Analytical performance of an LC-MS/MS based method covering more than 600 fungal metabolites

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Introduction

Similar to other contaminant classes, LC-MS/MS based multianalyte methods have become more and more popular in the field of mycotoxins, as they are able to simultaneously cover all toxins addressed by regulatory limits, related derivatives and masked forms as well as "emerging" mycotoxins. However, there is still a need for official guidelines being adjusted to the methodological specifics of this approach. For instance, Commission Decision 2002/657/EC [1] does not address matrix effects at all, although these effects related to the ionization process are known to be the main limitation of the accuracy of LC-MS based methods. In addition, the term „recovery" is not specified in [1], which leaves room for speculation whether the related target range of 70-120% refers to the apparent recovery RA or to the recovery of the extraction step RE.

$$RA = \frac{area_{pre-extract\ spikes}}{area_{neat\ solvent\ standard}} \quad RE = \frac{area_{pre-extract\ spikes}}{area_{post-extract\ spikes}} \quad SSE = \frac{area_{post-extract\ spikes}}{area_{neat\ solvent\ standard}}$$

In the field of multi-residue analysis for pesticides, SANTE 11945/2015 [2] specifies these terms and states a target range of 70-120% for the recovery of the extraction step (with an addendum of lower values still being acceptable provided they exhibit a reasonable precision). As regards matrix effects, no particular range for the absolute value of SSE is given.

As matrix effects may be compensated by matrix-matched calibration (the availability of stable isotope labelled internal standards is limited mainly to mycotoxins addressed by regulatory limits and a few derivatives), the absolute value of SSE is not the most critical aspect in view of the accuracy of the method. It is rather relative matrix effects (differences in the absolute SSE values between different lots, varieties etc. of a given matrix) that have been identified as the main limitation for quantitative bio-analytical methods [3, 4]. Whereas an FDA workshop on bioanalytical method validation has stated an RSD < 15% to be acceptable for SSE values deriving from at least 6 different lots of biological fluids [4], similar recommendations are missing for multi-analyte methods for food-/feedstuffs. Indeed, the investigation of relative matrix effects is often neglected in multimycotoxin methods, as validation data derives from replicates of a single or a pooled sample. For this reason, we have validated our LC-MS/MS based method for more than 650 analytes (mycotoxins, fungal and bacterial secondary metabolites and a few plant toxins) for 7 different matrices (wheat, maize, figs, raisins, almonds, pistachios, walnuts) including 7 different individual samples per matrix in order to investigate both relative and absolute matrix effects.

Experimental

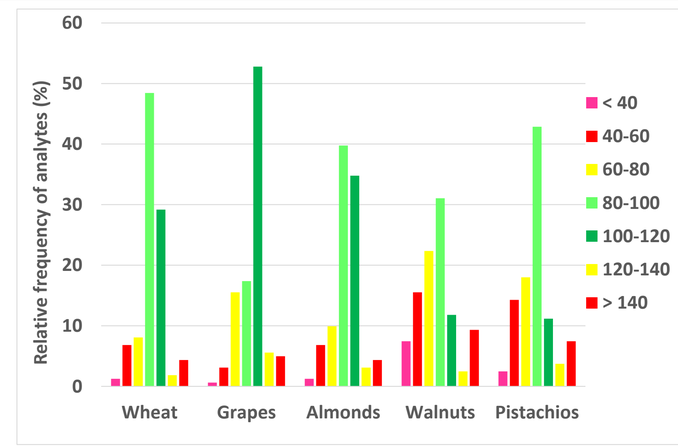
Individual samples were collected from different countries aiming at significant differences in variety, texture and colour (e.g. two white maize samples from Namibia and Ethiopia were included). Standards of mycotoxins and other microbial metabolites were purchased from Romer Labs (Tulln, Austria), AnalytiCon Discovery (Potsdam, Germany), BioAustralis (Smithfield, Australia), BioViotica (Dransfeld, Germany), Enzo Life Sciences (Lausen, Switzerland), Toronto Research Chemicals (Toronto, Canada), Fermentek (Jerusalem, Israel), Adipogen Life Sciences (Liestal, Switzerland) or were received as gifts from other researches.
Samples were spiked both before and after extraction on one concentration level (six concentration levels in case of maize and figs, pooled extract included). Samples are extraced using acetonitrile/water/acetic acid 79/20/1 at ratio of 4mL/g, followed by a 1+1 dilution using acetonitrile/water/acetic acid 20/79/1. External calibration is based on a multi-analyte stock solution serially diluted in acetonitrile/water/acetic acid 49.5/49.5/1. Injection volume was 5 µl.

