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Chiral HPLC analysis of formoterol stereoisomers and thermodynamic study of their interconversion in aqueous pharmaceutical formulations

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ABSTRACT

A chiral HPLC method was validated and successfully applied for the determination of formoterol stereoisomers and their inversion products in an aqueous matrix stored at 5–70 °C up to 3 weeks. Analysis was performed on a Chiral-AGP column (100 × 4-mm, 5-μm) using a variable mixture of mobile phase A (50-mM sodium phosphate buffer, pH 7.0) and B (10% v/v IPA) at a flow rate of 1.3 ml min^{-1}, and UV detection at 242 nm. All four formoterol stereoisomers were adequately resolved with acceptable detection and quantitation limits varying from 0.01–0.04 μg/ml and 0.04–0.1 μg/ml, respectively. The method showed acceptable accuracy (≥88%), precision (RSD ≤ 8.5%) and good linearity ($r^2 \geq 0.9999$) over the concentration range investigated. While interconversion at 5 ± 3 °C and 25 ± 2 °C/60% RH ±5% RH was too low to be determined accurately within the study period, chiral inversion of formoterol stereoisomers measured at high temperatures followed the first order rate kinetics and occurred at a single chiral center, resulting in the reversible formation of diastereoisomers, (*R*,*R*) ↔ (*S*,*R*) and (*S*,*S*) ↔ (*R*,*S*). No enantiomerization or diastereomerization occurred. There was no significant difference in inversion of the active components in racemic (*R*,*R*/*S*,*S*)-formoterol fumarate and the single isomer (*R*,*R*)-arformoterol tartrate drug formulations, and both drugs are expected to maintain their stereochemical integrity throughout the proposed shelf-life at the recommended storage condition (5 ± 3 °C).

1. Introduction

Racemates or mixtures of diastereoisomers account for about 25% of commercially available chiral drugs and differences in the biological activity of the stereoisomers are well recognized [1,2]. Knowledge of the stability and thermodynamics of interconversion of each isomer in such mixtures is critical to both the pharmaceutical industry and the regulatory agencies to assure the safety and efficacy of the drug [3,4]. Consequently, the industry regulators require the drug sponsor to provide assay methods capable of resolving and quantitating the stereoisomers to assess the stereochemical integrity of the drug substance and drug product [5].

Formoterol, ((*RR*)-(±)-*N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxypheny)-1-methylethyl]amino]ethyl]phenyl]formamide), is a highly potent and selective long-acting beta2-adrenoceptor agonist administered as a bronchodilator for patients with reversible obstructive airways disease [6]. As shown in Fig. 1, the compound contains two chiral centers with four possible stereoisomers: (*R*,*R*), (*R*,*S*), (*S*,*R*) and (*S*,*S*). Like other members of this group of drugs, formoterol is widely available as a racemate of the fumarate salt composed of a 50/50 mixture of (*R*,*R*)- and (*S*,*S*)-enantiomers. The order of potency with respect to relaxation of airways is (*R*,*R*) ≫ (*R*,*S*) = (*S*,*R*) > (*S*,*S*) [2,7,8].

Several studies have demonstrated that the (*R*,*R*)-formoterol is responsible for the β_2 agonist activity of the racemate, whereas the (*S*,*S*)-formoterol is virtually inactive [7–11]. In contrast, however, few studies have reported differences in the therapeutic effects of racemic vs. single isomer formoterol drug formulations. For instance, in the in vivo experiments conducted with sensitized guinea pigs, Handley et al. [12] indicated that the (*R*,*R*)-enantiomer is more potent than racemic (*R*,*R*/*S*,*S*)-formoterol in the inhibition of both histamine and antigen-induced bronchoconstriction while (*S*,*S*)-enantiomer was ineffective. While the (*S*,*S*)-enantiomer increased the levels of the inflammatory cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF) in human airway smooth muscle cells, the production of GM-CSF was decreased by (*R*,*R*)-enantiomer to about 40% of the control [13]. Other dissimilarity in biological activity was in urinary excretion of formoterol enantiomers and their glucuronide conjugates in male subjects, which was found to be stereoselective after oral administration of the racemic (*R*,*R*/*S*,*S*)-formoterol [14]. The (*R*,*R*)-formoterol glucuronide excretion was significantly higher than (*S*,*S*)-formoterol glucuronide (59.6% vs. 26.0% of the dose), and vice versa for the unchanged (*S*,*S*)- and (*R*,*R*)-formoterol (9.5% vs. 5.1% of the dose) [14]. In agreement with the published reports [8–11], Mhanna et al. [15] recently showed that the beta2-agonist activity of formoterol

