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Short communication

Determination of albuterol in plasma after aerosol inhalation by gas chromatography–mass spectrometry with selected-ion monitoring

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Abstract

Albuterol is a β_2-adrenergic agonist commonly used as a bronchodilator for the treatment of patients with asthma. We have developed an assay to determine plasma levels as low as 50 pg/ml of albuterol by gas chromatography–mass spectrometry (GC–MS). This assay utilizes isotopically labeled albuterol ([^{13}C]albuterol) as an internal standard. In this assay albuterol and the internal standard are recovered from 1 ml of plasma using solid-phase extraction. The samples are then derivatized to trimethylsilyl ethers using *N,O*-bis(trimethylsilyl)trifluoro-acetamide with 1% trimethylchlorosilane. The samples are then analyzed by GC–MS with selected-ion monitoring (SIM) for the ions *m/z* 369.15 and 370.15. The method has been validated for a concentration range of 50–10 000 pg/ml in plasma.

Keywords: Albuterol

1. Introduction

Albuterol (Fig. 1a) is commonly used by aerosol inhalation for the relief of bronchospasm in patients with asthma and other obstructive lung diseases. Blood levels of albuterol after inhalation are low and require a sensitive assay for determination. An assay to determine plasma concentration is necessary to study the pharmacokinetics and pharmacodynamics of the drug, which is absorbed systemically after delivery by inhalation [1,2].

Various assays have been developed to measure albuterol in plasma using techniques such as high-performance liquid chromatography (HPLC) with fluorescence [3–5] or electrochemical detection [6–8], mass spectrometry [9–13] and radioimmunoassay

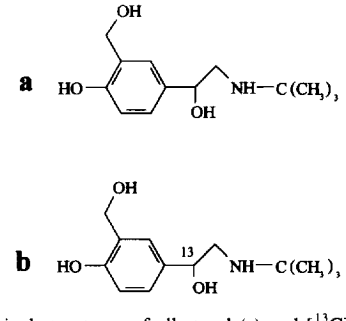

Fig. 1. Chemical structures of albuterol (a) and [^{13}C]albuterol (b).

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[14]. However, the currently available methods lack the necessary sensitivity to fully study the pharmacokinetics of albuterol after metered-dose inhalation. We describe here a sensitive GC–MS method suitable for the determination of albuterol in plasma at concentrations as low as 50 pg/ml, which is sufficient for detailed pharmacokinetic studies.

2. Experimental

2.1. Chemicals and materials

Crude [^{13}C]albuterol (Fig. 1b) was purchased from Icon (Summit, NJ, USA). Albuterol was obtained from Sigma (St. Louis, MO, USA). *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was supplied by Pierce (Rockford, IL, USA). HPLC grade methanol, acetonitrile, and water were purchased from Fisher (Pittsburgh, PA, USA); formic acid was also from Fisher. Zero grade helium was supplied by Standard Welders Supply (Memphis, TN, USA). 5% Dimethyl-dichlorosilane in toluene for glassware silanization was purchased from Supelco (Bellefonte, PA, USA). SPEC 15 mg C_{18} cartridges were purchased from Ansys (Irvine, CA, USA). Proventil® metered-dose inhalers were supplied by Schering (Kenilworth, NJ, USA). Aerochamber® spacers were obtained from Monaghan Medical (Plattsburgh, NY, USA). Vacutainer® tubes were purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

2.2. Purification of internal standard

Crude [^{13}C]albuterol was purified using a Waters HPLC system (Milford, MA, USA) including a 600E multisolvent delivery system, 490E multiwavelength detector, 746 data module and a 10 μm μBondapak C_{18} preparative column (300×19 mm I.D.). Mobile phase was a mixture of 0.01 *M* aqueous formic acid: methanol (9:1 in volume) at flow-rate of 14.4 ml/min. Ultraviolet absorbance detection was at 278 nm. The fraction for the peak at 19 min was collected. A white solid was obtained after evaporating the solvent. The isotopic purity of [^{13}C]albuterol determined using GC–MS in the SIM mode was 96.9%.

