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Colour Tests for Precursor Chemicals of Amphetamine-Type Substances

The Use of Colour Tests for Distinguishing between Ephedrine-Derivatives

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Abstract

Ephedrine, norephedrine and pseudoephedrine have in recent years been subject to extensive illicit trafficking in many countries of the world. A simple chemical method, based on a combination of three known colour tests, is presented to differentiate these and other related ephedrine derivatives. The underlying chemistry is discussed.

Introduction

Why colour tests for differentiating ephedrine derivatives?

Colour tests are usually the simplest and quickest chemical test that an analyst can apply to a sample. Most colour tests are quite sensitive; thus, only minute quantities of sample are necessary to complete a successful test, and often the best results are obtained with the smallest of sample quantities, frequently less than one mg. They are designed to provide an indication of the presence or absence of drug classes in the test sample and quickly eliminate negative samples. Good presumptive testing methods, as all analytical techniques, maximize the probability of a "true" result, and minimize false positives. However, presumptive tests are not considered sufficient for drug identification and results must be confirmed by additional laboratory tests [1].

While often declared obsolete by some, colour tests have their place not only in field test kits for police and customs personnel, but also as important constituents of analytical laboratory schemes.

Yet, some colour tests are more specific than others. Good examples to illustrate the considerable differences in specificity are the tests routinely used for the two illicit drugs most frequently encountered worldwide in the illicit market, the opiates and cannabis. The Marquis test (sulfuric acid-formaldehyde reagent) is one of those classical tests which produce colours with a very large variety of organic chemicals, both natural and synthetic origin, including several classes of drugs of abuse and their precursors, under various types of regulatory control. In contrast, Cannabis tests such as the Duquenois test and the diazo-dye formation between Fast Blue B salt and the important natural cannabinoids, combined with a solvent extraction step, appear to be a rather specific and reliable tool for the presumptive identification of cannabis products [2-4]. Due to their wide availability and low cost, both tests are standard constituents of practically all field testing kits worldwide. They are also popular in laboratories [5-8].

Most colour tests in actual use today have originally been developed for pharmaceutical purposes or as simple tools for the indication of certain functional groups in organic molecules during their synthesis. Subsequently, they have undergone extensive cross-trials against large sets of pharmaceuticals and other organic molecules during the first half of the last century. The vast experience of several decades´ work is made available in reviews such as the worldwide known "Feigl", a working tool appreciated by generations of analysts, synthetic and pharmaceutical chemists [9]. Little new has since been added to this body of extensive empirical knowledge in terms of new colour reactions.

A number of commercial test kits are available today for actual field application. Most of them were developed more than two decades ago and have since been in practical use (e.g. the United Nations Drug Testing Kit was launched in 1984). Their scope was originally

limited to the few significant drugs of abuse in the 1960s/1970s, i.e., opium, morphine, heroin, cannabis, cocaine, barbiturates and amphetamine /methamphetamine. Also, the colour tests used in the commercialized kits remained essentially the same for quite some time [10].

The need for extending the scope of field testing

With the gradual extension of controls to further drug types, such as methaqualone, designer amphetamines and the benzodiazepines, the scope of colour tests recommended for testing also started to grow. The addition of new colour tests to those originally recommended by the United Nations for field and laboratory use became imperative. Extensive consultations and laboratory trials preceded the inclusion of new colour tests (or odour tests – e.g., for cocaine) [11-13]. The consecutive issues of the United Nations publication "Rapid Testing Methods of Drugs of Abuse" (1988, 1995) are evidencing the expansion in scope [14].

With the enactment and entry-into-force of the 1988 Convention, 12 precursor chemicals were placed under a new type of control, thus adding a new dimension to the job of drug control personnel at the field level and in the laboratory as well. Upon request by governments, the United Nations launched in the 1990s a new field testing kit, "The United Nations Precursor Kit", specifically designed for the presumptive testing of precursor chemicals under international control. A total of almost 1,500 kits have since been provided for use to more than 80 countries. Field experience in many countries has documented their practicality.

