Asian Journal of Chemistry; Vol. 30, No. 6 (2018), 1383-1386

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21269

RP-HPLC-PDA Method for Simultaneous Quantification of Montelukast, Acebrophylline and Desloratadine Tablets

J. MAMATHA[1,*] and N. DEVANNA[2]

[1]Department of Chemistry, G. Pullaiah College of Engineering and Technology, Kurnool-518 002, India
[2]Department of Chemistry, Jawaharlal Nehru Technological University Ananthapur, Ananthapuramu-515001, India

*Corresponding author: E-mail: mamatha@gpcet.ac.in

Received: 6 February 2018; *Accepted*: 22 March 2018; *Published online*: 30 April 2018; AJC-18898

A simple, fast, accurate and specific RP-HPLC-PDA method has been developed for the simultaneous quantification of montelukast, acebrophylline and desloratadine in bulk and tablet dosage form. The chromatographic separation was performed on a reverse phase BDS C8 Column (150 × 4.6mm, 5 µm particle size) consisting mobile phase of potassium hydrogen ortho-phosphate buffer:acetonitrile (40:60 v/v), with a flow rate 1 mL/min, temperature 30 ºC and UV detection wavelength 280 nm. The retention times of montelukast, acebrophylline and desloratadine were observed as 2.04, 2.68 and 3.77 min, respectively. The developed method was validated by validation parameters such as linearity, range, accuracy, precision and robustness. The results obtained for validation parameters are within the limits as per ICH guidelines. The linearity of the drugs were obtained in the range of 5-30 ppm for montelukast, 100-600 ppm acebrophylline and 2.5-15 ppm for desloratadine. % RSD from precision studies were 0.4, 0.1 and 1.0 mean percentage recovery from accuracy studies were found to be 98.47, 98.71 and 100.11 for montelukast, acebrophylline and desloratadine respeactively. Limit of detection values were found as 0.01, 0.32 and 0.01 for montelukast, acebrophylline and desloratadine, respectively. Limit of quantification values were found as 0.03, 0.98 and 0.04 montelukast, acebrophylline and desloratadine, respectively. The method designed and validated can be successfully used for the regular quantification of montelukast, acebrophylline and desloratadine in tablet and bulk forms.

Keywords: Montelukast, Acebrophylline, Desloratadine, RP-HPLC-PDA.

INTRODUCTION

Montelukast (Fig. 1) chemically is (1-[[[1R-1-[3-(1E)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropane acetic acid sodium salt, is used in the treatment of asthma, allergic rhinitis which acts through inhibition of leukotriene D4(LTD4) [1,2]. Literature review indicates that few analytical methods like spectrophotometric [3], HPLC [4-7] and LC-MS [8] have been reported for the quantification of montelukast with other drugs. Acebrophylline (Fig.1) chemically is 4-[2-amino-3,5-dibromophenyl)methylamino]cyclohexan-1-ol; 2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid. It is having mucolytic expectorant and bronchodilating activity. It is used for treating asthma [9] and chronic obstructive pulmonary disease (COPD) [10]. It is a salt form of equimolar mixture of theophylline-7-acetic acid and ambroxol [11]. Various methods like spectrophotometry [12], RP-HPLC [13-15], LC-MS [16], HP-TLC [17] were reported for estimation of acebrophylline. acebrophylline is not official drug in IP, BP and USP [18].

Desloratadine (Fig. 1) is a non-sedative antihistamine agent whose IUPAC name is 8-chloro-6,11-dihydro-11-(4-piperidinylidene)5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine [19,20]. Desloratadine is partially soluble in water and completely soluble in ethanol [21]. Literature survey revealed that spectrophotometry [22], RP-HPLC [23-25] methods were used for estimation of desloratadine in combination with other drugs.

Montelukast, acebrophylline and desloratadine combination dosage form is available in the market for treating chronic asthma and chronic bronchitis. So far, there are no HPLC methods available in the literature for simultaneous quantification of montelukast, acebrophylline and desloratadine tablets. Hence, a new, simple, rapid, precise, accurate, robust and stability indicating that the developed method is for simultaneous quantification of montelukast, acebrophylline and desloratadine tablets.

