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Recommended methods for the **Identification and Analysis of Synthetic Cathinones in Seized Materials**

(Revised and updated)

MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES

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Laboratory and Scientific Section
UNITED NATIONS OFFICE ON DRUGS AND CRIME
Vienna

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Note

Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated and used in selected national laboratories as per the list of references. A number of alternative conditions and substitution of named commercial products may provide comparable results in many cases. However, any modification has to be validated before it is integrated into laboratory routines.

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Contents

1. Introduction

1.1 Background

New psychoactive substances (NPS) are defined by UNODC as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat". The term "new" does not necessarily refer to newly designed substances but to substances that have recently become available on the market. The global NPS market continues to be characterized by the emergence of large numbers of substances belonging to a diverse range of chemical groups. As of November 2019, over 950 different substances have been reported to the UNODC Early Warning Advisory (EWA) on NPS by Governments, laboratories and partner organizations [1].

Classification of NPS by similarities in their chemical structure greatly assists in understanding the scope and diversity of substances that have emerged as well as aiding the development of analytical methods for their identification. One of the predominant structural groups of NPS are synthetic cathinones. These are β-keto phenethylamines, that have similarities to amphetamine, methamphetamine and MDMA in structure and mechanism of action. As of November 2019, over 160 individual synthetic cathinones have been reported to the UNODC EWA [1] giving rise to a variety of analytical challenges requiring the need for sensitive, reliable and reproducible analytical methods to detect and identify these substances. This has been made even more challenging by the lack of affordable chemical reference standards, appropriate analytical methodologies and scientific literature in this subject area.

Aside from cathinone, methcathinone, and pyrovalerone, which were placed under international control prior to 2000, eight synthetic cathinones (3,4-methylenedioxypyrovalerone (3,4-MDPV), 4-methylethcathinone (4-MEC), *alpha*-pyrrolidinovalerophenone (α-PVP), ephylone, ethylone, mephedrone, methylone and pentedrone) have been placed under international control between 2015 and 2019.

1.2 Purpose and use of the Manual

The present *Manual* is one in a series of similar publications dealing with the identification and analysis of various classes of drugs under international control. These manuals are the outcome of a programme pursued by UNODC since the early 1980s,

aimed at the harmonization and establishment of recommended methods of analysis for national drug analysis laboratories.

The present *Manual* is a revision of the manual on *Recommended Methods for the Identification and Analysis of Synthetic Cathinones in Seized Materials* (ST/NAR/49), which was published in 2015. It has been prepared taking into account recent emergence of non-controlled synthetic cathinones and latest developments in analytical technology with a view to providing the basis for reliable forensic scientific evidence on synthetic cathinone-containing seized materials.

In accordance with the overall objective of the series, the present *Manual* suggests approaches that may assist drug analysts in the selection of methods appropriate for the sample under examination and provide data suitable for the purpose at hand, leaving room also for adaptation to the level of sophistication of different laboratories and various legal requirements. All methods included in this manual have been validated according to international standards and many have been also published in peer-reviewed scientific literature. **Any new method that is to be used in the reader's laboratory must be validated and/or verified prior to routine use.**

In addition, while there are several more sophisticated approaches, they may not be necessary for routine operational applications. Therefore, the methods described here should be understood as guidance; minor modifications to suit local circumstances should not affect the validity of the results. The choice of the methodology and approach to analysis, as well as the decision whether or not additional methods are required, remain with the analyst and may also be dependent on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works.

Attention is also drawn to the vital importance of the availability to drug analysts of reference materials and literature on drugs of abuse and the analytical techniques used for their identification. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

The Laboratory and Scientific Section of UNODC would welcome observations on the contents and usefulness of the present *Manual*. Comments and suggestions may be addressed to:

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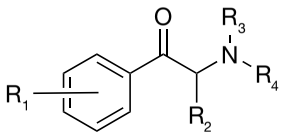
All manuals, as well as guidelines and other scientific-technical publications, may be requested by contacting the address above.

2. General aspects

2.1 Description of the pure compounds

Synthetic cathinones are normally present as white or off-white powders although they can come in a range of colours. Mephedrone for example, commonly appears as white or yellow powder/crystals, with a distinct odour described as ranging from fishy to vanilla or bleach. Although primarily encountered as a powder, mephedrone has also been known to take the form of capsules/tablets, of varying design [2]. Some of the more commonly encountered synthetic cathinones are presented in table 1.

Table 1. Commonly encountered synthetic cathinones



Common name/abbreviation	*Chemical name*	*Substituents*				*CAS number (where available)*
		R_1	**R_2**	**R_3**	**R_4**	
Benzedrone, 4-MBC	2-(benzylamino)-1-(4-methylphenyl) propan-1-one	4-CH_3	CH_3	H	C_7H_7	1225617-75-3
4-Bromoethcathinone, 4-BEC	1-(4-bromophenyl)-2-(ethylamino) propan-1-one	4-Br	CH_3	H	C_2H_5	135333-26-5 (HCl)
4-Bromomethcathinone, 4-BMC	1-(4-bromophenyl)-2-(methylamino) propan-1-one	4-Br	CH_3	H	CH_3	486459-03-4
Buphedrone	2-(methylamino)-1-phenylbutan-1-one	H	C_2H_5	H	CH_3	408332-79-6
Bupropion	1-(3-chlorophenyl)-2-[(1,1-dimethylethyl) amino]propan-1-one	3-Cl	CH_3	H	C_4H_9	34911-55-2
Butylone, βk-MBDB	1-(1,3-benzodioxol-5-yl)-2-(methylamino) butan-1-one	3,4-methylenedioxy	C_2H_5	H	CH_3	802575-11-7
4-Chloro-*N*,*N*-dimethylcathinone	1-(4-chlorophenyl)-2-(dimethylamino) propan-1-one	4-Cl	CH_3	CH_3	CH_3	-
4-Chloroethcathinone, 4-CEC	1-(4-chlorophenyl)-2-(ethylamino) propan-1-one	4-Cl	CH_3	H	C_2H_5	22198-75-0 (HCl)
4-Chloromethcathinone, 4-CMC, clephedrone	1-(4-chlorophenyl)-2-(methylamino) propan-1-one	4-Cl	CH_3	H	CH_3	1225843-86-6

4-Chloro-*α*-pyrrolidinopropiophenone, 4-chloro-*α*-PPP	1-(4-chlorophenyl)-2-(pyrrolidin-1-yl) propan-1-one	4-Cl	CH_3	pyrrolidinyl		93307-24-5 (HCl)
Dibutylone	1-(1,3-benzodioxol-5-yl)-2-(dimethylamino) butan-1-one	3,4-methylenedioxy	C_2H_5	CH_3	CH_3	802286-83-5
N,N-Dimethylcathinone, metamfepramone	2-(dimethylamino)-1-phenylpropan-1-one	H	CH_3	CH_3	CH_3	35026-77-8
2,4-Dimethylmethcathinone, 2,4-DMMC	1-(2,4-dimethylphenyl)-2-(methylamino) propan-1-one	2,4-CH_3	CH_3	H	CH_3	1225623-63-1
3,4-Dimethylmethcathinone, 3,4-DMMC	1-(3,4-dimethylphenyl)-2-(methylamino) propan-1-one	3,4-CH_3	CH_3	H	CH_3	1081772-06-6 (HCl)
Dimethylone, *β*k-MDDMA	1-(1,3-benzodioxol-5-yl)-2-(dimethylamino)-1-propan-1-one	3,4-methylenedioxy	CH_3	CH_3	CH_3	765231-58-1
Ephylone	1-(1,3-benzodioxol-5-yl)-2-(ethylamino)pentan-1-one	3,4-methylenedioxy	C_3H_7	H	C_2H_5	952016-47-6
Ethcathinone	2-(ethylamino)-1-phenylpropan-1-one	H	CH_3	H	C_2H_5	18259-37-5
2-Ethylethcathinone, 2-EEC	2-(ethylamino)-1-(2-ethylphenyl) propan-1-one	2-C_2H_5	CH_3	H	C_2H_5	-
3-Ethylethcathinone, 3-EEC	2-(ethylamino)-1-(3-ethylphenyl) propan-1-one	3-C_2H_5	CH_3	H	C_2H_5	-
4-Ethylethcathinone, 4-EEC	2-(ethylamino)-1-(4-ethylphenyl) propan-1-one	4-C_2H_5	CH_3	H	C_2H_5	1225619-32-8
N-Ethylhexedrone	2-(ethylamino)-1-phenylhexan-1-one	H	C_4H_9	H	C_2H_5	802857-66-5
2-Ethylmethcathinone, 2-EMC	1-(2-ethylphenyl)-2-(methylamino) propan-1-one	2-C_2H_5	CH_3	H	CH_3	-
3-Ethylmethcathinone, 3-EMC	1-(3-ethylphenyl)-2-(methylamino) propan-1-one	3-C_2H_5	CH_3	H	CH_3	-
4-Ethylmethcathinone, 4-EMC	1-(4-ethylphenyl)-2-(methylamino) propan-1-one	4-C_2H_5	CH_3	H	CH_3	1391053-87-4 (HCl)

Table 1. Commonly encountered synthetic cathinones (*continued*)

