DIVISION OF NARCOTIC DRUGS
Vienna

RECOMMENDED METHODS FOR TESTING AMPHETAMINE AND METHAMPHETAMINE

MANUAL FOR USE BY NATIONAL NARCOTICS LABORATORIES

UNITED NATIONS
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ST/NAR/9

C O N T E N T S

INTRODUCTION

Background

Over the past few years there has been a considerable increase in the number of scheduled substances newly included under international control. This increase reflects a rapid diversification of drugs of abuse, and the consequent increase of regulatory efforts results in turn in a larger number of controlled substances and in better but, at the same time, more stringent national legislation and sentencing provisions. At the same time, the seized quantities of drugs already under control, such as the opiates, cocaine and coca paste, cannabis products, amphetamine and related compounds have also shown an alarming and unprecedented increase in certain regions. This new situation, involving an increase both in the frequency and volume of seizures, presents a challenge not only to national law enforcement authorities, but also to the technical and scientific staff of forensic laboratories.

Owing to the ingenuity of illicit producers and promoters, unexpected new illicit drugs or combinations of drugs appear on the illicit market, requiring rapid and adequate action as well as ingenuity on the part of forensic chemists. Similarly, the increased number of controlled substances and of related legislative provisions place additional pressure on national forensic and narcotic laboratories and their staff. Analysts have to be able to deal with more substances and preparations and to use faster, more accurate and more specific methods of identification and analysis. In addition, the international character of drug trafficking requires the speedy exchange of analytical data between laboratories and law enforcement authorities both on the national and the international levels. Development of internationally acceptable methods of testing would contribute greatly to the achievement of these objectives, and this possibility has been under consideration for some time.

At its eighth special session in February 1984, the Commission on Narcotic Drugs requested the Secretary-General "to investigate the possibility of reaching agreement at the regional and interregional levels on recommended methods of analysis of drugs seized from the traffic". The Commission was of the opinion that closer scrutiny and harmonisation of the wide variety of analytical methods in use at the national level would not only ease the task of the staff of national institutions but would also facilitate the exchange of information at regional and interregional levels.

Purpose of the manual

In response to the Commission's request, a group of eleven experts and two consultants was convened in September 1986 by the Division of Narcotic Drugs in Kuala Lumpur at the invitation of the Government of

Malaysia. The present manual published by the United Nations Division of Narcotic Drugs reflects the conclusions of the group of experts and has been designed to provide practical assistance to national authorities by describing recommended methods to be used in forensic laboratories for the identification and analysis of amphetamine and methamphetamine products. The manual may also serve as a guide to national authorities in assessing existing methods used within their own government and university laboratories.

This manual is the fourth in a series of similar publications dealing with the identification and analysis of various groups of drugs under international control; it was preceeded by manuals on heroin (ST/NAR/6), cocaine (ST/NAR/7) and cannabis (ST/NAR/8) analysis.

These manuals suggest approaches that may help the forensic analyst to select a technique appropriate to the sample currently being examined. The analyst may then choose to follow any of the methods described in the manual, as each method can be expected to produce reliable analytical information with respect to the samples to which they are applied. Each method has been used for a number of years in reputable forensic laboratories and has been published in the scientific literature. In identifying these methods, the expert group was aware that many other useful and acceptable methods produce worthwhile analysis and information for the forensic analyst, and that a number of other acceptable options are recorded in the forensic scientific literature.

Use of the manual

Few methods are perfect, least of all in forensic drug analysis where the materials under examination are very likely to show significant variation both in their physical form and chemical composition. The choice of methodology and approach to analysis remains within the control of the analyst working within his or her own country. The analyst alone has seen the suspect material and can best judge the correct approach to the problem at hand. Furthermore the choice of methods may necessarily depend on the availability of reference materials and of instrumentation.

Not all the methods listed need to be applied to all samples suspected to contain amphetamine or methamphetamine. Requirements may vary, for example, as a result of local trends in samples encountered, facilities available, and the standard of proof acceptable in the prosecution system within which the analyst works. The more complex methods are needed only for certain forensic requirements, such as comparison of samples or for source determination.

In order to establish the identity of any controlled drug, it is suggested that the criteria should be at least two independent analytical parameters. The selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. For example, two uncorrelated TLC systems would count as two parameters. Uncorrelated TLC systems in this context means that either the solvent systems or the coating on the plates are

completely different. When possible, three entirely different analytical techniques should be used, for example: colour test, chromatography (TLC, GLC or HPLC) and spectroscopy (IR or UV). The actual choice of parameters is left to the discretion of the chemist.

Attention is also drawn to the vital importance of the availability of textbooks on drugs of abuse and analytical techniques. Furthermore, the analyst must continually keep abreast of current trends in analysis, consistently following current analytical and forensic science literature. For this purpose, attention is drawn to the Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control (ST/NAR/1), a vital tool for forensic laboratories, and to the Manual on Staff Skill Requirements and Basic Equipment for Narcotics Laboratories (ST/NAR/2), both published by the Division of Narcotic Drugs. The latter publication lists bibliographic references as well as a selection of well-known journals in the field. Analysts should refer to these and to previous manuals in this series for general descriptions of the analytical techniques included in this manual.

Close liaison with national law enforcement and judicial authorities as well as between national narcotic laboratories and those at the regional level can lead to greater awareness of the latest trends in drug presentation, the illicit traffic, smuggling techniques and the preparation of evidence to courts of law. These in turn will produce a more meaningful choice of analytical techniques to be applied to the latest submissions.

It is equally important that the latest information on changes in drugs available in the illicit traffic be quickly disseminated. This may often need to be done prior to publication in specialized periodicals dealing with forensic and other chemical analyses, since these publications are available to the forensic community some two to three years after the changes become known. The value of frequently published national reports on the latest information on such changes in drugs and on work being undertaken and analytical results obtained within individual laboratories cannot be over-emphasized.

The Division of Narcotic Drugs would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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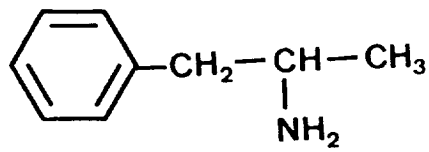
I. DESCRIPTION OF THE PURE COMPOUNDS

AMPHETAMINE		Boiling points (°C)
2-amino-1-phenylpropane	d	203 - 204
α-methylbenzeneethanamine	l	----
α-methylphenethylamine	dl	200 - 203

Scheduled under the "Convention on Psychotropic Substances 1971"

AMPHETAMINE = (dl), (+) racemic mixture
DEXAMPHETAMINE = (d-), (+), (S) isomer
LEVAMPHETAMINE = (l-), (-), (R) isomer



$C_9H_{13}N$
M Wt. = 135.2

AMPHETAMINE PHOSPHATE		Melting points (°C)
$C_9H_{13}N.H_3PO_4$	d	300 (decomp)
M Wt. = 233.2	l	----
	dl	300 (decomp)

AMPHETAMINE SULPHATE		Melting points (°C)
$(C_9H_{13}N)_2.H_2SO_4$	d	300 (decomp)
M Wt. = 368.5	l	----
	dl	280 - 281

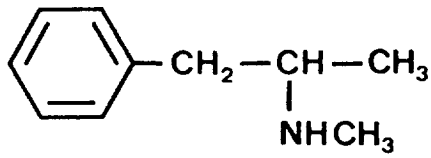
SOLUBILITIES

	Base	Phosphate	Sulphate
Water	slightly soluble	soluble	soluble
Ethanol	soluble	slightly soluble	slightly soluble
Diethyl ether	soluble	insoluble	almost insoluble
Chloroform	soluble	insoluble	insoluble

METHAMPHETAMINE		Boiling point (°C)
2-methylamino-1-phenylpropane	d	208 - 210
N,α-dimethylbenzeneethanamine	l	210
N,α-dimethylphenethylamine	dl	209 - 210
N,-methylamphetamine		
Methylamphetamine		
Phenylisopropylmethylamine		

Scheduled under the "Convention on Psychotropic Substances 1971"

METHAMPHETAMINE = (d-), (+), (S) isomer
LEVOMETHAMPHETAMINE = (l-), (-), (R) isomer



$C_{10}H_{15}N$
M Wt. = 149.2

METHAMPHETAMINE HYDROCHLORIDE		Melting point (°C)
$C_{10}H_{15}N.HCl$	d	170 - 175
M Wt. = 185.7	l	170 - 171
	dl	131 - 135

SOLUBILITIES

	Base	Hydrochloride
Water	slightly soluble	soluble
Ethanol	soluble	soluble
Diethyl ether	soluble	insoluble
Chloroform	soluble	soluble

II. PRODUCTION AND CHEMICAL CHARACTERISTICS OF ILLICIT AMPHETAMINE/METHAMPHETAMINE

Although a considerable amount has been diverted from licit manufacture, the majority of the amphetamine and methamphetamine products on the illicit market are produced by clandestine laboratories. The free bases of amphetamine and methamphetamine are liquids which are not very stable. Therefore, they are now more often found as powders in the form of amphetamine sulphate or phosphate or methamphetamine hydrochloride. Aqueous solutions of methamphetamine HCl commonly called "gold fish" are available in some countries and illicitly produced tablets are also in wide-spread use. Whereas illicitly produced amphetamine is still widely available in Europe, methamphetamine is more popular in North America and Japan.

Lack of quality control and variability in potency are characteristics of illicit amphetamine and methamphetamine samples. They often contain by-products and intermediates resulting from impure starting materials, incomplete reactions and inadequate purification of intermediates and the final synthetic product. These by-products and intermediates can provide valuable information concerning the illicit method of manufacture. Knowledge of the impurities is important for many reasons. The harmful effects of impurities can be evaluated, the potential danger publicized and treatment provided, if necessary. The presence or absence of specific impurities is useful in determining the synthetic route employed and in determining whether samples are from a common origin and/or from licit or illicit production. Awareness of the presence of particular impurities is important to the forensic analyst because of their potential interference in the analytical techniques employed for analysis of the amphetamine/methamphetamine exhibit.

The types and amounts of impurities present depend on the method of synthesis, the proportions and source of starting materials, the reaction time and temperature, the conditions of hydrolysis of intermediates and the purification procedures, if any, employed. Most impurities are weakly basic or neutral in nature and are normally present in the finished product at less than the 2 - 3% level.

Many methods are available for the illicit synthesis of amphetamine/methamphetamine. The "Leuckart" reaction has been the most popular, since the synthesis is simple, rapid, gives a good yield and does not involve any particularly hazardous procedure. It may be considered as a three step reaction involving a formylation stage, hydrolysis and then purification. For amphetamine, the condensation of phenyl-2-propanone (P-2-P, benzylmethylketone, BMK) with formamide, sometimes in the presence of formic acid, or by the use of ammonium formate gives rise to a number of side-reaction products. The hydrolysis step uses sulphuric acid to hydrolyse the N-formylamphetamine intermediate. The final purification stage involves either steam

distillation or the extraction of amphetamine base with ether and precipitation as the sulphate, followed by washing with one or more organic solvents and/or recrystallization of the amphetamine sulphate.

