Marmara Pharmaceutical Journal 20: 275-279, 2016
DOI: 10.12991/mpj.20162030844

ORIGINAL RESEARCH

A validated spectrophotometric method for determination of formoterol fumarate dihydrate in bulk and dosage form using methyl orange as ion pair reagent

Duygu TAŞKIN, Gizem ERENSOY, Sıdıka SUNGUR

ABSTRACT

In this study rapid, simple, accurate and sensitive spectrophotometric method has been developed for the determination of formoterol fumarate dihydrate in bulk and dosage forms. The method is based on the formation of yellow coloured ion pair complex due to the reaction of formoterol fumarate dihydrate (FF) and methyl orange (MO) at pH 4. Ion pair complex has a maximum absorption at 428 nm in chloroform and a linear calibration over the range of 4-20 µg/mL. The slope of the calibration curve was 0.0433, limit of detection was 0.22 µg/mL and limit of quantification was 0.66 µg/mL. The proposed method has been applied to the assay of formoterol fumarate dihydrate commercially available capsules. There was no significiant difference between the results obtained by proposed and reference methods in view of accuracy and reproducibility. No interference was observed from common excipients.

Keywords: Formoterol Fumarate dihydrate, spectrophotometry, ion pair complex, methyl orange

1. INTRODUCTION

Formoterol fumarate (*N*-[2-hydroxy-5-[1-hydroxy-2-[1-(4-methoxyphenyl) propan-2-ylamino]ethyl] phenyl] formamide) dihydrate (Figure 1) is a long acting β-2 agonist used for asthma and chronic obstructive pulmonary disease (1-3).

Some analytical methods for quantitative determination of formoterol fumarate in biological fluids by LC-MS/MS (3, 4). A survey of the literature also revealed that a few analytical methods have been reported for the determination of formoterol fumarate by HPLC (5-10), capillary electrophoresis (11), capillary electrophoresis with laser induced fluorescence after derivatization (12), derivative spectroscopic method for dosage form (13) spectrophotometry (14-15), chiral HPLC method (16), gas chromatography (17), HPTLC (18), quantitative NMR (19) for pure and pharmaceutical forms. But no ion pair extraction method has been reported for formoterol fumarate dihydrate.

Extractive spectrophotometric procedures are the most widely used techniques because of their simplicity, cost-effectiveness, sensitivity in many pharmaceutical analysis; ofloxacin and lomefloxacin (20), tadalafil (21), ranitidine (22), sertaconazole nitrate and miconazole nitrate (23), enoxacin (24), levofloxacin (25). Thus ion-pair extractive

Duygu Taşkın, Sıdıka Sungur
Istanbul Yeni Yüzyıl University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul-Turkey.

Gizem Erensoy
Istanbul Yeni Yüzyıl University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul-Turkey.

Correspondence:
Duygu Taşkın
Tel: +90 212 444 50 01
duygu.susuz@yeniyuzyil.edu.tr

Submitted / Gönderilme: 15.04.2016 **Revised / Düzeltme:** 06.06.2016
Accepted / Kabul: 12.06.2016

spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds.

In this study we report the development of accurate and precise extractive spectrophotometric method based on FF-MO ion-pair complex in chloroform and the measure the absorbance of coloured complex. The proposed method was applied successfully for the determination of the formoterol fumarate dihydrate in bulk and dosage form. No interference was observed from commonly used tablet excipients. The method was validated by the statistical data and can be easily adapted for industrial analysis.

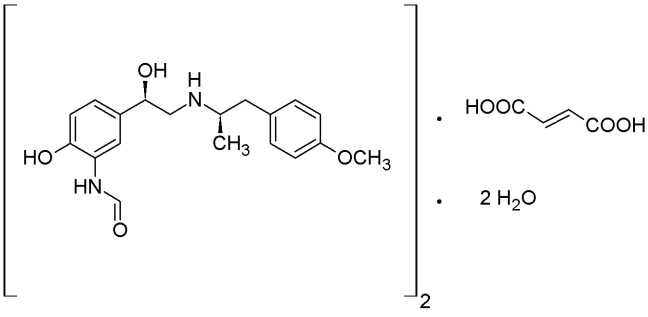

Figure 1. Chemical structure of formoterol fumarate dihydrate.

2. EXPERIMENTAL

2.1. Apparatus/instrumentation

Schimadzu UV-mini 1240 PC-UV visible spectrophotometer with 1 cm quartz cell was used for all spectral measurements. pH measurements were carried out with Jenco 6179 pH meter.

