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Short Communication

A liquid chromatographic method for the determination of hydrochlorothiazide in human plasma

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Introduction

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1, 1-dioxide) (HCT) is an antihypertensive-diuretic agent which is indicated in the management of hypertension either as the sole therapeutic agent or to enhance the effect of other antihypertensive drugs. Following oral administration, HCT is rapidly absorbed and is detected within 1 h in the urine [1]. Sensitive methods for the analysis of HCT exist using gas chromatography [2–4]. However, these methods involve lengthy extraction procedures and, consequently, are not very suitable for mass analyses. Liquid chromatography methods exist for the quantification of HCT, however, most assays do not achieve the sensitivity necessary for the pharmacokinetic analysis of a 12.5 mg oral dose [5–8]. Therefore, this prompted the development of an HCT method in plasma for production purposes, which is sensitive, specific, and robust. While developing this assay, Azumaya published a sensitive LC method for HCT using ion-pairing [9].

The method reported herein for the determination of HCT is linear over the range of 2.0–1000 ng ml^{-1} in human plasma. This range has been selected on the basis of its C_{max} of 70 ng ml^{-1} in plasma [10], and extrapolating more than five half-lives yields a lower limit of 2.0 ng ml^{-1}. Furthermore, this procedure was applied to ascertain the pharmacokinetics of a single 12.5 mg dose of HCT in humans.

Experimental

Materials

HCT was purchased from Sigma (St Louis, MO, USA). ACS grade sodium phosphate monobasic monohydrate and sodium carbonate were purchased from BDH (Ville St Pierre, QC, Canada). HPLC grade acetonitrile, ethyl acetate, and methanol were purchased from Caledon (Georgetown, ON, Canada). The water was deionized Type 1, reagent grade (Millipore, Ville St Laurent, QC, 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 Associates, Milford, MA, USA). A stainless-steel column (5 μm; 15 cm × 4.6 mm i.d.) was packed with Nucleosil C-8. The column was maintained at ambient temperature. The UV detector was set at 273 nm (0.02 aufs) to monitor the analytes. The mobile phase consisted of 10 mM sodium phosphate monobasic–acetonitrile–methanol (90:6:4, v/v/v), and was delivered at a flow rate of 1.2 ml min^{-1} and had a typical operating pressure of 1400 psi.

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Preparation of standards

A stock solution of HCT was prepared at 1.00 mg ml^{-1} in methanol. Appropriate dilutions of the stock were made with deionized water to prepared plasma standards at concentrations of 2.0, 5.0, 10.0, 50.0, 250, 500, 750 and 1000 ng ml^{-1}. Spiked plasma quality control samples (QCs) were prepared in pools of 30.0 ml at final concentrations of 20.0, 400, and 900 ng ml^{-1}. Individual aliquots of 500 µl were stored in 16 × 100 mm screw cap glass culture tubes and stored at −20°C until analysed. A stock internal standard solution of 6-bromo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide (HBT) (Boz Chem Engr., Dollard des Ormeaux, QC, Canada) was prepared at 1.00 mg ml^{-1} in methanol and diluted to 1.0 µg ml^{-1} with 0.50 M sodium phosphate monobasic. All stock solutions were stored at −20°C and were stable for at least 1 month.

Sample preparation

Aliquots of plasma (500 µl) were added to 16 × 100 mm screw cap glass culture tubes. Plasma samples were treated with 500 µl of 1.0 µg ml^{-1} HBT in 0.05 M sodium phosphate monobasic then 500 µl of 1.0 M sodium carbonate and vortexed briefly. The analytes were extracted with ethyl acetate (5 ml) on a reciprocating shaker (250 ± 25 oscillations min^{-1}) for 15 min. After centrifugation for 10 min at approximately 1500***g***, the organic layer was transferred into a clean disposable 16 × 100 borosilicate glass culture tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was dissolved in 300 µl of mobile phase and centrifuged at approximately 100***g*** for 3 min and 100 µl of the supernatant was injected onto the liquid chromatograph under the previously stated conditions. The reconstituted samples were stable at room temperature for at least 24 h.

