Reverse phase-high performance liquid chromatographic method for the analysis of paracetamol, cetirizine and pseudoephedrine from tablets

Lakshmi Sivasubramanian*, K. S. Lakshmi
Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur-603203, South India

Abstract

An accurate, simple, reproducible and sensitive method for the determination of paracetamol, cetirizine and pseudoephedrine was developed and validated. Paracetamol, cetirizine and pseudoephedrine were separated using a Hypersil C_{18} column by isocratic elution with a flow rate of 1.0 mL/min. The mobile phase composition was 25mM phosphate buffer (pH 5.0) – methanol – acetonitrile (30:60:10) (v/v/v) and 100 mg of heptane sulphonic acid was added for every 100 mL of mobile phase. Spectrophotometric detection was carried out at 240nm. The linear range of determination for paracetamol, cetirizine and pseudoephedrine were 100-600 µg/mL, 1-6 µg/mL and 12-72 µg/mL, respectively. The method was shown to be linear, reproducible, specific, sensitive and rugged.

Key Words: Paracetamol, Cetirizine, Pseudoephedrine, HPLC, Validation.

Introduction

Paracetamol (acetaminophen) is one of the most popular over-the counter analgesic and antipyretic drugs. Dosage forms of paracetamol and its combinations with other drugs have been listed in various Pharmacopoeias [1,2]. Numerous methods have been reported for the analysis of paracetamol and its combinations in pharmaceuticals or in biological fluids. Paracetamol has been determined in combination with other drugs using titrimetry [3,4], voltammetry [5], colorimetry [6], UV-spectrophotometry [7-9], quantitative thin layer chromatography (TLC) [10], high performance liquid chromatography (HPLC) [11-15] and gas chromatography (GC) [16] in pharmaceutical preparations.

Cetirizine is a second generation antihistamine drug and is official in BP [17]. A survey of literature revealed UV-Spectrophotometry [18] and HPLC [19-26] methods have been reported for the analysis of cetirizine individually and in combination with other drugs.

Pseudoephedrine, official in USP [27], BP [28] and IP [29] is widely used for symptomatic treatment of allergic rhinitis. All the three Pharmacopoeia describe HPLC method for estimation of pseudoephedrine hydrochloride from tablet formulation. Many methods have been reported in the literature for the determination of similar formulation with various other drugs using HPLC [30-34] and spectrophotometry [35].

Although there are few methods for the determination of paracetamol, cetirizine and pseudoephedrine individually or in combination with other drugs, a suitable HPLC method to determine the ternary mixture of all three drugs was not located in the literature survey. The objective of this study was to develop and validate a specific, accurate, precise and reproducible quality control method for paracetamol, cetirizine and pseudoephedrine in their ternary combination.

Results and Discussion

Method development

The mobile phase was chosen after several trials with methanol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of 25 mM KH_2PO_4 – methanol- acetonitrile (30:60:10) (v/v/v) was selected to achieve maximum separation and sensitivity.

Flow rates between 0.5 and 1.5 mL/min were studied. A flow rate of 1.0 mL/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C_{18} column, the retention times for paracetamol, cetirizine and pseudoephedrine were observed to be 3.23, 4.18 and 4.81 min respectively. Total time of analysis was less than 6 min. The maximum absorption of paracetamol, cetirizine and pseudoephedrine together was detected at 240 nm and this wavelength was chosen for the analysis. The chromatogram at 240 nm showed a complete resolution of all peaks (Fig. 1).

