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Short communication

High-performance liquid chromatographic determination of diclofenac in human plasma after solid-phase extraction

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Abstract

A novel high-performance liquid chromatographic (HPLC) method for the quantification of diclofenac in human plasma was set up. Samples, added with ibuprofen (used as internal standard) were purified by solid-phase extraction using Abselut Nexus cartridges (Varian) not requiring pre-conditioning. Drugs of interest were eluted directly into the autosampler vials and injected. The recovery of diclofenac was 92%, the analysis lasted 7 min with a sensitivity of 5 ng/ml and intra- and inter-day RSDs of 3 and 8%, respectively. The pharmacokinetics of diclofenac after oral and rectal administration in 10 healthy volunteers are reported.

Keywords: Diclofenac

1. Introduction

Diclofenac sodium or sodium[-*o*-(2,6-dichlorophenyl)-amino-phenyl] acetate (DIC), is the active substance of Voltaren and one of the most popular non-steroidal anti-inflammatory drugs (NSAIDs), exhibiting also analgesic and antipyretic properties. DIC is well absorbed orally and dissolves in the intestinal fluid [1,2]. Several methods have been described for the quantification of DIC in plasma based on different extraction procedures and coupled to gas chromatography–mass spectrometry [3,4] or high-performance liquid chromatography (HPLC) either with UV [5,6], fluorimetric [7] or electrochemical detection [8]. El-Sayed et al. [9] described a rapid procedure consisting of protein precipitation with acetonitrile and HPLC analysis with UV detection at 280 nm. Applying these conditions, it was possible to detect up to 25 ng/ml in plasma. The use of electrochemical detection, besides decreasing the limit of quantification to 10 ng/ml, permitted one to use a low amount of whole blood sample (100 μl) [8]. In 1998 Giagoudakis [2] proposed a HPLC method with liquid–liquid extraction and UV detection at 278 nm, reaching a sensitivity of 20 ng/ml.

In this paper we propose an alternative method for the determination of DIC in human plasma by HPLC and UV detection at 280 nm. The main features are

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an improved sensitivity with respect to previously reported methods based on the same technique and a pre-analytical phase with a high sample throughput. The potential of this method was tested on healthy volunteers to compare bioavailability from a semi-solid (suppositories) and a solid (slow-release tablets) DIC product available for rectal and oral administration.

2. Experimental

2.1. Standards

DIC and ibuprofen (IBP) standard powders were purchased from Sigma (St. Louis, MO, USA). DIC stock solution was prepared as 1 mg/ml in methanol. The working solutions (1, 5, 10 μg/ml) were obtained by diluting the stock with methanol. IBP, used as internal standard (I.S.) was prepared as 5 mg/ml in methanol.

2.2. Reagents

All reagents were of HPLC grade. Acetonitrile and hydrochloric acid (HCl) were purchased from BDH (Milan, Italy).

2.3. Solid-phase extraction (SPE)

SPE cartridges Abselut (Nexus bonded phase, 30 mg) (Varian, Harbor City, CA, USA) used in this study do not require pre-activation washings. To plasma samples (1 ml) were added I.S. (20 μl=100 μg), HCl 7 *M* (100 μl), then diluted with water (1 ml), centrifuged (4000 rpm, 10 min) and loaded onto the cartridges. After low vacuum application (5 in.Hg; 1 in.Hg=388.638 Pa) by a Visiprep Vacuum Manifold (Supelco, Bellefonte, PA, USA), cartridges were rinsed with 1 ml water, followed by 5, 20, 40% (v/v) methanol–water mixtures (1 ml each). After flushing with air for 1 min, DIC and IBP were recovered by applying 200 μl of methanol followed by a mixture of methanol–acetonitrile–ethyl acetate (35:35:30, v/v/v) (200 μl). These washings were directly collected into polypropylene vials and after loading the HPLC autosampler, 100 μl was injected into the column.

