Screening for Pharmaceuticals and Personal Care Products in Drinking Water Using a 3-D Ion Trap and Automated Online Sample Preparation

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Overview

Purpose: A fast, automated, inexpensive method of analysis for PPCPs in water – saving personnel, sample preparation time, data processing time and consumables.

Method: Automated online sample concentration full scan LC/MS detection and quantitation with targeted and timed data dependent MS^2 for confirmation using a 3-D ion trap mass spectrometer.

Results: PPCPs were detected down to levels as low as 10 ppt using automated online sample concentration and full scan detection, with MS^2 fragmentation for confirmation on a 3-D ion trap mass spectrometer.

Introduction

Pharmaceuticals and personal care products (PPCPs) consist of human and veterinary drugs, consumer products such as sun-screens, nutritional products and personal hygiene products. These compounds enter the environment through a variety of sources including wastewater effluent, landfill leachate, industrial effluent, and animal feed lots. Environmental impact of these compounds remains unclear. However, in 2007 the Environmental Protection Agency (EPA) released method 1694 addressing the analysis of these compounds. This method utilizes solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) monitoring a single transition. We will demonstrate automated online sample preparation with full scan quantitation and data dependent (DD) full scan MS^2 spectra for confirmation of PPCPs in drinking water using a 3-D ion trap mass spectrometer.

Method

Sample Preparation

A stock solution containing 10 µg/mL (10ppm) of 16 different PPCPs (Table 1) was prepared in water. Standards were prepared from the stock solution in concentrations ranging from 0.01 ppb to 50 ppb. Standards and samples were analyzed directly using volumes of 1 mL with no additional preparation.

LC Conditions

The Thermo Scientific EQuan MAX system (Figure 1) consists of a Thermo Scientific Accela 1250 UHPLC eluting pump, an Accela™ 600 loading high flow pump and an Open Accela Autosampler configured with a 5 mL injection loop and a 2.5 mL syringe (Figure 1). Solvents used for both pumps were A: water 4 mM ammonium formate and B: methanol with 4 mM ammonium formate. We used a Thermo Scientific Hypersil GOLD aQ loading column (2.1 x 20 mm, 12µm) and a Hypersil GOLD™ PFP analytical column (2.1 x 200 mm, 1.9µm). Chromatographic conditions were as follows – loading pump: 100% solvent A, 1 mL/minute during loading; eluting pump: 300 µL/minute 2% B; hold 2.5 minutes for loading; 9.5 minute gradient to 100% B; finalized with a 3.25 minute flush at 100% B and re-equilibration at 2% B. Total run time was 20 minutes.

Mass Spectrometry

The EQuan MAX system is equipped with the Thermo Scientific LCQ Fleet ion trap mass spectrometer source conditions: heated electrospray ionization (HESI) probe 450 °C; spray voltage 4 kV, capillary temp 250 °C, sheath gas 35, aux gas 20. Mass spectrometer method; scan event 1: Full scan MS from 145 - 850 amu. Scan event 2: timed DD MS/MS, dynamic exclusion with repeat count set to one and an exclusion duration of 3 seconds to facilitate compound confirmation (Figure 2). Data dependent MS/MS was performed with a 2.5 amu isolation width and a normalized collision energy of 35 for all PPCPs (Figures 2A and 2B).

Data Analysis

Data analysis was performed using Thermo Scientific TraceFinder software for quantitation and simultaneous compound confirmation using National Institute of Standards and Technology (NIST) library matching of DD MS/MS spectra.

Results

The initial step was full scan data acquisition of the PPCPs to determine retention times for the timed data dependent windows (Figure 3 and 2B). The use of timed data dependent windows improves the efficiency of the experiment and reduces the amount of MS^2 data to interrogate. In addition, the use of timed data dependent MS^2 experiments allows us to set low triggering thresholds for the maximum MS^2 sensitivity (Figure 4).

FIGURE 1. EquanMax Online Sample Pre-concentration System with the LCQ Fleet.

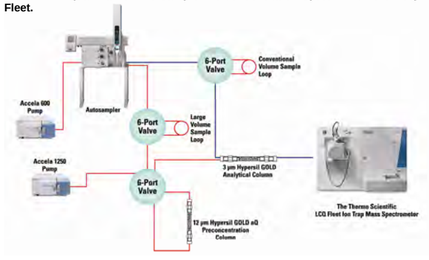


FIGURE 2 A. LCQ Fleet Mass Spectrometer Method. Data dependent MS^2 using global mass list for targeted screening.

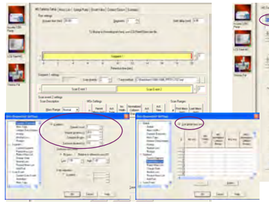


FIGURE 2 B. Caption. Global mass list MS^2 triggering.

FIGURE 3. Reconstructed Ion Chromatograms (RICs) for the 16 PPCPs that were analyzed, 5 ppb level. Note; diltiazem and miconazole appear in the same RIC.

