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Enantioselective HPLC-DAD method for the determination of etodolac enantiomers in tablets, human plasma and application to comparative pharmacokinetic study of both enantiomers after a single oral dose to twelve healthy volunteers

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ABSTRACT

An enantioselective high performance liquid chromatographic method with diode array detection (HPLC-DAD) was developed and validated for the determination of etodolac enantiomers in tablets and human plasma. Enantiomeric separation was achieved on a Kromasil Cellucoat chiral column (250 mm × 4.6 mm i.d., 5 μm particle size) using a mobile phase consisting of hexane: isopropanol: triflouroacetic acid (90:10:0.1 v/v/v) at a flow rate of 1.0 mL min^{-1}. The chromatographic system enables the separation of the two enantiomers and the internal standard within a cycle time of 8 min. The resolution between the two enantiomers was 4.25 and the resolution between each enantiomer and the internal standard was more than 2.0. Detection was carried out at 274 nm, and the purity assessment was performed using a photodiode array detector. Solid phase extraction technique using C-18 cartridge was applied to extract the analytes from the plasma samples, and the percentage recovery was more than 95% for the lower quantification limit. The method has been validated with respect to selectivity, linearity, accuracy and precision, robustness, limit of detection and limit of quantification. The validation acceptance criteria were met in all cases. The linearity range for the determination of each enantiomer in human plasma was 0.4–30.0 μg mL^{-1} and the limits of quantification of *R*-etodolac and *S*-etodolac were 0.20 and 0.19 μg mL^{-1}, respectively. The validated method was successfully applied to the determination of etodolac enantiomers in tablets and to a comparative pharmacokinetic study of the two enantiomers after the administration of 300 mg single oral dose etodolac racemate tablets to twelve healthy volunteers.

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1. Introduction

The new trend towards developing enantiopure drugs has increased the interest in enantiospecific pharmacokinetics of chiral drugs mainly in the case where only one of the two enantiomers is responsible for the pharmacological activity. The European (EU) guidelines on chiral active drugs hindered the development of chiral drugs as racemates in comparison to the development of the active enantiomer [1]. The development of pharmaceutical preparations containing the active enantiomer simplifies the achievement of pharmacokinetic/ pharmacodynamic relationships, which are particularly useful in characterizing the activity and the action mechanism of drugs.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of several rheumatic and inflammatory diseases. Most of the NSAIDs are used as racemates i.e. mixtures of *R* and *S* enantiomers. The pharmacodynamics, pharmacokinetic and toxicological properties of the two enantiomers are different in vivo [2,3]. Therefore, it is essential to monitor the drug enantiomers in biological fluids to keep the drug therapy safe and effective.

Etodolac (ET, Fig. 1), which is 2-[(1*RS*)-1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4–b]indol-1-yl]acetic acid [4], is a chiral NSAID with a single stereogenic carbon atom and exists in two enantiomeric forms; *R* and *S*. It is marketed as racemate and used as analgesic and for the treatment of the signs and symptoms of rheumatoid arthritis and osteoarthritis [5]. ET decreases the synthesis of peripheral prostaglandins involved in mediating inflammation by inhibition of the cycloxygenase (COX) enzyme [5]. The drug is a selective inhibitor for COX-2 enzyme and it has been reported that its selectivity towards COX-2 enzyme is 10 times that of

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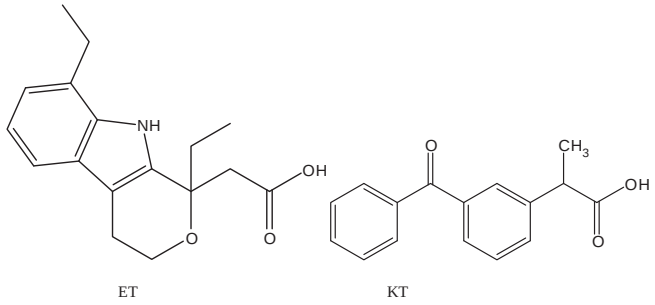

Fig. 1. Structure formulae of etodolac (ET) and ketoprofen (KT).

COX-1 enzyme [6]. It produces less gastrointestinal toxicity compared to the other NSAIDs [5]. The racemate drug is rapidly absorbed after oral administration and is highly bound to plasma protein (99.3%) [7]. It is metabolized in the liver to inactive metabolites that are primarily eliminated via the renal route. ET is administered orally for patients with rheumatoid arthritis at doses of 200–400 mg given twice daily in the form of tablets or capsules. The drug enantiomers possess virtually identical physical and chemical properties [8]. On the other hand, the pharmacological properties of the two enantiomers differ profoundly in which the *S*-form is responsible for the anti-inflammatory activity [8]. Most of the chiral NSAIDs mainly undergo in vivo unidirectional inversion mechanism; mediated by hepatic enzymes; from the inactive *R*-form to the active *S*-enantiomer [9]. ET does not show *in vivo* inversion of its enantiomers [10]. Since enantiomers of racemate drugs exhibit pronounced pharmacokinetic differences, resulting from stereoselective pharmacokinetic processes such as absorption, distribution, metabolism and excretion, it should be noted that the degree of enantioselectivity in pharmacokinetics is markedly species dependent and the data cannot be transposed between species [11]. In view of these differences, studies of the pharmacokinetics of chiral NSAIDs should always measure and quantify the separate enantiomers and not "total drug". The total drug concept fails to recognize that each enantiomer is a separate drug and when products are licensed as the racemic mixture they are products containing two drugs.

Several methods have been reported for the enantioselective determination of ET in pharmaceutical preparations and biological fluids. These methods included capillary electrophoresis (CE) [12, 13], capillary electrochromatography [14], gas chromatography (GC) [15–17] and HPLC [18–25] methods. The HPLC methods could be classified into two categories. The first category included methods using chiral stationary phases; Chiralcel OD [18], Pirkle (R)–DNBPG [18], Chiralpak [19] and Chiralpak IB [20] for separation of the enantiomers. The second category included the HPLC methods using reversed phase C-18 columns after pre-column derivatization with optically pure chiral reagents [21–25]. The procedures of the second category are time consuming due to pre-column derivatization and sample preparation. Moreover, derivatization procedures also affect the reproducibility of the assay and the purity of the analyte since strict control of the reaction conditions are required and removal of the excessive amounts of the derivatising agents before analysis is also necessary [26].

The present study describes the development of a simple and sensitive HPLC-DAD method for enantioselective determination of ET enantiomers in pharmaceutical preparations and human plasma using Kromasil cellucoat chiral column. Optimum conditions for the chromatographic separation of the enantiomers are presented. A solid phase extraction (SPE) of the enantiomers from plasma was carried out. The proposed SPE-HPLC-DAD method was validated and thereafter, the method has been successfully applied to a clinical pharmacokinetic study following a single oral dose of 300 mg ET tablets to twelve healthy Egyptian male volunteers.

2. Experimental

2.1. Materials and reagents

Racemate ET, ketoprofen (KT, Fig. 1), *R*-ET and *S*-ET standard materials were obtained from the European-Egyptian Co for pharmaceutical industries (Alexandria, Egypt) and certified to contain 99.59, 99.82, 99.80 and 99.77%, respectively. Methanol, ethanol, isopropanol and hexane were of HPLC grade and purchased from Sigma-Aldrich (Cairo, Egypt). HPLC grade triflouroacetic acid (TFA) was purchased from Merck (Darmstadt, Germany). Etodolac® tablets (European-Egyptian Co for pharmaceutical industries) labeled to contain 300 mg of racemate ET were purchased from a local drugstore. The octadecayl silane solid phase extraction (C-18 SPE) cartridges (500 mg, 6 mL) were supplied by Agilent technologies (Palo Alto, CA, USA). Pooled control plasma was obtained from the Medical Research Institute (Alexandria, Egypt) and stored at −20 °C until use.

2.2. Instrumentation and chromatographic conditions

The HPLC–DAD system (Agilent Technologies, Palo Alto, CA, USA) consisted of quaternary pump G1311A which comprises a solvent cabinet, vacuum degasser G1322A, a four-channel gradient pump and photodiode array. The chromatographic system is equipped with thermostated column compartment G1316A and manual injector that uses a Rheodyne 7725i 7-port sample injection valve fitted with a 20 μL sample loop. All are Agilent 1200 series. The chromatographic separations were performed on kromasil Cellucoat RP chiral column (tris-[3,4–b] carbamoylcellulose, 250 mm × 4.6 mm, 5 μm). The column was thermostated at 25 °C during analysis. Agilent chemstation software for LC was used for data acquisition and analysis.

