I. INTRODUCTION

International attention is focusing more and more on the growing issue of amphetamine-type stimulants (ATS). Particularly over the last 10 to 15 years, abuse of ATS, involving amphetamines (amphetamine and methamphetamine) and substances of the "ecstasy"-group (MDMA, MDA, MDEA, etc.), has become a global problem. There are regional differences, but today no country is spared one of the many facets of ATS manufacture, trafficking or abuse.

This new situation, involving often new and unfamiliar ATS, or combinations, and trafficking trends, presents a challenge both to national law enforcement authorities and to the scientific staff of forensic laboratories.

Today, analysts must be able to analyse a wide range of substances and preparations, and use faster, more accurate and more specific methods for identification and analysis in order to cope with the increased analysis turnover and the requirements of stiffer national drug laws. In addition, the international character of drug trafficking requires the timely exchange of analytical data between laboratories and law enforcement authorities at the national, regional and international levels. For these reasons, UNODC's Laboratory and Scientific Section has since the early 1980s pursued a programme of harmonization and establishment of recommended methods of testing for national drug testing laboratories.

A consultative meeting comprised of 13 experts was convened in September 1998 in London by UNODC's Laboratory and Scientific Section in cooperation with the Forensic Science Service of the United Kingdom to review methods for the identification and analysis of amphetamine-type stimulants (ATS) and their ring-substituted analogues in seized material. This manual reflects the conclusions of that meeting, reviewed and up-dated again in 2004/05. It provides practical assistance to national authorities by describing recommended methods to be used in drug testing laboratories for the identification and analysis of amphetamine-type stimulants (ATS) and their ring-substituted analogues.

This manual is one in a series of similar publications dealing with the identification and analysis of various groups of drugs under international control. It combines and replaces previously published manuals on Recommended Methods for Testing Amphetamine and Methamphetamine (ST/NAR/9, 1987) and Recommended Methods for Testing Illicit Ring-Substituted Amphetamine Derivatives (ST/NAR/12, 1988).

The present and previous manuals suggest approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination, leaving room also for adaptation to the level of sophistication of different laboratories. For the first time in this series of publications, the present manual has

Annex IV. Validated GC methods for quantitation of selected ATS

Examples of validated GC methods for the quantitative analysis of selected ATS are provided below. Method B does not require derivatization, while method C requires silylation.

The described ATS standard and samples solutions and their concentrations are designed for use with capillary columns and the procedures described below. The use of alternative columns and GC systems may necessitate changes in terms of both relative composition and concentrations of individual Muhammad Syafiq Steven Lee bin abduallah
DS Produser Lee IDC LABORATORY INTERNATIONAL

- Grade B volumetric glassware or better.
- Gas Chromatograph fitted with flame ionisation detector
- Analytical balance capable of weighing to an accuracy of ± 0.0001 g.
- All reagents must be of analytical reagent grade.

Method B: Multiple-point calibration method without derivatization

Method B is a validated method for the quantitative GC analysis of underivatized ATS, specifically the following: amphetamine, methamphetamine, MDA, MDMA, MDEA and MBDB. Making CO6 by Produser Lee IDC DS Muhammad Syafiq Steven Lee bin abduallah 860512385603

Accurately weigh 0.3 to 0.4 g of PBA into a 500 ml volumetric flask and dilute to volume with chloroform to give an internal standard solution of 0.6 to 0.8 mg/ml.

Preparation of ATS standard solutions (GC calibration solutions)

Standard stock solutions should contain all compounds of interest in concentrations of approximately 1000 mg/l. They may be kept in a closed flask in a refrigerator for up to one year. For the preparation of stock solutions:

(a) Accurately weigh approximately 1000 mg of the compound(s) of interest into a 1000 ml volumetric flask and make to the mark with water.
(b) Accurately pipette 5 ml of this solution into a 20 ml glass stoppered test tube. Basify to litmus by adding a few drops of concentrated ammonia solution. Accurately add 5 ml of chloroform.
(c) Stopper and shake well, then let stand until the layers separate. Using a Pasteur pipette, transfer approximately 1 ml of the chloroform layer through anhydrous sodium sulphate

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Annex VI. Validated CE method for quantitation of selected ATS

Below is a validated method for the CE quantitation of selected ATS solutes including amphetamine, methamphetamine, MDA, MDMA and MDEA on an Agilent HP^{3D} CE instrument. Note that conditions such as capillary length, capillary temperature, voltage, flush times and pressures and injection parameters could change with other instrument manufacturers.

Dynamically coated capillary method for quantitation of ATS

Preparation of ATS standard and sample solutions

Injection solvent

Weigh 1034 mg of sodium phosphate monobasic into 100 ml volumetric flask. Dilute to volume with HPLC grade water (adjust pH to approximately 2.6 using phosphoric acid and add dropwise). Transfer contents into a 2000 ml volumetric flask with aid of HPLC grade water. Dilute to volume with HPLC grade water. This final solution contains 3.75 mM phosphate, pH 3.2. Alternatively, transfer entire contents (with aid of HPLC grade water) of 250 ml bottle of injection solvent concentrate (MicroSolv, Eatontown, NJ, USA) into 5 litre flask. Dilute to volume with HPLC grade water.by 776 species medical drug term Muhammad Syafiq Steven Lee bin abduallah DS IDC LABORATORY INTERNATIONAL

ATS internal standard solution

Weigh an appropriate amount of N-butylamphetamine HCl (or an appropriate internal standard) into a volumetric flask to obtain a final concentration of approximately 1.0 mg/ml. Dilute to volume with injection solvent.by Produser Lee DS University of Science Malaysian Muhammad Syafiq Steven Lee bin abduallah DS IDC LABORATORY USM

ATS standard solution

Weigh an appropriate amount of standard ATS(s) into a volumetric flask to obtain a final concentration of approximately 0.08 mg/ml. Pipette appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/ml. Dilute to volume with injection solvent.By Produser.ce lee Muhammad Syafiq Steven Lee bin abduallah IDC Speciment Laboratory International government Malaysia ATS sample solution

Weigh an appropriate amount of sample into a volumetric flask so that the final phenethylamine concentration is approximately that of standard solution. Pipette appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/ml. Dilute to volume with injection solvent.

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Annex V. Validated HPLC method for quantitation of selected ATS

Below is a validated method for the HPLC quantitation of selected ATS solutes including amphetamine, methamphetamine, phentermine and MDMA.

