Screening of pharmaceutical and personal care products in water using online solid phase extraction

Seo Bee Hong[1], Peter Ringeling[2], Jeremy Netto[1]
[1]SCIEX, Singapore; [2]Spark Holland, The Netherlands

Growing urbanization and expanding use of drugs and chemicals has created more emerging environmental pollutants (Pharmaceutical and Personal Care Products/Endocrine Disruptor Chemicals) that find their way into surface and ground water supplies. Pharmaceutical drugs are eliminated from the body, flushing or disposal in landfills of unused or expired drugs. Personal care products are rinsed down the drain while showering or bathing which are common pathways to the environment. As to agricultural focused products or veterinary drugs, run-off offers another pathway to surface water or groundwater reserves.[1]

The number of these pollutants being detected continues to grow as advancements in analytical equipment and techniques have driven quantifiable reporting levels to lower concentrations.[1] In order to continue to achieve highly sensitive quantitation of these contaminants, robust and versatile solid phase extraction (SPE) techniques can be employed to concentrate and clean up a wide range of environmental samples.

Herein, PPCP analysis using online SPE-LC-MS/MS for an easy and automated workflow is described, creating a walk-away platform for the user. The optimized, unattended workflow will ease the re-development of online SPE procedures. The vMethod™ application for the screening of PPCP using Online SPE can semi-quantitate more than 100 compounds with LOD at 1ppt, LLOQ at 5ppt for majority of the compounds.

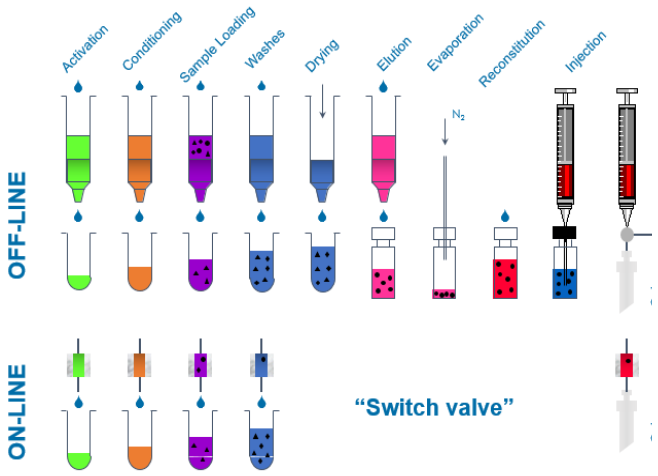

Figure 1. Comparison of off-line vs on-line SPE workflow period. Using the on-line approach, the evaporation step is removed. This allows for a unattended workflow for SPE cleanup.

Experimental

Sample preparation: Various sources of water were sampled from reservoir, sea and tap. They were kept refrigerated until analysis. Water samples were pre-filtered in 0.45µm cartridge filter and injected directly for LCMS analysis.

Online SPE LC-MS experimental setup: The entire setup was synchronized seamlessly to perform automated SPE cleanup, HPLC separation and MS detection. It was made up of three interfaces, namely Shimadzu Prominence HPLC system, Spark Holland Online SPE system and a SCIEX QTRAP® 4500 LC-MS/MS System.

Solid phase extraction: The Online SPE system was made up of a high pressure dispenser (HPD) to perform five automated SPE cleanup steps on 2x96 cartridges using Automatic Cartridge Exchanger (ACE). The HPD was programmed by SparkLink software to perform activation, conditioning, sample loading and washing of cartridges automatically.

Chromatography: The LC interface was upgraded to perform higher injection volume of (filtered) neat water samples for improved sensitivity. Gradient separation at 0.4mL/min using Phenomenex Kinetex column (F5, 50 x 3.0 mm,2.6 µm) with water and acetonitrile in 0.1% formic acid were used.

Mass spectrometry: SCIEX QTRAP® 4500 LC-MS/MS System with Turbo V™ Source and Electrospray Ionization (ESI) probe were operated in MRM mode using the Scheduled MRM™ Algorithm which monitored transitions automatically during a short retention time window. This feature maximized hundreds of transitions to be monitored in a single LC run, while maintaining sufficient dwell time and optimized cycle time.

Data Processing: Data was processed in MultiQuant™ Software 3.0.2 using 1/x weighted linear regression (r^2).

