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Forensic Analysis Divulged Triprolidine in Suspected Mephedrone

R.V. Phadke[1], K.S. Kapgate[2], A.A. Pande[3], J.T. Kohpare[4], V. J. Thakre[5]

[1,2]Assistant Chemical Analyser, Regional Forensic Science Laboratory, Amravati
[3]Assistant Director, Regional Forensic Science Laboratory, Nagpur
[4]Assistant Chemical Analyser, Regional Forensic Science Laboratory, Nagpur
[5]Director, Directorate, Forensic Science Laboratories, Mumbai

Abstract

Cathinone alkaloid is found in the khat plant and is used as a social drug by people in southern and eastern Africa. The structure of cathinone resembles amphetamine and shows psychological effects as a stimulant. Similar effects are also found in synthetically prepared 4-methyl methcathinone (Mephedrone) and come under the NDPS Act. Suspected seized drugs are sent to forensic science laboratories for detection of drugs under the NDPS ACT. During forensic examination, many times it is found that drugs are sold in an adulterated form. The present paper discussed a case where an antihistamine drug, Triprolidine, is detected in the seized sample instead of mephedrone. Triprolidine is an over-the-counter antihistamine with anticholinergic properties. During chemical analysis of the seized sample, it was found that some of the commonly used preliminary tests for detecting mephedrone give positive results. Still, further advanced analysis confirmed the presence of Triprolidine instead of mephedrone in the sample. The case thus proved that modern analytical techniques are inevitable in the forensic examination of drugs.

Keyword: - Mephedrone, NDPS Act, Triprolidine, Adulteration, TLC, GC-MS.

1.0 Introduction

Different drugs of abuse fall under the Narcotic Drugs and Psychotropic Substances Act (NDPS Act) 1985[1]. They are mainly categorized as narcotics, stimulants, hallucinogens, and depressants. The group of stimulants is a class of drugs that make the person feel more energetic, alert, and awake as it speeds up the message traveling between the brain and body. Recently, one of the stimulant drugs categorized under the NDPS Act is 4-methyl methcathinone, commonly called Mephedrone, meow-meow, white magic, drone, M-CAT, and bubble. Mephedrone is a synthetic drug and its effects resemble that of drugs such as amphetamines and methamphetamines[2]. A few years ago, it was popular as a legal high but then banned in many countries due to its potential for abuse and harmful health effects. Possession, selling, and consumption of these drugs fall under the NDPS Act 1 and have serious punishment. Hence drugs seized from the accused are submitted to the forensic science laboratory for confirmation.

During forensic examination of samples seized under the NDPS Act, drugs are found in adulterated form. The adulteration or mixing of drugs is done to increase the available quantity of drugs for making money. Similarly, to get the kick or to experience the high, they are mixed with low-cost medicines that have similar effects. Literature studies show the presence of drugs such as alprazolam, paracetamol,

chalk powder, sugar, and some other psychotropic drugs in heroin[3,4]. Cocaine and Mephedrone were also found to be adulterated[3]. Adulteration of monosodium glutamate (Ajinomoto) was also detected in mephedrone[5,6]. Adulteration in drugs is a common issue and at the same time difficult to detect. Mephedrone submitted in the laboratory is mainly white-colored or off-white-colored crystalline powder and becomes challenging as many drugs appear similar in color and form. Similarly, preliminary color tests also can just predict the presence of the drug. Thin-layer chromatography (TLC) and spectrophotometric analysis support the detection of drugs but advanced separation techniques such as gas chromatography-mass spectrometry play a very important role in the confirmation of single as well as mixed drugs.

Different chemical methods as well as analytical instrumental techniques are reported for the detection of Mephedrone. Various color tests are reported by authors in detail to distinguish different analogs of cathinone[6]. In one of the drug cases, a sample was seized as mephedrone by the Police authorities. The sample was accepted in a forensic science laboratory for chemical analysis. Preliminary spot tests show positive tests for mephedrone in the sample. The confirmatory tests for mephedrone were performed by thin-layer chromatography, UV-visible spectrophotometric, and GC-MS analysis. TLC and UV-visible spectrophotometric analysis reveals some unusual findings. Hence the presence of mephedrone was further confirmed by GC-MS analysis. The GC-MS analysis revealed the presence of a drug triprolidine instead of mephedrone in the sample.

