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High performance capillary electrophoresis for determination of the enantiomers of 2-arylpropionic acid derivatives in human serum Pharmacokinetic studies of ketoprofen enantiomers following administration of standard and sustained release tablets

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Abstract

A stereospecific capillary zone electrophoresis (CZE) method for determination of the enantiomers of some 2-arylpropionic acid derivatives (2-APA, profens) in human serum has been developed. Racemic-ibuprofen (rac-IBP), racemic-flurbiprofen (rac-FBP), racemic-ketoprofen (rac-KTP) and (+)-*S*-naproxen ((+)-*S*-NPX—an internal standard) were chosen for these studies. The 2-APA enantiomers were extracted from acidified serum samples using methylene chloride separated in a fused silica capillary. The capillary was filled with a background electrolyte, which consisted of 0.05 M heptakis 2,3,6-tri-*O*-methyl-β-cyclodextrin (TMβCD) (chiral selector) in 0.02 M triethanolamine-phosphate buffer of pH 5.0. Separation and resolution of the enantiomer mixture were obtained in one analytical run. The calculated electrophoretic parameters of the analytes were as follows: electrophoretic mobility, $\mu_{ep(-)\text{-}R} = -0.75 \times 10^{-4}$ to -0.30×10^{-4} cm^2/V s; $\mu_{ep(+)\text{-}S} = -0.83$–$(-0.38)$ cm^2/V s and electroosmotic mobility, $\mu_{EOF} = 2.35 \times 10^{-4}$ cm^2/V s, migration times, $t_{migr\,R} = 12.55$–16.07 min; $t_{migr\,S} = 13.08$–16.9 min, resolution factors, $R_S = 1.88$–3.70 and chiral selectivity, $\alpha = 1.16$–1.34. The method developed for the enantiomers was validated. The calibration curves were linear in the range of 0.5–50.0 μg/ml for FBP or KTP and of 1.0–50.0 μg/ml for IBP enantiomer concentrations. Recovery of the enantiomers from serum was about 90%. At the limit of quantification (LOQ) precision and accuracy were within 15%. The validated method was successfully applied to pharmacokinetic and bioavailability studies on KTP enantiomers in humans after administration of standard and sustained release tablets of rac-KTP. Significant differences in the pharmacokinetic parameters of both formulations were observed and the studied formulations were not bioequivalent.

Keywords: 2-Arylpropionic acid derivatives; Capillary zone electrophoresis; Validation parameters; Enantiomers' pharmacokinetics; Bioavailability

1. Introduction

2-Arylpropionic acid derivatives (profens) belong to the group of non-steroidal anti-inflammatory drugs

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(NSAIDs) with analgesic and antipyretic actions. The 2-APA propionic acid side chain possesses an asymmetric α-carbon and, therefore, occurs as (+)-*S* or (−)-*R* enantiomers. Although their pharmacological activity resides principally in the (+)-*S* enantiomer (eutomer), they are marketed in the form of their racemic (rac-) mixture (except for NPX). However, recently IBP and KTP have been marketed as the (+)-*S* enantiomer formulations. The in vivo inactive (−)-*R* enantiomer (distomer) of some profens can undergo a unidirectional chiral inversion to (+)-*S* enantiomer via the formation of its acyl CoA thioester [1]. 2-APAs inhibit activity of cyclooxygenase (COX-1, COX-2) of arachidonic acid with a reduced tissue production of prostaglandins such as PGE_2, $PGF_{2\alpha}$ [2].

Capillary electrophoretic methods for the determination of 2-APA derivatives have been developed for racemates or enantiomers in pure samples [3–5] and only rarely for enantiomers in biological fluids [6]. A suitable chiral selector plays a key role in a chiral resolution and dextrin derivatives are frequently used for the resolution of chiral drugs like profens. They are cyclic oligosaccharides consisting of 6, 7 or 8 D-(+) glucose units, linked by α-1,4 bonds and include α-, β- and γ-cyclodextrins. In order to improve their poor solubility methyl, carboxymethyl or hydroxypropyl derivatives are used with derivatization in the 2, 3 or 6 positions. Enantioselective recognition is explained by interactions between CDs, which have many chiral centers, and the guest enantiomer [7]. Heptakis 2,3,6-tri-*O*-methyl-β-cyclodextrin (TMβCD) was found to be an optimal compound for separation of profens [3–5]. The aim of the work was development of a suitable, rapid and stereospecific CZE method designed for pharmacokinetic and bioavailability studies on some 2-APA enantiomers: IBP, FBP, KTP and NPX in human serum.