Data acquisition

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

Results and Discussion

Chromatography

Typical chromatograms obtained from extracted plasma samples are illustrated in Fig. 1(a)–(d). Figure 1(a) shows a representative chromatogram of a processed plasma blank. This chromatogram indicates that no endogenous compounds exist at the retention times of HCT or internal standard. Figure 1(b) is a chromatogram amplified to the same degree as the blank showing the limit of quantification (LOQ, 2.0 ng ml^{-1}). Figure 1(c) is a plasma sample at 200 ng ml^{-1}. Figure 1(d) is a plasma sample obtained from a subject 1.0 h after a single 12.5 mg oral dose of HCT. The retention times of HCT and the internal standard were 11.3 and 13.3 min, respectively. The overall chromatographic run time was 15.0 min.

Validation

Linearity and quantification limit. A linear response in peak height ratio for HCT to internal standard over the range of 2.0–1000 ng ml^{-1} was observed with a minimum signal-to-noise ratio of 4:1. The correlation coefficients were 0.9998 or better (n = 6).

Recovery. The absolute recovery of HCT was evaluated by comparing the concentrations found in plasma samples spiked with known amounts of the analyte to the concentrations found in solution (adjusted for reconstitution). Spiked human plasma at two concentrations; one at four times the LOQ and the other at 90% of the upper limit of the assay in replicates of eight were extracted as described previously, except the internal standard was not added. The absolute peak heights from the extracted samples were compared to unextracted standard solution prepared in mobile phase. Similarly, the recovery of the internal standard was determined at the final recommended concentration. These results are provided in Table 1.

Specificity. Human plasma was collected from 10 healthy donors and screened for interference at the retention times of HCT and the internal standard. No significant interference had been observed in drug-free plasma samples. Also, the following over-the-counter (OTC) drugs were also tested for possible interference: caffeine, ibuprofen, aspirin, nico-

