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Drug screening in autopsy liver samples by over pressured layer chromatography

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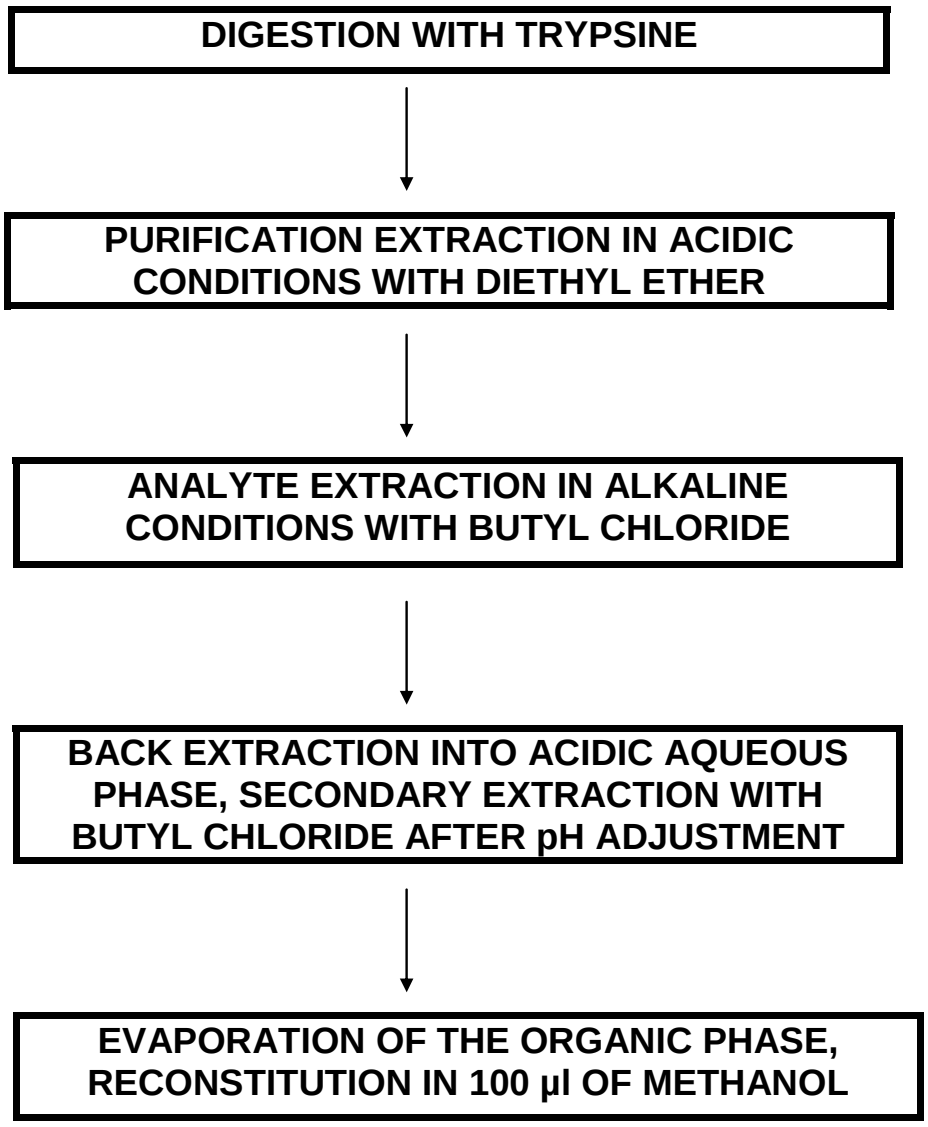
Background

