On-line extraction and ion trap mass spectrometry of drugs of abuse in urine

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In the last 5 years or so, liquid chromatography mass spectrometry (LCMS) has moved from being a specialist laboratory's tool into routine clinical use. The reduced sample preparation procedures compared to gas chromatography mass spectrometry (GCMS) have made it the instrument of choice for many toxicology laboratories, and LCMS is now frequently used for drugs of abuse testing. However, gas chromatography is still often considered the gold standard in drug testing, as the characteristic fragmentation pattern formed when an analyte is ionised gives a high degree of confidence in the identity of the analyte.

There are a number of different types of mass spectrometer, and the type generally found in clinical laboratories is a triple quadrupole system. In a triple quadrupole, the first quadrupole (Q1) separates the parent ions, allowing only the selected ion through to the second quadrupole (Q2) or collision cell. In Q2 the ions are excited by collision with a gas, so that they fragment. The third quadrupole (Q3) separates the daughter ions so that only the one selected reaches the detector , a process known as selected reaction monitoring (SRM). This gives a method of detecting a specific compound that is both very sensitive and reasonably selective, as the compound must have the selected parent mass and undergo the selected transition. Specificity is fairly good, but there are other materials that interfere, known as adiabatic interferences. In a paper in January 2009, (Fox, Twigger & Allen) quoted morphine as having a 16% false positive rate when using a single SRM transition. To increase the specificity, it is usual to use 2-3 SRM transitions per analyte, although this still does not give absolute specificity, particularly if the SRM transitions are not chosen carefully, and the identification is still not as good as found in GCMS.

An ion trap mass spectrometer uses an electric field to confine ions. Typical use of an ion trap is achieved by selecting a parent ion (as in Q1 above) and colliding it to produce daughter fragments (as in Q2). The whole spectrum of daughter fragments is then detected to give a characteristic mass spectrum that can be compared to a library spectrum to give a similarity score for the analyte. Like GCMS, the spectrum is specific to the target analyte, and the degree of similarity to the library spectrum can be expressed as a number.

An additional benefit of an ion trap is that this process can be repeated several times, so that a selected daughter ion can be collided to produce granddaughter ions, further confirming the identity of the analyte. This process is known as MSn, where n is the number of mass separations performed to detect the daughter ions.

Whichever system is used, there will be several difficulties to overcome. Ion suppression is a severe form of interference in any mass spectrometer, as the system relies on the analyte being present as an ion. Any conditions present in the MS source that reduce the ionisation efficiency of an analyte will raise the detection threshold, and can make detection of analytes much harder. Some degree of sample preparation is usually needed to reduce these effects, which are often caused by unwanted matrix components, such as salts. Ideally this will involve an extraction step, but this can increase

the cost and operator time significantly. A simpler alternative is to dilute the sample 1:20 in water, which reduces the ion suppression and increases the signal to noise ratio. However, this sort of dilution necessitates a more sensitive instrument, and can make some low concentration analytes, such as the primary heroin metabolite, 6-monoacetylmorphine, very difficult to detect.

Additionally, many drugs undergo conjugation reactions in the body to increase water solubility and allow excretion. The conjugates, mainly glucuronic acid, will generally be the only fragment lost in an SRM, so that there is only a single SRM to monitor. In GCMS systems, this is often solved by performing a hydrolysis step to remove the conjugate and release the parent drug. Unless sample preparation time is increased, analysts must detect the unconjugated drug which is often present at low concentrations, for example unconjugated morphine only accounts for about 9% of a dose, whereas morphine glucuronide accounts for over 85% of the dose. This further increases the required sensitivity of a triple quadrupole system.

The aim of this project is to use an HPLC system coupled to an ion trap mass spectrometer system to confirm a range of drugs of abuse in urine samples. On-line solid phase extraction (SPE) and the use of the MS3 capability of an ion trap should enable minimal sample pre-treatment and give a high degree of confidence in the results obtained. This method will be used for routine clinical drugs of abuse screening for a population consisting mostly of drug users on an NHS drug treatment program.

A medline search performed in March 2009 showed no publications using ion trap LCMS systems for drugs of abuse screening, and no publications using on-line SPE for the LCMS analysis of drugs of abuse. There are two methods published for screening of unknown drugs that use an ion trap MS. One is an application note from Thermo Fisher Scientific, but this does not use MS3 spectra and requires manual extraction prior to analysis. The other is a paper using the Applied Biosystems Q-Trap for screening, but this system is not capable of MS3 analyses and also uses a prior extraction step. This research will be a new use of both ion trap LCMS and in-line SPE for drugs of abuse, and will have significant benefits to both the Toxicology Unit and to the addiction division of South London and Maudsley NHS Foundation Trust