wherever possible. Ions that are a result of common losses (water loss, neutral ion loss) may be less specific and should be avoided.

In GC-MS, electron impact mass spectra for cathinones are dominated by the formation of stable immonium ions, often resulting in poorly specific mass spectra. In LC-MS, electrospray ionization (ESI) conditions such as collision induced dissociation (CID) energies, ionization source temperatures and voltages can be optimized to enhance mass spectral quality. This is particularly important for the cathinones, because their mass spectra are highly dependent on the various functional substituents. The general cathinone structure is shown in **Figure 1.**

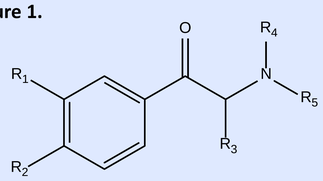


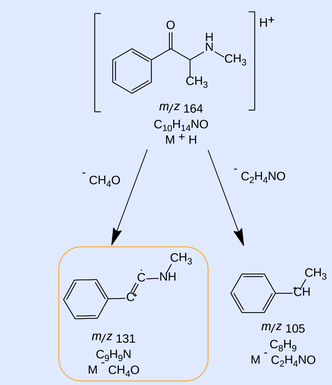
Figure 1. General structure for synthetic cathinones with substituent locations indicated as R_1-R_5.

RESULTS

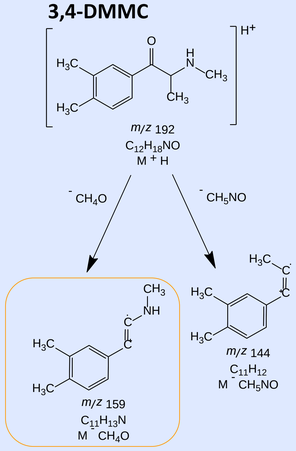
Cathinone	Transitions	Loss
Methcathinone	164.1070-->131.0731	-CH4O
	164.1070-->105.0703	-C2H4NO
3-FMC	182.0975-->149.0634	-CH4O
	182.0975-->123.060	-C2H4NO
4-FMC	182.0976-->149.0636	-CH4O
	182.0976-->123.0605	-C2H4NO
Methylone	208.0968-->160.0757	-CH4O2 (-MD)
	208.0968-->132.0807	-C3H9NO
Ethcathinone	178.1226-->131.0721	-C2H6O
	178.1226-->117.0586	-C3H6NO
	178.1226-->105.0700	-C2H6NO
Ethylone	222.1125-->174.1222	-CH4O2 (-MD)
	222.1125-->146.0958	-C3H9NO
Methedrone	194.1178-->**161.0833**	-CH4O
	194.1178-->146.0598	-C2H7O
	194.1178-->135.0803	-C2H4NO
Buphedrone	178.1226-->**131.0731**	-C2H6O
	178.1226-->91.0549	Tropylium
	178.1226-->145.0880	-CH4O
Butylone	222.1125-->174.0914	-CH4O2 (-MD)
	222.1125-->146.0964	-C3H9NO
Mephedrone	178.1226-->145.0889	-CH4O
	178.1226-->119.0853	-C2H4NO
Eutylone	236.1281-->188.1069	-CH4O2 (-MD)
	236.1281-->174.0547	-C3H11N
	236.1281-->161.0598	-C3H8NO
4-MEC	192.1383-->145.0886	-C2H6O
	192.1383-->131.0738	-C2H6NO
MDPBP	262.1438-->**161.0597**	-C5H10NO
	262.1438-->191.0704	-C4H9N (-PYR)
	262.1438-->112.1125	-C8H5O3
Pentedrone	192.1383-->**132.0810**	-C3H7O
	192.1383-->161.0958	-CH4N
	192.1383-->91.0546	Tropylium
Pentylone	236.1281-->188.1070	-CH4O2 (-MD)
	236.1281-->175.0682	-C3H8O
3,4-DMMC	192.1387-->159.1043	-CH4O
	192.1287-->144.0802	-CH5NO
alpha-PVP	232.1696-->161.0954	-C4H9N (-PYR)
	232.1696-->91.0549	Tropylium
4-EMC	192.1383-->**145.0889**	-CH4NO
	192.1383-->105.0701	-C4H8NO
MPBP	232.1696-->161.0960	-C4H9N (-PYR)
	232.1696-->133.1010	-C5H8NO
	232.1696-->112.1120	-C8H7O
MDPV	276.1594-->205.0857	-C4H9N (-PYR)
	276.1594-->126.1277	-C8H5O3
	276.1594-->175.0756	-C5H10NO
Pyrovalerone	246.1852-->175.1110	-C4H9N (-PYR)
	246.1852-->126.1280	-C8HYO
	246.1852-->105.0701	-C8H16NO
Naphyrone	282.1852-->211.1122	-C4H9N (-PYR)
	282.1852-->126.1280	-C11H7O
	282.1852-->141.0701	-C11H8

Table 1. Ion transitions for the twenty-two synthetic cathinones.

