EVALUATION OF A UPLC-TOF METHOD USING PROTONATED MOLECULAR IONS AND THEIR FRAGMENTATION PATTERNS FOR URINE SCREENING IN HOSPITALS

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INTRODUCTION

- Since its introduction in 1992, the REMEDi HS drug profiling system (DPS) (Bio-Rad) has been used in laboratories worldwide providing automated analysis for basic drugs
- For the last 10 years this system has been used by our consortium of 4 hospitals *i.e.* TMH, PWH, QEH and PYNEH, in Hong Kong to complement other screening techniques for routine uranalysis.
- Currently we handle a combined total of ~13,500 specimens/year. A survey of the drugs reported by the 4 hospitals (REMEDi data, 1 yr period) revealed the involvement of 132 common drugs.
- The decision of Bio-Rad to discontinue the support of the DPS, has necessitated its replacement with an alternative broad screening technique.
- In collaboration with laboratories in Copenhagen, Denmark (CPN) and Manchester, UK (MCR), we have developed a method based on UPLC-TOF mass spectrometry[1].
- We present the method, in addition to a preliminary evaluation of both the transferability of the technique and its performance, with authentic samples, in comparison to the REMEDi DPS.

METHODS

CHROMATOGRAPHY

ACQUITY® UPLC system (Fig 1).

Column:	Waters ACQUITY UPLC HSS T3 (2.1 x 100mm, 1.8µm)
Column temp:	35°C
Mobile phases	A: 0.05% formic acid B: methanol Gradient elution (17 min cycle time)
Flow rate:	0.3mL/min
Injection vol:	5µL

MASS SPECTROMETRY

Waters LCT Premier™ XE mass spectrometer (Fig 1).

Ionisation mode:	Electrospray +ve
Capillary voltage:	3000V
Aperture 1:	10 (V1) and 45V (V2)
Mass range:	50-1000 Da
Resolution:	10,000 (W mode)
Acq time:	0.15 sec
Lockspray™ reference:	Leucine enkephalin $[M+H]^+$ = *m/z* 556.2771

Fig 1. ACQUITY® UPLC and LCT Premier™ XE

REFERENCE LIBRARIES

Libraries were created based on retention time (RT) in combination with theoretical (generated from the elemental formula) and/or acquired spectra. For the latter, spectra were collected using 2 different voltages within the source region. A low voltage (V1) enabled exact mass measurement of the protonated molecular species; a higher voltage (V2) was used to generate fragments within the source (collision-induced dissociation; CID) for additional confirmatory purposes.

DATA PROCESSING

ChromaLynx™ application manager (Waters).

RESULTS AND DISCUSSION

To evaluate the transferability of the method, ~30 drugs (ranging in molecular weight and chromatographic retention) were selected and used by TMH to create a spectral library. For each analyte, 2 spectra were recorded in addition to RT:

- Theoretical spectra (no fragmentation condition)
- Spectra acquired at 45V (generating fragments)

Each laboratory analysed the same drug mixture using their own instrumentation; data were matched against the TMH library.

RTs were demonstrated to be highly reproducible (Fig 2).

ChromaLynx™ calculated the spectral match factor (MF) for each drug, against the TMH library. Overall, an average of 92% of all drugs examined were identified as 'positive' *i.e.* MF >500 (max MF = 1000); 75% of these drugs had MF >700. The mean MF (all drugs, 6 labs) was 751. These data indicated that spectra were very reproducible between instruments.

To evaluate the utility of the method, 23 authentic urine samples were analysed and matched to an extended library (237 drugs and metabolites). A total of 87 compounds were detected. Data were compared to REMEDi results; all analytes found by the DPS were identified with UPLC-TOF. However an enhanced detection was observed with the latter method. An additional 21 positives, involving 15 drugs, were found including: zolpidem, sulpiride, chlorpheniramine, ketamine, chlorpromazine, methadone, meclopramide and zopiclone.

