Joint FAO, IAEA and Latin American and Caribbean Analytical Network Workshop on Quality Assurance and Quality Control Measures in Food Testing Laboratories

Sample preparation alternatives for food contaminants in milk

LAPRW 2019, 5th May 2019, Rafain Palace Hotel and Convention Center, Foz de Iguazu, Brazil

CENUR Litoral Norte

UNIVERSIDAD DE LA REPÚBLICA URUGUAY

DQL

GACT

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- Livestock raising cohabits with agriculture. Therefore, animals are problably exposed to pesticides used to protect crops.
- In addition, during cattle raising veterinary drugs are used to prevent diseases.

The use of these compounds may result in the presence of their residues in edible tissues or organs, mainly in muscle, liver, kidneys and milk where most xenobiotics are metabolized or excreted, contaminating the final product.

Exposing consumers health to these contaminants and affecting international trade.

Milk industry in Uruguay

↑ greater chemotherapeutic and antiseptic pressure on animals

↓

Milk contamination

Pozo Lora et al., 1977; Khaniki, 2007; Acosta, 2008; Thomas et al., 2008; Boggio, 2010; Cajarville et al., 2012; Zumbado y Romero, 2015.

Main Contaminants in milk

Insecticides

Herbicides

Antibiotics

Antihelminthics

Acaricides

Fungicides

Anti/Ecto parasitic

Mycotoxins

Anti-inflammatory

PCBs

PAHs

Dioxins

Problems related to chemical contamination of milk

Ten liters of milk with an ATB (thermally stable) can altered the processing of 80,000 L of milk

Pozo Lora et al., 1977; Khaniki, 2007; Thomas et al., 2008; Boggio, 2010

Problems during tecnological processes

During the industrial process some of these contaminants could be concentrated in the final product, being detected in a higher concentration compared to the raw material.

Bajwa y Singh, 2011

EU Maximum Residue Levels (LMRs)

REGLAMENTO (CE) Nº 1881/2006 DE LA COMISIÓN

de 19 de diciembre de 2006

por el que se fija el contenido máximo de determinados contaminantes en los productos alimenticios

(Texto pertinente a efectos del EEE)

(DO L 364 de 20.12.2006, p. 5)

REGLAMENTO (CE) Nº 396/2005 DEL PARLAMENTO EUROPEO Y DEL CONSEJO

de 23 de febrero de 2005

relativo a los límites máximos de residuos de plaguicidas en alimentos y piensos de origen vegetal y animal y que modifica la Directiva 91/414/CEE del Consejo

COMMISSION REGULATION (EU) No 37/2010

of 22 December 2009

on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

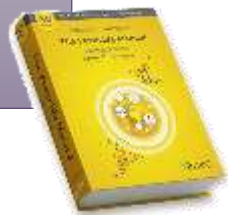
(Text with EEA relevance)

Objective

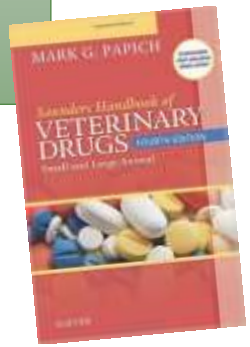
Development and validation of a multiclass methodology for the determination of aflatoxin M1, pesticides and veterinary drugs in milk

The analytical challenge…

Pesticide Manual: 1468 pesticides (excl. metabolites)