Common name/abbreviation	*Chemical name*	*Substituents*				*CAS number (where available)*
		R_1	R_2	R_3	R_4	
Ethylone	1-(1,3-benzodioxol-5-yl)-2-(ethylamino) propan-1-one	3,4-methylenedioxy	CH_3	H	C_2H_5	1112937-64-0
Eutylone, βk-EBDB	1-(1,3-benzodioxol-5-yl)-2-(ethylamino) butan-1-one	3,4-methylenedioxy	C_2H_5	H	C_2H_5	802855-66-9
Flephedrone, 4-FMC	1-(4-fluorophenyl)-2-(methylamino) propan-1-one	4-F	CH_3	H	CH_3	447-40-5
4-Fluoroethcathinone, 4-FEC	2-(ethylamino)-1-(4-fluorophenyl) propan-1-one	4-F	CH_3	H	C_2H_5	-
2-Fluoromethcathinone, 2-FMC	1-(2-fluorophenyl)-2-(methylamino) propan-1-one	2-F	CH_3	H	CH_3	1346599-37-8 (HCl)
3-Fluoromethcathinone, 3-FMC	1-(3-fluorophenyl)-2-(methylamino) propan-1-one	3-F	CH_3	H	CH_3	1346600-40-5 (HCl)
4-Fluoro-α-pyrrolidinohexanophenone, 4-fluoro-α-PHP	1-(4-fluorophenyl)-2-(pyrrolidin-1-yl) hexan-1-one	4-F	C_4H_9	pyrrolidinyl		-
4-Fluoro-α-pyrrolinovalerophenone, 4-fluoro-α-PVP	1-(4-fluorophenyl)-2-(pyrrolidin-1-yl) pentan-1-one	4-F	C_3H_7	pyrrolidinyl		850352-31-7 (HCl)
Mephedrone, 4-methylmethcathinone, 4-MMC	1-(4-methylphenyl)-2-(methylamino) propan-1-one	4-CH_3	CH_3	H	CH_3	1189805-46-6
Methcathinone	2-(methylamino)-1-phenylpropan-1-one	H	CH_3	H	CH_3	5650-44-2
Methedrone, βk-PMMA	1-(4-methoxyphenyl)-2-(methylamino) propan-1-one	4-OCH_3	CH_3	H	CH_3	530-54-1
2-methoxymethcathinone, 2-MeOMC	1-(2-methoxyphenyl)-2-(methylamino) propan-1-one	2-OCH_3	CH_3	H	CH_3	-

3-methoxymethcathinone, 3-MeOMC	1-(3-methoxyphenyl)-2-(methylamino) propan-1-one	3-OCH_3	CH_3	H	CH_3	1435933-70-2 (HCl)
4-Methoxy-*α*-pyrrolidinopropiophenone, MOPPP	1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl) propan-1-one	4-OCH_3	CH_3	pyrrolidinyl		478243-09-3
4-Methylbuphedrone	2-(methylamino)-1-(4-methylphenyl) butan-1-one	4-CH_3	C_2H_5	H	CH_3	1337016-51-9
4-Methyl-*N*,*N*-dimethylcathinone, 4-MDMC	1-(4-methylphenyl)-2-(dimethylamino) propan-1-one	4-CH_3	CH_3	CH_3	CH_3	1448845-14-4 (HCl)
2,3-Methylenedioxymethcathinone, 2,3-MDMC	1-(1,3-benzodioxol-4-yl)-2-(methylamino) propan-1-one	2,3-methylenedioxy	CH_3	H	CH_3	-
2,3-Methylenedioxypyrovalerone, 2,3-MDPV	1-(1,3-benzodioxol-4-yl)-2-(pyrrolidin-1-yl) pentan-1-one	2,3-methylenedioxy	C_3H_7	pyrrolidinyl		-
3,4-Methylenedioxypyrovalerone, 3,4-MDPV	1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl) pentan-1-one	3,4-methylenedioxy	C_3H_7	pyrrolidinyl		687603-66-3
3,4-Methylenedioxy-*α*-pyrrolidinopropiophenone, 3,4-MDPPP	1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl) propan-1-one	3,4-methylenedioxy	CH_3	pyrrolidinyl		783241-66-7
2-Methylethcathinone, 2-MEC	2-(ethylamino)-1-(2-methylphenyl) propan-1-one	2-CH_3	CH_3	H	C_2H_5	-
3-Methylethcathinone, 3-MEC	2-(ethylamino)-1-(3-methylphenyl) propan-1-one	3-CH_3	CH_3	H	C_2H_5	-
4-Methylethcathinone, 4-MEC	2-(ethylamino)-1-(4-methylphenyl) propan-1-one	4-CH_3	CH_3	H	C_2H_5	1225617-18-4
4-methyl-*α*-ethylaminobutiophenone, 4-MEABP	2-(ethylamino)-1-(4-methylphenyl) butan-1-one	4-CH_3	C_2H_5	H	C_2H_5	18268-19-4 (HCl)
4-Methyl-*α*-ethylaminopentiophenone, 4-MEAPP	2-(ethylamino)-1-(4-methylphenyl) pentan-1-one	4-CH_3	C_3H_7	H	C_2H_5	18297-05-7 (HCl)

Table 1. Commonly encountered synthetic cathinones (*continued*)

Common name/abbreviation	*Chemical name*	*Substituents*				*CAS number (where available)*
		R_1	R_2	R_3	R_4	
2-Methylmethcathinone, 2-MMC	1-(2-methylphenyl)-2-(methylamino) propan-1-one	2-CH_3	CH_3	H	CH_3	1246911-71-6
3-Methylmethcathinone, 3-MMC	1-(3-methylphenyl)-2-(methylamino) propan-1-one	3-CH_3	CH_3	H	CH_3	1246911-86-3
Methylone	1-(1,3-benzodioxol-5-yl)-2-(methylamino) propan-1-one	3,4-methylenedioxy	CH_3	H	CH_3	186028-79-5
4-Methyl-α-pyrrolidinobutiophenone, MPBP	1-(4-methylphenyl)-2-(pyrrolidin-1-yl) butan-1-one	4-CH_3	C_2H_5	pyrrolidinyl		1214-15-9 (HCl)
4-Methyl-α-pyrrolidinohexiophenone, MPHP	1-(4-methylphenyl)-2-(pyrrolidin-1-yl) hexan-1-one	4-CH_3	C_4H_9	pyrrolidinyl		1391052-36-0 (HCl)
4-Methyl-α-pyrrolidinopropiophenone, MPPP	1-(4-methylphenyl)-2-(pyrrolidin-1-yl) propan-1-one	4-CH_3	CH_3	pyrrolidinyl		1313393-58-6 (HCl)
Mexedrone	3-methoxy-2-(methylamino)-1-(4-methyl-phenyl)propan-1-one	4-CH_3	CH_2OCH_3	H	CH_3	-
Naphyrone	1-(2-naphthalenyl)-2-(pyrrolidin-1-yl) pentan-1-one	naphthyl	C_3H_7	pyrrolidinyl		850352-53-3
Pentedrone	2-(methylamino)-1-phenylpentan-1-one	H	C_3H_7	H	CH_3	879722-57-3
Pentylone	1-(1,3-benzodioxol-5-yl)-2-(methylamino) pentan-1-one	3,4-methylenedioxy	C_3H_7	H	CH_3	698963-77-8
α-Pyrrolidinobutiophenone, α-PBP	1-phenyl-2-(pyrrolidin-1-yl)butan-1-one	H	C_2H_5	pyrrolidinyl		13415-54-8 (HCl)

α-Pyrrolidinoheptiophenone, α-PHPP, PV8	1-phenyl-2-(pyrrolidin-1-yl)heptan-1-one	H	C_5H_{11}	pyrrolidinyl		13415-55-9 (HCl)
α-Pyrrolidinohexiophenone, α-PHP	1-phenyl-2-(pyrrolidin-1-yl)hexan-1-one	H	C_4H_9	pyrrolidinyl		13415-86-6
α-Pyrrolidinovalerophenone, α-PVP	1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one	H	C_3H_7	pyrrolidinyl		14530-33-7
3,4-Tetramethylene-α-pyrrolidinovalerophenone, TH-PVP	2-(pyrrolidin-1-yl)-1-(5,6,7,8-tetrahydro-naphthalen-2-yl)pentan-1-one	tetrahydronaphthalen-2-yl	C_3H_7	pyrrolidinyl		-
4-Trifluoromethylmethcathinone, 4-TFMMC	2-(methylamino)-1-[4-(trifluoromethyl) phenyl]propan-1-one	4-CF_3	CH_3	H	CH_3	-

2.2 Use and abuse

Synthetic cathinones are commonly taken by insufflation (snorting) or orally, although in recent years, the injection of synthetic cathinones has also been reported. Insufflation doses typically range from 20 to 80 mg, although it can be as low as 5 mg or as high as 125 mg in some cases, with peak effects experienced in less than 30 minutes. The peak effects of mephedrone, which requires a high dose when insufflated, have been reported to occur within 45 minutes to 2 hours after ingestion and effects are reported to last for up to 2 to 3 hours [2]. Users of NPS may often perceive them as safe and find them more attractive than traditional drugs of abuse. However the toxicity and health implications associated with these products are largely unknown [3]. Moreover, the descriptions on the package of these products regarding the constituents are often inaccurate and misleading. Identical packages have often been found to contain different psychoactive substances, thereby adding to the unpredictability of the effects of these products. A number of studies have demonstrated that these products may contain substances under international control and that the psychoactive constituents in the products are not consistent over time [4-6].

2.3 Pharmacology and toxicology

Some synthetic cathinones are structurally similar to the amphetamine-type stimulants amphetamine, methamphetamine and MDMA and are reported to have similar central nervous system (CNS) stimulant properties [7-10]. They can have a pronounced effect on the levels and action of neurotransmitters such as serotonin, dopamine and norepinephrine [7, 11-13]. Many cathinone derivatives have a single chiral centre and thus exist in two enantiomeric forms with differing potencies. For example, the *(S)*-enantiomers of cathinone and methcathinone have been reported as being more potent than the *(R)*-enantiomers [2].

Synthetic cathinones produce a variety of behavioural effects, and can affect locomotor activity, thermoregulation, learning and memory [7]. Short-term adverse effects reported following mephedrone use are variable and may include loss of appetite, blurred vision, anxiety, post-use depression, confusion, hallucinations, short-term psychosis and mania [14-16]. Similarly, clinical reports have noted that 3,4-MDPV use can result in anxiety, paranoia, memory loss and aggression [7]. Individuals intoxicated with ephylone displayed a variety of symptoms including palpitations, tachycardia, agitation, aggression, hallucinations, coma and, in some cases, death [17, 18]. Intoxication by synthetic cathinones may also lead to severe adverse effects including acute liver failure, acute kidney injury, high blood pressure and tremor [19, 20]. A number of synthetic cathinone users have also reported the development of tolerance, dependence or withdrawal symptoms with prolonged use [2].

