Determination of pesticides and mycotoxins in feed. Applicability of an analytical method in routine analysis.

F. Magni[a], N. Michlig[a], L. Demonte[a,b], M. Michlig[a,b], María R. Repetti[a]

[a]Programa de Investigación y Análisis de Residuosy Contaminantes Químicos (PRINARC), Facultad. Ingeniería Química (FIQ), Universidad Nacional de Litoral (UNL), Santa Fe, Argentina.
[b]Consejo Nacional de Investigaciones Científicas Técnicas (CONICET), C1033AAJ Buenos Aires, Argentina.

Introduction

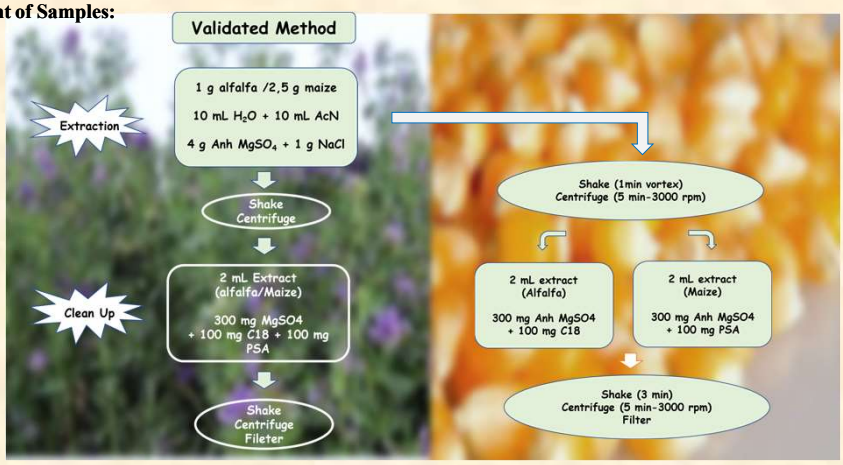
In this work, a previously validated QuEChERS method for the determination of pesticides and mycotoxins in feed samples was applied in routine analysis. At the moment of analyzing over 150 different types of feed samples, minor adjustments had to be made in the clean-up stage due to availability of C18 and PSA sorbents. To demonstrate the performance and robustness of the modified method, an on-going validation was carried out (SANTE/12682/2019) for 90 pesticides and 2 aflatoxins.

Materials y Methods

Quantitave analysis: 92 compounds

AFB1	Carboxin	Fenthion	Nitenpyram
AFB2	Chinimethionate	Fipronil	Phosmet
2,4 DB	Chlorantraniliprole	Flonicamid	Pirimicarb
2,4,D	Chlorimuron-Et	Flubendiamide	Pirimiphos-Me
4,6 dinitro-o-cresol	Chloryriphos	Fluodioxonil	Procloraz
Abamectin	Chloryriphos-Me	Flusilazole	Prophenophos
Acephate	Clethodim	Flutolanil	Propargite
Acetamiprid	Clofentezine	Haloxifop	Propiconazol
Acetochlor	Clothianidin	Imazalil	Pyraclostrobin
Alachlor	Cyproclonazole	Imazapyr	Pyrimethanil
Aldicarb	Cyromazine	Imazetapyr	S-Metolachlor
Amitraz	Diazinon	Imidacloprid	Spinosad A
Anilazine	Dicamba	Linuron	Spinosad D
Atrazina	Dichlorvos	Mecarbam	Tebuconazole
Azinphos-Me	Diclosulam	Metalaxyl	Terbufos
Azoxystrobin	Dicofol	Metamidiphos	Thiabendazol
Bendiocarb	Diflubenzuron	Methidathion	Thiacloprid
Benomyl	Dimetoato	Methomyl	Thiametoxam
Bentazone	Dinotefuran	Metoprene	Thophanate-Me
Bifrentrin	Epoxiconazole	Methoxifenozide	Triadimefon
Carbaryl	Fenhexamid	Metholachlor	Triadimenol
Carbendazim	Fenoxaprop-p-ethyl	Metribuzin	Triazophos
Carbofuran	Fenopropathrin	Metsulfuron-Me	Trifloxistrobin

Treatment of Samples:



Instrumental Parameters:

Waters ACQUITY UPLC
- ✓ Mobile Phase A: 5 mM NH4F + 0,1 % Acid Formic in Water.
- ✓ Mobile Phase B: 5 mM NH4F + 0,1 % Acid Formic in Methanol.
- ✓ Flow: 0,35 mL/min
- ✓ Injection Volume: 4 µL
- ✓ Column: ACQUITY UPLC® BEH C18 RP Shield (1.7 µm 2.1 x 100 mm) de Waters.
- ✓ Column Temperature: 40 ºC

