Asian Journal of Chemistry; Vol. 29, No. 1 (2017), 145-148

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2017.20164

Simultaneous Quantification of Olodaterol and Tiotropium Bromide by High Performance Liquid Chromatography

BHAIRAM BHOOMAIAH* and ANIREDDY JAYASREE

Centre for Chemical Sciences and Technology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad-500 085, India

*Corresponding author: E-mail: bhoomesh.analyst@gmail.com

Received: 1 July 2016; *Accepted*: 18 September 2016; *Published online*: 29 October 2016; AJC-18115

The present paper describes a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of olodaterol and tiotropium bromide. Chromatographic separations were carried out on Thermo Hypersil C8 analytical column (150 mm × 4.6 mm, 5 µ particle size). An isocratic elution system was developed using phosphate buffer:methanol [55:45 v/v]. The pH of the mobile was adjusted to 4.4 with orthophosphoric acid. The elution of the analytes was achieved with a flow rate of 1 mL/min. Detection was made by UV absorbance at a wavelength of 236 nm. The detector response was linear in the concentration range of 12.5-37.5 µg/mL for both the analytes. The limit of detection and limit of quantitation values were found to be 0.037 and 0.124 µg/mL for olodaterol and 0.023 and 0.079 µg/mL for tiotropium bromide, respectively. The method was validated following ICH guidelines. All the parameters of validation were found in the acceptance range.

Keywords: Olodaterol, Tiotropium bromide, HPLC, Analysis, Validation.

INTRODUCTION

Olodaterol [1-3], chemically known as 6-hydroxy-8-[(1R)-1-hydroxy-2-[[1-(4-methoxyphenyl)-2-methylpropan-2-yl]amino]ethyl]-4H-1,4-benzoxazin-3-one, is a adrenergic β-2 receptor agonist. Olodaterol is highly selective for β-2 receptor located in lungs. It has lower activity at the β-1 and β-3 adrenergic receptors that are commonly located in cardiac smooth muscle and adipose tissue, respectively. Binding of olodaterol to the β-2 receptor causes smooth muscle relaxation in the lungs and bronchodilation. Olodaterol is prescribed for the control of chronic obstructive pulmonary disease symptoms.

Tiotropium bromide [4-6], chemically described as (1α,2β,4β,7β)-7-[(hydroxidi-2-thienylacetyl)oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonane bromide, is a antimuscarinic agent or also referred as cholinergic antagonist. Tiotropium bromide mainly acts on M3 muscarinic receptors present in the smooth muscle cells, preventing smooth muscle contraction, as a result producing a bronchodilatory effect. The cholinergic antagonist effect of tiotropium bromide is to block the effect of cholinergic nerves, leading to the relaxation of muscles and dilation of airways. Tiotropium bromide is prescribed for the management of chronic obstructive pulmonary disease.

Olodaterol and tiotropium bromide combination is used in the treatment of airflow obstruction in patients with chronic obstructive pulmonary disease. The combination of these two drugs with different mechanisms of action improves breathing more than either drug alone [7,8]. Tiotropium bromide and olodaterol combination inhalation spray (Stiolto Respimat, Boehringer Ingelheim Pharmaceuticals, Inc., USA), was approved by the Food and Drug Administration in 2015 [9].

Literature survey revealed that no RP-HPLC method has been reported for simultaneous quantitation of olodaterol and tiotropium bromide. Hence, a RP-HPLC method has been developed in the present work for simultaneous estimation of olodaterol and tiotropium bromide in the presence of their stress degradants.

EXPERIMENTAL

Mobile phase: HPLC grade and analytical grade solvents and chemicals, respectively are used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used was phosphate buffer and methanol (Merck India Ltd., Mumbai) in the ratio of 55:45 v/v. Phosphate buffer was prepared by dissolving 1.3609 g of potassium dihydrogen phosphate (S.D. Fine Chemicals Ltd., Mumbai) in 300 mL of double distilled water in a 1000 mL volumetric flask and made up to the volume with the same solvent. pH of the buffer was adjusted to 4.4 with orthophoshoric acid (Sd. Fine Chemicals Ltd., Mumbai). Prior to use, the mobile phase was filtered through millipore membrane filter and degassed for 15 min by sonication.

Instrumentation and chromatographic conditions: In the present study Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used. Thermo Hypersil C8 (150 × 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of olodaterol and tiotropium bromide. The column temperature was maintained at 30 ± 1 °C. Isocratic elution with a flow rate of 1 mL/min was used. The injection volume was 10 µL. The eluents were detected at 236 nm.

