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High-performance liquid chromatographic method for the determination of indapamide in human whole blood

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ABSTRACT

A sensitive, accurate, and reproducible high-performance liquid chromatographic procedure for the analysis of indapamide in human whole blood is reported. After a single-step liquid–liquid extraction at pH 6.6 using diethyl ether, indapamide was eluted from a Nucleosil C_{18} 5-μm column with 80 m*M* ammonium acetate, pH 3.5–acetonitrile–2-propanol (65:30:5, v/v/v). The peak height *versus* whole blood concentration was linear over the range 10.0–500 ng/ml using ultraviolet detection. The mean absolute recovery of indapamide using the described assay was 87.4%. The inter- and intra-day accuracy and precision were within 9.6% of the actual values for all concentrations investigated. Furthermore, this procedure was applied to the analysis of whole blood samples from healthy subjects receiving a single 2.5-mg oral dose of indapamide.

INTRODUCTION

Indapamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)benzamide (Fig. 1), is an antihypertensive agent administered to individuals with mild to moderate hypertension. A significant reduction in blood pressure can be achieved with a daily oral dose of 2.5 mg [1]. HPLC methods exist for the determination of indapamide in serum, plasma and urine [2–5]. The analysis of indapamide was performed in whole blood because it is preferentially bound to the red blood cells [6]. To our knowledge, the only method for quantifying indapamide in human whole blood is that of Choi *et al.* [4]. However, it is anticipated that an assay which is five times more sensitive than that reported by Choi *et al.* [4] will be necessary to follow the pharmacokinetics of a 2.5-mg oral dose of indapamide. Consequently, this prompted the development of an assay in whole blood which is sensitive, specific and robust.

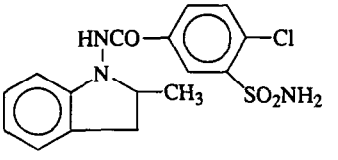

Fig. 1. Structure of indapamide.

The method reported herein for the determination of indapamide is linear over the range 10.0–500 ng/ml in human whole blood. This range was predicted on the basis of a reported maximum concentration (C_{max}) of 333 ng/ml in whole blood for a 5-mg oral dose of indapamide [7]. Extrapolating the C_{max} four half-lives yields a lower limit of 10.0 ng/ml. Furthermore, this procedure was successfully applied to ascertain the pharmacoki-

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netics of a single 2.5-mg dose of indapamide in humans over a 48-h period.

EXPERIMENTAL

Materials

Indapamide and the internal standard, glipizide, were purchased from Sigma (St. Louis, MO, USA). HPLC-grade potassium dihydrogenphosphate, hydrochloric acid and phosphoric acid were from Fisher Scientific (Montreal, Canada) and ACS-grade ammonium acetate was from J. T. Baker (Phillipsburg, NJ, USA). HPLC-grade acetonitrile, 2-propanol and diethyl ether were purchased from Caledon (Georgetown, Canada). The water was deionized Type 1, reagent grade (Millipore, Ville St. Laurent, Canada). All reagents were used without further purification.

Instrumentation

The chromatographic system consisted of a Waters Model 590 pump, a WISP 710B autosampler and a Lambda Max Model 481 UV detector (Waters Assoc., Milford, MA, USA). A stainless-steel column (15 cm × 4.6 mm I.D.) was packed with Nucleosil C_{18}, particle size 5 μm (prepared in-house). The column was maintained at ambient temperature. The UV detector was set at 241 nm, to monitor the analytes. The mobile phase, consisting of 80 m*M* ammonium acetate, pH 3.5 (adjusted with concentrated hydrochloric acid)–acetonitrile–2-propanol (65:30:5, v/v/v), was delivered at a flow-rate of 1.0 ml/min and had a typical operating pressure of 90 bar. Under these conditions, the retention times for indapamide and the internal standard were 5.2 and 5.9 min, respectively.