2.0 EXPERIMENTAL

2.1 Chemicals and solvents: Chemicals and solvents used for different tests were of analytical grade obtained from E-Merck India Ltd. Sample preparation was also done in methanol as well as in 0.1 N HCl for different analyses.

2.2 Preparation of Reagent for Preliminary spot tests

Dragendorff's Reagent

Solution (a): 2g Bismuth subnitrate + 25 ml Glacial Acetic acid+ 100 ml Distilled water.

Solution (b): 40 g Potassium Iodide + 100 ml Distilled water.

Mix -10 ml of Solution (a) + 10 ml of Solution (b) + 20 ml of Glacial Acetic acid 100 ml of distilled water.

Ninhydrin Reagent: Dissolve 0.5 g of ninhydrin in 40 ml of Acetone.

Marquis reagent –1ml 40% formaldehyde in 100 ml concentrated sulphuric acid.

Liberman reagent: 1 gm sodium nitrite in 10 ml Conc. sulphuric acid with cooling and swirling to absorb brown fumes.

Simon test reagent: a)1 gm sodium nitroprusside in 100ml water and 2 ml acetaldehyde. b) Freshly prepared 2% sodium carbonate solution.

Ehrlich reagent: 2.0 gm p-dimethylaminobenzaldehyde in 50 ml 95% ethanol and 50 ml concentrated hydrochloric acid.

Alkaline $KMnO_4$ Test: 0.1gms $KMnO_4$ in 50 ml 0.1N NaOH solution.

2.3 Thin layer chromatography (TLC):

HPTLC was performed on glass plates of size (10 cm x 10cm) precoated with 0.25 mm layer silica gel $60F_{254}$. The plate was activated in an oven at 110^0 C for about 30 minutes, then removed and cooled at room temperature. Then methanol extract of reference Mephedrone and the suspected sample to be tested were spotted on TLC plates with the help of fine capillaries. The plate was developed in a TLC

chamber pre-saturated with a mobile phase. Solvent systems used in the analysis are presented in **Table 1.** The plate was then removed, dried in air for 10 min, and sprayed with the visualizing reagent. The Rf values of the spots observed were noted.

Table 1: Solvent systems used in the study

Sr. No.	Solvent Systems	Ratio
1	Methanol	100
2	Methanol: Ammonia	100:1.5
3	Ethyl Acetate: Methanol: Ammonia	17:2:1

2.4 UV Visible spectrophotometry

Samples under analysis are prepared in 0.1 N HCl, and analyzed on a UV-Visible spectrophotometer instrument model analytik jena Specord S-600. A cell of 1 cm path length and a wavelength in the UV range of 200-400 nm was used for scanning. The spectra were compared with reference methamphetamine and mephedrone.

2.5 GC MS TOF analysis

Methanol extract from the samples was used for GC-MS analysis. GC–MS analysis was performed using an Agilent GC and a Leco Pegasus HT- GCMS with a Time-of-flight detector (TOF)

Restek Rxi-5Sil MS Capillary column dimensions 10 M, 0.15 mm ID was used for separation in this analysis with Helium@ 1.5ml/min as carrier gas. The analysis was carried out at the following conditions: Oven Temperature- Initial temp. 125ºC for 1 min- 35ºC/min -300ºC- 5 min hold. Source Temperature was 200ºC and Interface Temperature 260ºC was kept throughout the analysis. Mass spectra were obtained in the full scan mode (50–550 amu).