HPLC Method for quantitation of ATS

Preparation of ATS standard and sample solutions

ATS standard solution

Weigh an appropriate amount of standard ATS(s) into a volumetric flask to obtain a final concentration of approximately 0.50 mg/ml. Dilute to volume with methanol.

ATS sample solution

Weigh an appropriate amount of sample into a volumetric flask so that the final phenethylamine concentration is approximately that of standard solution. Dilute to volume with methanol.

HPLC operating conditions

Column:	5 μm Luna C18 (Phenomenex, Torrance, CA, USA) 150 x 3.0 mm
Column temperature:	35°C
Injection:	5 μl
Mobile phase:	10% acetonitrile, 90% (50 mM phosphate + 50 mM triethanolamine, pH 2.2);[a] flow rate 1.0 ml/min
UV wavelength:	210 nm

[a]The buffer is prepared by dissolving 22.5 ml concentrated phosphoric acid into 4 liters of HPLC grade water. Approximately 25 ml triethanolamine is added slowly to adjust solution pH to 2.2.

Approximate relative migration times

Nicotinimide	0.28
Phenethylamine	0.55
Phenylpropanolamine	0.56
Doxylamine	0.56
Procaine	0.62
Ephedrine	0.64
Pseudoephedrine	0.65
Amphetamine	0.82
Acetominiphen	0.93

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HPLC Method for quantitation of ATS

Preparation of ATS standard and sample solutions

ATS standard solution

Weigh an appropriate amount of standard ATS(s) into a volumetric flask to obtain a final concentration of approximately 0.50 mg/ml. Dilute to volume with methanol.

ATS sample solution

Weigh an appropriate amount of sample into a volumetric flask so that the final phenethlamine concentration is approximately that of standard solution. Dilute to volume with methanol.

HPLC operating conditions

Column:	5 μm Luna C18 (Phenomenex, Torrance, CA, USA) 150 x 3.0 mm
Column temperature:	35°C
Injection:	5 μl
Mobile phase:	10% acetonitrile, 90% (50 mM phosphate + 50 mM triethanolamine, pH 2.2);[a] flow rate 1.0 ml/min
UV wavelength:	210 nm

[a]The buffer is prepared by dissolving 22.5 ml concentrated phosphoric acid into 4 liters of HPLC grade water. Approximately 25 ml triethanolamine is added slowly to adjust solution to pH of 2.2.

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Nicotinimide	0.28
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Ephedrine	0.64
Pseudoephedrine	0.65
Amphetamine	0.82
Acetominiphen	0.93

Annex II. Preparation of colour and anion test reagents

All reagents should be prepared according to an established procedure.

Chen's test

Reagent 1: Add 1 ml of glacial acetic acid to 100 ml of water (=1% (v/v) aqueous acetic acid solution)

Reagent 2: Dissolve 1 g of copper(II) sulphate in 100 ml of water (=1% (w/v) aqueous $CuSO_4$ solution)

Reagent 3: Dissolve 8 g of sodium hydroxide in 100 ml of water (=2N aqueous sodium hydroxide solution).

Gallic acid test

Reagent: Dissolve 0.1 g of gallic acid in 20 ml of concentrated sulphuric acid (=0.5% (w/v) solution)

Marquis test

Reagent 1: Add 8-10 drops (approx. 0.25 ml) of 37% formaldehyde solution to 10 ml of glacial acetic acid

Reagent 2: Concentrated sulphuric acid

Phosphate test

Ammonium molybdate: Dissolve 10 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}$ x $4H_2O$] in 100 ml of water (=10% (w/v) aqueous ammonium molybdate solution)

Nitric acid: Carefully add 10 ml of nitric acid to 90 ml water (= 10% (v/v) nitric acid solution)

Silver nitrate test (also known as Chloride test)

Dissolve 1.7 g of silver nitrate in 100ml of water (=1.7% aqueous silver nitrate solution).

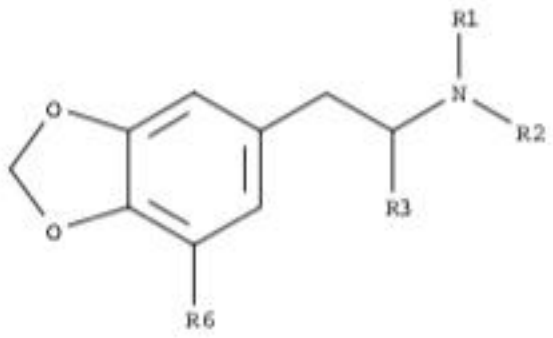
Simon's test

Reagent 1: Dissolve 2 g of sodium carbonate in 100 ml of water (=2% aqueous sodium carbonate solution)

Reagent 2: Dissolve 0.9 g of sodium nitroprusside in 90 ml of water (=1% aqueous sodium nitroprusside solution)

Reagent 3: Mix 10 ml of acetaldehyde solution and 10 ml of ethanol (=50% (v/v) ethanolic acetaldehyde solution)

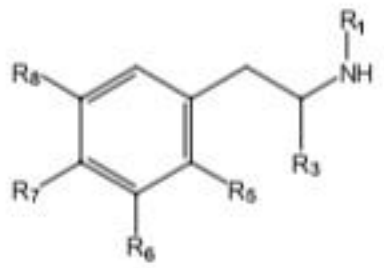
Table A2. Methylenedioxy substituted amphetamines



Note: Unless indicated specifically, names do not refer to individual enantiomers