2.4. Chromatographic conditions

A HPLC system (Kontron Instruments, Zurich, Switzerland) composed of two pumps (Model 420), an autosampler (Model 460), a double-beam UV detector (Model 430) was used. The KromaSystem 2000 (Bio-Tek Instruments, Milan, Italy) software was employed for storage and manipulation of data. A reversed-phase column C_{18} (250 mm×4.6 mm I.D., 5 μm) (Merck, Darmstadt, Germany) was eluted in isocratic mode with a mixture of dihydrogenphosphate potassium salt (KH_2PO_4, 25 m*M*, pH 3.5)–acetonitrile (30:70, v/v) at a flow-rate of 1 ml/min. Detection was at 280 nm. To verify the chromatographic performance, a mixture of DIC and IBP (20 μl, 50 ng DIC and 50 μg IBP) was injected daily into the column (Fig. 1).

2.5. Clinical study

After an overnight fast, at 08.00, before breakfast, 10 healthy volunteers (five men and five women) aged 20–35 years, were allocated in a randomized cross-over protocol to receive either a slow-release tablet (1×100 mg Voltaren Retard; Novartis Farma S.p.a, Italy) or a single suppository (1×100 mg Voltaren; Novartis Farma S.p.a). Blood samples were collected in heparinized Vacutainer tubes before drug administration (time 0) and after 30, 60 min, 2, 4, 6, 8, 10 and 24 h. Plasma was obtained by centrifugation at 3000 *g* for 10 min and immediately frozen at −20°C. Body mass index was normal in both men and women. Women received treatments within the fifth day of the menstrual cycle and were not under oral contraceptive therapy. After 1-week wash-out period all subjects received DIC at the same dose but by a different administration route.

2.6. Statistics

Statistical analysis was performed by using the Sigma Stat statistical package (Jandel Scientific, Erkrat, Germany). The area under the concentration–time curve in plasma (AUC_{plasma}) was calculated by the trapezoidal rule. To locate significant differences between treatments, the Student's *t*-test was used to compare the parameters C_{max}, T_{max} and AUC_{plasma}. Statistical significance was assumed at $P<0.05$.

