Identification of Synthetic Cathinones from Electron Impact Mass Spectra

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ABSTRACT

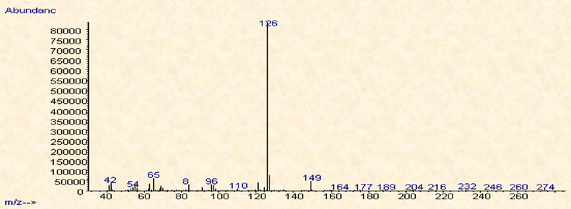
The popularity of synthetic cathinones and the diverse number of drugs within this relatively new class has increased considerably in recent years. Gas chromatography-mass spectrometry (GC-MS) is still the most widely used technique in routine forensic toxicology investigations. Due to the proliferation of structural analogs and limited cross-reactivity towards the entire class of drugs, chromatographic-based screening is of great importance for the synthetic cathinones. Chromatographic separation of analytes can be readily achieved using multi-component mixtures. However, the electron impact (EI) mass spectral properties of some of the forensically important synthetic cathinones can present a challenge due to the limited number of diagnostic ions.

The characteristic fragmentation pathways synthetic cathinones are described and discussed for nineteen secondary and tertiary amines within this class. These include buphedrone, ethcathinone, methcathinone, pentedrone, 4-EMC, 4-MEC, flephedrone, mephedrone, methedrone, α-PVP, MPBP, naphyrone, pyrovalerone, butlyone, ethylone, methylone, pentylone, MDPBP and MDPV. Although protonated molecular ions are readily observed using hyphenated electrospray ionization (ESI) techniques, parent ions are hard to obtain using EI-GC-MS. Molecular ions when they are present are odd, due to the "nitrogen rule". The mass spectra of synthetic cathinones are dominated by two characteristic cleavages to form iminium and acylium ions that are associated with the side chain and the core benzene ring (which is often substituted). The presence of the carbonyl bond on the α-carbon and the lone pair of electrons on the oxygen of the ketone moiety plays an important role in electron impact ionization. In addition to the rationalization of non-derivatized cathinones, acylation, silylation and two-step reductive silylation and reductive acylation methods will also be presented and discussed in terms of their mass spectral properties. Although fragmentation is largely predictable, it presents some practical limitations in terms of the specificity of some diagnostic ions, necessitating careful attention to chromatographic separation and identification criteria.

INTRODUCTION

Confirmation of unknown drugs using hyphenated mass spectroscopic techniques such as GC-MS or LC-MS rely upon reproducible chromatographic separation (retention time) and characteristic fragmentation (mass spectra). As a general rule, ions with a higher m/z ratio are preferred due to increased specificity, but abundance (intensity) of the ion is highly relevant from the standpoint of detectability. When selected ion monitoring (SIM) is used in place of full scan acquisition in GC-MS, at least three characteristic ions should be selected and ion ratios should be evaluated. This can present a challenge due to the relatively poor mass spectral quality of many of the synthetic cathinones, particularly the pyrrolidine species. This is illustrated in the electron impact (EI) mass spectrum of MDPV, which is heavily dominated by the m/z 126 base peak (Figure 1).

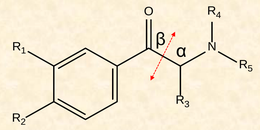
Figure 1. EI mass spectrum of MDPV



INTRODUCTION Cont'd

The extensive fragmentation leaves very few qualifier ions to choose from. The general structure of the synthetic cathinone species is shown in Figure 2. When R_4 and R_5 are occupied by the pyrrolidine group, these tertiary amines lack the active hydrogen that is necessary for commonly used and widely accepted approaches to derivatization such as acylation or silylation. In-situ decomposition of cathinones has been described and must be considered during GC-MS analysis. In this report we explore the limitations of EI mass spectra and describe how an active hydrogen can be introduced universally for the purposes of derivatization via the ketone functional group.

Figure 2. General structure of the synthetic cathinones



MATERIALS AND METHODS

Chemicals

Synthetic cathinone reference standards and internal standards were purchased from Cerilliant (Round Rock, TX). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and trimethylsilylimidazole (TMSI) were purchased from Thermo Scientific (Waltham, MA).

