ASMS 2015

TP 319

Highly Sensitive Detection of PPCPs in Water using Direct Injection

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Introduction

PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics. Government agencies, such as EPA and Euprpean Water Framework, have proposed regulations to monitor the water supply system. The analysis of contaminants in water, especially in drinking water, poses significant challenge due to low levels. Highly sensitive triple quadrupole LC/MS is the perfect tool to meet the challenge.

In this study, 141 PPCPs in water were detected at parts per trillion level using Agilent 1290 Infinity UHPLC coupled to Agilent 6495 triple quadrupole LC/MS by direct injection of water samples. Several modifications to the triple quadrupole MS have resulted in better performance. Improvements were achieved by the new MS1 ion optics, an improved curved and tapered collision cell, a new ion detector operating at dynode accelerating voltages of up to 20 kV, and a new autotune optimized for speed and sensitivity. Enhanced sensitivity gives improved peak area precision, leading ultimately to lower detection limits.

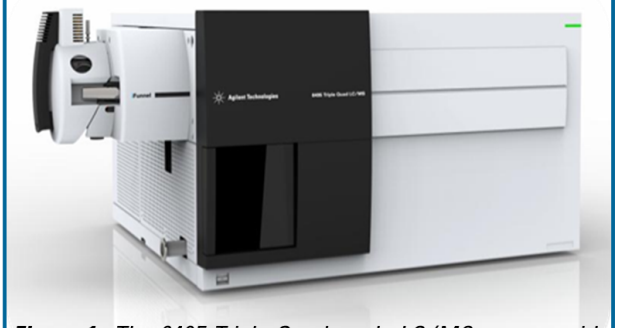

Figure 1. *The 6495 Triple Quadrupole LC/MS system with new mass filter one ion optics, improved collision cell and new ion detector for enhanced sensitivity.*

Experimental

Samples and sample preparation

The stock analyte standards and internal standards are at 25 ppb in acetonitrile. The internal standards were kept at constant concentration of 250 ppt, while analyte standards were spiked at 10, 25, 50, 100, 250, 500 and 1000 ppt in MilliQ water. Two of the three unknown samples were from collaborators – one cleaner and one from the contaminated water source. Another sample was local tap water. 250 ppt internal standards were spiked into unknown samples after filtration.

Experimental

Equipment and Method Setup

An Agilent 1290 Infinity UHPLC was coupled to an Agilent 6495 triple quadrupole LC/MS equipped with an Agilent Jet Stream electrospray ionization source. 119 and 22 analytes were detected in positive mode and in negative mode respectively with different mobile phases. The precise and accurate screen and quantitation of PPCPs were accomplished by dynamic multiple reaction monitoring (DMRM) with 316 MRM transitions in positive mode and 62 MRM transitions in negative mode. **40 uL** water samples was injected directly without tedious analyte enrichment.

Table 1. *Analytes in this study*

10,11-dihydro-10-hydroxycarbamazepine	Codeine	Hydromorphone	Montelukast	Propranolol	(±)11-nor-9-carboxy-delta-THC
6-Acetylmorphine	Cotinine	Hydroxybupropion	Morphine	Pseudoephedrine	Bezafibrate
Acebutolol	DEET	Ketoprofen	Nifedipine	Quetiapine	Celecoxib
Acetaminophen	Dehydroaripiprazole	Lamotrigine	Nifedipine oxidized	Ritalinic acid	Chloramphenicol
Albuterol	Desmethylcitalopram	Levorphanol	Norfentanyl	Sertraline	Diclofenac
Amitriptyline	Desmethylvenlafaxine	Lidocaine	Norfluoxetine	Sildenafil	Diclofenac 4-hydroxy
Amitriptyline metabolite	Dextromethorphan	Loratadine	Norfluoxetine	Simvastatin	Fenbufen
Amphetamine	Diltiazem	Lorazepam	Normeperidine	Sotalol	Furosemide
Aripiprazole	Diphenhydramine	MDA	Norquetiapine	Sulfamethazine	Gemfibrozil
Atenolol	Disopyramide	MDEA	Norsertraline	Sumatriptan	Hydrochlorothiazide
Atorvastatin	Donepezil	MDMA	Norverapamil	Tadalafil	Ibuprofen
Atrazine	Duloxetine	Mefenamic acid	Omeprazole	Temazepam	Methylparaben
Benzoylecgonine	Ecgonine methyl ester	Meperidine	Oxazepam	Thiabendazole	Modafinil acid
Buprenorphine	EDDP	Meprobamate	Oxcarbazepine	Tramadol	Naproxen
Bupropion	Erythromycin	Metformin	Oxycodone	Trazadone	n-Butylparaben
Caffeine	Erythromycin-anhydro	Methadone	Oxymorphone	Triamterene	Phenobarbital
Carbamazepine	Escitalopram	Methamphetamine	Oxymorphone glucuronide	Trimethoprim	Phenytoin
Carbamazepine 10,11 epoxide	Famotidine	Methotrexate	Paroxetine	Tylosin	Pravastatin
Carisoprodol	Fentanyl	Methylphenidate	Phenmetrazine	Valsartan	Sulfamethoxazole
Chlorpheniramine	Fluoxetine	Metoprolol	Phentermine	Venlafaxine	Triclocarban
Clenbuterol	Fluticasone propionate	Mevastatin	Phenylpropanolamine	Verapamil	Triclosan
Clopidogrel carboxylic acid	Gabapentin	m-Hydroxybenzoylecgonine	Pioglitazone	Zolpidem	Warfarin
Cocaethylene	Glyburide	Modafinil	Pregabalin	Zolpidem phenyl-4-carboxylic acid	
Cocaine	Hydrocodone	Monoethylglycinexylidide	Primidone		

