Comparison of different quantification approaches to deal with matrix effects in LC-ESI-MS/MS based determinations of mycotoxins in selected spices

A.F. Roig-Navarro[1], N. Fabregat-Cabello[1], Juan V. Sancho[1], H.G.J. Mol[2], P. Zomer[2]

[1]Research Institute for Pesticides and Water, Universitat Jaume I, E-12071, Castellón, Spain

[2]RIKILT Institute of Food Safety, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands

e-mail: roig@uji.es, phone: + 34 964387359, FAX: + 34 964387368

ThPS37-17

20th IMSC International Mass Spectrometry Conference Geneva, Switzerland August 24–29, 2014

Overview

Mycotoxins are products of the metabolism of some fungi usually found in substrates of vegetable origin. Due to the health problems they can induce, the European Union establishes maximum levels of some mycotoxins in food and feed.

In order to determine the mycotoxins at the levels required by legislation a wide range of analytical methods are developed each year. A clear trend can be seen to develop multi-mycotoxin methods based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the simultaneous identification and quantification of the sought compounds.

Quantitative performance of LC-MS/MS can be affected by the matrix effect, especially when an electrospray ionization source (ESI) is used. The evaluation of the matrix effect can be performed by the post-extraction addition method, based on the comparison of peak areas in solvent and sample matrix, and by post-column infusion, where fluctuations in the signal of the target compound, added to the eluent, is monitored after injection of a blank sample extract.

A wide variety of strategies can be employed in order to overcome the matrix effect. Among them, compensating matrix effect through calibration approaches includes the standard addition method and the use of internal standards (preferably the isotope-labelled analogue).

In standard addition method, a priori knowledge of the sample composition is not required which facilitates the analyses of composite products (e.g. multi-ingredient food products, compound feed). The use of isotopically labelled analogues as internal standards could be an efficient way to compensate for matrix effects, although availability and cost may limit the applicability in routine practise. A common approach is to normalize the areas, both in samples and standards, to that of the labelled compound. A more straightforward way of using the isotope labels is one-point isotopic internal calibration (OPIC), where the labelled compound is added to the sample as well but no external calibration standards are used. The concentration in the sample is calculated based on the response of the label in that same sample. One more method, the isotope pattern deconvolution (IPD) quantification procedure is based in multiple linear regression. For the last two methodologies only one injection is required for both analysis of the sample and calibration.

An alternative approach is the use of the signal suppression (or enhancement) measured for one specific 'matrix-marker' substance monitored throughout the chromatographic run, to compensate for the matrix effect. This idea from Stahnke (Stahnke et al., 2009) relies on their observation that different compounds experience almost the same matrix effect at the same retention time.

Here we present a comparative study of different strategies to account for matrix effects in LC-ESI-MS/MS. First, the applicability of the approach proposed by Stahnke et al. to mycotoxins in highly complex matrices was investigated. Subsequently, we compared the following calibration approaches to compensate for matrix effects with respect to accuracy and efficiency: multi-level external calibration using isotopically labelled internal standards, the standard addition method (multi-level and single level), and two single-injection methods (OPIC and IPD).

Stahnke, H., Reemtsma, T. and Alder L. ,2009. Compensation of matrix effects by postcolumn infusion of a monitor substance in multiresidue analysis with LC-MS/MS. Analytical chemistry 81: 2185–92.

Experimental

Compounds and matrices selected

Compounds:

aflatoxin B_1, deoxynivalenol, fumonisins B_1, B_2 and B_3, ochratoxin A, T-2 and HT-2 toxins and zearalenone

Matrices:

curcuma

nutmeg

Sample treatment

2.5g of sample
10 ml extraction solution:
ACN/water/formic acid (86:16:1)

1. Extraction: 2 h
2. Centrifugation: 5min/3000 rpm

200 µL extract
200 µL water

3. Vortexing: 3 s.
4. Refrigerator:30 min
5. Filter the extracts by pressing down the filter

Instrumental conditions

Instrumentation:
Waters Micromass Quattro Ultima (ESI+/ESI- (ZEA in curcuma))