Standard solutions: Olodaterol and tiotropium bromide reference standard samples were obtained from Lara Drugs Pvt. Ltd., Hyderabad. The standard stock solution was prepared by dissolving 100 mg each of olodaterol and tiotropium bromide in 100 mL mobile phase. Working standard solutions equivalent to 12.5, 18.75, 25.0, 31.25, 37.5 µg/mL olodaterol and tiotropium bromide was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

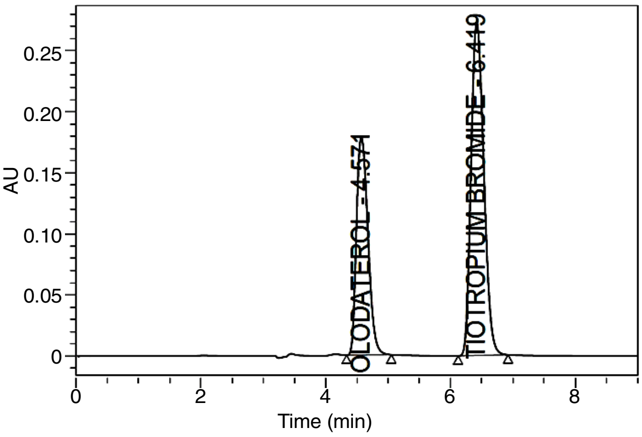
Calibration curve: 10 µL of working standard solutions (12.5-37.5 µg/mL olodaterol and tiotropium bromide) was injected automatically into the column in triplicate under the described chromatographic conditions. The chromatograms were recorded. The calibration curve was prepared by plotting the mean peak area *versus* concentration of analyte in µg/mL. The five concentrations of each drug were subjected to regression analysis to calculate the regression equation and regression coefficients.

RESULTS AND DISCUSSION

HPLC method optimization: The chromatographic conditions (analytical column, composition of the mobile phase, its pH, its flow rate and detection wavelength) were optimized through several trials to achieve the better sensitivity, good symmetric peak shape for olodaterol and tiotropium bromide and ability to give better separation between the olodaterol, tiotropium bromide and its possible degradation products. Different combination ratios of phosphate buffer at different pH and methanol were tested. The best chromatographic separation was achieved on Thermo Hypersil C8 (150 × 4.6 mm; 5 µm particle size) using a phosphate buffer and methanol (55:45 v/v) as mobile phase. pumped with a flow rate of 1 mL/min. The column temperature was kept constant at 30 ± 1 °C. The maximum absorption of olodaterol, tiotropium bromide together was detected at 236 nm and this wavelength was chosen for the analysis. Using the above described conditions, the retention times for olodaterol and tiotropium bromide was observed to be 4.571 and 6.419 min, respectively (Fig. 1). Total run time of analysis was less than 7 min.

HPLC method validation: Method validation was done in accordance with ICH recommendation [10].

System suitability: Chromatographic parameters associated to the developed method must pass the system suitability limits before the analysis of sample. The relative standard deviation of peak area, theoretical plates, resolution and tailing factor for olodaterol and tiotropium bromide peaks was evaluated using a solution containing 25 µg/mL of olodaterol and tiotropium bromide. All the results (Table-1) are satisfactory and the proposed method for routine analysis of olodaterol and tiotropium bromide simultaneously.



Peak name	Retention time	Peak area	USP plate count	USP tailing	USP resolution
Olodaterol	4.571	2287489	2922	1.30	–
Tiotropium bromide	6.419	3864639	4689	1.22	5.15

Fig. 1. Chromatogram of olodaterol and tiotropium bromide by the developed method

TABLE-1
SYSTEM SUITABILITY RESULTS

Parameters	Olodaterol	Tiotropium bromide	Recommended limits
Peak area	2286945 (% RSD – 0.082)	3865011 (% RSD – 0.040)	RSD ≤ 2
USP resolution	–	5.15	> 1.5
USP plate count	2893	4689	> 2000
USP tailing factor	1.29	1.22	≤ 2

Specificity: The specificity study was assessed to verify the absence of interference by the components of mobile phase. For this study, standard solution (25 µg/mL) and mobile phase blank solution were injected into the chromatographic system. The chromatograms were recorded shown in Figs. 2 and 3. The chromatogram demonstrated the specificity of the proposed method, since there were no peaks at the retention time of olodaterol and tiotropium bromide in the chromatogram of mobile phase blank.