Table 2. *UHPLC and 6495 LC/MS Parameters*

Column	Agilent ZORBAX RRHD Eclipse Plus C18 2.1 x 100 mm, 1.8 µm (p/n 959758-902) @ 40°C	
Injection volume	40 µL	
Mobile phase	A: Water: 5 mM NH_4OAc+ 0.02% HOAc (+); 0.005% HOAc (-) B: Acetonitrile	
Flow rate	0.3 mL/min	
Gradient program	Time 0 0.5 11 13 13.1 Stop Time	B% 5 5 100 100 5 15 min
Ion mode	Positive and negative ESI with Agilent Jet Stream	
Total number of MRMs	316 positive / 62 negative	
MS1 and MS2 resolution	Unit	

Results and Discussion

Methods Performance

The 6495 LC/MS triple quadrupole design enhancements have demonstrated a significant increase in ion transmission and sensitivity. Figure 2 and figure 3 shows the response of 119 analytes in positive ion mode and 22 analytes in negative ion mode at 10 ppt. All the compounds can be detected at method reporting level of 10ppt. Most of the analytes can be detected at the concentrations much lower than 10ppt.

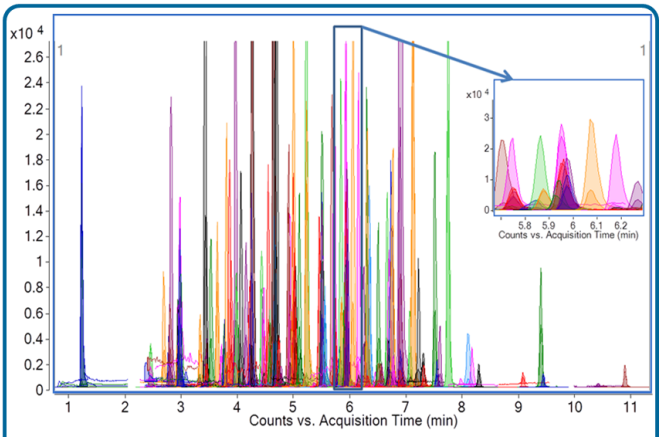

Figure 2. *Chromatograms of 119 PPCPs spiked into water at 10 ppt in positive ion mode.*

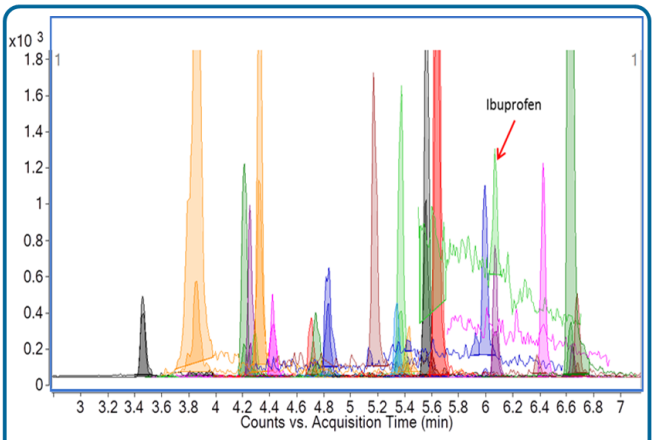

Figure 3. *Chromatograms of 22 PPCPs spiked into water at 10 ppt in negative ion mode.*

Linearity

Linearity was assessed covering a concentration range from 10ppt to 1000ppt. Calibration curves for metformine in positive mode and ibuprofen in negative mode are shown in figure 4 and figure 5. The equations were generated as the quadratic fit with weighting factor 1/x including the origin. The correlation coefficients (R^2) for all target analytes were greater than 0.99, most were greater than 0.995, except for one compound quetiapine in positive mode (R^2=0.982) due to an interfering systematic peak nearby.