2. Experimental

2.1. Materials

Rac-KTP (melting point (m.p.) 94–97 °C) and (+)-*S*-KTP (m.p. 75–78 °C), optical purity (o.p.) 99.0% (*S*:*R* was 99:1%), (+)-*S*-NPX (internal standard, IS) (m.p. 154–155 °C) (o.p. 98.0%), rac-FBP (m.p. 110–112 °C) and (+)-*S*-FBP (m.p. 109–110 °C) (o.p. 98.0%) and heptakis TMβCD were purchased from Sigma (St. Louis, MO), (−)-*R*-IBP (m.p. 54 °C) (o.p. 100%) and (+)-*S*-IBP (m.p. 54 °C) (o.p. 99.6%) from Ethyl Corporation (Orangeburg, S.C.), rac-IBP (m.p. 75–78 °C) from Polfa (Pabianice, Poland), rac-indobufen (rac-INDB) (m.p. 183 °C) and (+)-*S*-INDB (m.p. 198 °C) (o.p. 99.6%) from Pharmacia and Upjohn (Milan, Italy). Eighty-five percent of *ortho*-phosphoric acid (P.O.CH., Gliwice, Poland) and triethanolamine (Applied Science Laboratories Inc., State College, PA), 1.0 and 0.1 M NaOH (Agilent Technologies, Waldbroon, Germany) were used. Methanol (Merck, Darmstadt, Germany) was of HPLC grade. Demineralised water was always used (Seradest USF 1900, USF Seral, Germany).

2.2. Equipment and CE conditions

2-APA enantiomers were determined on an Agilent model ^{3D}CE apparatus (Agilent Technologies, Waldbronn, Germany) with the UV detector set at $\lambda = 253$ nm. Serum samples after methylene chloride extraction and reconstitution in methanol–water solution were automatically injected using hydrodynamic injection at the anode end. The temperature of the capillary was maintained by a thermostatic system at 35 °C. An Agilent fused silica capillary of 75 μm i.d. and of a 60-cm total length (effective length of 51.5 cm) was used for separation of 2-APA enantiomers. The apparatus was equipped with ChemStation software for instrument control, data acquisition and data analysis. The system was controlled by Windows NT software. All experiments were carried out at 20 kV and 50 × 5 mbar s injection (35 nl injected volume). The volume of sample loaded on to the capillary (V) was calculated using the Hagen–Poiseuille equation: $V = \Delta P d^4 \pi t / 128 \eta L$, where ΔP (in Pa) is the injection pressure, d is the inside diameter (m), t is the time of applied pressure, η is the buffer viscosity (Pa s) and L is the total length of the capillary (m) [8].

2.2.1. Background electrolyte

The profen enantiomers were determined using a 0.02-M background electrolyte (BGE) with 0.05 M TMβCD. The BGE, pH 5 was prepared as a mixture of appropriate volumes of aqueous 0.2 M orthophosphoric acid and 0.2 M triethanoloamine. The solution

was passed through 0.45 μm filter and degassed by ultrasound before introducing it into the capillary.

2.2.2. Capillary preparation

A new capillary was flushed with 1.0 M NaOH, 0.1 M NaOH, water and BGE for 10, 10, 5 and 8 min, respectively. The prepared capillary was washed with 0.1 M NaOH, demineralised water and BGE with chiral selector for 5, 5 and 6 min, respectively.

2.3. CE calculations

CE parameters such as electroosmotic (μ_{EOF}) and electrophoretic (μ_{ep}) flow, resolution factor (R_s) and chiral selectivity (α) were calculated for each enantiomer according to equations given in literature [3,6,8]. Relative migration times ($t_{R,S}$) were determined, with respect to NPX as internal standard, from the equation:

$$t_{R,S} = \frac{t_{\text{migr } R,S}}{t_{\text{IS}}}.$$

2.4. Calibration curves of IBP, FBP and KTP enantiomers in human serum

Stock solutions of IBP, FBP, KTP and (+)-*S*-NPX were prepared in methanol, at 10 mg/ml each. Then, standard solutions of 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0 and 1000.0 μg/ml rac-IBP, rac-FBP, rac-KTP and 100.0 μg/ml (+)-*S*-NPX (IS) were prepared in methanol. Fifty microliter of the standard solution of each profen was transferred to 4 ml glass screw vials, each containing 0.5 ml of blank serum. The resulting serum contained 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 μg/ml of each enantiomer. Then, the samples were processed according to the extraction procedure (2.5). The peak areas of IBP, FBP and KTP enantiomer to IS ratio as a function of each enantiomer concentration were considered. Equations of the calibration curves were used to calculate unknown profen enantiomer concentrations in human serum.

