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Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 663–667

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Stability indicating HPTLC method for the simultaneous determination of pseudoephedrine and cetirizine in pharmaceutical formulations

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Received 2 September 2000; received in revised form 14 November 2000; accepted 17 November 2000

Abstract

The combination of pseudoephedrine and cetirizine is widely used in the treatment of allergic rhinitis. A rapid, selective and stability indicating high performance thin layer chromatographic method was developed and validated for their simultaneous estimation in pharmaceutical dosage forms. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of ethyl acetate–methanol–ammonia (7:1.5:1, v/v/v). This system was found to give compact spots for both pseudoephedrine (*Rf* value of 0.69 ± 0.01) and cetirizine (*Rf* value of 0.38 ± 0.01). Also the degraded products were well separated from the pure drugs. Spectrodensitometric scanning-integration was performed at a wavelength of 240 nm. The polynomial regression data for the calibration plots showed good linear relationship with $r^2 = 0.9947$ in the concentration range of 10–26 μg for pseudeophedrine and 200–1200 ng for cetirizine with $r^2 = 0.9973$. The method was validated for precision, accuracy, ruggedness and recovery. The minimum detectable amounts were found to be 2 μg and 500 pg for pseudoephedrine and cetirizine, respectively. The limits of quantitation were found to be 6 μg for pseudoephedrine and 800 pg for cetirizine. Both the drugs do not undergo degradation under acidic and basic conditions. The samples degraded with hydrogen peroxide showed additional peaks at *Rf* values of 0.75 and 0.28 for pseudoephedrine and cetirizine, respectively. This indicates that both the drugs are susceptible to oxidation. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of pseudoephedrine and cetirizine. As the method could effectively separate the drugs from their degradation products, it can be employed as a stability indicating one.

Keywords: Pseudoephedrine; Cetirizine; HPTLC; Stability indicating; Degradation

1. Introduction

The combination of cetirizine, a long acting antihistaminic and slow release pseudoephedrine, a sympathomimetic decongestant is widely used in

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PII: S0731-7085(00)00586-0

the comprehensive management of allergic rhinitis, the symptoms of which include itching, sneezing, lacrimation and nasal congestion. Literature reveals a variety of analytical methods viz. colorimetry, HPLC [1–5], TLC [6–8], GC [9–13] and spectrophotometric techniques [14–17] for the analysis of the individual drugs. None of these methods are stability indicating.

Most of these methods are often time-consuming, expensive and cumbersome. The advantage of high performance thin layer chromatography (HPTLC) is that a large number of samples can be simultaneously analysed in a shorter time period. Unlike HPLC, this method utilises less quantities of solvents, thus lowering the cost of analysis.

An ideal stability indicating chromatographic method should estimate the drug and also be able to resolve the drug from its degradation products. Hence an attempt has been made to develop an accurate, rapid, specific and reproducible method for the determination of pseudoephedrine and cetirizine in presence of their degradation products for the content analysis during stability studies from pharmaceutical dosage forms containing this combination.

2. Experimental

2.1. Materials

Pseudoephedrine hydrochloride and cetirizine dihydrochloride were gifted by Ipca Laboratories Ltd, India and Bayer India Ltd, respectively. All other solvents and reagents were purchased from Ranbaxy chemicals, India and were of analytical grade.

2.2. Instrumentation

Spotting was done in the form of 6 mm bands with Camag microlitre syringe on precoated silica gel aluminium plate 60 F-254 (20×10 cm with 250 μ, thickness; Merck, Germany) using a Camag Linomat IV (Switzerland). The solvent system consisted of ethyl acetate–methanol–ammonia (20%) (7:1.5:1, v/v/v). Chromatogram was developed in a Camag twin trough chamber using a linear ascending technique. The chamber saturation time for mobile phase was optimised to 30 min. The length of chromatogram run was 8 cm. Subsequent to the development, the TLC plates were dried in a current of air. The densitometric analysis was performed on a Camag TLC scanner III in the absorbance mode at 240 nm. Densitograms were obtained by integration performed using a Perkin Elmer integrator system LCI-100.

2.3. Calibration plots

Stock solutions of pseudoephedrine hydrochloride (10 mg/ml) and cetirizine dihydrochloride (1 mg/ml) were prepared in methanol. A series of standard curves were prepared over a concentration range of 10–26 μg for pseudoephedrine hydrochloride. For cetirizine dihydrochloride the stock solution was spotted to give concentrations in the range of 200–1200 ng. The procedure for the same is discussed in Section 2.2. The data of spot area versus drug concentration was treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility.