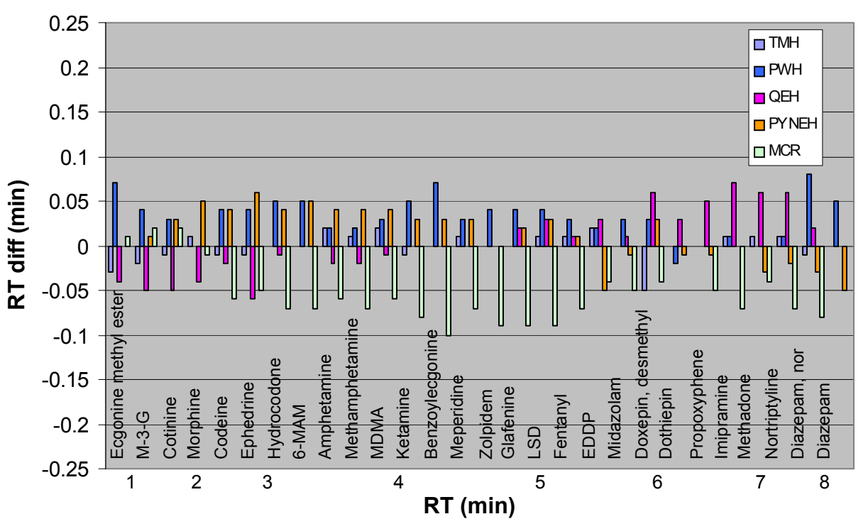

Fig 2. RT was highly reproducible between laboratories with an average deviation of 0.12% (from the mean RT of each drug).*

** CPN RT data excluded due to slight difference in configuration of the UPLC system.*

Three UKNEQAS samples from 2007 (DAU #223, #225 and #226) were prepared according to a previously published procedure[2] and analysed. The following analytes were identified; amphetamine, methamphetamine, EDDP, morphine, methadone, desmethyldiazepam, oxazepam, PCP, propoxyphene, buprenorphine (BUP) and LSD. With the exception of BUP and LSD, these results were in accordance to conventional analytical findings using HPLC-DAD and GC-MS. BUP and LSD (both at 10µg/L urine) could not be detected by GC-MS or HPLC but were detected using UPLC-TOF. Fig. 3 shows the results for one of the positive UKNEQAS samples.

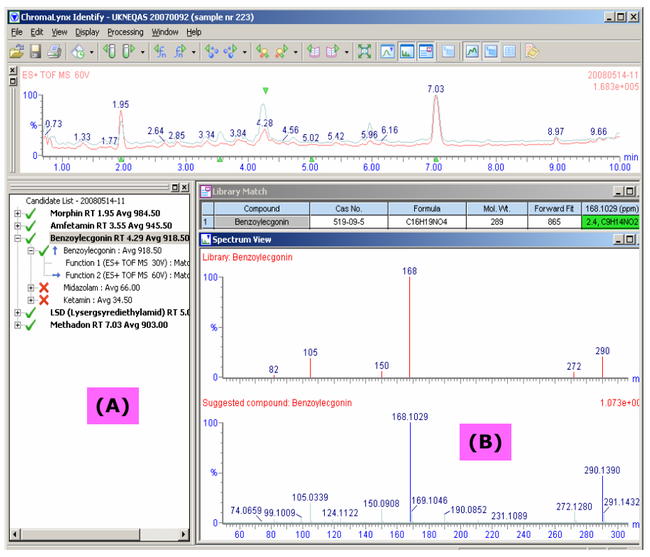

Fig 3. UKNEQAS sample #223. This sample showed positive matches for morphine, amphetamine, benzoylecgonine, LSD and methadone (3A). The spectrum view allows direct comparison of acquired spectra with library data (3B). Elemental formulae can be predicted from exact masses of found ions (library match Table).

CONCLUSIONS

- We have developed a screening method based on UPLC-TOF.
- Identification is based on a combination of RT, exact mass and fragments. Examination of the isotope ratios (I-Fit)[3] can also be used for further confidence.
- The transferability of the method between the 6 laboratories has been assessed and found to be very good.
- Analysis of UKNEQAS samples demonstrated good agreement with existing, conventional techniques.
- The developed method was demonstrated to be a suitable alternative to the REMEDi DPS system and provided an enhanced detection.

References

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