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A simple method for the study of salbutamol pharmacokinetics by ion chromatography with direct conductivity detection

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Abstract

A simple method for the study of the pharmacokinetics of salbutamol using ion chromatography with direct conductivity detection without chemical suppression is presented in this paper. Baseline separation of salbutamol from plasma components was achieved by using diluted HNO_3 (2 mmol l^{-1}) as mobile phase with acetonitrile (6%, v/v) as modifier. Atenolol was utilized as internal standard. Under the optimal conditions, the linear regression coefficients of the calibration curves are 0.996 within the concentration range of 1000–3 ng ml^{-1} salbutamol. The detection limit (signal-to-noise ratio = 3) was 1 ng ml^{-1} salbutamol. The noncompartmental pharmacokinetic parameters for salbutamol following administration of 8 mg salbutamol to 18 subjects were tested. The results coincided most satisfactorily with the results obtained by using reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection.

Keywords: Salbutamol; Ion chromatography; Pharmacokinetics

1. Introduction

Salbutamol {2-(*tert*-butylamino)-1-(4-(hydroxy-3-hydroxymethylphenyl)ethanol} is a drug with β_2-adrenoceptor activities. This compound possesses a phenylethanolamine nucleus, with a common β-hydroxylamino group on the side chain (Fig. 1). The drug was originally developed for the treatment of chronic obstructive pulmonary diseases. Recently, it was also studied as a banned substance in sport testing and as a growth promoter in animals. Some studies have demonstrated clear relationships between plasma concentrations of the drug after subcutaneous or oral administration and bronchodilator effects. However, there is quite a large variation in pharmacokinetics response for a given plasma concentration among different patients [1], so individual patients require different amounts of salbutamol for producing a therapeutic response [2]. A simple method for the determination of salbutamol in plasma is therefore required for clinical treatment of individual patients.

Determination of salbutamol in biological samples has been well documented [3–15]. Due to its low concentration in plasma, salbutamol in samples from pharmacokinetic studies was preferably analyzed by GC–MS and LC–MS [3–9]. However, many investigators do not possess these advanced analytical capabilities. Thus, high performance liquid chromatography (HPLC) was currently developed to determine salbutamol in biological samples [10–12] and to study its pharmacokinetics in plasma [12,13]. Nevertheless, this compound does not sufficiently absorb UV light, hence when in low concentration—as it usually exists in plasma—an HPLC method coupled to a UV detector is insufficient to detect salbutamol in the biological samples. Nowadays, many authors carry out their analytical studies by using HPLC coupled to a fluorescent detector employing excitation/emission wavelengths at around 280/310 nm [10–12]. A lowest detection limit of 1.0 ng ml^{-1} for a 1 ml sample could be achieved applying fluorescent detection for the determination of salbutamol levels in plasma [10]. In literature, electrochemical detection (ED) and enzyme immunoassay is also being described for salbutamol studies in order to improve the sensitivity of detection.

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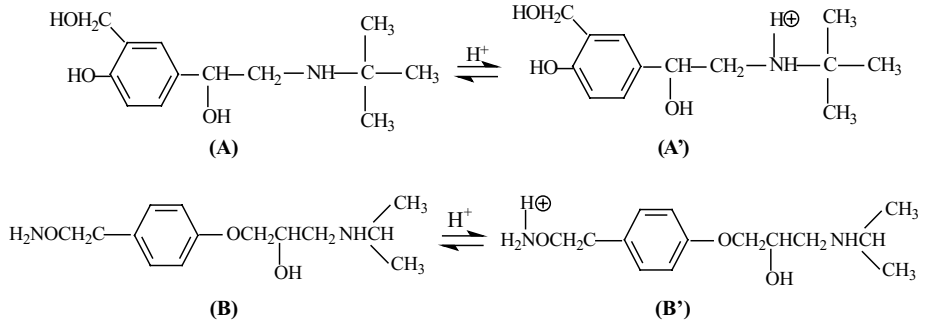

Fig. 1. Chemical structure of salbutamol and atenolol: (A) salbutamol; (A′) cationic salbutamol; (B) atenolol; (B′) cationic atenolol.

