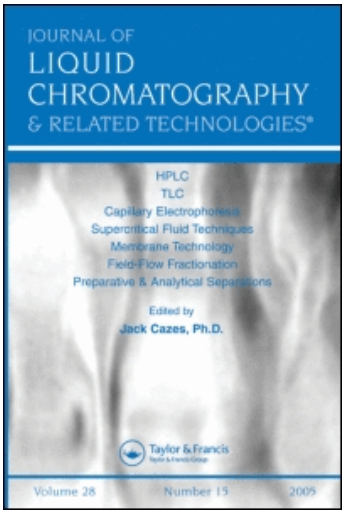

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SIMULTANEOUS DETERMINATION OF PYRIDOXINE HYDROCHLORIDE AND DOXYLAMINE SUCCINATE IN TABLETS BY HPTLC

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ABSTRACT

A new, simple, precise, accurate, rapid, and stability indicating high performance thin layer chromatography (HPTLC) method has been developed for the simultaneous determination of pyridoxine hydrochloride and doxylamine succinate in tablets. The stationary phase was silica gel $60F_{254}$ HPTLC plates and the mobile phase was acetone - chloroform - methanol - 25% ammonia solution (7 : 1.5 : 0.3 : 1.2, v/v). Detection and quantification was carried out densitometrically at 269 nm. The linearity ranges were 0.5-2.0 µg/spot for both pyridoxine hydrochloride and doxylamine succinate. Assays for pyridoxine hydrochloride and doxylamine succinate were 10.20 mg/tab (RSD 0.73%) and 10.00 mg/tab (RSD 1.93%) respectively for the brand analyzed. Percentage recoveries of pyridoxine hydrochloride and doxylamine succinate were in the range of 99.30 -103.00% and 97.70 - 101.00%, respectively.

INTRODUCTION

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

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

Various methods for simultaneous determination of PYR in combination with isoniazide by HPTLC,[5] spectrophotometry,[6] HPLC,[7] in combination with riboflavine and thiamine by fluorimetry,[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 HPTLC have not been reported. In this paper, we report here a new, simple HPTLC method for simultaneous determination of PYR and DOX in tablets.

EXPERIMENTAL

Instrumentation and Layers

CAMAG (Muttenz, Switzerland) HPTLC system equipped with LINOMAT-IV automatic sample applicator, twin trough chamber, TLC scanner II controlled by Cats software (V 3.17) was used. Merck $60F_{254}$ silica gel HPTLC plates (20 x 10 cm, 0.2 mm thickness) were used as the stationary phase. A Mettler AE200 balance was used for weighing.

Reagents and Chemicals

Standard of PYR was procured from USV Ltd, India. Its purity was checked as per BP and found to be 99.71%. Standard of DOX was procured from Sigma laboratories Ltd, India. Its purity was checked as per USP and

found to be 99.77%. Analytical reagent grade chloroform, methanol, acetone, 25% ammonia solution were used, which were supplied by S. D. Fine chemicals Ltd, India. Tablets containing PYR and DOX were purchased from the market.

Mobile Phase

Acetone - Chloroform - Methanol - 25% Ammonia Solution (7 : 1.5 : 0.3 : 1.2, v/v).

Standard Stock Solution

A combined standard stock solution of PYR and DOX was prepared by dissolving accurately weighed 25 mg of PYR and 25 mg of DOX in 50 mL of methanol (0.5 mg/mL PYR and 0.5 mg/mL DOX).

Working Standard Solution

Working standard solution was prepared by diluting 5 mL of standard stock solution to 10 mL with methanol.

Sample Solution

Twenty tablets were weighed, powdered, and an amount of the powdered sample equivalent to 50 mg of PYR and 50 mg of DOX was taken in a 50 mL volumetric flask, about 30 mL of methanol was added to it, sonicated for 10 minutes, and diluted to the mark with methanol. This solution was then filtered through Whatman No. 42 filter paper. Five mL of filtrate was further diluted to 10 mL with methanol and the resulting solution was used for assay analysis.

Procedure for Calibration

Aliquots of standard stock solution of PYR and DOX were taken in different volumetric flasks and diluted to the mark with methanol to obtain concentrations in the range of 0.5 - 2.0 µg/spot of PYR and DOX. Five µL of each of these solutions was applied on Merck $60F_{254}$ silica gel HPTLC plates in 6 mm bands with the CAMAG (Muttenz, Switzerland) Linomat IV sample applicator. The plates were developed for 60 mm in a twin trough chamber containing the mobile phase and after development, plates were dried with a hot air blower. Densitometric evaluation was performed at 269 nm using a deuterium lamp and the scanner described above. Peak areas were recorded for

all the tracks. Calibration curves were constructed by plotting peak areas (Y-axis) against the amount of the drug in µg/spot (X-axis) and the linear relationship was evaluated by calculation of the linear regression line by the method of least squares.

