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Sensitive and selective methods for determination of antipsychotic drug olanzapine in pharmaceuticals

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Abstract Two simple and sensitive spectrophotometric methods have been developed for analysis of the antipsychotic drug olanzapine in pharmaceuticals. Method A is based on liberation of iodine by reaction between the drug and potassium iodate, followed by reaction with leuco crystal violet (LCV), the color of oxidized LCV being measured at 598 nm. Method B is based on oxidation of olanzapine with chloramine-T (CAT) in acidic medium, the unconsumed CAT being determined with rhodamine B, measuring the absorbance at 550 nm. Calibration graphs were linear over the ranges of 0.05–2.0 and 0.1–1.6 μg mL^{-1} olanzapine for method A and B, respectively. The molar absorptivity, Sandell's sensitivity, detection limit, and quantitation limit were found to be 1.59×10^5, 0.00132, 0.038, and 0.117, respectively, for method A and 0.953×10^5, 0.00221, 0.064, and 0.192, respectively, for method B. The optimum conditions and other analytical parameters were evaluated. The proposed methods have been applied successfully for analysis of olanzapine in pure form and its dosage forms, and no interference was observed from common excipients present in pharmaceutical formulations.

Keywords Olanzapine · Chloramine-T · Rhodamine B · Leuco crystal violet

Introduction

Olanzapine, chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine, is a typical antipsychotic agent, also known as a second-generation antipsychotic. It belongs to the thienobenzodiazepine class and

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has been widely used in treatment of schizophrenia and other psychotic syndromes [1]. Since its introduction in 1996 in over 84 countries, several workers have reported high-performance liquid chromatography (HPLC) methods for determination of olanzapine in plasma, serum, human breast milk, and rat brain [2–12]. HPLC has also been used for assay of olanzapine in pharmaceutical formulations when present either alone [13, 14] or in combination with fluoxetine [15, 16]. Various other techniques including high-performance thin-layer chromatography (HPTLC) [16], nonaqueous titrimetry and ultraviolet (UV) spectrophotometry [17], derivative spectrometry [13], capillary zone electrophoresis, and linear voltammetry [13] have also been reported for assay of olanzapine in pharmaceuticals.

There are few reports on use of visible spectrophotometry in the assay of olanzapine [18–26]. Jasinka and Nalewajko [18] developed one indirect and two direct flow-injection spectrophotometric methods using hexacyanoferrate(III) and cerium(IV) sulfate as reagents. Recently, *N*-bromosuccinimide (NBS) and cerium sulfate have been suggested as oxidimetric reagents for determination of olanzapine by direct and indirect methods in conjunction with Celestine blue [19]. Mohamed [20], very recently, reported two kinetic spectrophotometric methods for determination of olanzapine in its dosage forms and spiked serum samples. However, the reported methods suffer from disadvantages such as poor sensitivity, low stability of colored products, complicated experimental setup, or meticulous control of experimental variables (Table 1). Basavaiah et al. [23] used potassium iodate for indirect determination of olanzapine. Basavaiah et al. studied the forced degradation products of olanzapine [27].

The present investigation aims to develop more sensitive and cost-effective methods for determination of olanzapine in pure form, pharmaceutical formulations, and spiked human serum samples. The methods employ potassium iodate and chloramine-T (CAT) as oxidizing agents, and leuco crystal violet (LCV) and rhodamine B as auxiliary reagents. The proposed methods have been demonstrated to be superior to reported methods with respect to speed, simplicity, sensitivity, and cost-effectiveness.

Experimental

Apparatus

A Varian Carry 50 Bio UV spectrophotometer with 1-cm matched quartz cell was used for all absorbance measurements, and a Systronics type 331 pH meter was employed for the pH measurements.

Reagents and solutions

All chemicals used were of analytical reagent grade, and double-distilled water was used throughout the experiments.

Standard drug solution

Pharmaceutical-grade olanzapine certified to be 99.85 % pure was procured from Cipla India Ltd., Mumbai, India and used as received. A 1,000 μg mL^{-1} standard solution of olanzapine was prepared by dissolving accurately weighed 100 mg pure drug in 0.1 M solution of H_2SO_4 and diluting up to 100 mL with the same acid. The stock solution was diluted stepwise with 0.1 M H_2SO_4 solution to obtain working concentrations.