EXPERIMENTAL

Montelukast, acebrophylline and desloratadine standards were obtained from Spectrum Pharma Pvt. Ltd. Hyderabad, India. Montelukast, acebrophylline and desloratadine tablets with brand name ACMON-DM manufactured by Aar Ess Remedies Pvt. Ltd. Noida, India was purchased from local pharmacy at Hyderabad, India. Both acetonitrile and methanol (HPLC-grade) were procured

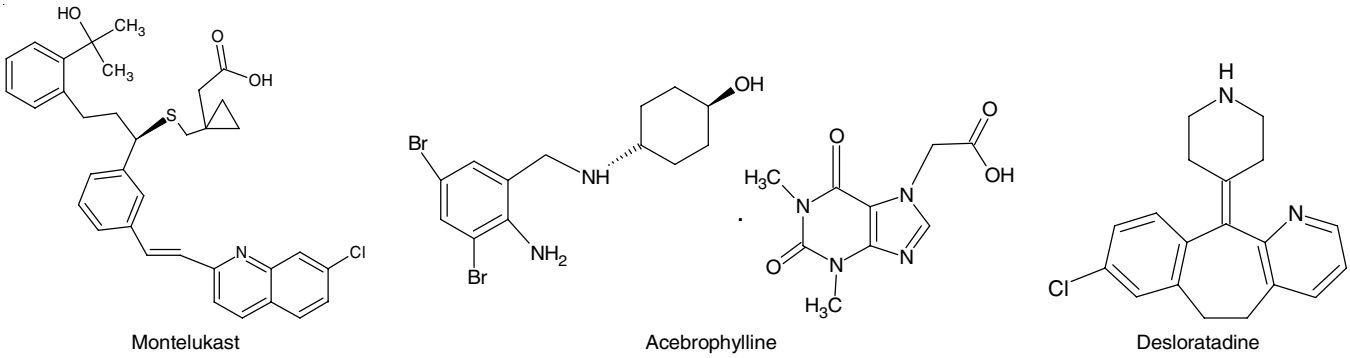

Fig. 1. Chemical structure of montelukast, acebrophylline and desloratadine

from Thermo Fischer Scientific India Pvt. Ltd. Milli-Q water was used in the mobile phase preparation. Waters HPLC system with Empower-2 software was used for chromatographic separations.

Chromatographic conditions

Chromatographic separation was carried out by emloying BDS C8 column (150 mm × 4.6 µm particle size) using 0.01N potassium dihydrogen ortho-phosphate buffer and acetonitrile in the ratio 40:60 % v/v as mobile phase. The flow rate was adjusted to 1 mL/min with run time 7 min. The coloumn temperature was maintained at 30 ºC. The column effluents were detected at isobestic point 280 nm and injection volume was 20 µL.

Preparation of 0.01N KH_2PO_4 buffer: Weighed 1.36g of KH_2PO_4 was transferred into a 1000 mL of volumetric flask and dissolved in 900 mL of Milli-Q water and made up to the volume with water and filtered through membrane filter (pore size 0.45 µ). The pH of above solution was adjusted to 3 with ortho-phosphoric acid.

Preparation of mobile phase: KH_2PO_4 buffer (0.01N, 600 mL) and acetonitrile (400 mL) mixture was used as mobile phase, degassed by ultrasonication and filtered through membrane filter of pore size 0.45 µ.

Preparation of diluent: The diluent used in the study was prepared by mixing 50:50 v/v of water and acetonitrile.

Preparation of standard solutions: Montelukast (10 mg), acebrophylline (200 mg) and desloratadine (5 mg) working standards were transferred into three separate 50 mL dry volumetric flasks. Then added about 30 mL of diluent to the flasks and dissolved by means of ultrasonication, make up the volume by adding the diluent. From the above solution, 1 mL was pipetted out and transferred into 10 mL dry volumetric flask to prepare the concentrations of 20, 400 and 10 ppm of montelukast, acebrophylline and desloratadine, respectively.

Preparation of sample solution: An amount equivalent to 10 mg of montelukast, 200 mg of acebrphylline and 5 mg of desloratadine was transferred into a 50 mL volumetric flask. The contents of the flask were dissolved in 30 ml of diluent, sonicated for 25min and made upto the final volume with diluent. 1 mL of above solution was pipetted and transferred into 10 mL volumetric flask and the final volume was made upto the mark using diluent. The final concentrations of montelukast, acebrophylline and desloratadine sample solution was found to be 20, 400 and 10 ppm, respectively.

