DIVISION OF NARCOTIC DRUGS
Vienna

RECOMMENDED METHODS FOR TESTING CANNABIS

MANUAL FOR USE BY NATIONAL NARCOTICS LABORATORIES

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ST/NAR/8

CONTENTS

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 harmonization 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 cannabis 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 third 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) analysis, and will be followed by a similar publication dealing with amphetamine/methamphetamine 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 of suspected cannabis. 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 quantitation of one of the cannabinoids present in the material, comparison of samples or the development of typology.

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 and any two of the available chromatography techniques (TLC, GLC or HPLC). The analysis of cannabis products represents a special problem to the forensic chemist.

Because cannabis and cannabis resin are plant material it is mandatory that the analyst includes macroscopic and/or microscopic examination of the material as part of the testing protocol. The choice of the two other techniques or more, is left to the discretion of the forensic 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. Analytsts 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. PRODUCTION OF ILLICIT CANNABIS PRODUCTS

A. Herbal products (Marijuana)

Cannabis (Cannabis sativa L.) is a plant widely distributed throughout the temperate and tropical zones of the world, and most countries have reported illegal growth and traffic of the herbal products. Large scale illicit cultivation of the cannabis plant to make herbal cannabis products occurs in North and South America, the Carribean, Africa and South East Asia. The presentation of the herbal material in the illicit traffic varies not only from region to region, but also within the countries of each region.

It is the traditional belief that only the fruiting and flowering tops and leaves of the cannabis plant contain significant quantities of the psychoactive constituents (e.g. tetrahydrocannabinol); they are known as the "drug-containing parts", and generally it is only these parts of the plant that are sold in the illicit traffic. These parts may be stripped from the plant while it remains growing. The central stem and main side stems of the plant are not removed and play no part in the production of illicit cannabis products. Alternately the entire plant may be removed by cutting the main stem at a point below the lowest leaf-bearing side stems. The separated herbal material, or whole plants, are allowed to air dry, usually by being spread out on the ground or, if in relatively small quantity, by being placed in shallow trays. Whole plants can be dried while suspended upside down and when dried, the drug-containing parts of the plant are stripped from the central and main side stems. A wide range of herbal presentations are made depending on the process subsequently used on the dried material. The separated parts may be highly compressed to make blocks of herbal materials (West African and Caribbean cannabis is frequently trafficked in this form). Alternately the cannabis may be left as a loose herbal material (samples from some Central and Southern African countries and from countries of South West and South East Asia are often in this form). A less frequently encountered presentation is produced when the herbal material is rolled into a "corn-cob" shape and wrapped in coarse vegetable fibre (Central Southern Africa).

If a high quality product is to be made for trafficking the fruiting and flowering tops alone are used. They are most often made into sticks; frequently the fruiting and flowering top is tied using twine around a central bamboo cane. Such sticks weigh about 2 grammes (gross), are approximately 8 centimeters long and are known in the illicit traffick as "Buddha-sticks" (South East Asia). Often when seized from the illicit traffic these sticks are found in bundles of up to 20 sticks. Alternately the fruiting and flowering top is often in a small roll wrapped in brown paper (South Africa). These rolls are considerably smaller than the South East Asian type. Usually there is less than 0.5 grammes of cannabis per roll, and there are few, if any, seeds within the material.

High quality product can be made by sieving herbal cannabis to remove those parts of the plant which contain relatively low levels of cannabinoids, or no cannabinoids. Essentially, this removes seeds and all but the most insignificant stem material. All that passes through the sieving process has been derived from the flowering and fruiting tops or the leaves of the cannabis. The material resembles finely chopped herbal material. In the illicit traffick it is known as "Kif". It is a characteristic product of North Africa. Such material has a high cannabis resin content and can be compressed into slabs which bear some physical resemblence to cannabis resin slabs made in the same region. However, when subjected to microscopic examination, such slabs are found to have retained essentially herbal characteristics. This material, whether loose or compressed into small blocks, has the same cannabinoid profile as cannabis resin slabs made in the same region.

An alternative high quality product is Sinsemilla. The word Sinsemilla derives from two Spanish words which mean "without seeds". Sinsemilla is produced by removal of male cannabis plants from the environment of female cannabis plants before the male plant has released its pollen. The female plants never become fertilized and therefore produce no seeds. It is claimed by those involved in the illicit cultivation of cannabis that the resin bearing parts of such plants contain a higher level of the psychoactive chemicals (e.g. THC) than ordinary female plants which have been allowed to become fertilized in the normal way. Forensic analysis would support this contention, Sinsemilla is found to contain higher levels of the cannabinoids, especially THC.

It is worth noting that the removal of male plants from the environment of female plants before fertilization has occured, has been practiced for many years in, for example, the Indian subcontinent. It was known that if this was not done, the female plants would run to seed, and a very poor yield of "ganja" would be produced. Invariably, however, a few seed bearing flowering tops were present in such material. This may have occured because cannabis is not entirely a dioecious plant. In any large field of cannabis plants, a number will be monoecious, that is bearing both male and female flowers.

Sinsemilla remains, at the time this booklet was prepared (October 1986), a product cultivated only in the Americas, although seizures of Sinsemilla have also been made outside the Americas. The seized material in these cases had, however, been cultivated within the Americas.

B. Resin products (Hashish)

The production of cannabis resin is centered on two main regions of the world. The countries around the Southern and the Eastern part of the Mediterranean form one region, and the countries of the Indian subcontinent form another. A variety of processes have been used in both regions to make cannabis resin. However, in general, the countries of one region use similar techniques. This has resulted in two "families" of cannabis resin. Countries around the Southern and Eastern parts of the Mediterranean make one group of cannabis resin products, and the countries of the Indian subcontinent produce a second group of products. However, there is some similarity in the methods used to make cannabis resin in both regions, for example, there are methods in both regions in which sieving is an important part of the process.

Resin from a single country within either of these regions will show much more similarity in physical appearance to resin from another country of the same region, than it will to a resin from the other region. (There may be significant differences in the cannabinoid profile of resins from one region).

Cannabis resin from Mediterranean countries

The herbal material is threshed, often against a wall. This process is done to separate the resin producing parts of the plant from those parts which do not produce resin, and are therefore low in psychoactive constituents. Particles of cannabis resin and of cannabis leaves, as well as cannabis seeds become detached from the more fibrous parts of the plant. The latter are discarded. The material is then sieved (seeds and minor fibrous parts are eliminated). The product remaining is now even higher in resin content. At this stage macroscopic herbal characteristics are virtually destroyed, but microscopically the material still exhibits many herbal traits. Physically it resembles a fine powder and at this stage it is compressed into slabs. In some countries (Eastern Mediterranean) the material is placed in cloth bags prior to compression, in other countries (North Africa) cellulose wrappings are added before compression. In one area (North Eastern Mediterranean) the material is trafficked occasionally as this fine powder without having been made into slabs.

Cannabis resin from the Indian Subcontinent

A different approach to the production of cannabis resin is used in the countries of the Indian subcontinent. The fruiting and flowering tops of the cannabis plants grown in the countries of the Indian subcontinent contain high levels of resin, to an extent that makes these parts of the plant sticky to the touch. When the fruiting and flowering tops of these plants are rubbed between the palms of the hand the resin is transferred from the plant to the palm.

Production of cannabis resin in the countries of the Indian subcontinent is, therefore, based on a rubbing or kneading process rather than a threshing process. A variety of methods may be used to achieve this. The ones described here may be taken as representative of the process.

A slow and laborious method involves the resin bearing parts of the cannabis plant being rubbed between the palms of the hand. A thin layer of cannabis resin forms on the palms of the hand as the material is rubbed. When all the resin has been transferred from the batch being rubbed, the plant is discarded (It may be used as a second class product, by for example, being made into an infusion similar to tea). The resin that has transferred to the palms of the hand is removed by scraping with the edge of a metal instrument. It may be transferred to a collecting bowl and the next batch of cannabis is subjected to the rubbing process. Gradually, separated cannabis resin builds up in the collecting bowl. A suitable quantity of the resin is then removed from the bowl and then pressed or rolled into slabs, rods, balls or whatever shape is favoured in the particular locality.

An alternative approach is to rub the flowering and fruiting tops of the cannabis against rubber sheeting. The cannabis resin is transferred to the rubber sheeting and from this it can be scraped off and collected into quantities suitable for production of slabs. This approach can be varied by the person who is harvesting the cannabis resin wearing rubber sheeting, or leather or similar fabric, while walking through a field of cannabis plants. Resin accumilates on the rubber sheeting as it brushes against the fruiting and flowering tops of the plants and, when sufficient has been collected, the sheeting may be scraped clean. Production of slabs , etc. then follows as described above.

The flowering and fruiting tops may be collected in a similar way to that used in herbal cannabis production. These are then allowed to dry, and broken and crushed between the hands into a coarse powder. This powder is then passed through sieves so that it attains a fineness similar to that obtained in the Mediterranean. The fine powder, which is still green, is stored in leather bags for four to five months until the weather becomes hot again. The powder is then exposed to the sun for a short time - sufficient for the resin to melt. The powder is replaced in the leather bags for a few days, after which it is removed and kneaded well by means of wooden rods so that a certain amount of oily material appears on its surface. Kneading is continued until a material suitable for pressing into slabs has been produced.

Finally a fundamentally different method is used in some localities of the Indian Subcontinent. By quantity, little cannabis resin is made in this way. The plant material, apart from the main stems, is immersed in boiling water. This removes the resin from the fruiting and flowering tops (Cf. the rending of meat whereby when meat is boiled the animal fats are removed from the flesh). The cannabis which has been extracted is discarded (it may be used for culinary purposes), and when the extracting liquid cools, a layer of solidified resin forms on its surface. This resin may be removed and formed into slabs or whatever shape is favoured. The problem with this method is that water is introduced into the resin. This results in the slabs of resin frequently turning mouldy as they age.

C. Liquid cannabis (Hashish oil)

Liquid cannabis is a liquid extract of either herbal cannabis material or of cannabis resin; the extract is often concentrated prior to trafficking. The reason for making liquid cannabis is to concentrate the psychoactive ingredients (e.g. THC). This may help the trafficker evade interdiction, because more psychoactive material can be contained in a smaller concealment. Of equal value to the trafficker is the ability to insert the liquid cannabis into concealments which cannot easily accommodate herbal or resin cannabis. Furthermore, it is easy to seal hermetically the liquid cannabis, thereby overcoming the possibility of detection by the odour emitted by the material.

Liquid cannabis, whether made from herbal or resin material, is prepared by a process similar to that used to percolate coffee. Alternately the process can be considered as being similar to soxhlet extraction undertaken in chemical laboratories to extract chemicals from solid materials, with continual refluxing of the extracting solvent.

The essential parts of the extraction apparatus are as follows:

(a) THE BOILING FLASK

A flask in which the extracting solvent can be boiled.

(b) THE EXTRACTION CONTAINER

A perforated basket which contains the material to be extracted (Cf. coffee grounds); the extracting solvents, once they have passed through the material to be extracted, are returned to the boiling flask.

(c) THE CONDENSER

A condenser which cools the extracting solvent and allows it to fall onto the material to be extracted.

METHOD

A suitable quantity of chopped herbal material or small pieces of.cannabis resin is placed in the extraction container. Organic solvent is placed in the boiling flask. Suitable organic solvents include ethanol, methanol, acetone, and petroleum ethers. The solvent is heated to boiling and the refluxing process commences. When the batch of cannabis or cannabis resin is fully extracted, heating is stopped and the apparatus allowed to cool. The extracted material in the perforated basket is discarded, as with spent coffee grounds. If necessary, a second fresh batch of cannabis or cannabis resin may be placed in the perforated basket and extracted with the same batch of solvent that had been used for the first extraction. This process can be repeated as often as required, using a number of batches of cannabis or cannabis resin with a single batch of extracting solvent. After the final batch of cannabis or cannabis resin has been extracted, the solvent in the

boiling flask may be concentrated by evaporation to the required consistency. The apparatus is dismantled and the unrequired solvent is boiled away. In some clandestine laboratories, especially in those countries where organic solvents are expensive or difficult to purchase, the excess solvent may be condensed for future use. In general, liquid cannabis, whether made from cannabis or cannabis resin, is prepared to have the consistency of a thick oil.

Alternately, if extraction of a single batch of cannabis or cannabis resin is thought to have made an extract of acceptable strength, the liquid in the boiling flask may be evaporated as described above.

II. DESCRIPTION OF ILLICIT CANNABIS PRODUCTS

A. Names and synonyms for illicit cannabis products

There are so many synonyms used for the various illicit cannabis products that it is beyond the scope of this manual to list them all. The reader is referred to the United Nations publication dealing with this subject - "The Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control", (ST/NAR/1).

B. Physical appearance and chemical characteristics of illicit cannabis products

It must be stressed that no two cannabis products have exactly similar physical appearances. Produced from a highly variable natural product, by a batch process capable of wide variation, and subsequently subjected to processing and transformation for trafficking purposes, it is not surprising that cannabis products occur in such a multitude of forms. Those described here are just a selection, albeit the most common. Because material submitted for forensic examination bears no physical relationship to any type described here, that does not mean, of course, that it is not cannabis or a cannabis containing product.

This section should be read in conjunction with Chapter I "PRODUCTION OF ILLICIT CANNABIS PRODUCTS".

1. Herbal products (Marihuana)

(a) Cannabis grown in a temperate climate

Cannabis cultivated in Europe, the North Americas, and the southern parts of the Southern Hemisphere is bright green when growing; after harvesting some samples lose their green colour and turn yellow, but rarely brown coloured. Generally the fruiting and flowering tops are devoid of resin - unlike herbal cannabis from the Indian Subcontinent they are not sticky when compressed in the palm of the hand. For the same reason it is difficult to compress this material into slabs as can be easily done with, for example, West African Cannabis. Seeds are invariably present. European cannabis will contain a higher leaf content than North American cannabis, in which fruiting and flowering tops predominate.

Chemical characteristics: very variable, because the seeds have been imported, often illicitly, from many different regions where cannabis grows wild. Different cannabinoid profiles, with and without both CBD and THV, are encountered.

(b) Cannabis grown in tropical climate

North African cannabis

Rarely trafficked out of the region; a finely chopped light green or yellow green herb which contains no seeds or fibrous material. Chemical characteristic: identical to the resin produced in the region, i.e. both THV and CBD are low relative to THC.

West African and Caribbean Cannabis

When growing, the material is green; on harvesting and drying, it turns brown. Some samples retain their green colour. Generally, Caribbean cannabis retains its green colour more than West African. It is rare to find a dried sample of West African cannabis which is not brown. Colour apart, these two types of cannabis are physically and chemically very similar. In some samples of West African cannabis the fruiting and flowering tops have been destroyed in processing; many dark brown seeds are visible within the compressed mass of herbal material.

Until recent years Caribbean cannabis was of a low quality, containing many stems and stalks which are low in or completely devoid of the psychoactive constituents of cannabis. A recent trend has been the attempt to produce Sinsemilla; no samples completely free of seeds have yet been detected, but the amount of non-psychoactive containing material in these seizures is greatly reduced, and the fruiting and flowering tops of some seizures are comparable to those found in North American Sinsemilla.

