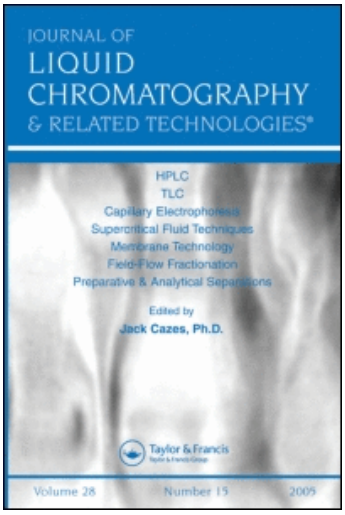

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SENSITIVE HIGH PRESSURE LIQUID CHROMATOGRAPHIC ASSAY METHOD FOR FORMOTEROL FUMARATE

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ABSTRACT

The HPLC method for determination of formoterol uses a 3-μm octyl bonded phase column, 4.6 X 50 mm, and a mobile phase of water-acetonitrile-trifluoroacetic acid in a ratio of 800:200:0.5 (v/v/v) at a flow rate of 2.0 ml/min. The limit of quantitation is 0.02 μg/mL, based on injection of 200-μL aliquots and the average between day coefficient of variation was 1.0%.

INTRODUCTION

In contrast to most measures of uniformity of drug dosage units, unit spray content of inhalation aerosols is based upon the mean of 4 to 12 sprays, depending on the drug (1). One reason for this is the lack of sufficiently sensitive analytical methods. For example, albuterol aerosols are typically labeled to deliver 100 μg per spray. Recent work has shown high variability

in the drug content of single sprays of albuterol. It has also shown that the drug content depends upon such factors as position of the canister prior to sampling and whether the canister has been primed or not (2). These results demonstrate the need for content uniformity test sampling based upon unit spray analysis.

The purpose of this note is to describe an assay method for formoterol fumarate, an anti-asthmatic, sufficiently sensitive for the assay of single 12 μg doses collected in the USP apparatus.

MATERIALS AND METHODS

Equipment

A Varian Star 9020 Series Workstation (Revision C software) was used with a model 9010 pump, a model 9095 autosampler equipped with the 1-ml syringe prep-option with a 200-μl loop (Valco Instruments Co. Inc.) and a model 9050 variable wavelength UV-VIS detector set at 214 nm. The detector was fitted with a 15-μl cell having a path length of 8 mm. Octyl bonded phase columns (Spherisorb, Chromatography Sciences Co., 50 x 4.6 mm, 3-μm, serial numbers 129143 and 039244) were used at ambient temperature.

Chemicals

Acetonitrile and methanol (J.T. Baker Co., Phillipsburg, NJ) were HPLC grade and trifluoroacetic acid (99+%, Aldrich Chemical Co., Milwaukee, WI) was spectrophotometric grade. Deionized water was prepared using a Sybron/Barnstead system. Formoterol fumarate, (±)-2'-hydroxy-5'-

[(RS)-1-hydroxy-2-[[(RS)-p-methoxy-α-methylphenethyl]-amino]ethyl] formanilide (I) and a related compound, (±)-2'-hydroxy-5'-[(RS)-1-hydroxy-2-[[(RS)-p-methoxy-α-methylphenethyl]-amino]ethyl] acetanilide (II) were obtained from Yamanouchi Pharmaceuticals, Tokyo, and Ciba-Geigy, Mississauga, Canada, respectively. The infrared (0.3% KBr), mass, and NMR spectra were concordant with the structure of the drug.

Mobile Phase

A solution of water-acetonitrile-trifluoroacetic acid in a ratio of 800:200:0.5 (v/v/v) was used at a flow rate of 2.0 ml/min.

Solutions

All solutions were prepared in methanol.
System suitability solution - 0.1 μg/ml each of formoterol fumarate and II.
Standard solution - 0.1 μg/ml formoterol fumarate.
Test solution - prepared to contain formoterol fumarate at a nominal concentration of 0.1 μg/ml.

