

## EMERGING CONTAMINANTS IN DRINKING WATER PART I: PAINKILLERS AND ILLICIT DRUGS

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### INTRODUCTION

The analysis of emerging contaminants (ECs) has become an area of significant interest in the field of environmental research. This increased interest was sparked by two factors: first, the availability of new analytical tools (GC/MS/MS or LC/MS/MS) with sub-ppb detection capability; and second, concerns about adverse effects of ECs on human health and wildlife. ECs are comprised of a large selection of chemicals such as pharmaceuticals, veterinary drugs, nano-materials, personal care products, and household chemicals. These chemicals differ from familiar pollutants such as pesticides, dioxins, heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs).

In the early 1990's, European scientists identified trace amounts of pharmaceutical drugs in surface and ground waters. This prompted national surveys throughout Europe and America. The survey results showed over 100 contaminants at unexpected ppb levels. ECs are defined as pollutants that are not currently registered in a routine monitoring program.

The selection of candidates for future regulations will depend on toxicity research, potential health issues, the persistence of ECs in the environment, and public awareness.

The term "emerging" can lead to the misconception that ECs are new chemical entities when in fact, the discharge of these contaminants into the environment occurred immediately after their introduction into the market. In this case, "emerging" refers to the scientific and public concerns over the presence about EC's in drinking water sources and the lack of knowledge on potential side effects.

Currently, over 3000 pharmaceutical drugs are registered worldwide, including illicit drugs, painkillers, beta-blockers, sedatives, anti-depressants, prescription medications, *etc.* These chemicals are usually discharged into the environment from private households and hospitals as a combination of unchanged and metabolized entities that ultimately reach local water treatment plants. The time-consuming preparation and identification of such a large number of chemically-diverse compounds would prove challenging for most waters testing laboratories that are already tasked with completing a high volume of analyses with limited resources.

The following method describes a low-level (sub-ppb) analysis of ECs in drinking water using the Waters® AquaAnalysis System, an ON-line SPE LC/MS/MS technology that provides automated sample preparation and analysis. The AquaAnalysis System allows water testing laboratories to significantly increase sample throughput while reducing manual intervention by analysts, thereby improving overall productivity and reproducibility.

### OFF-line SPE enrichment

In 2006, research groups in Europe and the U.S. published findings and analytical protocols for the analysis of pharmaceuticals in drinking water. Hummel et. al.<sup>1</sup> produced a multi-residue method for the analysis of opioids, tranquilizers, anti-epileptics, antidepressants, and beta-blockers (a total of 20 drugs) in surface water, ground water, and drinking water. They utilized LC/MS/MS technology with OFF-line SPE sample preparation. Vanderford et. al.<sup>2</sup> expanded on the list of pharmaceuticals with anticonvulsants, tranquilizers, anti-lipidemic, antipsychotics, and anti-microbials (a total of 15 drugs) in the same water matrices and analytical methodology. In both cases, the sample preparation protocols and trace level sensitivity (sub ppb) proved to be key challenges. Tables 1 and 2 compare both extraction protocols performed with Waters Oasis® HLB 200 mg cartridges.

Step 1: Pre-condition with 5 mL MTBE
Step 2: Pre-condition with 5 mL methanol
Step 3: Pre-condition with 5 mL water
Step 4: Load 500 mL sample at 15 mL/min – total time 30 min
Step 5: Dry under N <sub>2</sub> stream – total time 30 min
Step 6: Elute with 5 mL methanol
Step 7: Elute with 5 mL methanol/MTBE 10/90
Step 8: Evaporate to dryness with N <sub>2</sub> to 400 µL – total time 30 min
Step 9: Reconstitute to 500 µL – enrichment ratio 1000:1
Step 10: Inject 10 µL – total LC/MS/MS analysis 16 min

Table 1. Vanderford et. al. extraction method.