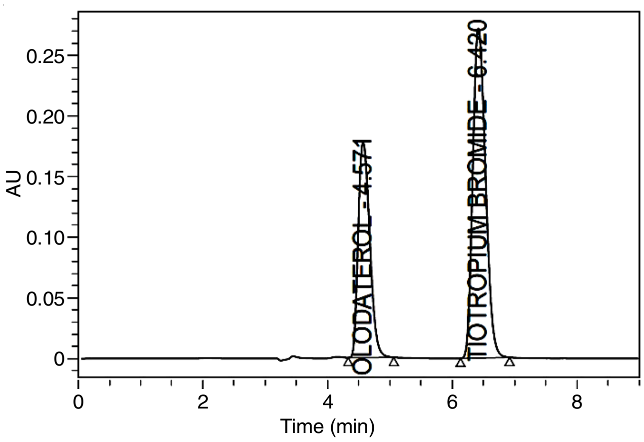
Linearity, limits of detection and quantification: Table-2 presents the equation of the regression line, regression coefficient (R^2), slope and intercept for each drug. Excellent linearity with good regression coefficient were found between the peak area and concentration in the range of 12.5-37.5 µg/mL for both olodaterol and tiotropium bromide. The high R^2 value was indicative of good linearity.

The limit of detection (LOD) and limit of quantitation (LOQ), which represents the sensitivity of the method, were calculated based on the signal-to-noise ratio. LOD and LOQ were experimentally verified by five injections of olodaterol and tiotropium bromide at the LOD and LOQ concentrations.

TABLE-2
LINEARITY RESULTS, LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTICATION (LOQ)

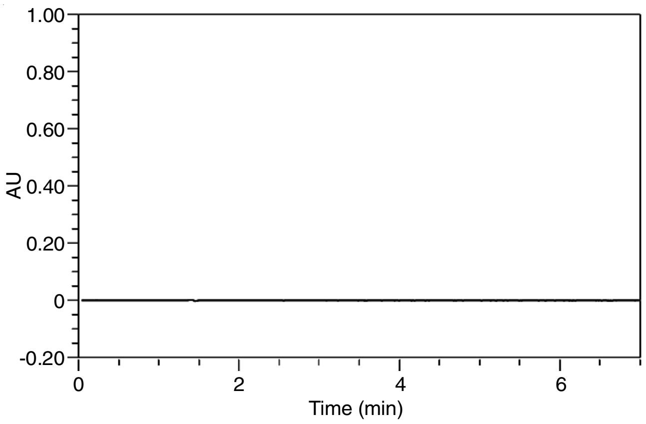
Drug	Regression equation (Y = mX + c)	Regression coefficient (R^2)	LOD (µg/mL)	LOQ (µg/mL)
Olodaterol	Y = 15814 X – 21429	0.9990	0.023	0.079
Tiotropium bromide	Y = 91080 X + 12531	0.9999	0.037	0.124

X = Concentration (µg/mL); Y = Area; m = slope; c = intercept



Peak name	Retention time	Peak area	USP plate count	USP tailing	USP resolution
Olodaterol	4.571	2286699	2906	1.30	–
Tiotropium bromide	6.420	3862153	4654	1.22	5.13

Fig. 2. Chromatogram of standard solution



Peak name	Retention time	Peak area	USP plate count	USP tailing	USP resolution
Olodaterol	–	–	–	–	–
Tiotropium bromide	–	–	–	–	–

Fig. 3. Chromatogram of mobile phase blank

The results (Table-2) indicated the adequate sensitivity of the method for the simultaneous assay of olodaterol and tiotropium bromide.

Precision: The precision of the method (within-day variations of replicate determinations) was checked by injecting olodaterol and tiotropium bromide standard solution 6 times at the 25 µg/mL concentration level. The precision of the method, expressed as the % RSD, was 0.410 and 0.117 % for olodaterol and tiotropium bromide, respectively (Table-3). The low percent RSD values indicated the precision of the method.

TABLE-3
METHOD PRECISION RESULTS

Olodaterol		Tiotropium bromide	
Peak area		Peak area	
2268007	Mean peak area:	3874157	Mean peak area:
2256525		3869788	
2279592	2271938	3880394	3873468
2268717		3867215	
2280252	%RSD:	3874102	%RSD:
2278537	0.410	3875156	0.117

Accuracy: A standard working solutions containing olodaterol and tiotropium bromide, at concentration levels 12.5, 25.0 and 37.50 µg/mL was prepared. The prepared standards were injected 3 times in the HPLC system as a test sample. From the respective area counts, the concentrations of the olodaterol and tiotropium bromide were calculated using the detector responses. The accuracy represented in terms of percentage recovery is listed in Table-4. The good percent recovery values indicated the accuracy of the method.

