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Sensitive spectrophotometric determination of lansoprazole in pharmaceuticals using ceric ammonium sulphate based on redox and complex formation reactions

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Abstract: Two simple sensitive and cost-effective spectrophotometric methods are described for the determination of lansoprazole (LPZ) in bulk drug and in capsules using ceric ammonium sulphate (CAS), iron (II), orthophenanthroline and thiocyanate as reagents. In both methods, an acidic solution of lansoprazole is treated with a measured excess of CAS followed by the determination of unreacted oxidant by two procedures involving different reaction schemes. The first method involves the reduction of residual oxidant by a known amount of iron(II), and the unreacted iron(II) is complexed with orthophenanthroline at a raised pH, and the absorbance of the resulting complex measured at 510 nm (method A). In the second method, the unreacted CAS is reduced by excess of iron (II), and the resulting iron (III) is complexed with thiocyanate in the acid medium and the absorbance of the complex measured at 470 nm (method B). In both methods, the amount CAS reacted corresponds to the amount of LPZ. In method A, the absorbance is found to increase linearly with the concentration of LPZ where as in method B a linear decrease in absorbance occurs. The systems obey Beer's law for 2.5-30 and 2.5-25 μg mL^{-1} for method A and method B, respectively, and the corresponding molar absorptivity values are 8.1×10^3 and 1.5×10^4 L $mol^{-1}cm^{-1}$. The methods were successfully applied to the determination of LPZ in capsules and the results tallied well with the label claim. No interference was observed from the concomitant substances normally added to capsules.

Keywords: lansoprazole determination; spectrophotometry; ceric ammonium sulphate; capsules.

Introduction

Lansoprazole(LPZ) is a substituted benzimidazole, chemically known as methyl-4-(2,2,2-trifluroethoxy)-2pyridyl]methyl]sulfinyl]benzimidazole(Fig.1). 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 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

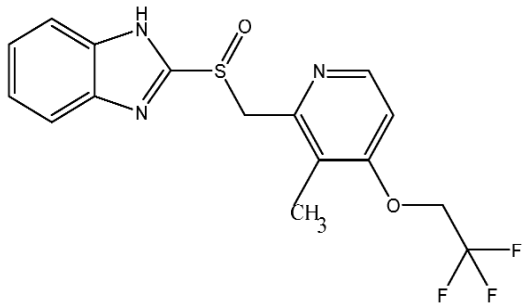

Figure 1. Structure of lansoprazole.

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[4,5]. In a recent communication, Yeniceli *et al*. [6] have reported the UV-spectrophotometric determination of 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. The present investigation aims to develop sensitive and cost-effective methods for the determination of LPZ in pure form and in capsule form by spectrophotometry. The methods utilize cerium ammonium sulphate, 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

Apparatus

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

Reagents and materials

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

Ceric ammonium sulphate, CAS (600 and 640 μg mL^{-1}), ferrous ammonium sulphate, FAS (370 and 400 μg mL^{-1}), orthophenanthroline (0.25%), ammonium thiocyanate (3 mol L^{-1}), sulphuric acid (5 mol L^{-1}) and ammonia (1:1) were prepared in the usual manner.

Standard solution of LPZ. Pharmaceutical grade LPZ, reported 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 1000 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 to 100 μg mL^{-1} with water. The standard solutions were kept in amber colored bottle and stored in a refrigerator when not in use.

Procedures

Method A. Different aliquots (0.25—3.0 mL) of standard 100 μg mL^{-1} LPZ solution were accurately measured and transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 3.0 mL by adding water. To each flask was added 1mL each of 5 mol L^{-1} H_2SO_4 and CAS (600 μg mL^{-1}), the last being added using microburette. The content was mixed and the flasks were let stand for 15 min. Then, 1 mL of 370 μg mL^{-1} FAS was added to each flask (micro burette), and again the flasks were let stand for 10 min followed by 1 mL of 0.25 % phenanthroline and 1 mL of 1:1 ammonia. The volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 510 nm against reagent blank after 15 min.