The HPLC system was used as a reference method. HPLC system (Schimadzu Corporation Analytical System) consisting of Rheoydne syringe sample injector (20 µL), LC-20AT pump system, DGU-20A5R degassing unit, SPD-M20A PDA detector, GL Sciences Inertsil ODS-3 column (46x260 mm, 5µm), CTO-10AS column oven.

2.2. Materials and reagents

Formoterol fumarate dihydrate (FF) was obtained from Neutec Pharmaceuticals. Ventofor-Combi containing 12 µg in a rotacap was obtained from local pharmacy. All the chemicals and reagents were obtained from Merck (Darmstadt, Germany) and used without any further purification.

2.3. Preparation of standard solutions and reagents

100 µg/mL standard solution of FF was prepared in methanol. Working standard solutions were prepared by appropriate dilution of the standard solution with methanol. 0.12 g methyl orange (MO) was dissolved in distilled water and diluted 100 mL with the same solvent. The phospate buffer solutions were prepared according to European Pharmacopoeia 8th Edition.

2.4. General Procedure

2.4.1. FF-MO method

1 mL of standard solution of FF (20-100 µg/mL), 1 mL of MO solution and 1mL pH 4 phosphate buffer solution were added in a 15 mL centrifuge tube and extracted with 5 mL chloroform after vortexing for 1 minute and centrifuging at 3000 rpm for 2 minutes. The absorbance of organic layer was measured at 428 nm aganist a reagent blank.

2.4.2. Procedure for capsules

120 rotacap capsules (Ventofor Combi® 12 mcg) were accurately weighed, mixed with 15 mL methanol and diluted to 25 mL with the same solvent. The solution was filtered. To 1 mL of the clear solution, 1 mL MO and 1mL pH4 phosphate buffer solution were added and extracted with 5 mL chloroform, then proceeded as described in section FF-MO method. These concentrations were calculated using the regression equation of calibration curve.

3. RESULTS

The proposed method is based on the formation of ion pair complex between a nitrogenous drug formoterol fumarate dihydrate and an anionic dye methyl orange at pH4. Absorbance of the yellow coloured ion pair complex was measured at 428 nm after extraction with chloroform.

3.1. Optimization of conditions

3.1.1 Effect of pH on ion pair formation

The effect of the pH was investigated in the interval of 2.0 to 8.0. Maximum absorbance was observed when the aqueous solution was buffered at pH 4.0 (Figure 2).

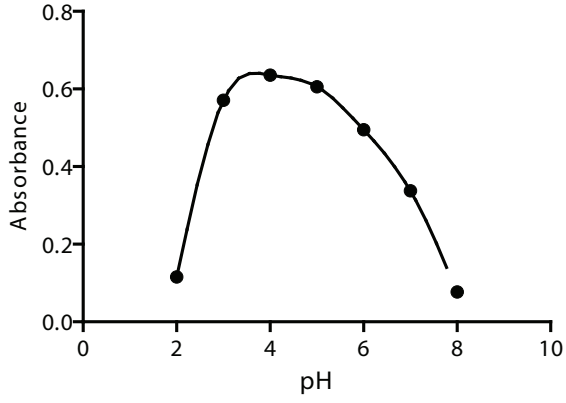

Figure 2. Effect of the pH on ion-pair complex formation between FF and MO reagent.

3.1.2 Effect of the extracting solvents

Various organic solvents such as chloroform, dichloromethane, benzene, carbon tetrachloride and toluene were tested for the extraction of the ion-pair and the highest absorbance was obtained with chloroform.

3.1.3 Effect of reagent concentration

The effect of the concentration of MO on the intensity of colour developed at 428 nm was studied and 1 mL of 0.12% MO reagent was sufficient to produce maximum and reproducible absorbance.

3.1.4 Stoichiometric Ratio

Stoichiometric relationship was determined using Job's Continuous Variations Method. Job's Curve prepared at the total concentration of $1x10^{-3}$ M solutions of FF and MO, indicated a stoichiometric ratio of 1:1 (Figure 3).