- 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

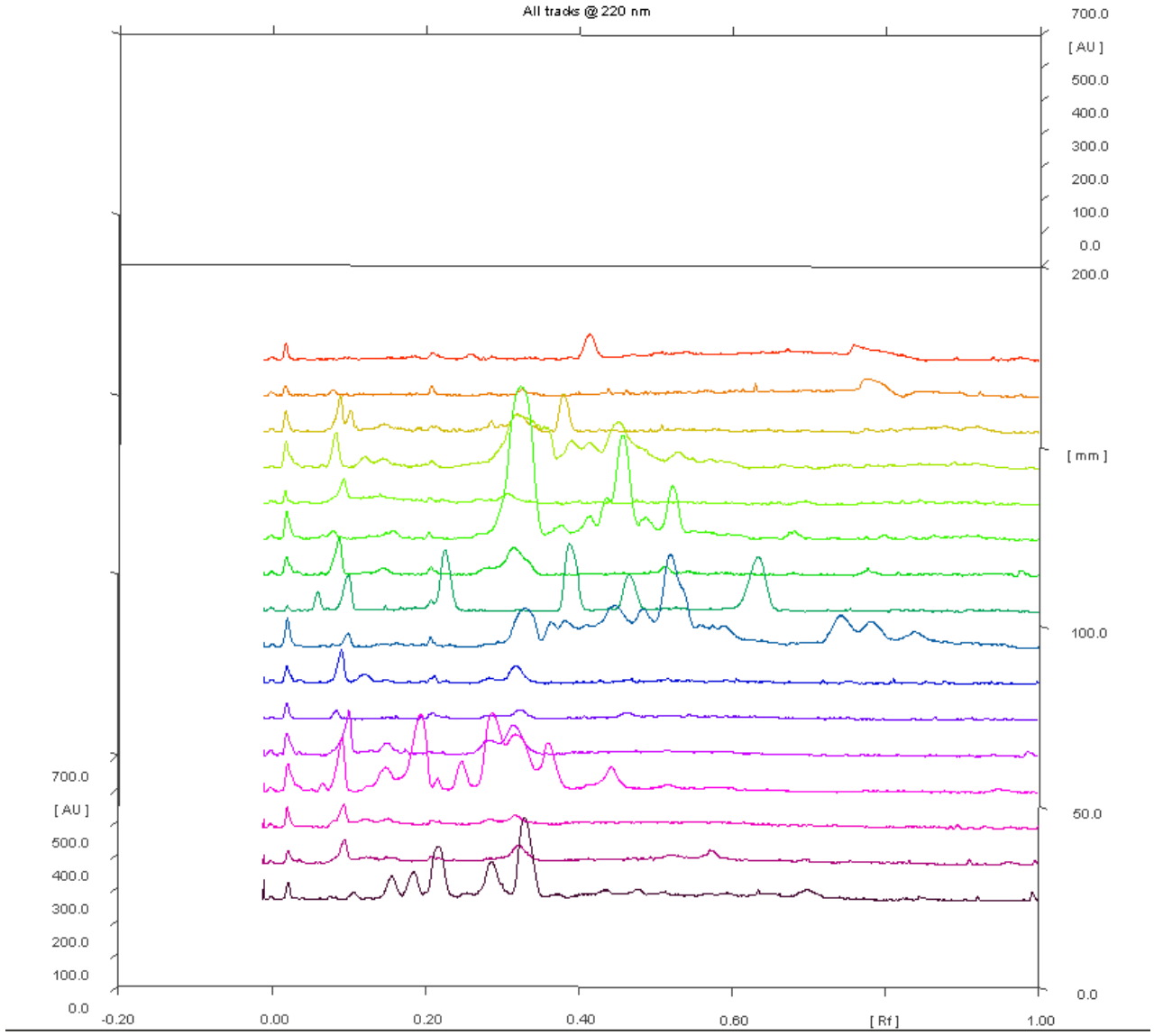


- 10 µl of the final extract applicated to two silica gel OPLC plates with an autosampler in band mode (Camag ATS III).