Column:
Restek, Ultra aqueous C18, 3 µm, 100x2.1 mm

Mobile phase:
MeOH/H_2O modified with ammonium formate solution (1mM) and formic acid (1% v/v)

Compound	Rt (min)	Precursor ion	Cone vol. (V)	SRM transitions [1] Native compound	SRM transitions Isotopic label	SRM transitions IPD
DON	2.8	$[M+H]^+$	20	297.0 > 249.1 (10) 297.0 > 231.1 (10)	312.0 > 263.1 (10)	297.0 > 249.1 (10) 298.0 > 250.1 (10) 312.0 > 263.1 (10)
AFB_1	5.2	$[M+H]^+$	30	313.1 > 285.1 (20) 313.1 > 269.1 (30)	330.1 > 301.1 (20)	313.1 > 285.1 (20) 330.1 > 301.1 (20) 330.1 > 300.1 (20)
HT-2	5.2	$[M+NH_4]^+$	20	442.2 > 263.1 (10) 442.2 > 215.1 (15)	464.2 > 278.1 (10)	442.2 > 263.1 (10) 443.2 > 264.1 (10) 464.2 > 278.1 (10)
FB_1	5.5	$[M+H]^+$	30	722.2 > 334.2 (40) 722.2 > 352.2 (30)	756.2 > 356.2 (40)	722.2 > 334.2 (40) 723.2 > 335.2 (40) 756.2 > 356.2 (40)
T-2	5.8	$[M+NH_4]^+$	20	484.2 > 185.1 (20) 484.2 > 305.1 (10)	508.2 > 198.1 (20)	484.2 > 185.1 (20) 485.2 > 186.1 (20) 508.2 > 198.1 (20)
FB_3	5.9	$[M+H]^+$	30	706.2 > 336.2 (40) 706.2 > 318.2 (40)	740.2 > 358.2 (40)	706.2 > 336.2 (40) 707.2 > 337.2 (40) 740.2 > 358.2 (40)
FB_2	6.1	$[M+H]^+$	30	706.2 > 336.2 (40) 706.2 > 318.2 (40)	740.2 > 358.2 (40)	706.2 > 336.2 (40) 707.2 > 337.2 (40) 740.2 > 358.2 (40)
OTA	6.6	$[M+H]^+$	30	404.2 > 239.1 (30) 404.2 > 221.1 (35)	424.2 > 250.1 (30)	404.2 > 239.1 (30) 406.2> 241.1 (30) 424.2 > 250.1 (30)
ZEA (+)	6.6	$[M+H]^+$	20	319.3 > 283.0 (10) 319.3 > 185.1 (30)	337.3 > 301 (10)	319.3 > 283.0 (10) 337.3 > 301.0 (10) 336.3 > 300.0 (10)
ZEA[2] (-)	6.8	$[M-H]^-$	20	317.1 > 175.1 (25) 317.1 > 273.1 (20)	335.1 > 185.1 (25)	317.1 > 175.1 (25) 335.1 > 185.1 (25) 334.1 > 184.1 (25)

[1]Information in brackets: (Collission energy (eV), bold = Quantifier; IPD: Isotope Pattern Deconvolution.
[2]Zearalenone analysed by ESI^- with a different mobile phase composition compared with the positive run

"Matrix marker" used to quantify ME

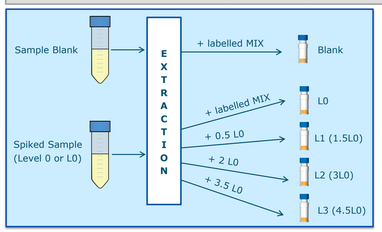
Carbendazim, ^{13}C-caffeine and chlormequat (conc. 2.5 ng/mL) added to mobile fase

$$ME(\%) = \left[\frac{Signal\ intensity\ (sample\ extract)}{Signal\ intensity\ (in\ solvent)} \cdot 100\right] - 100$$

ME by post-extraction addition

$$ME(\%) = \left[\frac{Area\ (in\ matrix)}{Area\ (in\ solvent)} \cdot 100\right] - 100$$

Comparison of quantification approaches



Multi-level external calibration using IL-IS (^{13}C-labelled analogues, at L0 level)

Multi-level standard addition

Single-level standard addition

One-point isotopic internal calibration (OPIC)

Isotope Pattern Deconvolution (IPD)

L0 (µg/g in samples): AFB_1, 0.005; DON, 1; FB_1, 0.5; FB_2, 0.2; FB_3, 0.1; HT-2, 0.1; OTA,0.01; T-2, 0.1; ZEA, 0.25.

