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Optimization and validation of a gas chromatographic method for analysis of (*RS*,*SR*)-diastereoisomeric impurity in formoterol fumarate

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Abstract

A gas chromatographic method for the determination of formoterol (*RS*,*SR*)-diastereoisomer, a process impurity, in formoterol fumarate was optimized and validated. The method involves silylation of formoterol fumarate with *N*-(trimethylsilyl)imidazole in *N*,*N*-dimethylformamide at room temperature in an autosampler vial to produce trimethylsilyl derivatives of the enantiomers prior to GC analysis. The optimized silylation and separation conditions, respectively, produced good yield and resolution of the analytes. The method appears to be convenient and fast, and permits accurate determination of (*RS*,*SR*)-diastereoisomer in formoterol fumarate with adequate precision (R.S.D. = 3.0%, $n = 9$) and sensitivity (DL < 0.01%) when compared with the official liquid chromatographic limit test method of Pharmeuropa. The method was successfully applied to quality control of commercial formoterol fumarate for their (*RS*,*SR*)-diastereoisomer contents.

Keywords: Validation; Diastereomer; Formoterol fumarate

1. Introduction

Formoterol is a new β2-adrenoceptor agonist with prolonged duration of action when inhaled for the treatment of recurrent obstructive respiratory disease [1–3]. The compound, which contains two chiral centers in the molecule with four possible stereoisomers, is sold as the fumarate salt of the enantiomeric mixture (*R*,*R* + *S*,*S*). Studies have shown that the therapeutic efficacy of inhaled formoterol resides essentially on the *R*,*R*-isomer, and is not affected by the *S*,*S*-isomer when present in the mixture [4,5]. The order of potency for the isomers is $R,R \gg R,S = S,R > S,S$ [5,6].

A draft limit test liquid chromatographic method was recently published in Pharmeuropa monograph for the determination of (*RS*,*SR*)-diastereoisomer in formoterol fumarate [7]. However, the sample components were poorly resolved thereby limiting its use to qualitative determination of the analyte in the material. An alternate simple approach is gas chromatography via derivatization of the hydroxyl groups in the compound with a suitable silylating agents; e.g. TMSI. In addition to producing quantitative reactions, TMSI has been shown to be superior for silylation of hydroxyl groups in the presence of aliphatic amines compared with other silylating agents; e.g. *N*,*O*-bis(trimethylsilyl)trifuoroacetamide (BSTFA), *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and *N*-methyl-*N*-trimetylsilyltrifluoroacetamide (MSTFA) [8–11]. These properties favor its use as a silylating agent for formoterol, which contain the aforementioned groups in its molecular structure [12].

The aim of this work is to optimize and validate a gas chromatographic method for the determination of (*RS*,*SR*)-diastereoisomer in formoterol fumarate after derivatization with TMSI. The derivatization reaction was performed in situ in a gas chromatographic autosampler vial at room temperature by adding a known amount of TMSI to a solution of the sample in the vial. The resulting solution was analyzed directly by GC, and the analytical procedure was evaluated for linearity, accuracy, precision, sensitivity, robustness and its suitability for isomeric purity determination of formoterol fumarate.

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2. Experimental

2.1. Chemicals

Formoterol fumarate dihydrate, *N*-[2-hydroxy-5-[(1*RS*)-1-hydroxy-2-[[(1*RS*)-2-(4-methoxyphenyl)-1-methylethyl] amino]ethyl]phenyl]formamide(*E*)-2-butenedioate dihydrate, formoterol fumarate (*RS*,*SR*)-diastereoisomer, *N*-[2-hydroxy-5-[(1*RS*)-1-hydroxy-2-[[(1*SR*)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide, and a sample of formoterol fumarate/formoterol fumarate (*RS*,*SR*) mixture (43%/54%) were obtained from Vinchem (Chatham, NJ, USA). Nine lots of formoterol fumarate commercially available for therapeutic use were obtained from three different manufacturers and evaluated during the course of this study for their isomeric purity. *N*-(Trimethylsilyl)imidazole (TMSI) and *N*,*N*-dimethylformamide (DMF) were obtained from Aldrich (Milwaukee, WI, USA) and Burdick & Jackson (Muskegon, MI, USA), respectively, and used as received. Autosampler vials with PTFE lined crimp-caps were purchased from Alltech (Deerfield, IL, USA).