Methamphetamine can be prepared in a similar manner employing methylamine and formic acid or N-methylformamide in the condensation step. The Leuckart reaction has been extensively studied and N-formylamphetamine or N-formylmethamphetamine and 4-methyl-5-phenyl-pyrimidine have been identified as the major impurities specific to this method. They are present normally at levels of less than 1%. Lately, formic acid has been used in the reaction and this results in the formation of N,N-di(β-phenylisopropyl)amine (DPIA) and N-formyl DPIA as major impurities in amphetamine and N,N-di(β-phenylisopropyl)methylamine (DPIMA) and N-formyl-DPIMA in methamphetamine synthesized by the Leuckart method. These have been detected at levels up to 3%. Many other impurities, including higher boiling pyridines have been identified. Although P-2-P is commercially available, its supply is controlled in some countries. One clandestine synthesis, from phenylacetic acid and acetic anhydride, gives dibenzylketone as a by-product. Impurities such as dibenzylketone introduce other impurities into the final product. Thus alpha-benzylphenethylamine and alpha-benzyl-N-methylphenethylamine have been detected in amphetamine and methamphetamine synthesized from impure P-2-P. Both of these by-products have lower LD_{50} values than does amphetamine pointing out the potential danger of impure street drugs.

Other methods for the synthesis of amphetamine and methamphetamine do not give as many route-specific impurities.

The reductive amination method in which P-2-P and a suspension of Raney nickel is reacted with a mixture of ammonia gas and hydrogen has been used in some countries for the preparation of amphetamine. To date, only low-pressure and low-temperature aminations have been reported. Other reducing agents known to have been used are platinum, aluminium powder with $HgCl_2$, and nickel plated Zn. Methamphetamine may also be prepared by this procedure using methylamine. The major impurities are Schiff bases, one postulated as being formed by the condensation of P-2-P and amphetamine. Thus, they are not route specific but could be present in any synthetic procedure involving P-2-P. Inorganic impurities from use of particular catalysts may serve as markers.

Two other common methods are the "oxime" route, in which hydroxylamine is reacted with P-2-P giving the oxime which is subsequently hydrogenated, and the "nitrostyrene" route in which condensation of P-2-P with nitroethane yields the nitrostyrene intermediate. Hydrogenation of the double bond and reduction of the nitro group of the intermediate give amphetamine. Both of these routes give benzylmethyl ketoxime as the major impurity. Its presence in the case of the oxime route is due to incomplete reaction whereas, in the nitrostyrene route, it results from partial reduction. Reductions have been carried out using both electron-transfer reagents such as sodium amalgam and sodium/ethanol, and hydride transfer reagents such as $LiAlH_4$ and $NaBH_3CN$. Aziridines have been detected in amphetamine syntheses involving hydride transfer reagents and may be of some use in

linking samples of common origin. The oxime route and subsequent electrochemical reduction is presently a popular illicit procedure in some countries.

All of the clandestine methods mentioned above employ C-N bond formation and produce in a non-stereospecific way a racemic mixture of dl-amphetamine or dl-methamphetamine. Because of the controls placed on the licit movement of P-2-P, ephedrine and pseudoephedrine have become popular starting materials for the illicit synthesis of methamphetamine. Their reduction with hydrogen iodide and red phosphorus, or by hydrogen and $Pd/BaSO_4$, directly or through the intermediate chlorephedrine (or chlorpseudo- ephedrine) formed with thionyl chloride, give methamphetamine in good yield. Impurities detected in reactions carried out by these procedures include P-2-P, iodine, chlorephedrine, ephedrine and inorganics such as Pd and Ba. If optically active (1R, 2S)-ephedrine (also known as l- or (-) ephedrine), which is readily available in some countries, is used in the reaction, d-methamphetamine is obtained. This is because the stereochemistry of the C-2 carbon remains unaffected during the dehydrohalogenation reaction sequence and retains the optical chirality at this carbon in methamphetamine. The confirmation of optical activity in the finished product together with the presence of l-ephedrine as an impurity is strong proof that this particular reaction procedure was employed. Amphetamine can be formed from phenylpropanolamine in a similar way.

The purity of the uncut drug may range from 90 - 99 percent. For trafficking purposes it is usually cut to 40% or less with a carbohydrate (glucose, lactose, sucrose, mannitol), magnesium sulphate, sodium glutamate, caffeine, ephedrine, procaine, antipyrine or phenazone. Aqueous solutions of methamphetamine HCl called "gold fish" are available in some countries.

III. PHYSICAL APPEARANCE OF ILLICIT AMPHETAMINE/METHAMPHETAMINE

Amphetamine products from licit manufacture contain the drug in the form of the sulphate or phosphate salt. They are marketed in different countries as tablets, spansule capsules, syrups and elixirs.

Methamphetamine, as the hydrochloride salt, is availale as a tablet and as a sterile solution for injection. Analysts should refer to various pharmaceutical compendia for a pictorial description and information on specific products legally available in their country.

Illicit amphetamine sulphate varies in colour from a white powder, similar to material from licit manufacture, to pink to yellow to brown depending upon the type and amount of impurities and adulterants. It is often damp with a characteristic unpleasant odour owing to the presence of solvent residues.

Illicit methamphetamine HCl is usually in the form of a caked or gummy powder. It may be white, brown or violet in colour, again depending on the presence of certain impurities.

IV. THE ANALYSIS OF MATERIALS CONTAINING AMPHETAMINE/METHAMPHETAMINE

A. Sampling

The principal reason for a sampling procedure is to produce a correct and meaningful chemical analysis. Because most methods - qualitative and quantitative - used in forensic science laboratories for the examination of drugs require very small aliquots of material, it is vital that these small aliquots be entirely representative of the bulk from which they have been drawn. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by such organizations as the Association of Official Analytical Chemists.

There may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed if, for example, the analyst wishes to preserve some part of an exhibit as visual evidence. Alternatively, it may be necessary to perform separate assays on two powder items, rather than combining the powders prior to a single assay being performed on the mixture, because each has been separately exhibited by the seizing officer, and the legal system within which the analyst works requires individual results on every exhibit which is to be taken before the courts.

To preserve valuable resources and time, forensic analysts should seek, on all possible occasions, to use an approved sampling system and thereby reduce the number of quantitative determinations needed. To facilitate such an approach, the forensic analyst may need to discuss individual situations with both seizing officers and the legal personnel with whom he works.

Amphetamine exhibits may be encountered as tablets and capsules both from the licit market by diversion and from illicit manufacture, and as fine or gummy powders. Methamphetamine is usually in the form of a powder or gummy substance, although tablets and capsules from licit and illicit sources are available in some countries. Both amphetamine base and methamphetamine base are liquids and these, as well as solutions of the salt forms, are frequently encountered in the illicit market.

1. Powders

(a) Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material - in the case of amphetamine or methamphetamine, the material will most often be a powder. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. The material should be

thoroughly homogenized prior to the application of the sequence of chemical tests, although presumptive testing may be applied at this stage if it is thought that the sampling or homogenization process will be lengthy and there is still some doubt as to the identity of the material. The simplest way to homogenize a powder is to shake it thoroughly within the clear plastic bag to which it has been transferred. If the powder contains aggregates these may be broken down by passing through successively finer sieves, or by pounding with a mortar and pestle, or by use of an adapted commercial food-mixer or food-processor.

Alternatively, the technique of coning-and-quartering can be applied, as follows: the sample is mixed by shaking or stirring. Large fragments are reduced if necessary; the material is then poured on a flat surface to form a cone. The "cone" is flattened and the material is then divided at right angles, forming quarters. Opposite quarters are taken for a sample; the remainder of the material is returned to the receptacle from which it was removed. Should further coning-and-quartering be desired to reduce sample size, particle sizes are further reduced, the material mixed thoroughly, poured onto a flat surface, and divided as before.

(b) Sampling of items consisting of more than one package

The analyst should examine the contents of all packages by eye, and possibly screen by using a simple colour test or TLC to determine:

1. If all packages contain suspect amphetamine or methamphetamine material, and/or

2. If one or more packages contain material different to that of the majority of packages. The simplest indicator is the physical appearance of the powder. If one or more packages obviously differ in content, these should be segregated and subjected to separate analysis.

The compositing of multiple container items is as follows:

(a) If there are less than 10 packages - all packages should be sampled.

(b) If there are 10 - 100 packages, randomly select 10 packages.

(c) If there are more than 100 packages - randomly select a number of packages equal to the square root of the total number of packages rounded to the next highest integer.

If the powders are found to be the same then the contents of a number of packages may be combined; the combined bulk material may then be homogenized in, for example, an adapted commercial food-processor. Alternately, the bulk may be subjected to coning-and-quartering.

When different types of material have been identified in the various packages, then each sub-group should be composited in an identical fashion to that previously outlined.

Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the dissolving solvent. If the total sample size is large, this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary to add the solvents by pipette to avoid error due to insoluble materials. Insoluble adulterants are a frequent occurrence in "street" samples seized within all countries.

(c) Sampling of materials containing gummy or large aggregates

If the particles can be easily reduced to powder, then this approach should be used and sampling procedure followed as outlined previously. Powdering may be achieved by mortar and pestle, commercial food-processor/mixer, or industrial grinder. If the material cannot be easily broken down, then random sized particles should be drawn from at least three different parts of the item. A minimum of 1 gramme should be collected, weighed accurately and subjected to assay.

2. Tablets and capsules - Commercial or licit preparations

The preliminary determination of commercial origin is a subjective one. Clear-cut examples of products of commercial origin would be dosage units resembling descriptions as pictorial representations in national compendia of pharmaceutical preparations. Commercial preparations usually undergo quality control by the manufacturer; therefore, little useful information would be gained by screening a large number of units from each package. The amount of ingredient per tablet or capsule determined will be statistically valid for the entire lot.

(a) Single container

1. 1-50 dosage units -- Randomly select 1/2 total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.

2. 51-100 dosage units -- Randomly select 20 units, proceed as above.

3. 101-1,000 dosage units -- Randomly select 30 units, proceed as above.

4. Greater than 1,000 dosage units -- Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) Multiple containers

Segregate containers by lot numbers and treat each group as described in 1.b above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalent to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, sieving through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

3. Tablets and capsules - Illicit origin

For illicit preparations, quality control may be regarded as non-existent. Wide variations may be suspected in tablet make-up, although in most instances, some of the active constituent will be present in each tablet. Some screening of individual units or containers is, therefore, necessary.

(a) Single container

Determine the total number of dosage units and the average weight per dosage unit (du).

For sample sizes up to 10 du -- Screen all dosage units.

For sample sizes from 11 du to 27 du -- Randomly select and screen 3/4 of all dosage units, rounding upward to the next higher integer.