Results and Discussion

"Matrix marker" used to quantify ME

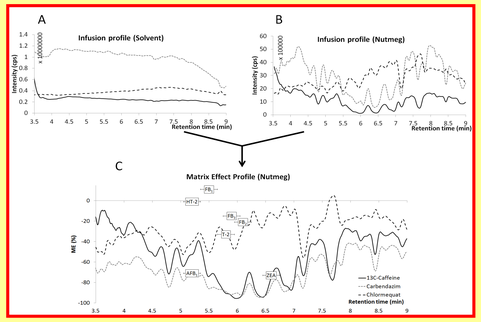


Figure 1. (A) Signal profile for ^{13}C-caffeine, carbendazim and chlormequat after the injection of the reference solvent and (B) after the injection of a nutmeg extract in a second run. (C) Matrix Effect Profile calculated as the ratio of the two infusion profiles together with the matrix effects obtained by post-extraction addition at each retention time for each mycotoxin. Line length for each mycotoxin matches its peak width

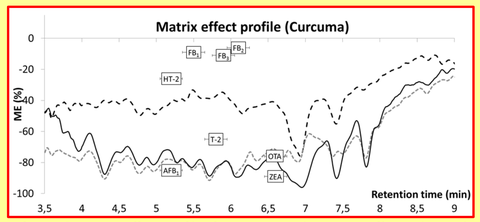


Figure 2. Matrix effect profile for curcuma. The matrix effects obtained by post-extraction addition at each retention time for each mycotoxin are also included.

Comparison of quantification approaches

Table 1. Matrix effect and recoveries[a] in percentage of mycotoxins in **nutmeg** using different calibration approaches.

		Multi-level calibration		Single-level calibration					
					Standard addition			Internal calibration	
Compound	Matrix effect±SD	Solvent STD w/o IL-IS	Solvent STD with IL-IS	L1-L3	L1	L2	L3	OPIC	IPD
		Recovery (RSD) n=3, three different days)							
Deoxynivalenol	-54 ± 5	**41** (2)	92 (9)	92 (17)	103 (**28**)	92 (9)	95 (15)	104 (12)	97 (12)
Aflatoxin B_1	-71 ± 5	**28** (20)	95 (13)	102 (14)	115 (**46**)	99 (**29**)	103 (20)	94 (16)	100 (17)
HT-2 toxin	-1 ± 13	85 (9)	103 (6)	94 (3)	128 (**32**)	95 (18)	102 (5)	98 (10)	107 (9)
Fumonisin B_1	11 ± 5	97 (10)	90 (9)	95 (25)	115 (30)	89 (12)	102 (29)	108 (20)	99 (20)
T-2 toxin	-33 ± 10	65 (16)	96 (10)	100 (25)	**142** (**37**)	101 (10)	112 (**32**)	111 (5)	99 (5)
Fumonisin B_3	-15 ± 9	90 (9)	105 (13)	113 (25)	108 (23)	105 (12)	118 (30)	**125** (10)	111 (10)
Fumonisin B_2	-21 ± 8	72 (1)	104 (7)	90 (23)	94 (13)	92 (12)	93 (21)	**125** (9)	120 (9)
Ochratoxin A[b]	-	-	-	-	-	-	-	-	-
Zearalenone	-73 ± 4	**28** (10)	112 (11)	100 (4)	**175** (**29**)	112 (17)	110 (4)	112 (12)	114 (13)

[a]Figures in red/bold: recoveries or RSDr outside range EU 519/2014. [b]The chromatographic peak was overlapped by an isobaric interference.
STD = standard, IL-IS = isotopically labelled internal standard; L1-L3 standard addition levels (see Experimental); OPIC: One-point isotopic internal calibration, IPD: Isotope Pattern Deconvolution.

