Appendix A.1 Project Approval

DBMS Project proposal submitted to the University of Portsmouth and to King's College Hospital for assessment and approval.

a. Proposal Title (no more than 20 words, avoid acronyms and abbreviations)
Screening for urinary drugs of abuse by liquid chromatography with ion trap mass spectrometric detection using Turboflow®

b. Objectives (typically 4-6)

Objective number	Objective description
01	Develop a Turboflow / LC method giving suitable extraction and separation of the opiate and amfetamine classes of drugs
02	Develop an ion trap MS procedure to reliably identify opiate and amfetamine class drugs
03	Evaluate the LC-MS method developed for detection of synthetic opioids (e.g. methadone, buprenorphine) and cocaine metabolites, with other analytes that are required by the laboratory service
04	Introduce the methodology into routine use within the laboratory, providing sufficient training to staff to allow continued operation and understanding
05	Consult with the local drug addiction specialists to provide a drugs of abuse service that meets their current and future needs

c. Importance (no more than 1000 words)

Clinical drugs of abuse testing is normally a two stage process designed to allow drug-free samples to be reported as negative quickly and cheaply, allowing resources to be concentrated on samples thought to contain drugs of interest (Levine, 2003, p. 34). An immunoassay "screen" tests for the presence of whole classes of drugs, such as opiates, without identifying the specific drug present. These presumptive positive samples are further tested by a chromatographic method to both confirm the presence of a drug and also identify the drug present. This is important to identify the difference between legal over the counter medications (e.g. codeine), prescription medication (morphine), or illicit drugs (heroin). All of these will produce a positive result by immunoassay.

There are different confirmatory tests, each with its benefits and limitations (Maurer, 2004). Traditionally the "gold standard" assay has been gas-chromatography with mass spectrometric detection (GC-MS), as this gives very high chromatographic resolution coupled with characteristic fragmentation (Levine, 2003, p. 9). The combination of retention time and mass spectrum confirm the presence of a drug when compared to a standard

with matching spectrum and retention time.

For GC-MS analysis, drugs need to be in the gas phase in order for chromatography to occur. The sample most commonly used for drugs of abuse testing is urine, an aqueous matrix which cannot be directly injected onto a GC column (Smith, Athanaselis, & Siegel, 2005, p. 375). To overcome this limitation, analytes are normally extracted into an organic solvent prior to analysis, and may also require derivatisation to improve the chromatographic or MS properties (Penton, 2004). Several different techniques are available: liquid–liquid extraction (LLE) is quick and cheap, but is difficult to automate, and solid phase extraction (SPE) is easy to automate and gives a clean extract, but at a higher cost (Flanagan, Taylor, Watson, & Whelpton, 2007, pp. 58, 68).

A further difficulty is that many drugs are present in the urine as metabolites, commonly glucuronic acid conjugates (Payne-James, Busuttil, & Smock, 2003, p. 612). Glucuronides are highly water soluble to allow easy removal from the body, but this also means that the conjugated drugs remain in the aqueous phase during both LLE and SPE. Hydrolysis is necessary to remove the glucuronide, and this increases sample pre-treatment time.

Recently liquid chromatography-mass spectrometry (LC-MS) has moved from the specialist laboratory into clinical use (Lord & Bralley, 2008, p. 9). The simplified sample preparation procedures have made it the instrument of choice for many toxicology laboratories, and LC-MS methods are frequently used for drugs testing.

Recent developments in 2-dimensional LC have included the production of the Turboflow® system. The Turboflow uses a large particle size packing with a high flow rate to wash proteins and salts out of a sample while retaining analytes on the column (Ashton, Allan, Ardrey, McDonnell, & Robinson, 2009). The turbulent flow achieved at high flow rates paradoxically minimises band broadening, and the extracted analytes are eluted onto a conventional HPLC column. This technology has been used in the pharmaceutical sector for a few years, but is only recently finding applications in routine analytical laboratories.

Most LC-MS systems in clinical laboratories are triple quadrupole instruments (MS/MS), which use single reaction monitoring (SRM) transitions to identify drugs and metabolites. SRM transitions do not give the characteristic mass spectrum obtained by GC-MS, and can only identify a drug based on some of the fragments obtained and the LC retention time. Additionally, LC systems do not give the same high resolution as GC, so that chromatographic peaks are wider and less pure (James & Nordby, 2005, p. 72).

A potential advantage of LC-MS is that aqueous samples can be introduced directly into the system, allowing more automation than can be used for GC-MS (James & Nordby, 2005, p. 72). Since extraction is not needed, neither is hydrolysis. However this reduces the specificity of the analysis, as there is normally only one fragment produced from glucuronides, meaning only one SRM transition can be monitored.

