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Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 1035–1045

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of ketoprofen enantiomers in human serum by capillary zone electrophoresis: man pharmacokinetic studies after administration of rac-ketoprofen tablets

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Received 5 March 2002; received in revised form 29 May 2002; accepted 22 June 2002

Abstract

A rapid and stereospecific capillary zone electrophoresis (CZE) method to quantify ketoprofen (KTP) enantiomers was developed. The KTP enantiomers and (+)-*S*-naproxen [(+)-*S*-NPX] as an internal standard (IS) were extracted with methylene chloride from serum acidified. Recovery of both enantiomers was in the range of 85–91%. The enantiomers were determined using a background electrolyte (BGE), consisting of 0.05 M heptakis 2,3,6-tri-*O*-methyl-β-cyclodextrin (TMβCD) in a phosphate-triethanolamine buffer, which filled a fused silica capillary of 75 μm i.d. The linear range of calibration curves was between 0.25 and 50 mg l^{-1}, with detection limit of 0.1 mg l^{-1} (signal-to-noise baseline ratio (S/N) >4). Intra- and interday precision and accuracy of the calibration curves, expressed by the coefficient of variation (CV), did not exceed 15.0%. The validated method has been successfully applied for pharmacokinetic studies of KTP enantiomers from tablets with rac-KTP in man.

Keywords: Racemic ketoprofen; Enantiomers' separation; Serum liquid extraction; 2-Arylpropionic acid derivatives; High performance capillary electrophoresis; Enantiomers' pharmacokinetics

1. Introduction

(±) Ketoprofen (KTP), (±)(*R*,*S*)-2-(benzoylphenyl)propionic acid is a chiral non-steroidal antiinflammatory drug (NSAID) of $pK_a = 4.55$, with analgesic and antipyretic actions. It inhibits cyclooxygenase (COX 1 and COX 2) activity with a reduction in the tissue production of prostaglandins such as $PGF_{2\alpha}$ and PGE_2. Nevertheless its therapeutic activity resides exclusively in the (+)-*S*-enantiomer, it is still marketed as a racemic mixture [1,2]. 2-Arylpropionic acid derivatives (2-APA, profens) undergo a unidirectional bioinversion of (−)-*R* enantiomer (distomer) to the pharmacologically active (+)-*S* enantiomer (eutomer) via formation of the acyl CoA tioester of 2-arylpropionate. The rate and extent of the inversion have been shown to be species dependent [3,4]. In rats, 46% or more of (−)-*R*-KTP was inverted to (+)-*S*-KTP during a single passage

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00439-9

through the liver [5]. However, human inversion was limited to 9–12% [6,7].

Direct and reversed phase HPLC are widely used for determination of KTP enantiomers in biological fluids [8]. However, capillary electrophoresis (CE) is also recently applied for determination of 2-APA derivatives as well as its enantiomers in racemic mixtures in pure authentic samples, tablets and capsules [9–11]. The application of CE for the analysis of chiral drugs in biological fluids was recently reviewed [12]. KTP as a racemic mixture only was determined in biological fluids, mainly in serum and/or in urine [13–17]. A suitable chiral selector performs a key role in a chiral resolution. Dextrin derivatives like linear maltodextrin oligosaccharides [18] or cyclodextrins (CDs), cyclic oligosaccharides consisting of six, seven or eight D-(+) glucose units linked by α-1,4 bonds, forming α, β, and γ CDs are used as chiral recognition agents for resolution of chiral drugs like profens. The subunit glucose differences result in a change of some of their physical properties, a feature, which can be exploited to obtain a good separation. Enantioselective recognition can be explained by interaction between CDs, which have many chiral centres, and the guest enantiomer [19]. The CDs can be derivatised in 2, 3 or 6 position to receive compounds with new features. Heptakis 2,3,6-tri-*O*-methyl-β-cyclodextrin (TMβCD) was found to be the best compound for separation of rac-KTP and other profens [9,20,21]. Highly sulfated cyclodextrins (HSCDs) are new and very expensive chiral selectors, which resolved KTP enantiomers at pH 2.5 with short migration time [22]. A study is available, where also other profens, like ibuprofen (IBP) enantiomers, are determined in vitro, in spiked serum samples [18].

