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Determination of Formoterol by Capillary Electrophoresis and its Application to Inhaler Capsules

A capillary electrophoretic (CE) method for the determination of formoterol (FOR) in a pharmaceutical preparation is described. Analysis was made in a background electrolyte consisting of 20 % acetonitrile and 50 mM phosphoric acid at pH 2.5, using fused silica capillary (86 cm × 75 μm ID), 27 kV potential, and detecting at 200 nm. Under these electrophoretic conditions 3,4-dihydroxybenzylamine used as an internal standard (IS) and FOR showed symmetrical peaks at 6.1 and 8.3 min., respectively. The inter-day and intra-day precision was examined in the concentration range of 2.98×10^{-6} M to 8.94×10^{-6} M. Good correlation and accuracy were obtained. Limit of detection, (LOD) and limit of quantitation (LOQ) values were 3.71×10^{-7} M and 1.11×10^{-6} M, respectively. The method was applied for the analysis of FOR in pharmaceutical inhaler capsules. The proposed method is reliable, precise, accurate, fast, and cost effective.

Keywords: Formoterol; Capillary electrophoresis; Pharmaceutical analysis; Inhaler capsule

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Introduction

Formoterol (FOR) [N-[2-hydroxy-5-[(RS)-1-hydroxy-2-[(RS)-2-(4-methoxyphenyl)-1-methylethylamino] ethyl] phenyl] formamide] as hemifumarate salt, is an active β_2-adrenoreceptor agonist and it is used for treatment of asthma and as a bronchodilator [1–3]. It possesses low side-effects and a longer action than other β_2-adrenoreceptor agonist [2–5]. Its chemical structure is shown in Figure 1.

Formoterol is administered in the racemic form and it has two stereogenic centers therefore four enantiomers are available which possess different pharmacological activity. The most active enantiomer is RR-formoterol [6–10].

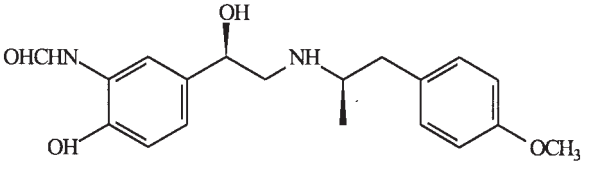

Figure 1. The chemical structure of FOR.

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Limited number of studies have been reported for the determination of FOR in pharmaceutical preparations and biological fluids. These include HPLC with electrochemical detection and UV detection [11–14]. Besides, capillary electrophoretic methods for the separation of formoterol enantiomers using laser induced fluorescence (LIF) detection has been reported [8]. Song et al. [15] also described a validated capillary electrophoretic method of the analysis of FOR in pharmaceutical dry syrup formulation. Recently, an electrochemical method was described for the analysis of FOR in capsules and human serum using differential pulse and square-wave voltammetry [16].

The aim of this study is to develop a validated method for the determination of FOR in the pharmaceutical preparations by capillary electrophoresis. Since FOR is used at very low doses, it needs a sensitive method for its analysis. Internal standard technique was employed to increase the repeatability and 3,4-dihydroxybenzylamine was found to be a suitable compound for this proposed method.

Results and discussion

Optimization of the method

The presence of an organic solvent in the background electrolyte enhances the formoterol solubility. Acetonitrile proved to be an appropriate solvent for FOR

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analysis. When acetonitrile percentage was lower than 20 % in the background electrolyte, an asymmetrical peak with a shoulder appeared. Therefore, it seems that the concentrations of the organic solvent is essential for the peak shape.

Since FOR is a basic compound, it would be recommended to start the CE analysis using an acidic background electrolyte. This acidic background electrolyte will also decrease the interaction of the silanol groups with the analyte. A background electrolyte consisting of 50 mM phosphoric acid and 20 percent acetonitrile at pH 2.5 was efficient for the proposed method. Standard and sample solutions were injected electrophoretically for 0.5 s and the analyte was detected at 200 nm. The resolution was performed applying 27 kV. The experiments were performed at room temperature (23 °C ± 1 °C). A solution of 7.5×10^{-6} M FOR tested in the background electrolyte gave a peak at 8.3 min. which is considered a reasonable analysis time.