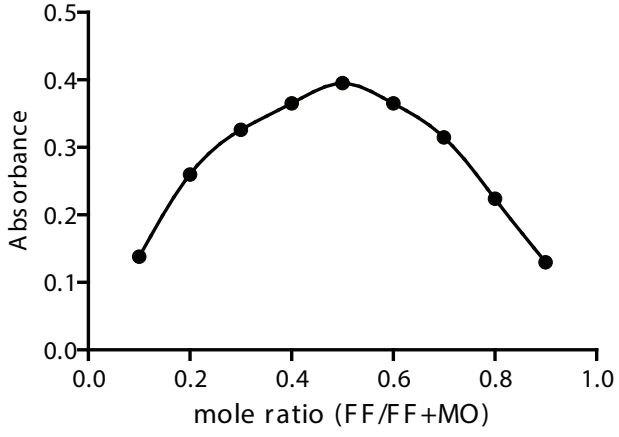

Figure 3. Job's method of continuous variation plot for the reaction of FF and MO.

3.2. Method validation

The proposed method was validated according to ICH guidelines (26) for validation of analytical procedures in order to determine linearity, limit of dedection, limit of quantification, precision and recovery.

3.2.1 Linearity

Under the optimum conditions a linear relationship was obtained from five points covering the concentration range of 4.0-20.0 µg/mL. The regression equation of the calibration curve was A=0.0434c - 0.0206 (R^2= 0.9981).

3.2.2 Sensitivity

The limit of dedection was calculated by LOD=3.3σ/S, where σ is the standart deviation of the intercept of the calibration curve and S is the slope of the calibration curve. The limit of quantification was calculated as LOQ= 3xLOD. LOD and LOQ values were found to be 0.22 and 0.66 µg/mL respectively. The statistical data are given in Table 1.

Table 1. Statistical analysis of the calibration graphs and analytical data in the determination of FF using the proposed method.

Parameters	
Wavelengths λ_{max} (nm)	428
Concentration range (µg/mL)	4-20
Regression equation	A= 0.0434c-0.0206
Slope	0.0434
Intercept	-0.0206
Determination coefficent (R^2)	0.9981
LOD (µg/mL)[a]	0.22
LOQ (µg/mL)[b]	0.66

[a]LOD, limit of dedection; [b]LOQ, limit of quantification.

3.2.3 Precision

Intra-day (three times a day operation under the same conditions) and inter-day (three different days) variations were examined using determined concentration levels. The results are summarized in Table 2.

Table 2. Intra-day and inter-day precision and accuracy data for FF obtained using the proposed methods.

Inter-day				**Intra-day**			
Conc. taken (µg/mL)	Conc. found (µg/mL)	Recovery (%)	RSD%[a] n=3	Conc. taken (µg/mL)	Conc. found (µg/mL)	Recovery (%)	RSD%[a] n=3
8.00	8.01	100.12	0.97	8.00	8.01	100.12	1.22
12.00	12.07	100.58	0.41	12.00	12.07	100.58	0.41
16.00	15.98	99.88	0.26	16.00	16.04	100.25	0.26

Recovery studies		
Concentration taken (µg/mL)	Concentration found (µg/mL)	Recovery
17.28	17.18	%99.46±0.49

[a]Mean of three determinations, RSD%, percentage relative standard deviation.

3.2.4 Recovery

Recovery studies were carried out by standard addition method. In this study, definite concentration of bulk drug was added to a known preanalyzed sample and total concentration was determined using the proposed method (Table 2).

3.2.5 Interferences

No interference was observed from commonly used tablet excipients such as lactose monohydrate and gelatin.

3.2.6 Application to formulation

The proposed method was applied to the determination of FF in commercially available capsules (Ventofor-Combi ® 12 mcg). The same samples were also analyzed simultaneously by the HPLC reference method (5). The results obtained from the analyses were compared statistically. The Student's t- values and F-values at the 95% confidence level did not exceed the tabulated values. Table 3 summarizes the results.

Table 3. Results of analysis of formoterol fumarate dihydrate capsules

Sample number	Proposed Method Concentration (μg/capsule)	Reference method (5) Concentration (μg/capsule)
1	11.35	11.68
2	11.02	11.57
3	11.30	11.99
4	11.07	11.32
5	11.40	11.48
6	11.51	11.20
X	11.28	11.54
SD	0.36	0.28
RSD %	3.19	2.42
CI[a]	10.90-11.66	11.24-11.84
F test[b]	1.66 [b](F=5.05 for p=0.05)	
t test[c]	1.30 [c](t=2.23 for p=0.05)	

[a] Confidence interval (95%)

DISCUSSION

Comparison of the proposed method with those of published on spectrophotometric determination of formoterol fumarate dihydrate (FF), showed no significant difference in respect of sensitivitiy, accuracy and precision.