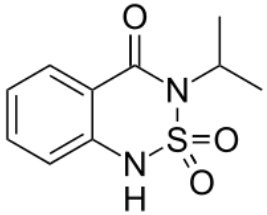
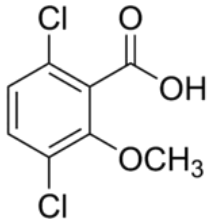
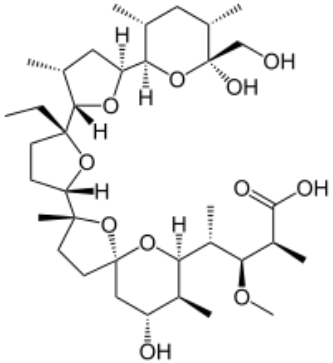
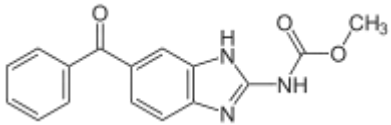
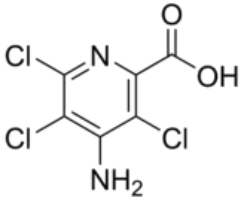
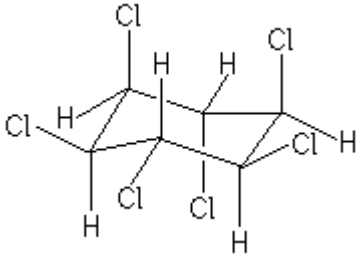
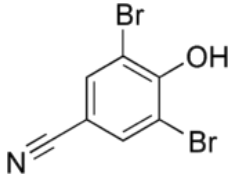
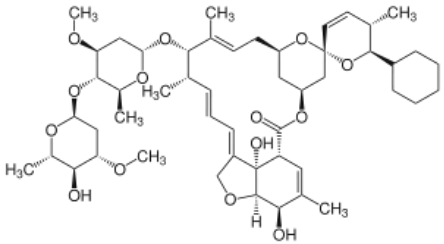
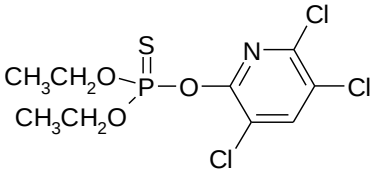
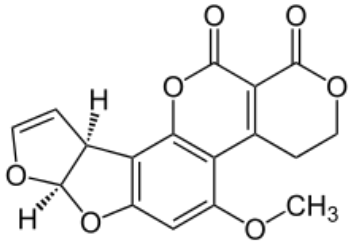
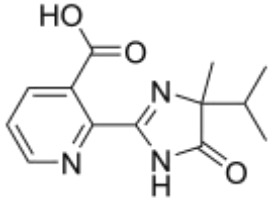
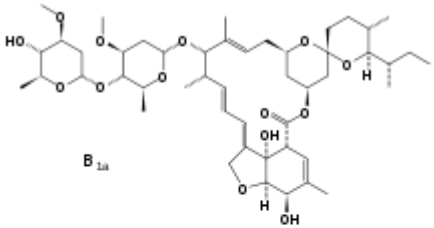
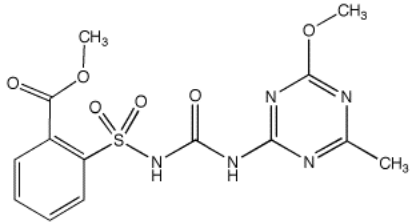
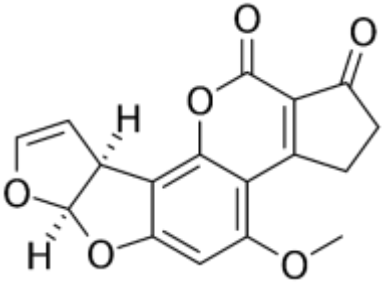
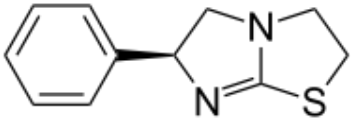
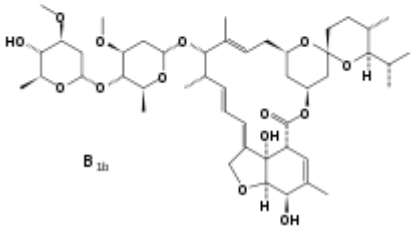


