

DETECTION OF MEPHEDRONE AND OTHER 'LEGAL HIGH' DRUGS IN BIOLOGICAL FLUIDS

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Summary: Mephedrone (4-Methylmethcathinone) first appeared in Scotland in 2009 with a large number of 'Bubbles' cases being reported in the Dundee and surrounding area¹. Mephedrone is a synthetic cathinone derivative with stimulant properties. Following a series of suspected mephedrone deaths in the UK, media interest quickly increased and it became necessary to provide a service with the ability to confirm the presence of this compound in biological fluids including blood and urine. Case samples were received into the Toxicology Section in relation to sudden and suspicious deaths, road traffic offences, murders, assaults and drug assisted sexual assaults. It was quickly realised that the production and use of New Psychoactive Substances (NPS), otherwise known as legal highs, was a fast growing market and that the laboratory required the development of a suitable screening protocol which could be adapted with the changing drugs trends. As a result of the SIPR award, a screening method was developed. The method included the ability to quantify the drugs where appropriate and gave scope for future work to add more drugs as required. The result is a robust and sensitive technique that is widely used to meet the operational needs within the SPA Toxicology Section.

INTRODUCTION

The original aims of our research proposal were to identify and validate suitable methods to detect mephedrone in biological fluids; to identify suitable cases to analyse from those submitted to the laboratory; and to gather information from Police Forces and hospitals in Scotland regarding mephedrone use/deaths.

Due to constantly evolving trends in the drugs scene and therefore changes in user consumption during the duration of this research, it was felt that the focus of the project should concentrate solely on the identification and validation of a suitable method to detect not only mephedrone but other similar 'legal high' drugs.

WHAT ARE 'LEGAL' HIGHS?

Legal highs are psychoactive substances which are synthetic chemicals, plant or fungal material and are intended to elicit a psychoactive response, being either stimulant, hallucinogenic, sedative or a combination of the three phenomena.² These drugs are often perceived as legal however, many of them are now controlled under the Misuse of Drugs Act in the UK. Due to the term legal being misleading, these drugs are now commonly referred to as New Psychoactive Substances (NPS) and it is a constant battle for scientists and legislators alike to keep up with the increasing number, which appear each year. New modified 'designer' drugs are constantly being manufactured to fill the gaps left by those now illegal in order to evade the law and to further the effects for the user.

MEPHEDRONE

Mephedrone became popular in the UK in 2010 and quickly hit the media headlines due to its club scene popularity and implication in several UK deaths. It was also popular internationally, however, little was known about the long-term side effects and toxicity. Methylmethcathinone (MMC) is a stimulant and is a compound belonging to the cathinone category of drugs. It is found in three forms: 2-MMC, 3-MMC and 4-MMC (commonly known as mephedrone). It has been compared with ecstasy, amphetamine and cocaine, and in 2010 was readily obtained online. Following the high level of social and media interest, the Advisory Council on the Misuse of Drugs (ACMD) and the UK government rushed through legislation to make it illegal, however, several problems were encountered due to lack of evidence of risks to health and loop-holes were established.

OTHER DRUGS SELECTED FOR INCLUSION IN THE SCREENING METHOD

Drug name	Also known as	Category
1-Benzylpiperazine	BZP	Piperazine
Beta keto N-methylbenzodioxolylbutanamine	Butylone/Bk-MBDB	Cathinone
4-Bromo-2,5-dimethoxy-phenethylamine	2-CB	Phenethylamine
1-(Chlorophenyl)piperazine	mCPP	Piperazine
2-Diphenylmethyl piperadine	2-DPMP	Pipradrol
3-Fluoromethcathinone	3-FMC	Cathinone
Ketamine		Miscellaneous
Fentanyl		Opioid
3,4-methylene-dioxyamphetamine	MDA	Phenethylamine
Methylenedioxypropylamphetamine	MDPV	Cathinone
3,4-methylene-dioxyethylamphetamine	MDEA	Phenethylamine
3,4-Methylenedioxy-N-methylcathinone	Methylone/Bk-MDMA	Cathinone
2-naphthylpropylamphetamine	Naphyrone	Cathinone
β -Keto-Methylbenzodioxolylpentanamine	Pentylone/Bk-MBDP	Cathinone
Para-methoxymethylamphetamine	PMMA	Phenethylamine
1-(Trifluoromethylphenyl)piperazine	mTFMPP	Piperazine

Amphetamine and methamphetamine were also included.

