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Determination of (*S*)(−)-cathinone by spectrophotometric detection

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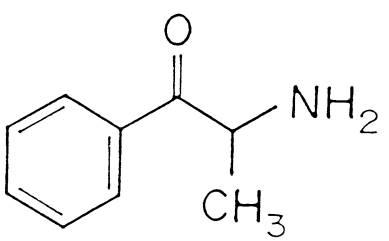
Abstract

Previous studies on the Khat plant (*Catha edulis*, Celastraceae) illustrated the importance of using freshly harvested young shoots and leaves such that cathinone, the principle active component and Schedule I controlled drug contained within the plant, could be suitably isolated and identified. The purpose of this work was to develop a quantitative analytical technique for the determination of cathinone. The proposed method is based on treating the reductant cathinone with copper(II)–neocuproine reagent in sodium acetate-buffered medium followed by measuring the absorbance of the copper(I)–neocuproine complex at 455 nm. The calibration plot is linear in the range 0.08–25 μg ml^{-1} with a detection limit of 0.08 μg ml^{-1}. The precision of the method, expressed as the relative standard deviation, is 1.35% for 10 μg ml^{-1} cathinone. Good recoveries have been obtained in applying the method to the analysis of cathinone in Khat leaves.

Keywords: Spectrophotometry; Cathinone; Neocuproine; Khat leaves

1. Introduction

The chewing of khat, which typically consists of the young leaves and stems of the *Catha edulis* plant, for its stimulant effects has in the past been largely confined to its area of cultivation, namely, East Africa and the Arab Peninsula. The phenylalkylamine alkaloid (−)-cathinone (α-aminopropiophenone) (**I**), which has been isolated from the plant, is thought to be its active principle since this compound not only produces behavioural effects that are analogous to those of amphetamine, but is also of a similar potency [1–4].



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It has been found that during the maturation of the leaves or decomposition of the plant through drying and storage, cathinone contained within the plant is enzymatically converted to cathine, a compound which historically was erroneously identified to be the active substance in khat which was responsible for the observed pharmacological effects. Therefore, cathinone was placed into Schedule I of the controlled substances Act in the United States [1,5,6], and khat is Schedule I substance when cathinone is present [7].

Fresh leaves are traditionally chewed for its stimulating effects as the cut khat loses its potency about 3–4 days after harvesting [8]. Despite the short period of drug effectiveness, the availability of rapid air freight service has resulted in the appearance of khat in non-origin countries, for example, United States, Italy, Great Britain, and Switzerland.

Several studies performed on khat such as phytochemical [8,9] and animal experiments [10,11] to show that the phenylalkylamine (*S*)(−)-cathinone is the main psychoactive alkaloid of khat. Other methods, such as gas chromatography [12–14], high performance liquid chromatography [15–17] and NMR studies [18], have been utilized for the chiral resolution of such alkaloid drugs.

In the present work, it is aimed establishing a novel protocol, which is an accurate, sensitive, more convenient and less time-consuming to determine cathinone in seized khat drug which could be readily available at seizure sites. The method is based on treating the reductant cathinone with copper(II)–neocuproine reagent in sodium acetate-buffered medium. Then, the colored complex product is quantified spectrophotometrically at 455 nm.

2. Experimental

2.1. Apparatus

2.1.1. Spectrometer

A Perkin-Elmer Model Lambda 2S UV-visible and an LKB Model 4050 UV-visible spectrophotometers, both with 1.0 cm silica cells were used.

2.1.2. pH Meter

A Metrohm 632 pH-meter with glass-calomel electrodes was used for pH measurements.

2.1.3. Heating apparatus

A water-bath GFL Gesellschaft Labortechnik, type 1D 23 was used.

2.2. Reagents

2.2.1. Cathinone standard solution

A 1000 μg ml^{-1} cathinone solution was prepared by dissolving 0.1000 g of hydrochloride of cathinone in 100 ml of distilled water. Further solutions were prepared by serial dilution.

2.2.2. Neocuproine (2,9-dimethyl-1,10-phenanthroline) solution

A 0.02 M solution was prepared by dissolving 1.0414 g of neocuproine hydrochloride trihydrate in 250 ml of 0.015 M HCl.