The main aim of this work was to develop a fast and validated method of analysis of KTP enantiomers in small volumes of human serum. Pharmacokinetic studies of KTP enantiomers, after administration of tablets with its racemic mixture to healthy volunteers will be conducted. The analytical parameters and in vivo results will be also presented and discussed to confirm in vivo usefulness of the proposed method.

2. Experimental

2.1. Materials

Rac-KTP and (+)-*S*-KTP (optical purity (o.p.) 99.0%), TMβCD and (+)-*S*-NPX (o.p. 98.0%), rac-flurbiprofen (rac-FBP) and (+)-*S*-FBP (o.p. 98.0%) were purchased from Sigma (St. Louis, MO). Rac-indobufen (rac-INDB), (+)-*S*-INDB (o.p. 98.5%) (Pharmacia & Upjohn, Milan, Italy) and (−)-*R*-IBP (o.p. 100.0%) and (+)-*S*-IBP (o.p. 99.6%) (Ethyl Corporation, Orangeburg, S.C.) were obtained free of charge. Eighty five percent ortophosphoric acid (P.O.CH., Gliwice, Poland) and triethanolamine (Applied Science Laboratories, Inc., State College, PA) were of reagent grade, whereas methanol (Merck, Darmastadt, Germany) was of HPLC grade. 1.0 and 0.1 M NaOH solutions were purchased from Agilent Technologies, Waldbroon, Germany. Demineralised water was always used.

2.2. Equipment and CE conditions

CE determinations of KTP enantiomers were conducted on an Agilent model ^{3D}CE apparatus (Agilent Technologies, Waldbronn, Germany) with diode array UV detector set at $\lambda = 253$ nm. Samples were automatically injected using hydrodynamic injection at the anode. 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 54 cm total length (effective length of 45.5 cm) was used for separation of KTP enantiomers. ^{3D}CE apparatus was equipped with ChemStation used for instrument control, data acquisition and data analysis. The system was controlled by WINDOWS NT software.

2.2.1. Background electrolyte (BGE) and capillary preparation

BGE was prepared as a mixture of appropriate volumes of 0.2 M ortophosphoric acid and 0.2 M triethanoloamine at pH 5.0. A chiral selector TMβCD was added to BGE to receive 0.05 M solution. The solution was passed through 0.45 μm filter and degassed by ultrasounds before inserting it into the capillary. 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, 3 and 6 min, respectively. The samples to be separated were injected onto the capillary after prior 5 min stabilisation time. All experiments were carried at the 25 kV voltage and 50 × 5 mbar s injection (35 nl injected volume).

2.3. CE calculations

Electrophoretic flow (μ_{eo}) was calculated using the equation:

$$\mu_{eo} = \frac{lL}{t_{eo}U}$$

where l, capillary length between the injection and detector sites (cm); L, capillary overall length (cm); t_{eo}, migration time for a peak of electroosmotic flow (s); U, applied voltage (V).

Resolution (R_s) was calculated from:

$$R_s = \frac{2(t_{2migr} - t_{1migr})}{w_1 + w_2}$$

where $t_{1:2migr}$, apparent migration times of 1, (−)-*R*- and 2, (+)-KTP enantiomers, $w_{1:2}$, widths at the peak base.

Relative migration time:

$$t_{R:S} = \frac{t_{R:Smigr}}{t_{IS}}$$

where t_{IS}, internal standard (IS) migration time.

Chiral selectivity:

$$\alpha = \frac{t_{Smigr} - t_0}{t_{Rmigr} - t_0}$$

where t_0, electroosmosis time.

