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Initial-rate method for the determination of pantoprazole in pharmaceutical formulations using 1-fluoro 2,4-dinitrobenzene

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A simple and selective kinetic spectrophotometric method for the determination of pantoprazole in pharmaceutical preparations is described. The procedure is based upon a kinetic investigation of the reaction of the drug with 1-fluoro-2,4-dinitrobenzene in DMSO at room temperature. The absorbance of the coloured product was measured at 420 nm. The plot of the logarithm of the initial rate of the reaction vs. the logarithm of molar concentration of pantoprazole is linear over the range 10–20 $\mu g \cdot ml^{-1}$. The procedure retains its accuracy in the presence of a large excess of its degradate, sulfenic acid, which is prepared by degradating the pure drug in borate buffer of pH 8 at room temperature for seven days. The results are validated statistically and through recovery studies. The method has been successfully applied to the determination of pantoprazole in commercial tablets. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

1. Introduction

Pantoprazole, 5-difluromethoxybenzimidazole-2-yl 3,4-dimethoxy-2-pyridylmethyl sulphoxide, is an irreversible proton pump inhibitor which has been developed for the treatment of acid-related gastrointestinal disorders.

Literature survey reveals few HPLC (Huber et al. 1990; Mansour and Sorour 2001; Cass et al. 2001) methods for the determination of the drug in biological fluids and tablet formulations. Chiral resolution of pantoprazole and the related sulphoxide by capillary zone electrophoresis (Eberle et al. 1997) has been reported. A voltammetric method (Radi 2003) was described for the determination of pantoprazole by differential pulse adsorptive stripping voltammetry at a carbon paste electrode. Under optimised conditions, the current showed a linear dependence with concentration in the range 1.0×10^{-7}–1.0×10^{-5} M and the detection limit was 2.0×10^{-8} M. Recently spectrophotometric procedures for the determination of pantoprazole have been developed: two methods based on charge transfer complexation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and iodine and third method depending on ternary complex formation with eosin and copper(II) (Moustafa 2000). The drug is quantified by a stability indicating procedure through chelation with Fe(III) in aqueous-ethanol medium to form an orange chelate which absorbed maximally at 455 nm (Salama et al. 2003). Beer's law was obeyed in the concentration range of 30–300 $\mu g \cdot ml^{-1}$. A first-order UV-derivative spectrophotometry using zero-crossing method (Rajic et al. 2003) was developed for the determination of pantoprazole in methanol-ammonia (4.0 v/v) where sufficient spectra resolutions of drug and its impurity were obtained. A compensation method (Wahbi et al. 2002) and other chemometric methods such as derivative, orthogonal function and difference spectrophotometry for direct determination of pantoprazole in dosage forms have been reported.

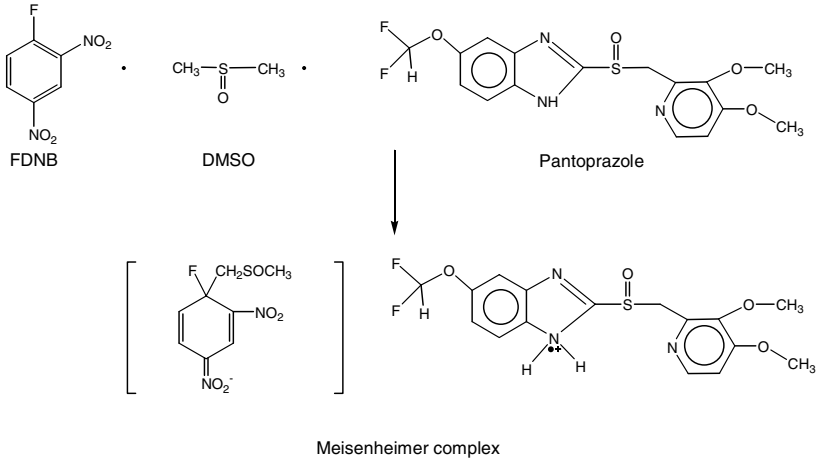
This paper describes a stability-indicating kinetic spectrophotometric method based on the reaction of the pantoprazole with 1-fluoro-2,4-dinitrobenzene (FDNB) in dimethylsulphoxide (DMSO) medium.

2. Investigations, results and discussion

A coloured product was obtained when FDNB was added to pantoprazole in DMSO medium, which absorbed maximally at 420 nm. At room temperature, the reaction was slow and more than 1 h was required to attain the maximum absorbance. Therefore, a kinetically based method was developed to quantify the drug. The method has been extended to the determination of intact drug in the presence of its degradation product.