Chemical characteristics: Both types lack CBD and have low THV:THC ratios.

Cannabis from Central Africa

Most samples are similar to West African cannabis, but a few are similar to those produced in the southern part of Africa. Chemical characteristic: Brown samples similar to West African cannabis in cannabinoid profile; green samples similar to southern African cannabis in cannabinoid profile.

Cannabis from Southern Africa

When dried, and prepared for trafficking, this material generally resembles cannabis grown in temperate areas. It is both much greener and contains a higher proportion of leaves than West African cannabis.

Chemical characteristics: No CBD. THV and THC in roughly equal amounts.

Cannabis from South America

Similar to Caribbean cannabis; samples vary enormously in quality from products containing high proportion of fibrous, non psychoactive containing material, to Sinsemilla type products consisting of only fruiting and flowering tops.

Chemical characteristics: Similar to Caribbean. The occasional sample contains a small amount of CBD.

Cannabis from the Indian Subcontinent

Three types may be trafficked: (1) brown fruiting and flowering tops which are high in resin and sticky to the palm of the hand; (2) dark green-brown material similar to some samples from West Africa; (3) green, largely leafy material devoid of fruiting and flowering tops.

Chemical characteristics: (1) CBD present, THC and THV approximately equal; (2) Resembles West African cannabis; (3) Similar to type (1) but low levels of cannabinoids.

Cannabis from South East Asia

"Buddha Sticks" - see Chapter I "PRODUCTION OF ILLICIT CANNABIS PRODUCTS".

Chemical characteristics: Normally only THC, no CBD and negligible THV.

2. Cannabis resin products

North African cannabis resin

Yellow brown, thin rectangular slabs wrapped in cellophane which rarely bears a mark. Coin imprints occur from time to time.

A recently introduced product is superficially similar to cannabis resin from the Indian subcontinent - it is almost black on the surface, and internally is much darker than the yellow brown slabs. This type is in the shape of blocks of toilet soap, and is wrapped in cellophane. No markings but coin imprints on some samples.

Chemical characteristics: CBD generally low relative to THC, and THV very low. Cannabinoid acids present in variable amounts from seizure to seizure.

East Mediterranean cannabis resin

Red-brown and powdery. Invariably trafficked inside cloth bags, which, until a few years ago were always white, but which occasionally bore an ink stamp. Nowadays the cloth bags are sometimes brightly coloured, with or without ink stamps. Slabs up to 0.5 kg in weight, occasionally 1 kg. The resin bears the imprint of the cloth when unwrapped.

Chemical characteristics: CDB present to greater extent than in any other cannabis resin product. THV very low. Acids, mostly CBDA, are also present to greater extent than in any other cannabis resin product.

North Eastern Mediterranean cannabis resin

Greenish-brown powder or (rarely) as small thin wafers of brittle material wrapped in cellophane.

Chemical characteristics: CBD much less than THC. THV low. Acids present in high amounts.

Cannabis resin from the Indian Subcontinent

A great variety of products are made. In quantity the rectangular slabs, black on the surface, and dark green within, which originate from the north west part of the subcontinent, predominate over all other types. These slabs, which frequently bear an embossed mark on the surface, are often wrapped in dark cellophane prior to trafficking. A few slabs are square. The slabs vary in thickness from 5 mm to 20 mm and are odorous and pliable when freshly made. On aging they lose their odour and become brittle. Typically the slabs weigh 0.25, 0.5 or 1 kg, but higher weights are occasionally encountered. Slabs from the northern part of the Indian Subcontinent are often mouldy, and crumble readily.

Other cannabis resin products from the Indian Subcontinent include sticks, often in bundles, small balls (1 cm in diameter), large balls (8 cm in diameter), and irregular shaped pieces of resin. All of these products are dark brown or black on the surface and dark green or dark brown internally.

Chemical characteristics: Varies as greatly as the physical variation. Generally, cannabinoid acid content is lower than for the Mediteranean cannabis resin. The cannabidiol content of the slab variety is less than that of the Eastern Mediteranean resin, but greater than that of the North African resin; it can be very low or absent in some other types. Generally THV is low, but some types contain more THC than any other cannabis resin, and accordingly reach a higher value when sold in the illicit traffic.

3. Liquid cannabis (Hashish oil)

Liquid cannabis is a dark viscous oil with a characteristic odour. When diluted with organic solvents, it becomes either a green coloured or brown coloured solution. The colour is not necessarily an indication of origin because the maturity of the plant material and the solvent used to prepare the liquid cannabis may influence its colour. Generally, liquid cannabis, which on dilution produces a green coloured solution, has been made from herbal cannabis, and liquid cannabis, which on dilution, produces a brown solution, has been made from cannabis resin. Liquid cannabis cannot be diluted with water; if water is added to liquid cannabis which has been diluted with, for example, ethanol, an emulsion is formed.

Some liquid cannabis is not concentrated before being trafficked; this product has the consistency (and often the odour) of an organic solvent, and may be green or brown coloured.

Chemical characteristics: The cannabinoid profile is, with one important difference, similar to that of the cannabis or cannabis resin from which the liquid cannabis has been made. The difference is that liquid cannabis is devoid of cannabinoid acids. The major producing regions of liquid cannabis are the resin producing countries of the Mediterranean and of the Indian Subcontinent, and the herbal cannabis producing Caribbean. The neutral cannabinoid profiles of the liquid cannabis from these regions are similar to those of the resin or herbal products produced in these regions. However, the cannabinoids form a much higher proportion of the material.

Typical THC levels in the three illicit cannabis products:

Herbal cannabis:	0,5 - 5%
Resin cannabis:	2 - 10%
Liquid cannabis:	10 - 30%

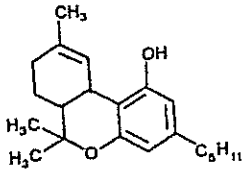
It should be noted that these values are only a guide to levels likely to be encountered by the forensic analyst. Many samples of herbal, resin or liquid cannabis will have a THC content outside these limits.

In addition to the neutral cannabinoids, seized cannabis material may also contain, in greatly varying levels, the corresponding cannabinoid acids (see Chapter III) as well. Although there does not seem to be a consistent relationship between the origin of the material and the actual cannabinoid acid content and composition, the forensic chemist may be called upon, depending on national legislation, to demonstrate the presence and/or determine the content of these acids separately in the sample under examination.

III. CHEMICAL CONSTITUENTS OF FORENSIC SIGNIFICANCE

TETRAHYDROCANNABINOL

THC, Δ^9-THC
(-)- Δ^9-trans-Tetrahydrocannabinol



$C_{21}H_{30}O_2$
M.Wt = 314.5

Melting points (°C)

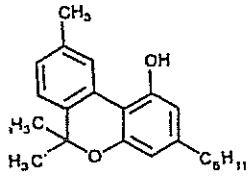
Viscous oil

Solubilities

Water	insoluble
Ethanol	soluble
Chloroform	soluble
Hexane	soluble

CANNABINOL

CBN



$C_{21}H_{26}O_2$
M.Wt = 310.4

Melting points (°C)

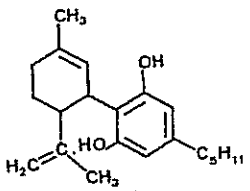
76 - 77

Solubilities

Water	insoluble
Ethanol	soluble
Chloroform	soluble
Hexane	soluble

CANNABIDIOL

CBD



$C_{21}H_{30}O_2$
M.Wt = 314.5

Melting points (°C)

66 - 67

Solubilities

Water	insoluble
Ethanol	soluble
Chloroform	soluble
Hexane	soluble

Other cannabinoids which are referred to in this booklet, together with their abbreviations, are as follows:

Cannabinolic acid	CBNA
Cannabidiolic acid *	CBDA
Cannabichromene	CBCh
Cannabichromenic acid *	CBChA
Cannabigerol	CBG
Cannabigerolic acid *	CBGA
Cannabivarin	CBV
Tetrahydrocannabinolic acid *	THCA
Tetrahydrocannabivarin	THV
Tetrahydrocannabivarinic acid.*	THVA

* In the section dealing with the TLC of cannabis products, reference is made to "cannabinoid acids". This means any mixture of cannabinoid acids which may be encountered in a cannabis product.

The reader is referred to the following books and review papers which deal with cannabinoid chemistry at length:

1. Mechoulam, R., (1973) Marijuana, Academic Press, New York and London.
2. Mechoulam, R., Marijuana Chemistry, Science, 168 (1970), pp. 1159-1166.
3. Turner, C.E. et al., (1980) Constituents of Cannabis sativa L., XVII. A Review of the Natural Constituents, J. of Natural Products, 43 (1980) pp. 169 - 234.

IV. THE ANALYSIS OF ILLICIT CANNABIS PRODUCTS

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. Alternately, it may be necessary to perform separate chemical analyses on two slabs of cannabis resin, rather than a single analysis on one slab which is representative of both, because each has been separately exhibited by the seizing officer, and the legal system within which the analyst works requires an individual result 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 tne legal personnel with whom he or she works.

Cannabis products represent a special problem to the analyst in that the items under examination are often of enormous size and the chemical tests used require only a very small aliquot. Homogenization is not appropriate nor useful in such situations. The analyst must ensure that the entire item or seizure is a controlled drug. The importance of visual examination, which plays a very minor role in the examination of the powder drugs, cannot be over-emphasized in the analysis of cannabis products.

Cannabis is most frequently encountered as a loose herbal material, although in recent years there has been a distinct trend to trafficking herbal cannabis in compressed slabs. Because a much greater quantity of cannabis can be compacted into a compressed slab, there is much less risk of detection when trafficking. Compressed slabs are also much easier to wrap, for example in thick masking tape which prevents the release of the distinctive odour associated with cannabis. Moreover, the compressed cannabis can be formed to fit exactly into commercially made tin-cans, which are then labelled to give the impression that they contain the foodstuffs of legitimate commerce.

Cannabis resin is almost always in slab form. In large scale illicit traffic the slabs are invariably wrapped. The materials used to wrap cannabis resin may be applied at the point of manufacture (e.g. Eastern Mediterranean resin) or it may be applied prior to trafficking (e.g. South West Asian resin). For trafficking purposes other wrappings may be added to those traditionally associated with the resin produced in a particular country. Almost always these wrappings are either plastic bags or thick plastic adhesive tape, or a combination of both. A seizure of cannabis may be of material within a single container or package, or the material may be inside a number of packages.

1. Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material - for cannabis most often the material will be a loose herb. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. The analyst should then carefully use visual testing to ensure that all the exhibit is material which is controlled under the legislation within which he or she works. The sequence of chemical tests can then follow. Homogenization of the material need only be applied in certain analytical situations, e.g. if the analyst wishes to quantify a particular cannabinoid. The simplest way of homogenizing cannabis (herbal or resin forms) is to pass the material through progressively finer sieves. In quantifying cannabinoids care should be taken to relate the content found to the total amount of cannabis plant material which was originally taken for analysis i.e. the content should not be quoted as a percentage of the weight of the final sieved material which was subjected to extraction.

2. Sampling of items consisting of more than one package

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

1. If all packages contain suspect cannabis or cannabis-containing 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 material. 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 material in all the packages is found by visual examination to be the same then the analyst may adopt one of two approaches:

(1) the contents of a number of packages may be combined and the combined bulk material may then be homogenized;

(2) alternately, chemical testing may be applied to a number of the packages.

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 extraction solvent. If the cost of solvent presents no problem and if the taking of a large aliquot will not significantly reduce the size of the exhibit to be taken to court, then this approach may be adopted. However when large amounts of material are used for the first extraction, it may be necessary that the solvent should be added by pipette to avoid error due to insoluble materials.

3. Sampling of materials containing large aggregates

If the aggregates can be easily reduced to small particles then this should be the approach, and sampling procedure followed as outlined previously. If the material cannot be easily broken down, then random samples should be drawn from at least two different parts of the item. In the case of large compressed blocks of herbal material, the analyst should ensure that the block is entirely composed of cannabis. This is achieved by breaking open the block.

B. Physical examination

1. Macroscopic characteristics

Many of the morphological characteristics of individual cannabis plants are greatly influenced by environmental factors such as room for growth, amount of light, nutrients and water, and by hereditary factors such as the seed strain from which it is derived. There is an enormous variation in size and shape. Typically 1 to 3 meters represent the height that most plants attain (when cultivated in open ground it can grow to a height of 6 meters in a four to six month growing season) but some strains produce plants which are rarely more than 1 meter in height. The plant is erect and the extent of branching, like the plant height, depends on both environmental and hereditary factors. The side branches are opposite on the main stem. However, on the extremities of the plant, the leaf arrangement reverses from decussate to alternate (see Figure 1).

The compound leaves vary in size according to the overall size of the plant. Each leaf has a slender stalk up to 6 cm in length. The three to eleven (mostly five, seven or nine) thin and soft-textured leaflets are narrowly lanceolate. The leaflet has a narrow wedge-shaped base, a coarsely saw-toothed edge and a long drawn-out pointed tip; the teeth, are sharp and point towards the tip of the leaflet; the veins run out obliquely from the midrib to the tips of the teeth. The leaflets of a single leaf are uneven in size, the largest being up to 15 cm. They are covered with glandular hairs (trichomes) on the upper surface, more profuse and longer hairs on the underside.

The flowers are very abundant and they are either male (staminate) or female (pistillate). Most plants are dioecious, but some are monoecious. Female plants are very leafy up to the top, whereas male plants have the leaves on the inflorescence fewer and much further apart.

The male inflorescence is loosely arranged, much branched and many flowered, standing out from the leaves, with individual flowering branches up to 18 cm long; it is covered by minute bristly hairs.

The female inflorescences do not project beyond the leaves; they are compact, short and contain fewer flowers. The bract or calyx completely covers the ovary, and forms a basally swollen tubular sheath about 2 mm long, out of which two stigmas project. This sheath is covered with slender hairs and short-stalked or stalkless circular glands.

Figure 1. Cannabis sativa L.

1 flowering shoot
2 male inflorescence
3 male flower
4 female inflorescence
5 female flower
6 fruit
7 seed

2. Microscopic characteristics

The very abundant trichomes which are present on the surface of the fruiting and flowering tops of cannabis are the most characteristic features to be found in the microscopic examination of cannabis products (Figure 2).

The diagram shows these various features, as follows:

A. Non glandular hairs (trichomes), numerous, unicellular, rigid, curved, with a slender pointed apex and an enlarged base, usually containing a cystolith but frequently broken and the cystolith freed (especially in cannabis resin) (NC. TR. and C.TR.).

B. The glandular thrichomes occur in three forms:

- sessile glands with one-celled stalk (generally on lower epidermis) (S.G.)

- long multicellular stalk form (generally on the bracteoles surrounding the female flowers)(M.G.TR.).

The head in both forms is globular consisting of eight to sixteen cells. It is frequently detached (especially in cannabis resin).

