Sensitive and Robust Screening of Hundreds of PPCP Compounds Using Online SPE-LC-MS/MS

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Pharmaceutical waste products have become emerging pollutants as a result of growing drug consumption, excretion and incorrect waste disposal. Human and veterinary pharmaceutical compounds that are not broken down and degraded can persist in soil and water, thereby exposing humans to potential hazards and ruining natural ecosystems.

The detection and identification of such substance residues – for example from painkillers, antibiotics or antidepressants - in water, soil and biota requires large-scale monitoring, and the method development can be challenging [1]. The analysis needs to be able to accommodate a wide range of molecular weights, polarities and solubilities, along with very low concentrations of known and unknown analytes in complex matrices.

The method of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is well suited for environmental analysis. By combining the selectivity of LC with the sensitivity of MS, this approach has the potential to detect, identify and quantify all compounds in a sample – even unknown analytes and those compounds that are present at very low concentrations [2].

However, interference remains a common complexity in environmental samples due to the wide variety of compounds typically present; moreover, co-elution of matrix substances can have negative effects on the ionisation [2]. To tackle this, additional separation techniques can be performed prior to MS introduction. Sample preparation methods can also be applied to clean up the sample and minimise co-elution of different molecules, though this has the disadvantage of adding to the overall cycle time.

Although sample preparation can significantly reduce matrix interferences, its main purpose in environmental analysis is to pre-concentrate the analytes of interest in order to enhance sensitivity. A suitable method to separate the analytes from interfering compounds is solid phase extraction (SPE). An offline SPE typically consists of several manual preparation steps such as conditioning of the SPE cartridge, injection of the liquid sample onto the cartridge, removal of the matrix in a washing step and finally the elution of the analytes from the cartridge. Online SPE provides clean-up and pre-concentration of the sample by loading it onto the online SPE cartridge where the target analytes then elute directly onto the analytical LC column. Innovative software algorithms can be applied that help to perform SPE in parallel and can thus reduce the overall cycle time for sample preparation and analysis.

Table 1

LC Gradient.

Time (min)	%A	%B
0	97	3
1.0	97	3
1.1	85	15
9.5	25	75
9.6	5	95
11.5	5	95
11.6	97	3
13.5	97	3

Here we present a new method for analysis of pharmaceuticals and personal care products (PPCP) using a customised online SPE-LC-MS/MS configuration for an easy and automated workflow that creates a walk-away platform for the user.

Method

Experimental sample preparation

Various sources of water were sampled from

reservoir, sea and tap and kept refrigerated until analysis. Each water sample was pre-filtered in 0.45 µm cartridge filter and 500 µl injected directly for LC-MS analysis.

Online SPE LC-MS Experimental Setup

The workflow was designed to perform automated SPE clean-up, HPLC separation and MS detection. Three interfaces made up the complete workflow automation: the Spark Holland SPE system, Shimadzu Prominence HPLC system and the SCIEX QTRAP® 4500 LC-MS/MS system.

Online SPE Procedure

The online SPE system proceeds automatically through a series of five automated clean-up steps using the automated cartridge exchange (ACE) module, which holds two trays of 96 extraction cartridges. The high-pressure dispenser (HPD) was programmed with SparkLink software to perform activation, conditioning, sample application and cartridge clean-up automatically.

SPE cartridges were activated using 2mL methanol, followed by conditioning with 2 mL water. 500 µL sample was injected and trapped at the cartridge sorbent, which was further washed with 2 mL water to remove matrix interferences. The trapped analytes were desorbed from the cartridge sorbent by diverting the fluidic path to the LC-MS, whereby the LC gradient desorbs the analytes to the HPLC column for LC separation.

Due to the diverse chemical characteristics of the panel of compounds, each sample was run through three different methods, taking 18 mins (including SPE) per method. Polar compounds were extracted and analysed using a Resin GP cartridge; mid- to non-polar compounds were extracted and analysed using a C18 cartridge, both in positive mode. Non-polar compounds were extracted and analysed using a C18 cartridge in negative mode. The extracted and concentrated analytes were eluted from the cartridge onto the analytical separation column.