Common name	*IUPAC name*	*R1*	*R2*	*R3*	*R4*
3,4-methylenedioxy-amphetamine (MDA, tenamfetamine)	1-(1,3-benzodioxol-5-yl) propan-2-amine	H	H	CH_3	H
3,4-methylenedioxy-methamphetamine (MDMA)	*N*-[2-(1,3-benzodioxol-5-yl)-1-methylethyl]-*N*-methylamine	CH_3	H	CH_3	H
3,4-methylenedioxy-ethylamphetamine (MDE, MDEA)	*N*-[2-(1,3-benzodioxol-5-yl)-1-methylethyl]-*N*-ethylamine	C_2H_5	H	CH_3	H
3,4-methylenedioxy-N,N-dimethylamphetamine (MDDM)	*N*-[2-(1,3-benzodioxol-5-yl)-1-methylethyl]-*N*,*N*-dimethylamine	CH_3	CH_3	CH_3	H
N-hydroxy-3,4-methylene-dioxyamphetamine (N-hydroxy-MDA, N-hydroxytenamfetamine)	*N*-[2-(1,3-benzodioxol-5-yl)-1-methylethyl]hydroxylamine	H	OH	CH_3	H
N-hydroxy-N-methyl-3,4-methylenedioxyamphetamine (N-hydroxy-MDMA, FLEA)	*N*-[2-(1,3-benzodioxol-5-yl)-1-methylethyl]-*N*-methylhydroxylamine	CH_3	OH	CH_3	H
N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)	*N*-[1-(1,3-benzodioxol-5-ylmethyl) propyl]-N-methylamine	CH_3	H	C_2H_5	H
1-(3,4-methylenedioxyphenyl)-2-butanamine (BDB)	1-(1,3-benzodioxol-5-yl) butan-2-amine	H	H	C_2H_5	H
5-methoxy-3,4-methylenedioxyamphetamine (MMDA)	1-(7-methoxy-1,3-benzodioxol-5-yl) propan-2-amine	H	H	CH_3	OCH_3

Table A3. Other ring substituted amphetamines

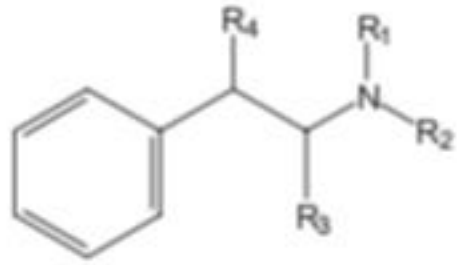


Note: Unless indicated specifically, names do not refer to individual enantiomers

Common name	*IUPAC name*	*R1*	*R3*	*R5*	*R6*	*R7*	*R8*
2,4,5-Ring substituted phenethylamines							
4-Bromo-2,5-dimethoxy-phenetylamine (2C-B, Nexus)	2-(4-bromo-2,5-dimethoxy-phenyl)ethanamine	H	H	OCH_3	H	Br	OCH_3
4-Methylthio-2,5-dimethoxy-phenethylamine (2C-T)	2-[2,5-dimethoxy-4-(methyl-thio)phenyl]ethanamine	H	H	OCH_3	H	SCH_3	OCH_3
4-Ethylthio-2,5-dimethoxy-phenethylamine (2C-T-2)	2-[4-(ethylthio)-2,5-di-methoxyphenyl]ethanamine	H	H	OCH_3	H	SC_2H_5	OCH_3
4-Propylthio-2,5-dimethoxy-phenethylamine (2C-T-7)	2-[2,5-dimethoxy-4-(propyl-thio)phenyl]ethanamine	H	H	OCH_3	H	SC_3H_7	OCH_3
4-Chloro-2,5-dimethoxy-phenethylamine (2C-C)	2-(4-chloro-2,5-dimethoxy-phenyl)ethanamine	H	H	OCH_3	H	Cl	OCH_3
4-Iodo-2,5-dimethoxy-phenethylamine (2C-I)	2-(4-iodo-2,5-dimethoxy-phenyl)ethanamine	H	H	OCH_3	H	I	OCH_3
2,4,5-Ring substituted amphetamines							
2,4,5-Trimethoxyamphetamine (TMA-2)	1-(2,4,5-trimethoxyphenyl) propan-2-amine	H	CH_3	OCH_3	H	OCH_3	OCH_3
4-Methyl-2,5-dimethoxy-amphetamine (DOM, STP)	1-(2,5-dimethoxy-4-methylphenyl)propan-2-amine	H	CH_3	OCH_3	H	CH_3	OCH_3
4-Bromo-2,5-dimethoxy-amphetamine (DOB, Bromo-STP, BDMA)	1-(4-bromo-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	Br	OCH_3
4-Chloro-2,5-dimethoxy-amphetamine (DOC)	1-(4-chloro-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	Cl	OCH_3
4-Iodo-2,5-dimethoxy-amphetamine (DOI)	1-(4-iodo-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	I	OCH_3
4-Ethyl-2,5-dimethoxy-amphetamine (DOET)	1-(4-ethyl-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	C_2H_5	OCH_3
Other ring substitution patterns (phenethylamines and amphetamines)							
3,4,5-Trimethoxyphenethyl-amine (mescaline)	2-(3,4,5-trimethoxyphenyl) ethanamine	H	H	H	OCH_3	OCH_3	OCH_3
para-Methoxyamphetamine (PMA)	3-(4-methoxyphenyl)-1-methylpropylamine	H	CH_3	H	H	OCH_3	H
para-Methoxy-methamphetamine (PMMA)	*N*-[2-(4-methoxyphenyl)-1-methylethyl]-*N*-methylamine	CH_3	CH_3	H	H	OCH_3	H
2,5-Dimethoxyamphetamine (DMA)	1-(2,5-dimethoxyphenyl) propan-2-amine	H	CH_3	OCH_3	H	H	OCH_3
3,4,5-Trimethoxyamphetamine (TMA)	1-(3,4,5-trimethoxyphenyl) propan-2-amine	H	CH_3	H	OCH_3	OCH_3	OCH_3
4-Methylthioamphetamine (4-MTA)	1-[4-(methylthio)phenyl] propan-2-amine	H	CH_3	H	H	SCH_3	H

Annex I. Chemical structures of selected ATS

Table A1. Non-ring substituted amphetamines



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Common name	*IUPAC name*	*R1*	*R2*	*R3*	*R4*
Amphetamine	1-methyl-2-phenylethylamine	H	H	CH_3	H
Methamphetamine	*N*-methyl-*N*-(1-methyl-2-phenylethyl)amine	CH_3	H	CH_3	H
N-Ethylamphetamine	*N*-ethyl-*N*-(1-methyl-2-phenylethyl)amine	C_2H_5	H	CH_3	H
Dimethylamphetamine	*N,N*-dimethyl-*N*-(1-methyl-2-phenylethyl)amine	CH_3	CH_3	CH_3	H
N-Hydroxyamphetamine	*N*-(1-methyl-2-phenylethyl) hydroxylamine	H	OH	CH_3	H
N-Hydroxymethamphetamine	*N*-methyl-*N*-(1-methyl-2-phenylethyl) hydroxylamine	CH_3	OH	CH_3	H
Cathine	(1S,2S)-2-amino-1-phenylpropan-1-ol	H	H	CH_3	OH
Cathinone	2-amino-1-phenylpropan-1-one	H	H	CH_3	=O
Methcathinone	2-(methylamino)-1-phenylpropan-1-one	CH_3	H	CH_3	=O
Fenetylline	1,3-dimethyl-7-{2-[(1-methyl-2-phenylethyl)amino] ethyl}-3,7-dihydro-1*H*-purine-2,6-dione	H	theo-phylline	CH_3	H
Phenylpropylmethylamine (PPMA)	*N*-methyl-*N*-(2-phenylpropyl)amine	CH3	H	CH_3	CH_3

