Postmortem brain and liver tissue analysis on filter paper for the rapid LC-MS/MS target screening of drugs

Julien Déglon, François Versace, Patrice Mangin, Marc Augsburger, Christian Staub

Unit of Toxicology - University Center of Legal Medicine, University Hospitals of Geneva, Switzerland

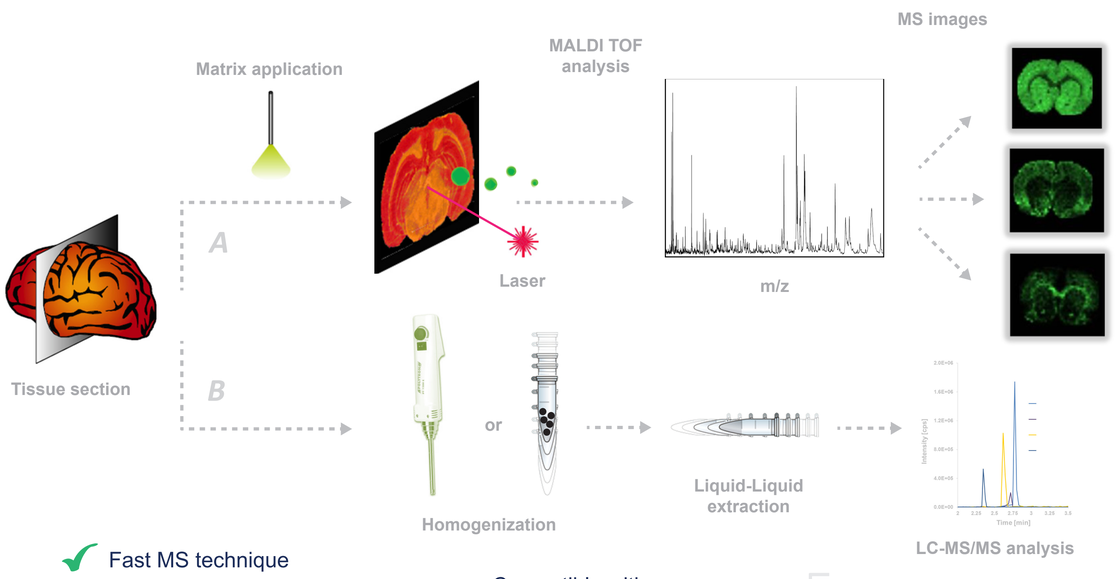
julien.deglon@hcuge.ch
www.curml.ch

1. Overview

- To use small amount of biological material and to reduce sample pretreatment to a minimum
  - ***Filter paper sampling + automated 96-well plate extraction***
- To develop an efficient strategy to rapidely identify drugs in tissue sections
  - ***LC-MS/MS platform using QqQ(LIT) + library search software***

2. Introduction

Whole blood still remains the preferred postmortem matrix in forensic toxicology. However, the use of alternative matrices such as **tissue sections** can provide additional information. Traditionally, tissue analysis is performed by matrix laser desorption ionization (MALDI)-MS (*A*) or by homogenization, extraction and cleanup steps followed by GC or LC-MS (*B*).



A
- ✓ Fast MS technique
- ✓ Mapping
- ✗ Dedicated instruments required

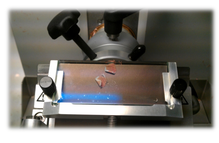
B
- ✓ Compatible with conventional intruments
- ✗ Tedious
- ✗ Solvent and material consumption

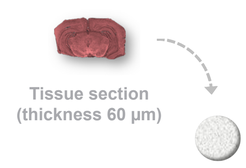
We proposed an alternative pretreatment technique for the rapid analysis of tissue sections.

3. Sample preparation

Tissue cutting

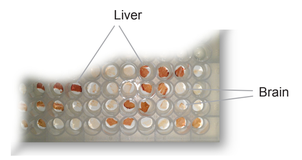
Frozen brain or liver tissue were cut using a **microtome cryostat** (Leica) and directly applied onto a disk (Ø 6 mm) of filter paper (Whatman 903).