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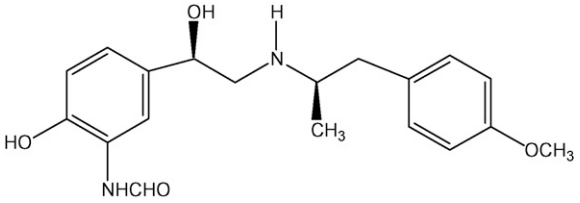

Fig. 1. Chemical structure of formoterol.

resides in the (*R*,*R*)-enantiomer. Although the clinical implication of their findings was not investigated, the observed high activity of the single (*R*,*R*)-formoterol on airway relaxation compared with racemic (*R*,*R*/*S*,*S*)-formoterol in the study was attributed to the presence of (*S*,*S*)-formoterol in the mixture.

In the light of the aforementioned, it is worthwhile to evaluate the thermodynamic profiles of possible interconversion of the four isomers of formoterol, which to the best of our knowledge has not been published. Likewise, with the exception of a few biological assay methods for formoterol enantiomers [16–19] and the isocratic HPLC method described by Aboul-Enein and Al-Duraibi [20] for separation of several racemic drugs including formoterol, our literature survey did not reveal any standard procedure for resolving and quantitating the four formoterol stereoisomers both in the active pharmaceutical ingredient (API) and the drug product. The present study provides a simple and sensitive chiral HPLC assay method for complete separation of formoterol stereoisomers in the drug substance and examine in vitro the thermodynamic profiles of their interconversion in an aqueous solution to mimic the commercial formulations and prevent the degradation of the active compound. Furthermore, the potential for chiral inversion in commercial nebulized solution of racemic (*R*,*R*/*S*,*S*)-formoterol fumarate 20 μg/2 ml (Perforomist™ (Dey, L.P., Napa, CA)) compared with the single isomer (*R*,*R*)-arformoterol tartrate 15 μg/2 ml drug (Brovana™ (Sepracor Inc., Marlborough, MA)) was also evaluated.

2. Materials and methods

2.1. Chemicals

Formoterol fumarate (99.8%, calculated on anhydrous basis) was obtained from Mylan (formerly Merck) Development Center (Taloja, India) and qualified as a reference standard according to *European Pharmacopoeia* (PhEur 6.0) monograph [21]. A sample of formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) mixture (44%/56%) was supplied by Sicor, S.p.A. (Vacallo, Switzerland) and used as received. Samples of racemic 20 μg/2 ml (*R*,*R*/*S*,*S*)-formoterol fumarate formulation (Perforomist™, a registered trademark of Dey, L.P., Napa) was obtained in-house, while the single isomer 15 μg/2 ml (*R*,*R*)-arformoterol tartrate formulation (Brovana™, a registered trademark for Sepracor Inc., Marlborough, MA) was obtained commercially. Methanol (HPLC grade) and analytical grade isopropyl alcohol (IPA), sodium phosphate monobasic monohydrate, and sodium phosphate dibasic (anhydrous) were purchased from EMD (Gibbstown, NJ). All other chemicals were of reagent grade or better and used without further purification. In-house purified water (USP grade) was used throughout the course of this study.

2.2. Standard preparation

A standard solution of 220 μg/ml formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) mixture (44%/56%) was prepared by dissolving 11 mg of the mixture in 50 ml of a placebo solution (0.006 M citrate buffer, 0.9% NaCl, pH 5.0) to mimic the commercial drug products. Appropriate dilutions were made in a similar placebo solution to obtain the desired concentration and used during the course of the study.

2.3. Mobile phase

A 50-mM sodium phosphate buffer solution (pH 7.0) was prepared by dissolving 2.69 g sodium phosphate monobasic monohydrate and 4.33 g anhydrous sodium phosphate dibasic in a few volume of purified water and diluted to volume in 1 l standard volumetric flask. The buffer solution was filtered through a 0.45-μm (90 mm diameter) nylon membrane (Pall Corporation, Ann Arbor, MI) and degassed under vacuum before use as mobile phase A. The 10% v/v IPA was prepared by diluting 100 ml of the solvent to volume with purified water in 1 l standard volumetric flask. This solution was used as mobile phase B.