As of 1992, the scope of controlled precursor substances was extended to 22 with the addition of four ATS precursors and six other precursor chemicals to the Tables of the 1988 Convention. The last addition so far was in 2000, when norephedrine was placed in Table 1 of that Convention. With three representatives of the ephedrine group (ephedrine, pseudoephedrine and norephedrine) being now in Table I of the 1988 Convention, a unique situation emerged: different members of a structurally and pharmacologically rather homogenous group of substances are subject to different control measures. Ephedrine, pseudoephedrine and norephedrine are listed in Table I of the 1988 Convention, while norpseudoephedrine (cathine) is controlled under Schedule III of the 1971 Convention as a psychotropic substance. Two close ephedrine-relatives, cathinone and metcathinone, are Schedule I psychotropic substances. In addition, two available ephedrine-derivatives, N-methylephedrine and chloropseudoephedrine which are subject to national control in some countries, e.g. Japan, were also included. The regulatory status of the ephedrine-related substances is summarized in Table I.

Table I
The regulatory status of ephedrine-related substances tested

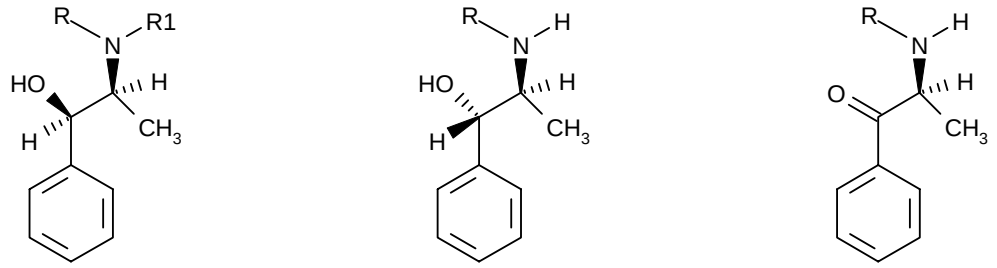
Precursor chemicals (1988 Convention)	*Psychotropic substances (1971 Convention)*	*Non-controlled derivatives*
Ephedrine	***Norpseudoephedrine***	***Chloropseudoephedrine***
Pseudoephedrine	***Methcathinone***	***N-Methylephedrine***
Norephedrine	***Cathinone***	

The ephedrine group as an analytical challenge: objectives of the present study

Ephedrine, norephedrine and pseudoephedrine have in recent years been subject to extensive illicit trafficking in many countries of the world. Hence, correct laboratory analysis and the availability of reliable rapid testing methods for use in the field for this group of precursor substances of various amphetamine-type substances have become high priority for many governments.

The chemistry of the ephedrine group (see Figure 1) requires that the specification of a suspected material be made at three distinct levels:

(i) a group specification (i.e., any member of the 'ephedrine' group),
(ii) a specification concerning the nature and degree of substitutions on carbon atoms C_1 and C_2 in the aliphatic chain, i.e.:
    i. Carbon atom C_1 may be substituted by a hydroxyl, carbonyl or chloro moiety;
    ii. Carbon atom C_2 may be substituted with -NH_2, -NHR or –NR2
(iii) a diastereomeric specification (see Figure 1).



Normal-series
Norephedrine (R=R1=H)
Ephedrine (R=CH_3, R1=H)
N-methylephedrine (R=R1=CH_3)

Pseudo-series
Norpseudoephedrine (R=H)
Pseudoephedrine (R=CH_3)

Chloropseudoephedrine (R=CH_3, R1=H; -Cl instead of -OH)

Cathinone (R=H)
Methcathinone (R=CH_3)

Figure 1
Chemical and stereo-structures of the ephedrine derivatives

The differentiation of the individual ephedrine derivatives is a complex and difficult task. This is reflected in the available scientific literature on this subject, which typically describes specific sample preparation and/or use of relatively sophisticated analytical techniques and conditions for an optimal separation and identification of this group of closely related substances [15-23].

It became therefore desirable, that systematic tests on all available ephedrine derivatives be carried out starting with using the colour reagents contained in the UN Precursor Test Kit with the aim to explore the possibility of a substance-by-substance differentiation of the various ephedrine-derivatives with simple colour tests.