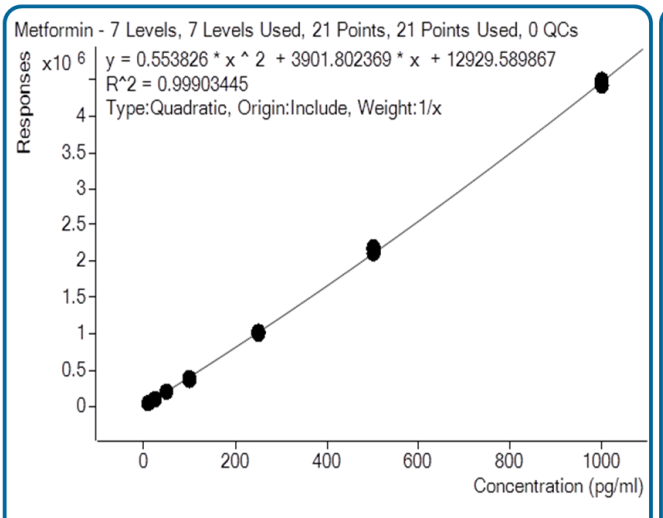

Figure 4. *Calibration curve for metformin in positive mode covering concentrations from 10ppt to 1000ppt.*

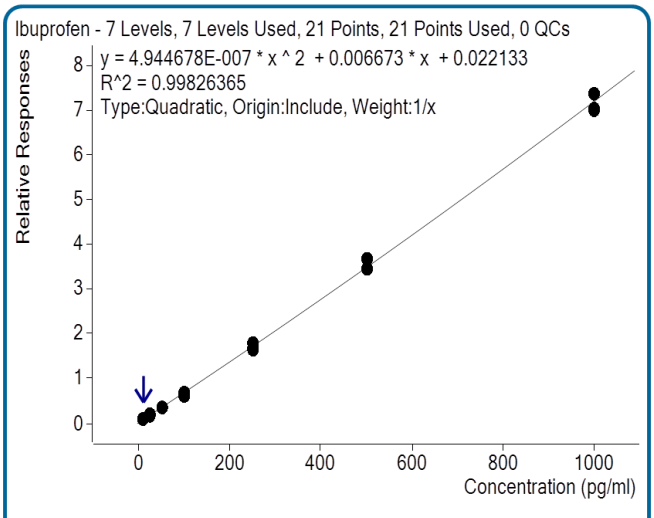

Figure 5. *Calibration curve for ibuprofen in negative mode covering concentrations from 10ppt to 1000ppt.*

Results and Discussion

Precision and Accuracy

Triplicate injections were made for calibration curves at each level. In most cases, the precision were very good. There were occasional cases that accuracy was beyond the 80-120% range, but most of them were close to 120% or 80%. 5-6 very hydrophobic compounds gave more accuracy outliers at the low levels. This may be due to the surface absorption of the compounds at lower spike level. Overall, 2.3% of measurements have an accuracy outlier beyond 80-120% (<1 accuracy outlier per two compounds with 21 measurements per compound) if 5 outlier compounds are removed from accuracy consideration in positive ion mode. In negative ion mode, accuracy was excellent for all compounds except for celecoxib. The accuracy issue for celecoxib may be caused by the uneven surface adsorption at low levels without corresponding internal standard.

Real World Samples

Three real world samples were tested. The first was the local tap water. The other two were from collaborators. One sample is cleaner and the other is from the contaminated water source. Duplicate injections were run per sample. The compound was regarded as "positive" if the average concentration of the two runs was greater than 10ppt. The results are listed in table 3-5.