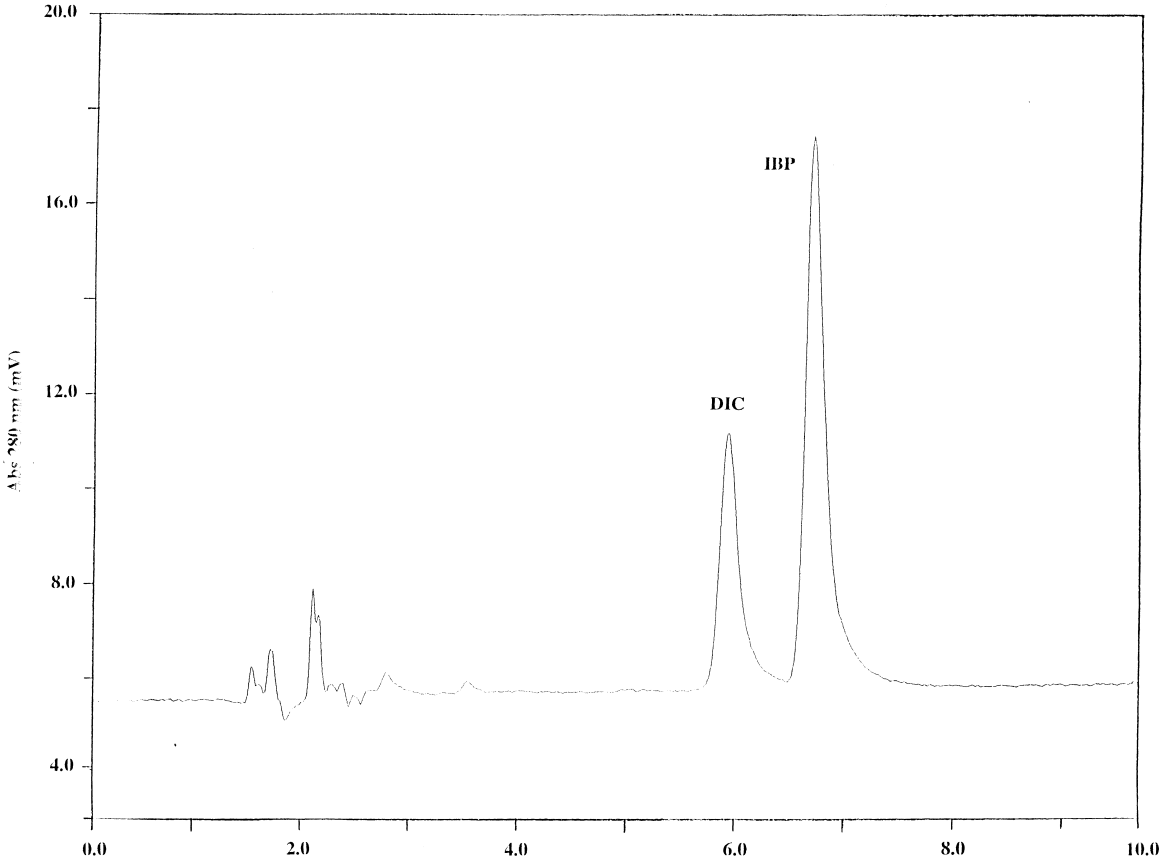

Fig. 1. Chromatographic profile of diclofenac (DIC) and ibuprofen (IBP) standard mixture (20 μl=50 ng DIC, 50 μg IBP). The column was eluted at 1 ml/min in isocratic mode with a mixture of acetonitrile–25 m*M* KH_2PO_4, pH 3.5 (70:30, v/v). Detection at 280 nm.

3. Results and discussion

Fig. 1 reports the chromatographic profile of a pure standard mixture of DIC and of IBP which retention times (t_R) were 5.87 and 6.75 min, respectively. In Fig. 2 are superimposed the chromatograms obtained from a blank plasma sample and from a patient's sample collected 30 min after 100 mg Voltaren Retard tablet administration (DIC concentration 50 ng/ml). DIC and IBP peaks were well resolved from the typical endogenous components of plasma that elute with the solvent front at the beginning of the chromatogram. The sample extracted without addition of DIC and IBP did not evidence interfering peaks at the t_R of the compounds of interest. Specificity of the chromatographic analysis was also confirmed by the fact that none of the compounds listed in Table 1 showed a retention time similar, or co-eluted with DIC and IBP.

The HPLC method proposed here showed some significant advantages with respect to those previously published. The pre-analytical procedure was cost- and time-saving and easy to perform. The solid-phase cartridges used do not require pre-conditioning or particular attention to matrix drying between washings, and allowed the technician to manually process up to 24 samples in less than 60 min. The possibility to elute the compounds of interest in a small volume of organic solvent collected directly into the HPLC autosampler vials was even more attractive and allowed one to skip the long and tedious drying step necessary with the classic liquid–liquid extraction.

