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CAN MULTIPLE INSTRUMENTAL ANALYSES BE REPLACED BY A SINGLE ANALYSIS?

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Introduction

Mass spectrometry in combination with gas or liquid chromatography (GCMS or LCMS) is the analytical technique of choice for drug testing in sport. It provides the benefit of both high sensitivity and high selectivity which is essential in today's anti doping laboratories. It is generally true to say however that the breadth of coverage of individual methods is limited by the way in which the techniques are employed.. There is also very little scope for the detection of new or designer compounds. The analyst will normally only find what he is looking for. Current applications of LCMS are based predominantly on the utilisation of tandem mass spectrometry (MS/MS) where methodologies are constructed to detect a limited range of compounds. In such methods, the mass spectrometric parameters are generally optimised for each compound and there is a finite number of compounds that can be covered using this approach. With GCMS, the situation is similar. Using selected ion monitoring (SIM) as the acquisition mode, a limited number of compounds can be covered in one analysis. When operated in full scan mode, GCMS requires considerable prior knowledge of the fragmentation of the compounds covered. This is usually in the form of mass spectral libraries to aid in the identification process.

We have performed a preliminary evaluation of high resolution accurate mass LCMS to provide a screen with improved selectivity and analyte coverage with the capability of detecting an unlimited number of analytes injected at low picogram levels.

Experimental

- Thermo LTQ Orbitrap coupled to a Surveyor MS pump and autosampler

- 0.1% formic acid/acetonitrile gradient on a Hypersil Gold aQ 2.1mm x 50mm column
- Electrospray source with the orbitrap collecting positive ion full scan data at 30,000 resolution and the LTQ collecting full scan and dynamic exclusion data dependant MS/MS data in negative ion mode
- A mixture of drugs was prepared containing 70 compounds representing a wide range of drug classes and chemistries including stimulants, sedatives, NSAIDs, narcotic analgesics, β agonists, corticosteroids and diuretics.

The initial instrument performance was assessed using 5 ng injections of drug mixture. Subsequent experiments were performed using extracted blank equine plasma spiked with different levels of the drug mix. The blank equine plasma extracts were prepared using liquid/liquid extraction at pH 9.6 into a mixture of dichloromethane: hexane:ethyl acetate.

Results

Sensitivity and selectivity of accurate mass

The use of high resolution accurate mass detection dramatically improves the signal to noise for all the > 90% of analytes ionised in positive mode. In the example in figure 1, data acquired for a 50 pg injection of sildenafil on the orbitrap is displayed as an ion chromatogram for the accurate mass of protonated sildenafil - m/z 475.2128 - with a mass width of 1 amu and 0.002 amu. The 1 amu mass window mimics the data that would be seen on a conventional low resolution system operating in full scan mode.

The markedly improved selectivity provided by the use of accurate mass ion chromatograms is clearly evident in the 0.002 amu mass range ion chromatogram. The sensitivity of this approach is also demonstrated as only 50 pg of sildenafil was injected in the spiked blank extracted matrix.

The effect seen in figure 1 is mirrored by all of the other compounds detected in +ve ion mode. As demonstrated in figures 2 and 3, when the first 32 compounds detected in +ve ion mode are displayed with high resolution and low resolution ion chromatograms, the only peaks seen in the 0.002 amu high resolution windows are the individual drug peaks. The drug peaks are not easily identifiable in many of the 1 amu low resolution ion chromatograms. The level of drug injected in the single sample analysed was 50 pg for all analytes.

Negative ion compounds

The orbitrap, due to the stability required in the power supplies, does not currently support polarity switching in the analytical run, hence can only operate in one polarity. The linear

ion trap (LTQ) is therefore utilised to detect those compounds such as the thiazide diuretics that are only ionised in negative ion mode.

Figure 4. shows data acquired, in effect, simultaneously by the orbitrap in +ve mode and the LTQ in -ve mode. The orbitrap is operating in full scan mode whilst the LTQ is using dynamic exclusion data dependant scanning . The top two traces represent data from the LTQ. The top trace is an extracted ion chromatogram for m/z 296 for hydrochlorthiazide whilst the second trace is an extracted ion chromatogram for the product ion m/z 269 generated by the data dependant scan function. The bottom two traces show the detection of two compounds, ranitidine and etamiphylline simultaneously by the orbitrap.