For sample sizes from 28 du -- Randomly select and screen 1/2 of all dosage units rounding upward to the next higher integer and selecting a minimum of 21 du and a maximum of 50 du.

Based on the results of the screening tests, proceed as follows:

1. If all dosage units appear to be identical, form a composite of screened dosage units as directed for licit preparations and analyze.

2. If the sample contains two dosage forms, subdivide the sample. If necessary, screen additional dosage units until both subsamples contain material for analysis, then form two composites and analyze.

3. If more than two dosage forms are present, the strategy is to make a composite of the most abundant dosage form, then to screen additional units until a sample of the same size is formed that contains only the less abundant dosage forms. This procedure is repeated until a composite is formed for each dosage form or until the sample is exhausted.

The percentage of dosage units containing a given controlled substance or other active constituent may be estimated by using the percent of units found to contain that substance out of the total number of units which were randomly selected and screened.

(b) Multiple containers

Randomly select a number of dosage units from each of a randomly selected number of containers, as determined in the compositing procedures for licit preparations, above. Screen each unit.

Based on the results of the screening test, proceed as follows:

1. If all screened units appear the same, combine screened units from all containers and form a composite.

2. If all screened units do not appear the same, each container should be treated as a separate exhibit or entity. Thus for each container, proceed according to the direction above for a single container.

4. Aqueous solutions - Illicit origin

Aqueous solutions of methamphetamine HCl are illicitly available in some countries. Since solutions by their very nature are homogeneous, a relatively small sample (10 ml) represents the entire volume.

(a) Single container

If sample size permits, pipet an amount for assay of at least 10 ml.

(b) Multiple containers

Segregate containers by lot numbers or other characteristics and treat each group as described under 1.b above. Report results separately for each group.

Determine the square root of the total number of containers in each group. Randomly select a number of containers equivalent to the square root rounded to the next higher integer.

From each of the selected containers withdraw a 10 ml or larger sample (if size permits) for a composite.

If size permits, pipet at least 10 ml of the composite for assay.

5. Residues from syringes or clandestine laboratory glassware

Because of the trace amounts of amphetamine/methamphetamine usually present on hypodermic syringes seized from individuals or on glassware and other equipment found in clandestine laboratories, the analyst should not attempt to perform presumptive tests but should proceed directly with conclusive analytical procedures.

Wash the syringe or glassware with a minimum amount of methanol and concentrate it to dryness under a stream of nitrogen. Proceed with selected tests.

B. Presumptive tests

1. Colour tests

It must be stressed that positive results to colour tests are only presumptive indications of the possible presence of amphetamine or methamphetamine. Many other materials, both substituted amphetamines as well as those which are harmless and uncontrolled by national legislation or international treaties, may give similar colours with the test reagents. Some cutting agents may also result in the sample giving false positives or negatives. This is particularly true for the Simon's reagent. It is mandatory for analysts to confirm such results by the use of alternative techniques.

(a) Marquis reagent

Prepare by adding 8-10 drops of 40% formaldehyde solution to 10 ml of concentrated sulphuric acid. Since paraformaldehyde is more stable than formaldehyde, the mixture of concentrated sulphuric acid and paraformaldehyde (10:1 v/v) can be used as an alternative.

METHOD

Place a small amount of the sample (1 to 2 mg of powder; one or two drops if a liquid) in a depression of a spot plate, add the reagent dropwise (no more than three drops). Both amphetamine and methamphetamine give an immediate orange colour turning to brown. The lower limit of detection is about 1 μg.

(b) Ninhydrin reagent

Dissolve 0.5 g of ninhydrin in 40 ml of acetone. Prepare this solution daily.

METHOD

Dissolve a small amount of the sample (1 to 2 mg of powder) in methanol. Place one drop of the solution on a filter paper and add one drop of the reagent. Heat on a hot-plate at 110°C. The colour turns to pinkish-orange with heating. This is not a particularly sensitive test.

(c) Simon's reagent

Solution 1. 20% aqueous sodium carbonate solution

Solution 2. 50% ethanolic acetaldehyde solution

Solution 3. 1% aqueous sodium nitroprusside solution

METHOD

Place a small amount of the sample on a spotting tile and mix it with a drop of solution 1. Then add one drop of solution 2. Addition of a few drops of solution 3 produces a blue colour for methamphetamine and other secondary amines. Amphetamine and other primary amines yield a slow pink to cherry-red colour. This test may be used to distinguish methamphetamine from amphetamine. Note, however, that the presence of some cutting agents may result in a false negative.

C. Thin layer chromatography

DEVELOPING SOLVENTS

SYSTEM A:	Methanol	100
	Concentrated ammonia	1.5
SYSTEM B:	Cyclohexane	75
	Toluene	15
	Diethylamine	10
SYSTEM C:	Methylethylketone	130
	Dimethylformamide	19
	Concentrated ammonia	1
	Isopropanol	30

Preparation of solutions to be applied to the TLC plate

Powder: Prepare a solution at a concentration of 5 mg per ml in methanol.

Capsules: Remove the contents of a representative sample of capsules (see sampling procedure above) and prepare a solution containing the equivalent of approximately 5 mg of the drug per ml in methanol.

Tablets: Grind a representative number of tablets to a fine powder and prepare a solution containing the equivalent of approximately 5 mg of the amphetamine/methamphetamine per ml in methanol.

Aqueous solutions: Spot directly or the equivalent of 5 mg/ml if the concentration of the drug is known.

Standard solutions: All made at a concentration of 5 mg per ml in methanol.

Because of the general insensitivity of the amphetamines to most visualization reagents, it is suggested that 5 µl of a 5 mg per ml solution of the drug in methanol be applied giving about 25 µg of drug on the TLC plate.

In those cases where it is suspected that the concentration of the amphetamine/methamphetamine in the sample is very low due to adulteration etc., it may be necessary to prepare a ten-times more concentrated solution for the analysis.

The form of standards and exhibits used, salt or base, is unimportant. Either form will be satisfactory. Because of the basic nature of the developing solvents the compounds move as the free bases.

VISUALIZATION

The plates must be dried prior to visualization. This can be done at room temperature or, more quickly, by use of a hot air blower. In the latter case, due care must be taken because of the volatility of the amphetamine/methamphetamine free bases. It is important for proper colour development, however, that all traces of ammonia or other bases be removed from the plate.

Visualization methods

1. UV light at 254 nm.
2. Ninhydrin reagent.
3. Acidified potassium iodoplatinate reagent.

First observe the plate under UV light. Then spray with the ninhydrin reagent and heat in an oven at 110°C for 5 minutes. Violet or pink spots are given by primary amines such as amphetamine and lighter spots for secondary amines such as methamphetamine. The plate may then be oversprayed with the acidified iodoplatinate solution. Amphetamine and methamphetamine give dirty grey-violet-brown spots on a pink background.

Warning - Because of the volatility of the amphetamine free bases, the temperature employed in evaporating solvents from the plate and the number of structurally similar amphetamines available on the illicit market, thin layer chromatography is of limited use and caution is recommended when interpreting the results.

Preparation of spray reagents

ACIDIFIED POTASSIUM IODOPLATINATE REAGENT

Dissolve 0.25 g of platinic chloride and 5 g of potassium iodide in water and make up to 100 ml. Add 2 ml of concentrated hydrochloric acid for the acidified version.

NINHYDRIN REAGENT

Prepare a 0.1% solution in isopropanol.

RESULTS

R_f x 100 values:

COMPOUND	DEVELOPING SYSTEM		
	A	B	C
Amphetamine	46	34	49
Methamphetamine	28	42	16
Ephedrine	30	--	12
Caffeine	68	6	75

D. Gas liquid chromatography

1. Packed column technique

(a) System A - without derivatization

Operating conditions:

Detector:	FID
Column:	6 ft (or 2 m), 2 to 4 mm ID glass.
Packing:	10% Apiezon L and 2% KOH on 80-100 mesh Chromosorb W HP or 3% SE-30 or OV-1.
Carrier gas:	Nitrogen at 30 ml per minute.
Column temperature:	Programmed from 130° to 260°C.
Internal standards:	n-Tetradecane or other n-alkanes with an even number of carbon atoms or diphenylamine or propylamphetamine HCl.

METHOD

Solutions of the standards (1 mg base/ml) are prepared by dissolving an accurately weighed portion of the salt in water. Make the solution alkaline by the addition of a few drops of 1.0N NaOH. Add an equal volume of the extracting solvent (hexane or ethylacetate), shake and allow the layers to separate. The final concentration should be about 1 mg of base and 1 mg of internal standard per ml.

Treat the illicit sample in a similar manner using sufficient sample to give an amphetamine/methamphetamine concentration approximately equal to that of the standard solution.

Inject 1 to 2 µl of the organic layer as appropriate.

For quantitation, include the internal standard in the initial aqueous solution (if an amine salt such as propylamphetamine HCl is employed) or in the ethylacetate extracting solvent (if n-alkanes or diphenylamine are used).

The content (%) of any component can be calculated using the general formula:

$$C_x\% = \frac{C_{r.\ std.}}{C_{sam.}} \times \frac{A_x / A_{int.std.in\ sam.\ chrom.}}{A_{r.st.} / A_{int.std.in\ std.\ chrom.}} \times 100$$

Where:

$C_x\%$ = content of component x in the sample (w/w %).

C_r std. = concentration of substance x in the standard reference solution (w/w %).

A_x = peak area for substance x during the sample chromatography.

$A_{int.\ std.\ in\ sam.\ chrom.}$ = peak area of the internal standard obtained during the sample chromatography.

$A_{int.\ std.\ in\ std.\ chrom.}$ = area of the internal standard obtained during the standard chromatography.

$C_{sam.}$ = concentration of the sample (w/v %).

(b) <u>System B - with derivatization</u>

Operating conditions:

Detector:	FID
Column:	6ft (2 m), 3 mm ID, glass.
Packing:	3% OV-17 on 80-100 mesh Chromosorb W HP or equivalent.
Carrier gas:	Nitrogen at 30 ml per minute.
Column temperature:	145°C or programmed from 130° to 270°C.
Internal standard:	n-Tetradecane or other n-alkanes with an even number of carbon atoms.
Derivatizing agent:	trifluoroacetic anhydride.

METHOD

Prepare an ethylacetate solution of the liberated base as in the method described above and dry a portion of it over anhydrous magnesium sulphate. Transfer 0.1 - 0.5 ml of the ethylacetate solution and 100 µl of trifluoroacetic anhydride to the tightly sealed reaction vessel and heat at 55°C for 20 minutes. Evaporate the solvent invacuo, and dissolve the residue in 100 µl of ethylacetate. Inject 1 µl into the gas chromatograph.

ELUTION PROFILES ON SELECTED COLUMNS

	COLUMN		
SYSTEM	10% Apiezon L 2% KOH	SE-30	3% OV-17
	without derivatization		TFA derivatives
Compound			
Amphetamine	1134[a]	1129	1536
Methamphetamine	1200	1176	1722
Ephedrine	1386	1363	1467
Caffeine	1862	1810	2376

a/ Figures are retention indices. These values will vary depending upon laboratory conditions (e.g. temperature, humidity, drafts) and other instrumental parameters.

