KINETIC DETERMINATION OF CAFFEINE IN DRUG FORMULATIONS

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الخلاصـــة :

وصفت في هذا البحث طريقتين حركيتين جديدتين للتحديد الكمي للكافيين أولهما طريقة الزمن الثابت والأخرى طريقة التركيز الثابت وفي الطريقتين استُـخـدِم (٠,٠٠٣٢٠) مولار من محلول السيريوم الرباعي في (٠,٠٠٨٠) مولار حمض الكبريتيك . هذا وقد تتبع معدل التفاعل عند طول موجة (٤٠٥) نانوميتر عند الزمن الثابت (٣٥٠) ثانية في الطريقة الأولى مستخدمين المعادلة التالية :

$$A = 2.31 - 0.00355\ C$$

أما في الطريقة الثانية فقد قِيس الزمن عند امتصاص ثابت مقداره (١٫٧٠) مستخدمين المعادلة التالية :

$$1/t = -0.00125 + 0.0000248\ C$$

وقد أُجريت مقارنة إحصائية بين الطريقتين مع الطريقة القياسية البريطانية وذلك لتركيز الكافيين في المستحضرات الطبية .

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ABSTRACT

Two simple and accurate kinetic methods, the fixed time and the fixed concentration methods, for the determination of caffeine were described. The two methods involve the use of 3.20×10^{-3} M cerium(IV) solution and 8.0×10^{-2} M sulfuric acid. Reaction rates were followed at 405 nm; absorbance measurements for the fixed time method were taken at 350 s; and the following calibration equation was used for calculating unknown concentrations of caffeine:

$$A = 2.31 - 3.55 \times 10^{-3} C$$

For the fixed concentration method, time was measured at a fixed absorbance of 1.70 and the following calibration equation was used:

$$1/t = -1.25 \times 10^{-3} + 2.48 \times 10^{-5} C$$

The two methods were applied to the determination of caffeine in proprietary drugs, interferences were studied, and a statistical comparison with the results obtained by the official BP method was made.

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INTRODUCTION

Caffeine is 1,3,7-trimethylpurine-2,6 (3*H*, 1*H*)-dione [1]. It is an alkaloid commonly known for its stimulant and analeptic effect on the spinal centres and cerebral cortex. It is also anticonvulsive and angiospasmodic. It produces a peripheral effect on the heart and the blood vessels. Caffeine is usually formulated in tablets prescribed for influenza, migraine attacks, nervous tension, and allergic gastroenteritis. Numerous methods for the determination of caffeine in tablets have been reported. Potentiometric titration methods [2–7] were reviewed by Kucharsky and Safarik [8], and all were carried out in nonaqueous solvents such as acetic anhydride and anhydrous acetic acid. Chromatographic methods [9–13] are lengthy and require tedious extraction procedures. Spectrophotometric methods [14–20] are nonspecific. The Standard BP method [20] for the determination of caffeine in tablets is also nonspecific. It requires extraction of caffeine in chloroform, dissolving it in water, and measuring absorbance at 273 nm. The method described in this paper is dependent upon measurement of the rate of oxidation of caffeine by cerium(IV) in sulfuric acid media.

EXPERIMENTAL

Apparatus

A Cary Model 2300 UV-V-NIR spectrophotometer connected to a Varian Model S-15 data station and Epson LX-86 printer was used for all absorbance measurements. Matched sets of 10.00 mm cells were used throughout.

Reagents and Samples

Highly purified distilled water was used for all preparations. All chemicals and reagents were of analytical reagent or pharmaceutical grade.

Caffeine (Analytical Sample)

A solution of 1 mg ml^{-1} was prepared by dissolving 500 mg in about 400 ml water, heated in a water bath thermostatted at 70 ± 2°C for 10 min, stirred for 5 min and diluted to volume in a 500 ml calibrated flask after cooling.

Caffeine (in Tablets)

A solution of 1 mg ml^{-1} was prepared by weighing 10 tablets, crushed them into fine powder and dissolving an exact mass of powder containing about 500 mg of caffeine, mixed with about 300 ml water, heated in a water bath thermostatted at 70 ± 2°C for 10 min, stirred for 5 min and filtered through an ordinary filter paper No. 4, and washed with hot water. The filtrate plus washings were diluted to 500 ml in a calibrated flask after cooling.

