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SENSITIVE AND SELECTIVE SPECTRO-PHOTOMETRIC DETERMINATION OF PANTOPRAZOLE SODIUM IN PHARMACEUTICALS USING PERMANGANATE

A simple visible spectrophotometric method is described for the determination of pantoprazole sodium sesquihydrate (PSS). The method is based on the formation of a brown colored product on treating PSS with permanganate in neutral medium, the absorbance being measured at 350 nm. The experimental conditions for the assay were optimized. The absorbance is found to increase linearly with the concentration of PSS and the calibration graph is linear in the range of 2.5-40.0 $\mu g\ ml^{-1}$ with a linear regression coefficient of 0.998. The calculated molar absorptivity value is $1.27 \times 10^4\ l\ mol^{-1}\ cm^{-1}$ and the corresponding Sandel sensitivity is 0.0341 $\mu g\ cm^{-2}$. The limits of detection (LOD) and quantification (LOQ) are calculated to be 0.49 and 1.47 $\mu g\ ml^{-1}$, respectively. Intra-day and inter-day accuracy expressed as relative error were better than 2.0% and the corresponding precision (RSD) was less than 2.5 %. The developed and validated method was applied to the determination of the active ingredient in a tablet dosage form and the results obtained agreed well with those of the reference method. The accuracy and reliability of the method were ascertained by performing recovery experiments via standard-addition procedure.

Key words: pantoprazole sodium sesquihydrate; assay; spectrophotometry; permanganate; pharmaceuticals.

Pantoprazole sodium sesquihydrate (PSS) is chemically known as sodium 5-(difluoromethoxy)-2-{[(3,4-dimethoxy-2-pyridinyl) methyl]sulfinyl}-1*H*-benzimidazole sesquihydrate (Fig. 1). It is used as an antiulcerative agent [1] by inhibiting the gastric acid secretion. Pantoprazole sodium sesquihydrate is immensely used for the cure of erosion and ulceration of esophagus caused by a gastroesophageal reflux disease. It is pharmaceutically formulated as gastro-resistant tablets containing 40 or 20 mg pantoprazole sodium sesquihydrate.

PSS is a non-official drug substance and there are only a few reports on the determination of this drug in pharmaceutical substances including a dosage form. A few methods based on HPLC [2-5], densitometric HPTLC [6], derivative UV-spectrophotometry [7] and difference UV-spectrophotometry [8] have been reported for the assay of PSS in commercial dosage forms. Several manipulation steps are involved in some

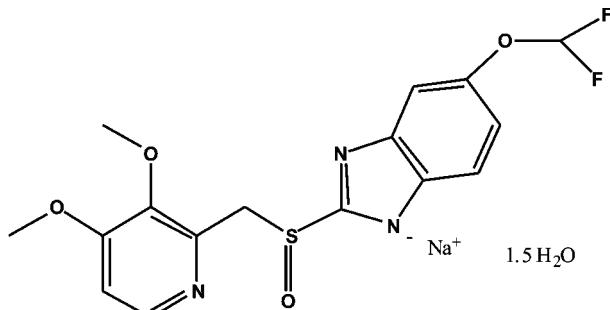of these methods which are not simple for the routine analysis of dosage forms.

Figure 1. The structure of pantoprazole sodium sesquihydrate.

Visible spectrophotometry is still considered to be a very convenient and low-cost technique, and hence widely used for the determination of pharmaceuticals in bulk and dosage forms [9-13]. The reported spectrophotometric methods for PSS [14-18] suffer from one or the other disadvantages such as a narrow linear range of applicability [16], lack of selectivity [17,18], lower sensitivity and for use of organic solvents as the reaction medium/expensive reagents

[14,15] (Table 1). Hence, there is a need to develop a simple, selective and sensitive procedure for the determination of PSS in pharmaceuticals.

The present communication describes the development and validation of a visible spectrophotometric method for the determination of PSS in pure form and in dosage forms. The method involves treating the drug with potassium permanganate in neutral medium and subsequent measurement of the absorbance at 350 nm. Permanganate has earlier been used for the assay of thioxanthines [19], isoniazid [20], methyl thiouracil [21], chloramphenicol [22], amidopyrine [23], valdecoxib [24], nicotine [25], tramodol HCl [26], cefuroxime [27], diloxamide [28] and pentacozine [29], to mention a few, and its use for the determination of PSS in neutral medium has not been reported. The method developed offers the advantages of simplicity, speed, accuracy and precision without the need for costly equipment/chemicals.

EXPERIMENTAL DATAILS

Apparatus

All absorbance measurements were performed using a Systronics model 106 digital spectrophotometer (Ahmedabad, India) provided with 1-cm matched quartz cells.