The methods have been applied to biological samples [14,15].

Sample pretreatment has also been an item of interest for the analysis of salbutamol in biological samples, because generally the sample matrix strongly interferes with the separation and detection processes. Based on the chemical structure of salbutamol, one can easily predict that a non-polar solvent such as benzene (though not to be applied because of health risks) could extract salbutamol effectively, but most compounds with non or weak polar structures would also be co-extracted into this solvent. These co-extracted compounds interfere with the HPLC separation and subsequent fluorescent detection. Therefore, solid-phase extraction utilizing an immunoaffinity column was introduced to clean-up the samples before injection into the HPLC column [14,16,17]. As the commercially available column for solid-phase extraction is relatively expensive, especially for the study of pharmacokinetics where many samples are to be collected and treated, utilizing weak or strong cation-exchange columns [7,14], the octadecyl (C_{18}), octyl (C_8), and cyano (CN) columns for solid-phase extraction were studied as well by several authors, although a relatively complicated procedure for elution of salbutamol from the column is required [3,4,8,9,11,12,14].

Our preliminary experiments demonstrated that salbutamol could be ionized in aqueous solution at relatively low pH values as shown in Fig. 1. It was therefore extracted by a diluted HNO_3 aqueous solution. This extract was then injected into a cationic exchange column for separation. As the ionized salbutamol molecule shows strong conductive properties, it could be on-line detected by a conductivity detector available in the commercial ion-chromatographic instrument. To avoid interferences, salbutamol could be first extracted from plasma by benzene (comments: see Section 2.4) and then reversely extracted by using the diluted HNO_3 solution. The latter solution was then utilized as mobile phase for the ion chromatographic separation. With this procedure, a simple method for the determination of salbutamol utilizing ion chromatography with conductivity detection could presently be established.

2. Experimental

2.1. Instrumentation

A Metrohm ion chromatography apparatus (Metrohm, Switzerland) was used for the present study, which consists of a 733 IC separation center, a 709 IC pump and a 732 IC detector. A Metrohm cation 1–2 separation column (4.0 mm × 125 mm) with a cationic pre-column was used throughout for the separation. All instrument control and data collection was performed by Metrohm chromatography software IC Metrodata for Windows.

HPLC-fluorescence detection was carried out by using an HPLC system consisting of a modular unit from Agilent Technologies (USA), model HP 1100 series liquid chromatograph with autoinjector, a solvent-delivery system and column oven. The separation was done on a C18 analytical column (250 mm × 4.6 mm). A model HP 1100 fluorescence detector (Agilent Technologies, USA) was operated at 273 nm for excitation and at 310 nm for emission wavelengths.

2.2. Reagents and materials

All reagents were of analytical grade, dilutions being performed with deionised water (Millipore MilliQ Water System, USA). Benzene, methanol, and acetonitrile (Beijing Chemical Company, Beijing, China) were of analytical HPLC grade. The mobile phase was 2.0 mmol l^{-1} nitric acid (Beijing Yili Fine Chemical Company) containing 6% (v/v) of acetonitrile. For HPLC-fluorescence detection, a mobile phase mixture of 0.01 mol l^{-1} ammonium acetate (pH 3.0) containing 6% (v/v) methanol was delivered at a flow-rate of 0.8 ml min^{-1}.

The pure standards of salbutamol, terbutaline, and atenolol (internal standard) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing China). Salbutamol sulfate controlled-release tablets (Volmax®) were obtained from Glaxo (Wellcome, UK).

2.3. Subject selection

A total of 18 healthy male subjects 18–25 years of ages and weighing 64.2 ± 4.0 kg were selected as volunteers, in accordance with the physical and clinical laboratory examinations. Subjects with a history of hyperthyroidism, diabetes, hypertension, cardiac disease or seizure disorders were excluded. Evidence of any clinically significant abnormalities that might interfere with metabolism or excretion of salbutamol was regarded an exclusion criteria. Subjects avoided alcoholic drinks and smoking during the study period.