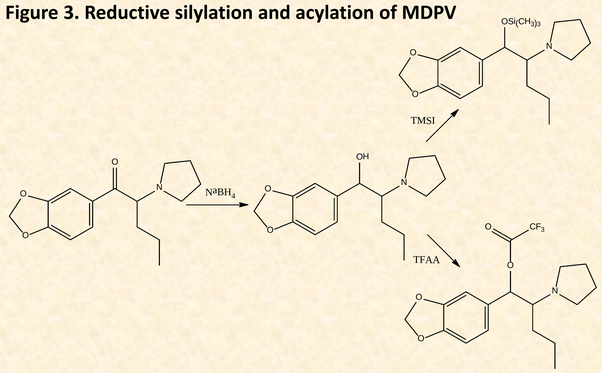
Instrumentation

Research was conducted using an Agilent 7890A GC system with a 5975 VL MSD equipped with a DB-5MS column (30 m x 0.25 mm x 0.25 µm). Helium was used as the carrier gas (1.3 ml/min). Sample (2 µL) was injected in split mode using a 25:1 split ratio at an injector temperature of 175°C. The initial GC temperature was 140ºC (hold 0.5 min), ramped to 265ºC at 10°C/min. For biological extracts, a post run burnout of 290°C was used. The total run time was 15.5 minutes.

Ketone Reduction

Sodium borohydride (30 µL) (2% w/v in ethanol) was added to cathinone standards that had been evaporated to dryness. Tubes were capped, vortex mixed, and heated (70°C, 15 min) and evaporated to dryness at room temperature under nitrogen. Hexane (30 µL) was added, vortex mixed and decanted from the reaction vial before being evaporated to dryness. Samples were then subject to traditional derivatization such as silylation (using TMSI) or acylation (using TFAA). A schematic for the reductive silylation and reductive acylation of MDPV is shown below in Figure 3.

Figure 3. Reductive silylation and acylation of MDPV



RESULTS AND DISCUSSION

Although the GC programming described here can be used to effectively separate cathinones of interest (Figure 4), the EI mass spectra of synthetic cathinones are dominated by the formation of iminium and acylium ions, with relatively minor secondary and tertiary fragmentations (Figure 5). The principal fragmentation occurs by dissociation of the α and β-carbon bond, which results in relatively few diagnostic ions and minimal or no molecular ion. The dominance of the pyrrolidinium species ($C_nH_{2n}N^+$) among the tertiary analogs presents a real challenge for selected ion monitoring. The base peak is determined by the R_3, R_4 and R_5 substituents, which means that positional isomers with the same side chain constituents produce very similar mass spectra (Table 1). This is depicted in Figure 6, which shows EI mass spectra for the structural isomers butylone and ethylone. This highlights the importance of efficient and definitive chromatographic separation during analysis.

