On-Line SPE LC-MS/MS Drug Screening in Serum on a Hybrid Quadrupole/ Linear Ion Trap Instrument

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INTRODUCTION

Comprehensive screening for the detection of drugs and toxic compounds in biological samples is an important function of toxicological analysis. As the demand to monitor the ever increasing number of drugs continues to rise, so too does the need to detect and quantify these compounds in a simple, single and automated run; providing a fast turn- around time for the results. This presentation describes the rapid cleanup of serum samples and detection of over 100 drugs using On-line SPE LC-MS/MS screening and confirmation. The LC-MS/MS was operated in Multiple Reaction Mode (MRM) for detection. Dependent MS/MS spectra were acquired in the Enhanced Product Ion (EPI) mode after being triggered from a *Scheduled* MRM™ Pro Algorithm Information Dependent Acquisition (IDA) survey scan. Combining MRM and product ion spectral acquisition allows for compound identification with highest confidence based on mass spectral library matching. The automated workflow monitors large panels of analytes; detecting and quantifying these compounds in a single run.

MATERIALS AND METHODS

Sample Preparation:

130 individual drug standards were obtained from Cerilliant and blank serum was spiked with drug mixture stock solutions making concentrations ranging from 0.5 to 2000 ng/mL to prepare the calibrators; 10, 1000 ng/mL for QCs.

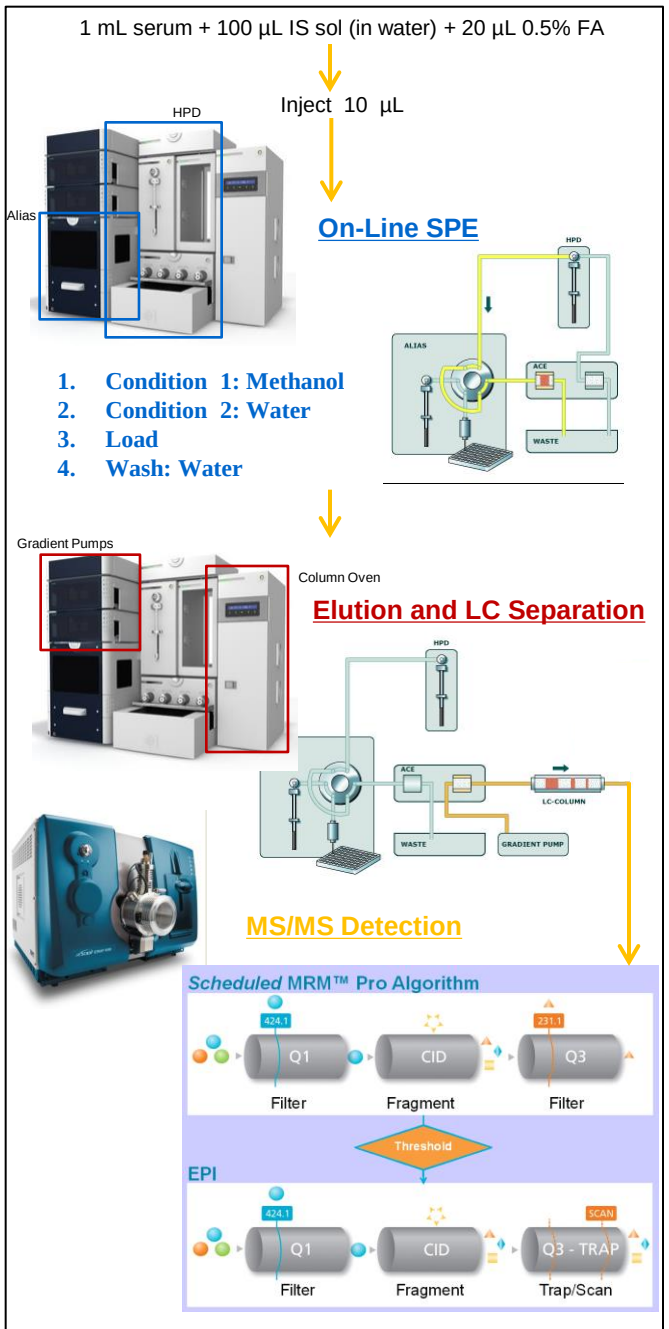

Figure 1. Performing On-Line SPE LC-MS/MS using the Spark Holland Pico and AB SCIEX QTRAP® 4500 LC/MS/MS System

Sample Clean up and LC separation was performed using a Spark Holland Pico System.