- small glandular trichome, with one-celled stalk (G.TR.)

Note

Macro- and/or microscopic examination are inappropriate in the forensic examination of some cannabis products. Both micro- and macroscopic features of herbal cannabis will not be present in liquid cannabis. The forensic examination of liquid cannabis is essentially based on chemical techniques, although the forensic chemist should be aware of the physical appearance and properties of liquid cannabis. The macroscopic characteristics and, to a lesser degree, the microscopic characteristics of cannabis products are also destroyed when the material is smoked. Generally, chemical analysis will produce more useful results in the examination of cannabis products which have been smoked, although there are occasions when microscopic evidence is still available.

For detailed descriptions of the morphological and microscopic characteristics of cannabis, the reader is referred to the following books and review papers:

1. Graham, J.D.P. (1976) Cannabis and Health. Academic Press, New York and London.
2. Nahas, G.G. (1973) Marihuana - Deceptive Weed. Raven Press, New York.
3. Mechoulam, R. (1973) Marihuana. Academic Press. New York and London.
4. Quimby, M.W. et al., (1973). Econ. Bot. 27, pp. 117 - 127.
5. Jackson, B.P. and D.W. Snowdon (1968) Powdered Vegetable Drugs. Churchill, London.

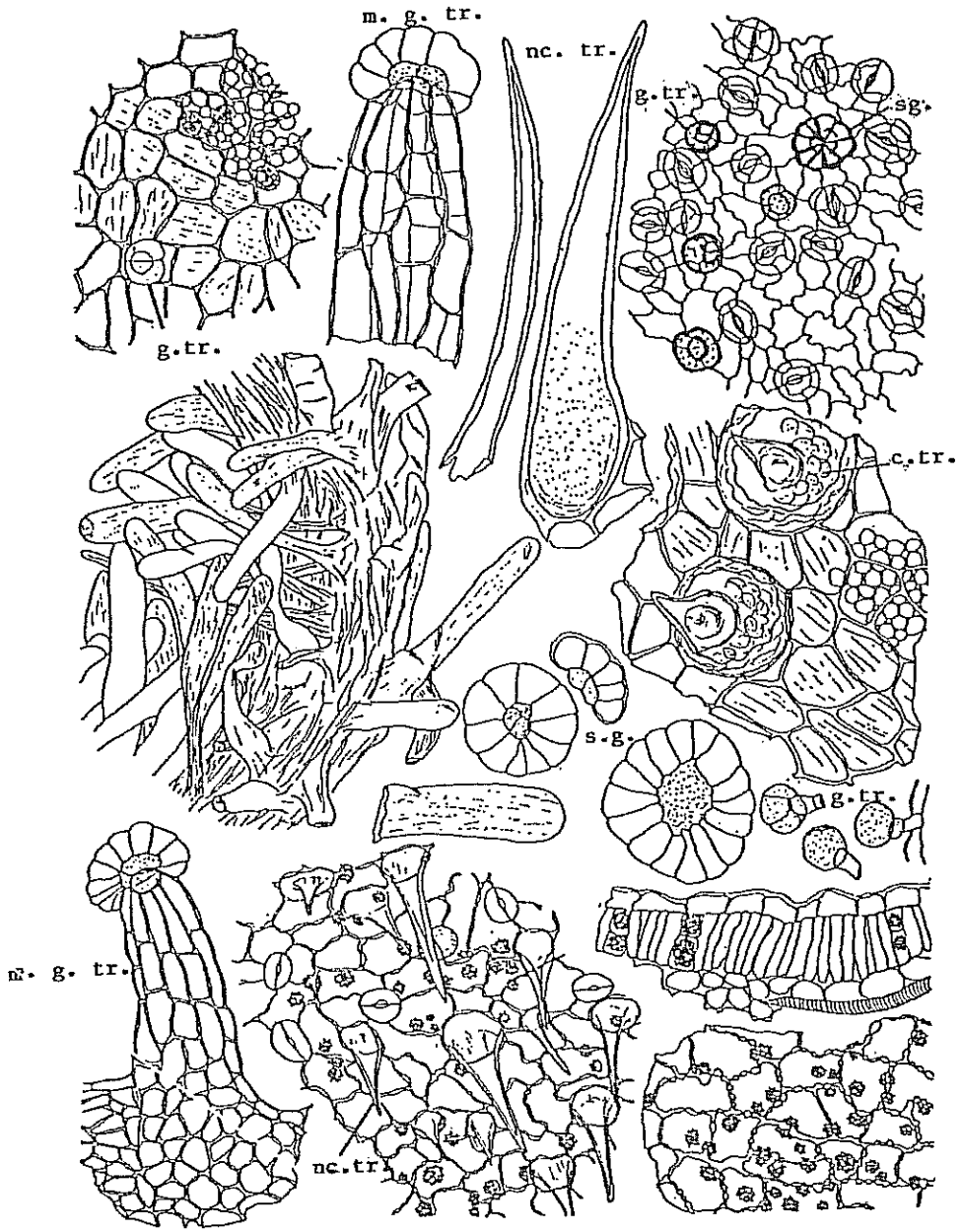


Figure 2. Microscopic characteristics of Cannabis

G.TR.	small glandular trichomes
M.G.TR.	multicellular multiseriate glandular trichomes
S.G.	sessile glands
NC.TR.	non-cystolithic trichomes
C.TR.	cystolithic trichomes

C. Presumptive tests

1. Colour tests

It must be stressed that positive results to colour tests are only presumptive indications of the possible presence of cannabis products or material containing cannabis products. A few other materials, often harmless and uncontrolled by national legislation or international treaties, may give similar colours with the test reagents. It is mandatory for the analysts to confirm such results by the use of an alternative technique.

All colour test reagents should be carefully scrutinized to ensure that they have not decomposed. Colour test reagents that are themselves coloured may lead to erroneous conclusions about the nature of the substance under test.

(a) Fast blue B salt test

METHOD 1. (test performed on filter paper)

REAGENTS

Solid reagent.	Fast blue B salt (di-o-anisidinetetrazolium chloride). The solid reagent is made by diluting Fast blue B salt with anhydrous sodium sulphate (1:100).
Solution 1.	Petroleum ether.
Solution 2.	A 10% w/w aqueous solution of sodium bicarbonate.

METHOD

Fold two filter papers into quarters and open partly to form a funnel; place a small amount of pulverized cannabis plant or resin or a very small drop of liquid cannabis into the centre of the upper paper; add two drops of solution 1 allowing the liquid to penetrate to the lower filter paper; separate the two filter papers, discarding the upper and allowing the lower filter paper to dry; add a very small amount of the solid reagent to the lower filter paper and then add two drops of solution 2.

RESULTS

A purple-red coloured stain at the centre of the filter paper is indicative of a cannabis product; this colour is a combination of the colours of the different cannabinoids which are the major components of cannabis: THC = red, CBN = purple, CBD = orange.

NOTES

1. When freshly made the solid reagent will be almost white or a very pale yellow colour. It should be stored in a cold dry place - within a plastic bag; inside the ice-making compartment of a refrigerator is ideal. If this reagent decomposes, it assumes a grey colour and should be discarded.

2. Fast blue B salt is claimed by some authorities to be a potential carcinogen; the same authorities assert that the dye Fast blue BB is less suspect as a potential carcinogen. Fast blue BB gives equally acceptable results in either of the two methods and, if possible, should be the dye used for colour tests for cannabis products.

3. To increase the specificity of this test, it is important to use an amount of suspect material no larger than the size of a match-head, and to use two filter papers in the test. The upper filter paper, which is discarded before colour production is initiated, prevents coextracted dyes present in other vegetable materials from reaching the lower filter paper and producing a false positive reaction.

4. The 10% solution of sodium bicarbonate (solution 2) produces the alkaline conditions which enhance the intensity of the colour reaction between the cannabinoids and the Fast blue B salt.

METHOD 2. (test performed in a test tube)

Solid reagent. The solid reagent is made by diluting Fast blue B salt with anhydrous sodium sulphate (2.5:100).

Solution 1. Chloroform.

Solution 2. 0.1N aqueous sodium hydroxide solution.

METHOD

Place a small amount of the suspected material (as described in test 1) in a test tube; add a very small amount of the solid reagent and 1 ml of Solution 1; shake the test tube for one minute; add 1 ml of solution 2; shake the test tube for two minutes; allow the test tube to stand for two minutes.

RESULTS

Colours, as described in Test 1 above, in the lower chloroform liquid layer indicate a positive result. The colour of the upper layer should be ignored.

NOTES

Please see Notes 1 and 2 dealing with the Fast blue B salt test when performed on filter papers.

(b) The rapid Duquenois test (Duquenois-Levine test)

REAGENTS

Solution 1. Five drops of acetaldehyde and 0.4 g of vanillin are dissolved in 20 ml of 95% ethanol.

Solution 2. Concentrated hydrochloric acid.

Solution 3. Chloroform.

NOTE

Solution 1 must be stored in a cool dark place and discarded if it assumes a deep yellow colour.

METHOD

Place a small amount of the suspect material in a test tube and shake with 2 ml of solution 1 for one minute; add 2 ml of solution 2 and shake the mixture and then allow it to stand for ten minutes; if a colour develops add 2 ml of solution 3.

RESULTS

If the lower (chloroform) layer becomes violet coloured this indicates the presence of a cannabis product.

D. Thin layer chromatography

DEVELOPING SOLVENTS

SYSTEM A	Petroleum ether	80
	Diethyl ether	20
SYSTEM B	Cyclohexane	52
	Di-isopropyl ether	40
	Diethylamine	8
SYSTEM C (for canna-binoid acids)	N-hexane	70
	Dioxane	20
	Methanol	10

Preparation of solutions to be applied to the TLC plate

Illicit cannabis samples

1. It should be noted that if the sole purpose of the TLC examination is qualitative (i.e. to confirm the micro- or macroscopic evidence that the suspect material is cannabis) then homogenization of herbal material need not be undertaken. Those parts of the cannabis plant known to contain the highest levels of cannabinoids (i.e. the flowering and fruiting tops and the leaves) may be selected for extraction for TLC examination. Little, if any, cannabinoids are present in the seeds and major stems of the plant.

The material (herbal or resin) should be reduced to small aggregates homogenized and pulverized to ensure as rapid and complete an extraction as possible. For cannabis resin and liquid cannabis the forensic scientific literature indicates that these materials are essentially homogeneous as a result of their production.

2. Suitable quantities for extraction for TLC analysis are about 1 g of herbal cannabis, 0.5 g of resin cannabis and 0.1 g of liquid cannabis. The extraction scheme should be designed to produce final solutions at strengths of 0.5 mg of tetrahydrocannabinol per ml. Typical levels of tetrahydrocannabinol in materials were listed in Chapter II "DESCRIPTION OF ILLICIT CANNABIS PRODUCTS".

3. Since cannabinoids are easily soluble in most organic solvents, petroleum ether, n-hexane, toluene, chloroform, methanol or methanol:chloroform 9:1 are equally suitable solvents for their extraction. It should, however, be noted that petroleum ether and n-hexane will give a relatively clean extract but will extract only the neutral cannabinoids quantitatively while the other solvents and their combinations give quantitative extraction of the cannabinoid acids as well. The final selection of the extracting solvent will be left to the decision of the forensic chemist (see also Chapter IV (E) "Gas liquid chromatography").

4. It should not be necessary to filter solutions prepared only for TLC or GC; application of the supernatant liquid will produce reliable results.

A suitable extraction procedure is as follows:

1 g of herbal cannabis (or 0.25 g of cannabis resin or 0.1 g of liquid cannabis) is extracted with 20 ml of acetone (or n-hexane or toluene or chloroform or methanol or methanol:chloroform 9:1) for 30 minutes at room temperature by shaking or for 15 minutes in an ultrasonic bath. The extract is filtered and its volume is adjusted to 25 ml by washing the filter paper and the residue with the extracting solvent.

Standard solutions

The cannabinoid standard solutions should be prepared to be 0.5 mg per ml in methanol (or in the internal standard solution) and should be stored in a dark, cold place, preferably in a refrigerator.

VISUALIZATION

The plates must be dried prior to visualization. This can be done at room temperature or, more quickly, by use of hot air. In the latter case care must be exercised that no component of interest is decomposed.

Visualization method:

Spray reagent: Solution of Fast blue B salt.

This may be prepared in two ways:

Method 1: Approximately 50 mg of Fast blue B is dissolved in 20 ml of 0.1 N NaOH.

Method 2: Approximately 50 mg of Fast blue B is dissolved in 1 ml of water and 20 ml of methanol; to facilitate solution the material may be first dissolved in the 1 ml of water to which the 20 ml of methanol are added.

N.B. Whichever method is used, the solution of Fast blue B salt must be freshly made. An acceptable frequency is once per day.

Note

The reader is referred to the health-risk warning given about Fast blue B salt in the colour tests section.

It is important for proper colour development that the TLC plate be made alkaline. One way of achieving this is to dissolve the Fast blue B dye in 0.1N sodium hydroxide (see method 1 above). Alternately, diethylamine may be sprayed on the TLC plate before the Fast blue B solution.

Of equal importance in the forensic field is the ability to store the visualized plate, often for years, after it was developed. Preservation is best achieved by subjecting the plate to a third and final spraying, this time with the same diethylamine solution that was initially used on the plate. Thus the spraying sequence is:

Diethylamine
Fast blue B solution
Diethylamine

The plates are dried by hot air, or overnight at room temperature. Finally, the plates are sealed inside clear plastic bags. Such plates have a long lifetime without darkening.

RESULTS

R_f x 100 values*:

Compound	DEVELOPING SYSTEM		
	A	B	C
CBCh	24	17	-
CBV	27	24	-
CBN	27	28	68
THV	32	35	-
THC	32	39	73
CBD	36	44	62
THCN	s	s	28
CBDA	s	s	20

(s) = streak, not spot, produced on the TLC plate.

* These values are subject to variation depending on laboratory conditions (e.g. temperature, humidity, drafts) and other parameters (e.g. age and quality of materials used).

E. Gas liquid chromatography

1. Packed column technique

Detector	FID (Hydrogen 30 ml per minute, air 300 - 450 ml per minute).
Column	6 ft (or 2 m), I.D. 2 to 4 mm.
Packing	3% OV-17 or SE-30 or OV-1
Carrier gas	Nitrogen at 30 ml per minute.
Operating conditions:	Injector temperature: 270°C. Oven temperature: between 240-260°C isotherm, (depending upon the actual packing) Detector temperature: 300°C
Internal standard	n-tetradecane or n-docosane or other suitable n-alkene; other standards frequently used: androst-4-ene-3,17-dion, dibenzylphthalate or cholestane.

Preparation of solutions for gas chromatography

METHOD 1. - without derivatization

For packed column GC analysis a commonly used method is that an injection of 5 µl of solution will result in 1 µg of THC being injected into the column. The dilution scheme should take account of the likely cannabinoid content of the starting material (see chapter II). A typical scheme would be as follows:

Illicit cannabis samples

For qualitative GC analysis the extracts prepared for TLC may be used directly. Suitable injection volumes may be 1-5 µl depending upon the actual THC concentration of the sample.

