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Spectrophotometric determination of lansoprazole in pharmaceuticals using bromate-bromide mixture based on redox and complexation reactions

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Abstract: Two sensitive spectrophotometric methods are described for the determination of lansoprazole (LPZ) in bulk drug and in capsule formulation. The methods are based on the oxidation of lansoprazole by *insitu* generated bromine followed by determination of unreacted bromine by two different reaction schemes. In one procedure (method A), the residual bromine is treated with excess of iron (II), and the resulting iron (III) is complexed with thiocyanate and measured at 470 nm. The second approach (method B) involves treating the unreacted bromine with a measured excess of iron (II) and remaining iron (II) is complexed with orthophenanthroline at a raised pH, and measured at 510 nm. In both methods, the amount of bromine reacted corresponds to the amount of LPZ. The experimental conditions were optimized. In method A, the absorbance is found to decrease linearly with the concentration of LPZ (r = -0.9986) where as in the method B a linear increase in absorbance occurs (r = 0.9986) The systems obey Beer's law for 0.5-4.0 and 0.5-6.0 μg mL^{-1} for method A and method B, respectively. The calculated molar absorptivity values are $3.97\mu10^4$ and $3.07\mu10^4$ L mol^{-1}cm^{-1} for method A and method B, respectively, and the corresponding Sandell sensitivity values are 0.0039 and 0.0013 μg cm^{-2}. The limit of detection (LOD) and quantification (LOQ) are also reported for both methods. Intra-day and inter-day precision, and accuracy of the methods were established as per the current ICH guidelines. The methods were successfully applied to the determination of LPZ in capsules and the results tallied well with the label claim and the results were statistically compared with those of a reference method by applying the Student's t-test and F-test. No interference was observed from the concomitant substances normally added to capsules. The accuracy and validity of the methods were further ascertained by performing recovery experiments *via* standard-addition method.

Keywords: lansoprazole; determination; bromate-bromide mixture; complexation reactions; capsules.

Introduction

Lansoprazole(LPZ) is a substituted benzimidazole, chemically known as methyl-4-(2,2,2-trifluroethoxy)-2pyridyl]methyl]sulfinyl]benzimidazole(Fig1). LPZ is a proton pump inhibitor[1] which inhibits the ultimate step in gastric acid secretion. Even the stimulus-independent acid secretion is suppressed. Both basal and stimulus acid is inhibited. Peptic activity is reduced secondary to acid inhibition. LPZ has a greater inhibitory effect on *H. pylori* than omeprazole, and is thus widely used in

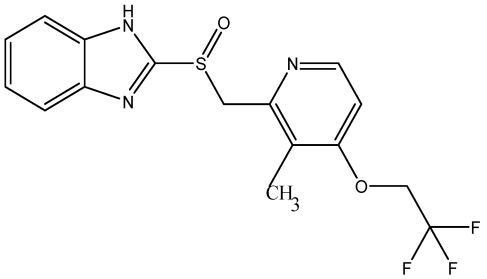

Figure 1. Structure of lansoprazole

the treatment of benign gastric ulcer associated with *H. pylori*; duodenal ulcer and reflux oesophagatis. LPZ is also indicated for Zollinger-Ellison Syndrome and acid related Dyspepsia.

The therapeutic importance of LPZ justifies research to develop analytical methods for its determination in body fluids and in pharmaceuticals. High-performance thin-layer chromatographic(HPTLC) method for the detection and determination of LPZ in human plasma[2] has been reported by Satin *et al.* A sensitive and quantitative method was developed for the estimation of reactive metabolite[3] formation *in vitro*, the analysis being completed by HPLC coupled with a fluorescence detector and a mass spectrometer. However, no study (or little study) has been done to determine LPZ in pharmaceuticals. There are two reports on the determination of LPZ in pharmaceuticals by HPLC. The method reported by Badwe *et al*[4] was performed on a C_{18} column with acetonitrile-H_3PO_4-KH_2PO_4 _acetic acid-triethylamine-water (380:100:50:2.5:2.5:465, v/v/v/v/v/v) as the mobile phase, at a flow rate of 0.9 mL min^{-1} and UV detection at 254 nm, and it is reported to be applicable in the concentration range 10-50 mg mL^{-1}. Very recently[5], a some what sensitive method(applicable over 0.3-60 μg mL^{-1} range) has been described by Sankar *et al.* The separation and analysis were performed on a RP C_{18} column in isocratic mode with mobile phase comprising 0.02 M KH_2PO_4(pH 4.0)-methanol(40:60) at a flow rate of 1 mL min^{-1} and UV detection at 254 nm. In a recent communication, Yeniceli *et al.*[6] have reported the UV-spectrophotometric determination of LPZ. The method is reported to be applicable for $5.4x10^{-6}$-$5.4x10^{-5}$ M (2-20 μg mL^{-1} LPZ).