HPLC Conditions:

- Phenomenex Kinetex column (C18, 3 x 50 mm, 2.6 µm, 100 Å); 30° C
- Mobile phase A: Water + 10 mM ammonium formate
- Mobile Phase B: Acetonitrile + methanol (1:1)
- Flow rate: 400 µL/min flow rate.

MS/MS Conditions:

An AB SCIEX QTRAP® 4500 LC/MS/MS System operating in *Scheduled* MRM™ Pro Algorithm mode was used for detection; in positive TurboIonSpray® probe mode.

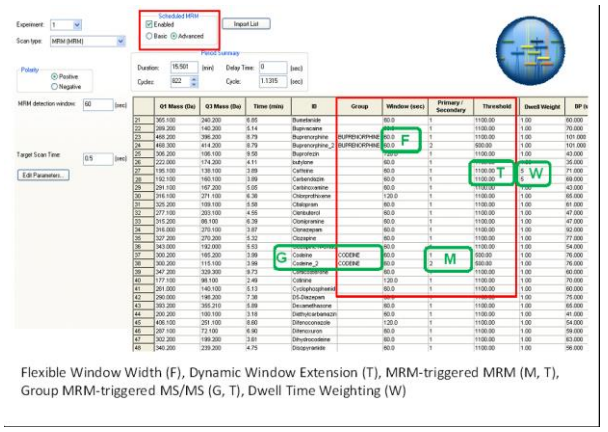

Figure 2. Analyst® Software version 1.6.2 with *Scheduled* MRM™ Pro Algorithm

EPI spectra at a scan speed of 10000 Da/s were triggered from the MRM survey scan and acquired using a dynamic fill time and dynamic background subtraction for optimal MS/MS quality. EPI spectra were generated using standardized Collision Energy (CE) of 35 V with Collision Energy Spread (CES) of 15 V to ensure a characteristic MS/MS pattern independently on the compound's fragmentation efficiency. MS/MS spectra were searched against the iMethod™ application Meta Library.

RESULTS AND DISCUSSION

Despite the high selectivity that MRM provides, there is always a risk of false positive findings due to endogenous compounds that have the same mass. Typically a second MRM is monitored per analyte and the ratio of quantifier to qualifier transition is calculated for each unknown sample and compared to the MRM ratio of standards for identification. However, targeted compounds with low fragmentation efficiencies (i.e. Low Intensity Ions) have been reported to produce false positive results for compound identification (1-3)

For improved accuracy, compound identification was performed using full scan MS/MS experiments in Enhanced Product Ion (EPI) mode with automated library searching capabilities to compare the unknown with a standard spectrum. The dependent MS/MS spectra were acquired using the EPI mode of the Q TRAP® system after being triggered from a *Scheduled* MRM™ Pro Algorithm IDA survey scan and searched against mass spectral libraries for compound identification. The information of the complete molecular fingerprint saved into EPI spectra significantly reduces the risk of false positive and negative results (4-7).

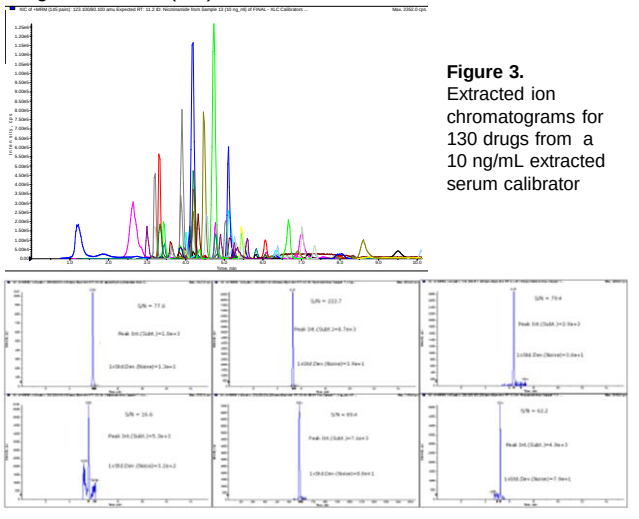

Figure 3. Extracted ion chromatograms for 130 drugs from a 10 ng/mL extracted serum calibrator

Figure 4. Chromatogram of select analytes in spiked serum at 1 ng/mL. The signal to noise ratio was calculated by dividing the average background signal intensity from the peak by a 1 times the standard deviation of the noise region.

Figure 3 shows representative *Scheduled* MRM™ chromatograms for over 100 different drugs. Figure 4 shows the signal-to-noise levels for selected compounds obtained from 1 ng/mL spiked serum. Extracted spectra and library search Purity Score values using an MS/MS library search algorithm are shown in Figure 7 for extracted serum samples with low analyte concentrations. Quantitative analysis was performed in the same run allowing for both quantification and qualitative data to be collected simultaneously. Figure 5 shows example calibration curves (0.5-1000 ng/mL) created from the same run for compounds identified from the library matching.