2.3. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed using an HP system (Hewlett-Packard, Palo Alto, CA, USA) consisting of an HP 5890 gas chromatograph connected to an HP 5988 quadrupole mass spectrometer. The GC was equipped with an HP7673 autosampler and a DB-5 column (30 m×0.25 mm I.D., 0.25 μm film thickness) from J&W (Folson, CA, USA). Helium was used as carrier gas with a head pressure of 15 p.s.i. (1 p.s.i.=6890 Pa) The injector and transfer line were maintained at 250°C. The column temperature was maintained at 120°C for 2 min followed by a gradient of 10°C/min to 230°C and then a gradient of 20°C/min to 285°C. Then the temperature was held for 15 min. The mass spectrometer conditions were as follows: electron impact, ion source temperature 210°C, ionization voltage 70 eV for scan mode and 60 eV for SIM mode. In the SIM mode the ions at *m/z* 369.15 and *m/z* 370.15 were detected.

2.4. Assay

Frozen plasma samples (1 ml) were warmed to room temperature and spiked with 1000 pg of [^{13}C]albuterol. After centrifugation (10 000 *g*, 20 min) the supernatant was applied to a SPEC 15 mg C_{18} cartridge, which was preconditioned using 0.5 ml methanol, 0.25 ml water and 0.25 ml 0.006 *M* phosphate buffer (pH 7.2). The cartridges were washed with 2×0.5 ml of water and the analytes were eluted using three 0.25 ml portions of methanol–acetonitrile (1:1). The eluate was collected in a 2 ml silanized amber vial and dried under nitrogen at 70°C. The residue was reconstituted in 150 μl methanol and transferred to an 100 μl silanized insert in an autosampler vial. The autosampler vials were placed in a manifold to be dried under nitrogen. The dried residue was derivatized with 30 μl BSTFA+1% TMCS at 80°C for 15 min. The derivatized sample (3 μl) was then injected into the GC column by splitless injection using the autosampler.

2.5. Calibration

An eight-point calibration curve was established using 1 ml samples of drug-free plasma spiked with methanolic albuterol to produce concentrations of 50, 300, 750, 1000, 3000, 5000, 7500 and 10 000 pg/ml. [^{13}C]albuterol (1000 pg) was added to each sample. The samples were extracted and analyzed as described above. Weighted linear regression (weighting function=$1/y^2$) was performed between the peak-area ratio of albuterol to [^{13}C]albuterol versus albuterol concentration.

2.6. Extraction recovery

Extraction recovery of albuterol was determined by adding 1000 pg of albuterol to 1 ml of plasma. The sample was extracted, derivatized, and analyzed as described above (Section 2.4) with the exception that 1000 pg of [^{13}C]albuterol were added before drying and derivatization. The extraction recovery was calculated as the ratio of albuterol concentration to [^{13}C]albuterol concentration.

2.7. Samples from dosed subject

In order to demonstrate the applicability of the present method to analyze biological materials from human subjects, the concentration of albuterol in plasma after aerosol inhalation by a human volunteer was analyzed. The subject was healthy, with normal laboratory profile, pulmonary function and electrocardiogram (ECG). All xanthine-containing foods and drinks were withheld 24 h before starting and throughout the study. The subject inhaled four puffs (90 μg of albuterol per puff) of a commercial Proventil metered-dose inhaler using an Aerochamber spacer. A 30 s interval separated each puff. The subject drank a 10 g slurry of activated charcoal in 240 ml of water 2 min before and after dosing and at 1 and 3 h post dosing. Venous blood samples were collected from a suitable forearm vein in Vacutainer tubes containing ethylene–diaminetetraacetic acid (EDTA). A blank sample was taken just prior to administration of albuterol followed by samples taken at 5, 10, 15, 30, 45 min, and 1, 2, 3, 4 and 6 h post administration. An indwelling heparinized venous catheter allowed venous access through