VD Handbook: 550 drugs



Mycotoxins: 300 identified

Contaminants diversity

Sample treatment approaches for pesticides, veterinary drugs, mycotoxins

QuEChERS

MSPD

SFE

Mini-Luke

Luke

ASE

PLE

SWEet

QuPPe

EtAc method

SPE

Method Validation

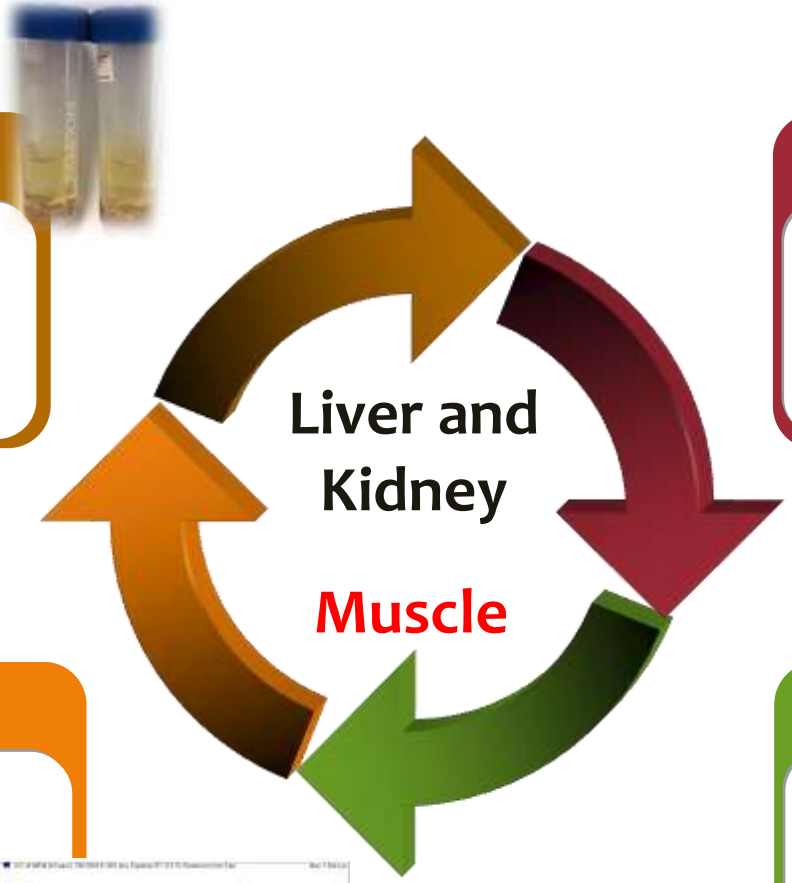
Method validation is the process of demonstrating that analytical procedures are suitable for their intended use.

DG-SANTE Guidelines
Document No. SANTE/11813/2017

2002/657/EC: Commission Decision implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

Codex, CAC/GL 90-2017: Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food and Feed

Experimental: Final Method



Extraction

5 g sample + 15 mL EtAc + 5 mL H_2O
Salting out $MgSO_4$ + NaCl

Clean-up 1

Overnight Freeze-out

Clean-up 2

60 mg C-18,
100 mg Al_2O_3,
600 mg $MgSO_4$

Analysis

HPLC-MS/MS
GC-MS

Milk: Extraction conditions

Eight different combinations of solvents were compared to optimize the best extraction approach (n=5, 100 µg/L)

MeOH

EtAc

MeCN

MeCN: MeOH (50:50)

