Color Tests for the Preliminary Identification of Methcathinone and Analogues of Methcathinone

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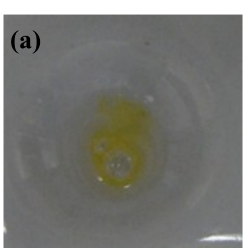
ABSTRACT: The preliminary identification of methcathinone and analogues of methcathinone presents a new and growing challenge to law enforcement. Color tests remain an important tool for the preliminary testing of suspected illicit drug samples. In this study, the applicability of a range of color tests to the preliminary identification of methcathinone and analogues of methcathinone was explored. It was found that Marquis reagent is suitable for the preliminary identification of methylenedioxy-substituted analogues of methcathinone. Liebermann's reagent was identified as an appropriate test for the preliminary identification of cathinone, methcathinone and 4-methylmethcathinone. Both of these color tests give rise to intensely yellow colored products. Liebermann's reagent also produced yellow and orange products upon reaction with N,N-dimethylcathinone and 4-methoxy-methcathinone respectively, although these products were less intensely colored. A testing sequence incorporating these tests was utilized on seized illicit drug samples and found to be suitable for use in routine casework.

KEYWORDS: color test, methcathinone, mephedrone, designer drugs, preliminary testing, drug testing, forensic chemistry.

The abuse of methcathinone (MCAT) and analogues of methcathinone has increased markedly in jurisdictions worldwide in recent years. For example, in Australia the rate of recent use of 4-methylmethcathinone (4-MMC) by regular methylenedioxymethamphetamine (MDMA) users rose from less than 1% in 2009 to 16% in 2010 [1,2]. These β-keto analogues of amphetamines [3], are stimulants with empathogenic effects [4]. The structures of analogues of methcathinone included in this study are summarised in Table 1.

Color tests still remain an important tool for the preliminary identification of illicit drugs in spite of developments in instrumental technology and the increased portability of this technology which enables its use in the field. As recently as 2000, 86% of laboratories surveyed still frequently used color tests when testing for illicit drugs [5]. The popularity of color tests arises from the fact that they are generally simple, quick, inexpensive, and quite sensitive [6]. They are readily available and require minimal materials. These factors enable color tests to be used in the field and can be employed by those without extensive chemical backgrounds.



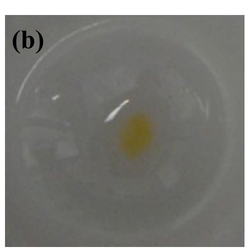


Figure 1 - Yellow-colored products of the reaction of Marquis reagent with 3,4-MDMC (a) and Liebermann's reagent with CAT (b).

Results of various color tests for methcathinone and its analogues have been described in the literature [6-13]; however, no comprehensive survey has been published. Furthermore, conflicting results from different sources were identified [6,8-13]. This study assesses the suitability of a range of color test methods for the preliminary identification of methcathinone and its analogues and recommends color tests for utilisation by law enforcement.

Experimental

Illicit Drugs

Certified reference materials of the hydrochloride salts of CAT, MCAT, 4-MMC, N,N-DMC, 3-FMC, 4-MOMC, 3,4-MDMC, 3,4-MDPV, BUT, MDA, and MDMA were supplied by the National Measurement Institute's Australian Forensic Drug Laboratory (NMI-AFDL).

Ten casework samples of illicit drugs were screened using attenuated total reflectance Fourier transform infrared spectrometry (ATR-FTIR). These were preliminary identified as containing 4-MMC (8 samples), 3,4-MDMC (1 sample) and 3,4-MDPV (1 sample). The physical form of these samples included coarse and finely divided white powders and colorless crystals in a range of shapes.

Chemicals

Color test reagent formulations are summarised in Table 2. Simon's reagent, Scott's test Solution 1, Chen-Kao test, and ferric chloride test were supplied by NMI-AFDL. Dille-Kopanyi Solutions 1 and 2 were from a testing kit produced by nik Public Safety. All other reagents were prepared from their constituent chemicals.

Hydrochloric acid was from BDH. Chloroform, methanol, ethanol, and pyridine were from Sigma-Aldrich. Sulfuric acid

Table 1 - Analogues of methcathinone commonly encountered by law enforcement.

