Journal of Chromatography, 416 (1987) 150–153
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3530

Note

Quantitative analysis of dicyclomine in human plasma by capillary gas chromatography and nitrogen-selective detection

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(First received July 24th, 1986; revised manuscript received November 28th, 1986)

Dicyclomine, β-diethylaminoethyl 1-cyclohexylcyclohexanecarboxylate hydrochloride (Fig. 1), is an antispasmodic, anticholinergic agent used in the treatment of functional bowel/irritable bowel syndrome [1–4]. Previous methods of analysis proved to be tedious [5] or lacked sufficient sensitivity [6]. One method required the routine use of gas chromatography–mass spectrometry (GC–MS) [6] and another GC method utilized a column packed with OV-225 (nitrogen-containing phase) with a nitrogen-selective detector [7]. All involved liquid–liquid extractions of alkaline plasma with diethyl ether.

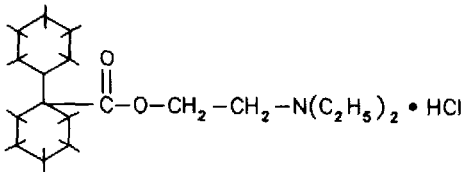

Fig. 1. Structure of dicyclomine hydrochloride.

The method presented in this communication is a fast and simple liquid–solid extraction of plasma with analysis by capillary GC. The use of a nitrogen-selective detector with capillary GC increases both sensitivity and selectivity. Automatic injection makes the method useful for pharmacokinetic or bioavailability studies which produce large numbers of samples.

EXPERIMENTAL

Materials

Methanol and ethyl acetate (both glass-distilled) were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Sodium hydroxide pellets, reagent

0378-4347/87/$03.50

grade, were purchased from Matheson Coleman & Bell (Norwood, OH, U.S.A.). Glass-distilled water was used in the preparation of aqueous solutions. A Baker-10 SPE™ system (J.T. Baker, Phillipsburg, NJ, U.S.A.) was used for the liquid–solid extraction, utilizing octadecyl reversed-phase 3-ml SPE columns.

Dicyclomine hydrochloride and the internal standard (the 1-benzyl analogue of dicyclomine hydrochloride) were supplied by the Merrell Dow Research Institute (Cincinnati, OH, U.S.A.). Standard solutions of dicyclomine hydrochloride and the internal standard were prepared in methanol.

Validation

To test the precision and accuracy of the assay, a validation study was performed. On six days, duplicate eight-point dicyclomine standard curves were prepared in human plasma. Seven randomized plasma standards, with concentrations unknown to the analyst, were also assayed in duplicate on each day.

Extraction procedure

For each standard or unknown, a Baker column was conditioned with two column volumes of methanol, followed by two column volumes of 0.3 *M* sodium hydroxide. Plasma (1.0 ml) was applied to the column with the vacuum off, then internal standard was added. After the addition of 0.5 ml of 1 *M* sodium hydroxide, the vacuum was reapplied and the sample pulled through the column. The column packing was washed with three column volumes of water–methanol (75:25) wash, then dried with the vacuum on for approximately 10 min. Dicyclomine and internal standard were then eluted with two 0.5-ml aliquots of methanol, collecting the eluate in a disposable glass tube. The eluate was evaporated to dryness with nitrogen in a 40°C water bath. The residue was dissolved in 50 μl of an ethyl acetate–methanol (95:5) mix and 5 μl were injected into a capillary gas chromatograph.

Instrumentation

Analyses were performed on a Hewlett-Packard 5790A capillary gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.). A 7-m, DB-5 fused-silica capillary column (0.25-μm film, 0.32 mm I.D., J&W Scientific, Rancho Cordova, CA, U.S.A.) was used. The GC apparatus was equipped with a nitrogen–phosphorus detector and a Hewlett-Packard 7672A automatic sampler was utilized. Each sample was injected at 120°C and temperature programmed to 250°C at a rate of 20°C/min. The injector temperature was set at 250°C and the detector at 300°C. Helium was used as the carrier and make-up gas. The column pressure was adjusted to 13.8 kPa and all samples were injected in the splitless mode.