Methods A and B described by Gousuddin and co-workers (14) are based on the formation of coloured chromogens of Fe^{2+} ions produced by the reduction of Fe^{3+} with FF. Formation of Fe^{2+} can be easily interfered by the other reduction agents. The method of Prasad (15) requires an extraction to eliminate the interference of the additives used in the preparation of dosage forms. Hence the proposed method can be considered as superior to these methods in terms of selectivity. Also, the reagent used in the proposed method is simple and readily available.

CONCLUSIONS

The proposed and validated spectrophotometric method for formoterol fumarate dihydrate is simple, rapid and sensitive. The reagent used commonly available. Since there is no interference with common excipients, this method can succesfully be applied for FF quantification in pharmaceutical products,

İyon çifti reaktifi olarak kullanılan metil oranj ile formoterol fumarat dihidratın saf ve dozaj formunun valide edilmiş spektrofotometrik yöntemle tayini

ÖZ

Bu çalışmada formoterol fumarat dihidratın saf ve dozaj formlarındaki tayini için hızlı, basit, doğru ve duyarlı bir spektrofotometrik yöntem geliştirilmiştir. Geliştirilen yöntem, formoterol fumarat dihidrat ile metil oranjın pH 4'te sarı renkli iyon çifti kompleksi oluşturması esasına dayanmaktadır. Kloroform fazında bulunan iyon çifti kompleksinin maksimum absorbsiyon yaptığı dalga boyu 428 nm olarak tespit edilmiş ve doğrusallık aralığı 4-20 μg/mL olarak bulunmuştur. Kalibrasyon eğrisinin eğimi 0,0433 μg/mL, dedeksiyon limiti 0,22 μg/mL ve kantitasyon limiti ise 0,66 μg/mL olarak bulunmuştur. Geliştirilen yöntem, ticari kapsüllerde bulunan formoterol fumarat dihidrat tayini için uygulanmıştır. Geliştirilen ve referans yöntemden elde edilen sonuçlar arasında doğruluk ve tekrarlanabilirlik açısından anlamlı bir fark gözlenmemiştir. Yardımcı maddelerin girişimi bulunmamaktadır.

Anahtar kelimeler: Formoterol fumarat dihidrat, spektrofotometri, iyon çifti kompleksi, metil oranj