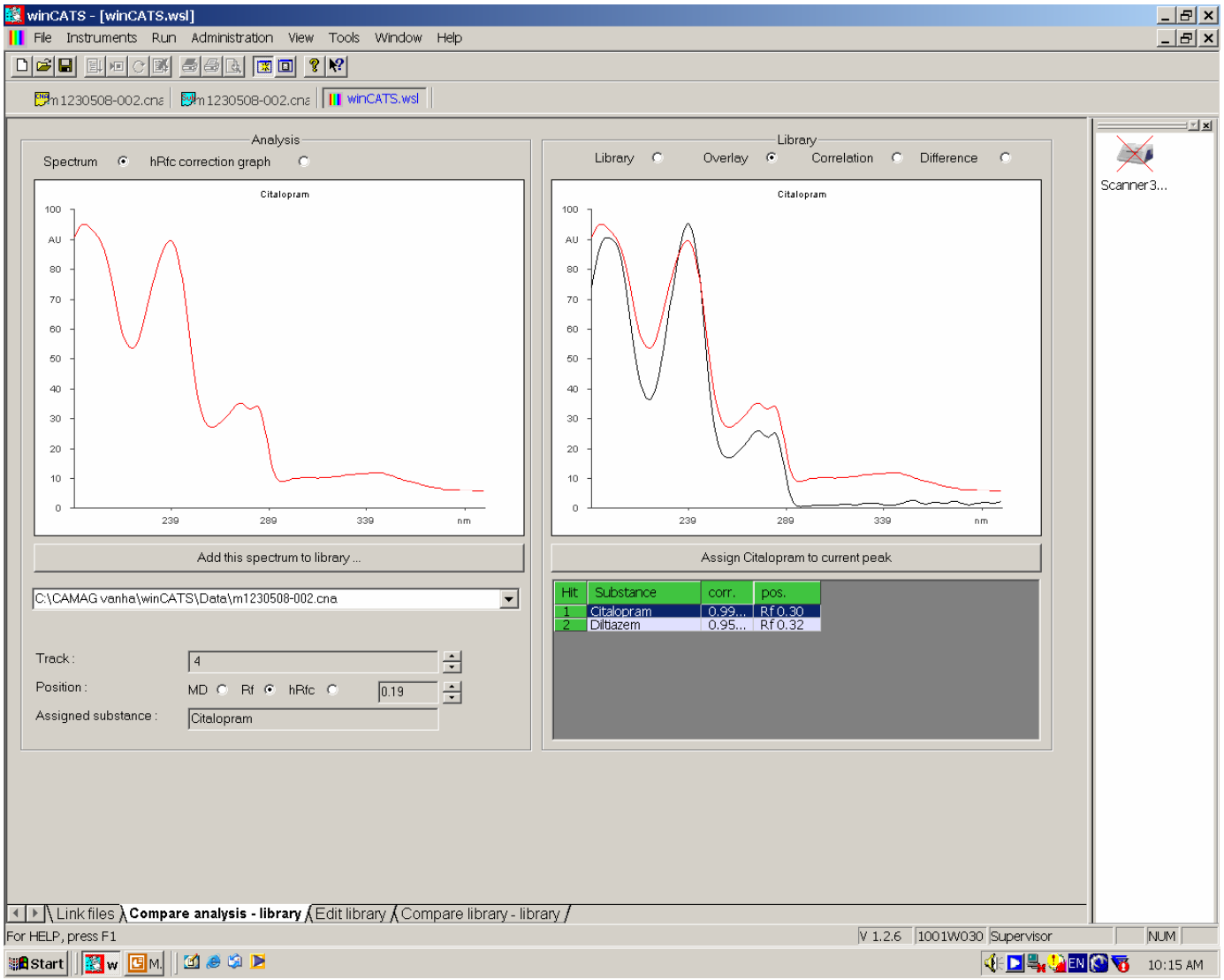
- Chromatography in two elution systems:
  - OPLC1: trichloroethylene-methyl ethyl ketone, n-butanol, acetic acid-water (17+8+25+6+4, v+v), development time 12 min 19 sec.
  - OPLC2: butyl acetate- ethanol-tripropyl amine- water (85+9.25+5+0.75, v+v), developmet time 11 min 12 sec.
  - A 5-compound R_f-correction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

