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The application of UHPLC and Ultrafast-LCMSMS to the analysis of small volume biological samples for drug residues

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Introduction:

The analysis of specimens such as blood spots, hair and saliva for the presence of drug residues is limited by the sample size and the need to perform both screening and confirmatory analyses. Sample preparation techniques for such samples benefit from micro-scaled approaches that minimise the exposure of the sample to diluents and possible contaminants, poor recovery and the increased processing time associated with scale. These benefits are also highly desirable for high-throughput analysis of urine for measuring clinical and illicit drug residues and for metabolomic profiling where they simplify sample processing and for reducing transport and storage costs.

We couple Noviplex cards for the isolation of small molecules from hydrolysed urine, with UHPLC and Ultrafast-LCMSMS (UFMS) to detect low level drug residues and metabolites in small volume biological specimens with subsequent identification of unknowns using MS scan events..

Figure 1: A combination of Nexera LC-30 with LCMS-8050 Ultrafast Triple Quadrupole Mass Spectrometer enables high sensitivity multiple analyte drug analysis on limited volume specimens. The scanning and switching speed supports UHPLC and high throughput. Analysis.

Methods

Enzyme hydrolysed urine specimens (10 µl + 90 µl water) were applied directly to the Noviplex card as proscribed for blood samples. The sample disc was exposed and dried for 30 minutes. Discs were 'eluted' with 100 µL of mobile phase. The eluted samples were compared with a 20-fold more concentrated specimen of hydrolysed urine extracted through a conventional extraction scheme.

Injection (1 µL) was onto a Nexera-LCMS-8050 (Shimadzu Corporation) in ESI mode with a Shimpak ODS-III column Shim-pack XR-ODS III (2.1 x 50 mm, 1.6 µm)operated at 40°C and a binary gradient of water (A) and acetonitrile (B); both containing 0.1% formic acid as the modifier. Solvent B was held at 5% from 0-0.5 min then ramped to 95% from 0.5-4.5 min and held at 95% from 4.5-7.0min. The flowrate was 400 µL/min. The interface was 300°C and the heater block 400°C. Drying, heating and nebulising gases were 10, 10 and 2 L/min respectively. Scanning was 98-450 Da at 15,000 Da/sec and triggered PIS was continuous at 30,000 amu/sec. All MRM events were 1 msec pause and 1 msec dwell time and polarity switching was used in each loop at 5 msec.

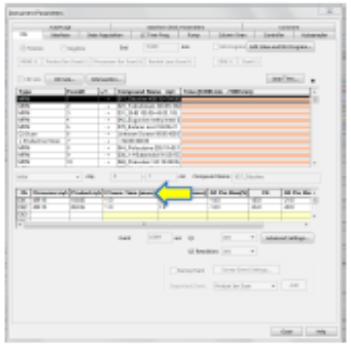
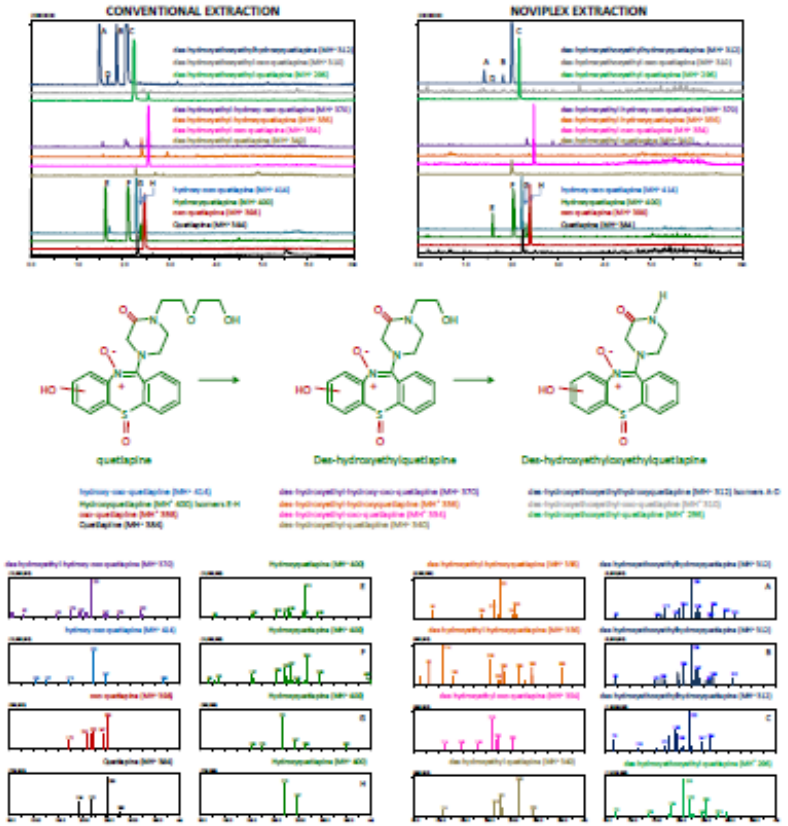


