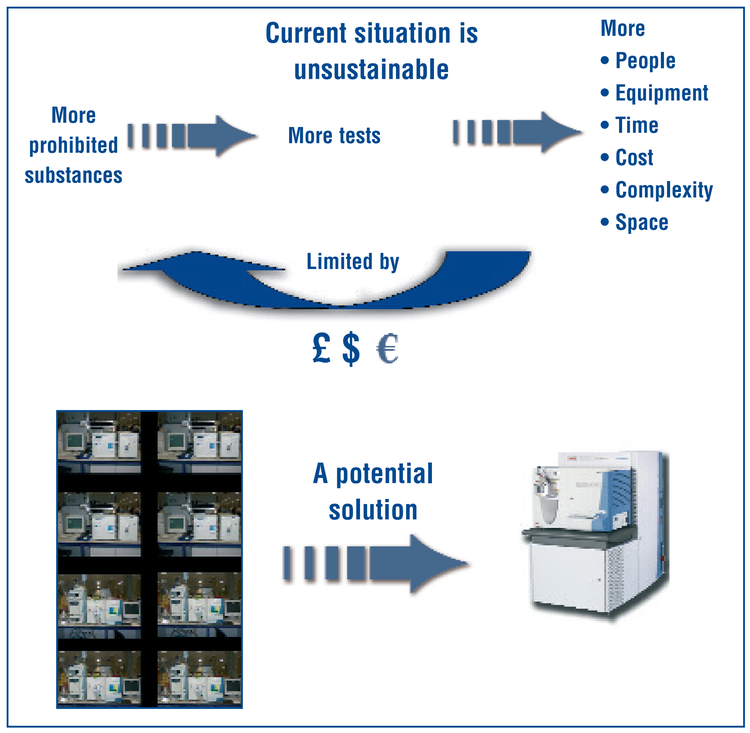
Can multiple instrumental analyses be replaced by a single analysis?

Rapid and sensitive screening for known and unknown prohibited substances using high resolution selectivity on a hybrid linear and orbitrap mass spectrometer

Simon Hudson[1], Steve Maynard[1] and Mark Harrison[2]

[1] HFL
Newmarket Road
Fordham
Cambridgeshire
CB7 5WW
UK

[2] ThermoElectron
1 Boundary Park
Boundary Way
Hemel Hempstead
HP2 7GE
UK



What we need:

- **fewer tests**
- **simpler data processing**
- **the ability to detect designer compounds**

Experimental

- Thermo LTQ Orbitrap coupled to a Surveyor MS pump and autosampler.
- Chromatography was performed using 0.1% formic acid/acetonitrile gradient on a Hypersil Gold aQ 2.1mm x 50 mm column. Gradient 100% aqueous to 100% organic in 5 minutes.
- Electrospray source with Orbitrap collecting positive ion full scan data at 30,000 resolution and the LTQ collecting full scan and dynamic exclusion data dependant MS^2 data in negative ion mode.
- A mixture of 70 drugs prepared representing a wide range of drug classes and chemical properties.
- Initial instrument performance assessed through injection of 5 ng of drug.
- Subsequent experiments performed on blank equine plasma extracts spiked with different levels of drug mix.
- Tetrahydrogestrinone spiked blank extract analysed to evaluate detection of 'new' compounds.

Results

Accurate mass – selectivity and sensitivity

Most of the substances selected for the drug mixture ionise in positive mode. The use of high resolution accurate mass detection dramatically improves the signal to noise (Figure 1).

The 1,000 mmu (1 amu) mass window mimics the data that would be presented by a conventional low resolution system operating in full scan mode.

The high selectivity conferred by the application of the 2 mmu mass width is shown by the much clearer signal at a retention time of 3.46 minutes. As only 50 picograms was injected this also demonstrates the sensitivity of this approach.

The effect demonstrated in Figure 1 is reproduced for all of the other compounds detected in +ve ion mode (Figure 2). The data from the 2 mmu high resolution chromatographic windows show only signals from the individual drug peaks. There is no significant 'noise' to complicate the interpretation.

The data from the 1,000 mmu low resolution chromatographic windows are more complicated. Overall there is an increase in the background noise and for many substances there is no signal that can clearly be ascribed to the substance concerned.

Detection of negative ion compounds

The Orbitrap does not currently support polarity switching in a single analytical run. This study used the Orbitrap in positive ion mode only. This current limitation can be overcome by using the linear ion trap (LTQ) to detect compounds that are ionised in negative ion mode only (Figure 3). Thiazide diuretics were chosen to demonstrate this.

Novel/designer drugs

Assuming an 'unknown' substance is extracted from the sample matrix and ionises in positive mode, full scan data acquired by the Orbitrap will contain mass spectral information about this substance. The challenge is to locate such substances in the data collected. This can be achieved by the comparison of data from multiple samples to identify any signal that is unique to an individual sample.

The SIEVE bioinformatic software from Thermo was used to compare data files and to highlight any differences between samples (output shown in Figure 4). Accurate mass information from any unique peak identified can be used to propose an elemental composition.

The step from elemental composition to structural identification is not straightforward. Further investigations using accurate mass MS^n on the Orbitrap may provide additional structural information leading to the identification of the unknown substance.

Conclusion

A high resolution accurate mass instrument operating in full scan mode offers a sensitive screening test with a broad coverage of substances in a single test. The range of substances detected depends on the extraction procedure and the ionisation characteristics of individual substances. In this study some ionisation limitations were overcome by using the LTQ to detect substances that ionise in negative mode.