2.4. Chromatography

Chiral HPLC separation was performed using Agilent 1100 HPLC System (Palo Alto, CA) consisting of a quaternary gradient pump, autosampler and variable wavelength UV detector with data collected and processed using a PerkinElmer Turbochrom Client/Server Data System—Version 6.1.2. The separation column was a Chiral-AGP 5 μm column (100 mm × 4.0 mm i.d.) (ChromTech Inc., MN). The analytes were monitored with UV detection at 242 nm under the following gradient conditions: 5% B (0 to 10 min, isocratic) to 25% B in 10 to 12 min, held at 25% B in 12 to 16 min (isocratic), and decreased from 25% to 5% B in 16 to 20 min at a flow rate of 1.3 ml min^{-1} with column temperature set at 25 ± 2 °C. Peak identification was achieved by retention time matching from parallel analysis of formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) mixture, aliquots of the commercial drug products (Perforomist™ and Brovana™), and the isolated isomers.

2.5. Isolation of formoterol isomers

HPLC fractions of the four isomers were collected from multiple injections of a 220 μg/ml solution of formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) mixture prepared in the placebo as described in Section 2.2. The collected fractions were separately purified by solid phase extraction (SPE) using two-stacked Alltech Maxi-Clean™ 600 mg C_{18} cartridges (Deerfield, IL) to remove buffer salts and concentrate the isolates. The cartridges were conditioned with methanol and then water before loading the isolated isomer at a flow rate of about 2 ml min^{-1}. The cartridges were then rinsed with water and the retained formoterol isomer was eluted with methanol. After drying the eluant under a stream of nitrogen, the concentrated isomer was re-dissolved in the placebo solution. The concentrations of the isomers in these solutions ranged from 20 to 35 μg/ml, and the chemical purity was approximately 97.2%, 98.5%, 97.6% and 99.1% for (*R*,*R*), (*S*,*S*), (*R*,*S*) and (*S*,*R*) formoterol, respectively.

2.6. Chiral inversion

For the determination of chiral inversion, separate solutions of the pure isomers placed in teflon lined screw capped amber glass vials were stored in stability chambers at 5 ± 3 °C, 25 ± 2 °C/60% RH ±5% RH, and 40 ± 2 °C/75% RH ±5% RH, and at 50, 60 and 70 °C in VWR Signature™ gravity convection ovens (West Chester, PA) with only temperature control capability. The solutions were sampled periodically for chiral HPLC analysis over a 3-week storage period. Similarly, sample solutions of the commercial products were stored under the same conditions and analyzed at the same time. In a separate experiment, a 40 μg/ml solution of formoterol fumarate

Table 1
System suitability criteria.

Parameter	Acceptance criteria[a]	Result
Precision for standard solution (RSD for 5 injections)		
(*R*,*R*)-formoterol peak area	NMT 2.0%	0.2%
(*S*,*S*)-formoterol peak area	NMT 2.0%	0.2%
Tailing factor for (*R*,*R*)-formoterol	NMT 3.0	2.2
Column efficiency for (*S*,*S*)-formoterol	NLT 1000	2700
Resolution between (*R*,*R*) and (*S*,*S*) peaks	NLT 1.2	2.9

[a] NMT = not more than; NLT = not less than.

prepared in the saline solution was exposed to white fluorescent light and UV light at 25 ± 2 °C in a photostability chamber and analyzed over a 2-week period to examine the potential for any chiral inversion under these conditions. The chiral lability of a solid sample of racemic formoterol fumarate salt was also investigated at temperatures up to 70 °C in the appropriate storage chamber/oven as specified above.

2.7. Chiral HPLC method validation

The method was validated by evaluating the following parameters: specificity/selectivity, accuracy, linearity, precision, robustness and sensitivity. Specificity was assessed from the chromatographic profiles of separate and combined solutions of the four-formoterol isomers prepared in the placebo and the blank (placebo solution). The chromatograms were examined for co-elution or any interference from the sample matrix. The detection and quantitation limits for each isomer was determined from analysis of a series of dilute solutions with known concentrations at a signal-to-noise ratio of 3:1 and 10:1, respectively.

The accuracy, expressed as percent recovery, and precision of the method was determined from analysis of triplicate preparations of formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) standard solutions at five different target concentrations, i.e., 0.1, 1.0, 4.0, 10.0, and 20.0 μg/ml. Linearity was assessed from the plot of peak area against the concentration of these solutions using linear least square regression analysis. Two different analysts examined the method ruggedness from five replicate preparations of a standard solution at the nominal concentration of 4 μg/ml on a different day. The robustness of the method was determined by evaluating the system suitability parameters following a small, but deliberate changes in chromatographic conditions including the composition of mobile phase B (±1%), flow rate (±0.1 ml min^{-1}), buffer pH (±0.2 unit), column temperature (±2 °C), and detection wavelength (±2 nm). The system suitability parameters and the acceptance criteria monitored through the course of the study are summarized in Table 1.