The method showed good linearity both at high

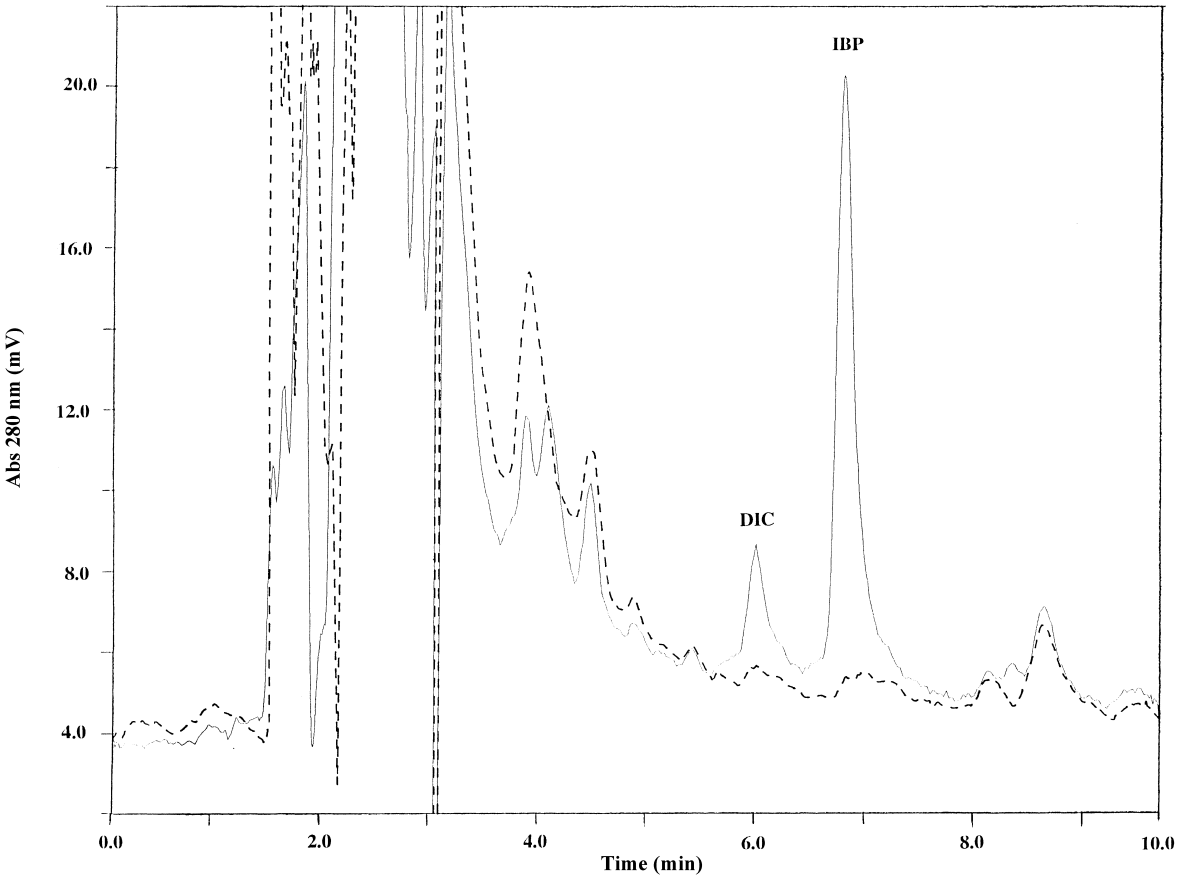

Fig. 2. HPLC profile of a control plasma extracted without DIC and IBP addition superimposed to a plasma sample collected 30 min after oral administration of a Voltaren Retard 100 mg slow-release tablet. The estimated DIC concentration was 50 ng/ml. The analysis was carried out under the chromatographic conditions described in Fig. 1.

and low DIC concentrations. Aqueous and plasma standard curves were set up in low (5–80 ng/ml) and high (100–3000 ng/ml) concentration ranges. Both curves gave good performance: the linear regression equations for the low concentration range were $y=0.00321(\pm0.0001)x+0.0029(\pm0.0015)$ and $y=0.00294(\pm0.0003)x+0.0043(\pm0.004)$ (r^2 0.999) for water and plasma ($n=5$), respectively. In the high concentration range equations were: $y=0.00281(\pm0.0002)x+0.0076(\pm0.0001)$ and $y=0.00271(\pm0.0001)x+0.0019(\pm0.0001)$ (r^2 0.999) for aqueous and plasma curves ($n=5$), respectively. The recoveries of pure aqueous standard of DIC from the solid-phase extraction were 103±12% and 100±5% at 100 and 2000 ng/ml, respectively ($n=19$). The recoveries of DIC from plasma at the same concentrations were 92±11% and 95±6% ($n=11$). IBP (100 μg/ml) was recovered 107±8% ($n=19$) and 95±8% ($n=12$) from water and plasma, respectively. The intra-day imprecision (RSD) was tested on samples with DIC concentration at 50, 100 and 1000 ng/ml and was 5.1, 3.2 and 2.2%, respectively ($n=5$). The inter-day imprecision on the same samples gave RSDs 7.8, 8.6 and 2.5% ($n=5$).

