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Validation of a RP-HPLC method for the assay of formoterol and its related substances in formoterol fumarate dihydrate drug substance

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Abstract

A stability-indicating reversed-phase high performance liquid chromatographic (HPLC) method has been developed and validated for the assay of formoterol fumarate and the related substances, namely, formoterol fumarate desformyl and formoterol fumarate acetamide analogs, in the active pharmaceutical ingredient. The separation was achieved by isocratic elution using an Alltech Alltima C_{18} (150 × 4.6 mm) column, a mobile phase consisting of ammonium acetate (50 mM; pH 5.0)–ethanol (65:35, v/v), a flow rate of 1.0 ml/min and UV detection at 242 nm. The detection and quantitation limits were 0.03 and 08 μg/ml, respectively, while the linear range of detection was between 0.03 and 255 μg/ml. Comparative determinations of formoterol fumarate in three lots of bulk drugs using the proposed HPLC method and the standard potentiometric titration method of pharmacopoeia show that both methods are equivalent for pure drug substance assay. However, the HPLC method allowed the separation and quantitation of the impurities not achievable with the official methods in the bulk drugs. This study shows that the proposed method is accurate, linear, and sensitive as stability indicating assay method for formoterol fumarate in the bulk drug.

Keywords: Formoterol fumarate; Formoterol fumarate desformyl analog; Formoterol fumarate acetamide analog; HPLC; Assay; Validation

1. Introduction

Formoterol is a long-acting beta2-adrenoceptor agonist with demonstrated bronchodilatory effects and rapid onset of action [1]. As a result of its prolonged duration of action, formoterol appear to be more effective than shorter-acting beta2-agonist in the treatment of noctural and exercise-induced asthma [1–3]. Structurally, formoterol, ((RR)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxypheny)-1-methylethyl]amino]ethyl]phenyl] formamide) is a phenylethylamine derivative with one phenolic hydroxyl and one secondary amino group, and is widely marketed as a racemate of the enantiomers, which have the RR+SS configuration. The anti-bronchoconstrictor activity of for-

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moterol lie with the (R,R) enantiomer and the (S,S) enantiomer does not exert any contractile effects when present in the racemate [4].

There have been limited reports on chromatographic assay of formoterol fumarate in the bulk drugs. Thus Graham et al. [5] described a liquid chromatographic assay method for formoterol fumarate in which the drug and a related compound, formoterol fumarate acetamide analog were nearly baseline separated on a short octyl bonded silica column using water–acetonitrile–trifluoroacetic acid (800:200:0.5, v/v/v) as the mobile phase. Despite its highly sensitivity, the method appears not to be a good choice for potency assay of formoterol fumarate in the bulk drug because of the poor hydrolytic stability of drug in low pH environment (pH < 4.0) [6]. Other chromatographic techniques available in the literature are primarily developed for the determination of formoterol in biological fluids [7–10]. Beside the non-specific potentiometric titration method described in the Japanese Pharmacopoeia (JP) monograph for the drug [11], our literature searches did not reveal the existence of standard methods of pharmacopoeias for potency assay of formoterol fumarate in active pharmaceutical ingredients. Consequently, alternate simple, fast and specific chromatographic methods for purity assay of formoterol fumarate in the bulk drugs must be developed and validated because of the increasing attention to develop the drug in various dosage forms for the treatment of asthma.

This paper, therefore, describes an isocratic liquid chromatographic method for quantitative determination of formoterol fumarate and its related substances, e.g. formoterol fumarate desformyl and acetamide analogs in the bulk drug. Unlike formoterol fumarate acetamide analog, which is present as a process impurity, formoterol fumarate desformyl analog is a process impurity as well as a major degradation product primarily produced by hydrolysis of the drug. The proposed method has been validated and found to be linear, accurate, precise, sensitive, specific and stability indicating. The structures of formoterol fumarate and the related substances examined in this study are shown in Fig. 1.

2. Experimental

2.1. Chemicals

Samples of formoterol fumarate dihydrate, formoterol fumarate desformyl acetamide analogs were obtained from Vinchem, Inc. (Chatham, NJ) and used as received. A primary reference standard of formoterol fumarate dihydrate CRS (Batch/lot number 1a, 100% purity) was purchased from European Directorate for the Quality of Medicines (EDQM). Acetonitrile (HPLC grade), methanol (HPLC grade), and analytical grade ammonium acetate, glacial acetic acid, sodium hydroxide 1.0 N, hydrochloric acid 1.0 N and hydrogen peroxide 30% were purchased from VWR (West Chester, PA). In-house purified water (USP grade) was used throughout the study.

