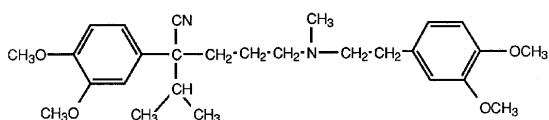


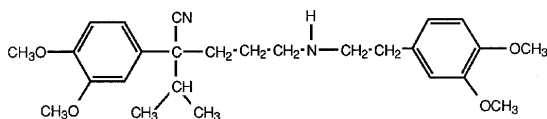
## Chiral LC/MS: Verapamil and norverapamil on CHIRAL-AGP. UV-method converted to MS-method.

A stereoselective bioanalytical method for the simultaneous quantification of the enantiomers of verapamil and its active main metabolite has been described in a publication by M. Hedeland et al in *J. Chromatogr.B*, vol.804 (2004), pp 303-311. It is an LC-ESI-MS/MS method using CHIRAL-AGP as the chiral column. The method was made LC/MS-compatible by replacing the phosphate buffer based mobile phase used with UV detection with an ammonium acetate buffer.

Verapamil is used for the treatment of cardiovascular diseases like hypertension and angina pectoris. Verapamil is administered as a racemate although it has been shown that the two enantiomers are different concerning pharmacological potency and pharmacokinetics. Verapamil is extensively metabolized, mainly through N-demethylation to norverapamil.



*Verapamil*



*Norverapamil*

The aim of the study was to develop and validate a new enantioselective LC-MS/MS method for the simultaneous quantification of the enantiomers of verapamil and norverapamil in human plasma samples without the need for achiral preseparation or chemical derivatization.

Column: CHIRAL-AGP 150x4.0 mm  
 Mobile phase: 15% acetonitrile in 20 mM ammonium acetate pH 7.4  
 Flow rate: 0.6 ml/min  
 Inj.vol.: 50 µl  
 Detection: Quattro LC quadrupole-hexapole-quadrupole mass spectrometer with electrospray interface (ESI), run in the Selected Reaction Monitoring (SRM) mode.

In an earlier publication from the same group, verapamil and norverapamil were analyzed using CHIRAL-AGP and UV-detection. The mobile phase could not be used directly in a LC-MS method, as it contained nonvolatile phosphate buffer. The retention and the enantioselectivity using a 20 mM sodium phosphate buffer pH 7.0 (A) and a 20 mM ammonium acetate buffer pH 7.0 (B) are demonstrated in the table below:

Sample	Mobile phase	$k'_1$	$k'_2$	$\alpha$
Verapamil	A	5.35	6.74	1.26
Verapamil	B	4.43	5.48	1.24
Norverapamil	A	5.50	6.38	1.16
Norverapamil	B	5.43	6.26	1.15

The data in the table demonstrates that the change of buffer did not affect the retention and enantioselectivity to a great extent. In both mobile phases there was an interference between the second eluted enantiomer of verapamil and the first eluted enantiomer of norverapamil. With MS detection verapamil could be separately detected from norverapamil, due to differing molecular masses, although they coelute during the chromatography. It was discovered that with MS-detection the chiral resolution for both compounds was slightly decreased due to band broadening in the MS interface. To improve the enantioselectivity pH was increased to 7.4.

Usually the electrospray interface requires a high concentration of organic modifier to produce a good spray. The relatively low acetonitrile concentration of 15%, could be compensated with a higher desolvation gas flow-rate (1000 l/h).

D<sub>3</sub>-verapamil was used as internal standard for both verapamil and norverapamil. The method was validated and the standard curves were linear with  $r^2 > 0.99$  at all concentrations except one. Verapamil enantiomers were quantified with acceptable precision and accuracy down to a level of 100 pg/ml plasma. The accuracies were not as good for some of the lower validation levels of norverapamil, probably due to the fact that D<sub>3</sub>-verapamil worked better as an internal standard for verapamil than for norverapamil. Still both accuracy and precision was acceptable at levels of 120 pg/ml plasma for norverapamil.

As a conclusion, with this method it was possible to obtain a higher sensitivity compared to earlier published methods. The use of MS-detection provided a substantial simplification in method operation, as detection selectivity could be obtained between verapamil and norverapamil without preseparation or chemical derivatization.