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Drug screening in autopsy liver samples by over pressured layer chromatography

Anna Pelander, Daniel Backstöm, and Ilkka Ojanperä
Department of Forensic Medicine, University of Helsinki, Finland

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Background

- Approximately 6500 post mortem cases analysed annually at the Department of Forensic Medicine, University of Helsinki.
- In approximately 500 cases no urine or vitreous humour available: qualitative drug screening analysis on liver.
- Liver is a good matrix for the detection of basic, lipophilic substances.
- Complex matrix, often fatty
 - Planar chromatographic methods suite well, as the plates are disposable.

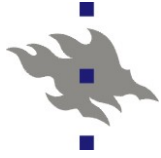


Materials and methods

- Sample prep by tissue digestion and four-phase liquid-liquid extraction.

- Chromatography by dual-plate OPLC.
 - OPLC: over pressured layer chromatography
 - Planar chromatography with constant flow, enhanced separation efficiency compared to capillary flow techniques.

- Identification by automated *in situ* UV-spectral comparison and reporting.



LIVER SAMPLE EXTRACTION SCHEME

DIGESTION WITH TRYPSINE



**PURIFICATION EXTRACTION IN ACIDIC
CONDITIONS WITH DIETHYL ETHER**



**ANALYTE EXTRACTION IN ALKALINE
CONDITIONS WITH BUTYL CHLORIDE**



**BACK EXTRACTION INTO ACIDIC AQUEOUS
PHASE, SECONDARY EXTRACTION WITH
BUTYL CHLORIDE AFTER pH ADJUSTMENT**

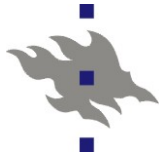


**EVAPORATION OF THE ORGANIC PHASE,
RECONSTITUTION IN 100 μ l OF METHANOL**



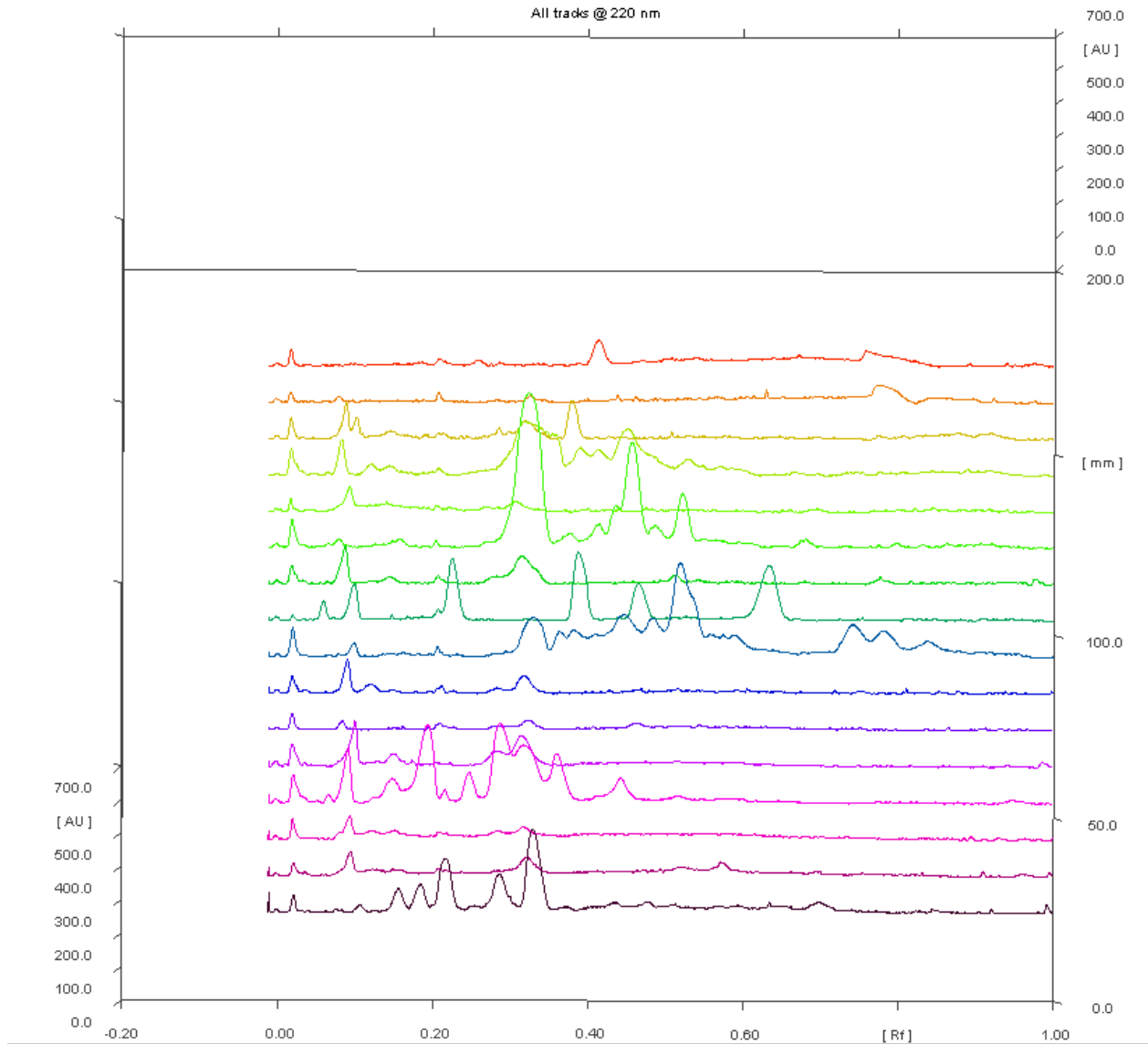
- 10 μl of the final extract applicated to two silica gel OPLC plates with an autosampler in band mode (Camag ATS III).