2.2. Preparation of standard solutions

A standard solution of formoterol fumarate dihydrate at the target concentration of 170 μg/ml chosen for this study was prepared by transferring 85 mg of the reference standard into a 100-ml volumetric flask containing about 25 ml purified water. About 10 ml of acetonitrile was added and the solution was sonicated for 10 min or until the solid completely dissolved keeping the water in the sonicator at ambient temperature. When dissolved, the volumetric flask was filled to mark with purified water. A 10.0-ml portion of the resulting solution was then transferred into a 50-ml volumetric flask, filled to volume with purified water, and mixed thoroughly prior to use. A blank solution was prepared by mixing 2 ml of acetonitrile with 98 ml of purified water in a 100 ml volumetric flask.

A diluted solution containing 0.17 μg/ml of formoterol fumarate dihydrate corresponding to 0.1% of the target concentration was prepared and used as the sensitivity solution. The resolution solution was prepared by dissolving 1.0 mg each of formoterol fumarate desformyl and formoterol fumarate acetamide analogs in 10 ml of the standard solution in a 10-ml volumetric flask. Unless stated otherwise, all sample and standard

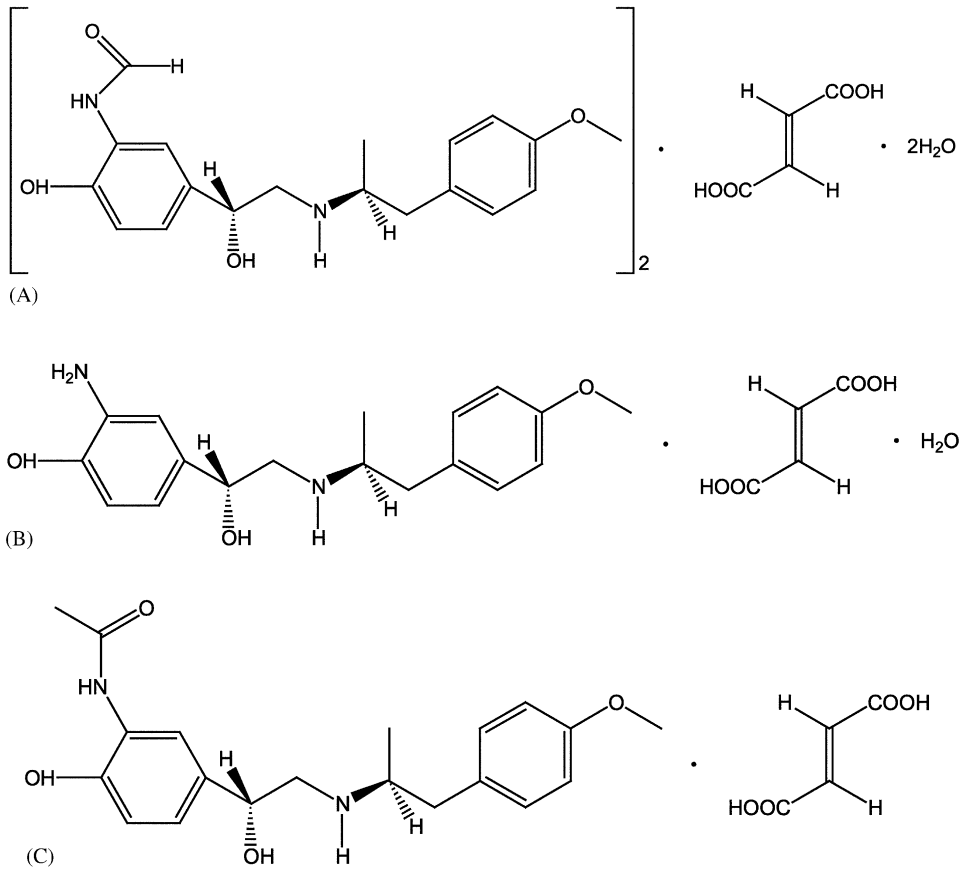

Fig. 1. Chemical structures of formoterol fumarate and its related substances: (A) (RR)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxypheny)-1-methylethyl]amino]ethyl]phenyl] formamide fumarate (formoterol fumarate), (B) (1RS)-1-(3-amino-4-hydroxyphenyl)-2-[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethanol (formoterol fumarate desformyl analog), and (C) (1RS)-1-(3-N-acetylamino-4-hydroxylphenyl)-2-[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethanol (formoterol fumarate acetamide analog).

solutions were refrigerated and analyzed within 48 h.