Specifically, the objectives of our studies were as follows:

1. To verify the suitability, practicality and selectivity of the colour tests contained in the United Nations Precursor Test Kits for the presumptive identification of ephedrine 'derivatives' of all three sub-groups, i.e., controlled substances (ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine/cathine), the two substances of the cathinone subgroup (cathinone and methcathinone), and non-controlled ephedrine derivatives (chloropseudo-ephedrine, N-methylephedrine).

2. To explore the possibility of identifying and proposing a combination of colour tests and a flow-chart for the differentiation of the following sub-groups of ephedrine derivatives:

i) nor-derivatives and N-substituted derivatives (primary and secondary amines);

ii) C_1-OH derivatives (ephedrine group) and C_1=O derivatives (cathinone sub-group); and

iii) the "normal"- and the "pseudo"-subset of derivatives.

It was hoped, that in an ideal case, a rational combination of two or three simple colour reactions could provide for a substance-by-substance identification or, at the minimum, an unequivocal assignment to one of the three sub-groups.

Experimental

Substances tested

The substances tested were those listed in Table I, above. In all cases, a few crystals of the individual substances were used for the tests, and the testing procedures of the Information Leaflets attached to the Precursor Kits were followed (see Annex I).

Reagents

The reagents in the UN Precursor Test Kit, which was provided by the Laboratory and Scientific Section of UNODC, were used to carry out colour tests. For specific modifications of some of the tests, which are not included in the UN Precursor Test Kit, procedures from the literature were used. The preparation of reagents is summarized in Annex I and II.

1. **Chen-Kao test** (also known as **Chen**; Test T in UN Precursor Test Kit) [24] is used in the UN testing procedure to distinguish ephedrine, pseudoephedrine, norephedrine, and methcathinone from amphetamine and methamphetamine. The latter two do not react with Chen's test reagent. The Chen's test results are shown in Table II, below.

2. To test the hypothesis, if further specificity could be introduced into the **Chen-Kao procedure**, we executed solvent extractions by extracting the chelate complex into organic solvents, as proposed in the literature [25, 26]. The results of this modification, which is not included in the UN Precursor Test Kit, are shown in Table III, below.

3. **Simon's test (with acetaldehyde)** is generally used as a test for secondary amines, such as methamphetamine, and secondary ring-substituted amphetamines, including MDMA and MDE [1, 8, 12]. In the UN Precursor Test Kit it is included (Test G) for the presumptive identification of piperidine. It was to be seen whether the reagent can be used for the detection of ephedrine-type secondary amines.

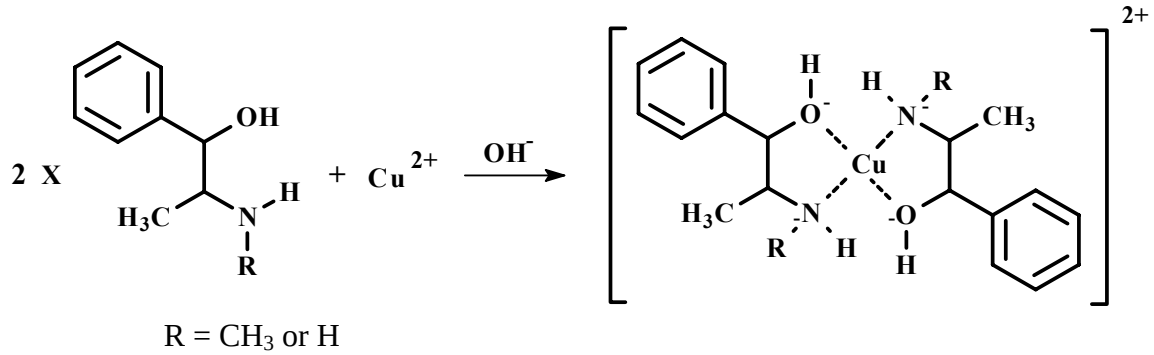
4. **Simon test with acetone** is proposed for the detection of primary amines, e.g. amphetamine [1, 8]. Although not included in the UN Precursor Test Kit, it was added to our testing sequence to enhance selectivity for the nor-derivatives.

Procedure

Tests were carried out in duplicate under laboratory conditions. The colours obtained were compared to those in the colour chart included in the UN Precursor Test Kit.

