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Stereospecific determination of tiaprofenic acid in plasma: problems with drug degradation

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Abstract

A sensitive stereospecific high-performance liquid chromatographic assay for the quantification of tiaprofenic acid in human plasma was developed. The procedure involved extraction of tiaprofenic acid from acidified plasma into hexane–diethyl ether (8:2, v/v). Stereospecific separation was achieved with a prepacked α_1-acid glycoprotein column without derivatization. The mobile phase consisted of 2% 2-propanol in 0.01 *M* phosphate buffer, pH 6.5. Tiaprofenic acid was detected at 317 nm. The limit of quantification was found to be 25 ng/ml for each enantiomer using a 0.5 ml plasma sample. The assay was reproducible and accurate to be applied to the stereoselective pharmacokinetic analysis of tiaprofenic acid in plasma. Because of photoinstability of tiaprofenic acid plasma sampling and sample extraction should be performed under light protection.

Keywords: Tiaprofenic acid

1. Introduction

Tiaprofenic acid [(±)5-benzoyl-α-methyl-2-thiophene acetic acid; TIA] is a chiral 2-arylpropionic acid (2-APA) derivative with analgesic, antipyretic and anti-inflammatory activity [1]. As the enantiomers of many 2-APAs are shown to possess different pharmacodynamic and pharmacokinetic behaviour it is of clinical relevance to determine TIA in biological fluids stereoselectively [2–5]. Indirect stereospecific HPLC assays by formation of diastereomers [6] and a direct stereospecific HPLC assay of TIA using immobilized human serum albumin as chiral stationary phase are described [7]. The pharmacokinetics of the enantiomers resulting from indirect and direct analytical methods, however, are not consistent probably due to racemization during derivatization procedure as discussed by Muller et al. [7].

Consequently, we developed another simple and rapid stereospecific HPLC method using a chiral α_1-acid glycoprotein column (AGP) which proved to be suitable for the highly sensitive HPLC quantification of the enantiomers of other 2-APAs, especially ketoprofen, ibuprofen, fenoprofen and flurbiprofen [8,9].

Moreover, TIA, which is suspected to cause phototoxic side effects, has been shown to be a photo-unstable compound with various degradation products of known structure [10]. Published papers on chromatography did not take into account this important issue of photoinstability although instabili-

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ty at room temperature was reported [11]. We, therefore, investigated the kinetics of degradation of TIA enantiomers under normal laboratory-light conditions and light protection.

2. Experimental

2.1. Chemicals

The enantiomers of TIA were generously supplied by PharmaTrans Sanaq (Basel, Switzerland). The optical purity of the *S*- and *R*-enantiomers as determined by optical rotation and HPLC was 95.4% and 94.9%, respectively. The internal standard (I.S.) (−)-indoprofen was a gift from Carlo Erba (Freiburg, Germany). All the chemicals and organic solvents used were of HPLC or reagent grade. The mobile phases were freshly prepared, filtered (0.45 μm) and degassed under vacuum prior to use. Stock solutions (concentration 1 mg/ml) were prepared by dissolving an appropriate amount of the TIA enantiomers, respectively in 0.01 *M* phosphate buffer (pH 6.5). Standard solutions were prepared in drug-free plasma from the stock solutions to yield concentrations from 25 ng/ml to 20 μg/ml. All the drug solutions were stored at 4°C in brown glass tubes in order to prevent light-induced degradation.

2.2. Stereospecific determination of tiaprofenic acid

The HPLC system consisted of a Model SP 8810 pump (Spectra Physics, Darmstadt, Germany), a Model SP 100 UV monitor (Spectra Physics) fitted with a Model 231 diluter-autosampler (Gilson/Abimed, Langenfeld, Germany) and a CR 3A Shimadzu integrator (Shimadzu, Egling, Germany). Stereospecific separation was achieved with an AGP column (100 mm×4.0 mm I.D., 5 μm, Grom, Herrenberg, Germany). To avoid temperature-dependent fluctuations in retention time and selectivity, a column thermostat (Chemdata, Sinsheim, Germany) was used and set up at 17°C. The mobile phase consisted of 2% 2-propanol in 0.01 *M* phosphate buffer pH 6.5. The flow-rate was 0.7 ml/min. Detection wavelength was set at 317 nm.