Method B. Varying aliquots (0.0—2.5 mL) of standard LPZ solution (100 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} sulphuric acid before adding 1mL of CAS (640 μg mL^{-1}). The content was mixed well and allowed to stand for 15 min with occasional shaking. To each flask was then added 1mL of 400 μg mL^{-1} FAS, and after 5 min, 1 mL of 3 mol L^{-1} thiocyanate was added and diluted to the mark with water. The absorbance of each solution was measured at 470 nm against a reagent blank .

In either spectrophotometric method, a standard graph was prepared by plotting the increasing absorbance values in method A or decreasing 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. Thirty one brands of LPZ capsules in 15 mg and 30 mg doses are currently available in the Indian market. Three brands were purchased from commercial sources and used in this investigation. 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 mark 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 the required concentration, was subjected to analysis by either method.

Results and discussion

The proposed methods are indirect and are based on the determination of the residual CAS after the reaction between LPZ and the oxidant is ensured to be complete in acid medium. The amount of oxidant reacted corresponds to the amount of drug in both methods.

Method development

Method A. The drug in varying concentrations, when treated with a fixed and known concentrations of CAS in acid medium, consumes the latter in proportionate concentrations for its oxidation, and there will be a concomitant decrease in the amount of the oxidant. When the decreasing concentrations of oxidant are treated with a fixed and known concentrations of iron (II) in the same acidic conditions, there will be a proportional increase in the concentration of iron (II). This is shown by the increase in the absorbance of orthophenanthroline complex formed with the residual iron(II). The absorbance measured at 510 nm is found to increase linearly with the increasing concentration of LPZ (Fig. 2) serving as the basis for the determination of drug.

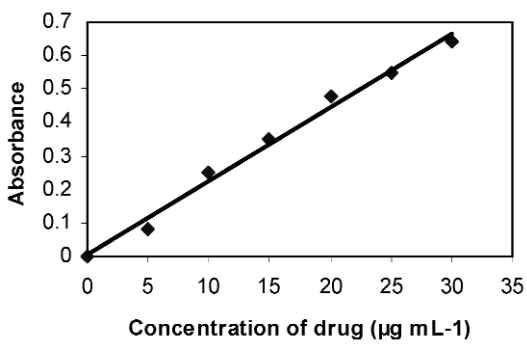

Figure 2. Linearity curve for method A(2.5-30 μg mL^{-1}LPZ+1 mL 5mol L^{-1} H_2SO_4 + 1 mL CAS(600μg mL^{-1}) + 1 mL FAS(370μg mL^{-1}) + 1 mL 0.25% orthophenanthroline+1mL of 1:1 ammonia, made upto 10 mL).

The optimum conditions were established to produce maximum colour through variation of such parameters as nature of acid and its concentration, reaction time and quantity of ammonia required to raise the pH to about 4, the optimum value for iron (II) –

orthophenanthroline complex formation. One mL of 5 mol L^{-1} sulphuric acid in a total volume of ~ 5 mL was used for the oxidation step, which was found to be complete in 15 min.

Considering 52 µg as the upper limit of iron that could be determined by the phenanthroline method, stoichiometrically, 600 µg of CAS would produce it from 370 µg of FAS. Hence, different concentrations of LPZ were reacted with 1 mL of 600 µg of CAS followed by determination of unreacted oxidant. This enabled to fix the concentration range of LPZ. One mL of 1:1 ammonia was used in a total volume of 10 mL to raise the pH required for iron (II) – phenanthroline complex formation.

Method B. This method is based on the oxidation of LPZ by a known excess of CAS in sulphuric acid medium, reducing the unreacted oxidant by iron (II) and subsequent formation of iron (III) – thiocyanate complex, which is measured at 470 nm. When a fixed concentration of CAS is made to react with increasing concentration of LPZ, there occurs a concomitant fall in the former's concentration. When the unreacted CAS is reduced by a fixed concentration 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. 3), which formed the basis for the determination of drug.