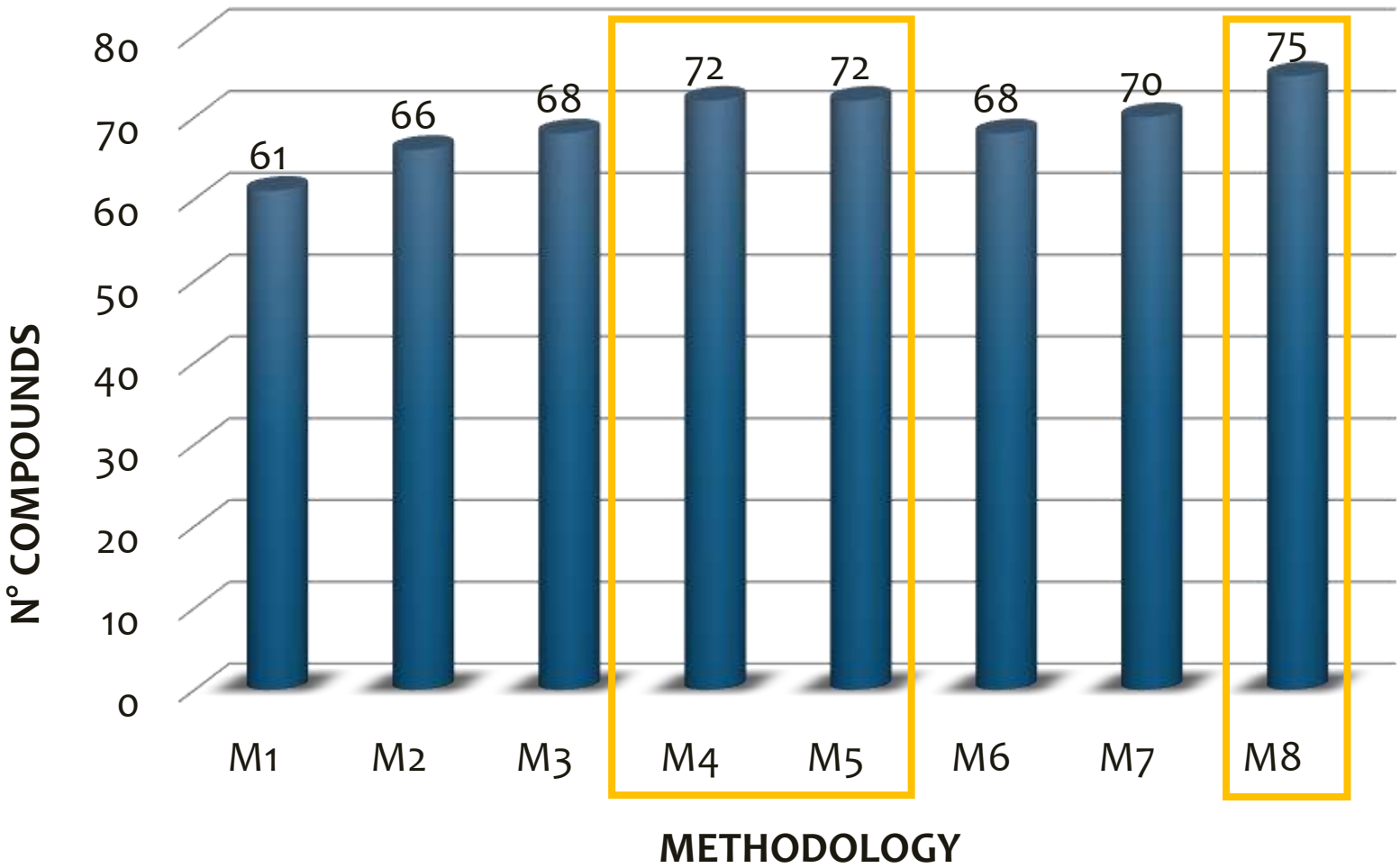
MeCN: EtAc (50: 50)

EtAc: MeOH (50: 50)

MeCN: H_2O (50:50)

MeCN: H_2O (citrate buffer) (75:25)

Extraction conditions



Clean-up conditions

Extraction

MeCN: MeOH (50: 50)	MeCN: EtAc (50: 50)	MeCN: H_2O (citrate buffer) (75: 25)

Clean-up

Overnight freeze-out + dSPE 60 mg C-18, 600 mg $MgSO_4$

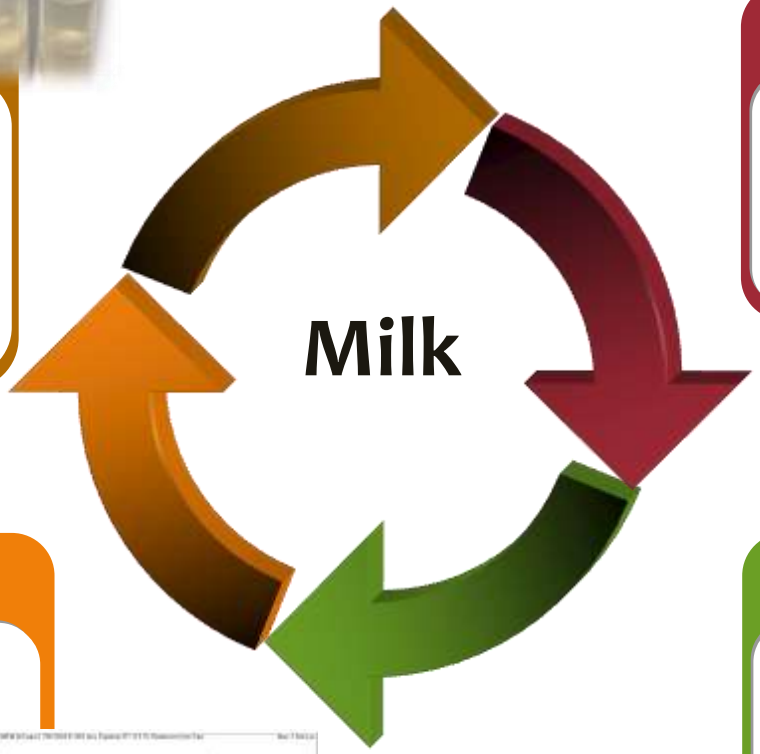
Analysis

GC-MS	LC-MS/MS

Final Method

Extraction

5 g sample + 15 mL MeCN:H_2O (75:25)
Salting out $MgSO_4$ + NaCl+Citrate buffer salts



