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Short communication

Quantitative analysis of bencynonate in human plasma using a deuterated internal standard by gas chromatography–mass spectrometry with selected-ion monitoring

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Abstract

A gas chromatographic–mass spectrometric method is described for the quantitative analysis of bencynonate in human plasma. Deuterated bencynonate served as the internal standard and selected-ion monitoring of the fragments of bencynonate and internal standard permitted the quantitation of bencynonate down to 25 pg/ml of plasma. The assay is linear for plasma bencynonate concentrations in the range 25 pg/ml–3 ng/ml. At 0.25 ng/ml the recovery and coefficient of variation are 54.3% and 19.1%, respectively. Application of the method to clinical studies gave data for the pharmacokinetics and relative bioavailability of bencynonate in man.

1. Introduction

Bencynonate is a new anticholinergic drug developed by our institute. After dosing (2 mg/man or 4 mg/man) of bencynonate, the plasma concentrations are so low that therapeutic drug monitoring has previously not been possible. A radioisotope assay and a radioacceptor assay have been used in rat experiments [1], but the detection limits of these methods were not sensitive enough for therapeutic drug monitoring. Furthermore, the two methods were unsuitable for human studies. In 1989, a gas chromatographic–mass spectrometric method with selected-ion monitoring (GC–MS-SIM) has been reported for the study of a drug which has a structure similar to that of bencynonate [2]. For evaluation of the pharmacokinetics and the relative bioavailability of bencynonate in man, we developed a sensitive GC–MS-SIM method for the analysis of therapeutic plasma concentrations of bencynonate in human.

2. Experimental

2.1. Chemicals and reagents

Bencynonate and deuterated bencynonates were obtained from the Synthetic Laboratory in our institute. Other solvents used were analytical

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grade reagents and were purchased from Beijing Chemical Company (Beijing, China).

2.2. Plasma sample preparation

To 2 ml of plasma were added 100 ng of [$^2H_3$]bencynonate and 0.2 ml of 0.2 *M* sodium hydroxide. After shaking for 1 min on a horizontal shaker, the mixture was extracted with 4 ml of diethyl ether by vortex-mixing for 30 s. The organic phase was transferred to a centrifuge tube (10 ml) after centrifugation at 1000 *g* for 5 min. The aqueous phase was once more extracted with 4 ml of diethyl ether. The two organic phases were combined and evaporated under a stream of nitrogen at 50°C, and then the tube was capped until analysis.

2.3. Instrumentation and analysis

For all GC–MS analyses a VG TRIO-2 gas chromatograph–mass spectrometer (Manchester, UK) interfaced to a PDP11-53 computer was used. Fragmentation was accomplished by electron-impact ionization at 70 eV and a current of 180 μA. Source temperature was 200°C. The instrument was calibrated using heptercosa (perfluortri-*n*-butylamine). The data system used two selected-ion current channels: that at *m/z* 138 for bencynonate and *m/z* 141 for [$^2H_3$]bencynonate. The sample time and interval were 80 ms and 20 ms, respectively.

The prepared samples were each reconstituted with 50 μl of methanol immediately prior to injection and 1 μl was injected onto the GC column. Chromatographic separation was performed on a 10 m × 0.25 mm I.D., 0.25 μm DB-5 capillary column (Hewlett-Packard, USA). The oven temperature was programmed from 60°C (initial time, 1 min) to 250°C (final time, 15 min) at 20°C/min. The injection port and interface oven temperatures were 250°C and 330°C, respectively. Splitless injection with a split valve off-time of 1 min was employed. The helium carrier gas flow-rate was 1 ml/min.

3. Results and discussion

3.1. Choice of internal standard

There are three synthetic deuterated bencynonates ([$^2H_3$]bencynonate, [$^2H_4$]bencynonate and [$^2H_5$]bencynonate) which can be used as internal standard. Fig. 1 shows the structures of bencynonate, [$^2H_3$]bencynonates and two main fragments. As *m/z* 138 is the base peak in the spectrum of bencynonate, which advantageous to obtain adequate sensitivity, it was selected as the monitoring ion. Therefore, only [$^2H_3$]bencynonate, the monitoring ion of which was *m/z* 141 (Fig. 2B), could be used as internal standard according to the structure of *m/z* 138 [2]. In the experiments, the amount of [$^2H_3$]bencynonate added to plasma as internal standard, was more higher than the amount of bencynonate in plasma to compensate the losses in occurring in the extraction and chromatographic separation.