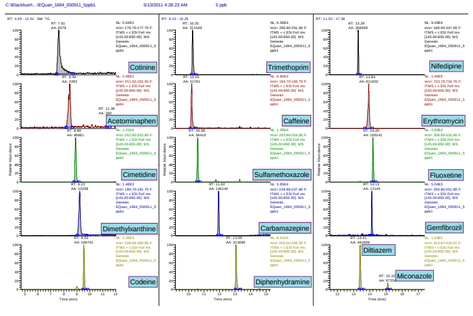
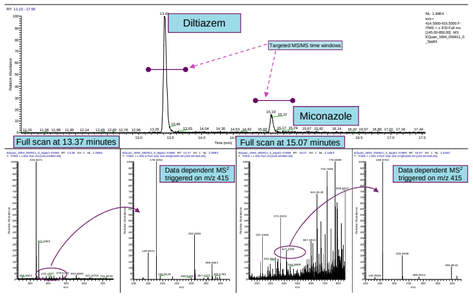


FIGURE 4. Upper pannel; RIC for m/z 415, depicting the chromatographic peaks for diltiazem and miconazole. Lower panel; full scan and corresponding data dependent MS^2 spectra for diltiazem and miconazole respectively. A library search can be performed on the resulting MS^2 data for confirmation.



Results (continued)

To further optimize the method the dynamic exclusion parameters were set to facilitate MS^2 acquisition not only at the inception of the peak (low intensity) but also at the apex of the peak by employing a repeat count of 1 and an short exclusion period of 3 seconds, which is approximately one half of chromatographic peak width (Figure 4). The MS^2 spectra in Figure 4 were triggered early and are from relatively low intensity precursor ions but still have excellent quality. However, utilizing the short exclusion period results in the MS^2 trigger occurring again at a higher intensity (data not shown). The utility of MS^2 data is illustrated with diltiazem and miconozole. Both compounds have the same nominal molecular weight, 414 amu (precursor [M+H]=415, see Figure 3) and therefore need additional information such as retention time and MS^2 data to differentiate them. Using the timed data dependent MS^2 acquisition gives us both retention time and fragmentation information (Figure 4)

FIGURE 5. Erythromycin results displayed in TraceFinder. LOD=0.05ppb, LOQ=0.1ppb, R^2 0.9963. Yellow flags denote compounds that were not detected for this specific sample at this level.

FIGURE 7. Panel A depicts the quantitative results for the Iced Tea Lemon samples full scan data and reference spectrum. Panel B depicts the automated confirmation results (Qual mode) showing the NIST library search results.

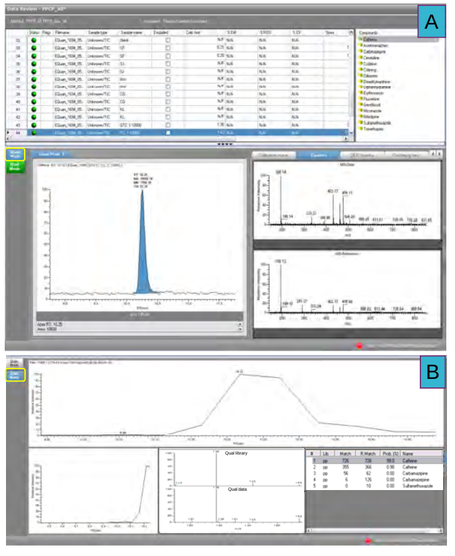


Table 1. Quantitation results summary for the PPCPs analyzed. LOD, LOQ, R^2 and calculated concentration for samples. SF- San Francisco water, SJ- San Jose water, AW- Arrowhead bottled water, CG- Crystal Geyser sparkling water, KL- Kirkland water, GTC- Green Tea, ITL- Iced Tea.

Compound (Group	LOD ppb	LOQ ppb	R^2	SF water	SJ water	AW water	CG water	KL water	GTC *	ITL*
1,7-dimethyixanthine (1)	0.50	1.00	0.9989	ND	ND	ND	ND	ND	ND	ND
Acetominephen (1)	0.50	0.50	0.9926	ND	ND	ND	ND	ND	ND	ND
Caffeine (1)	0.05	0.10	0.9990	ND	ND	ND	ND	ND	1.3	1.42
Carbamazepine (1)	0.01	0.05	0.9994	ND	ND	ND	ND	ND	ND	ND
Cimetidine (4)	0.01	0.05	0.9988	ND	ND	ND	ND	ND	ND	ND
Codeine (1)	0.05	0.10	0.9954	ND	ND	ND	ND	ND	ND	ND
Cotinine (1)	0.50	0.50	0.9972	ND	ND	ND	ND	ND	ND	ND
Diltiazem (1)	0.05	0.10	0.9983	ND	ND	ND	ND	ND	ND	ND
Diphenhydramine (1)	0.50	1.00	0.9959	ND	ND	ND	ND	ND	ND	ND
Erythromycin (1)	0.05	0.10	0.9962	ND	ND	ND	ND	ND	ND	ND
Fluoxetine (1)	0.10	0.50	0.9976	ND	ND	ND	ND	ND	ND	ND
Gemfibrozil (3)	0.50	0.50	0.9980	ND	ND	ND	ND	ND	ND	ND
Miconazole (1)	0.05	0.10	0.9982	ND	ND	ND	ND	ND	ND	ND
Nifedipine (pro 1)	0.50	0.50	0.9965	ND	ND	ND	ND	ND	ND	ND
Sulfamethoxazole (1)	0.05	0.05	0.9995	ND	ND	ND	ND	ND	ND	ND
Trimethoprim (1)	0.05	0.05	0.9984	ND	ND	ND	ND	ND	ND	ND