2.3. HPLC

The chromatographic separations were performed using a Hitachi system consisting of L-7100 pump, L-7300 oven, L-7200 autosampler, L-7450 photodiode array detector, and D-7000 SYSTEM MANAGER DATA ACQUISITION software, version 3.1. The mobile phase consists of ammonium acetate (50 mM; pH 5.0) and methanol in the ratio 65:35 v/v, filtered through a nylon membrane and degassed under vacuum before use. The chromatographic column used was an Alltech Alltima C_{18} 5 μm silica column (15 cm × 4.6 mm). The analytes were monitored with UV detection at 242 nm. Unless stated otherwise, all separations were performed at ambient temperature using a 1.0 ml/min flow rate, a 25 μl injection volume, and a 20 min run time. The system suitability parameters displayed in Table 1 were evaluated throughout the study.

2.4. Method validation

The test samples varying in concentration from 0.03 to 255 μg/ml of the selected lot of formoterol fumarate dihydrate were prepared and analyzed to evaluate the linearity of the method. Linearity for

Table 1
System suitability

Parameter	Acceptance criteria	Result
%R.S.D. for formoterol peak areas in five standard injections	NMT 2.0%	0.3%
Resolution (formoterol desformyl analog/formoterol)	NLT 1.5	4.2
Resolution (formoterol/formoterol acetamide analog)	NLT 1.5	3.1
Tailing factor (formoterol peak)	NMT 2.0	1.4
%R.S.D. for three injections of sensitivity solution (0.1% of target concentration)	NMT 10%	2%

each related substance was evaluated from standard solutions of the drug substance spiked with the related substance at 0.05–0.75% of the target concentration.

The accuracy of the method was assessed from the recovery data at concentrations varying from 85 to 255 μg/ml formoterol fumarate dihydrate corresponding to 50–150% of the target concentration. System precision was determined from the relative standard deviation (R.S.D.) of five replicate injections of the standard solution of formoterol fumarate dihydrate. The method repeatability/intermediate precision was evaluated from the assay results of two five-sample sets prepared from the same lot of the drug substance by two different analysts. Each set of samples was independently prepared by each analyst and assayed on different days using different high performance liquid chromatographic (HPLC) systems.

Forced degradation study was performed to assess the specificity and stability indicating properties of the method. In this study, solid or aqueous solution of the drug was deliberately exposed to acid, base, hydrogen peroxide, heat or light for a given period of time that produces adequate degradation of the samples. At the end of each experiment, the degraded samples were analyzed against a freshly prepared solution of formoterol fumarate dihydrate of equal concentration (control), and the purity of the formoterol peak was evaluated using a PDA detector.

Method robustness was determined by evaluating the effect of small, but deliberate variations in the chromatographic conditions such as mobile phase flow rate, column temperature, column type, UV detection wavelength, mobile phase methanol content and buffer pH on the method performance.

3. Results and discussion

3.1. Method development

Prior to chromatographic method development, the detection wavelength was determined by obtaining the UV spectra of solutions of the drug and the two related substances as described in JP monograph. As expected, all the analytes show maxima absorbance between 242 and 248 nm, and between 282 and 286 nm with slight variations in absorbance values, and hence the peak intensity. From the spectra obtained, a wavelength detection of 242 nm was chosen in order to achieve a good sensitivity for simultaneous determination of all the analytes as well as any other unknown impurities in the bulk drug.