Pantoprazole was degraded completely in borate buffer of pH 8 at room temperature after seven days to yield two degradation products; a sulfinamide and a sulfenic acid derivative. The sulfinamide is insoluble in aqueous media and is separated by filtration for soluble sulfenic acid derivative. These degradation products were tested by TLC using silica gel G plate and chloroform-methanol (10 : 0.4) as mobile phase. The spots were detected under an UV lamp at 366 nm. Each degradate gave a single spot at Rf values of 0.75 and 0.26 for sulfinamide and sulfenic acid, respectively. The Rf value of the intact drug was 0.54.

Scheme 1



To study the effect of the concentration of FDNB on the initial rate of reaction, varying volumes of 0.2% reagent were mixed with a fixed amount of pantoprazole ($16\ \mu g \cdot ml^{-1}$) in a 5 ml volumetric flask. The absorbance of each solution was measured at a fixed time of 35 min. The absorbance increased with increasing volume and became constant at 0.8 ml; above this volume the absorbance remained unchanged. Therefore, 1.0 ml was used throughout the experiment.

The stoichiometry of FDNB to pantoprazole was established by a limiting logarithmic method (Roso 1964). For this two sets of experiments were performed. In the first set, the concentration of the drug was varied while keeping a constant concentration of the FDNB. In the second set, the concentration was kept constant while varying the concentration of FDNB. The combining ratio was calculated from the slopes of the plots of logarithm of absorbance vs. logarithm of the respective concentration and found to be 1 : 1 between pantoprazole and FDNB.

Interesting colours are produced on addition of base to a polynitro aromatic which have been attributed to a variety of interactions (Buncel et al. 1968; Crampton 1969; Strauss 1970). In this study, pantoprazole acts as a base when it is added to FDNB. A proton transfer from FDNB to base is apparently responsible for colour formation. The early study has shown that DMSO stabilises the conjugation base of FDNB derivative and hence, a Meisenheimer complex is proposed for this colour formation (Scheme 1).

The rate of reaction was found to be dependent on pantoprazole concentration. The rates were followed at room temperature with different drug concentrations (Fig.). The initial rates of the reaction were obtained from the slope of the initial tangents to the absorbance-time curves, which indicated that the initial rate increases with increasing pantoprazole concentration.

The order of the reaction with respect to the pantoprazole concentration was evaluated from the plot of logarithm of initial rate vs. logarithm of molar concentration of pantoprazole and found to be one.

Under the established experimental conditions, the quantitative determination of pantoprazole in the presence of a large excess of FDNB results in a pseudo-zero order condition with respect to the reagent concentration. However, the initial rate follows a pseudo first order reaction and

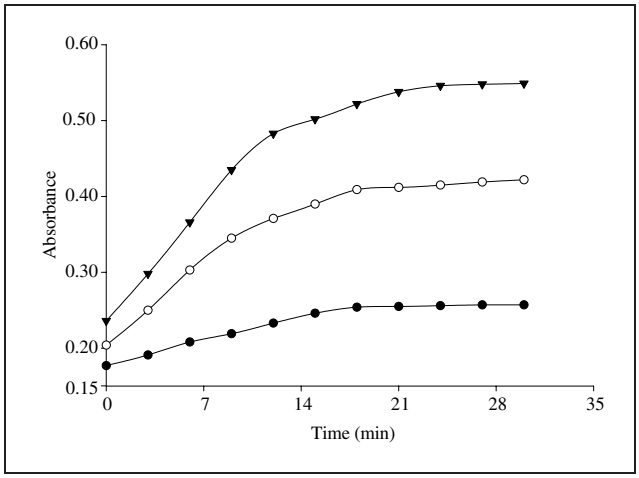


Fig.: Absorbance vs. time graph for the reaction between pantoprazole and FDNB, showing the dependence of the reaction on pantoprazole concentration (●) 2.608×10^{-5} M (o) 4.173×10^{-5} M (▼) 5.216×10^{-5} M.

obeys the equation

$$\text{rate} = \Delta A/\Delta t = k' C^n \quad (1)$$

where k' is the pseudo-order rate constant, C is the concentration of pantoprazole , n is the order of reaction.