Preparation of standards

A stock solution of indapamide was prepared at 1.00 mg/ml in methanol. Appropriate dilutions of the stock solutions were made with deionized water to prepare whole blood standards at concentrations between 10.0 and 500 ng/ml. Quality control (QC) samples spiked in whole blood were prepared in pools of 30.0 ml at final concentrations of 35.0, 150 and 330 ng/ml. Individual 1.00-ml aliquots of spiked blood were stored in 100 mm × 16 mm screw-cap glass culture tubes at −20°C until analyzed. A stock internal standard solution of glipizide was prepared at 1.00 mg/ml in methanol and diluted to 4.0 μg/ml with potassium dihydrogenphosphate (pH 6.6, 0.05 *M*). All stock solutions were stored at −20°C and were stable for at least one month.

Sample preparation

Aliquots of whole blood (1.00 ml) were added to 100 mm × 16 mm screw-cap glass culture tubes. Whole blood samples were treated with 1.00 ml of 4.0 μg/ml glipizide in potassium dihydrogenphosphate (pH 6.6, 0.05 *M*) and vortex-mixed briefly. The analytes were extracted with diethyl ether (6 ml) on a reciprocating shaker (150 ± 20 oscillations per min) for 15 min. After centrifugation for 10 min at *ca.* 1500 *g*, the organic layer was transferred into a disposable 100 mm × 16 mm borosilicate glass culture tube. The organic layer was evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was dissolved in 200 μl of mobile phase and centrifuged at *ca.* 1000 *g* for 3 min. A 40-μl aliquot of the supernatant was injected onto the liquid chromatograph under the previously stated conditions. The reconstituted extracted samples were stable at room temperature for at least 24 h.

Data acquisition

The peak heights of indapamide and the internal standard were measured with a Spectra-Physics Model 4270 integrator and down-loaded to a Chrom-Station (Spectra-Physics, Mountain View, CA, USA). The chromatographic data were automatically processed for peak-height ratios of indapamide to the internal standard and fitted to a weighted ($1/C$) linear regression.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from extracted whole blood samples are illustrated in Fig. 2A–D. Fig. 2A shows a representative chromatogram of a processed whole blood blank.