The chromatographic separations of formoterol fumarate, formoterol fumarate desformyl and acetamide analogs were investigated at 242 nm wavelength using different mobile phases consisting of citrate, phosphate and/or acetate buffers in combination with methanol or acetonitrile on different analytical C_{18} columns. The separation of the analytes varied substantially with the chromatographic conditions examined. For instance, a composition of 55:45 v/v of acetonitrile–buffer solution (citric acid (0.1 M)–disodium phosphate (0.2 M) (52:48 v/v, pH 5.0)) produced no resolution between formoterol and the adjacent peaks with formoterol eluting at or near the column void volume under isocratic conditions. A trial with gradient elution using a mobile phase consisting of potassium phosphate (20 mM), 1-octane sulfonic acid sodium salt (5 mM) and acetonitrile on Phenomenex Luna C_{18} 3 μm column (15 cm × 4.6 mm) (Torrance, CA) did not produce good separation between formoterol and formoterol desformyl analog peaks (separation factor, $\alpha < 1.0$). Finally, a mobile phase consisting of 65:35 v/v of 50 mM ammonium

acetate (pH 5.0)–methanol on Alltech Alltima C_{18} 5 μm column (15 cm × 4.6 mm) (Deerfield, IL) offered a good separation of the analytes at ambient temperature. Under these conditions, and using a flow rate of 1.0 ml/min and a run time of 20 min, formoterol elutes at about 10.2 min.

3.2. Method validation

3.2.1. Quantitation limit (QL) and detection limit (DL)

The QL (signal-to-noise ratio = 10) and DL (signal-to-noise = 3) [12] were determined from the signal to noise ratios of standard solutions of formoterol fumarate dihydrate at low concentrations (0.01–0.20 μg/ml). The quantitation and detection limits for formoterol fumarate dihydrate were found to be 0.08 μg/ml (S/N = 10.3), and 0.03 μg/ml (S/N = 2.9), respectively, corresponding to approximately 0.05 and 0.02% of the target concentration.

3.2.2. Linearity

The plot of peak area responses against concentration of formoterol is shown in Fig. 2. It can be seen that the plot is linear over the concentration range of 0.03–255 μg/ml yielding a regression equation $y = 27\,692x - 3830$ ($n = 10$) with a correlation coefficient $r^2 = 1.000$. A P-value of 0.5068 for the y-intercept indicates that the intercept was statistically equal to zero ($P > 0.05$). A similar plot at low concentrations (0.03–8.5 μg/ml) (see insert in Fig. 2) gave a slope value of 27 110 (n = 5), which is within 2% of the slope value for the entire concentration range examined. These results demonstrate that a single point calibration can be used for potency assay of formoterol fumarate in the bulk drug.

3.2.3. Accuracy/recovery

The data presented in Table 2 show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50, 100, and 150% levels were 100.1, 100.5 and 100.8%, with R.S.D. of 0.3, 0.3 and 0.4%, respectively. The R.S.D. value for overall mean recovery was 0.5%. Furthermore, a plot of actual versus determined concentrations of formoterol shows excellent linearity with a slope and intercept not significantly different (t-test, $P = 0.05$) from unity and zero, respectively, and a correlation coefficient $r^2 = 0.9999$. Excellent recovery and low R.S.D. value showed that the method is suitably accurate for potency assay of formoterol fumarate in the drug substance.

3.2.4. Precision

The R.S.D. of peak area responses for five replicate standard injections was 0.2%, which met the acceptance criterion established for the method. The intraday precision of the method was 0.3% with an average recovery of 100.7%. The R.S.D. value for intermediate precision performed by a second analyst on different day using a different instrument was 0.6% with an average recovery of 100.3% (Table 3). A comparative analysis of the mean results using the Student's t-test at 95% confidence interval gave a t value of 0.2 (t critical = 2.8) indicating that there is no significant difference between the mean values, thus the method is suitably precise and reproducible.

3.2.5. Specificity and selectivity

Fig. 3 demonstrates the specificity of the method in which formoterol and its related substances were well resolved with no interference from the sample matrix. The forced degradation data are presented in Table 4. Acceptable mass balance was obtained at each stressed condition. The formoterol peak purity index, determined with photodiode array detector, was unity for all the stressed samples indicative of a single, pure peak. The ability of the method to separate the drug from its degradation products further demonstrates the specificity and the stability indicating property of the method.

Though not shown in this report for the sake of brevity, the chromatogram of a sample mixture of formoterol fumarate and its related substances showed good separation ($R_s \geq 3.0$) of the analytes with good peak shape indicating that the method is selective for its intended purpose.

