Comprehensive Drug Screening in Urine by OPLC

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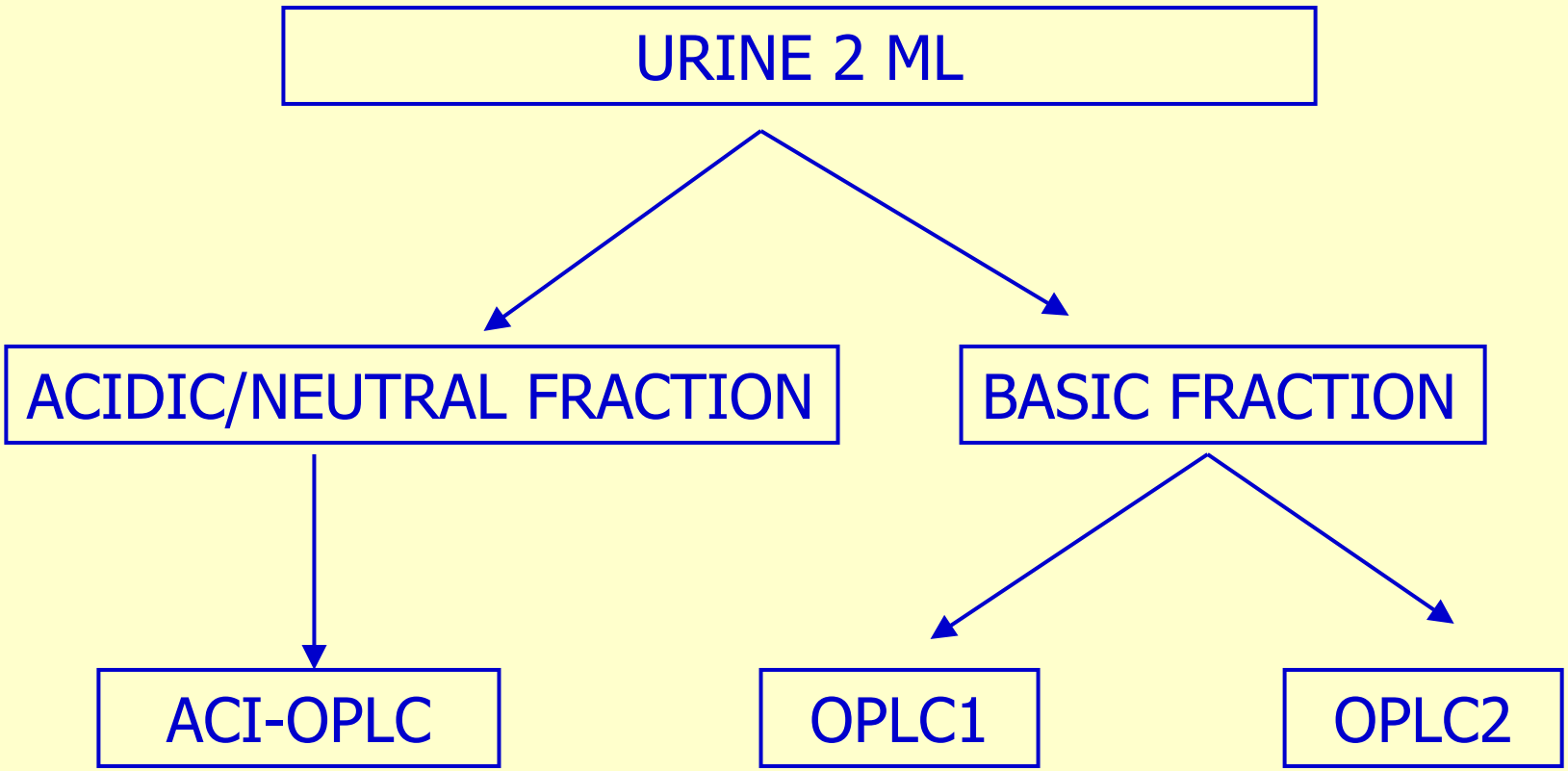
Introduction

- Overpressured Layer Chromatography (OPLC) = forced-flow planar chromatography in a closed separation system.
- Constant flow rate is generated by an external mechanical pump.
- Combines high throughput and good separation efficiency.