1. Chen-Kao Test

The formation of a violet-coloured chelate complex with copper sulfate in alkaline medium is considered to be characteristic "selective" for phenylakylamines with vicinal amino- and hydroxyl-groups and has been in use in pharmaceutical analysis for the simple identification of ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine (see for example [12, 26]). The result of the Chen-Kao reaction is a symmetrical (tetravalent) chelate complex (Figure 2). The colour, solubility and the stability of this complex appear to be affected by the structural and steric differences of the alkylamine part of the molecule. As seen in Tables II and III, of all tested ephedrine-type compounds only ephedrine and pseudoephedrine give the typical violet solution. All other derivatives produce a blue precipitete.



R = CH_3 or H

Figure 2
Chen-Kao complex formation between ephedrine and copper sulfate

A number of unrelated organic compounds, *inter alia*, pharmaceuticals, have been found to produce coloured, most often blue, complexes with Chen-Kao reagent, and minor modifications in the execution of the test have been introduced, e.g. variations in the nature and amount of the acid, the amount of copper sulfate and alkali used. To further improve selectivity, literature suggests the extraction of the complex formed in the first step of the reaction with ethyl ether or n-butanol. In this step, the violet complex is transferred to the organic layer, and the water phase turns usually blue [25, 26]. To our knowledge, no systematic evaluation of the value of this modification has been made to date for ephedrine-type substances.

Results

The results of the Chen-Kao reaction alone and after solvent extraction are summarized in Table II and III, respectively.

Table II
The results of the Chen-Kao test

Compound tested	Result	Remark
Ephedrine	***Violet***	+
Pseudopehedrine	***Violet***	+
Norephedrine	***Bright blue precipitate***	*
Norpseudoephedrine	***Blue precipitate***	*
Chloropseudoephedrine	***Bright blue (light greenish) precipitate***	*
N-Methylephedrine	***Pale blue precipitate (the crystals in the precipitate are violet)***	*
Cathinone	***Pale blue precipitate, turns to violet, through gray (greenish) in 2-3 minutes it turns to orange (brownish)***	*
Methcathinone	***Bright blue precipitate, through green it turns to brown, after 10 minutes the precipitate and the solution is orange***	*

Legend
\+ indicates full correspondence to the colour as specified in the UN test kit and the colour chart.
* indicates that the colour test for the compound has not been previously included in the UN test kit.

When reviewing Tables II to IV, it should be borne in mind that colour evaluations are always subjective. However, within this study, colour descriptions have been systematically applied. (The colour sequence applied evolves from pink, which reflects a shade of red, via purple to violet, i.e., towards the blue end of the visible spectrum.)

Table III
The results of solvent extractions of the complex formed in the Chen-Kao test

Compound tested	Chen-Kao	Ethyl ether extraction		n-butanol extraction	
		ether	water	n-butanol	water
Ephedrine	***Violet***	***Purple***	***Blue***	***Purple***	*--*
Pseudoephedrine	***Violet***	***Purple***	***Blue***	***Purple***	***Blue***
Norephedrine	***Blue***	*--*	***Pale blue, prec.***	***Blue-violet***	***Opaque***
Norpseudo-ephedrine	***Blue***	***Prec., forming a ring between phase layers***	***Blue***	***Blue-violet***	*--*

Legend
prec. refers to a precipitate
-- indicates no colour in the solvent or aqueous phase

Discussion

A brief analysis of the Chen-Kao test results allows the following conclusions:

1.1. The execution of the Chen-Kao reaction is simple, needs little practice and limited skills. Also, the violet colour obtained in the reaction is easy to define. For a correct execution, it is important to note that the typical colours develop relatively slowly, and that a good colour intensity requires a sample of a few milligrams of the substances tested (i.e., more than what would typically be required for most other tests included in the UN test kits).

1.2. Of all ephedrine-related compounds, only **ephedrine** and **pseudoephedrine** produced the typical, stable violet colour required by the testing procedure and the colour reference in the UN test kit.

1.3. All other compounds tested produced a blue to greenish-blue precipitate. This precipitate could be seen as characteristic for the members of the ephedrine group other than pseudoephedrine and ephedrine itself. Hence, the Chen-Kao test appears to show a significant specificity within the ephedrine group. However, it is known from previously published cross-testing work that various pharmaceuticals not related to the ephedrine group may produce similar blue copper complexes.