3.0 Results and Discussion

The samples were submitted to the laboratory for the detection of Mephedrone. The results of the analysis of samples are as follows:

3.1 Preliminary examination: The sample for detection of mephedrone was off-white colored crystalline powder. Preliminary analysis shows that the sample was soluble in water, methanol, and hydrochloric acid. Similarly, the test for chloride as well as sugar was also found positive for the sample. A microcrystal test using an aqueous extract of Mephedrone and mercuric chloride solution in water (10g/ml) shows a few paddle wheels and rosette of blade-like crystals under the microscope for Mephedrone and was found to be negative for the sample in this case. The results of the spot tests are presented in **Table 2** and are shown in **Figure 1.**

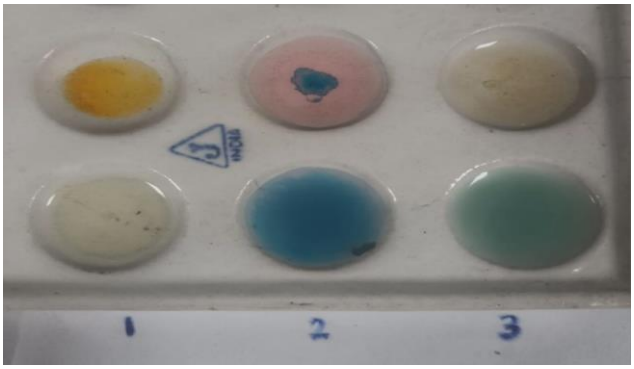
The results were also confirmed by using the reference Mephedrone. Color tests of exhibits such as cobalt thiocyanate and Liebermann tests give positive results that match with Mephedrone. Cobalt thiocyanate test is positive for a wide range of drugs[7,8]. Lieberman test gives yellow coloration for a sample and is also positive for many nitrogen-containing drugs. The Ehrlich test used for the detection of methamphetamine was negative for the sample.

Table 2: Colour tests/ spot tests for the sample

Sr. No	Tests	Sample	Ref Mephedrone
1.	Marquis test	Negative	Negative

2.	Cobalt thiocyanate	Blue coloration	Dark Blue coloration
3.	Liebermann test	No coloration	Dark Yellow coloration
4.	Ehrlich test	Negative	Negative
5.	Alkaline $KMnO_4$ test	Blue coloration	No coloration

Figure 1: Colour tests for the sample with reference to Mephedrone



Sample
1. Liberman test 2. Cobalt thiocyanate test 3. Alkaline KmnO4 test

3.2 Thin layer chromatography

Methanol ammonia (100:1.5) is the widely used solvent system for the separation of drugs using thin-layer chromatography[9]. Mephedrone can be successfully detected as an orange-colored spot by visualizing agent Dragendorff using this solvent system. The spot observed for the sample shows different rf values as shown in **Table 3,** which does not match the TLC of mephedrone and methamphetamine. Similarly, literature is also available for the adulteration of Ajinomoto in Mephedrone, hence the samples were also tested for the presence of Ajinomoto (monosodium glutamate-MSG)[5,6] and methamphetamine using TLC and found negative for it. As mentioned in Table 3, different solvent systems were also used to separate the spots to find the traces of mephedrone in the exhibit. All the tested solvent systems show a single spot. Thus, outcomes of the TLC results revealed the presence of a single component other than mephedrone in the sample as presented in **figure 2.**

Table 3: TLC results of drugs in different solvent systems

Sr. No.	Solvent Systems	Ratio	Mephedrone	Methamphetamine	Sample
1	Methanol: Ammonia	100:1.5	0.71	0.34	0.51
2.	Ethyl Acetate: Methanol: Ammonia	17:2:1	0.86	0.71	0.91
3	Methanol	100	0.61	0.32	0.2

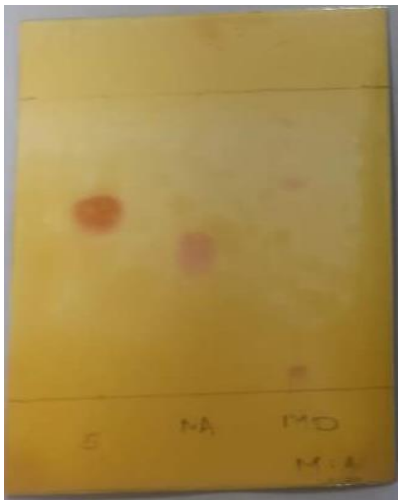


Figure 2: A photograph of thin layer chromatography plate

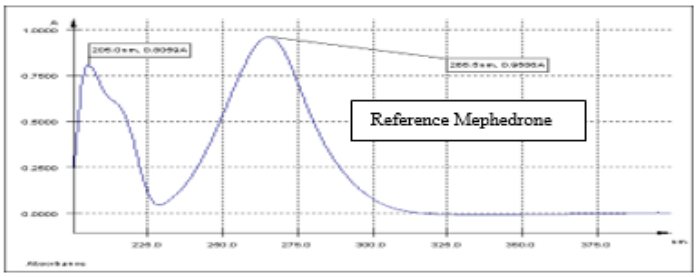
(1. Sample, 2. Reference Methamfetamine, 3. Reference MD**)**