Reagents and standards

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

Standard PSS solution. Pharmaceutical grade PSS which was certified to be 98.98 % pure was re-ceived as a gift from Cipla India Ltd., Mumbai, India, and used as received. Standard PSS solution (100 µg ml^{-1}) was prepared by dissolving a calculated quantity of a pure drug in water.

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

Potassium permanganate (300 µg ml^{-1}). An approximately 0.02 M solution was prepared by dissolving about 0.80 g of $KMnO_4$ (Merck, Mumbai, India) in water, diluting to 250 ml in a calibrated flask and standardized using H.A Bright's procedure [30]. The stock standard solution was then diluted appropriately with water to get a 300 µg ml^{-1} working concentration.

Procedures

Preparation of calibration graph

Different aliquots of 0.0, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 ml of standard PSS solution (100 µg ml^{-1}) were transferred into a series of 10 ml standard volumetric flasks and the total volume in each flask was adjusted to 5 ml with water. To each flask, 1 ml of 300 µg ml^{-1} $KMnO_4$ was added. The contents of each flask were mixed well and kept aside for 10 min with occasional shaking. The volume was made up to the mark with water and the absorbance was measured at 350 nm *vs.* reagent blank prepared in a similar manner.

The calibration graph was prepared by plotting the increasing absorbance values *vs.* concentrations of PSS. The concentration of the unknown was read from the calibration graph or deduced from the regression equation derived using the Beer's law data.

Table 1. The comparison of the Performance characteristic of the existing spectrophotometric methods with the proposed method

Sl. No.	Reagent(s) used	Methodology	Linear range, µg ml^{-1} (ε / 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.	14,15
2.	a) DDBQ	C-T complex measured at 457 nm.	10-60	Narrow linear range, use of organic solvent.	16
	b) Iodine	C-T complex measured at 293 and 359 nm.	18-142	Measured at shorter wavelength, use of organic solvent.	16
	c) Cu (II)-eosin	Ternary complex measured at 549 nm.	4-26	Narrow linear range, involves liquid-liquid extraction and use of organic solvent medium.	16
3.	BrO_3^--Br^-/MO, IC	Unbleached colour of MO/IC measured at 510/610 nm.	0.12-1.5 (1.8×10^5) 0.5-6.0 (4.1×10^4)	Less selective, accurate concentration of bromate and dyes to be known.	17
4.	BrO_3^--Br^-/Fe(II)--SCN$^-$ or Fe(II)-tiron	Fe(II)-SCN$^-$ complex or Fe(II)-tiron complex measured at 470 or 670 nm.	0.12-1.25 (2.2×10^5) 0.25-2.5 (1.2×10^5)	Less selective.	18
5.	$KMnO_4$ in neutral medium	Brown color measured at 350 nm.	2.5-40.0 (1.27×10^4)	Wide linear dynamic range and high sensitivity.	This work

Procedure for tablets

Twenty tablets were weighed accurately and ground into a fine powder. A quantity of the powder containing 10 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; the volume was diluted to the mark with water and mixed and filtered using a Whatman No. 42 filter paper. The filtrate containing PSS at a concentration of 100 μg ml^{-1} in PSS was subjected to analysis by the 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 "assay of tablets" and then subjected to analysis.

Procedure for the determination of PSS in a synthetic mixture

To the placebo blank of the composition described above, 10 mg of PSS was added and homogenized, transferred to a 100 ml standard flask and the solution prepared as described under "assay of tablets". The solution was mixed well and filtered using a Whatman No. 42 filter paper. The resulting solution containing PSS at the concentration of 100 μg ml^{-1} was assayed (n = 5) by the same procedure described above. The analysis was done to study the interferences of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

RESULTS AND DISCUSSION

In neutral medium, potassium permanganate quantitatively oxidizes PSS resulting in the formation of MnO_2 [31], which is brown in color with an absorption maximum at 350 nm. In a neutral solution, permanganate is only reduced to give MnO_2, wherein Mn is in a +4 oxidation state. The initial study showed that the absorbance of the brown color was linearly dependent on the concentration of PSS. Based on the above observations, a simple spectrophotometric method to the determination of PSS was developed and validated as per the current ICH guidelines [32].

The various experimental parameters affecting the formation of the reaction product were optimized.