*GTC, and ITL samples were diluted 1:50,000 for analysis, calculated final concentration was 65 and 70 ppm respectively.

Results: Quantitation

The manual detection and evaluation of the confirming MS^2 spectra for numerous samples (Figure 4) is laborious and time consuming. To expedite both the quantitative analysis and qualitative confirmation we utilized TraceFinder™ , a software package designed for the production laboratory setting. As such, TraceFinder software has a full suite of quantitative tools designed to process large numbers of compounds and samples efficiently. In addition, the software performs simultaneous qualitative analysis using a NIST-based library search for additional confirmation and non-targeted screening. The quantitative results for erythromycin, one of the 16 compounds analyzed, are shown in Figure 5. The displayed level, 0.05 ppb is the limit of detection (LOD) for erythromycin. Criterion for calibration curve acceptance was goodness of fit = R^2 0.99 or greater. LODs were determined by %difference less than 20% [(Calculated - Specified amount)/Specified] x 100 and %RSD less than 10%. Limits of quantitation (LOQs) were determined by %difference less than 15% and %RSD less than 10% (Figure 5 and Table 1). It is significant to note that while the %RSD criterion for LOD was set at 10%, for most compounds the variability was less than 5%, as shown for erythromycin in Figure 5.

A red flag (not present) on a compound indicates one of the calibration criterion had not been met. A yellow flag indicates the calibration criterion were not detected in the sample (Figures 5 and 6) below the LOD for those compounds. Thus, the software makes it easy to quickly quantitate and evaluate large numbers of compounds and samples. A summary of the LODs, LOQs, R^2 and sample results are shown in Table 1.

Results: Confirmation

The caffeine in two bottled tea products was quantitated and determined to be 0.065 mg/mL for the green tea product and 0.070 mg/mL for the iced tea product (Table 1). Compound confirmation was automatically performed by comparison to full scan reference spectrum (Figure 7, Panel A) and by an automated NIST library search (Figure 7, panel B). The NIST library search is performed for all data dependent MS^2 spectra associated with a chromatographic peak, as shown in Figure 7, Panel B. The library search results show the high quality and reproducibility of the MS^2 spectra. Library search results can also be automatically printed as part of the results. The NIST library search was performed using a unique user library of MS^2 spectra from PPCP compounds. These spectra were acquired on the LCQ Fleet ion trap mass spectrometer using mid-level standards. The library was created using the NIST MS Search 2.0 software. The normalized collision energy of the LCQ Fleet mass spectrometer, and other Thermo Scientific ion trap MS, insures that spectra will be consistent from day to day and instrument to instrument. Therefore, once a library is created, it can be used for years and shared between different Thermo Scientific ion trap MS.

Discussion

EPA method 1694 outlines a method for the analysis of numerous PPCPs. This method is both labor and time intensive, involving acidic extractions, basic extractions and multiple chromatographic methods. We have demonstrated a method that analyzes compounds from both basic and acidic extractions using a single chromatographic method with no sample preparation using the EQuan MAX online sample preparation system equipped with the LCQ Fleet 3-D ion trap mass spectrometer. Quantitation was performed on full scan MS with confirmation achieved with DD MS^2 all processed in an automated matter utilizing the TraceFinder routine quantitation software.

Conclusions

- We have successfully demonstrated an automated online sample preparation system utilizing a 3-D ion trap mass spectrometer to provide a cost-effective, time-efficient method for targeted PPCP monitoring in water.
- Limits of detection as low as 10 ppt in spiked water is possible using the EQuan MAX system with the LCQ Fleet 3-D ion trap mass spectrometer, however this is compound dependent, with 50 ppt or greater being the norm.
- Caffeine was successfully quantitated in two commercially-available products.
- The EQuan MAX system powered by the Accela pumps allows the loading of large volumes of samples (up to 20 mL), which enables the detection of lower compound concentrations.
- Advantages of using a 3-D ion trap mass spectrometer for MS analysis include:
  - Full scan sensitivity and scan rate
  - Timed DD MS^2 acquisitions for confirmation
- TraceFinder software facilitated the quantitation and automated confirmation of a large number of compounds in a large number of samples.