Because of simplicity, reasonable accuracy and precision, speed and sensitivity, visible spectrophotometry has withstood the test of time and remained competitive with the newer analytical methods. Literature survey revealed that the only visible spectrophotometric method[7] reported is based on the formation of a blue chromogen measurable at 810 nm when LPZ was reacted with iron(III) chloride and ferricyanide in HCl medium. Bromate-bromide mixture and two dyes, methyl orange and indigo carmine have successfully been used for the sensitive titrimetric, spectrophotometric and kinetic determination of many bioactive substances[8-16]. The present investigation aims to develop sensitive and cost-effective methods for the determination of LPZ in pure form and in tablet form by spectrophotometry. The methods utilize bromate-bromide mixture, and ammonium thiocyanate and orthophenanthroline as reagents. The methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quality control.

Experimental details

Apparatus

A Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and materials

All chemicals were of analytical reagent grade and distilled water used to prepare solutions.

Bromate-bromide mixture (20 and 35 µg mL^{-1} in $KBrO_3$). A stock standard solution equivalent to 1000 μg mL^{-1} $KBrO_3$ and a large excess of KBr was first prepared by dissolving accurately weighed 100 mg of $KBrO_3$ and 1g of KBr in water and diluting to the mark with water in a 100 mL calibrated flask. This was diluted stepwise to obtain working concentrations containing 20 and 35 μg mL^{-1} $KBrO_3$ for use in method A, and method B, respectively.

Ferrous ammonium sulphate, FAS (400 and 350 µg mL^{-1}). A stock solution equivalent to 0.01 mol L^{-1} FAS was prepared by dissolving about 400 mg of the salt (S.d. Fine Chem, Mumbai, India) in 50 mL of water containg 1mL of dil H_2SO_4 and diluted to 100 mL with water,

and standardized[17] using pure potassium dichromate. The stock solution was then diluted appropriately with water to get 400 μg mL^{-1} (for method A) and 350 μg mL^{-1} (for method B) FAS.

Orthophenanthroline (0.25%). About 250 mg of orthophenanthroline monohydrate (S. d. Fine Chem. Ltd., Mumbai, India) was dissolved in 100 mL of water with the aid of heat.

Ammonium thiocyanate (3 mol L^{-1}). Prepared by dissolving 23 g of the chemical (S.d. Fine Chem. Ltd., Mumbai, India) in 100 mL water.

Hydrochloric acid (5 mol L^{-1}). Concentrated hydrochloric acid (S.d. Fine Chem.. Mumbai, India Sp. Gr. 1.18,) was diluted appropriately with water to get 5 mol L^{-1} acid.

Ammonia (1:1). Ammonia was prepared by diluting 50 mL of strong ammonia (Qualigens fine chemicals Galxo India Ltd., Mumbai) with 50 mL of water.

Standard solution of lansoprazole. Pharmaceutical grade lansoprazole, cerified to be 99.8% pure was received from Cipla Ltd, Bangalore, India, as gift and was used as received. A stock standard solution equivalent to 200 μg mL^{-1} LPZ was prepared by dissolving accurately weighed amount of pure drug in 1 mol L^{-1} hydrochloric acid and diluting with the same acid to a known volume. The stock solution was diluted appropriately with water to get working concentrations of 10 and 20 μg mL^{-1} for use in method A and method B, respectively. The standard solutions were kept in amber colored bottle and stored in a refrigerator when not in use.

