DETECTION OF PHENYUREA RESIDUES IN OYSTERS USING EFFECTIVE PURIFICATION AND LIQUID CHROMATOGRAPHY-ELECTROSPRAY-MASS SPECTROMETRY

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Introduction

Phenylurea herbicides are extensively used in several European countries to control weed on crops, railways and garden. Scientific studies demonstrated the toxicity of both phenylurea and aromatic amines. Thus, several methods are available to analyse these compounds in water and soil samples (80/776/EEC directive). However, pesticide analysis on animal matrices stay few studied.

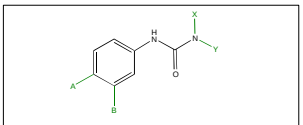
In this survey, we investigated the transfer possibility of phenylurea residues between application zones and estuarine bays where marine molluscs lives. We developed and validated a specific method to analyse these herbicides in oysters.

Phenylurea residues

Four phenylurea herbicides were studied, chlortoluron, diuron, isoproturon and linuron (cf fig 1a), with their demethylated and demethoxylated degradation products (X and Y losses).

The aniline residue was not followed because of its lack of specificity as phenylurea residue.

An internal standard, 4-bromoacetanilide (cf fig 1b), has been selected to control the analytical process.



herbicide	A	B	X	Y
chlortoluron	CH_3	Cl	CH_3	CH_3
diuron	Cl	Cl	CH_3	CH_3
isoproturon	$(CH_3)_2CH$	H	CH_3	CH_3
linuron	Cl	Cl	CH_3	OCH_3

Fig 1a : phenylurea herbicides

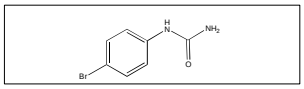


Fig 1b : 4-bromoacétanilide

Analytical strategy

Fig 2a : lyophilisation system

Fig 2b : ASE system

Sample preparation
- 20 g of oyster tissue + internal standard
- Lyophilisation (cf fig 2a) during 48 h
- Pulverisation of the lyophilised sample

Extraction
- Accelerated Solvent Extraction (cf fig 2b) - 100 bar / 60 °C - dichloromethane/acetone (50:50, v/v)

Purification
- Solid Phase Extraction 1 : CN/SiOH (0,5 g/1 g, 6 mL) elution with 6 mL of ethylacetate/methanol (20/80, v/v) followed by 6 mL of methanol
- Liquid/liquid partition : lipids are eliminated with hexane
- Solid Phase Extraction 2 : OASIS MCX (150 mg, 3 mL) elution with 3 mL of methanol followed by 3 mL of methanol with ammoniac 2 %
- Solid Phase Extraction 3 : C18 (1 g, 6 mL) elution with 6 mL of methanol/water (80:20, v/v) + external standard

Detection
- LC-ESI-MS/MS detection : octadecyl column, 2 transitions per compound

LC-MS/MS Detection

Fig 3 : LC-MS/MS system

HPLC gradient

A : methanol
B : 0,5 % of acetic acid

Time	0	10	15	25 min
A	40	55	55	100 %
B	60	45	45	0 %

Column

Uptispher ODS (50 x 2 mm, 3 µm) (Interchim-France)

Interface

Electrospray in positive mode (ESI+)

capillary voltage : 4,5 kV
cone voltage : 15 - 35 V

nebulisation flow : 90 L/h
desolvatation flow : 600 L/h with nitrogen

Mass spectrometer

Detection in multireaction monitoring mode (MRM)

gas cell pressure : 4.10^{-4} mbar
collision energy : 15 - 40 V

two transitions per compound

Validation

1 - Detection and identification limits determination

On the basis of ten blank sample extracts and a calibration curve, we determined both limits of detection and identification for each of ten phenylurea residues. The mean limits are :

LOD : 2,6 ppb **LOI : 2,7 ppb**

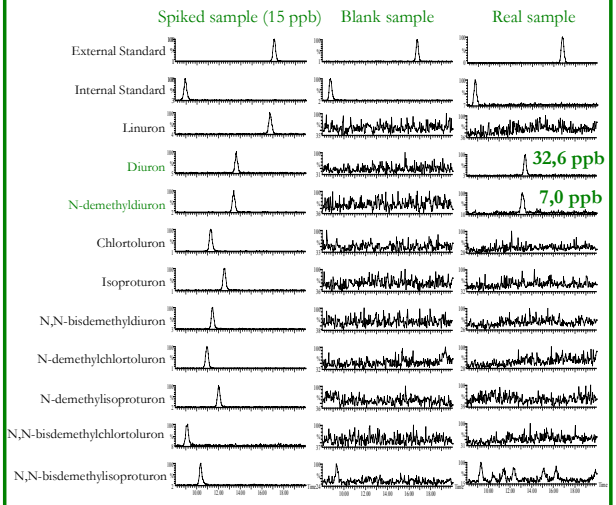
Retention time and transition ratio are used as identification criteria, according to the DG/SANCO 1805/2000 decision of the European Union.

2 - Validation of performance characteristics

Analytical performances of the method have been evaluated at the quantification limit (**3 ppb**). A calibration curve and ten blank samples surrogated at the quantification limit led to the results presented hereafter :

repeatability deviation : 11,6 %
trueness deviation : 5,1 %
recovery yield : 41,9 % with CV : 11,6 %
linearity of the extracted calibration curve : R^2 : 0,989

Sample analysis



The most intense transition of each monitored analyte is represented above.

Conclusion

We developed an effective method to quantify phenylurea in oyster; repeatability, trueness and quantification limit were evaluated. Few samples found to be contaminated by phenylurea residues, where both herbicide and the respective dealkylated phenylurea are present for each case.

It would be relevant to follow aniline residues to compare the behaviour of the different degradation products. The technique will be applied to other exposed shellfish, to compare differences between eventually contamination profiles.