2.5. Serum sample preparation procedure

Serum samples with profen enantiomers and IS were acidified using 0.2 ml 1 M orthophosphoric acid. Two milliliter of methylene chloride was added to the samples and, after shaking for 10 min the mixture was cooled at 4 °C. The lower layer was transferred to a clean glass tube and evaporated to dryness at 40 °C under a gentle flow of nitrogen. The resulting residue was reconstituted in 50 μl of methanol and 150 μl of water, respectively and injected into the capillary. Volunteer serum samples were processed in the same manner, except that each 0.5 ml serum was spiked with 50 μl IS.

2.6. Validation parameters

2.6.1. Recovery

Recoveries of 1.0 and 10.0 μg/ml KTP enantiomer standards were examined. The first series consisted of five 0.5 ml blank serum samples, spiked with 50 μl of 20.0 or 200.0 μg/ml rac-KTP and 50 μl of 100 μg/ml IS. The samples were extracted according to the above procedure. Then, up to five serum samples of a second series were supplemented with 50 μl IS only. KTP enantiomers in methanol were added to a dry residue after the extraction. Before injecting it into the capillary every sample volume was diluted with 150 μl water. The recoveries were calculated as the area ratio of either (−)-*R*- or (+)-*S*-KTP to IS using the formula:

$$\text{Recovery (\%)} = \frac{P_{\text{KTP extr/IS}}}{P_{\text{KTP non-extr/IS}}} \times 100$$

where $P_{\text{KTP extr/IS}}$, $P_{\text{KTP non-extr/IS}}$ are peak area of extracted or non-extracted KTP enantiomers to peak area of IS, respectively.

2.6.2. Linearity of the calibration curve

The linearity was assessed for the peak area of IBP, FBP and KTP enantiomer/IS ratio as a function of each enantiomer concentration. The correlation coefficient *r* was calculated to confirm the linearity of the calibration curves.

2.6.3. Limits of detection (LOD) and LOQ

The LOD for the enantiomers was determined as a signal to noise baseline ratio of 4:1. The LOQ is defined as the lowest concentration of each 2-APA enantiomer of the calibration curve at which the coefficient of variation CV $\leq$ 15.0% of the nominal concentration.

2.6.4. Precision

Precision of the method was evaluated for concentrations within the calibration curve range. For each serum sample concentration of 2-APA derivatives calibration curves were prepared three times in a period of one week. The assay precision was expressed as %CV.

2.6.5. Accuracy

Accuracy was estimated for the same range of enantiomer concentrations as for the evaluation of the method precision. It was expressed by the percent difference between the mean determined concentration and the nominal concentration as presented in the formula below:

$$\text{Error (\%)} = \frac{C_{\text{mean}} - C_{\text{nom}}}{C_{\text{nom}}} \times 100$$

2.7. Bioavailability studies on KTP enantiomers

The applicability of the methodology was estimated in bioavailability studies on KTP enantiomers in five healthy volunteers. One male and four female (44 ± 11 years old) weighing, 69 ± 12 kg were selected for the studies. They were non-smokers and did not take any medication or alcohol during the studies. All the volunteers were fully informed of the nature of the studies, signed informed consent forms and could discontinue their participation at any time. The protocol was accepted by the Human Investigations Ethical Committee at the University of Medical Sciences in Poznań. The preparations were given using an open, randomized, two-way cross-over design with an interval of one week between each administration. Single dose administered to the volunteers consisted of two tablets containing 100 mg rac-KTP and, then, one sustained release tablet containing 200 mg rac-KTP. Blood samples (4–5 ml) were obtained (in serum gel tubes S/4.7 ml, Sarstedt, Monovette, Germany) at the following times: immediately before administration of each formulation and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0 and 24.0 h after administration of two standard tablets and 1.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 24.0 and 30.0 h after the administration of one sustained release tablet. The blood samples were centrifuged for 30 min at $1800 \times g$ to obtain serum, which was kept frozen in plastic vials at $-27\,^{\circ}\text{C}$ until analysed.

2.8. Pharmacokinetic parameters

The serum KTP enantiomer concentrations were used to calculate pharmacokinetic parameters. Topfit 2.0 software package (Gustav Fischer, Stuttgart, 1993) was used as the data analysis system. Total area under the concentration–time curve, $AUC_{0\rightarrow\infty}$ was estimated by trapezoidal rule with extrapolation to infinity using $C_{\text{last}}/k_{\text{el}}$ (k_{el}—the elimination rate constant calculated from the terminal linear segment of the log serum concentration–time data). The elimination half-life ($t_{0.5}$) was estimated from $\ln 2/k_{\text{el}}$. $C_{\max}$ and $t_{\max}$ were read from individual enantiomer concentration–time curves. Clearance (Cl) was calculated by dividing the dose (D) of each enantiomer by $AUC_{0\rightarrow\infty}$ (assuming complete bioavailability according to literature data [9]). The volume of distribution (V_{d}) was estimated from $D/AUC_{0\rightarrow\infty}k_{\text{el}}$ and mean residence time (MRT) was calculated from the area under the first moment curve (AUMC) divided by AUC. In calculations of Cl and V_{d} for KTP from sustained release tablets a lower than 100% bioavailability (F) was taken into consideration.