Figure 1: OPLC instrument



207 drugs in dual-plate systems OPLC1 and OPLC2

Pelander A., Ojanperä I., Sistonen J., Rasanen I., and Vuori E., *J. Anal. Toxicol.* **27** (2003), 226-232.

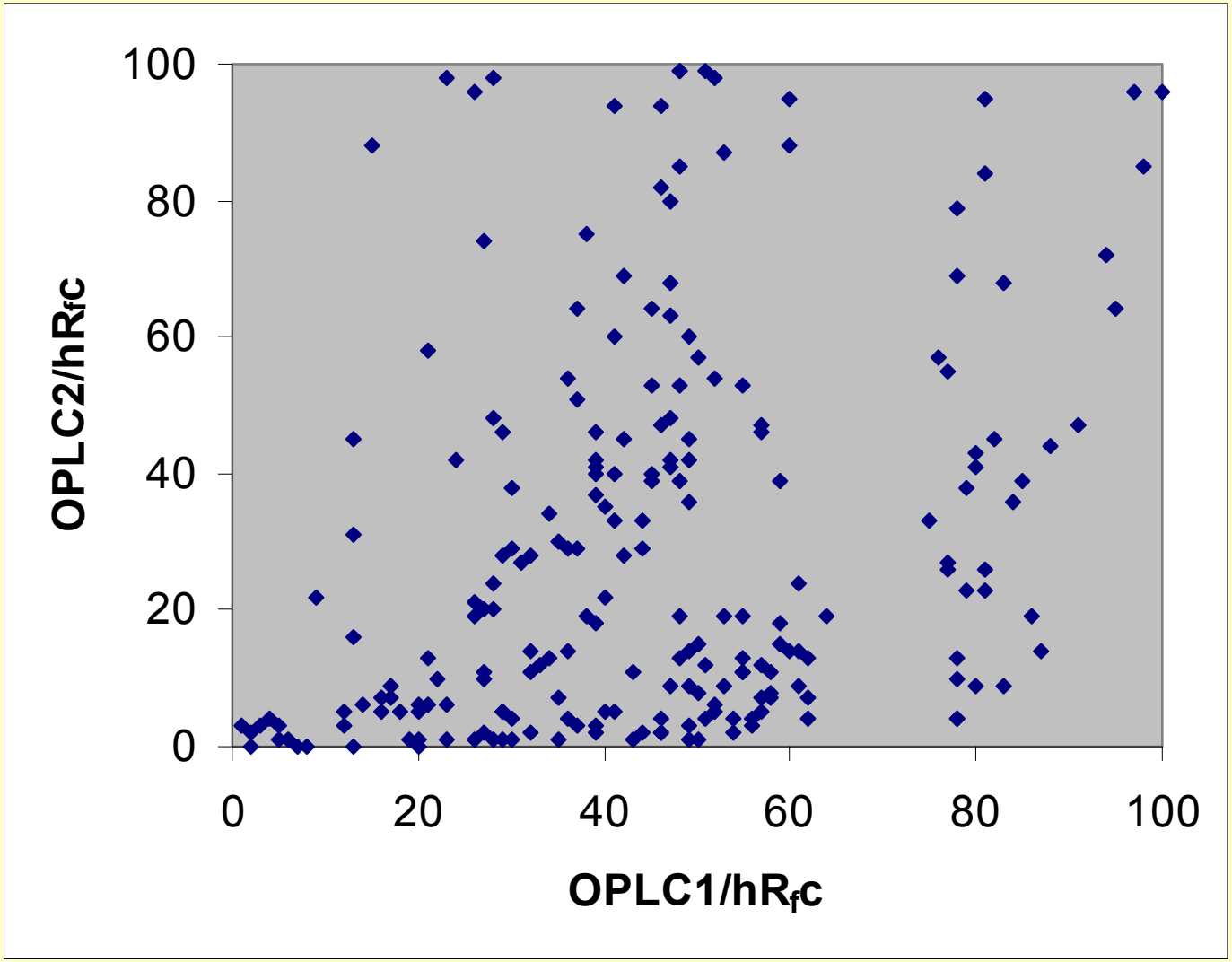


Figure 2: Correlation fo hR_fc values in dual-plate system for basic drugs.