2. Capillary column technique

Operating conditions:

Detector:	FID
Column:	Fused silica, chemically bonded and cross-linked methyl silicone or methyl phenylsilicone, such as SE-54, DB-1, DB-5 or equivalent).
Film thickness:	0.25 µm
Length:	10 to 30 m, ID 0.25 mm.
Carrier gas:	Helium, 40 cm/sec.
Split ratio:	40:1
Column temperature:	Programme: 2 min. at 75°C, increase to 280°C at 10°/min.
Internal standard:	n-Tetradecane or other n-alkane with an even number of carbon atoms or propylamphetamine HCl.

METHOD

Prepare drug standard solutions and unknown sample solutions at a concentration of 1 mg of the free base per 1 ml H_2O. The solution is made alkaline by the addition of a few drops of 1.0 N NaOH and the mixture shaken with 1 ml of ethylacetate. After filtering over $MgSO_4$, 1 μl is injected. On an 11m SE-54 column, retention times for amphetamine and methamphetamine are 1.6 and 1.9 minutes respectively.

For alternative GC systems see:

1. J. Forensic Sciences 31 (1986) pp 1102 - 1107.
2. J. Chromatography 90 (1974) pp 19 - 33.
3. J. Chromatography 258 (1983) pp 65 - 72.

E. High performance liquid chromatography

1. Isocratic technique

(a) Normal phase

Column:	125 mm by 4.9 mm ID.
Packing material:	Silica HPLC grade, 5 µm diameter (Spherisorb S5W or equivalent).
Mobile phase:	Equally good separations can be achieved by using either mobile phase A or B.
	A: A solution containing 1.17 g (0.01M) of ammonium perchlorate in 1000 ml of methanol. Adjust to pH 6.7 by adding 0.1M sodium hydroxide in methanol (ca. 1 ml).
	B: Methanol: aqueous ammonium nitrate buffer solution (90:10 v/v). To prepare the buffer solution add 94 ml of concentrated ammonia and 21.5 ml concentrated nitric acid to 884 ml water and then adjust the pH to 10 with ammonia.
Flow rate:	2.0 ml per minute.
Detection:	UV at 254 nm.
Sample preparation:	All materials are dissolved in methanol to give an approximate concentration of 1 mg free base per ml.
Standard solutions:	Dissolve a sufficient amount of methamphetamine or amphetamine salt to give a solution containing 1 mg free base per 1 ml of methanol.
Injection volume:	1 to 5 microlitres by syringe or loop-injector.
Quantitation:	By peak areas, external standard method.

(b) Reverse phase

Column:	250 mm by 4 mm ID.
Packing material:	Octadecyl-silica HPLC 5 µm diameter (LiChrosorb RP-18 or equivalent).

Mobile phase: C: Acetonitrile: 1% aqueous ammonium acetate: 2.5% aqueous diethylamine (40:45:15). The pH is adjusted to 8 - 9 by addition of ammonia or acetic acid.

Flow rate: 1.5 ml per minute.

Temperature: 35°C

Detector: UV at 254 nm.

Sample preparation: All materials are dissolved in a mixture of 2 parts water and 1 part acetonitrile to give an approximate concentration of 2 - 6 mg per ml.

Standard solutions: Dissolve a sufficient amount of amphetamine or methamphetamine standards in a mixture of 2 parts water and 1 part acetonitrile to give a concentration of 2 mg per ml.

Injection volume: 10 - 20 µl by loop injector.

Quantitation: By peak areas, internal standard method using lidocaine or procaine or external standard method.

RESULTS

The capacitance ratios (K'values) or retention times (in minutes) are as follows:

	NORMAL PHASE		REVERSE PHASE
SYSTEM	A	B	C
Compound			
Amphetamine	0.9	0.98	6.0 min.
Methamphetamine	2.0	2.07	11.0 min.
Ephedrine	1.0	1.79	----
Caffeine	0.2	0.26	----

Alternative HPLC methods for the analysis of amphetamine/methamphetamine:

1. J. Chromatography 218 (1981) pp 639 - 646.
2. Microgram XVIII (1985) pp 134 - 143.

F. Infrared spectroscopy

Sample preparation

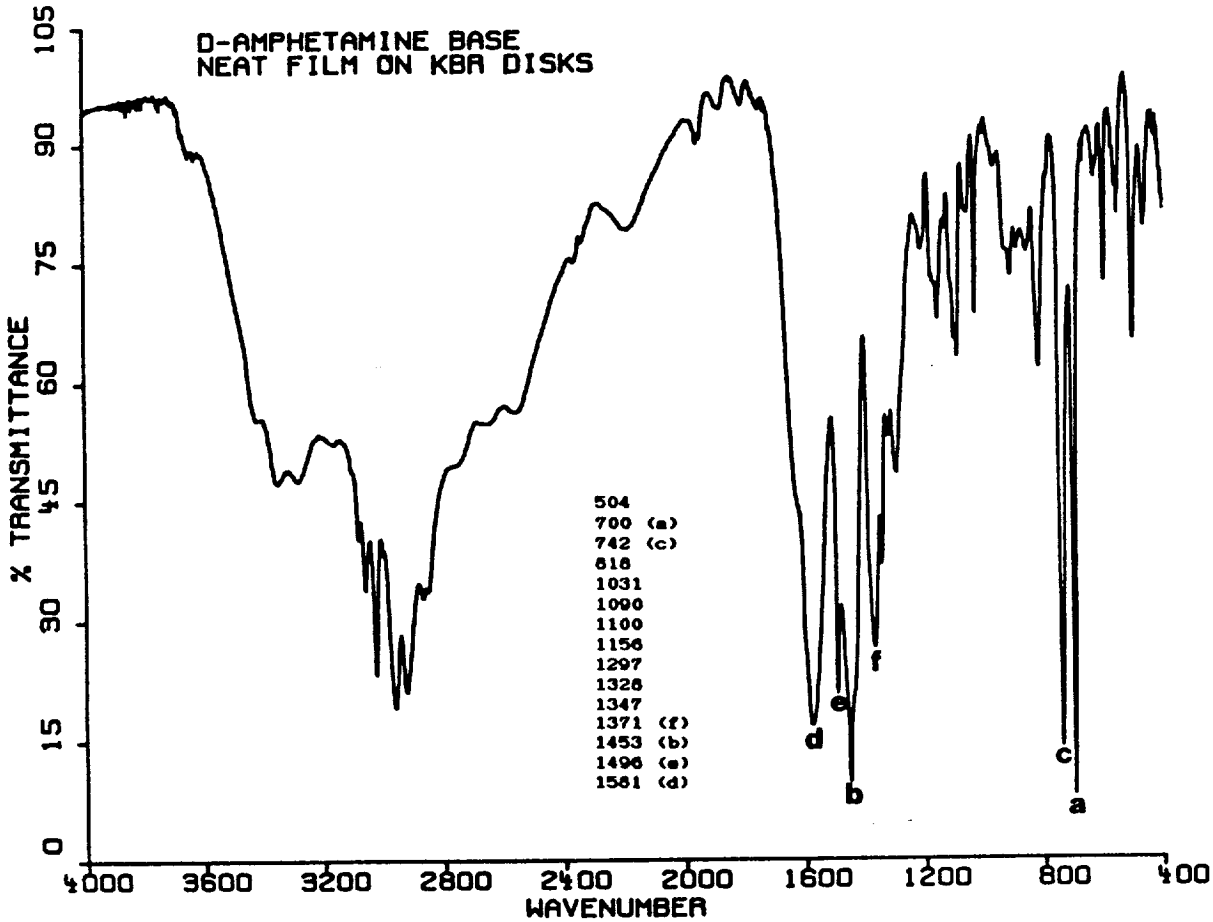
For description of the standard techniques (halide disc method, micro halide disc method and nujol mull method) see previous manuals of this series.

