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TIAFT 2024 ST.GALLEN
SOCIETY OF HAIR TESTING
SEPTEMBER 2ND – 6TH

Application of an UHPLC-HRMS/MS screening using Dried Blood Spot in post-mortem cases

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Fig 1 : HemaXis 903 ™

Introduction

Originally developed for neonatal screening for phenylketonuria, the Dried Blood Spot (DBS) sampling technique is increasingly being used in a wide range of areas, including the environment (pesticide and contaminant detection), doping, pharmaceuticals, and clinical and post-mortem toxicology.
This work present the development of a toxicological screening in LC-HRMS/MS using the DBS technique and its application in forensic toxicology and more specifically in **post-mortem cases**. This method was applied to 30 real cases, including 28 post-mortem cases in very different contexts (suicide, discovery of a putrefied body, accident, etc.).

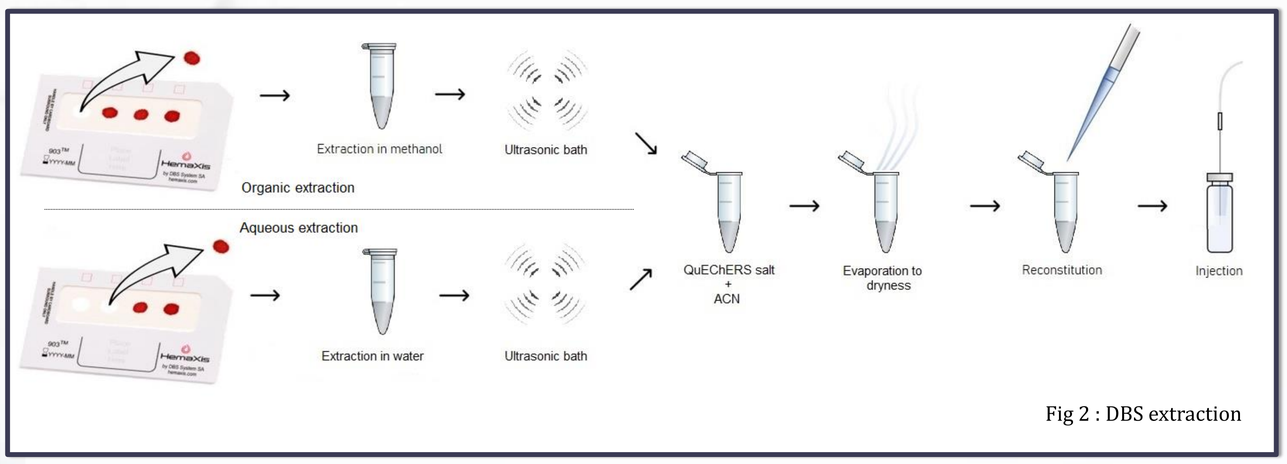

Fig 2 : DBS extraction

Materials & Methods

- Paper : Whatman™ 903 (HemaXis)
- LC-HRMS/MS : Qexactive ™ (ThermoFisher Scientific)
- Column : Accucore phenyl-hexyl (100 mm x 2.1 mm i.d ; 2.6 µm particle size)
- Total run time : 15.5 min
- Acquisition mode : ESI + & - ; $ddMS^2$
- Identification : ThermoFisher spectral library (5 ppm mass tolerance)
- Matrix : **2 spots** of **10 µL** of whole blood
- Drying period : 1 hour
- Incubation : MeOH or water
- Extraction : ACN + QuEChERS salt
- Injection : 10 µL of reconstituted residue

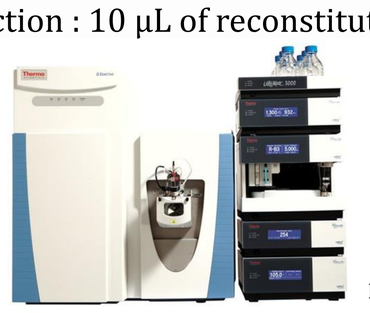

Fig 3 : QExactive ™

Post-mortem cases report

This developed method was applied to **30 real cases.** The following **3 cases** were chosen either because of **the number of molecules found** (case 1) or **the state of the body** (**decomposition**, cases 2 and 3).

Case 1 : a 46 y-o male found dead in his hotel room.