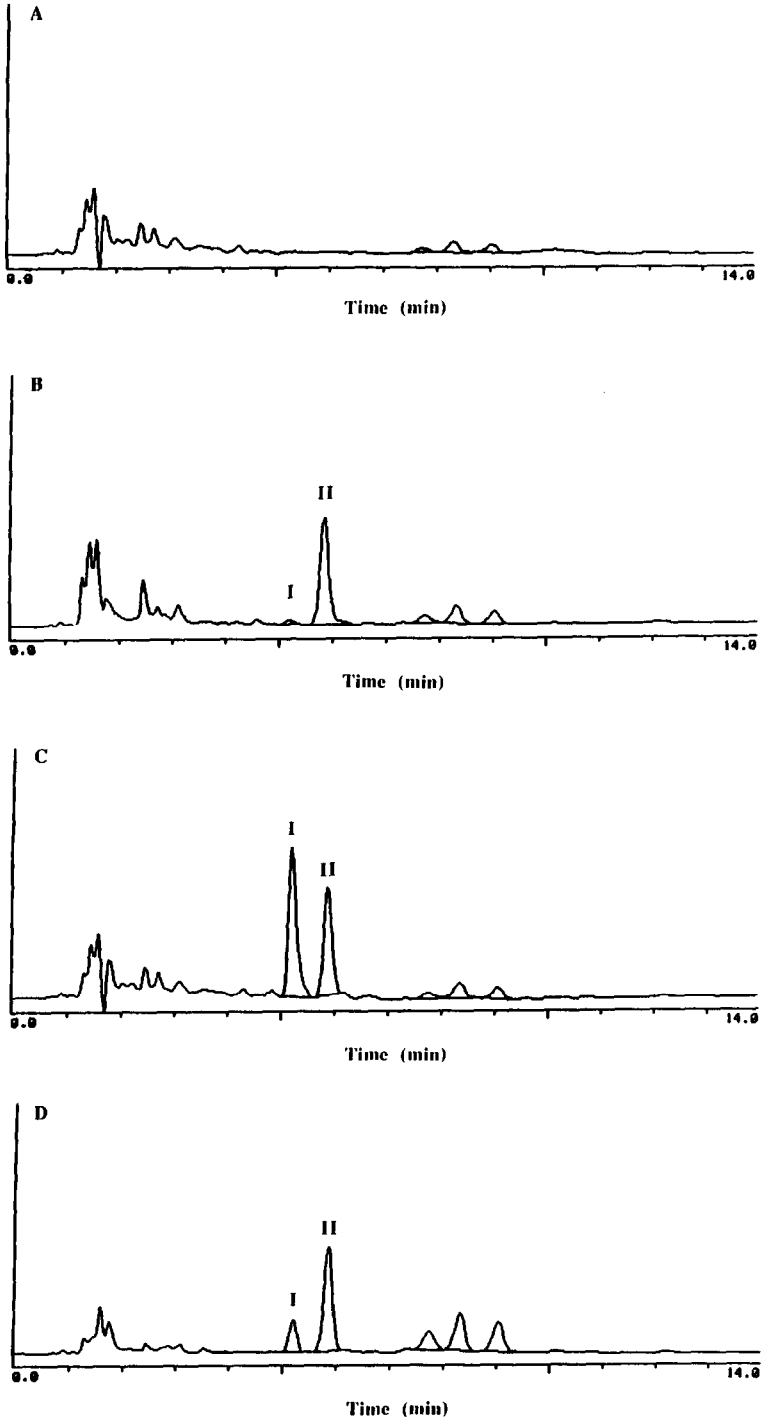

Fig. 2. Chromatograms of samples prepared according to the described procedure. (A) Whole blood blank; (B) whole blood spiked with 10.0 ng/ml indapamide; (C) whole blood spiked with 500 ng/ml indapamide; (D) whole blood of a subject (88.0 ng/ml) 4 h after a 2.5-mg oral dose of indapamide. All chromatograms are shown with an attenuation of 16. Peaks: I = indapamide; II = internal standard.

This chromatogram indicates that no endogenous compounds interfered at the retention time of indapamide or the internal standard. Fig. 2B is a chromatogram amplified to the same degree as the blank showing the limit of quantification (LOQ, 10.0 mg/ml). Fig. 2C is a whole blood sample at the upper limit (500 ng/ml) of the calibration range. Fig. 2D is a whole blood sample obtained from a subject 4 h after a single 2.5-mg oral dose of indapamide. The retention times of indapamide and the internal standard were 5.2 and 5.9 min, respectively. The overall chromatographic run time was 14.0 min.

Linearity and quantification limit

A linear response in peak-height ratio for indapamide to internal standard over the range 10.0–500 ng/ml was observed with a minimum signal-to-noise ratio of 4:1. The correlation coefficients were 0.9989 or better.

Recovery

The absolute recovery of indapamide was evaluated by comparing the concentrations found in whole blood samples spiked with known amounts of the analyte, which had been processed through the entire extraction procedure, to the same concentrations found in the reference solution (adjusted for reconstitution). The recovery of spiked human whole blood was evaluated at all three QC concentrations in replicates of six. These samples were extracted as described previously except the internal standard was not added. The absolute peak heights from the extracted samples were compared with those from the unextracted standard solutions prepared in mobile phase. Similarly, the recovery of the internal standard, glipizide, was determined at the final recommended concentration. These results are provided in Table I.

TABLE I

RECOVERY OF INDAPAMIDE AND INTERNAL STANDARD FROM HUMAN WHOLE BLOOD (n = 6)

Drug	Concentration (ng/ml)	Recovery (%)	R.S.D. (%)
Indapamide	35.0	83.5	8.8
	150.0	87.1	4.6
	330.0	91.6	2.2
Internal standard	200.0	36.9	2.0

Selectivity

Human whole blood was collected from ten healthy donors and screened for interference at the retention times of indapamide and the internal standard. No significant interference had been observed in drug-free whole blood samples. Also, the following over-the-counter (OTC) drugs were tested for possible interference: caffeine, ibuprofen, aspirin, acetaminophen, theophylline, phenylpropanolamine, nicotine and dextromethorphan. These OTC drugs did not interfere with the analysis of indapamide.