ACI-OPLC

Experimental

- Instrumentation:
  - sample application by Camag ATS III.
  - chromatography by Bionisis Personal OPLC Basic System 50.
  - UV-scanning densitometry by Camag TLC Scanner 3 operated with Cats 4.03 software.
  - automated reporting by Sunicom Cats Spectrum Library 1.50 software.

- Sample pretreatment
  - solid phase extraction of hydrolysed 2 ml urine samples with IST HCX-5 mixed mode SPE cartridges.
  - cartridges conditioned with methanol, water and pH 6 buffer.
  - after sample application cartridges rinsed with pH 6 buffer and 1 M acetic acid.
  - acidic-neutral fraction eluted with ethyl acetate-hexane (25+75, v+v).

- evaporation to dryness under nitrogen, reconstitution in 30 μl methanol.
- 10 μl applicated on a 20x20 cm silica gel aluminium sheet sealed for OPLC (Bionisis).
- 15 samples and 1 R_f correction standard per plate.
- R_f correction standard paracetamol (hR_fc 9), temazepam (hR_fc 30), fenobarbital (hR_fc 48), salisylic acid (hR_fc 72), and diclofenac (hR_fc 90) applicated on track 9.

- Chromatographic conditions:
  - plate saturation 0.5 h, toluene-acetic acid-isobutylmethylketone (6+1.5+1, v+v+v).
  - mobile phase toluene-acetic acid-isobutylmethylketone (6+0.75+1, v+v+v).
  - mobile phase volume 3500 μl, flow rate 450 μl/min, external pressure 50 bar.

- UV-scanning densitometry
  - plates dried in a stream of warm air.
  - scanning at 220 nm.
  - *in situ* UV spectra of detected peaks measured at 190-400 nm.
- Reporting
  - automated R_f correction and UV-spectral library search in ± 7 hR_fc window.
  - improved UV-spectral identification by spectrum maxima criteria.

Results

- hR_fc and UV-spectral library:
  - hR_fc and UV spectrum for 96 acidic and neutral drugs saved in a library.
  - hR_fc mean calculated from 5 parallel measurements.
- Repeatability of chromatography was evaluated by analysing 15 drug reference substances once a week for a period of 14 weeks.

- CV% of R_f values was 21%.
- CV% of hR_fc values was 7.4%.
- The corresponding figures were 13.9% and 2.4% in OPLC 1, and 11.0% and 3.4% in OPLC2.
- Air humidity varied from 36% to 69% during the study.

- Detection limits
  - detection limit was determined for 15 drugs in spiked urine.
  - criteria for detection limit was positive identification in the automated results report for three parallel samples.
  - detection limits varied from 0.25 mg/l to 2 mg/l.

- Method comparison to HPLC with authentic samples
  - 29 autopsy urine samples were analyzed by ACI-OPLC and HPLC-DAD.
  - no false positives observed by ACI-OPLC.
  - in seven cases a false negative for caffeine by ACI-OPLC.
  - in one case a false negative for oxazepam by ACI-OPLC.