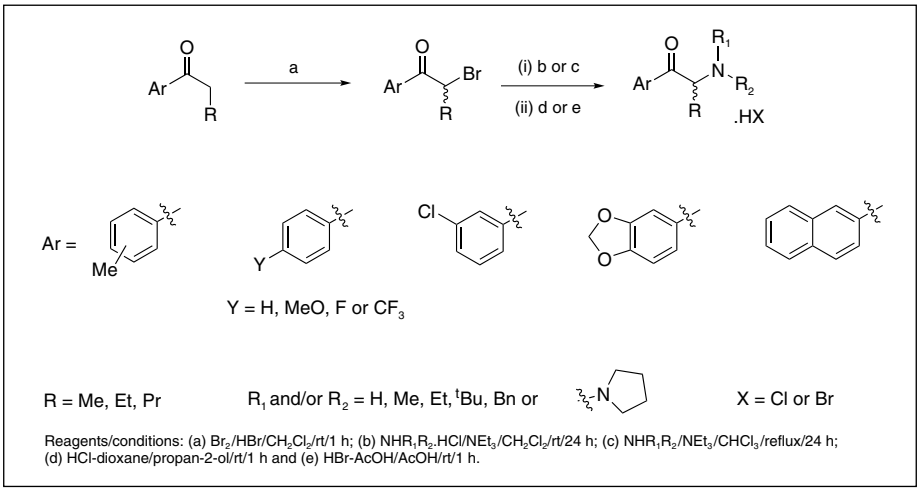
With regard to the metabolism of synthetic cathinones, mephedrone has been extensively studied and a number of metabolites have been characterized [15, 16, 21].

The major Phase I metabolites have been shown to be products of simple oxidative, reductive and *N*-dealkylation reactions [15]. The Phase I metabolites of mephedrone subsequently undergo extensive Phase II metabolism to form glucuronides as a prelude to excretion [21].

3. Synthesis of synthetic cathinones

The chemical synthesis of cathinones is facile and usually follows a two-step process. Typically the initial synthesis is of an α-bromoketone (from the pre-requisite arylketone) followed by a nucleophilic substitution with an appropriate amine to give the corresponding freebase of the cathinone. Due to the instability of the freebase, the cathinones are conveniently isolated as their corresponding hydrochloride or hydrobromide salts [22]. The first reported synthesis of mephedrone was by Saem de Burnaga Sanchez in 1929 [23], where 1-tolylpropan-1-one was α-brominated and then reacted with methylamine to produce racemic 4-methylmethcathinone. This method could be adapted for the synthesis of a wide range of cathinones as shown in figure I.

Figure I. Example of synthetic routes for selected cathinone derivatives [24].



Reagents/conditions: (a) Br_2/HBr/CH_2Cl_2/rt/1 h; (b) NHR_1R_2.HCl/NEt_3/CH_2Cl_2/rt/24 h; (c) NHR_1R_2/NEt_3/$CHCl_3$/reflux/24 h; (d) HCl-dioxane/propan-2-ol/rt/1 h and (e) HBr-AcOH/AcOH/rt/1 h.

These synthetic routes can be used in clandestine manufacture. However, if 4-methylephedrine is available, this can also be utilized via an oxidation step to produce mephedrone. This method is believed to be stereoselective if the reactant is a single enantiomer. However, due to the complexity of producing the pure enantiomers of 4-methylephedrine it is unlikely that this route is used routinely in clandestine laboratories. [9]

4. Qualitative and quantitative analysis of seized materials containing synthetic cathinones

4.1 Introduction

Generally, in attempting to establish the identity of a controlled drug or NPS in suspect material, the analytical approach must entail the determination of at least two uncorrelated parameters, one of which should provide information on the chemical structure of the analyte, for example infrared spectroscopy (IR), mass spectrometry (MS), or nuclear magnetic resonance (NMR). The choice of parameters in any particular case needs to be contextualized to the drug involved and the resources available. Judicial requirements may also dictate the analytical requirements.

4.2 Sampling

The principal reason for a sampling procedure is to permit an accurate and meaningful chemical analysis and interpretation. Because most qualitative and quantitative methods used in forensic drug analysis laboratories require very small quantities of material, it is vital that these small quantities are representative of the bulk from which they have been drawn. Sampling should conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by regional or international organizations. For general aspects of qualitative sampling of multi-unit samples, refer to the *Guidelines on Representative Drug Sampling* (www.unodc.org/documents/scientific/Drug_Sampling.pdf) [25].

4.3 Extraction and sample preparation

Samples should be prepared according to their morphology as follows:

Powders: A solution should be prepared at a concentration of approximately 1 mg/mL in methanol or ethanol.

Tablets/Capsules: Select a representative number of tablets according to the procedure in *Guidelines on Representative Drug Sampling* [25]. The sample should be ground to a fine powder, if not already in powdery form, and solution(s) prepared.

Syringes or glassware: Should be washed with a minimum amount of solvent e.g. methanol or ethanol.

Synthetic cathinones are typically present in their hydrochloride forms, and hence the following extraction using a base may result in a better peak shape during GC-MS analysis for some synthetic cathinones (e.g. ethylone).

Basic extraction procedure: Add an appropriate volume of water to an appropriate amount of powder (~1-2 mg/mL), basify the solution to approximately pH 11 using a suitable base (e.g. 1N NaOH solution) and extract with an equal volume of organic solvent such as ethyl acetate or chloroform.

Analytical notes

- Synthetic cathinones are less stable in their base forms and are sensitive to air, moisture and pH [26]. It is advisable not to basify the solution to greater than pH 12.
- Synthetic cathinones that degrade at high pH (pH >12) are the halocathinones such as 4-BEC, 4-BMC, 4-CMC, 4-FEC and flephedrone. In particular, the ortho positional isomers of the halocathinones, such as 2-FMC, are highly unstable in their base forms and multiple degradation peaks can be observed in the gas chromatogram of the sample solution after basification.
- The rate of degradation can be minimized by storing the solutions at -20°C.

4.4 Presumptive colour tests

Presumptive tests are non-specific tests, which can be used to identify which class of compounds a substance belongs to. However, they cannot be used to identify a specific compound within that class. Therefore, confirmatory tests must always be carried out in conjunction with these preliminary tests. Presumptive colour tests give a positive result simply by a colour change being observed on the addition of reagents to the substance of interest.

A negative control is required when undertaking presumptive testing to ensure that any colour change observed is due to the reaction between the substance and the reagents, and not to the reagents alone. In addition, it also ensures that the apparatus being used is thoroughly clean with no possibility of contamination. If possible, a positive control should be carried out on a reference standard or a known sample

of the compound expected to be present in the sample to give an indication of the colour change that should occur.

One of the most suitable presumptive tests for synthetic cathinones is the Zimmermann test (also sometimes referred to as the Janovsky test), which provides a clear and unambiguous response for both the hydrochloride and hydrobromide salts in most cases.

The Zimmermann colour test can also be used to differentiate between the halomethcathinones (such as 4-BMC and 4-CMC) and ketamine. While the halomethcathinones give the same colour test response as ketamine for colour tests such as Marquis, Simon's and the modified cobalt thiocyanate test, different colour test responses are observed for the Zimmermann colour test.

Zimmermann test reagents

A small amount of the sample to be tested should be added to a dimple well of a spotting tile and the reagents added sequentially. Negative controls should be used. Any colour change or other noticeable effect occurring immediately on addition of the following reagents should be noted and observations made again after five minutes.

- Add 2 drops of 1% w/v 1,3-nitrobenzene in methanol, then
- add 2 drops of 15% w/v potassium hydroxide in water.

The results observed with the Zimmermann test for a variety of cathinones are presented in table 2.

Table 2. Typical results obtained for a variety of cathinones and ketamine using the Zimmermann test

Drug	*Immediate colour change*	*Colour after 5 minutes*
Benzedrone	No colour change	Pale pink
4-BMC	Yellowish-green	Brown
Bupropion	No colour change	No colour change
Butylone	(After ~10 secs.) Very pale pink	Dark purple
4-CMC	Yellowish-green	Brown
Ethylone	Light pink	Dark pink
Eutylone	No colour change	Slight purple
Flephedrone	Light purple	Dark purple
Ketamine	Light pink	Purple

Table 2. Typical results obtained for a variety of cathinones and ketamine using the Zimmermann test (*continued*)

Drug	*Immediate colour change*	*Colour after 5 minutes*
3,4-MDPV	Yellow	Yellow
Mephedrone	Light purple	Dark red/purple
Methcathinone	Dark purple	Dark purple
Methedrone	(After a few secs.) Dark purple	Dark purple
Methylone	(After ~10 secs.) Light purple	Dark purple
2-MMC	Dark purple	Dark purple
3-MMC	Purple	Dark purple
4-MEC	(After ~10 secs.) Light purple	Purple with dark purple specs
α-PVP	Light yellow	Light yellow (no change in colour)
4-TFMMC	Dark purple	Dark purple

4.5 Raman spectroscopy

Raman spectrometers are available both as bench-top spectrometers as well as hand-held devices. While, bench-top Raman devices can be used for the identification and characterization of cathinones [27], handheld Raman devices are also useful for rapid and non-destructive presumptive field testing of cathinones [28].

Handheld Raman devices are suited to operate in field conditions as analysis can be performed directly through transparent or translucent packaging material (e.g. plastic, glass) with minimum or no sample preparation. It is also important to recognize and understand the limitations of such devices, for example, interferences of fluorescence from sample. As such, these devices should only be utilized as a preliminary screening tool to provide information on the possible identity of the seized material and should always be complemented by another confirmatory technique.

