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COMMUNICATION

Simultaneous Determination of Pyridoxine Hydrochloride and Doxylamine Succinate from Tablets by Ion Pair Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

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ABSTRACT

A new, simple, precise, and rapid ion pair reversed-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous determination of pyridoxine hydrochloride (PYR) and doxylamine succinate (DOX) in tablets. The stationary phase was a Microbondapak C18 column (10 μ, 300 mm × 3.9 mm i.d.). The mobile phase was water : methanol (60 : 40) containing 10 mM heptanesulfonic acid and 0.25% triethylamine and adjusted to pH 2.2 with orthophosphoric acid. Detection was carried out at 263 nm using an ultraviolet (UV) detector. The flow rate was 1.0 ml/min, and retention times were 3.65 min and 7.32 min for PYR and DOX, respectively. The linearity was obtained in the concentration range 0.5–500 μg/ml for PYR and DOX. Mean percentage recoveries were 100.20% and 101.20% for PYR and DOX, respectively.

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INTRODUCTION

Pyridoxine hydrochloride (PYR) is chemically 3-hydroxy-4,5-bis(hydroxymethyl)-2-picoline hydrochloride. It is a water-soluble vitamin and is involved principally in amino acid, carbohydrate, and fat metabolism (1). It is official in the IP (2), BP (3), and USP (4).

Doxylamine succinate (DOX) is chemically *N*,*N*-dimethyl-2-[α-methyl-α-(2-pyridyl)benzyloxy]ethylamine hydrogen succinate. It is an antihistamine with antimuscarinic and pronounced sedative effects (1). It is official in the USP (4).

Various methods for simultaneous determination of PYR in combination with isoniazide by high-performance thin-layer chromatography (HPTLC) (5), spectrophotometry (6), and high-performance liquid chromatography (HPLC) (7); in combination with riboflavine and thiamine by fluorimetery (8); in combination with maclozin hydrochloride and caffeine by spectrophotometry (9); and in combination with metronidazole by HPLC (10) are reported in the literature.

Simultaneous determination of DOX in combination with carbinoxamine maleate by spectrophotometry (11), in combination with dextromethophan hydrobromide by HPTLC (12), and in combination with pseudoephedrine hydrochloride and dextromethophan hydrobromide by HPLC (13) are reported in the literature. However, simultaneous determination of PYR and DOX by HPLC has not been reported. In this paper, we report a new, simple HPLC method for simultaneous determination of PYR and DOX in tablets.

EXPERIMENTAL

Instrumentation

A liquid chromatographic system comprised of a Rheodyne injector, ternary gradient pump (9012), and ultraviolet (UV) detector (9050) connected to star chromatography workstation software (version 4.51) for processing the data generated and a photodiode array (PDA) detector (9065) for determining peak purity were used. This system was supplied by Varian (Walnut Creek, CA).

Reagents and Chemicals

Reference standards for PYR were obtained from USV Limited (Mumbai, India), and the reference standard for DOX was procured from Sigma Laboratories, Mumbai, India. These standards were tested per the USP monograph and found to be 99.71% and 99.35 % pure, respectively. HPLC-grade methanol, heptanesulfonic acid sodium salt, and AR-grade triethylamine and orthophosphoric acid were supplied by S.D. Fine Chemicals Limited (Thane, India). Tablets containing PYR and DOX were procured from the market.

Chromatographic Conditions

The mobile phase consisted of a mixture of water : methanol (60 : 40, v/v) containing 10 mM heptanesulfonic acid and 0.25% triethylamine adjusted to pH 2.2 with orthophosphoric acid. A Microbondapak C18 column (10 μ, 300 mm × 3.9 mm) (Waters, Milford, MA) was used as stationary phase. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using a UV detector at 263 nm.

Standard Stock Solution

A combined standard stock solution of PYR and DOX was prepared by dissolving accurately weighed 50 mg of each standard in 100 ml of mobile phase (0.5 mg/ml of PYR/DOX).

Working Standard Solution

Standard stock solution (1 ml) was diluted to 10 ml with mobile phase (50 μg/ml of PYR/DOX) and was used as the working standard for assay analysis.

Sample Solution

Twenty tablets were weighed and crushed to fine powder. An accurately weighed portion of the powder equivalent to 50 mg of PYR and 50 mg of DOX was put in a 100-ml volumetric flask, about 80 ml of mobile phase was added to it, and the flask was kept in an ultrasonic bath for 10 min. This solution was then diluted to the mark with mobile phase and filtered through Whatman no. 42 filter paper, and 1 ml of the filtrate was diluted to 10 ml with the mobile phase and used for assay analysis.

Calibration

Aliquots of standard stock solution of PYR and DOX were taken in different standard volumetric flasks and diluted with mobile phase to obtain the final concentration in the range 0.5–500 μg/ml of PYR and DOX. Then, 20 μl of each solution was injected into the chromatograph. Peak areas were recorded for all the chromatograms. A calibration curve was constructed by plotting peak areas

(*Y* axis) against the amount of drug in micrograms/milliliter (*X* axis), and the linear relationship was evaluated by calculation of the regression line by the method of least squares.