For quantitating the main neutral cannabinoids a 10 µl aliquot is taken of the same extract. After evaporating the solvent in vacuo the residue is redissolved in 10 ml of methanol:chloroform (1:1) containing 2 mg/ml n-tetradecane as internal standard.

Injection volume: 1-5 µl.

Standard solutions - same as for TLC

An alternative approach is:

0.5 g of herbal cannabis (0.1 g of cannabis resin; 0.05 g of liquid cannabis) is extracted with 5 ml of acetone containing 0.5 mg/ml n-docosane in a stoppered flask at room temperature by frequently shaking the flask for 30 min. 1 µl is injected of the clear supernatant. This extract can also be used for TLC and HPLC analysis.

METHOD 2 - with derivatization

Illicit cannabis samples

2 ml aliquots of the extracts prepared for TLC or GC analysis without derivatization (Method 1) can be used for silylation. Derivatizing agents frequently used are:

N,O-bis(trimethylsilyl)acetamide (BSA)
N-methyl-N-trimethylsilytrifluoreacetamide (MSTFA)
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

An alternative approach is:

1 g of herbal cannabis (0.25 g of cannabis resin or 0.1 g of liquid cannabis) is extracted with 40 ml methanol:chloroform (9:1) using ultrasonic agitation for 15 minutes. The cannabis is removed by filtration. 4 ml aliquot of the filtered extract is taken and the solvent is removed under vacuum until a paste is produced. 1.5 ml of anhydrous pyridine containing 1 mg per ml of androst-4-ene-3,17-dione is added and the solution is subjected to ultrasonic agitation until the paste has redissolved. 0.5 ml of N,O-bis(trimethylsilyl)trifluoro-acetamide (BSTFA) with 1% trimethylchlorosilane is added. The reaction mixture is heated for 10 minutes at 80°C. 2.5 µl of the reaction mixture is injected.

Standard solutions

2 ml aliquots of the cannabinoid standards are treated the same way.

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

$$C_X\% = \frac{C_{r.\,std.}}{C_{samp.}} \times \frac{A_X \,/\, A_{int.std.in\ samp.\ chrom.}}{A_{r.std} \,/\, A_{int.std.in\ std.\ chrom.}} \times 100$$

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%)

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

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

$A_{r.std.}$ = peak area for substance x obtained during the standard solution chromatography.

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

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

For alternative packed column GC systems see:

1. J. Pharm. Sci. 63 (1974) pp. 1872-1876; 64 (1975) pp 810-814.
2. J. Pharm. Pharmacol. 33 (1981) pp. 369-372.
3. J. Chromatography 129 (1976) pp. 347-354.
4. Pharm. Acta Helv. 59 (1984) pp. 247-259.
5. Bull. Narcotics 37 (1985) pp. 87-94.

2. Capillary column technique

Detector	FID.
Column	OV-1 - chemically bonded fused silica capillary 10 m by 0.52 mm I.D.
Film thickness	1 µm
Carrier gas	Helium
Flow Rate	2 ml per min.
Injection technique	split-splitless
Operating temperatures:	Injector: 290°C. Oven: 240°C. Detector: 290°C.

Preparation of solutions for chromatography

See the section dealing with preparation of solutions for TLC or gas chromatography in the packed column section; both non-derivatization and derivatization methods may be used with capillary column GC analysis of cannabis products.

For alternative capillary GC systems see:

1. Anal. Chem. 48 (1976) pp. 24-29.
2. Bull. Narcotics 33 (1981) pp 45-54.
3. Forensic Sci. Int. 24 (1984) pp. 37-42.
4. Acta Univ. Palack. Olomouc. 97 (1981) pp. 157-166; 108 (1985) pp. 29-38.
5. Pharm. Acta Helv. 59 (1984) pp. 247-259.

F. High performance liquid chromatography

1. Isocratic technique

METHOD 1

Operating conditions

Column	250 mm by 4.6 mm i.d.
Packing material	Octadecyl-silica (medium load of C_{18} on Partisil 5)
Mobile phase	0.02 N H_2SO_4 20% v/v Methanol 80% v/v
Flow rate	2.0 ml per minute
Detection	UV at 220 nm or UV at 254 nm
Injection volume	10 µl by syringe or loop injector.
Quantitation	by peak areas, internal standard method.
Internal standard	di-n-octyl phthalate.

Preparation of solutions for chromatography

Illicit cannabis samples

Aliquots corresponding to 200 mg of herbal cannabis, 50 mg of cannabis resin or 20 mg of liquid cannabis of any of the extracts prepared for TLC or GC analysis are evaporated in vacuo and the residue is redissolved in 1 ml of methanol:chloroform (9:1) containing 0.8% (g/v) di-n-octyl phthalate as internal standard.

Standard solutions

Using stock solutions of cannabinoids a series of calibration solutions are prepared in the range 0.1 to 10 µg per ul. A constant addition of 13 µg per ul of di-n-octylphthalate is made to each standard solution.

METHOD 2

Operating conditions

Column	150 mm by 4.6 mm I.D.
Packing material	Octadecyl-silica (Spherisorb S3 ODS 2) HPLC grade 3 μm
Mobile phase	Methanol 85 Water 14.2 Acetic acid 0.8
Flow rate	1.5 ml per minute
Operating temperature	ambient
Detection	UV at 230 μm
Injection volume	2-3 ul
Quantitation	by peak areas, internal or external standard methods

Preparation of solutions for chromatography

Illicit cannabis samples

See Method 1.

An alternative approach is:

150-200 mg of herbal cannabis, 50-100 mg of cannabis resin or 5-10 mg of liquid cannabis are extracted for 15 minutes (ultrasonic agitation) in a 2.5 ml screw-topped bottle with heat-stable PTFE seal. The extracting solvent is 1 ml of methanol:chloroform (9:1) containing 0.8% (g/v) di-n-octyl phthalate as internal standard. The mixture is centrifuged for 5 minutes at 3500 r.p.m. and the supernatant liquid is used for analysis.

Standard solutions

Using stock solutions of cannabinoids a series of calibration solutions are prepared in the range 0.1 to 10 μg per μl. A constant addition of 13 μg per μl of di-n-octylphthalate is made to each standard solution. 5 μl of each concentration is injected.

RESULTS

Elution orders are as follows (retention times in minutes)*:

COMPOUND	METHOD 1	METHOD 2
OBV	—	4.0
CBD	2.5	4.1
CBG	2.5	4.1
THV	—	4.6
CBDA	3.5	4.6
CBGA	5.0	5.5
CBN	5.0	5.7
THC	6.0	6.4
THVA	—	7.7
CBCh	8.0	7.7
CBNA	12.0	—
THCA	14.0	11.4
CBChA	17.0	12.7
Int. std.	19.0	17.6

* These values are subject to variation depending on laboratory conditions (e.g. temperature, humidity, drafts) and other parameters (e.g. age and quality of materials used).

2. Gradient technique

METHOD 1

Operating conditions

Column	250 mm x 4.6 mm I.D.
Packing material	Ultrasil-Octyl HPLC grade 10 µm
Mobile phase	A. Acetonitrile B. Water (deionized and filtered through 0.45 µm filter)
Gradient programe	(1) At the start of chromatographic development: 25% A, 75% B. (2) 36 minute linear gradient. (3) Final composition: 85% A, 15% B.

Flow rate	2.0 ml per minute
Detection	UV at 254 nm
Oven temperature	40°C
Injection volume	20 µl
Quantitation	by peak areas, internal standard method
Internal standard	di-n-octyl phthalate.

Preparation of solutions for chromatography

See previous section: Isocratic technique, Method 1.

METHOD 2

Operating conditions

Column

Two columns are used in this method under identical operating conditions.

Column (1) Packing material	150 mm x 4.6 mm I.D. Spherisorb S3 ODS2 HPLC grade 3 µm
Column (2) Packing material	250 mm x 5.0 mm I.D. Spherisorb S5 ODS HPLC grade 5 µm

Mobile phase:	A. Methanol B. 0.02N H_2SO_4
Solvent programme:	(1) At the start of chromatographic development: 80% A, 20% B. (2) 20 minute linear gradient. (3) Final composition: 90% A, 10% B.
Flow rate:	1.5 ml per minute
Operating temperature:	Ambient
Detection:	UV at 230 nm
Injection volume:	2-3 µl

Preparation of solutions for chromatography

See previous section: Isocratic technique, Method 2.

RESULTS

Elution profile is as follows (retention times in minutes)*:

CBV	7.4
CBD	7.8
CBG	8.3
THV	8.6
CBDA	9.4
CBGA	11.8
CBN	12.0
THC	13.7
THVA	15.6
CBCh	16.9
THCA	21.5
CBChA	23.1

* These values are subject to variation depending on laboratory conditions (e.g. temperature, humidity, drafts) and other parameters (e.g. age and quality of materials used).

For alternative HPLC techniques see:

1. Pharm. Acta Helv. 59 (1984) pp. 247-259.
2. Forensic Sci. Int. 21 (1983) pp. 129-137.

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are seen only rarely. Therefore, this section will be limited to the following substances:

Marijuana (cannabis)
Cocaine
Heroin
Phencyclidine (PCP)
Amphetamines
Barbiturates
Selected hallucinogens (LSD, mescaline, psilocybin)

An examination of the literature would reveal that there are many articles, books, research reports, and so on, on all aspects of these drugs. Because of the breadth of the subject, it will be necessary to limit this discussion in scope to those matters that are of direct concern to their analysis and presentation in court by the drug analyst. Within the discussion on each drug, the following topics will be covered:

1. Legal definition (according to Title 21, Food and Drugs Act, Part 1308, revised, 1982)
2. Occurrence (natural or synthetic)
3. Active ingredient(s)
4. Extraction/preparation
5. Methods of analysis
6. Legal/scientific problems

This material is by no means exhaustive of all the information available on each drug. The reader is referred to the extensive Bibliography at the end of the chapter for further information.

Marijuana

Legal Definition

The term "marijuana" means

all parts of the plant *Cannabis sativa* L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resins; but shall not include the mature stalks of such plant, fiber produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt derivative, mixture or preparation of such mature stalks (except the resin extracted therefrom) fiber, oil, or cake, or the sterilized seed of such plant which is incapable of germination

as defined in Federal Schedule I.

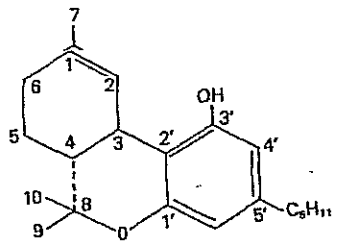
Occurrence

As defined above, marijuana is synonymous with the plant *Cannabis sativa* L. In fact, as will be seen later, there are purported to be at least two other species of *Cannabis*, *indica* and *ruderalis*, whose possible existence have caused some problems with the analysis of marijuana for forensic purposes. *Cannabis sativa* was originally cultivated in the Orient for its fibers but now grows worldwide, including the United States, where it is more of a weed than anything else. Of the other proposed species of marijuana, it is stated that *Cannabis indica* is not grown for its fibers but strictly for psychoactive purposes. *Cannabis ruderalis* is not native to the West, growing only in the USSR and surrounding areas.[10]

Active Ingredients

Regardless of the species of *Cannabis*, the active ingredients are all the same, although they may vary in quantity. Collectively, these substances are known as cannabinoids. They are practically unique among the naturally occurring psychoactive substances in that they do not contain nitrogen and hence are not alkaloids. There are over 20 of these cannabinoids that have been isolated and identified, but only a few are of any forensic importance.

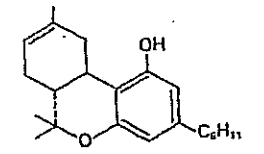
It should be noted that confusion has arisen because of the two different numbering systems used to identify particular carbon atoms in the naming of the cannabinoids. The one that will be used here is the one that treats all these substances as substituted monoterpenoids. This approach has the advantage of being applicable to virtually every member of this family.



Δ^1-trans-Tetrahydrocannabinol

The primary psychoactive agent in marijuana and its preparations is Δ^1-*trans*-tetrahydrocannabinol (THC). In American-grown *Cannabis sativa* the Δ^1-THC content is usually less than 1% in the leaves and 1 to 5% in the flowers. Leaves from purported *Cannabis indica* contain more psychoactive ingredients.[10] Hashish, a resinous extract of the flowering tops of marijuana, is much more concentrated in the resin and thus in THC.

Other major cannabinoids of interest are Δ^6-THC, cannabidiol (CBD), and cannabinol (CBN). Δ^6-THC is a minor constituent of marijuana, usually comprising less than 10% of the total THC content. CBD and CBN are both usually found in naturally occurring samples of marijuana and can easily be separated from THC chromatographically. Their presence will normally allow for reliable differentiation between naturally occurring and synthetic THC samples.



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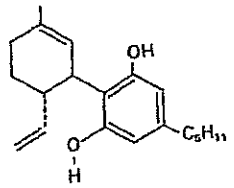
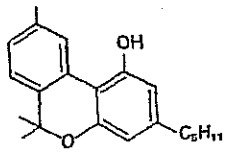


Cannabidiol



Cannabinol

Preparation of Δ^1-trans-THC

Although there are a number of syntheses for THC, the most practical one for nonbiologic purposes (where it is not necessary to isolate pure levo-THC) is the reaction of citral (I) and olivetol(II) in 1% BF_3 and methylene chloride. This gives *d,l*-Δ^1-*trans*-THC in 20% yield and some *d,l*-Δ^1-*cis*-THC, as depicted in Figure 3-1.[11] Synthetic THC is not usually prepared for abuse purposes because of the ready availability of marijuana and the difficulty of the synthesis.

There are a number of methods for extraction of the isomeric THCs from marijuana and/or hashish for later confirmation or experimentation. One such method involves petroleum ether extraction of an aqueous methanolic mixture of plant material followed by preparative TLC using silica gel as the stationary phase and methanol–ammonium hydroxide-saturated chloroform (1:18) as the mobile phase. The THC is then removed from the substrate with chloroform.[12]

Methods of Analysis

The majority of forensic science laboratories employ low-power microscopy and a color test (the modified Duquenois-Levine test) for the analysis of marijuana and its preparations, such as hashish. In addition,

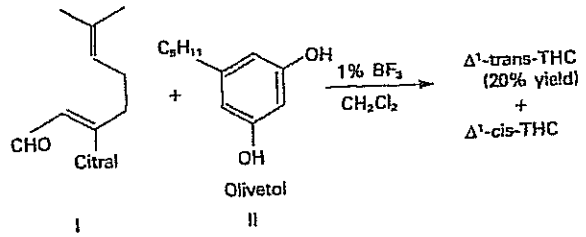


FIGURE 3-1 Synthesis of Δ^1-*trans*-THC.

many also employ GC or TLC as a further test for THC. On rare occasions even GC-MS will be used for confirmation. In those states where the percentage of THC in a sample is important for legal purposes, the quantitative analysis is usually obtained by GC.