LC Separation

High-resolution LC using an F5 core-shell column was used for separation. The autosampler was upgraded to 1000 µl loop volume, to perform higher injection volume of the water samples (500 µl) for improved sensitivity. Gradient separation (Table 1) was at 0.4 mL/min using a Phenomenex Kinetex column (F5, 50 x 3.0 mm, 2.6 µm) with water and acetonitrile in 0.1% formic acid.

MS/MS Detection

The Sciex QTRAP 4500 LC-MS/MS system with Turbo V™ source and Electrospray Ionisation (ESI) probe was operated in multiple reaction monitoring (MRM) mode using the Scheduled MRM™ algorithm, which monitored transitions automatically during a short retention time window.

Data Processing

Data were processed in MultiQuant™ software 3.0.2 using 1/x weighted linear regression (r2).

Results and Discussion

An automated, walk-away platform for screening over 100 PPCP compounds in reservoir, sea and tap water was developed using online SPE and validated. The vMethod™ application was shown to semi-quantitate more than 100 compounds with a limit of detection (LOD) at 1 ppt (one part per trillion, 1×10^{-12}) and lower limit of quantification (LLOQ) at 5 ppt for the majority of the compounds (Figure 1).

The large panel of analytes was prepared and extracted using a generic online SPE procedure. High-resolution LC was combined with the highly sensitive MS/MS; over 100 compounds were screened with two MRM transitions monitored in each compound. The method included Scheduled MRM for enhanced selectivity and sensitivity, along with optimised dwell times and cycle times to deliver the best sensitivity and reproducibility.