Cerium(IV) Stock Solution

0.020 M Ce(IV) solution was prepared in 0.50 M sulfuric acid.

Recommended Procedure

The following procedures should be followed for the two kinetic methods at 25°C.

Method I: The Fixed Time Method

4.00 ml of Ce(IV) stock solution was prepared in a 25 ml calibrated flask into which the appropriate amount of caffeine solution was added. The solution was diluted to the mark with water, and swirled gently while the stop watch was turned on. Some of this solution was transferred into the photocell and the decrease of absorbance of cerium(IV) solution was followed at 405 nm against a water blank (cerium(IV) solution could not be used as a blank to avoid negative absorbance readings). The absorbance at the fixed time of 350 s was measured and the unknown concentration of caffeine could be calculated from the corresponding calibration equation (Equation 4).

Method II: The Fixed Concentration Method

4.00 ml of Ce(IV) stock solution was prepared in a 25 ml calibrated flask into which the appropriate amount of caffeine solution was added. The solution was diluted to the mark with water, and swirled gently while the stop watch was turned on. Some of this solution was transferred into the photocell and the decrease of absorbance of cerium(IV) solution was followed at 405 nm against a water blank (cerium(IV) solution could not be used as a blank to avoid negative absorbance readings). The exact time was measured and recorded when the absorbance

value reaches 1.70, and hence the unknown concentration of caffeine could be calculated from the corresponding calibration equation (Equation 5).

RESULTS AND DISCUSSION

Optimization

The method is based on the slow oxidation of caffeine with Ce(IV) and on following the rate of the reaction by measuring the depletion of Ce(IV) absorbance at 405 nm. Optimum conditions for the reaction were reached by studying the reaction rate at different concentrations of cerium(IV), caffeine, and sulfuric acid. The low value of the extinction coefficient of cerium(IV) solution, ($750\ l\ mol^{-1}\ cm^{-1}$), limits its highest concentration to 3.20×10^{-3} M. The reaction rate seemed to decrease with increasing sulfuric acid concentration and accelerate with decreasing acid concentration. Sulfuric acid in the range of 0.03 to 0.15 M was found to be adequate. Above 0.15 M the rate was too slow to follow and below 0.03 M cerium(IV) sulfate precipitate was formed. Reaction rate is also dependent upon the caffeine concentration; it accelerates as the caffeine concentration increases and slows down as it decreases. The rate was found to be reasonable in the range 120–450 $\mu g\ ml^{-1}$ of caffeine concentration.

Kinetic Methods [21, 22]

For analysis of caffeine in the range 120–450 $\mu g\ ml^{-1}$ taking a constant concentration of 3.2×10^{-3} M cerium(IV) and 0.08 M sulfuric acid, the following equation is applied:

$$\text{Rate} = k'[\text{caffeine}]^n , \qquad (1)$$

where k' is a pseudo n-th order rate constant and n is the order of the reaction with respect to caffeine, which was found to be unity. The equation indicates that the reaction under constant and high concentrations of cerium(IV) and sulfuric acid relative to caffeine is dependent only upon the caffeine concentration. Therefore, it is expected that rate is different for each different analyte concentration. By taking rate as $\Delta A/\Delta t$ and using Equation (1), therefore, at a fixed time the following equation applies:

$$\Delta A = k''[\text{caffeine}]_o , \qquad (2)$$

indicating that absorbance at a fixed time is directly proportional to the initial concentration of caffeine (referred to as Method I). Again by using Equation (1) and at a fixed absorbance, the following equation applies:

$$1/t = k'''[\text{caffeine}]_o , \qquad (3)$$

that is reciprocal of the time for a fixed absorbance is directly proportional to the initial concentration of caffeine (referred to later as Method II). So, absorbance *versus* time curves were drawn taking 140, 160, 219, 259, 319, and 399 $\mu g\ ml^{-1}$ of caffeine keeping cerium(IV) and sulfuric acid concentrations constant each time at 3.20×10^{-3} M and 0.08 M respectively to manifest the basis of the two methods from which calibration equations were obtained (a typical example is represented by Figure 1). Using Figure 1 and applying Equations (2) and (3), Methods I and II were applied as follows.

