Iodimetric Assay of Pantoprazole Sodium Sesquihydrate in Pharmaceuticals Using Iodate and Iodide as Reagents

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Titrimetric and spectrophotometric assay of pantoprazole sodium sesquihydrate (PSS) using iodate as the oxidimetric reagent is described. In titrimetry, PSS is treated with a measured excess of iodate in H_2SO_4 medium followed by the reaction of unreacted oxidant with iodide and the liberated iodine was back titrated with a standard thiosulphate solution. The spectrophotometric method is based on the oxidation of PSS with iodate in H_2SO_4 medium followed by the extraction of resulting iodine into chloroform and measurement of absorbance at 520 nm. In both the methods, the amount of iodate reacted corresponds to the PSS content. Experimental conditions that provide wide linear range, maximum sensitivity and selectivity, and accuracy and precisions have been optimized. In titrimetry, the calculations are based on a 2:3 (IO_3^- : PSS) reaction stoichiometry and the method is applicable over 1.0-10.0 mg range. In spectrophotometry, the absorbance is found to increase linearly with concentration and the Beer's law is obeyed in the range 25.0-500.0 $\mu g\ ml^{-1}$ with a correlation coefficient (r) of 0.999 (n=7). The apparent molar absorptivity and Sandell sensitivity values are 0.692 x $10^3\ l\ mol^{-1}$ cm^{-1} and 0.658 $\mu g\ cm^{-2}$, respectively. The slope and intercept of the equation of the regression line are 0.0015 and 0.0055, respectively. The limits of detection (LOD) and quantification (LOQ) are 3.92 and 11.87 $\mu g\ ml^{-1}$, respectively. Accuracy and precision of the assays were determined by computing the intra-day and inter-day variations at three different levels of PSS. The proposed methods were applied to the analysis of tablet forms of PSS and the results tallied well with the label claim. No interference was observed from concomitant substances normally added to tablets. The results were statistically compared with those of a literature method by applying the Student's t-test and F-test. The accuracy and validity of the methods were further ascertained by placebo blank and synthetic mixture analyses and also by recovery studies *via* spike method.

Key Words: Pantoprazole Sodium Sesquihydrate; Assay; Titrimetry; Spectrophotometry; Iodate; Pharmaceuticals

Introduction

Pantoprazole sodium sesquihydrate (PSS), sodium 5-(difluoromethoxy)-2 – [[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1*H*-benzimidazole sesquihydrate (Fig. 1) is a proton pump inhibitor that inhibits gastric acid secretion by altering the activity of H^+/K^+ ATPase, the final common stage of acid secretion in parietal cells [1-3]. It is pharmaceutically formulated as gastro-resistant tablets containing 40 or 20 mg PSS. PSS is a non-official drug substance and there are only a few reports on the determination of this drug in pharmaceutical substances including dosage form.

Methods for the determination of PSS in pharmaceutical formulations and biological materials which have been reported previously included high performance liquid chromatography (HPLC) [4-7],

densitometric HPTLC [8], capillary electrophoresis [9, 10], derivative UV-spectrophotometry [11] and difference UV-spectrophotometry [12].

Visible spectrophotometry has withstood the test of time and remained competitive with the newer analytical methods because of its inherent simplicity, adequate sensitivity, reasonable accuracy and precision, and availability in all quality control laboratories. Unfortunately, the spectrophotometric methods reported for determination of PSS by Salama *et al*. [13, 14], Moustafa [15] are associated with some drawbacks such as longer reaction times, use of heating step, narrow linear ranges, and measurement at shorter wavelength (Table 1). The literature survey revealed that no titrimetric assay has ever been reported for PSS.