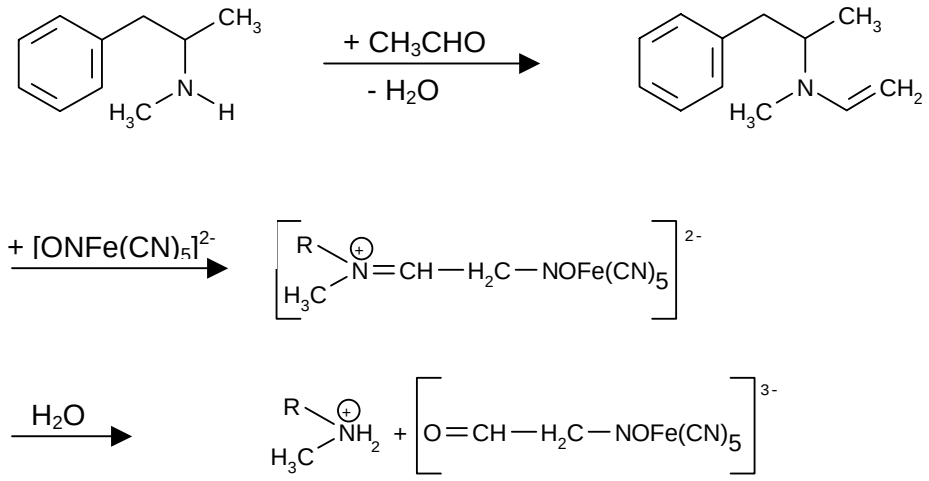
1.4. The two keto-amines, **cathinone** and **methcathinone**, initially also produce blue-coloured complexes with the Chen-Kao reagent. However, a slow transition of the initial colour into yellow, followed by an orange-brown colour can be observed with both compounds, thus indicating the instability of the complexes initially formed, and an obvious decomposition of the two compounds under the alkaline conditions of this colour reaction.

1.5. The results of the solvent extractions, summarized in Table III, appear to add little novelty to the results of the original Chen-Kao reactions. However, in cases of doubts, they may serve as confirmatory steps.

2. Simon test
(a) with acetaldehyde (Simon-1)
(b) with acetone (Simon-2)

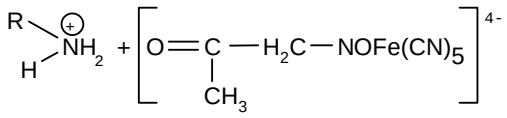
The formation of various colours between amines and sodium nitroprusside in alkaline medium, in the presence of an aldehyde or a ketone (Simon test), is considered to be characteristic for the amine partners. The formation of a deep blue colour with the reagent containing acetaldehyde as reactive partner (Simon-1; Figure 3) is considered to be characteristic for secondary amines, such as methamphetamine and other N-substituted amphetamine derivatives. This reagent has been in use by laboratories and also under field conditions, and a simple test tube system (“pocket test”) has also been developed for on-site urine testing for methamphetamine [27]. However, many other secondary amines, for example, such common chemicals like diethylamine and piperidine, may give similar colours. In general, colours are intense but may fade quickly in the presence of some impurities. For these reasons, it is essential for the analyst to confirm the Simon’s test result by performing supplementary colour tests.

A variant of the Simon test using acetone instead of acetaldehyde as reaction partner (Simon-2) enhances selectivity for primary amines (e.g., purple colour with amphetamine). The reaction mechanisms [12] proposed for this and the reaction with acetaldehyde are depicted in Figures 4 and 3, respectively. In view of the usefulness and obvious simplicity of both reactions, it is somewhat surprising that the suitability of the Simon test for the distinction between various ephedrine derivatives has not been explored previously in any systematic manner.



(Note: R = aromatic rest of molecule)

Figure 3
Adduct formation between a secondary amine (e.g., methamphetamine) and sodium nitroprussid-acetaldehyde [12]



(Note: R = aromatic rest of molecule)

Figure 4
Adduct of chemical reaction (Schiff-base formation) between a primary amine (e.g. norephedrine) and sodium nitroprussid-acetone [12]

Results

The results of the two variants of Simon reaction are summarized in Table IV.