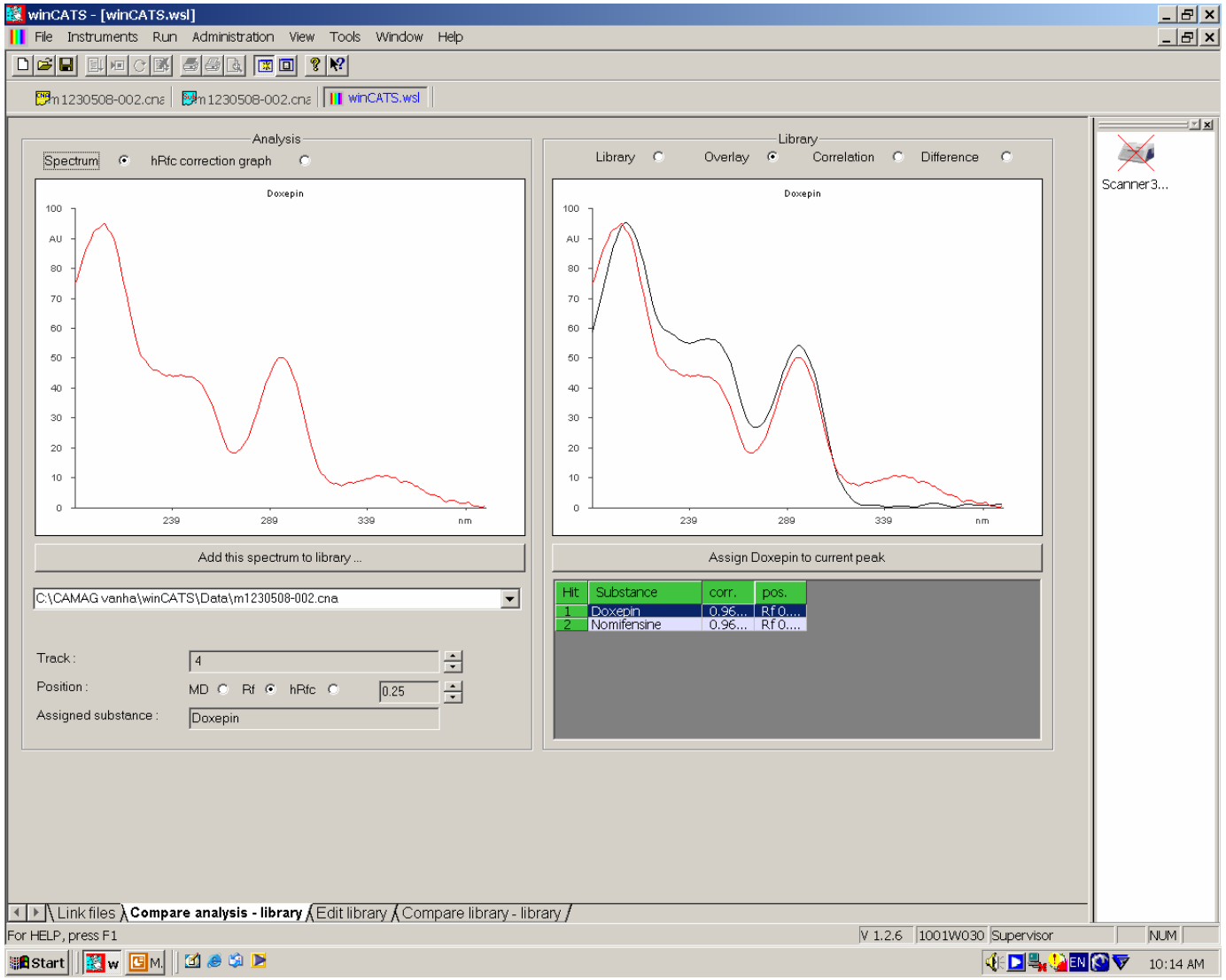
- Identification parameters:
  - Corrected hR_f-values from two independent elution systems
  - Spectral comparison with UV-spectra library
  - Software: winCats 1.2.6 (Camag)
  - Visualization reactions