Clean-up 1

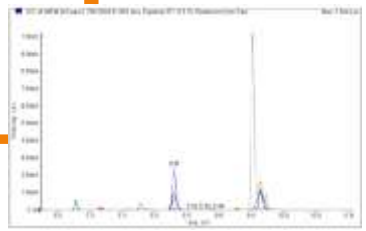
Overnight
Freeze-out

Clean-up 2

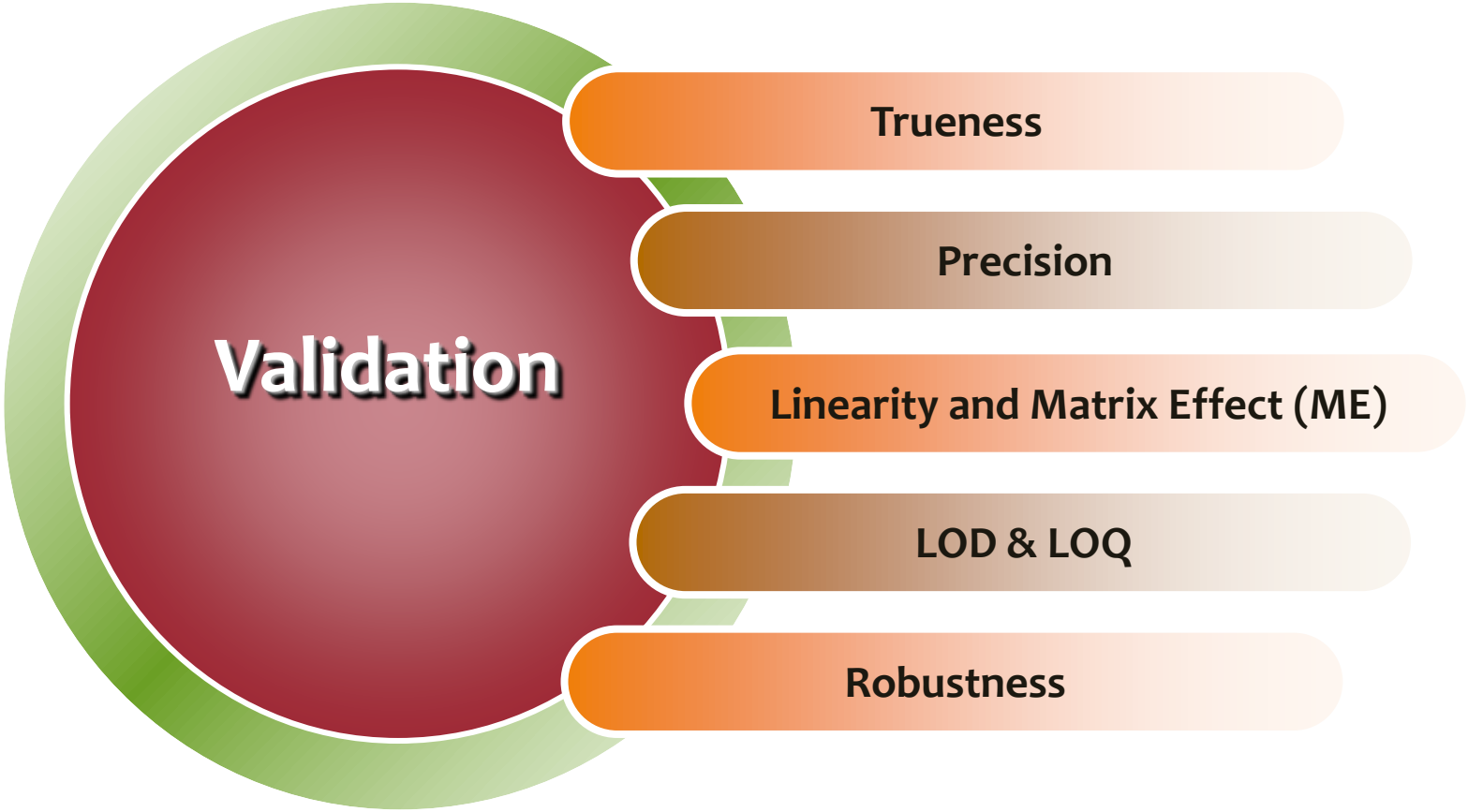
60 mg C-18,
600 mg $MgSO_4$

Analysis

HPLC-MS/MS
GC-MS



Method Validation