Hashish or other preparations of marijuana are usually examined under a high-power (100 to 200×) microscope. Seeds are also examined microscopically and may be subjected to the Duquenois-Levine test.

In the microscopic test for marijuana one is normally examining crushed, dried leaf material. There are two major morphological features of importance in this low-power examination. The first is the presence of cystolithic hairs. These are claw-shaped, usually curved structures with a broad circular base. Within the hair is a cystolith of calcium carbonate. The presence of the cystolith can (and should) be confirmed by adding a drop of acidified chloral hydrate to the leaf material on a microscope slide, quickly covering it with a coverslip, and then observing under a high-power microscope. Evolution of bubbles of carbon dioxide confirms the presence of a true cystolith. It should be noted that cystolithic hairs have been identified in some 80 species of plants, so this test is not confirmatory for marijuana. On the reverse side of the leaf containing the cystolithic hairs can be found numerous short, wide nonglandular covering hairs. These are also common to many species of plants.[13]

Finely powdered cannabis should be examined under a high-power microscope. In this state one finds less of the cystolithic and covering hairs intact on the leaf, but many broken ones are present, as are pieces of epidermis, resin sacs, and other structures. Some of the same materials may be present in hashish, depending on the efficiency of the resin extraction or isolation.[13]

The modified Duquenois-Levine test (see Appendix 3-1) can be run on virtually any sample of cannabis, although fresh or wet leaf material should be dried first. A bag of suspected cannabis seeds can be tested for traces of THC clinging to the surface of the seeds by washing them with petroleum ether, evaporating, and running the test on the residue. The results of the Duquenois-Levine test will quickly deteriorate if fresh, po-

tent concentrated hydrochloric acid is not used. The test should be run at least once a day on known cannabis to make sure that the reagents are potent.

The Duquenois-Levine test is by no means specific for marijuana. One study lists some 12 oils and extracts of vegetable origin that respond positively to the test.[14] A false-positive test with certain coffee extracts has also been reported, a fact that is often brought out in court by knowledgeable defense attorneys.

The question of whether or not the combination of the microscopic test and the Duquenois-Levine test is specific for marijuana has been debated for years within the forensic science community. A common scenario is that a mixture of hops, which contain cystolithic hairs, and camphor, which would give semipositive results to the Duquenois-Levine test, would behave the same as marijuana when subjected to this scheme. The unlikelihood of encountering such a mixture on the street notwithstanding, some laboratories have added some form of chromatography to the analytical protocol.

Unless quantitative analysis is to be performed, the chromatography test of choice is TLC. Not only is it quicker and less expensive than GC or HPLC, but with the proper choice of mobile phase, one can separate THC from CBD and CBN. In addition, the visualization reagent, Fast Blue BB (preferred to Fast Blue B because of the carcinogenicity of the latter), colors the cannabinoids various shades of orange or pink. This strong presumption of the presence of THC is enough to identify marijuana to a degree of reasonable scientific certainty when coupled with the microscopic and color tests.

In those cases where a preparation of marijuana is present, such as hashish oil, the microscopic examination may not reveal any useful plant morphology. Here a chromatographic test must be performed along with the Duquenois-Levine test.

Legal/Scientific Problems

There are two major areas where problems have occurred in court with cannabis. They are the question concerning the number of species of marijuana and the definition of hashish. It should be noted that although many states still define marijuana as *Cannabis sativa* L., the species argument is not often pursued anymore because of its lack of success in court. However, to avoid the issue, some states have changed the definition of marijuana to "a member of the genus *Cannabis*." The problem with the definition of hashish arises only in those cases where a statute differentiates between hashish and marijuana for sanctioning purposes and where possession of THC is a separate offense.

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TECHNICAL NOTE

David J. Hauber,[1] *M.S.*

Marijuana Analysis with Recording of Botanical Features Present and Without the Environmental Pollutants of the Duquenois-Levine Test

REFERENCE: Hauber, D. J., "**Marijuana Analysis with Recording of Botanical Features Present and Without the Environmental Pollutants of the Duquenois-Levine Test,**" *Journal of Forensic Sciences*, JFSCA, Vol. 37, No. 6, November 1992, pp. 1656–1661.

ABSTRACT: In order to properly document the botanical features present in a sample submitted as suspected marijuana and to reduce the problems of the disposal of the hazardous wastes produced with the use of the Duquenois-Levine Test, a protocol is described that involves recording the morphological features of *Cannabis* found in a sample and two thin-layer chromatography systems for determining the cannabinoids present. This protocol provides more information on a sample than was obtained with other, previous protocols involving the Duquenois-Levine Test.

KEYWORDS: criminalistics, marijuana analysis, Duquenois-Levine Test, TLC of cannabinoids

Much of the analytical work performed for the identification of marijuana, *Cannabis sativa* L., has been based on the articles of Nakamura [*1*] and Thornton and Nakamura [*2*]. These reports are excellent, thorough, compilations of data for the use of the microscopic identification of trichomes in the identification of fragments of *Cannabis*. The latter article, in addition to reporting on the authors' study of trichomes, delves deeply into the significance of the Duquenois-Levine Test in confirming the identification of *Cannabis*.

Since these reports were written, however, this laboratory has noted an increase in the numbers of submissions of marijuana samples that have flowers and relatively intact leaves rather than the fragments of leaflets that were common 15 years ago. Several years ago, it was realized by many of our analysts that documentation of the morphological features observed in marijuana samples was very advantageous in training analysts and was far superior to the "Micro +" documentation once used frequently. With detailed documentation, one can report on the witness stand exactly what was seen rather than being unsure whether a plethora of *Cannabis* features were found or just a *Cannabis* leaflet fragment with minimal features was present. Only recording the presence of the particular types of characteristic trichomes on leaflet fragments ignores a large amount

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Materials and Methods

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[2]Harris, J. R., Kentucky S
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of data on the sample that is extremely useful in identification. Because what is being identified is a plant or plant parts, the morphological examination of the sample with a stereoscope, therefore, is the best available mechanism for such an identification, and the documentation of the findings is imperative.

In addition, compliance with the new regulations on the disposal of chemical wastes has been added to the duties of the forensic scientist since these reports were written. Waste from performing the Duquenois-Levine Test [2,3] involves a complex mixture of chloroform, concentrated hydrochloric acid, ethanol, and reaction products involving cannabinoids, vanillin, acetaldehyde, and miscellaneous other chemicals. Reduction of the chlorinated hydrocarbon wastes is a primary environmental concern [4]; therefore, analysis protocols should develop in the direction of eliminating the Duquenois-Levine Test.

In addition to the problem of waste disposal, the Duquenois-Levine Test cannot distinguish individual cannabinoids or other compounds with similar functional groups. One of these other compounds, olivetol, is used by me to treat leaves of various plants for use as training samples. By dissolving olivetol in petroleum ether and pouring this solution on plant material other than marijuana and allowing the petroleum ether to evaporate, the sample can then be extracted with petroleum ether and produce the same result as marijuana with the Duquenois-Levine Test. Such a sample will also produce the same result as marijuana when the test is run on the unextracted plant sample directly. It would be preferable to use tests that distinguish among the cannabinoids and compounds reacting similarly to them.

This article describes a protocol for marijuana analysis that involves detailed notation of features found with stereoscopic examination of the plant material and two thin-layer chromatography systems to examine the cannabinoids present. While there is chemical waste with these TLC systems, the wastes are all clean burning solvents; their disposal is, therefore, less expensive economically and environmentally.

Materials and Methods

Microscopic Feature Description

To achieve the goal of recording the botanical features present in a submission of marijuana, the sample is visually examined, and as much as possible is viewed stereoscopically with magnifications of 10 to 40 times. The features observed are then recorded either in a number code[2] or in an abbreviated written form depending upon the preference of the examiner. Many of these botanical features have been mentioned in the U.S. Treasury Department's Marijuana, Its Identification, of 1948 [5] and Schultes and Hofmann's book of 1973 [6]; however, in Table 1 is the more detailed listing of features that I use. To give an example, the description of a marijuana leaflet on a palmate petiole would be described in my abbreviations as "Cnb 11 on palm pet," which means, "Cannabis leaflet on a palmate petiole." This one short statement documents the presence of a leaflet with cystolithic trichomes on one side, unicellular trichomes on the other side, leaflet serrations, the pinnate and marginal pattern of the veins, and the attachment of this leaflet to a palmate petiole. A notation of whether the achenes are reticulate or marbled in appearance is also of aid in any multiple species argument the defense might bring up, since the very common reticulated appearance of the achenes is indicative of the narrow definition of *Cannabis sativa* L. in Professor Schultes' key [7–9].

[2]Harris, J. R., Kentucky State Police Southeastern Regional Laboratory, personal communication, 1983.

TABLE 1—*Botanical characteristics that are recorded by abbreviations.*

Cannabis leaflet fragment (includes *Cannabis*-like cystolithic trichomes and unicellular trichomes on opposite sides of the leaflet particle that, itself, has a general *Cannabis* appearance)
Cannabis leaflet fragment with various features individually listed as follows: serrations, pinnate venation (that is, the pattern of the leaflet veins branching individually off of the midrib or middle vein in a featherlike pattern), marginal venation (that is, veins running along the serrated edges but vanishing before reaching the very tip)
Cannabis leaflet (the whole leaflet, or one with all the features mentioned for a leaflet fragment)
Cannabis leaf (at least one leaflet on an obvious palmate petiole)
palmate petiole
staminate flowers or parts (anthers, sepals)
pistillate flowering tops or an isolated pistillate flower
enveloping bract of the pistillate flower
stigmatic styles of the pistillate flower (when broken off from the rest of the flower)
glandular trichome
glandular trichome with head
hull (husk)
achene with reticulate or marbled pattern; the achene, which actually is the fruit of the marijuana plant, is commonly, but incorrectly, referred to as the seed
Cannabis seedling, with cotyledons, at the first, second, or third leaf stage
Cannabis stalks or stems
foreign plant material (including approximate percentage or amount)
roots
Aspergillus fruiting bodies
fungal mycelia
soil

Chemical Features Determined

Two different chemical tests in addition to the stereoscopic examination are required for reporting the identification of marijuana in the Kentucky State Police Forensic Laboratories System. For one test, it uses the hexane:ethyl ether (4:1) solvent system [*10*] with E. Merck 0.25-mm Kieselgel 60 F_{256} plates, or the equivalent, for separation and characterization of the cannabinoids. Traditionally, the Duquenois-Levine Test has been used as the other chemical test. Replacement of the Duquenois-Levine Test with a second TLC with the hexane:acetone (4:1) solvent system occurred at the Northern Regional Laboratory after five years of dual testing with these two TLC systems and the Duquenois-Levine Test. This latter system was an inadvertent modification of one used at another laboratory.[3] This modification was found to have no significant effect upon separation but it did increase the absolute R_f values by approximately 20%.

Method Testing

Various spices, condiments, and members of the botanical order, Urticales, to which *Cannabis* belongs, were examined by stereoscope at magnifications of 10 and 40 times and extracted with petroleum ether for TLC analysis. Various chemicals, including many reported to give positives with the Duquenois-Levine Test or used in the synthesis of THC [*2,3,10*] were also tested. Table 2 contains a list of the material tested. Samples were spotted about 14 mm from the bottom of a 10-cm long TLC plate; the solvent was allowed to rise up the entire plate before being removed. The plates were then sprayed with Fast Blue 2B in a 1:1 mixture of methyl alcohol and water for visualization of the chromatographic zones.

[3]Skowronski, G. T., U.S. Drug Enforcement Administration North Central Laboratory, personal communication on a TLC system with six parts hexane to one part acetone.

TABLE 2—*A list*

Allspice
Bay leaves
Black pepper corns
Black pepper, ground
Catnip
Cinnamon, ground
Cinnamon, stick
Citral
Cloves, ground
Cream of tartar
Cumin seed
Curry powder
Elm (*Ulmus american*
leaves
Eugenol
Garlic powder
Garlic salt
Ginseng root
Glutamate, monosodi

Results and Discussi

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TABLE 2—*A list of spices, condiments, and plants examined for features of marijuana and extracted for TLC.*

Allspice	Ginger, ground	Onion, minced and flakes
Bay leaves	Guaiazulene	Orcinol
Black pepper corns	Hackberry (*Celtis occidentalis*), leaves	Oregano leaves
Black pepper, ground	Hops (*Humulus lupulus*), leaves	Parsley flakes
Catnip	Jimson weed (*Datura stramonium*), seeds	Patchouli oil
Cinnamon, ground	Mace, ground	Red peppers, crushed
Cinnamon, stick	Metamucil	Red peppers, ground cayenne
Citral	4-methylresorcinol	Sage leaves
Cloves, ground	Morning glories seed	Sage, rubbed
Cream of tartar	Mulberry, red (*Morus rubra*), leaves	Savory, ground
Cumin seed	Mustard seed	Summer savory
Curry powder	Nutmeg	Thymol
Elm (*Ulmus americana*) leaves	Osage orange (*Maclura pomifera*), leaves	Thymolphthalein
Eugenol	Olivetol	Tobacco
Garlic powder		Tumeric, ground
Garlic salt		
Ginseng root		
Glutamate, monosodium		

Results and Discussion

Microscopic examination of the listed samples produced no sample with which an experienced examiner should have trouble. In Table 3 are listed the color and relative R_f values, normalized to delta-9-tetrahydrocannabinol (delta-9-THC), of colored chromatographic zones of the specimens and chemicals from Table 2 that produced such zones. No plant product nor other chemical was found to produce a colored chromatographic zone that could be confused with THC. The facts that delta-9-THC and cannabidiol switch relative positions as do cannabichromene and cannabinol when run on these two systems are of significance. Also, while the hexane:ether system cannot adequately separate cannabichromene from cannabigerol, it can separate cannabidiol well. The reverse occurs with the hexane:acetone system but cannabidiol and cannabichromene frequently overlap some as do cannabinol and cannabichromene. The sum of these facts allows a greater confidence in the determination of the major cannabinoids present. In addition, I have not found any mechanism to make a test sample that is not marijuana produce a confusing TLC result for either system without actually using a cannabinoid, as can be done with the Duquenois-Levine Test. It should be noted that hashish samples and ground, compressed marijuana samples are examined by instrumental means in addition to stereoscopic and TLC analysis because of the lack of many of the key botanical features.

Conclusion

Adequate stereoscopic examination with thorough documentation of morphological features present and a more detailed determination and recording of the cannabinoids present in a sample with the two TLC systems discussed provide an unambiguous identification of *Cannabis sativa* L. This combination of methods is both rapid and inexpensive and involves less toxic materials that are easier to dispose of in an environmentally safe way than testing with the Duquenois-Levine Test. Because the problem is the identification of plant parts present and not a chemical, the method emphasizes documentation of these plant parts and does not tie up instrumentation designed to identify chemicals.