Calibration and calculations

Peak areas of dicyclomine and internal standard were integrated by a computer-automated laboratory data system (Computer Inquiry Systems, Englewood Cliffs, NJ, U.S.A.). For each run, a calibration curve was generated, plotting peak-area ratios (expressed as percents) of dicyclomine divided by the internal

TABLE I

DICYCLOMINE STANDARD RESULTS FOR SIX RUNS

Standard concentration (ng/ml)	Peak-area ratio (mean ± S.D.) (%)	C.V. (%)
0	0.15 ± 0.36	–
5	7.20 ± 1.00	13.89
10	15.33 ± 0.90	5.87
25	42.68 ± 2.38	5.58
50	83.62 ± 2.92	3.49
100	166.47 ± 5.53	3.32
150	263.58 ± 10.70	4.06
200	352.74 ± 18.04	5.11

TABLE II

ANALYSIS OF PLASMA CONTAINING UNKNOWN ADDED CONCENTRATIONS OF DICYCLOMINE HYDROCHLORIDE ($n=6$)

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)	Recovery (%)
0	0.77 ± 1.20	–	–
7	8.30 ± 1.97	23.73	118.62
40	39.76 ± 1.73	4.35	99.39
67.5	68.54 ± 2.52	3.68	101.54
105	99.79 ± 2.78	2.79	95.04
140	134.28 ± 6.51	4.85	95.91
180	178.13 ± 5.62	3.16	98.96

standard against dicyclomine concentrations. Peak-area ratios of each unknown were applied to this curve to determine concentrations.

RESULTS AND DISCUSSION

Composite results for the six validation runs are tabulated in Table I. The assay was linear over the range 5–200 ng/ml and had acceptable precision. Of the six blank plasma standards, run in duplicate, only one showed a small endogenous peak. All others were clean, with no interfering peaks. Typical chromatograms of a blank and standard are shown in Fig. 2.

In order to demonstrate the extraction and chromatography of dicyclomine in a real patient, a blood sample was drawn from a volunteer 1.5 h after receiving a 20-mg tablet of Bentyl® prescribed for spastic colon. The blood sample was centrifuged, plasma was removed, and 1.0 ml was treated as described above. The chromatogram of this sample is shown in Fig. 3.

The precision and accuracy of the method as demonstrated by the analysis of the coded unknowns is shown in Table II. The mean overall recovery was 101.6%,

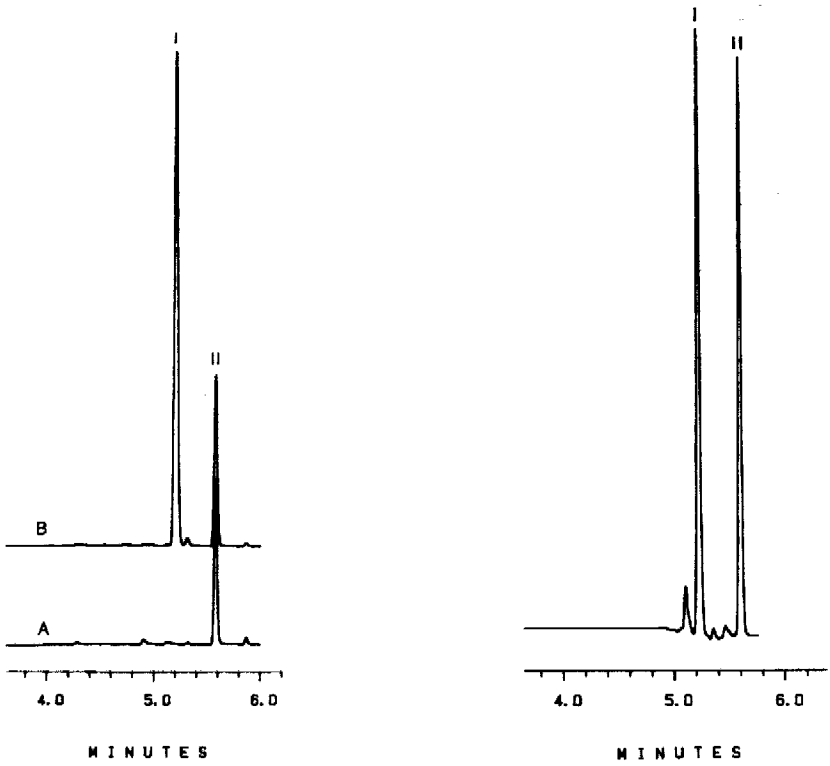

Fig. 2. Chromatograms of extracted plasma standards. Samples: (A) blank; (B) 200 ng/ml; Peaks: I = dicyclomine; II = internal standard.

Fig. 3. Chromatogram of extracted plasma from a human subject 1.5 h after receiving 20 mg of dicyclomine hydrochloride (Bentyl). Peaks: I = dicyclomine; II = internal standard.

with values ranging from 95.04% (105 ng/ml added) to 118.62% (7 ng/ml added). The coefficient of variation (C.V.) was below 5% for all levels except the lowest (23.73% for 7 ng/ml added). The overall C.V. averaged 7.1%.

In conclusion, the validity of the method to measure dicyclomine in human plasma has been demonstrated. The method is faster and simpler than analytical methods previously available and is adequate for analysis of plasma concentrations as low as 5 ng/ml. The method can be used for pharmacokinetic and bioavailability studies of Bentyl or related products.

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