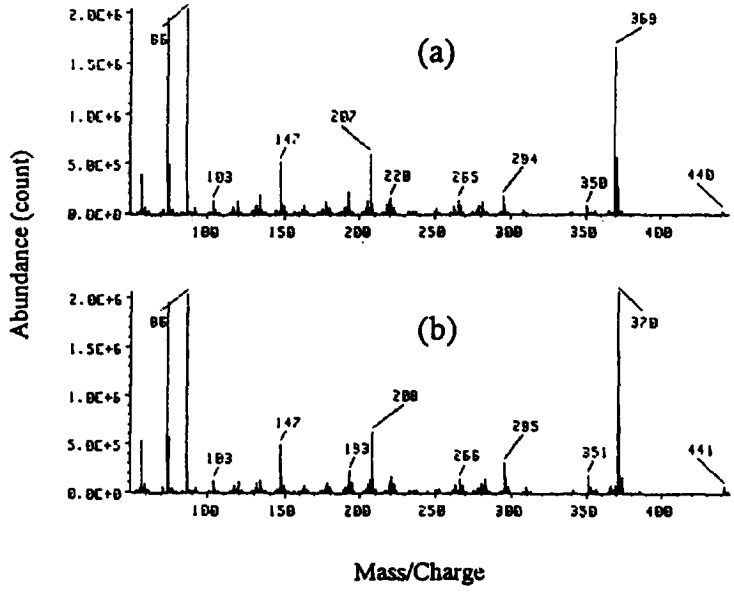

Fig. 2. Mass spectra of the TMS ethers of albuterol (a) and [^{13}C]albuterol (b), obtained using gas chromatography–mass spectrometry with electron-impact (70 eV) ionization.

the 6 h sampling. Plasma was immediately separated by centrifugation and stored at −72°C in two polypropylene tubes until analysis.

3. Results and discussion

3.1. Mass spectra

Mass spectra of trimethylsilyl (TMS) ethers of albuterol and [^{13}C]albuterol are shown in Fig. 2 and a proposed fragmentation pattern is presented in Fig. 3. The prominent peaks at m/z 369 in albuterol and m/z 370 in [^{13}C]albuterol were used for quantitation. However, isotopic correction was needed because the fragment $C_{17}H_{33}O_3Si_3$ (m/z 369) of albuterol has a 34.92% isotopic peak at m/z 370. The isotopic correction factor (0.3492) is the ratio between m/z

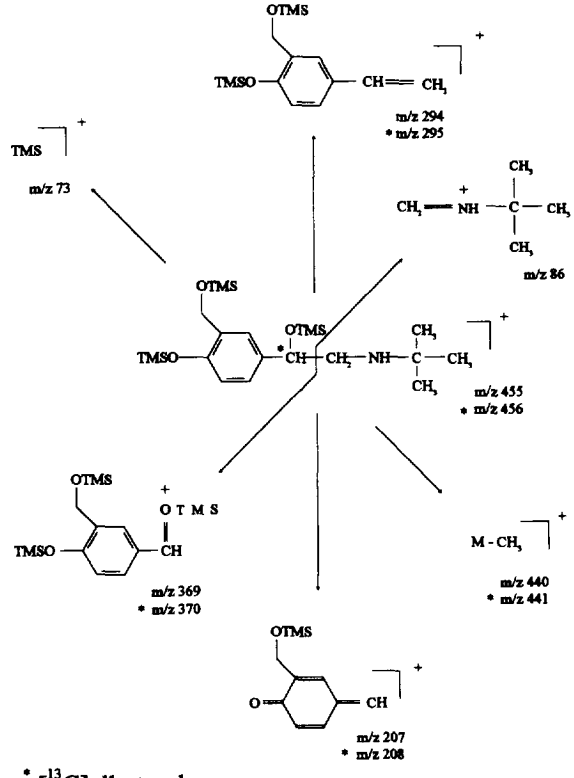

Fig. 3. Proposed mass spectra fragmentation pattern for TMS ethers of albuterol and [^{13}C]albuterol using electron-impact mass spectrometry.