TABLE 3—*Relative R_fs of various cannabinoids, spices, condiments, and chemicals that produced colored chromatographic zones with Fast Blue 2B on the two TLC systems used.*

Compound or Spice	Hexane:Ethyl Ether (4:1) TLC System		Hexane:Acetone (4:1) TLC System	
	Color with Fast Blue 2B	Relative Rf	Color with Fast Blue 2B	Relative Rf
Delta-9-THC	red	1.00	red	1.00
Delta-8-THC	red	1.10	red	1.08
Cannabichromene	purple	0.80	purple	0.89
Cannabidiol	orange	1.14	orange	0.93
Cannabigerol	orange	0.78	orange	0.72
Cannabinol	purple	0.90	purple	0.86
Allspice	yellow	0.67	yellow	0.77
Black pepper	yellow	0.0	yellow	0.27
	pink	0.63	purple	0.90
			purple	1.48
Cloves	yellow	0.86	yellow	0.87
	pink	1.08	purple	1.04
			yellow	1.54
Curry	yellow	0.0	yellow	0.08
	yellow	0.92	yellow	1.00
Eugenol	yellow	0.78	yellow	0.89
Ginger	orange	0.27	orange	0.53
	yellow	0.40	yellow	0.63
			yellow	0.74
Guaiazulene	purple	1.66	purple	1.65
Mace	red-purple	0.0	purple	0.0
	purple	0.44	purple	0.09
	purple	0.75	purple	0.30
			purple	0.70
4-methylresorcinol	red-brown	0.11	red-brown	0.34
Nutmeg	purple	0.0	purple	0.09
	purple	0.17	purple	0.16
	purple	0.25	purple	0.63
	purple	0.35	purple	0.81
Olivetol	red-brown	0.07	red-brown	0.28
	red	0.48	red purple	0.72
Orcinol	red-brown	0.05	red-brown	0.21
Red pepper	yellow	0.0	yellow	0.0
	yellow	0.25	yellow	1.05
	tan	0.10	yellow	2.00
	purple	0.73	pink	1.28
	yellow	1.73	pink	1.77
Sage	grey-green	0.10	green	0.58
			green	0.75
Savory	grey-green	0.10	green	0.50
Thymol	yellow	0.95	yellow	0.96
Tumeric	yellow	0.0	yellow	0.04
	yellow	0.96	yellow	0.75
			yellow	1.06

Acknowledgments

The author wishes to th
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, and chemicals that produced o TLC systems used.

Hexane:Acetone (4:1) TLC System	
Color with Fast Blue 2B	Relative Rf
red	1.00
red	1.08
purple	0.89
orange	0.93
orange	0.72
purple	0.86
yellow	0.77
yellow	0.27
purple	0.90
purple	1.48
yellow	0.87
purple	1.04
yellow	1.54
yellow	0.08
yellow	1.00
yellow	0.89
orange	0.53
yellow	0.63
yellow	0.74
purple	1.65
purple	0.0
purple	0.09
purple	0.30
purple	0.70
red-brown	0.34
purple	0.09
purple	0.16
purple	0.63
purple	0.81
red-brown	0.28
red purple	0.72
red-brown	0.21
yellow	0.0
yellow	1.05
yellow	2.00
pink	1.28
pink	1.77
green	0.58
green	0.75
green	0.50
yellow	0.96
yellow	0.04
yellow	0.75
yellow	1.06

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Validation of twelve chemical spot tests for the detection of drugs of abuse

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Abstract

Validation procedures are described for 12 chemical spot tests including cobalt thiocyanate, Dille–Koppanyi, Duquenois–Levine, Mandelin, Marquis, nitric acid, *para*-dimethylaminobenzaldehyde, ferric chloride, Froehde, Mecke, Zwikker and Simon's (nitroprusside). The validation procedures include specificity and limit of detection. Depending on the specificity of each color test, between 28 to 45 drugs or chemicals were tested in triplicate with each of the 12 chemical spot tests. For each chemical test, the final colors resulting from positive reactions with known amounts of analytes were compared to two reference color charts. For the identification of unknown drugs, reference colors from the Inter-Society Color Council and the National Bureau of Standards (ISCC-NBS) and Munsell charts are included along with a description of each final color. These chemical spot tests were found to be very sensitive with limits of detection typically 1 to 50 μg depending on the test and the analyte.

Keywords: Chemical spot test; Color test; Drug detection

1. Introduction

Chemical spot tests (sometimes referred to as color tests) provided toxicologists and criminalists with one of the earliest tools for the presumptive identification of drugs and

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poisons [1–3]. These tests continue to be popular for several reasons. They rely on simple chemical reactions and produce visible results that can be interpreted with the naked-eye. The reagents and laboratory materials needed to perform the tests are inexpensive and readily available. The tests can be performed by technicians without extensive training. Since the tests require minimal reagents and materials, small and even on-site laboratories can perform the tests. They can also be employed in the field by law enforcement agents. The utility of these tests is demonstrated by the fact that even today, when the use of sophisticated analytical instrumentation is so pervasive, they are still an integral part of the testing arsenal of forensic laboratories.

In two US National Institute of Justice (NIJ) standards, NILECJ-STD-0604.00 [4] and NILECJ-STD-0605.00 [5], the procedures for the use and validation of eleven different chemical spot-testing reagents were described. To better address the current needs of drug testing laboratories, the information in these documents was reviewed and updated or revalidated as needed. The need to include additional tests/analytes or remove existing tests/analytes from the original standards was assessed. An informal questionnaire addressing the use of these chemical spot tests in forensic laboratories was mailed to approximately 325 laboratories/individuals selected from the rosters of American Society of Crime Laboratory Directors (ASCLD), the Regional Association of Forensic Scientists and the Criminalistics Section of the American Academy of Forensic Sciences (AAFS). This mailing was performed in a semi-random fashion with an attempt to contact at least two laboratories or drug chemists in each state and to include the regional Drug Enforcement Administration laboratories. We received 121 responses. They indicated that chemical spot tests are still frequently used by 86% (104) of the responding laboratories. Greater than 90% of these laboratories used at least four of the tests; cobalt thiocyanate for cocaine, Duquenois–Levine for marijuana, Marquis for many basic drugs and *para*-dimethylaminobenzaldehyde (*p*-DMAB) for LSD. Ten of the chemical spot tests described in the NIJ documents were still routinely used by more than one third of the laboratories. Twenty-five percent of respondents suggested adding the Simon's or nitroprusside test for the detection of secondary amines, such as methamphetamine and methylenedioxymethamphetamine, to the battery of spot tests described in the original NIJ documents. Additional drugs that had become more prevalent since the publication of the standards such as acetaminophen, alprazolam, diazepam, ephedrine, hydrocodone and pseudoephedrine were added to the original list of analytes to be tested. This article reviews the data presented in the two original NIJ documents and presents new validation data for an expanded list of drugs using 12 chemical spot tests (CSTs).

2. Materials and methods

2.1. Chemicals

Cobalt thiocyanate, cobalt acetate dihydrate, glacial acetic acid, isopropylamine, acetaldehyde, ammonium vanadate, formaldehyde, *para*-dimethylaminobenzaldehyde, ferric chloride, vanillin, sodium molybdate, selenius acid, copper sulfate pentahydrate,

sodium nitroprusside, 2-chloroacetophenone and sodium carbonate were purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA). Methanol, hexane and chloroform were obtained from Burdick and Jackson (Muskegon, MI, USA). Hydrochloric acid, sulfuric acid, nitric acid and pyridine were purchased from Mallinckrodt Baker, (Paris, KY, USA). Ethanol was obtained from Quantum Chemical (Tuscola, IL, USA). The drugs were purchased in powder form from Sigma–Aldrich Chemical (St. Louis, MO, USA), Alltech-Applied Science (State College, PA, USA) or Research Triangle Institute (RTI, NC, USA).

2.2. Materials

Porcelain plates with 12 wells, glass culture tubes (12×75 mm) and Pasteur pipettes were purchased from VWR Scientific Products (Denver, CO, USA). The Munsell Book of Color (Volumes 1 and 2) were purchased from GretagMacbeth (New Windsor, NY, USA). The centroid color charts, published by the Inter-Society Color Council and the National Bureau of Standards, were obtained from Nick Hale (Naples, FL, USA).

2.3. Chemical spot test procedures

The procedures for preparing the chemical spot test reagents and performing each test are described below. One or two drops of reagent(s) were added to the drug using a Pasteur pipette unless otherwise noted.

A.1 Cobalt thiocyanate

Dissolve 2.0 g of cobalt (II) thiocyanate in 100 ml of distilled water.

A.2 Dille–Koppanyi reagent, modified

Solution A: Dissolve 0.1 g of cobalt (II) acetate dihydrate in 100 ml of methanol. Add 0.2 ml of glacial acetic acid and mix.

Solution B: Add 5 ml of isopropylamine to 95 ml of methanol.

Procedure: Add two drops of solution A to the drug, followed by one drop of solution B.

A.3 Duquenois–Levine reagent, modified

Solution A: Add 2.5 ml of acetaldehyde and 2.0 g of vanillin to 100 ml of 95% ethanol.

Solution B: Concentrated hydrochloric acid.

Solution C: Chloroform.

Procedure: Add three drops of solution A to the drug and shake for 1 min. Then add three drops of solution B. Agitate gently, and determine the color produced. Add nine drops of solution C and note whether the color is extracted from the mixture to A and B.

A.4 Mandelin reagent

Dissolve 1.0 g of ammonium vanadate in 100 ml of concentrated sulfuric acid.

A.5 Marquis reagent

Carefully add 100 ml of concentrated sulfuric acid to 5 ml of 40% formaldehyde (formaldehyde:water, v:v).

A.6 Nitric acid

Concentrated nitric acid.

A.7 Para-dimethylaminobenzaldehyde (p-DMAB)

Add 2.0 g of *p*-DMAB to 50 ml of 95% ethanol and 50 ml of concentrated hydrochloric acid.

A.8 Ferric chloride

Dissolve 2.0 g of anhydrous ferric chloride or 3.3 g of ferric chloride hexa-hydrate in 100 ml of distilled water.

A.9 Froehde reagent

Dissolve 0.5 g of molybdic acid or sodium molybdate in 100 ml of hot concentrated sulfuric acid.

A.10 Mecke reagent

Dissolve 1.0 g of selenious acid in 100 ml of concentrated sulfuric acid.

A.11 Zwikker reagent

Solution A: Dissolve 0.5 g of copper (II) sulfate pentahydrate in 100 ml of distilled water.

Solution B: Add 5 ml of pyridine to 95 ml of chloroform.

A.12 Simon's reagent

Solution A: Dissolve 1 g of sodium nitroprusside in 50 ml of distilled water and add 2 ml of acetaldehyde to the solution with thorough mixing.

Solution B: 2% sodium carbonate in distilled water.

Procedure: Add one drop of solution A to the drug, followed by two drops of solution B.

2.4. Validation of chemical spot tests

2.4.1. Test color and specificity

The drugs and other analytes were classified and prepared as either a drug standard, crystal, powder, tablet, or extract (Table 1). Drug standards were prepared in either chloroform or methanol at a concentration of 2.0 or 4.0 mg/ml (free-base). Crystals were tested without further processing. Tablets were crushed into a fine powder and leaf

Table 1
The classification of chemical spot test analytes

Analyte	Classification	Analyte	Classification
Acetaminophen	Drug standard	MDA HCl	Drug standard
Alprazolam	Drug standard	Meperidine HCl	Drug standard
Amobarbital	Drug standard	Mescaline HCl	Drug standard
Aspirin	Tablet	Methadone HCl	Drug standard
Baking soda	Powder	Methaqualone	Drug standard
Benzphetamine HCl	Drug standard	Methyiphenidate HCl	Drug standard
Brompheniramine maleate	Drug standard	Morphine monohydrate	Drug standard
Chlordiazepoxide HCl	Drug standard	Nutmeg	Extract
Chlorpromazine HCl	Drug standard	Opium	Powder
Cocaine HCl	Drug standard	Oxycodone HCl	Drug standard
Codeine	Drug standard	Pentobarbital	Drug standard
Contac	Tablet	Phencyclidine HCl	Drug standard
D-Amphetamine	Drug standard	Phenobarbital	Drug standard
D-Methamphetamine	Drug standard	Procaine HCl	Drug standard
Diacetylmorphine HCl	Drug standard	Propoxyphene HCl	Drug standard
Diazepam	Drug standard	Pseudoephedrine HCl	Drug standard
Dimethoxymethaphetamine HCl	Drug standard	Quinine HCl	Drug standard
Doxepin HCl	Drug standard	Salt	Crystals
Dristan	Tablet	Secobarbital	Drug standard
Ephedrine HCl	Drug standard	Sugar	Crystals
Exedrine	Tablet	Tea	Extract
Hydrocodone tartrate	Drug standard	THC	Extract
LSD	Drug standard	Tobacco	Extract
Mace	Crystals		

material was extracted with hexane. For all of the CSTs (except A.3), 500 μg of each analyte (125 or 250 μl of drug standard) was added to each of three wells on the porcelain test plate. For the drug standards and extracts, the organic solvent was evaporated and the residue was reconstituted in 100 μl of chloroform before the CST was performed. The CST reagents were then added with a Pasteur pipette as described in Section 2.3 for each test. For A.3, 500 μg of analyte was added to a glass culture tube. If organic solvent was present, it was evaporated and the test reagents were added as described for A.3. The final color was observed after 5 min and compared to reference colors in the Munsell and centroid color charts. Each analyte was tested in triplicate.

2.4.2. Drug detection limit

A working 1.0 μg/μl solution (or lower if necessary) of each analyte to be tested was prepared. The limit of detection (LOD) for each analyte was determined by testing serial dilutions of the working solution until the lowest concentration of analyte that was detectable in five replicates ($n=5$) was identified. This concentration was then multiplied by ten and recorded as the 'operational drug detection limit'. All tests were performed in the porcelain test plates except for A.3 which was performed in glass culture tubes.

3. Results and discussion

Chemical spot tests are widely accepted as presumptive tests for drug detection. These CSTs provide information that allows the analyst to select the appropriate testing procedures to confirm the identity of the suspected drug. The information listed in the tables is intended as a guide for using CSTs and for preparing quality control materials for chemical spot tests when they are performed in the laboratory or in the field. The actual color produced by the reagents for each drug may vary depending on many factors: the concentration of the drug, whether the drug is in salt or free base form, which salt form is present, any additional diluents or contaminants present in the sample, the color discrimination of the analyst and the conditions under which the test is performed [2,6].

The original NIJ standards [4,5] used centroid color charts published by the Inter-Society Color Council and the National Bureau of Standards (ISCC-NBS) for color comparison. These charts include almost 270 colors logically grouped and listed numerically. However, these color standards are obsolete and are no longer considered to be an international standard for color description or comparison. Therefore, the ISCC-NBS numbers are listed for historical comparison purposes only. The ISCC-NBS charts have been replaced by the Munsell Color charts. The Munsell Book of Color (Volumes 1 and 2) is a master atlas of color that contains almost 1600 color comparison chips. The colors are prepared according to the specifications contained in the final report of the subcommittee of the Optical Society of America. Each page of the Munsell book presents one hue. There are 40 pages, each is 2.5 hue steps apart. On each page, the color chips are arranged by Munsell value and chroma. The standard way to describe a color using Munsell notations is to write the numeric designation for the Munsell hue (H) and the numeric designation for value (V) and chroma (C) in the form of H V/C. Since there are considerably more colors in the Munsell charts than in the centroid charts, two or more Munsell notations may correspond to the same previously used ISCC-NBS number.