3. Results and discussion

3.1. Chiral HPLC method validation

While the detail of the analytical method development will not be discussed for the sake of brevity, the separation of formoterol stereoisomers under the optimized chromatographic conditions described in Section 2.4 is shown in Fig. 2. All the four isomers were adequately separated with no interference from the sample matrix indicating the specificity of the method. Presumably due to differences in chromatographic modes, the (*S*,*R*) was the least retained followed by (*R*,*R*), (*S*,*S*) and (*R*,*S*) in the present study compared with the retention order (*R*,*R*) < (*R*,*S*) < (*S*,*R*) < (*S*,*S*) reported by Gu et al. [18,19] under normal phase separation condition.

As summarized in Table 2, the calibration curves were linear (coefficient of correlation, $r^2 \geq 0.9999$) over the concentration

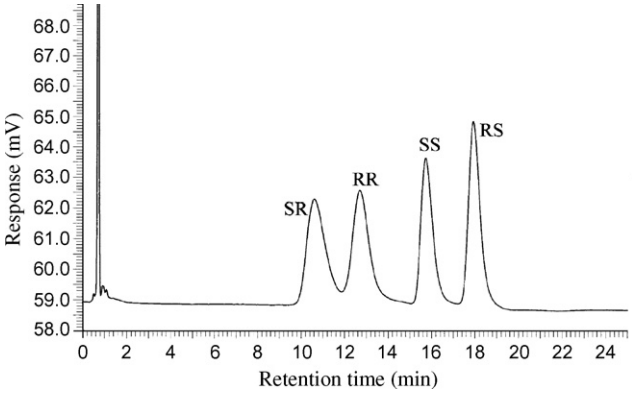

Fig. 2. Typical chromatographic separation of formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) mixture (44%/56%) on Chiral-AGP.

range investigated yielding almost identical slopes with an average value of 1.86×10^4 (RSD = 1.0%, $n = 4$). Analytes' recoveries were within acceptable range of 88.4–98.2%, and the precision data from triplicate determinations at each target concentration yielded acceptable RSD values of ≤8.5% (see Table 2). The intra- and inter-day precision at the nominal concentration of 4.0 μg/ml was less than 1.8% ($n = 5$). The limits of detection (3× signal-to-noise) and quantitation (10× signal-to-noise) varied from 0.01–0.04 μg/ml and 0.04–0.1 μg/ml, respectively. Furthermore, the method showed acceptable robustness as small but deliberate changes in the chromatographic conditions stated in Section 2.7 did not adversely affect the system suitability parameters (see Table 3).

In general, the results presented in Tables 2 and 3 were deemed satisfactory for quantitative analysis of the stereoisomers in the API and drug product using the chiral HPLC method. The analysis of a 4.0 μg/ml solution of the formoterol fumarate (*R*,*R*/*S*,*S*)/formoterol fumarate (*R*,*S*/*S*,*R*) mixture used during the course of this study indicates that it contain approximately 19.3%, 26.0%, 29.7% and 25.0% of (*R*,*R*), (*S*,*S*), (*R*,*S*) and (*S*,*R*) formoterol, respectively.

3.2. Interconversion of stereoisomers

The results for the individual isomer solutions stored at 5–70 °C showed that interconversions of formoterol isomers occurred at only one chiral center as the (*R*,*R*)-enantiomer only inverted to the (*S*,*R*)-diastereomer, while the (*S*,*S*)-enantiomer only inverted

Table 2
Summary of chiral HPLC method validation data.

Parameter	(*R*,*R*)	(*S*,*S*)	(*R*,*S*)	(*S*,*R*)
Accuracy (% recovery ± RSD) ($n = 3$)				
0.1 μg/ml[a]	94.4 ± 4.3	98.2 ± 2.4	90.2 ± 5.3	91.3 ± 8.5
1.0 μg/ml	94.4 ± 1.4	95.6 ± 2.1	90.7 ± 0.3	88.4 ± 1.2
4.0 μg/ml	92.6 ± 0.3	92.5 ± 0.7	93.5 ± 0.2	92.5 ± 0.6
10 μg/ml	94.7 ± 0.3	94.9 ± 2.1	94.9 ± 0.1	94.9 ± 0.1
20 μg/ml	94.6 ± 0.1	94.6 ± 0.2	95.7 ± 0.1	96.0 ± 0.1
Linearity				
Range (μg/ml)	0.1–15	0.1–21	0.1–24	0.1–20
Slope	1.84×10^4	1.85×10^4	1.88×10^4	1.87×10^4
Intercept	3.35×10^2	1.75×10^2	1.19×10^3	1.55×10^3
Correlation coefficient, r^2	1.0000	1.0000	1.0000	0.9999
Ruggedness (4.0 μg/ml)				
Intra-day precision ($n = 5$)	0.3%	0.7%	0.2%	0.5%
Inter-day precision ($n = 5$)	0.6%	1.8%	0.9%	0.6%
Sensitivity				
Limit of detection (μg/ml)	0.04	0.01	0.02	0.02
Limit of quantitation (μg/ml)	0.1	0.04	0.06	0.06

[a] Spiked concentration.

