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Validation of the determination of organophosphorus and carbamate pesticides in milk, liver and meat using LC-MS/MS

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Introduction

According to Council Directive 96/23/EC, EU member states are required to establish an annual plan for monitoring of various residues and contaminants in products of animal origin. This includes carbamates (group B2c) and organophosphorus pesticides (OPPs) (group B3b). The latter have also been included in the EU coordinated monitoring program as laid down in 901/2009. In this work, a recently developed generic extraction/dilution procedure has been validated to replace traditional group-specific methods for determination of OPPs, carbamates and other residues in milk, meat and liver.

Experimental

Validation test set:
5 blank; 5-fold at 0.005 and 0.025 mg/kg
Milk: raw cow milk
Meat and liver: 4 species (pig, cow, chicken, horse)

Extraction [1]

5 g sample (add 1 ml water for meat/liver)
Add 15 ml MeCN/1% formic acid
Shake 30 min head-over-head
Centrifuge (=> 0.25 g matrix equivalent/ml)

[1] H.G. J. Mol, P. Plaza-Bolanõs, P. Zomer, T.C. de Rijk, A.A.M. Stolker, P.P. J. Mulder, Anal.Chem., 80 (2008) 9450–9459

LC-MS/MS analysis

5 µl injection on a Restek Ultra aqueous C18 (3µm; 100x2.1 mm) column.
Mobile phase: a gradient of H_2O:MeOH,
1 mM ammonium formate and 20 µl/L formic acid
Flow rate 400 µl/min.

MS/MS: AB Sciex 5500 Qtrap, two transitions for each analyte.

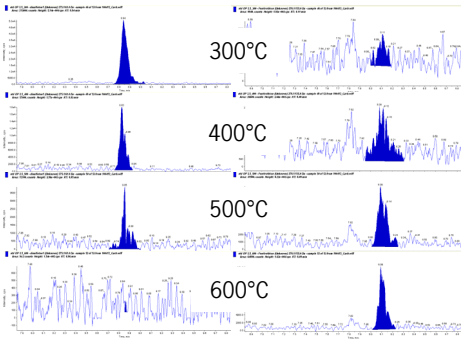
Quantification

1-point calibration at equivalent of 0.025 mg/kg, matrix-matched (cow extract in case of meat and liver).

Results

Limitation in OPP scope

For some OPPs LC-MS/MS is not very sensitive. This can be improved by adjustment of the source conditions but with an opposite effect on other analytes.



Effect of temperature of heater gas of the TurbolonSpray source on S/N of disulfoton (left) and fenitrothion (right), 2.5 ng/ml.

Quantitative aspects

Recoveries and RSDs

Pesticide	MILK 0.005 mg/kg rec (n=5)	RSD	MEAT 0.005 mg/kg rec (n=5)	RSD	LIVER**** 0.005 mg/kg rec (n=5)	RSD
aldicarb	91	5	78	8	*106*	*8*
aldicarb sulfon	95	10	80	7	96	10
aldicarb sulfoxide	88	10	79	13	89	11
amitraz***	non consistent results					
amitraz metabolite	87	4	84	7	71	8
azamethifos	99	5	11	158	80	7
azinphos-ethyl (M)	91	6	80	7	91	9
carbaryl	92	6	80	7	97	5
chlorfenvinphos***	88	7	81	4	94	11
chlorpyriphos (M)	77	11	76	8	84	17
chlorpyriphos-methyl (M)	83	6	88	6	83	10
cyromazine	68	7	68	10	57	19
diazinon (M)	87	11	80	3	109	4
dichlorvos	88	15	54	49	121	5
disulfoton	75	10	78	3	91	7
disulfoton-sulfone	98	3	86	6	96	13
disulfoton-sulfoxide	89	8	84	6	98	4
edifenphos	85	4	81	8	95	10
ethiofencarb	92	6	82	4	106	5
ethiofencarb sulfon	97	12	85	8	91	10
ethiofencarb sulfoxide	91	7	80	11	96	9
fensulfothion	86	4	75	13	94	7
fensulfothion-O	86	5	64	22	98	8
fensulfothion-O sulfon	80	7	74	12	95	13
fensulfothion-sulfon	87	7	90	6	99	8
fenthion (M)	97	11	80	8	86	11
fenthion sulfon (M)	84	7	88	14	96	19
fenthion sulfoxide (M)	106	5	77	9	87	18
fenthion-O (M)	non consistent results					
fenthion-O sulfon (M)	99	8	106	17	96	19
fenthion-O sulfoxide* (M)	81	8	82	7	92	9
methidathion (M)	88	2	80	3	98	10
methomyl	88	8	84	10	87	9
parathion-(ethyl)** (M)	139	15	75	16	122	10
parathion-methyl (M)	103	11	90	18	115	17
paraoxon-methyl*** (M)	87	7	62	43	91	11
phorate	84	5	79	7	104	10
phorate sulfon	94	4	85	8	94	12
phorate sulfoxide	87	3	83	10	96	10
phorate-O sulfon*	76	14	73	10	85	4
phorate-O sulfoxide	86	5	71	20	84	6
phoxim	82	5	79	8	97	10
pirimiphos-methyl (M)	91	6	79	4	98	15
profenofos (M)	81	9	81	5	85	14
propetamphos	90	6	88	6	98	9
propoxur	85	7	78	7	103	10
pyrazophos (M)	90	6	85	7	95	8
thiabendazole	88	2	72	8	96	7
triazophos (M)	88	2	89	2	105	16
trichlorphon	95	6	87	12	79	6

* 0.01 mg/kg; **0.025 mg/kg; ***0.1 mg/kg
**** values with underscore = degradation observed in homogenised liver
(M) = included in mandatory scope of EU coordinated monitoring program for animal products (901/2009)

Matrix effects

	response matrix vs solvent			
	average	median	min	max
cow's whole milk	0.95	0.94	0.42	1.87
cow meat extract	1.01	0.99	0.84	1.37
cow liver extract	0.76	0.77	0.49	1.10

➢Suppression most abundant in liver, but not significant for most pesticides in milk and meat. Exceptions are within factor 2.

Qualitative aspects

Retention time

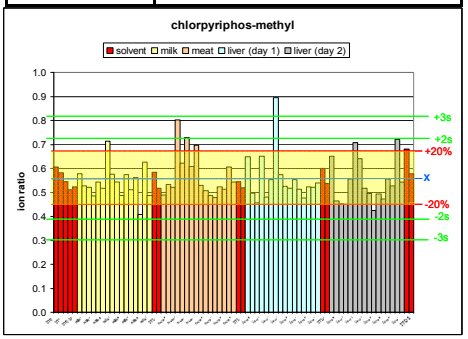
	Reproducibility of absolute retention times (n≈3700)			
	average	median	min	max
SD (min)	0.019	0.014	0.010	0.154
RSD%	0.29	0.19	0.13	3.37

Reproducibility of ion ratios

A second transition was available for 45 analytes (three only at the higher spike level). In total ~3349 values were obtained and tested against current legislation (2002/657/EC)

At a compound level, strictly applying the identification criterion would result in up to 23% false negatives. The overall false negative rate is ~5%.

Criterion	pesticides present but not meeting ion ratio criterion	
	number	%
2002/657/EC	150	4.5
± 2SD	161	4.8
± 3SD	31	0.9



Conclusions

A very straightforward method for OPPs and carbamates in animal products was succesfully validated for the majority of the analytes. In order to avoid false negatives during identification, criteria from 2002/657 should not be applied without experimental verification.

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