2.9. Statistical analysis

Differences between the pharmacokinetic parameters of the enantiomers were analysed using ANOVA test. Standard error of the mean (S.E.M.) was used to express a tendency of the data. Differences between means were considered statistically non-significant (NS) if the P value was >0.05. When $0.05 > P \geq 0.01$ the parameters taken as significantly (S) different and when $0.01 > P \geq 0.001$ they were regarded to be highly significantly (HS) different.

3. Results and discussion

3.1. CE conditions for the resolution of 2-APA derivative enantiomers

The 0.02 M triethanolamine-phosphate buffer (pH 5), with the chiral selector, TMβCD, is particularly suitable for resolution of enantiomers of 2-APA derivatives [4,5]. This system has previously been used to resolve enantiomers of KTP following extraction from human serum [6], enantiomers of IBP, feno-

Table 1
CZE parameters calculated from electropherograms of IBP, FBP, KTP and (+)-*S*-NPX enantiomers obtained from extracted human serum samples

Compound	$t_{migr\ R}/t_{migr\ S}$ (min)	μ_{EOF} (cm^2/Vs)	$\mu_{ep\ R}/\mu_{ep\ S}$ (cm^2/Vs)	R_S	t_R	t_S	α
IBP	12.55/13.08	2.35×10^{-4}	$-0.30 \times 10^{-4}/-0.38 \times 10^{-4}$	2.19	0.95	0.99	1.34
FBP	13.60/14.02		$-0.46 \times 10^{-4}/-0.51 \times 10^{-4}$	2.02	1.03	1.06	1.16
KTP	16.07/16.90		$-0.75 \times 10^{-4}/-0.83 \times 10^{-4}$	3.70	1.22	1.28	1.16
(+)-*S*-NPX[a]	13.21		$-/-0.40 \times 10^{-4}$	1.88	–	0.97	1.18

BGE composed of 20 mM orthophosphoric acid and 20 mM triethanolamine, 50 mM TMβCD, temperature 35 °C, voltage 20 kV, current 17–18 μA.

[a] Parameters for (+)-*S*-NPX calculated with regard to (−)-*R*-FBP peak.

profen and KTP in authentic samples [3]. To improve resolution of analytes in the developed method the voltage was decreased from 25 [6] to 20 kV. Instead of the extended light-path capillary, where some enantiomers of 2-APA derivatives (IBP, fenoprofen, KTP) were separated [3], a longer normal capillary was used for the separation and resolution of other 2-APA derivatives in mixture from endogenous compounds. In the present CZE method, the concentration of the chiral selector was increased from 25 [3] to 50 mM to obtain complete resolution of the analytes. Using TMβCD-containing buffer (0.05 M), enantiomers of 2-APA derivatives: IBP, FBP, KTP and of (+)-*S*-NPX in mixture were separated from each other after their extraction from serum. This result confirms the high efficiency and resolution of CE, and the exceptional value of heptakis TMβCD, for the separation of enantiomers of 2-APA derivatives. Generally, 2-APA derivatives are weak acids and at pH 5 they are present also in an undissociated form, which allows for the formation of complexes with CDs resulting in their selective resolution [3]. A complete resolution of all three pairs of the enantiomers of the studied profens, together with the IS, was obtained in a relatively short period of time. This demonstrates the resolution power of CE as compared to LC, where the results were not obtained in one analytical run. Moreover, the latter method required pre-column derivatization with L-leucinamide and the use of two internal standards: (+)-*S*-NPX and indomethacin [10]. The derivatization procedure for 2-APA enantiomers considerably extends the analysis time for these analytes. Direct HPLC with normal phases using very expensive chiral columns does not always secure complete resolution to base line [11] or the retention time is very long. The Whelk O1 phase, with (3*S*,4*S*)-4-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrofenantren as chiral selector, possesses a special feature of enantioselectivity for the separation of 2-APA derivatives. IBP and FBP enantiomers were resolved in one analytical run taking up over 20 min [12], but the retention time of KTP was even longer than 40 min [13]. For the CZE method, at a normal polarity mode of the electrode during injection, the migration time of the first peak, ((−)-*R*-IBP) was 12.55 min while that of the last peak, ((+)-*S*-KTP) was 16.90 min (Table 1, Fig. 1). The resolution of enantiomers of the 2-APA illustrated the stereoselectivity of the CZE methodology and the lack of interference from co-extracted endogenous compounds demonstrated the potential of the methodology. The best resolution was obtained for the KTP enantiomers, as indicated by the relatively high value of the resolution coefficient ($R_S = 3.70$). This probably reflected an extensive difference in stability of CDs complexes formed with (−)-*R* or (+)-*S*-KTP [3]. Lower R_S values were manifested by IBP ($R_S = 2.19$) and FBP ($R_S = 2.02$) (Table 1). We also attempted to separate the enantiomers of INDB, however, this proved impossible since the unresolved peak of rac-INDB showed a similar migration time to that of (+)-*S*-IBP and, thus, peaks of the two compounds partially overlapped. In the developed CZE method, only 35 nl of sample was required, in comparison to LC where considerably larger volumes (10–50 μl) had to be injected. Regardless of the differences in injection volume, the LOQs of the validated LC method and the CZE developed for the IBP, FBP and KTP enantiomers were identical (0.5–1.0 μg/ml) [10,12]. Moreover, the volume of buffer used in CE during one analytical run was about 1.0 ml while in HPLC the solvent requirements are higher [10,12]. Thus, the use of CE in chiral analysis is associated with lower cost