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

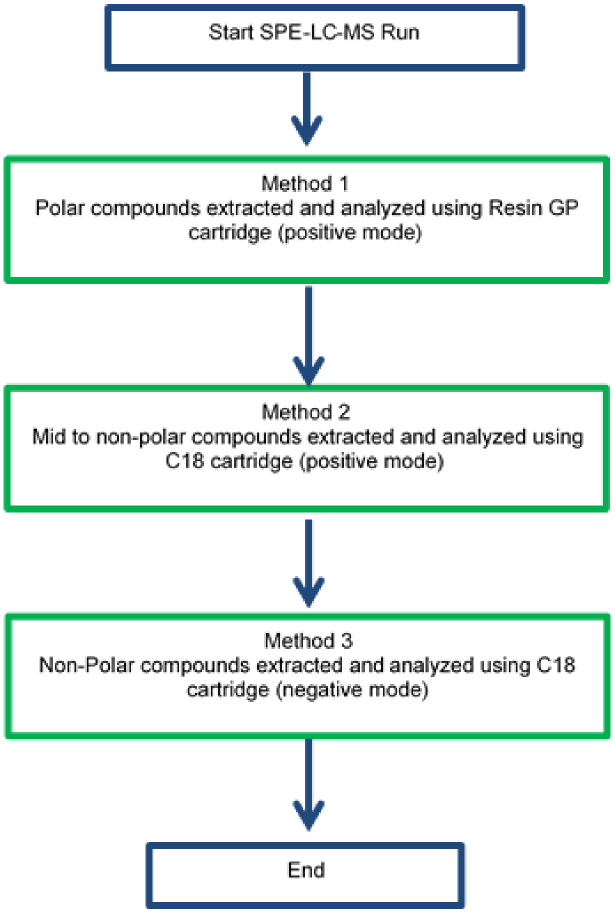

Figure 2. On-line SPE-LC-MS workflow for positive and negative polarities.

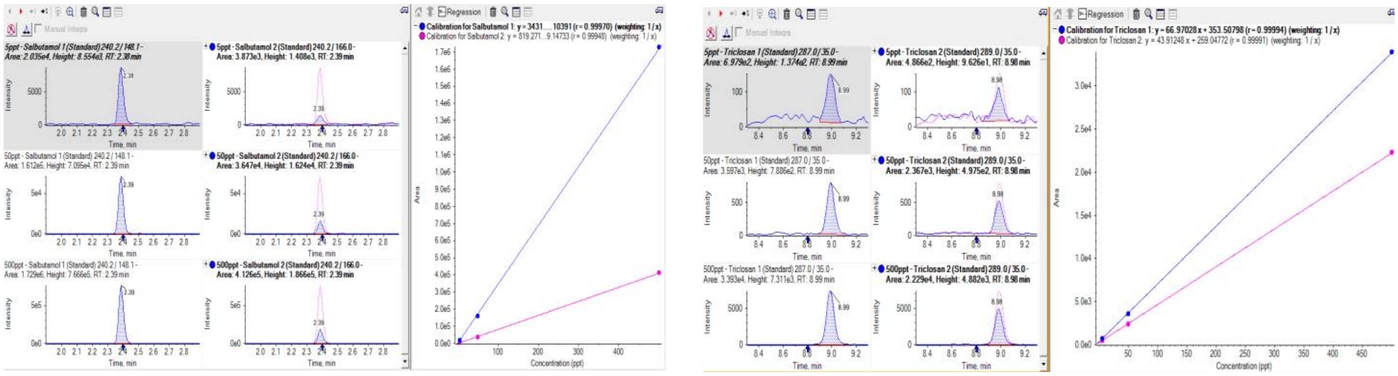
5ppt - Salbutamol 1 (Standard) 240.2 / 148.1 - Area: 2.035e4, Height: 8.554e3, RT: 2.38 min
5ppt - Salbutamol 2 (Standard) 240.2 / 166.0 - Area: 3.873e3, Height: 1.408e3, RT: 2.39 min
50ppt - Salbutamol 1 (Standard) 240.2 / 148.1 - Area: 1.612e5, Height: 7.095e4, RT: 2.39 min
50ppt - Salbutamol 2 (Standard) 240.2 / 166.0 - Area: 3.647e4, Height: 1.624e4, RT: 2.39 min
500ppt - Salbutamol 1 (Standard) 240.2 / 148.1 - Area: 1.729e6, Height: 7.666e5, RT: 2.39 min
500ppt - Salbutamol 2 (Standard) 240.2 / 166.0 - Area: 4.126e5, Height: 1.866e5, RT: 2.39 min
Calibration for Salbutamol 1: y = 3431 .... 10391 (r = 0.99970) (weighting: 1 / x)
Calibration for Salbutamol 2: y = 819.271...9.14733 (r = 0.99948) (weighting: 1 / x)
5ppt - Triclosan 1 (Standard) 287.0 / 35.0 - Area: 6.979e2, Height: 1.374e2, RT: 8.99 min
5ppt - Triclosan 2 (Standard) 289.0 / 35.0 - Area: 4.866e2, Height: 9.626e1, RT: 8.98 min
50ppt - Triclosan 1 (Standard) 287.0 / 35.0 - Area: 3.597e3, Height: 7.886e2, RT: 8.99 min
50ppt - Triclosan 2 (Standard) 289.0 / 35.0 - Area: 2.367e3, Height: 4.975e2, RT: 8.98 min
500ppt - Triclosan 1 (Standard) 287.0 / 35.0 - Area: 3.393e4, Height: 7.311e3, RT: 8.99 min
500ppt - Triclosan 2 (Standard) 289.0 / 35.0 - Area: 2.229e4, Height: 4.882e3, RT: 8.98 min
Calibration for Triclosan 1: y = 66.97028 x + 353.50798 (r = 0.99994) (weighting: 1 / x)
Calibration for Triclosan 2: y = 43.91248 x + 259.04772 (r = 0.99991) (weighting: 1 / x)