The effect of the KMnO₄ concentration

To study the effect of the $KMnO_4$ concentration, varying volumes of 300 μg ml^{-1} $KMnO_4$ (0.25-1.5 ml) were made to react with 4 ml of 100 μg ml^{-1} PSS into

a series of 10 ml volumetric flasks. It is apparent from Fig. 2 that the absorbance increased with increasing the volume of the $KMnO_4$ solution and it became constant at 0.75 ml. Thus, the adoption of 1.0 ml of 300 μg ml^{-1} $KMnO_4$ in the final solution proved to be adequate for the maximum concentration of PSS used in the calibration curve.
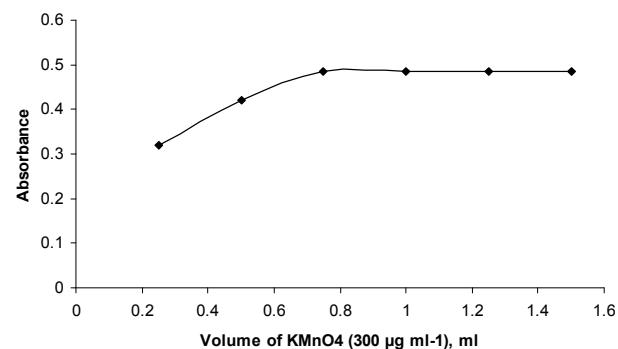

Figure 2. The effect of $KMnO_4$ on absorbance of the reaction product (MnO_2).

The effect of time

To study the effect of time, a fixed concentration of PSS (40 μg ml^{-1}) was made to react with 1 ml of 300 μg ml^{-1} $KMnO_4$ solution; absorbance readings were recorded at different time of 5, 10, 15, 20 and 30 min. The oxidation reaction was completed in 10 min, and the color was stable up to 30 min in the presence of the reaction product(s) (Fig. 3).
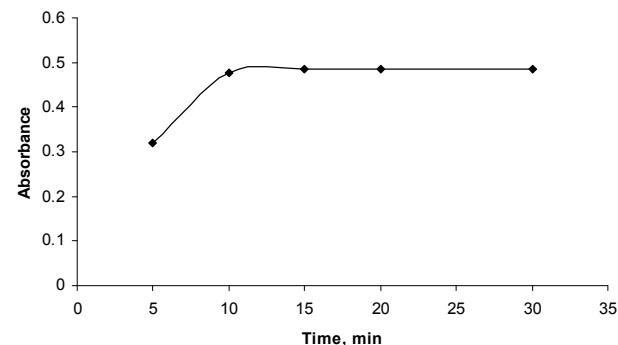

Figure 3. The effect of time on absorbance of the reaction product (MnO_2).

Method validation procedures

The proposed method has been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity

Under optimum conditions, a linear relation was obtained between absorbance and concentration of PSS in the range 2.5-40.0 μg ml^{-1} (Fig. 4). The calibration graph is described by the equation:

$$Y = a + bX$$

where Y = absorbance, a = intercept, b = slope and X = = concentration, obtained by the method of least squares. The 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 calculated as per the current ICH guidelines are compiled in Table 2. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas:

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

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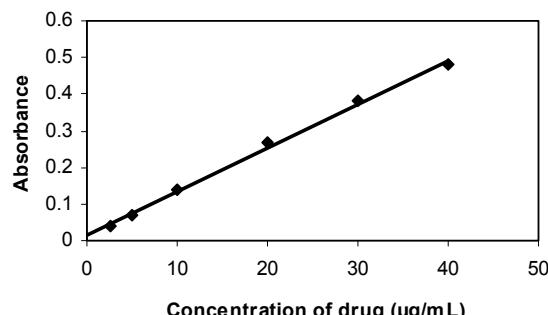

Figure 4. Calibration curve.

Table 2. Sensitivity and regression parameters

Parameter	Method
λ_{max} / nm	350
Linear range, μg ml^{-1}	2.5-40.0
Molar absorptivity(ε), l mol^{-1} cm^{-1}	1.27×10^{4}
Sandell sensitivity[a], μg cm^{-2}	0.0341
Limit of detection (LOD), μg ml^{-1}	0.49
Limit of quantification (LOQ), μg ml^{-1}	1.47
Intercept (a)	0.0172
Slope (b)	0.0119
Standard deviation of a (S_a)	0.0376
Standard deviation of b (S_b)	0.0010
Regression coefficient (r)	0.9983

[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

Precision and accuracy

Intra-day precision and accuracy of the proposed method 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 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 method was 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 according to the recommended procedure. There was no interference by the inactive ingredients.

A separate experiment was performed with the synthetic mixture. The analysis of the synthetic mixture solution prepared above yielded recoveries ranging between 107.27 and 110.51%, with a standard deviation of 1.36-1.47%. The results of this study are presented in Table 4 indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy, as well as the precision of the proposed methods.