2.4. Calibration curve of KTP enantiomers in serum

Stock solutions of KTP and (+)-*S*-NPX were prepared with 10 mg l^{-1} each in methanol. Then, standard solutions of 5.0; 10.0; 20.0; 50.0; 100.0; 200.0; 500.0 and 1000.0 mg l^{-1} rac-KTP and 200.0 mg l^{-1} IS were prepared in the same solvent. The standard solutions were stable for a month when refrigerated. 50 μl sample of each KTP and IS were transferred by automatic pipette (Eppendorf, Hamburg, Germany) to a 4 ml glass screw vials containing 0.5 ml blank serum. The resulting serum, containing 0.25; 0.5; 1,0; 2.5; 5.0; 10.0; 25.0 and 50.0 mg l^{-1} each KTP enantiomers and 20.0 mg l^{-1} IS were processed according to the procedure specified below. The peak area ratio of (+)-*R*- and/or (+)-*S*-KTP to IS plotted versus KTP enantiomer concentration established a calibration curves required. The regression equation obtained was used to calculate a serum concentration in volunteer samples after peroral administration of 200 mg rac-KTP tablet.

2.5. Extraction procedure of serum samples

0.5 ml human serum samples with 50 μl IS, were acidified using 0.2 ml 1 M ortophosphoric acid. After shaking for 1 min, 2 ml methylene chloride were added. The mixture was shaken for 10 min (horizontal position, amplitude 1 cm and frequency 300 cycles per min) and then cooled for 20 min and subsequently centrifuged for 5 min. The lower layer was transferred to a clean glass tube and evaporated to dryness at 40 °C under a gentle nitrogen flow. The residue obtained was reconstituted in 50 μl of methanol and 150 μl water. Thirty-five nano litres of the above solution were injected into the capillary. Serum volunteer 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

The recovery of 1.0 and 10.0 mg l^{-1} KTP enantiomer standards were determined. First series consisted of five 0.5 ml blank sera, spiked with 50 μl of 20.0 mg l^{-1} and/or 200.0 mg l^{-1} rac-KTP and 50 μl of 200 mg l^{-1} IS. The samples were extracted according to the above procedure. Then, up to five serum samples were supplemented with IS only (II series). KTP enantiomers were added to a dry residue obtained after the extraction. The dissolved samples were injected into the capillary.

The recoveries were calculated as the area ratio of either (−)-*R*- or (+)-*S*-KTP to IS using the formula:

$$\%\text{Recovery} = \frac{P_{\text{KTPextr./IS}}}{P_{\text{KTPnon-extr./IS}}} \times 100$$

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

2.6.2. Linearity of the calibration curve

The linearity was assessed for the peak area of a KTP enantiomer/IS ratio as a function of an enantiomer concentration. KTP enantiomer concentrations in serum samples covered the range of 0.25–50.0 mg l^{-1} and after extraction were injected directly into the capillary of the CE system used.

2.6.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD was determined as a signal to noise baseline ratio of 4:1. The LOQ is defined as the lowest concentration of KTP enantiomers of the calibration curve within the coefficient of variation (CV) $\leq 15.0\%$ of its nominal value.

2.6.4. Precision

Intra-day precision (repeatability) was evaluated for three known concentrations (1.0, 2.5 and 10.0 mg l^{-1}) within the calibration curve. Each concentration injected was prepared five times. Inter-day precision (reproducibility) was assessed for concentration within the calibration curve range. The precision was calculated using the formula:

$$\%\text{CV} = \frac{\text{S.D.}}{C_{\text{mean}}} \times 100$$

where S.D., standard deviation; C_{mean}, mean KTP enantiomers concentration determined.

2.6.5. Accuracy

The intra-day and inter-day accuracy ($\%\Delta X$) was estimated for the same range of KTP enantiomer concentrations as for the evaluation of the method precision. The accuracy of the results was expresseded by the percent difference between the C_{mean} determined and the nominal concentration (C_{mean}):

$$\%\Delta X = \frac{C_{\text{mean}} - C_{\text{nom}}}{C_{\text{nom}}} \times 100$$

2.6.6. Specificity of a chiral method established

This is described as the potential ability of a method to discriminate the enantiomers among all potential interfering chiral or achiral substances. It confirms that the signal measured is caused exclusively by enantiomers of the analyte. Some chiral drugs, derivatives of 2-APA were spiked to human serum with rac-KTP and injected after extraction to a CE capillary to determine any potential interference.