Dosage forms. The following dosage forms were purchased from local commercial sources and subjected to analysis:

Lanzole capsules(15 and 30 mg) from Cipla Ltd., India, Lanzopen capsules (15 and 30 mg) from Morepen Labs Ltd, India, and Propilan capsules (15 and 30 mg) from Glenmark Pharm. Ltd, India.

Procedures

Spectrophotometric method A. Different aliquots (0.5, 1.0———-4.0 mL) of standard 10 μg mL^{-1} LPZ solution were accurately transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 4 mL by adding water. To each flask was added 1mL each of 5 mol L^{-1} HCl and bromate-bromide mixture (20 μg mL^{-1} in $KBrO_3$), the last being added using microburette. The contents were mixed and the flasks were let stand for 5 min. Then, 1 mL of 400 μg mL^{-1} FAS (micro burette) was added to each flask, and again the flasks were let stand for 15 min followed by 1 mL of 3 mol L^{-1} ammonium thiocyanate. The volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 470 nm against water blank.

Spectrophotometric method B. Varying aliquots (0.5, 1.0…3.0 mL) of standard LPZ solution (20 μg mL^{-1}) were accurately measured into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. The solution in each flask was acidified by adding 1 mL of 5 mol L^{-1} hydrochloric acid before adding 1mL of bromate-bromide mixture. (35 μg mL^{-1} in $KBrO_3$) The contents were mixed well and allowed to stand for 5 min with occasional shaking. To each flask was then added 1mL of 350 μg mL^{-1} FAS, and after 15 min, 1 mL each of 0.25 % orthophenanthroline and 1:1 NH_3 solutions were added and diluted to the mark with water. The absorbance of each solution was measured at 510 nm against a reagent blank after 15 min.

In either spectrophotometric method, a standard graph was prepared by plotting the decreasing absorbance values in method A or increasing absorbance values in method B versus concentration of LPZ. The concentration of the unknown was read from the standard graph or computed from the respective regression equation derived using the Beer's law data.

Procedure for Capsule. The contents of twenty capsules were accurately weighed and ground into a fine powder. A quantity of the powder equivalent to 100 mg of LPZ was accurately weighed into a 100 mL calibrated flask, 60 mL of 1 mol L^{-1} HCl added and shaken for 20 min; the volume was finally diluted to the mask with 1 mol L^{-1} HCl, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded, The filtrate (1000 μg mL^{-1} LPZ) was appropriately diluted with water to get 10 and 20 μg mL^{-1} LPZ concentrations was subjected to analysis by either method.

Results and Discussion

Bromate-bromide mixture is a valuable

oxidimetric reagent widely used in the assay of several pharmaceutical substances both by titrimetric and spectrophotometric methods[8-16]. The present communication deals with the spectrophotometric assay of LPZ using bromate-bromide mixture as the oxidimetric reagent. The proposed methods are indirect and are based on the determination of residual bromine after allowing the reaction between LPZ and oxidant to go to completion, and rely on two different reaction schemes.

Method development

Spectrophotometric method A. Complex formation reaction involving iron(III) and thiocyanate is a well known reaction that has been widely used for trace level determination of iron [18]. The present method is based on the oxidation of LPZ by a known excess of bromate-bromide mixture in hydrochloric acid medium, reduction of the residual oxidant by a fixed amount of iron(II) and subsequent formation of iron(III)–thiocyanate complex which is measured at 470 nm. When a fixed amount of bromate-bromide mixture is made to react with increasing amounts of LPZ, there occurs a concomitant fall in the oxidant concentration. When the unreacted oxidant is reduced by a fixed amount of iron(II), there will be a proportional decrease in the concentration of iron (III). This is observed as a proportional decrease in the absorbance of iron(III)-thiocyanate complex on increasing the concentration of LPZ (Fig. 2 & 3) which formed the basis for the assay of drug by the present method.