2.3. Sample preparation procedure

Similar to the analytical procedure described for flurbiprofen recently [9], a 500-μl aliquot of human plasma spiked with I.S. (either 1 μg/ml or 10 μg/ml) was acidified by adding 200 μl 2 *M* hydrochloric acid, followed by extraction into 5.5 ml ice-cooled hexane–diethyl ether (8:2, v/v). After centrifugation (5 min at 1500 *g*) 5.0 ml of the organic layer were removed and evaporated to dryness under dry nitrogen. The residue was redissolved in 500 μl (1–20 μg/ml) or 250 μl (25–500 ng/ml) 0.01 *M* phosphate buffer (pH 6.5), respectively. The injection volume was 50 μl (high concentrations) and 100 μl (low concentrations), respectively. Standard curves were prepared by injecting extracted plasma samples spiked with the *R*- and *S*-enantiomers of TIA to yield concentrations in a range between 25–500 ng/ml (1 μg/ml I.S.) and 1–20 μg/ml (10 μg/ml I.S.). The validation data were obtained for both enantiomers together, i.e. each plasma sample was spiked with *R*- and *S*-TIA from different stock solutions, respectively. For all extraction steps brown glass tubes were used.

2.4. Precision of the assay

Five concentrations ($n=4$) of plasma standards in the range 25–500 ng/ml and 1.0–20.0 μg/ml, and four quality control samples were determined in a day in order to achieve the intra-day precision values. The inter-day precision was determined by performing single measures of spiked plasma samples over six days in the range 25–500 ng/ml and 1.0–20.0 μg/ml, respectively. Each day, two quality control samples were analyzed as well. Concentrations were back-calculated after plotting peak-area ratios of TIA enantiomers to I.S. versus concentrations of the standards.

2.5. Recovery values

The recoveries of the enantiomers of TIA and of internal standard (at a concentration of 1 μg/ml and 10 μg/ml) were determined by comparing extracted spiked samples with those of equivalent standards injected into the HPLC without prior extraction in the range of 25 ng/ml–20 μg/ml plasma.

2.6. Stability of tiaprofenic acid

TIA enantiomers (10 μg/ml) in 0.01 *M* phosphate buffer (pH 7.4) were stored in colourless glass tubes and in brown glass tubes under laboratory-light conditions. Samples were drawn at different time points (0, 0.5, 1, 2, 3, 4, 5, and 6 h) and analyzed with the assay described above. Furthermore spiked plasma samples (10 μg/ml, $n=3$) were treated according to the same scheme like buffer samples and were extracted in colourless glass tubes and analyzed under laboratory-light conditions in comparison with drug extraction under complete light protection. Long-term stability was determined for TIA samples stored in brown glass tubes at −30°C over twelve weeks.

2.7. Application

The utility of the method was demonstrated after oral administration of 300 mg of racemic TIA (Surgam) to three healthy volunteers. Blood samples were collected up to 8 h under light protection. Plasma was frozen immediately and stored at −30°C in brown glass tubes.

3. Results and discussion

Typical chromatograms of blank human plasma, plasma obtained from a volunteer 5 h following oral administration of racemic TIA (300 mg) are shown in Fig. 1. The retention times were 20.0 and 24.9 min for *R*- and *S*-TIA and 15.8 min for the internal standard (−)-indoprofen, respectively. The limit of quantification (the lowest concentration that could be determined during the intra-day and inter-day validation with precision and accuracy of less than or equal to 15%) was 25 ng/ml for each enantiomer. The recovery values (Table 1) were within 93.0 and 106.2% (mean) for TIA enantiomers and 86 and 82% (mean) for I.S. at a concentration of 10 μg/ml and 1 μg/ml, respectively. The peak-area ratios of the compounds were linearly related ($r>0.999$) to the amount of TIA enantiomers added to human plasma in the ranges of 25–500 ng/ml and 1–20 μg/ml using two calibration curves. The intra-day and inter-day validation data, summarized in Table 1, demonstrate that the method is sufficiently precise and sensitive for the quantification of TIA enantiomers in plasma provided that complete light protection was performed. Although there are no problems with optical stability of TIA enantiomers like racemization [7], there are substantial problems with light-induced drug degradation which are not mentioned in methodological papers on TIA quantification published previously. In contrast, Nilsen et al. found that the drug is stable in dialysis buffer solutions during 24 h [12].

