

# Research of Cyamemazine and its principal metabolites in urine using positive mode electrospray LC/MS

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## Introduction

The phenothiazine derivative cyamemazine is the most widely used anti-psychotic neuroleptic drug in France. This medicine is used in order to treat Schizophrenia and lot of psychoses. The Pharmaco-Toxicology Laboratory of Edouard Herriot Hospital had noticed interference between this drug and the amphetamine Emit® test on automatic (in urine). Indeed, one patient who took cyamemazine had false-positive results for this test (confirmed by GC/MS).

The aim was to identify and to characterize cyamemazine (CMZ) and its two metabolites S-oxide cyamemazine (SOCMZ) and demethylcyamemazine (DMCMZ) in urine using LC/MS and to try the amphetamine test on the same patients.

## Experimental methods

Analytical procedure:

- 1) Enzymatic hydrolysis
- 2) Liquid-liquid extraction
- 3) Evaporation under nitrogen and resumption in mobile phase
- 4) Injection of 10 µL for LC/MS analysis

Cyamemazine and metabolites were eliminated from the body as such conjugated carbohydrate product which must be hydrolysis before extraction.

Chlorobutane was used in order to extract cyamemazine and metabolites. The analysis was performed by high-performance liquid chromatography coupled with mass spectrometer : "Agilent 1100 series LC/MSD" (electrospray source, quadrupole analyzer).

## Experimental methods

Column XTerra® MS C18 3.5 µm, 150 x 2.1 mm (Waters®)

Solvent A: buffer formiate 5 mM pH 3.55

Solvent B: Acetonitrile

LC Program:	Time (min)	% B	Flow (mL/min)
	0	10	0.20
	10	30	0.20
	20	35	0.20
	30	40	0.20
	47	50	0.20

Injection volume: 10 µL ; Column temperature: 40°C

## Results and discussion

Molecules were identified using UV and MS spectra: being able to use spectra is a great advantage of LC/MS. Moreover, the electrospray ionization source is soft. Consequently, molecular ions are detected (protonated molecules  $[M+H]^+$ ). Thanks to injection of standard solutions, metabolic compounds were characterized (retention time, UV and MS spectrum) so as to identify this in urine. The urine of one patient treated with cyamemazine was analyzed. This analysis indicates the presence of CMZ, DMCMZ and SOCMZ. Seven other patients were also tested with similar results. No patient tested positive for amphetamine.

Figure 1: The Cyamemazine and its metabolites S-oxide cyamemazine and demethylcyamemazine.

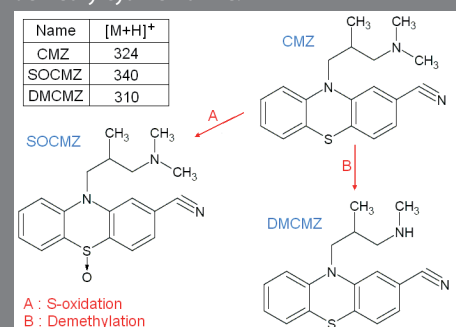
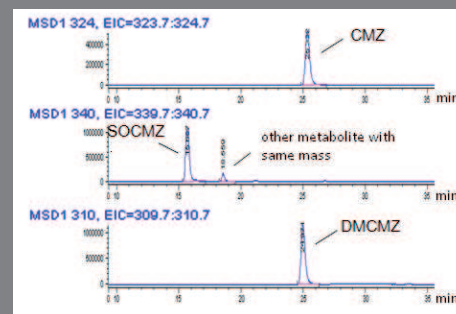


Figure 2: Urine analysis by LC/MS:

Molecular ions  $[M+H]^+$  of CMZ, SOCMZ and DMCMZ extract by the pseudo SIM mode. This attests their presence in patient's urine.



## Conclusion

The process so as to identify CMZ, SOCMZ and DMCMZ is established with success. It is going to help the phenothiazine qualitative research, frequently requested by doctors.



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