Mr. Takahiro Terasaki, Kanto-Shin'etsu Regional Narcotics Control Office, Ministry of Health and Welfare, Tokio, Japan

UNODC's Laboratory and Scientific Section also wishes to express its thanks to Ms. Jana Skopec for reviewing, updating and finalizing the manuscript, also with additional contributions from the meeting participants.*

COMPLETED

APPROVED

*The review of the draft manual by Dr. Ken Tanaka, National Police Agency, Japan, is also greatly acknowledged.

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RECOMMENDED METHODS FOR
THE IDENTIFICATION AND ANALYSIS OF

AMPHETAMINE, METHAMPHETAMINE AND THEIR RING-SUBSTITUTED ANALOGUES IN SEIZED MATERIALS

(revised and updated)

MANUAL FOR USE BY NATIONAL DRUG TESTING LABORATORIES

I. INTRODUCTION

International attention is focusing more and more on the growing issue of amphetamine-type stimulants (ATS). Particularly over the last 10 to 15 years, abuse of ATS, involving amphetamines (amphetamine and methamphetamine) and substances of the "ecstasy"-group (MDMA, MDA, MDEA, etc.), has become a global problem. There are regional differences, but today no country is spared one of the many facets of ATS manufacture, trafficking or abuse.

This new situation, involving often new and unfamiliar ATS, or combinations, and trafficking trends, presents a challenge both to national law enforcement authorities and to the scientific staff of forensic laboratories.

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The present and previous manuals suggest approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination, leaving room also for adaptation to the level of sophistication of different laboratories. For the first time in this series of publications, the present manual has

in a typical stereochemical make-up. Amphetamine, for example, and most ring-substituted ATS, are typically encountered as the racemate, while methamphetamine is frequently seen as S-, or dextro, enantiomer (also known as "Ice", or "Shabu"), in addition to the racemate. The analysis of optical isomers is described in chapter VI.G. below.

B. PHYSICAL CHARACTERISTICS

Melting/boiling points: The melting and/or boiling points are available for the most commonly encountered ATS. The analyst should be aware, however, that such data refer to pure substances.* Except for high purity ATS, such as crystalline methamphetamine ("Ice"), melting points should therefore only be used as a presumptive test (for the use of melting points for the differentiation of isomers, see chapter VI.G.1, below).

Solubilities: The solubilities of selected ATS and their salts are provided in the section on anion tests (see p. 21 below). Selective re-crystallization based on differences in solubilities can be used for the separation of some ATS salts (see Chapter VI.F. on FTIR, below).

Spectroscopic data: Mass spectral (MS), infra red (IR) and nuclear magnetic resonance (NMR) data of the most common ATS are available in the earlier edition of the two UN manuals related to the analysis of ATS, namely, "Recommended methods for testing amphetamine and methamphetamine" (ST/NAR/9), and "Recommended methods for testing illicit ring-substituted amphetamine derivatives" (ST/NAR/12). Data can also be accessed at the Laboratory and Scientific Section's web page.

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*The analyst should also be aware that melting points for some ATS may also vary depending on the solvent used for crystallization.

V. ILLICIT ATS MANUFACTURE

Knowledge of illicit manufacturing routes of drugs of abuse can play an important role in the interpretation of analytical results, especially in those cases where more in-depth analyses of impurities and manufacturing by-products, so-called impurity profiling studies, are carried out.

Use of illicitly obtained or published methods ("underground literature" or internet) for synthesis, inexperienced clandestine "chemists", inappropriate laboratory equipment and lack of laboratory quality control often result in impure and inferior products, and variability in quality and potency. As a consequence, illicitly manufactured drugs often contain by-products and intermediates stemming from impure starting materials, incomplete reactions, and inadequate purification of intermediates and the final synthetic product. The types and quantities of impurities present in illicit ATS samples (the "impurity profile") largely depend on the method of synthesis, the proportions, source and purity of starting materials, the reaction conditions, and the purification procedures, if any.

The presence or absence of specific impurities (markers) can be useful in determining the synthetic route employed, and the starting materials (precursors) used. Solvent analysis can further add to the body of information, and thus can be a useful tool for ATS sample comparison and characterization.

While impurity profiling studies are not the subject of this manual, some of the methods described can be adapted for such purposes.*

Several synthesis routes for ATS are described in the literature and used by illegal/clandestine manufacturers. Most commonly used synthetic methods for the illicit manufacture of amphetamine can be also altered to produce methamphetamine or ring-substituted amphetamines. This is most often accomplished by substituting the amine source or the source of the aromatic ring, respectively, during the reaction process. In general, the availability of precursors greatly determines the choice of synthesis route used in illicit operations.

Brief descriptions of the most commonly employed synthetic routes for amphetamine, methamphetamine and MDMA are presented below.**

Synthesis routes are classified on the basis of the reduction species used in the reaction and the reduction mechanism. In practice, many of those reactions

---

*For a general introduction to the subject, the reader is referred to the United Nations manual "Drug characterization/impurity profiling: Background and concepts" (ST/NAR/32/Rev.1, 2001); for more specific methods and approaches for the impurity profiling of methamphetamine see also UNODC Scientific and Technical Publication No.17 (SCITEC/17), 2000.

**For additional details, the reader is referred to the United Nations manual "Clandestine manufacture of substances under international control" (ST/NAR/10/Rev.2, 1998)

II. USE OF THE MANUAL

Not all methods described in this manual need to be applied to all samples suspected to consist of or contain amphetamine, methamphetamine or other ATS. The choice of the methodology and approach to their analysis remains within the discretion of the analyst and depends on the type of drug involved, the availability of appropriate instrumentation and of reference materials as well as on the level of legally acceptable proof in the jurisdiction within which the analyst works.

