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DETERMINATION OF SALBUTAMOL IN HUMAN PLASMA WITH BIMODAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND A ROTATED DISC AMPEROMETRIC DETECTOR

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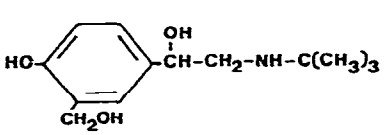
SUMMARY

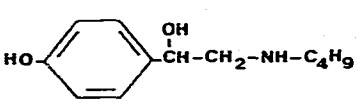
The sensitivity of electrochemical detection was combined with the selectivity of a bimodal high-performance liquid chromatographic system for the successful determination of salbutamol in human plasma. Following initial sample clean-up using Sep-Pak® cartridges, analytes were passed first through a cation-exchange column, and then, after column switching, through a reversed-phase column. An amperometric detector with a rotated disc working electrode was used for detection. The detection limit was 0.5 ng/ml when 1.0 ml of plasma was used. The coefficient of variation was 9.8% at an average concentration of 4.7 ng/ml. The method was adequate for pharmacokinetic studies and for clinical applications.

INTRODUCTION

Salbutamol, 2-*tert.*-butylamino-1-(4-hydroxy-3-hydroxy-methylfenyl)-ethanol (Fig. 1) is a sympathicomimetic drug with a selective effect on β-2-adrenoceptors. Its pharmacological action is well documented [1], and as a bronchodilator the drug is widely used in the treatment of respiratory diseases.



salbutamol



bamethan

Fig. 1. Chemical structure of salbutamol and of bamethan.

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However, pharmacokinetic information on salbutamol is restricted, apparently due to the rather limited analytical techniques currently available for its determination [2, 3]. Nevertheless, the pharmacokinetics of this drug could be of clinical relevance in various disease states and in connection with problems concerning its first-pass metabolism [2]. The high-performance liquid chromatographic (HPLC) method presented in this paper may facilitate investigations in this field.

MATERIALS AND METHODS

Chemicals

All aqueous solutions were prepared with double-distilled water.

Salbutamol sulphate and bamethan sulphate were kindly supplied by Glaxo (Hoofddorp, The Netherlands) and Boehringer Ingelheim (Alkmaar, The Netherlands), respectively. All other chemicals were of analytical reagent grade and obtained from Merck (Darmstadt, G.F.R.). They were used as received.

Aqueous solutions of standards were prepared containing 0.5 μg/ml salbutamol or 1.0 μg/ml bamethan. For purposes of preservation these solutions were slightly acidified with formic acid (± 0.01%, v/v).

Sep-Pak® cartridges were from Waters (Etten-Leur, The Netherlands). Spektralkohle Ringsdorff RW-A (Ringsdorff-Werke, Bonn–Bad Godesberg, G.F.R.) was used for the preparation of carbon paste.

Sep-Pak manipulation

Sep-Pak cartridges were connected to 5-ml disposable PVC syringes, from which the plunger had been removed, to serve as an eluent reservoir. The outlets of the cartridges were fixed to 10-ml reagent tubes as recipients for the eluate, leaving a vent for the air to be displaced (Fig. 2). In this form twelve cartridges could be handled in a Sorvall centrifuge in one run.

Chromatographic system

A schematic representation of the chromatographic system is given in Fig. 3. Eluents were delivered by two Kipp 9208 HPLC pumps (Kipp Analytica, Emmen, The Netherlands). Valve 1 was a Rheodyne 7120 injection valve, equipped with a 1.0-ml loop. Valve 2 was a Rheodyne 7000 six-port rotatory valve. Column 1 (50 × 4.6 mm) was home-packed with Partisil SCX (10 μm). Packing was performed by suction of a Partisil SCX suspension in water into the empty column at a vacuum bottle. Column 2 (250 × 4.6 mm) contained LiChrosorb RP-2 (10 μm) and was obtained as a prepacked column from Brownlee Labs. (Santa Clara, CA, U.S.A.). The composition of the eluent solutions was as follows. Eluent 1: phosphate buffer (pH 7.5) containing 2.58 g of $Na_2HPO_4 \cdot 2H_2O$ and 0.3 g of KH_2PO_4 per liter. Eluent 2: sodium perchlorate (40 g/l) and 2-propanol (45 ml/l) in a phosphate buffer (pH 7) containing 14.0 g of $Na_2HPO_4 \cdot 2H_2O$ and 7.5 g of KH_2PO_4 per liter. The flow-rate of pump 2 was 2.0 ml/min.