Step 1: Pre-condition with 2 mL heptane  
 Step 2: Pre-condition with 2 mL acetone  
 Step 3: Pre-condition with 6 mL methanol  
 Step 4: Pre-condition with 8 mL water  
 Step 5: Load 1000 mL sample at 20 mL/min – total time 50 min  
 Step 6: Dry under N<sub>2</sub> stream – total time 60 min  
 Step 7: Elute with 8 mL acetone  
 Step 8: Evaporate to dryness N<sub>2</sub> to 50 µL – total time 30 min  
 Step 9: Reconstitute to 500 µL – enrichment ratio 2000:1  
 Step 10: inject 25 µL – total LC/MS/MS analysis 30 min

Table 2. Hummel et. al. extraction method.

As shown in Tables 1 and 2, both protocols start with a thorough cleaning of the SPE sorbent with a wide selection of polar and non-polar solvents. These steps are crucial for trace analysis. The next step is sample loading of between 0.5 and 1.0 L of water, immediately followed by a drying step. The purpose of nitrogen drying is to remove water from the final elution. The organic phase is evaporated to near dryness and reconstituted to a final volume of 500 µL.

## ON-line SPE enrichment

With an ON-line SPE system, all of the steps can be automated, eliminating many of the manual processes from the extraction protocol. Tables 3 and 4 illustrate the ON-line SPE protocol used for the analysis of painkiller and illicit drugs in drinking water.

Step 1: Pre-condition with 100 % water  
 Step 2: Pre-condition with 50/50 methanol/acetone  
 Step 3: Pre-condition with 40/40/20 ethyl acetate/hexanes/acetone  
 Step 4: Pre-condition with 50/50 methanol/acetone  
 Step 5: Pre-condition with 100 % water  
 Step 5: Load 15 mL drinking water sample  
 Step 6: Wash 5/95 methanol/water  
 Step 7: Elute with acidic 70/30 ACN/methanol gradient

Table 3. AquaAnalysis extraction method steps.

Step 1: aqueous flush – 3 min (parallel)  
 Step 2: organic flush – 3 min (parallel)  
 Step 3: organic wash – 3 min (parallel)  
 Step 4: organic flush – 3 min (parallel)  
 Step 5: aqueous flush – 3 min (parallel)  
 Step 6: Load 15 mL – at 4 mL/min + 2 % NH<sub>4</sub>OH – 6 min (sequential)  
 Step 7: Wash – at 4 mL/min – 3 min (sequential)  
 Step 8: Elute – acidic + 0.5 % FA gradient – 12 min (sequential)

Table 4. AquaAnalysis time chart.

The ON-line SPE protocol shows equal robustness in comparison with the OFF-line SPE protocols with the same selection of organic solvent. The added benefits are as follows:

- Sample volumes (20 mL versus 500 mL).
- Pre-SPE cleaning is automated.
- Cleaning is performed in parallel for time saving.
- Custom cleaning blends by software control.
- Decreased solvent consumption.

Since smaller sample volumes (20 mL) are required, the loading times can be drastically reduced as seen in Step 6 of Table 4. Once the analytes of interest are trapped on the extraction column, the analysis proceeds with a direct elution in backflush mode and refocuses on the analytical column for further separation. As shown in Table 3, the drying, evaporation, and reconstitution steps were totally eliminated from the ON-line SPE protocol.

## EXPERIMENTAL

The MRM conditions for the analyses of illicit drugs and benzodiazepines are listed in Tables 5 and 6. The SPE extraction column used was the Oasis HLB 2.1 x 20 mm 25 $\mu$ m with a high pH loading mobile phase. The focusing column used was the Waters XBridge<sup>®</sup> C<sub>18</sub> 2.1 x 100 mm, 3.5  $\mu$ m with a low pH elution mobile phase. The washing and reconditioning parameters are listed in the SPE and LC conditions section..

Illicit drugs	Parent	Daughter
Benzoyllecgonine	290.1	→ 168.2
Cocaine	304.1	→ 182.2
Heroin	370.1	→ 169.2
Buprenorphine	468.1	→ 101.0
Codeine	300.2	→ 165.2
Amphetamine	136.2	→ 90.9
Morphine	286.1	→ 153.1
6-monoacetylmorphine	328.1	→ 165.2
Methamphetamine	150.2	→ 90.9
Methadone	310.2	→ 104.9
Norbuprenorphine	414.2	→ 101.0

Table 5. Illicit drugs MRM conditions.