2.4. Method validation

The accuracy and precision of the assay were tested at 18 μg and 600 ng of pseudoephedrine and cetirizine, respectively. For recovery, the analysed samples were spiked with 50%, 100% and 150% of the standard drugs and the mixtures were reanalysed by the proposed method ($n = 3$). The extraction solvent employed was methanol. Samples were analysed in the same way as described in Section 2.2. In order to estimate the limit of detection and limit of quantification, blank methanol was spotted six times following the same method as explained in Section 2.2. The noise level was determined. The limit of detection was calculated to be three times the standard deviation and ten times the standard deviation value gave limit of quantification. The ruggedness of the proposed method was studied using reagents from different lots and different manufacturers.

2.5. Analysis of the developed formulation

To determine the content of both the drugs from the bilayered formulation (label claim: 120 mg/tablet as extended release of pseudoephedrine and 5 mg/tablet of cetirizine as conventional), 20 tablets were powdered and powder equivalent to 20 mg of pseudoephedrine and 0.8 mg of cetirizine was weighed. Methanol was used for extraction. To ensure complete extraction of the drug it was sonicated for 15 min and the solution was made upto 25 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and the supernatent was analysed for the drug content. Solution (1 μl) was spotted onto the plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate. A placebo tablet was also subjected to the same extraction process as discussed above and spotted. The possibility of excipient interference in the analysis was studied.

2.6. Stability indicating method

The drugs were subjected to forced degradation under acidic conditions (1 M HCl), basic conditions (1 M NaOH) and oxidation (H_2O_2) by heating at 70°C for 2 h. A 200 mg/ml aqueous solution of both drugs was prepared and accordingly treated. These solutions were further neutralised, diluted to a final concentration of 2 mg/ml and then spotted on to TLC plates. The chromatogram was run as described in Section 2.2.

3. Results and discussion

3.1. Development of the optimum mobile phase

Both the pure drug and the degraded products were spotted on the TLC plates and run in different solvent systems. Initially n–butanol–ethanol-water-acetic acid in varying ratios was tried. However, with all the ratios tried diffused spots were obtained for both pseudoephedrine and cetirizine. Then another mobile phase which was reported for pseudoephedrine (ethyl acetate–cyclohexane–methanol–ammonia in varying ratios, 7:1.5:1:0.5, 8:1.5,1:0.5, 7:1:1:0.5, v/v/v/v) was tried. Although a compact spot was obtained for cetirizine the spot for pseudoephedrine was diffused. When cyclohexane was eliminated from this mobile phase the spots were found to improve. Hence ethyl acetate–methanol–ammonia (7:1:0.5, v/v/v) was tried. Here again spot for pseudoephedrine was slightly diffused. Increasing methanol and ammonia concentration improved the spot characteristics. Finally the mobile phase ethyl acetate–methanol–ammonia (7:1.5:1, v/v/v) gave good resolution of the two components with a Rf value of 0.69 for pseudoephedrine and 0.38 for cetirizine. Well defined spots for both the drugs were obtained when the chamber was saturated with the mobile phase for 30 min.

3.2. Calibration curves

The polynomial regression data for the calibration plots ($n = 3$) showed a good linear relationship over a concentration range of 10–26 μg for pseudoephedrine and 200–1200 ng for cetirizine. No significant difference was observed in the slopes of standard curves (ANOVA; $P > 0.05$) Table 1.

3.3. Validation

The results in Table 2 revealed excellent accuracy and high precision of the assay method. The proposed method when used for extraction and subsequent estimation of the drug combination from pharmaceutical dosage forms after spiking with 50%, 100% and 150% of additional drug afforded recovery of 98–100% as listed in Table 3.

Table 1
Polynomial regression data for the standard curves ($n = 3$)

Drugs	$r^2 \pm$ S.D.	Slope $\pm$ S.D.	Intercept $\pm$ S.D.
[a]	0.9947 ± 0.0045	50.6135 ± 1.2354	32.1643 ± 0.9911
[b]	0.9973 ± 0.0015	1.5685 ± 0.0949	74.4643 ± 1.3223

[a] Pseudoephedrine: linearity range:10–26 μg.
[b] Cetirizine: linearity range: 200–1200 ng.

Table 2
Accuracy and precision of the method ($n = 6$)

Drugs	S.D. of areas	RSD (%)
Accuracy		
[a]	21.54	1.95
[b]	37.91	3.59
Precision		
[a]	27.61	2.64
[b]	32.89	3.09

[a] Pseudoephedrine:18 μg.
[b] Cetirizine: 600 ng.