The oral salbutamol dose (8 mg) was administered with 250 ml water at morning time. The breakfast was taken 2 h after administration. Blood samples were collected through an intravenous catheter 1 h before dose application and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, and 72 h after the dose. Next the plasma was separated from blood and the plasma samples were immediately stored below −30 °C for further treatments.

2.4. Sample clean-up procedure

The extraction procedure was as following: 400 μl plasma was added to a 2-ml polypropylene extraction tube (Ding-Guo Company, Beijing, China). Atenolol (100 μl of 80 μg ml^{-1} solution) was added to each tube as the internal standard. Benzene (300 μl) was then added for extraction. This procedure was repeated for three times. The merging extracts were evaporated with nitrogen stream in a water bath at 40 °C. The residue was then redissolved by 2.0 mmol l^{-1} of nitric acid containing 6% (v/v) acetonitrile for further chromatographic separation. Toluene was also tested as it possesses less toxicity than benzene. However, the extraction efficiency appears 10% lower than when using benzene for salbutamol extraction. Hence, as a general comment, it should be stated that similar analysis as presented in the presented work *can be carried out employing the less toxic toluene solvent*, taking into account the cited extraction efficiency decrease and consequent influence upon method validational parameters.

The extraction procedure for analysis by HPLC-fluorescence detection was as following. Aliquots (1 ml) of samples containing 20 μl terbutaline (1 μg ml^{-1}) as the internal standard were applied to C18 (Supelco, USA) cartridges (1.0 ml), pre-conditioned with 4 ml of methanol–water (50:50). The washing sequence involved 1 ml of water and 0.2 ml of acetonitrile. The column was dried under vacuum for 5 min and elution of the analytes was performed using twice 1 ml of methanol. This extract was dried under a nitrogen stream in a water bath at 40 °C. The dry residue was dissolved in 100 μl of ammonium acetate (0.01 mol l^{-1}) containing 6% (v/v) methanol, then injected (50 μl) into the HPLC column.

3. Results and discussion

3.1. Optimization of the ion chromatographic separation

Fig. 1 shows that salbutamol can be protonated in acidic medium. This provides a possibility to retard this pharmaceutical compound onto the ion-exchange column. Due to its cationic form in acidic medium, a cation-exchange column was utilized for the separation of salbutamol from plasma samples. Atenolol was selected as internal standard because this compound has been described in literature as internal standard for salbutamol separations [18]. The mobile phase used in the present study is a diluted HNO_3 solution, containing acetonitrile as modifier to obtain sharp and symmetrical peaks. As shown in Fig. 2, the baseline separation could be achieved at HNO_3 concentrations as low as 2.0 mmol l^{-1}.