2.2. Gas chromatograph

Chromatographic separations were performed on Agilent 6890 series gas chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector, a Gerstel MultiPurposeSampler MPS (Baltimore, MD, USA), and Perkin-Elmer Turbochrom Client/Server Data System—Version 6.1.2 (Shelton, CT, USA). Unless stated otherwise, the gas chromatograph, fitted with Phenomenex ZB-5 capillary column [(poly(diphenyldimethyl)siloxane containing 5% diphenylsiloxane monomer, 30 m × 0.32 mm i.d., 0.25-μm film thickness)] (Torrance, CA, USA), was operated under the following conditions: carrier gas (ultra-high-purity helium) flow rate, 2 ml/min; make-up gas (nitrogen) flow rate, 32.5 ml/min; split ratio, 1:20; injection volume, 1 μl; injection port, column oven and detector temperatures were kept at 280, 225 and 300 °C, respectively.

2.3. Stock (RS,SR)-diastereoisomer solution

A stock solution of the diastereoisomer of formoterol fumarate (0.1 mg/ml) in DMF was prepared and refrigerated until use. The solution was found to be stable for up to thirty days of storage.

2.4. Standard solution

About 10 mg of formoterol fumarate reference standard was weighed into an autosampler vial, and 1.0 ml of the stock (*RS*,*SR*)-diastereoisomer solution prepared in Section 2.3 was added into the vial. The mixture was shaken gently until all the solids dissolved. Then, 100 μl of TMSI was added as the silylating agent, and the vial was capped immediately. The resulting solution was thoroughly mixed and allowed to stand at room temperature in the autosampler tray for at least 10 min to complete the derivatization prior to GC analysis.

A typical blank solution was prepared by adding 100 μl of TMSI to 1 ml of DMF in autosampler vial, capped immediately, and mixed thoroughly prior to use.

2.5. Sample solution

Analysis solution of each sample of formoterol fumarate (10 ± 1 mg) examined was directly weighed into separate GC autosampler vials and 1.0 ml of DMF was added into each vial. The resulting solutions were silylated with TMSI as described in Section 2.4.

2.6. Sample preparation for method validation

A stock spiking solution (0.05 mg/ml) of formoterol (*RS*,*SR*)-diastereoisomer was prepared in DMF and appropriate volumes were diluted with DMF to concentrations varying from 0.005 to 0.03 mg/ml of spiking solutions.

The accuracy of the method was determined by evaluating the recovery of formoterol (*RS*,*SR*)-diastereoisomer in standard solutions prepared by spiking 10 ± 0.1 mg of formoterol fumarate reference standard in separate autosampler vials with 1 ml of the appropriate concentrations of the spiking solutions to obtain the desired levels (0.05, 0.30 and 0.5%, w/w; (*RS*,*SR*)-diastereoisomer) of the analyte. The resulting solutions were silylated with TMSI as described in Section 2.4. The linearity of the method was assessed from a series of standard solutions prepared in the same manner as described above over a concentration range of 0.03–0.5% (w/w) (*RS*,*SR*)-diastereoisomer.

System precision was determined from the relative standard deviation of five replicate injections of the standard solution. The method precision was performed by five repeat determinations of (*RS*,*SR*)-diastereoisomer in a selected lot of commercial formoterol fumarate. A second analyst on a different instrument and different day repeated the experiment with the same lot of the material to evaluate the method ruggedness (intermediate precision). Method robustness was assessed from the changes in the separation profile of the analytes with deliberate variations in the experimental conditions such as carrier gas flow rate, column oven temperature, and column type.

