Analyte-Dependent Stability of Synthetic Cathinones

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ABSTRACT

Synthetic cathinones are a class of designer drugs that have been increasing in popularity since the late 2000s according to the National Forensic Laboratory Information System (NFLIS). Numerous case reports have identified synthetic cathinones in both ante-mortem and post-mortem toxicology investigations. Several studies to date have documented the instability of certain cathinone species in biological samples. In this report we describe a systematic approach to identify analyte dependent differences in stability between cathinone species using twenty-two synthetic cathinones with different structural features.

INTRODUCTION

Synthetic cathinones continue to present a challenge to forensic toxicology laboratories due to the constant introduction of new analogs. The numerous synthetic cathinones identified in forensic toxicology casework are differentiated primarily by substituents on the aromatic ring or amine group (Figure 1). These structural features influence their pharmacological effects, chemical properties, and subsequently their stability in biological matrices.

Previous studies have demonstrated that these drugs are highly unstable in basic conditions, especially at elevated temperatures. However, information is limited regarding how the structure (secondary or tertiary amine) and various ring substituents impact stability. Understanding these analyte dependent differences, specifically the introduction of functional groups that may exert a stabilizing influence, can help predict stability of future cathinones.

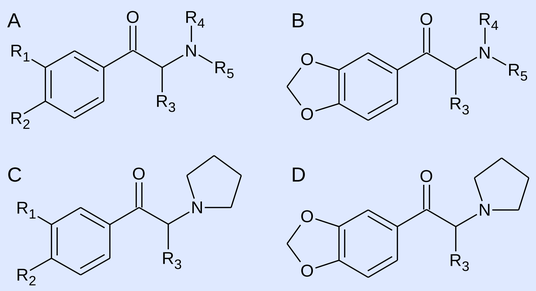

Figure 1. General structure for A) unsubstituted and substituted secondary amine cathinones, B) secondary amine, methylenedioxy cathinone, C) tertiary amine, non-methylenedioxy substituted, D) tertiary amine with methylenedioxy substituent.

RESULTS

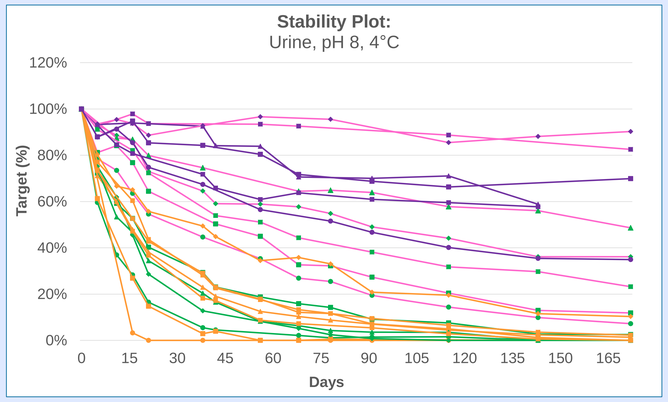

Figure 2. Representative stability plot of twenty-two cathinones in urine (pH 8) stored at 4°C.

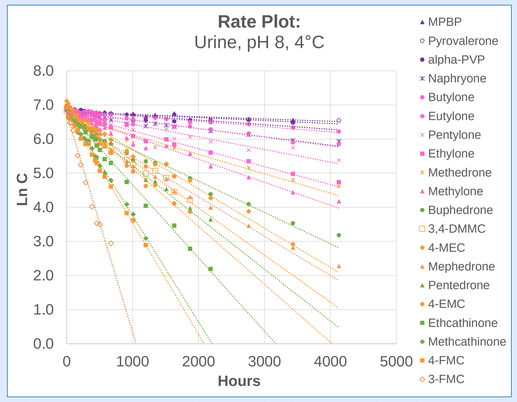

Figure 3. Representative rate plot from urine (pH 8) stored at 4°C.