CE is a technique related to the migration time of the positive and negative particles in an electric field. Although, the mobility of the particles is directly affected by the surface of the silica, the use of a suitable IS compensates for possible fluctuation in migration time. 3,4-dihydroxybenzylamine HBr at a final concentration of 7.6×10^{-6} M in a solution was injected and it appeared at 6.1 min. The electropherogram of FOR and IS is shown in Figure 2.

Selectivity and validation of the method

Figure 2 shows that both compounds appeared at a reasonable time, with almost symmetrical sharp peaks. The integration data of 7.5×10^{-6} M of FOR and 7.6×10^{-6} M of IS of are given in Table 1. The resolution values reflect the selectivity of the method.

The validation of the method was examined by injecting 6 samples under the above-mentioned experimental conditions. Integration values were evaluated statistically. They were examined employing peak normalization which is the ratio of peak area (A) to migration time (Mt) (PN = A/Mt) and also the ratio of peak normalization of FOR to peak normalization of IS (PN(FOR)/PN(IS)). The results are shown in Table 2.

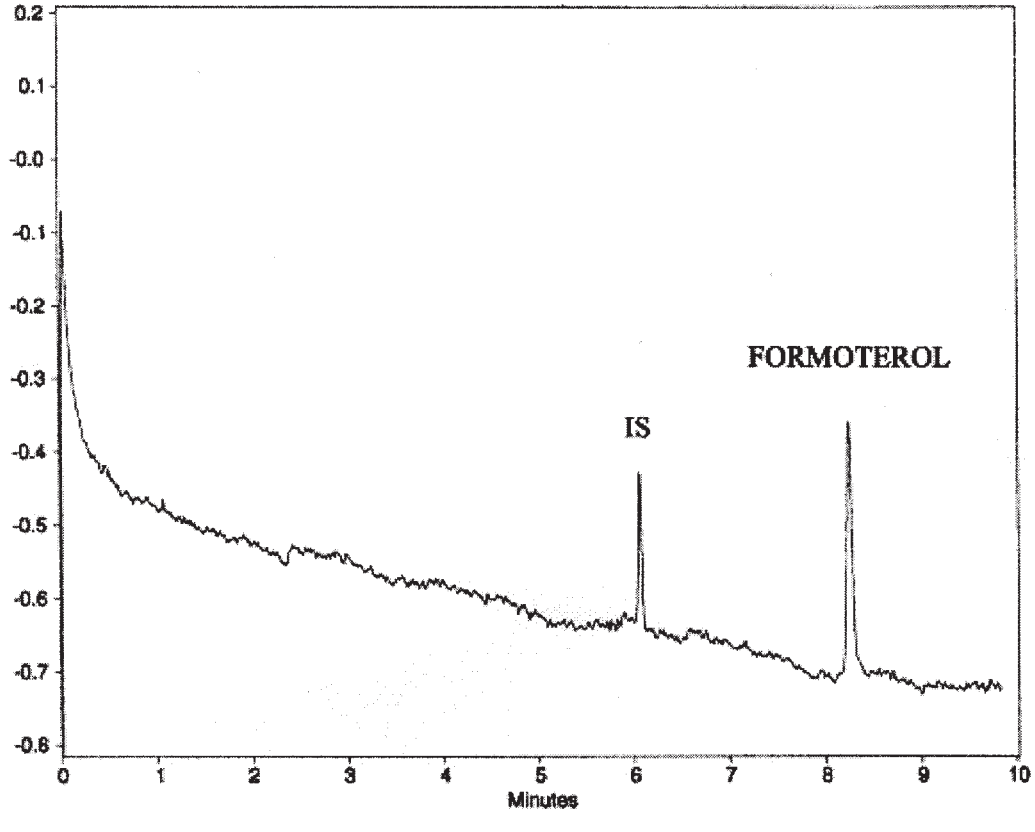

Figure 2. The electropherogram of FOR (7.5×10^{-6} M) and IS (7.6×10^{-6} M) in the background electrolyte consisting of 50 mM phosphoric acid and 20 percent acetonitrile, pH 2.5. See "Experimental" for electrophoretic conditions.