Electrochemical detection

The electrochemical detector used in this method has been described earlier

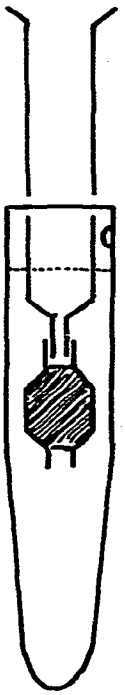

Fig. 2. Construction for the handling of Sep-Pak columns in a centrifuge.

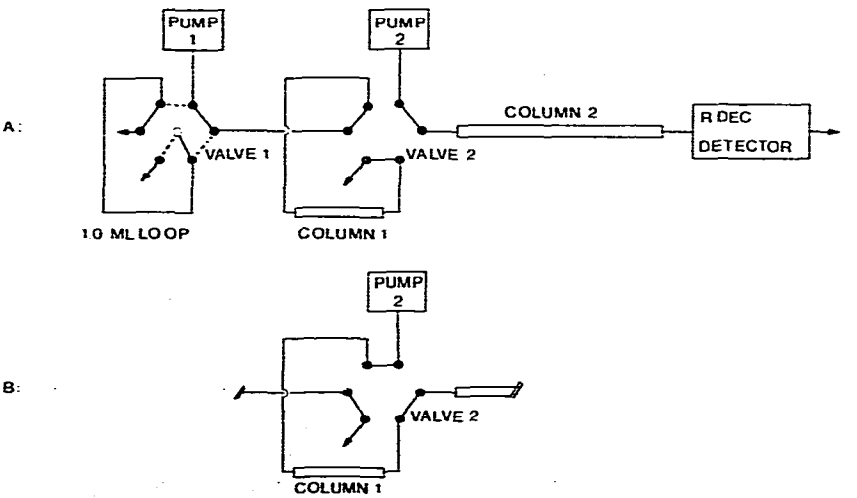

Fig. 3. Schematic representation of the bimodal HPLC system.

[4]. It differs from more common types of electrochemical detectors in that the working electrode is a rotated disc electrode. Carbon paste was used as the electrode material. The paste was prepared by mixing 15.0 g of graphite powder with 9.0 g of nujol [5]. A saturated calomel electrode (SCE; K 401, Radiometer, Copenhagen, Denmark) was used as the reference electrode and a platinum wire as the auxiliary electrode.

The three-electrode potentiostat was home-made and comparable in performance with commercially available instruments. The potential of the working electrode was maintained at +950 mV vs. SCE.

Each time the system was started up, the electrode was initially conditioned at a potential of +1600 mV vs. SCE for 15 min with eluent 2 flowing.

Sample preparation

In small glass tubes plasma samples (1.0 ml) were spiked with 20 μl of the internal standard solution.

Before use Sep-Pak columns were washed with 5.0 ml of methanol followed by 5.0 ml of water. The spiked samples were transferred with pasteur capillary pipettes and passed through the prepared columns. Sample tubes and pasteur pipettes were rinsed with 1.0 ml of water, which was then passed through the columns. The columns were then centrifuged at 1500 *g* for 10 min. The aqueous eluates were discarded and the columns transferred to clean glass tubes, to be eluted with 5.0 ml of a mixture of methanol—diethyl ether (25:75). The eluates were evaporated under nitrogen at about 40° C.

Calibration curves for salbutamol in the range 2.5—20 ng/ml were obtained by adding 5—40 μl of the standard solution ot 1.0 ml of blank human plasma samples and utilizing the above procedure.

Bimodal HPLC procedure

The residue from the Sep-Pak procedure was redissolved in 500 μl of eluent 1. Of this solution 250 μl were injected via valve 1 while valve 2 was in position A and the flow-rate of pump 1 was set at 3.0 ml/min. After about 5 min pump 1 was stopped. Valve 2 was switched to position B when the previous chromatogram was finished. When the solvent front appeared, valve 2 was switched back to position A and pump 1 was started again. A further sample could then be injected.

RESULTS

Representative chromatograms of plasma samples obtained with the method are shown in Fig. 4. When the elution time for column 1 was chosen correctly no interfering peaks were observed. Blank human plasma from several pools was tested in this respect.

The constancy of the detector response towards a test mixture containing salbutamol and bamethan was examined during a four-day period and a coefficient of variation of 6.9% was found (n = 8). The average of seven calibration plots made during a nine-day period was described by the equation $y = 0.0769x + 0.0213$. The standard deviations of the slope and intercept were 0.0063 and 0.00022, respectively. The coefficient of correlation ranged from 0.9987 to 0.9999 with a mean of 0.9994.

