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Determination of *S/R* ratio of mephenytoin in human urine by chiral HPLC and ultraviolet detection and its comparison with gas chromatography[1]

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KEY WORDS mephenytoin; cytochrome P-450 CYP2C19; high pressure liquid chromatography; pharmacogenetics; gas chromatography

AIM: To improve HPLC method for rapid determination of urinary *S/R*-ratio of mephenytoin, a widely used metabolic index for cytochrome P-450 2C19 (CYP2C19) activity. **METHODS**: Aliquots of 0 - 8-h urine sample after dosing racemic mephenytoin 100 mg underwent one-step extraction with dichloromethane. Analysis was performed on a chiral column (250 mm × 4 mm, 5 μm) at λ = 207 nm. The eluent was a mixture of acetronitrile and water containing both 0.1 % glacial acetic acid and 0.2 % triethylamine (14:86, vol/vol) at a flow-rate of 0.9 mL·min^{-1}. **RESULTS**: The enatiomers of mephenytoin in urine were well separated within 9 min. A linear correlation was observed between 50 - 5000 μg·L^{-1} with the detection limit of 12.5 μg·L^{-1} for both enantiomers of mephenytoin. This HPLC analysis was comparable to gas chromatography in accuracy and sensitivity, but with much shorter retention time and better resolution. **CONCLUSION**: The present HPLC method is good for rapid determination of the ability of subjects to hydroxylate *S*-mephenytoin after oral administration of the racemic drug.

S-Mephenytoin 4′-hydroxylase (cytochrome P-450 2C19, CYP2C19) catalyzes the oxidation metabolism of *S*-mephenytoin and other substrates, of which some are clinically important drugs[1]. The genetically polymorphic expression of the enzyme CYP2C19 is one of the determinants responsible for the interracial and interindividual differences in the disposition of and/or the response to the affected drugs[1-3]. The impaired hydroxylation of *S*-mephenytoin appears to be very important in Oriental subjects since the population frequency of the deficient or poor metabolizers (PM) is approximately 3.52 % of European Caucasians[3] but a higher prevalence (14.32 %) in Chinese populations[4]. For rapid determination of the catalytic activity levels of this enzyme, a simple, sensitive, and reproducible assay is required, especially in a large body of population-based epidemiologic and experimental investigation[5]. In general, the urinary *S/R* ratio of racemic mephenytoin and the excreted amount of 4′-hydroxymephenytoin are considered the two specific phenotypic traits or activity markers for the CYP2C19, and the enatiomers have been separated by chiral capillary gas chromatography[6,7], and also by chiral liquid chromatography using β-cyclodextrin as a mobile phase additive[8] or by a chiral column in the HPLC system[9]. These methods, however, have some drawbacks such as long retention times and incomplete resolution. The aim of this study was to develop a more rapid method.

MATERIALS AND METHODS

Materials *R*- and *S*-mephenytoin was kindly donated by Dr G R Wilkinson (Vanderbilt University School of Medicine, Nashville TN, USA). The oral tablet of racemic mephenytoin (Mesantoin®, 100 mg/tablet) was purchased from Sandoz Pharmaceutical (East Hanover NJ, USA). HPLC-grade acetonitrile (Huangyan, Zhejiang, China) and doubly distilled water were required for HPLC and UV detection. All other chemicals were of AR grade unless specified otherwise.

HPLC The HPLC system consisted of an HP series 1050 solvent-delivery system, an on-line degasser, a manual injector, a chiral column (ChiraDex®, 250 mm × 4 mm, 5 μm), and a variable-wavelength detector. The data system

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was an HP Vectra DOS ChemStation controller with an HP LaserJet printer. The apparatus were purchased from Hewlett-Packard (Palo Alto CA, USA). The mobile phase consisted of acetronitrile and water containing both 0.1 % glacial acetic acid and 0.2 % triethylamine (14:86, vol/vol) at a flow-rate of 0.9 mL $\cdot min^{-1}$. The UV signal was monitored at λ = 207 nm. Chromatograms and data were recorded using the HP ChemStation.

Sample preparation Aliquots of urine specimen or spiked samples (1.0 mL) were pipetted into 10 mL screw-capped centrifuge tubes in which 4.0 mL of dichloromethane were added. This mixture was vortexed for 4 min, and then centrifuged at 1400 × *g* for 10 min. The organic phase was transferred into a conical tube, and was evaporated to dryness at 37 ℃. The residue dissolved in 50 μL was injected into the ChiraDex column.

Calibration curve and detection limit The standards were prepared by spiking an aliquot of each standard solution into blank urine. Seven calibration samples containing both *S*- and *R*-mephenytoin were made at 50 – 5000 $\mu g \cdot L^{-1}$ for standard curves by ploting the peak area against each spiked concentration.

Application and comparison with GC Using this method , the results of the randomly selected 31 urine samples from 24 extensive (EM) and 7 poor metabolisers (PM) after they orally took racemic mephenytoin 100 mg were compared with those by the chiral capillary gas chromatography. All samples were analyzed in duplicate by GC and LC.