Table IV: The results of the Simon reactions
Simon-1 (with acetaldehyde); Simon-2 (with acetone)

Compound tested	Simon-1		Simon-2	
	Result	Remark	Result	Remark
Ephedrine	***Light blue, in 5 min. deeper***	*	***NR***	*
Pseudoephedrine	***NR***	*	***NR***	*
Norephedrine	***Olive green***	*	***Light pink***	*
Norpseudoephedrine	***NR***	*	***Light pink***	*
N-methylephedrine	***Light pink***	*	***Light orange***	*
Chloropseudoephedrine	***Bright blue, in 5 min. grey***	*	***NR***	*
Cathinone	***Brown solution -> white prec.***	*	***NR***	--
Methcathinone	***Greyish blue -> in 5 min. brown***	*	***NR***	--

Legend
* indicates that the colour test for the compound has not been previously included in the UN test kit
-- indicates no colour in the solvent or aqueous phase
NR indicates that the compound gives no colour reaction with the reagent
prec. refers to a precipitate

Discussion

The reasons for the inclusion of both modifications of the Simon tests into our experimental scheme were:

a) to test the suitability of the Simon reagents for the detection of secondary amines with vicinal hydroxyl-, keto-, or chloro-groups, such as the ephedrine derivatives;
b) to complement the Chen-Kao reaction with more specific tools for discriminating between the nor- and the N-substituted-derivatives, as well as between the substances of the "normal" and the "pseudo" series.

Based on mechanistic considerations, it was expected that (i) the a vicinal hydroxyl (keto, or chloro) group would considerably influence the outcome of the reactions; and (ii) the steric orientation of the OH and NH(CH_3) would affect the outcome of the adduct formation. Indeed, the tests yielded some unexpected results (see Table IV, above), which are summarized as follows:

Simon reagent with acetaldehyde (Simon-1 in Table IV)

2.1. **Ephedrine** reacts with the expected blue colour, but the intensity of the colour is weak as compared to methamphetamine and to other secondary amines such as piperidine. The full development of the colour requires 5 to 10 minutes. This may be due to the effect of the

vicinal hydroxy functional group. **Chloropseudoephedrine** also results in a blue colour. However, this colour is unstable and changes into grey after a few minutes.

2.2. **Norephedrine** and ***N*-methylephedrine** react with distinct colours: olive green and pale pink, respectively. The keto-analogues cathinone and methcathinone react with unstable initial colours. The brown solution of **cathinone** subsequently turns into a white precipitate, while the initial greyish-blue colour of **methcathinone** turns into brown within about 5 minutes. These colour sequences may indicate the decomposition of the two compounds under alkaline conditions of the Simon reaction. It is known that both cathinone and methcathinone are rather unstable as free bases in alkaline conditions giving rise to a series of decomposition products (benzaldehyde, ethylamine, phenylpropanedione and a pyrazine dimer).

2.3. Importantly, **pseudoephedrine** and **norpseudoephedrine** produce no colours. This is a significant finding since it offers an opportunity for distinguishing between the individual substances of the normal- and the pseudo-series within the ephedrine group.

Simon reagent with acetone (Simon-2 in Table IV)

2.4. As expected, this variant of Simon reagent gives no reaction with the secondary amines ephedrine and pseudoephedrine, while a light purple or light pink colour develops with the two nor-derivatives **norephedrine** and **norpseudoephedrine**. The colours are weak and develop slowly (3 to 5 minutes).

2.5. **Chloropseudoephedrine** gives no colour, while the tertiary amine ***N*-methylephedrine** gives a distinct light orange colour with this variant of the Simon reagent.

Final conclusions

1. Two colour tests included in the UN Precursor Test Kit: Test T (Chen-Kao test)[1] and Test G (Simon test with acetaldehyde)[2], are suitable for field level presumptive differentiation of ephedrine-type compounds. The execution of the colour tests and the interpretation of the results following the instruction leaflet and the manual are simple. If executed correctly, with sufficient material, the risk of false negatives is small. However, it is important to note that the full development of the colours generally requires larger amounts (approximately 1-5 mg) of the suspected material than the amounts needed of amphetamines.