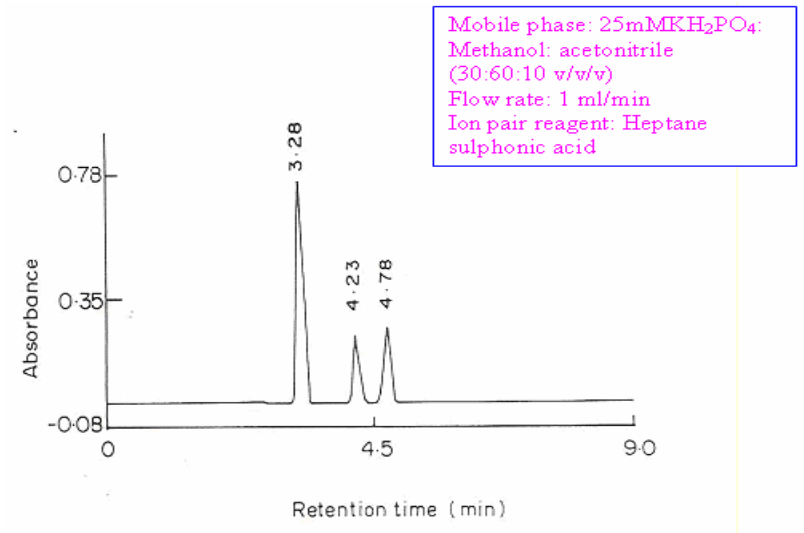

Figure 1: Chromatogram showing the separation of paracetamol, cetirizine and pseudoephedrine

Linearity

Table 1 presents the equation of the regression line, correlation coefficient (r^2), relative standard deviation (RSD), values of the slope and intercept for each compound. Excellent linearity was obtained for compounds between peak areas and concentrations of 100-600 µg/mL with r^2 = 0.9897, 1-6 µg/mL with r^2=0.9994 and 12-72 µg/mL with r^2=0.9999 for Paracetamol, Cetirizine and Pseudoephedrine respectively.

Table 1. Results of Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

S.No.	Parameters	Paracetamol	Cetirizine	Pseudoephedrine
1	Linearity range (µg/mL)	100-600	1-6	10-60
2	Correlation coefficient (r^2)	0.9897	0.9994	0.9999
3	Regression Equation [Y = mX + C] Slope (m) Intercept (C)	 71885.97 2990051	 3161463 126116.8	 347810.8 - 13675.7
4	LOD (µg/mL)	0.921	0.151	0.321
5	LOQ (µg/mL)	2.512	0.502	0.836

X- Conc. in µg/mL, Y- Peak area, LOD– Limit of Detection, LOQ – Limit of Quantitation

Limits of detection and quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3. Limits of quantification (LOQ) were established at a signal-to-noise ratio (S/N) of 9. LOD and LOQ were experimentally verified by six injections of paracetamol, cetirizine and pseudoephedrine at the LOD and LOQ concentrations. The LOD was calculated to be 0.921, 0.151 and 0.321 µg/mL and the LOQ was calculated to be 2.512, 0.502 and 0.836 µg/mL for paracetamol, cetirizine and pseudoephedrine (Table 1).

Suitability of the method

The chromatographic parameters such as resolution, selectivity and peak asymmetry were satisfactory for these compounds (Table 2). The calculated resolution values between each peak pair were no less than 2.0 and the selectivity was not less than 1.30. K' values were found to be 1.31, 2.27 and 3.12 for paracetamol, cetirizine and pseudoephedrine respectively.

Table 2. System Performance Parameters

Compound	t_r ± S.D (n=6, mean)	Area ± S.D (n=6, mean)	k'	R	α	T
Paracetamol	3.23 ± 0.63	3757732 ± 0.62	1.14	2.11	1.68	1.44
Cetirizine	4.18 ± 0.86	6519841 ± 0.33	1.92			1.50
Pseudoephedrine	4.81± 0.47	8317886 ± 0.41	2.21	1.41	1.15	1.37

K' – Capacity factor, R – Resolution, α – Separation factor, T – Tailing factor, t_r – retention time.

Precision

The precision of the method (within-day variations of replicates determinations) was checked by injecting paracetamol, cetirizine, pseudoephedrine 6 times at the LOQ level. The precision of the method, expressed as the RSD% at the LOQ level was 2.32, 2.72 and 3.64% for paracetamol, cetirizine and pseudoephedrine respectively (Table 3).