2.2.3. Cu(II) solution

A 0.0125 M solution was prepared by dissolving 0.7550 g copper(II) nitrate trihydrate in 250 ml of distilled water. Working solutions were prepared by further dilution.

2.2.4. Acetate buffer

A 2.50 M stock solution was prepared by dissolving 51.2688 g of acetic acid sodium salt, anhydrous in 250 ml of distilled water. Working solutions were prepared by further dilution.

All reagents and solvents of analytical-reagent grade.

2.3. Extraction

Khat leaves were dried at 40°C for ca. 5 h and were desiccated. Then, the dried leaves were cut into small pieces, dimensions of approximately 0.5 cm squares and a representative amount for analysis was weighed and placed in a dry, methanol-washed separatory funnel. Then, the prepared sample was mixed with 25 ml of ethanol and left to stand for 30 min with intermittent shaking.

The green methanolic extract was then filtered, through Whatmann filter paper no. 41, into a

500-ml volumetric flask diluted and completed with distilled water. A representative 1-ml volume was used for the analysis following the established optimized procedure.

2.4. Procedure

To a 50-ml beaker, add 1 ml of 1.25×10^{-2} M of copper(II) solution. An aliquot containing the desired amount of cathinone solution is added to the beaker and mixed gently with the copper(II) solution, left to react approximately 30 s. Then, 1 ml of 0.02 M neocuproine solution was added to the mixture, followed by 10 ml of distilled water. The mixture was heated in a boiling bath for 1 min, and allowed to cool, then add 1.25 ml of 2.5 M sodium acetate buffer solution and heat again in a boiling bath for about 10 min and set aside to cool. Transfer the solution, after cleaning beaker sides with distilled water, to a 25-ml volumetric flask and complete to the mark with distilled water. The absorbance of the yellow solution was measured at 455 nm against a blank prepared without the cathinone. The concentration of the analysed compound was calculated, as usual, from a calibration graph constructed under identical experimental conditions.

3. Results and discussion

3.1. Order of addition of reagents

In order to obtain optimum results, the sequence of addition of reagents should be the same as that mentioned in the procedure, otherwise a loss in colour intensity is observed as can be seen in Fig. 1.

Preliminary investigation of the suitable buffer as a medium for the reaction between the cathinone and the Cu(II)–neocuproine reagent revealed the buffer dependency of the reaction. Therefore, various buffer solutions (0.1 M of the sodium salt) of acetate, borate, carbonate, oxalate and phosphate were prepared. It was noticed that as the buffer solution was added last to the reaction mixture, absorbance was strongly increased as shown in Table 1. Best results were obtained when acetate used, therefore, it was chosen for further work.