The above equation can be written in logarithmic form as:

$$\log (\text{rate}) = \log k' + n \log C \quad (2)$$

The regression analysis using the method of least square was performed to estimate the slope, intercept and correlation coefficient. Under the established experimental condi-

Table 1: Determination of pantoprazole in laboratory prepared mixtures with its degradate, sulfenic acid derivative, by the proposed method

Intact-taken ($\mu g \cdot ml^{-1}$)	Degraded taken ($\mu g \cdot ml^{-1}$)	Found ($\mu g \cdot ml^{-1}$)	Recovery of intact (%)
10	80	10.002	100.02
10	120	10.00	100.00
10	160	9.98	99.82
10	280	9.95	99.51
10	320	10.01	100.10
10	400	10.02	100.25

Table 2: Intra day assay: evaluation of accuracy and precision of the proposed method

Amount ($\mu g \cdot ml^{-1}$)			
Taken	Found ± SD	Recovery ± RSD (%)	SAE[a]
12	11.86 ± 0.10	98.84 ± 0.85	0.041
16	16.04 ± 0.08	100.25 ± 0.52	0.034
20	19.90 ± 0.12	99.52 ± 0.60	0.049

[a] Standard analytical error

Table 3: Inter day assay: evaluation of accuracy and precision of the proposed method

Amount ($\mu g \cdot ml^{-1}$)			
Taken	Found ± SD	Recovery ± RSD (%)	SAE[a]
12	11.88 ± 0.07	99.01 ± 0.61	0.029
16	15.86 ± 0.09	99.14 ± 0.60	0.038
20	19.80 ± 0.14	98.98 ± 0.71	0.057

[a] Standard analytical error

tions, a calibration graph was constructed by plotting log initial rate vs. log molar concentration of pantoprazole showing a linear response over the concentration range of $10-20\ \mu g \cdot ml^{-1}$.

The regression analysis yielded the following regression equation,

$$\log (\text{rate}) = 3.570 + 1.222 \log C \qquad (3)$$

with a correlation coefficient of 0.9979.

The confidence limit for the intercept value at 95% confidence level was calculated using the relation $a \pm t\ S_a$ and was found to be $3.570 \pm 2.37 \times 10^{-2}$ which pointed towards a high reproducibility of the method. The limit of detection (Morelli 1983) and variance were evaluated and found to be $1.65 \times 10^{-2}\ \mu g \cdot ml^{-1}$ and 1.113×10^{-4}, respectively. The small value of variance confirmed the negligible scattering of the calibration data point around the line of the regression.

Table 6: Interval hypothesis: comparison of the results of the proposed method with a reference method

Formulation	θ_L	θ_U
PAN–40	0.990	1.004
Pantodac®	0.994	1.009
Pantop®	0.988	1.003
Pantocid®	0.982	1.011

The selectivity of the proposed method for pantoprazole was checked by direct determination of the intact drug in presence of its degradate, sulfenic acid, in different amounts. The percent recovery (Table 1) is quite satisfactory and thus the proposed method can be used for stability indication.

The accuracy and precision of the proposed method was evaluated by determining the pantoprazole content of a pure sample six times within one day (Table 2). The interday precision was measured by assaying the pure sample on five consecutive days and the results are summarised in Table 3. The Standard deviation, relative standard deviation and standard analytical errors encountered in intraday and interday assays can be considered to be very satisfactory.

The developed method was successfully applied to the assay of pantoprazole in tablets; the results are given in Table 4. The same batch tablets were also analysed by the reference method (Moustafa 2000). The results of the proposed method were compared with those of the reference method using point hypothesis. It is apparent form Table 4 that the calculated t- and F-values are less than

Table 4: Comparison of proposed method with a reference method

Formulation	Labelled amount (mg)	Proposed method		Reference method		t-value[b]	F-value[b]
		Recovery[a] (%)	RSD[a] (%)	Recovery[a] (%)	RSD[a] (%)		
PAN – 40	40	99.66	0.78	99.95	0.45	0.7829	2.96
Pantodac®	40	100.04	0.84	99.89	0.48	0.3597	3.08
Pantop®	40	99.78	0.80	100.23	0.61	1.089	1.74
Pantocid®	40	99.85	0.70	100.18	0.39	1.006	3.06

[a] Average of six independent analyses.
[b] Theoretical t-value and F-value at 95% confidence level are 1.812 and 5.05, respectively.