RESULTS AND DISCUSSION

This method achieved a complete baseline separation and gave rapid elution (Fig 1) with the retention time of < 9 min, which was much shorter than those of the previous ones[6-9]. No potential interference peaks were found from urine. (Fig 1)

The linearity of the urinary concentrations was observed at the range of 50 – 5000 $\mu g \cdot L^{-1}$ with a correlation coefficient of 0.999 for both enantiomers of mephenytoin, and the lower limit of detection was approximately 12.5 $\mu g \cdot L^{-1}$ when a ratio of signal to noise was > 3. The *R/S* ratios of mephenytoin in 0 – 8-h urine after a single oral dose of racemic mephenytoin were compared with those by previous GC method[10]. There was an excellent correlation (r_s = 0.9665, $P < 0.01$, Fig 2) in *S/R* ratios derived from these two methods for each of 31 subjects, verifying the validity and accuracy of the present HPLC assay.

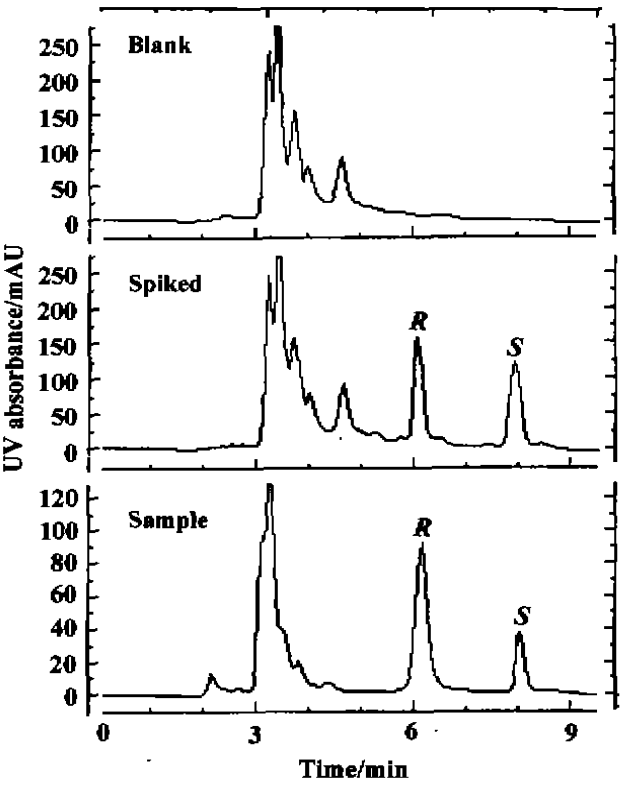

Fig 1. Chiral liquid chromatograms of *S*- and *R*-mephenytoin enantiomers in human urine.

The intraday and interday reproducibilities were confirmed based on 5 and 10 assays of urinary *S/R* ratio from 3 different subjects (2 EM, 1 PM) within 1 d or in 7 d, respectively. The intraday and interday variations (RSD %) of this method were both < 8.5 %.

In summary, this method is suitable for the routine determination of the catalytic activity levels of CYP2C19 in humans.

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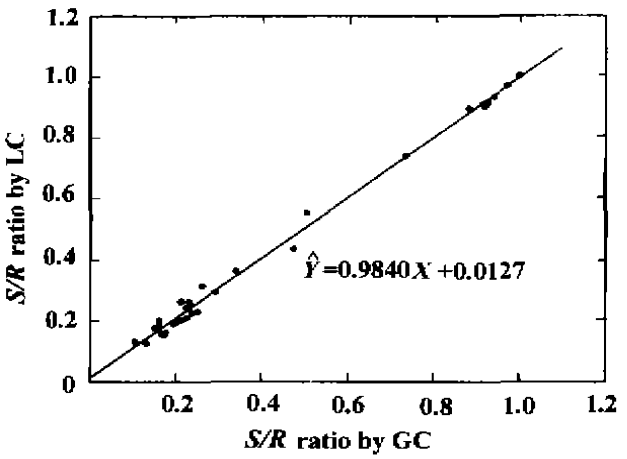

Fig 2. Correlation of 0 – 8-h urinary *S/R* ratio determined by LC with that by GC after a single dose of racemic mephenytoin 100 mg in 31 subjects.

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液相色谱手性分离与紫外检测人尿中美芬妥英 *S/R* 比值及其与气相色谱的比较[1]

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关键词 美芬妥英；细胞色素 P-450 CYP2C19；高压液相色谱法；药物遗传学；气相色谱法

目的：建立人尿中 *R*,*S*-美芬妥英的液相色谱手性分离与检测方法，用于 CYP2C19 酶活性的快速测定. **方法**：受试者口服消旋美芬妥英后的 0 – 8 h 尿液标本用二氯甲烷提取后用手性色谱柱进行分离，流动相为乙腈和水(14:86，体积比)，其中含有 0.1 %的冰醋酸和 0.2 %的三乙胺，流速为 0.9 $mL \cdot min^{-1}$，紫外检测波长为 207 nm. **结果**：在选定的色谱条件下 *R*,*S*-美芬妥英能很好地分离，尿中其他物质无干扰. 用外标法定量，线性范围在 50 – 5000 $\mu g \cdot L^{-1}$，最小检出浓度为 12.5 $\mu g \cdot L^{-1}$，保留时间在 9 min 内. 液相色谱分析结果和气相色谱分析具有良好的一致性. **结论**：该法样本制备简便、分析时间短、线性范围宽、干扰少、灵敏和准确，可用于人体内美芬妥英代谢的研究和肝药酶 CYP2C19 酶活性的检测.