Robustness: The method robustness was established at a concentration of 25 µg/mL olodaterol and tiotropium bromide. In order to measure the method robustness, the HPLC parameters were deliberately varied. The studied parameters were: column temperature (± 2 °C) and flow rate (± 0.1). The system suitability parameters were measured to demonstrate the robustness of the method. The results (Table-5) indicated that

TABLE-4
METHOD ACCURACY RESULTS

Olodaterol				Tiotropium bromide			
Taken (µg/mL)	Found (µg/mL)	Recovery (%)	Mean (%)	Taken (µg/mL)	Found (µg/mL)	Recovery (%)	Mean (%)
12.51	12.51	99.97	99.79	12.51	12.47	99.63	99.56
12.51	12.43	99.34		12.51	12.53	100.17	
12.51	12.52	100.04		12.51	12.37	98.87	
25.00	24.90	99.61	99.79	25.00	24.99	99.95	99.92
25.00	24.87	99.49		25.00	25.05	100.18	
25.00	25.07	100.28		25.00	24.91	99.63	
37.50	37.62	100.31	100.12	37.50	37.38	99.68	100.01
37.50	37.42	99.80		37.50	37.58	100.21	
37.50	37.66	100.42		37.50	37.55	100.14	

TABLE-5
METHOD ROBUSTNESS RESULTS

Parameter varied	Retention time	Peak area	USP plate count	USP tailing	USP resolution
Olodaterol					
Column temperature – 29 °C	4.502	2276150	2892	1.31	–
Column temperature – 31 °C	4.510	2276282	2955	1.30	–
Flow rate – 0.9 mL/min	5.606	2838605	3142	1.31	–
Flow rate – 1.1 mL/min	3.773	1895235	2514	1.29	–
Tiotropium bromide					
Column temperature – 29 °C	6.388	3847419	4686	1.22	5.30
Column temperature – 31 °C	6.390	3848004	4687	1.22	5.30
Flow rate – 0.9 mL/min	5.332	3205397	4421	1.23	4.98
Flow rate – 1.1 mL/min	6.958	4793079	4981	1.21	5.50

the small change in the conditions did not significantly affect the system suitability. Therefore, the method is robust.

Conclusion

The RP-HPLC-PDA detector system with C8 reversed-phase column (150 mm × 4.6 mm, 5 µm, particle size) was used in this study. Phosphate buffer and methanol in the ratio of 55:45 (v/v) was chosen as the mobile phase and a detection wavelength of 236 nm was used with a flow rate of 1.0 mL/min. The method validation was performed according to the guidelines of the ICH. The HPLC method for the simultaneous quantification of olodaterol and tiotropium bromide was successfully developed and validated. The method was validated in terms of linearity, accuracy, precision, selectivity, robustness, detection limit and quantitation limit. All the validation parameters were found to be within the acceptance limits.

ACKNOWLEDGEMENTS

The authors expresses their gratitude to Aurobindo Pharma Pvt. Ltd., Bachpally, Hyderabad, India for providing facilities in the laboratory.

REFERENCES

1. T. Bouyssou, P. Casarosa, E. Naline, S. Pestel, I. Konetzki, P. Devillier and A. Schnapp, *J. Pharmacol. Exp. Ther.*, **334**, 53 (2010).
2. T. Bouyssou, C. Hoenke, K. Rudolf, P. Lustenberger, S. Pestel, P. Sieger, R. Lotz, C. Heine, F.H. Büttner, A. Schnapp and I. Konetzki, *Bioorg. Med. Chem. Lett.*, **20**, 1410 (2010).
3. J.A. van Noord, J.J. Smeets, B.M. Drenth, J. Rascher, A. Pivovarova, A.L. Hamilton and P.J.G. Cornelissen, *Pulm. Pharmacol. Ther.*, **24**, 666 (2011).
4. M. Kato, K. Komamura and M. Kitakaze, *Circ. J.*, **70**, 1658 (2006).
5. S.J. Keam and G.M. Keating, *Treat. Respir. Med.*, **3**, 247 (2004).
6. G.M. Keating, *Drugs*, **72**, 273 (2012).
7. M. Cazzola, P. Rogliani, J. Ora and M.G. Matera, *Expert Rev. Clin. Pharm.*, **8**, 529 (2015).
8. G. Milano, V. Zavan, W.M. Natta, A. Martelli and F. Mattioli, *Pharm. Therap.*, **41**, 97 (2016).
9. Stiolto Respimat (Tiotropium Bromide/Olodaterol Inhalation Spray) Prescribing Information; Ridgefield, Connecticut: Boehringer Ingelheim Pharmaceuticals, Inc.; Jun, 2015; Available at: http://docs.boehringer-ingelheim.Com/Prescribing%20 Information/PIs/Stiolto%20Respimat/stiolto.pdf. Accessed October 15, 2015.
10. ICH Validation of Analytical Procedures; Text and Methodology; Q2(R1), International Conference on Harmonization (2005).