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Table 1: Comparison of the performance characteristic of the existing spectrophotometric methods with the proposed methods

Sl. No.	Reagent(s) used	Methodology	Linear range, µg ml^{-1} ($\in$ in l mol^{-1}cm^{-1})	Remarks	Ref.
1.	Trivalent iron	1:2 chelated in EtOH medium measured at 455 nm.	30-300	Ethanolic medium used, less sensitive and requires heating in a thermostated water bath at 60^{0}C for 30 min.	[13, 14]
2.	a) DDBQ	C-T complex measured at 457 nm.	10-60 (5.65 X 10^3)	Narrow linear dynamic range.	[15]
	b) Iodine	C-T complex measured at 293 and 359 nm.	18-142 (1.46 X 10^3)	Measurement at shorter wavelength.	[15]
	c) Cu (II)-eosin	Ternary complex measured at 549 nm.	4-26 (1.62 X 10^4)	Narrow linear range and requires heating in a water bath at 70^{0}C for 25 min.	[15]
3.	KIO$_3$	Extraction of resulting iodine into chloroform and measurement of absorbance at 520 nm.	25-500 (0.692 x 10^3)	Wide linear dynamic range, moderate sensitivity, highly stable colour species	This work

DDBQ: Dichlorodicyanobenzoquinone

The present work aims to develop two new, rapid and sensitive methods, using titrimetry and spectrophotometry for the determination of PSS based on its ability to undergo oxidation. The procedures are based on the oxidation of the drug by iodate in acid medium. In titrimetry, the residual oxidant was determined by iodometric back titration with thiosulphate and in spectrophotometry, the iodine is quantitatively extracted into chloroform and absorbance measured at 520 nm. These methods are obviously simpler than those using sophisticated instruments, expensive devices and reagents. Furthermore, as it involves extraction step, better selectivity is attained and interferences commonly associated with other methods are absent. 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.

Materials and Methods

Apparatus

A Systronics model 106 digital spectrophotometer with 10 mm quartz cells were used for absorbance measurements.

Reagents

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

Potassium Iodate: A 0.002 M potassium iodate solution was prepared by dissolving 0.428 g of the reagent (Sarabhai M. Chemicals, Baroda, India) in water and diluting to one litre in a volumetric flask and used in titrimetric method. A 5 % iodate solution was prepared for spectrophotometric method.

Sodium Thiosulphate: A 0.01 M thiosulpahte solution was prepared by dissolving 2.5 g of the chemical (S. d. Fine Chem. Ltd., Mumbai, India) in one litre of water for use in titrimetric method.

Sulphuric Acid (2M): First, 5M sulphuric acid was prepared by adding 278 ml of concentrated acid (S. d. Fine Chem. Ltd., Mumbai, India; sp. gr. 1.83) to 722 ml of water with cooling. This stock solution was appropriately diluted to get 2M acid.

Potassium Iodide: A 10 % solution was prepared by dissolving 10 g of the chemical in 100 ml of water and used in titrimetric work.

Starch Indicator: One g of the reagent (Merck, Mumbai, India) was made into a paste and poured into 100 ml of boiling water, boiled for 1 min and cooled and used for titrimetric method.

Standard PSS Solution: Pharmaceutical grade PSS certified to be 99.98 % pure was received as gift from Cipla India Ltd, Mumbai, India, and used as received. Standard PSS solutions (1 mg ml^{-1} and 500 µg ml^{-1}) were prepared by dissolving calculated quantity of pure drug in water.

Table 2: Sensitivity and regression parameters

Parameter	Method
λ_{max}, nm	520
Linear range, μg ml^{-1}	25-500
Molar absorptivity(ε), l mol^{-1} cm^{-1}	0.692 x 10^3
Sandell sensitivity[a], μg cm^{-2}	0.6575
Limit of detection (LOD), mg ml^{-1}	3.92
Limit of quantification (LOQ), mg ml^{-1}	11.87
Intercept (a)	0.0055
Slope (b)	0.0015
Standard deviation of a (S$_a$)	0.0128
Standard deviation of b (S$_b$)	0.00003
Regression coefficient (r)	0.999

[a]Limit of determination as the weight in μg per ml of solution, which corresponds to an absorbance of
A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.
[b]Y=a+bX, Where Y is the absorbance, X is concentration in μg ml^{-1}, a is intercept, b is slope.