Assay

Twenty µl each of working standard and sample solution were injected into the chromatograph, and the peak areas were recorded. The amount of PYR/DOX was computed by external standard quantification as given below.

$$\text{Amt. PYR/DOX per tablet} = (Aspl \times C \times D \times \text{Avg. wt.})/(Astd \times W)$$

where *Aspl* is the area of PYR/DOX in sample solution, *Astd* is the area of PYR/DOX in standard solution, *C* is the concentration of standard, *D* is the dilution factor, and *W* is the weight of powdered tablet used.

RESULTS AND DISCUSSION

Chromatography

Reversed-phase liquid chromatography alone with mobile phases of methanol and water used in different combinations could not retain PYR satisfactorily. Any attempt to retain PYR for a longer time by increasing the aqueous content of the mobile phase resulted in very high retention times for DOX.

The use of an ion pairing agent, sodium salt of heptanesulfonic acid, helped to achieve a longer retention for PYR, but tailing factors for both the peaks (i.e., PYR and DOX) were high. Therefore, a study was carried out by addition of small amounts of triethylamine in the mobile phase and adjusting the pH of the mobile phase to 2.2. The addition of 0.25% triethylamine and adjusting the pH to 2.2 could help in solving the tailing factor problem.

PYR and DOX were well resolved in a reasonable time (10 min). The resolution between PYR and DOX was 10.0. A typical chromatogram is shown in Fig. 1. The dilution of analytes with mobile phase helped to minimize the peaks that appeared as a result of the diluent and facilitated quantification of PYR.

The wavelength of 263 nm was selected for the UV detection because at this wavelength there was maximum overlap of the spectra of PYR and DOX. The peak purity of the peak due to PYR and DOX was tested using a PDA detector and were found to be pure.

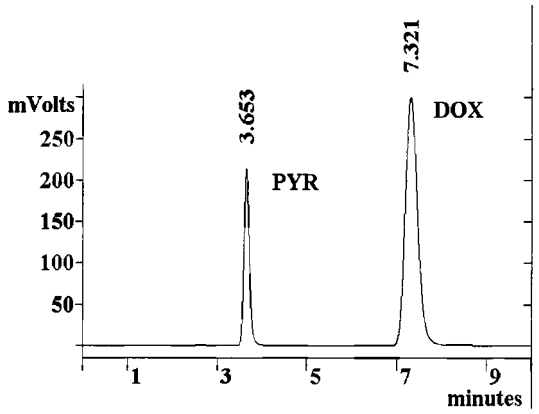

Figure 1. Typical chromatogram of PYR and DOX.

System Suitability

To ascertain the effectiveness of the system suitability test, six replicate injections of freshly prepared working standard solution of PYR and DOX (50 µg/ml) were injected into the chromatograph, and the relative standard deviation (RSD) of peak areas was calculated. System suitability parameters such as tailing factor, resolution factor, and theoretical plates applied to a typical chromatogram are given in Table 1.

Linearity, Limit of Detection, and Limit of Quantification

The plot of the peak area versus the respective concentration of PYR and DOX was found to be linear in the range 0.5 µg/ml–500 µg/ml. The calibration curve is shown in Fig. 2, and it could be represented by the following linear regression equations.

$$y \text{ PYR} = 3398.1x + 7399.9 \qquad (r = .9999)$$

$$y \text{ DOX} = 11135x + 12320 \qquad (r = .9999)$$

Table 1

System Suitability Parameters

No.	Parameters	PYR	DOX
1	Theoretical plates	4563	3446
2	Resolution factor	—	10.0
3	Tailing factor	1.21	1.21
4	RSD (%)	0.55	0.55

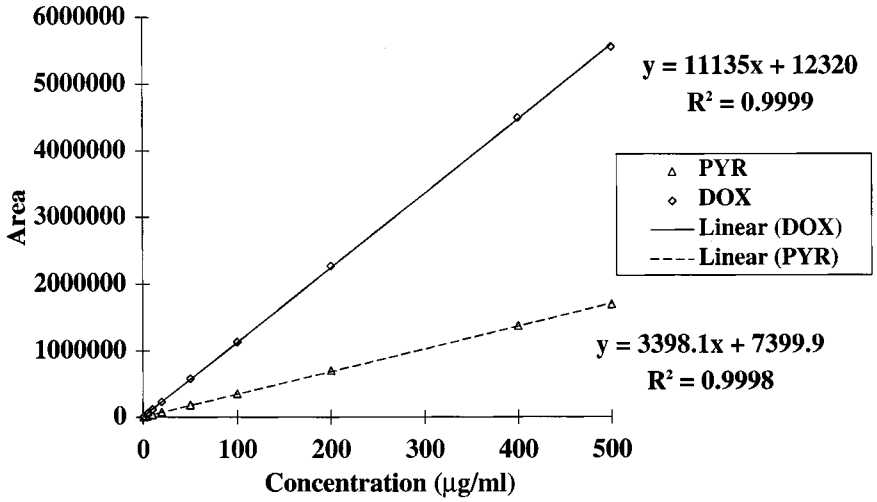

Figure 2. Linearity of PYR and DOX.

where *y* is the area, *x* is the concentration in micrograms per milliliter, and *r* is the correlation coefficient.