LCV (Eastman Kodak Co): 250 mg LCV was dissolved in 200 mL distilled water containing 3 mL 85 % phosphoric acid (Merck), and the volume was made up to 1 L with distilled water for storage in amber-colored bottle away from sunlight.
Potassium iodate solution: 5×10^{-2} M aqueous solution was prepared in double-distilled water.
CAT solution: 2×10^{-3} M aqueous solution was prepared.
Rhodamine B solution: aqueous solution of 0.02 % was prepared in double-distilled water.
Hydrochloric acid solution: 1 M aqueous solution of hydrochloric acid.
Sodium hydroxide: 0.1 M aqueous solution of sodium hydroxide.

Procedure

Preparation of calibration curve

Method A Different aliquots containing 0.05–2.0 μg olanzapine were accurately measured and transferred into a series of 25-mL standard flasks, and the volume was adjusted to 5.0 mL by adding distilled water. To each flask, 1 mL 1 M HCl, 1 mL LCV, and 1 mL potassium iodate were added. The content was mixed well, and flasks were let stand for 10 min with occasional shaking, after which the pH of each mixture was adjusted with sodium hydroxide solution. The volume was diluted to the mark with water and mixed well, and absorbance was measured at 598 nm against reagent blank.

Method B Varying aliquots of standard olanzapine solution containing 0.1–1.6 μg olanzapine were accurately measured and transferred into a series of 25-mL calibrated flasks, and the total volume was adjusted to 5.0 mL with distilled water. To each flask, 1 mL 2 M HCl and 0.5 mL CAT were added. The content was mixed well, and flasks were let stand for 10 min with occasional shaking, after which 1 mL rhodamine B was added to each flask, the volume was adjusted to the mark with distilled water, the contents was mixed well, and the absorbance was measured against a reagent blank at 550 nm after 5 min.

Procedure for the dosage forms

Twenty tablets each containing 10 or 20 mg olanzapine were weighed accurately and ground to fine powder. An amount of powder equivalent to 100 mg olanzapine was accurately weighed into a 100-mL volumetric flask, 60 mL 0.1 M H_2SO_4 was

added, and the contents were shaken thoroughly for about 15 min. The volume was adjusted to the mark with 0.1 M H_2SO_4, mixed well, and filtered. The first 10-mL portion of the filtrate was rejected, and a convenient aliquot of filtrate was taken for analysis following the procedures described earlier.

Results and discussion

Olanzapine is reported to be rapidly oxidized to its sulfoxide via a red-colored radical cation [28]. This observation has been used for direct as well as indirect assay of olanzapine using KIO_3 and CAT, respectively.

Acidic aqueous solution of olanzapine has no absorption maxima in the visible range of the spectrum. The mechanism of olanzapine oxidation was fixed from UV–Vis spectra of the unoxidized form of the drug and its oxidized form directly and within 15 min after addition of KIO_3 and CAT. Unoxidized olanzapine exhibits one characteristic absorption band at 260 nm; however, its oxidized form directly after addition of oxidants (KIO_3 and CAT) exhibits three absorption bands in the UV region, at 225, 270, and 340 nm, and two absorption bands in the visible region, at 425 and 532 nm. In the absorption spectrum recorded within 15 min after addition of oxidants, the band in the visible region disappeared. In the UV region, a hypsochromic shift of 5 nm from 225 to 230 nm and hyperchromic shifts at 270 and 340 nm were observed. The presence of these shifts at 230, 270, and 340 nm for oxidized olanzapine is characteristic of sulfones in many pharmaceutical forms (Fig. 1) [29].

Based on the spectral investigations and literature reports [30], it can be concluded that the drug is oxidized with CAT and KIO_3 through S-atom in its thiophene structure in two steps involving two electrons. In the first step the unstable colored radical cation is formed, followed by its reduction to colorless sulfoxide. With the aim of determining the olanzapine S-oxide product, the sensitive color reaction with acetyl chloride in the presence of nitrite ions was used [31]. This