Three brands of tablets containing PSS, pantodac-20 (Aristo Pharmaceuticals Ltd., Mumbai, India), pantop-40 (Cipla Ltd, Mumbai, India) and pantosec-40 (Zy. Alidac, Mumbai, India), used in the investigation were purchased from local commercial sources.

General Procedure

Titrimetry

Different volumes (1-10 ml) of standard solution containing 1 mg ml^{-1} PSS were taken in a 100 ml titration flask and the volume was made up to 10 ml with water. The solution was acidified by adding 5 ml of 2M H$_2$SO$_4$. Then, 10 ml of 0.002 M iodate solution was added by means of pipette, the contents were mixed well and kept aside with occasional shaking. After 10 min, 5 ml of 10 % potassium iodide solution was added and the liberated iodine was titrated with 0.01 M thiosulphate to a starch end-point. A blank titration was performed simultaneously, and then the amount of PSS in the aliquot was computed from the following formula:

Amount (mg) $\quad = \quad$ VM$_w$S/n

where V $\quad = \quad$ ml of KIO$_3$ consumed
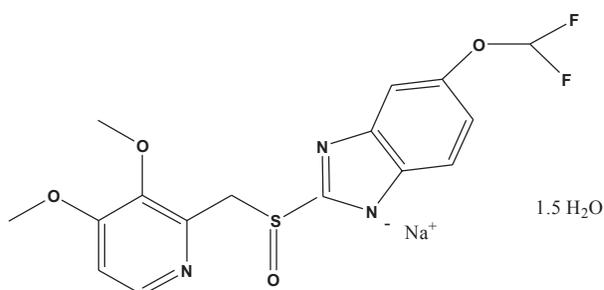
Fig. 1: Structure of pantoprazole sodium sesquihydrate

M$_w$ = relative molecular mass of drug

S $\quad=\quad$ strength of KIO$_3$, mol/l

n $\quad=\quad$ number of moles of KIO$_3$ reacting with per mole of PSS.

Spectrophotometry

Into a series of 125 ml separating flasks, 0, 0.5, 2, 4, 6, 8 and 10 ml of 500 μg ml^{-1} standard PSS solution was delivered with the help of a microburette and the total volume was brought to 15 ml with water. 1 ml 2M H$_2$SO$_4$ followed by 2 ml of 5% KIO$_3$ solution were added to each flask. The content was mixed well and allowed to stand for 15 min with occasional shaking. Finally, 5 ml of chloroform were accurately measured and transferred into each flask and shaken for 1 min, and the two layers were allowed to separate. The chloroform layer was dried over anhydrous Na$_2$SO$_4$ and its absorbance measured at 520 nm *Vs* a reagent blank.

A calibration graph was prepared by plotting the measured absorbance *Vs* concentration of PSS, and the concentration of the unknown was read from the calibration graph or deduced from the regression equation derived using the Beer's law data.

Assay of Tablets

Twenty tablets were weighed accurately and ground into a fine powder. A quantity of the powder containing 100 mg of PSS was accurately weighed into a 100 ml calibrated flask and 60 ml of water added. The content was shaken for about 20 min; volume diluted to the mark with water and mixed, and filtered using a Whatman No. 42 filter paper. First 10 ml portion of the filtrate was discarded, and a convenient aliquot was taken and the assay completed according to the titrimetric procedure described

earlier. The tablet extract containing PSS at a concentration of 1 mg ml^{-1} was then diluted with water to obtain working concentrations of 500 µg ml^{-1} in PSS for spectrophotometric method. A convenient aliquot was then subjected to analysis by spectrophotometric procedure described above.

Placebo Blank Analysis

A placebo blank of the composition: talc (20 mg), starch (10 mg), acacia (15 mg), methyl cellulose (10 mg), sodium citrate (10 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under 'tablets', and then subjected to analysis.