Benzodiazepines	Parent	Daughter
Alprazolam	309.1	→ 281.1
Bromazepam	316.2	→ 182.2
Clobazam	301.0	→ 299.1
Clonazepam	315.9	→ 270.0
Diazepam	285.0	→ 154.1
Flunitrazepam	314.0	→ 268.1
Lorazepam	320.9	→ 275.0
Oxazepam	287.0	→ 241.0
Triazolam	343.0	→ 308.1
Nitrazepam	282.1	→ 236.2
Prazepam	325.1	→ 271.1

Table 6. Benzodiazepines MRM conditions.

## SPE and LC conditions

<b>Loading pump:</b>	line A: 100% Aqueous (2% NH <sub>4</sub> OH) (4 mL/min) line B: 100% Methanol
<b>Eluting pump:</b>	line A: 100% Aqueous + 0.5 % formic acid (0.4 mL/min) line B: 100% Methanol/Acetonitrile (30/70) + 0.5% FA
<b>Recondition pumps:</b>	A: 50/50 Methanol/Acetone (4 mL/min) B: 80/20 Ethyl Acetate/Acetone C: 80/20 Hexanes/Acetone
<b>Extraction column:</b>	2.1 x 20 mm Oasis HLB 25 $\mu$ m
<b>Analytical column:</b>	2.1 x 100 mm XBridge C <sub>18</sub> 3.5 $\mu$ m

## RESULTS AND DISCUSSION

The extracted chromatograms for benzodiazepines and illicit drugs at 1000 ppt and 10 ppt respectively, are shown in Figures 1 and 2. All benzodiazepines showed excellent sensitivity at 10 ppt, as did all illicit drugs, except morphine and norbuprenorphine. By increasing the sample volume to 60 mL the enrichment ratio was improved and provided a quantifiable signal.