Table 3. Precision of the developed method at the LOQ level (n=6) measured at 240nm

Compound	**Mean Peak area***	**% RSD**
Paracetamol	31206	4.66
Cetirizine	21303	3.21
Pseudoephedrine	24682	2.74

* Mean of six observations

Accuracy

A standard working solution containing paracetamol, cetirizine and pseudoephedrine yielding final concentrations of 150µg/mL, 5µg/mL and 50µg/mL respectively was prepared. The prepared mixture of standards was injected 6 times as a test sample. From the respective area counts, the concentrations of the paracetamol, cetirizine and pseudoephedrine were calculated using the detector responses. The accuracy, defined in terms of % deviation of the calculated concentrations from the actual concentrations is listed in Table 4.

Table 4. Accuracy of the developed method (n=6)

Compound	**Spiked Concentration µg/mL**	**Measured Concentration µg/mL Mean ± S.D**	**RSD (%)**	**Deviation (%)**
Paracetamol	100	104.62 ± 0.72	0.521	4.62
Cetirizine	10	10.38 ± 0.35	0.462	3.80
Pseudoephedrine	50	49.54 ± 0.24	0.311	0.92

$$\% \text{ Deviation} = \frac{(\text{Spiked Concentration} - \text{Mean measured concentration})}{\text{Spiked Concentration}} \times 100$$

Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using a standard working solution, the same chromatographic system and the same column on different days. The prepared mixture of standards was injected 6 times as a test sample. Small differences in areas and good constancy in retention times were observed after 60 hours. A RSD of less than 0.82% for areas and 0.36% for retention times were obtained (tables 5 and 6). The comparable detector responses obtained on different days indicate that the method is capable of producing results with high precision on different days.

Table 5. Variability according to Area

	Day 1			**Day 6**		
	Paracetamol	**Cetirizine**	**Pseudoephedrine**	**Paracetamol**	**Cetirizine**	**Pseudoephedrine**
Area	25129307	9772673	12497919	25467218	9864232	12568020
S.D	156052.99	30490.73	51991.34	181071.92	51491.29	47884.15
RSD (%)	0.621	0.312	0.416	0.711	0.522	0.381

Table 6. Variability according to Retention time

	Day 1			**Day 6**		
	Paracetamol	**Cetirizine**	**Pseudoephedrine**	**Paracetamol**	**Cetirizine**	**Pseudoephedrine**
R_t	3.23	4.18	4.81	3.61	4.32	4.14
S.D	0.010	0.014	0.015	0.013	0.013	0.018
RSD %	0.332	0.345	0.331	0.373	0.324	0.351

Analysis of formulation

To determine the content of paracetamol, cetirizine and pseudoephedrine simultaneously in conventional tablets (label claim: 500 mg paracetamol, 5 mg cetirizine and 60 mg pseudoephedrine). The twenty tablets were weighed, their mean weight determined and they were finely powdered. The powder equivalent to 100 mg of paracetamol was weighed and transferred in to a 100 mL volumetric flask containing 50mL of methanol, sonicated for 30 min and diluted to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant containing 1 mg/mL of paracetamol, 10 µg/mL of cetirizine and 120 µg/mL of

pseudoephedrine was taken and filtered using 0.45 µm filter (Millipore,). The 20 µl of each samples were injected thrice and amount present in each tablet were determined (Table 7).

Table 7. Applicability of the proposed method in commercial tablet

Parameters	**Paracetamol**	**Cetirizine**	**Pseudoephedrine**
Label Claim (mg)	500	5	60
Drug content (%) ± S.D	99.6 ± 1.23	99.32 ± 1.34	99.71 ± 1.32
SE (95% Confidence level)	0.66	0.87	0.68

Recovery studies

Recovery studies was carried out by applying the method to drug sample to which known amount of drugs corresponding to 80, 100, 120% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with expected results (Table 8).