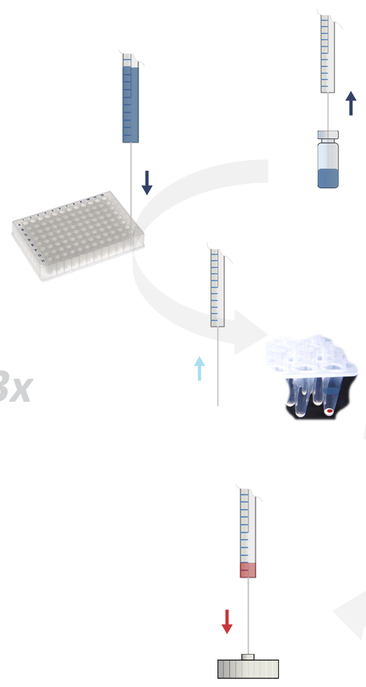




96-well plate extraction

1. Tissue section onto filter paper disk
2. Load filter paper disk into well
3. Draw 100 µL MeOH as extraction solvent
4. Dispense 100 µL MeOH into well
5. Draw air and dispense into well for mixing (3x)
6. Draw 5 µL supernatant Injection into LC-MS/MS
7. Needle cleanup Ready for next injection



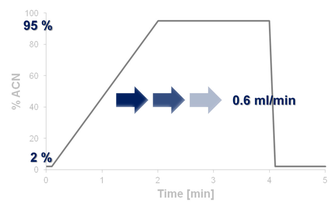


Automated tissue extraction was performed by a Thermo Scientific Dionex WPS-3000 autosampler using **user-defined program**.

- ✓ Automated handling
- ✓ Cycle duration 2 min.

4. LC-MS/MS strategy

1. LC separation



Chromatographic separation was achieved on a Kinetex® fused-core (Phenomenex) **C18** 2.6 µm (2.1 x 50 mm) using a mixture of water and acetonitrile as mobile phase. The LC method featured a **5-min** total run time.

2. MS/MS detection

Detection was performed in **information dependent acquisition** (IDA) mode using a 5500 QTrap® system (AB Sciex) with positive ESI source.

SRM

Transitions	203
Dwell time	1 ms
Resolution Q1 & Q3	unit
Pause between mass ranges	5 ms

Cycle duration 1.4 s

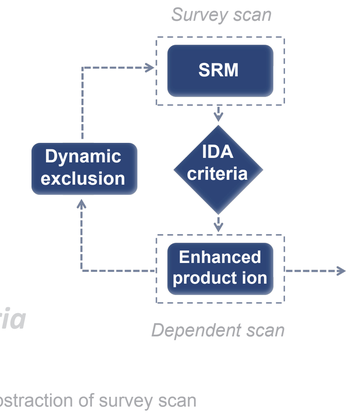
EPI (enhanced product ion)

Scan	50 → 600 Da
Scan rate	10000 Da/s
Collision energy	50 eV
Collision energy spread	± 20 eV

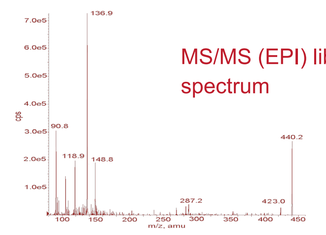
IDA criteria

Threshold 5000 cps

- ☒ after dynamic background substraction of survey scan
- ☒ exclude former target ions for 10 s after 2 occurence



3. Identification



EPI spectra were treated by SmileMS (GeneBio) and compared with Cliquid 2.0 (AB Sciex) library