The standard deviation of the method was calculated from duplicate measurements using the formula S.D. = $\sqrt{d^2/2n}$, in which d is the difference between the duplicates and n is the number of duplicates. Thus the coefficient of variation was found to be 9.8% (n = 13) at an average concentration of 4.7 ng/ml.

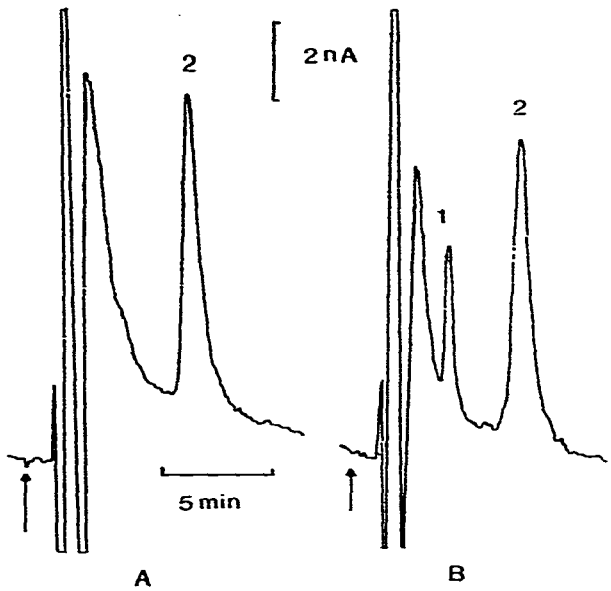

Fig. 4. Chromatograms of a blank plasma sample (A) and a plasma sample containing 5.8 ng/ml salbutamol (1 = salbutamol, 2 = internal standard).

The signal-to-noise ratio indicates a detection limit of about 0.5 ng/ml plasma.

The recovery of salbutamol and the internal standard was in all instances between 95 and 101%.

Ten plasma samples from a healthy volunteer after oral administration of 8 mg of salbutamol were analysed with the present method and a newly modified gas chromatographic—mass spectrometric (GC—MS) method in which salbutamol was converted to its trimethylsilyl derivative [3, 6]. The correlation plot of the GC—MS results (y) vs. the results of the present method (x) is described by the equation $y = 1.0002x + 1.02$ ($r = 0.9925$; $n = 10$; concentration range 4—25 ng/ml), the GC—MS method consistently giving somewhat higher values (Table I).

TABLE I

RESULTS OF THE PRESENT HPLC METHOD COMPARED WITH GC—MS

Sample time (h)	HPLC result (ng/ml)	GC—MS result (ng/ml)
0 (blank)	Not detectable	Not detectable
1.5	13.6	13.0
2.0	8.8	10.4
2.5	8.1	10.0
3.0	12.6	14.0
3.5	22.7	23.9
4.0	17.3	18.4
5.0	11.8	12.3
7.5	7.0	8.3
12.0	3.8	5.5

No interference with our method was observed from the following drugs when they were given to patients whose salbutamol levels were monitored: theophylline, prednisone, beclomethason, furosemide, triamterene, atenolol, methyldopa, acenocoumarol and nitrazepam.

A plasma concentration—time curve for a patient who received 2 mg of salbutamol orally is shown in Fig. 5.

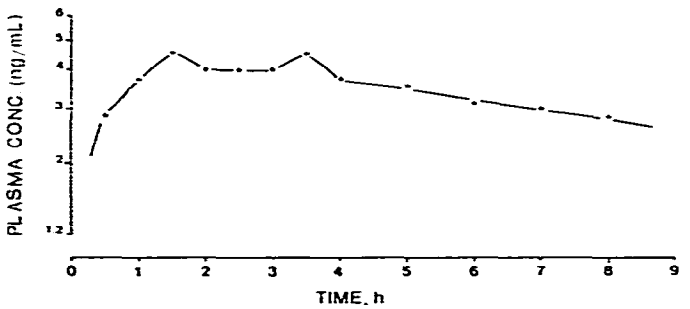

Fig. 5. Semilogarithmic plot of plasma concentration vs. time in a patient who received 2 mg of salbutamol orally at $t = 0$.

DISCUSSION

Due to the low therapeutic concentrations of salbutamol a sensitive detection method is required for its determination. Since the salbutamol molecule contains a phenolic hydroxyl group, electrochemical detection was a viable choice.