Case number	Findings by ACI-OPLC	Findings by HPLC-DAD
4401	furosemide	furosemide, caffeine
4402	NDD	NDD
4403	ibuprofen	ibuprofen
4404	caffeine, lorazepam/oxazepam	caffeine, lorazepam
4405	caffeine	caffeine
4407	NDD	NDD
4408	oxazepam, temazepam	oxazepam, temazepam
4409	caffeine	caffeine
4410	NDD	caffeine
4411	NDD	NDD
4412	caffeine	caffeine, oxazepam
4413	caffeine	caffeine
4414	NDD	caffeine, temazepam
4415	oxazepam, temazepam	oxazepam, temazepam
4416	NDD	NDD
4417	NDD	caffeine
4418	NDD	NDD
4419	oxazepam	oxazepam
4420	NDD	caffeine
4421	lorazepam	lorazepam, caffeine
4423	NDD	NDD
4425	ibuprofen	caffeine, ibuprofen
4426	caffeine, ibuprofen	caffeine, ibuprofen
4428	NDD	caffeine
4429	caffeine	caffeine
4430	an undefined benzodiazepine	NDD
4431	caffeine	caffeine
4432	NDD	NDD
4433	oxazepam, temazepam, oxycarbazepine, ketoprofen, ibuprofen	oxazepam, oxycarbazepine, ketoprofen, ibuprofen