Table 1. The integration values of the 7.5×10^{-6} M FOR and 7.6×10^{-6} M IS peaks in the background electrolyte consisting of 50 mM phosphoric acid and 20 percent acetonitrile at pH 2.5.

	FOR	IS
migration time (Mt)	8.3	6.1
capacity factor	11.02	15.26
area (A)	478	794
peak width at baseline (in min.)	0.066	0.099
symmetry factor	1.0158	1.0779
plate number	132208	107190
plate/meter	1322082	1071897
resolution	25.6–26.3	25.6–26.3

Table 2. The integration values for capillary electrophoretic parameters of 7.5×10^{-6} M FOR and 7.6×10^{-6} M IS.

	Mt (FOR)	A (FOR)	Mt (IS)	A (IS)	PN (FOR)	PN (IS)	PN (FOR)/ PN (IS)
Mean	8.07	1019.2	5.95	462.7	126.3	77.8	1.65
RSD	0.47	2.75	0.57	2.87	2.38	2.92	2.07
SD	0.04	28.06	0.03	13.28	3.00	2.27	0.03

Average of 6 determinations; Mt: migration time, A: area, PN: peak normalization.

Linearity and precision

Inter-day and intra-day precision of linearity was investigated in the range of 2.98×10^{-6} M to 8.94×10^{-6} M FOR concentrations and a fixed amount of IS, in five dilutions and three groups. The dilutions of the groups were injected at different days. Table 3 indicates the linearity and precision of the proposed method for the determination of FOR preparations.

The limit of detection (LOD) (S/N = 3.3) and limit of quantitation (LOQ) (S/N = 10) values were 3.71×10^{-7} M and 1.11×10^{-6} M, respectively.

The application of method to the pharmaceutical formulation

The analysis of FOR in pharmaceutical formulations was carried out. The results for these analysis are shown in Table 4 which indicate the reliability of the proposed method. In conclusion, the proposed validated method is selective, simple as it does not require tedious sample preparation and can be adopted in the quality control laboratories.

Table 3. Linearity and Precision of inter-day and intra-day experiments.

Regression parameters	Inter-day (l = 3; n = 15)	Intra-day (l = 1; n = 5)
Slope ± SD	204667 ± 4926	235165 ± 6522
Slope ± CI (0.5)	204667 ± 7005	235165 ± 3637
Intercept ± CI (0.5)	0.026	0.128
Correlation coefficient	0.9994	0.9992

l and n designate days and number of experiments respectively. SD is the standard deviation and CI is the confidence interval.

Table 4. The quantification values of FOR capsules under the experimental conditions described.

	% FOR
mean ± SD	98 ± 2.2
t-test of significance	0.5
F-test of significance	0.5

Experimental

Apparatus

The CE experiments were conducted using a spectrophoresis 100 system equipped with modular injector, a SpectraFOCUS ultraviolet and visible scanning detector (all Thermo Separation Products, CA, USA) cabled to an Etacomp 486 DX4-100 computer. The data processing was done using a PC 1000 (Version 2.6) working under OS/2 Warp program (Version 3.0). As capillary we used a 86 cm × 75 µm ID (50 cm to detector) fused silica capillary tube (Phenomenex, CA, USA). All the solutions used during the experiments were filtered through a Phenex microfilter (25 mm, 0.45 µm; Phenomenex, CA, USA) and degassed using a ultrasonic bath (model B-220, Branson, Shelton, CT, USA). The pH meter for measuring the pH of the solutions was a model P 114 pH glass electrode from Consort (Turnhout, Belgium).

Chemicals

The standard materials of formoterol and 3,4-dihydroxybenzylamine HBr as IS were supplied by Novartis Sağlık, Gida ve

Tarım Ürünleri Sanayi ve Ticaret A.Ş. (Istanbul, Turkey) and Aldrich (Milwaukee, WI, USA), respectively. The pharmaceutical preparation of Foradil® Inhaler Capsule with 12 µg dose each was manufactured by Novartis and was supplied from a local drugstore. All the other chemicals (analytical grade) used in the experiments were the products of Merck Co. (Darmstadt, Germany). Double distilled water was used throughout the entire study.