2. Only ephedrine and pseudoephedrine produce a copper complex with the expected violet colour with the Chen-Kao reagent, all other ephedrine-related compounds tested give blue colours, often with a precipitate. The two diastereomeric forms (ephedrine and pseudoephedrine) cannot be distinguished by this reaction alone. While this test can, therefore, be considered rather specific for the two N-methyl substituted derivatives, it is less so for the entire ephedrine group. The reagent is also known to produce similar blue colours with chemically unrelated pharmaceuticals. It always has to be borne in mind, therefore, that all colour reactions are presumptive tests, and that it can never be excluded that other

[1] Included in the UN Precursor Test Kit for the presumptive identification of ephedrine and pseudoephedrine.
[2] Included in the UN Precursor Test Kit for the presumptive identification of piperidine.

unrelated substances provide similar colours. Final confirmation in a laboratory is therefore essential.

3. Extraction of the chelate complex with organic solvents of different polarity (ethyl ether or n-butanol) provides additional confirmation of the original results, but no further differentiation.

4. The Simon test of the Precursor Test Kit (Simon-1, Test G) and the Simon test with acetone (Simon-2; not included in UN precursor test kit) may be used for the following purposes:

a) to complement the information obtained in the Chen-Kao reactions
b) to produce a distinction between N-methyl and nor-derivatives
c) to distinguish the "normal" and the "pseudo" series of the ephedrine-derivatives

5. On the basis of the results of the three colour reactions, a flow chart is proposed (Figure 5), which may assist laboratory and field personnel in the pre-selection of unknown suspected specimens of the ephedrine group before further laboratory investigation. In view of the inherent difficulties encountered during the unequivocal identification of unknowns suspected to be ephedrine derivatives even by laboratory-based, chromatographic techniques the preliminary assignment of the samples to a particular sub-group by the simple and quick use of a sequence of two or three colour tests may save precious time and resources.

6. To our knowledge, this is the first time that the suitability of the three reagents for the differentiation of ephedrine derivatives has been explored to any systematic extent.

Figure 5
Flow chart for the distinction of ephedrine-related compounds using the Chen-Kao, the Simon-1 and Simon-2 colour tests

Reaction	**Analyzed compounds**					
	Ephedrine	***Pseudo-ephedrine***	***Norephedrine***	***Norpseudo-ephedrine***	***N-Methyl-ephedrine***	***Chloropseudo-ephedrine***
Chen-Kao test	↓ Violet	↓ Violet	↓ Bright blue precipitate	↓ Blue precipitate	↓ Pale blue precipitate	↓ Bright blue (light greenish) precipitate
Simon test I (with acetaldehyde)	↓ Light blue (in 5 min. deeper)	↓ N.C.	↓ Olive green (brownish)	↓ N.C.	↓ Light pink	↓ Bright blue, after 5 min. it turns to grey (brownish sediment)
Simon test II (with acetone)	↓ N.C.	↓ N.C.	↓ Light pink	↓ Light pink	↓ Light orange	↓ N.C.

Legend: N.C. = no colour

7. The colours and the stability of the reaction products produced by cathinone and methcathinone are influenced by the vicinal ketoamino structural moiety. Both drug molecules are unstable, especially in aqueous alkaline solution, resulting in sequences of transitional colours. On the other hand, both compounds are rather inert in the usual colour tests used for the presumptive identification of phenylalkylamine derivatives (e.g. Marquis, Froehde's reagent). Therefore, the colour sequences produced by the three reagents (Chen-Kao, Simon-1 and Simon-2), rather than the initial colours, may prove useful in the presumptive identification of these two drugs.

8. The potential of a few other tests commonly used for the presumptive identification of phenylalkylamines (KOH, Marquis) ([8] and Annex II) was also evaluated by us with the entire set of ephedrine-related compounds, but did not add any substantial new information to those yielded by the three reagents above (Chen-Kao, Simon-1 and Simon-2) alone. Most ephedrine derivatives are rather inert in strong acidic conditions and do not produce characteristic colours with the reagents in concentrated acids (for reference see also Appendix 20.1. in [8]).