2.6.7. Internal standard

As the most important factor, IS should be carefully selected for assuring repeatability at the optimisation stage of the CE method. The IS was chosen from other derivatives of 2-APA to improve the determination of KTP enantiomers in serum especially in the course of the extraction step.

2.7. In vivo application—pharmacokinetic studies

The practical potential of the worked out method was demonstrated in healthy volunteers. A single dose consisted of two tablets with 100 mg rac-KTP each was administered to five volunteers. Five male and female 44 ± 11-years-old healthy volunteers, 69 ± 12 kg body weight, were selected for the studies. The volunteers were judged healthy, as determined by physical examination and standard laboratory tests. Moreover, the volunteers were non-smokers and did not take any medications and alcohol during the studies. All the volunteers were fully informed of the nature of these studies and signed informed consent forms and could discontinue their participation at any time. The protocol was accepted by the Human Investigations Ethical Committee at the K. Marcinkowski University of Medical Sciences in Poznań. Blood samples (4–5 ml) were

obtained (in serum gel tubes S/4.7 ml, Sarstedt, Monovette, Germany) from the right antecubital fossa at the following times: immediately before the administration of tablets and 0.5; 1.0; 1.5; 2.0; 3.0; 4.0; 6.0; 9.0; 12.0 and 24.0 h after the administration. Within 30 min following blood withdrawal, the samples were frozen in plastic vials at −27 °C, until analysed. The serum KTP enantiomer concentrations were used to calculate pharmacokinetic parameters. The elimination half-life ($t_{0.5}$) was calculated from $\ln 2/\lambda_z$ (the terminal elimination rate constant, λ_z was estimated by linear segment of the log serum enantiomer concentration–time data). The $AUC_{0\rightarrow\infty}$ were estimated by trapezoidal rule with extrapolation to infinity using C_{last}/λ_z. T_{max} was calculated from an enantiomer concentration-time curve and C_{max} was read at t_{max}. Serum drug clearance (Cl) was calculated dividing the dose (D) of each enantiomer by $AUC_{0\rightarrow\infty}$, assuming complete biological accessibility of KTP administered in the form of tablet.

$$Cl = \frac{D}{AUC_{0\rightarrow\infty}} \quad (lh^{-1})$$

The volume of distribution (V_d) was calculated using the formula:

$$V_d = \frac{D}{AUC_{0\rightarrow\infty}\lambda_z} \quad (1)$$

Mean residence time (MRT) was calculated from formula:

$$MRT = \frac{AUMC}{AUC} \quad (h)$$

where AUMC is area under the first moment curve.

The TOPFIT 2.0 software package [23] was used for calculation of pharmacokinetic parameters.

2.8. Statistical analysis

The standard error of the mean (S.E.M.), derived from averaged individual fittings, was used to express the variability of the data sets. Comparisons of parameters with probabilities (P) of no difference $0.001 \leq P < 0.01$ is termed very significantly (VS) different, when $0.01 \leq P < 0.05$, the differences between parameters are termed significantly (S) different. Values $P > 0.05$ do not permit rejection of the null hypothesis of no difference and these differences are considered to be non significant (NS). Mean pharmacokinetic parameters of the KTP enantiomers were tested using one way ANOVA test.

3. Results and discussion

3.1. CE conditions for KTP enantiomers resolution

A chiral selector-TMβCD, as the best one for separation of profen's enantiomers [9,20,21], and rac-KTP pure samples were separated from 25 mM TMβCD [9]. Then, the selector was successfully used for analysis of extracted KTP enantiomer serum samples but at higher (50 mM) concentration. The resolution of KTP enantiomers at TMβCD concentrations lower than 50 mM could not be obtained. Despite the voltage augmented from 20 to 25 kV, the injection volume increased to 35 nl, the applied concentration assured complete separation of KTP enantiomers, IS and endogenous compounds, at relatively short migration times (9.18 and 9.56 min) (Fig. 1). The above conditions corroborated with the other CE parameters showed in Table 1. The use of a normal fused silica capillary of 75 μm i.d. warranted a sufficient LOQ at the level of 0.25 mg l^{-1} and permitted to obtain a correct pharmacokinetic profile (Fig. 2) of KTP enantiomers to calculate pharmacokinetic parameters. An attempt was also made to apply a high sensitivity detection cell (HSDC) in order to increase LOQ but lack of repeatability and an unstable base line prevented its application. Therefore, the normal fused silica capillary 75 μm i.d. was found to be the optimal solution in these studies.