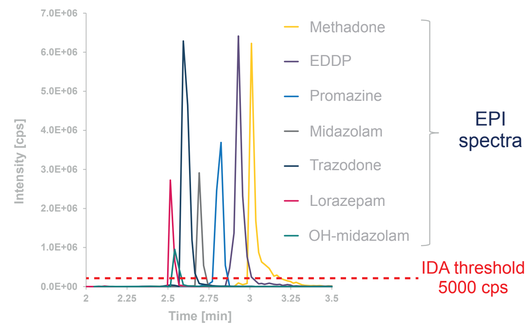
5. Results

Extraction

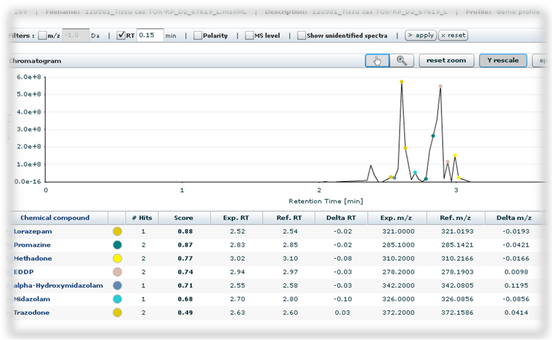
with filter paper *without filter paper*

The combination of the **filter paper** with the **organic solvent** allowed for the tissue section to be fixed onto the cellulosic disk while allowing interest compounds to be extracted.

Identification



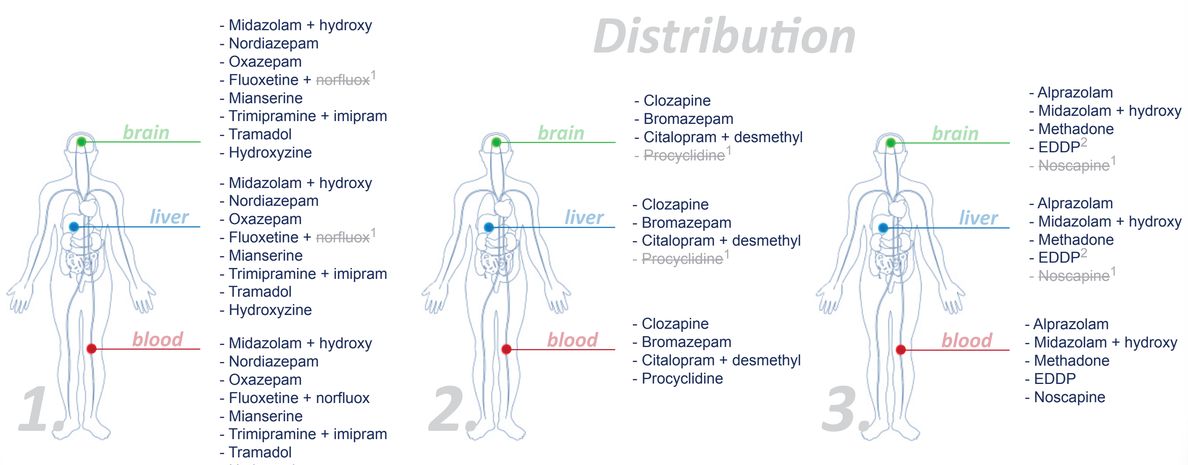
EPI spectra →



SRM chromatogram of postmortem liver section

SmileMS identification window based on EPI spectra acquired when SRM signal > IDA threshold

Distribution



Screening comparision between blood and tissue sections (brain and liver) performed on three postmortem cases. Blood screening was carried out by GC-MS and LC-DAD.

[1] SRM transition not included in survey scan

[2] Peak intensity 100 time lower in brain tissue than in liver

6. Conclusions

- ✓ *Automated* Tissue sample pretreatment was reduced to a minimum by using **automated 96-well plate** extraction coupled with **conventional** LC autosampler.
- ✓ *Efficient* Concomitant use of **filter paper** as sampling support and **MeOH** as extraction solvent provided clean extract with satisfying sensitivity.
- ✓ *Fast* Target screening of **200 drugs** from tissue sections in less than **8 minutes**, including sample pretreatment.
- ✓ *ID* Reliable and fast identification of target compounds using **SmileMS** based on EPI spectra.
- ✓ *Distribution* **Good correlation** was observed between brain / liver tissue analysis and corresponding blood screening.

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