Depending on the specificity of each color test, between 28 to 45 drugs or chemicals were tested in triplicate with each of the CSTs. For each CST, the final color resulting from a positive reaction with a known amount of analyte was compared to two reference color charts. These results are shown in Table 2. Reference colors from the ICSS-NBS and Munsell charts were included along with a description of each final color.

A positive CST may indicate a specific drug or class of drugs is present in the sample, but the tests are not always specific for a single drug or class. For this reason, laboratories must rely on a battery of CSTs for the preliminary identification of an unknown drug. For example, Cobalt thiocyanate (A.1) is used to detect cocaine. However, many other drugs will also react with this reagent (Tables 2 and 3) and each analyte that tested positive with cobalt thiocyanate produced a strong blue color. Also, the nitric acid test produced variations of yellow and orange colors with a variety of analytes including acetaminophen, diacetylmorphine, dimethoxymethamphetamine and mescaline.

Six of the CSTs are indicated for the detection of opioids and other amines. These include Mandelin (A.4), Marquis (A.5), nitric acid (A.6), ferric chloride (A.8), Froehde

Table 2
Final colors produced by reagents A.1 through A.12 with various drugs and other substances

	Analyte	Solvent	ICSS-NBS[g]	Color	Munsell
A.1	Benzphetamine HCl	$CHCl_3$	168	Brilliant greenish blue	5B 7/8
A.1	Brompheniramine Maleate	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Chlordiazepoxide HCl	$CHCl_3$	168	Brilliant greenish blue	2.5B 6/8
A.1	Chlorpromazine HCl	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Cocaine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Diacetylmorphine HCl	$CHCl_3$	169	Strong greenish blue	7.5B 6/10
A.1	Doxepin HCl	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Ephedrine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Hydrocodone tartrate	$CHCl_3$	168	Brilliant greenish blue	5B 6/8
A.1	Meperidine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Methadone HCl[a]	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Methylphenidate HCl	$CHCl_3$	168	Brilliant greenish blue	10BG 6/8
A.1	Phencyclidine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Procaine HCl[a]	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Propoxyphene HCl[a]	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Pseudoephedrine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Quinine HCl	$CHCl_3$	178	Strong blue	2.5PB 5/12
A.2	Amobarbital	$CHCl_3$	222	Light purple	5P 7/8
A.2	Pentobarbital[a]	$CHCl_3$	222	Light purple	5P 7/8
A.2	Phenobarbital[a]	$CHCl_3$	222	Light purple	5P 7/8
A.2	Secobarbital[a]	$CHCl_3$	222	Light purple	5P 7/8
A.3	Mace[f]	Crystals	237[b]	Strong reddish purple[b]	2.5RP 5/12[b]
			237[c]	Strong reddish purple[c]	2.5RP 5/12[c]
			221[d]	Very light purple[d]	5P 8/4[d]
A.3	Nutmeg	Extract	244[b]	Pale reddish purple[b]	10P 6/4[b]
			244[c]	Pale reddish purple[c]	10P 6/4[c]
			261[d]	Light gray purplish red[d]	5RP 7/4[d]
A.3	Tea	Extract	119[e]	Light yellow green	5GY 8/6
A.3	THC[a]	EtOR	204[b]	Gray purplish blue[b]	7.5PB 4/4[b]
			199[c]	Light purplish blue[c]	7.5PB 7/8[c]
			219[d]	Deep purple[d]	7.5P 4/12[d]
A.4	Acetaminophen	$CHCl_3$	107	Moderate olive	10Y 5/8
A.4	Aspirin	Powder	127	Grayish olive green	2.5GY 4/2
A.4	Benzphetamine HCl[a]	$CHCl_3$	116	Brilliant yellow green	2.5GY 8/10
A.4	Brompheniramine	$CHCl_3$	50	Strong orange	7.5YR 7/14
A.4	Maleate Chlorpromazine HCl	$CHCl_3$	108	Dark olive	10Y 3/4
A.4	Cocaine HCl[a]	$CHCl_3$	69	Deep orange yellow	10YR 7/14
A.4	Codeine[a]	$CHCl_3$	108	Dark olive	5Y 3/4
A.4	Contac	Powder	84	Strong yellow	2.5Y 6/10
A.4	D-Amphetamine HCl[a]	$CHCl_3$	164	Moderate bluish green	5BG 5/6
A.4	D-Methamphetamine HCl[a]	$CHCl_3$	137	Dark yellowish green	10GY 4/6
A.4	Diacetylmorphine HCl[a]	$CHCl_3$	43	Moderate reddish brown	10R 3/6
A.4	Dimethoxy-meth HCl	$CHCl_3$	96	Dark olive brown	5Y 2/2
A.4	Doxepin HCl	$CHCl_3$	44	Dark reddish brown	10R 2/4
A.4	Dristan	Powder	110	Grayish olive	7.5Y 4/4
A.4	Exedrine	Powder	108	Dark olive	7.5Y 3/4
A.4	Mace[f]	Crystals	125	Moderate olive green	5GY 4/8

Table 2. Continued

	Analyte	Solvent	ICSS-NBS[g]	Color	Munsell
A.4	MDA HCl	$CHCl_3$	193	bluish Black	10B 2/2
A.4	Mescaline HCl[a]	$CHCl_3$	78	Dark yellowish brown	10YR 3/4
A.4	Methadone HCl	$CHCl_3$	187	Dark grayish blue	5B 3/2
A.4	Methaqualone	$CHCl_3$	66	Very orange yellow	10YR 8/14
A.4	Methylphenidate HCl	$CHCl_3$	67	Brilliant orange yellow	2.5Y 8/10
A.4	Morphine monohydrate[a]	$CHCl_3$	47	Dark grayish reddish brown	10R 3/2
A.4	Opium[a]	$CHCl_3$	59	Dark brown	7.5YR 2/4
A.4	Oxycodone HCl	$CHCl_3$	103	Dark greenish yellow	10Y 6/6
A.4	Procaine HCl	$CHCl_3$	51	Deep orange	5YR 5/12
A.4	Propoxyphene HCl	$CHCl_3$	44	Dark reddish brown	10R 2/4
A.4	Quinine HCl	$CHCl_3$	100	Deep greenish yellow	10Y 9/6
A.4	Salt	Crystals	50	Strong orange	5YR 7/12
A.5	Aspirin	Powder	13	Deep red	5R 3/10
A.5	Benzphetamine HCl[a]	$CHCl_3$	41	Deep reddish brown	7.5R 2/6
A.5	Chlorpromazine HCl	$CHCl_3$	256	Deep purplish red	2.5RP 3/8
A.5	Codeine[a]	$CHCl_3$	225	Very dark purple	7.5P 2/4
A.5	D-Amphetamine HCl[a]	$CHCl_3$	35 to 44	Strong reddish orange	10R 6/12 to 7.5R 2/4
A.5	D-Methamphetamine HCl[a]	$CHCl_3$	36 to 44	Dark reddish brown	10R 4/12 to 7.5R 2/4
A.5	Diacetylmorphine HCl[a]	$CHCl_3$	256	Deep reddish orange	7.5RP 3/10
A.5	Dimethoxy-meth HCl	$CHCl_3$	107	Dark reddish brown	7.5Y 5/8
A.5	Doxepin HCl	$CHCl_3$	21	Deep purplish red	7.5R 2/2
A.5	Dristan	Powder	20	Moderate olive	5R 3/2
A.5	Exedrine	Powder	16	Blackish red	5R 3/8
A.5	LSD	$CHCl_3$	114	Dark grayish red	10Y 2/2
A.5	Mace[f]	Crystals	87	Dark red	7Y 7/8
A.5	MDA HCl[a]	$CHCl_3$	267	Olive Black	Black
A.5	Meperidine HCl	$CHCl_3$	56	Moderate yellow	5YR 3/6
A.5	Mescaline HCl[a]	$CHCl_3$	50	Black	5YR 6/12
A.5	Methadone HCl	$CHCl_3$	28	Deep brown	2.5YR 8/4
A.5	Methylphenidate HCl	$CHCl_3$	71	Strong orange	10YR 8/8
A.5	Morphine monohydrate[a]	$CHCl_3$	239	Light yellowish pink	10P 3/6
A.5	Opium[a]	Powder	47	Moderate orange yellow	10R 3/2
A.5	Oxycodone HCl[a]	$CHCl_3$	214	Very deep reddish purple	
A.5	Propoxyphene HCl	$CHCl_3$	230	Dark grayish reddish brown	2.5P 6/4
A.5	Sugar	Crystals	59	Pale violet	2.5RP 2/2
A.6	Acetaminophen	$CHCl_3$	67	Blackish Purple	5YR 2/4
A.6	Chlorpromazine HCl	$CHCl_3$	98	Dark brown	
A.6	Codeine[a]	$CHCl_3$	101	Brilliant orange yellow	2.5Y 8/12
A.6	Diacetylmorphine HCl[a]	$CHCl_3$	89	Brilliant greenish yellow	7.5Y 8.5/10
A.6	Dimethoxy-meth HCl	$CHCl_3$	82	Light greenish yellow	7.5Y 9/6
A.6	Doxepin HCl	$CHCl_3$	83	Pale yellow	5Y 9/6
A.6	Dristan	Powder	51	Very yellow	2.5Y 8/14
A.6	Exedrine	Powder	67	Brilliant yellow	5Y 8.5/3
A.6	LSD	$CHCl_3$	55	Deep orange	5YR 6/12
A.6	Mace[f]	Crystals	102	Brilliant orange yellow	2.5Y 8/12
A.6	MDA HCl	$CHCl_3$	101	Strong brown	5YR 5/10
				Moderate greenish yellow	10Y 7/6
				Light greenish yellow	7.5Y 9/6

Table 2. Continued

	Analyte	Solvent	ICSS-NBS[g]	Color	Munsell
A.6	Mescaline HCl[a]	$CHCl_3$	16	Dark red	5R 3/6
A.6	Morphine monohydrate[a]	$CHCl_3$	67	Brilliant orange yellow	2.5Y 8/12
A.6	Opium[a]	Powder	72	Dark orange yellow	10YR 6/10
A.6	Oxycodone HCl	$CHCl_3$	83	Brilliant yellow	5Y 8.5/8
A.7	LSD[a]	$CHCl_3$	219	Deep Purple	7.5P 3/10
A.8	Acetaminophen	MEOH	103	Dark greenish yellow	10Y 6/10
A.8	Baking soda	Powder	51	Deep orange	5YR 6/14
A.8	Chlorpromazine HCl	MEOH	48	Very orange	5YR 7/14
A.8	Dristan	Powder	200	Moderate purplish blue	10PB 4/2
A.8	Exedrine	Powder	200	Moderate purplish blue	10PB 4/2
A.8	Morphine monohydrate[a]	MEOH	146	Dark green	5G 3/6
A.9	Aspirin	Powder	228	Grayish purple	7.5P 5/2
A.9	Chlorpromazine HCl	$CHCl_3$	14	Very deep red	5R 3/10
A.9	Codeine[a]	$CHCl_3$	147	Very dark green	7.5G 2/6
A.9	Contac	Powder	95	Moderate olive brown	2.5Y 4/6
A.9	Diacetylmorphine HCl[a]	$CHCl_3$	256	Deep purplish red	5RP 3/10
A.9	Dimethoxy-meth HCl	$CHCl_3$	115	Very yellow green	5GY 6/10
A.9	Doxepin HCl	$CHCl_3$	41	Deep reddish brown	7.5R 2/8
A.9	Dristan	Powder	163	Light bluish green	5BG 7/6
A.9	Exedrine	Powder	177		10B 6/10
A.9	LSD	$CHCl_3$	120	Moderate yellow green	5GY 6/6
A.9	Mace[f]	Crystals	70	Light olive yellow	10YR 8/8
A.9	MDA HCl[a]	$CHCl_3$	157	Greenish black	7.5G 2/2
A.9	Morphine monohydrate[a]	$CHCl_3$	256	Deep purplish red	5RP 3/10
A.9	Opium[a]	Powder	65	Brownish black	7.5R 2/2
A.9	Oxycodone HCl	$CHCl_3$	84	Strong yellow	2.5Y 7/10
A.9	Propoxyphene HCl	$CHCl_3$	20	Dark grayish red	2.5R 3/2
A.9	Sugar	Crystals	83	Brilliant yellow	5Y 8.5/8
A.10	Chlorpromazine HCl	$CHCl_3$	21	Blackish red	5R 2/2
A.10	Codeine[a]	$CHCl_3$	166	Very dark bluish green	2.5BG 2/4
A.10	Contac	Powder	95	Moderate olive brown	2.5Y 4/6
A.10	Diacetylmorphine HCl[a]	$CHCl_3$	161	Deep bluish green	2.5BG 3/8
A.10	Dimethoxy-meth HCl	$CHCl_3$	59	Dark brown	5YR 2/4
A.10	Doxepin HCl	$CHCl_3$	17	Very dark red	5R 2/4
A.10	Dristan	Powder	94	Light olive brown	2.5Y 6/10
A.10	Exedrine	Powder	91	Dark grayish yellow	5Y 6/4
A.10	Hydrocodone tartrate	$CHCl_3$	165	Dark bluish green	5BG 3/6
A.10	LSD	$CHCl_3$	157	Greenish black	7.5G 2/2
A.10	Mace[f]	Crystals	111	Dark grayish olive	10Y 3/4
A.10	MDA HCl[a]	$CHCl_3$	166	Very dark bluish green	2.5BG 2/4
A.10	Mescaline HCl[a]	$CHCl_3$	107	Moderate olive	7.5Y 5/8
A.10	Morphine monohydrate[a]	$CHCl_3$	166	Very dark bluish green	2.5BG 2/4
A.10	Nutmeg	Extract	65	Brownish Black	10YR 2/2
A.10	Opium[a]	Powder	114	Olive black	10Y 2/2
A.10	Oxycodone HCl	$CHCl_3$	107	Moderate olive	7.5Y 5/8
A.10	Propoxyphene HCl	$CHCl_3$	41	Deep reddish brown	10R 2/6
A.10	Sugar	Crystals	98	Brilliant greenish yellow	10Y 8.5/10

Table 2. Continued

	Analyte	Solvent	ICSS-NBS[g]	Color	Munsell
A.11	Baking soda	Powder	181	Light blue	2.5PB 7/6
A.11	Exedrine	Powder	144	Light green	5G 7/6
A.11	Pentobarbital[a]	$CHCl_3$	222	Light purple	7.5P 7/6
A.11	Phenobarbital[a]	$CHCl_3$	222	Light purple	7.5P 7/6
A.11	Secobarbital[a]	$CHCl_3$	222	Light purple	7.5P 7/6
A.11	Tea	Extract	120	Moderate yellow green	2.5GY 7/8
A.11	Tobacco	Extract	136	Moderate yellowish green	10GY 6/6
A.12	D-Methamphetamine HCl[a]	$CHCl_3$	183	Dark blue	2.5PB 2/6
A.12	Dimethoxy-meth HCl[a]	$CHCl_3$	179	Deep blue	2.5PB 3/8
A.12	MDMA HCl	$CHCl_3$	183	Dark blue	2.5PB 2/6
A.12	Methylphenidate HCl	$CHCl_3$	214	Pale Violet	2.5P 6/4

[a] Usual kit reagent for that particular drug.
[b] Aqueous phase.
[c] Aqueous phase after chloroform extraction.
[d] Chloroform phase (marijuana extraction usually rapid compared to other materials).
[e] Not extracted into chloroform.
[f] 2-Chloroacetophenone.
[g] Abbreviations: ICSS-NBS=Inter-Society Color Council and the National Bureau of Standards, Munsell= Munsell color notation, $CHCl_3$=chloroform, EtOH=ethanol, MEOH=methanol, B=blue, G=green, P=purple, Y=yellow, R=red.