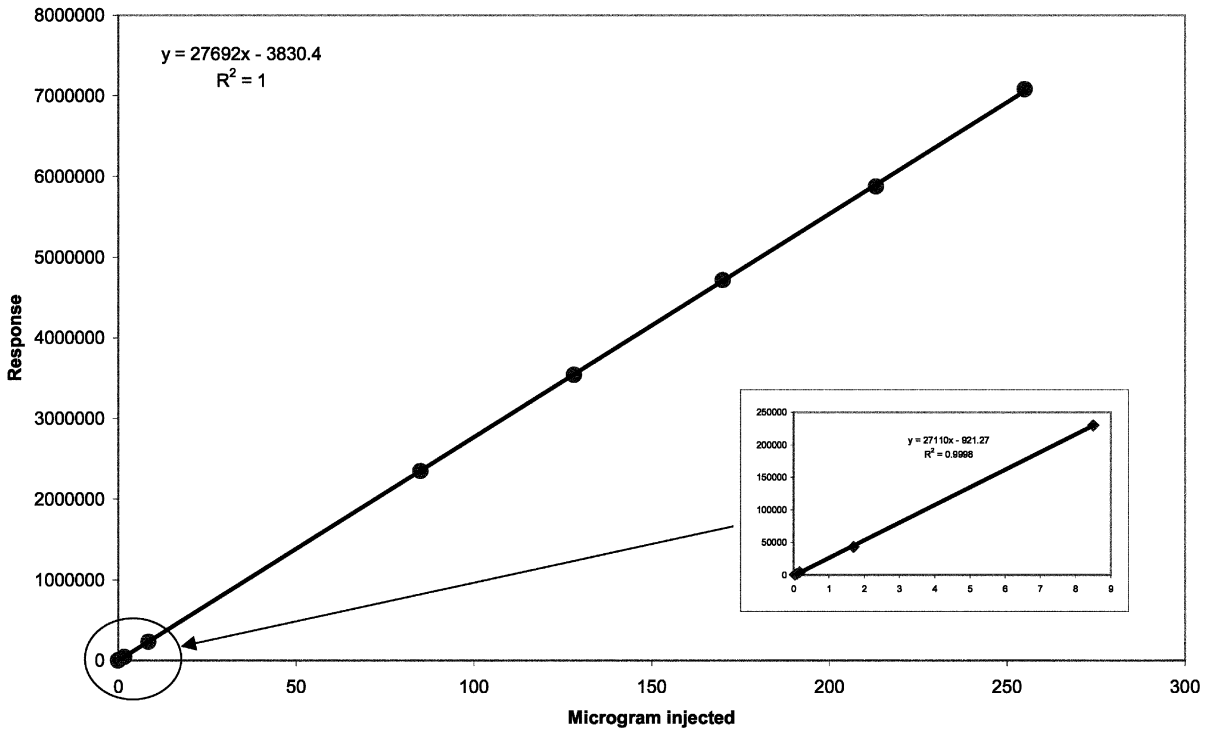

Fig. 2. Linearity plot for formoterol fumarate drug substance.

3.2.6. Robustness

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance (see Table 5). As expected, the retention time of the analytes decreased with increasing mobile phase flow rate and vice versa. With the exception of the slight shift in the analytes' retention time, the chromatographic properties of the method remained constant when an alternate C_{18} silica column from a different manufacturer was used. A slight decrease in retention factor (k)

Table 2
Accuracy/recovery for formoterol fumarate

Level (%)	Sample	Actual amount (μg)	Determined amount (μg)	% Recovery	Mean (n = 3)	%R.S.D.
50	1	84.83	85.17	100.4	100.1	0.3
	2	84.49	84.49	100.0		
	3	84.15	83.98	99.8		
75	1	127.3	128.5	100.9		
100	1	170.0	170.7	100.4	100.5	0.3
	2	169.7	171.2	100.9		
	3	169.5	170.0	100.3		
125	1	211.5	214.5	101.4		
150	1	254.5	255.5	100.4	100.8	0.4
	2	254.0	256.2	100.9		
	3	254.2	257.0	101.1		
Mean (n = 11)					100.6	0.5

Table 3
Repeatability/intermediate precision of the assay method

Sample	% Formoterol fumarate	
	Analyst 1, day 1	Analyst 2, day 2
1	100.4	100.0
2	100.9	100.9
3	100.3	99.9
4	100.8	101.0
5	101.0	99.7
Mean	100.7	100.3
%R.S.D.	0.3	0.6
Grand mean	100.5	
%R.S.D.	0.5	

of the analytes was observed with increasing column oven temperature. Although peak heights increased by about 3% with decreasing UV detection wavelength from 242 to 239 nm, this has no adverse effect on the method since standard and samples would generally be analyzed concurrently and at the same wavelength for routine quality control analysis. Changes in pH of the buffer solution did not alter the chromatographic profile of the sample components, which remained constant with 0.2 U increase or decrease in pH from the normal experimental condition. As expected, increasing the methanol content of the mobile phase proportionally decreased the retention time of the analytes, and vice versa when the methanol concentration was decreased.