### *Procedure for the Determination of PSS in Synthetic Mixture*

To the placebo blank of the composition described above, 100 mg of PSS was added and homogenized, transferred to a 100 ml standard flask and solution prepared as described under tablets. The solution was mixed well and filtered using a whatman No. 42 filter paper. The resulting solution was assayed (n = 5) by titrimetry according to the same procedure described

above. The synthetic mixture solution (1 mg ml^{-1} in PSS) was then diluted with water to obtain working concentration of 500 µg ml^{-1} in PSS for spectrophotometric method. A convenient aliquot was then subjected to analysis. The analysis was used to study the interferences of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

Results and Discussion

Method Development

The proposed methods are based on the oxidation of PSS molecule by iodate. In titrimetry, the drug was reacted with known excess of iodate, and after oxidation, the residual iodate was determined by iodometric back titration with thiosulphate. In spectrophotometric method, the drug was treated with a large excess of iodate and the iodine released is extracted into chloroform and measured at 520 nm. The measured absorbance of iodine, extracted into $CHCl_3$, formed due to reduction of iodate on its reaction with PSS in H_2SO_4 medium, was found to be proportional to the concentration of drug. The probable reaction mechanism is shown in Fig. 2.
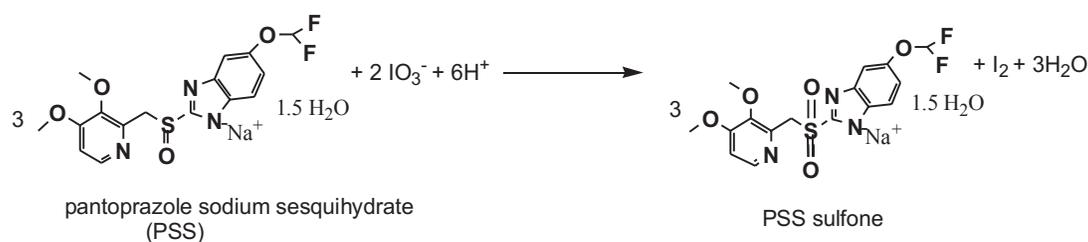
Scheme 1
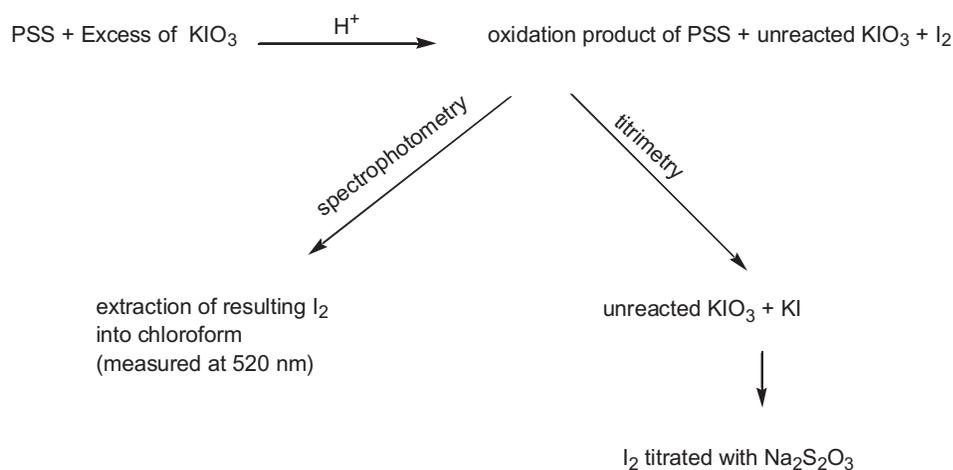
Scheme 2

Fig. 2: Probable reaction schemes

Optimization of Variables

Titrimetry

Potassium iodate was found to react quantitatively with PSS in H_2SO_4 medium. A 5 ml volume of 2 M acid in a total volume of 25 ml was found adequate; although 3-10 ml resulted in the same value of 'n'. Stoichiometric study revealed that three moles of PSS reacted with two moles of iodate.