The handheld Raman device makes use of a laser beam and as such, it is important to be aware of the associated safety issues and take necessary precautions, for example, the use of protective gloves and eye protection, handling the device at the recommended distance. Scanning dark-coloured material directly must be avoided as the laser beam can potentially cause the material to burn or ignite. Never use the device on samples suspected to contain explosives.

For general aspects of the use of handheld Raman devices, refer to the *Guidelines on Handheld Raman Field Identification Devices for Seized Material* (https://www.unodc.org/unodc/en/scientists/guidelines-on-raman-field-identification-devices.html) [28].

4.6 Microcrystal tests

Microcrystal tests are quick, simple, and extremely sensitive tests for the identification of substances. They involve the formation of crystals from the reaction of the target compound with a chemical reagent, followed by the analysis of the resulting crystals by means of a microscope and comparison with reference material. The use of a polarized light microscope is recommended in accordance with the procedure used. The reference material may be photographs of known microcrystals, or reference standards/known drug samples which are analysed similarly.

Procedures have been reported using mercury chloride [29] and mephedrone was observed to form characteristic "paddlewheels and rosettes of blades" (figure II).

Reagent

The reagent is an aqueous solution of mercury chloride at a concentration of 10 mg/mL.

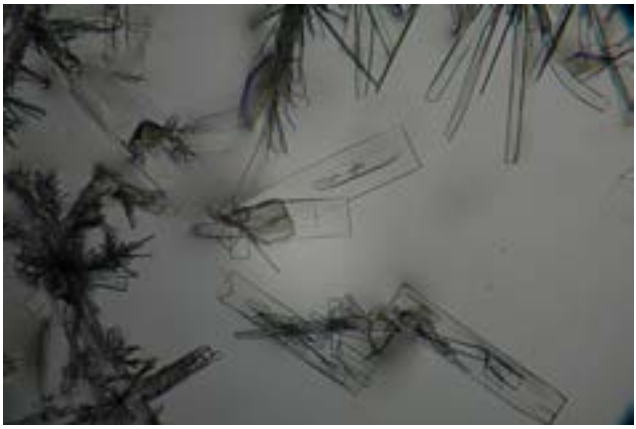
Reference standards should be prepared as aqueous solutions at concentrations of 10 mg/mL.

Method

Mix an aliquot (10 µL) of the test solution (10 mg/mL) with 10 µL of the reagent on a glass slide. Gently swirl with a plastic pipette tip a few times to aid nucleation and crystal formation and let the mixture settle for 5-10 minutes. When possible, a positive control using reference standards should be carried out alongside the test solution.

Structurally similar substances may show similar looking microcrystal patterns at higher concentrations. Hence, care should be taken in interpreting microcrystal patterns.

Figure II. "Paddlewheel and rosette of blades"-shaped crystal observed during a microcrystalline test for mephedrone [30]



4.7 Thin layer chromatography

Thin layer chromatography (TLC) is a commonly used technique for the separation and identification of drugs. It is inexpensive, rapid, sensitive, flexible in the selection of both the stationary and mobile phase and amenable to a wide variety of substances, in base and salt form, ranging from the most polar to non-polar materials. A retention factor (R_f) can be calculated for each compound within a sample to provide a tentative discrimination of compounds within a drug class.

$$R_f \text{ value} = \frac{\text{Distance from origin to sample spot}}{\text{Distance from origin to solvent front}}$$

TLC provides chromatographic separation and identification of the components when used in conjunction with a drug reference standard but TLC does not provide any structural information about the drug. Subsequently, it is used in combination with other techniques to confirm unequivocally the identity of the drug.

TLC Plates (stationary phases)

Coating: Silica gel G with a layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (Silica gel GF_{254}).

Typical plate sizes: 20 x 20 cm, 20 x 10 cm, 10 x 5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

Solvent systems

The following solvent system should be prepared as accurately as possible by use of pipettes, dispensers and graduated (measuring) cylinders. The solvent system should be placed in a glass TLC tank for a sufficient time to allow vapour phase saturation to be achieved prior to the analysis.

Ethyl acetate, methanol and (25%) ammonia - (89.5 : 10.0 : 0.5 v/v/v).

Preparation of standard solutions

These should be prepared at a concentration of between 1 to 5 mg/mL in methanol (or according to laboratory protocol) and stored in a dark and cool place.

Sample solutions

Prepared as per section 4.3.

Spotting and developing

The samples, together with suitable negative and positive controls, are applied as separate spots. Apply approximately 1 µL and 5 µL aliquots of the sample solution, 2 µL of the standard solution(s) and 2 µL of solvent (as a negative control) onto the TLC plate. Spots should be applied carefully to avoid damaging the plate's surface.

Analytical notes

- The starting point of the run, i.e. the "spotting line" should be at least 2 cm from the bottom of the plate.
- The spacing between applications of sample (spotting points) should be at least 1 cm and spots should not be placed closer than 1.5 cm to the side edge of the plate.
- To avoid diffuse spots during development, the size of the sample spot should be as small as possible (2 mm) by applying solutions in several aliquots rather than a single discharge.
- Allow spots to dry and place plate into solvent-saturated tank (saturation of the vapour phase is achieved by using solvent-saturated pads or filter paper as lining of the tank).
- The solvent in the tank must be below the spotting line.
- Remove plate from the development tank as soon as possible after the solvent reaches the development line (at least 1 cm from the top of the plate) marked beforehand; otherwise, diffused spots will occur.

Visualization/detection

Reagent: Dissolve 2 g ninhydrin in 100 mL ethanol (2% ninhydrin)

Plates must be dried prior to visualization. The solvent can be allowed to evaporate at room temperature or removed with a hot air blower. The plate should be viewed under UV light (254 nm) with any spots being noted before being sprayed with ninhydrin reagent (2%). The plate should then be placed into an oven at 80°C and until all spots have developed (~40 min). Once removed from the oven, the spots should be marked with a pencil and then the R_f value calculated for each.

When TLC is carried out on substituted cathinone compounds, various colours and shapes of spots are observed. The spots produced by each compound vary in colour (black/blue/purple) when viewed under UV light (table 3).

Table 3. TLC results for a variety of cathinone compounds (spray reagent ninhydrin 2%; UV = 254 nm)

Drug	*Spot colour under short λ UV light*	*R_f value*
Benzedrone	Black line	0.83
Bupropion	Black line	0.60
Butylone	Light blue spot	0.20
Ethylone	Faint spot	0.24
Eutylone	Light blue spot	0.32
Flephedrone	Black spot	0.15
4-MDMC	Black spot	0.33
3,4-MDPV	Light blue spot	0.55
4-MEC	Black spot	0.25
Mephedrone	Black spot	0.13
Methcathinone	Black spot	0.17
Methedrone	Black spot	0.14
Methylone	Faint spot	0.14
2-MMC	Black spot	0.18
3-MMC	Black spot	0.16
Naphyrone	Bright blue/purple spot	0.44
Pentedrone	Black spot	0.34
α-PVP	Black spot	0.58
4-TFMMC	Faint line	0.27

Analytical notes

- R_f values are not always reproducible due to small changes in plate composition and activation in solvent systems, tank saturation or development distance. Therefore, the R_f values provided are indications of the chromatographic behaviour of the substances listed.
- It is essential that reference standards are run simultaneously on the same plate.
- For identification purposes, both the R_f value and the colour of the spots after spraying with the appropriate visualization reagents should always be considered.

4.8 Gas chromatography with mass spectrometry

Gas chromatography-mass spectrometry (GC-MS), one of the most commonly used hyphenated techniques for the identification of drug samples of forensic significance,

is commonly used as a confirmatory test for the cathinones. It affords two independent means of analysis (chromatographic separation and mass fragmentation data). There are a wide range of instruments available and analysis should be undertaken using standard analytical capillary columns.

Preparation of the standard and sample solution

A reference standard of the drug should be prepared at a concentration of 1 mg/mL in methanol or ethanol, or prepared using basic extraction as mentioned in section 4.3. For the sample solution, a representative sample of the drug to be analysed is prepared as above.

There are various GC-MS methods emerging within the peer-reviewed literature for the analysis of cathinone compounds and forensic science laboratories may adopt these or carry out the appropriate research to generate their own method. It is critical that, whatever method is used, it is appropriately validated. Two general methods for the analysis of synthetic cathinones are presented in this *Manual*.

GC-MS operating conditions

	Method 1	Method 2 [24]
GC oven conditions:	80°C for 3 minutes, increased to 280°C at a rate of 40°C/min and then held isothermal at 280°C for 4 minutes	90°C for 1 minute, increased to 300°C at a rate of 8°C/min and then held isothermal at 300°C for 10 minutes
Column:	5% phenyl/95% methyl silicone column (HP-5MS), 12.5 m length x 0.25 mm i.d., 0.33 µm film thickness	5% phenyl/95% methyl silicone column (HP-5MS), 30 m length x 0.25 mm i.d., 0.25 µm film thickness
Injection parameters:	1 µL aliquot of sample injected with a split ratio of 40:1.	2 µL aliquot of sample injected with a split ratio of 75:1.
Injector temp:	280ºC	225ºC
Carrier gas:	Helium, inlet pressure: 8 psi	Helium, inlet flow: 1 mL/min
Detector:		
Ionization mode:	EI mode, 70 eV	EI mode, 70 eV
Scan parameters:	TIC Full scan 35-600 amu	TIC Full scan 50-550 amu
GC Interface temp:	280ºC	300ºC
MS source temp:	230°C	230°C
MS quadrupole temp:	150°C	150°C

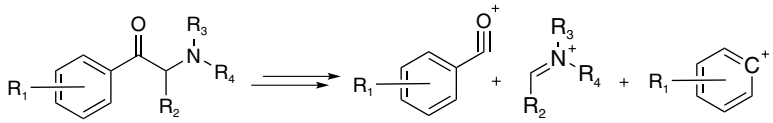
Identification using GC-MS is accomplished by comparing the retention time and mass spectrum of the analyte with that of a reference standard. All compounds identified by GC-MS and reported by the analyst must be compared to a current mass spectrum of the appropriate reference standard, preferably obtained from the same instrument, operated under the same conditions.

