EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 2
418-424
Year: 2018

COMBINED QUANTIFICATION OF FORMOTEROL FUMARATE AND GLYCOPYRROLATE BY STABILITY INDICATING RP-HPLC METHOD WITH PHOTODIODE ARRAY DETECTOR

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Article Received on 11/12/2017 **Article Revised on 02/01/2018** **Article Accepted on 23/01/2018**

ABSTRACT
The current study involves development and validation of a stability-indicating RP-HPLC method with photodiode array detector for the combined estimation of formoterol fumarate and glycopyrrolate. Formoterol fumarate and glycopyrrolate was subjected to acid, alkaline, oxidative, thermal and photo degradation conditions. The chromatographic separation was carried out using a Sunsil C18 (250 × 4.6 mm; 5 µm particle size) column and phosphate buffer (pH 4.5): methanol (60:40 *v/v*) as the mobile phase. The formoterol fumarate, glycopyrrolate and their degradation products were detected at 289 nm. The linearity was observed in the range of 18-54 µg/mL (formoterol fumarate) and 9.6-28.8 µg/mL (glycopyrrolate). The limit of quantitation for formoterol fumarate and glycopyrrolate were found to be 1.116 µg/mL and 0.386 µg/mL, respectively. The percentage recovery was found in the range of 100.15-100.44% for formoterol fumarate and 99.66-99.74% for glycopyrrolate. Formoterol fumarate and glycopyrrolate was degraded under all the forced conditions applied. The method was able to resolve the degradation product from the peaks of analytes. Therefore, the developed and validated method can be applied for simultaneous estimation of formoterol fumarate and glycopyrrolate in the presence of the forced degradation products.

KEYWORDS: Formoterol fumarate, Glycopyrrolate, RP-HPLC, Forced degradation, Analysis.

INTRODUCTION
Formoterol fumarate is a long acting selective β2 adrenergic agonist. While giving treatment to patients with chronic obstructive pulmonary disease, formoterol fumarate is given to manage the asthma.[1,2] Chemically, formoterol fumarate is described as (E)-but-2-enedioic acid; N-[2-hydroxy-5-[(1S)-1-hydroxy-2-[[(2S)-1-(4-methoxyphenyl)propan-2-yl] amino] ethyl] phenyl] formamide (Fig. 1).

Glycopyrrolate is an anticholinergic agent with antispasmodic activity. Glycopyrrolate is used in the treatment of gastrointestinal conditions related with intestinal spasm and hyperhidrosis, and to lessen the secretions during anesthesia.[3,4] Chemically glycopyrrolate is described as (1,1-dimethylpyrrolidin-1-ium-3-yl) 2-cyclopentyl-2-hydroxy-2-phenylacetate (Fig. 1).

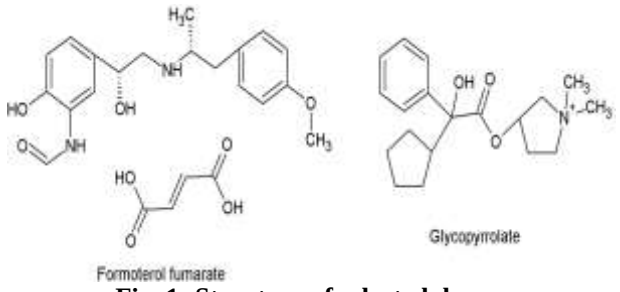

Fig. 1: Structure of selected drugs.

The combination of formoterol fumarate and glycopyrrolate is used for the long-term treatment of airflow obstacle in patients having chronic obstructive pulmonary disease.[5,6] The combination of formoterol fumarate and glycopyrrolate is not official in any pharmacopeia. To best of our data, there is only one RP-HPLC method that quantifies glycopyrrolate and formeterol fumarate simultaneously has been reported.[7] The reported method employed Xterra column as the stationary phase and 0.1% orthophosphoric acid: methanol (60:40 *v/v*) as the mobile phase. Till date no

stability indicating RP-HPLC method has been reported for the simultaneous quantification of glycopyrrolate and formeterol fumarate. The aim of the current study is to develop a stability indicating RP-HPLC method with photodiode array detector for the simultaneous determination of glycopyrrolate and formeterol fumarate. Quantitative estimation was done by photodiode array detector set at 289 nm wavelength.