Method I: The Fixed Time Method

The fixed times, 150, 200, 250, 300, 350, and 400 seconds were preselected, and their corresponding intersections at the absorbance ordinate for each reaction rate drawn for the different concentrations of caffeine are recorded in Table 1. Regression

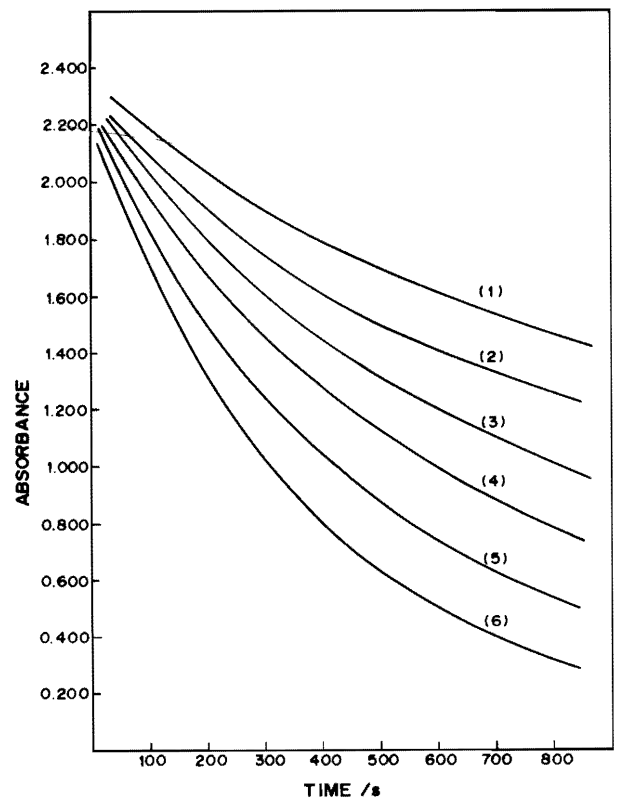

Figure 1. Absorbance versus *Time Curves for the Reaction of 3.20×10^{-3} M Ce(IV) Solution with Different Initial Concentrations of Caffeine in $\mu g\ ml^{-1}$ as Follows: (1) 140; (2) 160; (3) 219; (4) 259; (5) 319; (6) 399; in all 0.08 M H_2SO_4 at 25°C.*

equations were included with their values of correlations coefficients r indicating good linearity of absorbance *versus* initial concentrations of caffeine. On the basis of intercept and r, the fixed time 350 seconds was found to be more appropriate and therefore chosen as the fixed time for measurements by this method; hence the corresponding calibration equation of:

$$A = 2.31 - 0.00355\ C\ , \qquad (4)$$

was used for calculating unknown concentrations of caffeine.

Method II: The Fixed Concentration Method

1.50, 1.60, 1.70, and 1.80 were preselected as fixed absorbances, and their corresponding intersections at the time abscissa for each reaction rate drawn for the different concentrations of caffeine were measured and are reported in Table 2. Regression of reciprocal of time *versus* initial concentration of caffeine are given in the same table, with (r) values indicating good linearity for all. The following calibration equation:

$$1/t = -0.00125 + 0.0000248\ C\ , \qquad (5)$$

corresponding to the fixed absorbance of 1.70 was chosen, on basis of (r) as the best straight line that could be used for calculating unknown concentrations of caffeine.

APPLICATIONS AND INTERFERENCES

Both methods were applied to the determination of caffeine in a combination with other drugs in tablet forms. The proprietary drugs investigated such

Table 1. Calibration Equations Obtained by the Fixed Time Method from Absorbance *versus* Time Curves as in Figure 1.

Caffeine $\mu g\ ml^{-1}$	Absorbance at fixed times					
	150 s	200 s	250 s	300 s	350 s	400 s
140	2.11	2.04	1.97	1.92	1.86	1.81
160	2.01	1.91	1.84	1.77	1.70	1.63
219	1.93	1.82	1.72	1.63	1.54	1.46
259	1.81	1.69	1.58	1.48	1.38	1.30
319	1.66	1.51	1.37	1.25	1.15	1.04
399	1.52	1.34	1.18	1.02	0.92	0.81
Calibration equation	$A = 2.39 - 2.22\times10^{-3}\ C$	$A = 2.37 - 2.63\times10^{-3}\ C$	$A = 2.36 - 2.99\times10^{-3}\ C$	$A = 2.35 - 3.39\times10^{-3}\ C$	$A = 2.31 - 3.55\times10^{-3}\ C$	$A = 2.28 - 3.77\times10^{-3}\ C$
r	−0.99	−0.99	1.00	−1.00	−0.99	−0.99

Table 2. Calibration Equations Obtained by the Fixed Concentration Method from Absorbance *versus* Time Curves as in Figure 1.