The aim of detecting 'unknown' substances may be achieved by the application of this type of instrument aided by bioinformatic software developments.

The cost of these instruments is high but the capital investment could be justified by a significant reduction in the total number of screening tests employed and by a high sample throughput.

Cycle times around five minutes were achieved for this study, indicating that the throughput goal can be met.

Initial results also suggest that such an approach could meet the challenge to screen for an increasingly wide range of prohibited substances at low concentrations using significantly fewer tests. The total capital investment may therefore be reduced by the requirement to deploy significantly fewer individual instruments.

FIGURE 1: Data acquired from a 50 picogram injection of sildenafil on the Orbitrap.
Data is displayed as 2 separate ion chromatograms for the accurate mass of protonated sildenafil (m/z 475.2128), with mass widths of 1000 mmu and 2 mmu respectively.

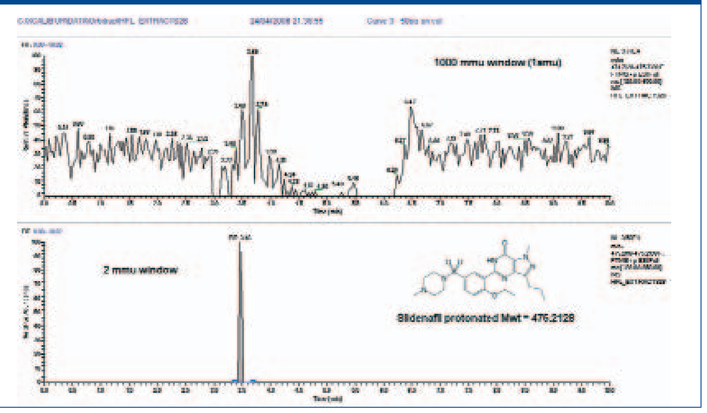


FIGURE 2: Data from an injection of a single blank extract spiked with 32 compounds, all detected in +ve ion mode.
Data is displayed using 1000 mmu resolution (blue trace) and 2 mmu resolution (red trace). 50 picograms of each substance was injected.

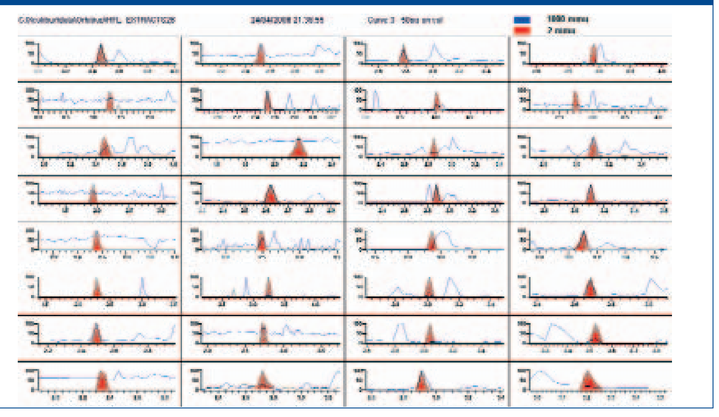


FIGURE 3: Data acquired simultaneously by the Orbitrap in positive mode and the LTQ in negative mode.
The Orbitrap is operating in full scan mode whilst the LTQ is using dynamic exclusion data dependant scanning.
1000 picograms of each substance injected.

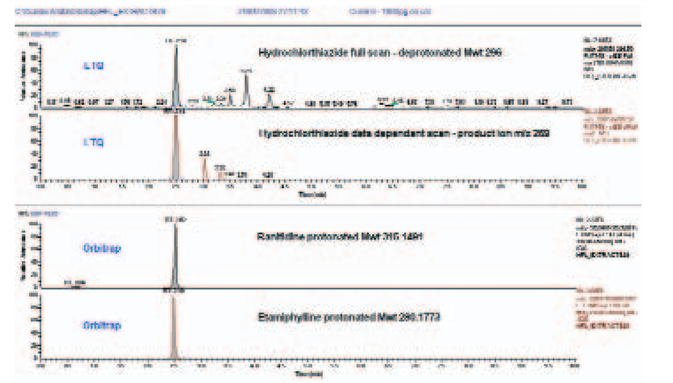
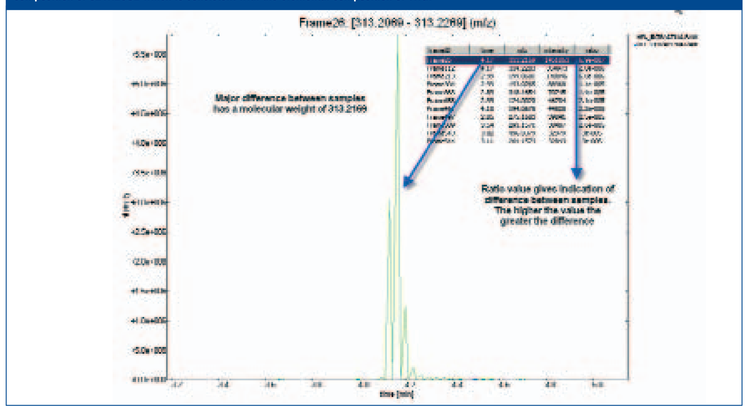


FIGURE 4: Output from automated comparison of a THG spiked extract and a blank sample showing the major difference between the two data files as a peak in the THG spiked extract with the accurate mass of 313.2169.
A proton is removed from the elemental composition calculation to reflect proton addition during ionisation, giving a proposed elemental composition of C21H28O2 which is consistent with the elemental composition of THG.



Ask anyone … HFL