At laboratory temperature (30 ± 2°C), depending on the amount of drug involved, the time required for the complete oxidation of the drug was 15 min, contact times up to 30 min had no effect on the stoichiometry or the results.

Spectrophotometric Method

Several substances of pharmaceutical interest have been determined by measuring the iodine released in the redox reaction between the substrate and iodate in acid medium [16-23]. Several parameters involved in the redox reaction and subsequent measurement of iodine released, were optimized. To find a suitable reaction medium for the oxidation step, HCl, H_2SO_4 and H_3PO_4 media were investigated, and the reaction was found to be rapid and quantitative in H_2SO_4 medium. For a given concentration of PSS, constant absorbance readings were obtained when the overall concentration was 0.05-0.15 M with respect to H_2SO_4 (Fig. 3). Hence, 1 ml of 2M H_2SO_4 in a total volume of 20 ml aqueous phase with an overall acid concentration of 0.1 M was used in all subsequent work. To study the effect of iodate concentration, a fixed concentration of PSS was reacted with 0.5-5 ml 5% KIO_3 solution in a total volume of 22 ml; maximum and constant absorbance readings were recorded with 2 to 5 ml of iodate solution (Fig. 4).
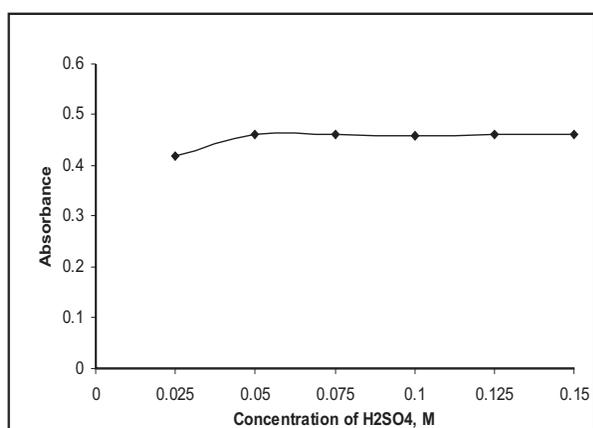
Fig. 3: Effect of H_2SO_4, M (300 µg ml⁻¹ PSS)

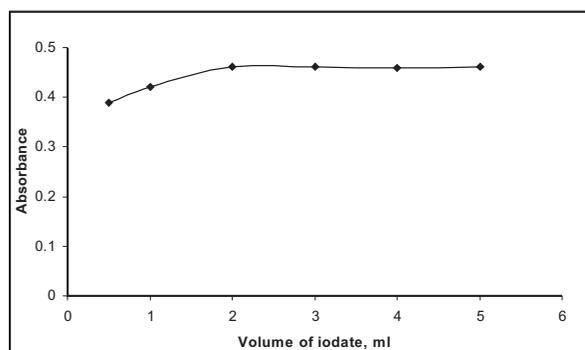**Fig. 4: Effect of KIO_3, 5 % (300 µg ml⁻¹ PSS)**

Hence 2 ml of 5 % KIO_3 solution was fixed as the optimum. The oxidation reaction was complete in 15 min and the absorbance readings were constant when the reaction time was varied from 15 to 45 min (Fig. 5).
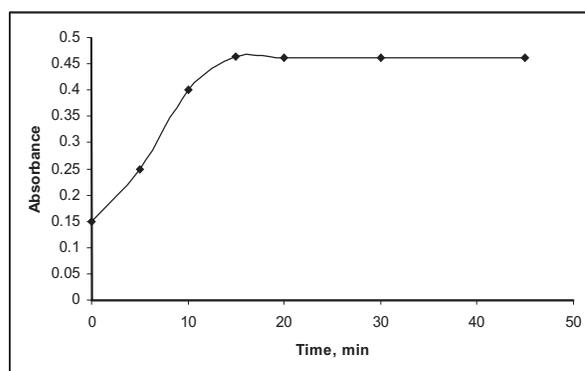
Fig. 5: Effect of reaction time (300 µg ml⁻¹ PSS)

