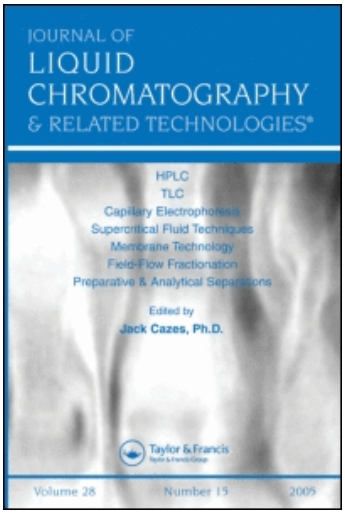

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DETERMINATION OF DICLOFENAC SODIUM IN HUMAN PLASMA BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Following a detailed study, a simple, rapid and accurate reversed-phase liquid chromatographic method has been developed for the determination of diclofenac sodium in human plasma. The sample is separated isocratically within 7 min using an octadecyl-bonded silica column and a mobile phase of methanol and sodium acetate buffer (68:32, v/v; pH 4.2). The compounds were quantitated using a ultraviolet detector operated at 274 nm which allowed determination of 0.10 - 2.50 μg/ml of diclofenac sodium with high reproducibility. The limit of detection is 0.03 μg/ml. Intra-day and inter-day coefficients of variation for assaying the plasma sample containing 0.20 μg/ml concentration of diclofenac sodium were 7.4% (n=9) and 7.6% (n=7), respectively. The extraction efficiency of diclofenac sodium were 91.3 - 93.2% for plasma. The method has been used to determine diclofenac sodium in the plasma samples from ten volunteers and provide data on the pharmacokinetics of the drug.

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INTRODUCTION

Diclofenac sodium (sodium [o-(2,6-dichloroanilino)-phenyl] acetate) is a relatively safe and effective non-steroidal drug with pronounced antirheumatic, antiinflammatory, analgesic, and antipyretic properties (1). It has been widely used for several years in the treatment of degenerative joint diseases and other arthritic conditions (2,3).

A few procedures have been reported for quantitation of diclofenac sodium in human plasma, including thin-layer chromatography (TLC) (4), gas chromatography (GC) (5-7) and high-performance liquid chromatography (HPLC) (8-15). TLC lacks the sensitivity and accuracy required for the analysis of diclofenac sodium in pharmacokinetic investigation. The GC methods are highly sensitive and specific. However, all require extensive sample preparation by extraction and derivatization prior to GC separation. In recent years, HPLC methods have been used for the determination of diclofenac sodium alone or together with its metabolites in body fluids. Nevertheless, some procedures have to employ the complex instrumentation and installation (8,9) or use expensive electrochemical detection (10,11) or fluorimetric detection (12,13). These hardly meet the needs of simplicity and rapidity for clinical drugs monitoring.

This paper describes a simple and accurate method based on one organic extraction step with hexane-isopropyl alcohol and quantitation by reversed-phase liquid chromatography with ultraviolet detection. It has been used to determine diclofenac sodium in the plasma of ten volunteers who had taken diclofenac sodium tablets, and provides data on the pharmacokinetics of the drug.

EXPERIMENTAL

Apparatus

The analysis was performed with a high-performance liquid chromatograph consisting of a Waters Model 510 pump, a U6K injector, a 490E programmable multiwavelength detector operated at 274 nm

and 0.015 AUFS and a Baseline 810 Chromatography Workstation (Waters Assoc., Milford, MA, U.S.A.). Chromatographic separations were carried out on a Spherisorb ODS column (200 x 4.6 mm I.D.; particle size 10 μm; Dalian, China) at ambient temperature. A Model Φ71 pH meter (Beckman Instruments, Fullerton, CA, U.S.A.) with a pencil combination Beckman eletrode was employed for the pH measurement of the different solutions.

Standards and reagents

Diclofenac sodium (99.9%) and Diphenylamine (the internal standard) were supplied by Mingxing Pharmaceutical Factory (Guanzhou, P.R.China) and Nanxian Reagent Factory (Shanhai, P.R.China), respectively. Diclofenac sodium tablets were provided by BEIJING CIBA-GEIGY Pharmaceutical (Beijing, P.R.China). HPLC-grade methanol (Linhai Chemicals Factory, Zhejiang, P.R.China) and sodium acetate (Shanhai Chemical Reagent Factory, Shanhai, P.R.China) were used to prepare the mobile phase. All chemicals, except where otherwise stated, were of analytical grade, and water used in this assay was doubly distilled.