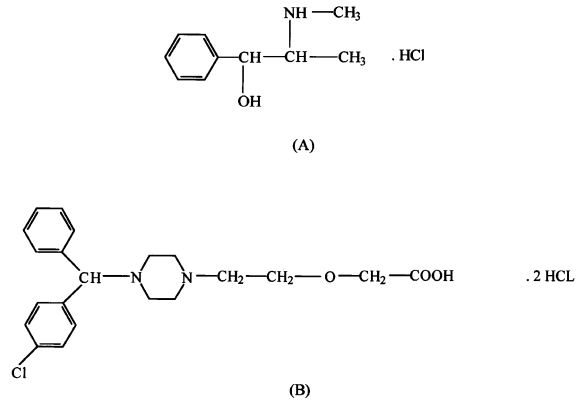

Fig. 1. Structure of pseudoephedrine hydrochloride (A), cetirizine dihydrochloride (B).

The minimum detectable amounts with a signal to noise ratio of 3:1, were found to be 2 μg and 500 pg for pseudoephedrine and cetirizine, respectively. The limits of quantitation, with a signal to noise ratio of 10:1, were found to be 6 μg for pseudoephedrine and 800 pg for cetirizine. In the ruggedness study, the RSD for system precision and recovery studies was found to be 1.45% and 1.24% for different lots of reagents and 1.56% and 1.05% for different manufacturers respectively.

3.4. Analysis of the formulation

Spots of the pure drugs were observed in the chromatogram of the drug samples extracted from the developed bilayered tablets. There was no interference from the excipients present in the tablet, as evidenced from the chromatogram of the placebo formulation (Figs. 1 and 2). The drug content was found to be more than 98% as is evidenced from the Table 4.

3.5. Stability indicating method

The chromatogram of the acid and base degraded samples for both pseudoephedrine and cetirizine showed only the spots of the pure drug. This indicates that both the drugs do not undergo degradation under acidic and basic conditions. The samples degraded with hydrogen peroxide (Fig. 2) showed additional peaks at *Rf* values of 0.75 and 0.28 for pseudoephedrine and cetirizine respectively. This indicates that both the drugs are susceptible to oxidation. The spots of the degraded products were well resolved from the drug spots. In case of pseudoephedrine the hypothetical degraded product would result from the oxidation of the hydroxyl group to a ketone. This compound being more non polar in nature has an *Rf* value (0.75) higher as compared to the pure drug. For cetirizine the formation of an alcohol by oxidation at the carbon atom attached to the

Table 3
Recovery studies ($n = 6$)

Drug	Excess drug added to the analyte (%)	Theoretical content (mg)	Recovery (%)	RSD (%)
Pseudoephedrine	50	180	99.43	1.75
	100	240	100.14	1.62
	150	300	98.14	1.12
Cetirizine	50	7.5	100.96	1.21
	100	10.0	101.23	1.52
	150	12.5	99.70	1.27

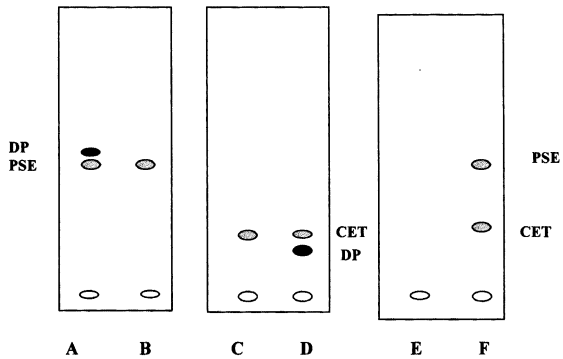

Fig. 2. Schematic representation of separation of pseudoephedrine (PSE) and cetirizine (CET) from their degraded oxidised products. A, pseudoephedrine + oxidised products; B, pseudoephedrine, C, cetirizine, D, cetirizine + oxidised product, E, placebo, F, formulation.

Table 4
Applicability of the HPTLC method for the analysis of the pharmaceutical formulations ($n = 6$)

Drug	Label claim	Drug content (%)	RSD(%)
Pseudoephedrine	120 mg	102.47	0.27
Cetirizine	5 mg	98.57	1.65

piperazine ring would result in a more polar compound which has a lower *Rf* value of 0.28 compared to cetirizine.

4. Conclusion

The developed HPTLC technique is precise, specific, accurate and stability-indicating. The statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of pseudoephedrine and cetirizine in pharmaceutical formulations. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one.

Acknowledgements

The authors are highly thankful to University Grant Commission for the financial assistance.

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