3.2. Extraction and recovery

The extraction procedure for bencynonate has been applied to rat plasma. The results indicated

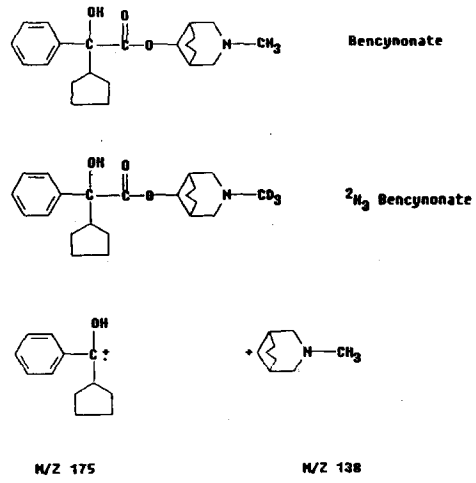

Fig. 1. The structures of bencynonate, [$^2H_3$]bencynonate, and two main fragments.

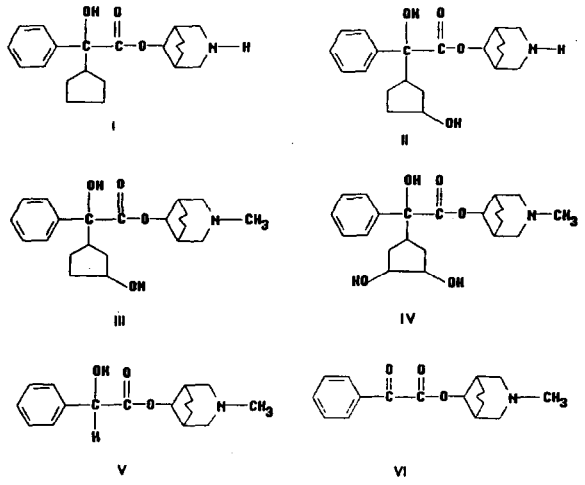

Fig. 2. The structures of six bencynonate metabolites.

that the extraction was both validated and convenient, especially for the extraction of large numbers of samples and thus we used this procedure for the extraction of bencynonate from human plasma. The extraction recovery and coefficient of variation (intra-day C.V.) of bencynonate from plasma spiked at 0.25 ng/ml ($n = 5$) were 54.3% and 19.1%, respectively.

3.3. Chromatography and detection

An earlier study [2] showed that bencynonate could produce six metabolites in human. The structures of the six metabolites are listed in Fig. 2. Three of these structures, III, V, VI, could produce chromatographic peaks and m/z 138 fragments, which could interfere with the detection of bencynonate in our method. Fig. 3 shows the total-ion current chromatogram and the m/z 138 mass chromatogram of an extract from rat urine after administration of bencynonate. In Fig. 3B, peak BCT is the bencynonate and peaks VI, V, and III are the three metabolites of bencynonate according to their mass spectra. Thus, the peaks of the bencynonate metabolites were well separated from the bencynonate peak. This indicated that the monitored compound was bencynonate only, and not the sum of bencynonate and its metabolites as monitored in the radioisotope array mode.

Under the selected GC operation conditions, the chromatogram gave a good peak and a smooth background (Fig. 4), which are important to obtain a high sensitivity. In addition, the chromatogram of a control sample which only

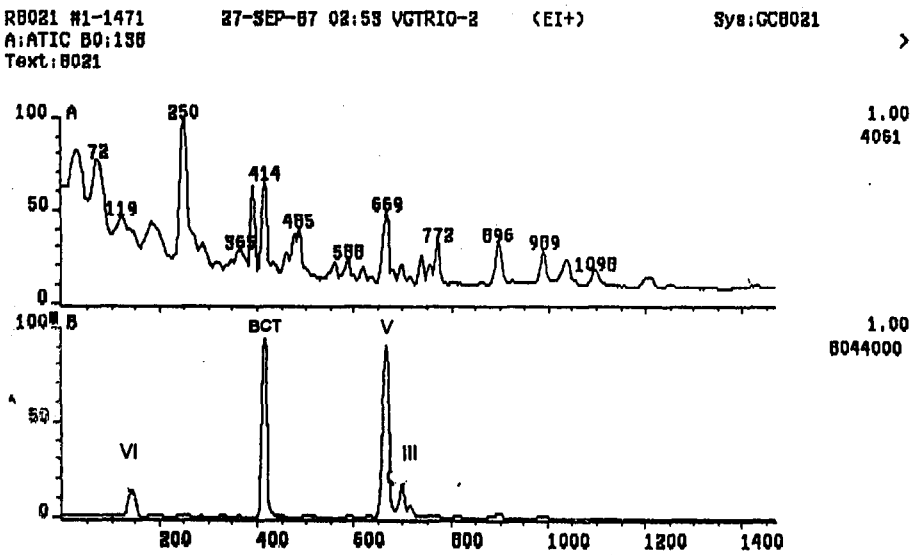

Fig. 3. Chromatograms of extracts from rat urine after administration of bencynonate. (A) Total-ion current chromatogram; (B) the m/z 138 mass chromatogram.