For the chromatography of the two enantiomers of ET and the internal standard KT, the mobile phase consisted of hexane: isopropanol: TFA (90:10:0.1 v/v/v). The flow rate of the mobile phase under isocratic elution was kept at 1.0 mL min^{-1}. The injection volume was 20 μL and the chromatographic run time was 10.0 min. Detection of analytes and internal standard was performed at 274 nm. The mobile phases used were degassed and filtered through a 0.45 μm membrane filter (Millipore, Milford, MA, USA) prior to use. The samples solutions were filtered using 0.45 μm disposable filters.

2.3. Analysis of tablets

2.3.1. Preparation of stock and standard solutions

Stock standard solutions of *R*-ET and *S*-ET were prepared by dissolving the enantiomer in ethanol to a final concentration of 250 μg ml^{-1}. Standard solutions of *R*-ET (50 μg mL^{-1}) and *S*-ET (50 μg ml^{-1}) were prepared by the appropriate dilution of the stock standard solutions with ethanol.

2.3.2. Preparation of tablets sample solution

ET tablets sample solution was prepared by weighing 20 tablets, pulverizing them and the average weight of a tablet was calculated. A quantity of the mixed powdered tablets equivalent to 50 mg of ET was weighed, transferred into a 50 mL volumetric flask and mixed with 25 mL of ethanol. The contents of the flask were shaken for 5 min, diluted to volume with ethanol, mixed well and filtered. An aliquot (5 mL) of the filtrate was transferred into a 50 mL volumetric flask and diluted to volume with ethanol. The solution is the ET tablets sample solution.

2.4. Analysis of plasma samples

2.4.1. Preparation of stock standard solutions and quality control (QC) samples

The stock standard solutions of racemate ET (1000 μg mL^{-1}), *R*-ET (750 μg mL^{-1}), *S*-ET (750 μg mL^{-1}) and the internal standard KT (100 μg mL^{-1}) were prepared in ethanol and stored at 4 °C. A series of working standard solutions of *R*-ET and *S*-ET at 10, 20, 40, 80, 160, 200, 300, 400, 500 and 750 μg mL^{-1} was prepared by diluting the stock standard solutions with ethanol and used for preparation of the QC samples. Standard plasma samples were prepared by spiking 20 μL of appropriate working standard solutions of the analyte to 480 μL of control pooled human plasma. Standard plasma for the calibration curve were prepared at concentrations 0.4, 0.8, 1.6, 3.2, 6.4, 8.0, 12.0, 16.0, 20.0 and 30.0 μg mL^{-1} of each enantiomer. Five levels of ET quality control samples of each enantiomer were prepared in blank human plasma at concentrations 1.2, 4.8, 7.2, 14.0 and 25.0 μg mL^{-1}. All samples were stored at −20 °C until analysis. One set of standards and quality controls were analyzed on each analysis day with the same procedure applied to the plasma samples.

2.4.2. Sample preparation

Plasma samples frozen at −20 °C were thawed on the day of analysis at room temperature followed by vortex shaking to ensure homogeneity. 500 μL aliquots were transferred to polypropylene tubes, spiked with 20 μL of KT (100 μg mL^{-1}) as internal standard and mixed for 30 s. The spiked plasma samples were diluted to 1 mL with water before being submitted into the C-18 SPE cartridges that had been previously conditioned by eluting 2 × 1 mL of methanol followed by 2 × 1 mL of water. The samples were eluted slowly at a rate of 2 mL min^{-1} under vacuum and after that, the cartridges were washed with 3 × 1 mL of water. The cartridges were dried under vacuum (−15 mmHg) for 20 min to eliminate the residual water. ET and KT (I.S) were eluted from the SPE cartridges with 2 × 1 mL of methanol and recovered in conical glass tubes. The elution was carried out under atmospheric pressure and negative pressure was applied only to recover the remaining methanol. The eluate was distilled under reduced pressure then the residue was dissolved in 100 μL of absolute ethanol and 20 μL aliquots were injected into the HPLC system.

2.5. Method validation

The developed HPLC-DAD method for the determination of ET enantiomers in tablets was validated following the elements of the guidelines of ICH for the validation of analytical method [27], while the validation of ET determination in human plasma was done following the FDA guidelines for validation of bioanalytical methods [28]. The tested parameters were the matrix effect and selectivity, accuracy, precision, linearity, recovery, limit of detection, limit of quantification and stability.

2.5.1. Matrix effect and selectivity

The selectivity of the method for the determination of ET enantiomers in tablets and the effect of the co-formulated adjuvant added to ET tablets formulation were tested. A portion of placebo tablets containing no active ingredient was processed in the same manner as indicated for sample preparations. Solutions of the co-formulated adjuvant (100 μg mL^{-1}) were prepared in absolute ethanol. The chromatograms of the standard solution, the sample solution and the placebo solution were compared. DAD also supports the selectivity of the method through peak purity assessment using purity plots and match plots for the analyte in both sample and standard solutions.

The selectivity of the method for the determination of ET enantiomers in human plasma was investigated by analyzing at least six different batches of blank human plasma samples. Double blank samples (processed without internal standard) and blank samples (processed with internal standard only) were prepared and tested for confirmation that the endogenous components did not interfere with the peak region for the analytes and the internal standard.

2.5.2. Linearity

The linearity of the HPLC detector response with the concentrations of ET enantiomers in tablets matrix was evaluated using seven concentration levels between 50% and 150% of the analytical method concentration (50 μg mL^{-1}). Triplicate injections made for each concentration. Peak areas plotted versus the concentration for construction of calibration curve and regression data analysis. The acceptance criterion for each back-calculated standard concentration was 100 ± 2% with RSD% lower than 2% [27]. The acceptance criterion for the regression line is that the correlation coefficient (r) must be higher than 0.999 [27].

Calibration curves of plasma samples were constructed by plotting the peak area ratio of etodolac enantiomers to internal standard against the individual enantiomers concentration. Nine concentrations between 0.4 μg mL^{-1} and 30.0 μg mL^{-1} of ET enantiomers in plasma were used to obtain the linearity data that were used in statistical analysis. The acceptance criterion for each back-calculated standard concentration was 100 ± 10% (RSD% < 10%) except at the lower limit of quantification (LLOQ), where the RSD% should not exceed 15% [28]. The acceptance criterion for the regression line is that the correlation coefficient (r) must be higher than 0.99 [28].

2.5.3. Accuracy and precision

The accuracy and precision of the method described for the determination of ET enantiomers in tablets were calculated as the percent recoveries and RSD%, respectively. Known amount of standard ET were added to tablet placebo to reach concentrations of 80%, 100% and 120% of the expected amount of the analyte in real tablet sample. Five replicates were prepared for each concentration level. The accuracy and precision samples solutions were analyzed in the same manner as indicated above for tablets sample preparation. Accuracy was expressed as the percentage of observed value to true value, and precision was expressed as the relative standard deviation (RSD). The criteria for acceptability of the data included accuracy within ± 2% deviation from the nominal values and precision within 2% RSD [27].

The accuracy and precision of the method described for the determination of ET enantiomers in human plasma was evaluated by analysis of plasma samples contained ET enantiomers at the LLOQ and at each concentration of the QC samples in sets of five replicates on the same day and on three consecutive days. Accuracy was expressed as the percentage of observed value to true value, and precision was expressed as the relative standard deviation (RSD). The criteria for acceptability of the data included accuracy within ± 15% deviation from the nominal values and precision within ± 15% RSD [28].

2.5.4. Robustness

The robustness of the method was performed by testing its capacity to be unaffected by a slight change of the minor component of the mobile phase i.e isopropanol (± 5% absolute) and the column's temperature (± 2 °C). the results were evaluated in terms of capacity factor of each peak, the resolution between two consecutive peaks and the percentage recoveries of the analytes.

2.5.5. Extraction recovery

Recovery presents the extraction efficiency of a method, which was determined at the LLOQ (0.4 μg mL^{-1}) and at the five QC levels (1.2, 4.8, 7.2, 14.0, 25.0 μg mL^{-1}; $n=5$). The recoveries were evaluated by comparing the peak-area ratios of ET enantiomers in spiked plasma samples with those of samples to which the ET enantiomers had been added after extraction.

2.5.6. Stability

The stability of ET enantiomers in plasma and the prepared solutions was tested using the QC samples at the five different concentrations under different conditions. The QC samples were subjected to (a) short-term stability by thawing the frozen samples and maintaining them at room temperature for 8 h before analysis, (b) long-term stability by storing the samples at −20 °C for 15 days before analysis, (c) three freeze-thaw stability by allowing the frozen samples at –20 °C to thaw at room temperature and then the samples were refrozen at −20 °C and the process is repeated three times before analysis and (d) post-preparative stability of the processed samples by storing them for 24 h at 4 °C before analysis. All samples were processed using the procedure described under the sample preparation section. Samples were considered to be stable if assay results were within the acceptable limits of accuracy (100 ± 10%) and precision (15% RSD %). All stability samples were estimated by analyzing five replicates ($n=5$).