Procedure for Assay

Five µL each of working standard and sample solution was applied onto the HPTLC plate in 6 mm band and plate was developed, dried, and scanned. The peak areas were recorded as described in the calibration procedure. The amounts of PYR and DOX were computed by external standard quantification using the relationship

$$W = \frac{A_T \cdot C \cdot D}{A_s \cdot W_a}$$

where, W = content of the drug (PYR / DOX) per tablet.
W_a = weight of the powdered tablets taken for analysis.
A_T = area of the test sample.
A_s = area of the standard.
C = concentration of the standard.
D = dilution factor.

RESULTS AND DISCUSSION

Chromatography

The mobile phase resolved PYR and DOX very efficiently as shown in Figure 1. The Rf values were 0.28 and 0.86 for PYR and DOX, respectively. The resolution factor was 7.3 and tailing factors at 5% peak height were 0.8 for PYR and 1.0 for DOX. The wavelength of 269 nm was selected for the densitometric evaluation because at this wavelength there was maximum overlap of absorption spectra of PYR and DOX as shown in Figure 2.

Linearity, Limit of Detection, and Limit of Quantification

The plot of the peak area versus the concentration of PYR and DOX were found to be linear in the range of 0.5 - 2.0 µg/spot. The calibration curve could be represented by the following linear regression equations:

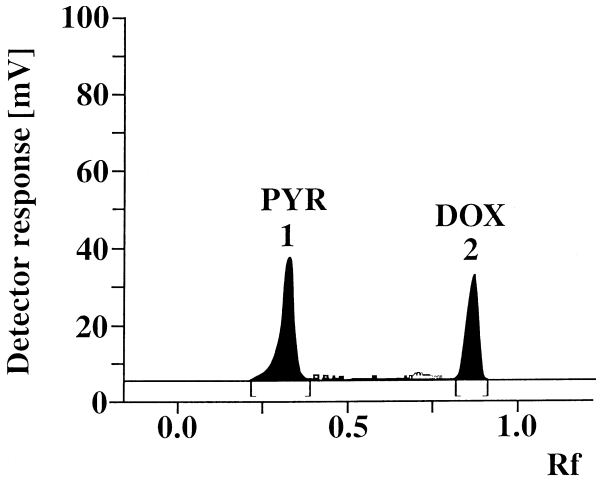

Figure 1. Typical chromatogram of PYR and DOX.

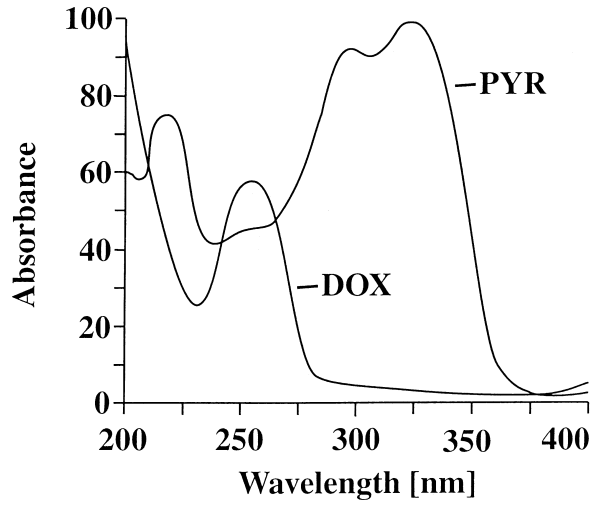

Figure 2. Absorbance spectra of PYR and DOX.

Table 1

Results of Assay of PYR and DOX in Tablets

Sample	PYR Label Claim (mg/tab)	Amount Found[b] (mg/tab)	RSD (%) n = 6	DOX Label Claim (mg/tab)	Amount Found[b] (mg/tab)	RSD (%) n = 6
Doxinate[a] Tablets	10	10.20	0.73	10	10.00	1.93

[a] Mfg. by Sigma Laboratories Ltd., India, B. No. DX805, Mfg. Dt. - Feb 98.
[b] Average of six experiments.

$$yPYR = 529.53x + 161.73 \quad (r = 0.999)$$
$$yDOX = 440.07x + 128.78 \quad (r = 0.999)$$

where y =area and x = concentration of PYR / DOX in µg/spot.

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

$$LOD = 3.3 \times \delta/S \quad \text{and} \quad LOQ = 10 \times \delta/S$$

where δ, the noise estimate, is the standard deviation of responses of blank samples (n=11) and S is slope of the corresponding calibration curve.

Limits of detection for PYR and DOX were found to be 0.01 µg (S/N = 2.3) and 0.01 µg (S/N = 2.9), respectively. The limits of quantification were 0.03 µg for PYR and 0.03 µg for DOX, respectively.

Assay

The content of PYR and DOX found in "Doxinate" tablets by the proposed method are as shown in Table 1. The amounts found by proposed method were 10.20 mg/tab and 10.00 mg/tab with RSD 0.73% and 1.93% respectively. The low values of RSD indicates that the proposed method is precise and accurate.