3.2. Extraction recovery

KTP enantiomers were extracted from 0.5 ml acidified human serum using methylene chloride. The solvent was found suitable for that process and its small volume of 2 ml gave a good

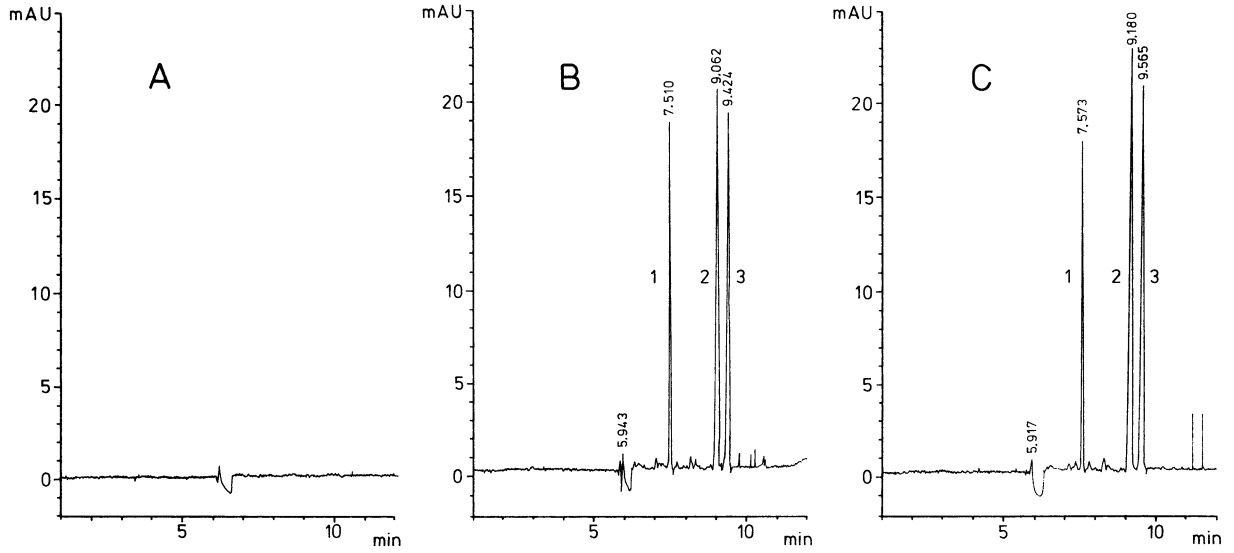

Fig. 1. Electropherograms of KTP enantiomers and (+)-*S*-NPX (IS) after extraction of human serum samples; A, blank serum; B, blank serum spiked with 10 mg l^{-1} (−)-*R*- and (+)-*S*-KTP and 20 mg l^{-1} of IS; C, serum sample of a healthy volunteer at 1 h elapsed from the administration of 2 × 100 mg rac-KTP tablets (11.76 mg l^{-1} of (−)-*R*- and 11.34 mg $l^{-1}$of (+)-*S*-KTP). Peaks: 1, 2 and 3 peaks correspond to IS, (−)-*R*- and (+)-*S*-KTP, respectively.

extraction results. The recovery for 1.0 and 10.0 mg l^{-1} each enantiomer concentration have been quite high, ranging from 85 to 91% (Table 2). KTP enantiomers and other 2-APA derivatives are generally easily extracted from serum using liquid or solid phase extraction (SPE) [24,25].

