STABLE ISOTOPE DILUTION ANALYSIS OF ORGANIC POLLUTANTS USING LC-MS/MS (QqQ) AND ISOTOPE PATTERN DECONVOLUTION

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INTRODUCTION

LC-MS/MS equipped with a triple quadrupole (QqQ) in the Selected Reaction Monitoring (SRM) has been widely accepted as the main tool in the identification, structural characterization and quantitative determination of semi-polar and polar organic pollutants in food and environmental samples The main limitation of these technique for quantitative purposes is the ion suppression caused by matrix components when using an electrospray ion source. The use of stable isotopically labelled compounds as internal standards (deuterated or ^{13}C-labelled) is normally the preferred strategy to correct for such problem. To this end, a calibration graph is required using the natural and labelled intensity ratio of the more abundant ions. Because of that, the labelled compound in organic dilution is usually selected to provide no mass overlap with the unlabelled analogue.

Isotope Pattern Deconvolution (IPD) appears as a promising alternative to improve the determination of organic pollutants, since allows spectral overlap between natural and labelled clusters as well as not require any isotope dilution calibration graph. Briefly, this alternative approach is based on the determination of the molar fractions for each pure isotope pattern (natural abundance and labelled) contributing to the isotope pattern observed in the mixture by multiple linear regression. A simple equation is employed for the determination of the concentration of the analyte as the ratio of molar fractions is equal to the ratio of molar concentrations in the mixture.

Nevertheless, the application of Isotope Dilution Mass Spectrometry (IDMS) using LC-MS/MS (QqQ) in SRM mode is not straightforward. In such a case, each precursor ion selected in the first Q may contain different isotope compositions owing to the presence of ^{13}C, ^{15}N, ^{37}Cl… atoms in the molecule, which may result in different product ions measured in the second quadrupole, for the same neutral loss of mass. Consequently, in order to carried out the accurate deconvolution of the isotope patterns of the natural and labelled analyte, the distribution of the above mentioned product ions has to be taken into account.

In the present work, an Isotope Dilution Analysis methodology based on the IPD approach has been applied for the first time to LC-ESI-MS/MS (QqQ) in SRM mode. The results obtained from the deprotonated and fragment ion clusters were compared to evaluate the suitability of the proposed methodology. In addition, the developed method was also tested in order to improve the confirmation at low concentrations of the target compound. As a proof of concept, we have selected the determination of the pharmaceutical diclofenac, since the labelled analyte diclofenac-d_4 provides spectral overlap. It has to worth noting that only 3 transitions were used in the calculation, making possible the application of the present methodology to the simultaneous determination of other pharmaceuticals.

EXPERIMENTAL

LC-MS/MS

Instrument: Waters Acquity Binary Solvent and Sampler Manager

Column: Acquity UPLC BEH C18, 1.7 µm, 50 x 2.1 mm i.d. (Waters)

Flow rate: 0.3 mL/min

Injection volume: 20 µL

Solvent A: H_2O 0.1 mM NH_4Ac; 0.01% HCOOH

Solvent B: MeOH 0.1 mM NH_4Ac, 0.01% HCOOH

Gradient:

Time (min)	0	1.5	2	3	5	6	7	7.1	9
%Solvent B	5	5	30	50	70	90	90	5	5

Analyzer: QqQ(TQD, Waters) using an orthogonal Z-spray-electrospray interface

ESI: 3.0 kV (ES-)

