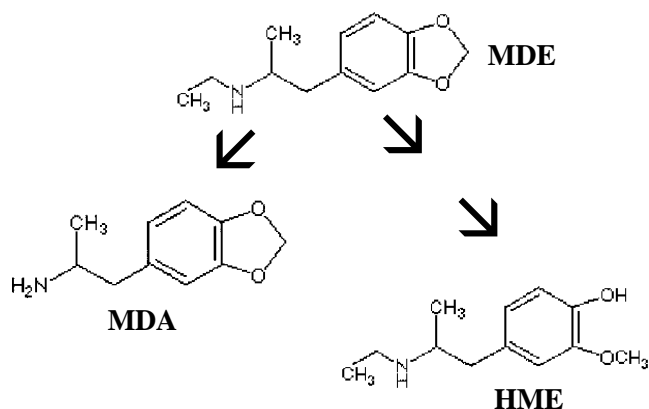


## Chiral bioanalysis of an ecstasy compound and metabolites on CHIRAL-CBH

An enantioselective HPLC method for the simultaneous determination of N-ethyl-3,4-methylenedioxyamphetamine (MDE) and its major metabolites N-ethyl-4-hydroxy-3-methoxyamphetamine (HME) and 3,4-methylenedioxyamphetamine (MDA) has been developed by J. Buechler et al. The article "Enantioselective quantitation of the ecstasy compound (R)- and (S)-N-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma and urine" was published in *J. of Chromatogr. B*, 793 (2003) 207-222.

The most commonly abused illicit recreational drugs in the western world are ecstasy compounds such as N-methyl-3,4-methylenedioxyamphetamine (MDMA, "Ecstasy", "Adam"), N-ethyl-3,4-methylenedioxyamphetamine (MDE, "Eve") and 3,4-methylenedioxyamphetamine (MDA). Many users believe that these drugs are harmless, however acute adverse effects as hypothermia, cardiovascular complications, renal and hepatic failure (occasionally leading to death) and unwanted neuropsychiatric reactions are reported in increasing amounts.

Ecstasy compounds are being used in psychiatric research. MDE, with a lower neurotoxicity than MDMA, is preferred in human studies. MDE is metabolized by two routes, one is dealkylation leading to MDA and the other is cleaving of the methylenedioxy group followed by methylation, leading to N-ethyl-4-hydroxy-3-methoxyamphetamine (HME)



The pharmacokinetic properties after administration of the pure enantiomers of MDE in humans is described in the publication. The stereospecific HPLC method was developed using a CHIRAL-CBH column, 150x4.0 mm (together with a CHIRAL-CBH guard column). Both urine and plasma were analyzed using the same method, with a mobile phase consisting of 7% 2-propanol in 20 mM sodium dihydrogenphosphate buffer pH 6.4 with 50  $\mu$ M disodium EDTA. The flow rate used was 0.7 ml/min. Fluorometric detection was used with an excitation wavelength of 286 nm and an emission wavelength of 322 nm.

An internal standard, N-ethyl-3,4-methylenedioxybenzylamine NEMDBA, is used. Below the retention times are listed:

	Tr (R)	Tr(S)
MDE	5.70	6.49
HME	7.43	8.07
MDA	9.02	11.95

Retention time for the internal standard was 16.50.

It is very important to control the pH as it has a large effect on retention time and resolution.

The method required only 1000  $\mu$ l plasma and 200  $\mu$ l urine for accurate determination due to an LOQ lower than in earlier described methods. The use of fluorometric detection provided a high selectivity and less interference with endogenous compounds. A simple solid-phase extraction step could be used.

The method was validated according to ICH guidelines. Some validation data are shown in the table below:

Plasma	MDE	HME	MDA
	Range (ng/ml)	5-800	10-2000
Linearity	0.9997-0.9999	0.9993-0.9998	0.9999
LOQ (ng/ml)	5	10	5
Recovery (%)	95.37-96.91	94.43-95.36	92.57-93.07
Urine	MDE	HME	MDA
	Range (ng/ml)	5-800	10-2000
Linearity	0.9997-0.9999	0.9996-0.9998	0.9999
LOQ (ng/ml)	5	10	5
Recovery (%)	95.74-96.54	92.86-94.86	95.46-98.40

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