Analytical Method Development and Validation of Pseudoephedrine Sulphate and Desloratidine from Bulk and Dosage Form Using 0.01N *Ferric chloride*

[1]*S. Sharma* and [2]*M.C. Sharma*

[1]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) 477001 India
[2]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India

Abstract: Three simple, rapid, accurate, precise and cost-effective methods, I; formation and solving of Simultaneous estimation equation method, II; absorbance ratio method, III; using 0.01N ferric chloride have been developed for simultaneous estimation of Pseudoephidrine Sulphate[PSD] and Desloratidine[DSL] in tablet dosage form. Pseudoephidrine Sulphate showed absorbance maxima at 274 nm and Desloratidine showed at and 288 nm in 0.01N Ferric Chloride solution. Beer's law was obeyed in concentration range 5-35 µg/ml for PSD and 5-40 µg/ml for DSL, respectively for all proposed three methods. The sampling wavelengths for method I,II and III selected for both the drugs were 274nm, 288nm, 255nm, 268nm and 422 nm on trial and error basis using 0.01N Ferric Chloride solution as solvent. All the Three methods allowed rapid analysis of binary pharmaceutical formulation with accuracy. Results of analysis for six methods were tested and validated for various parameters according to ICH guidelines.

Key words: Pseudoephidrine Sulphate · Desloratidine · 0.01N Ferric Chloride

INTRODUCTION

Pseudoephedrine sulphate (PSD) is an orally active direct and indirect sympathomimetic upper respiratory tract decongestant drug. It is widely used in allergicrhinitis, cold, coughs and sinusitis and to prevent otisis barotrauma. Chemically the drug is (1S, 2S) -2-methylamino-1- phenylpropan-1-ol sulphate. Several methods such as HPLC [1-2], HPTLC [3], packed column supercritical fluid chromatography [4] and Spectrophotometry [5-8] have been reported in the literatures. Desloratidine (DES) is 8- Chloro-6; 1- dihydro-11 (-4 piperidinylidine) -5H- benzo [5, 6] Cycloheptal [1, 2-b] pyridine, descarboethoxyloratidine. It is not yet official in any Pharmacopoeia. It is non sedating peripheral histamine H1 receptor antagonist, active metabolite of loratidine. A survey of literatures reveals that DSL is estimated by HPLC [9-10], HPTLC [11], Spectrophotometry [12] and special data analysis [13-15]. The present groups of authors have already reported UV Method development different pharmaceutical dosage form [14-30] using Ferric chloride [31]. The purpose of this study was to develop spectrophotometric procedure for the selective determination of Pseudoephidrine Sulphate, Desloratidine in presence of common excipients.

MATERIALS AND METHODS

Instruments and Chemicals: UV/Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells.HPLC grade, sodium hydroxide AR grade, potassium hydrogen orthophosphate AR grade of Rankem ltd. Water HPLC grade of Milli-Q were used. Pharmaceutical formulation Commercial tablets, each containing Pseudoephedrine sulphate (120mg) and Desloratidine (5 mg); were procured from the local market. 1.0M Hydrochloric acid, 3 % sodium nitrite in water, 1 M sodium hydroxide, 3% ammonium sulphamate solution, 0.01 N ferric chloride.

Preparation of Standard Stock Solution: Standard stock solution of PSD and DSL having concentration 100 µg/ml prepared by dissolving separately 10 mg of each drug in 100 ml volumetric flask using 0.01 N ferric chloride solution. Beer's law was obeyed in concentration range 5-35 µg/ml for PSD and 5-40 µg/ml for DSL, respectively for all the proposed methods. The sampling wavelengths for method II, selected for both the drugs were 231 nm and 247nm, on trial and error basis using 0.01 N ferric chloride

---

Corresponding Author: S. Sharma, Department of Chemistry Chodhary, Dilip Singh Kanya Mahavidyalya,
Bhind (M.P.) India. E-mail: smitasharma@yahoo.com.