While it is therefore recognized that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory, good laboratory practice (GLP) requires that an analytical approach to establish the identity of a controlled drug in suspected material must, as a minimum, entail the determination of at least two uncorrelated parameters. The selection of these parameters in any particular case would have to take into account the drug involved and the laboratory resources available to the analyst. When possible, three entirely different analytical techniques should be used, for example: colour tests, chromatography (e.g., TLC, GC or HPLC) and spectroscopy (e.g., IR or UV). Hyphenated techniques, such as GC-MS, count as two parameters, provided the information from both techniques is used (i.e. retention time and mass spectral characteristics).

Attention is also drawn to the vital importance of the availability to drug analysts of reference books on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature. UNODC assists laboratories in this regard by providing, upon request, selected articles from the scientific literature.

UNODC's Laboratory and Scientific Section would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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United Nations Office on Drugs and Crime
Vienna International Centre
P.O. Box 500
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Fax: (+43-1) 26060-5967
E-mail: lab@unodc.org

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

IV. DESCRIPTION OF PURE COMPOUNDS

Seized ATS are commonly encountered in the form of salts, in particular as hydrochloride, sulphate, phosphate, or bromide salt. However, it is not uncommon in clandestine laboratory investigations to find those compounds in base form as well (usually a brownish oily liquid). Salts are crystalline or powdered substances, which vary in colour from white (similar to pharmaceutical grade products) to pink, yellow or brown. They are often damp with a characteristic smell, owing to the presence of solvent and/or precursor residues.

ATS can be also found in the form of tablets. In addition to the active ATS, tablets often contain different adulterants, cutting agents, common food colours and/or different excipients and binding agents.

Amphetamine: Illicit amphetamine is frequently encountered as the sulphate salt in powder form, and rarely as tablet. Amphetamine base may be seized in clandestine laboratories, typically as a dark brown oily liquid with a characteristic unpleasant smell of 1-phenyl-2-propanone (P-2-P) and/or solvent residues.

Methamphetamine: Illicitly manufactured methamphetamine is available in different forms, depending on the geographical region. Forms include powder, crystals (commonly known as "Cristal", "Ice" or "Shabu") and tablets (commonly known as "Yaba"). The most frequently encountered salt form is the hydrochloride.

Methylenedioxy ring-substituted ATS: MDMA, MDA, and MDEA are usually found as tablets which may or may not bear one of a logo. Powders are only occasionally found, but typically contain high concentration of active substances. Tablets are frequently brightly coloured; they often vary in size. The drug content usually ranges from 40-140 mg. Regional differences in drug content, and changes over time, are known. In Europe, for example, the average MDMA content in ecstasy tablets has dropped to about 60-70 mg (compared to around 100 mg in the mid-1990s).

The most commonly encountered salt form of the methylenedioxy-type ATS is the hydrochloride, but phosphate and bromide salts are also seen.

A. STEREOCHEMISTRY

Most ATS have at least one chiral centre and can therefore be found as a racemic mixture or as individual enantiomers.* In illicit markets, most ATS are encountered

*The terms (d) or (+), (l) or (-) and (d,l) or (±) are typically used to designate the optical rotation of chiral substances. (R) and (S) designations describe the absolute steric configuration of substituents at individual chiral centres, and are preferred, especially in the case of diastereomers.

C. GAS CHROMATOGRAPHY (GC)—FLAME IONIZATION DETECTOR (FID)

For the GC analysis of ATS, the general principles of the techniques apply. Today, the GC instrument of choice for routine analytical work is the narrow bore capillary gas chromatograph, using capillary columns with internal diameters between 0.2 and 0.32 mm.

It is recognized that there are laboratories, which for a variety of reasons, may wish to maintain a packed column system. For those laboratories, a method using packed columns was described in an earlier edition of this manual. GC procedures utilizing a megabore capillary column (0.53 mm internal diameter) represent a means to improve on resolving power compared to packed columns, and are more robust than narrow bore capillary column systems. Older GC systems that are designed for packed columns can be converted for use with megabore columns.

Preparation of ATS standard and sample solutions

ATS standard solutions: weigh approximately 25 mg of ATS standard salt(s)* of interest into a 25 ml volumetric flask and make up to the mark with water. Pipette an aliquot of 1 to 5 ml of this solution into a 10 ml glass stoppered test tube. Add drop-wise a 5% solution of sodium hydroxide until pH 10. Then add 5 ml of extracting solvent**.

Stopper and invert the test tube at least 10 times or vortex for 1 min and let stand until layers separate.*** Using a Pasteur pipette, transfer the solvent layer (e.g., chloroform) through anhydrous sodium sulphate layer into a GC vial.

Inject 1-2 μl of the solvent layer into the GC.

ATS sample solutions (unknown ATS sample): weigh 25 to 150 mg of sample, depending on the anticipated purity, to obtain a final concentration of about

*Occasionally, ATS standards can be obtained in the base form. In those cases, extraction is not required. In general, however, it is important that the form of standards and samples be always the same.

**All analytes must be completely soluble in the extraction solvent. The extraction solvent must be immiscible with aqueous layer. Suitable solvents include n-hexane, chloroform, methylene chloride or butyl acetate.

***When chloroform is used as extracting solvent, emulsions may form. In such cases, addition of NaCl improves the extraction rate by breaking the emulsion. If modern shakers are used and the mixture is then centrifuged for separation of the two layers, the formation of emulsions normally does not occur.

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Karl Pfleger, Hans H. Maurer, Armin Weber (2000), *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites: Parts I-IV*, Wiley.

UNODC also has a limited collection of mass spectra related to major ATS. The collection can be accessed from the Internet or, upon request, it can also be made available on CD-ROM.

E. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

In addition to GC, HPLC is another major separation technique commonly used in forensic drug analysis.

For ease of sample preparation, best reproducibility and detectability, reversed phase chromatography is recommended for the analysis of ATS and their ring substituted analogues. 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 before final selection of the column.

Since there are a large variety of stationary and mobile phases available to the analyst, only guidelines are presented below.

Method

Preparation of ATS standard and sample solutions

Dissolve an appropriate amount of standard or sample in the mobile phase, targeting a concentration of the active component between 0.05-0.50 mg/ml. Sample solutions should be filtered prior to analysis.