NDD = no drugs detected

Table 2: Comparison of findings in urine samples by ACI-OPLC and HPLC-DAD

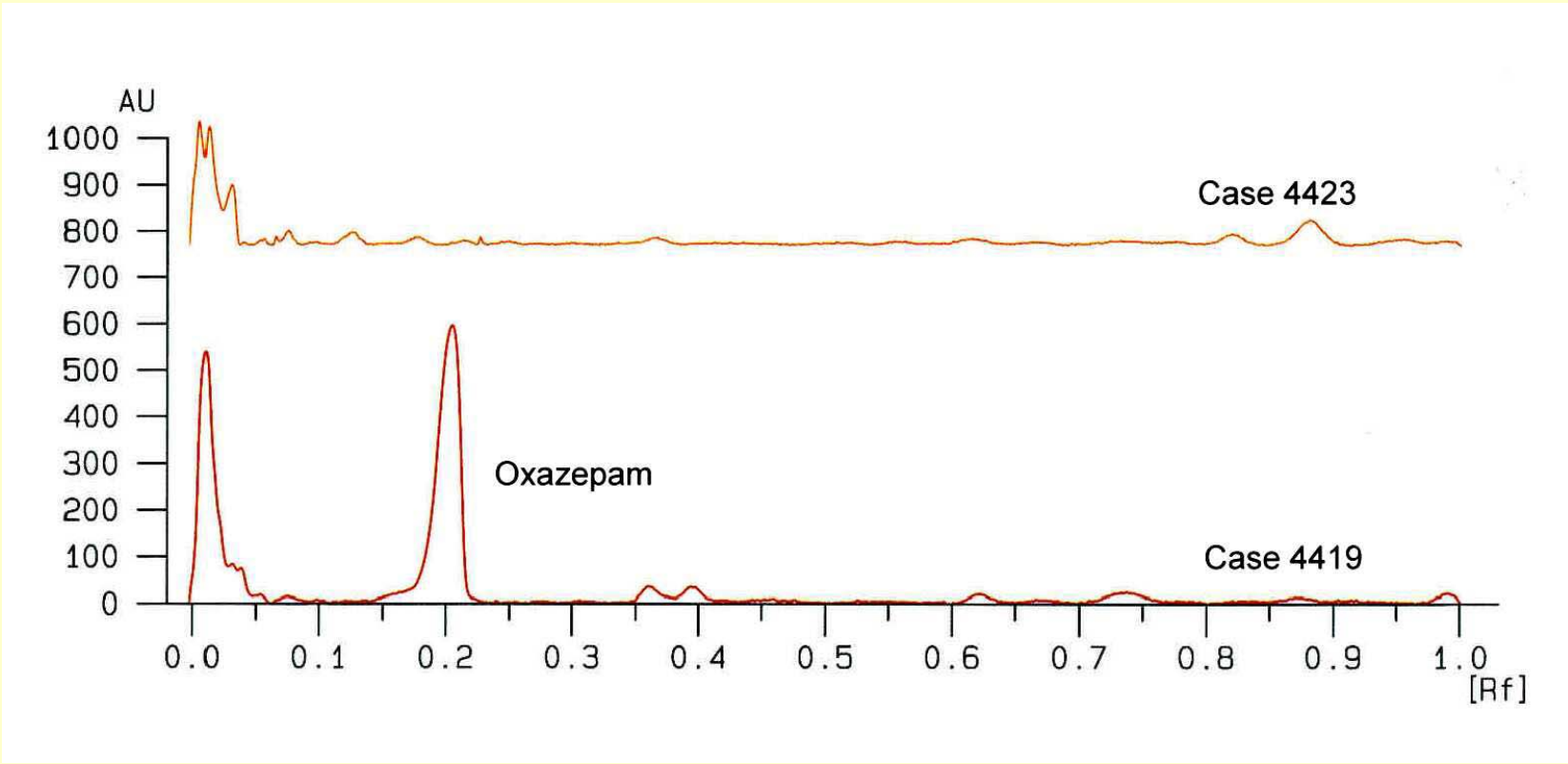
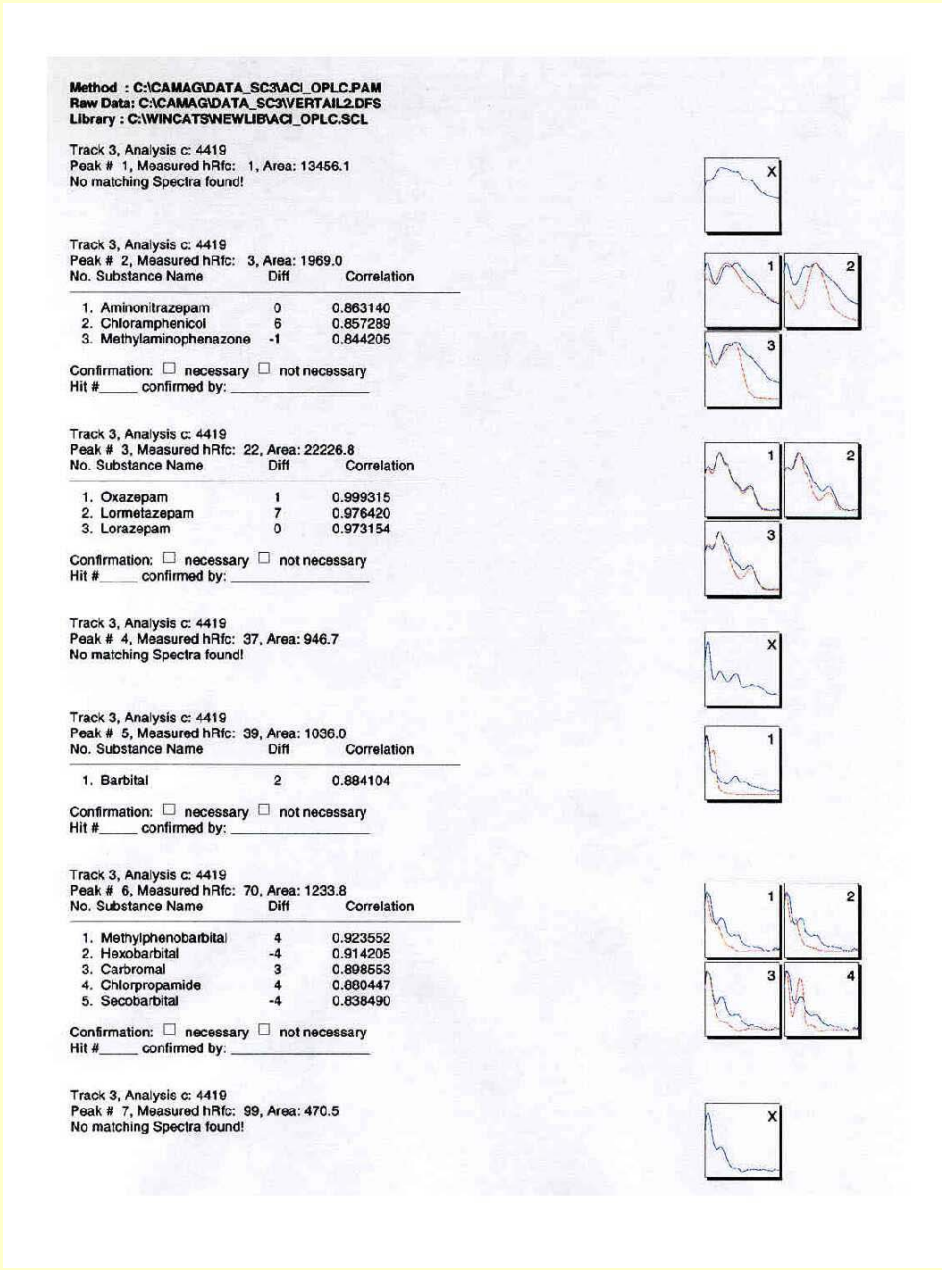