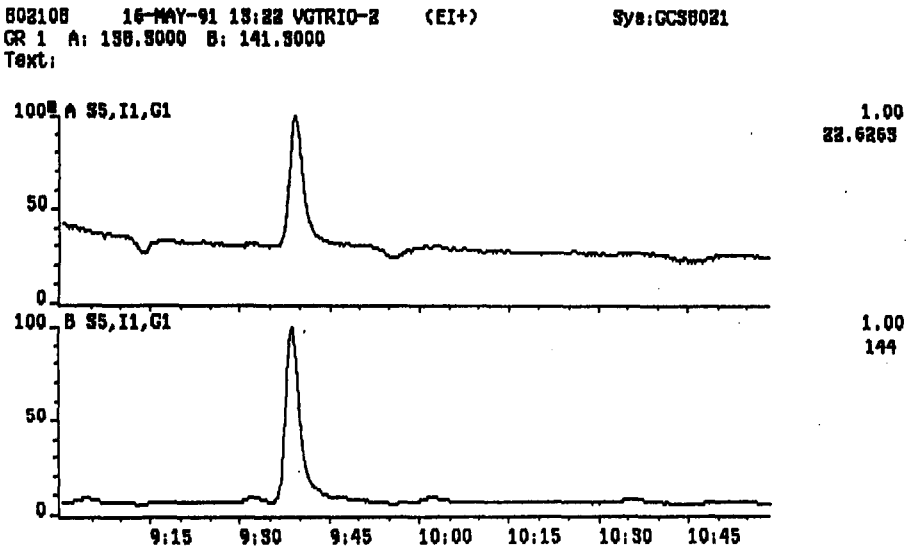

Fig. 4. The selected-ion monitoring chromatograms of extracts from human plasma after administration of bencynonate. (A) The m/z 138 chromatogram; (B) the m/z 141 chromatogram.

contained $[^2H_3]$bencynonate indicated that $[^2H_3]$bencynonate did not interfere with the detection of bencynonate. The stability of the instrument was determined by repeatedly injecting the same sample (plasma bencynonate concentration 0.5 ng/ml) on five separate days. The coefficient of variation (inter-day C.V.) was 6% ($n = 9$). indicating satisfactory instrumental reproducibility.

3.4. Standard curve, detection limit and accuracy

The ion-current ratios generated by monitoring the characteristic ions for bencynonate/$[^2H_3]$bencynonate were determined from their peak heights at the appropriate GC retention times. The concentrations of bencynonate in unknowns or quality control samples were then calculated from their ratios using the slope of the standard curve; $y = 0.0033x + 0.0027$, $n = 7$, $r = 0.992$, where y is the ratio of the height of the bencynonate peak to the height of the $[^2H_3]$bencynonate peak, and x is the concentration of bencynonate in plasma (ng/ml).

The detector response was linear over the bencynonate plasma concentration range 0.025–3 ng/ml. The reliable detection limit of bencynonate in plasma was 25 pg/ml, based on a signal-to-noise ratio observed for the 25 pg/ml sample. The accuracy of the assay, as determined by the analysis of control samples with two different concentrations, is shown in Table 1.

3.5. Human data

In the clinical study twelve men were divided into two groups. In the first group each man was administered two 2-mg tablets of bencynonate orally, while in the second group each men received only one 2-mg tablet of bencynonate. For a period of at least 10 h after dosing, clinical samples were quantitatively analyzed using the method described above. The bencynonate plasma concentration–time profiles are shown in Fig. 5. After administration of two 2-mg tablets of bencynonate for a 1-week period, the men in the first group were administered 4 mg bencynonate in aqueous solution again. The plasma concentration–time profile is also shown in Fig. 5. The relative bioavailability of bencynonate was then obtained. The detailed explanation of the pharmacokinetics and the relative bioavailability of

Table 1
Assay of bencynonate plasma control samples ($n = 5$)

Bencynonate added (ng/ml)	Mean concentration detected (ng/ml)	Relative differences (%)	C.V. (%)
2.00	2.10	5.0	19.0
0.25	2.70	8.0	19.9

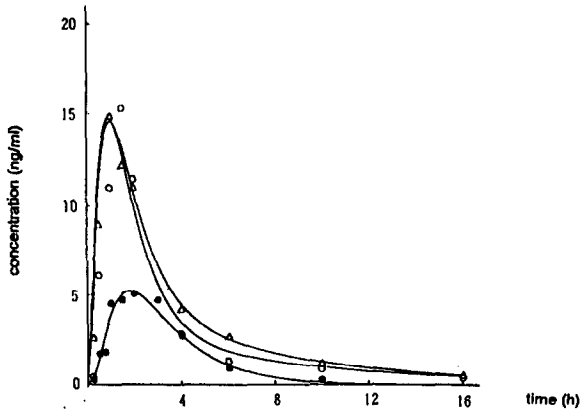

Fig. 5. Plasma concentration–time profiles of bencynonate. (●) Profile after administration of one 2-mg tablet of bencynonate orally; (○) profile after administration of two 2-mg tablets of bencynonate orally; (△) profile after oral administration of 4 mg of an aqueous solution of bencynonate.

bencynonate was reported in another publication [1].

Acknowledgement

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