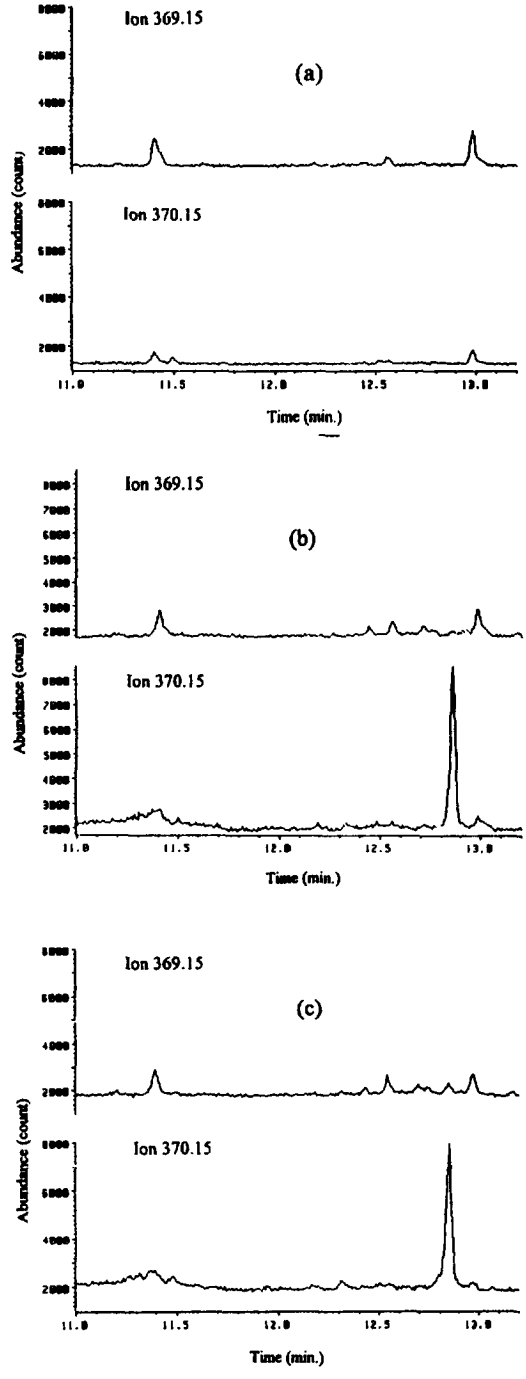

Fig. 4. Selected-ion monitoring (SIM) signals of (a) drug-free plasma, (b) plasma spiked with [^{13}C]albuterol (1000 pg/ml), and (c) plasma spiked with albuterol (50 pg/ml) and [^{13}C]albuterol (1000 pg/ml).

Table 1
Within-day precision and accuracy for the determination of albuterol in spiked plasma ($n=5$)

Spiked concentration (pg/ml)	Measured concentration (mean±S.D.) (pg/ml)	Coefficient of variation (%)	Relative error (%)
50	45.6±6.08	13.3	−8.8
5000	4825±138	2.9	−3.5
9500	9469±437	4.6	−0.3

370 and m/z 369 for the fragment $C_{17}H_{33}O_3Si_3$ of albuterol calculated using the relative abundance of natural isotopes [15].

3.2. Solid-phase extraction and recovery

The micro cartridges gave reasonable flow-rates without using vacuum. In some cases where the flow was too slow, low vacuum was applied to get a flow-rate between 0.5–1.5 ml/min. Flow-rates higher than 1.5 ml/min resulted in lower recovery, and should be avoided. The extraction recovery was 82.5±3.54% ($n=2$) for plasma samples spiked with 1000 pg of albuterol.