RP-HPLC method: The current analytical method developed was validated according to ICH Q2(R1) guidelines [26]. The validation parameters engaged in the current study were specificity, linearity, precision, accuracy, robustness, LOD, LOQ and solution stability.

Specificity: The interferences of components of tablet sample matrix with the analyte peaks were determined by performing forced degradation studies. The sample matrix is subjected to various stress conditions such as acid, base, peroxide, heat and UV light. HPLC chromatogram of standard solution of montelukast, acebrophylline and desloratadine was presented in Fig. 2.

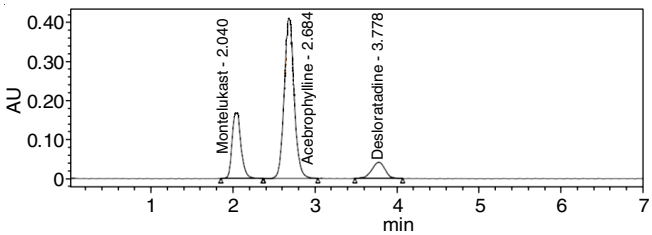

Fig. 2. Standard chromatogram of montelukast, acebrophylline and desloratadine

Linearity: Linearity of the analytes were determined by injecting six diverse concentrations of working standard solutions of montelukast (5-30 ppm), acebrophylline (100-600 ppm) and desloratadine (2.5-15 ppm) into HPLC system. The peak areas were recorded and the correlation coefficient was calculated.

Accuracy: Accuracy of the developed method was established by standard addition method at 50, 100 and 150% levels for montelukast, acebrophylline and desloratadine. The sample solutions were injected thrice into HPLC system and the percentage recovery was calculated.

Precision: System precision was established by injecting six different solutions of montelukast having concentration equal to 20 ppm, acebrophylline having concentration equal to 400 ppm and desloratadine having concentration equal to 10 ppm. The mean and % RSD values of peak area and retention time were calculated accordingly.

Robustness: Robustness of the method is established by intentionally changing the selected chromatographic conditions like flow rate, mobile phase ratio and temperature of the column. The flow rates selected for study were 0.8, 1.0 and 1.2 mL/min. The column temperature was changed from 25 to 30 ºC and

35 ºC. The sample solution was injected into the HPLC system in triplicate and the peak areas were recorded.

RESULTS AND DISCUSSION

Linearity was obtained in the concentration range 5-30 ppm for montelukast, 100-600 ppm for acebrophylline and 2.5-15 ppm desloratadine for the developed method. The linearity curve obtained for montelukast, acebrophylline and desloratadine was linear with correlation coefficient 0.999 for all the three analytes (Table-1).

TABLE-1
LINEARITY RESULTS FOR MONTELUKAST, ACEBROPHYLLINE AND DESLORATADINE

Parameter	Regression equation parameters		
	Montelukast	Acebrophylline	Desloratadine
Linearity range (ppm)	5-30	100-600	2.5-15
Correlation co-efficient	0.999	0.999	0.999
Slope	62549	8492	46066
Y-intercept	9835	17955	2145
LOD (ppm)	0.01	0.32	0.01
LOQ (ppm)	0.03	0.98	0.04

Accuracy of the developed method was established by standard addition method by preparing 50, 100 and 150 % level solutions, the results were presented in Table-2. Precision of the proposed method was assessed by system precision and method precision studies. System precision values were analyzed by injecting standard solution six times in to HPLC system.

TABLE-2
ACCURACY RESULTS

Drugs	Spiked concentration (ppm)		Recovery (%)
Montelukast	10	50 %	98.61
	20	100 %	98.47
	30	150 %	99.94
Acebrophylline	200	50 %	98.43
	400	100 %	98.71
	600	150 %	99.14
Desloratadine	5	50 %	100.18
	10	100 %	100.11
	15	150 %	98.61

The % RSD values of system precision was observed to be 0.4, 0.1 and 1.0 as shown in Table-3. The % RSD values of method precision was observed to be 0.7, 0.1 and 0.5.The results implied that the method developed was accurate and precise. LOD and LOQ values of montelukast, acebrophylline and desloratadine are given in the Table-1.

Robustness: The proposed method is unaffected by small variations in method parameters and influential environmental factors (room temperature, air humidity, *etc*.) and characterize its reliability during normal usage. The peak areas were recorded and shown in Table-4.