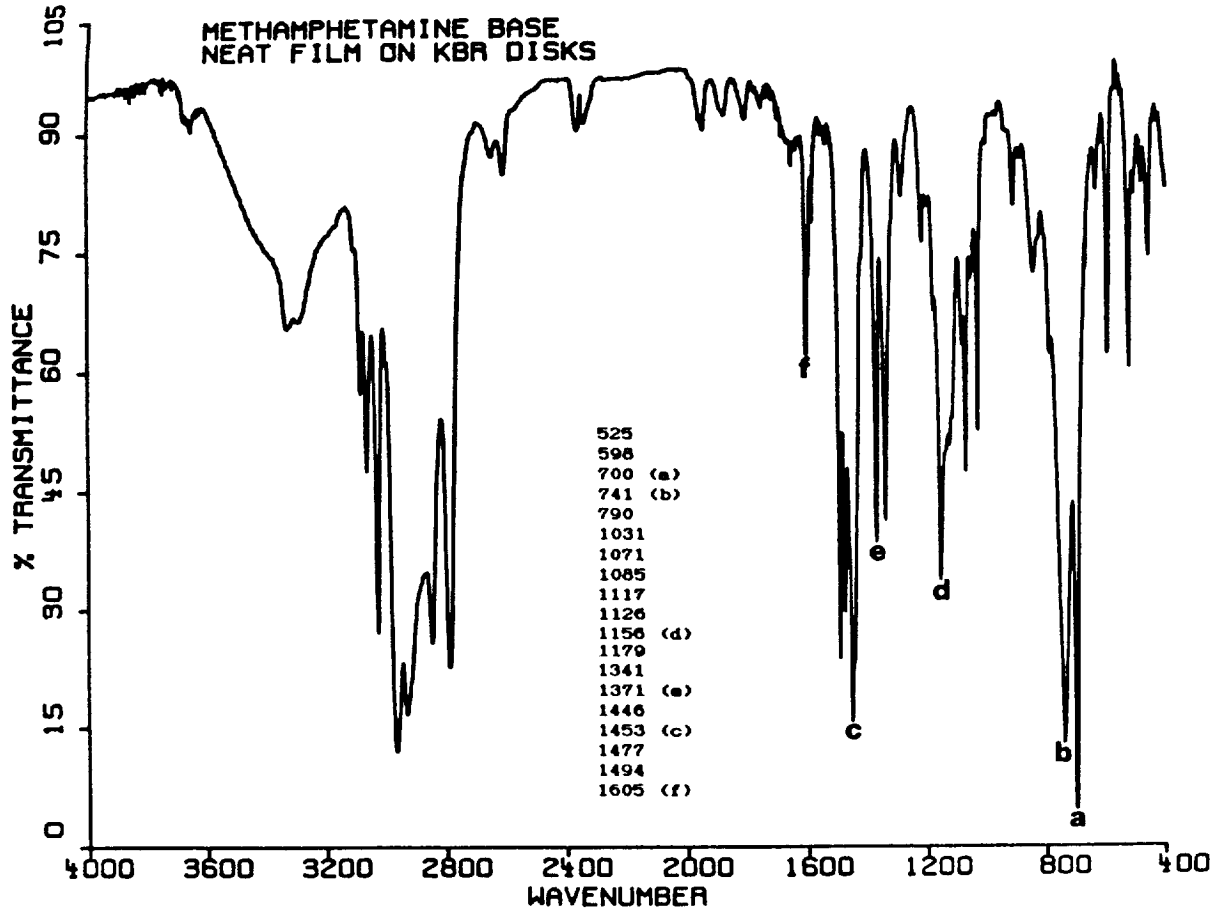
Thin film method

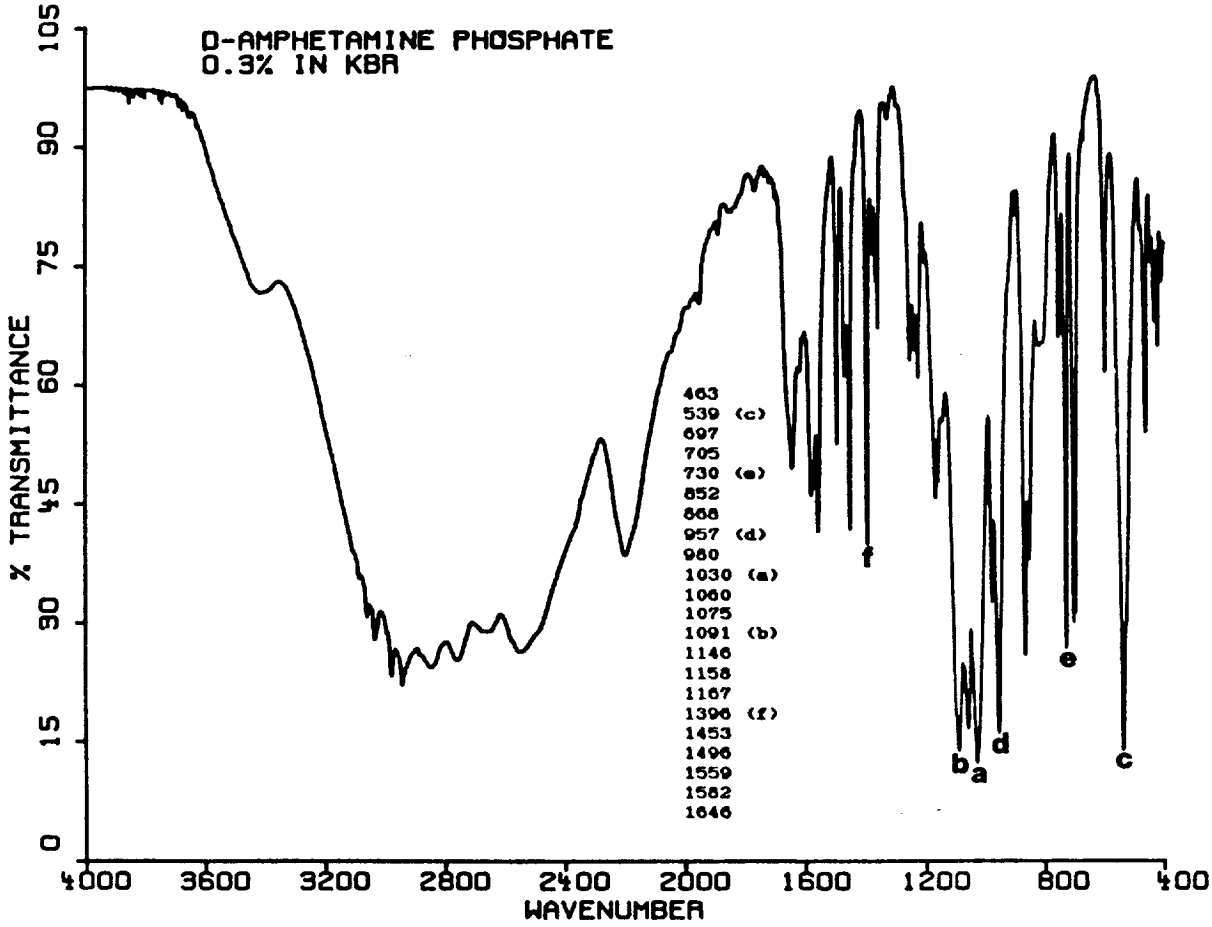
This method is particularly useful for obtaining spectra of the amphetamine/methamphetamine free bases which are liquids. A drop of the amine is sandwiched between two alkali halide plates forming a thin liquid film.

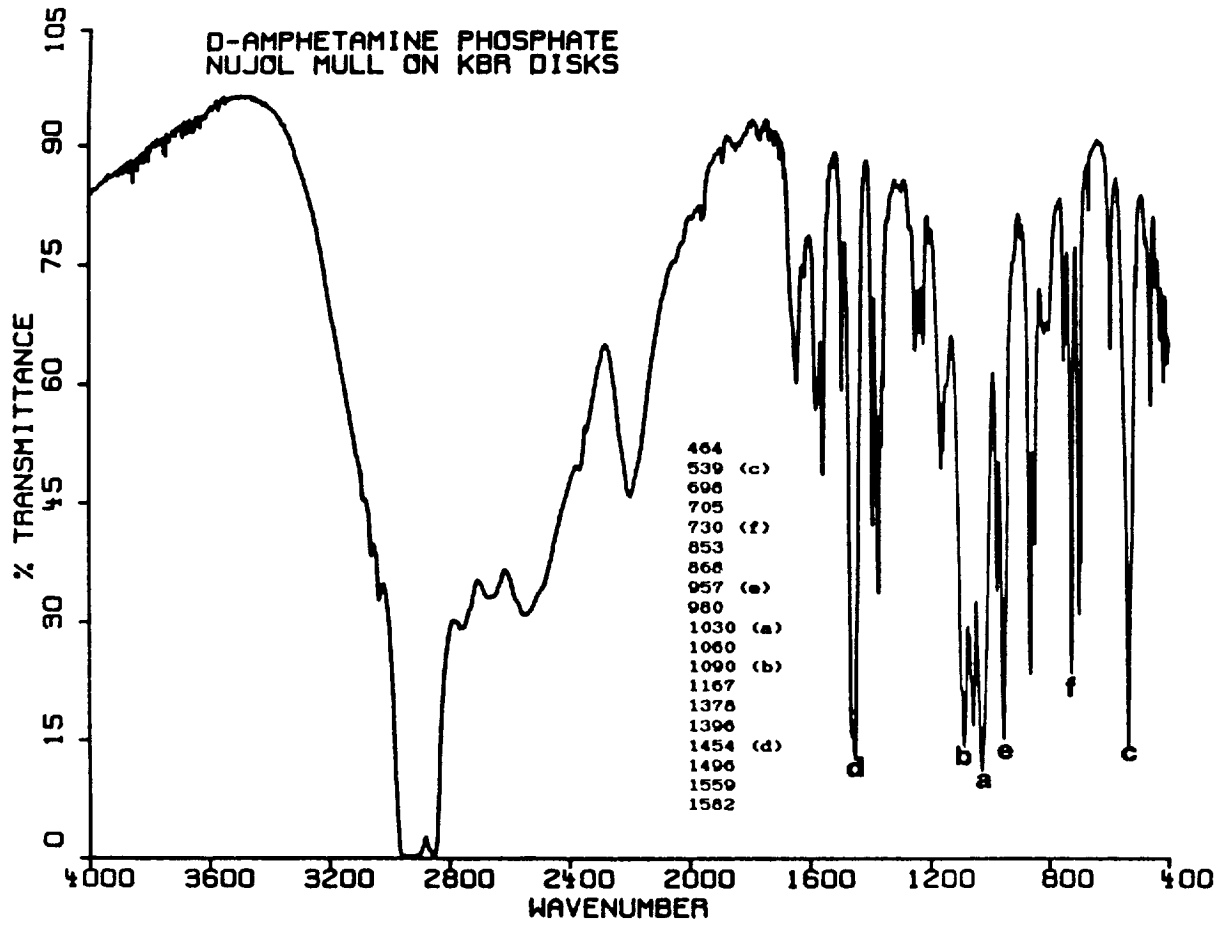
RESULTS

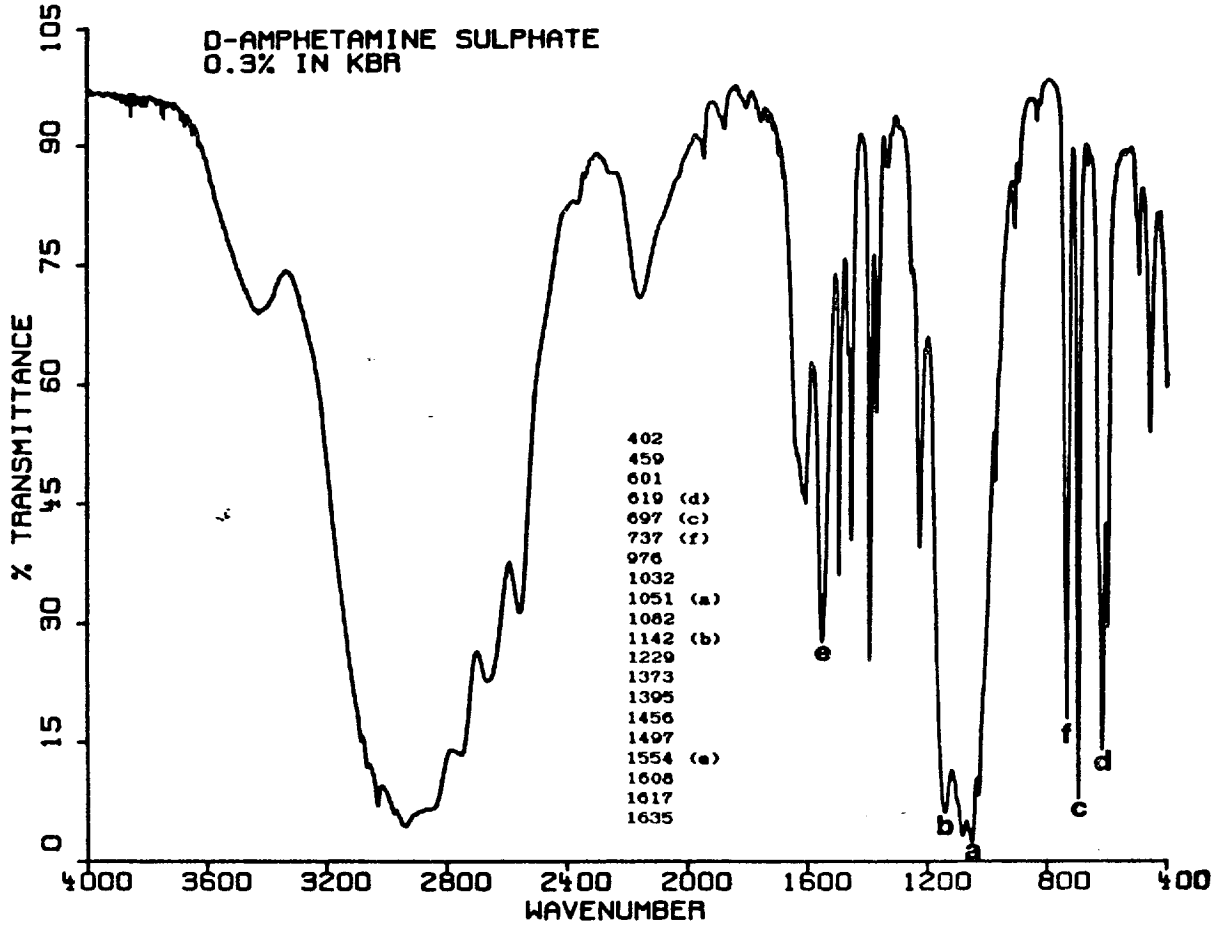
In general, spectra of the amphetamine and methamphetamine salts are recorded using samples prepared by the halide disk or nujol mull methods and the free bases are run as thin films. The major peaks occuring in the IR spectra of amphetamine sulfate, amphetamine phosphate, amphetamine base, methamphetamine hydrochloride and methamphetamine free base are listed in order of decreasing magnitude of absorbance on each figure. The sequence, however, may vary from sample to sample.