2.6. Application to pharmacokinetic study

The pharmacokinetics of etodolac tablets was studied in healthy subjects in accordance with the Declaration of Helsinki for biomedical research involving human subjects [29] and good clinical practice (GCP) [30]. The protocol and associated informed consent statements were reviewed and approved by the Pharmacokinetic Unit, Faculty of Pharmacy, University of Alexandria (Egypt). The informed consent form was signed by the volunteers. Twelve Egyptian male subjects, aged from 25 to 35 years, non-smokers and non-drinkers were enrolled in this study. The mean weight and height of these subjects were 65.5 kg and 173 cm, ranging from 60 kg to 71 kg and from 169 cm to 177 cm, respectively. On the basis of medical history, physical examination, clinical examination and laboratory investigations (hematology, blood and urine analysis), all subjects were judged to be in good health. The subjects were instructed to abstain from taking any medication including over the counter (OTC) drugs for at least two weeks prior to and during the study period and to avoid any alcohol or xanthine containing food and beverages 36 h prior to, or during the course of the study. The subjects were under direct medical supervision at the study site.

On the day of the clinical study, following an overnight fast, each subject received a single 300 mg oral dose of ET (Etodolac® 300 mg tablets, European Co for Pharmaceutical Industries, Egypt) with 240 mL of water. Subjects were maintained in the fasting state for 4 h after the drug administration. Blood samples (4 mL) were collected at 0.0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. The samples were transferred to heparinized tubes and centrifuged at 4000 rpm for 20 min. Plasma was separated and stored at −20 °C until analysis. A 500 μL aliquot of the thawed plasma was spiked with the internal standard and processed as described under sample preparation.

The pharmacokinetic parameters were calculated from the plasma concentration–time data. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the concentration-time data. The terminal elimination rate constant (k_e) was obtained from linear regression analysis of the ln terminal linear phase of the concentration-time curve. The elimination half-life ($t_{1/2}$) was calculated as $0.693/k_e$. The area under the plasma concentration–time curve from zero to the last measurable plasma concentration point (AUC_{0-24}) was calculated using the linear trapezoidal method. Extrapolation to time infinity ($AUC_{0-\infty}$) was estimated by the combination of AUC_{0-t} and $AUC_{t-\infty}$, where $AUC_{t-\infty}$ represents the residual area of drug from time t to infinity and were calculated by dividing the last plasma concentration value measured by the elimination rate constant, thus ($AUC_{0-\infty}$) was calculated by the equation $AUC_{0-\infty}=AUC_{0-t}+C_t/k_e$ where C_t is the last measurable plasma concentration value. The oral clearance rate (CL/F) was calculated as $CL/f=\text{dose}/AUC_{0-\infty}$. The apparent (oral) volume of distribution (V_d/F) was calculated as $V_d/f=\text{dose}/(AUC_{0-\infty}\times K_e)$. The mean residence time (MRT) was obtained by dividing the area under the first moment–time curve ($AUMC_{0-\infty}$) by the area under the curve ($AUC_{0-\infty}$).

3. Results and discussion

3.1. Method development and optimization

During development and optimization of an HPLC method for the determination of ET enantiomers in tablets and human plasma using Cellucoat chiral column, several mobile phases were tested to obtain an acceptable resolution between the drug enantiomers and to satisfy the HPLC system suitability. These included the use of hexane containing different proportions of methanol, ethanol or isopropanol. It was found that a mobile phase consisted of hexane and isopropanol (60:40 v/v) would separate ET enantiomers but the retention of both enantiomers and the resolution between them were poor (Fig. 2A). Increasing the proportion of isopropanol in the mobile phase would decrease the resolution between the 2 enantiomers and their retention while using isopropanol as a mobile would elute both enantiomers with the solvent front. Gradual decrease of the % isopropanol in the mobile phase resulted into an improvement of both the resolution between the enantiomers and their retention (Fig. 2B,D) while using n-hexane as a mobile would result into distortion of the chromatogram (Fig. 2E). Therefore, a mobile phase composed of hexane: isopropanol (90:10 v/v) gave acceptable retention of the two enantiomers and good resolution between them (Fig. 2D). Addition of TFA (0.1%) in the mobile phase improved the symmetry of the peaks. The proposed chromatographic method is suitable for the determination of ET enantiomers in tablets.

The direct application of the proposed method for the determination of ET enantiomers in human plasma needed some modifications as the sensitivity of the method could not cope with the concentrations of the drug in plasma and the chromatographic separation was carried out using a normal phase. The modifications included the extraction of the drug enantiomers together with a suitable internal standard from human plasma followed by distillation of the extract under reduced pressure and reconstitution of the residue in minimum amount of absolute ethanol prior to chromatography. KT was selected as an internal standard. It possesses similar chemical structure to ET as both drugs posses free carboxylic acid group (Fig. 1). It is eluted as a single almost symmetric peak between the peaks of ET enantiomers and its peak is well resolved from both enantiomers of ET (Fig. 3A). Moreover, it gave sufficient response at 274 nm. Solid phase extraction (SPE) using C-18 cartridge was applied for the extraction of the drug and the internal standard from the plasma samples. It was found that washing of the plasma sample after application to the C-18 cartridge with 5 mL of water would elute only the biomaterial. Elution of the retained substances from the SPE cartridge with 2 mL of methanol followed by distillation of the eluate and reconstitution of the residue in absolute ethanol proved