Table 5: Standard addition method for the determination of pantoprazole in dosage forms

Formulation	Amount ($\mu g \cdot ml^{-1}$)			Recovery ± RSD, (%)	SAE[a]
	Taken	Added	Found ± SD		
PAN – 40	5	5	9.89 ± 0.15	98.85 ± 1.56	0.063
	6	10	16.01 ± 0.08	100.0 ± 0.50	0.033
	12	6	17.99 ± 0.15	99.99 ± 0.83	0.061
Pantodac®	5	5	9.97 ± 0.08	99.71 ± 0.87	0.035
	6	10	16.00 ± 0.16	100.01 ± 1.06	0.069
	12	6	17.98 ± 0.11	99.92 ± 0.62	0.045
Pantop®	5	5	9.98 ± 0.07	99.87 ± 0.74	0.030
	6	10	15.96 ± 0.11	99.77 ± 0.69	0.450
	12	6	18.02 ± 0.12	100.13 ± 0.69	0.050
Pantocid®	5	5	9.97 ± 0.12	99.69 ± 1.28	0.052
	6	10	15.98 ± 0.10	99.93 ± 0.66	0.043
	12	6	18.04 ± 0.13	100.27 ± 0.75	0.055

[a] Standard analytical error

the theoretical ones at a 95% confidence level, which showed no significant difference with regard to accuracy and precision. The reliability and accuracy of the proposed method was further confirmed by a recovery study through the standard addition method. A known amount of the pure drug was added to the preanalysed tablets at three different levels and the total amount was determined by the proposed method. Each level was repeated six times using four different market formulations. The percent recoveries and RSD (Table 5) indicated that the commonly encountered tablet excipients did not interfere with the determination.

The interval hypothesis tests (Hartman et al. 1995) have also been carried out for comparison of the results obtained by proposed and reference methods at a 95% confidence level (Table 6). For pharmaceutical analysis, a bias of $\pm 2\%$ is acceptable and thus the limit of acceptance interval is within $\theta_L = 0.98$ and $\theta_U = 1.02$. Table 6 shows that the true bias of all samples is smaller than $\pm 2\%$.

3. Experimental

3.1. Apparatus

Spectral runs and absorbance were recorded on Spectronic 20 D^+ spectrophotometer (Milton Roy, USA) with matched glass cuvettes.

3.2. Material and reagents

Pantoprazole sodium sesquihydrate was kindly provided by Concept Pharmaceuticals, India Ltd. Its purity was checked by TLC and melting point (Merk Index 1996).

Commercial preparations of pantoprazole were purchased from the local market. All other reagents used were of analytical reagent grade.

3.3. Standard solutions

FDNB solution, 0.2% (v/v), was prepared in DMSO. It was protected from light and stored in refrigerator. Standard pantoprazole solution ($0.25\ mg \cdot ml^{-1}$) was prepared in DMSO.

Laboratory-degraded pantoprazole solution: 100 mg of pantoprazole was dissolved in 200 ml of borate buffer (pH 8) and kept at room temperature ($\cong$ 30 °C) for 7 days. The reddish brown precipitate of sulfinamide degradate was filtered and the filtrate was evaporated to dryness under vacuum. The residue was extracted three times with 20 ml ethanol. The combined ethanolic extracts were evaporated to dryness under vacuum and the residue was dissolved in distilled water and diluted to 50 ml. The resulting solution was labeled to contain the sulfenic acid degradate obtained from $2\ mg \cdot ml^{-1}$ of pantoprazole. This degradate solution was examined by TLC using silica gel and a mobile phase of chloroform-methanol (10 : 0.4) which confirmed that there was no undegraded pantoprazole.

3.4. General procedure

An aliquot from DMSO stock pantoprazole solution ($0.25\ mg \cdot ml^{-1}$) corresponding to 50–100 µg was pipetted into a 5 ml volumetric flask, followed by 1 ml of 0.2% FDNB solution and diluted to volume with DMSO. The mixture was shaken well and immediately transferred to spectrophotometric cell. The absorbance was measured at 420 nm as a function of time against the reagent blank. The initial rates of the reaction at different concentrations were obtained from the slope of the initial tangent to the absorbance-time curves.

The calibration curve was constructed by plotting the logarithm of initial rate of reaction vs. the logarithm of molar concentration of pantoprazole. The amount of the drug was computed either from the calibration curve or regression equation.

3.4.1. Determination of mixtures of intact and degraded pantoprazole

Aliquots of laboratory-degraded solution corresponding to 0.2–2.0 mg degraded pantoprazole were transferred into a series of 5 ml volumetric flasks. To each flask, 0.2 ml of pure pantoprazole ($0.25\ mg \cdot ml^{-1}$) prepared in DMSO was added and mixed well. The amount of pantoprazole was determined by the proposed method.

3.4.2. Procedure for the determination of pantoprazole in commercial dosage forms

Ten tablets were accurately weighed and finely powdered. The amount of the powder equivalent to 50 mg pantoprazole was dissolved in about 40 ml DMSO. Filtration was carried out through Whatman filter paper No. 42 and the filtrate was diluted to 100 ml with DMSO. It was further diluted according to the need. The assay was completed by following the recommended procedure.

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