Quantification of Pesticides at Low ppt Levels in Water Using Direct Aqueous Injection LC/QQQ Mass Spectrometry

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Introduction

Analysis of pesticides in drinking water has traditionally been performed using liquid-liquid extraction or off-line solid phase extraction followed by GC/MS or GC/MS/MS analysis. Lately, with the introduction of robust LC/MS/MS systems offering high sensitivity analysis of pesticides, this technology has become the preferred technique for the analysis of polar to intermediate polar pesticides. The scope of this work was to develop fully automated methods for the quantitative analysis of some 31 pesticides, including both active compounds and breakdown products.

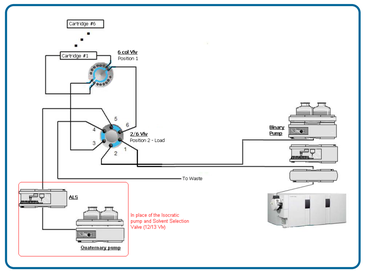
This work was done as a direct response to a new legislation in Denmark taken into action January 1, 2012.

Denmark, with about 2/3 of cultivated area, is seen as a pioneer in the field of pesticide metabolite analysis. Almost all drinking water comes from ground water, opposite the situation in the neighboring countries like Sweden, Norway and Finland.

The method requirements featured extraordinary sensitivity with a Lower Limit of Quantification (LLOQ) at or below 10ppt, and a coefficient of variation below 5% at 10-20 times the LLOQ.

In order to achieve the required sensitivity an LC/QQQ system equiped with optional on-line SPE system (Fig.1) was selected. The instrument setup below can be used both for large volume injections into a reusable SPE cartridge or with direct injectons on an analytical column. The valve setup allows for up to 6 SPE cartridges.

Fig.1. Instrument setup of Agilent on-line SPE LC/QQQ system

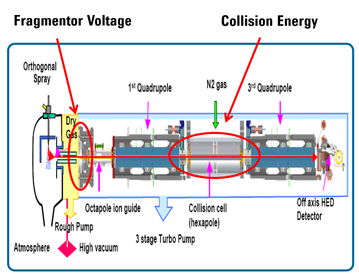


Experimental

Drinking water samples were collected and analyzed using a fully automated high performance liquid chromatography triple quadrupole mass spectrometry system. The modular HPLC/QQQ system consisted of a degasser, a binary high pressure mixing pump, an automated liquid sample handler, a thermostatted column compartment (Agilent Technologies) and a triple quadrupole mass spectrometer model 6460 equipped with a Jet Stream ion source (Agilent Technologies). An aliquot of 100µL of water was injected on a Zorbax Eclipse Plus C18, 2.1x150mm, 3.5µm (Agilent Technologies) column and separated using gradient conditions. Data was collected using acquisition in MRM mode acquiring one or two transitions per compound. Data was evaluated using MassHunter Quantitative Analysis software version B.05.00 (Agilent Technologies). In total three separate methods were developed. Method one consisted of compounds ionizing in positive ion mode, method two compounds ionizing in negative ion mode. Finally method three was used for the analysis of glyphosate and AMPA post derivatization using fmoc. The methods developed were setup and implemented on four additional contract laboratories and results were compared. The compounds evaluated and method performance was according to table 1. Values are an average of the method performance at the four laboratories tested.

Optimisation of MRM conditions were done using the automated MassHunter Optimizer software version B.04.00 (Agilent Technologies) allowing optimization of fragmentor voltage and collision energy of multiple compounds in one single injection.

Fig. 2. Cross sectional of an Agilent LC/QQQ system



Method Development

As the compounds were highly related consisting of both active components and several metabolites (e.g. Atrazine and Metribuzine) it was of crucial importance to avoid any possibility to detecting the wrong compound. Hence all compounds were injected as single standards and every single standard were checked for contamination or coelution to a potential metabolite sharing the same product ion. In addition the sensitivity requirement stressed optimization of additional source parameters and settings of the ion optics. In figure 3 below the effect of optimizing the Nozzle voltage can be seen. Figure 4 to the right is an overview of the Agilent Jet Stream ion source.

Fig. 4. Agilent Jet Stream ion source

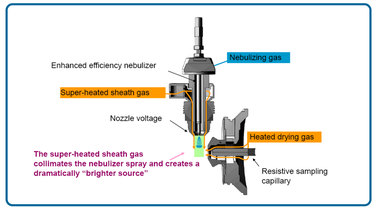
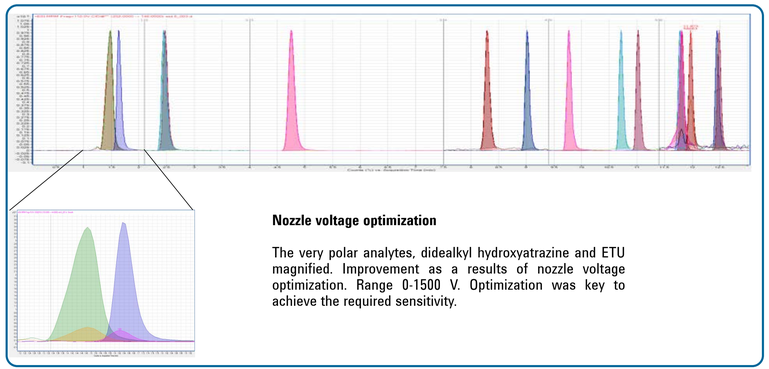