The conditions for the determination of iron(III) with thiocyanate are well established[18]. Hence, various parameters associated with the oxidation of LPZ by bromate-bromide mixture and subsequent reduction of residual oxidant by iron(II) were optimized. Although nitric acid or hydrochloric acid medium can be used for the complexation of iron(III) with thiocyanate,[18] the latter was selected, since nitric acid, being an oxidizing agent itself, would interfere with the oxidation step of the reaction scheme. Sulphuric acid medium, although convenient for the oxidation step, was not preferred since it is reported to reduce the colour intensity of iron(III)-thiocyanate complex. One mL of 5 mol L^{-1} hydrochloric acid in a total volume of about 6 mL was used for the oxidation step which was complete in 5 min and the same acidic condition was used to reduce the residual bromine by iron(II) which was complete in 15 min and resulting iron(III) was complexed with thiocyanate in the same acid medium.

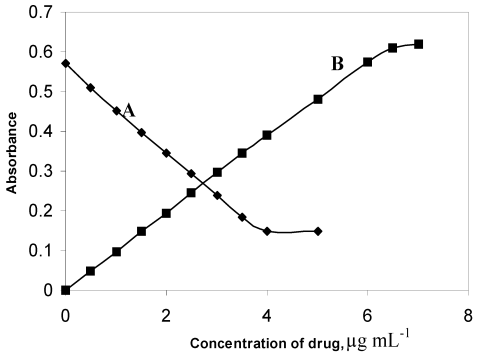

Figure 2. Beer's law curves for method A and method B

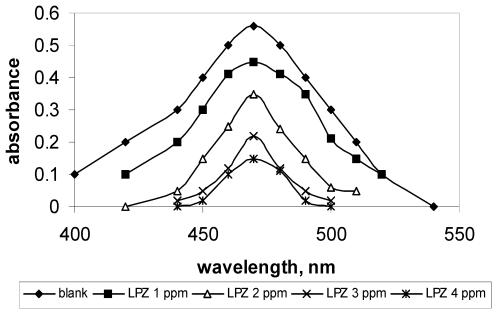

Figure 3. Absorption spectrum of lansoprazole for method A

Fixing 5.5 μg mL^{-1} as the upper limit of iron(III) that could be determined by the thiocyanate method,[18] bromate-bromide mixture equivalent to 200 μg of $KBrO_3$ was required to generate it form 400 μg of FAS. However, although a fixed amount of FAS is not really required, large amounts are undesirable since iron(II) tends to undergo aerial oxidation. Hence, a fixed amount (400 μg) of FAS enough to reduce the total *insitu* generated bromine was employed. The oxidation of LPZ by bromine was complete in 5 min and subsequent reduction of residual oxidant by iron(II) was complete in 15 and finally complex formation reaction between the resulting iron(III) and thiocyanate was instantaneous under the described experimental condi-

tions. Developed colour was stable for at least 60 min in the presence of reaction product.

Spectrophotometric method B. The spectrophotometric method based on the use of orthophenanthroline as the complexing agent continues to be one of the sensitive methods for the determination of iron in a variety of matrices[19] This reaction coupled with the oxidizing property of *insitu* generated bromine has been made use in developing a sensitive indirect method for the assay of LPZ. The drug in varying amounts, when treated with a fixed and known amount of bromate-bromide in acid medium, consumes the latter in proportionate amounts for oxidation, and there will be a concomitant decrease in the amount of the oxidant. When the decreasing amounts of oxidant are reacted with a fixed amount of iron(II) in the same acidic conditions, there will be a proportional increase in the concentration of iron(II). This is reflected in increase in absorbance of orthophenanthroline complex formed with residual iron(II). The absorbance measured at 510 nm is found to increase linearly with LPZ concentration (Fig. 2 & 4) serving as the basis for the assay procedure.

The conditions were optimized to produce a maximum colour through variation of such parameters as acid concentration, reaction time and amount of ammonia required to raise the pH to about 4. One mL of 5 mol L^{-1} hydrochloric acid in a total volume of about 5 mL was used for the oxidation step which was complete in 5 min and the same acidic condition was used to reduce the residual bromine by iron(II). Larger amounts of acid are not preferable since they would require large quantities of ammonia to raise the pH to 4, required for iron(II)-phenanthroline complex formation.