The limit of detection (LOD) and the limit of quantification (LOQ) of PYR and DOX were calculated using equations given in the International Conference on Harmonization (ICH) guideline (14).

$$LOD = 3.3 \times \sigma/S; \qquad LOQ = 10 \times \sigma/S$$

where σ, the noise estimate, is the standard deviation of the blank responses (10 injections), and *S* is the slope of the corresponding calibration curve of the drug. The LOD and LOQ were found to be 0.011 μg/ml and 0.007 μg/ml, respectively, for PYR and 0.04 μg/ml and 0.02 μg/ml, respectively, for DOX.

Assay

The contents of PYR and DOX found in the commercial brand of tablets by the proposed method are shown in Table 2. The low values of RSD indicate that the method is precise and accurate.

Accuracy and Precision

To confirm the accuracy and the precision of the proposed method, recovery experiments were carried out by the standard addition technique. Three different levels of standards were added to preanalyzed tablet samples, and each level was repeated three times. The mean percentage recoveries of PYR and DOX were 100.20% and 101.20%, respectively. The results are shown in Table 3, which indicates that the method is accurate and precise, and also that there is no interference due to the excipients present in the tablets.

Stability of the Sample Solution

Sample solution injected after 12 hr did not show any appreciable change in assay value.

Table 2

Assay of PYR/DOX in Tablets

	PYR			DOX		
Brand	Label Claim (mg/tablet)	Amount Found[a] (mg/tablet)	RSD (%) ($n = 6$)	Label Claim (mg/tablet)	Amount Found[a] (mg/tablet)	RSD (%) ($n = 6$)
Doxinate tablets[b]	10	10.40	0.70	10	10.20	0.33

[a] Average of six experiments.
[b] Manufactured by Sigma Laboratories Limited, B.No. DX 805, manufactured date, February 1998.

Table 3

Results of Recovery Analysis

Brand Parnozine Tablets	Amount of Drug From Tablets (mg)	Amount of Drug Added (mg)	Total Amount of Drug (mg)	Amount Found (mg)	% Recovery
PYR	40	0	40.00	40.00	100.00
	40	3.50	43.5	43.50	100.00
	40	13.50	53.5	53.70	100.40
	40	18.50	58.5	58.80	100.50
Average					100.20
DOX	40	0	40.00	40.90	102.25
	40	3.50	43.50	44.10	101.40
	40	13.50	53.50	54.20	101.30
	40	18.50	58.50	58.40	99.80
Average					101.20

Stability-Indicating Ability

The stability study is an integral part of pharmaceutical product development. The data collected during the stability study decide the shelf life, storage conditions, and impurity profile of the product. If the assay method itself is stability indicating, it is considered to be versatile. Therefore, an attempt has been made to investigate the stability-indicating ability of the proposed method. The powdered samples of tablets equivalent to 12.5 mg of PYR and DOX were kept at 80°C, in 1 ml of 0.1N HCl, in 1 ml of 0.1N NaOH, and in 1 ml of 30% H_2O_2 separately for 8 days. Simultaneously, 12.5 mg of PYR, 12.5 mg of DOX, and a mixture of 12.5 mg of PYR and 12.5 mg of DOX standards were exposed to the above stress conditions. All the exposed standards and tablet samples then were analyzed by the proposed method.

In the chromatogram of samples exposed to heat, alkali, and acid, no degradation peak was observed, and the assay value was not changed significantly for both the ingredients. However, in the case of samples exposed to a strong oxidizing agent, assay values of both the ingredients were found to be decreased significantly. These results are given in Table 4. Peaks due to PYR/DOX in the chromatogram of all exposed samples were also investigated using a PDA detector and were found to be pure.

CONCLUSION

The proposed method is simple, precise, accurate, and rapid for the simultaneous determination of PYR and

Table 4

Assay Results of Samples Exposed to Stress Conditions

	Amount of Drug Found (mg/tablet)	
Degradation with Respect to	PYR	DOX
Initial (unexposed sample)	10.41	10.08
Heat at 80°C for 8 days	10.41	10.15
Acidic condition (0.1 N HCl) for 8 days	10.38	10.90
Strong oxidizing condition (30%H_2O_2) for 8 days	5.22	5.81
Alkaline condition (0.1 N NaOH) for 8 days	10.36	10.58

DOX from tablets. Hence, it can be easily and conveniently adopted for routine quality control analysis.

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