3.2.7. Stability of standard solution

The stability of a standard solution of the drug substance was examined by analyzing separate portions of the solution stored at room temperature, and at 4 °C in a refrigerator for 7 days against a freshly prepared standard solution. Both

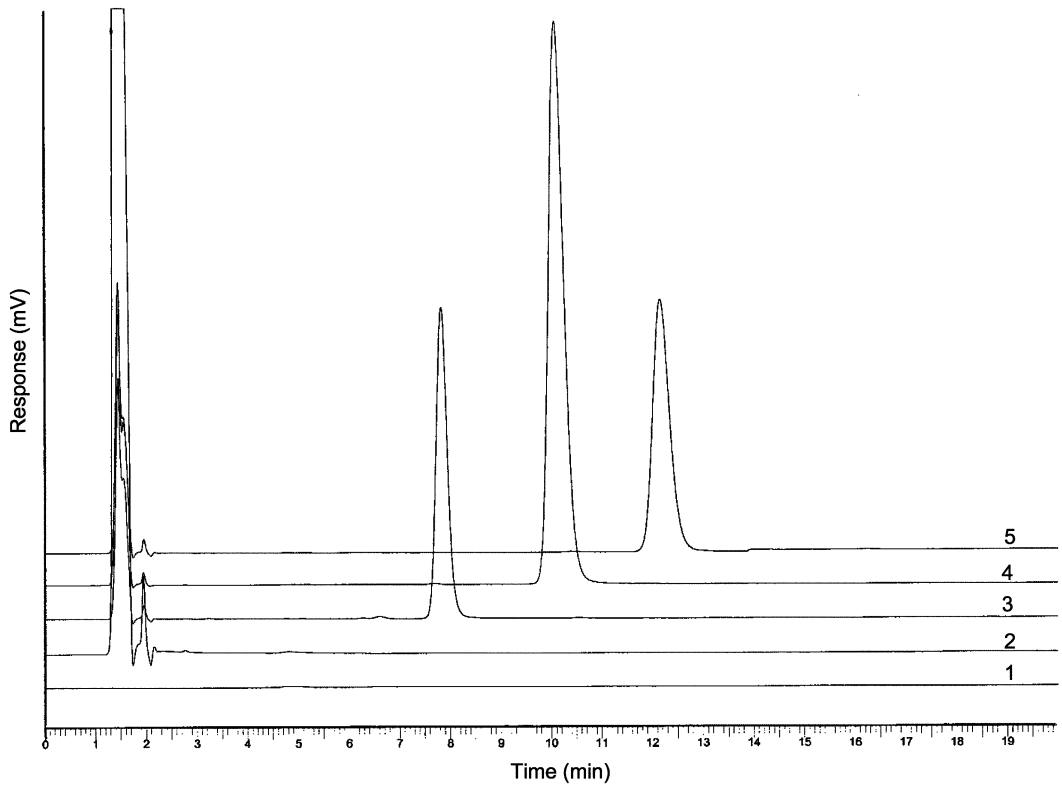

Fig. 3. Chromatographic profiles of formoterol and its related substances: (1) mobile phase, (2) blank, (3) formoterol fumarate desformyl analog, (4) formoterol fumarate, and (5) formoterol fumarate acetamide analog.