Precision and accuracy

The inter-day precision and accuracy were assessed by the analysis of two samples at each QC concentration (low, medium and high) together with a single calibration curve on six separate days (Table II). The precision was based on the calculation of the relative standard deviation

TABLE II

INTER-DAY PRECISION AND ACCURACY OF INDAPAMIDE IN HUMAN WHOLE BLOOD

Nominal concentration (ng/ml)	n	Mean found concentration (ng/ml)	R.S.D. (%)	R.E. (%)
Standard				
10.0	6	10.2	3.5	1.9
15.0	6	14.5	3.3	−3.2
30.0	6	29.8	5.0	−0.5
100	6	104.3	2.9	4.3
200	6	193.2	2.9	−3.4
300	6	299.2	2.2	−0.3
500	5	502.6	1.2	0.5
Quality control				
35.0	12	34.5	9.6	−1.6
150	12	150.1	6.1	0.1
330	12	330.0	7.0	0.0

TABLE III

INTRA-DAY PRECISION AND ACCURACY OF INDAPAMIDE IN HUMAN WHOLE BLOOD

Nominal concentration (ng/ml)	*n*	Mean found concentration (ng/ml)	R.S.D. (%)	R.E. (%)
Standard				
10.0	6	9.1	8.0	−9.1
Quality control				
35.0	6	35.3	7.0	1.0
150	6	145.6	1.9	−2.9
330	6	333.6	1.3	1.1

(R.S.D.). An indication of accuracy was based on the calculation of the relative error (R.E.) of the mean found concentration as compared to the nominal concentration. The R.S.D. for all samples analyzed were within 9.6% and the R.E. ranged from −3.4 to 4.3% of the nominal concentrations.

The intra-day precision and accuracy were determined by the evaluation of a typical production run. Whole blood samples spiked with indapamide at concentrations of 10.0, 35.0, 150 and 330 ng/ml were evaluated in replicates of six. The R.S.D. for all samples analyzed were within 8.0% and the R.E. ranged from −9.1 to 1.1% of the nominal concentrations. These results are presented in Table III.

Application

Whole blood samples were obtained prior to dosing and at fifteen subsequent time points following a 2.5-mg oral dose of indapamide. Following collection, the samples were stored at −20°C until analyzed. All samples were analyzed by the method presented here. The time course of whole blood indapamide concentrations is depicted in Fig. 3. The assay allows the quantification of whole blood levels of indapamide for at least 48 h following a single 2.5-mg oral dose and permits complete characterization of the resulting whole blood profile.

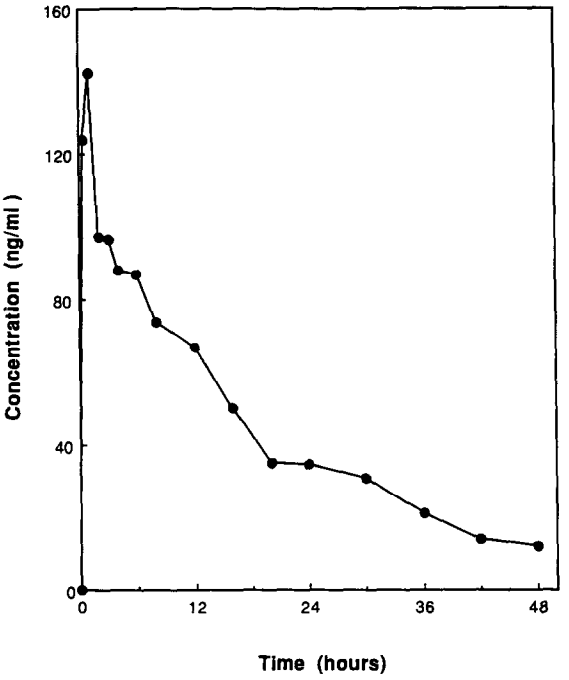

Fig. 3. Representative concentration–time profile of a subject following a single 2.5-mg oral dose of indapamide.

CONCLUSION

The described method for the analysis of indapamide in human whole blood was specific, sensitive and robust. The intra- and inter-assay precision of the method was below 9.6%, while the accuracy of the method was within 9.1% even at the LOQ. The inter-day means of the R.E. for the standards and QCs for indapamide ranged from −3.4 to 4.3%, exemplifying the accuracy of the assay. Furthermore, a relatively large number of samples can be processed daily (*ca.* 80). From the results presented here, this method could be used to determine the pharmacokinetics of a single 2.5-mg oral dose of indapamide from human whole blood.

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