Preliminary voltammographic experiments with a rotated disc carbon-paste electrode showed in principle sufficient sensitivity (Fig. 6).

An anodic shift of the half-wave potential was observed with decreasing pH. This shift was expected as protons are liberated upon oxidation of the phenolic hydroxyl group. Therefore a pH of 7.0 was chosen for eluent 1, being near the upper limit allowed by the silica-based column material. The rotation speed of the electrode was so chosen that the current was under kinetic control [5].

When a constant mixture containing salbutamol and related drugs having a resorcinol group (orciprenaline and fenoterol) was injected repeatedly into column 2 over a longer period of time, it was observed that the detector response ratio of salbutamol towards the other compounds changed with the lifetime of the electrode. Hence it was concluded that in order to obtain reliable quantitative results the internal standard should contain an electroactive group very similar to that of salbutamol. This requirement was fulfilled by bamethan which also has a phenolic hydroxyl group.

The described pretreatment of the electrode at +1600 mV resulted in an improved sensitivity of the electrode, and reduced the conditioning period. The lifetime of an electrode varied from one to several weeks. A high electrode potential was required for the oxidation of salbutamol (Fig. 6). Thus selectivity had to be sacrificed for the purpose of sensitivity.

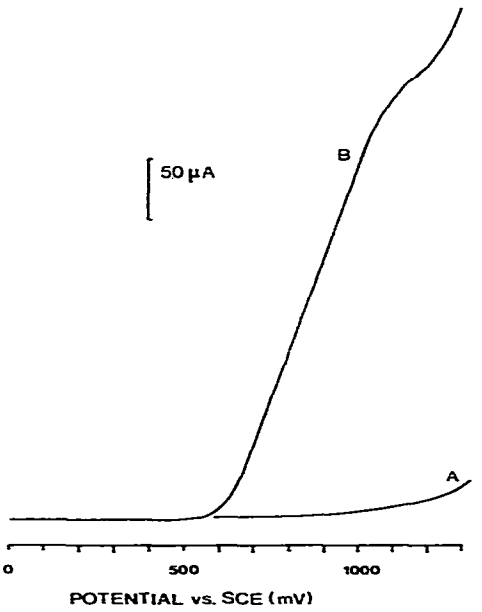

Fig. 6. Voltammograms as obtained with a rotated disc carbon-paste electrode. (A) pH 7.0 phosphate buffer; (B) pH 7.0 phosphate buffer containing 100 µg/ml salbutamol. (Rotation speed approx. 2000 rpm, scanning rate 5 mV/sec.)

Since it appeared very difficult to gain adequate selectivity with conventional sample preparation techniques, including ion-pair extraction [7], we have investigated the feasibility of mode-sequencing HPLC. Principles and applications of this concept have been reviewed recently [8]. In the approach used, compounds of interest remain on the ion-exchange column during elution with eluent 1, due to their high capacity factor in this system. Elution focusing is achieved on transferring the analytes to the reversed-phase column as a result of the high ionic strength of eluent 1. Resolution from an interfering compound in plasma was increased by ion-pair formation of the analytes with perchlorate ion [9]. After approximately every 40 injections column 1 needed to be repacked.

As salbutamol was eluted earlier than bamethan with eluent 1, its capacity factor on column 1 was determined at intervals by connecting the efflux of column 1 to the detector.

Our bimodal HPLC procedure can easily be automated with commercially available components. As sample pretreatment (column 1) and analysis (column 2) occur at the same time, five to seven samples can be analysed per hour.

The selectivity of the chromatographic system allowed direct injection of plasma samples after deproteinisation with perchloric acid and neutralization with sodium bicarbonate. However, a poor recovery was obtained due to co-precipitation of the analytes with plasma proteins. Furthermore, in the Sep-Pak procedure not only proteins were removed, but also electrolytes that would cause band spread on the ion-exchange column. Evaporation of the organic eluate from the Sep-Pak columns was facilitated as the aqueous eluate was almost quantitatively removed by centrifugation. Sep-Pak columns were reused

approximately 15 times with no measurable loss in performance.

The difference between the results obtained with GC—MS and the present method is not explained. However, we feel that the lower values found with our method (consistently about 1 ng/ml difference over the whole concentration range) cannot be due to standard solution errors, interfering peaks or recovery problems.

The above-described method can easily be modified for the assay of related drugs such as fenoterol and terbutaline, thus providing useful and relatively simple methods for pharmacokinetic studies of β-sympathicomimetics in man.

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