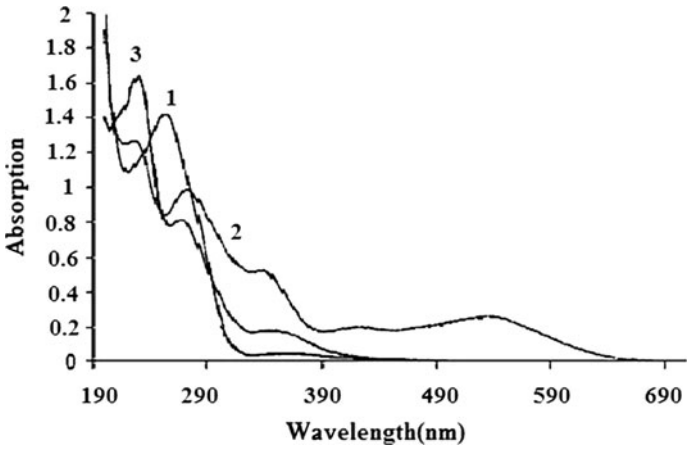


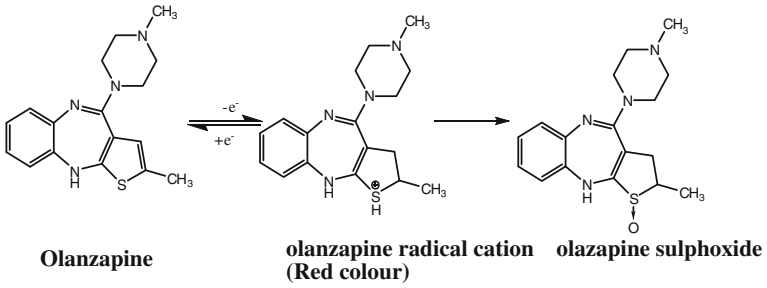
Fig. 1 Absorption spectra of **a** unoxidized olanzapine, **b** its oxidized form directly and within 15 min, and **c** after oxidant addition

reaction is used for quantitative sulfoxide determination. It was found that olanzapine S-oxide reacts with acetyl chloride with the formation of a yellow compound. The sensitivity of this reaction can be increased by using UV light. Therefore, thin-layer chromatography on TLC plates coated with silica gel was performed. The sulfoxide solution of olanzapine spotted on the TLC plate was sprayed with acetyl chloride solution. The yellow spot that appeared was developed under UV light. The mechanism of olanzapine oxidation is shown in Scheme 1.

Oxidized product of olanzapine was unstable. This observation has been used for direct as well as indirect assay of olanzapine using KIO_3 and LCV (method A) and CAT and rhodamine B (method B).

The proposed method A is based on liberation of I_2 by reaction of olanzapine with KIO_3 in acidic medium. The liberated iodine is treated with LCV to form CV dye. The color intensity of the dye is directly proportional to the concentration of olanzapine (Scheme 2).

Method B is based on oxidation of olanzapine with known excess of CAT in hydrochloric acid medium followed by determination of residual oxidant with rhodamine B and measurements of absorbance at 550 nm (Fig. 2). This method makes