Table 1: Optical Characteristics of Pseudoephedrine Sulphate and Desloratidine

Calculation	Method 1	Method 2	Method 3
PSD			
Regression equation			
Y = mx + c			
a. Slope (m)	0.0276	0.0026	0.098
b. Intercept (c)	0.0412	0.0017	0.064
Correlation coefficient (r²)	0.9985	0.9997	0.9995
Linearity range (μg/ml)	5-35	5-35	5-35
LOD (μg/ml)	0.376,	0.265,	0.225
LOQ (μg/ml)	1.274	2.314	0.143
DSL			
Regression equation			
Y = mx + c			
a. Slope (m)	0.0543	0.0028	0.0398
b. Intercept (c)	0.034	0.0054	0.0242
Correlation coefficient (r)	0.9991	0.9993	0.9990
Linearity range (μg/ml)	5-40	5-40	5-40
LOD (μg/ml)	0.126	0.166	0.332
LOQ (μg/ml)	1.021	0.325	0.659

Method I: Simultaneous Equation Method, Method II: Absorbance ratio method, Method III: Using Ferric Chloride

solutions as solvent. For methods I, II, seven mixed standards solutions with concentration of PSD and DSL in the μg/ml of 5:35, 10:30, 15:25, 20:20, 25:15, 30:10 and 35:5, were prepared by diluting appropriate volumes of standard stock solutions.

Preparation of Sample Stock Solution: Twenty Tablets were taken, there average weight was determined and crushed to a fine powdered, powdered equivalent to 120 mg of PSD and 5 mg of DSL was weight and dissolved in 100 ml of 0.01 N ferric chloride with vigorous shaking for 15 minute. The solution was filtered through Whatmann filter paper No. 41 to a 100ml of volumetric flask and volume was made up to mark with 0.01 N ferric chloride to get sample stock solution which was further diluted with 0.01 N ferric chloride to get required concentration in linearity range. Sample solutions were scanned using proposed six methods and the results were obtained and reported in (Table 1).

Methods

Method I: Simultaneous Estimation Method: Simultaneous estimation method [32-34] of analysis was based on the absorption of drugs (PSD and DSL) at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations was 274nm and 288 nm, λmax of PSD and DSL, respectively. The absorbances of both the drugs were

measured at 274 nm and 288 nm. The absorptivity values E (1%, 1cm) determined for PSD at 274 nm and 282 nm were 296.0 and 312 while respective values for DSL were 288.0 and 305 nm. These values were means of six estimations. The concentration of two drugs in mixture was calculated by, using following equations

$$C_{PSD} = \frac{(A_2 a y_1 - A_1 a y_2)}{(a x_2 . a y_1 - a x_1 . a y_2)} \quad (1)$$

$$C_{DSL} = \frac{(A_1 a x_2 - A_2 a x_1)}{(a x_2 . a y_1 - a x_1 . a y_2)} \quad (2)$$

Where A_1 and A_2 were the absorbances of sample at 274 nm and 288 nm respectively, a_{x1} and a_{x2} were the absorptivity E (1%, 1cm) of PSD at 296 nm and 312 nm respectively; a_{y1} and a_{y2} were the absorptivity of DSL at 288 nm and 305 nm respectively.

Method II: Absorbance Ratio Method: Absorbance ratio method [35-36] of analysis was based on the absorbance's at two selected wavelengths, one of which is an isobestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig. 1) 266 nm (Isobestic point) and 241 nm (λmax of PSD) were selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorbances at 255 nm and 268 nm for PSD and DSL were measured. The absorptivity values of each drug at both wavelengths were determined which was the mean of six independent values. The absorbances and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$CPSD = \frac{(Q_M - Q_Y)}{(Q_X - Q_Y)} . \frac{A_1}{a x_1} \quad (3)$$

$$C_{DSL} = \frac{(Q_M - Q_X)}{(Q_Y - Q_X)} . \frac{A_1}{a x_1} \quad (4)$$

Q_M, Q_X and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1}, \quad Q_X = \frac{a x_2}{a x_1}, \quad Q_Y = \frac{a y_2}{a y_1}$$

Where A_1 and A_2 were the absorbance of the sample at 255 nm and 268 nm respectively, a_{x1} and a_{x2} were the absorptivity of PSD at 255 nm and 268 nm respectively and a_{y1} and a_{y2} were the absorptivity of DSL at 255 nm and 268 nm respectively.

Table 2: Analysis data of tablet formulation

	Method 1	Method 2	Method 3
PSD Mean* (%) ± S.D.	99.97 ± 0.021	99.87±0.12	99.92±0.32
% RSD	0.213	0.213	0.322
DSL Mean* (%) ± S.D.	99.98 ± 0.023	100.09±0.21	100.01± 0.18
% RSD	0.121	0.187	0.298

*Mean of six observations, S.D.: Standard deviation, R.S.D: Relative standard deviation,