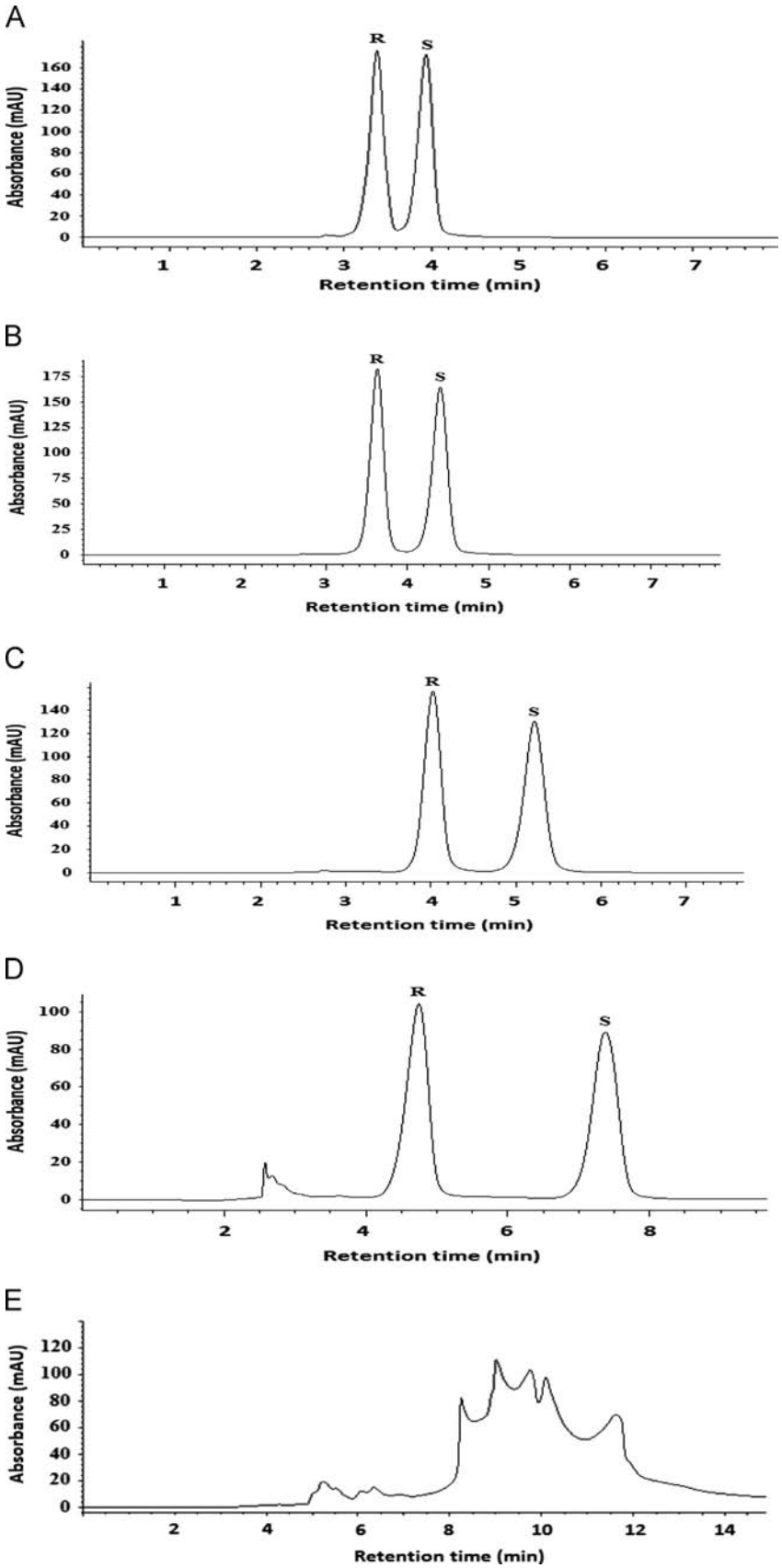

Fig. 2. Representative chromatograms of the effect of % isopropanol in the mobile phase on the chromatography of a mixture containing *R*-ET (R, 51.24 μg mL^{-1}) and *S*-ET (S, 50.45 μg mL^{-1}) using celucoat chiral column (A): (hexane:isopropanol 60:40 v/v), (B): (hexane:isopropanol 70:30 v/v), (C): (hexane:isopropanol 80:20 v/v), (D): (hexane: isopropanol 90:10 v/v) and (E): (hexane:isopropanol 100:0 v/v).

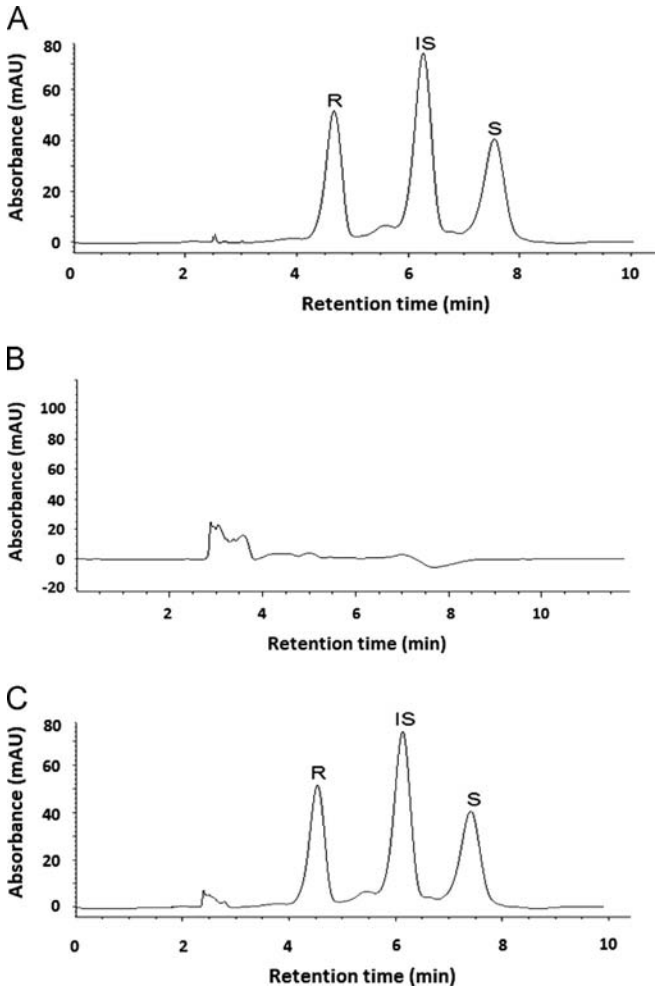

Fig. 3. Chromatograms of (A) a mixture containing *R*-ET (R, 25.62 μg mL^{-1}), *S*-ET (S, 25.20 μg mL^{-1}) and KT internal standard (IS, 20 μg mL^{-1}), (B) a blank plasma, and (C) a plasma sample spiked with *R*-ET (R, 25.62 μg mL^{-1}), *S*-ET (S, 25.20 μg mL^{-1}) and KT (IS, 20 μg mL^{-1}) after SPE procedure using celucoat chiral column and hexane: isopropanol:TEA (90:10:0.1 v/v/v) mobile phase.

that the components of blank plasma would not interfere with the method (Fig. 3B), meanwhile application of extraction procedure to a plasma sample spiked with ET and KT showed a chromatogram (Fig. 3C) that is identical to that of standard shown in fig. 3A.

3.2. Method validation

3.2.1. Selectivity and matrix effect

The selectivity of the method was assessed by applying the proposed chromatographic procedure to tablets placebo and different lots of blank plasma samples (after SPE procedure). No interfering peaks were detected at the retention times of the analytes (*R*-ET and *S*-ET) and internal standard (KT), indicating no interference from the co-formulated adjuvants of the tablets or the endogenous substances of plasma (Fig. 3B). In addition, the spectral purities of *R*-ET, *S*-ET and KT chromatographic peaks were evaluated using the data of the DAD. The similarity of the UV spectra extracted at different time intervals throughout the elution time of each peak, in addition to the findings that the purity angles were within the purity threshold limits for all of the analyzed samples, indicating that no peaks were co-eluted with the analytes and evidencing the ability of the method to assess the analytes of interest in plasma or tablets without interference from the co-formulated adjuvants of the tablets or the endogenous components of the plasma.

3.2.2. Linearity and range

The linearity of an analytical method is defined as the ability of the method (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample, while the range is the interval between the upper and lower analyte concentrations (including these values) for which the method has a suitable level of precision, accuracy and linearity [27]. The linearity of the HPLC detector response with the concentrations of the *R*-ET and *S*-ET in tablets matrix was evaluated using 7 concentration levels between 50% and 150% of the working concentration of the method while that for the plasma matrix was evaluated using 9 concentrations ranged between 0.4 μg mL^{-1} and 30 μg mL^{-1}. Under the optimal experimental chromatographic conditions for the

Table 1
Linearity parameters used for the determination of *R*-ET and *S*-ET in plasma samples and tablets using the proposed HPLC method.

Parameter	Matrix			
	Plasma		Tablets	
	R-ET[a]	*S*-ET[a]	*R*-ET[b]	*S*-ET[b]
Linearity range (μg mL^{-1})	0.4–30.0	0.4–30.0	25.0–75.0	25.0–75.0
LOD (μg mL^{-1})	0.06	0.06	0.12	0.14
LOQ (μg mL^{-1})	0.20	0.19	0.41	0.47
Regression parameters[c]				
Slope (b)	0.086	0.133	42.886	42.442
Standard deviation of slope (S_b)	0.002	0.003	0.060	0.047
Upper confidence limit of slope[d]	0.0914	0.1401	43.0.0770	42.5924
Lower confidence limit of slope[d]	0.0798	0.1249	42.6951	42.2916
Intercept (a)	0.0018	−0.0052	−3.802	5.701
Standard deviation of intercept (S_a)	0.012	0.016	0.312	0.246
Upper confidence limit of intercept[d]	0.0362	0.0398	6.1219	13.5148
Lower confidence limit of intercept[d]	−0.0326	−0.0502	−13.7219	−2.1148
Standard deviation of residuals ($S_{y/x}$)	0.014	0.018	1.897	1.494
Correlation coefficient (r)	0.9988	0.9991	0.9999	0.9999

[a] Peak area ratio using ketoprofen as internal standard.
[b] Peak area.
[c] $Y=a+bC$, where C is the concentration (μg mL^{-1}) and Y is the peak area ratio or peak area.
[d] 99% confidence limit.

determination of ET enantiomers in plasma, linear relationships were obtained between the peak area ratios of the analyte (*R*-ET or *S*-ET) to that of the internal standard within the concentration ranges stated in Table 1. Meanwhile, the determination of *R*-ET and *S*-ET in tablets depends on the use of peak area to construct the calibration graphs within the concentration ranges presented in Table 1. The lower limit of quantification (LLOQ) of ET enantiomers in plasma was determined as the concentration at which the signal-to noise (*S*/*N*) ratio was greater than 10, with precision: calculated as RSD%; lower than 10% and the % accuracy observed for the mean of back-calculated concentration was within 90–110. In this study, the LLOQ for ET enantiomers in plasma matrix was 0.4 μg mL^{-1}. The results of the statistical analysis [31] of the data are summarized in Table 1. The high values of the correlation coefficient (*r*) of the regression lines, small values of the standard deviation of residuals ($S_{y/x}$), the slope (S_b) and the intercept (S_a) indicated good linearity of the calibration graphs.