MATERIALS AND METHODS

Apparatus and chromatographic conditions

Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used. Sunsil C18 (250 × 4.6 mm; 5 µm particle size) analytical column was utilized for separation and simultaneous quantification of glycopyrrolate and formeterol fumarate. The column temperature was maintained at 30±1°C. Isocratic elution with a flow rate of 1.0 mL/min was used. The injection volume was 10 µl. The eluents were detected at 289 nm.

Mobile phase

HPLC grade and analytical grade solvents and chemicals, respectively are used in the preparation of mobile phase. The mobile phase used was phosphate buffer and methanol (Merck India Ltd., Mumbai) in the ratio of 60:40 *v/v*. Phosphate buffer was prepared by dissolving 1.3609 g of potassium dihydrogen phosphate (Sd. 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 4.5 with orthophoshoric acid (Sd. Fine Chemicals Ltd., Mumbai). The mobile phase was filtered through 0.45 µm pore size membrane filter and also degassed for 15 min by sonication.

Standard solutions

Glycopyrrolate and formeterol fumarate reference standard samples were obtained from Lara drugs pvt Ltd., Hyderabad. The standard stock solution was prepared by dissolving 900 mg and 480 mg of formeterol fumarate and glycopyrrolate in 100 mL mobile phase in a 100 mL volumetric flask. Working standard solutions equivalent to 18, 27.0, 36.0, 45.0 and 54.0 µg/mL formeterol fumarate and 9.6, 14.4, 19.2, 24.0 and 28.80 µg/mL glycopyrrolate was prepared from stock solution by suitably diluting the stock standard solution with the mobile phase.

Calibration curve

10 µL of working standard solutions (18-50 µg/mL formeterol fumarate and 9.6-28.80 µg/mL glycopyrrolate) was injected into the column in triplicate under the described chromatographic conditions. The chromatograms and peak areas of glycopyrrolate and formeterol fumarate were recorded. The calibration curve was prepared by plotting the mean peak area versus concentration of drug (µg/mL). The concentration and peak areas of glycopyrrolate and formeterol fumarate were subjected to regression analysis to calculate the regression equation and regression coefficients. The concentration of glycopyrrolate and formeterol fumarate in unknown sample can be calculated using corresponding calibration curve or regression equation

Forced degradation studies

Forced degradation study was carried out to assess the specificity and stability indicating nature of the proposed method.[8] During forced degradation study, glycopyrrolate and formeterol fumarate was subjected to degradation conditions such as acid, alkaline, oxidative, thermal and photolytic.

During acid hydrolysis, 900 mg and 480 mg of formeterol fumarate and glycopyrrolate, respectively was mixed with 10 mL of 0.1N HCl in 100 mL volumetric flask. The solution was sonicated for 30 min. The solution was neutralized with sufficient volume of 0.1 N NaOH and diluted to the mark with mobile phase.

During alkaline hydrolysis, 900 mg of formeterol fumarate and 480 mg glycopyrrolate was mixed with 10 mL of 0.1N NaOH in 100 mL volumetric flask and sonicated for 30 min. The solution was neutralized with enough volume of 0.1 N HCl and diluted with mobile phase up to the mark.

Oxidative degradation was carried out by mixing 900 mg of formeterol fumarate and 480 mg of glycopyrrolate with 10 mL of H_2O_2 (3% *v/v*) in a 100 mL volumetric flask and the resultant solution was sonicated for 30 min. After oxidation, the solution was diluted with mobile phase up to the mark.

Photo degradation studies were carried out by the exposure of formeterol fumarate (900 mg) and glycopyrrolate (480 mg) to direct sunlight for 24 hrs. The sample was cooled and transferred to a 100 mL volumetric flask containing 30 mL of mobile phase, mixed well and completed up to mark with mobile phase.

Dry heating was performed by keeping formeterol fumarate (900 mg) and glycopyrrolate (480 mg) in hot air oven maintained at a temperature of 105 °C for 30 min. The treated sample was cooled and dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. The contents of the flask were mixed well and diluted up to the mark with mobile phase.

All the samples were diluted with mobile phase to get a final concentration of 36 µg/mL of formeterol fumarate and 19.2 µg/mL of glycopyrrolate. All the degradation samples were analyzed using the chromatographic conditions described.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The chromatographic conditions such as analytical column, composition of the mobile phase, mobile phase pH, mobile phase flow rate and detection wavelength were optimized by means of a number of trials to achieve

the good sensitivity, symmetric peak shape and good resolution for formeterol fumarate, glycopyrrolate and forced degradation products. Different combination ratios of phosphate buffer at different pH with methanol and acetonitrile were tested. Good chromatographic separation was achieved on Sunsil C18 (250 × 4.6 mm; 5 µm particle size; temperature 30˚C) using a phosphate buffer and methanol (60:40 *v/v*) as mobile phase with a flow rate of 1 mL/min. Good peak area response for formeterol fumarate and glycopyrrolate was detected at 289 nm and hence the same wavelength was preferred for the analysis. Using the described conditions, the retention times for formeterol fumarate and glycopyrrolate was observed to be 3.267 min and 3.847 min, respectively (Fig. 2). Total run time of analysis was 6 min.