Conclusion

The use of high resolution accurate mass instrumentation operating in full scan mode offers a highly sensitive very broad coverage of compounds in potentially a single analysis. Drug coverage will be dependant to an extent on the extraction procedure used and the ability of the ion source to ionise the compounds although the use of the LTQ in this study to detect the -ve ion compounds aids in the single analysis coverage. The use of full scan acquisition means that no data is lost. This in turn allows the detection of novel/designer drugs and also the possible retrospective review of data rather than a total re-analysis of the sample.

The cost of such instrumentation may at first appear prohibitive but if you consider that in a typical anti doping analysis, there may be up to 7 or 8 instrumental analyses then the concept of being able perform all the work in one or two analyses of 5 minutes would easily justify the cost of the equipment.

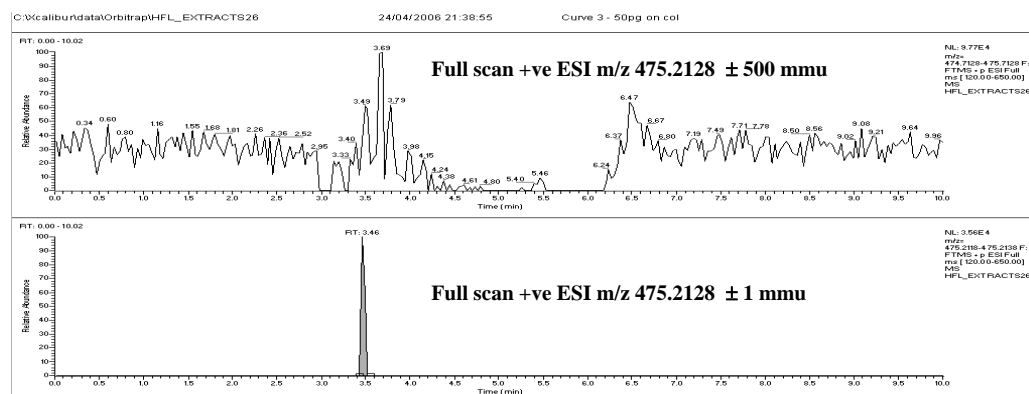


Figure 1: Data acquired from a 50 picogram injection of sildenafil on the Orbitrap.

Data is displayed as two separate ion chromatograms for the accurate mass of protonated sildenafil (m/z 475.2128), with mass widths of 1000 mmu and 2 mmu respectively.

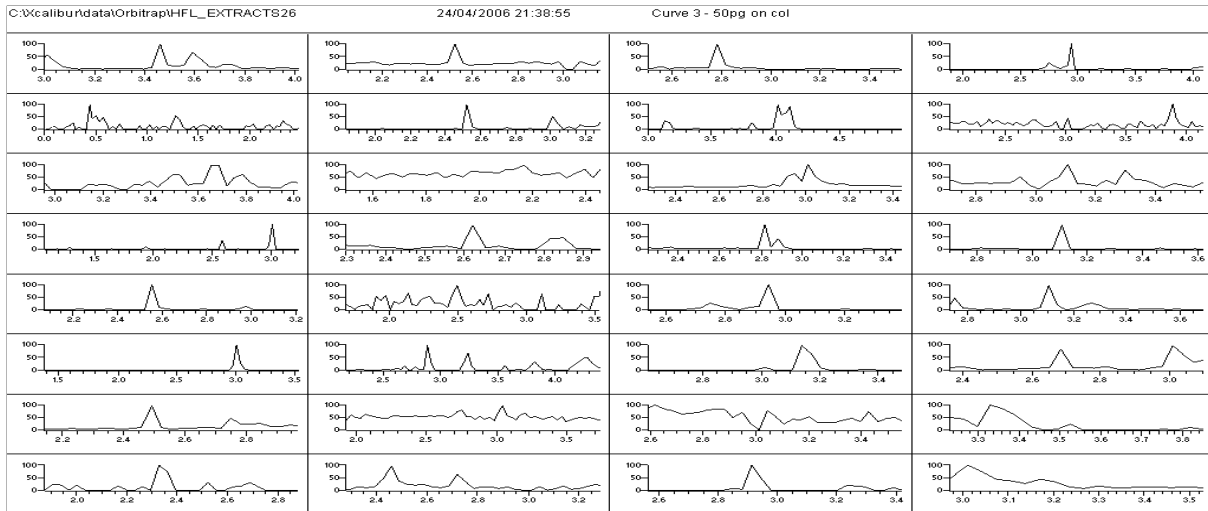


Figure 2: Data from an injection of a single blank extract spiked with 32 compounds, all detected in +ve ion mode. Data is displayed using 1000 mmu resolution.