The range of linearity of ET-enantiomers in plasma matrix (0.4–30 μg mL^{-1}) is larger compared to that in tablets matrix (25–75 μg mL^{-1}). Therefore, the homoscedasticity assumption of the calibration data of ET-enantiomers in plasma matrix was tested using the residual plots (plots of residuals versus concentration) and by analysis of variance (ANOVA) *F*-test [31–33]. The residual plots showed a random distribution over the X-concentration axis and the residuals were more or less within a band parallel to the X-concentration axis indicated that the calibration data in plasma matrix are homoscedastic [31–33]. Moreover, the homoscedasticity of the calibration data was further confirmed by applying ANOVA *F*-test statistical method. The experimental *F* value; expressed as the ratio between the variances at the lowest SD^2 and the highest SD^2 of the working concentration range; was calculated. The lowest SD^2 corresponded to the upper limit of quantification (ULOQ) while the highest SD^2 corresponded to the lower limit of quantification (LLOQ). The tabled F value is obtained from the *F*-table at confidence level 99% for $f1=f2=(n-1)$ degree of freedom. The results (Table 2) indicated that the calibration data for the determination of ET enantiomers in plasma matrix is homoscedastic as the experimentally calculated *F*-values are lower than the table one. Consequently, ordinary linear regression is appropriate for calibration of the data of ET-enantiomers in plasma matrix and there was no need to use weighted linear regression.

Table 2
Test of homoscedasticity of ET calibration data in plasma matrix using F-test.

Standard in plasma matrix (μg mL^{-1})	*R*-ET		*S*-ET	
	Peak area ratio	SD	Peak area ratio	SD
0.4	0.0722	0.0048	0.0977	0.0062
	0.0699		0.1089	
	0.0678		0.1101	
	0.0766		0.1099	
	0.0639		0.0989	
30	2.5811	0.0141	3.9701	0.0178
	2.5622		4.0101	
	2.5977		3.9888	
	2.5707		3.9711	
	2.5888		4.0012	
F[a]	8.6289		8.2425	

[a] The theoretical value of $F_{4,4}$ ($p=0.01$) is 15.98.

3.2.3. Accuracy and precision

Accuracy of an analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found [27] but precision of an analytical method is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [27]. The accuracy of the developed method was confirmed by measuring the recovery and the percentage error (E_r %) of known added amounts of each enantiomer into a blank matrix (tablets placebo or blank plasma). The results (Table 3) indicated that good accuracy was obtained as the mean % recoveries were 99.40 to 100.92 and 95.02 to 100.88 and the E_r% were less than 1.00% and 5.00% for the determination of *R*-ET and *S*-ET enantiomers in tablets matrix and plasma, respectively. Application of the Student's *t*-test; to compare the within sample mean with its corresponding standard value; indicated that the method is accurate as the calculated *t*-values were lower

Table 3
Accuracy and precision of the determination of ET enantiomers in tablets and human plasma ($n=5$).

Matrix	Drug	Added concentration (μg mL^{-1})	Intra-day				Inter-day			
			Mean recovery (%) ± SD	RSD (%)	E_r (%)	*t*-value[a]	Mean recovery (%) ± SD	RSD (%)	E_r (%)	*t*-value[a]
Tablets	*R*-ET	40.0	99.40 ± 0.64	0.64	−0.60	2.10	100.82 ± 0.84	0.84	0.82	2.18
		50.0	100.33 ± 0.52	0.52	0.33	1.42	100.54 ± 0.71	0.71	0.54	1.42
		60.0	100.10 ± 0.87	0.87	0.10	0.26	100.69 ± 0.99	0.98	0.69	1.56
	S-ET	40.0	100.32 ± 1.01	1.00	0.32	0.72	100.92 ± 0.94	0.93	0.92	2.21
		50.0	100.26 ± 0.82	0.82	0.26	0.71	100.60 ± 0.67	0.66	0.60	2.00
		60.0	99.81 ± 0.91	0.91	−0.19	0.47	100.37 ± 0.55	0.55	0.37	0.46
Plasma	*R*-ET	0.4	96.47 ± 3.82	3.96	−3.53	2.07	95.55 ± 4.80	5.02	−4.45	2.07
		1.2	95.66 ± 3.96	4.14	−4.34	2.45	95.02 ± 4.22	4.45	−4.98	2.64
		4.8	97.85 ± 2.22	2.27	−2.15	2.17	96.91 ± 3.11	3.21	−3.09	2.22
		7.2	97.69 ± 2.09	2.14	−2.31	2.47	97.49 ± 2.77	2.84	−2.51	2.03
		14.0	99.38 ± 1.88	1.89	−0.62	0.74	98.60 ± 2.29	2.32	−1.40	1.37
		25.0	99.10 ± 1.71	1.72	−0.90	1.18	98.98 ± 2.07	2.09	−1.02	1.10
	S-ET	0.4	95.72 ± 4.31	4.50	−4.28	2.22	95.27 ± 5.17	5.43	−4.73	2.05
		1.2	96.12 ± 4.11	4.28	−3.88	2.11	95.10 ± 5.33	5.61	−4.90	2.06
		4.8	98.00 ± 3.01	3.07	−2.00	1.49	97.55 ± 2.71	2.78	−2.45	2.02
		7.2	98.55 ± 2.61	2.65	−1.45	1.24	97.88 ± 2.78	2.84	−2.12	1.71
		14.0	99.01 ± 1.88	1.90	−0.99	1.18	98.65 ± 1.99	2.02	−1.35	1.52
		25.0	100.51 ± 1.59	1.58	0.51	0.72	100.88 ± 1.78	1.76	0.88	1.15

[a] The theoretical value for *t*-value ($p=0.01$) is 4.60.

than the theoretical value (Table 3). The within-day and between-day precision for the five replicate determination (Table 3) indicated that the method is precise for the determination of ET enantiomers in tablets and human plasma as the percentage relative standard deviation values were less than 1.25% and 6.00%, respectively. The assessment of precision was also performed using ANOVA. The calculated *F*-values for the intra- and inter-day determination of ET enantiomers in tablets were lower than 2.80 and 3.00, respectively. The calculated *F*-values for the intra- and inter-day determination of ET enantiomers were lower than 7.40 and 8.50, respectively. The calculated *F*-values were lower than the theoretical *F*-value (15.98, $p=0.01$) indicating good precision. These finding fulfils to the requirements and acceptance criteria for the accuracy and precision of analytical [27] and bioanalytical [28] methods of analysis.

3.2.4. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [27]. The parameters that varied in order to test the robustness of the method were the composition of the mobile phase and the temperature. Each parameter was varied on three levels while keeping all other parameters constant. For each measurement set, the values of %RSD of the capacity factor, the resolution, and the percentage recovery of each enantiomer were calculated using replicate measurements for each level. The obtained results are summarized in Table 4 proving the robustness of the developed method.

3.2.5. Limit of detection and limit of quantification

The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can reliably be detected (signal-to-noise ratio is 3:1) while the limit of quantification (LOQ) was determined by establishing the lowest concentration of analyte that can be determined with acceptable precision and accuracy (signal-to-noise ratio is 10:1) [27]. As shown in Table 1, the LOD and LOQ proved that the proposed method is sensitive for the detection and the determination of very small concentrations of ET enantiomers in both tablets and human plasma.

3.3. Extraction efficacy

Solid phase extraction (SPE) and liquid–liquid extraction (LLE) techniques are commonly used in the preparation of biological samples due to their ability to improve the sensitivity and robustness of the assay. In the present work, SPE method was adopted. It provided acceptable high recovery, the desired sensitivity, stability of both ET enantiomers (analytes) and KT (internal standard) and simplicity as the SPE procedure used was an automated one. At the five QC concentration levels, the percent extraction recovery for both

Table 4
Robustness data.

Mobile phase	Composition		Temperature (°C)	Capacity factor (k')			Resolution (Rs)[a]		Mean % recovery (RSD %)[b]	
	Hexane	Isopropanol		*R*-ET	KT	*S*-ET	*R*-ET/ KT	KT/ *S*-ET	*R*-ET	*S*-ET
A	90	9.5	25	0.761	1.322	1.678	2.896	2.053	100.12 (0.56%)	100.23 (0.73%)
B[c]	90	10	25	0.752	1.317	1.659	2.890	2.042	99.87 (0.81%)	99.92 (0.68%)
C	90	10.5	25	0.741	1.306	1.647	2.884	2.035	99.79 (1.10%)	99.57 (0.97%)
D	90	10	23	0.756	1.319	1.663	2.890	2.041	100.07 (1.12%)	99.84 (0.93%)
B[c]	90	10	25	0.752	1.317	1.659	2.890	2.042	99.87 (0.81%)	99.92 (0.68%)
E	90	10	27	0.748	1.313	1.654	2.898	2.048	100.28 (1.38%)	100.37 (0.99%)

[a] Resolution between 2 consecutive peaks.
[b] Mean and the % relative standard deviation of three determinations.
[c] Chromatographic method parameters.