Table 3: Results of Recovery (Accuracy) Studies

Method	Drug	Label claim (mg)	%Amount Added	% Recovery*	S.D. (±)	% RSD	S.E.
			80	99.21	0.312	0.250	0.120
	PSD	120	100	99.94	0.128	0.680	0.750
			120	100.14	0.277	0.480	0.081
1			80	100.11	0.321	0.400	0. 34
	DSL	5	100	100.09	0.355	0.680	0.690
			120	99.97	0.187	0.350	0.890
			80	100.13	0.109	0.620	0.970
	PSD	120	100	99.98	0.438	0.490	0.940
1			120	99.93	0.765	0.330	0.820
			80	100.05	0.376	0.470	0.330
	DSL	5	100	100.04	0.411	0.470	0.746
			120	99.98	0.650	0.560	0.431
			80	100.32	0.443	0.990	0.520
	PSD	120	100	100.09	0.658	0.016	0.530
3			120	99.90	0.547	0.120	0.940
			80	100.06	0.317	0.610	0.410
	DSL	5	100	99.97	0.437	0.390	0.490
			120	100.12	0.587	0.550	0.436

*Recovery is mean of three observations, S.D.: Standard deviation, R.S.D.: Relative standard deviation, S.E.: standard error

Method III - Using Ferric Chloride: Aliquots of standard stock solution ranging from 5-35 g/mL were transferred to a series of 25 ml volumetric flask. To each flask, 1 mL of 0.01 N ferric chloride was added. The flasks were allowed to stand in water bath for 25 min. The flasks were then cooled to room temperature and the solutions were made up to the volume with water. The absorbance of the pink coloured chromogen was measured at 376 nm and 422 nm against reagent blank.

Validation: The developed methods for the simultaneous estimation of PSD and DSL were validated as per ICH guidelines [37].

Linearity: For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. To establish linearity of the all proposed six methods, six separate series of solutions of PSD and DSL (5-35 µg/ml and 5-40µg/ml in 0.01 N ferric chloride) were prepared from the stock solutions and analyzed.

Accuracy: To check the accuracy of proposed method, recovery studies were carried out from the pre-analyzed sample at three deferent level of standard addition 80%, 100% and 120% of the level claim. The results of the recovery studies are given in (Table 3).

Precision

Repeatability: To the check of degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times at all concentrations in linear range. The standard deviation, relative standard deviation and standard error were calculated. The results of statistical evaluation are reported in (Table 2).

Intermediate Precision (Intra-Day and Inter-Day Precision): The Intra and Inter-day precision was determined by assay of the sample solution on the same day and different days at different time intervals respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ of PSD and DSL by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. The results of the same are shown in (Table 1).

RESULT AND DISCUSSION

The two methods selected for multi-component analysis were given the satisfactory results. This method utilizes the active analogue principle that lies at the spectroscopic method [14-31]. A novel approach to use 0.02% SLS as solvent proven beneficial with many respects such as reduction in cost, no use of organic solvent and stable pharmaceutical solvent for analysis. The spectra of PSD and DSL exhibit ë max of 274 nm and 288 nm, respectively. Additionally one isoisorptive point was observed at 232.2 nm. These wavelengths were selected for simultaneous estimation and Q analysis PSD and DSL and are assumed to be sensitive wavelengths. For method I, the LOD, LOQ values for PSD and DSL was found to be 0.376, 1.274 and 0.126, 1.021 (μg/ml) respectively; for method II, 0.265, 2.314 & 0.166, 0.325 (μg/ml) respectively; for method III, 0.225, 0.143 & 0.332, 0.659 (μg/ml) respectively; Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicated the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, RSD and standard error was calculated of PSD and DSL. The results of stastical evaluation are given (Table 2). Intermediate precision study expresses within laboratory variation in different days. In intermediate precision study, %RSD values were not more than 2.0% in all the cases (Table 3). The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The means of %recovery (%RSD) were found to be low values (<2.0) for all the six proposed methods (Table 3). These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods. linear regression equations obtained were; absorbance at 274 and 288 nm= [$0.023\times$conc. in μg/ml]+0.143 (Method I and II for PSD, DSL, r^2 = 0.9985 and 0.9991), 288 nm = [$0.324\times$conc. in μg/ml]+0.054 (Method II, 255 and 268nm for PSD and DSL, r^2 = 0.9997,0.9993), 255nm = [$0.0214\times$conc. in μg/ml]+0.0265

(Method II,268nm for DSL, r^2 = 0.9989), (Method III for 376 nm, PSD, r^2 = 0.9995) and = [$0.011\times$ conc. in μg/ml]+0.0044 (Method III for 422 nm, DSL, r^2 = 0.9990). The method was validated according to ICH guidelines. The accuracy of the method was evaluated by percentage recovery (by standard addition method) of both the drugs.

