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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Guaifenesin and Pseudoephedrine HCl in Extended Release Tablet Dosage form

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ABSTRACT

A new simple, sensitive, rapid, precise, and accurate reverse phase high performance liquid chromatographic method had developed for the simultaneous estimation of guaifenesin and pseudoephedrine HCl in ER tablet dosage form. The components separated by using Zorbax SB Phenyl (150 × 4.6 mm i.d × 5 µm) column with UV detection at 210 nm. The mobile phase solvent A and B in the ratio of 950:50 v/v, flow rate was found to be 2 ml/min and run time detected in 15 min. Guaifenesin and pseudoephedrine HCl were eluted with retention time of 10.64 min and 2.635 min. The method validated for linearity, accuracy, precision, robustness, and ruggedness as per ICH guidelines and results found within the acceptable limits. The linearity for guaifenesin and pseudoephedrine HCl had found in the range of 96-721 µg/ml and 10-72 µg/ml. The validated method had applied for the routine analysis of these compounds in ER tablet dosage form.

Keywords: RP- HPLC, Method development, Validation, Guaifenesin, Pseudoephedrine HCl

INTRODUCTION

Guaifenesin (GUA), chemically (+)-3-(2-methoxyphenoxy)-propane-1,2-diol (Figure 1A), it is used as an expectorant, which increases the output of phlegm and bronchial secretions by reducing surface tension and adhesiveness [1]. Chemically, Pseudoephedrine hydrochloride (PSE) is (S,S)-2-methylamino-1-phenyl-1-ol hydrochloride (Figure 1B). It acts as an indirect sympathomimetic agent by stimulating adrenergic nerve endings to release norepinephrine [2]. From literature survey, it reveals many analytical methods for estimation of guaifenesin and pseudoephedrine HCl individually and in combination with other drugs such as UV Spectrophotometry [3-7], Reversed-phase High-performance Liquid Chromatography (RP-HPLC) [8-18], High Performance Thin Layer Chromatography (HPTLC) (HPTLC) [19], Liquid Chromatography Tandem-mass Spectrometry (LC-MS/MS) [20] methods. The present work illustrated a validated reverse phase HPLC method for simultaneous estimation of Guaifenesin and Pseudoephedrine HCl by RP-HPLC in ER tablet dosage form.

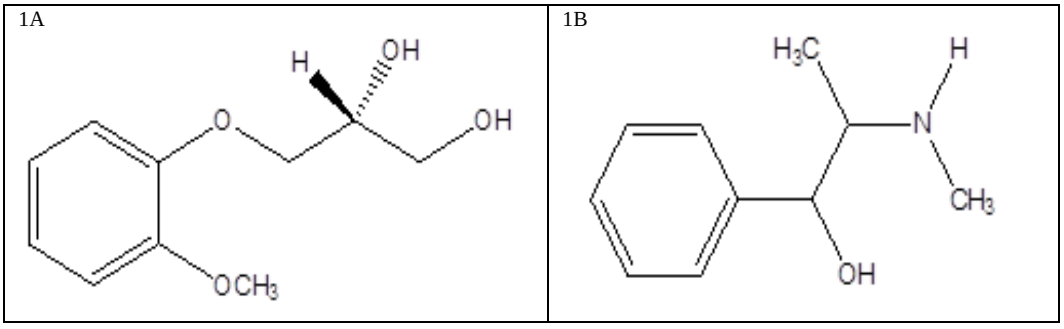

Figure 1: (1A) Guaifenesin, (1B) Pseudoephedrine

MATERIALS AND METHODS

Reagents and chemicals

Pure drug samples of guaifenesin and pseudoephedrine HCl received from Aurobindo Laboratories Limited, Hyderabad, India. Analytical grades of orthophosphoric acid and HPLC grade of tetrahydrofuran, acetonitrile and methanol were manufactured by Merck, Mumbai, India and commercial ER tablet of Guaifenesin and Pseudoephedrine HCl, Mucinex D were procured from local drug market.

Instrumentation

Chromatography was performed with Shimadazu 2010 HPLC system coupled with UV detector was used.

Chromatographic condition

Column: Zorbax SB Phenyl (150 × 4.6 mm i.d × 5 µm), Pump mode: Isocratic, Flow rate: 2 ml/min, Detection: UV, 210 nm, Injection volume: 10 µl, Column temperature: 50°C, Run time: 15 min

Preparation of solutions

Preparation of mobile phase

Prepared a degassed mixture of solution A and solution B was in the ratio of 950: 50 v/v.