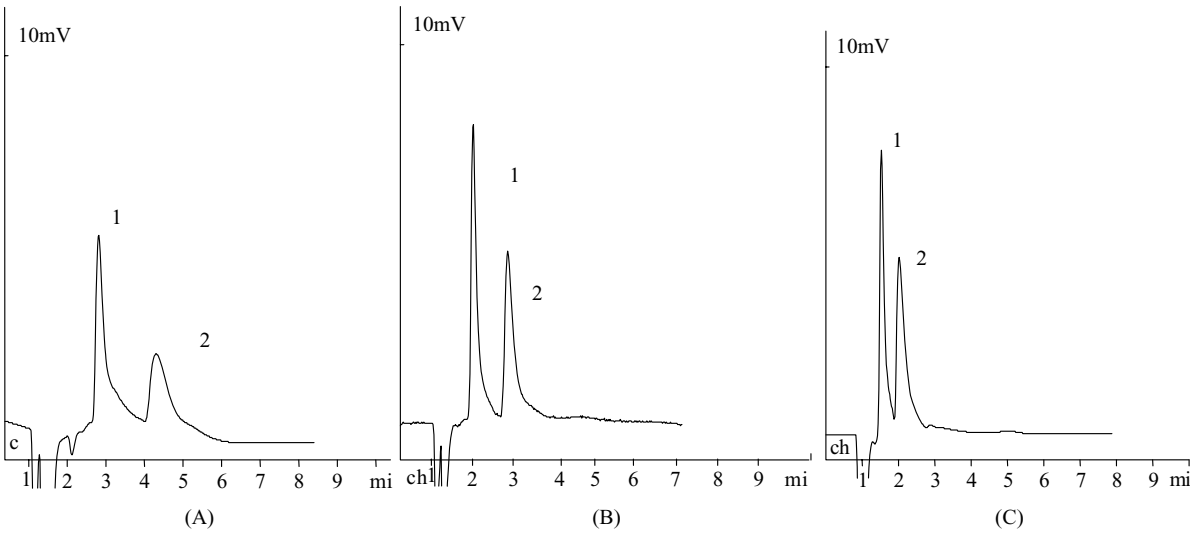

Fig. 2. Effect of nitric acid concentrations on retention times of salbutamol and atenolol: (1) salbutamol; (2) atenolol. Chromatographic conditions: IC column, Metrohm cation 1–2 separation column (4.0 mm × 125 mm); flow-rate, 1.0 ml min^{-1}; sample injection volume, 20 μl. Mobile phase: 6% (v/v) acetonitrile with: (A) 0.5 mmol l^{-1} of HNO_3; (B) 2.0 mmol l^{-1} of HNO_3; (C) 3.0 mmol l^{-1} of HNO_3.

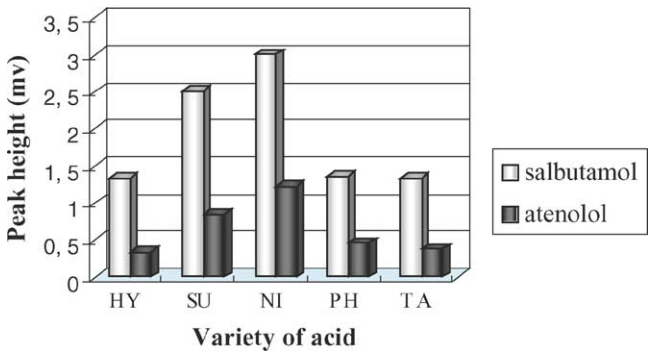

Fig. 3. Effects of acid nature on sensitivity of salbutamol and atenolol determinations. HY, hydrochloric acid (2.0 mmol l^{-1}); SU, sulfuric acid (1 mmol l^{-1}); NI, nitric acid (2.0 mmol l^{-1}); PH, phosphoric acid (2.0 mmol l^{-1}); TA, tartaric acid (3.0 mmol l^{-1}); chromatographic conditions: IC column, Metrohm cation 1–2 separation column (4.0 mm × 125 mm); flow-rate, 1.0 ml min^{-1}; sample injection volume 20 μl. Mobile phase: the acids containing 6% (v/v) of acetonitrile.

Lowering the HNO_3 concentration caused broadening of salbutamol and atenolol peaks. A concentration higher than 2.0 mmol l^{-1}, however, caused both peaks to overlap. Therefore, 2.0 mmol l^{-1} of HNO_3 was applied to the subsequent study. Fig. 2(A–C) shows the chromatograms of salbutamol and atenolol at three different concentrations of HNO_3, mentioned above.

Other acids such as HCl, H_2SO_4, H_3PO_4, and tartaric acid within the same range of concentrations were also examined to investigate whether there was any acid that achieved a better separation. The results are shown in Fig. 3. No significant improvement of resolution was achieved by substituting these acids for HNO_3. Moreover, the peak heights of both salbutamol and internal standard decreased. Considering the detection sensitivity of the salbutamol determination in plasma, nitric acid was finally selected in the mobile phase for further study.

In the preliminary experiments, a peak broadening was observed for the salbutamol elution. This peak broadening could be caused by the interaction between the phenyl-group of salbutamol with the framework of the stationary phase. Therefore acetonitrile was added to modify the polarity of the mobile phase. It was found that peak broadening was greatly decreased by adding 6% (v/v) of acetonitrile to the mobile phase, leading to sharp and symmetrical peaks for salbutamol and internal standard. Therefore, 6% (v/v) of acetonitrile was added throughout to the mobile phase for the separation. The effect of acetonitrile upon the retention time is illustrated in Fig. 4.