CONCLUSIONS

All two drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed methods and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed methods are new, simple, accurate, sensitive and precise and can be adopted for routine analysis of Pseudoephidrine sulphate and Desloratidine in tablet dosage form.

REFERENCES

1. Vasudevan, M., S. Ravishankar and R.S. Chandan, 2001. Simultaneous estimation of Pseudoephidrine and Loratidine by HPLC. Ind. Drugs., 38: 276.
2. Feyyaz, O. and D. Erdal, 2006. Liquid chromatographic and Spectrophotometric determination of Pseudoephidrine, Acrivastine in pharmaceutical preparation and human plasma. J. Anal. Chem., 2: 199-211.
3. Sane, R.T., M. Francis, S. Khedkar and S. Pawar, 2001. A simultaneous HPTLC for Pseudoephidrine and Loratidine in combined dosage form. Ind. Drugs., 38: 433.
4. Patil, K.M. and I.C. Bhoir, 2001. Concurrent assay of Aetamenophen, Pseudoephidrine, Guaitensin by packed column supercritical fluid chromatography. Ind. J. Pharm. Sci., 63: 274-77.
5. Nalini, C.N. and K. Kavitha, 2003. Simultaneous determination of Pseudoephedrine hydrochloride and cetrizine hydrochloride by Derivative spectroscopy. Ind. Drugs., 40: 658.

6. Palabiyik, I.M. and E. Dinc, 2004. Simultaneous Spectrophotometric determination of pseudoephedrine and Ibuprofen in combined dosage form. J. Pharm. Biomed. Analysis., 34: 473-483.

7. Gazzy, A.A. and E.L. Sayed, 2003. Spectrophotometric determination of binary mixture pseudoephedrine with some H_1 receptor antagonist by using derivative ratio spectrophotometric method in combined dosage form. J. Pharm. Biomed. Analysis, 31: 81-90.

8. Mabrouk, M.M. and S. Hammad, 2003. Simultaneous determination of loratidine and pseudoephedrine sulphate in pharmaceutical formulation by RP- LC and derivative Spectrometric method. J. Pharm. Biomed. Analysis, 33: 597-604.

9. The Merck Index, 2001. XIIIth edition, Merck Research laboratories, Division of Merck and Co. Inc., white house station N.J., pp: 514.

10. Martindale, 2002. The complete Drug Reference, 33rd edition, Pharmaceutical Press, London, Chicago, pp: 415.

11. More, A.R., A.J. Vaidya and V.V. Vaidya, 2005. Determination of Loratidine and its active metabolite Desloratidine from plasma by HPLC for bioequivalence study. Ind. Drugs., 42: 519.

12. Kenie, M.C. and L. Donald, 2004. HPLC method for bioequivalence evaluation of Desloratidine fumarate tablet in dog. J. Pharm. Biomed. Analysis, 34: 1013-1019.

13. Talele, S.G., P.S. Jain and S.J. Surana, 2005. Validated HPTLC method for estimation of Desloratidine from tablet dosage form. Ind. Drugs., 42: 671.

14. Talele, S.G., J.M. Patel and R.A.Fursule, 2006. Extractive Spectrophotometric determination of Desloratidine from bulk and tablet dosage form. Ind. Drugs., 43: 507.

15. Hamoka, S., 2002. Spectral data analysis and identification of Desloratidine. J. Chromat. B., 774: 105-113.

16. Sharma, M.C. and S. Sharma, 2010. Validated Simultaneous Spectrophotometric Estimation of Paroxetine HCl Bulk and Tablet Dosage Form using Ferric Chloride. J. Optoel. and Biomed. Mater., 2(4): 185-189.

17. Sharma, M.C. and S. Sharma, 2010. UV-Densitometric Determination of Sparfloxacin and its application to the Assay in Pharmaceutical Dosage Forms. J. Optoel. and Biomed. Mater., 2(4): 191-195.

18. Sharma, S., M.C. Sharma, R. Sharma and A.D. Sharma, 2010. Spectrophotometric Analysis of Nebivolol Hydrochloride in Tablet Dosage form using 5.0 M Niacinamide solution as hydrotropic solubilizing agent. J. Pharm. Res., 3(5): 1074-1076.

19. Sharma, S., M.C. Sharma, R. Sharma and A.D. Sharma, 2010. Simultaneous Estimation and Validation of Ezetimibe and Simvastatin in Combined Tablet Dosage Forms by Hydrotropic Solubilization Technique Using 3.0 M Urea. J. Pharm. Res, 3(5): 1063-1067.