DG-SANTE Guidelines
Document No. SANTE/11813/2017

Results & Discussion

GC-MS & LC-MS/MS

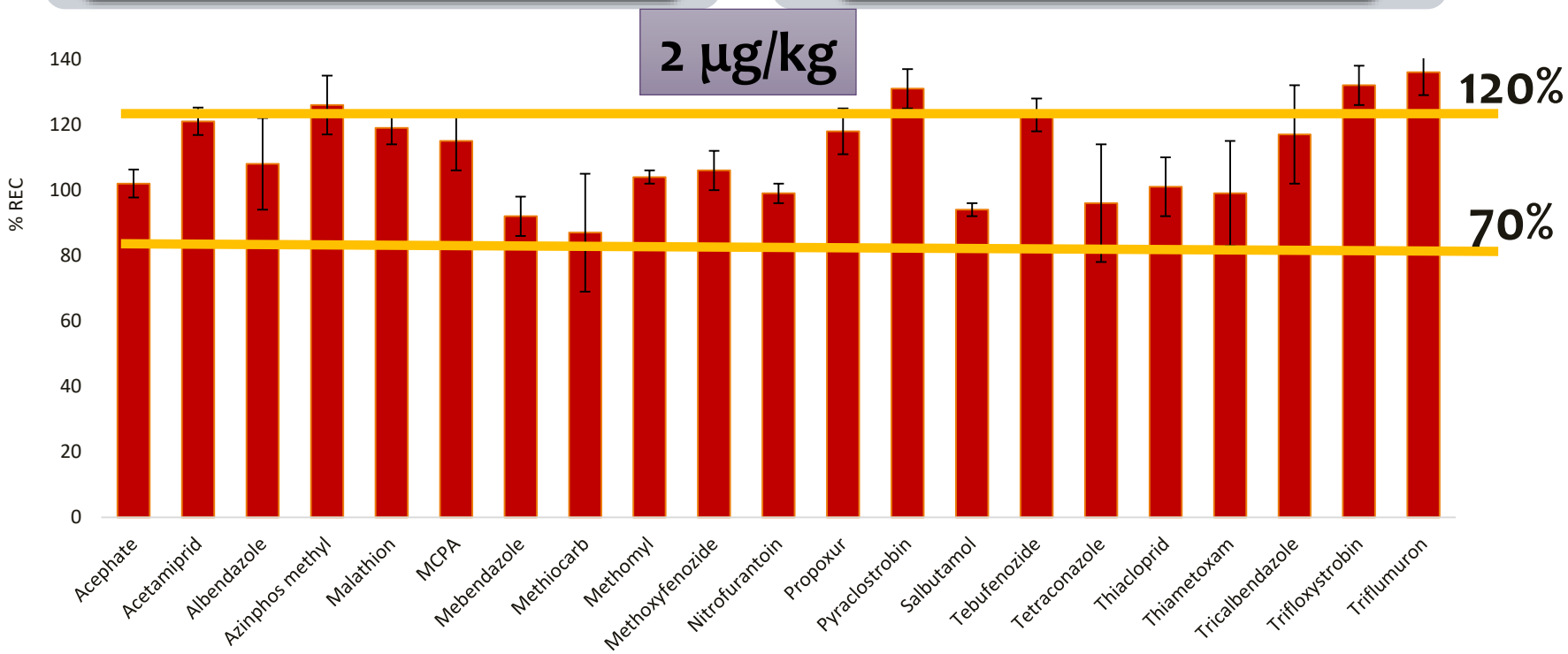
Spiking levels assayed for trueness and precision evaluation (*n*=5)