Scheme 1 Mechanism of oxidation of olanzapine

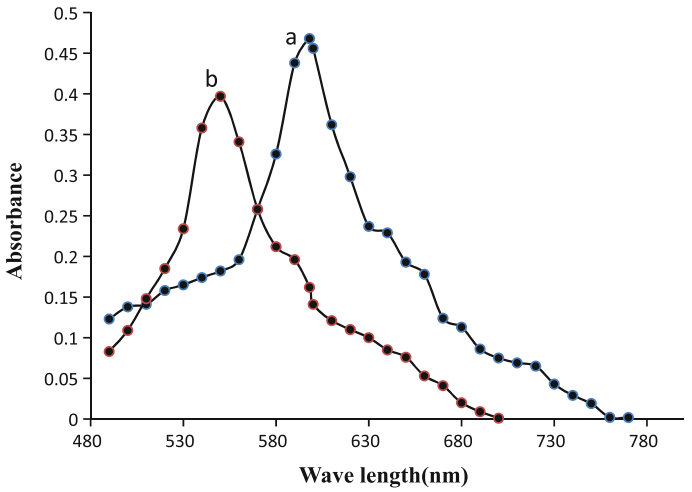
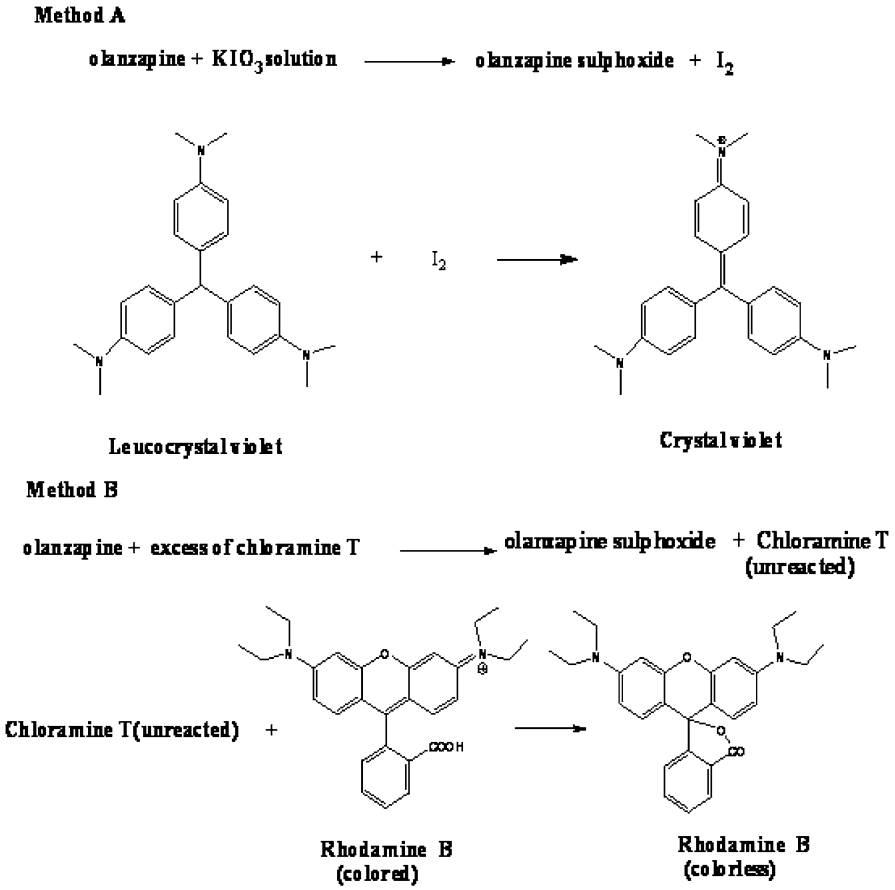


Fig. 2 Absorption maxima for **a** method A and **b** method B



Scheme 2 Color reactions

use of the bleaching action of CAT on rhodamine B, the decolorization being caused by oxidative destruction of the dye. Olanzapine, when added in increasing concentrations to a fixed concentration of CAT, consumes the latter, and there will be a concomitant decrease in concentration of CAT. With addition of a fixed concentration of dye to decreasing concentrations of CAT, a concomitant increase in absorbance is obtained. Consequently, a proportional increase in absorbance at the respective wavelength is observed with increasing concentration of olanzapine (Scheme 2).

Optimization of reaction variables

Effect of reagent concentrations

For method A the influence of iodate and LCV concentrations on the colored reaction was verified. Optimization revealed that 4.5×10^{-2} to 6×10^{-2} M of

KIO_3 gives the maximum and stable absorbance, and the intensity decreases with increasing iodate concentration. Hence, 1 mL 5×10^{-2} M KIO_3 solution was used for studies. The concentration of LCV based on its absorbance showed that the maximum absorbance was achieved after addition of 0.8 mL LCV, remaining constant at higher concentrations; hence, 1 mL LCV was used for further work.

For method B the influence of excess oxidant with respect to olanzapine and rhodamine B and the concentration of rhodamine B on the course of the reaction was checked. Optimization experiments revealed that one- to sixfold excess of oxidant with respect to drug and dye ensured complete oxidation of olanzapine, and also the amount of unreacted CAT was sufficient to oxidize the rhodamine B. Finally, 1 mL 2×10^{-3} M aqueous solution of CAT was used for further investigations. Testing the effect of concentration of rhodamine B on absorbance showed a linear relation between 0.7 and 1.6 mL (0.02 %). Hence, 1 mL was selected for studies.

Effect of time, temperature, and pH

For method A, a reasonable reaction time was 10 min, and a delay of up to 30 min had no effect on the absorbance; the developed color remained stable for several days. For method B, the effect of a waiting period when the dye was added to the mixture of the drug with CAT with respect to the minimum blank, and the reproducibility and stability of the final color, were ascertained. It was found that the rhodamine B could be added 10 min after the CAT was added in the first step.

The oxidation of olanzapine with iodate (method A) and CAT (method B) was studied in the temperature range between 20 and 40 °C. The rate of oxidation decreased slightly with increasing temperature. Therefore, working at room temperature, i.e., 25 °C, was adopted for both procedures.