Forced degradation studies: Montelukast, acebrophylline and desloratadine sample solutions were exposed to stress conditions like acid, base, peroxide, heat, water and UV light. The three drugs were degraded enough in the applied stress conditions. Forced degradation studies indicated that montelukast, acebrophylline and desloratadine are not stable in acidic and basic stress conditions and showed 3-4 % degradation. There was no interference of degraded products in the analyte peaks, hence

TABLE-3
SYSTEM PRECISION VALUES FOR MONTELUKAST, ACEBROPHYLLINE AND DESLORATADINE

S. No.	Peak name	Rt (min)	Area	USP plate count	USP tailing	Rt (min)	Area	USP plate count	USP tailing	Rt (min)	Area	USP plate count	USP tailing
		MONTELUKAST				ACEBROPHYLLINE				DESLORATADINE			
1	Inj-1	2.037	1274217	2031	2031	2.676	3475141	2521	1.10	3.759	476398	2831	1.01
2	Inj-2	2.040	1282706	2099	2157	2.684	3471338	2552	1.09	3.772	465728	2676	0.99
3	Inj-3	2.042	1286826	2109	1771	2.684	3471542	2482	1.11	3.778	467715	2504	0.99
4	Inj-4	2.042	1283710	1.24	1.20	2.687	3473153	2393	1.07	3.778	465620	2848	0.99
5	Inj-5	2.043	1281615	1.24	1.22	2.688	3472831	2531	1.10	3.784	466225	2880	0.99
6	Inj-6	2.044	1289977	1.16	1.19	2.688	3483102	2531	1.08	3.789	472877	2655	0.99
Mean			1283175				3474518				469094		
Std. Dev.			5343.8				4421.6				4498.7		
% RSD			0.4				0.1				1.0		

TABLE-4
RESULTS OF ROBUSTNESS STUDY

Chromatographic conditions	Rt (min)			Average area		
	MON	ACE	DES	MON	ACE	DES
Buffer:Acetonitrile 45:55(v/v)	2.026	2.554	3.354	1294316	3485593	482179
Buffer:Acetonitrile 40:60(v/v)	2.041	2.685	3.777	1283175	3474518	469094
Buffer:Acetonitrile 35:65(v/v)	2.037	2.770	4.158	1308581	3500083	474600
Flow rate (0.8 mL/min)	2.148	2.790	3.884	1265273	2996594	331663
Flow rate (1.0 mL/min)	2.041	2.685	3.777	1283175	3474518	469094
Flow rate (1.2 mL/min)	1.945	2.552	3.589	1222223	3287345	447875
Temperature (25 °C)	2.049	2.716	3.922	1230066	2856676	317339
Temperature (30 °C)	2.041	2.685	3.777	1283175	3474518	469094
Temperature (35 °C)	2.041	2.603	3.512	1228822	2845522	324081

TABLE-5
RESULTS OF FORCED DEGRADATION STUDY

Stress conditions	Assay of active ingredients (%)					
	MON	Degradation (%)	ACE	Degradation (%)	DES	Degradation (%)
Acid, 2 N HCl	95.05	4.92	95.48	4.52	95.06	3.25
Base, 2 N NaOH	97.28	4.07	97.33	3.91	97.02	2.32
H_2O_2 (20 %, v/v)	98.01	1.99	98.35	2.69	98.04	1.80
Dry heat (105 °C)	99.4	1.85	99.23	0.89	99.32	1.00
UV	99.27	0.48	99.32	0.68	99.34	0.52
Water	99.23	0.28	99.27	0.73	99.42	0.35

the method was proved to be stability indicating method. The results of stress studies were provided in Table-5.

Conclusion

A simple, sensitive, fast, accurate, precise and specific RP-HPLC-PDA method has been developed for the simultaneous quantification of montelukast, acebrophylline and desloratadine in bulk and tablet dosage form. The developed method was validated by linearity, precision, accuracy, robustness, limit of detection and limit of quantification. The proposed method is satisfactorily applied for the separation of analyte peaks in presence of degradation products. The chromatograms showed that there were no interference peaks due to degradants, hence the method is stability indicating and can be applied for quality control of montelukast, acebrophylline and desloratadine combination tablets.

ACKNOWLEDGEMENTS

The authors are grateful to Spectrum Pharma Research Solutions, Hyderabad, India for providing the gift samples, and laboratory facilities to carry out the research work.