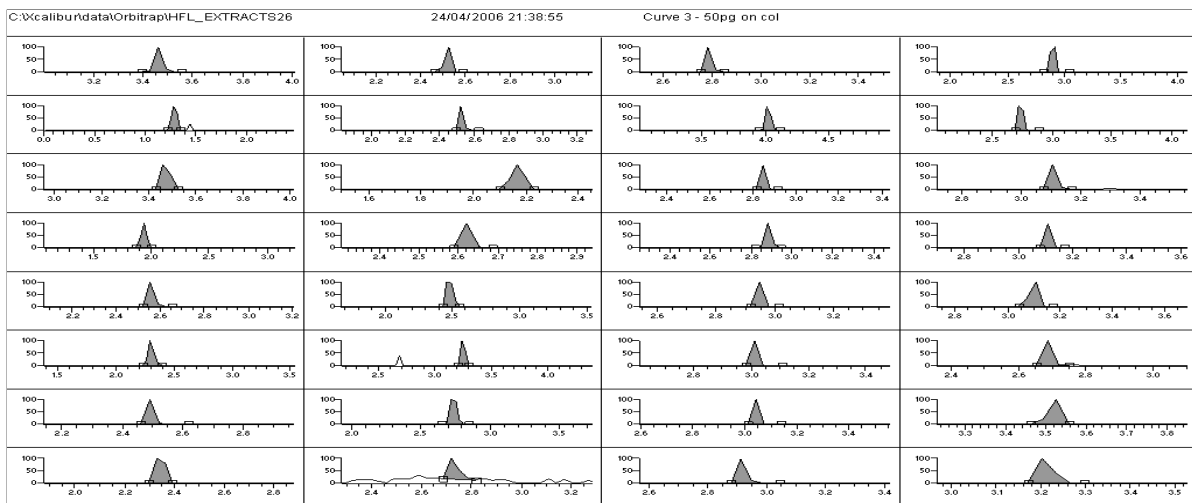


Figure 3: Data from an injection of a single blank extract spiked with 32 compounds, all detected in +ve ion mode. Data is displayed using 2 mmu resolution.

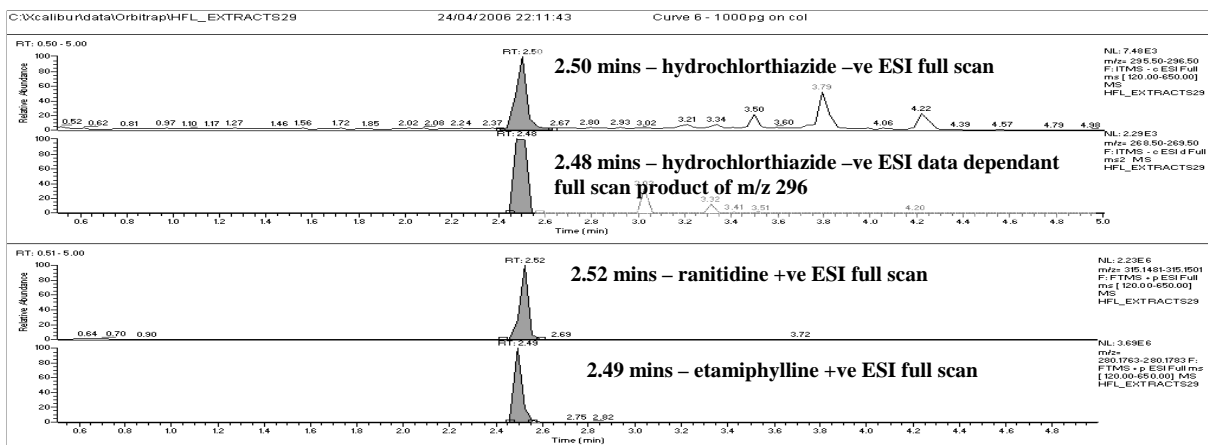


Figure 4: Data acquired simultaneously by the Orbitrap in positive mode and the LTQ in negative mode. The Orbitrap is operating in full scan mode whilst the LTQ is using dynamic exclusion data dependant scanning. 1000 picograms of each substance injected.