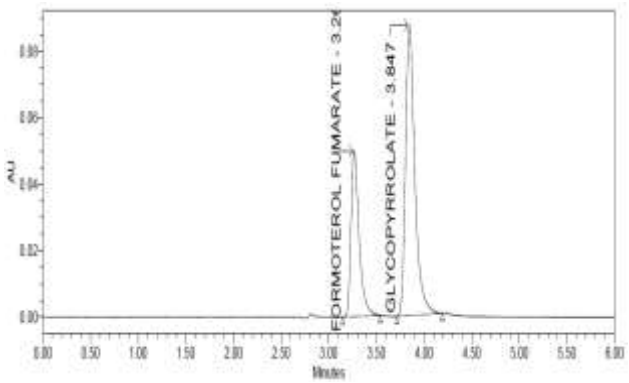

Fig. 2: Chromatogram of formeterol fumarate and glycopyrrolate by the proposed method.

Method validation

Method validation was completed following ICH recommendation.[9]

The relative standard deviation of peak area and retention time, theoretical plates, resolution and tailing factor for formeterol fumarate and glycopyrrolate peaks was determined to assess the system suitability. The system suitability studies were performed using a solution containing 36 µg/mL of formeterol fumarate and 19.2 µg/mL of glycopyrrolate. All the results (Table 1) declare the adequacy of the method for routine analysis of formeterol fumarate and glycopyrrolate simultaneously.

Table 1: Results of system suitability for the analysis of formeterol fumarate and glycopyrrolate

Parameters	Formeterol Fumarate	Glycopyrrolate	Recommended limits
Retention time	3.266 (% RSD – 0.093)	3.843 (% RSD – 0.091)	RSD ≤2
Peak area	293933 (% RSD – 0.522)	594765 (% RSD – 0.040)	RSD ≤2
USP resolution	-	3.496	> 1.5
USP plate count	7662	8249	> 2000
USP tailing factor	1.522	1.432	≤ 2

All the values given the table are average of five determinations

The method selectivity was done to verify the interference by the components of mobile phase. For this, working standard solution (36 µg/mL formeterol fumarate and 19.2 µg/mL glycopyrrolate) and mobile phase blank solution were injected into the HPLC system. The chromatograms were recorded (Fig. 3). The chromatogram confirmed the specificity of the proposed method, as no peaks were observed at the retention time of formeterol fumarate and glycopyrrolate in the chromatogram of mobile phase blank.

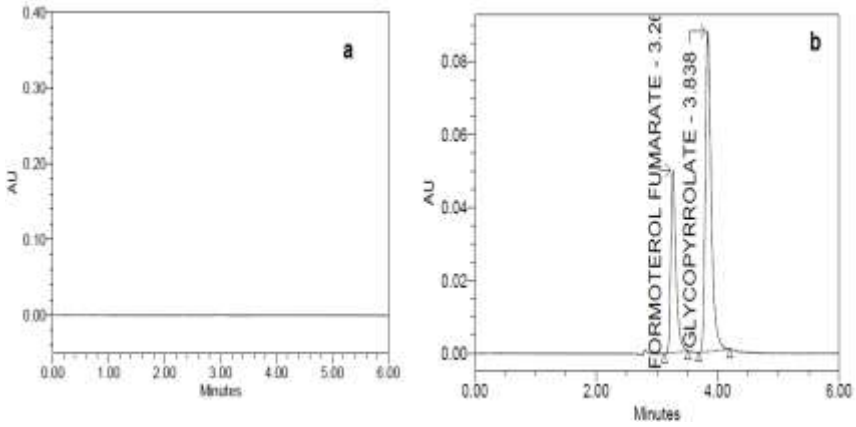

Fig. 3: Chromatogram of (a) Mobile phase blank (b) Standard solution of formeterol fumarate and glycopyrrolate.

The regression line equation, slope, intercept and regression coefficient (R^2) for formeterol fumarate and glycopyrrolate are presented in Table 2. Good linearity with excellent regression coefficient was established between the peak area and drug concentration in the range of 18-54 µg/mL for formeterol fumarate and 9.6-28.8 µg/mL for glycopyrrolate.