Table 5
Stability of ET enantiomers in human plasma ($n=5$).

Stability condition	Targeted concentration (μg mL^{-1})		Mean recovery (%) ± SD		Error (E_r %)		Precision (RSD %)	
	R-ET	*S*-ER	*R*-ET	*S*-ET	*R*-ET	*S*-ET	*R*-ET	*S*-ET
Short-term stability	1.20	1.20	95.17 ± 4.02	95.10 ± 4.54	−4.83	−4.90	4.22	4.79
	4.80	4.80	96.88 ± 3.61	96.49 ± 4.04	−3.12	−3.51	3.73	4.19
	7.20	7.20	97.33 ± 3.11	97.65 ± 3.89	−2.67	−3.35	3.20	3.98
	14.00	14.00	98.77 ± 2.11	98.23 ± 1.99	−1.23	−1.73	2.14	2.03
	25.00	25.00	98.48 ± 2.45	98.51 ± 1.88	−1.52	−1.49	2.49	1.91
Freeze–thaw stability	1.20	1.20	95.13 ± 4.82	95.75 ± 4.71	−4.87	−4.25	5.08	4.92
	4.80	4.80	96.59 ± 3.90	95.88 ± 4.22	−3.41	−4.12	4.04	4.40
	7.20	7.20	97.82 ± 3.55	97.59 ± 3.09	−2.18	−2.41	3.63	3.17
	14.00	14.00	98.44 ± 2.48	98.57 ± 2.17	−1.56	−1.43	2.52	2.20
	25.00	25.00	98.88 ± 2.12	98.18 ± 1.96	−1.12	−1.82	2.14	2.00
Long-term stability	1.20	1.20	95.47 ± 4.92	95.32 ± 4.83	−4.53	−4.68	5.15	5.07
	4.80	4.80	95.99 ± 4.11	96.09 ± 4.15	−4.01	−3.91	4.28	4.32
	7.20	7.20	97.88 ± 3.56	97.81 ± 3.00	−2.12	−2.19	3.64	3.07
	14.00	14.00	98.22 ± 2.71	98.40 ± 1.39	−1.78	−1.60	2.76	1.41
	25.00	25.00	98.85 ± 2.01	98.19 ± 1.72	−1.15	−1.81	2.03	1.75
Post-preparative stability	1.20	1.20	95.01 ± 5.01	95.05 ± 4.88	−4.99	−4.95	5.27	5.13
	4.80	4.80	96.75 ± 3.89	96.00 ± 4.82	−3.25	−4.00	4.02	5.02
	7.20	7.20	97.90 ± 3.34	98.03 ± 3.39	−2.10	−1.97	3.41	3.46
	14.00	14.00	98.65 ± 2.22	98.11 ± 2.31	−1.35	−1.89	2.25	2.35
	25.00	25.00	98.31 ± 2.08	98.98 ± 1.87	−1.69	−1.02	2.11	1.89

enantiomers (mean ± % standard deviation) after five replicates (Table 3) indicated that the extraction efficacy for the tested analytes using SPE was satisfactory and no significant matrix effects were evident.

Table 6
Assay results for the determination of ET enantiomers in preparations.

Preparation	*R*-ET			*S*-ET			Ratio *R*-ET/ *S*-ET
	Mean recovery (%) [a]	Er (%)	CV (%)	Mean recovery (%) [a]	Er (%)	CV (%)	
Raw material	49.86	−0.14	0.52	49.88	−0.12	0.60	≈1
Tablets	50.07	0.07	0.44	49.95	−0.05	0.71	≈1

[a] Mean of five determinations.

3.4. Stability

Three freeze–thaw cycles of the QC samples (freeze–thaw stability) did not appear to affect the quantification of ET enantiomers. Thawing the frozen samples and maintaining them at room temperature for 8 h (short-term stability) had no effect on quantification of ET enantiomers. The QC samples were stored in a freezer at −20 °C and remained stable for at least 15 days (long-term stability). The prepared samples were also analyzed after at least 24 h at 4 °C (post-preparative stability). The results of the stability studies (Table 5) indicated that human plasma samples containing ET enantiomers can be handled under normal

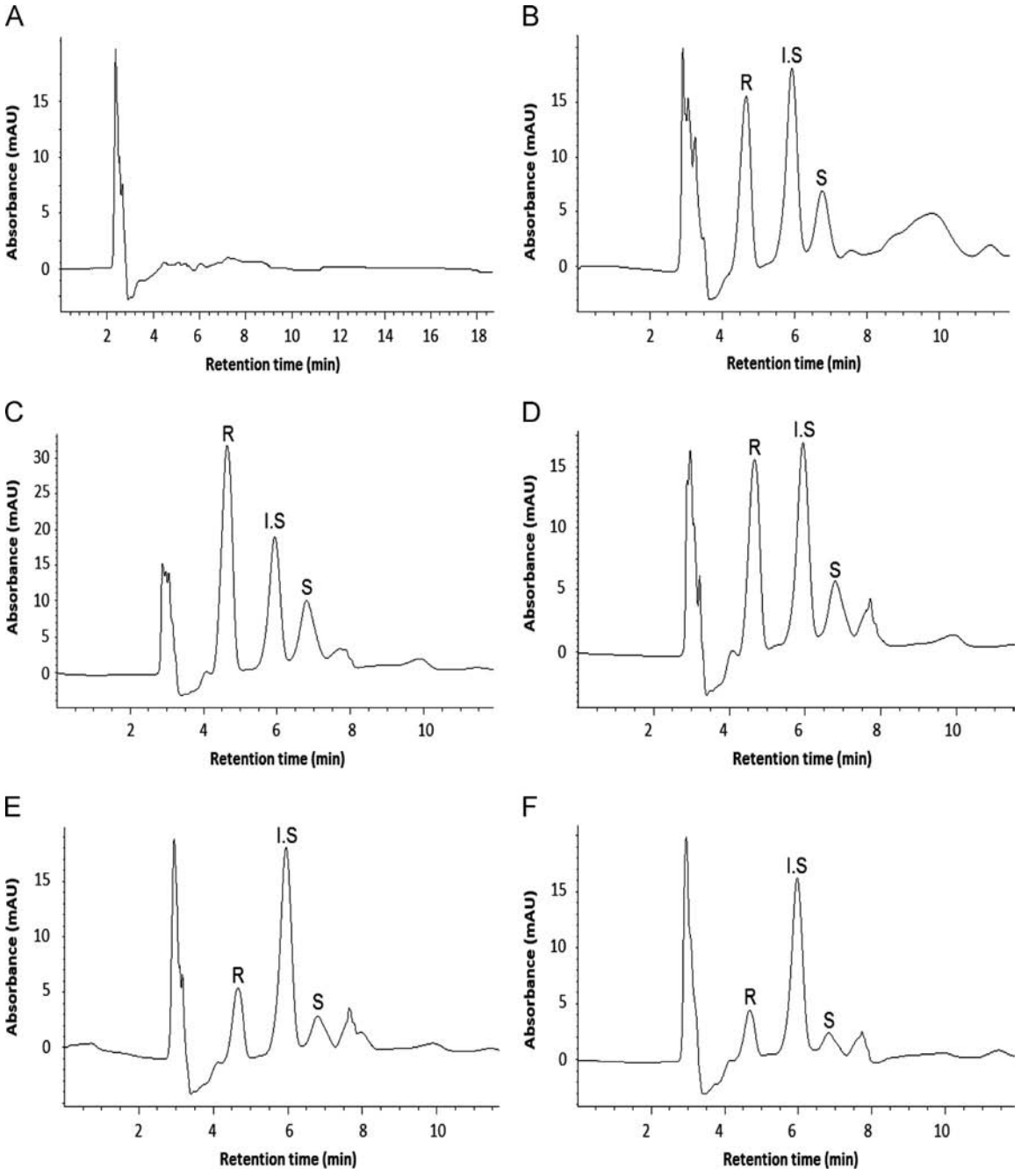

Fig. 4. Typical chromatograms of plasma samples collected at (A) pre-dosing, (B) 0.5 h, (C) 1.5 h, (D) 3.0 h, (E) 8.0 h and (F) 12 h after 300 mg oral dose of racemate ET tablets to a volunteer. *R*: *R*-ET., *S*: *S*-ET., I.S.: KT internal standard.

laboratory conditions and could be stored at −20 °C for 15 days without any significant compound loss.