Regression analysis for the analyzed samples within this method resulted in R^2 values of 0.99 or greater. Typical recoveries were greater than 80% (Table 1). Matrix effects were evaluated at 10 ng/mL concentrations, using one lot of serum, and % accuracy differences were typically less than 20%. Figure 6 shows some specific examples with % CV and % Accuracy values across the calibration curve concentrations.

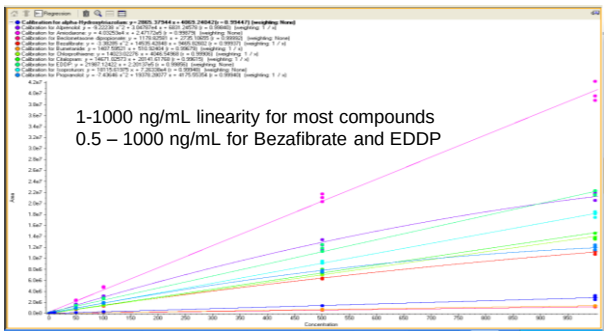

Figure 5. Representative calibration curves for spiked serum calibrators

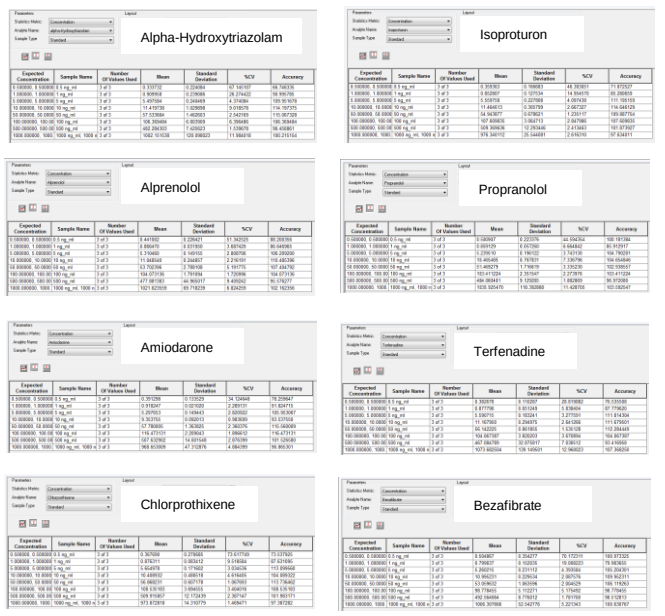

Figure 6. % CV and % Accuracy values obtained for selected drugs, across the calibration curve concentrations.

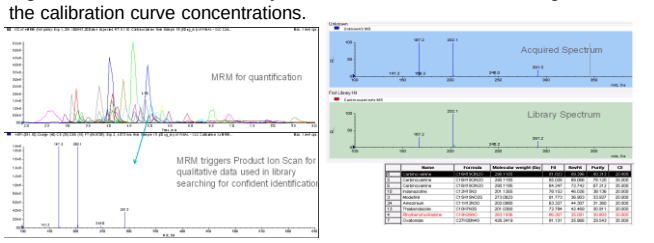

Figure 7. Example of extracted spectra and library search purity score values using an MS/MS library search algorithm

Analyte	%CV at 1ng/mL LOQ (n=3)	% Accuracy	Matrix Effect (% accuracy difference)	Recovery (%)
Acetominophen	0.9	102	15	98
Beclometasone	8.5	109	6	61
Bezafibrate	20	80	23	55
Clonazepam	20	112	4	112
Nalorphine	9	87	1	81
Norfentanyl	5	99	3	14
Oxycodone	3	97	11	86
Propanolol	6	90	49	61
Sotalol	2	103	15	97
Terfenadine	5	87	16	76

Table 1. Typical Recoveries and matrix contribution values as represented by these selected examples

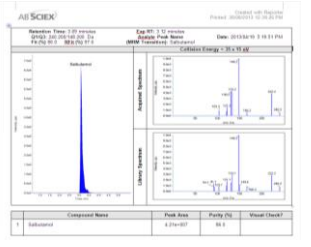

Figure 8. The Analyst® Reporter software was used to generate a report after automated library search. In this report, the integrated MRM signal of Salbutamol was extracted and the comparison of the acquired EPI spectrum and the spectrum from the library are shown.

CONCLUSIONS

Analysis for large panel of drugs in serum with automated sample preparation, detection and confident identification in a single injection was achieved

References

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