3. Results and discussion

3.1. Derivatization reaction

Fig. 1 shows the reaction of formoterol with TMSI at room temperature in the absence of acid catalysts. The by-product, imidazole, is weakly amphoteric and has been shown to prevent the formation of enol-silyl ether [13]. Because of the presence of water in DMF and the drug

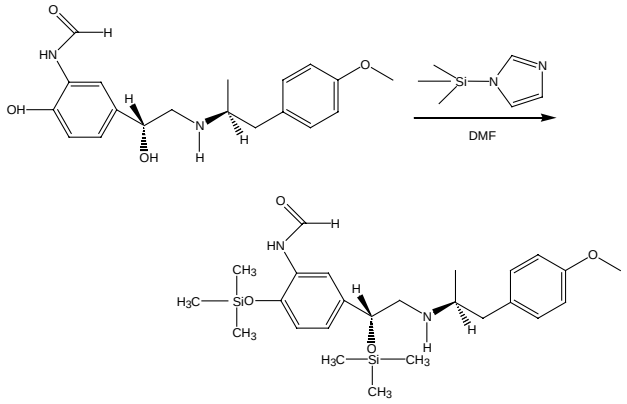

Fig. 1. Silylation of formoterol fumarate with *N*-(trimethylsilyl)imidazole (TMSI).

substance, which varied from 4.0 to 5.0% in the latter, the quantity of TMSI required for maximum yield of trimethylsilyl derivatives was determined empirically. Fig. 2 shows the formation of the trimethylsilyl derivatives of formoterol fumarate reference standard spiked with about 0.5% formoterol (*RS*,*SR*)-diastereoisomer. While no derivative was detected in sample silylated with 10 and 25 μl of TMSI, maximum conversion was reached when 50–100 μl

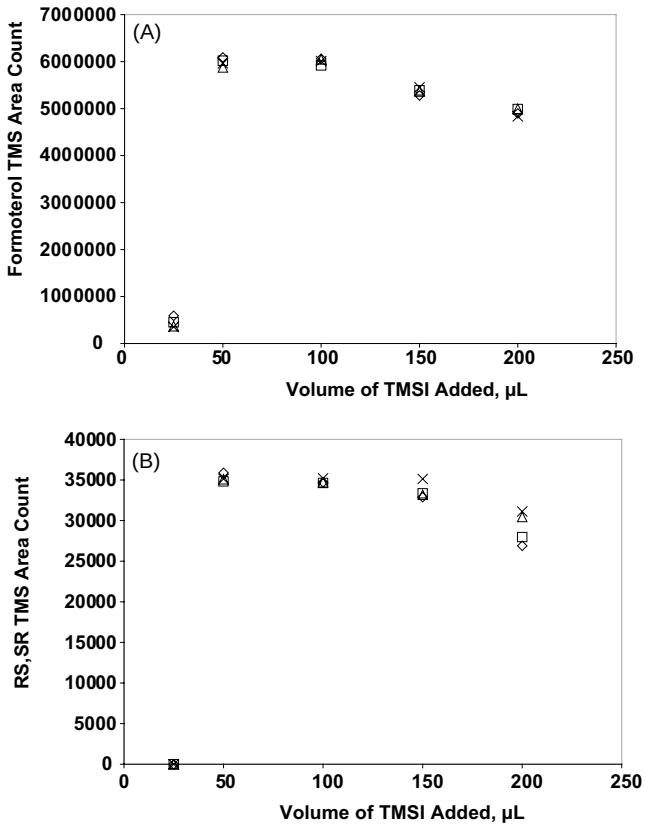

Fig. 2. Effect of amount of silylating reagent on the formation of (A) formoterol, and (B) formoterol (*RS*,*SR*)-diastereoisomer trimethylsilyl derivatives. Reaction time: (◇) 10 min; (□) 70 min; (△) 130 min and (×) 190 min.