Various acids such as HNO_3, H_2SO_4, HCl, $HClO_4$, etc. were used; the best results were found in HCl medium for both methods. In method A, pH 2–3 was required for liberation of I_2, giving a blue color with LCV at pH 4–4.5. After pH 5, the solution becomes turbid. The initial pH was maintained by 1 mL 1 M HCl, and later the pH was adjusted up to 4 by using 0.1 M NaOH solution. In method B, 0.8–2 mL 2 M HCl gave the maximum and stable absorbance; hence, 1 mL HCl was used.

Method validation

Absorbance was plotted versus time, and a linear correlation was found. Beer's law was obeyed over the concentration range from 0.05 to 2.0 μg mL^{-1} for method A and from 0.1 to 2.0 μg mL^{-1} for method B (Fig. 3); the molar absorptivity and Sandell's sensitivity are given in Table 1. The limits of detection, limits of quantification, regression equation, and correlation coefficients values are given in Table 1.

The percentage relative error between the measured concentrations and taken concentration for olanzapine was used to evaluate the accuracy. The precision of the method was calculated in terms of intermediate precision (intraday and interday).

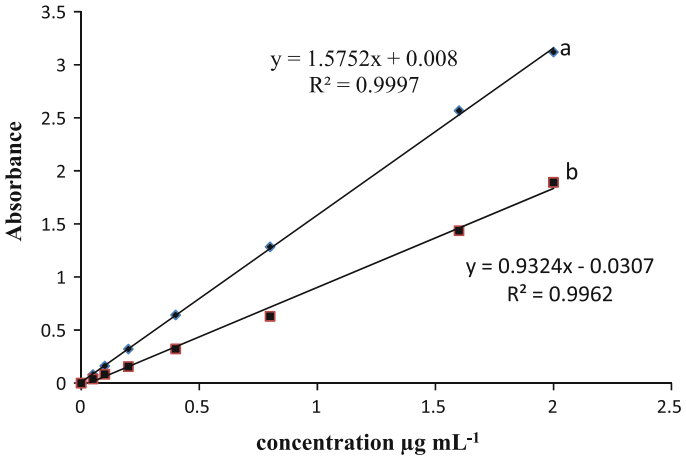


Fig. 3 Calibration graph for **a** method A and **b** method B

Table 1 Analytical and regression parameters for spectrophotometric determination

Parameter	Result ($n = 6$)	
	Method A	Method B
λ_{max} (nm)	598	458
Beer's law limits (µg mL^{-1})	0.05–2.0	0.1–2
Molar absorptivity (L mol^{-1} m^{-1})	5.29×10^5	2.49×10^5
Sandell's sensitivity* (µg cm^{-2})	0.00059	0.00125
Limit of detection (µg mL^{-1})	0.02	0.032
Limit of quantification (µg mL^{-1})	0.057	0.096
Regression equation ($Y = bX^* + a$)		
Slope (b)	1.575	0.936
Standard deviation of slope (S_b)	0.119	0.0839
Intercept (a)	0.008	0.004
Standard deviation of intercept (S_a)	0.018	0.0195
Variance (S_a^2)	3.2×10^{-4}	3.8×10^{-4}
Correlation coefficient	0.998	0.996

* Concentration of analyte

Three different concentrations of olanzapine (within the working limits) were analyzed in seven replicates during the same day and on five consecutive days. The SD and RSD values of intraday and interday studies showed that precision was good (Table 2).

Selectivity

To determine the selectivity of the proposed methods, an analytical placebo was prepared and subjected to analysis by the proposed methods. It was confirmed that

Table 2 Interday and intraday precision and accuracy studies for method A

Olanzapine taken (μg mL^{-1})	Intraday ($n = 7$)			Interday ($n = 5$)		
	Found	Precision[a]	Accuracy[b]	Found	Precision[a]	Accuracy[b]
Method A						
0.5	0.51 ± 021	4.26	1.71	0.52 ± 0.028	5.62	2.8
1	1.02 ± 018	1.82	2.14	1.01 ± 0.015	1.49	1.4
2	2.04 ± 031	1.51	2.14	2.06 ± 0.021	1.018	1.8
Method B						
0.5	0.51 ± 028	5.62	2.57	0.50 ± 0.020	4.09	1.2
1	1.01 ± 033	3.30	1.14	1.02 ± 0.022	2.19	2.0
1.5	1.52 ± 025	1.689	1.15	1.52 ± 0.027	1.77	1.6

[a] Relative standard deviation (%)

[b] Bias %: (found − taken/taken) × 100

the change in absorbance with respect to water blank was caused only by the analyte. To identify the interference by common tablet excipients, a synthetic mixture composed of olanzapine (50 mg), talc (80 mg), starch (160 mg), calcium gluconate (80 mg), lactose (80 mg), sodium alginate (40 mg), and magnesium stearate (40 mg) was prepared and subjected to analysis by the proposed methods after solution preparation using the procedure described for tablets. The percentage recoveries suggested no interference by the excipients in the assay of olanzapine under the described optimum conditions.