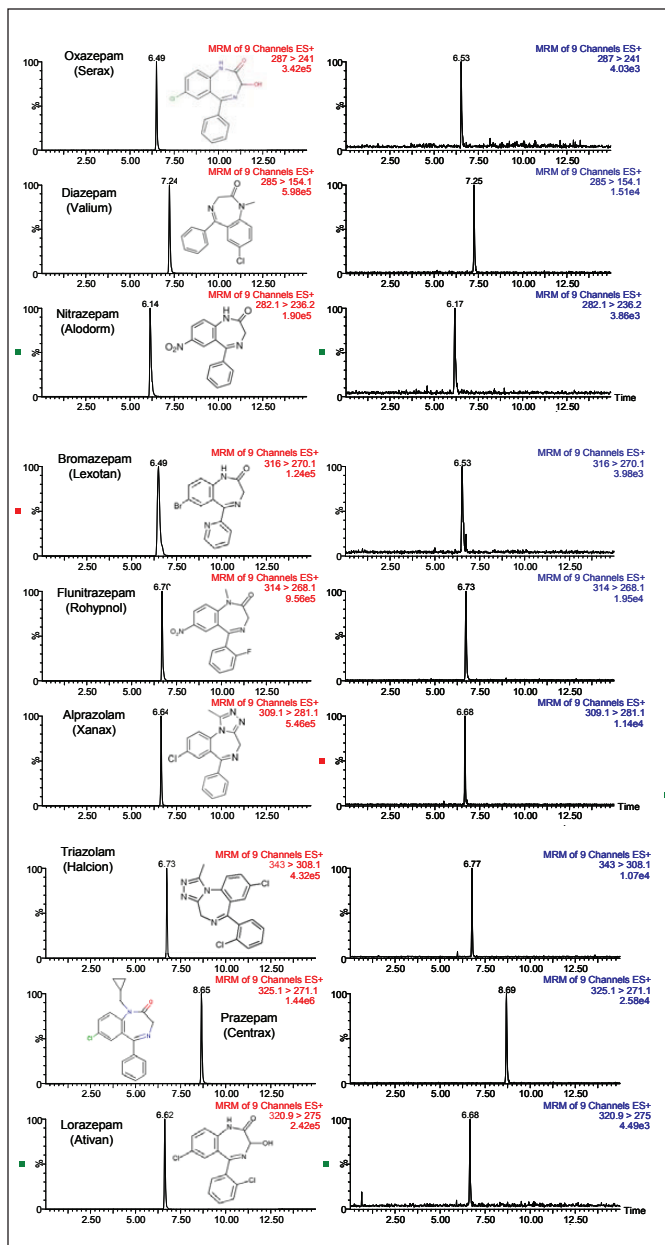


Figure 1. Extracted chromatograms of benzodiazepines in 15 mL of drinking water at 1000 ppt (red) and 10 ppt (blue).

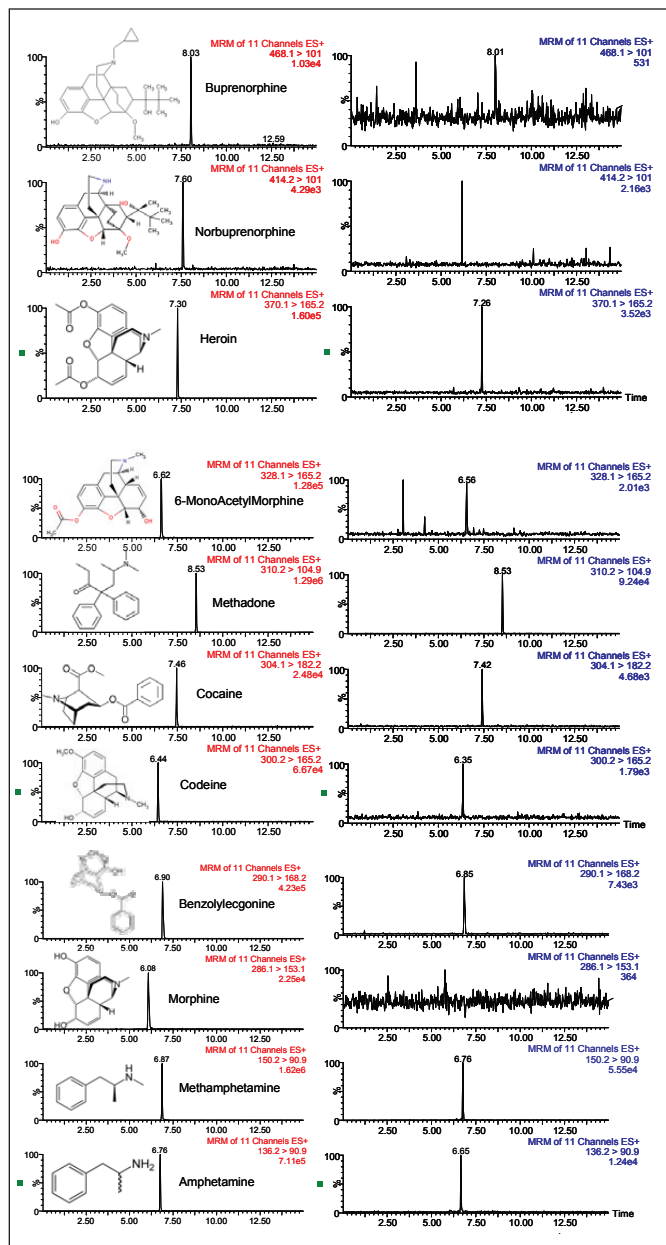


Figure 2. Extracted chromatograms of illicit drugs in 15 mL of drinking water at 1000 ppt (red) and 10 ppt (blue).

## Carryover

The AquaAnalysis System is equipped with a parallel extraction column reconditioning feature. During the elution and analysis of a sample, the second extraction column is cleaned with a user selected blend of water and organic solvents to remove any remaining interferences from the previous injection. Figures 3 and 4 show negligible amounts of compounds of interest in water blanks following a high concentration standard. For example, with the benzodiazepines, prazepam gave a 9.7 ppt carryover signal with the recondition OFF. With the recondition ON, the prazepam signal is eliminated from the blank.