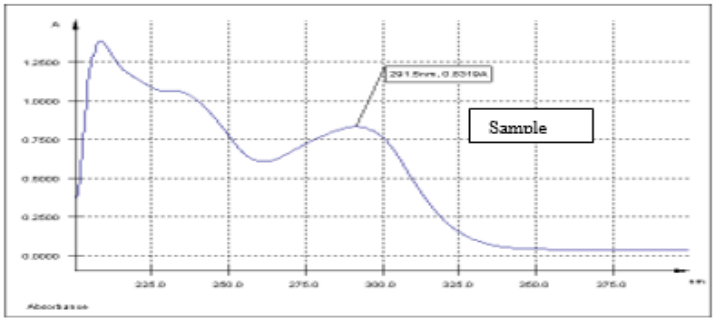
3.3. UV Visible spectrophotometric analysis: UV Visible spectrophotometric analysis of the sample in aqueous HCl shows absorbance maxima **(λ_{max})** as shown in **Table 4** and is presented in **figure 5**. The values of absorbance maxima for mephedrone tally with the literature value for mephedrone in aqueous HCl[10]. The absorbance maxima for the sample do not tally with reference methamphetamine or mephedrone.

Table 4: UV Visible spectrophotometric analysis results of different drugs

Name of drug	**Absorbance maxima (λ_{max})**
Reference Mephedrone	264.5 nm
Reference methamphetamine	253.0nm, 258.5 nm, 264.0 nm
Sample	291 nm

Figure 3: UV spectra of (a) Reference Mephedrone (b) Sample





3.4 Gas chromatography-mass spectrometry

Tests performed as above, such as chemical analysis or spot test, were not sufficient to rule out or confirm the other drug in the sample. The sample was analyzed on GC MS TOF instrument, and the spectra results were screened using mass spectral library of National Institute of Standard and technology mass spectral library. The sample shows m/z fragments at 55, 96, 152, 193, 208, and 278 and show 925 similarity matches with triprolidine and do matches with reference mephedrone (reference mephedrone show m/z fragments at 58, 91, and 119). The results are depicted in figure 4. Thus, presence of triprolidine, a histamine drug commonly used in medicinal formulations[11] instead of mephedrone was identified and confirmed by GC MS TOF in the sample.