Application to formulations

The proposed methods were applied for determination of olanzapine in three representative tablets. The results in Table 3 show that the methods were successful for determination of olanzapine and that the excipients in the dosage forms do not interfere. A statistical comparison of the results by the proposed methods and literature method has been carried out [17]. The literature method consisted of measurement of the methanolic extract of the tablets at 226 nm. The results agreed well with the claimed values and also are in agreement with the results obtained by the literature method. Statistical analysis of the results using Student's t test for accuracy and F-test for precision revealed no significant difference between the proposed method and the literature method at the 95 % confidence level with respect to accuracy and precision (Table 3).

Conclusions

The proposed methods are simple, rapid, cost-effective, and validated. They are more sensitive than many existing methods and are free from experimental processes such as heating or extraction steps. The stability of the color system is an advantage over earlier methods (Table 4). The methods provide sensitivity

Table 3 Results of assay of tablets by the proposed methods and statistical evaluation

Tablet brand name	Nominal amount (mg/tab)	Literature method	Proposed method A	Proposed method B
Oliza	10	9.79 ± 0.040	9.84 ± 0.049	9.83 ± 0.021
			$t = 2.12$	$t = 2.56$
			$F = 1.5$	$F = 3.628$
Oleanz	10	9.81 ± 0.037	9.86 ± 0.075	9.9 ± 0.049
			$t = 1.124$	$t = 2.39$
			$F = 4.09$	$F = 1.753$
Olimit	10	9.88 ± 0.053	9.96 ± 0.08	9.99 ± 0.069
			$t = 1.350$	$t = 2.04$
			$F = 2.28$	$F = 1.69$

Mean value of five determinations. Tabulated *t*-value at the 95 % confidence level is 2.78. Tabulated *F*-value at the 95 % confidence level is 6.39

Table 4 Performance characteristics of the existing spectrophotometric methods and the proposed methods

Reagent(s) used	Methodology	λ_{max} (nm)	Linear range (μg mL^{-1})	LOQ (μg mL^{-1})	Remarks
(a) Hexacyanoferrate(III)	Unreacted oxidant measured	425	2.5–40.0		Reaction requires 1:1
(b) Hexacyanoferrate(III)	Radical cation measured	540	0.5–250		mixture of H_2SO_4 and H_3PO_4, FIA assembly
(c) Cerium(IV) sulfate [18]		540	0.05–300		required
(a) NBS	Radical cation measured	532	10–120 ($\varepsilon = 4.2 \times 10^4$)	7.0	Uses 1:1 mixture of H_2SO_4 and H_3PO_4 as the reaction medium; color stable for only 30 s
(b) NBS–Celestine blue	Unbleached dye color measured	538	0.5–6.0 ($\varepsilon = 6.4 \times 10^4$)	0.30	Highly acidic conditions
(c) Cerium(IV)–Celestine blue [19]	-do-	-do-	0.6–3.0 ($\varepsilon = 1.5 \times 10^5$)	0.37	required
(a) KIO_3	Initial rate of formation of radical cation measured	537	0.4–7.0		Scrupulous control of experimental variables and special equipment for kinetic

Table 4 continued

Reagent(s) used	Methodology	λ_{max} (nm)	Linear range (μg mL^{-1})	LOQ (μg mL^{-1})	Remarks
(b) KIO_3 [23]	Maximum absorbance measured	537	0.4–7.0		measurement required
(a) KIO_3 + LCV	Colored product formed	598	0.05–2.0 ($\varepsilon = 5.3 \times 10^5$)	0.02	Colored product stable, mild acidic conditions, wide linear
(b) CAT + rhodamine B (proposed)	Unbleached dye color measured	458	0.1–2.0 ($\varepsilon = 2.49 \times 10^5$)	0.032	dynamic ranges, highly sensitive

LOQ Limit of quantitation, *FIA* Flow Injection analysis

comparable to that achieved by sophisticated and expensive techniques such as HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk samples and tablets.

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