Analytical notes

- When reference standards are not available, the use of reference data from external sources or user generated libraries, may be considered depending on the purpose at hand and national legal requirements.
- Reference data must be properly validated, for example, validation study of the external reference data, comparability with different analytical conditions, peer review.
- The use of reference data must be documented and, where applicable, their impact and limitations, for example, compounds with similar fragmentation patterns, should be clearly stated.

Typical fragments of synthetic cathinones are as shown in figure III.

Figure III. Characteristic mass fragmentation pattern for synthetic cathinones



acylium ion iminium ion aryl ion

Table 4 provides reference data from GC-MS analysis using methanol, ethanol or basified water/ethyl acetate as the extracting solvent.

Table 4. Nominal mass, GC retention times and major GC-MS ions for selected synthetic cathinones

Drug	*Nominal mass*	*Approximate GC RT (min)*		*Characteristic mass fragmentation ions*			
		Method 1	*Method 2*	*Iminium ion (base peak)*	*Acylium ion*	*Aryl ion*	*Other major ions*
4-BEC	255	6.51	-	72	183/185	155/157	44
Benzedrone	253	-	18.2	134	119	91	65
Buphedrone	177	5.6	-	72	105	77	57
Bupropion	239	-	11.9	44	139	111	57
Butylone	221	6.85	14.3	72	149	121	57
4-CEC	211	6.22	-	72	139/141	111/113	44
4-Chloro-*N,N*-dimethylcathinone	211	6.17	-	72	139/141	111/113	42
4-CMC	197	5.9	-	58	139/141	111/113	75
Dibutylone	235	6.87	-	86	149	121	71
N,N-Dimethylcathinone	177	5.47	-	72	105	77	42
2,4-DMMC	191	5.87	-	58	133	105	77
2-EEC	205	5.91	-	72	133	105	44
3-EEC	205	6.11	-	72	133	105	44
4-EEC	205	6.23	-	72	133	105	44
2-EMC	191	5.72	-	58	133	105	77
3-EMC	191	6.02	-	58	133	105	77
4-EMC	191	6.28	-	58	133	105	77
Ephylone	249	7.29	-	100	149	121	58
Ethcathinone	177	5.55	-	72	105	77	44
N-Ethylhexedrone	219	6.45	-	114	105	77	58
Ethylone	221	6.8	-	72	149	121	44
Eutylone	235	6.94	14.9	86	149	121	58
Flephedrone	181	5.4	7.6	58	123	95	75

Table 4. Nominal mass, GC retention times and major GC-MS ions for selected synthetic cathinones (*continued*)

Drug	*Nominal mass*	*Approximate GC RT (min)*		*Characteristic mass fragmentation ions*			
		Method 1	*Method 2*	*Iminium ion (base peak)*	*Acylium ion*	*Aryl ion*	*Other major ions*
4-Fluoro-α-PHP	263	7.05	-	140	123	95	84
4-Fluoro-α-PVP	249	6.73	-	126	123	95	84
3-FMC	181	5.09	-	58	123	95	75
4-MDMC	193	-	10.3	72	119	91	56
3,4-MDPPP	247	7.45	-	98	149	121	56
3,4-MDPV	275	8.15	18.8	126	149	121	65
4-MEAPP	219	6.53	-	100	119	91	58
4-MEC	191	6.01	10.5	72	119	91	44
2-MeOMC	193	6.03	-	58	135	107	77
3-MeOMC	193	6.13	-	58	135	107	77
Mephedrone	177	5.73	9.7	58	119	91	65
Methcathinone	163	5.26	7.8	58	105	77	51
Methedrone	193	6.59	12.3	58	135	107	77
4-MEABP	205	6.12	-	86	119	91	58
Methylone	207	6.61	13.5	58	149	121	65
Mexedrone	207	6.44	-	88	119	91	162
2-MMC	177	5.34	8.7	58	119	91	65
3-MMC	177	5.51	9.4	58	119	91	65
MPBP	231	6.87	-	112	119	91	70
MPHP	259	7.35	-	140	119	91	84
Naphyrone	281	8.16	20.8	126	155	127	96
α-PBP	217	6.61	-	112	105	77	70
Pentedrone	191	5.92	-	86	105	77	44
Pentylone	235	6.94	-	86	149	121	44
α-PVP	231	6.8	-	126	105	77	96
4-TFMMC	231	-	7.4	58	173	145	95

Note: "-" denotes not determined

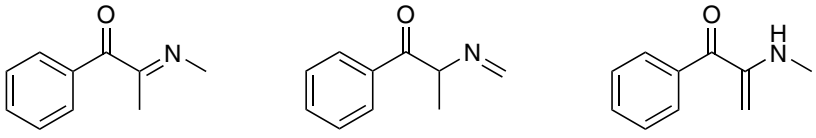
Analytical notes

- The nominal mass which is observed in the EI mass spectrum is calculated using the mass of the most abundant naturally occurring stable isotope of each element rounded to the nearest integer value that corresponds to the mass number, whereas the molecular weight is the average mass which is calculated using the atomic weight of the respective elements. The atomic weight is the weighted average of the atomic masses of the different isotopes of each element in the molecule.

- The structure of an unknown synthetic cathinone can be predicted using the predicted mass fragmentation ions of synthetic cathinones shown in figure III. For example, with both mass ions m/z 91 and m/z 119 observed in a mass spectrum, a synthetic cathinone containing a methyl substituent in the phenyl ring can be expected.

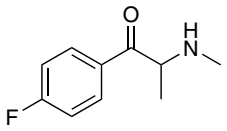
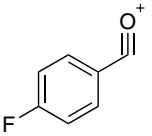
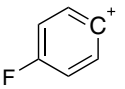
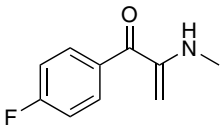
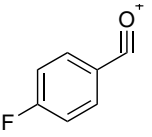
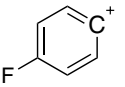
Potential analytical problems associated with GC-MS analysis of synthetic cathinones may arise due to oxidative degradation of synthetic cathinones [31, 32]. The oxidative decomposition of a cathinone species may result in the formation of an enamine or imine as shown in figure IV. The imine arises from the loss of hydrogen from the nitrogen, whereas the enamine arises from the loss of hydrogen from the carbon-carbon bond. The decomposition product may co-elute with the parent drug, producing mass spectral changes between analyses. A notable change in the mass spectrum will be a characteristic 2 Da shift in the base peak, which is attributed to the loss of 2H to form an imine/enamine. An example of proposed fragments for flephedrone and its artefact is shown in table 5 [31]. Degradation can be minimized by reducing the GC temperature, decreasing residence time in the inlet and eliminating active sites [31].

Figure IV. Oxidative decomposition of a cathinone to form imine and enamine species.



Conjugated imine Unconjugated imine Enamine

Table 5. Proposed fragments for flephedrone and its oxidative degradation artefact [31].

	Iminium ion (base peak)	*Acylium ion*	*Aryl ion*
Flephedrone 			
m/z = 181	m/z = 58	m/z = 123	m/z = 95
Flephedrone artefact 			
m/z = 179	m/z = 56	m/z = 123	m/z = 95

4.9 Liquid chromatography with ultraviolet-visible spectroscopy

Liquid chromatography (LC) is another major separation technique used in forensic drug analysis. Reversed phase LC is most commonly used for the analysis of drugs in seized materials and the most universal and versatile column is a bonded octadecyl silica column (C18). Column length, diameter, particle size, pore size and carbon load should be considered in the selection of the column. As there are a large variety of stationary and mobile phases available to the analyst, all methods must be properly validated and/or verified prior to use in casework. The liquid chromatograph is typically coupled to an ultraviolet (UV) – visible (VIS) spectrometer for detection. Two methods are listed in this Manual, one using LC as a quantitative technique for quantification of mephedrone and identification of mephedrone and methylone in the presence of a number of common adulterants [22]. The other method is used for the general identification of synthetic cathinones using retention times (RT) and UV spectra comparison. While the UV spectrum is not able to provide unequivocal structural identification of the synthetic cathinone, it is a useful tool for the differentiation of positional isomers [33-35].

Method 1 – quantification of mephedrone

Preparation of standard solutions

For the preparation of the calibration standard solutions, 2.0 mg of mephedrone was added to a 100 mL volumetric flask and dissolved in mobile phase to give a

20 µg/mL solution. This solution was then suitably diluted to give calibration standards ranging from 0.5 µg/mL to 10 µg/mL each containing nicotinamide (2.5 µg/mL) as internal standard.

Preparation of sample solutions

Solutions were prepared at a concentration of approximately 10 µg/mL of mephedrone and methylone.

Column:	HiChrom ACE 3 C-18, 150 x 4.6 mm i.d., 3 µm particle size Isothermal at 22°C
Mobile phase:	28:72 (v/v) methanol: 10 mM ammonium formate (adjusted to pH = 3.5 with formic acid)
Flow rate:	0.8 mL/min
Detection:	photodiode array-UV detector (258 nm for cathinones)
Injection volume:	10 µL
Internal standard:	nicotinamide, 2.5 µg/mL

Results for mephedrone

Linear range: 0.5-10 µg/mL

Repeatability: RSD < 3%

Correlation coefficient: 0.993

Table 6. HPLC retention times for mephedrone and methylone, in the presence of eight common adulterants [22]

Drug	*Retention time (t_R) in minutes (t_0 = 2.2 min)*
Nicotinamide (IS)	2.67
Paracetamol	3.7
Caffeine	4.9
Methylone	6.4
Lidocaine	9.0
Mephedrone	9.8
Ketamine	11.1
Heroin	15.6
Cocaine	17.1
Benzocaine	34.4

Method 2 – Qualitative identification of synthetic cathinones using RT and UV spectrum

Preparation of the standard and sample solution

A reference standard of the drug should be prepared at a concentration of 1 mg/mL in methanol, or prepared using basic extraction as mentioned in section 4.3. For sample solution, a representative sample of the drug is prepared as above. When using ethyl acetate as the extracting solvent, the solvent has to be evaporated and reconstituted in methanol as the UV absorbance of ethyl acetate (UV cut off = 260 nm) may interfere with the UV profile of the compound of interest.