(A.9) and Mecke (A.10) reagents. Unlike the cobalt thiocyanate reaction, different colors were produced with different drugs making it easier to presumptively identify the specific drug present. For example, a selected battery of tests to identify heroin (diacetylmorphine) might include the Mandelin, Marquis and Froehde tests because they would produce reddish brown, deep purplish red and purplish red colors, respectively. Codeine, a second opiate, could be identified with the same battery of CSTs because it produced olive, dark purple and dark green colors, respectively. These three CSTs were reactive to many opioids with LODs as low as 1–5 μg, Table 4, but as mentioned the colors produced and the sensitivity was dependent on the many factors listed above.

Since positive reactions were dependent on the functional groups present in the chemical structure of the tested analytes, several of the CSTs were specific for certain classes of drugs. The *p*-DMAB reagent (A.7) reacted only with LSD, producing a deep purple color. This test had an LOD of 6 μg for LSD. Although mace, nutmeg and tea reacted with the modified Duquenois–Levine test (A.3), as shown in Table 2, only tetrahydrocannabinal (THC) produced a deep purple color that was extracted into chloroform. The Simon's test is reported to be specific for secondary amines like methamphetamine and MDMA. It did not react with ephedrine or pseudoephedrine because their structure contains an hydroxyl group that is in close proximity to the amine. Barbiturates can be detected by both the Dille–Koppanyi (A.2) and Zwikker (A.11) reagents. However, the Dille–Koppanyi test was more sensitive with LODs of 25 μg or lower whereas the LOD for phenobarbital with the Zwikker test was 1000 μg.

Table 3
Specificity of color tests. (+) Indicates that a color reaction occurs[a]

	Reagent											
	A.1	A.2	A.3	A.4	A.5	A.6	A.7	A.8	A.9	A.10	A.11	A.12
Acetaminophen	–	–	–	+	–	+	–	+	–	–	–	–
Alprazolam	–	–	–	–	–	–	–	–	–	–	–	–
Aspirin	–	–	–	+	+	–	–	–	+	–	–	–
Baking soda	–	–	–	–	–	–	–	+	–	–	+	–
Brompheniramine maleate	–	–	–	–	–	–	–	–	–	–	–	–
Chlordiazepoxide HCl	+	–	–	+	–	–	–	–	–	–	–	–
Chlorpromazine HCl	+	–	–	+	+	+	–	+	+	+	–	–
Contac	+	–	–	+	–	–	–	–	+	+	–	–
Diazepam	–	–	–	–	–	–	–	–	–	–	–	–
Doxepin HCl	–	–	–	–	–	–	–	–	–	–	–	–
Dristan	+	–	–	+	+	+	–	–	+	+	–	–
Ephedrine HCl	+	–	–	+	+	+	–	+	+	+	+	–
Exedrine	–	–	–	+	+	+	–	–	–	+	–	–
Hydrocodone tartrate	+	–	–	–	–	–	–	–	–	+	–	–
Mace[b]	–	–	+	+	+	+	–	–	+	+	–	–
Meperidine HCl	+	–	–	–	+	–	–	–	–	–	–	
Methaqualone	–	–	+	–	–	–	–	–	–	–	–	+
Methylphenidate HCl	+	–	–	+	+	–	–	–	–	–	–	–
Nutmeg[b]	–	–	+	–	–	–	–	–	–	+	–	–
Phencyclidine HCl	+	–	–	–	–	–	–	–	–	–	–	–
Propoxyphene HCl	+	–	–	+	+	–	–	–	+	+	–	–
Pseudoephedrine HCl	+	–	–	–	–	–	–	–	–	–	–	–
Quinine HCl	+	–	–	+	–	–	–	–	–	–	–	–
Salt	–	–	–	+	–	–	–	–	–	–	–	–
Sugar	–	–	–	–	+	–	–	–	+	+	–	–
Tea[b]	–	–	+	–	–	–	–	–	–	–	+	–
Tobacco	–	–	–	–	–	–	–	–	–	–	+	–

[a] Substances that gave no colors with these reagents are: D-galactose, glucose, mannitol, oregano, rosemary and thyme.

[b] Tea, mace and nutmeg may interfere with the Duquenios test, but not the Duquenois–Levine modified test (A.3).

4. Conclusions

Chemical spots tests are valuable tools for the presumptive identification of drugs in unknown samples. These tests are very sensitive with LODs typically 1 to 50 μg depending on the CST and the analyte. The methods and validation procedures for 12 chemical spot tests for use in the laboratory or in the field were described. For the identification of unknown drugs, reference colors from the Munsell and ICSS-NBS centroid color charts representing positive reactions for the 12 CSTs were included. Although these tests are sensitive and can be relatively specific, the actual color observed by the analyst performing the CST depends on many factors such as the concentration of the drug, whether the drug is a salt or free base, which salt form is

Table 4
Drug detection limits[a]

Reagent	Analyte	Drug detection limit (μg)
A.1	Cocaine HCl	60
A.1	Methadone HCl	250
A.2	Amobarbital	25
A.2	Pentobarbital	10
A.2	Phenobarbital	15
A.2	Secobarbital	25
A.3	THC	5
A.4	D-Amphetamine HCl	20
A.4	D-Methamphetamine HCl	100
A.4	Codeine	20
A.4	Diacetylmorphine HCl	20
A.4	Morphine monohydrate	5
A.5	D-Amphetamine HCl	10
A.5	Codeine	1
A.5	Diacetylmorphine HCl	10
A.5	LSD	5
A.5	Mescaline HCl	10
A.5	Methadone HCl	20
A.5	D-Methamphet HCl	5
A.5	Morphine monohydrate	5
A.6	Mescaline HCl	1
A.7	LSD	6
A.8	Morphine monohydrate	200
A.9	Codeine	50
A.9	Diacetylmorphine HCl	200
A.9	LSD	50
A.9	Mescaline HCl	100
A.9	Morphine monohydrate	25
A.10	Codeine	25
A.10	Diacetylmorphine HCl	200
A.10	LSD	50
A.10	Mescaline HCl	50
A.10	Morphine monohydrate	50
A.11	Phenobarbital	1000
A.12	D-Methamphetamine HCl	10
A.12	Methylphenidate HCl	300

[a] The solvent was chloroform except for A.8 which was methanol.

present, the presence of contaminants in the sample, the color discrimination of the analyst and the conditions under which the CST is performed.

Acknowledgements

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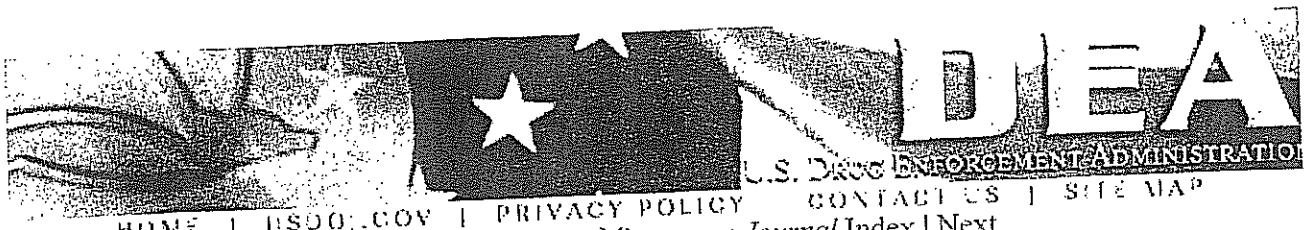

Technical Note

Specificity of the Duquenois-Levine and Cobalt Thiocyanate Tests Substituting Methylene Chloride or Butyl Chloride for Chloroform

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ABSTRACT: The use of alternative solvents in the Duquenois-Levine and Cobalt Thiocyanate tests were explored substandard results with recently purchased lots of chloroform. Methylene chloride provided satisfactory results wh for chloroform in both tests. Butyl chloride provided satisfactory results in the Duquenois-Levine test.

KEYWORDS: Duquenois-Levine, Cobalt Thiocyanate, Marijuana, Cocaine, Chloroform, Methylene Chloride, n-But

Introduction

The Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test (Scott test) are proven screening tests for of marijuana and cocaine, respectively. The organic solvent traditionally used in these tests is chloroform. Howeve recently purchased by this laboratory produced little or no color change when performing the Duquenois-Levine an Thiocyanate tests. Shortly after opening, this chloroform became yellow to green in color, at which point it was uns perform these tests. According to the manufacturer, this unusual decomposition of the chloroform was due to insuf of preservatives. This experience led to the investigation of using alternative organic solvents, specifically methyle n-butyl chloride, in the Duquenois-Levine and Cobalt Thiocyanate tests.

Experimental

Reagents and Solvents

Hydrochloric acid, methylene chloride, and n-butyl chloride were obtained from Fisher Scientific. Acceptable quality was obtained from OmniSolv. The Duquenois reagent was prepared by adding 10 grams of vanillin and 5 milliliters acetaldehyde to 500 milliliters of ethanol. The vanillin, acetaldehyde, and ethanol were obtained from Kodak, EM S Fisher Scientific, respectively. The cobalt thiocyanate reagent was prepared by dissolving ten grams of cobalt (II) t mixture of 490 milliliters of distilled water and 500 milliliters of glycerin. The cobalt (II) thiocyanate and glycerin wer from Aldrich Chemical and Fisher Scientific, respectively.

Procedures

The Duquenois-Levine test was performed on 17 different substances using chloroform, methylene chloride, or bu the organic solvent. The test was performed by placing approximately 10 to 20 milligrams of a target substance in tube, then 10 drops of the Duquenois reagent. After shaking, 10 drops of concentrated hydrochloric acid were add

tube was again shaken. Any color that resulted after the hydrochloric acid step was recorded. Twenty drops of chlc then added, and the tube was vortexed, then allowed to settle and separate into two layers. Any color that transfer organic layer was recorded (Table 1). This procedure was repeated for each target substance by substituting meth or butyl chloride for chloroform.

The cobalt thiocyanate test was performed on 14 different substances using chloroform, methylene chloride, or bu The test was performed by placing approximately 2 to 4 milligrams of a target substance in a glass test tube, then cobalt thiocyanate reagent. After shaking,1 or 2 drops of concentrated hydrochloric acid were added, and the tube shaken. Ten drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate layers. The final color of the chloroform (organic) layer was recorded (Table 2). This procedure was repeated for e substance by substituting methylene chloride or butyl chloride for chloroform.

Results and Discussion

The results for the Duquenois-Levine test using either methylene chloride and butyl chloride were consistent with r using chloroform. The marijuana became purple with the addition of the Duquenois reagent and hydrochloric acid. of the organic solvent, the purple color transferred to the organic layer, indicating a positive test for cannabinoids. consistent in all tests involving marijuana, regardless of the solvent used. None of the remaining 16 substances te characteristic purple color in the organic solvent layer.

Similarly, the results of the Cobalt Thiocyanate test were equivalent whether chloroform or methylene chloride was However, the results for the butyl chloride were mixed. Addition of the cobalt thiocyanate reagent to cocaine hydro resulted in the surface of the particles turning a bright blue (faint blue for cocaine base). The solution changed bac adding one or two drops of hydrochloric acid and mixing. Addition of 10 drops of chloroform, vortexing, and allowin to settle resulted in a blue organic layer for both cocaine hydrochloride and cocaine base. The test had similar resu methylene chloride was substituted for chloroform. In the case of butyl chloride, however, the organic layer stayed inconclusive test. Diphenhydramine and lidocaine also gave blue organic layers with either chloroform and methyle These compounds are known false positives for cocaine. However, in the case of butyl chloride, the organic layers diphenhydramine and white for lidocaine. The other ten materials had consistent negative test results for all three solvents.

Conclusions

Methylene chloride may be substituted for chloroform in both the Rapid Modified Duquenois-Levine test and Cobal test. Similarly, butyl chloride may be substituted for chloroform in the Duquenois Levine test. However, butyl chlori reliable substitute solvent for use in the Cobalt Thiocyanate test. Methylene chloride also works well as an extracti place of chloroform.

[Tables 1 and 2 Follow.]

Table 1. Duquenois-Levine Test Results

	Chloroform	Methylene Chloride	Butyl Chloride
Material	aqueous/organic	aqueous/organic	aqueous/organic
Allspice	brown/clear	brown/clear	brown/clear
Celery Flakes	yellow/clear	yellow/clear	yellow/clear
Chamomile	yellow/clear	yellow/clear	yellow/clear
Chamomile Tea	yellow/clear	green/clear	green/clear
Coffee	brown/clear	brown/clear	brown/clear
Dill Seed	yellow/clear	yellow/clear	yellow/clear
Hops	yellow/clear	yellow/clear	yellow/clear
Ginger	orange/orange	orange/orange	orange/clear

Ginseng	brown/brown	green/clear	green/clear
Marijuana	purple/purple	purple/purple	purple/purple
Marjoram	yellow/clear	green/clear	green/clear
Mint	green/clear	green/clear	green/clear
Sage	yellow/clear	yellow/clear	yellow/clear
Salvia Divinorum	green/clear	brown/clear	green/clear
Thyme	yellow/clear	green/clear	green/clear
Tobacco	brown/clear	brown/clear	brown/clear
White Pepper	orange/yellow	orange/yellow	orange/yellow

Table 2. Cobalt Thiocyanate Test Results

	Chloroform	Methylene Chloride	Butyl Chloride
Material	organic layer	organic layer	organic layer
Benzocaine	clear	clear	clear
Cocaine	blue	blue	clear
Cocaine Base	blue	blue	clear
Dextrose	clear	clear	clear
Diphenhydramine	blue	blue	clear
Heroin	clear	clear	clear
Inositol	clear	clear	white
Lidocaine	blue	blue	clear
Methamphetamine	clear	clear	clear
MDMA	clear	clear	clear
Morphine	clear	clear	clear
Procaine	clear	clear	clear
Soap	clear	clear	clear
Sodium Bicarbonate	clear	clear	clear

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