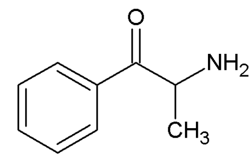
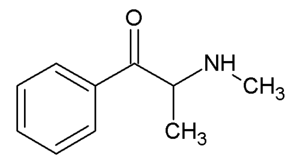
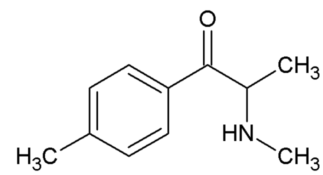
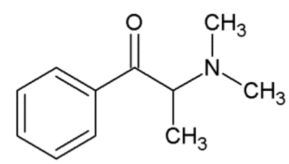
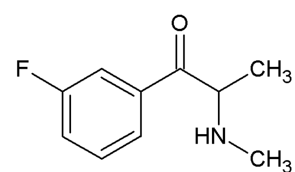
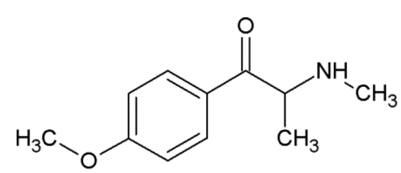
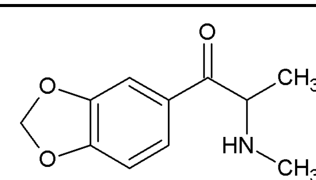
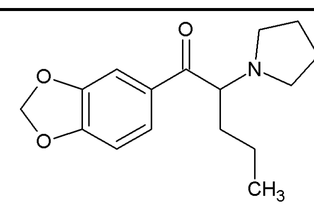
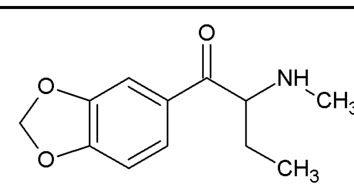
Compound	Abbreviation	Common Name	Structure
Cathinone	CAT	-	
Methcathinone	MCAT	-	
4-Methylmethcathinone	4-MMC	Mephedrone	
N,N-Dimethylcathinone	N,N-DMC	-	
3-Fluoromethcathinone	3-FMC	Flephedrone	
4-Methoxymethcathinone	4-MOMC	Methedrone	
3,4-Methylenedioxymethcathinone	3,4-MDMC	Methylone	
3,4-Methylenedioxypyrovalerone	3,4-MDPV	-	
Butylone	BUT	-	

Table 2 - Color test reagent formulations.

Colour Test	Ref	Reagent Formulation	Solution Quantities
Marquis Reagent	18	9:1 sulfuric acid and 37% formaldehyde	2-3 drops
Liebermann's Reagent	11	10% w/v sodium nitrite in sulfuric acid, added with cooling in water bath and swirling to absorb brown fumes	2 drops
Simon's Reagent	18	Solution 1: 10% v/v acetaldehyde	1 drop
		Solution 2: 10% w/v sodium nitroprusside	2 drops
Scott's Test (modified)	18	Solution 1: 2% thiocyanate solution, glycerol, glacial acetic acid	5 drops
		Solution 2: hydrochloric acid	Until precipitate disappears
		Solution 3: chloroform	10 drops
Chen-Kao Test	18	Solution 1: 1% v/v acetic acid	2 drops
		Solution 2: 1% w/v copper (II) sulfate	2 drops
		Solution 3: 8% w/v sodium hydroxide	2 drops
Ferric Chloride Test	18	5% w/v ferric chloride	5 drops
Cobalt Thiocycante	19	10% w/v cobalt thiocyanate in methanol	2-3 drops
Froehde's Reagent	19	5% w/v sodium molybdite in hot sulfuric acid	2-3 drops
Mecke Reagent	19	1% w/v selenious acid in sulfuric acid	2-3 drops
Ehrlich's Reagent	5, 6	2% w/v p-methylaminobenzaldehyde in 1:1 95% ethanol and sulfuric acid	2-3 drops
Mandelin's Test	11	0.5% w/v ammonium vanadates in sulfuric acid	2-3 drops
Gallic Acid Test	6	0.5% w/v gallic acid n-propyl ester in sulfuric acid	2-3 drops
Zwikker Reagent	5, 6	Solution 1: 0.5% w/v copper (II) sulfate	1 drop
		Solution 2: 1:19 pyridine and chloroform	1 drop
Sodium Nitroprusside	5, 6	Solution 1: 1% w/v sodium nitroprusside	2 drops
		Solution 2: 8% w/v sodium hydroxide	1 drop
Dille-Kopanyi Reagent	5, 20	Solution 1: 0.1% w/v cobalt acetate in ethanol	2 drops
		Solution 2: 1:19 isopropylamine and ethanol	1 drop

was sourced from both BDH and Sigma-Aldrich. Formaldehyde was from Unilevar. Gallic acid n-propyl ester was from TCI. Sodium nitrite and copper (II) sulfate pentahydrate were from Univar. Sodium molybdite was from Mallinckrodt. Selenious acid was from Unilab. p-Methylamino-benzaldehyde was from Fisher. Sodium nitroprusside was from M&B.

Methodology

All color tests were conducted in clean white porcelain spot trays, with the exception of Scott's test which utilized clean glass culture tubes. The reagent solution(s), in quantities described in Table 2, were applied to a pin-head sized sample of drug and a paired blank well. The tray was gently agitated and any color change immediately observed and recorded. Where

Table 3 - Results of color tests.