3.5. Assay of raw material and tablets

Commercially available pharmaceutical grade raw material and tablets (Etodolac® tablets) were analyzed for the contents of the *R*- and *S*-ET enantiomers using the proposed validated HPLC-DAD method. The results (Table 6) indicated that the raw material and the tablets contain equal concentrations of the *R*- and *S*- enantiomers of the drug and were in a good agreement with the label claim. The RSD (%) and E_r (%) of the assay results (Table 6) indicated high precision and accuracy of the proposed method.

3.6. Pharmacokinetic application

The proposed method was successfully applied to a pharmacokinetic study of ET enantiomers in 12 healthy Egyptian male volunteers following single oral administration of 300 mg of ET tablets. The concentrations of each enantiomer in the plasma samples prepared from the blood samples collected from each volunteer throughout a period of 24 h were calculated. Fig. 4 shows representative chromatograms for the analysis of *R*-ET and *S*-ET at the pre-dose and five different sampling time intervals. The concentration-time profile of *R*-ET and *S*-ET in these volunteers is shown in Fig. 5 and the mean estimated non-compartmental pharmacokinetic parameters derived from the plasma-concentration profiles are summarized in Table 7. The *R*-ET and *S*-ET plasma concentrations calculated at different time intervals for the twelve volunteers showed that both enantiomers are readily absorbed after oral administration and the concentration of the *R*-enantiomer was generally higher than that of the *S*-enantiomer (Figs. 4 and 5). The two enantiomers reached the peak plasma concentration at approximately the same time after oral administration of ET i.e. 1 h which reflects a similar absorption profile of both enantiomers. The mean C_{max} of *R*-ET was higher than that of *S*-ET by 3.8 folds which come in agreement with the in-vivo study conducted on rats [34]. The $t_{1/2}$ values of both enantiomers were found to be 5.73 and 5.46 h for *R*-ET and *S*-ET, respectively. The values of AUC_{0-24} and $AUC_{0-\infty}$ were found to be higher for the *R*-enantiomer than that of the *S*-enantiomer by 3.93 and 3.92 folds, respectively. The values of the apparent (oral) volume of distribution and the oral clearance were found to be higher for the *S*-enantiomer than that of the *R*-enantiomer by 3.74 and 3.91 folds, respectively. The repetitive dosing pharmacokinetic study of etodolac carried out using only two volunteers [23] gave lower values of oral clearance for both enantiomers and higher distribution of the active *S*-enantiomer than that in the current study conducted on single oral dose. The results revealed that the pharmacokinetics of etodolac enantiomers are dose dependent since repetitive dosing of etodolac racemate gives continue increasing concentrations of both enantiomers in plasma that may affect the capacity of the metabolizing enzymes and hence alter the values of the pharmacokinetic parameters [35]. Therefore, determination of the pharmacokinetic parameters of ET using repeated dosing approach will not reflect accurately the clinical behavior of ET as it is analgesic drug commonly prescribed for acute disease (pain) and rarely used for chronic diseases.

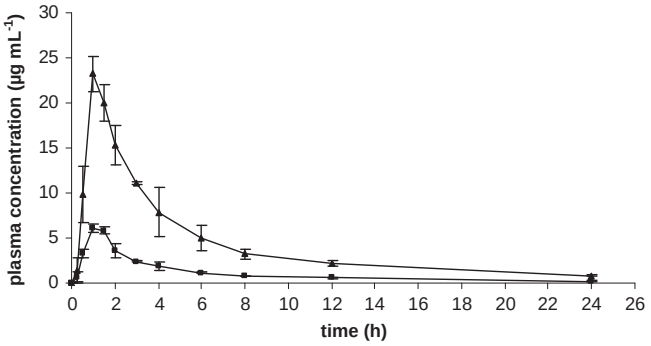

Fig. 5. Mean plasma concentration–time profile of *R*-etodolac (▲) and *S*-etodolac (■) following single oral administration of a dose of 300 mg of racemic etodolac to 12 Egyptian subjects.

Table 7
Main pharmacokinetic parameters of ET enantiomers following single oral dose of 300 mg to healthy volunteers ($n=12$).

Parameter	Mean ± SD	
	R-ET	*S*-ET
C_{max} (µg mL^{-1})	23.26 ± 1.58	6.12 ± 0.39
T_{max} (h)	1.13 ± 0.23	1.08 ± 0.19
$t_{1/2}$ (h)	5.73 ± 0.59	5.46 ± 0.41
MRT (h)	7.50 ± 0.47	7.40 ± 0.43
AUC_{0-24} (µg h mL^{-1})	102.49 ± 7.25	26.06 ± 0.52
$AUC_{0-\infty}$ (µg h mL^{-1})	109.33 ± 8.34	27.87 ± 0.95
CL/f (L h^{-1})	2.76 ± 0.22	10.78 ± 0.37
V_d/f (L)	22.64 ± 0.95	84.73 ± 4.32

The interpretation of the findings that the relative plasma concentration of the *R*-enantiomer is higher than the *S*-enantiomer, for the first while without considering any factors, could be due to a higher absorption rate of the *R*-enantiomer than the *S*-enantiomer and/or extensive metabolism of the *S*-enantiomer compared to the *R*-enantiomer. Jing-min et-al [34] studied the pharmacokinetic differences between the two enantiomers in rats and proposed without evidences that the *S*-enantiomer may undergo extensive metabolism compared to the *R*-enantiomer. If that proposal is the case and as the drug interacts with the COX enzyme by competitive inhibition which is concentration dependent, so the inactive *R*-enantiomer would block the enzyme and the availability of pharmaceutical preparations containing racemate ET must be evaluated which is not accepted for such pharmacologically active drug. The same authors (Jing-min et-al) [34] proposed without evidences that the higher concentration of the *R*-enantiomer in plasma is due to the high distribution of the *S*-enantiomer in the synovial fluid compared to the *R*-enantiomer. The proposal could be accepted if synovial fluid is enantioselective which is not the case. It is suggested that the difference in pharmacological activity of ET enantiomers could be due to the interaction (binding) with the COX enzyme.

Non-steroidal anti-inflammatory drugs as indomethacine (IN, Fig. 6), sulindac (SU, Fig. 6), diclofenac (DI, Fig. 6), ibuprofen (IB, Fig. 6), and Ketoprofen (KT, Fig. 6) possess a carboxylate anionic group separated from a planar hydrophobic moiety (benzene ring, indole ring, …,etc) by one carbon atom attached to a hydrophobic side chain. These are the essential structural requirements for the pharmacological activity for NSAIDs [36]. COX enzyme; the receptor of NSAIDs; possesses a cationic argenine residue to interact with the carboxylate group of the drug, a hydrophobic planar area to interact with the planar hydrophobic moiety of the drug and a hydrophobic trough to fit the hydrophobic side chain of the drug [36]. The distance between the cationic argenine residue and the hydrophobic planar area is exactly the same as that between the carboxylate anionic group and the hydrophobic planar area of NSAIDs. ET (ET, Fig. 6) is different from the other NSAIDs as the distance between the carboxylate group and the indole planar moiety is two carbon atoms, one of them is a part of alicyclic tetrahydropyrane ring. The tetrahydropyran ring could undergo

