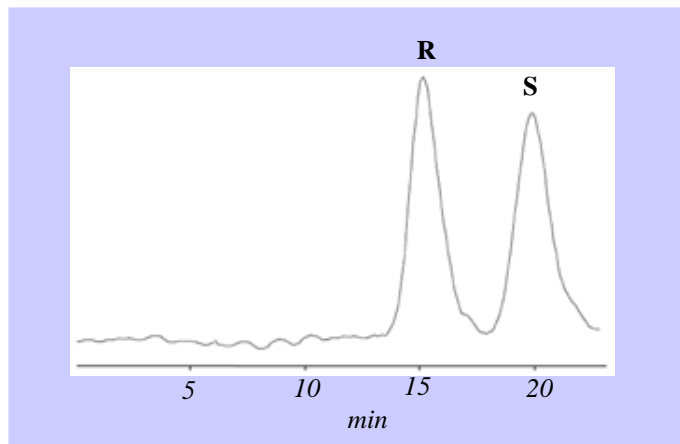


Chiral LC-MS: Amlodipine in human plasma on CHIRAL-AGP

A sensitive, accurate and precise HPLC method with MS-detection for enantiomeric determination of amlodipine in human plasma has been developed by B.Streel et al. The method is published in *J. Biochem. Biophys Methods*, 54 (2002) 357-368.

Amlodipine is a potent dihydropyridine calcium channel blocker used to treat hypertension and angina pectoris. It is used as a racemic mixture although the vasodilating effect is connected to the S-(-)-enantiomer. Thus it is important to study the pharmacokinetic behaviour of the enantiomers in order to understand relationships between drug levels and therapeutic response.



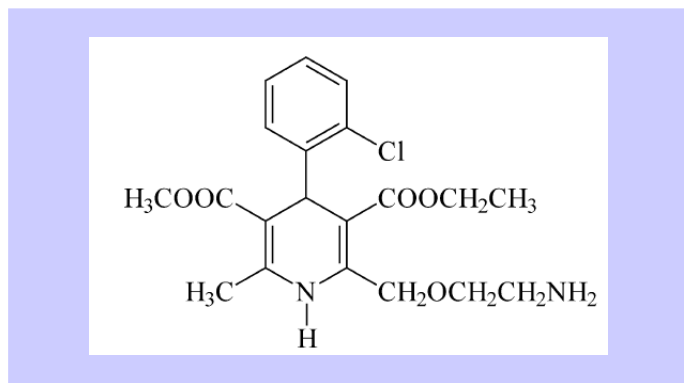
Chromatogram of the enantiomers of amlodipine using the method described in the publication

The resolution between the amlodipine enantiomers is 3.0 and the R-enantiomer is eluted before the S-enantiomer. Selective reaction monitoring (SRM) mode was used to determine S- and R-amlodipine. SRM spectra produce a very clean ion chromatogram, due to the selectivity and sensitivity of this operational mode. No interfering compounds could be found at the retention times of the amlodipine enantiomers.

Validation

The method was validated concerning linearity, detectability, precision and accuracy and was found to be both accurate and precise. Validation results can be found in the table below.

	R-amlodipine	S-amlodipine
Linearity (r^2)	0.999	0.999
LOD (ng/ml)	0.03	0.03
LOQ (ng/ml)	0.09	0.09
Repeatability (%)	7.1	6.2
Precision (%)	9.8	8.5
Accuracy (%) (1.0 ng/ml)	98.9±5.5	101.3±7.4



A method for the stereoselective determination of amlodipine requires a highly sensitive method as each enantiomer needs to be determined at subnanogram per milliliter concentration. For this purpose the MS-detector is very suitable.

The column used for the determination of the amlodipine enantiomers was CHIRAL-AGP 150x4.0 mm. The mobile phase was 1% 1-propanol in 10 mM ammonium acetate buffer pH 4.5, flow rate 0.9 ml/min. The mobile phase was kept at 30°±0.1.

The mass spectrometer used for the detection was equipped with an APCI interface in the positive-ion mode. The mass spectrometer was setup as follows:

- to generate and select the pseudomolecular ion $[M+H]^+$ via the first quadropole filter (Q1) the MS was set at m/z 409 for amlodipine and at m/z 260 for the internal standard (IS) S-propranolol
- to achieve MS-MS fragmentation the pseudomolecular ions were introduced into the collision cell (Q2) with a collision energy of 15 eV
- to detect the product ions the signals at m/z 238 for amlodipine and at m/z 116 for IS were monitored via the third quadropole mass filter (Q3)

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