Table 2. Matrix effect and recoveries[a] in percentage of mycotoxins in **curcuma** using different calibration approaches.

		Multi-level calibration		Single-level calibration					
					Standard addition			Internal calibration	
Compound	Matrix effect±SD	Solvent STD w/o IL-IS	Solvent STD with IL-IS	L1-L3	L1	L2	L3	OPIC	IPD
		Recovery (RSD) n=3, three different days)							
Deoxynivalenol	-42 ± 5	**56** (11)	95 (10)	92 (9)	104 (**22**)	99 (5)	95 (2)	108 (16)	100 (16)
Aflatoxin B_1	-85 ± 1	**13** (**26**)	107 (**26**)	100 (19)	69 (**29**)	88 (18)	92 (23)	111 (**39**)	119 (**36**)
HT-2 toxin	-26 ± 12	**56** (17)	101 (20)	91 (16)	81 (**53**)	84 (**36**)	86 (28)	96 (14)	106 (13)
Fumonisin B_1	-9 ± 9	82 (15)	65 (**35**)	77 (13)	102 (30)	92 (13)	84 (15)	84 (29)	77 (29)
T-2 toxin	-65 ± 1	**36** (6)	88 (6)	97 (13)	100 (**39**)	107 (9)	97 (9)	102 (6)	91 (6)
Fumonisin B_3	-11 ± 12	80 (10)	86 (**35**)	85 (25)	101 (25)	89 (7)	88 (12)	109 (28)	99 (29)
Fumonisin B_2	-6 ± 9	91 (11)	80 (**34**)	94 (11)	120 (17)	98 (3)	99 (9)	95 (**37**)	90 (**37**)
Ochratoxin A	-76 ± 11	**21** (20)	92 (18)	-	-	-	-	84 (**48**)	-[c]
Zearalenone	-89 ± 4	**6** (**34**)	77 (11)	82 (4)	86 (22)	76 (12)	77 (7)	96 (11)	100 (11)

[a]Figures in red/bold: recoveries or RSDr outside range EU 519/2014. [b]Unsatisfactory linearity were obtained for standard addition method. [c]No second transition available for IPD calculations.
STD = standard, IL-IS = isotopically labelled internal standard; L1-L3 standard addition levels (see Experimental); ICAL: Isotopic Internal Calibration, IPD: Isotope Pattern Deconvolution.

Conclusions

- The correction of the matrix effect by monitoring the signal of a continuously added substance was studied but without satisfactory results. The assumption that matrix effect mainly depends on retention time is not applicable for the mycotoxins and matrices in the present study. Otherwise, this approach permitted a qualitative evaluation of the signal suppression and enhancement phenomena at each retention time.
- The evaluation of absolute matrix effect exhibited by the electrospray source in the LC-MS/MS system showed that the signal was particularly supressed for DON and AFB_1 and ZEA.
- The great majority of recovery and RSD values were between 70-120% and below 20% respectively for standard addition method (both for multiple or single addition at higher concentration levels) and calibration curve with internal standard. Thus, those methodologies compensate the matrix effect suitably and trueness and precision meet the EU 519/2014 acceptance criteria. When suitable internal standard is not available, single standard addition methods can be the choice as it reduces considerably the total analysis time.
- This study has also demonstrated that single-point calibration approaches (OPIC and IPD) provide similar results, in terms of recovery and precision, to the values obtained with the whole calibration curve. Nevertheless, recoveries for single-point calculations with isotope labelled internal standards lead occasionally to unacceptable high recoveries.

The Dutch Ministry of Economic Affairs is acknowledged for financially supporting this work. The authors acknowledge the financial support from Generalitat Valenciana (Research group of excellence Prometeo 2009/054 and Collaborative Research on Environment and Food Safety ISIC/2012/016). N.Fabregat-Cabello also acknowledges the Generalitat Valenciana for her Ph.D. research grant under the Program VALi+D.