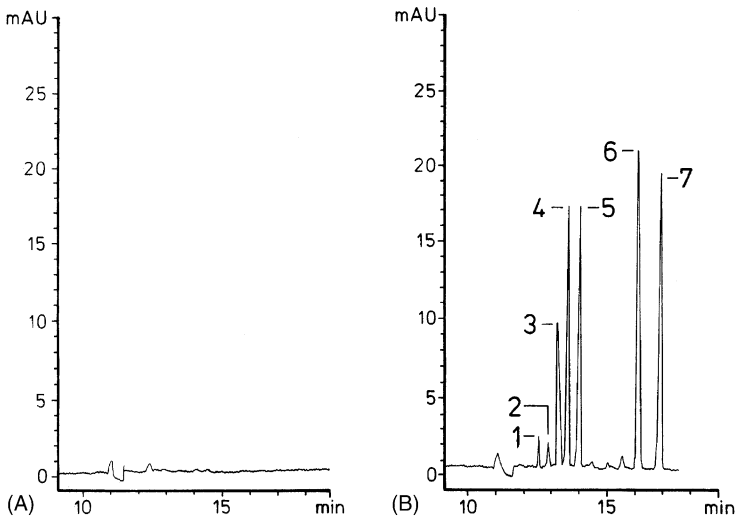

Fig. 1. Electropherograms obtained after extraction of 0.5 ml human serum samples: (A) blank serum; (B) serum sample of a healthy volunteer at 0.5 h following oral administration of 200 mg rac-KTP tablets and spiked with rac-IBP, rac-FBP and (+)-*S*-NPX resulting in 5 μg/ml concentration of each enantiomer; (1) (−)-*R*-IBP; (2) (+)-*S*-IBP; (3) (+)-*S*-NPX; (4) (−)-*R*-FBP; (5) (+)-*S*-FBP; (6) (−)-*R*-KTP; (7) (+)-*S*-KTP.

and is more efficient than HPLC in separating highly charged and polar species [14].

3.2. Validation parameters

Standard curves for the enantiomers of the analysed 2-APA derivatives were linear in the concentration range of 0.5–50.0 μg/ml for FBP and KTP and from 1.0 to 50.0 μg/ml for IBP. Equations of the standard curves manifested a relatively low value for the intercept, which did not significantly differ from zero. The equations of the standard curves for the KTP enantiomers were used for the quantification of the analytes in volunteers' serum following oral administration of a standard or a sustained release tablet (Table 2). The LOD (a signal to noise baseline ratio, S/N = 4:1) was 0.25 μg/ml for the FBP and KTP enantiomers and 0.5 μg/ml for IBP enantiomers. On the other hand, the LOQ was 0.5 μg/ml for the FBP and 1.0 μg/ml for the KTP enantiomers, respectively. The higher value of the lower quantitation threshold for IBP enantiomers (1.0 μg/ml) as compared to values for the remaining compounds reflected the lower value of the specific absorption coefficient, $A^{1\%}_{1\,\mathrm{cm}} = 18.5$, as compared to that for FBP ($A^{1\%}_{1\,\mathrm{cm}} = 800$) [15]. The use of a normal fused capillary of 75 μm i.d. permitted a high volume of injection (35 nl) and, therefore, a low value for the LOQ was reached. It thus allowed a correct pharmacokinetic model and to properly calculate the bioavailability parameters to be obtained. Similar effects can