Preparation of background electrolyte

The background electrolyte consisted of 50 mM phosphoric acid and 20 percent acetonitrile, pH 2.5. The background electrolyte and all the injected samples were filtered and degassed.

Preparation of standard and internal standard solutions

Stock solution of FOR 1.5×10^{-3} M concentration was prepared in an 20 percent ethanolic solution and dilutions were made using the background electrolyte. Stock solution of 3,4 dihydroxybenzylamine HBr (IS) 3.5×10^{-3} M was dissolved in distilled water and dilutions were prepared by using background electrolyte. The composition of the solution: 0.5 mL IS and 2.0 mL FOR in various concentrations.

Washing and conditioning of silica capillary

Washing and conditioning of the capillary was performed each day at the beginning of the study, in turn, 2 min. 0.1 M NaOH, 2 min distilled water, 2 min 0.1 M $HClO_4$, 2 min distilled water and background electrolyte.

Recovery of FOR from pharmaceutical preparation

The content of ten capsules was weighed and the average weight of a capsule was calculated. An amount corresponding to the average weight of one capsule was weighed and transferred into a tube. 2.5 mL background electrolyte were added and vortexed three times for three minutes. The solution was filtered, 2.0 mL FOR solution were pipetted and 0.5 mL IS added and vigorously shaken. This solution was injected into the CE.

References

[1] L. M. Hollingshead, K. L. Goa, *Drugs* **1991**, *42*, 115.

[2] A. J. M. Schreurs, H. E. J. Sinninghe Damste, C. S. Graaff, A. P. M Greefhorst, *Eur. Resp. J.* **1996,** *9*, 1678.

[3] E. Naline, Y. Zhang, Y. Quian, N. Mairon, G. P. Anderson, B. Grandordy, C. Advenier, *Eur. Resp. J.* **1994,** *7*, 914.

[4] G. P. Anderson, *Life Sci.* **1993**, *52*, 2145.

[5] K. Murase, T. Mase, H. Ida, K. Takahashi, M. Murakami, *Chem. Pharm. Bull* **1978,** *26*, 1123.

[6] B. Waldeck, *Chirality* **1993,** *5*, 350.

[7] J. Trofast, K. Osterberg, B. L. Kallstrom, B. Waldeck, *Chirality* **1991,** *3*, 443.

[8] S. Cherkaoui, M. Faupel, E. Francotte, *J. Chromatogr. A* **1995,** *715*, 159.

[9] E. R. Francotte, P. Richert, *J. Chromatogr. A* **1997**, *76*, 101.

[10] R. Hett, Q. K. Fang, G. Yun, H. Yapig, H. T. Butter, N. Xiaoyi, S. A. A Wald, *Tetrahedron Lett.* **1997,** *38*, 1125.

[11] J. Campestrini, J. B. Lecaillon, J. Godbillon, *J. Chromatogr. B* **1997**, *704*, 221.

[12] B. T. J. van der Berg, E. J. G. Portier, M. van der Berg, M. C. P. Braat, C. J. van Boxtel, *Therap. Drug Monit.* **1994,** *16*, 196.

[13] S. J. Graham, N. Beaulieu, E. G. Lovering, *J. Liq. Chromatogr.* **1993,** *16*, 1497.

[14] J. J. Butter, B. T. J. van der Berg, E. J. G. Portier, G. Kaiser, C. J. van Boxtel, *J. Liq. Chromatgr.& Rel. Technol.* **1996,** *19*, 993.

[15] J. Z. Song, J. Chen, S.-J. Tian, Z.-P. Sun, *J. Pharm. Biomed. Anal.* **1999,** *21*, 569.

[16] B. T. Demircigil, S. A. Özkan, Ö. Coruh, S. Yilmaz, *Electroanalysis* **2002,** *14*, 122.