The limit of detection for DIC was 1.2 ng, while the good signal-to-noise ratio allowed one to reach the limit of quantification of 5 ng/ml.

DIC plasma concentrations as a function of time for the slow-release tablets and for the lipophilic rectal formulation are shown in Fig. 3. Curves were traced on the the mean concentration calculated from the 10 healthy volunteers at each time. As expected,

Table 1
Retention times of some drugs tested as possible interferent compounds

Drug	Retention time (min)
Acetylsalicylic acid	2.3
Allopurinol	2.0
Atenolol	3.9
Benzensulfonamide	2.5
Betamethasone	2.5
Caffeine	3.6
Chlorodiazoepoxyde	2.5
Chlorpromazine	2.9
Dexamethasone	2.9
Fenquizone	2.0
Glibenclamide	4.5
Hydrocortisone	2.5
Hydantoin	2.8
Indomethacin	5.5
Lidocaine	n.d.
Ludiomil	n.d.
Nicardipine	4.0
Oxepam	3.0
Phenobarbital	3.7
Phenyl glyoxalic acid	2.3
Primidone	6.0
Theofilline	2.3
Warfarin	4.0

n.d.: Not detectable under the analytical conditions used.

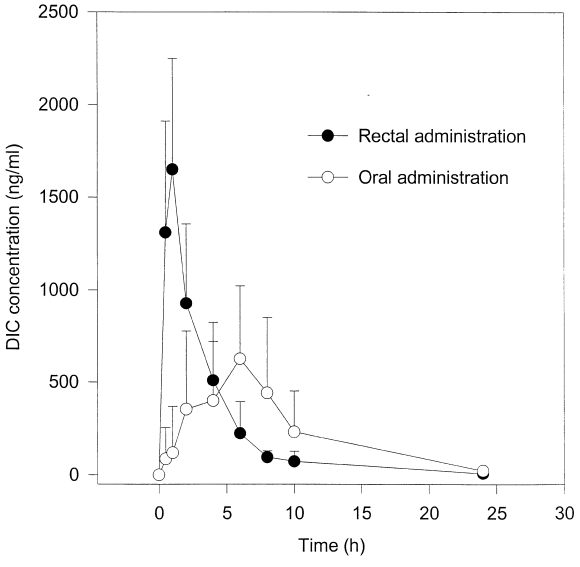

Fig. 3. Mean plasma concentrations of DIC after a single oral administration of a Voltaren Retard 100 mg slow-release tablet (○) or a single rectal administration of a Voltaren 100 mg suppository (●). Values are mean±SD of 10 subjects.

absorption occurred more quickly after rectal administration (T_{max}=1.0±0.5 h), with a concentration peak (C_{max}) significantly higher (1650±600 ng/ml) than after the slow-release oral one (C_{max}=630±390 ng/ml, T_{max}=6.0±2.0 h) ($P<0.05$ C_{max} oral vs. rectal). The between-subject variability observed in the pharmacokinetics curves was not related to sex. Ten hours after oral administration, DIC plasma concentration was still relevant (230±110 ng/ml) while after rectal administration DIC was mainly metabolised and/or excreted while the parent compound in plasma accounted for 73 ng/ml. Bioavailability of the two drug formulations, however, was not different, with a mean AUC_{plasma} of 5340±2840 ng h/ml after the oral administration and 5460±2220 ng h/ml after the rectal administration, in perfect agreement with previously published data [2,9].

The above pharmacokinetics findings may be relevant to the analgesic treatment of acute pain.

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