Table 3
Summary of robustness data[a].

Parameter	Resolution, R_s (NLT 1.2)			Precision (RSD) (NMT 2.0%)		Tailing factor, T (NMT 3.0)	Column efficiency, N (NLT 1000)
	(*S*,*R*)/(*R*,*R*)	(*R*,*R*)/(*S*,*S*)	(*S*,*S*)/(*R*,*S*)	(*R*,*R*)	(*S*,*S*)	(*R*,*R*)	(*S*,*S*)
Normal conditions[b]	1.5	3.6	2.7	0.2	0.5	1.7	5658
Mobile phase							
4% B	1.5	3.2	2.7	0.1	0.1	1.7	7145
6% B	1.4	3.8	2.5	0.1	0.1	1.7	2548
Flow rate							
1.2 ml min^{-1}	1.5	3.2	2.7	0.3	1.1	1.7	6387
1.4 ml min^{-1}	1.4	3.9	2.7	0.2	0.2	1.7	4485
Buffer pH							
6.8	1.4	3.2	2.1	0.2	0.8	1.7	1355
7.2	1.5	3.1	3.0	0.3	0.4	1.6	7049
Column temperature							
23 °C	1.5	3.3	2.6	0.2	0.2	1.7	5828
27 °C	1.5	4.0	2.8	0.2	0.3	1.7	4960
Detection wavelength							
240 nm	1.5	3.7	2.7	0.2	0.2	1.7	5452
244 nm	1.5	3.7	2.7	0.2	0.2	1.7	5396

[a] Values in parentheses are the system suitability acceptance criteria. NMT = not more than; NLT = not less than; R_s = resolution between the adjacent peaks as identified in Fig. 2.
[b] Analysis performed under normal chromatographic conditions as specified in Section 2.4.

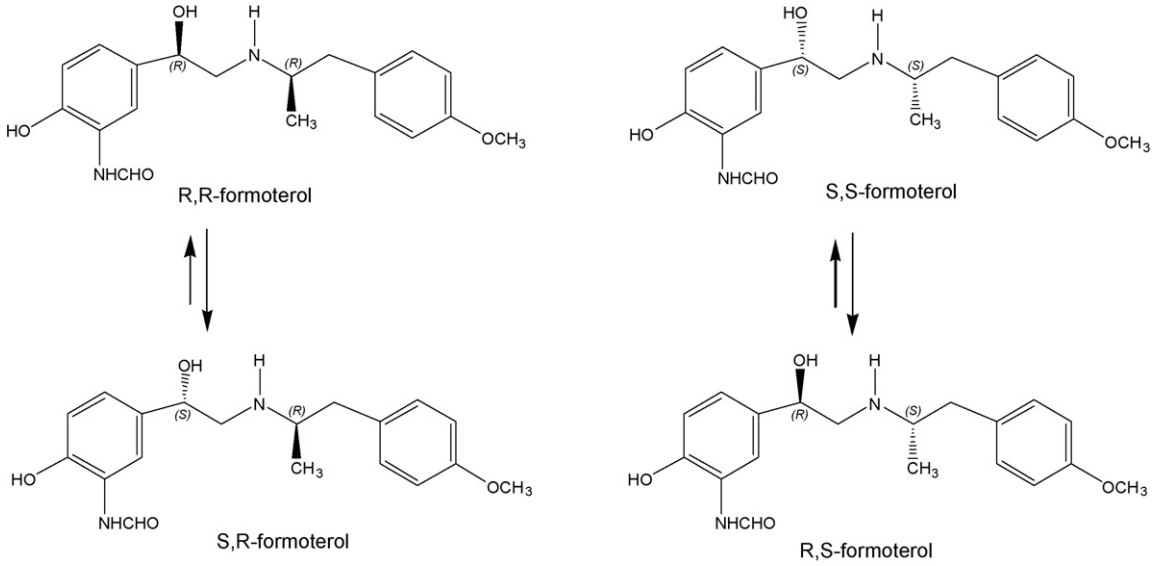

Fig. 3. The chemical structures and conformational equilibria for formoterol stereoisomers.

to the (*R*,*S*)-diastereomer, and vice versa for both diastereoisomers. The structures and conformational equilibria for formoterol stereoisomers are shown in Fig. 3. No enantiomerization or diastereomerization occurred due to absence of inversion of one enantiomer or diastereoisomer into another. As illustrated in Fig. 4, the inversions most likely occurred at the C-1 suggesting that the hydroxy (OH^-) group, though not a good leaving group but, was protonated in acidic medium allowing for the removal of water to produce the inverted compound [22].

