

# Evaluation of a drugs screening method using an hybrid LC-MS/MS system in clinical toxicology

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## Introduction

- Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) combined with library search can be a very effective alternative to the existing screening method used in clinical routine labs (LC-DAD-Remedi® instrument). Evaluation of a new LC-MS/MS approach was performed by comparison of results obtained on more than hundred real serum samples.

- Screening procedures are commonly employed by clinical toxicology laboratories to analyze drugs, drugs of abuse and other compounds in serum, urine or other matrices. LC-MS has been considered for this application because of its sensitivity, specificity and ability to detect a broad range of compounds. However, a generic method that supports all of the compounds of interest in a clinical toxicology laboratory has to be investigated.

## Materials and Methods

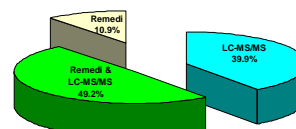
- LC-MS system: 3200 Qtrap (Applied Biosystems)
- Analytical column: Hypersil Gold 100 x 2.1mm, 1.9 µm particle size (ThermoScientific)
- HPLC solvents: A: 0.1% formic acid, ammonium formate 1 µm
- Sample preparation:**
  - After addition of deuterated internal standard (D3-Clomipramine & D5-Amphetamine), an automated SPE system ASPEC GX-274 (Gilson) and 30 mg Oasis HLB cartridges (Waters) were used for the sample clean-up. 10 µl of the collected fraction are injected onto the LC-MS.
- LC Conditions:**
  - 12 minutes gradient from 5%-70% B was employed with flow rate of 400 µl/minute.
- MS Conditions:**
  - Turbo ionspray source was used with a positive needle voltage of 5.0 kV, a spray heater at 360°C and gas flow rate at 35L/min.
  - An information dependent acquisition (IDA) process including as survey experiment a multiple reaction monitoring (MRM) and as dependent experiment an enhanced production ion (EPI) scan was used [1].
  - EPI scan were acquired at 20, 35 and 50eV as three distinct spectra.
- Automatic data analysis and library search:**
  - SmileMS a newly developed software based on EPI spectra similarity was used.

## Results and discussion

- A total of 146 real serum samples have been tested:

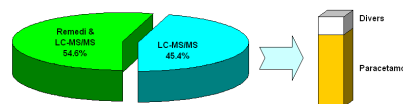
### Positive results:

77 different compounds were identified one or more times giving a total of 321 positive identifications with the following distribution:



### Negative results :

No compounds were identified according to Remedied® instrument in 11 samples.

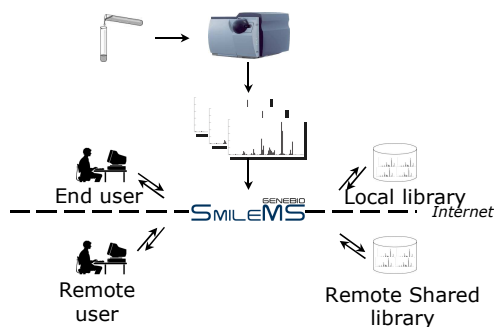
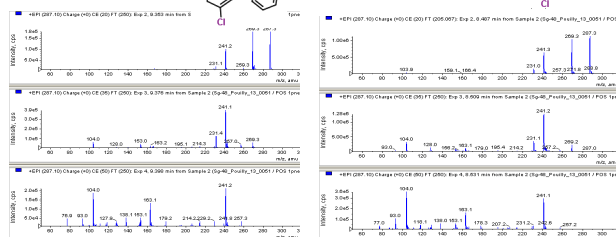
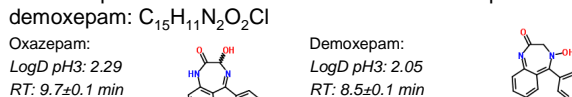


As paracetamol is not detected with the Remedied® instrument, the global result is quite good and we can consider about **91% of similarity** between the both techniques.

### Global evaluation :

In most of the cases, retention time (RT) were not needed to identify compounds.

One exception was observed to differentiate oxazepam and demoxepam:  $C_{15}H_{11}N_2O_2Cl$



## Conclusion

- LC-MS/MS offers higher sensitivity and/or specificity for number of compounds compared to LC-UV-DAD.
- A broader number of compound can be identified in a similar time delay compared to the results obtained via the Remedied® instrument.

- Use of LC-MS/MS with SmileMS offers an alternative to the Remedied® instrument in clinical toxicology laboratories as a front screening method.

## Reference

- [1] C.A. Mueller, W. Weinmann, S. Dressen, A. Schreiber, M. Gergov, Rapid Commun. Mass Spectrom. 19 (2005) 1332.