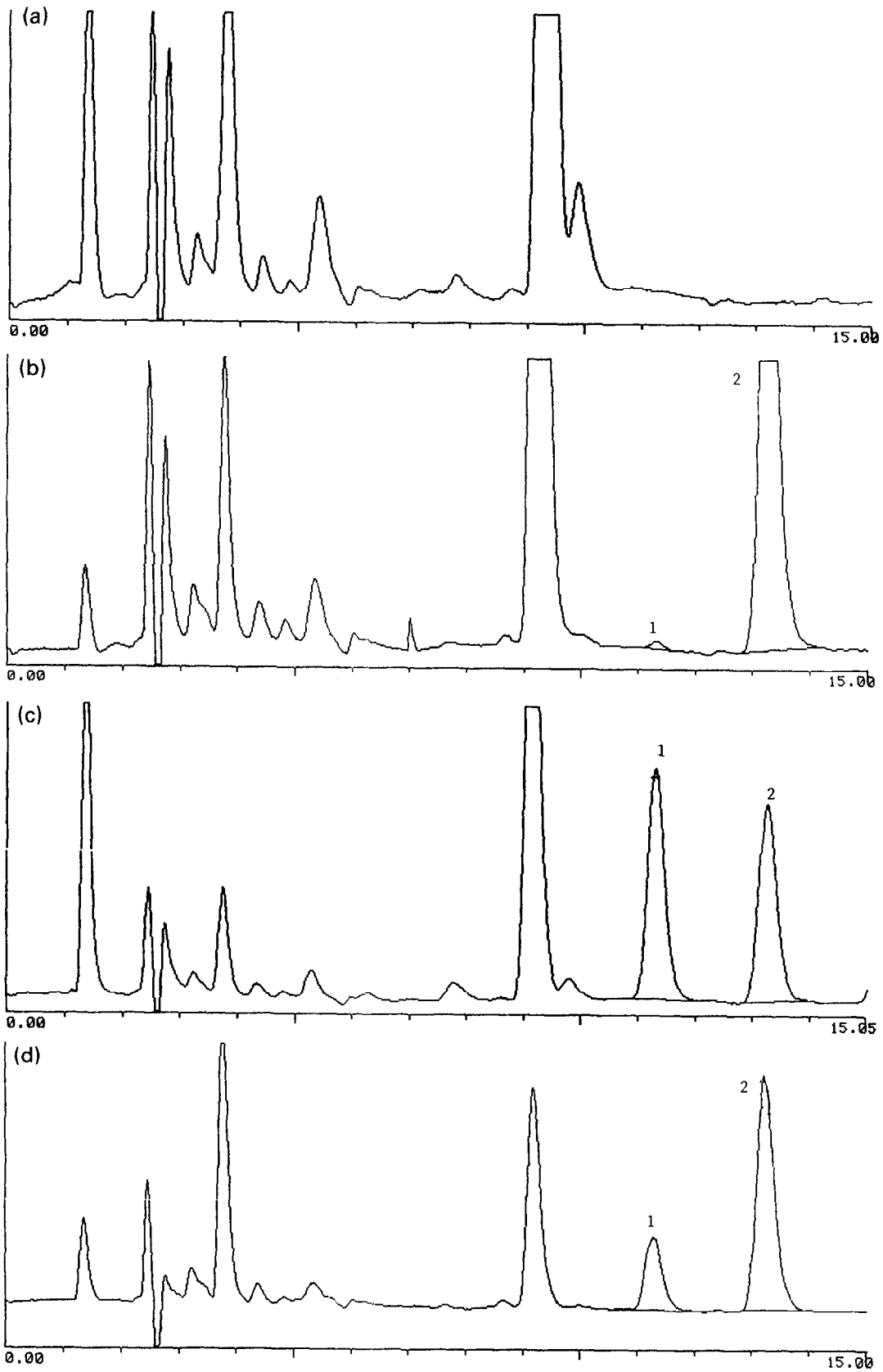

Figure 1
Samples prepared according to the described procedure (a) processed plasma blank, attenuation = 2, (b) plasma spiked at 2.0 ng ml^{-1} HCT, attenuation = 2, (c) plasma spiked at 200 ng ml^{-1} HCT, attenuation = 6, (d) subject 1.0 h after a 12.5 mg oral dose of HCT, attenuation = 5. Peak identification is as follows: HCT (1) and internal standard (2).

Table 1
Recovery of HCT and internal standard from human plasma*

Drug	Conc. (ng ml^{-1})	% Recovery	% RSD
HCT	8.0	94.6	8.5
	900.0	97.3	4.4
Internal standard	200.0	98.4	2.6

*n = 8.

tine, acetaminophen, theophylline, phenylpropanolamine, and dextromethorphan. These OTC drugs did not interfere with the analysis of HCT.

Precision and accuracy. The inter-day precision and accuracy was assessed by the repeated analyses of plasma specimens containing different concentrations of HCT (Table 2). The precision was based on the calculation of the RSD. The accuracy was based on the calculation of the relative error of the mean found concentration as compared to the actual concentration. Two samples at each QC concentration: low; medium; and high, together with a calibration curve were run as a single batch. To be regarded as a separate batch, the entire sample processing must take place in a time domain completely separate from one another. At spiked QC plasma concentrations of 8.0, 400 and 900 ng ml^{-1} for HCT the method gave RSD of 8.1, 0.8 and 1.6%, respectively. The % RE obtained from the calibration curve ranged from −7.3 to 7.5% of the nominal concentrations for HCT.

The intra-day precision and accuracy was determined by the evaluation of a typical production run. Plasma samples spiked with HCT at concentrations of 2.0, 8.0, 400 and 900 ng ml^{-1} were evaluated. The % RSD for all samples analysed were within 12.0% and the % RE ranged from 2.4 to 14.0% of the nominal concentrations. These results are presented in Table 3.