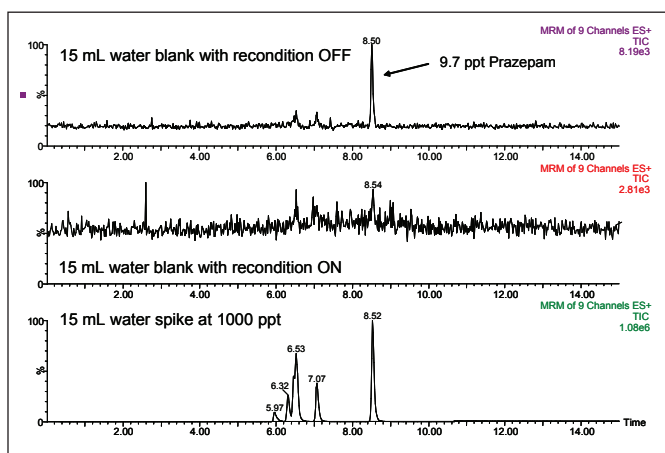


Figure 3. Total ion chromatogram of benzodiazepines in drinking water at 1000 ppt vs. two water blanks with the extraction column recondition ON and OFF.

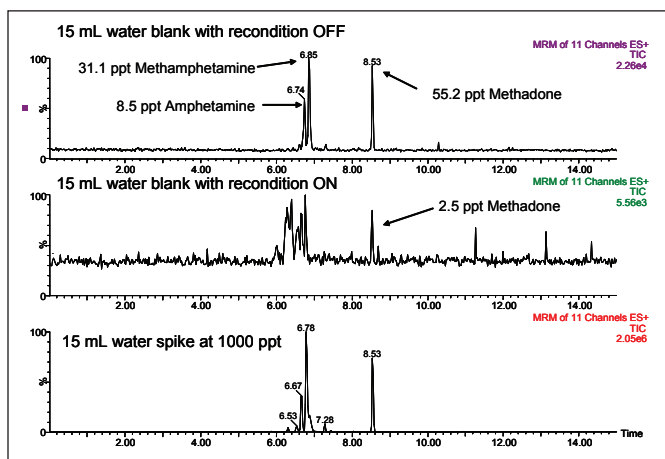


Figure 4. Total ion chromatogram of illicit drugs in drinking water at 1000 ppt versus two water blanks with the extraction column recondition ON and OFF.

The illicit drugs methadone and methamphetamine gave carryover signals with the recondition OFF at 31.1 ppt and 55.2, respectively. With the recondition ON, the carryover was measured at 2.5 ppt for methadone and below 1 ppt for methamphetamine.

## CONCLUSION

The presence of emerging contaminants in the environment is rapidly becoming a global concern. Furthermore, there has been little effort to determine the ecological risks of these contaminants mainly because it requires analytical tools with sub-ppb capabilities and time-consuming sample preparation protocols. The AquaAnalysis System streamlines both the extraction and analysis in a turn-key total solution platform.

When comparing the run times of OFF line SPE protocols currently used for pharmaceutical analysis in drinking water (106 minutes) and the AquaAnalysis system (20 minutes), the total analysis time is reduced five-fold from the Hummel protocol and by a factor of nine from the Vanderford protocol (170 min).

The small sample size (20 mL) and full automation afforded by the AquaAnalysis System enables analysis times to be reduced by as much as 80%. The resultant benefits to the water testing laboratory include significant reductions in the cost of consumables and sample shipping, as well as savings in time (increased productivity), and effort (minimal manual intervention), compared to OFF-line sample preparation methodologies.

## References

1. Hummel, D, Loffler, D, Fink, G, and Ternes, T A., *Envir Sci Technol.* 40: 7321-7328, 2006.
2. Vanderford, B J, Snyder, SA, *Environ Sci Technol.* 40: 7312-7320, 2006.

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