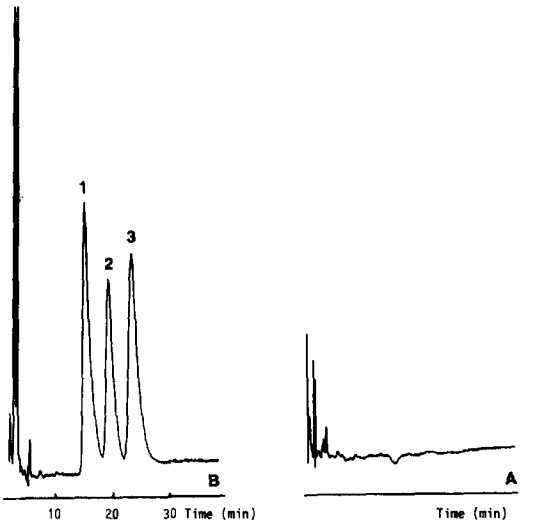

Fig. 1. Chromatograms of plasma extracts. (A) Blank human plasma. (B) Human plasma obtained 5 h after administration of racemic tiaprofenic acid (300 mg) to a healthy volunteer (I.S., (−) indoprofen). The retention times were 15.8, 20.0 and 24.9 min for (−) indoprofen (1), *R*-tiaprofenic acid (2) (concentration: 1.06 μg/ml), and *S*-tiaprofenic acid (3) (concentration: 1.64 μg/ml), respectively.

The concentration–time profiles of *R*-TIA in buffer solution and in plasma under normal laboratory-light conditions and light protection are shown in Fig. 2. No differences between the TIA enantiomers could be observed. After 2 h under normal daylight conditions more than 50% of the drug was degraded, whereas in brown glass tubes no instability was observed. Sample preparation under normal laboratory-light conditions without light protection and immediate HPLC analysis resulted in a drug loss of at least 10% as referred to analogous procedure with complete light protection. Furthermore, the substance

Table 1
Analytical recovery, intra-day (day 1) and inter-day precision of tiaprofenic acid enantiomers in human plasma

	Recovery (mean±S.D., $n=10$) (%)	Day 1 (mean±S.D., $n=5$) (%)	C.V. (%)	Days 2–7 (mean±S.D.) (%)	C.V. (%)
R-TIA					
25.0 ng/ml	101.8±2.6	24.1±3.4	14.1	26.4±2.2	8.3
50.0 ng/ml	106.2±3.9	54.6±7.0	12.8	50.4±4.7	9.3
100.0 ng/m	97.3±3.2	106.9±1.2	1.1	105.3±11.5	10.9
250.0 ng/m	93.2±2.2	248.6±0.8	0.3	244.6±21.3	8.7
500.0 ng/m	94.8±2.0	498.9±0.9	0.2	502.6±2.4	0.5
1.00 μg/ml	101.4±3.9	1.04±0.01	0.96	1.01±0.06	5.94
2.50 μg/ml	99.6±2.6	2.64±0.05	1.89	2.51±0.14	5.58
5.00 μg/ml	97.4±2.9	5.30±0.17	3.21	5.31±0.12	2.26
10.00 μg/ml	97.3±2.1	10.24±0.05	0.49	10.33±0.30	2.90
20.00 μg/ml	98.1±1.4	20.10±0.30	1.49	19.95±0.0	0.20
S-TIA					
25.0 ng/ml	97.1±6.2	24.6±3.7	15.0	25.0±3.0	12.0
50.0 ng/ml	100.7±1.7	59.2±6.1	10.3	47.1±3.1	6.6
100.0 ng/ml	98.9±2.1	110.7±6.5	5.9	98.3±10.0	10.1
250.0 ng/ml	93.0±1.2	244.6±0.9	0.4	239.0±22.4	9.4
500.0 ng/ml	94.5±1.7	499.3±1.4	0.3	495.9±13.2	2.7
1.00 μg/ml	98.0±1.9	0.96±0.01	1.04	0.95±0.07	7.37
2.50 μg/ml	100.3±2.3	2.45±0.04	1.63	2.44±0.20	8.20
5.00 μg/ml	96.7±2.6	5.01±0.19	3.79	5.22±0.33	6.32
10.00 μg/ml	98.7±1.7	9.94±0.05	0.50	10.07±0.43	4.27
20.00 μg/ml	99.5±1.3	19.96±0.31	1.55	19.95±0.05	0.25

