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Enantioselective assay of S(+)- and R(−)-propafenone in human urine by using RP-HPLC with pre-column chiral derivatization*

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Abstract: The enantioselective assay for S(+)- and R(−)-propafenone (PPF) in human urine that developed in this work involves extraction of propafenone from human urine and using S(+)-propafenone as internal standard, chiral derivatization with 2,3,4,6-tetra-*O*-*β*-D-glucopranosyl isothiocyanate, and quantitation by an RP-HPLC system with UV detection (λ=220 nm). A baseline separation of propafenone enantiomers was achieved on a 5-μm reverse phase ODS column, with a mixture of methanol:water:glacial acetic acid (25:12:0.02,v/v) as mobile phase. There was good linear relationship from 24.9 ng/ml to 1875.0 ng/ml for both of enantiomers. The regression equations of the standard curves based on $C_{S\text{-}PPF}$ (or $C_{R\text{-}PPF}$) versus ratio of $A_{S\text{-}PPF}/A_S$ (or $A_{R\text{-}PPF}/A_S$) were y=0.0032x−0.081, (r=0.999) for S-PPF and y=0.0033x+0.0039, (r=0.998) for R-PPF, respectively. The method's limit of detection was 12.5 ng/ml for both enantiomers, and the method's limit of quantitation was 28.2±0.52 ng/ml for S-PPF, 30.4±0.53 ng/ml for R-PPF (RSD<8%, n=5). The analytical method yielded average recovery of 98.9% and 100.4% for S-PPF and R-PPF, respectively. The relative standard deviation was no more than 6.11% and 6.22% for S-PPF and R-PPF, respectively. The method enabled study of metabolism of S(+)- and R(−)-propafenone in human urine. The results from 7 volunteers administered 150 mg racemic propafenone indicated that propafenone enantiomers undergo stereoselective metabolism and that in the human body, S(+)-propafenone is metabolized more extensively than R(−)-propafenone.

Key words: Enantioselective assay, Propafenone, Human urine, Chiral derivatization, High-performance liquid chromatography

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INTRODUCTION

Pharmacokinetic and pharmacodynamic differences involving stereoselective drug disposition are important for clinical evaluation of some chiral compounds that are rountinely administered in their racemic form (Zhou *et al.*, 2002). Propafenone (PPF), a kind of Ic antiarrhythmics drug, is now marketed as racemate. The two enantiomers of PPF are equipotent in terms of sodium channel-blocking activity, but the main side effect is the *β*-adreno receptor-blocking action of the S(+)-isomer (Malfatto *et al.*, 1988) so information on the stereoselective disposition of the racemate is of clinical relevance. Propafenone traverses three main metabolic pathways, including *N*-desalkylation via CYP3A4 and CYP1A2, 5-hydroxylation via CYP2D6, and glucuronidation via glucuronosyltransferase (Kroemer *et al.*, 1996). In this work, an analytical method for determination of PPF enantiomers was developed by RP-HPLC using GITC as chiral derivatization reagent to study the stereoselective metabolism of

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PPF enantiomers in human.

MATERIALS AND METHODS

Chemicals

Racemic PPF hydrochloride, S(−)-propranolol, S(+)-PPF, R(−)-PPF, 2,3,4,6-tetra-*O*-*β*-D-glucopranosyl isothiocyanate (GITC) were purchased from Sigma (St Louis, MO, USA). Concentrated ammonia, ether, methanol, glacial acetic acid, acetonitrile were analytical reagent or chromatographic grade and obtained from common commercial sources.

Instrumentation and chromatographic conditions

The modular HPLC equipment was composed of a LC-10AT *VP* with SPD-10A *VP* (Simadau, Japan) and N2000 data system (Zhejiang University, China). The analytical column was a Shimpack CLC-ODS (150 mm × 4.6 mm i.d., 5 μm). A mixture of methanol-water-glacial acetic acid (25:12:0.02, v/v) was used as mobile phase. The flow rate was set at 0.8 ml/min. The wavelength of the UV detector was set at 220 nm.