- Chromatography in two elution systems:
 - OPLC1: trichloroethylene-methyl ethyl ketone, n-butanol, acetic acid-water (17+8+25+6+4, v+v), development time 12 min 19 sec.
 - OPLC2: butyl acetate- ethanol-tripropyl amine- water (85+9.25+5+0.75, v+v), developmet time 11 min 12 sec.
 - A 5-compound R_f -correction standard mixture in both systems.
 - Instrument: OPLC-NIT Personal OPLC Basic System 50
 - External pressure 50 bars, flow rate 450 $\mu\text{l}/\text{min}$.

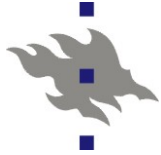


- Detection by UV-densitometry scanning at 220 nm, *in situ* UV-spectra of detected peaks measured between 190 and 400 nm (Camag TLC Scanner 3).

- Identification parameters:
 - Corrected hR_f -values from two independent elution systems
 - Spectral comparison with UV-spectra library
 - Software: winCats 1.2.6 (Camag)
 - Visualization reactions



Single wave-length scanning of all tracks at 220 nm.



winCATS - [winCATS.wsl]

File Instruments Run Administration View Tools Window Help

m1230508-002.cne m1230508-002.cne winCATS.wsl

Analysis

Spectrum hRfc correction graph

Citalopram

100 AU
90
80
70
60
50
40
30
20
10
0

239 289 339 nm

Add this spectrum to library ...

C:\CAMAG\venha\winCATS\Data\m1230508-002.cna

Track: 4

Position: MD Rf hRfc 0.19

Assigned substance: Citalopram

Library

Library Overlay Correlation Difference

Citalopram

100 AU
90
80
70
60
50
40
30
20
10
0

239 289 339 nm

Assign Citalopram to current peak

Hit	Substance	corr.	pos.
1	Citalopram	0.99...	Rf 0.30
2	Diltiazem	0.95...	Rf 0.32

Link files Compare analysis - library Edit library Compare library - library

For HELP, press F1

V 1.2.6 1001W030 Supervisor NUM

Start w M

10:15 AM

Identification of citalopram by UV-spectral and hR_{fc} comparison



winCATS - [winCATS.wsl]

File Instruments Run Administration View Tools Window Help

m1230508-002.cna m1230508-002.cna winCATS.wsl

Analysis

Spectrum hRfc correction graph

Doxepin

239 289 339 nm

Add this spectrum to library ...

C:\CAMAG\vanha\winCATS\Data\m1230508-002.cna

Track: 4

Position: MD Rf hRfc 0.25

Assigned substance: Doxepin

Library

Library Overlay Correlation Difference

Doxepin

239 289 339 nm

Assign Doxepin to current peak

Hit	Substance	corr.	pos.
1	Doxepin	0.96...	Rf 0...
2	Normifensine	0.96...	Rf 0...

Scanner3...