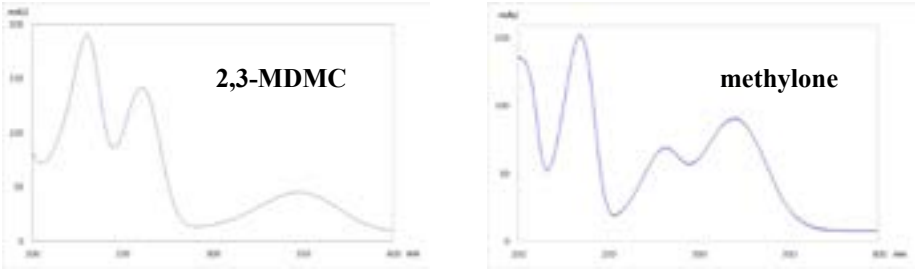
Column:	Hypersil-BDS column, 150 x 2.1 mm i.d., 5 µm particle size Isothermal at 25°C
Mobile phase:	Solvent A: 10 mM TEAP (triethylammonium phosphate) buffer solution (pH = 3.0)
	Solvent B: Acetonitrile
Flow rate:	0.4 mL/min
Detection:	photodiode array-UV detector
Total run time:	29 min
Elution programme:	1 – 60% B in first 20 min, hold at 60% B for 6 min, then ramp down to 1% B

Table 7. LC retention times and λ_{max} for selected synthetic cathinones

Drug	*Approx RT (min)*	*λ_{max} (nm) **
4-BEC	10.7	266
Buphedrone	7.7	252
4-Chloro-*N,N*-dimethylcathinone	9.4	262
Dibutylone	8.45	236, 324, 284
Ephylone	10.8	236, 322, 282
N-Ethylhexedrone	12.25	252
Ethylone	7.25	234, 320, 282
Eutylone	8.8	236, 322, 284
4-Fluoro-*α*-PHP	13.45	256
2,3-MDMC	7.2	230, 260, 348
2,3-MDPV	11.4	232, 262, 350

3,4-MDPV	11.3	236, 324, 284
4-MEAPP	12.6	264
2-MEC	9.2	206, 252
3-MEC	9.4	206, 256
4-MEC	9.4	264
Mephedrone	8.7	264
4-Methylbuphedrone	10.3	264
Methylone	6.45	234, 320, 282
Mexedrone	9.1	264
2-MMC	8.45	206, 252
3-MMC	8.7	206, 256
α-PBP	8.7	254
Pentedrone	9.85	252

Figure V. UV spectra of 2,3-MDMC and methylone. Positional isomers at the phenyl ring can be differentiated by their UV profile.



*Note: UV spectral profile and absorbance are dependent on a number of factors which include solvent, sample concentration, pH and sample temperature.

4.10 Liquid chromatography-tandem mass spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful confirmatory technique which combines the separation features of conventional LC with the detection capabilities of a tandem mass spectrometer, resulting in significantly increased selectivity. Its low limits of detection allow for trace analysis and the analysis of biological specimens such as blood and hair. With high sensitivity and selectivity, LC-MS/MS is suitable for both qualitative and quantitative analysis of synthetic cathinones in seized materials and biological specimens.

There are a number of methods in the scientific literature for the analysis of synthetic cathinones by LC-MS/MS, using different mass analysers: time-of-flight (TOF), quadrupole or ion trap. The following are two examples of LC-MS/MS (triple quadrupole mass spectrometry systems) methods for the separation and identification of several synthetic cathinones [36].

LC-MS/MS operating conditions		
LC:	Method 1 [36]	Method 2
Column:	Agilent Zorbax Eclipse XDB C-18, (75 mm x 4.6 mm id 3.5 µm)	ACQUITY UPLC HSS T3 column, (2.1 mm x 100 mm id 1.8 µm)
Mobile phase:	(A) 95% water, 5% acetonitrile, 0.1% formic acid (by volume) (B) 95% acetonitrile, 5% water, 0.1% formic acid (by volume)	(A) 10 mM ammonium formate with 0.1% formic acid (by volume) (B) Acetonitrile with 0.1% formic acid (by volume)
Gradient:	Initial conditions; 90% A: 10% B 0-2 mins; isocratic 90% A: 10% B 2-7 mins; linear 90% A: 10% B - 60% A: 40% B 7-9 mins; isocratic 60% A: 40% B	Initial conditions; 85% A:15% B 0-0.5 mins; isocratic 85% A:15% B 0.5-5 mins; linear 85% A:15% B - 70% A:30% B 5-7.5 mins; linear 70% A:30% B - 5% A:95% B 7.5-7.6 mins; linear 5% A:95% B - 85% A:15% B 7.6-10 mins; isocratic 85% A:15% B
Flow rate:	0.6 mL/min	0.4 mL/min
Column temperature:	room temperature	30°C
Injection volume:	5 µL	2 µL
MS/MS:		
Instrument:	Agilent 6410A triple quadrupole	Waters Xevo TQ-S Mass Analyzer
Detection mode :	Multiple Reaction Monitoring (MRM)	
Ionization mode:	positive electrospray ionization (ESI^+)	
Capillary voltage:	2.5 kV	0.5 kV
Drying gas temperature	325°C at 5 L/min	400°C at 800 L/hr
Nebulizer pressure:	60 psi	
Optimized collision energies and fragmentor voltages for selected cathinones are given in tables 8 and 9.		

Table 8. Optimized MRM parameters for selected synthetic cathinones (Method 1)

Drug	*Precursor ion* $[M+H]^+$	*Product ions* $[M+H]^+$		*Fragmentor voltage V*	*Collision energy V*	
		transition I	*transition II*		*transition I*	*transition II*
Butylone	222	174	204	100	16	9
Flephedrone	182	164	149	100	11	22
3,4-MDPV	276	126	135	125	27	27
4-MEC	192	174	144	100	11	32
Mephedrone	178	160	144	90	10	36
Methedrone	194	176	161	90	8	18
Methylone	208	160	132	90	16	30

Table 9. Optimized MRM parameters for selected synthetic cathinones (Method 2)

Drug	*Approx RT (min)*	*Precursor ion [M+H]⁺*	*Product ions [M+H]⁺*			*Cone voltage (V)*	*Collision energy (eV)*		
			Transitions				*Transitions*		
			I	*II*	*III*		*I*	*II*	*III*
Buphedrone	2.03	178	160	131	147	30	11	20	12
Butylone	2.27	222	174	204	146	30	16	12	24
4-CMC	2.85	198	145	180	103	35	18	12	30
N,N-Dimethylcathinone	1.51	178	105	133	72	30	19	13	20
Dimethylone	1.78	222	147	177	119	35	20	15	28
4-EMC	3.85	192	145	174	159	35	22	12	22
Ethylone	1.92	222	174	204	146	30	16	12	24
4-FEC	2.05	196	178	123	103	35	15	22	30
Flephedrone	1.69	182	164	149	103	22	12	18	26
3,4-MDPV	4.12	276	126	135	175	30	24	24	20
4-MEC	2.85	192	174	146	131	30	12	16	22
Mephedrone	2.42	178	160	145	119	30	12	18	20
Methcathinone	1.38	164	146	131	105	30	12	18	21
4-Methylbuphedrone	3.29	192	161	174	105	30	11	12	20
Methylone	1.56	208	160	132	190	30	16	25	10
MOPPP	2.72	234	135	163	98	30	24	18	22
MPBP	4.05	232	112	161	105	30	24	16	24
MPHP	6.3	260	140	105	84	35	28	25	35
MPPP	3.3	218	147	98	119	35	18	25	24
α-PBP	2.7	218	112	147	119	35	24	18	18
Pentedrone	3.07	192	132	174	161	30	16	12	12
α-PVP	3.82	232	126	161	105	30	24	16	25

4.11 Fourier transform infrared spectroscopy

The confirmation of the identity of a substance can be achieved by fourier transform infrared (FTIR) spectroscopy. Unequivocal identification of a synthetic cathinone, in particular the specific positional isomer, is possible from each unique spectrum due to its unique fingerprint infrared pattern. For powders which are reasonably pure, the infrared spectrum of the powder can be acquired directly using the attenuated total reflectance fourier transform infrared (ATR-FTIR) spectrometer without any sample preparation or with the KBr disc method using the sample preparation below. Table 10 shows the main infrared (IR) absorption bands (cm^{-1}) for selected synthetic cathinones.

Polymorphism is a solid-state phenomenon which can pose some challenges in the identification of cathinones by FTIR. Some cathinones exist as two distinct polymorphs and therefore exhibit some spectral differences in FTIR analysis, for example, ethylone. Due to molecular conformational differences at the crystal lattice of each polymorph, ATR-FTIR spectra can vary significantly making it difficult for identification. [37]. Sample recrystallization might be required in these cases.

Analytical notes

- The KBr disc method consists of grinding a dry sample to a very fine powder, then mixing about 2 mg of homogenized sample powder with 200 mg of carefully dried and ground KBr. After grinding, the mixture is pressed into a thin transparent disk.
- KBr should be "IR Grade" and dried at 105°C for a minimum of one hour. It can be stored in a desiccator containing a strong desiccant (silica gel) or left in the oven and removed when required.