Half Life in Blood					
Amine	**Ring Substitution**	**32°C**	**20°C**	**4°C**	**-20°C**
Tertiary	MD-substituted	10 - 21d	2.7m	-	-
Tertiary	-	1 - 8d	0.3 – 1.7m	≥10m	-
Secondary	MD-substituted	1 - 5d	9 - 31d	≥10m	-
Secondary	-	8 - 29h	1 - 7d	0.4 - 6m	2.6m (3-FMC)
Half-Life in Urine (pH 8)					
Amine	**Ring Substitution**	**32°C**	**20°C**	**4°C**	**-20°C**
Tertiary	MD-substituted	2 - 4m	4m	-	-
Tertiary	-	5 - 46d	0.4 - 1.4m	≥4m	-
Secondary	MD-substituted	19 - 72h	3 - 11d	1.4 - 6m	≥8m
Secondary	-	2 - 20h	0.4 - 4d	4 - 25d	≥1m
Half-Life in Urine (pH 4)					
Amine	**Ring Substitution**	**32°C**	**20°C**	**4°C**	**-20°C**
Tertiary	MD-substituted	-	-	-	-
Tertiary	-	-	-	-	-
Secondary	MD-substituted	≥2m	-	-	-
Secondary	-	0.3 - 2.3m	≥2m	-	-

Table 2. Half-lives for cathinones derived from constants from first order rate plots. Half-lives only generated when instability (>20% loss of target concentration) was observed for at least three days.

Instability (>20% loss of target concentration) was observed within hours of storage at 32°C and 20°C. The presence of the pyrrolidinyl group them significantly more stable than their secondary amine counterparts. The methylenedioxy group also contributes a significant stabilizing effect for secondary amine ($F(11,273)=8.74$, $p<0.0001$, 4°C) and tertiary amine ($F(5,116)=8.22$, $p<0.0001$, 4°C) cathinones. There were no significant differences between unsubstituted and ring substituted cathinones ($\alpha=0.05$), with the exception of 3-FMC (Figure 2). Half-lives derived from first order rate constants also highlighted analyte dependent differences in stability (Table 2, Figure 3)

CONCLUSIONS

PYR/MD > PYR > MD > Ring Substituted ≈ Unsubstituted > 3-FMC

The stability of synthetic cathinones is highly dependent on the chemical structure of the specific analogue. When considering cathinone stability, substitutions at the aromatic ring, α-carbon and nitrogen play an important role. Analyte dependent differences in cathinone stability should be carefully considered in toxicological investigations.

MATERIALS AND METHODS

Twenty-two synthetic cathinones, selected to include secondary and tertiary amine (pyrrolidinyl) cathinones, with and without aromatic ring substituents (unsubstituted, substituted, or methylenedioxy (MD) substituted). were evaluated in terms of stability (Table 1). Blood and urine, fortified with all twenty-two analytes, was monitored over a period of six months at 32°C, 20°C, 4°C and -20°C. Specimens were analyzed in duplicate, using appropriate sampling intervals (hours, days, weeks, and months) using a validated liquid chromatography quadrupole/time of flight mass spectrometry (LC-Q/TOF-MS) assay. Half-lives were determined for each of the cathinones in blood and urine at each temperature. When significant drug loss was evident (>20%), analyte dependent differences in stability were evaluated statistically using analysis of variance (ANOVA). One-way ANOVA was also used to determine if there was statistical significant *within* and *between* the cathinone subgroups.

Secondary Amine		
Unsubstituted	Substituted	Methylenedioxy
Methcathinone	3-FMC	4-MEC	Methylone
Ethcathinone	4-FMC	3,4-DMMC	Ethylone
Buphedrone	Methedrone	4-EMC	Butylone
Pentedrone	Mephedrone		Eutylone
			Pentylone
Tertiary Amine		
Non-Methylenedioxy		Methylenedioxy
alpha-PVP	Pyrovalerone	MDPBP
MPBP	Naphryone	MDPV

Table 1. Twenty-two synthetic cathinones organized by chemical structure.

REFERENCES

Lindsay Glicksberg, Kelsie Bryand and Sarah Kerrigan. Identification and Quantification of Synthetic Cathinones in Blood and Urine using Liquid Chromatography-Quadrupole/Time of Flight (LC-Q/TOF) Mass Spectrometry. Journal of Chromatography B, 1035, 91-103 (2016).

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