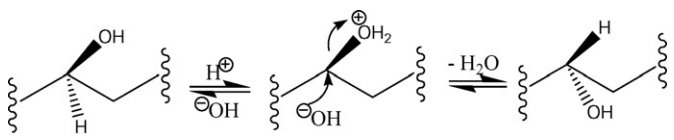

Fig. 4. A possible reaction scheme for inversion of configuration.

While no measurable inversion of the isomers was observed at 5 or 25 °C during the 3-week study period, the inversion of each isomer was relatively slow at temperatures up to 50 °C, varying from 2.1% to 4.9% after 21 days. At 70 °C, the amount of the individual isomer formed after 21 days was high, reaching about 26% for inversion of (*R*,*R*) to (*S*,*R*), 29% for (*S*,*R*) to (*R*,*R*), 36% for (*S*,*S*) to(*R*,*S*), and 33% for (*R*,*S*) to (*S*,*S*). The inversions of the stereoisomers shown in Fig. 5 indicate that the (*S*,*S*) inverts faster to (*R*,*S*) than (*R*,*S*) inversion to (*S*,*S*). However, the inversion of (*R*,*R*) to (*S*,*R*) and the reverse inversion were roughly equivalent.

The representative chromatogram of the commercial formulation of racemic (*R*,*R*/*S*,*S*)-formoterol fumarate presented in Fig. 6 at elevated temperatures showed chiral inversion of (*R*,*R*)- and (*S*,*S*)-enantiomers to (*S*,*R*)- and (*R*,*S*)-diastereoisomers, respectively. Similarly, the active component of the commercial single isomer (*R*,*R*)-arformoterol tartrate solution interconverted to the (*S*,*R*)-diastereoisomer at elevated temperatures (Fig. 6). As observed with

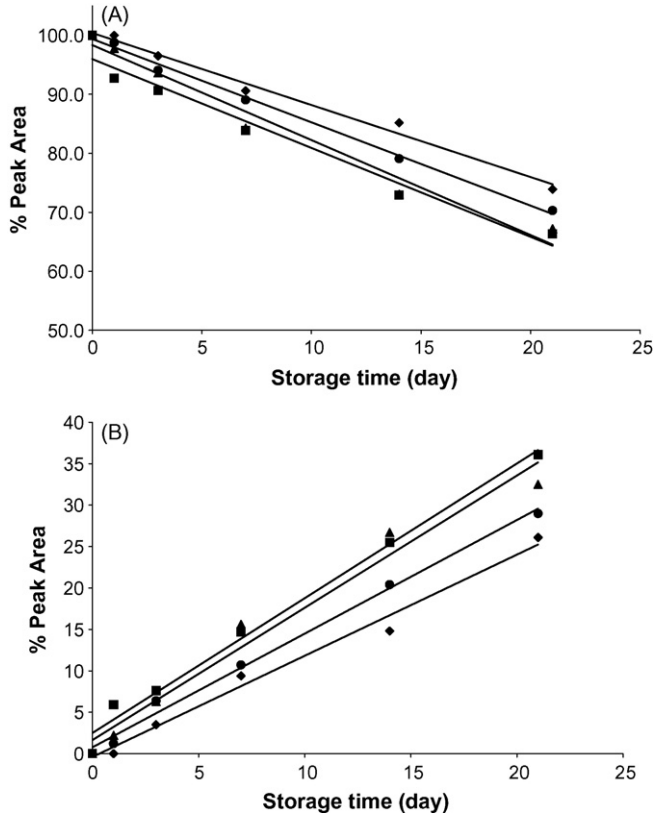

Fig. 5. Plots of storage time (day) against percent peak area for (A) inversion and (B) formation of individual isomers at 70 °C. Symbol: (●) *SR*, (♦) *RR*, (■) *SS*, (▲) *RS*.