Colour Test / Drug	Marquis Reagent	Liebermann's Reagent	Simon's Reagent	Cobalt Thiocyanate	Froehde's Reagent	Mecke Reagent	Mandelin's Test	Gallic Acid Test
CAT	-	Y (B)	-	Pr/B	-	-	O/R	-
MCAT	-	Y (B)	-	Pr/B	-	-	O/R	-
4-MMC	-	Y (B)	-	Pr/B	-	-	-	-
N,N-DMC	-	Y (F)	-	*	*	*	*	*
3-FMC	-	-	B (F)	Pr/B	-	-	O/R	-
4-MOMC	-	O (F)	-	*	*	*	*	*
3,4-MDMC	Y (B)	O/B	Bl (F)	B/Pr	Y (B)	Y (B)	G/Br	Y
3,4-MDPV	Y (B)	Y/G	-	B/Pr	Y (B)	Y (B)	G/Br	Y
BUT	Y (B)	G/Br	Bl (F)	*	*	*	*	*
MDA	Bl (D)	*	Pi	*	*	*	*	*
MDMA	Bl (D)	Br	Bl (D)	*	*	*	*	*

Key: - = no reaction; * = not tested ; Bl = blue; Br = brown; G = green; O = orange; Pi = pink; Pr = purple; R = red; Y = yellow; (B) = bright; (D) = dark; (F) = faint. *Note: All test not shown yielded no reaction or were not tested.*

more than one reagent solution was required for a test, the solutions were applied in the order listed in Table 2.

Results and Discussion

The results of the color tests are summarised in Table 3. Yellow colored products were yielded by the reaction of 3,4-methylenedioxy-substituted analogues of methcathinone (3,4-MDMC, 3,4-MDPV, and BUT) with Marquis reagent (Figure 1). These products likely arise from the reaction of the drug molecules with sulfuric acid in a mechanism analogous to that of the reaction of MDMA with Marquis reagent [14]. Here, the extension of the conjugation by the ketone group shifts the color of the products from blue-black observed for MDA and MDMA to yellow. Marquis reagent gave rise to no color change with any other methcathinone analogue tested. This reflects the deactivation of the aromatic ring by the electron withdrawing effects of the ketone group [10], which prevents formation of the orange color characteristic of the reaction of Marqius reagent with amines. The discriminating power (DP) of this test was calculated in order to objectively quantify the selectivity of this test. The discriminating power is the probability that two samples from two different sources (that is, samples of two different drugs) will not randomly match if a given test is performed on them. If a population contains one in N randomly matching pairs, the discriminating power is calculated using Equation 1. In this study, the pairing of each of the three methylenedioxy-substituted analogues of methcathinone with 20 "designer drug" standards yielded 60 pairs, nine of which could not be confidently discriminated using Marquis reagent. Thus, the discriminating power of Marquis reagent for methylenedioxy-substituted analogues of methcathinone was 0.85 for the sample of "designer drugs" studied, indicating that this is a highly discriminating preliminary test.

$$DP = 1 - \frac{1}{N}$$

Equation 1 - Calculation of discriminating power [15].

Yellow colored products also arose from the reactions of CAT, MCAT, N,N-DMC, and 4-MMC with Liebermann's reagent (Figure 1). The mechanism of this reaction remains unclear; Liebermann's reagent has, to date, been given little attention in the published literature. Although several possible mechanisms were considered, all entailed the persistence of unstable nitrous acid in solution and were thus deemed unlikely. An orange product was produced by the reaction of