Mobile phase

The mobile phase was a methanol-buffer mixture (68:32, v/v) which had been passed through a 0.45- μm membrane filter (Millipore, Bedford, MA, U.S.A.) and then degassed before use. The buffer was 0.05 M sodium acetate, prepared by dissolving 6.8 g of sodium acetate in 1000 ml of water and the pH adjusted to 4.2 with hydrochloric acid. Mobile phase flow-rate was 1.40 ml/min with a typical back-pressure of 11.5 MPa.

Preparation of solutions

A 1.00 mg/ml diclofenac sodium stock standard solution was prepared by dissolving 100 mg of diclofenac sodium in 100 ml of methanol and storing in a refrigerator. A 1.00 mg/ml diphenylamine stock solution was prepared by dissolving 100 mg of diphenylamine in 100 ml of methanol and storing in a black box in a refrigerator.

Analytical procedure

A 0.5-ml volume of a plasma sample was placed in a test tube, and 0.6 ml of 1 M phosphoric acid added. After vortex mixing for 10s, 5ml hexane-isopropyl alcohol (95:5, v/v, containing 30 ng/ml diphenylamine as an internal standard) were added and vortex mixed for 1 min, then centrifuged for 10 min at 1000 g. 4 ml of the organic layer was collected, evaporated to dryness with air at 40 °C and 150 µl of the HPLC mobile phase added to dissolve the residue. After 30 s of vortex mixing, 25 µl of this sample solution were injected into the HPLC system.

RESULTS

Chromatographic separation

Figure 1 shows typical chromatograms of a standard solution and plasma samples. Under the chromatographic conditions described, diclofenac sodium and diphenylamine (internal standard) had retention times of approximately 4.8 min and 6.4 min, respectively. It can be seen, from Figure 1, good separation and detectability of diclofenac sodium in serum was obtained with minimal interference from serum components. Hence it is relatively easy to estimate the peak area with accuracy.

Precision

Intra-day reproducibility studies, evaluated by assaying 9 plasma samples containing 0.20 µg/ml concentration of diclofenac sodium, yielded a coefficient of variation of 7.4%. Inter-day reproducibility studies, evaluated by assaying the same concentration 7 times over a 7-day period, was 7.6%. At a concentration of 0.80 µg/ml, the intra-day and inter-day coefficient of variation were 3.7% and 2.5 %, respectively.

Linearity and detection limit of method

A series of the solutions containing 0.10, 0.50, 1.00, 1.50, 2.00 and 2.50 µg/ml of diclofenac sodium were prepared to study

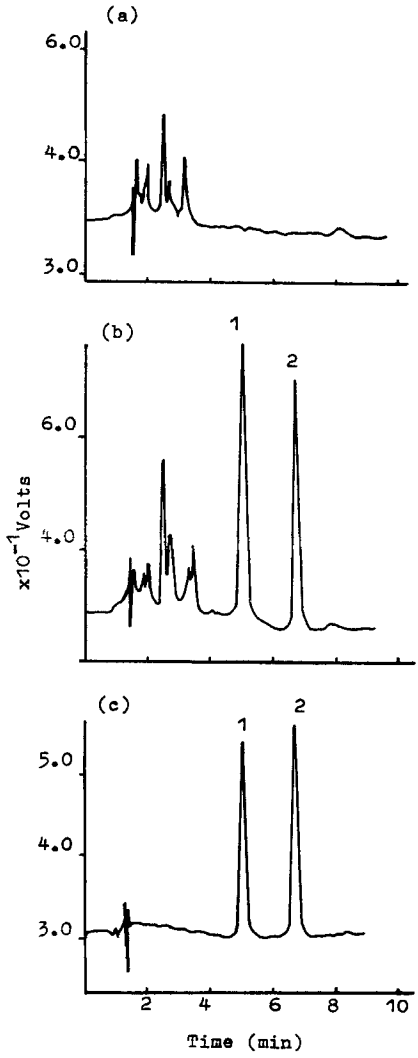

Fig. 1. Chromatograms of diclofenac sodium in human plasma and the standard solution containing 0.80 μg/ml diclofenac sodium.
(a) a plasma extract of a blank plasma.
(b) a plasma extract containing diclofenac sodium (2 h after oral administration of 50 mg of diclofenac sodium).
(c) standard solution.
1, diclofenac sodium; 2, diphenylamine.

the relationship between the peak area ratio of diclofenac sodium to diphenylamine and the concentrations of diclofenac sodium under selected conditions. The results showed that the peak area ratio was linearly related to the diclofenac sodium concentration for the range 0.10 - 2.50 μg/ml. The linear equation for the concentration versus the peak area ratio was Y=0.90X+0.02 with a correlation coefficient of 0.9994. The detection limit was 0.03 μg/ml.