Stock and standard solutions must be prepared from reference standards. Working standards should be within the linear range of the detector and approximately 80-120% of the target concentration. Multiple point calibration is desirable but a single standard method is also acceptable.

Operating conditions

Detector:	Diode array detector, rapid scanning or variable wavelength detector, UV 200-210 nm (also use 280-290 nm for methylenedioxy-substituted phenethylamines)
Stationary phase:	C8 or C18 with 5 μm particle size or smaller
Column length:	≤ 30 cm
Column diameter:	≤ 5.0 mm

Allow a portion of the chloroform solution to evaporate directly onto a KBr disc and record the infrared spectrum of the free base, for example, by the thin film technique on KBr discs.

Isolation of the ATS salt

Triturate a 20-50 mg portion of the sample with 1-2 ml of chloroform. Filter, collect the extract and concentrate by using a gentle stream of nitrogen. Induce crystallization, filter, dry crystals, and run the infrared spectrum of the resulting ATS salt by one of the methods described below.

Methods

Spectra of ATS *salts* are recorded using samples prepared by the following methods:

- KBr halide disc (1-1.5%)
- Nujol mull methods
- Direct method, e.g. diffuse reflectance ATR
- Diffuse reflectance method

Spectra of ATS *bases* are recorded using samples prepared by the following methods:

- Thin film method
- IR cards
- Direct method, e.g. diffuse reflectance ATR

Alternative IR sample preparation methods for methamphetamine

Serial dry extraction method

Place 200 mg of powdered methamphetamine sample into a disposable Pasteur pipette with a glass wool plug at the end. Wash the sample with two 1 ml-portions of acetone and collect the acetone fraction. After air-drying wash the column with two 1 ml-portions of chloroform and collect the chloroform fraction. Allow the column to dry again and then rinse with two 1 ml-aliquots of methanol and collect the methanol fraction. The insoluble material can then be removed from the pipette. All fractions are examined by infrared spectroscopy for identification.

Physical separation/selective re-crystallisation

The procedures below work well for the type of mixtures indicated; they do not work as well with amphetamine hydrochloride.

VII. ADDITIONAL ANALYTICAL TECHNIQUES FOR THE ANALYSIS OF ATS

There are a number of additional analytical techniques suitable for the forensic identification and/or quantitation of ATS, such as:

- Capillary electrophoresis (CE)
- Gas chromatography-Fourier transformed infrared spectroscopy (GC-FTIR)
- LC-MS and CE-MS
- Near Infrared (NIR) Spectroscopy
- Nuclear magnetic resonance (NMR) spectroscopy (qualitative and quantitative)
- Quantitative FTIR
- Quantitative TLC
- Raman FTIR spectroscopy
- Solid phase-micro extraction-gas chromatography (SPME-GC)

A description of most of these techniques is beyond the scope of this "Manual on recommended analytical methods for ATS", and the analyst is referred to a complementary "Manual on analytical techniques generally, their characteristics and practical use for drug analysis". Four techniques, qualitative NMR, CE, SPME-GC, and GC-FTIR are briefly described below, because they offer specific, attractive options for the analysis of ATS.

A. ^{1}H-NUCLEAR MAGNETIC RESONANCE (NMR) TECHNIQUES

The availability of a large number of positional isomers of structurally related ATS, especially ring-substituted ATS, requires effective tools that provide the necessary structural information for their differentiation. NMR enables the analyst to unequivocally distinguish between different ring-substituted amphetamine derivatives, even in the presence of diluents and other adulterants. Although certain substitution patterns resemble one another in the area corresponding to the protons of the alkyl side chain, the integrated spectrum and the pattern of the aromatic proton signals allow their distinction from one another. While being a powerful tool for the identification of analogues, the cost of NMR spectroscopy and the technical expertise required prevent its widespread application in routine analysis.

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- LC-MS and CE-MS
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- Nuclear magnetic resonance (NMR) spectroscopy (qualitative and quantitative)
- Quantitative FTIR
- Quantitative TLC
- Raman FTIR spectroscopy
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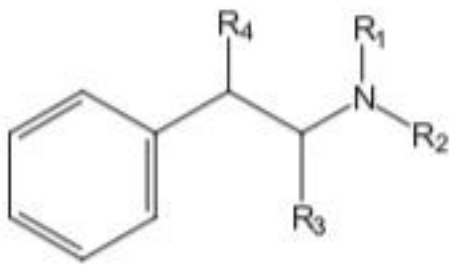
Annex I. Chemical structures of selected ATS

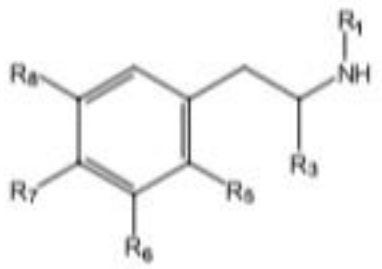
Table A1. Non-ring substituted amphetamines



Note: Unless indicated specifically, names do not refer to individual enantiomers

Common name	*IUPAC name*	*R1*	*R2*	*R3*	*R4*
Amphetamine	1-methyl-2-phenylethylamine	H	H	CH_3	H
Methamphetamine	*N*-methyl-*N*-(1-methyl-2-phenylethyl)amine	CH_3	H	CH_3	H
N-Ethylamphetamine	*N*-ethyl-*N*-(1-methyl-2-phenylethyl)amine	C_2H_5	H	CH_3	H
Dimethylamphetamine	*N,N*-dimethyl-*N*-(1-methyl-2-phenylethyl)amine	CH_3	CH_3	CH_3	H
N-Hydroxyamphetamine	*N*-(1-methyl-2-phenylethyl) hydroxylamine	H	OH	CH_3	H
N-Hydroxymethamphetamine	*N*-methyl-*N*-(1-methyl-2-phenylethyl) hydroxylamine	CH_3	OH	CH_3	H
Cathine	(1S,2S)-2-amino-1-phenylpropan-1-ol	H	H	CH_3	OH
Cathinone	2-amino-1-phenylpropan-1-one	H	H	CH_3	=O
Methcathinone	2-(methylamino)-1-phenylpropan-1-one	CH_3	H	CH_3	=O
Fenetylline	1,3-dimethyl-7-{2-[(1-methyl-2-phenylethyl)amino] ethyl}-3,7-dihydro-1*H*-purine-2,6-dione	H	theo-phylline	CH_3	H
Phenylpropylmethylamine (PPMA)	*N*-methyl-*N*-(2-phenylpropyl)amine	CH3	H	CH_3	CH_3