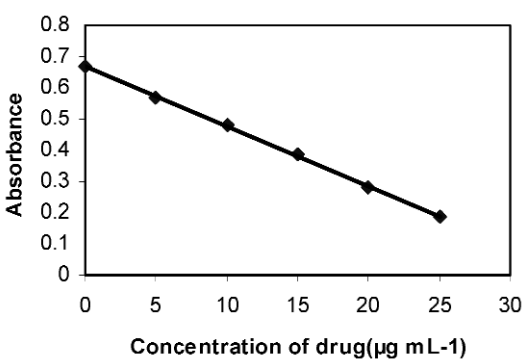

Figure 3. Linearity curve for method B(0-25 µg mL^{-1}LPZ+1 mL 5mol L^{-1} H_2SO_4 + 1 mL CAS(640µg mL^{-1}) + 1 mL FAS(400µg mL^{-1}) + 1 mL 3 mol L^{-1} thiocyanate,made upto 10 mL).

Various parameters associated with the oxidation of LPZ by CAS and subsequent reduction of the residual oxidant by iron (II) were optimized. One mL of 5 mol L^{-1} sulphuric acid in a total volume of ~ 5 mL was used for the oxidation step, which was found to be complete in 15 min.

Because of non-linearity at higher concentrations, 5.5 µg mL^{-1} was taken as the upper limit of iron(III) that could be determined by thiocyanate method. Stoichiometrically, 645 µg of CAS would be required to generate it from 400 µg of FAS in a total volume of 10 mL. However, 640 µg of CAS was actually used in the study to ensure the presence of a little of excess of iron (II). The oxidation of LPZ by CAS was complete in 15 min and subsequent reduction of residual oxidant by iron (II) and complex formation reaction between resultant iron (III) and thiocyanate were instantaneous under the described experimental conditions.

Analytical parameters

A linear relation is found between absorbance and concentration in the ranges given in Table 1. In method B, 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 [8] are also compiled in Table 1, and demonstrate the high sensitivity of the methods.

Method validation

Evaluation of 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 standard deviation (RSD) values for seven

Table 1. Analytical and regression parameters of spectrophotometric methods

Parameter	Method A	Method B
λ_{max}, nm	510	470
Beer's law limits, μg mL^{-1}	2.5-30	2.5 - 25
Molar absorptivity, L mol^{-1} cm^{-1}	8.1×10^3	1.5×10^4
Sandell sensitivity, μg cm^{-2}	0.046	0.024
Limit of detection, μg mL^{-1}	0.28	0.37
Limit of quantification, μg mL^{-1}	0.85	1.13
Regression equation, Y*		
Intercept (a)	0.009	0.670
Slope (b)	0.022	-0.019
Correlation coefficient, (r)	0.9908	-0.9994
S_a	0.031	0.115
S_b	0.002	0.006

*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.

replicate analyses at three different concentration levels were calculated. 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). To determine the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. Table 2 summarizes the intra-day precision and accuracy data for the determination LPZ by the proposed methods, which were ≤ 2%. The inter-day RSD values ranged from 1.5-3.5%.