Table 2
Calibration curve equations for IBP, FBP and KTP enantiomers

Compound (μg/ml)	Enantiomer	Calibration curve equation	Correlation coefficient, *r*
IBP (1.0–50.0)	(−)-*R*	$P_{(-)\text{-}R\text{-IBP}}/P_{(+)\text{-}S\text{-NPX}} = (0.0149 \pm 0.0002)C_{(-)\text{-}R\text{-IBP}} + 0.0057$	0.9997
	(+)-*S*	$P_{(+)\text{-}S\text{-IBP}}/P_{(+)\text{-}S\text{-NPX}} = (0.0151 \pm 0.0001)C_{(+)\text{-}S\text{-IBP}} + 0.0057$	0.9998
FBP (0.5–50.0)	(−)-*R*	$P_{(-)\text{-}R\text{-FBP}}/P_{(+)\text{-}S\text{-NPX}} = (0.2601 \pm 0.0039)C_{(-)\text{-}R\text{-FBP}} + 0.061$	0.9993
	(+)-*S*	$P_{(+)\text{-}S\text{-FBP}}/P_{(+)\text{-}S\text{-NPX}} = (0.2707 \pm 0.0059)C_{(+)\text{-}S\text{-FBP}} + 0.094$	0.9986
KTP (0.5–50.0)	(−)-*R*	$P_{(-)\text{-}R\text{-KTP}}/P_{(+)\text{-}S\text{-NPX}} = (0.1668 \pm 0.0022)C_{(-)\text{-}R\text{-KTP}} + 0.039$	0.9995
	(+)-*S*	$P_{(-)\text{-}R\text{-KTP}}/P_{(+)\text{-}S\text{-NPX}} = (0.1657 \pm 0.0021)C_{(+)\text{-}S\text{-KTP}} + 0.046$	0.9995

Table 3
Validation parameters of standard curves for the analysis of IBP, FBP and KTP enantiomers in human serum

Parameter	IBP		FBP		KTP	
	(−)-*R*	(+)-*S*	(−)-*R*	(+)-*S*	(−)-*R*	(+)-*S*
LOD (μg/ml)	0.50	0.50	0.25	0.25	0.25	0.25
LOQ (μg/ml)	1.00	1.00	0.50	0.50	0.50	0.50
Range of calibration curve (μg/ml)	1.0–50.0		0.5–50.0		0.5–50.0	
Precision (%CV)	4.5–8.3	0.7–12.9	3.3–11.3	1.5–14.4	2.4–13.6	2.8–13.5
Accuracy (%error)	0.4–13.4	0.2–11.2	0.2–6.0	2.5–8.0	0.5–13.0	0.3–14.0

be obtained using a 50-μm capillary with a bubble, but when the shorter part of capillary (to window of cassette) is shortened or is destroyed and the capillary must be changed. In contrast with the normal capillary, a small loss of capillary length does not stop it being used for further analysis.

Values for the coefficient of variability, CV = 0.6–14.4%, pointed to the relatively high precision in estimation of studied enantiomer concentrations. Thus, the method fulfils validation criteria of an analytical method designed for pharmacokinetic studies in which CVs not higher than 15% are acceptable [16]. Also, the accuracy of the estimations fitted the range required for testing drug content in body fluids (it ranged from 0.2 to 14.0%) (Table 3). Recovery of KTP enantiomers from serum following extraction with dichloromethane, at 1.0 and 10.0 μg/ml was relatively high (85–91%). In general, 2-APA derivatives are relatively easily extracted into organic solvents (dichloromethane, diethyl ether) as non-ionized species [6,17].