Table 4. Recovery of the drug from the synthetic mixture

PSS in synthetic mixture taken, μg ml^{-1}	PSS recovered[a]±SD, %
20.0	110.51±1.47
30.0	108.04±1.36
40.0	107.27±1.42

[a]Mean value of five determinations

Application to formulations

In order to evaluate the analytical applicability of the proposed method to the quantification of PSS in commercial tablets, the results obtained by the proposed method were compared to those of the reference method [5] by applying student's t-test for accuracy and F-test for precision. The reference method involves the HPLC determination of pantoprazole so-

Table 3. Evaluation of intra-day and inter-day accuracy and precision (RE: relative error; RSD: relative standard deviation)

PSS taken, μg ml^{-1}	Intra-day accuracy and precision			Inter-day accuracy and precision		
	PSS found, μg ml^{-1}	RE, %	RSD, %	PSS found, μg ml^{-1}	RE, %	RSD, %
10.0	9.90	1.00	1.15	9.79	2.10	1.28
20.0	19.53	2.34	1.26	19.65	1.75	1.37
30.0	29.36	2.13	1.74	29.60	1.33	1.42

dium in pharmaceuticals with UV-detection at 289 nm. 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 method and the reference method with respect to accuracy and precision.

Recovery studies

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed 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 method. The added PSS recovery percentage values ranged between 104.27 and 111.8% with the standard deviation of 1.04-1.48% (Table 6) indicating that the recovery was good and that the co formulated substance did not interfere with the determination.

CONCLUSIONS

A new method which is selective and sensitive has been developed and appropriately validated for the assay of pantoprazole sodium sesquihydrate in pharmaceuticals. The proposed method was found to be superior to some of the reported spectrophotometric methods with respect to simplicity, sensitivity, selectivity, a linear dynamic range of applicability and cost-effectiveness. In addition, the method is bestowed with twin advantages of simplicity of operations and

the possibility of carrying them out with the common laboratory instrument. Since the proposed method requires the use of only one reagent ($KMnO_4$), it certainly is the most cost-effective of all the existing spectrophotometric methods.

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Table 5. The results of the analysis of tablets by the proposed method

Tablet brand name	Label claim, mg/tablet	Found[a] (label claim ± *SD*), %	
		Reference method	Proposed method
Pantodac[b] 20	20	98.46 ± 0.78	99.55 ± 1.78 *t* = 1.35[c] *F* = 5.21[d]
Pantop[e] 40	40	103.5 ± 0.63	104.1 ± 1.24 *t* = 0.77 *F* = 3.87

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

Table 6. The accuracy assessment by recovery experiments

Tablet studied	PSS in tablet, µg ml⁻¹	Pure PSS added, µg ml⁻¹	Total found, µg ml⁻¹	Pure PSS recovered[a]±*SD*, %
Pantodac 20	9.96	5	15.55	111.80 ± 1.35
	9.96	10	20.87	109.10 ± 1.48
	9.96	15	25.98	106.80 ± 1.24
Pantop 40	10.41	5	15.80	107.80 ± 1.04
	10.41	10	20.96	105.50 ± 1.13
	10.41	15	26.05	104.27 ± 1.29

[a]Mean value of three measurements

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NAUČNI RAD

OSETLJIVO I SELEKTIVNO SPEKTROFOTOMETRIJSKO ODREĐIVANJE NATRIJUM PANTOPRAZOLA U FARMACEUTSKIM PROIZVODIMA KORIŠĆENJEM PERMANGANATA

Određivanje pantoprazolnatrijum-seskvihidrata (PSS) je izvršeno korišćenjem jednostavne vidljive spektrofotometrijske metode. Metoda je bazirana na formiranju braon obojenih proizvoda prilikom tretiranja PSSS sa permanganatom u neutralnoj sredini. Apsorbanca je merena na 350 nm. Eksperimentalni uslovi analize su optimizovani. Apsorbanca se linearno povećava sa povećanjem koncentracije PSS, a kalibracioni dijagram je linearan u opsegu 2,5 do 40,0 μg ml^{-1}, sa koefeicijentom linearne regresije od 0,998. Vrednost molarne apsorpcije je 1,27×10^4 l mol^{-1} cm^{-1} i odgovara Sandel-ovoj osetljivosti od 0,0341 μg cm^{-2}. Limiti detekcije (LOD) i kvantifikacije (LOQ) iznose 0,49 i 1,47 μg ml^{-1}, redom. Intra-dnevna i inter-dnevna tačnost izražene kao relativna greška su bolje od 2,0%, a odgovarajuća preciznost (RSD) je manja od 2,5%. Razvijena i validovana metoda je primenjena za određivanje aktivne komponente u dozama tableta i dobijeni rezultati su u skladu sa onim dobijenim referentnom metodom. Tačnost i pouzdanost metode su potvrđene izvođenjem eksperimenata standardnom metodom.

Ključne reči: pantoprazolnatrijum seskvihidrat; analiza; spektrofotometrija; permanganat; farmaceutski proizvodi.