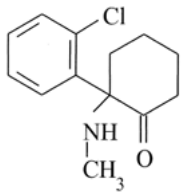
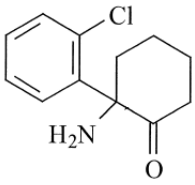
Chiral LC-MS: Bioanalysis of ketamine and norketamine on CHIRAL-AGP

M.E. Rodriguez et al have developed a sensitive HPLC assay with MS-detection for simultaneous determination of (R)- and (S)-ketamine and (R)- and (S)-norketamine in plasma. They published a paper entitled "Determination of the enantiomers of ketamine and norketamine in human plasma by enantioselective liquid chromatography - mass spectrometry" in J. of Chromatogr. B, 794 (2003) 99-108.



Ketamine



Norketamine

Ketamine is an analgesic agent. It is a racemic mixture where (S)-ketamine is a more potent analgesic than (R)-ketamine. However, side effects as post-hypnotic stimulatory properties and agitated behaviour are associated with (R)-ketamine. Ketamine is metabolized via N-demethylation to norketamine, which also is chiral and has similar pharmacological activity as ketamine. The described method is developed to be used in the analysis of ketamine in a clinical study where the compound is used as an analgesic agent for the treatment of neuropathic pain.

A CHIRAL-AGP column, 100x4.0 mm, was used for the enantioselective separations of ketamine, norketamine and bromoketamine (internal standard), together with a CHIRAL-AGP guard column. The mobile phase was 6% 2-propanol in 10 mM ammonium acetate buffer pH 7.6. Flow-rate was 0.5 ml/min, injection volume 20 µl and the assay was run at RT. A mass-selective detector supplied with an atmospheric pressure ionization electrospray (API-ES) was used. Mass spectra were recorded in the positive ion mode and the chromatograms were monitored at *m/z* 238.1 (ketamine), *m/z* 224.1 (norketamine) and *m/z* 284.0 (bromoketamine). The sensitivity of the ketamine and norketamine were primarily dependent on the MS experimental parameters.
The analysis was complete in 19.5 minutes. Retention times and enantioselectivity are listed in table below:

	k'(S)	k'(R)	(α)
Ketamine	9.0	10.5	1.17
Norketamine	5.2	8.2	1.58
Bromoketamine	9.8	12.8	1.31

The method was stable and reproducible. Over 350 plasma samples were analyzed on a single CHIRAL-AGP column with replacement of the CHIRAL-AGP guard column after around 100 samples.

Validation

The validation shows that the method has excellent accuracy, recovery and precision.
Standard curves were linear in the concentration range 1 - 125 ng/ml.
Other validation data are presented in the table below:

	(S)-Ket	(R)-Ket	(S)-Nket	(R)-Nket
LLOQ (ng/ml)	1.0	1.0	1.0	1.0
LLOD (ng/ml)	0.25	0.25	0.25	0.25
Accuracy (%)	100.1	100.0	100.2	100.3
Precision (%)	≤7.5	≤5.8	≤7.2	≤6.7

This bioanalytical assay is a simple, sensitive and reproducible method for the enantioselective and simultaneous determination of ketamine and norketamine in human plasma. Due to the short analysis time, up to 60 patient samples can be analyzed/day by the method.

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