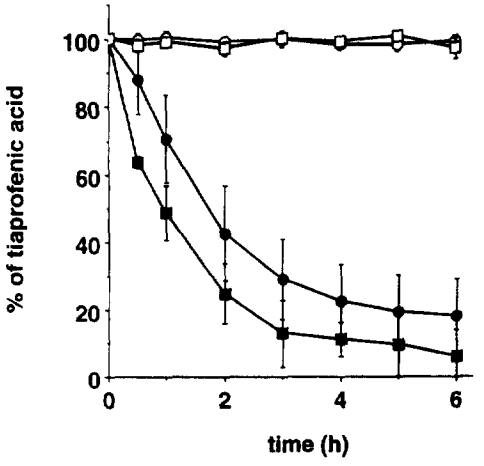

Fig. 2. Stability of *R*-tiaprofenic acid (10 μg/ml) in buffer solution (pH 7.4) and plasma under light protection and daylight conditions. For the determination of the light-induced degradation of TIA in plasma, samples were extracted without light protection. Values are mean±S.D. ($n=3$ days). As no differences between enantiomers were detected only data of the *R*-enantiomer are shown. □, light protection: buffer; ○, light protection: plasma; ■, daylight: buffer; ●, daylight: plasma.

was stable over a period of at least twelve weeks stored at −30°C in the dark.

Consequently, for accurate determination of plasma concentrations it is essential to complete plasma sampling and sample extraction as fast as possible and to use light protection wherever possible. Otherwise the plasma concentrations measured present an underestimation of real values. Fig. 3 shows a representative concentration time course of TIA enantiomers in plasma after oral administration of 300 mg racemic compound with rapid sample extraction under complete light protection. In line with the findings of Muller et al. [7] in the subject studied the concentrations of *S*-TIA were found to be higher than that of the *R*-enantiomer at every time point. The difference, however, was marginal during the first 4 h but became greater progressively with time (Fig. 3). Our method appears to be suitable for pharmacokinetic studies of TIA as the observed stereoselectivity was not detected by Singh et al. [13] who used a pre-column derivatization assay. In order to investigate whether chiral inversion is responsible for the observed stereoselectivity the pure enantio-

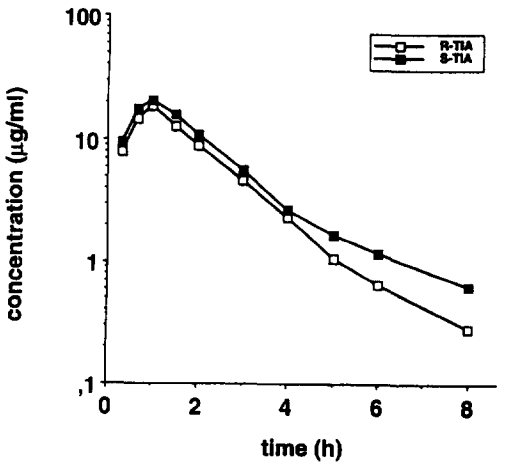

Fig. 3. Representative plasma concentration–time courses of *R*- and *S*-tiaprofenic acid following oral administration of 300 mg of racemic tiaprofenic acid to a healthy volunteer.

mers rather than the racemic compound have to be administered.

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