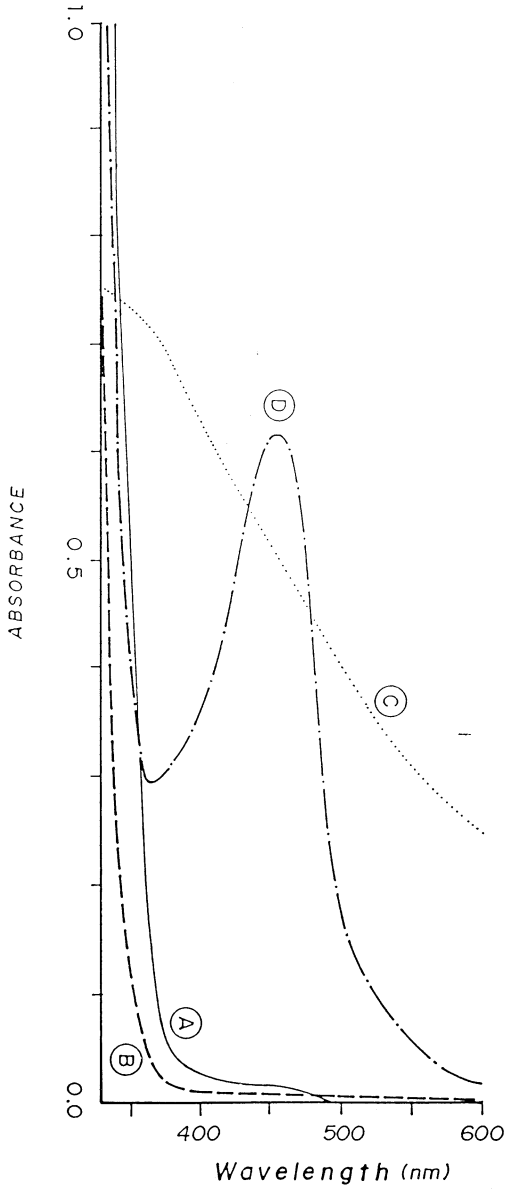

Fig. 1. The effect of order of addition of reagents. A: Cu(II) + cathinone + neocuproine; B: neocuproine + cathinone + acetate; C: Cu(II) + cathinone + acetate; D: Cu(II) + cathinone + neocuproine + acetate.

Table 1
Effect of buffer solutions on the absorbance of the cathinone–copper(II)–neocuproine reaction

Buffer (0.1 M)	Absorbance*	% Enhancement
No buffer	0.010	100
Acetate	0.727	7270
Borate	0.612	6120
Phosphate	0.250	2500
Oxalate	0.461	4610
Carbonate	0.630	6300

* Average of three determinations.

3.2. Concentration of neocuproine and copper ion

Colour developed strongly as the amount of neocuproine in the reaction mixture was increased to 8×10^{-4} M. The increase in sensitivity that occurred at higher neocuproine concentrations was inadequate to justify additional neocuproine in the reaction mixture. Neocuproine solutions were found to be stable for many weeks at room temperature. A solution of 8×10^{-4} M of neocuproine was used as the copper(I) ion chelating agent in the preparation of the colour reagent.

Absorbance values obtained using 10 μg ml^{-1} of cathinone were constant as the amount of copper ion was increased from 1×10^{-5} M to 5×10^{-3} M. This indicates an excess of the oxidation catalyst.

3.3. Effect of temperature

The reaction of cathinone with copper(II)–neocuproine reagent in acetate buffer to form a distinct yellow colour was carried out at elevated temperature using a boiling water-bath. Higher temperatures allowed the absorbance to develop more rapidly. At 100°C, which was selected for the procedure, a maximum and constant intensity of absorbance from cathinone was achieved by heating for 6–10 min (Fig. 2): heating for 10 min was adopted in the procedure.

3.4. Effect of surfactants

It is well established that the local microenvironment in a micellar aggregate is dramatically different from that in the homogeneous aqueous bulk solution. Therefore, growing interest is paid to the use of micelles and other organized media in chemical analysis.

So, various types of surfactants micelles were examined as potential enhancers for the reaction of copper(II)–neocuproine with cathinone in acetate buffer. Three surfactants of the three main types of surfactants were studied: CTABr (a cationic surfactant), SLS (an anionic surfactant), and Brij-35 (a nonionic surfactant). It was observed that none of the surfactants tested had any effect on the measured absorbance.

3.5. Analytical application

A study of the analytical application of the spectrophotometric procedure was carried out to establish the application range, reproducibility and detection limit.

Using the optimized reaction parameter, the absorbance was proportional to the amount of cathinone within the range 0.075–25 μg ml^{-1} with a correlation coefficient of 0.9998, a slope of 0.0726 and an intercept of 8.414×10^{-3}. Above this level there was a regular negative deviation from Beer's law as the cathinone level increased to 50 μg. Most analyses were performed on solutions containing 10 μg ml^{-1} of cathinone. The detec-

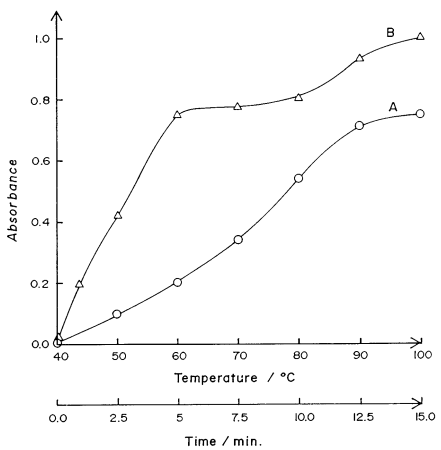

Fig. 2. Effect of (A) temperature and (B) heating time on the reaction of cathinone with copper (II)–neocuproine reagent in acetate buffer.