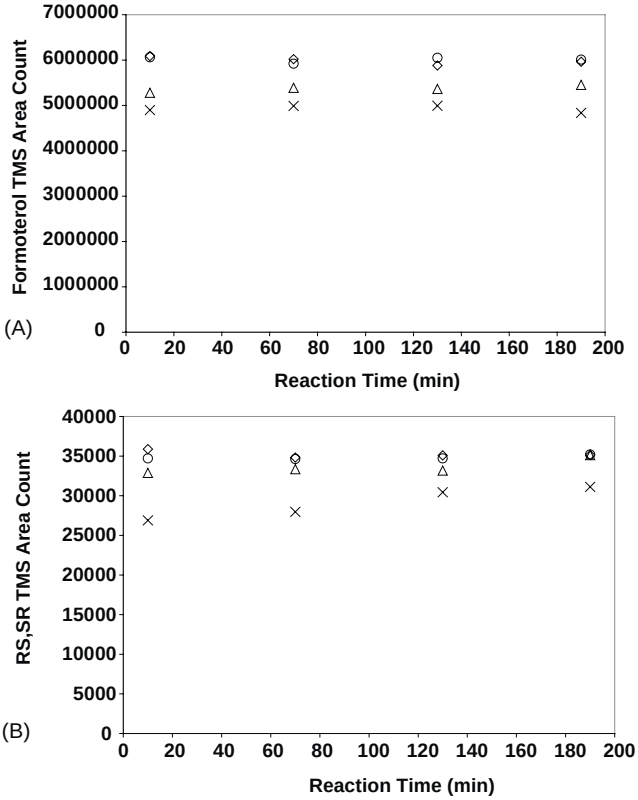

Fig. 3. Plot of reaction time (min) vs. peak area count of (A) formoterol and (B) formoterol (*RS*,*SR*)-diastereoisomer trimethylsilyl derivatives produced. Volume of silylating reagent: (◇) 50 μl; (○) 100 μl; (△) 150 μl and (×) 200 μl.

of TMSI was employed for silylation. It can be seen in Fig. 3 that the amount of each derivative, measured in terms of peak area (μV s), at different time intervals was almost constant suggesting that the reaction essentially depends on the amount of the derivatizing agent in the reaction medium. In general, the reaction approaches maximum conversion in less than 10 min; thus the hydroxyl groups in the compounds were rapidly and conveniently silylated at room temperature. As presented in Figs. 2 and 3, silylation with large volumes of TMSI (>100 μl) shows a noticeable decrease in trimethylsilyl derivatives of both analytes indicative of degradation of formoterol and its diastereoisomer in excess amounts of TMSI. This was also observed in a previous report where formoterol exhibited poor hydrolytic stability in acidic and basic solutions at room and elevated temperatures [12]. Therefore, a 100 μl of TMSI and a reaction time of not less than 10 min were selected as the optimum condition for silylation of all samples in the present study.

3.2. Gas chromatographic separation

After the initial screening of the GC columns, the following variables: column oven temperature, injection volume, and split ratio were selected for method optimization since these variables appear to affect the sensitivity of the method.

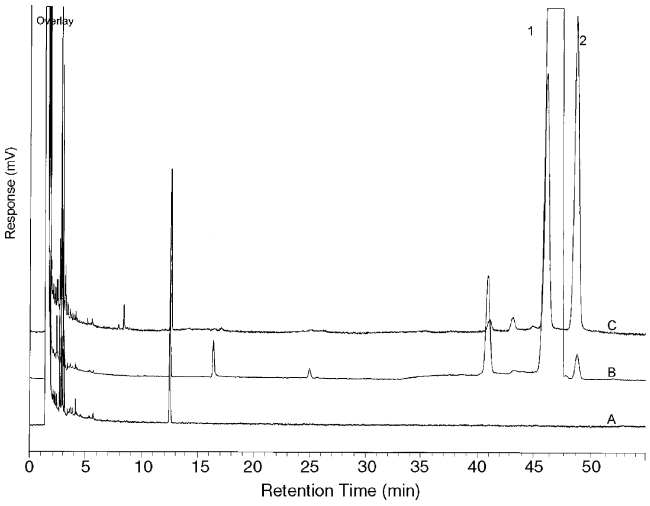

Fig. 4. Chromatographic profiles of trimethylsilyl derivatives of (A) blank solution, (B) formoterol fumarate reference standard spiked with 0.5% (*RS*,*SR*)-diastereoisomer, and (C) formoterol fumarate/formoterol fumarate (*RS*,*SR*) mixture (43%/54%). Peak (1) formoterol and (2) formoterol (*RS*,*SR*)-diastereoisomer.