Shaking times from 0.5 to 5 min were used to study the effect of shaking time on the extraction of iodine into the chloroform layer, and 1 min was found adequate for the maximum concentration of PSS employed. Hence, a shaking time of 1 min was used in subsequent work. The separation of aqueous and organic phases was complete in <1 min. several organic solvents such as cyclohexane, carbon tetrachloride, methylene chloride and chloroform were used to extract the iodine and best results with maximum absorbance, clear and quick separation of aqueous and organic phases were achieved with chloroform. A single extraction with 5 ml of chloroform was found sufficient to completely extract the iodine released from the maximum concentration of PSS.

Method Validation Procedures

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and Sensitivity

Over the range investigated (1-10 mg), a fixed stoichiometry of 2:3 (IO_3^- : PSS) was obtained in titrimetry which served as the basis for calculations. In spectrophotometry, under optimum conditions a linear relation was obtained between absorbance and concentration of PSS in the range 25-500 μg ml^{-1} (Fig. 6). The calibration graph is described by the equation:

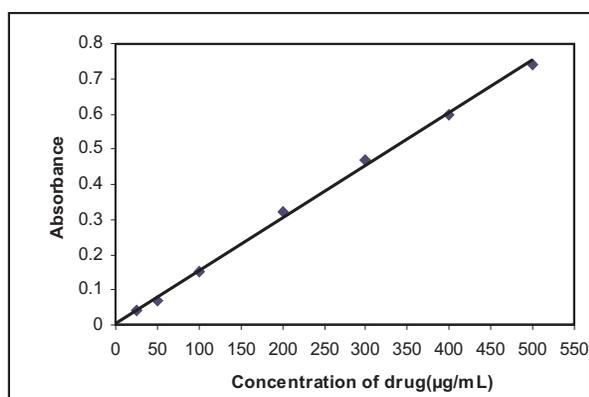**Fig. 6: Calibration curve**

$$Y = a + bX$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in μg ml^{-1}) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and sandell sensitivity values, the limits of detection and quantification are calculated as per the current ICH guidelines [24] are compiled in Table 2 speak of the excellent sensitivity of the proposed method. The limits of detection (LOD) and quantification (LOQ) were calculated according to

the same guidelines using the formulae:

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s$$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

Precision and Accuracy

Intra-day precision and accuracy of the proposed methods were evaluated by replicate analysis (n = 5) of calibration standards at three different concentration levels in the same day. Inter-day precision and accuracy were determined by assaying the calibration standards at the same concentration levels on five consecutive days. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively (Table 3).

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution prepared as described earlier was subjected to analysis by titrimetry and spectrophotometry according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution prepared above yielded percent recoveries which ranged between 98.06 and 103.8 with standard deviation of 0.82-1.16 in all the cases. The results of this study are presented in Table 4 indicating that the

Table 3: Evaluation of intra-day and inter-day accuracy and precision

Method[a]	PSS taken	Intra-day accuracy and precision			Inter-day accuracy and precision		
		PSS found	%RE	%RSD	PSS found	%RE	%RSD
Titrimetry	2.0	1.97	1.5	1.12	1.96	2.00	1.58
	4.0	4.05	1.25	1.36	3.93	1.75	1.76
	6.0	6.10	1.67	1.27	5.92	1.33	2.28
Spectrophotometry	100.0	103.6	3.6	1.58	96.17	3.83	2.63
	200.0	206.0	3.0	1.76	191.7	4.15	1.89
	300.0	311.0	3.7	2.14	291.8	2.73	2.14

RE. elative error and RSD. Relative standard deviation.
[a]In titrimetry, PSS taken/found are in mg and they are μg ml^{-1} in spectrophotometry.