EXTRACTION PROCEDURE AND INSTRUMENTATION

Materials and methods

A certified standard of mephedrone was obtained through LGC Standards. The drug received was mephedrone hydrochloride (4-Methylmethcathinone Hydrochloride mol.wt. 213.70) from Toronto Research Chemicals Inc. (The molecular weight of mephedrone is 177.242)

The internal standard Amphetamine-d11 (Cerilliant) was obtained from LGC Standards.

N.B. Methylmethcathinone (MMC) is a cathinone related compound found in three forms i.e. 4-MMC (commonly known as mephedrone), 2-MMC and 3-MMC. The authors have assumed that the compound detected in case samples matches mephedrone but are aware of the existence of the related isomers.

Standards for the other drugs were obtained as appropriate (information can be provided on request) and where necessary samples were obtained from the SPA Drugs Section.

Solid phase extraction (SPE) for GC/MS

Liquid-liquid extracts purified using Phenomenex StrataX High Performance Polymeric SPE cartridges and Zymark RapidTrace instruments.

Liquid-liquid extraction procedure for LC/MS and LC/MS/MS

- Blood or urine (1ml), amphetamine-d11 (0.2mg/L), water (1ml), NaOH (3%; 100µl) and ether (6ml) mixed, centrifuged and the ether recovered to a clean conical tube.
- Formic acid (1%; 200µl) was added, mixed, centrifuged and the ether aspirated to waste. After 30mins the formic acid was transferred to an auto-sampler vial and injected into LC/MS using the appropriate method.

GC/MS analysis

Instrument: Agilent 6890 GC system, HP5973 mass selective detector system (electron impact source in scan mode), Agilent 7683 injector and autosampler.

Column: Phenomenex ZB-5 fused silica column coated with 5% phenyl 95% dimethylpolysiloxane. 30m, 0.25mm x 0.25µm with 10m guard column.

LC/MS analysis

Instrument: Agilent 1100 series LC system with G1946B LC/MSD. (MSD electrospray in SIM/scan mode).

Column: Hichrom Inertsil ODS-3 5µm 2.1 x 150mm column.

LC/MS/MS analysis

Instrument: Agilent 1100 series LC system with 6410 Triple Quad LC/MS. (positive ESI in MRM mode).

Column: Zorbax Eclipse HPLC Column XDB-C8, 4.6 x 150mm.

STABILITY OF MEPHEDRONE

In order to study the stability of methylmethcathinone (MMC) spiked aliquots (2 milligram per litre) were stored within a freezer and a fridge. Aliquots were analysed in triplicate at the start of the study and at weekly intervals thereafter for a period of 4 weeks. The average of the triplicates was calculated and comparisons carried out.

MMC stored in the freezer remained stable up to two weeks and a marked loss was noted in week 4. A loss in detection of MMC stored in the fridge was noted in week one and continued to markedly diminish through to week 4. In conclusion MMC is not very stable. Samples should be stored in a freezer on receipt and analysed within approximately 3 weeks.

LIMITATIONS

It is recognised that the lack of availability of certified standards potentially limits the addition of NPS to analytical methods. It was not possible to differentiate between positional isomers of MMC (2, 3 and 4-MMC) using the current method. Positional isomers of other NPS are also known to exist and could cause similar limitations in identification.

OUTCOME

A robust and sensitive screen for 19 drugs has been established. The technique also enables the drugs to be quantified however this is only carried out where necessary since it is often difficult to interpret such results due to the lack of available data regarding toxicity and impairment.

There were few cases that were positive for MMC only. Poly drug use is common, particularly in relation to drugs deaths, therefore interpretation of results in relation to MMC can prove difficult.

FURTHER WORK

The screening technique developed as a result of this research has already been developed further. In a separate partnership with the University of Strathclyde, Glasgow, the Toxicology Section has working closely with an MSc student to further increase the screen capabilities and range of NPS detected.

The screening technique developed will be regularly updated, where certified standards are available, in order to keep up with changing drug trends and the operational demand within the SPA Forensic Services Toxicology Section.

The Toxicology Section and Drugs Sections within SPA will continue to work to best serve the needs of the Police and COPFS and will maintain links with other agencies such as the Scottish Drugs Enforcement Agency.

SOURCES OF FURTHER INFORMATION

1. Report to the Advisory Committee on the Misuse of Drugs. Mephedrone. CAIR Scotland. 14 February 2010.
2. Winstock, Adam. Legal Highs: The challenge of new psychoactive substances. Legislative Reform of Drug Policies, October 2011.