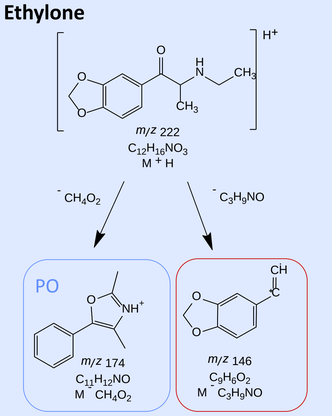
Methcathinone



3,4-DMMC



Ethylone



MDPV

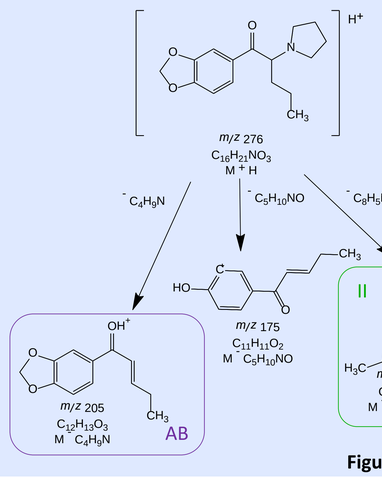


Figure 2.

DISCUSSION & CONCLUSIONS

Secondary Amine, Benzylic Unsubstituted or Substituted Cathinones

- Loss of ketone with subsequent rearrangement to form a cyclic cation was prevalent among the secondary amines. This fragment can be summarized as the loss of R_4R_5O.
- The majority of the quantification or qualifier ions for secondary substituted and unsubstituted cathinones were attributed to the CH_4O loss, indicated in orange in **Table 1**.

Secondary Amine, Methylenedioxy-Substituted Cathinones

- Formation of phenyloxazole (PO) cations arising from the loss of CH_4O_2 in methylenedioxy derivatives. Due to improved specificity, the quantification ion for all secondary methylenedioxy cathinones was the phenyloxazole cation, indicated in blue in **Table 1**.
- The second common fragmentation was the loss of $R_3R_4R_5NO$, indicated in red.

Tertiary Amine Cathinones (Pyrrolidine)

- Tertiary amine synthetic cathinones are also characterized by the formation of stable alkyldioxybenzoyloxonium (AB) ions arising from the loss of pyrrolidine. The ion resulting from the pyrrolidine loss was chosen as either the quantification ion or a qualifier ion for all tertiary amine cathinones, indicated in purple in **Table 1**.
- Other common qualifier ions for the pyrrolidine-type cathinones were the immonium ion (II), shown in green in **Table 1**.

MATERIALS AND METHODS

Chemicals and Reagents

Synthetic cathinone reference standards (1.0 mg/mL) were purchased from Cerilliant (Round Rock, TX).

Instrumentation

Analysis was performed using an Agilent Technologies 5630 Accurate-Mass Q-TOF LC/MS equipped with a Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm particle size). Ions were acquired in ESI positive mode using full scan, auto MS/MS mode with fixed collision energies at 10, 20, and 40 eV. Nitrogen drying gas was at 13 L/min, drying gas temperature was 200°C, the nebulizer was at 20 psi, the sheath gas temperature was at 250°C, nitrogen sheath gas flow was 12 L/min, capillary voltage was 4000 V, nozzle voltage was 0 V, fragmentor voltage was at 150 V, and the skimmer was at 65 V.

Transition Ion Selection

The transition ions were chosen by assessing the specificity and abundance of the diagnostic ions. Characteristic losses, such as water loss, were not chosen. Ions of high molecular weight were chosen to increase their specificity to synthetic cathinones. The ion transitions chosen for each synthetic cathinone and their proposed chemical loss are shown in **Table 1.**

Structure Identification

Fragmentation pathway and potential structures for each ion were determined with the use of ACD fragmentation software. The pathways are shown in **Figure 2.**

Figure 2 (left). Fragmentation pathways and structures for representative drugs. A (methcathinone), B (3,4-DMMC), C (ethylone), D (MDPV).

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