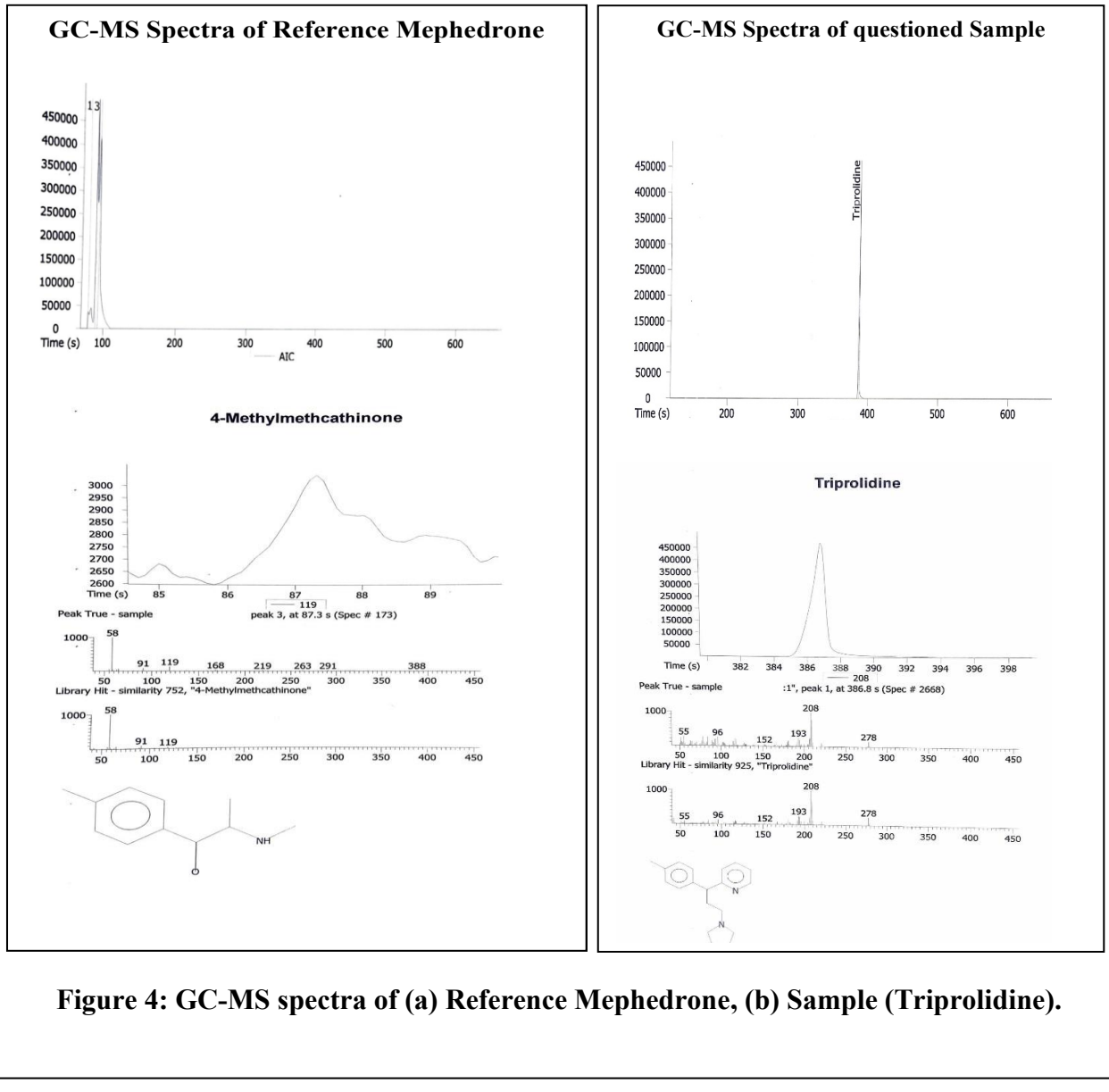


Figure 4: GC-MS spectra of (a) Reference Mephedrone, (b) Sample (Triprolidine).

Discussion

A drug for mephedrone testing submitted in FSL is white, off-white, and sometimes brownish-colored

crystalline powder. It is difficult to distinguish or identify the mixing of drugs based on hue and preliminary analysis only. The photographs in the figure show the positive Liberman test for reference mephedrone and negative for the sample, cobalt thiocyanate test positive for both reference mephedrone and sample, and bluish coloration for alkaline $KMnO_4$ test that is negative for reference mephedrone and positive for sample. Thin-layer chromatography analysis confirms the presence of a single component in the sample. Spectrophotometric analysis of the sample in Aqueous HCl shows absorbance maxima of 291nm for the sample, however, it is 265 nm for the reference mephedrone. GC MS analysis confirmed the presence of triprolidine in the sample. A literature study on triprolidine analysis shows that the compound triprolidine gives blue-green coloration with alkaline $KMnO_4$ and absorbance maxima at 291 nm in aqueous HCl. Thus, the literature supports the presence of triprolidine, an antihistamine with anticholinergic properties, drug in the sample[11,12]. Thus, the results obtained in forensic analysis confirmed the presence of some other drug instead of mephedrone.

Conclusion

As the drugs nowadays are sold in adulterated form, proper and full-proof detection or identification of drugs submitted to the FSL is the crucial part and thus needs all dimensional analysis to reach to conclusion. The study of the sample in the present case shows the presence of the unexpected drug triprolidine. It thus again proves the successful role of forensic science in the detection and identification of drugs.

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