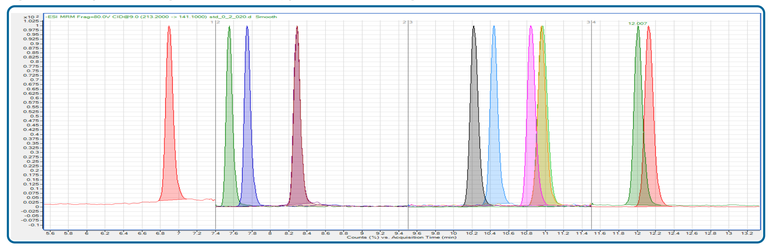
Fig 3. Chromatogram of the positive ion suite at 0.2µg/L level. All chromatograms normalized to 100%.



Nozzle voltage optimization

The very polar analytes, didealkyl hydroxyatrazine and ETU magnified. Improvement as a results of nozzle voltage optimization. Range 0-1500 V. Optimization was key to achieve the required sensitivity.

Fig 5. Chromatogram of the negative ion suite at 0.2µg/L level. All chromatograms normlized to 100%.



Results and Discussion

Summary of method developed

In total three separate methods were developed. The total length of the chromatographic run did not exceed 15 min on any method. The mobile phases used were as follows:

Method 1. Positive ion suite. A: 5mM ammoniumformiate B: Methanol
Method 2. Negative ion suite. A: 0.1% acetic acid B: Acetonitrile
Method 3. Glyphosate/AMPA. A: 2.2mM ammoniumacetate B: Acetonitrile

Table 1. Method performance

Compound Name	CAS. NR	RSD (%) 0.2µg/L n=20	RSD (%) 0.01µg/L n=10
2,4-D	94-75-7	1.6	7.9
MCPA	94-74-6	1.5	4.9
Mechlorprop	7085-19-0	1.7	3.5
Diuron	330-54-1	1.1	1.2
ETU	96-45-7	1.5	3.6
Metribuzin	21087-64-9	1.3	2.5
4-CPP	3307-39-9	1.3	6.4
Atrazin	1912-24-9	0.8	1.6
Bentazon	25057-89-0	1.6	5.1
2,6-DCPP	25140-90-3	2.0	4.7
DEIA	3397-62-4	1.9	4.7
2-Hydroxy-atrazin	2163-68-0	1.2	2.7
Desethylatrazin	6190-65-4	1.5	3.4
Hexazinon	51235-04-2	1.9	1.0
Glyphosate	1071-83-6	2.0	4.3
Dichlorprop	7547-66-2	1.7	7.0
Desisopropyl atrazin	1007-28-9	1.9	4.6
BAM	2008-58-4	1.4	3.9
AMPA	1066-51-9	2.4	5.1
Desethyl-2-hydroxyatrazin	19988-24-0	1.0	1.8
Desethyl-terbutylazin	30125-63-4	1.1	3.1
Simazin	122-34-9	1.1	2.4
Desisopropyl-atrazin	1007-28-9	2.2	3.5
Desisopropyl-hydroxy-atrazin	7313-54-4	1.9	4.5
Didealkyl-2-hydroxy-atrazin	645-92-1	3.1	11
2-hydroxysimazin	2599-11-3	1.4	2.6
Metribuzin-desamin-deketo	52236-30-3	1.7	10.1
Metribuzin-diketo	56507-37-0	4.1	10.3
Metribuzin-desamino	35045-02-4	0.9	2.3
2,6-Dichlorbenzoacid	50-30-6	2.6	8.7

Summary of method validation

Each standard solution containing all pesticides, was indiviually prepared at every single laboratory. Standard curves, ranging from 0.01 to 1.0µg/L, were linear using a linear curve fit and weightning 1/x with an r^2 value at or above 0.998 for all compounds. Reproducibility values for level 0.2µg/L and 0.01µg/L are based on consecutive injection of standards. RSD values are average values of the four laboratories where the methods were implemented. The absolute deviation of results based on two individually prepared standards ranging from 15.5% (didealkylhydroxy atrazine at 0.01µg/L level) down to 2.8% (2,6-dichlorobenzoic acid at 1.0µg/L level). The difference in signal between the laboratories is within 12% for all compounds using optimized conditions (tested at 0.2µg/L on same standard).

Conclusions

The methods developed shows an extraordinary reproducibility within as well as between laboratories. Sample results have, to a large extent, proven to be highly user independant. However, during method implementation on site some critical parameters have been indentified. First, it is of crucial importance that high quality solvents and buffers are used for preparations of mobile phases and standards. Second, standards and mobile phases need to be freshly prepared. Third, the importance of a well maintained ion sources, in particular for the analysis in negative ion mode, can never be overestimated.

References:
Journal of AOAC International, 1732-1747, Vol. 93, No 6, 2010. *Christer Jansson, Jenny Kreuger*