Table 4
Forced degradation of formoterol fumarate

Condition	Time (h)	% Recovery	Mass balance (%)	RRT[a] of degradation products
Acid 0.1 N HCl, RT[b]	5	79.9	98.0	0.76, 1.38
Acid 0.1 N HCl, 60 °C	1	58.6	98.7	0.42, 0.75, 1.37
Base 0.1 N NaOH, RT	5	93.5	100.1	0.39, 0.76
Base 0.1 N NaOH, 60 °C	1	90.0	100.5	0.40, 0.45, 0.61, 0.76, 1.39, 1.51, 1.58, 1.69
H_2O_2 5%, RT	5	91.3	94.7	0.39, 1.12, 1.29
H_2O_2 5%, 60 °C	1	82.9	92.2	0.38, 1.11, 1.76
Heat solid, 60 °C	72	100.3	100.5	None detected
Light solid, UV	168	98.9	100.5	0.54
Light solid, CWF[c]	168	100.3	100.4	0.39

[a] RRT, relative retention time.
[b] RT, room temperature.
[c] CWF, cool white fluorescent light.

solutions did not show any change in the concentration of the analyte after the storage period. The solution stored at room temperature gave an assay value of 100.1% of formoterol fumarate, while that stored in the refrigerator contained 100.0% formoterol fumarate. These values are in excellent agreement with 100.0% of formoterol fumarate detected in the initial (fresh) standard solution indicating that a standard solution of the drug substance in acetonitrile-purified water (2:98, v/v) is stable for at least 1 week when refrigerated or stored at room temperature.

Table 5
Chromatographic characteristics from changes in experimental conditions

Parameter	Variation	Relative retention time[a]		Resolution[b]		Formoterol tailing factor
		RRT (D)	RRT (A)	D/F	F/A	
Flow rate	0.8 ml/min	0.8	1.2	5.0	3.3	1.5
	1.0 ml/min	0.8	1.2	4.8	3.0	1.4
	1.2 ml/min	0.8	1.2	4.6	3.0	1.4
Column type	Alltima C_{18}	0.8	1.2	4.8	3.0	1.4
	Kromasil C_{18}	0.8	1.2	4.3	3.1	1.4
Column oven temperature	25 °C	0.8	1.2	4.8	3.1	1.4
	Ambient	0.8	1.2	4.8	3.0	1.4
	30 °C	0.8	1.2	4.4	3.2	1.3
Detection wavelength	239 nm	0.8	1.2	4.8	3.1	1.4
	242 nm	0.8	1.2	4.8	3.0	1.4
	245 nm	0.8	1.2	4.8	3.1	1.4
Buffer pH	4.8	0.8	1.2	4.8	3.1	1.4
	5.0	0.8	1.2	4.8	3.0	1.4
	5.2	0.8	1.2	4.8	3.1	1.5
Mobile phase methanol	34 %v/v	0.7	1.2	5.1	3.2	1.4
Content	35% v/v	0.8	1.2	4.8	3.0	1.4
	36% v/v	0.8	1.2	4.4	2.8	1.4

[a] RRT(D), relative retention time for formoterol desformyl analog; RRT(A), relative retention time for formoterol acetamide analog.
[b] D/F, resolution between formoterol and formoterol desformyl analog; F/A, resolution between formoterol and formoterol acetamide analog.

3.3. Related substances

The relative response factors for the related substances were determined from the triplicate preparations of a solution containing formoterol and the related substances in known amounts corrected for base fraction of each compound. Using formoterol as the reference component, the average response factors for formoterol desformyl and acetamide analogs were found to be 0.7 and 0.8, respectively. These values were subsequently used to correct the peak area response for the determination of the related substances in the test samples. A relative response factor of 1.0 was used for all other unknown peaks.

The recovery data for the related substances are presented in Table 6. As can be seen, the determined amounts of formoterol fumarate desformyl (corrected for its background level in the drug substance) and acetamide analogs in the spiked solutions are in good agreement, within method variability, with the actual spiked amounts. The average recoveries were 89.2% (12.2% R.S.D.) and 104.6% (9.5% R.S.D.) for formoterol fumarate desformyl and acetamide analogs, respectively, over a range of 0.05–0.75% of the target concentration. Acceptable precision was achieved for both compounds with the method as indicated by the R.S.D. data at 0.05 and 0.5% levels.

A plot of actual versus determined amounts of formoterol fumarate desformyl analog was linear with a correlation coefficient (r^2) of 0.9994, and slope and y-intercept values of 0.9552 and −0.0067, respectively. The correlation coefficient (r^2), slope and y-intercept for a similar plot for formoterol fumarate acetamide analog were 0.9993, 0.9607, and 0.0101, respectively. In addition to satisfying the acceptance criteria for related substance linearity (slope: 0.80–1.20; $r^2 \geq 0.95$), the P-values for the y-intercepts were greater than 0.05 indicating that each intercept was insignificantly different from zero.