Caffeine $\mu g\ ml^{-1}$	$1/t$ / (s^{-1}) at fixed absorbances (A)			
	$A = 1.50$	$A = 1.60$	$A = 1.70$	$A = 1.80$
140	0.00132	0.00160	0.00200	0.00250
160	0.00200	0.00241	0.00294	0.00370
219	0.00270	0.00323	0.00392	0.00488
259	0.00351	0.00426	0.00521	0.00667
319	0.00488	0.00578	0.00714	0.00909
399	0.00645	0.00769	0.00833	0.0115
Calibration equation	$1/t = -1.36\times10^{-3} + 1.94\times10^{-5}\ C$	$1/t = -1.56\times10^{-3} + 2.30\times10^{-5}\ C$	$1/t = -1.25\times10^{-3} + 2.48\times10^{-5}\ C$	$1/t = -2.23\times10^{-3} + 3.46\times10^{-5}\ C$
r	1.00	1.00	0.99	1.00

Table 3. Statistical Comparison of the Results of Analysis of the Proprietary Drugs Obtained by the Fixed Time Method (Method I) and the Fixed Concentration Method (Method II) with those Obtained by the Official BP Method (Method III).

Drug	Recovery ± sd*			t**
	Method I	Method II	Method III	
Systral	99.78 ± 0.35	99.98 ± 0.21	99.88 ± 0.25	0.64 1.1
Vandid–Caffein	99.96 ± 0.28	99.79 ± 0.40	99.8 ± 0.32	1.3 0.06
Cafergot	100.05 ± 0.41	99.86 ± 0.60	100.22 ± 0.41	0.93 1.3
Prontopyrin	510.2	508.5	40.8	

* Average of 5 determinations

** Theoretical value = 2.78 (p = 0.05), the second value obtained when Method II was compared with BP.

as Systral (Astra–Werke) containing 50 mg caffeine and 20 mg chlorphenoxamine HCl; Vandid–coffein (Chemie Linz AG); containing 50 mg caffeine and 20 mg vanilic acid diethylamine; Cafergot (Sandoz Ltd.), containing 100 mg caffeine and 1 mg ergotamine tartrate; and Prontopyrin (Heinrich Mack), containing 50 mg caffeine, 200 paracetamol, and 250 mg aluminum acetylsalicylate, were randomly collected from local dispensaries. Results of analysis of caffeine in these drugs are to be found in Table 3 together with the results obtained by the BP method [20]. The results obtained are very satisfactory and show the precision of the two methods. Excipients such as starch, glucose, and lactose did not show any interference with the methods and have no effect on caffeine determination by the two methods. Moreover, the presence of other drug components such as: chlorphenoxamine HCl, which is an antihistaminic and antiallergic; vanillic acid diethylamide, which is an analeptic; ergotamine tartarate, which is specific for migraine attacks; and paracetamol which is an analgesic, also showed no interferences. This conclusion was supported by the precision calculated as percent recovery and by the accuracy calculated as the Student t-test values when the method was compared with the official BP method as in Table 3. However, the presence of acetylsalicylate in Prontopyrin proprietary drug formulation together with the caffeine showed significant interference, giving high results. This was confirmed by observing the absorbance change of cerium(IV) under the conditions of the reaction, thus indicating fast reaction of the former with the latter, which agrees with previous work by the first author [23].

A statistical comparison of the results obtained by the two methods with that obtained by the BP method for the same batch of samples and from the Student t-test values, which did not exceed the theoretical value at the 95% confidence level, indicates no significant difference and high accuracy for the two methods. Our method seems to be very simple and highly specific compared with the official BP method and other previous methods.

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Paper Received 18 May 1991; Revised 28 December 1991.