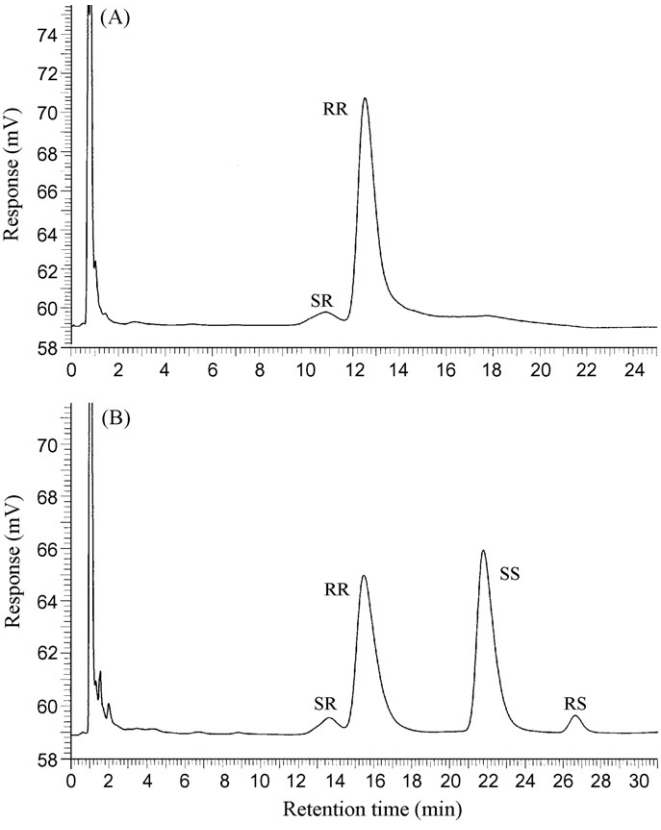

Fig. 6. Chiral inversions of (A) single isomer (*R*,*R*)-arformoterol tartrate and (B) racemic (*R*,*R*/*S*,*S*)-formoterol fumarate drug solutions after 2 weeks storage at 60 °C.

Table 4
The rate constant for interconversion of (*R*,*R*)- and (*S*,*S*)-isomers in racemic and single isomer drug formulations.

Temperature		Rate constant (k)		
°C	°K	rac-(*R*,*R*/*S*,*S*)-formoterol		(*R*,*R*)-arformoterol
		(*R*,*R*)	(*S*,*S*)	(*R*,*R*)
40	313	0.0229	0.0163	0.0330
50	323	0.0505	0.1048	0.1685
60	333	0.2281	0.2671	0.3851
70	343	0.7570	0.6152	1.3423

the individual isomers, no measurable chiral inversion was detected over the study period in both drug solutions stored at 5 and 25 °C. Thus, the stereochemical integrity of the active components in each drug is expected to remain intact when refrigerated or stored at ambient temperature.

It is worth mentioning that storage of solid racemic formoterol fumarate salt at the temperature range examined did not produce any detectable chiral inversions. Also, exposure of formoterol fumarate solution to white or UV lights in the photostability chamber did not yield any chiral inversion but led to degradation with formoterol desformyl analog as the main by-product supporting the mechanism for inversion that requires protonation and subsequent loss of water.

3.3. Kinetics of chiral inversions

The rate constants for interconversions of formoterol stereoisomers at each temperature were obtained by linear least square regression analysis. The activation energy of a reaction can be calculated from the Arrhenius equation, which represents the relationship between the rate constant k and the activation energy E_a of the reaction:

$$\ln\ k = -\frac{E_a}{RT} + \text{constant} \quad (1)$$

where T is the absolute temperature and R is the gas constant (8.315). The plot of $\ln k$ vs. $1/T$ is a straight line with a slope of E_a/R. The slope multiplied by 8.315 provides E_a in kJ/mol, which can be converted to kcal/mol by dividing kJ/mol by 4.2 [23]. The experimentally determined rate constants of interconversion for individual isomers in racemic (*R*,*R*/*S*,*S*)-formoterol fumarate and single isomer (*R*,*R*)-arformoterol tartrate are presented in Table 4. The results showed that the inversion of the isomers increased

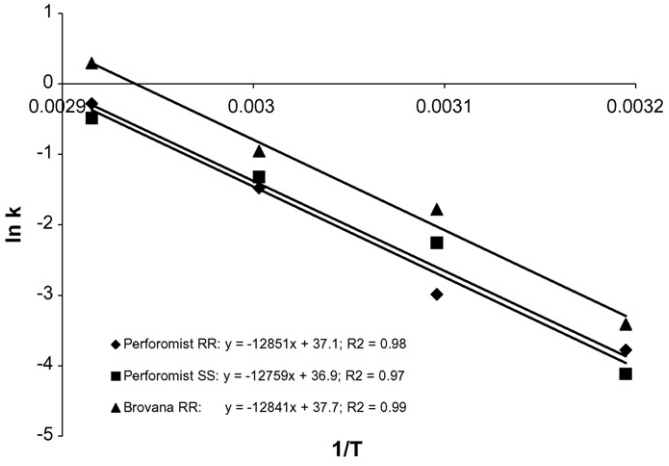

Fig. 7. Arrhenius plots for inversion of (*R*,*R*)- and (*S*,*S*)-enantiomers in Perforomist™ and Brovana™. Symbol: (♦) Perforomist™ *RR*, (■) Perforomist™ *SS*, (▲) Brovana™ *RR*.