Table 8. Recovery results for paracetamol, cetirizine and pseudoephedrine by proposed method

S.No	**Paracetamol**			**Cetirizine**			**Pseudoephedrine**		
	Added µg	**Found µg**	**Recovery %**	**Added µg**	**Found µg**	**Recovery %**	**Added µg**	**Found µg**	**Recovery %**
1	400	400.65	100.16	4	4.06	101.5	48	48.21	100.43
2	500	501.11	100.22	5	4.97	99.4	60	60.31	100.51
3	600	598.97	99.82	6	6.11	101.8	72	71.89	99.84
		Mean	100.06		Mean	100.9		Mean	100.26
		%RSD	0.21		%RSD	1.23		%RSD	0.65

Materials and Methods

The drug samples, cetirizine, pseudoephedrine and paracetamol were obtained as gift samples from Suven Life Sciences, Hyderabad. Chromatographic grade double distilled water, analytical

grade KH_2PO_4, Orthophosphoric acid, heptane sulphonic acid sodium salt, HPLC grade acetonitrile, methanol (Merck, Mumbai) were used.

The method development was performed with a LC system consisting of a Shimadzu model with LC-10AT pump, variable wavelength programmable UV/VIS detector SPD-10 AVP, operating software winchrome. Samples were injected with a 7725 Rheodyne injector system with a 20 µL sample loop. The detector was set to 240nm (0.03 au.f.s) and peak areas were integrated automatically by computer software program.

Separation was carried out at ambient temperature using a Hypersil C_{18} column (5 µ, 250 mm × 4.6 mm I.D). All the calculations concerning the quantitative analysis were performed with external standardization by the measurement of peak areas.

Stock and Standard Solutions

Paracetamol (100.00 mg), Cetirizine (10.00 mg) and pseudoephedrine (10.00mg) were accurately weighed in a 10 mL volumetric flask and dissolved in the mobile phase and filled up the volume with the mobile phase.

Standard working solution

Standard working solutions were prepared individually in mobile phase for cetirizine and pseudoephedrine. Aliquots from each working solution were combined and diluted with mobile phase to yield a solution with final concentrations of 1 mg/mL, 10 µg/mL, 120 µg/mL. Studies on the stability of the analytes in standard working solution showed that there were no decomposition products in the chromatogram or difference in areas during analytical procedure, even after storage for four days at $+4^0$C.

Chromatographic Conditions

HPLC analysis was performed by isocratic elution with a flow rate of 1.0 mL/min. The mobile phase composition was 25mM KH_2PO_4 – methanol – acetonitrile (30:60:10) (v/v/v). About 100 mg of heptane sulphonic acid sodium salt was added to each 100 mL of mobile phase. All solvents were filtered through a 0.45 µm Millipore filter before use and degassed in an ultrasonic bath. Volumes of 20 µL prepared solutions and samples were injected into the column. Quantification was effected by measuring at 240 nm as established from the chromatogram. The chromatographic run time was 10 min and the column void volume was 1.523 min.

Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor(k'), the resolution (R), the selectivity (α) and peak asymmetry (T).

Calibration

Mixed standard solutions containing paracetamol (100-600 µg/mL), cetirizine (1-6 µg/mL) and pseudoephedrine (12-72 µg/mL) were prepared in the mobile phase. Triplicate 20 µL injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of each drug was plotted against the concentration to obtain

the calibration graph. The five concentrations of each compound were subjected to regression analysis to calculate the calibration equation and correlation coefficients.

Conclusion

The developed method is suitable for the determination and quantification of the ternary combination of paracetamol, cetirizine and pseudoephedrine. A high percentage recovery shows that the method can be successfully used on a routine basis. The proposed method is simple, sensitive, rapid, specific and could be applied for quality and stability monitoring of paracetamol, cetirizine and pseudoephedrine combinations.

References

[1] J.E.F. Reynolds, "Martindale The Extra Pharmacopoeia", 31st ed., 27-28, Pharmaceutical Press, London, **1996**.

[2] The United States Pharmacopoeia, 24th revision, U.S. Pharmacopoeia Convention, Rockville, MD, **2000**.

[3] British Pharmacopoeia**,** Version 2, The Stationary Office Ltd., Norwich, **1998**.

[4] European Pharmacopoeia, third edition, Convention on the Elaboration of a European Pharmacopoeia, Strasbourg, **1997**.

[5] O.V. Lau, S.V. Luk, Y.M. Cheung, *Analyst*, **1989**, 114, 1047.