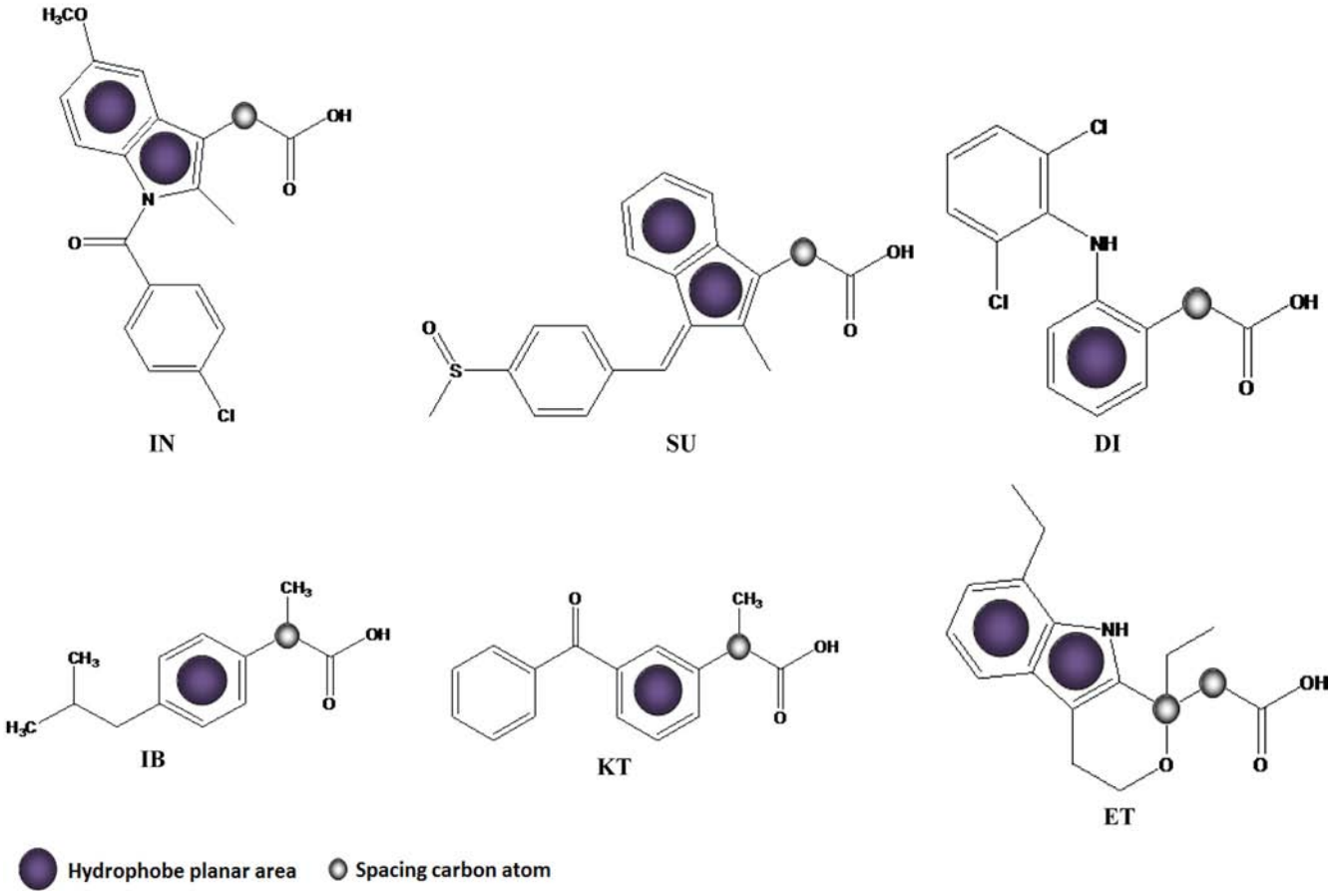

Fig. 6. Presentation of the chemical structures of indomethacine (IN), sulindac (SU), diclofenac (DI), ibuprofen (IB), ketoprofen (KT) and etodolac (ET).

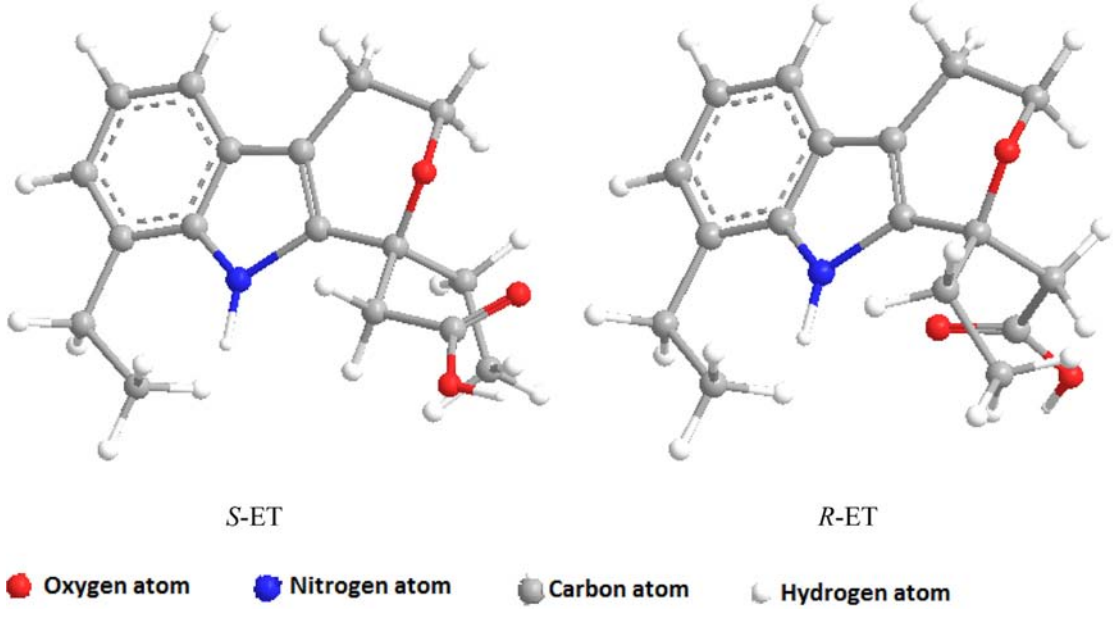

Fig. 7. Three-dimension conformational chemical structures of *S*-etodolac (*S*-ET) and *R*-etodolac (*R*-ET).

conformation (chair and boat). The conformation of the *S*-ET and *R*-ET of the lowest energy are presented in Fig. 7. The carboxylate and indole in the *S*-ET are on the same side and the distance between them is similar to that of the COX enzyme and so bind to the enzyme but the carboxylate and the indole in the *R*-ET are on opposite sides and could not bind to the enzyme. It is suggested that both ET enantiomers would enter the extra-vascular cells from blood plasma by passive transport to reach the extra-vascular COX enzyme where the conformer of *S*-ET is strongly bound to it but the conformer of *R*-ET could not bind to it. Therefore, the

concentration of free *R*-ET in the cells would be higher than *S*-ET and hence more *S*-ET would be transported from the blood plasma into the extra-vascular cells to compensate for the bound *S*-ET resulting into a decrease of *S*-ET in blood plasma. The proposal is strengthened by the fact that COX enzyme is an extra-vascular enzyme, the finding that the apparent volume of distribution (Vd/f) of *S*-ET is higher than that of *R*-ET and the elimination half-life of both enantiomers is the same. Another contributing factor to the stereoselectivity in pharmacokinetics is the stereoselective biotrasformation (metabolism) of chiral drugs. Metabolizing enzymes often display a preference for one enantiomer of a chiral drug over the other, resulting in enantioselectivity. The structural characteristics of enzymes dictate the enantiomeric discrimination associated with the metabolism of chiral drugs [37–40]. Chiral NSAIDs as ketoprofen, ibuprofen, flurbiprofen or 2-arylpropionic acids derivatives undergo unidirection stereoselective inversion mediated by the hepatic enzyme where the inactive *R*-enantiomer is stereoselectively biotransformed to the active ***S***-enantiomer [9,41–44]. ET is an NSAID of the same chemical category of the above mentioned drugs, yet it does not undergo the same in vivo inversion [10] and so the concentrations of ET enantiomers would not be affected. This could be due to the presence of alicyclic ring in ET chemical structure that allows the conformation of the drug at the physiological pH and might hinder such unidirectional inversion (Fig. 7). ET is metabolized in the liver to inactive metabolites that are primarily eliminated via the renal route [7]. Up to knowledge, the stereoselective metabolism (biotransformation) of ET and its impact on the concentration of its enantiomers in plasma has not been reported and still under investigation in our laboratories.

4. Conclusion

The enantiomers of a chiral drug may differ significantly in their pharmacokinetics, potency, selectivity for receptors and toxicity. The use of single enantiomer of racemate drugs could result in selective pharmacologic profiles, improved therapeutic indices, and simple pharmacokinetics due to different rates of metabolism of the different enantiomers. In this study, an Enantioselective HPLC-DAD method has been developed for the determination of ET enantiomers in tablets and in human plasma. The method proved to be selective, accurate, precise and robust for the determination of both ET enantiomers. The method was successfully applied to study the pharmacokinetics of each enantiomer in humans where the two enantiomers showed markedly different pharmacokinetic behavior. The concentrations of the inactive *R*-enantiomer in plasma samples were higher than that of the active *S*-enantiomer after oral administration of a single oral dose of the racemate ET tablets to healthy volunteers. The active *S*-enantiomer was assumed to be the form of the racemate ET that binds to the targeted cyclooxygenase enzyme which is an extra-vascular one. It is concluded that the use of the enantiopure *S*-ET would provide a superior medication compared to the racemate ET.

Acknowledgment

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