Extraction efficiency

Extraction efficiencies of diclofenac sodium and the internal standard were determined by comparing peak areas of the analytes from extracted plasma standards to those from a chromatographic standard solution prepared in mobile phase at the equivalent concentration and chromatographed directly. Mean (n=7) percent recoveries (S.D.) of diclofenac sodium were 91.3 (10.1) and 93.2 (3.9) for the low (0.20 μg/ml) and high (1.20 μg/ml) concentrations, respectively. The recovery of internal standard (0.20 μg/ml) from plasma was 97.3 (9.1).

Interferences

The interference of other commonly encountered medications on the HPLC chromatogram was studied using aspirinum, chlorprophenpyridamine, ibuprofen, acidum pipemidicum, norfloxacin, ofloxacin, lomefloxacin and ciprofloxacin. No interference was observed on the detection of diclofenac peak.

Application

Ten healthy male Chinese volunteers aged 22.9±s0.7 and weighing 67.7±s4.7 kg entered the study. All volunteers gave their written consent and underwent a physical examination. There were no abnormal findings in liver and kidney functions in particular. After 12 h of overnight fasting, the volunteers received an oral dose of single 75-mg diclofenac sodium, in a randomized crossover study design. Blood samples (2.0 ml) were taken before medication and after 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10h and the plasma separated by centrifugation and was then frozen. Fig. 2 illus-

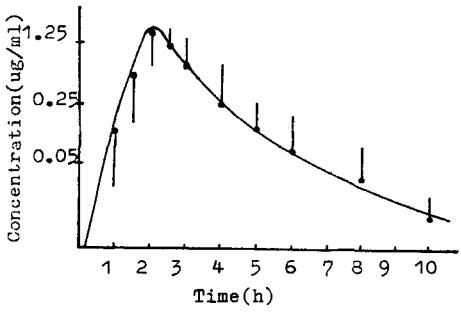

Fig. 2. Mean plasma levels of diclofenac sodium after oral administration of 75-mg of diclofenac sodium to ten human volunteers. n=10, x±s.

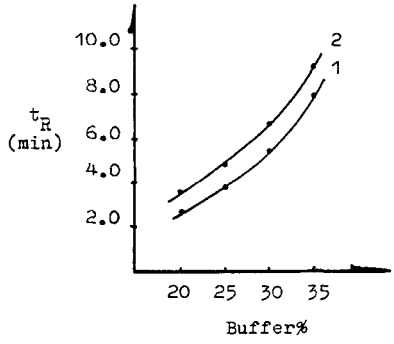

Fig. 3. Dependencies of the retention times of diclofenac sodium and diphenylamine on the composition of the mobile phase. 1, diclofenac sodium; 2, diphenylamine.

trates the plasma concentration versus time profile for diclofenac sodium in the ten volunteers.

DISCUSSION

To achieve optimum resolution, the composition of mobile phase, buffer concentration and pH were all studied systematically. A 200 x 4.6 mm I.D. octadecylsilane column was chosen, with various proportions of methanol in aqueous sodium acetate buffer and different pH values as the mobile phase. At a given buffer pH and composition of mobile phase, neither retention time changed noticeably with varying buffer salt concentration. Fig. 3 shows the dependency of the retention times of diclofenac sodium and diphenylamine on the composition of the mobile phase. Both retention times are reduced with increase in methanol content. On the other hand, the retention time of diclofenac sodium is also reduced with a increase in buffer pH (Fig. 4). However, the retention time of diphenylamine was unaltered. In addition, it has been found in the experiment that no changes in the detector response (peak area) were observed with varying composition of the mobile phase and pH. Thus, the retentions of diclofenac sodium and diphenylamine can be easily controlled within a large appropriate range by adjusting the pH or methanol content of the mobile phase. In this method the concentration of sodium acetate buffer solution was selected as 0.05 M and the pH was adjusted to 4.2 with hydrochloric acid. The ratio of sodium acetate buffer solution and methanol was selected as 32:68 (v/v), delivered at a flow-rate of 1.40 ml/min at ambient temperature.