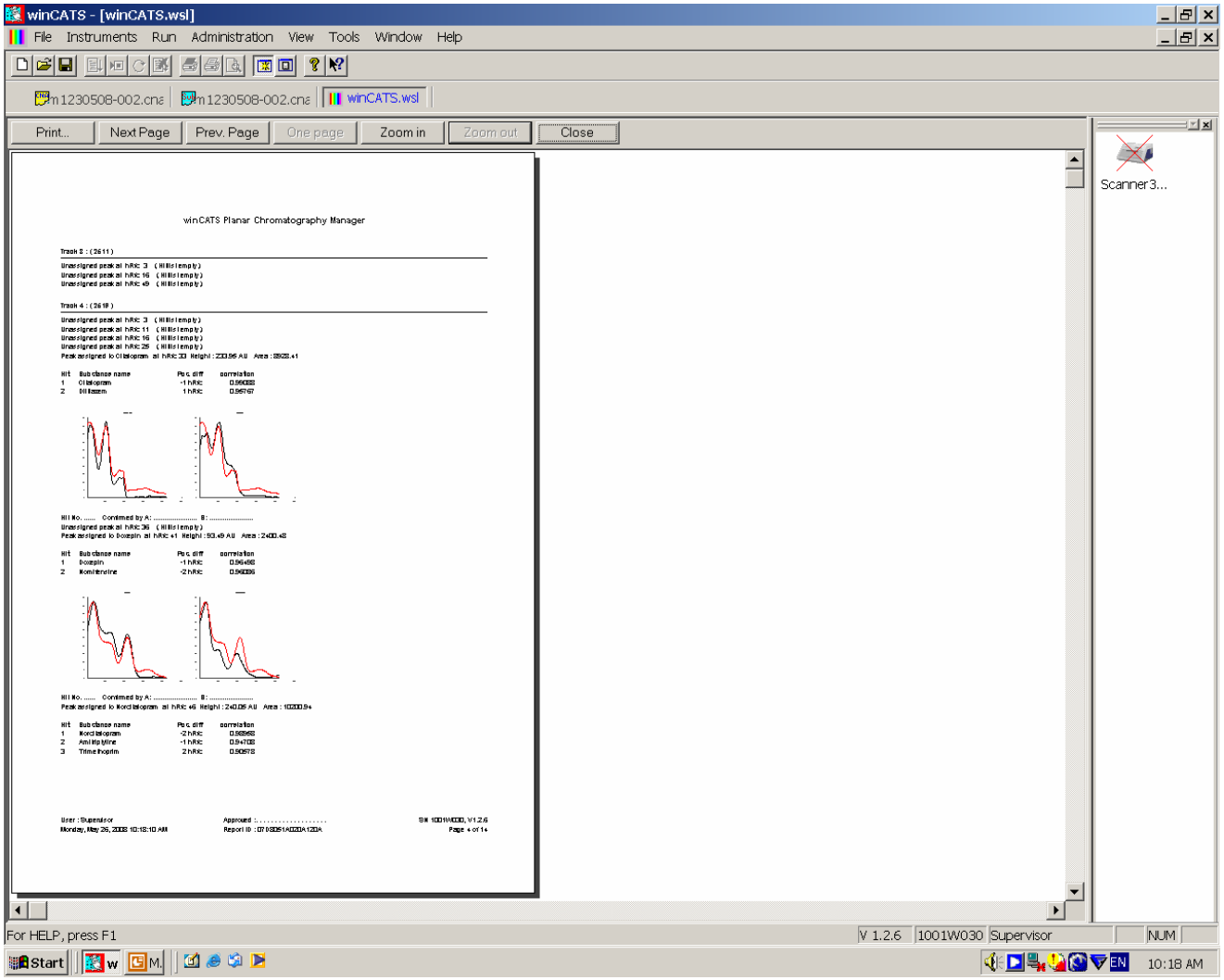
Single wave-length scanning of all tracks at 220 nm.



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



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



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 were urine or vitreous is not available.