3.3. Selectivity and sensitivity

Representative ion chromatograms of drug-free plasma, plasma spiked with [^{13}C]albuterol (1000 pg/ml), and plasma spiked with albuterol (50 pg/ml) and [^{13}C]albuterol (1000 pg/ml) are shown in Fig. 4. The retention time for albuterol and the internal standard is 12.85 min. In this region, there is no significant interference from blank plasma. At 50 pg/ml, albuterol produced a peak that can be quantitated with satisfactory precision and accuracy.

3.4. Calibration and linearity

An eight-point calibration graph was obtained by plotting the peak-area ratio between albuterol m/z 369 and [^{13}C]albuterol m/z 370 ($R_{a/I}$) versus concentration of albuterol. The peak-area ratio was calculated using the following expression:

$$R_{a/I} = \frac{A_{369}}{A_{370} - 0.3492A_{369}} \quad (1)$$

where A_{369} is the peak-area for ion m/z 369 from albuterol, A_{370} is the peak-area for ion m/z 370 and $0.3492A_{369}$ is the isotopic contribution of $C_{17}H_{33}O_3Si_3$ from albuterol. Over the concentration range of 50–10 000 pg/ml, the linearity was satisfactory as shown by the equation: $y=0.000931x+0.0477$, where x is the concentration of albuterol and y the peak-area ratio of albuterol to internal standard. The standard deviations for the slope and the intercept are 0.000023 and 0.00574 respectively. The correlation coefficient (r^2) was 0.996.

3.5. Precision, accuracy and limit of quantitation

The within-day and day-to-day precision and accuracy were determined by analyzing replicate

Table 2
Day-to-day precision and accuracy for the determination of albuterol in spiked plasma ($n=5$)

Spiked concentration (pg/ml)	Measured concentration (mean±S.D.) (pg/ml)	Coefficient of variation (%)	Relative error (%)
50	52.4±8.33	15.9	4.8
5000	4937±567	11.5	−1.3
9500	9598±420	4.4	1.0

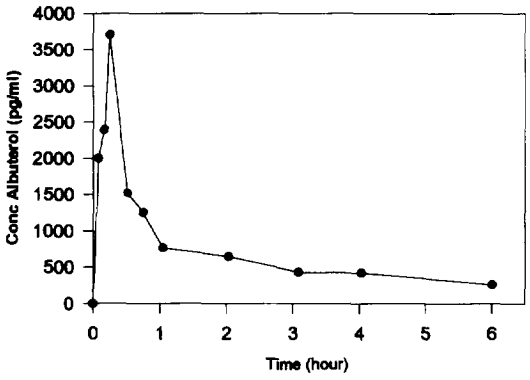

Fig. 5. Concentrations of albuterol in plasma from one subject receiving 360 μg of albuterol via metered-dose inhalation.

plasma samples ($n=5$) spiked with albuterol at 50, 5000 and 9500 pg/ml and the internal standard at 1000 pg/ml. As shown in Table 1, the within-day coefficient of variation (C.V.%) was lower than 14% for all three concentrations. The day-to-day coefficient of variation was 15.9% for 50 pg/ml (Table 2), indicating the assay was nearing its limit of quantitation. Bias was less than 9% for the concentrations tested.

3.6. Assay application

Plasma samples from a subject administered 360 μg of albuterol by metered-dose inhaler were analyzed. The pharmacokinetic profile over a 6 h period is shown in Fig. 5. The concentration of albuterol in plasma reached a peak of 3711 pg/ml at 15 min.

4. Conclusion

An assay to measure 50–10 000 pg albuterol in 1 ml plasma has been developed. This assay uses solid-phase extraction and a capillary gas chromatography–mass spectrometry procedure that yields high recovery and removes essentially all plasma interferences. The results of the analysis of a dosed subject indicate that this method is suitable to study the pharmacokinetics of albuterol administered by metered-dose inhalers.

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