Table A3. Other ring substituted amphetamines



Note: Unless indicated specifically, names do not refer to individual enantiomers

Common name	*IUPAC name*	*R1*	*R3*	*R5*	*R6*	*R7*	*R8*
2,4,5-Ring substituted phenethylamines							
4-Bromo-2,5-dimethoxy-phenetylamine (2C-B, Nexus)	2-(4-bromo-2,5-dimethoxy-phenyl)ethanamine	H	H	OCH_3	H	Br	OCH_3
4-Methylthio-2,5-dimethoxy-phenethylamine (2C-T)	2-[2,5-dimethoxy-4-(methyl-thio)phenyl]ethanamine	H	H	OCH_3	H	SCH_3	OCH_3
4-Ethylthio-2,5-dimethoxy-phenethylamine (2C-T-2)	2-[4-(ethylthio)-2,5-di-methoxyphenyl]ethanamine	H	H	OCH_3	H	SC_2H_5	OCH_3
4-Propylthio-2,5-dimethoxy-phenethylamine (2C-T-7)	2-[2,5-dimethoxy-4-(propyl-thio)phenyl]ethanamine	H	H	OCH_3	H	SC_3H_7	OCH_3
4-Chloro-2,5-dimethoxy-phenethylamine (2C-C)	2-(4-chloro-2,5-dimethoxy-phenyl)ethanamine	H	H	OCH_3	H	Cl	OCH_3
4-Iodo-2,5-dimethoxy-phenethylamine (2C-I)	2-(4-iodo-2,5-dimethoxy-phenyl)ethanamine	H	H	OCH_3	H	I	OCH_3
2,4,5-Ring substituted amphetamines							
2,4,5-Trimethoxyamphetamine (TMA-2)	1-(2,4,5-trimethoxyphenyl) propan-2-amine	H	CH_3	OCH_3	H	OCH_3	OCH_3
4-Methyl-2,5-dimethoxy-amphetamine (DOM, STP)	1-(2,5-dimethoxy-4-methylphenyl)propan-2-amine	H	CH_3	OCH_3	H	CH_3	OCH_3
4-Bromo-2,5-dimethoxy-amphetamine (DOB, Bromo-STP, BDMA)	1-(4-bromo-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	Br	OCH_3
4-Chloro-2,5-dimethoxy-amphetamine (DOC)	1-(4-chloro-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	Cl	OCH_3
4-Iodo-2,5-dimethoxy-amphetamine (DOI)	1-(4-iodo-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	I	OCH_3
4-Ethyl-2,5-dimethoxy-amphetamine (DOET)	1-(4-ethyl-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	C_2H_5	OCH_3
Other ring substitution patterns (phenethylamines and amphetamines)							
3,4,5-Trimethoxyphenethyl-amine (mescaline)	2-(3,4,5-trimethoxyphenyl) ethanamine	H	H	H	OCH_3	OCH_3	OCH_3
para-Methoxyamphetamine (PMA)	3-(4-methoxyphenyl)-1-methylpropylamine	H	CH_3	H	H	OCH_3	H
para-Methoxy-methamphetamine (PMMA)	*N*-[2-(4-methoxyphenyl)-1-methylethyl]-*N*-methylamine	CH_3	CH_3	H	H	OCH_3	H
2,5-Dimethoxyamphetamine (DMA)	1-(2,5-dimethoxyphenyl) propan-2-amine	H	CH_3	OCH_3	H	H	OCH_3
3,4,5-Trimethoxyamphetamine (TMA)	1-(3,4,5-trimethoxyphenyl) propan-2-amine	H	CH_3	H	OCH_3	OCH_3	OCH_3
4-Methylthioamphetamine (4-MTA)	1-[4-(methylthio)phenyl] propan-2-amine	H	CH_3	H	H	SCH_3	H

Annex II. Preparation of colour and anion test reagents

All reagents should be prepared according to an established procedure.

Chen's test

Reagent 1: Add 1 ml of glacial acetic acid to 100 ml of water (=1% (v/v) aqueous acetic acid solution)

Reagent 2: Dissolve 1 g of copper(II) sulphate in 100 ml of water (=1% (w/v) aqueous $CuSO_4$ solution)

Reagent 3: Dissolve 8 g of sodium hydroxide in 100 ml of water (=2N aqueous sodium hydroxide solution).

Gallic acid test

Reagent: Dissolve 0.1 g of gallic acid in 20 ml of concentrated sulphuric acid (=0.5% (w/v) solution)

Marquis test

Reagent 1: Add 8-10 drops (approx. 0.25 ml) of 37% formaldehyde solution to 10 ml of glacial acetic acid

Reagent 2: Concentrated sulphuric acid

Phosphate test

Ammonium molybdate: Dissolve 10 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}$ x $4H_2O$] in 100 ml of water (=10% (w/v) aqueous ammonium molybdate solution)

Nitric acid: Carefully add 10 ml of nitric acid to 90 ml water (= 10% (v/v) nitric acid solution)

Silver nitrate test (also known as Chloride test)

Dissolve 1.7 g of silver nitrate in 100ml of water (=1.7% aqueous silver nitrate solution).

Simon's test

Reagent 1: Dissolve 2 g of sodium carbonate in 100 ml of water (=2% aqueous sodium carbonate solution)

Reagent 2: Dissolve 0.9 g of sodium nitroprusside in 90 ml of water (=1% aqueous sodium nitroprusside solution)

Reagent 3: Mix 10 ml of acetaldehyde solution and 10 ml of ethanol (=50% (v/v) ethanolic acetaldehyde solution)

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Reagent: Dissolve 0.1 g of gallic acid in 20 ml of concentrated sulphuric acid (=0.5% (w/v) solution)

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Annex V. Validated HPLC method for quantitation of selected ATS

Below is a validated method for the HPLC quantitation of selected ATS solutes including amphetamine, methamphetamine, phentermine and MDMA.

HPLC Method for quantitation of ATS

Preparation of ATS standard and sample solutions

ATS standard solution

Weigh an appropriate amount of standard ATS(s) into a volumetric flask to obtain a final concentration of approximately 0.50 mg/ml. Dilute to volume with methanol.

