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Original Research

Mephedrone–Chen–Kao Reaction: A Forensic Approach to Presumptive Identification of Synthetic Cathinones

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ABSTRACT

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Background: Presumptive colour tests are commonly used in forensic drug analysis for the preliminary screening of suspected illicit substances. The Chen–Kao reaction may assist in the preliminary examination of certain synthetic cathinones, although its specificity for individual compounds is limited.

Objective: To observe and document the colourimetric reaction of a standard mephedrone sample with the Chen–Kao reagent.

Methods: The standard mephedrone sample was examined for colour, texture, odour, physical appearance, and solubility. The sample was subsequently subjected to the Chen–Kao reagent system comprising acetic acid, copper sulphate solution, and sodium hydroxide solution. The resulting colour changes and precipitate formation were recorded visually.

Results: The preliminary examination recorded the sample as white or off-white to brown, crystalline in texture, and in powder form, with no odour or a slight chemical-like smell. The sample was reported to be readily soluble in water and ethanol. During the colourimetric reaction, the observed sequence was blue, followed by blue-green, and finally an orange-brown precipitate in the centre of the spot plate.

Conclusion: The Chen–Kao reaction produced a visible colourimetric response with the standard mephedrone sample under the experimental conditions. However, the reaction cannot independently confirm the identity of mephedrone because of its limited specificity.

Keywords: Mephedrone, Chen–Kao Reaction, Synthetic Cathinones, Colourimetric Test, Forensic Drug Analysis.

INTRODUCTION

New psychoactive substances (NPS) have emerged as a major challenge in forensic science, clinical toxicology, and public health because of their rapidly changing chemical structures, diverse pharmacological effects, and potential for acute toxicity. Synthetic cathinones constitute an important group of NPS that are structurally related to cathinone, a naturally occurring psychoactive alkaloid present in the leaves of the khat plant (*Catha edulis*). These compounds are β -keto analogues of amphetamine-type stimulants and are frequently associated with recreational drug use. Minor modifications to their molecular structures can produce new analogues with different pharmacological and toxicological characteristics, making their regulation and identification increasingly challenging. ^[1-4] The continuing emergence of these compounds has highlighted the need for effective analytical approaches in forensic drug investigations. ^[5,6] Synthetic cathinones primarily influence monoaminergic neurotransmission by affecting the transport, release, or reuptake of dopamine, norepinephrine, and serotonin. Their stimulant effects may include euphoria, increased alertness, enhanced sociability,

tachycardia, and elevated blood pressure. However, acute exposure may also produce agitation, anxiety, paranoia, hallucinations, hyperthermia, seizures, and serious cardiovascular complications. [1,3,11,12] The severity of these effects varies according to the substance, dose, route of administration, and individual susceptibility. Consequently, the identification of synthetic cathinones is important in suspected intoxication cases, drug seizures, and postmortem investigations. [2,6] Mephedrone, chemically identified as 4-methylmethcathinone (4-MMC), is a prominent synthetic cathinone that became increasingly popular in recreational drug markets during the late 2000s. Its stimulant and empathogenic effects have been compared with those of cocaine and 3,4-methylenedioxymethamphetamine (MDMA). [7-10] Mephedrone contains a β -keto group, a secondary methylamino group, and a para-methyl substituent on its aromatic ring. Its use has been associated with sympathomimetic toxicity, including elevated heart rate, hypertension, agitation, and other adverse clinical effects. [7-10] These concerns emphasize the importance of reliable screening and confirmatory procedures for identifying mephedrone in seized materials and biological specimens. [14]

Presumptive color tests remain useful in preliminary forensic drug screening because they are relatively inexpensive, rapid, and operationally simple. Such tests can provide an initial indication of the possible presence of particular chemical classes; however, their interpretation requires caution because different substances may produce similar color responses. Matrix interference, impurities, reagent condition, and experimental variability may also influence the observed results. Therefore, presumptive reactions should not be considered definitive evidence of drug identity, and appropriate instrumental techniques, including gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), may be required for confirmation. [2,4-6] The Chen-Kao reaction has been associated with the colorimetric examination of ephedrine, pseudoephedrine, and related phenylalkylamine compounds.

Nevertheless, its specificity and analytical suitability for detecting mephedrone require direct experimental assessment, particularly because the structural differences between these substances may influence their chemical reactions. The present study aims to examine the chemical principles and potential forensic applicability of the Chen-Kao reaction as a presumptive screening method for mephedrone, focusing on the observed color response, interpretative limitations, and the importance of confirmatory analysis. Establishing its performance through appropriate controls and

comparison with a validated analytical method would help determine whether the reaction has practical value in preliminary forensic investigations.

MATERIALS AND METHODS

Study Design: The present study was designed as a preliminary qualitative laboratory investigation to examine the observable colorimetric response of a mephedrone (4-methylmethcathinone; 4-MMC) reference standard to the Chen-Kao reagent system. The investigation focused on documenting visible color changes and precipitate formation and assessing the potential applicability of the reaction as a presumptive screening approach in forensic drug analysis.

Materials and Reagents: A mephedrone reference standard was used as the test material. Methanol was used as the sample solvent. The Chen-Kao reagent components consisted of acetic acid (Reagent A), copper(II) sulfate solution (Reagent B), and sodium hydroxide solution (Reagent C). The source, purity, batch number, and preparation details of the reference standard and reagents should be documented to ensure analytical traceability.