Online SPE in PPCP analysis reduces the

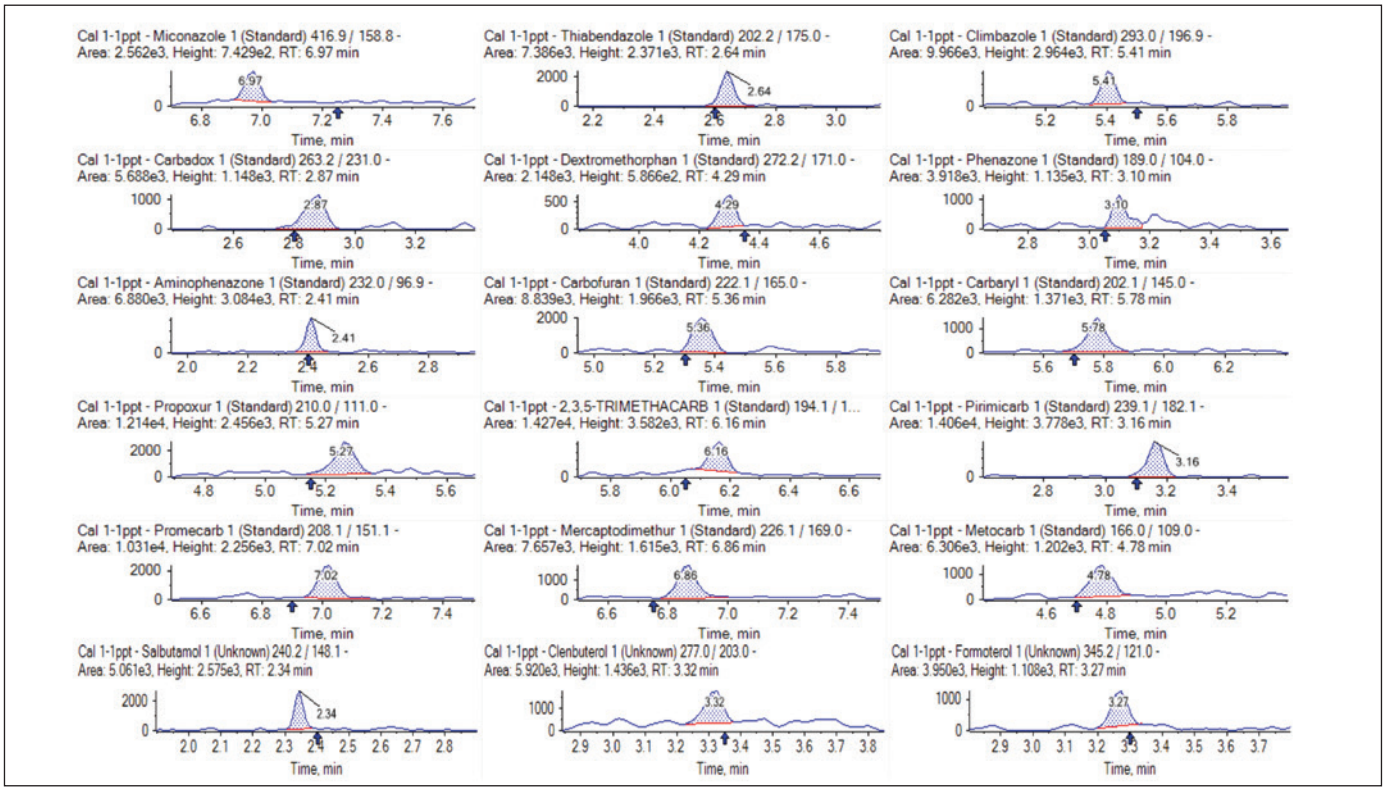

Figure 1

Detection limit of 18 compounds at 1 ppt.

overall cycle time for sample preparation and analysis, and provides an unattended workflow for the user to perform automated SPE clean-up. Traditional offline SPE requires multiple steps and larger solvent volume than online SPE techniques. Our method allows more than 100 PPCP compounds to be screened with a 13 minute separation. There is no evaporation in online SPE, as the cartridge is switched directly in line with the HPLC column after the wash step. The one-time use of disposable SPE cartridges effectively reduced matrix interferences for a wide variety of environmental samples, hence improving sensitivity.

The Scheduled MRM algorithm provides enough points across the chromatographic peak to give better peak detection and improved reproducibility. Using the knowledge of the elution time of the individual peptide, each MRM transition is monitored only during this short retention time window. This allows hundreds of MRM transitions to be monitored in a single LC run, while maintaining maximised dwell times and optimised cycle times.

The wide diversity of the PPCP compound classes and their chemical properties in our testing panel raised some significant challenges during the SPE method development and LC/MS analysis, including chemistry effects from the choice of SPE sorbent chemistry to the choice of elution solvent. In order to overcome these, three alternative screening workflow methods were developed (Figure 2). Two different cartridge chemistries, Resin GP and C18, were used to improve the recovery for different hydrophobicity compounds, with positive or negative ionisation modes accordingly.

In conclusion, we have developed a sensitive, reproducible and robust method for large-scale screening of diverse PPCP products in the environment. The use of online SPE enables 100 compounds to be extracted in 13 minutes and our LC-MS/MS method detects chemically diverse compounds at very low concentrations.

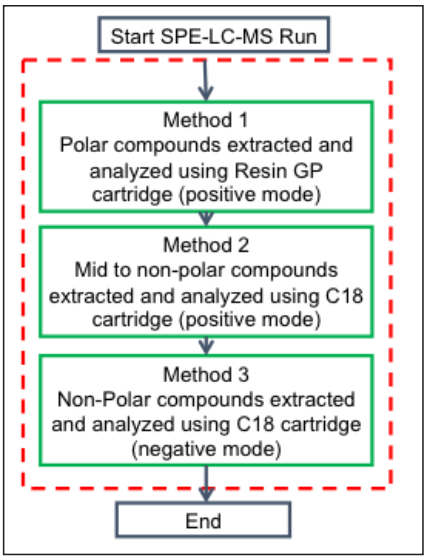

Figure 2

Online SPE-LC-MS workflow for positive and negative polarities

References

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