Table 4: Recovery of the drug from synthetic mixture

Method	PSS in synthetic mixture taken[a]	PSS recoverd[b] (Percent ±SD)
Titrimetry	3.0	99.74 ± 0.98
	6.0	101.2 ± 0.82
	9.0	102.7 ± 1.16
Spectrophotometry	100	98.06 ± 1.05
	200	101.5 ± 0.94
	300	103.8 ± 1.11

[a]mg in titrimetry and μg ml^{-1} in spectrophotometry.
[b]Mean value of five determinations

inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods.

Application to Formulations

In order to evaluate the analytical applicability of the proposed methods to the quantification of PSS in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method [7] by applying Student's t-test for accuracy and F-test for precision. The results (Table 5) show that the Student's t- and F-values at 95 %

confidence level are less than the theoretical values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Recovery Studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Preanalysed tablet powder was spiked with pure PSS at three concentration levels (50, 100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In all cases, the added PSS recovery percentage values ranged between 98.7 and 111.2 % with standard deviation of 0.63-1.38 (Table 6) indicating that the recovery was good, and that the co formulated substance did not interfere in the determination.

Conclusions

The methods described in this paper are simple, relatively specific, accurate and precise for the determination of PSS. In particular, the titrimetry is much simpler in technique, more rapid than all the methods reported so far for pantoprazole sodium sesquihydrate. It is applicable over a micro range (1-10 mg), requires inexpensive chemicals, and yet

Table 5: Results of analysis of tablets by the proposed methods

Tablet Brand name	Label claim, mg/tablet	Found[a] (Percent label claim ±SD)		
		Reference method	Titrimetry	Spectrophotometry
Pantodac[b] 20	20	104.5 ± 0.53	103.8 ± 0.81	104.1 ± 1.39
			t= 1.65	t= 0.66
			F= 2.34	F= 0.53
Pantop[c] 40	40	106.3 ± 0.85	105.8 ± 1.08	106.4 ± 1.21
			t= 0.82	t= 0.15
			F= 1.62	F= 2.03
Pantosac[d] 40	40	101.9 ± 0.63	101.2 ± 0.96	102.7 ± 1.19
			t= 1.39	t= 1.39
			F= 2.32	F= 3.57

[a]Mean value of five determinations.
[b]Aristo Pharmaceuticals Ltd., Mumbai, India.
[c]Cipla Ltd, Mumbai, India.
[d]Zy. Alidac, Mumbai, India
The value of t (tabulated) at 95 % confidence level and for four degrees of freedom is 2.77.
The value of F (tabulated) at 95 % confidence level and for four degrees of freedom is 6.39.

Table 6: Accuracy assessment by recovery experiments

Method	Tablet studied	PSS in tablet[a]	Pure PSS added[a]	Total found[a]	Pure PSS recovered[b], Percent±SD
Titrimetry	Pantodac 20	4.04	2.0	6.06	101.0 ± 0.63
		4.04	4.0	8.14	102.5 ± 1.17
		4.04	6.0	10.25	103.5±0.85
	Pantop 40	4.06	2.0	6.13	102.7 ± 1.05
		4.06	4.0	8.03	98.7 ± 0.93
		4.06	6.0	10.20	102.0 ± 0.64
Spectrophotometry	Pantodac 20	104.1	50.0	159.7	111.2 ± 1.2 4
		104.1	100.0	211.0	106.9 ± 1.38
		104.1	150.0	261.2	104.7 ± 1.32
	Pantop 40	106.4	50.0	160.2	107.6 ± 1.26
		106.4	100.0	211.5	105.1 ± 1.13
		106.4	150.0	268.9	108.3 ± 1.17

[a]mg in titrimetry and μg ml^{-1} in spectrophotometry.
[b]Mean value of three measurments.

provides accurate and precise results. The proposed spectrophotometric method employs mild working conditions without heating unlike the reported methods and as sensitive as many reported methods. The methods are also useful due to high tolerance limit for common excipients found in drug formulations. These merits coupled with the use of simple and inexpensive instrument, recommend the use of the methods in routine quality control Laboratories.

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