REFERENCES

1. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission: Vol II, Ministry of Health and Family Welfare, Government of India, India, pp 1704-05 (2010).
2. D.S. Kumar and Y.N. Allada Narayudu, *The Pharma Innov. J.*, **3**, 54 (2014).
3. T. Radhakrishna, A. Narasaraju, M. Ramakrishna and A. Satyanarayana, *J. Pharm. Biomed. Anal.*, **31**, 359 (2003); https://doi.org/10.1016/S0731-7085(02)00650-7.
4. T.H. Hoang, R. Farkas, C. Wells, S. McClintock and M. Di Maso, *J. Chromatogr. A*, **968**, 257 (2002); https://doi.org/10.1016/S0021-9673(02)00956-1.
5. L. Liu, H. Cheng, J.J. Zhao and D.D. Rogers, *J. Pharm. Biomed. Anal.*, **15**, 631 (1997); https://doi.org/10.1016/S0731-7085(96)01894-8.
6. R.M. Singh, P.K. Saini, S.C. Mathur, G.N. Singh and B. Lal, *Ind. J. Pharm. Sci.*, **72**, 235 (2010); https://doi.org/10.4103/0250-474X.65023.
7. C.J. Kitchen, A.Q. Wang, D.G. Musson, A.Y. Yang and A.L. Fisher, *J. Pharm. Biomed. Anal.*, **31**, 647 (2003); https://doi.org/10.1016/S0731-7085(02)00723-9.
8. R. Papp, P. Luk, W.M. Mullett and E. Kwong, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **858**, 282 (2007); https://doi.org/10.1016/j.jchromb.2007.08.003.
9. N.S. Jadhav and K.G. Lalitha, *Int. J. Pharm. Phytopharmacol. Res*, **4**, 113 (2014).
10. G. Agliati, *Curr. Therapeutic Res.*, **56**, 169 (1995); https://doi.org/10.1016/0011-393X(95)85042-2.
11. A.G. Susmita, G. Aruna, S. Angalaparameswari and M. Padmavathamma, *Asian J. Pharm. Res.*, **5**, 143 (2015).
12. J. Maniya, H. Raj, H. Vaghani, M. Mangukiya and P. Dudhat, *Indo Am. J. Pharm. Res.*, **2**, 1027 (2012).
13. S. Takkarusu, S. Thota, V.R. Kumar and V. Neerati, *Res. J. Pharm. Biol. Chem. Sci.*, **5**, 480 (2014).
14. S. Ramanjaneyulu, *Int. J. Inventions Pharm. Sci.*, **1**, 404 (2013).
15. R. Mohit Bauskar, *Res. J. Pharm. Technol.*, **4**, 1542 (2011).
16. K.-D. Nam, J.-H. Seo, S.-V. Yim and K.-T. Lee, *J. Pharm. Investig.*, **41**, 309 (2011); https://doi.org/10.4333/KPS.2011.41.5.309.
17. W.D.S. Solomon, M. Manu, R. Sivakumar, R. Venkatanarayanan and P.R. Vijai Anand, *J. Pharm. Res.*, **3**, 2561 (2010).
18. M.L. Kharodiya, T.P. Patel, L.M. Prajapati and A.K. Joshi, *World. J. Pharm. Res.*, **4**, 1808 (2015).
19. https://pubchem.ncbi.nlm.nih.gov/compound/Desloratadine.
20. http://www.drugbank.ca/drugs/DB00967.
21. N.V.S. Naidu and R. Challa, *Int. J. Pharm. Anal. Res.*, **5,** 294 (2016).
22. A.A. Gouda and M. Kasssem, *Arabian J. Chem.*, **9S2**, S1712 (2016); https://doi.org/10.1016/j.arabjc.2012.04.050.
23. R.B. Patel, M.R. Patel and J.B. Mehta, *Arabian J. Chem.*, **10S1**, S644 (2017); https://doi.org/10.1016/j.arabjc.2012.10.026.
24. M. Qi, P. Wang and Y. Geng, *J. Pharm. Biomed.*, **38**, 355 (2005); https://doi.org/10.1016/j.jpba.2005.01.003.
25. D.T. El-Sherbiny, N. El-Enany, F.F. Belal and S.H. Hansen, *J. Pharm. Biomed. Anal.*, **43**, 1236 (2007); https://doi.org/10.1016/j.jpba.2006.10.027.
26. ICH, Q2 (R1), Harmonized Tripartite Guidelines, Validation of Analytical Procedures, Text and Methodology, London (2009).