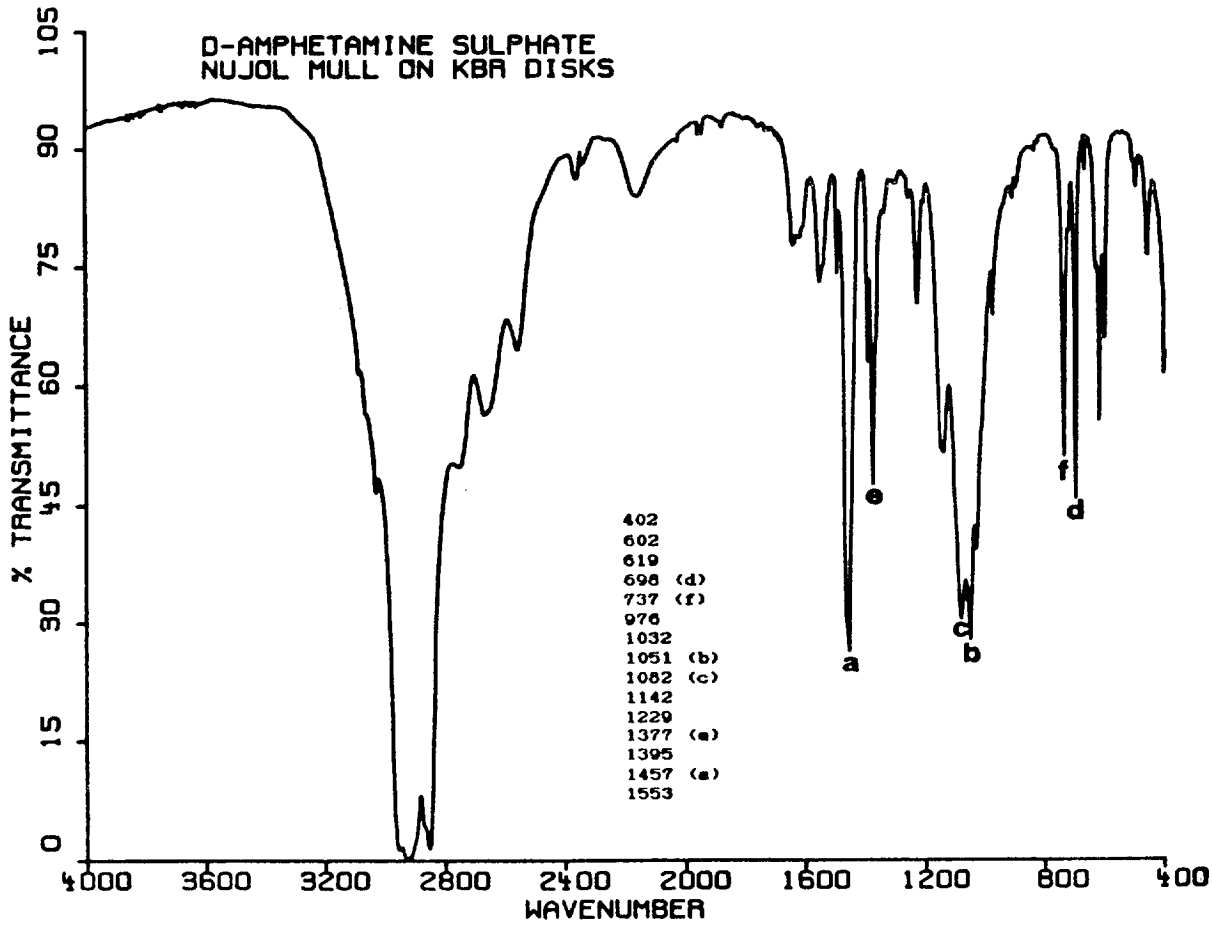

D-AMPHETAMINE BASE
NEAT FILM ON KBR DISKS
% TRANSMITTANCE
105
90
75
60
45
30
15
0
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER
504
700 (a)
742 (c)
818
1031
1090
1100
1156
1297
1328
1347
1371 (f)
1453 (b)
1496 (e)
1561 (d)
a
b
c
d
e
f

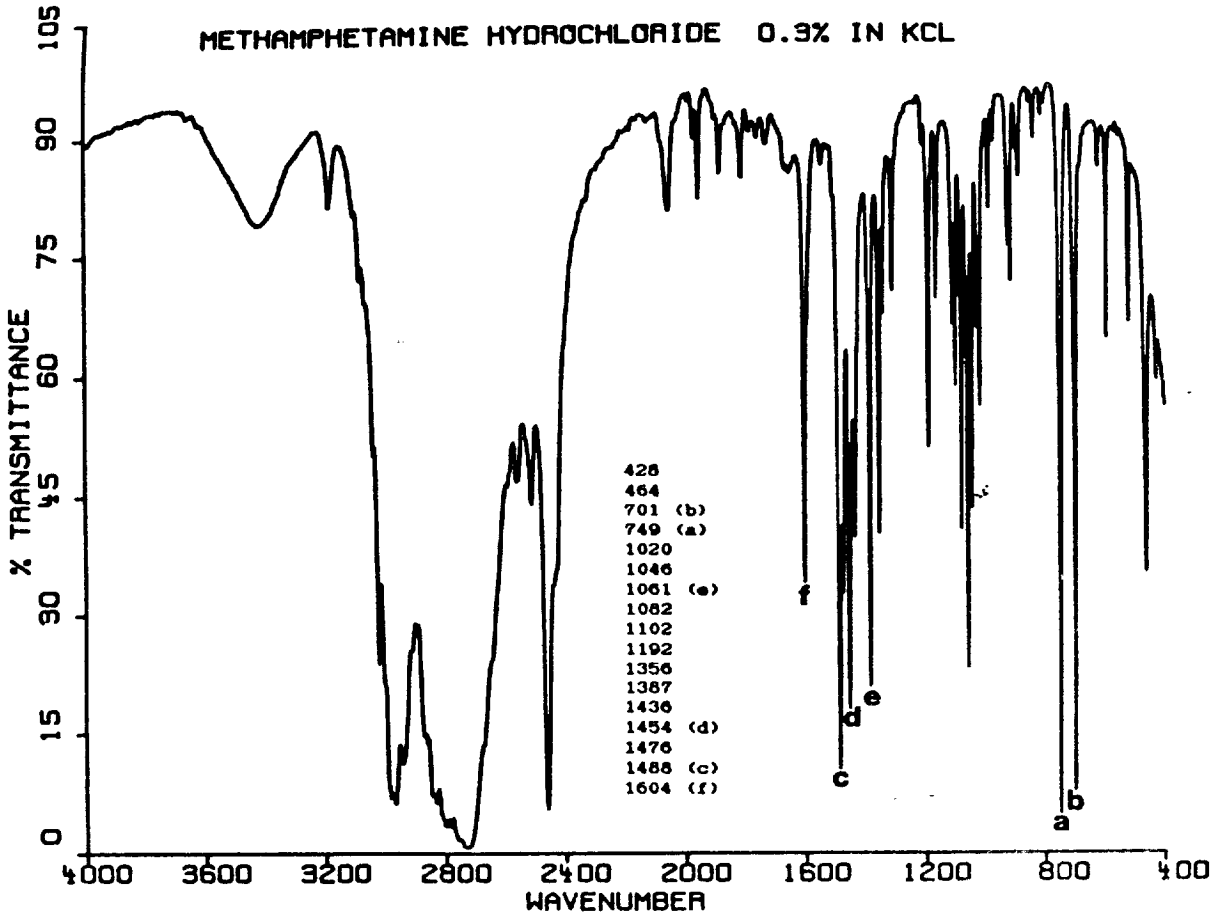

METHAMPHETAMINE BASE
NEAT FILM ON KBR DISKS
% TRANSMITTANCE
105
90
75
60
45
30
15
0
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER
525
598
700 (a)
741 (b)
790
1031
1071
1085
1117
1126
1156 (d)
1179
1341
1371 (e)
1446
1453 (c)
1477
1494
1605 (f)
a
b
c
d
e
f