Table 2

Results of Recovery Analysis

Sample	Drug	Equivalent Amt of Std Drug Added (mg/Tab)	Amt Found[a] (mg/Tab)	Recovery (%) n = 3	RSD (%) n = 3	Total Mean % Recovery
Doxinate Tabs	PYR	0	10.30	103.00	1.66	101.18
		1	11.10	100.90	1.63	
		3	13.20	101.50	1.95	
		4	13.90	99.30	0.50	
	DOX	0	10.10	101.00	1.36	100.10
		1	11.10	100.90	1.65	
		3	12.70	97.70	1.57	
		4	14.10	100.70	1.91	

[a] Average of three experiments.

Accuracy

The accuracy of the proposed method was confirmed by recovery experiments. Pre-analyzed tablet samples equivalent to 25 mg each of PYR and DOX were weighed on the balance described above and taken in four different 50 mL volumetric flasks. To these flasks different levels of standard stock solution of PYR and DOX (0 mL, 5 mL, 15 mL and 20 mL) were added. The contents were sonicated and diluted to the mark with methanol. This solution was then filtered through Whatman No. 42 filter paper. Five mL of filtrate was further diluted to 10 mL with methanol and then analyzed by the proposed method, repeating each level thrice. Amounts of drug found per tablet was calculated for each level. Percentage recoveries were calculated from the amount of drug added and amount of drug found. Percentage recoveries of PYR and DOX were between 99.30% - 103.00% and 97.70% - 101.00 % respectively for brand analyzed. The results are given in Table 2. These results indicate that the method is accurate and precise, and also there is no interference due to the excipients present in the brand of tablet analyzed.

Stability Indicating Ability

The stability study is an integral part of pharmaceutical product development. The data collected during stability study decides shelf life,

Table 3

Assay Results of Tablet Samples Exposed to Stress Conditions

Degradation With Respect to	Amount of Drug Found (mg/Tab) PYR	% of Label Claim	DOX	% of Label Claim
Initial (unexposed sample)	10.50	105.0	10.20	102.0
Heat (@ 80°C) for 8 days	10.10	101.0	10.20	102.0
Acidic condition (0.1 N HCl) for 8 days	9.80	98.0	10.80	108.0
Alkaline condition (0.1 N NaOH) for 8 days	9.80	98.0	10.40	104.0
Strong oxidising condition (30%H_2O_2) for 8 days	5.80	58.0	6.10	61.0

storage condition, and impurity profile of the product. If assay method itself is stability indicating, it is considered to be versatile. Therefore, an attempt has been made to investigate stability indicating ability of the proposed method. The separate samples of powdered tablets equivalent to 25 mg of PYR and 25 mg of DOX were subjected to various stress conditions like acid (in 1 mL 0.1 N HCl), heat (@ 80°C), alkali (in 1 mL 0.1N NaOH) and strong oxidizing agent (in 1 mL 30% H_2O_2) for eight days. Simultaneously, standards of 25 mg of PYR, 25 mg of DOX and mixture of 25 mg of PYR and 25 mg of DOX were exposed to the above stress conditions separately. After eight days, all the exposed standards and tablet samples were analyzed by the proposed method. The contents of PYR and DOX found in tablet samples are reported in Table 3 and observations are summarized below, which show that the proposed method is stability indicating.

Degradation with Respect to Heat, Alkaline and Acidic Conditions

In the chromatogram of sample exposed to this condition no degradation peak of PYR or DOX was observed.

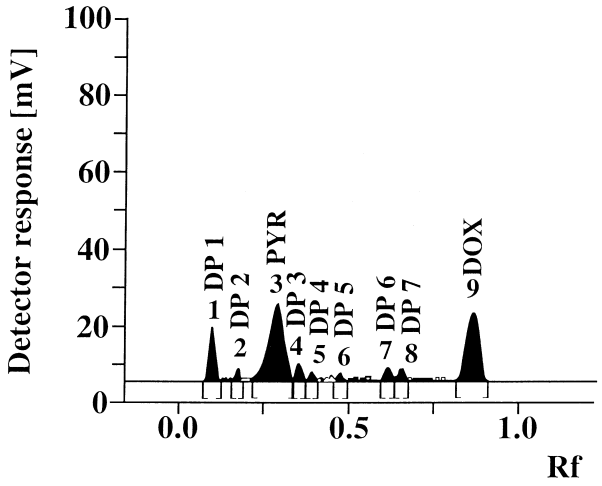

Figure 3. Degradation of PYR and DOX in strong oxidising condition.

Degradation with Respect to Strong Oxidising Condition

In this stress condition, both PYR and DOX were degraded. A typical chromatogram of tablet sample exposed to above stress condition is shown in Figure 3. The peaks marked as DP2, DP3, DP4, and DP6 were due to degradation products of DOX.

The peak marked as DP1 was due to degradation product of PYR and DOX. The peaks marked as DP4 and DP7 were due to degradation products of excipients present in the tablets. All the degradation peaks were well resolved from the principle peak of PYR and DOX.

CONCLUSION

The proposed HPTLC method is simple, precise, accurate, rapid, and stability indicating for the simultaneous determination of PYR and DOX from tablets. Hence, it can be easily and conveniently employed for the routine quality control analysis of these drugs.

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