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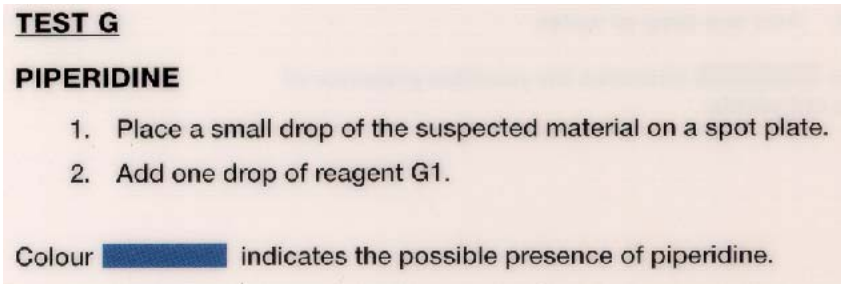
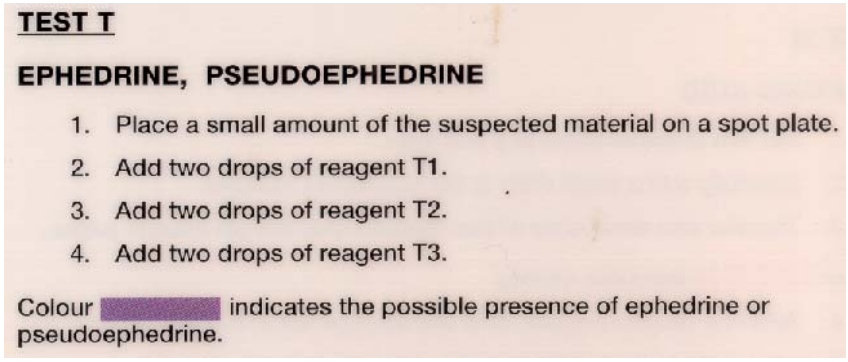
ANNEX I

United Nations Kits for the presumptive testing of precursors and essential chemicals: Testing procedures and reagents

1) Testing procedures for Simon (Test G)[3] and Chen-Kao (Test T) tests as included in the UN Precursor Test Kit:





2) Reagents for Simon and Chen-Kao tests (preparation of reagents as for UN Precursor Test Kit):

Simon-1 Test (with acetaldehyde): Test G

G1: Add 10% (vol./vol.) acetaldehyde to a 1% aqueous solution of sodium nitroprusside (dissolve 0.9g of sodium nitroprusside in 90ml of water, then add 10ml of acetaldehyde)

Chen-Kao Test: Test T

T1: 1% (vol./vol.) aqueous acetic acid solution (add 1ml of glacial acetic acid to 100ml of water)

T2: 1% aqueous solution of copper(II) sulphate (dissolve 1g of copper(II) sulphate in 100ml of water)

T3: Dissolve 8g of sodium hydroxide in 100ml of water (= 2N aqueous solution of sodium hydroxide)

[3] Note that the Simon test is not included specifically as a test for ephedrine-type substances, but for piperidine.

ANNEX II

Preparation of reagents not included in UN precursor Test Kit

Marquis test

*Mechanism***:** The formation of a carbonium ion is responsible for the colours.

Reagents

1A Add 8-10 drops (approx. 0.25 ml) of 37 % formaldehyde solution to10 ml of glacial acetic acid

1B Concentrated sulfuric acid

Amounts of reagents used for the test:

1A 1 drop

1B 3 drops

Results

We got the best reactions with 7-8 crystals, by pulverizing the crystals and then adding the reagents. After each step the reaction mixture was mixed by shaking.

Compound tested	**Result**
Ephedrine	Pale yellow
Pseudoephedrine	Pale yellow (greenish)
Norephedrine	Pale yellow (brownish)
Norpseudoephedrine	Yellow (brownish)
(1*R*,2*S*)-(-)-*N*-methylephedrine	Brownish red
Chloropseudoephedrine	N.C.

N.C.-No changes were observed

This reaction is not specific for ephedrine related compounds, and does not allow a differentiation of the six compounds according to any of the sub-groups.

KOH test

Reagent

Dissolve 0.2g of pulverized KOH in 2.5g of iso-propanol.

Amount of reagent used for the test: 2 drops

Results/Remarks

The six ephedrine-related compounds did not give any reaction (no colour) with KOH/iso-propanol (pulverized samples were used, as above).