Table 6
Accuracy/recovery for related substances

Level (%)	Spiked (%)	Area percent	Determined[a] (%)	Mean ± %R.S.D.[b]
Formoterol desformyl analog				
0.05	0.044	0.021	0.031	0.036 ± 19.4
	0.045	0.023	0.033	
	0.044	0.031	0.044	
0.1	0.088	0.059	0.085	
0.3	0.265	0.167	0.238	
0.5	0.442	0.286	0.409	0.414 ± 3.1
	0.442	0.283	0.404	
	0.442	0.300	0.428	
0.75	0.664	0.442	0.631	
Formoterol acetamide analog				
0.05	0.048	0.042	0.053	0.057 ± 8.3
	0.049	0.044	0.055	
	0.050	0.049	0.062	
0.1	0.097	0.085	0.107	
0.3	0.290	0.233	0.291	
0.5	0.484	0.381	0.476	0.470 ± 2.2
	0.492	0.380	0.475	
	0.497	0.367	0.458	
0.75	0.725	0.571	0.714	

[a] Determined (%) = Area %/relative response factor (RRF).
[b] Mean ± %R.S.D. for triplicate determinations.

Table 7
Comparison of HPLC with potentiometric titration and TLC methods

Sample	HPLC assay (%)			JP monograph	
	Formoterol	Desformyl	Acetamide	Titration (%)[a]	TLC[b]
Lot A	99.6	0.30	nd	99.8	None
Lot B	99.4	0.30	nd	99.8	None
Lot C	99.9	0.32	nd	99.9	None
Mean	99.6	0.31		99.8	
%R.S.D.	0.3	3.8		0.1	
Grand mean	99.7				
%R.S.D.	0.2				

[a] Potentiometric titration assay.
[b] TLC for impurity test.

3.4. Analysis of bulk drugs

Three lots of formoterol fumarate dihydrate obtained from Vinchem, Inc. were analyzed using the present HPLC method, the potentiometric titration and the TLC methods listed in the JP monograph for the drug substance. The results are presented in Table 7. While both the titration and the TLC methods could not detect any impurity, the HPLC method not only detects but also quantified the impurities present in the samples. Additional evaluations of the method performance with nine different lots of commercially available materials show the purity and quality of the drug substances, which varied from supplier to supplier (Table 8). With the exception of the three lots from supplier B, all other lots tested met the JP monograph purity specification for formoterol fumarate of not less than 98.5% [$(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4$ (mol. wt.: 804.89)], calculated on anhydrous basis. Furthermore, the results obtained clearly showed the differences in the impurity profiles of the samples, hence the suitability of the method for quantitative determinations of formoterol fumarate and its related substances in active pharmaceutical ingredients.

Table 8
HPLC assay of formoterol fumarate drug substances

Sample	Assay (%)				
	Formoterol	Desformyl	Acetamide	Unknowns	Total
Supplier A					
Lot number 1	99.8	0.17	0.02	nd	100.0
Lot number 2	100.2	0.22	nd[a]	nd	100.4
Lot number 3	99.8	0.23	nd	nd	100.0
Supplier B					
Lot number 1	97.1	1.60	1.12	0.05	99.9
Lot number 2	97.4	1.88	0.98	0.13	100.4
Lot number 3	97.1	1.93	0.98	0.12	100.1
Supplier C					
Lot number 1	98.7	0.26	1.03	1.04	101.0
Lot number 2	98.6	0.46	1.52	0.06	100.6
Lot number 3	98.6	0.45	1.51	0.08	100.6

[a] nd, not detected.

4. Conclusion

An isocratic liquid chromatographic method has been described and validated for qualitative and quantitative determination of formoterol fumarate and the related substances investigated in the bulk drug. Acceptable assay precision ($< 2\%$ R.S.D.) and accuracy ($< 1.0\%$ difference) were obtained at 50–150% of the analytical concentration of 170 μg/ml, and excellent linearity was achieved over a range of 0.03–255 μg/ml of formoterol fumarate. In addition to its high sensitivity and robustness, the proposed HPLC method proved reliable for impurity profiling of the bulk drug compared with the potentiometric titration and TLC methods described in the JP monograph for formoterol fumarate. The validation and application of this method for potency assay of formoterol fumarate in pharmaceutical dosage forms are in progress in the authors' laboratory.

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