[6] H.A. El-Obeid, A.A. Al-Badr, *Analytical Profiles of Drug Substances*, **1985**, 14, 551.

[7] H.K. Chan, D.J.W. Grant, *Int. J. Pharm*., **1989**, 57, 117.

[8] N. Erk, F. Onur, *Anal. Lett*., **1987**, 30 (6), 1201.

[9] H.N. Dogan, *Pharmazie*, **1996**, 51 (10), 773.

[10] P. Kahela, E. Laine, M. Anttila, *Drug Dev. Ind. Pharm*., **1987,** 13 (2), 213.

[11] M.S. Ali, S. Rafiuddin, M. Ghori, A.R. Kahtri, *J.AOAC Int*., **2008**, 91 (2), 344.

[12] R. Bhushan, D. Gupta, A. Mukherjee, *Biomed. Chromatogr*., **2007**, 21 (12), 1284.

[13] M. Hossain, J.W. Ayres, *Int. J. Pharm*., **1996**, 133, 223.

[14] K.H. Yuen, K.K. Peh, Y.L. Quah, K.L. Chan, *Drug Dev. Ind. Pharm*., **1997,** 23 (2), 225.

[15] D.D. Orsi, L. Gagliardi, A. Bolarsco, D. Toneli, *Chromatographia*, **1996**, 43 (9/10), 496.

[16] J.J. Bergh, A.P. Lotter, *Drug Dev. Ind. Pharm*., **1984**, 10 (1), 127.

[17] British Pharmacopoeia, Vol.II Her Majesty's Stationary Office, British Pharmacopoeia Commission, London, **1993**.

[18] P. Reddy, M.V. Suryanarayana, S. Venkatraman, M. S. Reddy, C.S. Sastry C.S. *Indian Drugs*, **1983,** 30, 286.

[19] J. Moncrieff, *J. Chrom. Biomed. Appln*., **1992,** 121, 128.

[20] S.S. Zarapkar, *Indian Drugs*., **1995,** 32, 232.

[21] M.T. Rosseel, R.A. Lefebvre, *J. Chrom. Biomed. Appln*., **1992,** 565, 504.

[22] M.V Suryanarayan, B.P. Reddy, G.L. Krupadanam, S. Venkatraman, C.S. Sastry *Indian Drugs*, **1995,** 29, 605.

[23] M.S. Arayne, N. Sultana, F.A. Siddique *Pak. J. Pharm.Sci*., **2005**, 18 (3), 7.

[24] P. Kowalski, A. Plenis, *Biomed. Chromatogr*., **2007**, 21 (9), 903.

[25] A.M. Jaber, A.L. Al Sherife, M.M. Al Omari, A.A. Badwan, *J.Pharm. Biomed. Anal*., **2004,** 36 (2), 341.

[26] B. Paw, G. Misztal, H. Hopkala, J. Drozd, *Pharmazie*., **2005**, 57 (5), 313.

[27] United States Pharmacopoeia, 18th Edition, USP Convention Inc., Rockville, MD, 1339, **1995.**

[28] British Pharmacopoeia, Vol.II Her Majesty's Stationary Office, British Pharmacopoeia Commission, London, 1085, **1993.**

[29] Indian Pharmacopoeia, Vol.II, Government of India, The Controller of Publication, New Delhi, 641, **1996.**

[30] A.K. Dash, *J. Pharm. Biomed. Anal*., **1986,** 121, 601.

[31] G.W. Fong, W.M. Eichoff, *Int. J. Pharm*., **1989,** 53, 91.

[33] J.H. Maierdan Wang, F.Z. Yan, Y. Jin, Y.Q. Cui, D.J. Sun, *Yaowu Fenxi Zazhi.*, **1984,** 14, 27.

[34] F.T. Noggle, C.R. Clark, *J. Assoc. Off. Anal. Chem*., **1984,** 67, 198.

[35] M.T. Paeeran, G.S. Baravani, V.K. Bhalla, *Indian Drugs.*, **1988,** 25, 242.

[36]I. Singhvi, Neela Bhatia, *Indian J. Pharm. Sci*., **2006,** 68 (1), 72.