MMC Standard



CHEN - KAO REAGENT

Apparatus and Laboratory Equipment: The laboratory apparatus included an analytical balance, beakers, measuring cylinders, droppers, a porcelain dish, and a spot plate. A digital camera was used to record the initial appearance of the reference material and any visible changes during the examination. Laboratory measurements and observations were documented in a standardized recording sheet.

Sample Preparation and Preliminary Examination: The reference standard was examined visually before analysis, and its physical appearance was documented. A 2 mg portion was weighed and dissolved in 2 mL of methanol, giving a nominal sample concentration of 1 mg/mL. The prepared solution was assessed for visible homogeneity, and any undissolved material was recorded. Direct smelling of the sample was avoided for safety reasons.

Colorimetric Examination: The prepared reference solution was examined using the Chen-Kao reagent components under appropriate laboratory safety conditions. The sequence of reagent application, reagent concentrations, volumes, and experimental conditions should follow a verified protocol applicable to the

analyte. The initial appearance and subsequent visual changes were recorded, including any change in color, color intensity, or precipitate formation. Observations were documented during the predefined 0–60-second interval. Photographs were obtained under consistent lighting conditions, where feasible, to support the visual record. Reagent blanks and appropriate positive and negative controls were required to assess background reactions and determine whether the observed response could reasonably be attributed to the sample.

Data Recording and Analysis: The results were recorded descriptively according to the initial appearance, observed color response, precipitate formation, and time of observation. As the investigation was qualitative, the observations were intended for descriptive interpretation rather than statistical inference. Any apparent reaction was treated as presumptive evidence only and not as definitive identification of mephedrone.

Quality Assurance and Safety: All laboratory work was conducted using appropriate personal protective equipment, including a laboratory coat, nitrile gloves, and protective goggles. Sample and reagent handling was performed in a suitable fume hood in accordance with institutional safety procedures. Chemical waste was managed according to applicable laboratory guidelines.

RESULTS

The standard mephedrone sample was examined for its physical characteristics, including colour, texture, odour, solubility, and physical appearance. The observations recorded during the preliminary examination are presented in Table 1.

Table 1. Preliminary physical examination of the standard mephedrone sample

Parameter	Observation
Colour	White or off-white to brown
Texture	Crystalline
Odour	No odour or a slight chemical-like smell
Solubility	Readily soluble in water and ethanol (polar solvents)
Physical appearance	Powder form

Colourimetric Reaction Between Chen–Kao Reagent and the Mephedrone Standard Sample: The colourimetric reaction between the Chen–Kao reagent and the standard mephedrone sample was examined using the reagent components described in the experimental procedure. The observed colour changes were recorded during the reaction.

Table 2. Reagent composition and observed colour changes in the Chen–Kao test

Component	Description
Test reagent	Chen–Kao reagent
Reagent A	Acetic acid (1% v/v aqueous solution)
Reagent B	Copper sulphate solution (1%)
Reagent C	Sodium hydroxide (NaOH) solution (approximately 2 N)
Initial colour	Blue
Intermediate colour	Blue-green
Final observation	Orange-brown precipitate in the centre of the spot plate

The recorded colour sequence was blue → blue-green → orange-brown precipitate. The final observation consisted of an orange-brown precipitate at the centre of the spot plate.



Figure 1. Colourimetric reaction between the Chen–Kao reagent and the standard mephedrone sample.

Observed colour sequence illustrated in the figure 1: blue → blue-green → orange-brown precipitate in the centre of the spot plate.



Figure 2. Observed colour sequence

DISCUSSION

The present experiment evaluated the colourimetric reaction between the standard mephedrone sample and the Chen–Kao reagent for its potential application in preliminary forensic drug screening. The preliminary examination recorded the sample as white or off-white to brown, crystalline in texture, with no odour or a slight chemical-like smell, and in powder form. The sample was reported to be readily soluble in water and ethanol. During the colourimetric examination, the observed colour sequence was blue, followed by blue-green, and finally an orange-brown precipitate in the centre of the spot plate. Mephedrone is a synthetic cathinone containing a β -keto group, a para-methyl-substituted benzene ring, and an N-methylamino group.

The observed colour change indicates a visible reaction between the sample and the reagent system under the experimental conditions. The Chen–Kao reaction may be useful in the presumptive screening of certain cathinones and in differentiating them from other substances during preliminary forensic analysis. However, the reaction is not specific to mephedrone, and the observed colour may be influenced by impurities, adulterants, reagent conditions, and subjective interpretation. Therefore, the results should be considered preliminary rather than conclusive. Further confirmation using validated instrumental techniques, such as gas chromatography–mass spectrometry (GC–MS) or liquid chromatography–mass spectrometry (LC–MS), is necessary to establish the chemical identity of the sample reliably.

CONCLUSION

The Chen–Kao test produced a colour change from blue to blue-green, followed by an orange-brown precipitate in the centre of the spot plate when tested with the standard mephedrone sample. The reaction may be useful for preliminary forensic screening; however, confirmatory instrumental analysis is essential for reliable identification due to its limited specificity.

DECLARATIONS

Ethical Approval: Not applicable, as the study involved an experimental examination of a standard reference sample and did not involve human participants or animals.

Funding: No external funding was received for this study.

Conflict of Interest: The authors declare no conflict of interest.

Author Contributions: The authors contributed to the experimental work, data interpretation, literature review, and preparation of the manuscript.

Data Availability: The observations recorded during the experiment are presented in this manuscript. Additional information may be obtained from the corresponding author upon reasonable request.

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