For instance, formoterol and its diastereoisomer eluted at about 31 and 32 min, respectively, under the following conditions: injection volume, 2 μl; split ratio, 1:75; column oven program, 220 °C for 5 min then ramped to 250 °C at 1 °C/min and held at 250 °C for 20 min. The injector port and detector temperatures were kept at 280 and 300 °C, respectively. However, the (*RS*,*SR*)-diastereoisomer could not be detected at concentrations less than 0.1% suggesting that the method is not sensitive under these conditions. The best selectivity and sensitivity were obtained under conditions given in Section 2.2. A typical chromatographic profiles of trimethylsilyl derivatives of (A) blank solution, (B) formoterol fumarate reference standard spiked with about 0.5% formoterol (*RS*,*SR*)-diastereoisomer, and (C) formoterol fumarate/formoterol fumarate (*RS*,*SR*) mixture (43%/54%) are shown in Fig. 4. The chromatograms show baseline separation of formoterol and the (*RS*,*SR*)-diastereoisomer trimethylsilyl derivatives at about 46 and 48 min, respectively, with no interference with the sample blank confirming the specificity of the method.

3.3. Accuracy/recovery

The accuracy data obtained for triplicate determinations at each concentration of formoterol (*RS*,*SR*)-diastereoisomer are presented in Table 1. The calculated amounts, corrected for the background level of (*RS*,*SR*)-diastereoisomer in the reference material, were in good agreement with the spiked amounts of the analyte in the test samples. The average recoveries for triplicate analyses of the reference material spiked with 0.05, 0.3 and 0.5% (w/w) of the analyte were 93.2% (5.0% R.S.D.), 95.5% (2.1% R.S.D.) and 92.9% (0.6% R.S.D.), respectively, with an average recovery value of 93.9% (3.0% R.S.D., $n = 9$). The accuracy of this method is acceptable for quantitative determination of the analyte in samples.

3.4. Linearity

The detector response was linearly related to the analyte concentration over a range of approximately 0.03–0.5% (w/w), which corresponds to about 15–250% of the proposed specification limit of not more than 0.2% [7]. A plot of peak area (μV s) versus spiked amounts (%, w/w) of formoterol (*RS*,*SR*)-diastereoisomer produced a linear regression equation $y = 56420x - 10.955$ with a correlation coefficient $r^2 > 0.999$ ($n = 6$).

3.5. Detection and quantitation limits

The detection limit (DL) (three times signal-to-noise) and quantitation limit (QL) (10 times signal-to-noise) were estimated from the slope and the standard deviation of the *y*-intercept of the regression line obtained in Section 3.4 since the prepared samples' concentrations are in the region of detection and quantitation limits [14]. The detection and quantitation limits for formoterol (*RS*,*SR*)-diastereoisomer in formoterol fumarate were found to be 0.01 and 0.03%

Table 1
Accuracy/recovery for formoterol (*RS*,*SR*)-diastereoisomer

Sample	Spiked amount (%)	Determined amount (%)	Recovery (%)	Mean ($n = 3$)	R.S.D. (%)
1	0.049	0.043	87.8	93.2	5.0
2	0.049	0.047	95.9		
3	0.049	0.047	95.9		
1	0.300	0.280	93.3	95.5	2.1
2	0.300	0.291	97.0		
3	0.297	0.286	96.3		
1	0.497	0.461	92.8	92.9	0.6
2	0.494	0.462	93.5		
3	0.498	0.460	92.4		
Mean ($n = 9$)				93.9	3.0

Table 2
Repeatability/intermediate precision

Sample	% Formoterol (*RS*,*SR*)-diastereoisomer	
	Analyst 1, day 1	Analyst 2, day 2
1	0.081	0.088
2	0.085	0.081
3	0.089	0.090
4	0.082	0.098
5	0.084	0.074
Mean	0.084	0.086
R.S.D. (%)	3.8	10.6
Grand mean	0.085	
R.S.D. (%)	7.7	

(w/w), respectively. The average recovery from triplicate determinations of the analyte at the QL level was 85.2% with an R.S.D. of about 13.0% which is satisfactory for low level determination of the analyte in formoterol fumarate.