Application

Plasma samples were obtained prior to dosing and at 15 subsequent time points following a 12.5 mg oral dose of HCT. Following collection, the samples were stored at −20°C until analysed. All samples were analysed by

Table 2
Inter-day precision and accuracy of HCT in human plasma

Nominal conc. (ng ml^{-1})	n	Mean found conc. (ng ml^{-1})	SD	RSD (%)	RE (%)*
Std 2.0	6	2.15	0.138	6.4	7.5
Std 5.0	6	5.08	0.440	8.7	1.6
Std 10.0	6	10.00	0.253	2.5	0.0
Std 50.0	6	46.32	1.212	2.6	−7.3
Std 250	6	244.72	2.767	1.1	−2.1
Std 500	6	496.85	1.735	0.3	−0.6
Std 750	6	746.78	4.732	0.6	−0.4
Std 1000	6	1015.10	3.578	0.4	1.5
QC 8.0†	10	8.69	0.702	8.1	8.6
QC 400†	12	420.35	3.483	0.8	5.1
QC 400†	12	920.74	14.546	1.6	2.3

*RE = Relative error.
†QC = Quality control samples at concentration indicated.

Table 3
Intra-day precision and accuracy of HCT in human plasma

Nominal conc. (ng ml^{-1})	n	Mean found conc. (ng ml^{-1})	SD	RSD (%)	RE (%)*
Std 2.0	9	2.28	0.273	12.0	14.0
QC 8.0†	8	8.24	0.472	5.7	3.0
QC 400†	9	418.52	3.593	0.9	4.6
QC 900†	9	921.41	12.219	1.3	2.4

*RE = Relative error.
†Quality control samples at concentration indicated.

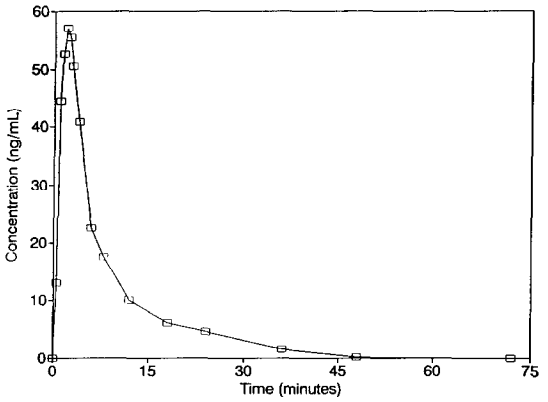

Figure 2
Representative concentration–time profile of a subject after a single 12.5 mg oral dose of HCT.

the method presented here. The time course of plasma HCT concentrations is depicted in Fig. 2.

Conclusions

The described assay for the analysis of HCT in human plasma is specific, sensitive, and robust. The intra- and inter-assay precision of the method is below 12.0%, while the accuracy of the method is within 14.0%, even at the LOQ. Furthermore, the assay is fast and requires a relatively simple sample preparation. A large number of samples can be processed daily (about 80). This method has been used to monitor plasma levels in clinical trials generating over 4000 samples.

This assay allows the quantification of HCT plasma levels for at least 48 h following a single 12.5 mg oral dose of HCT, and permits the complete characterization of the resulting plasma profile.

References

[1] M.C. Gillis (Ed.), *Compendium of Pharmaceuticals and Specialities*, 24th edn, pp. 460. Southam Murray, Toronto (1989).
[2] D. Lutz, E. Ilias and H. Jaeger, in *Glass Capillary Chromatography in Clinical Medicine and Pharmacology* (H. Jaeger, Ed.), pp. 453. Marcel Dekker, New York (1985).
[3] B. Lindstrom, M. Molander and M. Groschinsky, *J. Chromatogr.* **114**, 459–462 (1975).
[4] E. Redalieu, V.V. Tipnis and W.E. Wagner Jr, *J. Pharm. Sci.* **67**, 726–728 (1978).
[5] R.H. Barbhaiya, T.A. Phillips and P.G. Welling, *J. Pharm. Sci.* **70**, 291–295 (1981).
[6] M.J. Van Der Meer and L.W. Brown, *J. Chromatogr.* **423**, 351–357 (1987).
[7] M.J. Cooper, A.R. Sinaiko, M.W. Anders and B.L. Mirkin, *Anal. Chem.* **48**, 1110–1111 (1976).
[8] R. Weinberger and T. Pietrantonio, *Anal. Chim. Acta* **146**, 219–226 (1983).
[9] C.T. Azumaya, *J. Chromatogr.* **532**, 168–174 (1990).
[10] B. Beermann and M. Groschinsky-Grind, *Eur. J. Clin. Pharmacol.* **12**, 297–303 (1977).

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