The method sensitivity parameters, limit of detection (LOD) and limit of quantitation (LOQ), were calculated based on the signal-to-noise ratio. The results (Table 2) indicated the sufficient sensitivity of the developed method for the combined assay of formeterol fumarate and glycopyrrolate.

Table 2: Linearity and sensitivity data for formeterol fumarate and glycopyrrolate

Parameter	Formeterol fumarate	Glycopyrrolate
Linearity (µg/mL)	18-54	9.6-28.8
Regression equation (y = mx + c)	y = 8160 *x* - 127	y = 30846 *x* + 761.5
Slope (m)	8160	30846
Intercept (c)	-127	761.5
Regression coefficient (R^2)	0.9997	0.9990
LOD (µg/mL)	0.335	0.116
LOQ (µg/mL)	1.116	0.386

The method precision and accuracy were tested by injecting formeterol fumarate (36µg/mL) and glycopyrrolate (19.2µg/mL) standard solution 6 times into the system. The precision and accuracy of the method were expressed as %RSD and % assay (Table 3), respectively. The low percent RSD values (<0.5%) and good % assay (~100%) for formeterol fumarate and glycopyrrolate indicated the satisfactory precision and accuracy of the method, respectively.

Table 3: Results of precision and accuracy for formeterol fumarate and glycopyrrolate

Sample No.	Formeterol fumarate		Glycopyrrolate	
	Peak area response	Assay (%)	Peak area response	Assay (%)
1	293661	99.41	594774	99.70
2	293675	99.41	594138	99.59
3	293624	99.40	594685	99.69
4	293569	99.38	594152	99.60
5	293741	99.43	594706	99.69
6	293464	99.34	594739	99.70
Average	293622	99.39	594532	99.66
RSD	0.0328	0.0326	0.0507	0.0506

The method accuracy was further demonstrated via recovery study. Formeterol fumarate and glycopyrrolate at concentration levels (50%, 100% and 150%) was added to placebo. The prepared solutions were injected thrice in the HPLC system. From the respective peak area response, the % recovery of formeterol fumarate and glycopyrrolate were calculated. The good percent recovery values (Table 4) indicated the accuracy and the non interference of the common excipients in the analysis of formeterol fumarate and glycopyrrolate by the developed method.

Table 4: Recovery study results for formeterol fumarate and glycopyrrolate.

Spiked Level (%)	Concentration of drug (µg/mL)		Recovery (%)	Mean (%)
	Spiked	Found		
Formeterol fumarate				
50	17.82	17.90	100.44	100.15
	17.82	17.81	99.93	
	17.82	17.83	100.06	
100	35.64	35.76	100.33	100.34
	35.64	35.75	100.31	
	35.64	35.78	100.38	
150	53.46	53.70	100.44	100.44
	53.46	53.69	100.42	
	53.46	53.70	100.44	
Glycopyrrolate				
50	9.60	9.58	99.80	99.69
	9.60	9.57	99.65	
	9.60	9.56	99.63	
100	19.20	19.15	99.73	99.66
	19.20	19.13	99.64	
	19.20	19.13	99.61	
150	28.80	28.73	99.76	99.74
	28.80	28.72	99.71	
	28.80	28.73	99.76	

The method robustness was assessed at a concentration of 36 µg/mL formeterol fumarate and 19.2 µg/mL glycopyrrolate. In order to determine the method robustness, the chromatographic parameters were deliberately varied. The studied parameters include: column temperature (±2°C) and flow rate (±0.1). The system suitability parameters were determined to show the robustness of the method. The results (Table 5) indicated that the deliberately varied changes in the chromatographic conditions did not significantly affect the system suitability. Hence, the method is robust.

Table 5: Robustness study results for formeterol fumarate and glycopyrrolate.