3.3. *In vivo application—pharmacokinetic studies*

The method was used for pharmacokinetic and bioavailability studies on KTP enantiomers. Pharmacokinetic profiles were followed for up to 30 h after oral administration of standard and sustained release tablets. KTP enantiomers were rapidly absorbed from gastrointestinal tract after oral administration of the standard tablet (t_{max} = 0.5–3.0 h). On the other hand, the pharmacokinetic profile of KTP enantiomers obtained for each volunteer following administration of the sustained release tablet manifested a biphasic course of absorption associated with sustained release of the enantiomers from the formulation (Fig. 2). In the studied time interval of 0–30 h enantiomer concentrations reached maximum serum levels twice; $t_{max\,1,2}$ were in the range of 3–6 and 8–14 h, respectively. Apart from the mentioned sustained drug release from the formulation, the biphasic course of absorption might have resulted also from enterohepatic circulation of KTP following reabsorption of the drug from bile to blood. This is consistent with the fact that 10–20% of the dose is secreted into the bile in which the enantiomers may be released from conjugates with glucuronides and may return to the circulation. Plasma levels of KTP enantiomers were lower following administration of sustained release tablets as compared to standard tablets but fitted the range of therapeutic concentrations of 1–5 μg/ml [9]. Moreover, in the case of sustained release tablets therapeutic levels of KTP enantiomers in serum were maintained for a much longer period of time (approximately 12 h) as compared to standard tablets (around 7 h). Thus, the sustained release tablets seem to be more adequate in long-term treatment of rheumatic diseases as they require less frequent administration. On the other hand, if a rapid analgesic action is required as a significant aim of clinical practice, the standard tablets seem to be more appropriate. Differences in pharmacokinetic parameters (C_{max}, t_{max}, AUC, MRT, V_d, $t_{0.5}$) demonstrated between the two types of tablets by comparison of the same enantiomer proved to be statistically significant. Administration of the sustained release tablets yielded higher V_d and MRT values than those noted following administration of the standard tablets. This was typical for drug formulated as a sustained release preparation given to prolong the duration of action. However, the AUC was lowered by approximately 25%. This pointed to lower biological availability of sustained release tablets as compared to standard tablets. The differences in KTP bioavailability following administration

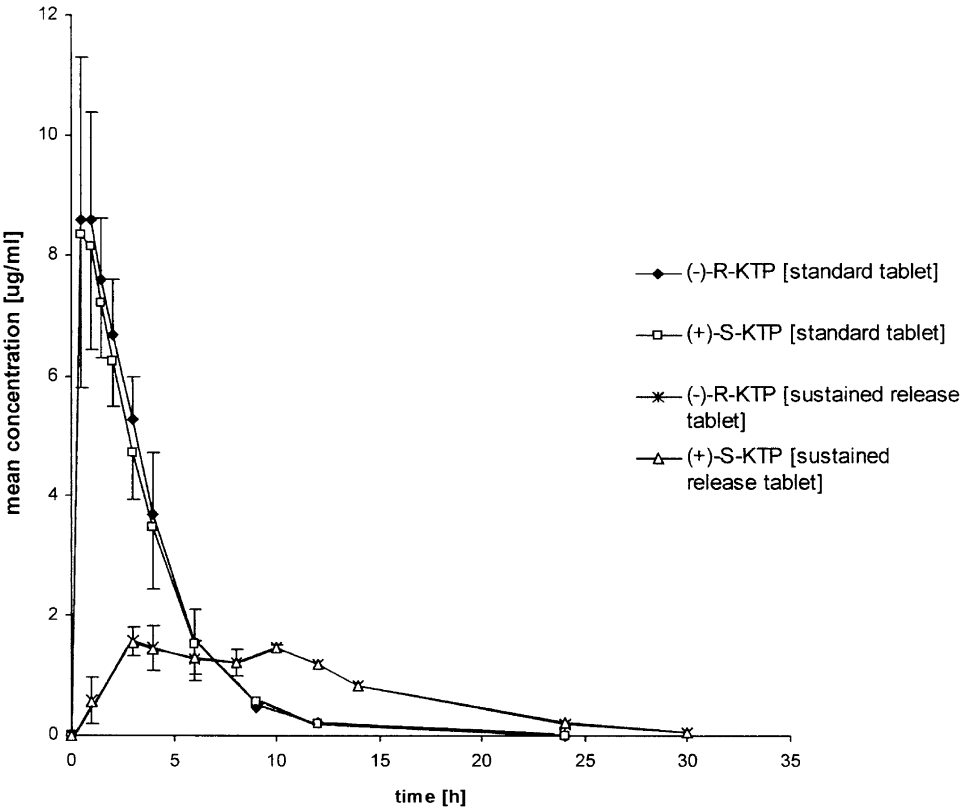

Fig. 2. Mean serum KTP enantiomer concentrations as a function of time after oral administration of 2×100 mg rac-KTP Profenid tablets and a 200-mg rac-KTP Profenid sustained release tablet to five healthy volunteers.

of the two types of tablets were taken into account in calculations of Cl and V_d. Values of clearance (Cl) estimated for KTP enantiomers following administration of standard tablets or sustained release tablets did not significantly differ from each other (Table 4).

In analysis of differences in serum concentrations of KTP enantiomers we observed slightly lower levels of (+)-*S* enantiomer at the adsorption stage following administration of either form of the drug. However, the differences were statistically non-significant. The higher concentrations of the (−)-*R* enantiomer as compared to those of (+)-*S* enantiomer could be explained by faster elimination of the (+)-*S* enantiomer. This has been confirmed by results of in vitro studies [18], in which total plasma concentrations of 35 μg/ml rac-KTP were followed by a more significant increase of protein-unbound fraction of (+)-*S* enantiomer. However, it is known that clearance of drugs of low hepatic extraction rate (such as KTP) is directly proportional to free concentration of a drug in blood [19].