Solution A

Transferred a 16 ml of 25% aqueous tetramethylammonium hydroxide solution in 1000 ml of water added a 5 ml of 88% orthophosphoric acid and mixed. Adjust the P^H of solution to 2.2 ± 0.05 with dilute orthophosphoric acid or 25% aqueous tetramethylammonium hydroxide.

Solution B

Prepared a degassed mixture of acetonitrile and tetrahydrofuran were in the ratio of 1000: 2 v/v.

Preparation of diluent

Methanol used as diluent.

Standard stock solution A

48 mg of Pseudoephedrine hydrochloride used as a working standard into a 100 ml volumetric flask and added about 70 ml of diluent, sonicated to dissolved and diluted to volume upto the mark and mixed.

Preparation of mixed standard solution

48 mg of Guaifenesin working standard into a 100 ml volumetric flask, added about 60 ml of diluent, sonicated and dissolved at room temperature. Added about 10 ml of standard stock solution A, diluted to volume with diluent and mixed carefully.

Preparation of sample solution

Accurately weighed 20 tablets of guaifenesin and pseudoephedrine HCl were powdered finely and equivalent to 2400 mg of guaifenesin was transferred into a 200 ml volumetric flask, diluted to volume with diluent, and mixed. Centrifuge the solution at 10,000 rpm for 10 min. Further, dilute 2 ml of supernatant solution to 50 ml diluent (Figure 2).

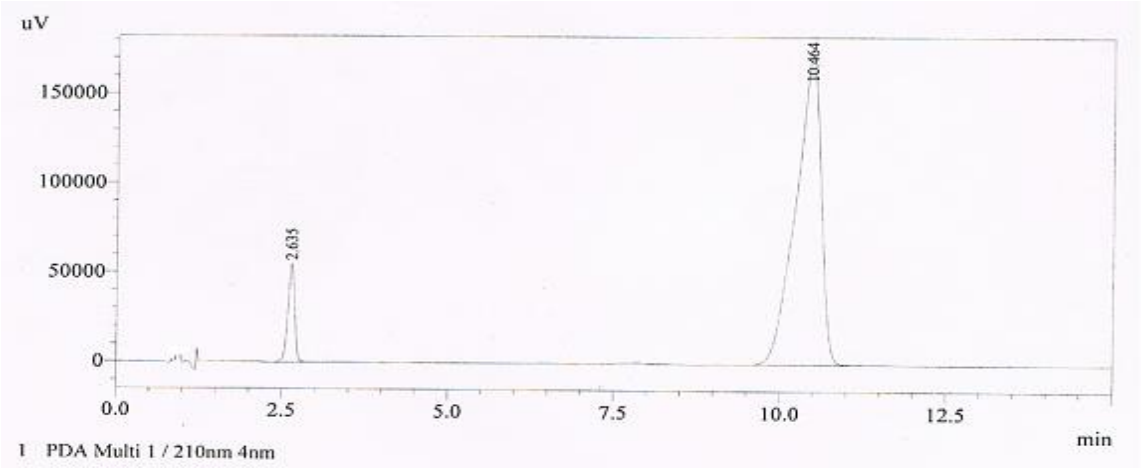

Figure 2: Chromatogram showing guaifenesin (R_t 2.635) and pseudoephedrine (R_t10.464)

RESULTS AND DISCUSSION

Validation

System suitability

Number of theoretical plates, HETP and peak tailing of guaifenesin and pseudoephedrine HCl were determined and results were obtained as shown in Table 1.

Table 1: System suitability parameters

Drug	Theoretical plates	Tailing factor
Guaifenesin	3810	0.9
Pseudoephedrine HCl	2185	0.9

System precision and method precision

%RSD of guaifenesin and pseudoephedrine HCl of system and method precision were calculated by using six replicate injections as shown in Tables 2 and 3. From the results, the developed method has considered as precise.