Table 2
Results obtained from using the standard addition method for cathinone determination

Cathinone (μg ml^{-1})	Absorbance*	
	A	B
4.0	0.309	0.771
8.0	0.596	1.061
12.0	0.890	1.330
16.0	1.177	1.608

* Average of four determinations.
A, pure cathinone.
B, mixed with khat leaves extract.

tion limit of the method for a signal-to-noise ratio of 3 is 0.075 μg ml^{-1}. The reproducibility of the method is satisfactory with the relative standard deviation of 1.4% for 10 μg ml^{-1} (based on ten repeated determinations). This level of precision is adequate for quality control purposes as might be required for the analysis of pharmaceutical preparations.

3.6. Determination of cathinone in Khat leaves

The proposed method according to the above-mentioned procedure was evaluated by determining cathinone in a freshly cut Khat leaves (Khat leaves were obtained with gratitude from the Forensic Laboratories, King Fahed Security College). Standard addition and direct calibration methods were used for the recovery study on a freshly cut Khat leaves.

In the standard addition method, standards of 0.0, 4.0, 8.0, 12.0 and 16.0 μg ml^{-1} were prepared from the stock solution (i.e. 500 μg ml^{-1}) in 25 ml solution. A similar set of standards was also prepared, which contained 20.6 ml of the khat leaves extract containing ca. 6.66 μg ml^{-1}. The results obtained are shown in Table 2. The standard addition graph was parallel to the normal linear calibration graph, and gave a value of 6.55 μg ml^{-1} of the cathinone in the khat leaves extract. This is amounted to ca. 98.3% recovery for the drug sample.

In the direct calibration method, a stock solution 500 μg ml^{-1} of the authentic cathinone was prepared by dissolving 0.05 g of the sample in 100 ml of deionized water. Then standards of 2.5, 5.0, 7.5 and 10.0 μg ml^{-1} solutions were prepared from the stock solution. From the freshly cut khat leaves a solution for cathinone was prepared by extraction 25 ml, containing ca. 8 μg ml^{-1}, to give a solution whose absorbance was measured. The results can be seen in Table 3. Fig. 3 shows that the concentration obtained from this graph is ca. 8.2 μg ml^{-1}, compared to 8.0 μg ml^{-1} cathinone in the extracted solution. This is amounted to ca. 102.5% recovery for the drug sample from the freshly cut khat leaves.

Table 3
Results obtained from using direct calibration method for cathinone determination

Cathinone (μg ml^{-1})	Absorbance*
2.5	0.20
5.0	0.37
7.5	0.52
10.0	0.71
Khat leave extract	0.56

* Average of four determinations.

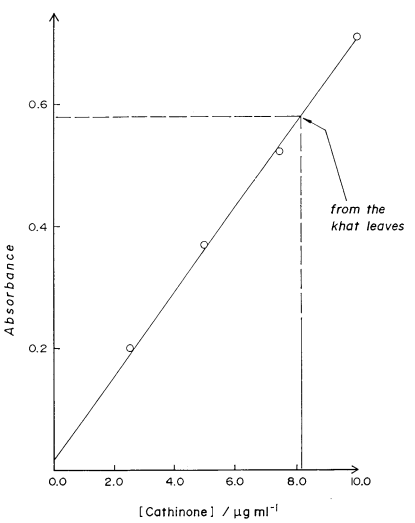

Fig. 3. Direct calibration method for the determination of cathinone in Khat leaves.

4. Conclusion

The salient features of the proposed procedure using neocuproine for the determination of cathinone are simplicity and excellent precision and sensitivity. The rate of development and stability of the colour make the procedure ideal for handling large numbers of samples.

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