Analysis was carried out with a QTrap 5500 MS/MS system (AB Sciex, Foster City, CA, USA) equipped with a TurboIon ESI-source and coupled to an 1290 series UHPLC system (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25°C on a Gemini C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 security guard cartridge, 4 × 3 mm i.d. (all from Phenomenex, Torrance, CA, USA). Elution was carried out in binary gradient mode with a flow rate of 1000 µl/min. Both mobile phases contained 5 mM ammonium acetate and were composed of methanol/ water/acetic acid 10:89:1 (v/v/v; eluent A) and 97:2:1 (v/v/v; eluent B), respectively. ESI-MS/MS was performed in scheduled multiple reaction monitoring (sMRM) mode both in positive and negative polarity in two separate chromatographic runs. Target scan time was 1 sec, retention time window width was 40 and 52 sec. in the positive and negative ionization mode, respectively. 2 MS/MS transitions are acquired per analyte, positive identification is verified based on retention time and ion ratios.

Results and Discussion

	LOQ[a](µg/kg)	maize	wheat	figs	raisins	almonds	pistachios	walnuts
Analyte	figs	maize	RA[b]	RE[b]	RA	RE	RA	RE	RA	RE	RA	RE	RA	RE	RA	RE
Aflatoxin B1	0.85	0.7	61±9	95±5	84±5	94±10	82±7	89±8	87±9	86±14	95±4	99±7	67±4	103±11	38±3	86±6
Aflatoxin G1	0.9	1.5	70±10	98±5	80±3	88±8	83±6	88±5	69±13	81±14	82±6	99±11	56±6	96±9	51±8	81±8
Ochratoxin A	1.4	1	98±3	90±3	97±5	93±8	87±10	85±8	112±7	96±9	98±4	96±7	86±3	83±4	86±6	81±4
Fumonisin B1	8.5	10	55±5	53±5	62±8	79±14	56±5	56±6	78±19	70±14	63±17	78±18	26±3	42±7	48±4	59±6
Fumonisin B2	6	6	62±4	59±4	74±10	89±11	74±7	72±7	94±12	91±18	80±18	92±18	31±2	53±4	46±8	68±8
Zearalenon	0.6	0.5	85±8	90±8	100±3	92±12	82±10	85±9	110±7	97±12	98±5	95±7	87±5	95±7	57±7	59±4
Deoxynivalenol	10	10	110±12	92±5	79±10	80±13	58±4	80±10	61±9	67±14	61±8	82±11	75±8	103±9	34±7	99±20
Nivalenol	6	4	72±5	85±5	60±8	70±12	12±2	15±2	43±2	49±9	61±7	66±15	101±13	111±18	63±6	73±8
T-2 Toxin	1.8	4	97±5	101±6	98±4	91±11	88±8	87±9	110±7	104±13	104±6	114±9	97±5	94±6	81±7	87±6
HT-2 Toxin	8	5	90±15	92±14	100±7	87±12	89±9	97±14	113±6	97±13	103±4	90±10	91±15	93±19	78±13	93±14
Citrinin	0.5	1.5	28±15	19±9	44±13	23±6	58±5	33±2	56±8	34±8	71±6	36±13	39±8	38±8	62±10	46±5
Moniliformin	2.2	n.a.	81±15	62±9	80±8	58±9	66±5	43±2	48±5	40±10	98±4	86±10	100±18	71±8	99±8	76±6
Enniatin B	0.03	n.a.	91±6	94±7	111±7	101±9	81±10	85±10	107±6	93±10	102±8	96±12	91±4	89±5	88±6	80±10
Sterigmatocystin	0.3	0.4	104±7	103±7	101±11	102±14	86±10	82±8	108±6	97±11	96±10	104±14	84±4	86±5	65±6	74±6
Ergometrin	11	13	117±11	102±18	105±8	108±15	80±8	69±10	115±12	131±16	146±15	141±21	71±5	69±7	69±7	75±7
Alternariol	0.35	2.3	80±12	86±10	86±7	97±12	86±8	96±6	104±12	97±18	102±3	99±12	75±9	85±8	22±3	68±9
Penitrem A	0.3	0.2	165±18	81±4	108±8	89±12	220±9	70±8	100±9	83±10	106±7	92±9	113±5	78±5	98±14	70±7