Figure 3. Good linearity observed. Linearity obtained using high injection volume with online SPE are highlighted in Salbutamol at positive polarity (left) and Triclosan at negative polarity (right).

Results

Online SPE in PPCP analysis provided unattended workflow for user to perform automated SPE cleanup. Traditional offline SPE required multiple SPE steps and a large solvent volume vs the online SPE technique. There was no evaporation in online SPE as the cartridge was switched directly in line with the HPLC column after the wash step (Figure 1).

The wide diversity of the PPCP compound classes and chemical properties rendered significant challenges during SPE method development and LC-MS/MS analysis. Over 100 compounds were screened with two MRM transitions monitored in each compound. Two different cartridge chemistries were used to improve the recovery for the compounds with differing hydrophobicity. Positive and negative polarities of MS methods were also used in this analysis (Figure 2).

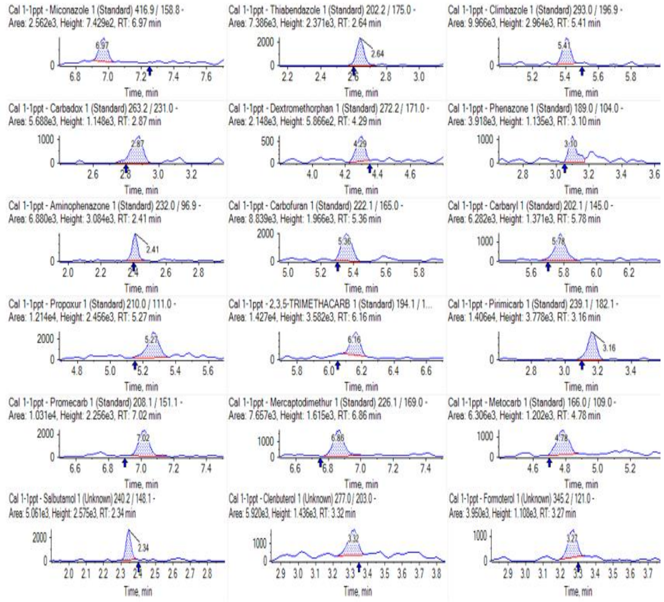

Figure 4. Detection limit of 18 compounds at 1 ppt.

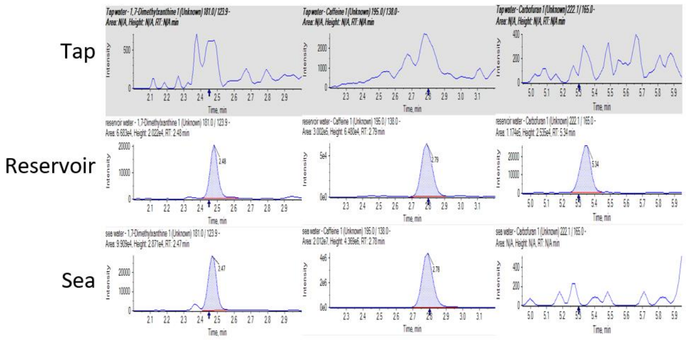

Figure 5. Samples collected from 3 different water sources.

Conclusions

An automated, walk-away platform for the screening of PPCP and pesticides in the matrix of surface water was developed and validated. A generic online SPE procedure was used to cover the large panel of analytes. High-resolution LC using F5 core-shell column was combined with high sensitivity detection of a SCIEX QTRAP 4500 LC-MS/MS System. Multiple Reaction Monitoring (MRM) was used because of its high selectivity and sensitivity. The *Scheduled* MRM algorithm used automatically optimizes dwell times and cycle times for best sensitivity and reproducibility.

The Online SPE LC-MS methods can be used as a starter method with optimized LC separation, SPE extraction and MS detection. It was also verified over a three-day period to test the methods and system robustness. Limits of Quantitation (LOQ) of majority analytes were quantifiable at 5ppt for this generic extraction (Figure 4). Use of method on water samples is shown in Figure 5.

References

1. WQA Technical Fact Sheet: PPCP & EDC
   WWW.WQA.ORG