Figure 4. Total ion chromatogram of 18 synthetic cathinones

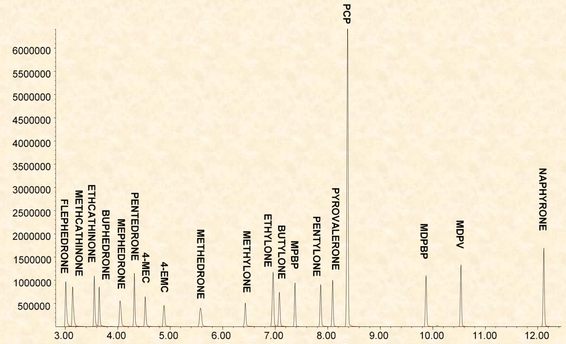


Figure 5. Fragmentation of synthetic cathinones

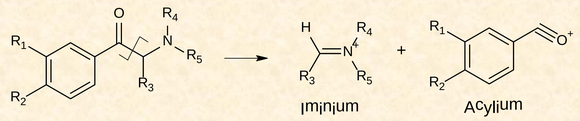
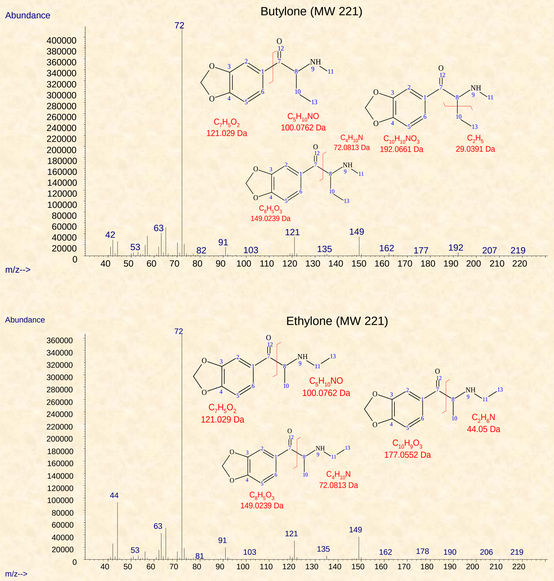


Figure 6. Mass spectra of butylone and ethylone



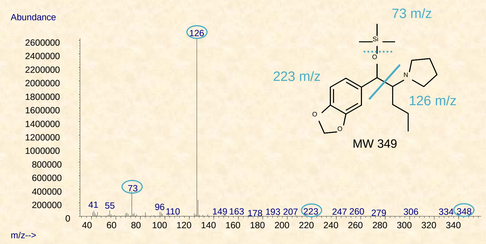
RESULTS AND DISCUSSION

Table 1. Structural influence on base peak

Name	R_1	R_2	R_3	R_4	R_5	Base Peak m/z	MW
Methcathinone	H	H	CH_3	**H**	CH_3	**58**	163
Mephedrone	H	CH_3	CH_3	**H**	CH_3	**58**	177
Methedrone	H	OCH_3	CH_3	**H**	CH_3	**58**	193
Methylone	3,4-Methylenedioxy		CH_3	**H**	CH_3	**58**	207
Flephedrone	H	F	CH_3	**H**	CH_3	**58**	181
4-EMC	H	C_2H_5	CH_3	**H**	CH_3	**58**	191
Ethcathinone	H	H	CH_3	**H**	C_2H_5	**72**	177
Buphedrone	H	H	C_2H_5	**H**	CH_3	**72**	177
4-MEC	H	CH_3	CH_3	**H**	C_2H_5	**72**	191
Ethylone	3,4-Methylenedioxy		CH_3	**H**	C_2H_5	**72**	221
Butylone	3,4-Methylenedioxy		C_2H_5	**H**	CH_3	**72**	221
Pentedrone	H	H	C_3H_7	**H**	CH_3	**86**	191
Pentylone	3,4-Methylenedioxy		C_3H_7	**H**	CH_3	**86**	235
MPBP	H	CH_3	C_2H_5	**Pyrrolidinyl**		**112**	231
MDPBP	3,4-Methylenedioxy		C_2H_5	**Pyrrolidinyl**		**112**	261
Pyrovalerone	H	CH_3	C_3H_7	**Pyrrolidinyl**		**126**	245
α-PVP	H	H	C_3H_7	**Pyrrolidinyl**		**126**	231
MDPV	3,4-Methylenedioxy		C_3H_7	**Pyrrolidinyl**		**126**	275
Naphyrone	Naphthyl		C_3H_7	**Pyrrolidinyl**		**126**	281

Although reductive silylation and reductive acylation was readily achieved for all nineteen cathinones tested, spectra of the tertiary amines were not significantly improved. Figure 7 illustrates the mass spectrum for MDPV following reductive silylation. Silylated and acylated derivatives still underwent extensive alpha cleavage when derivatized via the ketone functional group. Trimethylsilyl imidazole (TMSI) was selected for silylation because it is selective towards hydroxyls and carboxylic acids, is used without a catalyst and does not react with aliphatic amines. Its selectivity makes it useful in polyfunctional compounds and was capable of producing a single trimethylsilyl cathinone derivative for all of the secondary and tertiary cathinones included in the study. By comparison, the nonselectivity of TFAA (and similar reagents for acylation) produced multiple derivatives which is an inherent drawback, particularly if quantitative analysis is to be performed.

Figure 7. Mass spectrum of MDPV following reductive silylation



ACKNOWLEDGEMENTS

This project was supported by Award No. 2012-R2-CX-K003, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.