Block temperature : 120ºC

N_2 Desolvation flow : 1200 L/h

Desolvation temperature : 500ºC

Cone gas flow: 60 L/h

Collision gas: Ar C-50, 4e-3 mbar

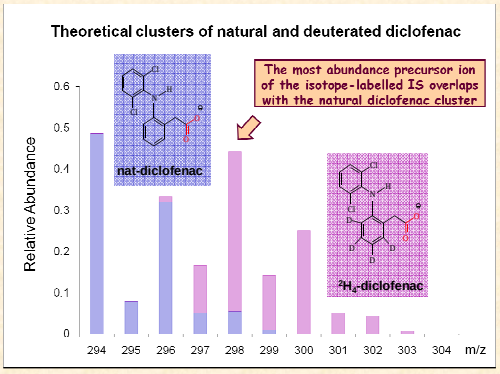
Dwell time: 0.01 s

MS/MS optimized conditions for nat and D4-diclofenac

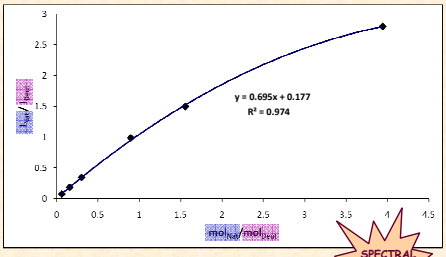
Compound	Mode (ES)	Q Transition	Cone (V)	C.E. (eV)	q Transition	C.E. (eV)
nat-Diclofenac	-	294.2 > 250.2	25	10	296.2 > 252.1	25
$^2H_4$-Diclofenac	-	298.2 > 254.1	25	10	300.2 > 256.2	25

RESULTS AND DISCUSSION

Theoretical clusters of natural and deuterated diclofenac



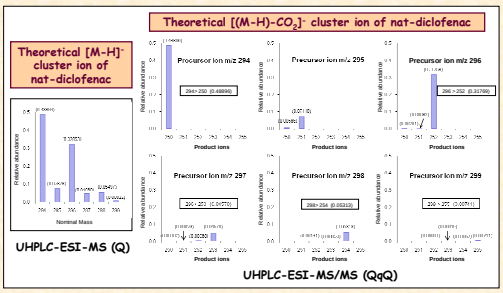
Calibration graph using intensity ratios



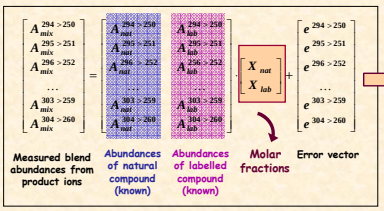
Steps for IDA using LC-ESI-MS/MS (QqQ)

1. Development of IPD equations: from single Q to QqQ
2. Calculation of the deuterium enrichment of diclofenac-d_4
3. Cluster purity of natural and diclofenac-d_4
4. Determination of the concentration of diclofenac-d_4
5. Study of the ionization behavior of natural and diclofenac-d_4
6. Validation of the procedure and calculation of uncertainty

1. Development of IPD equations: from single Q to QqQ



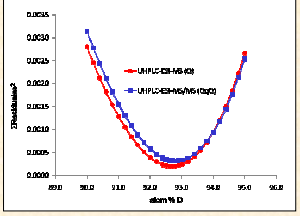
The isotopic composition of the SRM transitions measured in the QqQ is assumed to be a linear combination of two isotope patterns: the isotope pattern of the product ions selected of the natural abundance compound and the labelled abundance ones



Only 3 transitions were selected to calculate the molar fractions:
294 > 250
296 > 252
298 > 254

2. %D enrichment of diclofenac-d_4

The cluster of the labelled IS is calculated for different values of deuterium enrichment and compared with the measured one. The best match was obtained for 92.8 atom % D