Figure 2: The seven minute method was implemented without scheduled events to simulate a constant heavy duty cycle with 1 msec dwell and 1 msec pause times for 530 +MRM events and 20 – MRM events, and simultaneous Q3 + and – scan with triggered product ion scanning. In practice, the MRM list is scheduled and triggering is either from MRM or from scan. Even at this data rate, all channels were well defined and 3 PIS per trigger were collected.

Figure 3: The analysis of quetiapine and its metabolites in human urine was used to test a method that allowed for broad-spectrum drug screening using MRM channels for known compounds (polarity switching caters for positive and negative ion analytes) and Q3 s canning with triggered product ion spectra for profiling and investigating unknown compounds. In the presence of over 550 simultaneous MRM channels, polarity switching, simultaneous Q3 positive (shown top left and right for quetiapine and metabolites)and Q3-negative ion scans and triggered events for both (shown below are triggered PIS for quetiapine metabolites acquired at 30,000 Da/sec). The MRM channel for quetiapine provided the trigger for further investigation of the sample for putative metabolites.



Discussion and Conclusion:

UHPLC based methods for multiple analytes are difficult to adapt on slow scanning instruments without loss of peak definition, analyte drop-outs and loss of sensitivity. We have screened urine extracts from a routine compliance laboratory using a MRM based screen for >500 analytes and further investigated irregularities using full-scan data that was collected simultaneously. The UFMS events were enabled quadrupole mass filtering (30000 Da/sec at 0.1 Da mass definition (10 points per 1 Da rather than interpolated masses), Ultrafast polarity switching (5 msec), negligible mass axis displacement or loss of sensitivity for constant dwell time and negligible cross-talk.

Our identification of quetiapine metabolites is predictive of N- or S-oxidation, hydroxylation, pyrimidyl ring oxidation prior to cleavage and side chain cleavage. Other pathways have been investigated but are not reported here and positions of hydroxylation are not investigated.

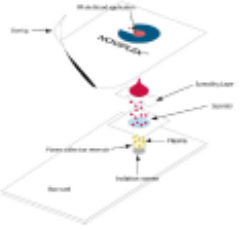


Figure 4: Noviplex is designed for isolating a standard aliquot of plasma from blood using a wicking reservoir and selective membrane. The reservior sorbent also offers some selectivity. We are interested in the technique for its ability to manage other biological matrices including urine.

Noviplex cards are designed for the isolation of plasma from blood samples. We were interested in the general utility of the device for the isolation of a range of small organic molecules of varying polarity and tested the device with a mixed drug panel. The successful isolation of analytes is illustrated by quetiapine and metabolites but was also observed for a range of molecules from methamphetamine and metabolites to benzodiazepines, opiates and methadone. The Noviplex isolation disc provided a degree of sample clean-up and we speculate that highly polar molecules from the urine were selectively retained on the sample disc or were not passed through the separation membrane.

Conclusion:

We have used successfully overlaid a Q3-scan with polarity switching and continuous triggered scanning over a comprehensive MRM based screening method for drug residues. Included in the MRM channels were both licit and illicit substances. In this example, a MRM response for the drug quetiapine allowed a comprehensive investigation of metabolism to be completed on the sample. The experiment is a demonstration of the ability to both screen a known target list with MRM and to profile a sample with non-target screening. The method is particularly powerful as it combines high sensitivity LCMS to allow a reduction in dwell time and UFMS to allow the method to be UHPLC compatible. We have attempted similar methods on non-UFMS capable LCMS platforms and found them unable to manage PIS or polarity switching in either UHPLC or conventional HPLC modes.

Noviplex cards provide an alternative sample preparation mechanism that offers a degree of clean up greater than dilute and shoot methodologies and also a potential method for transporting samples prior to analysis.