Parameter varied	Retention time	Peak area	Plate count	Tailing factor	Resolution
Formeterol fumarate					
Flow rate – 0.9 mL/min	2.704	244855	7017	1.51	-
Flow rate – 1.1 mL/min	4.054	370010	8450	1.52	-
Column temperature-29^{o}C	2.706	245726	7124	1.50	-
Column temperature-31^{o}C	4.051	367245	8537	1.56	-
Glycopyrrolate					
Flow rate – 0.9 mL/min	3.163	504926	7395	1.43	3.22
Flow rate – 1.1 mL/min	4.730	768205	8108	1.43	3.39
Column temperature-29^{o}C	3.170	505996	7590	1.41	3.27
Column temperature-31^{o}C	4.726	758747	8250	1.45	3.42

The forced degradation study indicated that formeterol fumarate and glycopyrrolate was susceptible to acidic, alkaline, oxidative, photolytic and thermal conditions. The chromatograms of acidic, alkaline, oxidative, photolytic and thermal sample of formeterol fumarate and glycopyrrolate showed an extra peak of the degradation product (Fig. 4-8). The degraded product was well resolved from the formeterol fumarate and glycopyrrolate with significantly different retention time. The results for the forced degradation study are summarized in Table 6.

Table 6: Summary of formeterol fumarate and glycopyrrolate degradation studies.

Stress condition	Formeterol fumarate			Glycopyrrolate			Retention time of degradants
	Peak area	Recovery (%)	Degra-dation (%)	Peak area	Recovery (%)	Degra-dation (%)	
Acid	272112	92.21	7.79	556135	93.22	6.78	2.807
Alkaline	277127	93.90	6.10	557131	93.39	6.61	2.805
Oxidative	274684	93.08	6.92	556999	93.37	6.63	2.809
Thermal	272016	92.17	7.83	564978	94.71	5.29	2.809
Photo	275250	93.27	6.73	561688	94.16	5.84	2.806

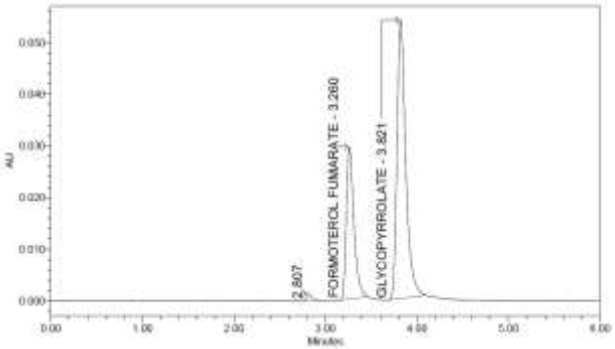

Fig. 4: Chromatogram of acid induced degradation of formeterol fumarate and glycopyrrolate

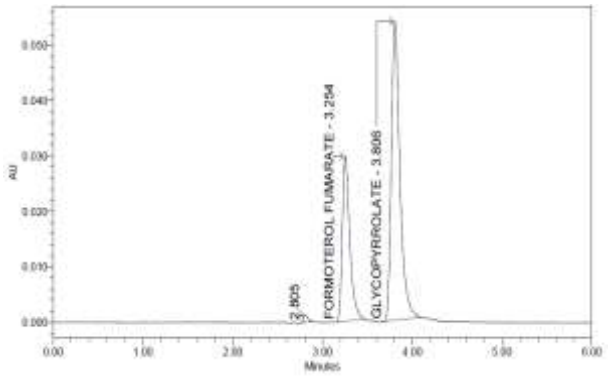

Fig. 5: Chromatogram of alkali induced degradation of formeterol fumarate and glycopyrrolate.

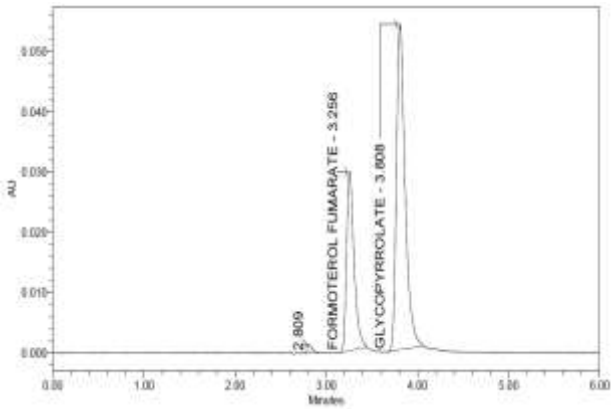

Fig. 6: Chromatogram of hydrogen peroxide induced degradation of formeterol fumarate and glycopyrrolate.

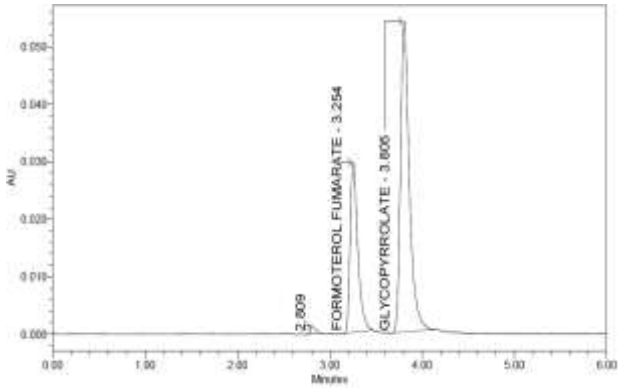

Fig. 7: Chromatogram of heat induced degradation of formeterol fumarate and glycopyrrolate.