3.6. Precision

The system precision was determined from the peak area responses for five replicate injections of a standard solution. The R.S.D. for the formoterol and formoterol (*RS*,*SR*)-diastereoisomer responses were 0.9 and 3.0%, respectively, which are acceptable for the study. The repeatability/intermediate precision of the method was assessed by determining the (*RS*,*SR*)-diastereoisomer content of two five-sample sets of a selected lot of formoterol by two different analysts. The results obtained by the two analysts on different day and different instrument are presented in Table 2. The mean concentration from five determinations of formoterol (*RS*,*SR*)-diastereoisomer in the sample was 0.084% (w/w) (3.8% R.S.D.) as determined by the first analyst, which was in good agreement with 0.086% (w/w) (10.6% R.S.D.) obtained by the second analyst. Statistical analysis of the two variances by *F*-test showed that there is no significant difference between the two results at the 95% confidence interval. Hence, the method is suitably precise and reproducible.

Table 4
Isomeric purity of formoterol fumarate

Sample	(*RS*,*SR*)-diastereoisomer (%)	
	GC	HPLC[a]
Supplier A	0.07 ± 0.01[b]	Passed
Supplier B	2.5 ± 0.9	Failed
Supplier C	0.04 ± 0.02	Passed

Note: NMT, not more than.
[a] Pharmeuropa monograph (specification limit, NMT 0.2%) [7].
[b] Mean ± S.D. ($n = 3$).

3.7. Robustness

The separation presented in Fig. 4 was repeated after slight but deliberate variations of the carrier gas flow rate, column type, and column oven temperature to demonstrate the robustness of the method. As presented in Table 3, deliberate changes in the parameters evaluated did not affect formoterol retention time (t_r), and the resolution (R_s) between formoterol and the (*RS*,*SR*)-diastereoisomer peaks. All peaks were baseline resolved without any interference from the sample matrix following these changes, which demonstrates the robustness of the method.

3.8. Application

The suitability of the GC method for the determination of formoterol (*RS*,*SR*)-diastereoisomer in commercial formoterol fumarate was assessed by analyzing different lots of the material obtained from different manufacturers for quality. While the results agreed with those obtained using the Pharmeuropa monograph, more reliable data were obtained with the present method. As presented in Table 4, the assay results clearly showed the differences in the isomeric purity of the samples examined. In addition, the method is simple, fast and requires fewer sample preparations when compared with the official liquid chromatographic method [7].

Table 3
Chromatographic characteristics from changes in experimental conditions

Parameter	Variation	*RS*,*SR*; t_r (min)	R_s	Formoterol R.S.D. (%)[a]	*RS*,*SR* assay (%)
Flow rate	1.9 ml/min	42.7	1.1	0.7	0.084
	2.0 ml/min	41.3	1.1	0.4	0.078
	2.1 ml/min	39.9	1.1	2.0	0.085
Column oven temperature	224 °C	43.2	1.1	0.7	0.085
	225 °C	41.3	1.1	0.4	0.078
	226 °C	39.5	1.1	1.3	0.079
Column type	Phenomenex ZB-5	41.3	1.1	0.4	0.078
	Alltech EC-5	47.7	1.0	0.7	0.082
	Agilent HP-5	44.6	1.1	0.6	0.084

Note: NMT, not more than.
[a] Acceptance criterion: R.S.D. for peak area in five standard injections, NMT 2.0%.

4. Conclusion

A simple gas chromatographic method for the determination of formoterol (*RS*,*SR*)-diastereoisomer in formoterol fumarate was investigated. Using TMSI as the derivatizing agent, the silylation reaction appeared to reach completion in a very short period of time (<10 min) at room temperature. The separation method showed good linearity from 0.03 to 0.5% (w/w) ($r^2 > 0.999$), and adequate recovery (3.0%, R.S.D., $n = 9$) over the concentration range examined. The estimated detection and quantitation limits were 0.01 and 0.03% (w/w), respectively. In addition to exhibiting excellent reproducibility and selectivity, the present method is convent and reliable for isomeric purity determination of formoterol fumarate.

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