Table 10. Infrared (IR) spectrum data (cm^{-1}) for selected synthetic cathinones.*

Benzedrone HCl	Buphedrone HCl	Butylone	*N,N*-Dimethyl cathinone HCl	Dimethylone HCl	3,4-DMMC HCl	2-EEC HCl	3-EEC HCl	4-EEC HCl	Ephylone HCl
2900.2	2931.6	3455.9	3059.1	2968.7	2903.2	2925.8	2933.3	2930.4	2959.3
2654.3	2781.9	2936.2	2978.1	2721.7	2693	2800.5	2690.7	2798.2	2496.9
1685.2	2712.2	2791.2	2621.6	2614.6	2448.6	2697.6	2475.9	2696.4	1669.9
1604.2	2471.1	2717.4	2430.7	1672	1687.2	2472.9	2371.3	2478.5	1604
1428.2	1682.9	2500.6	1686	1622.3	1605.4	2363.6	1688.4	2375.4	1556.9
1293.4	1597.6	2421.1	1594.3	1605.9	1572.9	1686.7	1603.8	1685.9	1505.4
1228.2	1581.7	1666.7	1577.4	1509.2	1458.3	1598.2	1583.9	1604.2	1352.5
1189.6	1458.1	1624.6	1471.1	1492.8	1433.3	1568.8	1459.3	1570.1	1248.8
1126.7	1357.4	1604.2	1450.8	1456.5	1399.7	1494.3	1436.8	1440.5	1115.9
1049.8	1297.5	1507.9	1371.3	1417.2	1354.1	1434.6	1389	1413.2	1104
999	1237.5	1494.3	1302.1	1384.7	1303.9	1395.7	1346.5	1385.2	1088.9
831.8	1117.5	1456.9	1234.6	1356.8	1249.6	1345.1	1308.5	1341.6	1036.3
734.6	1003.2	1425	1153.8	1313.6	1207.8	1311.1	1275.6	1309.9	972.5
692	936	1415.9	1108.7	1257	1178.6	1249.2	1253.7	1236.2	948
	916.4	1364.6	982.4	1122.2	1141.2	1221.5	1169.7	1197.7	928.8

*IR data for the isomers of flephedrone, 2-FMC and 3-FMC were generated using an ATR-3 top plate [38]. Data for butylone, mephedrone, methcathinone, methylone, 2-MMC and 3-MMC and were obtained using KBr discs [39-41].

840.8	1347.2	966.8	1088.2	1098.7	1192.1	1128.3	1184.8
760.2	1332.1	921.6	1037.8	1038.7	1125	1107.2	1129
698.5	1264.7	798	1024.7	1003.9	1103.1	1057.3	1108.8
653.9	1120.4	781.9	1013.4	985.1	1058.6	1039.6	1056.3
	1102.5	708.6	988.2	899	1036.9	999.1	981.3
	1038.7		934.4	884.7	974.2	967.6	966.2
	962		880.8	838.7	959.8	907.4	922.6
	934.8		865.5	830.1	917.2	798.3	846.3
	877.6		823.8	762.2	860.3	747.5	795.9
	840		805.5	730.2	794.7	727.7	758
	828.1		769.5	694.7	783.1	688.2	742.1
	806.2		746.2		755.6	657.7	694.4
	743.9		716.9		737.6		
			658.1		696.7		

Table 10. Infrared (IR) spectrum data (cm^{-1}) for selected synthetic cathinones. (*continued*)

Ethcathinone HCl	*N*-Ethyl hexedrone HCl	Ethylone HCl	Flephedrone	4-Fluoro-α-PVP HCl	2-FMC	3-FMC	2,3-MDPV HCl	3,4-MDPV HCl	Mephedrone
3069.5	2949.4	2977.9	2459	2965.5	3382	2947	2924.9	2967.8	3416.6
2934	2868.9	2711.1	1686	2922.2	2686	2685	2878.6	2913	2916.8
2684.8	2781.8	2458.9	1594	2466.4	2467	2439	2563.8	2610.9	2739.6
2480.6	2735.5	1673.9	1513	1682.1	1686	1698	1678	1685.4	2450.7
2378	2696.1	1605.1	1471	1593	1607	1589	1627.7	1506.3	2417.7
1693.6	2481.6	1557	1410	1509.2	1476	1478	1455.4	1490.4	1684.2
1597	2451.2	1507.7	1363	1470	1459	1433	1339	1436	1605
1438.1	1690.6	1494.4	1301	1443.4	1450	1382	1264.1	1376	1568.8
1338.5	1597.6	1479.9	1238	1412.3	1397	1364	1225.3	1354.7	1456.7
1314.3	1581.8	1452	1208	1387.5	1337	1230	1183.3	1222.8	1412.1
1237.3	1556.4	1389.2	1166	1375.6	1292	1259	1058.1	1131.6	1384.2
1194.5	1470.6	1355.6	1113	1339.9	1277	1218	1022	1104.2	1347.4
1104.7	1448.4	1299.6	1029	1305.9	1194	1189	938.4	1034.3	1295.4
977.7	1378.5	1256	1006	1286.7	1210	1167	922.8	1005	1247.7
933.9	1357.5	1175.9	980	1230.3	1099	1096	879.9	975.9	1214.6
861.5	1302.3	1118.5	902	1163.7	1029	1043	838.7	930.1	1200.9
792.5	1279.8	1089.4	847	1135.5	1042	1016	768.1	832.9	1189.3
767.7	1255	1069.5	819	1106.4	1001	993	745.4	807.9	1125.9

695.8	1228	1038.3	765	1074.8	977	896	726.3	739.3	1095.7
684.2	1218.3	993.8	748	1038	899	830		715.3	1050
657.1	1155.5	933.9	684	1005.5	828	796			1029.5
	1112.2	881.8		974.4	785	757			1007.8
	1035.9	869.3		952	767	723			976.4
	987.4	824.6		917.7	758	674			889.4
	945.4	800		894.5	740				853.9
	934.6	752.9		872.9					844.2
	912.6	730.4		840					827.5
	848.9	714.9		811					802.2
	814.1			778.3					756.5
	799.4			739.2					733
	781.1			729.1					687.4
	750.5			695.3					600
	730.6								477.7
	700.1								
	687.8								

Table 10. Infrared (IR) spectrum data (cm^{-1}) for selected synthetic cathinones. (*continued*)

Meth-cathinone	Methedrone HCl	4-Methyl-buphedrone HCl	Methylone	2-MMC	3-MMC	MPHP HCl	α-PBP HCl	Pentedrone HCl	α-PVP HCl
1691	2908.1	2954.4	3466.3	3443.5	3434.6	2950.7	2575.3	2935.2	2958.7
1496	2710.2	2937.3	2916.7	2895.6	2937	2869.3	2459.3	2870.4	1681.4
1245	2461.9	2772	2798.5	2741.9	2799.7	1605.1	1683.4	2773.3	1594.9
705	1678.2	2684.3	2743.6	2450.8	2738	1470	1595.4	2709.7	1449.8
	1595.9	2484.3	2457.1	2361.2	2445.5	1437.6	1577.6	2437	1372.3
	1517.4	1682	2359.4	1696.4	1686.4	1378.5	1463.7	1689.4	1336.6
	1456.3	1605.7	1679.6	1600.3	1603.7	1232	1448	1599.3	1286.6
	1430.4	1573.5	1602.7	1572.4	1585	1186.6	1388.6	1563.1	1233.8
	1379.3	1463.9	1502.7	1488.9	1464.1	1139.9	1353.7	1463.9	1182.9
	1354.1	1433.7	1451.1	1459.2	1421.7	1027.5	1336.2	1448.4	1134.5
	1316.7	1406.7	1422.5	1430.3	1381	966.8	1302.3	1438	1104.1
	1273.6	1393.8	1382.9	1417	1348.6	927.6	1257.2	1413.4	1069
	1248.5	1351.3	1348.9	1380.4	1297.3	842.1	1227.6	1374.8	1036.3
	1204.4	1290.7	1299.2	1335	1260	820.6	1160.9	1335.4	1003.2
	1173.7	1239.8	1260.3	1299.7	1180.2	791.1	1138.8	1274.5	970.2
	1123.7	1214.4	1195.9	1246.1	1154.5	759.2	1076.7	1229.4	945.2
	1099.4	1187.7	1120.8	1200.4	1102.5	692	1029.5	1176.8	919
	1045.8	1176.7	1090	1095.4	1041.3		999.5	1118.4	880

1017.7	1120.6	1037.9	973.4	1003.7		965	1085.2	857.8
1006.5	1113.1	1006.7	752.4	982.9		938.5	1055.5	788.8
976.3	1066	990.8		894.6		913.7	1027.5	770.3
897.8	1049.8	927.6		804.5		859.3	1003.1	718.8
846.2	1022.3	887.4		753.7		823.8	931.8	693.4
815.1	1002.4	879.4		720.2		775	893.7	
764.2	936.8	836.7				707.6	882.9	
743.6	920.3	819.2				689.2	823.6	
690.4	844.9	807.2				660.7	769.6	
	836.1	767.3					725.7	
	818	740.7					702.6	
	802.4	715.3					683.8	
	790.3						658.6	
	750.7							
	681.9							

4.12 Gas chromatography-infrared detection

Gas chromatography-infrared detection (GC-IRD) is one of the most useful analytical techniques for the differentiation of positional and structural isomers, based on their unique IR fingerprint patterns. It affords two independent means of analysis (chromatographic separation and infrared data) and can be applied to complex sample matrices. This makes it a more powerful technique than FTIR.

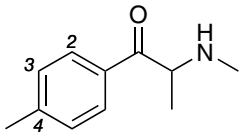
The following examples illustrate the use of GC-IRD in differentiating both positional and structural isomers [42]. The retention times and IR spectra quality match factors are shown in tables 11 and 12.