Taking 5 μg mL^{-1} as the upper limit of iron (II) that could be determined by orthophenanthroline method, 350 μg of FAS was used in this method. Quantitatively this was found to react with bromine equivalent to 350 μg of bromate . Hence, different amounts of LPZ were reacted with 1 mL bromate-bromide mixture equivalent to 35 μg mL^{-1} $KBrO_3$ before determining the unreacted bromine. This enabled to fix the concentration range of LPZ that could be determined by the method. The complex was stable for several days even in the presence of the reaction product.

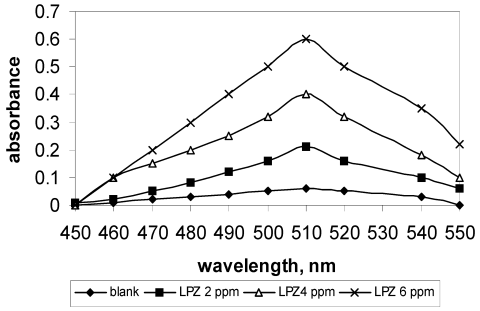

Figure 4. Absorption spectrum of lansoprazole for method B

Method Validation

Quantitative parameters

A linear relation is found between absorbance and concentration in the ranges given in Table 1. In method A, Beer's law is obeyed in the inverse manner. The calibration graphs are described by the equation:

$$Y = a + b\,X$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in μg mL^{-1}) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in Table 1. Sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification calculated according to ICH guidelines[20] are also compiled in Table 1, and demonstrate the high sensitivity of the methods.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the current ICH guidelines using the following formulae:

$$LOD = \frac{3.3\,\sigma}{S} \quad \text{and} \quad LOQ = \frac{10\,\sigma}{S}$$

where σ is the standard deviation of seven reagent blank determinations and S is the slope of the calibration curve.

Accuracy and precision

Intra-day and inter-day precision were assessed from the results of seven replicate analyses on pure drug solution. The mean values and relative

Table 1. Analytical and regression parameters of spectrophotometric methods

Parameter	Method A	Method B
λ_{max}, nm	470	510
Beer's law limits, μg mL^{-1}	0.5 – 4.0	0.5 - 6.0
Molar absorptivity, L mol^{-1} cm^{-1}	3.97×10^4	3.07×10^4
Sandell sensitivity, μg cm^{-2}	0.0039	0.0013
Limit of detection, μg mL^{-1}	0.07	0.095
Limit of quantification, μg mL^{-1}	0.21	0.36
Regression equation, Y*		
Intercept (a)	0.565	0.0013
Slope (b)	-0.1047	0.095
Correlation coefficient, (r)	-0.9986	0.9986
S_a	0.0084	0.0105
S_b	0.0027	0.00167

*Y = a+bX, where Y is the absorbance and X concentration in μg mL^{-1}
S_a. Standard deviation of intercept.
S_b. Standard deviation of slope.

standard deviation (RSD) values for replicate analyses at three different concentration levels were calculated. To calculate the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error (RE). Table 2 summarizes the intra-day precision and accuracy data for the determination LPZ by the proposed methods which were ≤ 3%. The inter-day precision was less than 4 %.

Table 2. Evaluation of accuracy and precision

Method	LPZ taken μg mL^{-1}	LPZ found* μg mL^{-1}	Range, μg mL^{-1}	RE %	SD μg mL^{-1}	SEM μg mL^{-1}	RSD[a], %	RSD[b], %	ROE,** %
A	1.0	0.97	0.045	3.0	0.017	0.006	1.75	3.2	±1.74
	2.0	1.96	0.07	2.0	0.024	0.009	1.22	2.5	±1.21
	3.0	2.96	0.04	1.7	0.015	0.005	0.51	2.8	±0.50
B	2.0	1.98	0.032	1.0	0.025	0.009	1.26	4.0	±1.25
	4.0	3.94	0.046	1.5	0.018	0.007	0.46	2.9	±0.45
	6.0	5.92	0.063	1.3	0.032	0.012	0.54	3.8	±0.53

RE relative error; SD. Standard deviation; SEM .Standard error of mean; RSD. Relative standard deviation; a. Intra-day precision, b. Inter-day precision
* Mean value of seven determinations
** At the 95% confidence level for 6 degrees of freedom.

