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Short communication

Determination of salmeterol in metered-dose and dry-powder inhalers by reversed-phase high performance liquid chromatography

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1. Introduction

Salmeterol or [(*RS*)-5{1-hydroxy-2-[6-(4-phenylbutoxy)-hexylamino] ethyl}salicyl alcohol is a β^2-agonist used as a bronchodilator in inhalation therapy [1,2]. It is prepared as a 1:1 salt of salmeterol and 1-hydroxy-2-naphthoic acid (characterised by its 250 MHz, ^{1}H-NMR spectrum in deuterated methanol) and exhibits remarkable bronchodilator activity when administered from either metered-dose inhalers (MDIs) or dry powder inhalers (DPIs). There are no known published methods for the determination of salmeterol in inhalers.

The purpose of this work was to develop a HPLC method for the determination of salmeterol in MDIs and DPIs.

2. Experimental

2.1. Apparatus

A liquid chromatograph (TOSOH Corporation, Japan) consisting of a CCPE dual-piston reciprocating pump, a UV-8011 UV–visible detector and a Rheodyne injector fitted with a 20 μl loop were used. The column used was a 150 mm × 4.6 mm i.d. column packed with 5 μm C18 TSK GEL from TOSOH. Detection was at 225 nm. Data acquisition was accomplished by using an integrator, Chromatocorder from TOSOH.

2.2. Reagents and chemicals

Analytical reagent grade triethylamine, orthophosphoric acid (85% w/w), HPLC grade acetonitrile, methanol (E. Merck India Ltd., Bombay, India) and distilled water prepared in the laboratory were used to prepare the mobile phase consisting of methanol–acetonitrile–wa-

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ter–diethylamine–orthophosphoric acid (85%) 55:10:35:0.1:0.1;/v/v/v/v/v. A flow rate of 1.0 ml min^{-1} was used.

2.3. Reference standard

A standard solution of salmeterol hydroxynaphthoate (purity 99.8%, 5.0 μg ml^{-1}) was prepared in methanol. The calibration curve for salmeterol hydroxynaphthoate was prepared in the range 5–25 μg ml^{-1}.

2.4. Sample preparation

2.4.1. Salmeterol metered dose inhaler

40 sprays of salmeterol were actuated into a 250 ml beaker containing 80 ml of methanol by immersing a cannister together with its adaptor body in the solution. Two appropriate washings (5 ml each) with methanol were applied to the adaptor body collected in the same solution. The contents were then warmed at 50°C for 10 min to expel propellents from the solution. The resulting solution was cooled to ambient temperature and diluted to 100 ml with methanol. The cannister was shaken after actuation of every 5 sprays to achieve uniform delivery of the drug into the solution. Each actuation of cannister delivered 25 μg of salmeterol equivalent to salmeterol hydroxynaphthoate.

2.4.2. Salmeterol dry powder inhaler

20 capsules of salmeterol were actuated into a 250 ml beaker containing 50 ml of methanol with the aid of a Rotahaler device by immersing in the solution. Two appropriate washings (5 ml each) with methanol were applied the device collected in the same solution. The contents were then sonicated for 10 min. The resulting solution was filtered through a Whatman No. 42 filter and diluted to 100 ml with methanol. Each actuation delivered 25 μg of salmeterol equivalent to salmeterol hydroxynaphthoate.

3. Results and discussion

The determination of salmeterol in aerosol formulations involved problems of investigating a

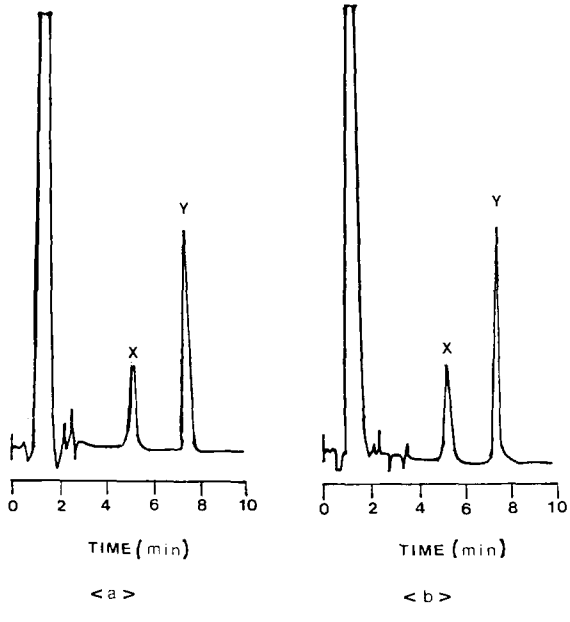

Fig. 1. Chromatograms obtained from (a) MDIs and (b) DPIs. All analyses were monitored at 225 nm. X = Salmeterol; Y = 1-hydroxy-2-naphthoic acid.