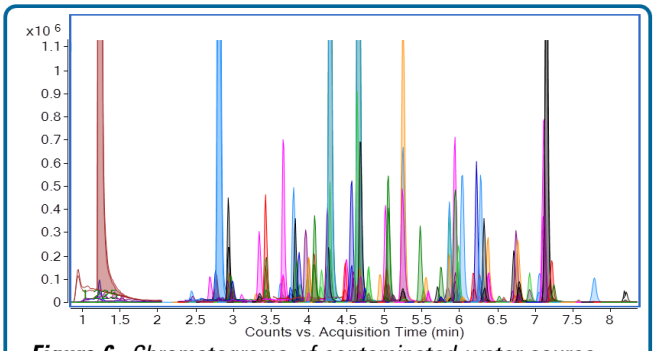

Figure 6. *Chromatograms of contaminated water source.*

Table 3 . *Compounds found in local tap water*

Name	Injection1 (ppt)	Injection2 (ppt)	Average (ppt)
Gabapentin	20.7	19.6	20.2
Metformin	31.2	30.1	30.6
Montelukast	12.7	12.3	12.5

Table 4 . *Compounds found in customer water-1*

Name	Injection1 (ppt)	Injection2 (ppt)	Average (ppt)
Montelukast	12.1	11.9	12.0
Ketoprofen	16.1	14.9	15.5
Caffeine	28.0	15.5	21.7
DEET	107.9	120.0	113.9

Table 5. Compounds found in customer water-2

Name	Average (ppt)	Name	Average (ppt)	Name	Average (ppt)	Name	Average (ppt)	Name	Average (ppt)
10,11-dihydro-10-hydroxycarbamazepine	882.3	Desmethylvenlafaxine	785.5	Lorazepam	139.7	Propranolol	70.2	Celecoxib	43.2
Amitriptyline metabolite	30.0	Dextromethorphan	36.4	Meprobamate	153.8	Pseudoephedrine	223.3	Chloramphenicol	12.0
Amitriptyline	29.2	Diltiazem	58.3	Metformin	3956.3	Ritalinic acid	118.6	Diclofenac 4-hydroxy	42.9
Atenolol	2405.4	Diphenhydramine	205.0	Methadone	48.5	Sertraline	45.8	Diclofenac	264.6
Atorvastatin	38.7	Ecgonine methyl ester	39.1	Methamphetamin	287.1	Sotalol	70.2	Furosemide	393.2
Atrazine	42.0	EDDP	101.0	Metoprolol	314.6	Sulfamethazine	11.3	Gemfibrozil	323.2
Benzoylecgonine	213.5	Erythromycin	44.3	Modafinil	15.2	Temazepam	86.1	Hydrochlorothiazide	494.5
Bupropion	161.8	Erythromycin-anhydro	34.3	Monoethylglycine xylidide	29.8	Thiabendazole	39.8	Ibuprofen	139.4
Caffeine	1357.0	Escitalopram	185.7	Montelukast	11.8	Tramadol	717.4	Modafinil acid	116.0
epoxide	37.0	Fluoxetine	28.8	Norquetiapine	28.5	Trazadone	32.8	Naproxen	350.4
Carbamazepine	221.3	Gabapentin	>>1000	Norsertraline	28.0	Triamterene	105.7	Phenobarbital	53.8
Carisoprodol	27.5	Hydrocodone	26.1	Oxazepam	28.0	Trimethoprim	299.3	Phenytoin	123.2
Clopidogrel carboxylic acid	213.6	Hydroxybupropion	256.7	Oxcarbazepine	43.6	Tylosin	11.5	Pravastatin	54.1
Cocaine	35.8	Ketoprofen	16.3	Oxycodone	89.1	Valsartan	495.6	Sulfamethoxazole	577.4
Codeine	67.4	Lamotrigine	940.2	Oxymorphone	15.5	Venlafaxine	415.2	Triclocarban	39.2
Cotinine	94.3	Levorphanol	208.9	Phentermine	117.4	Verapamil	10.6	Triclosan	255.1
DEET	536.3	Lidocaine	342.7	Pregabalin	442.5	Zolpidem phenyl-4-carboxylic acid	46.5		
Desmethylcitalopram	97.2	Loratadine	10.2	Primidone	67.9				

Conclusions

Fast and simple LC-MS/MS methods for the accurate screen and quantitation of the PPCPs in water have been developed. The methods leverage the full advantage of high sensitivity provided by the Agilent 6495 Triple Quadrupole mass spectrometer. This is of key importance for the goal of achieving low ppt level LLOQs for the quantitation of trace contaminants in water by direct injection. Tedious sample enrichment and cleanup process can be avoided.