The ruggedness/robustness of the methods was assessed by calculating the RSD for results obtained by performing the analysis using three different instruments and by three different persons. The inter-instrumental RSD values were in the range of 3.2 – 5.5 whereas the inter-personal RSD values varied from 2.6-4.2 (n= 3 in both instances) for three concentrations employed for accuracy and intra-day precision studies.

Application to capsules analysis

Thirty one brands of LPZ capsules in 15 mg and 30 mg doses are currently available in the Indian market. The validity of the methods was checked by applying them to assay in three brands of capsules. Table 3 gives the results of assay and reveal that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a reference method[6] by applying Student's t-test for accuracy and F-test for precision. The reference method consisted of the measurement of the absorbance of the drug solution in 0.01 mol L^{-1} NaOH at 292 nm. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) except in a couple of instances, suggesting that the proposed methods are as accurate and precise as the reference method.

Accuracy and validity of the methods were further ascertained by performing recovery experiments *via* standard addition technique. To a fixed and known amount of LPZ in capsule powder (pre analysed), pure drug was added at three levels and the total was found by the proposed methods. Each test was repeated three times. The recovery of pure LPZ added to capsule powder ranged from 96.8 to 102.5 % (Table 4) indicating that commonly encountered tablet excipients and additives such as talk, starch, lactose, sodium alginate, magnesium stearate, calcium gluconate and calcium dihydrogenorthophosphate did not interfere in the assay procedures.

Table 3. Results of determination of lansoprazole in formulations and statistical comparison with the reference method

Capsule Brand name[#]	Nominal amount, mg	% found* ± SD		
		Reference method	Method A	Method B
LANZOLE[a]	15	98.56±0.76	101.2±0.98 t=1.66 F=3.45	100.8±1.12 t=2.17 F=3.76
	30	101.5±1.14	99.1±0.89 t=1.64 F=3.74	97.8±1.10 t=0.77 F=5.22
LANZOPEN[b]	15	97.02±0.56	99.4±1.18 t=4.44 F=4.32	98.11±0.92 t=2.69 F=2.33
	30	100.6±0.85	102.8±1.21 t=2.03 F=3.38	101.5±1.34 t=2.48 F=1.30
PROPILAN[c]	15	99.66±0.96	97.51±1.41 t=2.16 F=2.87	102.1±1.35 t=1.98 F=3.34
	30	102.6±0.62	99.8±1.29 t=4.33 F=4.62	100.8±1.18 t=3.62 F=3.16

*Mean value of five determinations
#Marketed by: a. Cipla Ltd ; b. Morepen Labs. Ltd; c. Glenmark Pharm. Ltd.,
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.

Table 4. Results of Recovery Study by Standard addition method.

Method	Formulation studied	LPZ in formulation, µg	Pure LPZ Added, µg	Total Found, µg	Pure LPZ Recovered*, %
A	PROPILAN	9.75	5.0	14.68	98.61
	15 mg	9.75	15.0	14.68	100.42
		9.75	30.0	14.68	101.72
B		20.42	10.0	30.10	96.8
		20.42	20.0	40.26	99.2
		20.42	40.0	61.42	102.5

*Mean value of three determinations

Conclusions

Two new methods have been developed and appropriately validated for the assay of lansoprazole using bromate-bromide as the oxidimetric reagent. Both methods are based on well-characterised complexation reactions and are the most sensitive ever reported for lansoprazole in terms of linear range of response and molar absorptivity. An additional advantage of the spectrophotometric methods is that the absorbance is measured at longer wavelengths where the interference from excipients is less. The methods employ bromate-bromide as the oxidimetric reagent which is exceptionally stable in solution. And there is no risk of standardization. The methods should therefore find ready application in pharmaceutical industrial quality control.

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