ATS sample solution

Weigh an appropriate amount of sample into a volumetric flask so that the final phenethlamine concentration is approximately that of standard solution. Dilute to volume with methanol.

HPLC operating conditions

Column:	5 μm Luna C18 (Phenomenex, Torrance, CA, USA) 150 x 3.0 mm
Column temperature:	35°C
Injection:	5 μl
Mobile phase:	10% acetonitrile, 90% (50 mM phosphate + 50 mM triethanolamine, pH 2.2);[a] flow rate 1.0 ml/min
UV wavelength:	210 nm

[a]The buffer is prepared by dissolving 22.5 ml concentrated phosphoric acid into 4 liters of HPLC grade water. Approximately 25 ml triethanolamine is added slowly to adjust solution pH to 2.2.

Approximate relative migration times

Nicotinimide	0.28
Phenethylamine	0.55
Phenylpropanolamine	0.56
Doxylamine	0.56
Procaine	0.62
Ephedrine	0.64
Pseudoephedrine	0.65
Amphetamine	0.82
Acetominiphen	0.93

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Sulphate test

Dissolve 5 g of barium chloride dihydrate in 100 ml of water (= approx. 5% aqueous barium chloride solution).

There are other established procedures for the preparation of colour test reagents, for example, Clarke's, which show slight variation in recipes.

Narkotik Kimia JSJN Bukit Aman

University Of Science Malaysian USM

IT Data Science IDC Medical Speciment Laboratory

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ATLASSIAN Community

Produser.ce Lee (DS) (USM)

Narkotik Kimia (JSJN): up your team IDC by building University Of Science Malaysian >your IRC Card DS.
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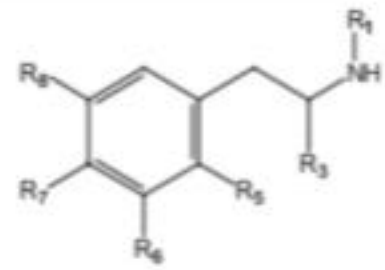
SYAFIQ STEVEN LEE
860512-38-5603

UNIVERSITY OF SCIENCE IDC LABORATORY MALAYSIAN

PRODUSER.CE LEE (DS)

Produser.ce Lee

Table A3. Other ring substituted amphetamines



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4-Methylthio-2,5-dimethoxy-phenethylamine (2C-T)	2-[2,5-dimethoxy-4-(methyl-thio)phenyl]ethanamine	H	H	OCH_3	H	SCH_3	OCH_3
4-Ethylthio-2,5-dimethoxy-phenethylamine (2C-T-2)	2-[4-(ethylthio)-2,5-di-methoxyphenyl]ethanamine	H	H	OCH_3	H	SC_2H_5	OCH_3
4-Propylthio-2,5-dimethoxy-phenethylamine (2C-T-7)	2-[2,5-dimethoxy-4-(propyl-thio)phenyl]ethanamine	H	H	OCH_3	H	SC_3H_7	OCH_3
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2,4,5-Trimethoxyamphetamine (TMA-2)	1-(2,4,5-trimethoxyphenyl) propan-2-amine	H	CH_3	OCH_3	H	OCH_3	OCH_3
4-Methyl-2,5-dimethoxy-amphetamine (DOM, STP)	1-(2,5-dimethoxy-4-methylphenyl)propan-2-amine	H	CH_3	OCH_3	H	CH_3	OCH_3
4-Bromo-2,5-dimethoxy-amphetamine (DOB, Bromo-STP, BDMA)	1-(4-bromo-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	Br	OCH_3
4-Chloro-2,5-dimethoxy-amphetamine (DOC)	1-(4-chloro-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	Cl	OCH_3
4-Iodo-2,5-dimethoxy-amphetamine (DOI)	1-(4-iodo-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	I	OCH_3
4-Ethyl-2,5-dimethoxy-amphetamine (DOET)	1-(4-ethyl-2,5-dimethoxy-phenyl)propan-2-amine	H	CH_3	OCH_3	H	C_2H_5	OCH_3
Other ring substitution patterns (phenethylamines and amphetamines)							
3,4,5-Trimethoxyphenethyl-amine (mescaline)	2-(3,4,5-trimethoxyphenyl) ethanamine	H	H	H	OCH_3	OCH_3	OCH_3
para-Methoxyamphetamine (PMA)	3-(4-methoxyphenyl)-1-methylpropylamine	H	CH_3	H	H	OCH_3	H
para-Methoxy-methamphetamine (PMMA)	*N*-[2-(4-methoxyphenyl)-1-methylethyl]-*N*-methylamine	CH_3	CH_3	H	H	OCH_3	H
2,5-Dimethoxyamphetamine (DMA)	1-(2,5-dimethoxyphenyl) propan-2-amine	H	CH_3	OCH_3	H	H	OCH_3
3,4,5-Trimethoxyamphetamine (TMA)	1-(3,4,5-trimethoxyphenyl) propan-2-amine	H	CH_3	H	OCH_3	OCH_3	OCH_3
4-Methylthioamphetamine (4-MTA)	1-[4-(methylthio)phenyl] propan-2-amine	H	CH_3	H	H	SCH_3	H

When the fibre is inserted into the heated GC injector port, the extracted amines are thermally desorbed. In HPLC, CEC and CE, the solvent mixture elutes the amines form the fibre.

(Trace) analysis from aqueous samples

The fibre is dipped directly into the aqueous sample solution, which was made alkaline in order to release the ATS free bases. The sample solution is stirred to increase the exchange of the compounds between the solution and the fibre for a speedier extraction. Sample analysis is carried out in the same way as described in the headspace method.

Further reading

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860512-38-5603

D. GAS CHROMATOGRAPHY-FOURIER TRANSFORM INFRARED SPECTROSCOPY (GC-FTIR)

GC-FTIR, as another hyphenated technique, unifies the advantages of a the GC separation technique with the high analyte specificity of IR. GC-FTIR with a lightpipe flow cell has no limitations as to the carrier gas flow, which makes it idealfor use with short wide bore columns, thus resulting in efficient and fast analy-sis. For instance, most common ATS can be analyzed within less than five min-utes. However, since the technique is rather insensitive, large amounts of substancehave to be injected, and the film thickness of the stationary phase is critical inorder not to overload the column.