REFERENCES

1. Szafranski W, Cukier , Ramirez A, Menga G, Sansores R, Nahabedian S, PetersonS, Olsson H. Effiacy and Safety of Budesonide/Formoterol in the Management of Chronic Obstructive Pulmonary Disease. Eur Respir J 2003;21: 74-81.
2. Fozard JR, Buescher H. Comparation of the Anti-Bronchoconstrictor Activities of Inhaled Formoterol, Its (R,R) and (S,S)-Enantiomers and Salmeterol in the Rhesus Monkey. Pulm Pharmacol Ther 2001; 14: 289-95.
3. Sardela VF, Deventer K, Pereira HMG, Aquino Netı FR, Van Eenoo P. Development and Validation of a Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometric Method for the Direct Detection of Formoterol in Human Urine. J Pharm Biomed Anal 2012; 70: 471-5.
4. Mascher DG, Zech K, Nave R, Kubesch KM, Mascher HJ. Ultra-Sensitive Determination of Formoterol in Human Serum by High Performance Liquid Chromatography and Electrospray Tandem Mass Spectrometry. J Chroma B Anal Technol Biomed Life Sci 2006;830: 25-34.
5. Assi KH, Tarsin W, Chrystyn H. High Performance Liquid Chromatography Assay Method for Simultaneous Quantitation of Formoterol and Budesonide in Symbicort Turbhaler. J Pharm Biomed Anal 2006; 41: 325-8.
6. Akapo SO, Asif M. Validation of a rapid RP-HPLC method for the assay of formoterol and its related substances in formoterol fumarate dihydrate drug substance. J Pharm and Biomed Anal 2003; 33: 935-45.
7. Nadrassan DK, Chrystyn H, Clark BJ, Assi KH. Validation of High Performance Liquid Chromatography Assay for Quantification of Formoterol in Urine Samples after Inhalation Using UV Detection Technique. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850: 31-7.
8. Trivedi RK, Chendake DS, Patel MCA. Rapid, Stability-Indicating RP-HPLC Method fort he Simultaneous Determination of Formoterol Fumarate, Tiotropium Bromide and Ciclesonide in a Pulmonary Drug Product. Sci Pharm 2012; 80: 591-603.
9. El-Bagary RI, Fouad MA, El-Shal MA, Tolba EH. Forced Degradation of Momatesone Furoate and Devolopment of Two RP-HPLC Methods for Its Determination with Formoterol Fumarate and Salcyclic Acid. Arab J Chem 2015- In Press.
10. Siraj A, Jayakar B, Aleem MA. Devolopment of Reverse Phase High Performance Liquid Chromatography Method and Its Validation for Estimation of Formoterol Fumarate Rotacaps 2011; 2: 319-24.
11. Song JZ, Chen J, Tian SJ, Sun ZP. Assay for the Determination of Low Dosage Form of Formoterol Dry Syrup by Capillary Electrophoresis with Head-Column Field-Amplified Sample Stacking. J Pharm Biomed Anal 1999; 21: 569-76.
12. Cherkaoui S, Faupel M, Francotte E. Seperation of Formoterol Enantiomers and Detection of Zeptomolar Amounts by Capillary Electrophoresis Using Laser-Induced Fluorescence. J Chromatogr A 1995;715: 159-65.
13. Gurjar NM, Sth AK, ZanwarA, Patel J, Deshmukh G. Development of First Derivative Spectroscopy Method For Estimation of Budesonide and Formoterol In Combined Dosage Form. An Int J Pharm Sci 2012; 3: 82-92.
14. Gousuddin M, Raju SA, Sultanuddin MS. Development and Validation of Spectrophotometric Methods for Estimation of Formoterol Bulk Drug and Its Pharmaceutical Dosage Forms. Int J Pharm Pharm Sci 2011; 3: 3-6.
15. Prasad AV. Simultaneous Spectrophotometric determination of Formoterol Fumarate and Budesonide in Their Combined Dosage Form. Indian J Chem Techn 2006; 13:81-3.
16. Akapo SO, McCrea C, Gupta J, Roach M, Skinner W. Chiral HPLC Analysis of Formoterol Stereoisomers and Thermodynamic Study of Their Interconversion in Aqueous Pharmaceutical Formulationsa. J Pharm Biomed Anal 2009;49: 632-7.
17. Akapo SO, Wegner M, Mamangun A, McCrea C, Asif M, Dussex JC. Optimization and Validation of a Gas Chromatographic Method for Analysis of (RS,SR) Diastereoisomeric Impurity in Formoterol Fumarate. J Chromatogr 2004;1045: 211-6.
18. Parmar VK, Patel HN,Patel BK. Sensitive and Robust Methods for Simultaneous Determination of Beclamethasone Dipropionate and Formoterol Fumarate Dihydrate in Rotacaps. J Chromatogr Sci 2014; 52:1-12.
19. Apperley DS, Harris RK, Larsson T, Malmstrom T. Quantitative Nuclear Magnetic Resonance Analysis of Solid Formoterol Fumarate and Its Dihydrate. J Pharm. Sci 2003;92: 2487-94.
20. Issa YM, Abdel-Gawad FM, Abou Table MA, Hussein HM. Spectrophotometric Determination of Ofloxacin and Lomefloxacin Hydrochloride with Some Sulphonphthalein Dyes. Anal Lett 1997; 30: 2071-84.
21. Kaf AA, Gouda AA. Spectrophotometric Determination of Tadalafil in Pure and Dosage Forms. Chem Ind Chem Eng Q 2011; 17: 125–132.
22. Ruiz TP, Lozano CM, Tomas V, Sanz A, Sahuquillo E. Flow-injection Extraction-spectrophotometric Method for the Determination of Ranitidine in Pharmaceutical Preparations. J Pharm Biomed Anal 2001; 26: 609-615.
23. Gouda AA, Sheikh RE, Amin AS, Ibrahim SH. Optimized and Validated Spectrophotometric Determination of Two Antifungal Drugs in Pharmaceutical Formulations using an Ion-pair complexation Reaction. J Taibah Univ Sci 2016; 10: 26-37.
24. Süslü I, Tamer A. Spectrophotometric Determination of Enoxacin as ion-pairs with Bromophenol blue and Bromocresol purple in Bulk and Pharmaceutical Dosage Form. J. Pharm Biomed Anal 2002; 29: 545-54.
25. Kassab NM, Amaral MS, Singh AK, Rocha MI, Santoro M. Development and Validation of UV Spectrophotometric Method for Determination of Levofloxacin in Pharmaceutical Dosage Forms. Quim Nova, 2010; 33: 968-71.
26. The European Agency for the Evaluation of Medical Products. ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures 1996.