The flow-rate of the mobile phase and the sample injection volume were also examined. A flow-rate of 1.0 ml min^{-1} was finally selected for the separation as this rate is currently used for ion-chromatographic separations. Decreasing the flow-rate could improve the separation of salbutamol and internal standard in the present experiment, however leading to longer run times. The sample volume injected into the column was also examined and results showed that the increase in sample volume improved the peak heights of both compounds, but decreased the resolution of salbutamol from the internal standard. To compromise sensitivity and selectivity items, a loop size of 20 μl was used throughout.

3.2. Linear ranges and detection limit

Under the optimal conditions described above, the linear regression coefficients of the calibration curves are 0.996 within the concentration range of 1000–3 ng ml^{-1} of salbutamol. The detection limit (signal-to-noise ratio = 3) was 1 ng ml^{-1} salbutamol. The relative standard de-

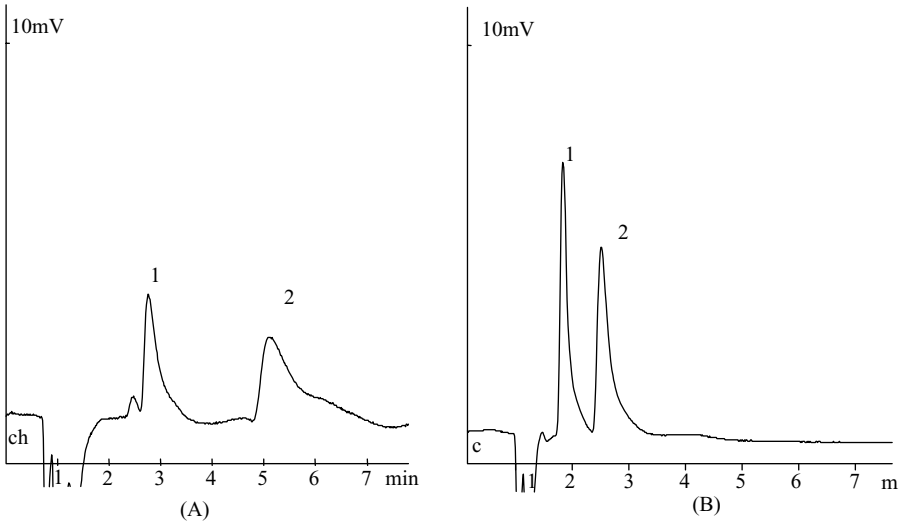

Fig. 4. Effect of acetonitrile concentration on the separation of salbutamol and atenolol; (1) salbutamol; (2) atenolol. Chromatographic conditions: IC column, Metrohm cation 1–2 separation column (4.0 mm × 125 mm); flow-rate, 1.0 ml min^{-1}; sample injection volume 20 μl. Mobile phase: (A) 2.0 mmol l^{-1} of HNO_3; (B) 2.0 mmol l^{-1} of HNO_3 containing 6% (v/v) acetonitrile.

