

focus on Laboratory Products

Development of a Method to Better Quantify Levels of the Persistent Organic Pollutants Iodinated X-Ray Contrast Media and Artificial Sweeteners Found in Water

Waldemar Ens MSc, IWB Water Laboratory, Basel, Switzerland.

Persistent organic pollutants (POPs) can cause long term damage to the environment. Those found in water supplies may affect many species. Two classes of organic molecules have come to the attention of regulators and water treatment authorities in recent years; iodinated X-Ray contrast media (XCM) and artificial sweeteners (AS). These molecules are designed to have a high degree of stability and to be unaffected by organic metabolic processes. A sweetener that is rapidly broken down in the mouth does not seem very sweet to the taster. An XCM that was metabolised in the body would not help a radiographer with their diagnosis. It is therefore easy to see how such molecules could become POPs when excreted from the body and passed into the waste water system. The stability and robust nature of these molecules is such that they are frequently unaffected by most treatment regimens used to process wastewater before discharge.

Aware of the increased abundance of these molecules in wastewater, the IWB Water Laboratory set out to develop a robust, simple method to analyse levels of these molecules in water from the river Rhine, groundwater and drinking water. A summary of this work is presented here.

Background Research

XCMs are typically heavily iodinated to provide good x-ray adsorption and are difficult to ionise in a mass spectrometer (MS). Artificial sweeteners are typically strongly polar.

Solid-phase extraction (SPE) combined with liquid chromatography (LC)-electrospray ionisation (ESI)-tandem mass spectrometry (MS/MS) is the leading analytical methodology for the determination of XCM and AS.

However, SPE methods can suffer from poor recovery, are time consuming, and expensive, especially when sequential SPE columns are used.

The aim of this study was to develop a simple, sensitive, and robust method for the quantification of XCM and AS to regularly monitor the presence of these compounds at all stages of the drinking water production process.

The molecules of interest within this study were:

Iodinated X-Ray Contrast Media

Diatrizoic acid
Iopamidol
Iopromide
Iomeprol
Iothalamic acid
Ioxitalamic acid
Iohexol

Artificial Sweeteners

Acesulfame
Saccharin
Cyclamate



Figure 1. EZ-2 evaporator

Method Development

The river Rhine carries many organic molecules within its flow. To analyse such a complex matrix a degree of separation before analysis was required, therefore High Performance Liquid Chromatography (HPLC) was used, coupled to an AB Sciex 5500 QTRAP MS.

Unfortunately a direct injection method without sample pre-concentration was not sensitive enough to detect the required levels of analyte and samples would therefore require enrichment.

In order to gain higher sensitivities, a 10-fold sample concentration step using a Genevac EZ-2 plus centrifugal vacuum evaporator, that provided excellent recoveries (90±6%), was selected for sample preparation.

Using the EZ2 Plus Evaporator (see Figure 1) many samples can be loaded, and dried under vacuum automatically with a predefined method that required no operator attention. The analytes of interest are not volatile under these conditions so may be completely dried.

- 10ml of water samples were placed into 15 mL Pyrex test tubes and internal standard was added.
- Samples were then evaporated overnight in a Genevac EZ2 Plus evaporator at a maximum temperature of 54°C.
- The precipitate was reconstituted in 1 mL of eluent (water, formic acid and ammonium formate).
- After 10 min sonification and 10 min shaking, the samples were filtered before submitting for LCMS/MS analysis.

One problem with vacuum concentration can be that all the matrix components are also concentrated. These can affect the ionisation of the analytes in MS, and therefore the results. The use of an internal standard was found to mitigate this risk. Deuterated samples of diatrizoic acid and iopamidol were used to validate the XCM analyses; deuterated acesulfame and ¹³C₆-labelled saccharin were used for the sweetener analyses. The use of isotope-labelled internal standards allowed accurate quantification, since these were shown to be affected similarly to the native analyte by the matrix elements when analysed in MS.

Results

A 10-point calibration curve was found to be linear over a working range of 10–500 ng/L for XCM and 10–1600 ng/L for AS. The coefficients of determination were higher than 0.999 for all compounds. Based on a sample volume of 10 mL, with a 10-fold concentration by evaporation, LOQs below 10 ng/L could be achieved for all compounds (see Table 1).

Table 1. Linear regression determined by a 10-point calibration curve (n=2) and LOQs calculated with a S/N ratio of 10.

	R2	LOQ (ng/L)
Diatrizoic acid	0.9997	1
Iopamidol	0.9997	5
Iomeprol	0.9994	5
Iopromide	0.9995	5
Iothalamic acid	0.9994	3
Ioxitalamic acid	0.9996	4
Iohexol	0.9997	8
Acesulfame	0.9999	2
Saccharin	0.9992	7
Cyclamate	0.9995	1

The precision of 10 replicates was excellent with relative standard deviation values ranging between 3 and 6% for the lower working range limit and 1 and 3% for the upper working range limit.

Sample preparation recoveries and matrix effects were investigated for all three matrix types: surface, ground, and drinking water. As shown in *Figure 2a*, sample preparation recovery was similar for all compounds with an excellent average recovery of 90 ± 4 % and an average RSD of 2% for triplicate injections.

To investigate the total recovery, calibration samples were subjected to sample enrichment. In addition, internal standards were taken into account for the recovery calculation.

As presented in *Figure 2b*, good results were obtained with mean recoveries from five independent measurement series ranging between 78 and 113%. In particular, for the three most important compounds, diatrizoic acid, iopamidol, and acesulfame, recoveries were found to be almost 100 % with relative standard deviations $\leq 5\%$ for all three matrices.

Conclusions

A sensitive LC-MS/MS method has been developed which allows the simultaneous quantification of seven XCM and three AS in water. Instead of using labour-intensive SPE, an easy sample preparation was performed by applying a sample enrichment step through centrifugal vacuum evaporation, achieving LOQs below 10 ng/L. The developed method supports increased sample throughput, is easier to perform and cheaper than comparable methods using SPE enrichment.

Validation confirmed the high linear working range, high sensitivity, and robustness of the method. The use of four isotope-labelled internal standards enabled compensation of matrix effects and facilitated accurate quantification. The method was successfully applied to Rhine, ground, and drinking water samples.

The final method was validated by use of internal standards over 10 repetitions, the sample preparation and analytical method were found robust, repeatable and consistent with a standard deviation across all samples of $<6\%$, and has been accepted into routine use.

About the Author

Waldemar Ens was MSc Student at FHNW University of Applied Sciences and Arts Northwestern Switzerland, and working at the IWB Water Laboratory, Neuhausstrasse 31, Postfach CH-4002 Basel, Switzerland for the development of this work.

References

Development, validation, and application of a novel LC-MS/MS trace analysis method for the simultaneous quantification of seven iodinated X-ray contrast media and three artificial sweeteners in surface, ground, and drinking water. Waldemar Ens, Frank Senner, Benjamin Gyga & Götz Schlotterbeck. *Analytical and Bioanalytical Chemistry*. May 2014, Volume 406, Issue 12, pp 2789-2798.

Table 2. Recoveries ($n=3$) for the sample enrichment step (a), matrix effects investigated by spiking 250 ng/L ICM and 500ng/L AS (b), and total recoveries (c) for Rhine, ground, and drinking water.