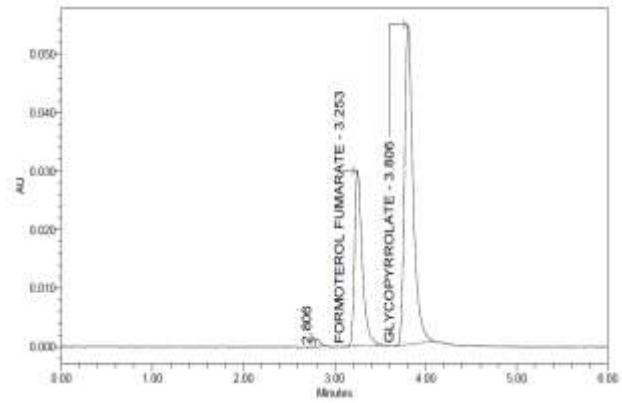

Fig. 8: Chromatogram of photo induced degradation of formeterol fumarate and glycopyrrolate.

CONCLUSION

A stability-indicating RP-HPLC method with photodiode detector for simultaneous estimation of formeterol fumarate and glycopyrrolate has been developed using Sunsil C18 analytical column as stationary phase and phosphate buffer: methanol (60:40 *v/v*) as the mobile phase. The method was validated following ICH recommendations for linearity, selectivity, specificity, precision, accuracy, LOD, LOQ and robustness. The method was successfully able to quantify formeterol fumarate and glycopyrrolate in the presence of their degradants formed under different forced degradation conditions. Hence, a developed method is specific and stability indicating for simultaneous estimation of formeterol fumarate and glycopyrrolate. The proposed method can be applied for the assay of combined pharmaceutical dosage forms of formeterol fumarate and glycopyrrolate.

ACKNOWLEDGEMENTS

The author, M.V. Reddy, expresses his deep sense of gratitude to the Department of Chemistry, GITAM Institute Technology, GITAM University, and Visakhapatnam for their encouragement and facilities provided throughout the project.

REFERENCES

1. Bartow RA, Brogden RN. Formoterol. An update of its pharmacological properties and therapeutic efficacy in the management of asthma. Drugs, 1998; 55(2): 303-22.
2. Berger WE. The use of inhaled formoterol in the treatment of asthma. Ann Allergy Asthma Immunol, 2006; 97(1): 24-33.
3. Howard J, Wigley J, Rosen G, D'mello J. Glycopyrrolate: It's time to review. J Clin Anesth, 2017; 36: 51-3.
4. Lee HH, Kim DW, Kim DW, Kim C. Efficacy of glycopyrrolate in primary hyperhidrosis patients. Korean J Pain, 2012; 25(1): 28-32.
5. Radovanovic D, Mantero M, Sferrazza Papa GF, Valenti V, Aliberti S, Di Marco F, Santus P. Formoterol fumarate + glycopyrrolate for the treatment of chronic obstructive pulmonary disease. Expert Rev Respir Med, 2016; 10(10): 1045-55.
6. Reisner C, Fabbri LM, Kerwin EM, Fogarty C, Spangenthal S, Rabe KF, Ferguson GT, Martinez FJ, Donohue JF, Darken P, St Rose E, Orevillo C, Strom S, Fischer T, Golden M, Dwivedi S. A randomized, seven-day study to assess the efficacy and safety of a glycopyrrolate/formoterol fumarate fixed-dose combination metered dose inhaler using novel Co-Suspension™ Delivery Technology in patients with moderate-to-very severe chronic obstructive pulmonary disease. Respir Res, 2017; 18(1): 8.
7. Akram NMd, Umamahesh M. A new validated RP-HPLC method for the determination of glycopyrrolate and formeterol fumarate in its bulk and pharmaceutical dosage forms. World J Pharm Pharm Sci, 2017; 6 (6): 903-17.
8. Geneva: Proceeding of the International Conference on Harmonization; 1996. ICH, Q1 (B), Harmonized Tripartite Guideline, Stability testing: Photostability Testing of New Drug Substances and Products, in.
9. International Conference on Harmonization, ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.