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, %	ROE, %
A	10.0	9.79	0.44	2.08	0.16	0.06	1.60	±1.60
	20.0	19.65	0.27	1.73	0.10	0.04	0.49	±0.49
	30.0	29.49	1.27	1.69	0.46	0.17	1.56	±1.56
B	10.0	9.92	0.42	0.79	0.13	0.05	1.34	±1.34
	15.0	14.91	0.78	0.63	0.26	0.10	1.76	±1.76
	20.0	19.58	0.45	2.13	0.19	0.07	0.98	±0.98

RE relative error; SD. Standard deviation; SEM .Standard error of mean; RSD. Relative standard deviation, and ROE. Range of error at the 95 % confidence level for six degrees of freedom.
* Mean value of seven determinations

Application

Table 3 gives the results of assay and reveals 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}

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

Capsule brand name[#]	Nominal amount, mg	% found* ± SD		
		Reference method	Method A	Method B
LANZOLE[a]	15	101.5±0.84	99.8±1.54 t=2.26 F=3.36	102.1±1.82 t=0.71 F=4.69
	30	100.6±0.85	101.8±1.24 t=1.81 F=2.13	99.76±1.02 t=1.42 F=1.44
LANZOPEN[b]	15	97.02±1.06	99.4±1.48 t=2.96 F=1.95	98.11±1.92 t=1.16 F=3.28
	30	100.6±0.85	102.8±1.61 t=2.83 F=3.59	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	100.8±1.29 t=2.98 F=4.33	101.3±1.18 t=2.28 F=3.62

*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

Capsule studied	Method A				Method B			
	Drug in capsule, μg mL^{-1}	Pure drug added, μg mL^{-1}	Total found, μg mL^{-1}	Pure drug recovered*, %	Drug in capsule, μg mL^{-1}	Pure drug added, μg mL^{-1}	Total found, μg mL^{-1}	Pure drug recovered * %
LANZOLE 15 mg	4.99	5	9.87	97.56	5.11	5	10.18	101.3
	4.99	10	15.45	104.6	5.11	10	15.33	102.2
	4.99	20	25.05	100.3	5.11	20	24.86	98.75
LANZOPEN 15 mg	4.97	5	10.13	103.2	4.91	5	10.02	102.1
	4.97	10	14.92	99.5	4.91	10	14.73	98.2
	4.97	20	25.03	100.3	4.91	20	24.97	100.2
PROPILAN 30 mg	4.88	5	9.84	99.28	5.11	5	10.09	99.6
	4.88	10	14.65	97.65	5.11	10	15.64	105.3
	4.88	20	25.78	104.5	5.11	20	25.37	101.3

*Mean value of three determinations

Table 5. Comparision of the proposed and reported methods.

Sl.No.	Reported methods	Reagents used	λ_{max}, nm	Range, µg mL^{-1}	Remarks	Ref.
1	HPTLC	-	-	0.05-0.25	Narrow linear dynamic range	2
2	HPLC-MS	-	-	-	Highly expensive instrumental set up required	3
3	HPLC	-	254	-	pH has to be carefully maintained, expensive instrumental set up.	4
4	HPLC	-	254	0.3-60	pH has to be carefully maintained, expensive instrumental set up.	5
5	UV-spectrophotometry	0.01 M NaOH	292	2-20	-	6
7	Vis-spectrophotometry	Iron(III) chloride-ferriccyanide	810	-	-	7
8	Vis-spectrophotometry	**a**.CAS-iron(II)-Orthophenanthraline	510	2.5-30	Wide linear dynamic ranges, stable coloured species, inexpensive instrumental setup, use of eco-friendly chemicals.	Present
		b.CAS-iron(II)-thiocyanate	470	2.5-25		

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 (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.

Conclusions

Two new methods have been developed and appropriately validated for the assay of lansoprazole. Both methods are based on well-characterised complexation reactions and are the most sensitive ever reported for LPZ in terms of linear dynamic concentration ranges and molar absorptivity values. An additional advantage of the methods is that the absorbance is measured at longer wavelengths where the interference from excipients is far less than at shorter wavelengths and also the present method requires hardly 30 min for analysis of the content whereas highly sophisticated instruments like HPLC, HPTLC etc. take 30-45 min for the stabilization itself(Table-5). The methods use CAS solution as a quantitative reagent which is highly stable in solution and are based on the measurement of stable coloured species. The methods should therefore find ready application in pharmaceutical industrial quality control.

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