20. Sharma, M.C. and S. Sharma, 2010. Simultaneous Estimation and Validation of Pseudoephidrine Sulphate and Desloratidine from Bulk and Tablets as hydrotropic solubilizing agent. J. Curre. Pharma. Res., 1: 26-30.

21. Sharma, S., M.C. Sharma and A.D. Sharma, 2010. Hydrotropic solubilization phenomenon Spectrophotometric estimation of Tenfovir disoproxil fumerate tablet. J. Chemic. Pharmac. Res., 2(2): 411-415.

22. Sharma, S., M.C. Sharma and A.D. Sharma, 2010. Novel application and spectrophotometric estimation of Melitracen HCl tablet dosage form using Niacinamide as hydrotropic solubilizing agent. J. Chemic. Pharmac. Res., 2(2): 416-420.

23. Sharma, M.C and S. Sharma, 2010. A Quantitative Estimation and Validation of Atorvastatin calcium and Pioglitazone in Pharmaceutical Tablet Dosage Form by Hydrotropic Solubilization Phenomenon. Intern. J. Chem. Tech. Res., 2(4): 2487-2491.

24. Sharma, M.C. and S. Sharma, 2010. Novel method for Spectrophotometric analysis of Simultaneous Estimation of Bisoprolol Fumarate Tablet Formulations using hydrotropy solubilization Agents. J. Optoelect. Biomed. Mat., 2(4): 223-225.

25. Sharma, M.C. and S. Sharma, 2010. Development and Validation of Simultaneous Estimation of Etoposide Solid Dosage form using hydrotropic Agents. J. Optoelect. Biomed. Mat., 2(4): 227-229.

26. Sharma, R., G. Pathodiya, G.P. Mishra and M. Sharma, 2010. Simultaneous Estimation and Validation of Cefixime Trihydrate and Ornidazole in Bulk and Tablets using Hydrotropic Solubilizing Agents. J. Pharm. Res., 3(12): 2953-2955.

27. Sharma, M.C. and S. Sharma, 2011. Spectrophotometric determination of Lamivudine in Bulk and Pharmaceutical Formulation using hydrotropic Solubilization. Intern. J. Chem. Tech. Res., 3(2): 988-991.

28. Sharma, S., R. Sharma and M.C. Sharma, 2010. Simultaneous Estimation and Validation of Poorly Water Soluble Drugs Rabeprazole Sodium and Itropide Hydrochloride Combined Tablet Dosage Form by Hydrotropic Solubilization Agents. Intern. J. Pure and Appl. Chem., 5(4): 305-311.

29. Sharma, M.C., S. Sharma and S.C. Chatuervedi, 2011. Spectrophotometric Methods for the Determination of Repaglinide in tablets Using Indigo Carmine. Intern. J. Pure and Appl. Chem., 6(1): 75-78.

30. Sharma, M.C. and S. Sharma, 2010. UV Spectrophotometric Methods for Estimation of Anastrazole Bulk and Tablet Dosage Form By derivative spectroscopy. J. Optoel. and Biomed. Mater., 2(4): 217-221.

31. Madhuri, D., K.B. Chandrasekhar, M. Ramakotaiah, G. Somasekhar, K. Harinadhababa and K.R. Kumar, 2010. Validation of Spectrophotometric determination of Rabeprazole using Ferric Chloride (FeCl3). Int. J. Res. Pharm. Sci., 1: 209-211.

32. Beckett, A.H. and J.B. Stenlake, 2002. Practical Pharmaceutical Chemistry, CBS Publishers and distributors, New Delhi. Part, 2: 275-307.

33. Shankar, M.B., M. Shah, F.A. Geetha and K.K. Bhatt, 2004. Simultaneous estimation of tizanidin and diclofenac in tablets. Indian. J. Pharm. Sci., 66: 332-335.

34. Puranik, M., A. Hirudkar, S.J. Wadher and P.G. Yoele, 2006. Development and validation of spectrophotometric methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in tablet dosage form. Indian J. Pharm. Sci., 68: 737-739.

35. Pernarowski, A.M. and J.E. Knevel, 1960. Christian Application of absorbance ratio to the analysis of pharmaceuticals I. Theory of analysis of binary mixture. Ind. J. Pharm. Sci., 1: 943-948.

36. Davidson, A.G., A.H. Beckett and J.B. Stenlake, 2001. Practical Pharmaceutical Chemistry; CBS Publishers and Distributor, New Delhi, 2: 284-288.

37. ICH, 1996. Q2B Validation of analytical procedures: Methodology, Consensus Guidelines; ICH Harmonized Tripartite Guidelines.