3.3. Method validation procedure

The linearity was observed in the entire 0.25–50.0 mg l^{-1} range of KTP enantiomer concentrations and covered all the KTP enantiomer levels determined in volunteers. The highest individual enantiomer concentrations determined in these studies were 17.2 and 16.2 mg l^{-1} for (−)-*R* and (+)-*S*-KTP, respectively. The LOD for the method was 0.1 mg l^{-1}. The LOQ in serum was found to be 0.25 mg l^{-1} for each KTP enantiomer with the precision of CV 6.1 and 7.1% for (−)-*R*- and (+)-*S*-enantiomer, respectively.

The inter-day precision and accuracy were estimated for studied concentrations of the calibration curve but the intra-day parameters were calculated from three chosen concentration in the range 1.0–10.0 mg l^{-1} The results are shown in Table 3. Percent CV expressing the intra-day (2.4–5.6%) and inter-day (0.5–14.2%) variations indicate that the method is quite precise. The accuracy was found to be $\leq$10%. The small differences of CV $\leq$ 10% noted between the nominal concentra-

Table 1
CZE parameters calculated from electropherograms of KTP enantiomers, (+)-*S*-NPX and electroosmotic flow peaks, obtained from human serum samples injected after the extraction procedure proposed

TMβCD (mM)	$t_{R\,migr}/t_{S\,migr}$ (min)	μ_{eo} (cm^2 V^{-1} s^{-1})	R_S	t_R	t_S	α
50.0	9.17/9.57	2.77×10^{-4}	4.32	1.21	1.26	1.12

μ_{eo}, electroosmotic mobility; $t_{R\,migr}/t_{S\,migr}$, enantiomers migration time; R_S, resolution; t_R, t_S, relative migration time; α, chiral selectivity. BGE composed of 20 mM phosphate and 20 mM triethanolamine, pH 5, temperature 35 °C, voltage 25 kV, current 26–28 μA.

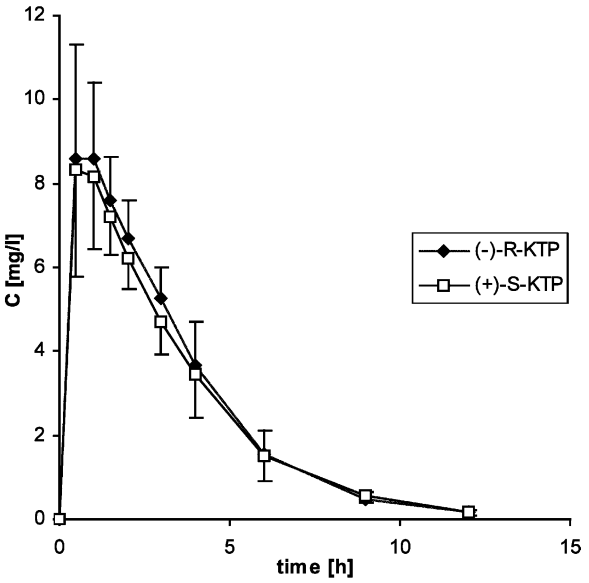

Fig. 2. Mean serum KTP enantiomer concentration as function of time after administration of 2 × 100 mg rac-KTP tablets to five healthy volunteers.

Table 2
Mean recovery of KTP enantiomers from a healthy volunteer's serum ($n = 5$)

Nominal (−)-*R*- or (+)-*S*-KTP concentration (mg l^{-1})	%Recovery ± S.E.M.	
	(−)-*R*-KTP	(+)-*S*-KTP
1.0	88.4 ± 2.2	85.1 ± 2.0
10.0	90.3 ± 2.9	91.0 ± 3.0

tion and the determined concentration documented an appropriate accuracy of the method.

The separated peaks of KTP enantiomers in samples extracted from volunteer sera and (+)-*S*-NPX are shown in Fig. 1. No peaks interfering with KTP enantiomers or IS were detected, which could originate from serum endogenous compounds. Also FBP and IBP enantiomers or rac-INDB peaks did not interfere with the peaks of KTP enantiomers. This indicated appropriate specificity of the worked out method.