Table 2: System precision

Sample	Area	
	Guaifenesin	Pseudoephedrine HCl
1	5719229	524364
2	5685139	521156
3	5691780	520878
4	5701605	521445
5	5767994	528276
6	5735063	524484
Mean	5716802	523434
SD	31047	2867
%RSD	0.5	0.5

Table 3: Method precision

Sample (guaifenesin)	Area	Assay	% Recovery	Sample (pseudoephedrine)	Area	Assay	% Recovery
1	5884130	1184.18	98.7	1	506829	119.62	99.7
2	5955226	1198.14	99.9	2	512999	121.07	100.9
3	5934993	1199.28	99.3	3	511411	120.38	100.3
4	5924047	1188.36	99.0	4	509704	119.91	99.9
5	5936144	1199.01	99.3	5	511546	120.37	100.3
6	5942743	1193.46	99.5	6	513585	120.96	100.8
Overall statistical analysis	Average	1191.12	99.3	Overall statistical analysis	Average	120.39	100.3
	SD	4.78	0.41		SD	0.57	0.48
	%RSD	0.4	0.4		%RSD	0.5	0.5

Linearity

Linearity was determined by constructing calibration curve and plotted the graph between area vs concentration. The concentration for Guaifenesin and Pseudoephedrine HCl were found in the range of 96-721 µg/ml and 9-72 µg/ml and co-relation coefficients were obtained 0.9997 and 0.9997 respectively as shown in Table 4 and Figure 3.

Table 4: Linearity

Guaifenesin% level	Conc (µg/ml)	Area	Pseudoephedrine % level	Conc (µg/ml)	Area
20	96.25	1159646	20	9.70	111325
50	240.61	2752390	50	24.25	256572
70	336.86	3914502	70	33.95	365397
90	433.10	5048377	90	43.65	470488
100	481.23	5511802	100	48.50	508840
110	529.35	6013249	110	53.35	566388
130	625.59	7306995	130	63.05	672140
150	721.84	8198884	150	72.75	761872
Overall statistical analysis	Slope	11380	Overall statistical analysis	Slope	10416
	Intercept	59384		Intercept	9481
	R^2	0.9997		R^2	0.9997

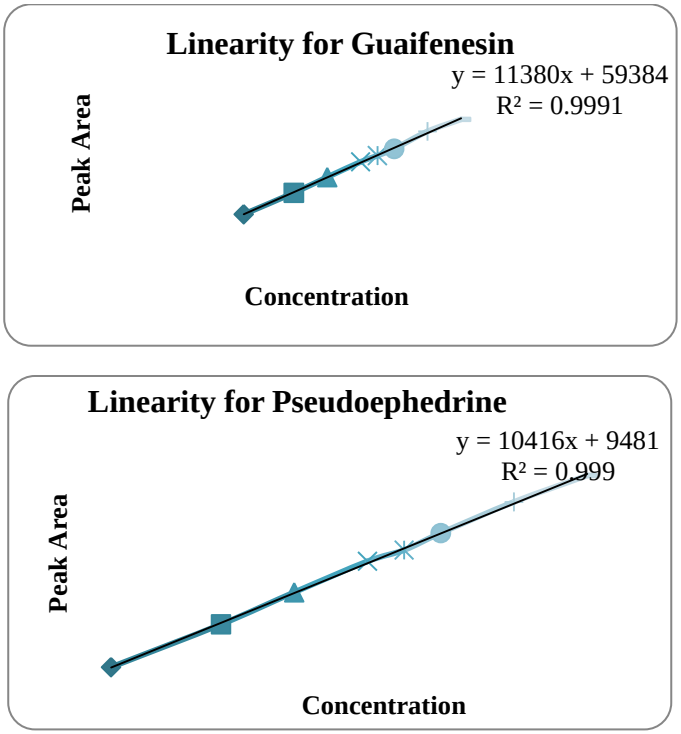

Figure 3: Linearity for guaifenesin and pseudoephedrine HCl

Accuracy

Accuracy was determined by recovery studies and a known concentration of standards in the series of 50, 75, 100, 125 and 150% were added to preanalyzed sample and subjected to the proposed HPLC analysis as shown in Table 5.

Table 5: Accuracy

Drug concentration		Area		Amount added (mg)		Amount found (mg)		% Recovery		Mean Recovery	
GUA	PSE	GUA	PSE	GUA	PSE	GUA	PSE	GUA	PSE	GUA	PSE
50%	50 %	2319971	198474	1203.38	120.06	1182.28	119.22	98.2	99.3	99.82	99.26
75%	75%	3514107	300384	1805.92	179.96	1790.82	180.43	99.2	100.3		
100%	100%	4748287	401864	2407.21	243.37	2403.81	241.38	99.9	99.2		
125%	125%	5917386	501923	3038.11	305.90	3078.24	301.48	101.3	98.6		
150%	150%	6976633	591657	3596.21	359.29	3555.35	355.38	100.5	98.9		

Robustness

Actual chromatographic conditions like pH ± 0.2, flow rate ± 10% were altered deliberately and the effect on system suitability parameters were not much affected and results of analysis were summarized in Table 6.