[a] calculated according to the EURACHEM guide [5] [b] average ± stdev in %



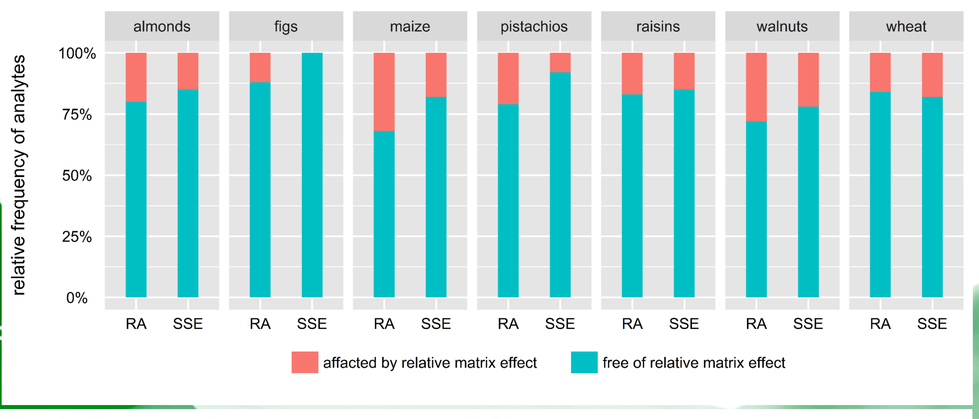


Fig.2: Fraction of analytes exhibiting RSDs exceeding current guidelines (> 20% for RA, > 15% for SSE).

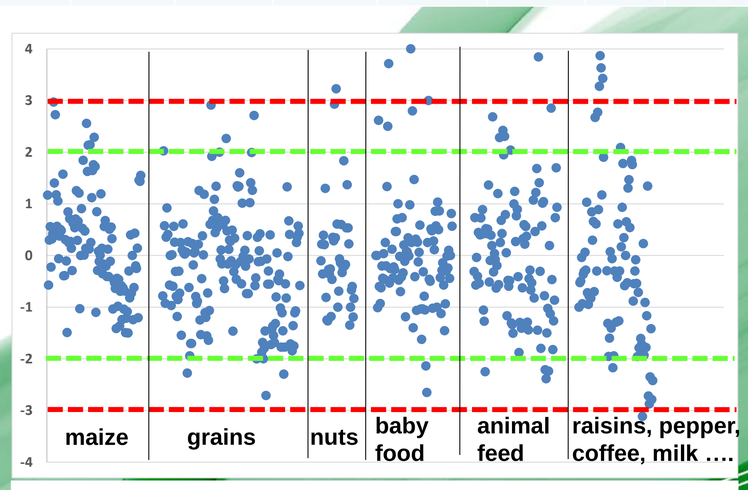


Fig.3: Performance in proficiency testing (BIPEA, 93.6% satisfactory z-scores, 746 results submitted)

Fig. 1 Distribution of apparent recoveries (top) and recoveries of the extraction step (bottom)

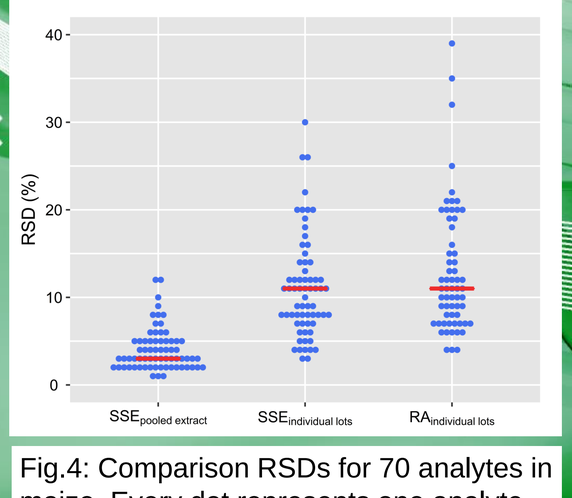


Fig.4: Comparison RSDs for 70 analytes in maize. Every dot represents one analyte.

Conclusion

- Only 57-83% of all RA values are within the target range of 70-120% (Fig. 1), whereas 84-93% of all values for RE comply to that criterium (exceptions being polar or strongly acidic compounds like moniliformin, nivalenol, fumonisins and citrinin).
- Results obtained in proficiency testing indicate that the method accuracy is fit for purpose even for analyte/matrix combinations exhibiting values outside 70-120% for RA (e.g. aflatoxins in nuts) or RE (fumonisins in maize). This suggests that the critical issue might be relative effects (i.e. unacceptable large variations betweenmatrix effects within individual samples of a given matrix) rather than the absolute value.
- The fraction of unacceptably high RSDs depends on the type of matrix (Fig. 2). It seems that relative matrix effects contribute significantly whereas extraction efficiency plays only a minor role (Fig. 4). This implies that method validation based on replicates from a single sample underestimates the measurement uncertainty.

Acknowledgement

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References

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