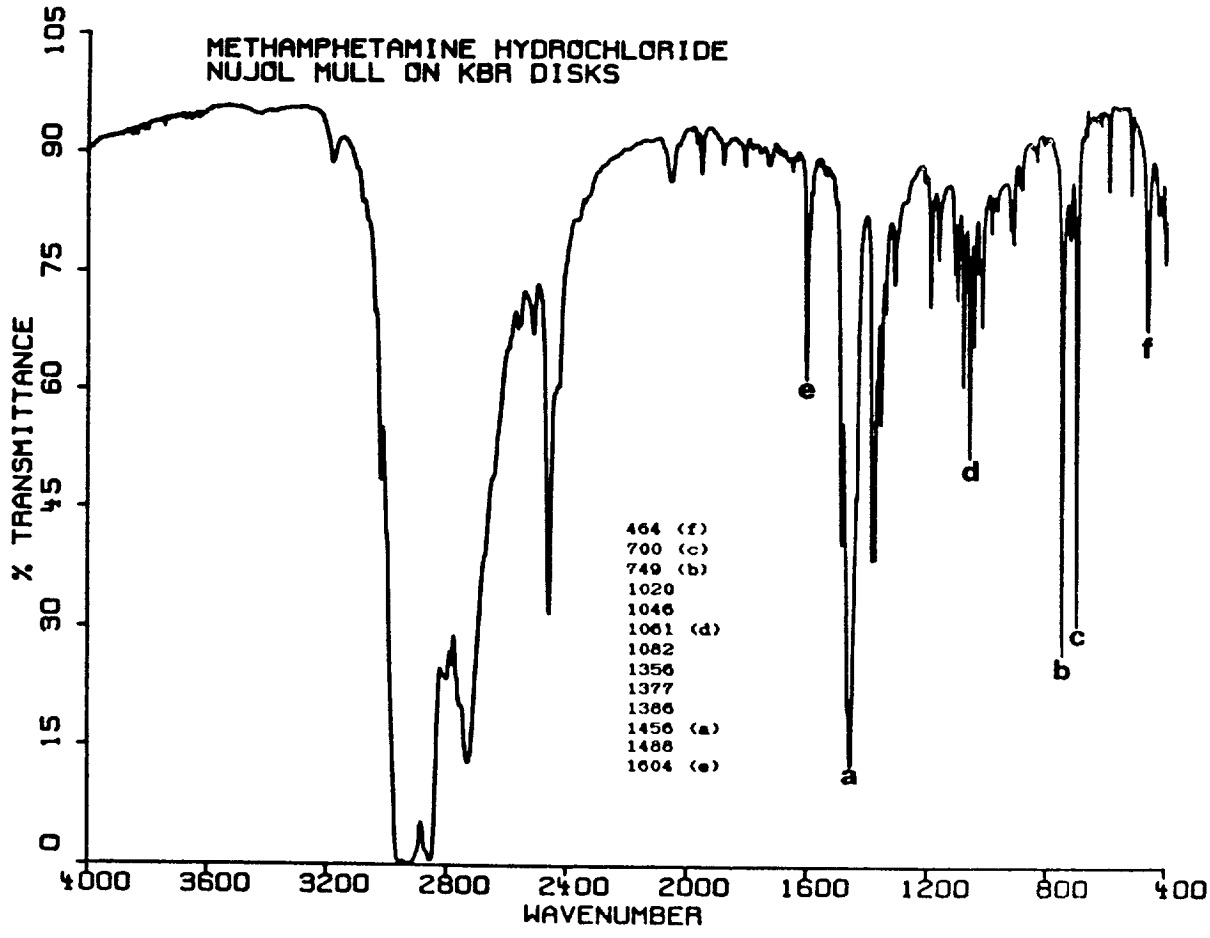

D-AMPHETAMINE PHOSPHATE
0.3% IN KBR
% TRANSMITTANCE
0
15
30
45
60
75
90
105
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER
463
539 (c)
697
705
730 (a)
852
868
957 (d)
980
1030 (a)
1060
1075
1091 (b)
1146
1158
1167
1396 (f)
1453
1496
1559
1582
1646
a
b
c
d
e
f


D-AMPHETAMINE PHOSPHATE
NUJOL MULL ON KBR DISKS
% TRANSMITTANCE
0
15
30
45
60
75
90
105
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER
464
539 (c)
696
705
730 (f)
853
868
957 (e)
980
1030 (a)
1060
1090 (b)
1167
1378
1396
1454 (d)
1496
1559
1582
a
b
c
d
e
f


D-AMPHETAMINE SULPHATE
0.3% IN KBR
% TRANSMITTANCE
WAVENUMBER
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
15
30
45
60
75
90
105
402
459
601
619 (d)
697 (c)
737 (f)
976
1032
1051 (a)
1082
1142 (b)
1229
1373
1395
1456
1497
1554 (e)
1608
1617
1635
a
b
c
d
e
f


D-AMPHETAMINE SULPHATE
NUJOL MULL ON KBR DISKS
% TRANSMITTANCE
WAVENUMBER
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
15
30
45
60
75
90
105
402
602
619
698 (d)
737 (f)
976
1032
1051 (b)
1082 (c)
1142
1229
1377 (a)
1395
1457 (a)
1553
a
b
c
d
e
f


METHAMPHETAMINE HYDROCHLORIDE 0.3% IN KCL
% TRANSMITTANCE
WAVENUMBER
428
464
701 (b)
749 (a)
1020
1046
1061 (e)
1082
1102
1192
1356
1387
1436
1454 (d)
1476
1488 (c)
1604 (f)


METHAMPHETAMINE HYDROCHLORIDE
NUJOL MULL ON KBR DISKS
% TRANSMITTANCE
WAVENUMBER
464 (f)
700 (c)
749 (b)
1020
1046
1061 (d)
1082
1356
1377
1386
1456 (a)
1488
1604 (e)

G. Analysis of optical isomers

Both amphetamine and methamphetamine have one asymmetric carbon atom resulting in a pair of enantiomers in each case. Depending upon the licit or illicit source of the material, l-, d- and dl-amphetamine or methamphetamine could be encountered in samples submitted for analysis.

These optical isomers differ to some extent in pharmacological activity and are subject to different regulatory measures in certain countries. Under the "Convention on Psychotropic Substances" each optical isomer (d-, or l-) as well as the racemic mixture (dl) of amphetamine is scheduled. However, with methamphetamine, only the d-(+)-isomer and the l-(-) isomer are scheduled. In those countries where national legislation requires that the specific optical isomer present be identified, the following analytical procedures may be used.

1. Microcrystal test to distinguish l-, d-, and dl-amphetamine

Both d- and l- amphetamine give identical microcrystals. The way to distinguish them is to form the racemate, which does give different crystals.

REAGENTS

1. Testing reagent 5% $HAuCl_4$ in H_3PO_4

Prepare by dissolving 1 g of commercial gold trichloride acid ($HAuCl_4.3H_2O$) in 20 ml of a solution containing one volume of concentrated H_3PO_4 and two volumes of water.

2. Volatilizing reagent

Prepare a 5% aqueous NaOH solution.

METHOD

For amphetamine and methamphetamine the "hanging drop" technique is employed. This requires a cavity slide, a cover glass, the testing reagent and a volatilizing reagent. Transfer a small quantity of the sample powder into the depression of the cavity slide, followed by one or two drops of the volatilizing reagent. This liberates the free amine in the form of a volatile free base which rises from the solution as a vapour. Immediately transfer a drop of the testing reagent onto a glass slide and invert the slide crosswise over the sample cavity. The reagent

then reacts with the amine vapour present in the cavity. After an appropriate interval, reinvert the reagent slide and examine for crystals in the reagent or at the edge of the reagent drop.

RESULTS

Both d- and l- amphetamine produce long yellow rods or coarse needles and long narrow blades. The racemate, dl-amphetamine, gives at first "oily" drops then coloured platy crystals. These crystals largely form after inversion.

Distinction of d- and l- amphetamine

If the above test indicates that the sample is d- or l- amphetamine, distinction should be undertaken as to which is present. To a little known d-amphetamine salt in a cavity and a little known l-amphetamine salt in another, add some sample powder. Repeat the test above. The mixture that is (d+d) or (l+l) will give the long yellow rods etc. The mixture that proves to be (d+l) will give the platy crystals of the racemate as previously described.

2. Microcrystal test to distinguish d- and dl- methamphetamine

REAGENTS

1. Testing reagent - H_3BiI_6 in H_2SO_4

Prepare by dissolving 1.25 g potassium iodide in 2.0 ml of water. Add 2.5 ml of a solution of H_2SO_4 diluted 1:7 with water, 0.5 ml of concentrated $Bi(NO_3)_3$ solution and 0.1 g of sodium hypophosphite. The concentrated $Bi(NO_3)_3$ stock solution is prepared by dissolving 50 g of bismuth subnitrate in 70 ml of a solution of HNO_3 (diluted 1:1 with water) and made up to 100 ml with water. The testing reagent can be kept for several months.

2. Volatilizing reagent - 5% aqueous NaOH solution

METHOD

Proceed with the hanging drop procedure as described above for amphetamine but using the H_3BiI_6 in H_2SO_4 testing reagent.

RESULTS

d-Methamphetamine gives long orange needles. dl-Methamphetamine gives characteristic orange-red rods with slanting ends.

For further information on crystal tests, readers are referred to:

1. Fulton, C.C. (1969) Modern Microcrystal Tests for Drugs, Wiley-Interscience, New York
2. U.S. Dept. of Justice (1986) Basic Training Program for Forensic Drug Chemists.

3. Infrared spectroscopic method for distinguishing optical isomers of amphetamine

Another method for distinguishing between the optical isomers of amphetamine is based on the fact that three distinct infrared spectra can be produced for d-, dl- and l- amphetamine as the d-mandelate salts.