Figure 6: Example of UV-scanning densitograms at 220 nm for two urine samples. Oxazepam was identified in case 4419, Case 4423 did not contain acidic or neutral drugs.



Method : C:\CAMAG\DATA_SC3\ACI_OPLC.PAM
Raw Data: C:\CAMAG\DATA_SC3\VERTAIL2.DFS
Library : C:\WINCATS\NEWLIB\ACI_OPLC.SCL

Track 3, Analysis c: 4419
Peak # 1, Measured hRfc: 1, Area: 13456.1
No matching Spectra found!

Track 3, Analysis c: 4419
Peak # 2, Measured hRfc: 3, Area: 1969.0

No.	Substance Name	Diff	Correlation
1.	Aminonitrazepam	0	0.863140
2.	Chloramphenicol	6	0.857289
3.	Methylaminophenazone	-1	0.844205

Confirmation: ☐ necessary ☐ not necessary
Hit #_____ confirmed by: _____

Track 3, Analysis c: 4419
Peak # 3, Measured hRfc: 22, Area: 22226.8

No.	Substance Name	Diff	Correlation
1.	Oxazepam	1	0.999315
2.	Lormetazepam	7	0.976420
3.	Lorazepam	0	0.973154

Confirmation: ☐ necessary ☐ not necessary
Hit #_____ confirmed by: _____

Track 3, Analysis c: 4419
Peak # 4, Measured hRfc: 37, Area: 946.7
No matching Spectra found!

Track 3, Analysis c: 4419
Peak # 5, Measured hRfc: 39, Area: 1036.0

No.	Substance Name	Diff	Correlation
1.	Barbital	2	0.884104

Confirmation: ☐ necessary ☐ not necessary
Hit #_____ confirmed by: _____

Track 3, Analysis c: 4419
Peak # 6, Measured hRfc: 70, Area: 1233.8

No.	Substance Name	Diff	Correlation
1.	Methylphenobarbital	4	0.923552
2.	Hexobarbital	-4	0.914205
3.	Carbromal	3	0.898553
4.	Chlorpropamide	4	0.880447
5.	Secobarbital	-4	0.838490

Confirmation: ☐ necessary ☐ not necessary
Hit #_____ confirmed by: _____

Track 3, Analysis c: 4419
Peak # 7, Measured hRfc: 99, Area: 470.5
No matching Spectra found!

Figure 7: Example of results report generated by the software for Case 4419.

Conclusions

- Combination of earlier developed dual-plate system for basic drugs (OPLC1 and OPLC2) and ACI-OPLC provides an efficient tool for comprehensive qualitative drug screening of urine samples.
- Efficient analysis method compared to HPLC, results equal.

- Simple compared to GC, no derivatization needed.
- The prize of the OPLC plates limits the applicability of the method.