There are several methods described that dichloromethane (10) and hexane (14) can be used as the organic solvents for the extraction of diclofenac sodium from plasma. However, it has been observed in the experiment that the emulsive phenomenon was too frequent to operate when dichloromethane was used as the extractant. When hexane was selected as the extractant, it was also not completely out of the emulsive phenomenon. However, when the hexane used was mixed with a small amount of isopropyl alcohol the emulsive phenomenon can be completely removed. In the present

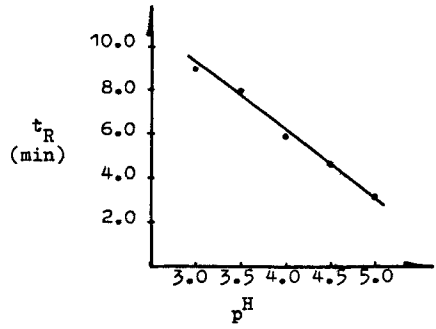

Fig. 4. Dependence of the retention time of diclofenac sodium on pH of the buffer solution.

method, the ratio of hexane and isopropyl alcohol was selected as 95:5 (v/v).

To obtain higher extraction efficiency for diclofenac sodium from plasma, a study was made of the effect of the amount of phosphoric acid added in the plasma on the extraction efficiency. The results indicated that the extraction efficiency is exceedingly low when the organic extractant is used for the extraction alone. However, it is favorable for improving the extraction efficiency to acidify the plasma before the extraction. The results showed that the extraction efficiency increased with increasing the volume of phosphoric acid (1 mol/L) added, but the responses were unchanged when the acid volume added above 0.5 ml. In this work the volume of phosphoric acid was selected as 0.60 ml.

The pharmacokinetics of diclofenac sodium were studied in 10 healthy Chinese volunteers. After single oral administration of 75 mg diclofenac sodium, the data obtained were fitted with PKBP-N1 program (16) on computer. In Table 1 are reported the pharmacokinetic parameters of diclofenac sodium administered orally to ten volunteers. The results showed that the dispostion of diclofenac sodium was conformed to a two-compartment model. Peak plasma drug concentration occur 1.8 h after ingestion and the

TABLE 1. Pharmacokinetic parameters of diclofenac sodium after administering an oral dose of single 75-mg to ten healthy Chinese volunteers

	α (1/h)	β (1/h)	K_a (1/h)	K_{12} (1/h)	K_{21} (1/h)	K_{10} (1/h)	$T_{a1/2}$ (h)	$T_{\alpha\,1/2}$ (h)	$T_{\beta\,1/2}$ (h)	$AUC_{0-\infty}$ (ug/ml.h)	T_{max} (h)	C_{max} (ug/ml)
1	2.37	0.60	3.07	0.57	1.34	1.06	0.23	0.29	1.16	2.92	2.08	1.39
2	1.91	0.45	2.00	0.34	0.61	1.40	0.35	0.36	1.55	2.66	2.00	1.48
3	2.03	0.34	2.24	0.37	0.45	1.55	0.31	0.34	2.01	3.22	2.06	1.82
4	1.86	0.41	2.13	0.26	0.50	1.51	0.32	0.37	1.70	4.09	1.58	2.26
5	1.31	0.47	1.91	0.21	0.71	0.85	0.36	0.53	1.49	4.39	1.50	1.72
6	1.72	0.25	2.89	0.53	0.43	1.01	0.24	0.40	2.73	8.31	1.86	4.12
7	1.17	0.36	1.40	0.12	0.42	0.99	0.49	0.59	1.94	4.06	2.28	1.42
8	1.90	0.44	3.10	0.35	0.59	1.39	0.22	0.37	1.59	2.95	1.32	1.82
9	2.53	0.51	2.60	0.58	0.77	1.69	0.27	0.27	1.36	2.69	1.50	1.60
10	1.35	0.32	1.70	0.24	0.43	0.99	0.41	0.52	2.17	2.84	1.63	1.04
$\bar{x}$	1.82	0.42	2.30	0.36	0.63	1.24	0.32	0.40	1.77	3.81	1.78	1.87
$\pm$SD	0.44	0.10	0.59	0.16	0.28	0.29	0.09	0.11	0.46	1.70	0.32	0.86

mean peak plasma concentration achieved is 1.87 ug/ml. Moreover, The results implied that diclofenac sodium is absorbed repidly, distributed widely in the body and also eliminated at a fairly rapid rate.

CONCLUSION

The method provided excellent recovery and good precision, and is simple and reliable in both its chromatographic conditions and sample preparation procedure. Furthermore, the analytical procedure is easy to handle and because of the short time between two injections it is very suitable for the routine determination of a large number of sample. It has been successfully applied to the analysis of plasma sample obtained during the pharmacokinetic study of diclofenac sodium in ten healthy volunteers participating in a diclofenac sodium single oral dose clinical trial.

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