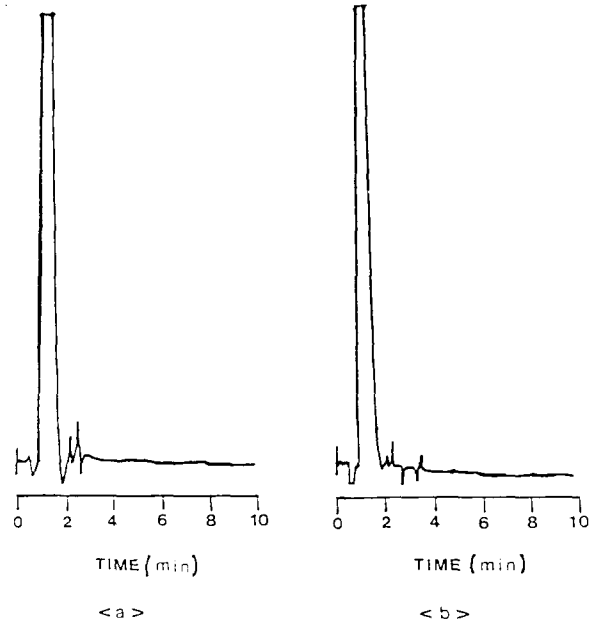

Fig. 2. Chromatograms obtained from placebos of (a) MDIs and (b) DPIs, all analyses were monitored at 225 nm.

Table 1
Recovery of salnuterol equivalent to salmeterol hydroxynaphthoate from MDIs and DPIs

Concentration added (μg per spray)	Concentration found (μg per spray)	%Recovery (Mean ± SD $n = 3$)	RSD (%)
From MDIs (within-day study)			
10.0	9.89	98.9 ± 1.62	1.64
15.0	15.13	100.9 ± 1.36	1.35
20.0	19.72	98.6 ± 1.19	1.21
From MDIs (day-to-day study)			
10.0	10.13	101.3 ± 1.78	1.76
15.0	14.86	99.1 ± 1.26	1.27
20.0	19.93	99.6 ± 1.44	1.44
From DPIs (within-day study)			
10.0	10.15	101.5 ± 1.36	1.34
15.0	15.10	100.7 ± 1.06	1.05
20.0	20.15	100.7 ± 1.49	1.48
From DPIs (day-to-day study)			
10.0	9.98	99.8 ± 1.17	1.17
15.0	15.08	100.5 ± 1.66	1.65
20.0	19.93	99.6 ± 1.03	1.03

suitable mobile phase that would separate the drug from the excipients and from 1-hydroxy-2-naphthoic acid. However, addition of triethylamine and the organic modifier acetonitrile to the mobile phase gave sufficient selectivity to achieve the separation, without interference from excipients. This is evident from a chromatogram from an MDI and a DPI as shown in Figs. 1 (a) and 1 (b) respectively. Determination was accomplished by the external standardisation method. The response of the detector was found to be linear; the regression equation was: $y = 15\ 241.06x - 3907.688$ ($r = 0.99955$) for salmeterol hydroxynaphthoate in the concentration range 5–25 μg ml^{-1}. Detection levels were estimated to be 2 μg ml^{-1} monitored at 225 nm and 0.05 A.U.F.S. There was no interference from the placebos of an MDI and a DPI. This is demonstrated in Figs. 2 (a) and 2 (b) respectively.

Table 2
Precision data for the analysis of standard solutions containing 25 μg of salmeterol

Replicate	Peak areas of salmeterol	
	Within-day study	Day-to-day study
1	360492	375521
2	361252	365432
3	359815	370098
4	365518	369876
5	358815	371249
Mean	361178.4	370435.4
SD	2586.175	3605.068
RSD (%)	0.175	0.98

The recovery of salmeterol equivalent to salmeterol hydroxynaphthoate was assessed by adding standard solutions of 10, 20 and 30 μg of salmeterol hydroxynaphthoate to previously analysed samples of an MDI and a DPI separately. The amount of salmeterol recovered equivalent to salmeterol hydroxynaphthoate was then determined using measurements of peak areas from the standard stock solutions of the drug added to the preanalysed samples of MDIs and DPIs. To confirm the precision of the assay method (using five replicate injections of standard salmeterol hydroxynaphthoate) reproducibilities for within-day and day-to-day variations were determined. The results are summarized in Tables 1 and 2.

The method described is rapid and precise and can be conveniently adapted for routine quality control analysis of MDIs and DPIs of salmeterol.

References

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[2] J. Palmer and B. Douglas, Eur. Pat. Appl. EP416,951, March 1991.