(+)-*S*-NPX represented the most suitable compound among analysed substances. Moreover, it is easily available since it had already been used for 20 years as a pure enantiomer in a non-steroidal anti-inflammatory and analgesic therapy. NPX among analysed substances, such as FBP, IBP and INDB, had a migration time, which is very close to that of KTP enantiomer peaks and was thoroughly separated from them. However, the migration times of IBP enantiomers were much shorter than that of NPX peak. Moreover, the relatively low UV absorption coefficient of IBP, $A_{1\ cm}^{1\%} = 18.5$, if compared with (+)-NPX, $A_{1\ cm}^{1\%} = 208$ [26], would have require its higher concentrations, e.g. 200 mg l^{-1} to obtain a similar peak area.

3.4. In vivo application—pharmacokinetic studies

The method proposed was successfully applied for pharmacokinetic studies of KTP enantiomers in vivo. KTP enantiomers were very fast absorbed in GI from tablets resulting in maximum serum concentration (C_{max}) 11.2 ± 1.7 mg l^{-1} (7.5–17.2) and 10.7 ± 1.5 mg l^{-1} (7.7–16.2) for (−)-*R* and (+)-*S* enantiomer, respectively. The C_{max} values were obtained at the same time $t_{max} = 1.3 \pm 0.4$ h (Table 4). The (−)-*R* levels in human serum were somewhat higher than the levels of (+)-*S*-KTP, $AUC_{(+)\text{-}S}/AUC_{(-)\text{-}R}$ ratios were in the range of 0.9–1.0 and confirmed it. The serum concentration differences were clearly predicted at the stage of absorption and distribution (Table 4, Fig. 2). The presented results confirmed those ones published in the literature, where *S*:*R* ratio was 0.8–1.0 [27]. The influence of a (−)-*R*-KTP enantiomer on pharmacokinetics of its antipode is not observed [6,28]. The (+)-*S*- to (−)-*R*- KTP chiral inversion should also be ruled out [6]. Unbound (+)-*S*-KTP concentrations at greater concentrations of total rac-KTP, above 35 mg l^{-1}, were slightly higher than those of its antipode [29]. Moreover, it is known that the clearance of drugs with low hepatic extraction such as KTP is proportional to the unbound fraction of the drug. Then, the (+)-*S* eantiomer fraction, conjugated with glucuronides can be eliminated faster from human body than its antipode conjugate. This seemed to be a main reason of higher levels of unbound (+)-*S*- than of (−)-*R*-KTP, resulting in higher levels of (+)-*S* than (−)-*R*-glucuronide

Table 3
Precision and accuracy data of standard curves for analysis of (−)-*R*- and (+)-*S*-KTP in human serum

Nominal concentration (mg l^{-1})	(−)-*R*-KTP			(+)-*S*-KTP		
	Mean assayed value (mg l^{-1})	Accuracy (error %)	Precision (CV, %)	Mean assayed value (mg l^{-1})	Accuracy (error %)	Precision (CV, %)
Intra-day repeatability ($n = 5$)						
1.00	0.99	1.0	5.6	0.99	1.0	5.3
2.50	2.64	5.5	3.1	2.65	6.8	4.3
10.00	10.16	1.6	2.4	10.22	2.2	2.4
Inter-day reproducibility ($n = 10$)[a]						
0.25	0.24	4.0	6.1	0.26	4.0	7.7
0.50	0.51	2.0	6.3	0.49	2.0	6.1
1.00	1.04	4.0	14.3	1.04	4.0	14.2
2.50	2.75	10.0	7.4	2.75	10.0	6.1
5.00	5.18	3.6	7.4	5.12	2.4	5.2
10.00	9.82	1.8	2.8	9.84	1.6	2.3
25.00	25.63	2.5	2.1	25.57	2.3	2.3
50.00	49.49	1.0	0.7	49.43	1.1	0.5

[a] Ten calibration curves were prepared during 2 months period.