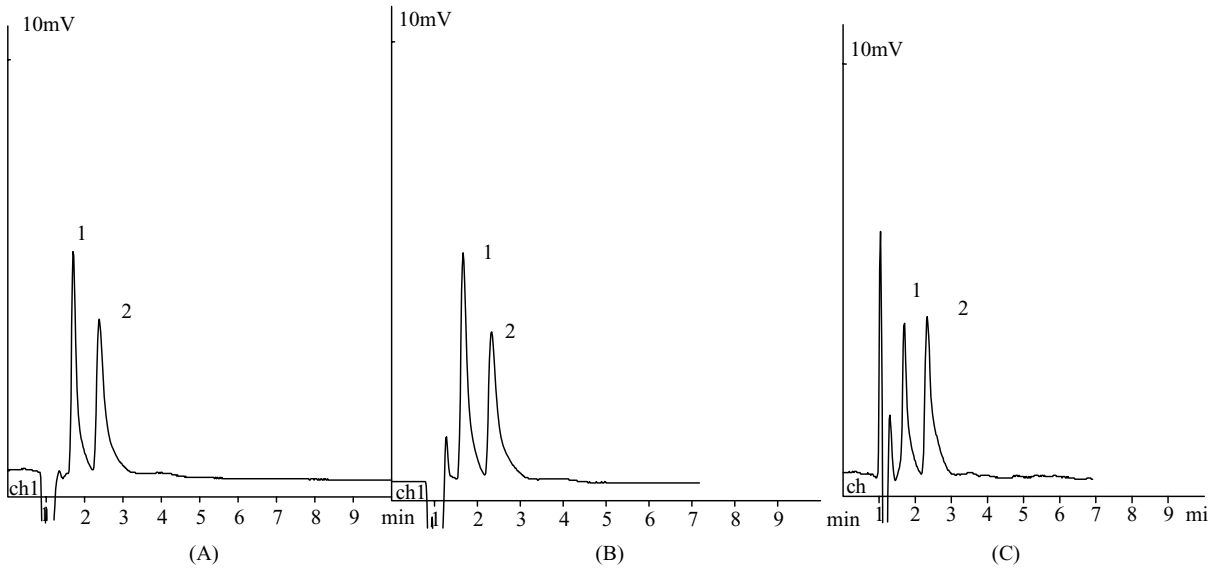

Fig. 5. Typical chromatograms of salbutamol in standard form and in spiked plasma samples. (1) salbutamol; (2) atenolol. Chromatographic conditions: IC column, Metrohm cation 1–2 separation column (4.0 mm × 125 mm); flow-rate, 1.0 ml min^{-1}; sample injection volume 20 μl. Mobile phase: 2.0 mmol l^{-1} of HNO_3 containing 6% (v/v) acetonitrile. (A) Chromatograms of salbutamol (10.0 ng ml^{-1}) and atenolol aqueous standard (50.0 ng ml^{-1}); (B) chromatograms of human plasma extract spiked with salbutamol (10.0 ng ml^{-1}) and atenolol (50.0 ng ml^{-1}); (C) chromatograms of human plasma 4 h after oral salbutamol dosing.

viation (R.S.D., $n = 10$) at three concentrations: 500, 50, and 5 ng ml^{-1} salbutamol were of 3.6, 4.2, and 8.1, respectively. The analytical procedure showed good precision for the determination of the standard solution at different concentrations.

3.3. Sample extraction

As described above, salbutamol is a relatively weak polar compound at neutral conditions. It can be easily extracted from plasma by benzene (see toluene comments above). This extraction procedure could effectively eliminate inorganic and organic ions from plasma, which would interfere with the separation of salbutamol from plasma matrix components by utilizing ion chromatography. However, the interfering species with non or weak polar properties would be co-extracted by benzene. A reverse extraction procedure by using diluted HNO_3 was carried out, since salbutamol could be protonated at low pH values in aqueous solution. It was effectively extracted into the HNO_3 solution. This extract could then be injected into the cationic exchange column for separation and detection. The extraction efficiency was around 85–93% in the present experiment for the tested plasma samples. In comparison to the procedures mentioned in the literature, this procedure is simple, fast, and relatively cheap.

3.4. Recovery test in plasma samples

In order to validate the method, a recovery test was carried out on samples to which known amounts of salbutamol were added. Fig. 5 shows the typical chromatograms of salbutamol in standard solutions and in spiked plasma samples. After a cleaning-up procedure, we observed that most interfering species were eliminated from the sample matrix. The intra-day and inter-day precision and accuracy values were determined by analyzing replicates of plasma samples ($n = 5$) spiked with salbutamol at 10.0 and 50.0 ng ml^{-1}, the internal standard at 80 ng ml^{-1}. As shown in Table 1, a good recovery was achieved for salbutamol in plasma. By testing the recovery at two salbutamol concentrations, recoveries were found of 92.2 and 95.3 for 10 and 50 ng ml^{-1} salbutamol, respectively, spiked in plasma samples.