with increasing temperature, suggesting that the conversion of the (*R*,*R*)- to (*S*,*R*)-, and (*S*,*S*)- to (*R*,*S*)-isomer is endothermic ($\Delta H^{\circ} > 0$). As shown in Fig. 7, the regression plots of natural log transformed data were linear indicating that the chiral inversions follow first order rate kinetics. The calculated energies of activation (E_a) for chiral inversion of the (*R*,*R*)- and (*S*,*S*)-enantiomers in racemic (*R*,*R*/*S*,*S*)-formoterol fumarate and the single isomer (*R*,*R*)-arformoterol tartrate was approximately 25 kcal/mol, which (within experimental error) was in close agreement with 28, 28, 22 and 30 kcal/mol obtained from similar plots (data not illustrated) for inversion of isolated (*R*,*R*), (*S*,*R*), (*S*,*S*), and (*R*,*S*) isomers, respectively.

The projected rates for (*R*,*R*)- and (*S*,*S*)-enantiomer inversion in racemic (*R*,*R*/*S*,*S*)-formoterol fumarate upon storage at 5 °C were 3.3×10^{-3}%/month and 3.6×10^{-3}%/month, respectively. For storage at 25 °C, the projected chiral inversion rates for (*R*,*R*)- and (*S*,*S*)-enantiomers were 7.2×10^{-2}%/month and 8.1×10^{-2}%/month, respectively. Thus, for a 24-month storage of racemic (*R*,*R*/*S*,*S*)-formoterol fumarate drug solution at the recommended long-term storage condition of 5 ± 3 °C [24], approximately 0.1% each of (*R*,*R*)- and (*S*,*S*)-formoterol are expected to interconvert to (*S*,*R*)- and (*R*,*S*)-formoterol, respectively. Also, approximately 0.2% of each enantiomer is expected to invert for a 3-month storage at room temperature (25 ± 2 °C) post-dispensing [24]. For the single isomer (*R*,*R*)-arformoterol tartrate drug solution, the projected rate of inversion upon storage at 5 and 25 °C were 6.3×10^{-3}%/month and 0.14%/month, respectively, corresponding to about 0.1% of (*R*,*R*)-formoterol inversion to (*S*,*R*) for a 18-month storage at the approved long-term condition of 5 ± 3 °C, and approximately 0.2% for storage at room temperature (25 ± 2 °C) up to 6 weeks [25]. These projections, which are equivalent in both formulations based on the molecular ratio of formoterol in the active drug, are well below the detection limits of the test method and are also lower than the FDA/ICH threshold of ≤1.0% for a degradant in both medications [26]. Therefore, in addition to the stability of the drug products at pH 5.0 [24,25], both drug solutions are expected to retain their stereochemical integrity; hence their potency throughout the proposed shelf-life at the recommended storage condition (5 ± 3 °C).

4. Conclusion

A validated chiral HPLC method was successfully applied for resolving and quantitating formoterol stereoisomers and their inversion products in racemic or enantiomerically pure pharmaceutical formulations. The validation data demonstrated that the method is specific, accurate, linear, precise, sensitive and robust. The results of the inversion study indicated that interconversions of formoterol is very slow at low temperatures (≤50 °C), but followed the first order rate kinetics and occurred at only one chiral center with no enantiomerization (*R*,*R* to *S*,*S*) or diastereomerization (*R*,*S* to *S*,*R*) occurring. Interconversions in both the racemic (*R*,*R*/*S*,*S*)-formoterol fumarate and a single isomer (*R*,*R*)-arformoterol tartrate pharmaceutical formulations are similar and the average energy of activation (E_a) for chiral inversions was about 25 kcal/mol. The projected chiral inversion using the Arrhenius equation for a drug containing a pure form of each formoterol isomer is approximately 0.1% for a 24-month storage at 5 ± 3 °C, and 0.2% for a 3-month storage at 25 ± 2 °C/60% RH ±5% RH. These projections, which are equivalent in both the racemic and single isomer drug formulations, are well below the FDA/ICH threshold of ≤1.0% for a degradant in the drug products.

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