Assay procedure

1.0 ml of urine was transferred to a 10 ml Teflon-lined, screw-capped test tube. Ten μl of 50 μg/ml S-propranolol was added as an internal standard. Two tenth ml of concentrated ammonia was added to alkalize urine samples. Propafenone and S-propranolol were extracted with 2 ml ether by gently rocking for 15 min. The ether was evaporated to dryness after the mixture was centrifuged at 10 000 ×g for 15 min and organic phase was separated. The residue was dissolved with 100 μl of GITC solution (1 mg/ml in acetonitrile). The chiral derivatization was allowed to react at 37 °C for 30 min. The reaction mixture was evaporated to dryness under a gentle nitrogen stream. The residue was reconstituted with 100 μl of the mobile phase and an aliquot of 20 μl of the resulting solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Chromatogrphic specificity

S-(+)- and R(−)-propafenone reacted with GITC and formed the S-(+)- and R(−)-propafenone-GITC derivatives (Fig.1).

The blank urine sample was prepared according to the *Assay Procedure*. No interferences appeared at the peak positions of S(+)-propafenone-GITC derivatives, R(−)-propafenone-GITC derivatives and internal standard-GITC derivatives (Fig. 2a). S(+)-propafenone-GITC derivatives and R(−)-propafenone-GITC derivatives were verified with pure enantiomer standards (Figs.2b–2d).

Calibration curves

Calibration curve for the assay was constructed by analyzing a series of blank urine samples spiked with racemic propafenone at concentration of 24.9, 49.5, 238.1, 454.5, 833.3, 1426.8, 1666.7 and 1875.0 ng/ml. The samples were prepared as described under *Assay Procedure*. Peak area ratios (y) of the S(+)-propafenone-GITC derivatives or R(−)-propafenone-GITC derivatives vs the internal standard-GITC derivatives were measured and plotted against the concentration (x) of propafenone enantiomer.

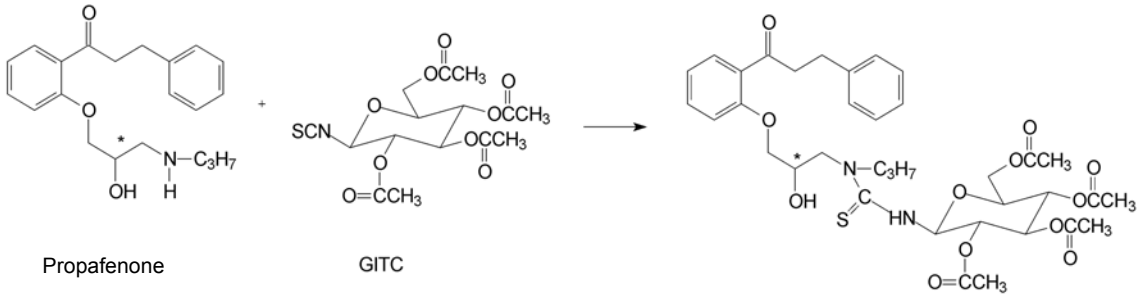

Fig.1 Structures of propafenone, propranolol(IS) and their GITC derivatives

Fig.2 Chromatograms of propafenone enantiomers

(a) blank urine; (b) blank urine spiked with S(+)-propafenone; (c) blank urine spiked with R(−)-propafenone; (d) urine of volunteer administered 150 mg propafenone. 1: S-propranolol; 2: S(+)-propafenone; 3: R(−)-propafenone

The linearity of the calibration curves was at the range from 24.9 to 1875.0 ng/ml for both of the propafenone enantiomers (Table 1). The regression equations of the calibration curve were y=0.0032x −0.081(r=0.999) for S(+)-propafenone and y= 0.0033x+0.0039, (r=0.998) for R(−)-propafenone.

Recovery studies

A series of blank urine samples, spiked with different concentration of racemic propafenone, were processed as described under *Assay Procedure*. The peak area ratios of S(−)-propafenone or R(+)-propafenone to internal standard were compared with those obtained by analyzing water sample containing equal concentration of S(+)-propafenone or R(−)-propafenone.

Table 1 Recovery for assay of propafenone enantiomers in urine

Conc. Spiked (ng/ml)	Recovery ($\bar{x}\pm s$%, n=5)	
	S-PPF	R-PPF
49.5	97.99±5.93	100.3±7.18
454.5	98.43±3.17	101.2±4.57
1428.6	100.40±1.1	99.6±1.18

The recoveries of S(+)-propafenone or R(−)-propafenone at 49.5, 454.5, 1428.6 ng/ml are summarized in Table 1. The average recovery of this assay was 98.9% and 100.4% for S(+)- and R(−)-propafenone, respectively (RSD 7.2%−1.2%, n=15).