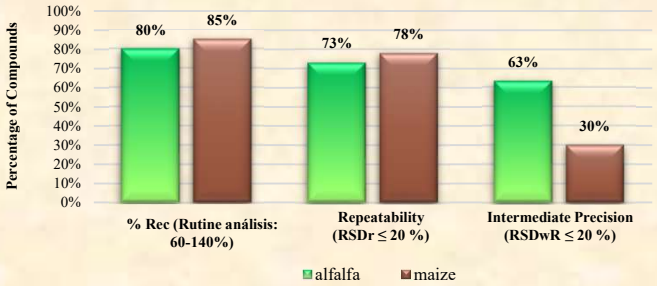
Waters ACQUITY TQD
- ✓ MRM ESI (+) and ESI (-)
- ✓ Cone Voltage: 40 V
- ✓ Capillary Voltage: 1 kV
- ✓ Dwell time: 0.008-0.05 (ESI +) / 0.05 (ESI -)
- ✓ Source temperature: 120 °C
- ✓ Desolvatation temperature: 390 °C
- ✓ Cone gas flow: 48 $L.h^{-1}$
- ✓ Desolvatation gas flow: 900 L.h-1
- ✓ Software: MassLynx 4.1

Criteria for Identification and Quantification:
- ✓ Presence of Q and q ions
- ✓ Retention time of Q y q ± 0,1 min
- ✓ S/N (Q) ≥ 10 and S/N (q) ≤ 3
- ✓ Ratio q/Q ± 30% (respect to standard in matrix)

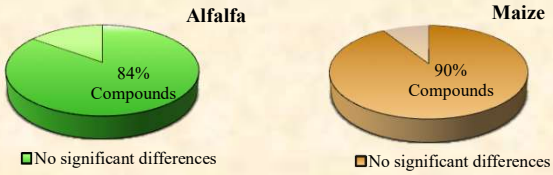
On-Going Validation (SANTE/12682/2019):
- ➢ The purpose of on-going method validation is to:
- ➢ Demonstrate robustness through evaluation of mean recovery and within-laboratory reproducibility (RSDwR).
- ➢ Demonstrate that minor adjustments made to the method over time do not unacceptably affect method performance.
- ➢ Demonstrate applicability to other commodities from the same commodity category.
- ➢ Determine acceptable limits for individual recovery results during routine analysis.

Results y Discussion

Estimated accuracy and precision from spiked samples (100 µg/kg) during on-going validation



Initial Method Validation vs On-going Validation: the %Rec (100 µg/kg) were compared using Student's t-test (α=0.05) for all the studied analytes.



Alfalfa

	% Rec Method Validated	% Rec Method Modificated
Dinotheluran	59	78
Fenthion	28	88
Methomyl	227	116
Metribuzin	ND	107
Terbufos	ND	74
2,4 D	131	59
2,4 DB	142	23
Aldicarb	66	25
Bifenthrin	85	58

Maize

	% Rec Method Validated	% Rec Method Modificated
Acephate	ND	85
Chlorpyriphos-Me	ND	90
Fenthion	40	77
Metribuzin	668	96
2,4 D	84	ND
2,4 DB	83	ND
Spinosad D	98	56
Thiabendazol	83	44

Proficiency Test on incurred and spiked pesticides in wheat
EU Reference Laboratory on Cereals & Feeding stuff
EUPT-CF8 2014

Recoveries evaluated at 10 µg/Kg

	Reference Value (mg/kg)	Results (mg/kg)	SD	Recovery (%)	z-score	Initial Validation % Rec (Alfalfa)	Initial Validation % Rec (Maize)
Azoxystrobin	0.211	0.219	0.037	45	0.2	82	98
Carbendazim	0.055	0.042	0.01	100	1.3	ND (25)	ND (25)
Epoxiconazole	0.122	0.113	0.008	90	0.4	ND (100)	ND (25)
Flonicamid	0.102	0.115	0.005	ND	2.6	ND	ND
Linuron	0.069	0.089	0.005	135	0.6	ND (50)	ND (50)
Pyraclostrobin	0.07	0.086	0.008	120	0.2	96	90

Z-scores will be interpreted in the following way:
$|z| \leq 2$ Acceptable
$2 < |z| \leq 3$ Questionable
$|z| > 3$ Unacceptable

ND: Not Detected (LOQ µg/Kg)

Conclusions

- ➢ The robustness of a validated method for the analysis of pesticides and mycotoxins in feed samples was demonstrated using on-going method validation strategies and statistical t-test analysis.
- ➢ It was demonstrated that changing the sorbent composition (C18 and PSA) of the clean-up step with respect to the initial validation didn't affect significantly the extraction performance of most of the studied analytes.
- ➢ The applicability of the modified method to a Proficiency Test in wheat flour was evaluated, and results allow to presume that even with minor changes the method can be used in feed types that are different from the initially validated (maize and alfalfa).

Aknowledgement