Link files Compare analysis - library Edit library Compare library - library

For HELP, press F1

V 1.2.6 1001W030 Supervisor NUM

Start w M. 10:14 AM

Identification of doxepin by UV-spectral and hR_{fc} comparison



winCATS - [winCATS.wsl]

File Instruments Run Administration View Tools Window Help

m1230508-002.cns m1230508-002.cns winCATS.wsl

Print... Next Page Prev. Page One page Zoom in Zoom out Close

winCATS Planar Chromatography Manager

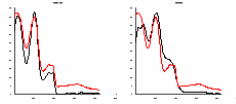
Track 2 : (2611)

Unassigned peak at RfR: 3 (HfR: empty)
Unassigned peak at RfR: 10 (HfR: empty)
Unassigned peak at RfR: 40 (HfR: empty)

Track 4 : (2618)

Unassigned peak at RfR: 3 (HfR: empty)
Unassigned peak at RfR: 11 (HfR: empty)
Unassigned peak at RfR: 10 (HfR: empty)
Unassigned peak at RfR: 20 (HfR: empty)
Peak assigned to Olfenopam at RfR: 23 Height: 233.96 AU Area: 18025.41

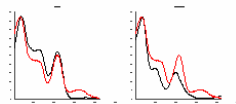
Hit	Sub class name	Pct diff	confirmation
1	Olfenopam	-1.74%	0.99023
2	Olfenopam	1.74%	0.98767



Hit No. Confirmed by A: B:

Unassigned peak at RfR: 30 (HfR: empty)
Peak assigned to Oxiprin at RfR: 11 Height: 33.45 AU Area: 12402.45

Hit	Sub class name	Pct diff	confirmation
1	Oxiprin	-1.74%	0.99405
2	Kontrastine	-2.74%	0.98235



Hit No. Confirmed by A: B:

Peak assigned to Kortefopam at RfR: 40 Height: 24025 AU Area: 10202.94

Hit	Sub class name	Pct diff	confirmation
1	Kortefopam	-2.74%	0.98235
2	Amifopam	-1.74%	0.99105
3	Tinefopam	-2.74%	0.98767

User: Supervisor Approved:
Monday, May 26, 2008 10:18:10 AM Report ID: 0163261422241234 SW: 1001W030, V1.2.6 Page: 4 of 14

For HELP, press F1 V 1.2.6 1001W030 Supervisor NUM

Scanner 3...

Start w G M

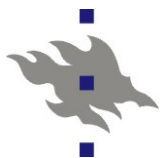
10:18 AM

Example of a results report page generated automatically by the software.



Results and statistics

- Limit of identification (LOI) was determined for 25 toxicologically relevant drugs.
- Pooled drug free liver spiked with reference standards.
- Starting level at 1 mg/kg, three replicates at the achieved LOI level.
- LOI varied between 1 and 10 mg/kg, median 2.5 mg/kg.



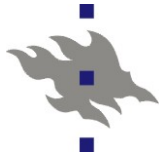
Identification limits of 25 basic drugs in liver by OPLC with UV scanning densitometry or visualisation.

Drug	Identification limit in liver by UV spectral comparison (mg/kg)	Identification limit in liver by visualisation (mg/kg)*
Amitriptyline	2.5	1.0 (Mq brown)
Chloroquine	1.0	native violet fluorescence when wet
Chlorpromazine	5.0	0.5 (Salkowski, Mq aniline red)
Citalopram	1.0	nd**
Clomipramine	1.0	0.5 (Salkowski light blue)
Codeine	5.0	5.0 (Mq dark violet)
Dextropropoxyphene	7.5	2.5 (Mq dark gray)
Diltiazem	2.5	nd
Doxepin	2.5	1.0 (Mq brown)
Fluoxetine	2.5	1.0 (FBK)
Fluvoxamine	5.0	nd
Levomepromazine	1.0	1.0 (Salkowski, Mq violet)
Melperone	2.5	nd
Mianserin	2.5	nd
Mirtazapine	1.0	nd
Moclobemide	1.0	nd
Nortriptyline	2.5	1.0 (FBK, Mq orange-brown)
Olanzapine	2.5	2.5 (FBK)
Orphenadrine	5.0	1.0 (Mq yellow)
Paroxetine	5.0	2.5 (FBK, Mq greenish)
Propranolol	1.0	1.0 (FBK)
Quinine	2.5	0.5 (Salkowski + 15 min UV(366 nm) blue fluoresc.)
Thioridazine	10.0	1.0 (Salkowski, Mq turquoise)
Tramadol	2.5	2.5 (Mq brownish)
Verapamil	2.5	nd

** nd = not detected



- In 2007 a total of 1495 cases analysed by the method.
- 86 different drugs identified
 - The most common findings were citalopram (n= 93), mirtazapine (n= 50), levomepromazine (n= 46), amitriptyline (n= 36), and amiodarone (n= 32).
- Total number of findings 1094.



Conclusions

- Dual-plate OPLC suits well for the qualitative screening analysis of basic, lipophilic drugs in liver samples.
- The LOIs were relatively high, but sufficient for levels typical for poisoning cases, and in many cases also at therapeutic levels.
- Detection of acidic and neutral drugs is still a challenge in cases where urine or vitreous is not available.