

Enantiospecific separation of mephenytoin and metabolites on CHIRAL-AGP MS/MS detection

A sensitive method using enantiospecific liquid chromatographic/tandem mass spectrometry detection for the quantification of S- and R- mephenytoin with metabolites S- and R-nirvanol and S- and R-4'-hydroxymephenytoin in plasma and urine was published by B. Jansson et al in *Rapid Commun.Mass Spectrom.* 2006, 20: 463-472.

With this method it was possible to simultaneously characterize the metabolic activities of two human drug-metabolizing enzymes, cytochrome P450 (CYP) 2C19 and 2B6. Mephenytoin is stereoselectively metabolized in humans. The enzyme CYP2C19 catalyzes the formation of 4'-hydroxymephenytoin (hydroxylation) and CYP2B6 catalyzes the formation of nirvanol (N-demethylation). R-mephenytoin is mainly metabolized by N-demethylation, while S-mephenytoin is 4'-hydroxylated with a difference between extensive and poor metabolizers. CYP2C19 can be phenotyped by measuring the S/R-mephenytoin ratio in plasma or the 4'-hydroxylated metabolite in urine. By measuring the area under the plasma concentration-time curve ratio of S-nirvanol/S-mephenytoin it is possible to determine the metabolic activity of CYP2B6.

Mephenytoin is an important probe in order to detect enzymatic induction or inhibition which could be the cause of drug-drug interactions. To be able to accurately characterize the pharmacokinetics of mephenytoin, stereoselective methods have to be used, as R-mephenytoin has a much longer half-life than S-mephenytoin.

The separations are performed using a **CHIRAL-AGP** 150x4.0 mm column with a mobile phase of 2% acetonitrile in 5 mM ammonium acetate. The detector is a mass spectrometer in the ESI positive ion mode. MRM (multiple reaction monitoring) was used which gave the following precursor-product ion pairs:

	<u>m/z</u>	<u>eV</u>
Mephenytoin (219)	134	18
Nirvanol (205)	134	17
4'-hydroxymephenytoin (235)	150	19

The flow-rate used is 0.9 ml/min. The flow is split to 0.25 ml/min before entering the mass spectrometer.

The advantage of using this LC/MS method is that it is possible to quantify all three compounds in one chromatogram, although S-mephenytoin and R-nirvanol are co-eluted as can be seen in the chromatogram below.



Validation

The full validation data described in the article show that the method is precise, accurate and reproducible.

Intraday precision		Interday precision	
<u>Plasma</u>	<u>Urine</u>	<u>Plasma</u>	<u>Urine</u>
1.2-6.9%	0.4-2.2%	2.1-7.9%	0.7-2.1%

LLOQ (lower limit of quantification)		
	<u>Plasma</u>	<u>Urine</u>
S-mephenytoin	3 ng/ml	3 ng/ml
R-mephenytoin	3 ng/ml	3 ng/ml
S-nirvanol	1 ng/ml	3 ng/ml
R-nirvanol	3 ng/ml	3 ng/ml
S-4'-hydroxymephenytoin	1 ng/ml	3 ng/ml
R-4'-hydroxymephenytoin	1 ng/ml	3 ng/ml

Calibration curve linearity (ng/ml)		
	<u>Plasma</u>	<u>Urine</u>
S-mephenytoin	3-1500	3-5000
R-mephenytoin	3-1500	3-5000
S-nirvanol	1-1000	3-5000
R-nirvanol	3-1500	3-5000
S-4'-hydroxymephenytoin	1-500	3-5000
R-4'-hydroxymephenytoin	1-500	3-5000