

A New Approach for Acute Clinical Toxicology Based on Ion Trap LC/MSMS Library Search

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Introduction

Medical doctors treating patients with acute toxicological symptoms need fast and reliable identification of the causing toxins. Existing screening methods in clinical routine labs often rely on spectral comparisons of LC-UV-DAD data (REMEDI). Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS) combined with library searching already applied in forensics and food testing applications can be a very effective and more information-rich alternative. The approach does not require any chemical derivatization steps during the sample preparation. Ease of use regarding both hardware and software operation is a very important factor, particularly in routine laboratories. Based on simple SOPs and a software that fully automates data acquisition, evaluation and reporting such a system should enable identification of toxins and drugs in less than 30 minutes.

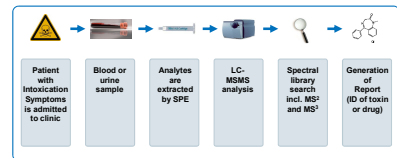


Fig. 1 Schematic of complete workflow.

Methods

Urine or plasma samples from patients showing acute toxicological symptoms are extracted by means of solid phase extraction. Subsequently the sample is analyzed by LC-MSMS. Resulting spectra are searched against a library containing ~130 of the most common drugs and toxins using a highly adapted search algorithm (SmileMS from GeneBio). The automatically generated report shows all identified substances. The complete analysis and evaluation of the data (using Bruker Compass OpenAccess software) is fully automated and can be performed by clinical staff without any LC-MS experience. The combination of a UHPLC and most recent ion trap MS technology enables a full analysis including automated acquisition of MS² and MS³ spectra, library search and reporting in less than 15 minutes.

Instrumentation:

- MS: Bruker HCT Ultra Ion Trap
- LC: Agilent 1200 Rapid Resolution
- SPE: Gilson Aspec GX-274

Library

- 127 selected compounds relevant for emergency clinical toxicology + controls
- Reference spectra acquired on an HCTUltra under validated SOPs
- Includes 127 MS² and 184 MS³ spectra (avg 2.45 spectra per compound)
- Annotated with CID, RT, precursor mass, instrument characteristics, etc
- Can be annotated to match local conditions (specific chromatography properties, ...)

Results

The proposed system combines a LC-MSMS enabling instrument with a sample processing management software and an efficient library search algorithm. It is able to identify a large number of compounds collected from samples in emergency room conditions. The graphical interface of the software is accessible for non expert technicians and is appropriate for routine analyses.

The scoring model searches MS² as well as MS³ spectra. It provides confidence values normalized between 0 and 1, which allows an easy interpretation by non expert technicians in routine analysis.

The sensitivity and specificity performances makes it a convenient and very robust replacement of the REMEDI system (LC-DAD-library search).

The quality and reproducibility of the library spectra was first tested with a leave-one-out procedure. The latter was performed on a subset of the library consisting of 160 fragmentation spectra corresponding to 28 compounds (>5 MS² spectra per compound on average). The result showed that the algorithm successfully ranked a correct spectrum as best match for all 28 compounds.

A population of 150 patients with various intoxications have been collected and are analyzed both with REMEDI and with MS.

2 internal controls (for ID and for RT) are used: D3-clomipramine and D5-amphetamine. Figure 2 shows the result from one of these patients. Amitriptyline, Nortriptyline (a metabolite of Amitriptyline), Metoclopramide, Zolpidem and Acetaminophen were found with high scores together with both controls.

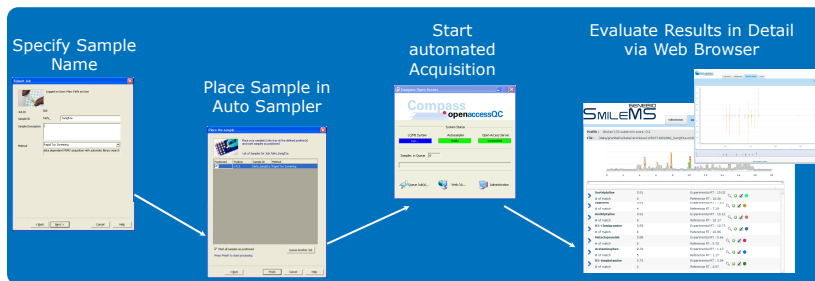


Fig. 2 Bioinformatics workflow: minimal input is required from sample to result.

● IMSC 2009, Poster PWA: 417

Summary

- Complete and robust system for Clinical Toxicology screening application
- Fast, reliable, simple to use and more information-rich (compared to REMEDI)
- It uses a powerful algorithm (SmileMS from GeneBio), robust against spectral variability
- Dedicated library exists for clinical toxicology applications
- Generation of highly confident results easy to interpret

Conclusions

- Powerful automated identification of drugs and toxins in acute clinical toxicology
- powerful library search platform
- simple push button solution, appropriate for use in routine analysis
- ID in 30 minutes