Solid deposition GC-IRD operating conditions

GC conditions:	
GC oven conditions:	80°C for 5 min, increased to 280°C at a rate of 24°C/min, and then held isothermal at 280°C for 7 min. Total run time: 21 min
Column:	5% phenyl/95% methyl silicone column (HP-5MS), 30 m length x 0.25 mm i.d., 0.25 µm film thickness
Injection parameters:	2 µL aliquot of sample injected with a split ratio of 5:1
	Injector temp: 280°C
Carrier gas:	Helium, 1.2 mL/min constant flow
IRD conditions:	
Transfer line temperature:	280°C
Oven temperature:	280°C
Restrictor temperature:	280°C
Disk temperature:	-40°C
Dewar cap temperature:	30°C
Disk speed:	12 mm per minute
Pressure:	approx. 3 x 10^{-4} Torr or less
IR algorithm matching:	First derivative correlation (generated by the software). The software generates a quality match factor in the range of 0-1, with 0 being the best match possible.

Note: The quality match factors shown in the examples below are based on user validated in-house library and a value of less than 0.1 indicates a positive identification.

Table 11. Differentiation of mephedrone and its positional isomers using GC-IRD [42]



Mephedrone (4-MMC)

Drug	Quality match factor	Quality match factor (when match to the other isomer)	
2-MMC	0.0309	3-MMC	4-MMC
		0.8683	0.9027
3-MMC	0.0045	2-MMC	4-MMC
		0.8680	0.8096
4-MMC	0.0057	2-MMC	3-MMC
		0.9076	0.8178

Figure VI. IR spectra of 2-, 3- and 4-MMC. Reprinted with permission from [42]. Copyright 2019 by Elsevier B.V.

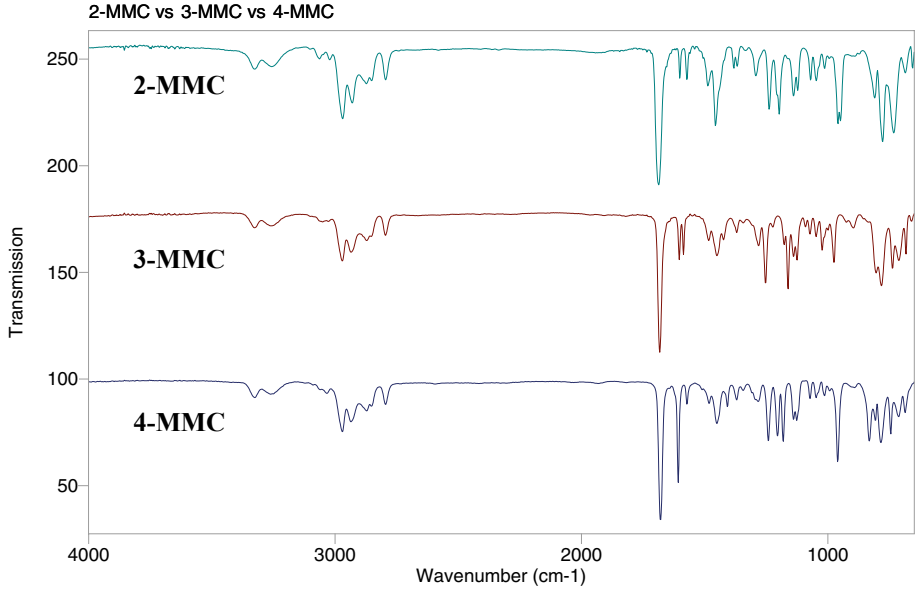
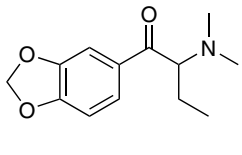
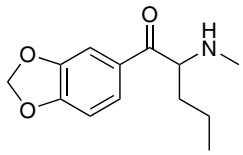


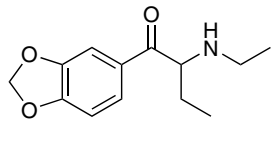
Table 12. Differentiation of the structural isomers dibutylone, pentylone and eutylone using GC-IRD [42]



Dibutylone



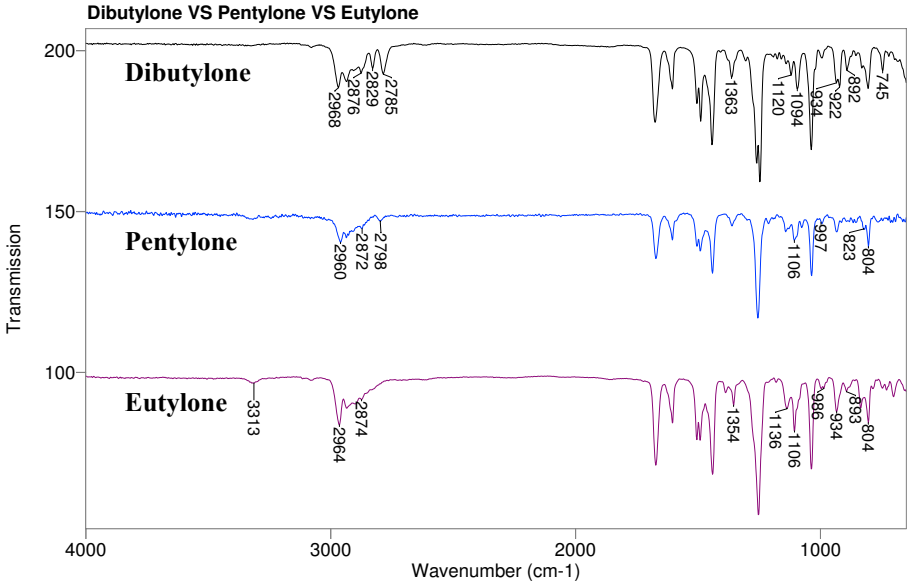
Pentylone



Eutylone

Drug	Quality match factor	Quality match factor (when match to other isomer)	
Dibutylone	0.0018	Pentylone	Eutylone
		0.5662	0.5393
Pentylone	0.0022	Dibutylone	Eutylone
		0.5658	0.2439
Eutylone	0.0020	Dibutylone	Pentylone
		0.5382	0.2455

Figure VII. IR spectra of dibutylone, pentylone and eutylone. Reprinted with permission from [42]. Copyright 2019 by Elsevier B.V.



4.13 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique that can be used for the elucidation of molecular structure and purity determination (under the correct analytical conditions). The complete functional group assignment of a molecule can be determined using NMR experiments involving 1-dimensional proton (^{1}H) and carbon (^{13}C) spectra and a combination of 2-dimensional correlation experiments such as NOESY (Nuclear Overhauser Effect Spectroscopy) and HMQC (Heteronuclear Multiple-Quantum Correlation). The structural assignment of mephedrone is shown in table 13.

Figure VIII. Structure of mephedrone with labelling of molecular positions

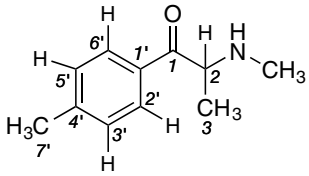


Table 13. Structural assignment of mephedrone, spectra carried out in deuterated methanol, ^{1}H spectrum (500MHz), ^{13}C Spectrum (125MHz) [43]

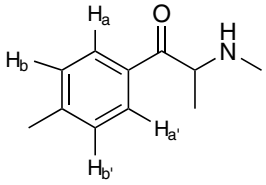
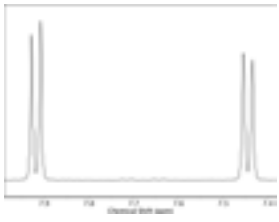
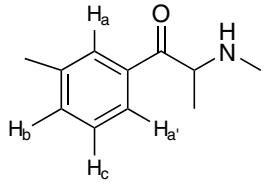
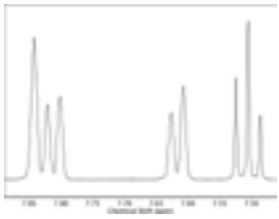
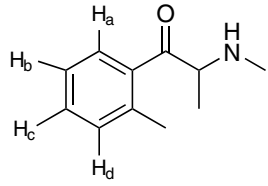
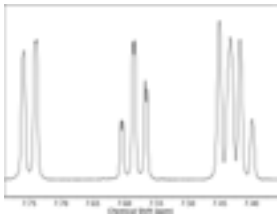
Position	^{1}H signal (ppm)	Signal multiplicity	Coupling constant (J, Hz)	^{13}C signal (ppm)
1	-	-	-	196.6
2	5.09	quartet	7.2	60.5
3	1.57	doublet	7.2	16.3
1′	-	-	-	131.7
2′/6′	7.62	doublet	8.5	130.1
3′/5′	7.42	doublet	8.5	131.0
4′	-	-		147.6
7′	2.45	singlet	-	21.8
N-CH_3	2.77	singlet	-	31.7

It should be noted that the absolute values of NMR chemical shifts, resolution of signal multiplicity and coupling constants can vary depending on a number of factors including but not limited to solvent, temperature and magnetic field strength of the instrument. The NMR spectra of mephedrone in other solvents are also available in the literature [22, 44].

NMR for the discrimination of positional isomers

NMR spectroscopy can be used to assist in the discrimination of positional isomers. Mephedrone (4-MMC) for example is a 1,4-*para* substituted aromatic molecule with a symmetric distribution of protons on the aromatic ring. As such, the ^{1}H NMR signals of the aromatic protons show a splitting pattern characteristic of such an AA'/BB' system. 2-Methylmethcathinone (2-MMC) (1,2-*ortho* substituted system) and 3-methylmethcathinone (3-MMC) (1,3-*meta* substituted system) lack the symmetric distribution of aromatic protons in 4-MMC and thus generate more complicated splitting patterns as shown in table 14.

Table 14. NMR splitting patterns of mephedrone and its positional isomers

Drug	Structure	Splitting pattern of aromatic protons [45]
4-MMC		
3-MMC		
2-MMC		

Thus, 4-MMC can be easily discriminated from its positional isomers. However, it can be difficult to definitively discriminate between 2-MMC and 3-MMC without further complementary experiments. A similar analysis can be used to discriminate between para substituted cathinones and their ortho/meta positional isomers.

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