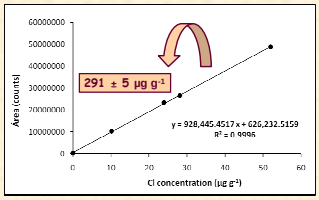


3. Cluster purity calculation

		Relative abundances (%) M-H	M	M-2H
UHPLC-ESI-MS (Q) nat-diclofenac	MilliQ	100.0 (1.1)	1.2 (2.2)	-2.3 (4.0)
	SW	100.4 (3.2)	0.5 (4.9)	-0.5 (6.1)
	EWW	100.4 (0.9)	0.2 (1.7)	-1.3 (3.2)
	IWW	101.2 (0.9)	-0.9 (1.6)	0.2 (2.9)
UHPLC-ESI-MS (Q) $^2H_4$-diclofenac	MilliQ	100.0 (2.1)	1.1 (1.4)	-1.6 (2.5)
	SW	101.0 (2.0)	0.4 (2.3)	-1.5 (2.7)
	EWW	100.0 (2.1)	1.1 (1.4)	-1.8 (1.5)
	IWW	97.5 (1.5)	1.6 (1.5)	0.0 (1.4)
UHPLC-ESI-MS/MS (QqQ) nat-diclofenac	MilliQ	99.7 (0.7)	0.3 (1.8)	0.9 (2.9)
	SW	100.5 (1.8)	-1.2 (3.9)	1.0 (3.9)
	EWW	99.5 (0.9)	1.8 (2.5)	-1.7 (4.8)
	IWW	99.7 (1.2)	0.8 (2.5)	-0.1 (4.3)
UHPLC-ESI-MS/MS (QqQ) $^2H_4$-diclofenac	MilliQ	99.5 (1.4)	0.9 (1.3)	0.0 (1.1)
	SW	100.0 (3.1)	-1.4 (3.2)	1.4 (1.9)
	EWW	99.5 (1.7)	1.3 (1.4)	-0.5 (1.5)
	IWW	98.9 (1.5)	1.2 (1.4)	0.1 (1.2)

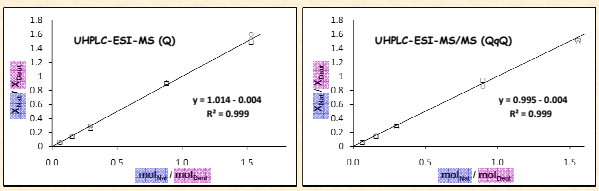
4. Concentration of diclofenac-d_4

To avoid possible isotopic effects in the ion source, the quantification of the spike was performed by external calibration using microHPLC-ICP-MS, monitoring the ^{35}Cl coming from the natural and isotope-labelled diclofenac



5. Study of the ionization behavior of natural and diclofenac-d_4

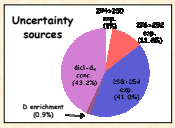
Application of multiple linear regression in the IDMS experimental calibration graph. The plot of the molar fractions X_{nat} and X_{lab} (calculated using both single Q and QqQ by triplicate) vs. the molar ratio between natural and labelled compound in the sample did not show any noticeable isotopic effect



6. Validation of the IPD methodology

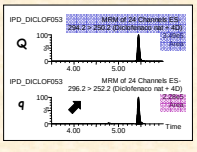
Instrumentation	Recovery (%) Milli-Q	SW	EWW	IWW
UHPLC-ESI-MS (Q)	101.2 ± 3.0	96.7 ± 4.2	-	-
UHPLC-ESI-MS/MS (QqQ)	105.7 ± 2.2	99.5 ± 3.2	99.1 ± 9.8	103.1 ± 4.5

The better selectivity of the MS/MS (QqQ) allows the accurate determination of diclofenac both in EWW and IWW



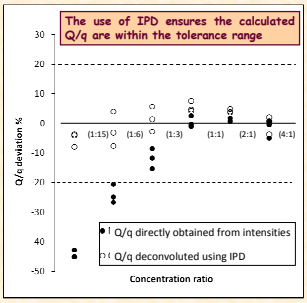
Application of IPD in confirmation

The contribution of the labelled compound to the confirmation transition (q) hampers the accurate calculation of the Q/q



Q/q correction with IPD

The use of IPD ensures the calculated Q/q are within the tolerance range



CONCLUSIONS

- For the first time, UHPLC-MS/MS (QqQ) in SRM mode has been employed for the development of an isotope dilution analysis methodology taking profit of the stable isotope-labelled internal standards (ISs) used to correct the ion suppression caused by matrix components in the electrospray ion source.
- Isotope Pattern Deconvolution provides the molar fractions without requiring a calibration curve and making possible mass overlapping between the natural and labelled compound, which allows minimal labelling and could improve the confirmation at low concentrations of the analyte.
- The methodology has been applied to the determination of diclofenac in influent and effluent waste water. To this end, the deuterium enrichment and concentration of diclofenac-d_4 as well as the theoretical abundances of the product ions selected were accurately calculated. The total combined uncertainty from the different uncertainty sources was also studied, being the concentration of the IS the major contributor to the total uncertainty.

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