Table 4
Pharmacokinetic parameters of KTP enantiomers from five healthy volunteers after administration of 2 × 100 mg rac-KTP tablets

Volunteers initials	Enantiomer	C_{max} (mg l^{-1})	t_{max} (h)	$AUC_{0\rightarrow t}$ (mg h l^{-1})	$AUC_{0\rightarrow \infty}$ (mg h l^{-1})	MRT (h)	Cl/F (ml min^{-1})	V_d (l)	$t_{0.5}$ (h)
(1) G.Sz.	(−)-*R*	9.8	1.0	29.6	30.2	3.0	55.1	11.5	2.4
	(+)-*S*	9.4	1.0	29.0	30.0	3.3	55.5	13.6	2.8
(2) S.G.	(−)-*R*	17.2	0.5	30.2	30.7	1.8	54.4	3.9	0.8
	(+)-*S*	16.2	0.5	27.8	28.2	1.8	59.0	4.9	1.0
(3) A.Z	(−)-*R*	9.5	1.0	39.9	40.8	3.4	40.8	7.9	2.2
	(+)-*S*	9.1	1.0	35.2	36.4	3.5	45.8	10.6	2.7
(4) B.K.	(−)-*R*	11.8	1.0	31.3	32.5	2.6	51.3	8.9	2.0
	(+)-*S*	11.3	1.0	29.8	31.1	2.6	53.6	10.0	2.2
(5) R.D.	(−)-*R*	7.5	3.0	36.6	37.4	4.6	44.6	6.7	1.8
	(+)-*S*	7.5	3.0	37.6	38.6	4.7	43.2	6.6	1.8
Mean ± S.E.M.	(−)-*R*	11.2 ± 1.7	1.3 ± 0.4	33.5 ± 2.0	34.3 ± 2.1	3.1 ± 0.5	49.2 ± 2.8	7.8 ± 1.2	1.8 ± 0.3
	(+)-*S*	10.7 ± 1.5	1.3 ± 0.4	31.9 ± 1.9	32.9 ± 2.0	3.2 ± 0.5	51.4 ± 3.0	9.1 ± 1.5	2.1 ± 0.3
ANOVA test		NS	NS	NS	NS	NS	NS	NS	NS

$\alpha = 0.05$; NS, non-significant differences between (−)-*R*- and (+)-*S*-KTP concentration.

conjugates [30]. The lower (+)-*S*-KTP total concentrations than those of its antipode were observed in conducted studies following a relatively high (200 mg) rac-KTP dose. Obviously, it should be kept in mind that 10–20% of the dose is excreted in bile and some enterohepatic circulation [1] ought to be taken into consideration to fully explain these differences. At the stage of elimination, at 6 h elapsed from the administration, (+)-*S*-KTP concentrations were slightly higher because its elimination rate was lower, $t_{0.5} = 2.1 \pm 0.3$ h for (+)-*S* and $t_{0.5} = 1.8 \pm 0.3$ h for (−)-*R* enantiomer, respectively. That $t_{0.5}$ data are approached to those published in the literature [7], where elimination half-life was provided in the range 2.2–2.4 and 2.2–3.5 h for (−)-*R*- and (+)-*S*-KTP, respectively. It could be explained by the fact that KTP enantiomers undergo a limited (−)-*R* to (+)-*S* inversion (9–12%) [6,7]. That conclusion was supported by fact that in urine was conjugated (+)-*S*-KTP following ingestion (−)-*R*, but the reverse was not observed [6]. The differences between other pharmacokinetic parameters presented in Table 4 were insignificant.

4. Conclusion

The worked out CE method fulfils the validation requirements for procedures used for determination of drugs in biological fluids. It is stereospecific, adequately accurate and precise and there is no doubt that it can be applied for pharmacokinetics and bioavalability studies of (−)-*R*- and/or (+)-*S*-KTP and other 2-APA derivatives like ibuprofen, flurbiprofen and naproxen enantiomers. The CE method can be an alternative method to HPLC for determination of KTP enantiomers and other 2-APA derivatives, mentioned above, and of indobufen.

Acknowledgements

The author thanks Professor T.W. Hermann (University of Medical Sciences in Poznań) for providing appropriate equipment to prepare this HPCE work, Professor R. Neubert from Martin Luther University in Halle and Professor B. Clement from Christian Albrecht University in Kiel for the chance to practice CE at their laboratories and K. Marcinkowski University of Medical Sciences authorities in Poznań for financial support of this work (grant #501-3-06-03).

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