System Suitability

A 200-μL aliquot of the resolution solution was injected. The system was deemed to be suitable for use if the efficiency of the column, calculated using the formoterol fumarate peak, was not less than 30 000 plates/meter, the

resolution between formoterol fumarate and II was greater than 1.2, and the coefficient of variation of five injections of the standard solution was less than 2%, all calculated according to USP procedures. The retention time of formoterol fumarate was about 5 min; the relative retention time of the II was about 1.1.

Procedure

Aliquots (200 μl) of the standard and test solutions were separately injected into the chromatograph and allowed to elute for 7 minutes. The concentration of formoterol fumarate in the test solution was calculated from $C_s(A_u/A_s)$, where A_u and A_s were the peak areas due to formoterol fumarate in the test and standard solutions, respectively, and C_s was the concentration, in μg/ml, of formoterol fumarate in the standard solution.

RESULTS AND DISCUSSION

Chromatography

A chromatogram showing the resolution between formoterol and II is presented in Figure 1.

Linearity and Sensitivity

The response of the HPLC system to the drug, determined on three different days, was linear over the range from 0.01 to 0.19 μg/ml, with the

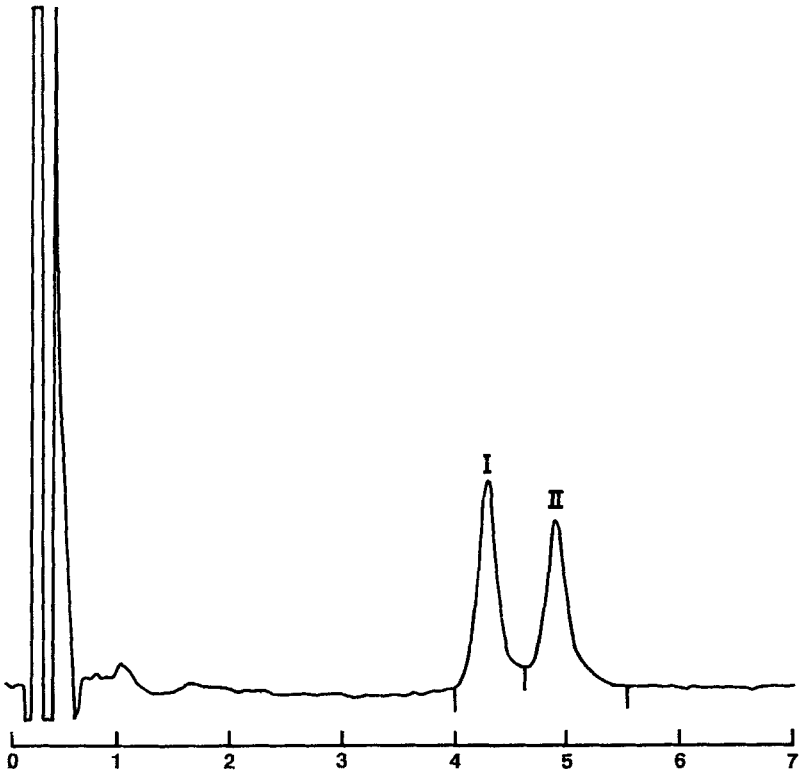

Figure 1. Chromatogram of formoterol fumarate (I) and II. The amounts on column were about 20 ng of each compound.

square of the correlation coefficient ranging from 0.9977 to 0.9996 with intercepts of zero. The limit of quantitation was 0.02 μg/ml, with a relative standard deviation of 8.3% at this concentration. The limit of detection was estimated to be 0.007 μg/ml.

Precision of the System

Six injections of a solution of 0.08136 μg/ml formoterol fumarate were made on four different days. The average within day and between day coefficient of variation was 1.0%.

Precision of the Method

Four portions of a drug raw material sample were assayed in sextuplet on four different days. The average within day precision was 2.3% and the between day precision was 5.6%.

Solution Stability

There was no detectable change in the chromatogram of a solution of formoterol fumarate in methanol after exposure to laboratory lighting and ambient temperature for 24 hours.

Ruggedness

Two columns used during the course of the work exhibited comparable resolution and stability characteristics. An increase in the percentage of water in the mobile phase led to an increase in resolution, but a decline in peak shape quality. Conversely, an increase in acetonitrile improved the peak shape but led to a decrease in resolution. An increase in the amount of trifluoroacetic acid reduced resolution and shortened retention times.

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