It is much easier to observe differences between concentrations of (−)-*R* and (+)-*S*-KTP enantiomers following administration of the standard tablet, as compared to administration of the sustained release tablet, due to several-fold higher KTP enantiomer levels following the former procedure (Fig. 2). The differences between tablet types and enantiomers could also be noted during the elimination phase. In volunteer sera, levels of (+)-*S* enantiomer were maintained for a slightly longer time than those of (−)-*R* enantiomer. Accordingly, higher values of $t_{0.5}$ were noted for (+)-*S* enantiomer than for (−)-*R* enantiomer, both following administration of the standard tablet ($t_{0.5\,(-)\text{-}R} = 1.8 \pm 0.3$ h and $t_{0.5(+)\text{-}S} = 2.1 \pm 0.3$ h) and following the sustained release tablet ($t_{0.5(-)\text{-}R} = 3.8 \pm 0.4$ h and $t_{0.5(+)\text{-}S} = 3.9 \pm 0.3$ h).

Table 4
Mean pharmacokinetic parameters of KTP enantiomers in five healthy volunteers after cross-over administration of 2 × 100 mg rac-KTP Profenid tablets and a 200-mg Profenid prolongatum tablet

Parameter	Enantiomer	Profenid (sustained release tablet)	Profenid (standard tablet)	ANOVA test ($\alpha = 0.05$)
$C_{max\,1}$ (μg/ml)	(−)-*R*	1.8 ± 0.3	11.2 ± 1.7	HS
	(+)-*S*	1.7 ± 0.2	10.7 ± 1.5	HS
$C_{max\,2}$ (μg/ml)	(−)-*R*	1.7 ± 0.3	–	–
	(+)-*S*	1.7 ± 0.3	–	–
$t_{max\,1}$ (h)* (range)	(−)-*R*	4.0 (3.0–6.0)	1.0 (0.5–3.0)	–
	(+)-*S*	3.0 (3.0–6.0)	1.0 (0.5–3.0)	–
$t_{max\,2}$ (h)* (range)	(−)-*R*	12.0 (8.0–14.0)	–	–
	(+)-*S*	12.0 (8.0–14.0)	–	–
AUC_{0-t} (μg h/ml)	(−)-*R*	23.4 ± 3.0	33.5 ± 2.0	S
	(+)-*S*	23.2 ± 2.9	31.9 ± 1.9	S
$AUC_{0-\infty}$ (μg h/ml)	(−)-*R*	25.3 ± 2.8	34.3 ± 2.1	S
	(+)-*S*	25.5 ± 1.9	32.9 ± 2.0	S
MRT (h)	(−)-*R*	13.5 ± 2.8	3.1 ± 0.5	HS
	(+)-*S*	14.7 ± 4.1	3.2 ± 0.5	HS
Cl (ml/min)	(−)-*R*	51.4 ± 5.3	49.2 ± 2.8	NS
	(+)-*S*	50.0 ± 3.7	51.4 ± 3.0	NS
V_d (l)	(−)-*R*	16.6 ± 1.5	7.8 ± 1.2	S
	(+)-*S*	16.5 ± 1.4	9.1 ± 1.5	S
$t_{0.5}$ (h)	(−)-*R*	3.8 ± 0.4	1.8 ± 0.3	HS
	(+)-*S*	3.9 ± 0.3	2.1 ± 0.3	HS
$F_{rel} = (AUC_{prolong}/AUC_{standard}) \times 100$	(−)-*R*	73.7 ± 8.3		
	(+)-*S*	77.5 ± 5.8		

Differences between enantiomers of each formulation: NS, non-significant; HS, highly significant ($0.001 \leq P < 0.01$); S, significant ($0.01 \leq P < 0.05$); F_{rel}, relative bioavailability; (*), median.

The differences, however, proved statistically insignificant. The higher levels of (+)-*S* enantiomer at the elimination phase probably reflected chiral inversion of (−)-*R* enantiomer to (+)-*S* enantiomer of KTP, the range of which amounts in humans to 9–12% [20].

4. Conclusion

The CZE method developed for some 2-APA derivative enantiomers is stereospecific, adequately accurate and precise and it can be suitable in pharmacokinetic, bioavailability and optical purity studies on these enantiomers. Moreover, CE with its high resolution of 2-APA enantiomers, suitable rapid analysis time, very small volume of injected sample and water-based solvents proves that it can be a valid alternative to LC methods.

As compared to standard the tablets, bioavailability of rac-KTP following administration of sustained release tablets was lowered by approximately 25%.

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