Table 6: Robustness

Condition	Variation	Mean		SD		% RSD	
		GUA	PSE	GUA	PSE	GUA	PSE
Flow Rate	-10%	6210948	567917	2270	308	0.1	0.1
	+10%	5107535	467214	1575	288	0.1	0.1
Buffer PH	-0.2 unit	5611616	512795	1903	364	0.1	0.1
	+0.2 unit	5605124	512550	1861	162	0.1	0.1

Ruggedness

Six samples of a single batch were analyzed by two different systems i.e., Shimadzu LC 2010 system 1 and Shimadzu prominence system 2 on different days and results were found within acceptable limits (RSD<2).

Limit of detection and limit of quantitation (LOD and LOQ)

LOD was found to be 0.654 µg/ml and 0.542 µg/ml, whereas LOQ was found to be 0.428 µg/ml and 0.482 µg/ml for guaifenesin and pseudoephedrine HCl.

CONCLUSION

The developed HPLC method was validated for various parameters like linearity, accuracy, precision as per ICH guidelines. The validation parameters were within the acceptable limits and thus the proposed method was applied for simultaneous estimation of guaifenesin and pseudoephedrine HCl in ER tablet dosage form.

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REFERENCES

[1] http://www.drugbank.ca/drugs/DB00874
[2] http://www.drugbank.ca/drugs/DB00852
[3] S. Rahul, S.H. Kumar, S. Vinod, T. Shruti, J. Nilesh, *Int. J. Res. Pharm. Sci*., **2011**, 1(3), 41-49.
[4] E. Pushpalatha, P. Tejaswini, M. Najboon, S. Vineesha, M.D. Madhanna, T.A. Kumar, M. Raju, *Int. J. Pharm. Res. Anal*., **2015**, 5(2), 90-95.
[5] K.A.S. Attia, M.W. Nassar, A.E. Olemy, *Eur. J. Biomed. Pharm. Sci.* **2014**, 1(2), 1-12.
[6] N.C. Patel, D.B. Patel, K.C. Pruthviraj, *Asian. J. Res. Chem*. **2013**, 6(4), 407-414.
[7] B. Koteswara Rao, I. Jyothsna Kumari, M. Nageswara Rao, C. Ram Babu, *Int. J. Pharm. Pharm. Res*., **2015**, 3(4), 139-148.
[8] V. Jain, M.C. Sharma, *J. Taibah. Uni. Sci*., **2016**, 10(1), 38-45.
[9] K.L. Narasimharao, B.C. Krishnaiaha, K. Sudheerbabua, K. Padmaja Reddya. *J. Saudi. Chem. Soc*., **2014**, 18(5), 593-600.
[10] T.V. Raghava Raju, N. Anil Kumar, S. Raja Kumar, *Chroma. Res. Int*., **2013**.
[11] A.B. Chaudhary, S.M. Bhadani, C.M. Shah, *World. J. Pharm. Pharm. Sci*., **2015**, 4(5), 1679-1694.
[12] M. Vasudevan, S. Ravisankar, A. Sathiyanarayanan, R.S. Chandan, *J. Pharm. Biomed. Anal*., **2000**, 25-31.
[13] M. Yolanda, S. Martono, A. Rohman. *Int. J. Pharma. Clin Res*., **2016**, 8(5), 476-480.
[14] K. Nalini, P. Narmada, G. Vijaya Lakshmi, Y. Gowtham, K.V. Jogi, *Int. J. Pharm. Sci. Res*., **2014**, 5(2), 410-460.
[15] G. Sailaja, B. Hari Babu, *J. Appl. Pharm. Sci*., **2015**, 5(12), 61-66.
[16] R. Vijay Kumar, U. Vinay, *Der. Pharma. Lettre*., **2015,** 7(7), 329-339.
[17] N.S. Abdelwahab, E.A. Abdelaleem, *Arab. J. Chem*., **2013**, 1-6.
[18] M.F. Farid, N.A.E. Ragehy, M.A. Hegazy, M. Abdelkawy, G.H. Metwally, *Beni-suef Uni. J. Bas. App. Sci*., **2014**, 260-268.
[19] P.S. Jain, N.K. Kale, S.J. Surana, *J. Chromatogr. Sep. Tech.*, **2015**, 6(4), 1-4.
[20] A.A. Asirvatham, D. Sridharan, A. Thenmzohi, M. Palanivelu, *Ann. Int. J. Pharm. Sci*. **2011**, 2(3), 73-82.