HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

IDENTIFICATION OF DESIGNER DRUGS USING A MULTI-TECHNIQUE APPROACH WITHOUT REFERENCE STANDARDS

Ilkka Ojanperä*, Suvi Ojanperä, Anna Pelander

Department of Forensic Medicine, P.O Box 40, FI-00014 University of Helsinki, Finland

INTRODUCTION

Availability of reference standards for designer drugs, metabolites or rare substances is often hindered by administrative requirements, and some substances are not available at all. In this study, designer drugs from seized samples were identified based on accurate mass by liquid chromatography - time-of-flight mass spectrometry (LC-TOFMS) without reference standards. High performance thin-layer chromatography (HPTLC), using Fast Black K salt (FBK) and fluorescamine staining, was used for distinguishing between primary, secondary and tertiary amines with identical molecular formula.

SAMPLES

•Two seized street drug samples, containing unknown designer drugs

ACCURATE MASS MEASUREMENT BY LC-TOFMS[1]

Experimental

•TOFMS: Bruker Daltonics MicroTOF equipped with electrospray ion source (ESI)

•LC: Agilent 1100 series with Phenomenex Luna C18(2) 100 × 2 mm (3 µm) column and precolumn

•LC conditons

– solvent A: 5 mM ammonium acetate in 0.1% formic acid; solvent B: acetonitrile

– gradient mode, run time 19 min

•MS instrument settings

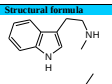
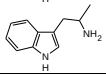
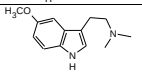
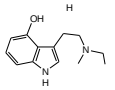
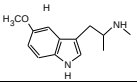
– positive ion polarity ESI

– automated postrun internal mass scale calibration was enabled by injecting the calibration solution in the beginning and at the end of each run

•Mass database consisted of exact monoisotopic masses of 925 compounds including drugs and their metabolites, and all designer drugs from Shulgins' TiHKAL and PiHKAL

•Automated identification was based on accurate mass and isotopic pattern

Results

Sample	Candidate	Structural formula	MH+
1	NMT		175,12298
	AMT		175,12298
2	5-MeO-DMT		219,14919
	4-OH-MET		219,14919
	NO-TMS		219,14919

•Compounds with identical molecular formula can not be differentiated based on accurate mass

DIFFERENTIATION OF AMINES BY HPTLC[2-4]

Experimental

•Plates: Precoated HPTLC silica gel 60, 10 cm × 20 cm (Merck)

•Development: 7 cm in a double trough tank (CAMAG)

•Mobile phase: 15 mL of toluene-acetone-ethanol-ammonia (45+45+7+3)

•Saturation with filter paper for 0.5 h

•Detection:

- •FBK reagent: Spraying with 1) 0.5% Fast Black K salt in water, 2) 0.5 M NaOH
- •Fluorescamine reagent: Spraying with 1) triethylamine – acetone (1:10) , 2) 0.01% fluorescamine in acetone, 3) again with triethylamine – acetone (1:10)

Results

•By HPTLC; Sample 1 R_f = 0.35, Sample 2 R_f = 0.28

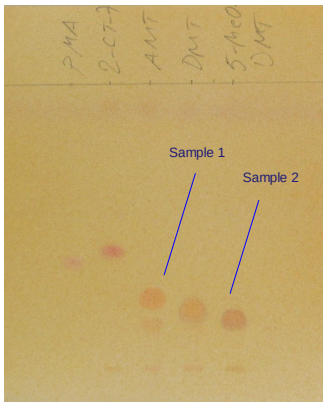
•Separate runs were visualized with the FBK reagent and fluorescamine reagent

•FBK: orange colour for sample 1 and mixed orange-violet colour for sample 2

•Fluorescamine: no greenish fluorescence for either sample

⇨Sample 1 contains NMT, as the aliphatic secondary amine structure gives a pure orange colour with FBK

•Fluorescamine confirmed that no primary amine was present



⇨Sample 2 contains 5-MeO-DMT, as this structure gives a mixed color due to formation of both a triazene (orange) and diazo compound (violet)

•The phenol 4-OH-MET and the aliphatic secondary amine were counted out, as the former should have given a violet colour due to predominant diazo coupling and the latter a pure orange colour

DISCUSSION

•The study emphasizes the use of multiple techniques in solving a complex analytical problem, e.g. the structure elucidation of street drugs without reference standards

•LC-TOFMS provides an elemental composition and a hit list based on a database of exact molecular masses

•Gas chromatography-mass spectrometry (GC-MS) analysis is largely dependent on the use of spectral libraries

⇨In this study, use of a dedicated GC-MS library, Mass Spectra of Designer Drugs 2006 (by Peter Rösner) with 5531 spectra, revealed 5-MeO-DMT in sample 2, but failed to identify sample 1 correctly giving AMT as the first hit, because NMT was not included in the library

•HPTLC analysis with use of two established visualization reagents was capable of differentiating between the correct and false findings on the LC-TOFMS hit list

www.helsinki.fi/yliopisto

[1]Ojanperä S, Pelander A, Pelzing M, Krebs I, Vuori E, Ojanperä I. *Rapid Commun. Mass Spectrom.* **20** (2006) 1161

[2]Ojanperä I, Vartiovaara J, Ruohonen A, Vuori E. *J. Liq. Chromatogr.* **14** (1991) 1435

[3]Ojanperä I, Wähälä K, Hase TA. *Analyst* **117** (1992) 1559

[4]Klein B, Sheehan JE, Grunberg E. *Clin. Chem.* **20** (1974) 272