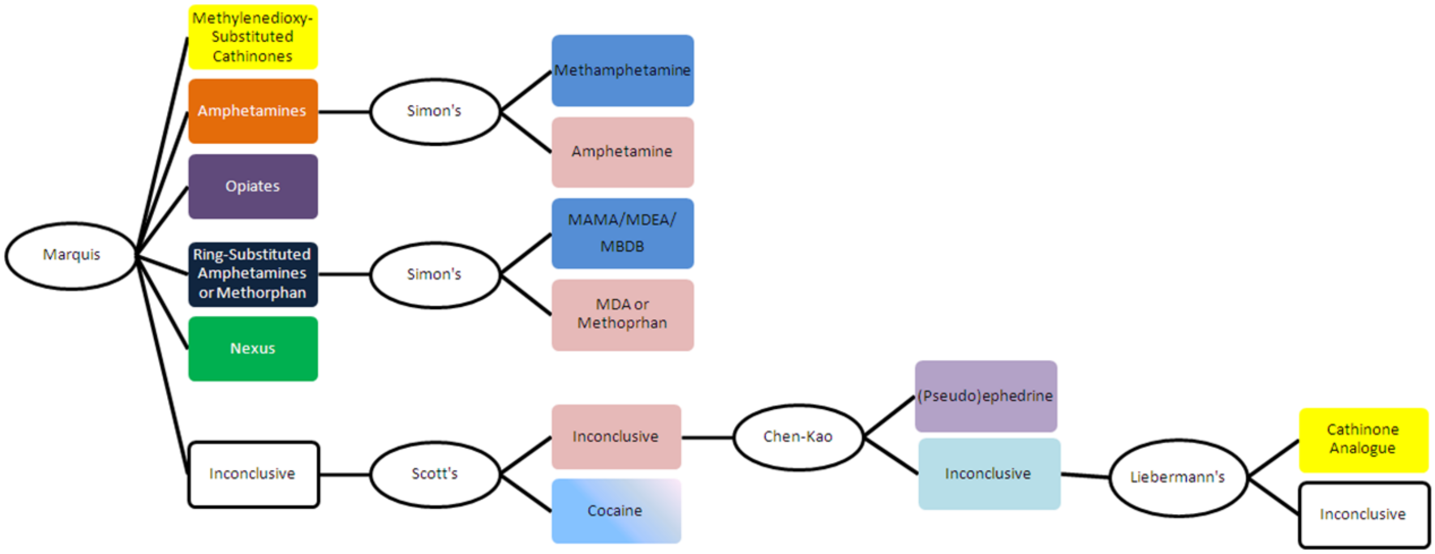


Figure 2 - Recommended color test procedure, incorporating color tests for methcathinone analogues.
Note: Ferric chloride test (after inconclusive Liebermann's reagent) omitted for purposes of clarity.

4-MOMC with Liebermann's reagent. However, this results should be confirmed by alternative preliminary identification techniques such as ATR-FTIR, with several "designer drugs" included in this study (results not shown) yielding similarly colored products. No colored product was observed when Liebermann's reagent was applied to 3-FMC.

In some cases, ambiguous color changes resulted from the reaction of methcathinone analogues with color test reagents. For example, some blue streaking of the solution occurred upon the reaction of Simon's reagent with 3-FMC, 3,4-MDMC and BUT; however, this was not comparable to the intense blue produced by this reagent with other secondary amines such as methamphetamine or MDMA. This likely arises from the electron withdrawing effects of the ketone group [10], which weakens the nucleophilic nature of the amine group sufficiently to prevent the nucleophilic addition of the amine to the aldehyde, the first step in enamine production [12,16,17]. Similarly, Mandelin's reagent turned from yellow to orange upon application to analogues of methcathinone. For both of these reagents, the color change was insufficiently definitive for utilisation in routine casework.

Methcathinone and its analogues were also shown in this study to not react with a wide range of color tests commonly utilized by law enforcement, including Simon's, Scott's, Chen-Kao, ferric chloride, cobalt thiocycante, Ehrlich's, Madelin's, gallic acid, Zwikker, sodium nitroprusside, and Dille-Kopanyi. Froehde's, and Mecke reagent reacted only with 3,4-methylenedioxy-substituted methcathinone analogues, producing yellow and orange-brown products respectively which, as with Marquis reagent, arise from the reaction of the drug molecule with sulfuric acid.

Given these results, Marquis reagent and Liebermann's reagent were identified as the most appropriate color tests for the preliminary identification of methcathinone and analogues of methcathinone. Both of these color tests are relatively simple to prepare and apply to case work, as they each include only one solution. Commercial test kits for both reagents are also available. Furthermore, these reagents can easily be incorporated into testing sequences of the type typically employed by law enforcement for the preliminary identification of illicit drugs.

Based on the results of this study, a testing sequence (shown in Figure 2) incorporating Marquis and Liebermann's reagent was been proposed and applied to casework samples. In all cases, results of the color tests were consistent with those of ATR-FTIR. These results suggested that these color tests were suitable for samples with a variety of physical characteristics, and were also sufficiently sensitive to detect the compounds of interest when sample sizes and concentrations are typical of those encountered by operational law enforcement.

Conclusions

This research has shown that color tests are a suitable method for the preliminary identification of methcathinone and analogues of methcathinone. Bright yellow colors are produced by both Marquis reagent and Liebermann's reagent, with Marquis reagent yielding a yellow product with methylenedioxy-substituted analogues of methcathinone and Liebermann's reagent with CAT, MCAT, N,N-DMC, and 4-MMC. The reaction of 4-MOMC with Liebermann's reagent produces an orange product. The mechanisms of reaction of these color tests, and the reasons for the failure of other color tests to yield colored products with these drugs, have been considered. The applicability of a color testing sequence incorporating these reagents to the preliminary identification of methcathinone and analogues of methcathinone has been successfully demonstrated on casework samples.

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