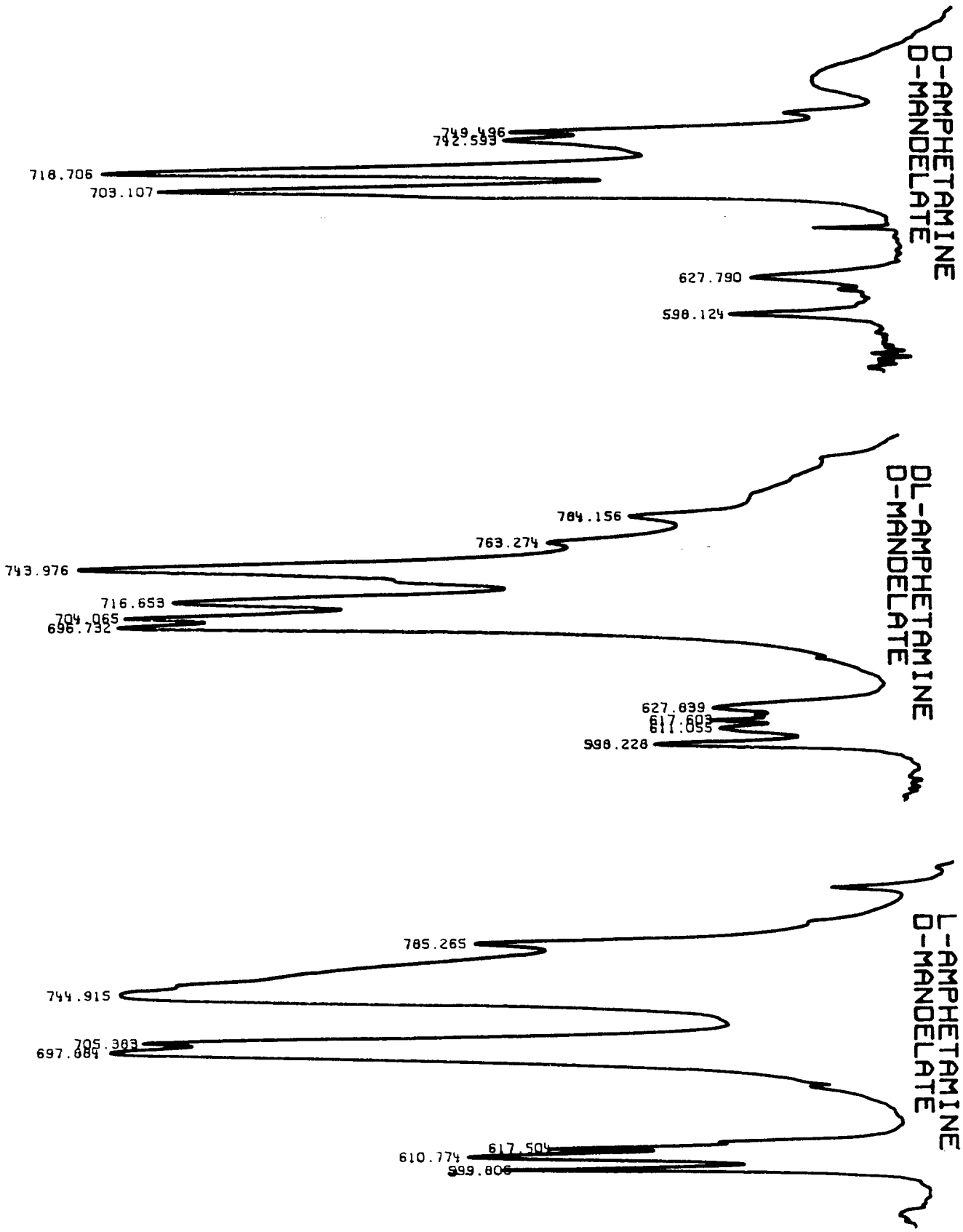
METHOD

A water solution of any amphetamine salt (10 - 50 mg) is made basic and the amphetamine extracted into methylene chloride. The methylene chloride is dried over anhydrous sodium sulphate and concentrated to ca. 2 ml. A saturated solution of d-mandelic acid in methylene chloride is added several drops at a time until the amphetamine is neutralized (pH paper). The d-mandelate salt is allowed to crystallize, the solution filtered through suction and the crystals washed with a small portion of methylene chloride. After drying, prepare a KBr disc of the crystals and run the infrared spectrum. Repeat the procedure using known optically pure isomers of amphetamine and compare the resulting spectra with those obtained from the pure standards.

RESULTS

The spectra (see Figures) of the different isomers are sufficiently distinct, particularly in the 800-600 cm^{-1} region, to distinguish d-, dl- and l- amphetamine.

Reference - Analytical Chemistry, 42 (1970) p 1459.


D-AMPHETAMINE
D-MANDELATE
749.496
742.593
718.706
703.107
627.790
598.124
DL-AMPHETAMINE
D-MANDELATE
784.156
763.274
743.976
716.653
704.065
698.732
627.639
617.603
611.055
598.228
L-AMPHETAMINE
D-MANDELATE
785.265
744.915
705.383
697.684
617.504
610.774
599.806

4. Alternative methods to distinguish between the optical isomers of amphetamine/methamphetamine

1. TLC - J. Chromatography 117 (1976) pp 442-444.
2. GLC - J. Forensic Sciences 27 (1982) pp 39-48.
3. HPLC - Analytical Chemistry 58 (1986) pp 1643- 1648.
4. NMR - Analyst 107 (1982) pp 544-549.
   - J. Pharm. Belg. 36 (1981) pp. 348-353.
5. HPLC-MS - Analytical Chemistry 58 (1986) pp 1349-1352.

H. Analysis of amphetamine/methamphetamine impurities

1. Extraction/sample preparation

Since most of the impurities are neutral or weakly basic their separation from an aqueous solution of the amphetamine or methamphetamine salt usually involves extraction with an organic solvent. For quantitative determination of some impurities such as DIPA, extraction from alkaline solution is necessary but a large amount of the drug is also extracted. For fingerprinting an amphetamine or methamphetamine sample, where it is preferred to separate the impurity from the bulk of the sample, the following procedure is quite satisfactory.

Sample solutions - Grind a 200 mg portion of the seized amphetamine or methamphetamine sample to a fine powder and dissolve in phosphate buffer (pH 7) to form a solution of 100 mg/ml concentration.

Liquid-liquid extraction - Extract 2 ml of sample solution with 0.2 ml of heptane (or n-octane) by vigorous shaking for 2-5 minutes. After phase separation, transfer the organic layer into a glass tube, leaving a small amount behind in order to avoid the transfer of any aqueous phase. For quantitative analysis diphenylamine (as an internal standard) may be added to the initial heptane solution at a concentration of 35 mg/L.

2. Thin layer chromatography

Developing solvents:

SYSTEM A:	Hexane	50
	Ether	50
SYSTEM B:	Cyclohexane	75
	Toluene	15
	Diethylamine	10
SYSTEM C:	Isopropanol	95
	Concentrated ammonia	5

Solutions for spotting - Prepare solutions of standards at a concentration of 2 mg per ml of acetonitrile. Spot 1 to 5 µl of these and the solution from the liquid-liquid extraction procedure outlined above.

VISUALIZATION

1. UV light at 254 nm.

2. Acidified potassium iodoplatinate.

References:

Bulletin on Narcotics 39 (1981) pp 37 - 54.
J. Forensic Sciences 30 (1985) pp 427 - 438.
Eisei Kagaku 29 (1983) pp 400 - 406.

3. Gas liquid chromatography

Operating conditions:

SYSTEM A: packed column technique - same as system A in section IV.D1. (see above).

SYSTEM B: Capillary column technique
Same as the method described in IV.D.2 (see above).

Procedure: Inject 1 to 5 µl of sample solution obtained from the liquid-liquid extraction procedure outlined above.

References:

System A - 1. J. Forensic Sciences 23 (1978) pp 693 - 700.
2. Eisei Kagaku 29 (1983) pp 400 - 406.

System B - 1. J. Chromatography 258 (1983) pp 65 - 72.
2. J. Chromatography 234 (1984) pp 499 - 502.

4. High performance liquid chromatography

Reverse phase system for amphetamine impurities:

This method allows automated extraction and analysis to be performed. It utilizes an on-line extraction column for enriching the impurities while amphetamine and polar diluents are washed out with H_2O. A six-port valve is then switched and the impurities are eluted from the C-8 extraction column onto a C-18 analytical column where they are separated.

Apparatus:	Isocratic HPLC pump, gradient HPLC pumping system, 6-port switching valve.
Extraction column:	15 mm by 3.2 mm ID Octasilane 7 µm diameter HPLC grade.
Analytical column:	100 mm by 4.6 mm ID Octadecylsilane HPLC grade 5 µm diameter.
Guard column:	30 mm by 3.2 mm ID Octadecylsilane HPLC grade 5 µm diameter.
Mobile phase:	Washing solution HPLC - grade water for absorption and separation. For desorption and separation Solvent A 0.2 M butylamine in water. The pH is adjusted to 8.0 with o-phosphoric acid. Solvent B 20% (v/v) of solvent A in acetonitrile.

The gradient is programmed linearly from 30 to 100% solvent B over 20 minutes, then isocratic 100% B for five minutes. At the end of the day wash the system with 75% acetonitrile in water for about 30 minutes.

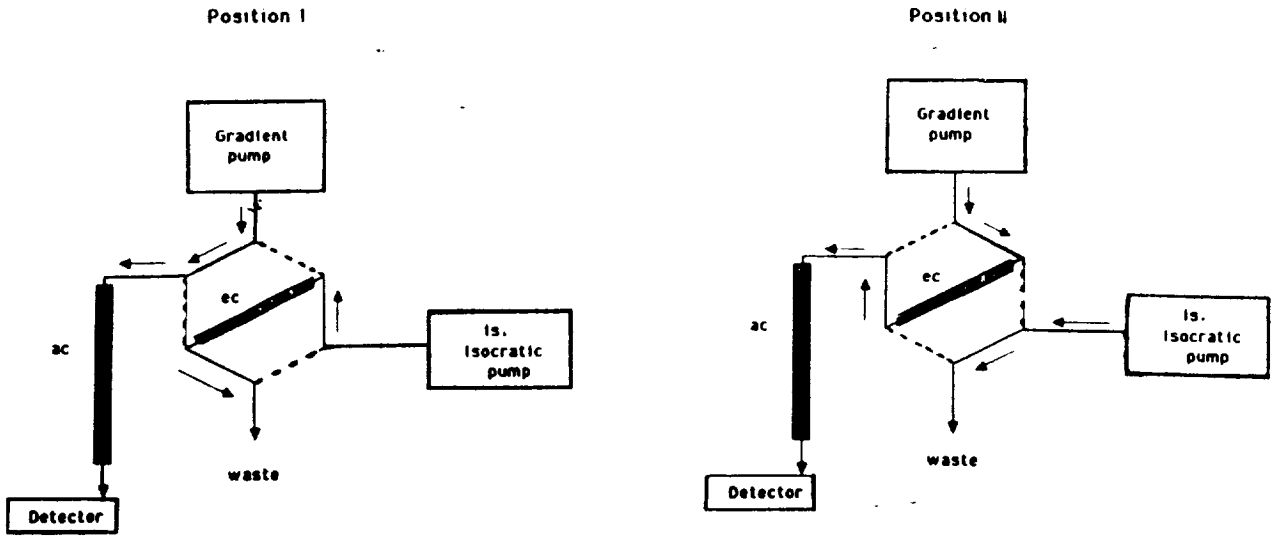
Flow rate:	1.0 ml per minute.
Detection:	UV at 220 and 254 nm.
Injection volume:	100 µl by loop injector.
Quantitation:	By peak heights or areas, external standard method.
Sample preparation:	Grind to a fine powder a portion of the seized sample and dissolve in acetonitrile - citrate buffer pH 3 (2:8) at a concentration of 50 mg/ml. If necessary, the solution may be set on an ultrasonic bath for 15 minutes.

METHOD

Column switching events - see Figure

Time 0:	100 µl Sample is injected onto the extraction column (ec) with water as mobile phase.
Time 1.5 min.:	Washing with water is finished. Switching valve is switched from position 1 to position 2. Gradient is started. Printer started. Trace impurities are desorbed from (ec) and separation on analytical column (ac) begins.

Time 16.5 min.:	Reset switching valve from position 2 to position 1 to allow reequilibration of (ec) with water before next injection.
Time 26.5 min.:	Elution and separation from (ac) are finished.
Time 30 min.:	(ac) re-equilibrated with initial gradient and ready for next injection.



Switching configuration for on-line pre-concentration and clean-up.

ec = extraction column

ac = analytical column

is = injection system

RESULTS

Compound	Capacity ratios K'
Amphetamine	1.7
N-formylamphetamine	4.2
4-methyl-5-phenylpyrimidine	6.2
N,N-di(β-phenylisopropyl)formamide	13.3
N,N-di(β-phenylisopropyl)amine	14.3
N,N-di(β-phenylisopropyl)methylamine	19.3
Caffeine	0.7
Ephedrine	1.5
Phenazone	1.8
Procaine	4.8
Glucose	nd*
Sucrose	nd

*nd - not detected

References

J. Chromatography 369 (1986) p 365.

For other HPLC methods:

1. Analytical Chemistry 58 (1986) pp 1643 - 1648.
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