3.5. Pharmacokinetics study

The pharmacokinetic profiles of salbutamol from the subjects obtained after administration of salbutamol for 72 h are

Table 1
Intra- and inter-day variation, accuracy and extraction recoveries of salbutamol in plasma

Concentration (ng ml^{-1})	Intra-day ($n = 5$)		Inter-day ($n = 5$)		Recovery (%)
	Found (ng ml^{-1})	R.S.D. (%)	Found (ng ml^{-1})	R.S.D. (%)	
10.0	10.3 ± 0.2	3.3	10.2 ± 0.5	5.0	92.2
50.0	50.2 ± 0.4	5.1	50.2 ± 0.3	4.4	95.3

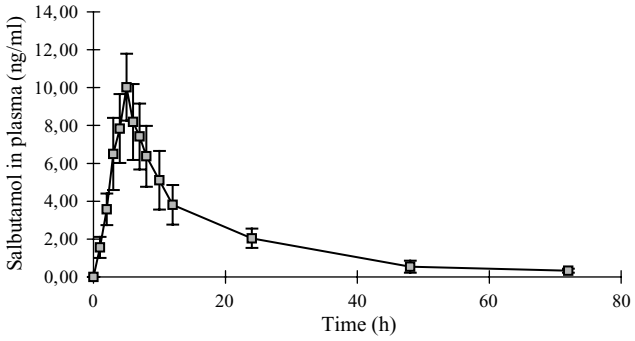

Fig. 6. Pharmacokinetic profiles of salbutamol from the subjects obtained after administration of salbutamol (mean values from 18 subjects). (Chromatographic conditions as listed in Fig. 5.)

shown in Fig. 6. All temporal data shown are relative to the time of the single oral dose. The relevant pharmacokinetic parameters from noncompartmental analysis of the data are listed in Table 2.

When these results were compared with those obtained by another Chinese group employing HPLC with fluorescence detection [19], we found that both results are well consistent after administration of salbutamol from the same pharmaceutical preparation (Volmax®), indicating that salbutamol pharmacokinetics are exactly coinciding for the same ethnic group. The T_{max}, C_{max} and AUC_{0-t} are 5.3 ± 1.0 h, 10.5 ± 2.6 ng ml^{-1}, and 116 ± 36 ng h ml^{-1}, respectively, in the cited reference. Also, we noted that the data obtained in the cited reference were obtained by reversed-phase HPLC with fluorescence detection. From these results we concluded that there were no significant differences between the analytical capacities of reversed-phase HPLC with fluorescence detection and ion chromatography applying conductivity detection for the study of salbutamol pharmacokinetics.

To confirm this conclusion, we also performed the pharmacokinetics study by using the same dose of salbutamol with reversed-phase HPLC-fluorescence detection. Table 2 lists the noncompartmental pharmacokinetic parameters for salbutamol following administration of 8 mg salbutamol to 18 subjects tested by using reversed-phase HPLC with fluorescence detection. In comparison to the results obtained using ion-chromatography with conductivity detection from the present paper, we could easily observe that no significant differences occur between both methods, indicating that the present method is reliable for the study of salbutamol pharmacokinetics.

Table 2
Noncompartmental pharmacokinetic parameters for salbutamol following administration of 8 mg salbutamol to 18 subjects by using ion-chromatography with conductivity detection and reversed-phase HPLC with fluorescence detection

Parameter	Ion-chromatography (mean ± S.D.)	Reversed-phase HPLC (mean ± S.D.)
T_{max} (h)	5.1 ± 0.6	4.7±1.6
C_{max} (ng ml^{-1})	10.3 ± 1.7	10.2±2.9
$T_{1/2ke}$ (h)	12.4 ± 4.7	15.6±9.3
AUC_{0-t} (ng h ml^{-1})	135.1 ± 23.0	127.5±30.1
$AUC_{0-\infty}$ (ng h ml^{-1})	143.9 ± 24.3	152.3±40.9
MRT_{0-t} (h)	14.4 ± 2.8	14.5±4.8
$MRT_{0-\infty}$ (h)	17.9 ± 4.8	22.7±12.0

4. Conclusion

The presently described method is an ion-chromatographic method applying conductivity detection for the study of salbutamol pharmacokinetics. The method is simple not only in terms of chromatographic separation but also regarding the sample clean-up procedures. The results obtained offer sufficient sensitivity and precision for salbutamol determinations in plasma. The present work demonstrates the potential of ion-chromatography for the study of pharmacokinetics of β_2-adrenoceptors agonists.

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