Precision studies

The drug-free incubates, spiked with different concentrations (49.5, 454.5, 1428.6 ng/ml) of racemic propafenone, were used for precision studies. Five samples for each concentration were analyzed according to the method described under *Assay Procedure*. The calculated relative standard devia-

tions are given in Table 2.

The precision in this assay was satisfactory. The relative standard deviations were 3.19%–6.22%.

Table 2 The assay precision for analysis of PPF enantiomers in urine

Enantiomeric Conc. (ng/ml)	RSD (%)	
	S-PPF	R-PPF
49.5	6.11	6.22
454.5	3.19	5.34
1428.6	4.67	5.30

Table 3 Ratio of S-PPF to R-PPF in 2 h human urine

Samples	Conc. in urine (ng/ml)		Ratio of S-PPF/R-PPF
	S-PPF	R-PPF	
1	252.1	292.1	0.863
2	189.8	321.9	0.590
3	96.4	156.0	0.618
4	124.6	170.8	0.730
5	312.8	391.3	0.799
6	871.3	1066.5	0.817
7	246.9	354.0	0.697

Sensitivity study

Drug-free urine samples spiked with relatively low concentrations of propafenone were prepared according to the method described under *Assay Procedure*. The limit of detection (LOD) and the limit of quantitation (LOQ) were measured based on S/N≥3 and S/N≥10, respectively. The results indicated LOD was 12.5 ng/ml for both of the enantiomers and LOQ calculated from calibration curves was 28.2±0.52 ng/ml for S-PPF and 30.4±0.53 ng/ml for R-PPF (RSD<8%, n=5) in this assay.

Determination of S-(+)- and R-(−)-propafenone in human urine

Seven healthy volunteers were orally administered 150 mg racemic propafenone after control urine was collected. 2 h after propafenone was administered; after total volume of urine was measured, 1.0 ml of urine was transferred to a 10 ml Teflon-lined glass tube, the urine samples were prepared and analyzed according to the *Assay Procedure*. The results are shown in Table 3.

CONCLUSION

Chiral chromatography has been very useful and helpful for studying the stereoselective metabolism of chiral drugs in vitro and in vivo (Yao *et al*., 2000; Li and Zeng, 2000; Zeng *et al*., 1999). S-(+)- Propafenone and R-(−)-propafenone were derivatized with GITC and separated by RP-HPLC with UV detection. Stereoselectivity and sensitivity were improved by using chiral derivatization. The method developed in this paper offered enantioselective, simple and economic approaches for analysis of propafenone enantiomers in human urine. The results from 7 volunteers administered 150 mg racemic propafenone indicated that propafenone enantiomers undergo stereoselective metabolism; and that S-propafenone is metabolized more extensively than its antipode in the human body.

References

Kroemer, H.K., Botsch, S., Heinkele, G., Schick, M., 1996. In vitro assessment of various cytochromes P450 and glucuronosyltransferases using the antiarrhythmic propafenone as a probe drug. *Methods in Enzymology*, **272**: 99-105.

Li, X., Zeng, S. 2000. Stereoselective propranolol metabolism in two drug induced rat hepatic microsomes. *World J Gastroenterology*, **6**:74-78.

Malfatto, G., Zaza, A., Forster, M., Sodowick, B., Danilo, P.J., Rosen, M.R., 1988. Electrophysiologic and antiarrhythmic effects of propafenone, 5-hydroxypropafenone and *N*-depropylpropafenone. *J Pharmacol Exp Ther*, **246**:419-426.

Yao, T.W., Zhou, Q., Zeng, S., 2000. Stereoselective determination of propafenone enantiomers in transgenic Chinese hamster CHL cells expressing human cytochrome P450. *Biomed Chromatogr*, **14**:498-501.

Zeng, S., Zhong, J., Pan, L., Li, Y., 1999. HPLC separation and quantitation of ofloxacin enantiomers in rat microsomes. *J Chromatogr B*., **728**:151-155.

Zhou, Q., Yao, T.W., Zeng, S., 2002. Effects of stereochemical aspects on drug interaction in Pharmacokinetics. *Acta Pharmacol Sin*, **23**:385-392.