Ion trap MS aims to combine the best points of LC-MS and GC-MS systems. LC can be used for the sample introduction and separation, meaning minimal

sample preparation, and increased opportunity for automation. The ion trap is capable of producing similar fragmentation patterns to GC-MS, giving reliable analyte identification (Polettini, 2006, p. 56). Additionally, an ion trap can produce secondary fragmentation of daughter ions. This enables the single ion produced from the fragmentation of intact glucuronides to be further fragmented, producing a spectrum equivalent to that provided by the hydrolysed glucuronide.

Since the ion trap monitors all daughter ions from a molecule, the time spent looking for each fragment is much less than the time spent on each SRM in MS/MS. The sensitivity of the instrument is therefore reduced, i.e. the limits of detection are higher (Polettini, 2006, pp. 54-56). To compensate for this, a larger sample volume is required, which in turn necessitates an extraction procedure.

Turboflow LC-MS analysis of drugs of abuse by ion trap MS should provide confirmation of the presence of drugs with a rapid turnaround time and minimal sample preparation. Results produced should be more robust than those from triple quadrupole systems, and give similar reliability to GC-MS systems. Minimal sample preparation will mean that the system can be automated, allowing the operator more time for other tasks.

This method will allow addiction services in south London to monitor a wider range of drugs than at present, allowing better treatment of some of the more difficult users with more timely results. The clinics likely to benefit from this most are the injectables clinic, where users are prescribed diamorphine, and the stimulants clinic for cocaine and amphetamine users. The emergency department and intensive care services of King's College Hospital will also benefit from better and faster drug screen results.

Although there are many methods for drugs of abuse using ion trap GC-MS, and triple quadrupole LC-MS/MS methods, there is little published work on ion trap LC-MS for drugs of abuse in urine (Maurer, 2004, 2007). Cheng, Yau, Wong, Chan, & Mok (2006) have published an ion trap LC-MS drugs of abuse method for 19 analytes after off-line SPE, and Wu & Fuh (2004) have published an on-line SPE method for amfetamines by performing SRM analyses on an ion trap. Application notes are available for the Turboflow for some drugs, using MS/MS detection, but this is a new application. Once established, ion trap and Turboflow methods can be modified to increase the number of analytes that may be analysed.

References

Ashton, P., Allan, A., Ardrey, B., McDonnell, S., & Robinson, S. (2009). Rapid Analysis of Opiates from Low Volume Whole Blood Samples by LC-MS/MS Utilizing Turboflow Methods. San Jose, CA: Thermo Fisher Scientific.

Cheng, W.C., Yau, T.S., Wong, M.K., Chan, L.P., & Mok, V. K.K. (2006). A high-throughput urinalysis of abused drugs based on a SPE-LC–MS/MS method coupled with an in-house developed post-analysis data treatment system. Forensic Science International , 95-107.

Flanagan, R. J., Taylor, A. A., Watson, I. D., & Whelpton, R. (2007).

Fundamentals of analytical toxicology. Chichester: John Wiley.

James, S. H., & Nordby, J. J. (2005). Forensic science : an introduction to scientific and investigative techniques (2nd ed.). Boca Raton, Fla. ; London: CRC Press.

Levine, B. (2003). Principles of forensic toxicology (2nd ed.). Washington, DC: AACC Press.

Lord, R. S., & Bralley, J. A. (2008). Laboratory evaluations for integrative and functional medicine (2nd ed.). Duluth, Ga.: Metametrix Institute.

Maurer, H. (2004). Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control. Clinical Chemistry and Laboratory Medicine, 42(11), 1310-1324.

Maurer, H. (2007). Current role of liquid chromatography–mass spectrometry in clinical and forensic toxicology. Analytical and Bioanalytical Chemistry, 388(7), 1313-1325.

Payne-James, J., Busuttil, A., & Smock, W. S. (2003). Forensic medicine : clinical and pathological aspects. San Francisco, Calif. ; London: GMM.

Penton, Z. (2004). Derivatization of Drugs Prior to GC/MS Analysis. Varian Application Note 69

Polettini, A. (2006). Applications of LC-MS in toxicology. London: Pharmaceutical Press.

Smith, F. P., Athanaselis, S. A., & Siegel, J. A. (2005). Handbook of forensic drug analysis. Amsterdam ; London: Elsevier Academic.

Wu, T.Y., & Fuh, M.R. (2004). Determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, and 3,4-methylenedioxymethamphetamine in urine by online solid-phase extraction and ion-pairing liquid chromatography with detection by electrospray tandem mass spectrometry. Rapid communications in mass *spectrometry* , 775-780.