Molecules	DBS	Combination of classical methods (µg/L)
Paracetamol	✓	✓
Benzodiazepines (diazepam, nordazepam, oxazepam, temazepam, nitrazepam, 7-NH2-nitrazepam, 7-NH2-clonazepam)	✓	✓
Clonazepam	X	✓ (< 5.0)
Antipsychotics (alimemazine, alimemazine sulfoxide, quetiapine, quetiapine metabolites, 9-OH-risperidone, levopromazine)	✓	✓
Noralimemazine ; Risperidone	X	✓ (< 0.50) ; ✓ (< 1.0)
Norlevomepromazine	Out of library	✓ (< 10)
Antidepressants (paroxetine, nortriptyline)	✓	✓
Substitution products (methadone , EDDP)	✓	✓
Other (buspirone, pregabalin)	✓	✓
Beclomethasone	X	✓ (< 0.50)

Case 2 : a 48 y-o male found dead in the woods in an advanced state of decomposition.

Molecules	DBS	Combination of classical methods (µg/L)
Benzodiazepines and related products (zopiclone, norzopiclone)	✓	✓
Antipsychotics (quetiapine, quetiapine metabolites, cyamemazine, norcyamemazine)	✓	✓

Case 3 : a 39 y-o male found dead in his bed in a state of decomposition.

Molecules	DBS	Combination of classical methods (µg/L)
Paracetamol	✓	✓
Benzodiazepines (diazepam, nordazepam, temazepam,)	✓	✓
Oxazepam	X	✓ (< 5.0)
Antipsychotics (quetiapine, quetiapine metabolites)	✓	✓
Narcotics (cocaine, BZE, EME, cocaethylene,	✓	✓
Substitution products (buprenorphine, norbuprenorphine)	✓	✓
Other (levamisole)	X	✓ (< 5.0)

Theoretical results

Preliminary tests were performed on 60 molecules (benzodiazepines, antipsychotics, substitution products, antidepressants and narcotics). The following parameters were tested : limit of detection, calibration model, stability at +4°C and +20°C over one week. All the LODs ranged from 5 to 10 µg/L, calibration models were linear for all molecules in the range 5 or 10 to 2,000 µg/L.

Molecules	LOD (µg/L)	Calibration range (µg/L)	Therapeutic range (µg/L)	Stability at +4°C ; +20°C
Alprazolam	5	5 – 2,000	5 - 50	loss < 20 %
Buprenorphine	5	5 – 2,000	0.50 - 5	**loss > 80 %**
Citalopram	5	5 – 2,000	50 - 180	loss < 20 %
Cocaine	5	5 – 2,000	-	loss < 20 %
Amphetamine	5	5 – 2,000	-	loss < 20 %
Methadone	5	5 – 2,000	70 - 100	loss < 20 %
Morphine	10	10 – 2,000	20 - 120	loss < 20 %
Oxazepam	5	5 – 2,000	200 – 1,500	loss < 20 %
Quetiapine	5	5 – 2,000	100 - 500	loss < 20 %
Risperidone	5	5 – 2,000	20 - 60	loss < 20 %

Tab. 1 : LOD, calibration & therapeutic range and stability over one week of some developed molecules

Concerning the stability at +4°C and +20°C, a loss < 20% was recorded for all molecules except for buprenorphine (Fig.4), clozapine and olanzapine.

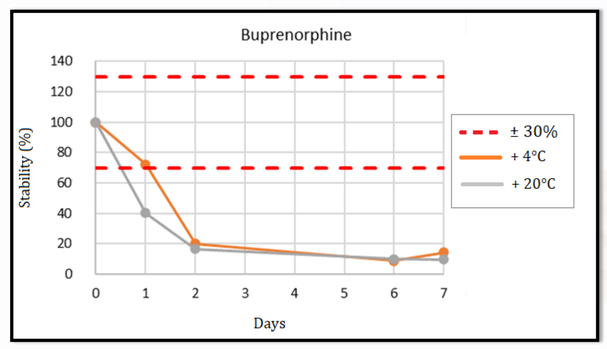

Fig 4 : stability assessment for buprenorphine

Conclusion

The use of DBS in forensic toxicology analysis is beginning to grow for several reasons :

- **Blood collection** (from cadaver or living person) **easier** than traditional methods
- **Reduced volume** (20µL) sufficient to perform the same analysis as with conventional methods which require 3-4 mL, with good sensitivity, which is very practical in certain cases such as infants, exsanguinated victims, constricted veins, etc.
- Ease of storage and transport.

The application of the DBS technique to post-mortem cases in very different contexts or to living subjects demonstrates the variability in the quality of the blood used and therefore the robustness of the method.

However, there is a very important issue to consider when interpreting the results obtained : the possible environmental contamination of the sample or by sweat, whatever the sampling site (finger, toe, etc.).

Forensic toxicological analysis using DBS is **very promising**, but further development and research is needed to address these grey areas.

With the financial support of the SFTA