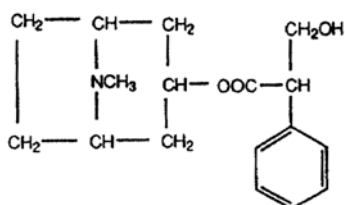


## Atropine on micro CHIRAL-AGP with MS and UV detection

A method for the stereoselective separation and quantification of atropine has been published by D. Breton et al in *J. Chromatography A*, 1088 (2005 104-109). The method is based on an enantioselective separation on a micro CHIRAL-AGP column and subsequent detection using UV and MS. Included is also a study on the effects on the MS detection in the presence of octanoic acid in the mobile phase.

Atropine is an alkaloid that can be found in belladonna roots. It is an anticholinergic agent with two main types of action. It causes respiratory stimulation on the central nervous system and it suppresses smooth muscles and secretory glands innervated by parasympathetic nerves. Atropine is also the preferred antidote for immediate management of toxicity associated with nerve agents.



Atropine is a mixture of optical isomers and is also referred to as dl-hyoscyamine.

The column used was **CHIRAL-AGP** 150x2.0 mm. In order to optimize enantioselectivity and retention a statistical experimental design was used. Six different factors were investigated at two different levels. 16 experiments were performed. The different factors are listed below:

- Flow rate
- Buffer concentration
- pH
- Charged modifier (octanoic acid) concentration
- Uncharged modifier (acetonitrile) concentration
- Temperature

The mobile phase was a mixture of ammonium acetate buffer, acetonitrile and octanoic acid. Two detectors were used, UV at 230 nm and MS with APCI interface in the positive-ion mode. The MS detector was used to confirm that there were no degradation products hidden under the peaks. Using these chromatographic conditions, L-atropine elutes before the D-enantiomer.

The factorial experiments showed that two main effects (pH and acetonitrile concentration) could be judged to be statistically significant on the resolution and the retention of D,L-atropine. The experiments led to the following optimized isocratic conditions for the efficient, economical and time saving separation of the atropine enantiomers:

Mobile phase:	3% acetonitrile and 1 mM sodium octanoate in 10 mM ammonium acetate pH 6.2
Flow rate:	0.2 ml/min
Temperature:	20°C

The retention times obtained were as follows:

L-atropine	10.1 min
D-atropine	11.5 min

A prerequisite in order to obtain resolution was that octanoic acid was included in the mobile phase. For this reason it was also demonstrated in the study that the presence of sodium octanoate did not seem to pose problems in the MS detector. The counter ion only had a minor effect on the detector response and did not decrease the sensitivity.

For quantification the chromatograms were monitored using  $m/z = 290.1$ .

The use of a narrow bore column with the I.D. of 2 mm at a low flow rate of 0.2 ml/min gives a low consumption of mobile phase, which is of advantage both of practical and economic reasons.

Small amounts of the diastomer (inactive enantiomer, D-enantiomer) could be detected using either UV or MS detection. MS detection is especially useful when studying pharmaceutical active ingredients decomposition during stress conditions. In these studies it must be shown that the method is specific and that no degradation products are hidden under the peaks.