LC

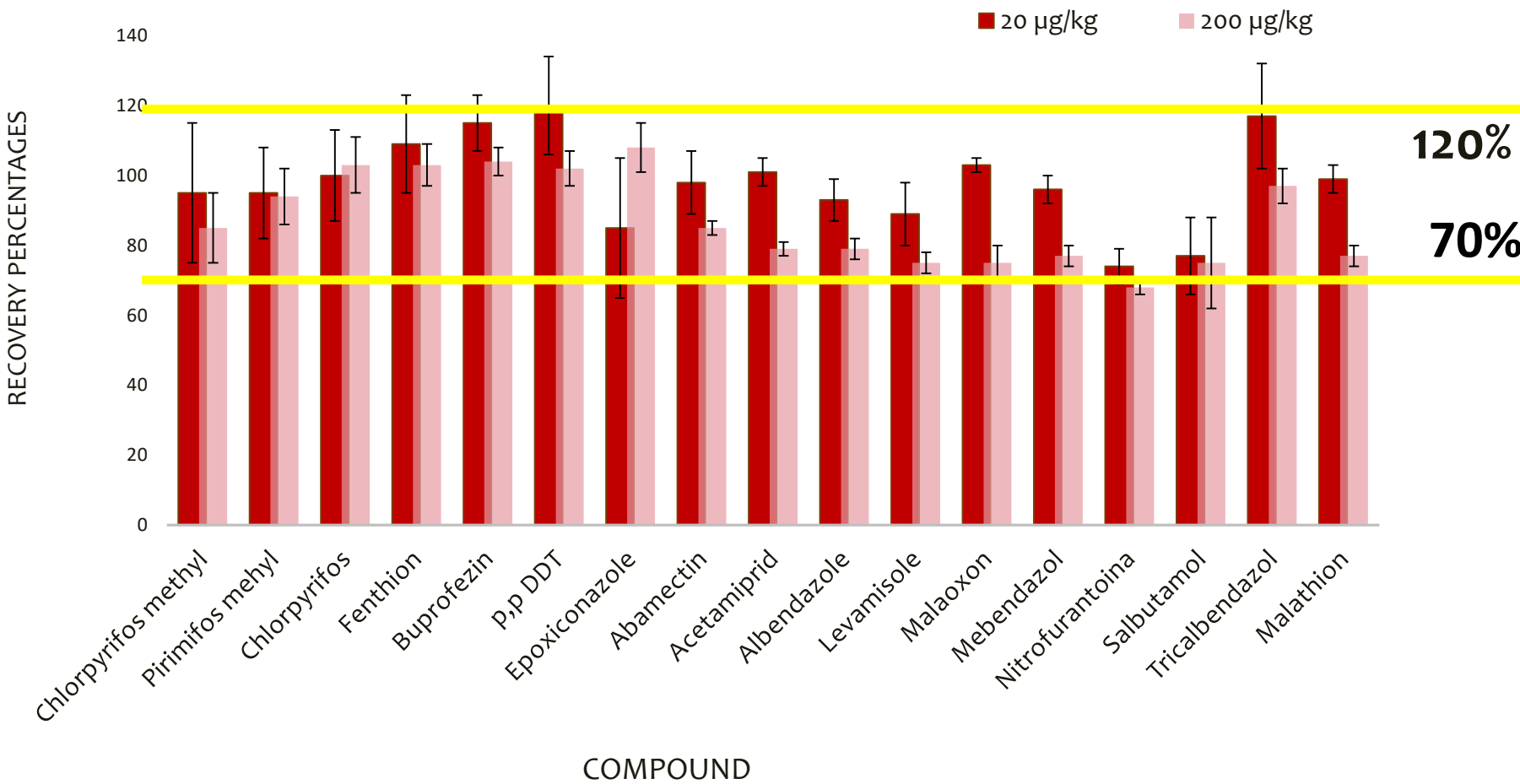
2, 10, 20 and 200 µg/kg

GC

20 and 200 µg/kg



GC-MS & LC-MS/MS


20 µg/kg
200 µg/kg
RECOVERY PERCENTAGES
140
120
100
80
60
40
20
0
120%
70%
Chlorpyrifos methyl
Pirimifos mehyl
Chlorpyrifos
Fenthion
Buprofezin
p,p DDT
Epoxiconazole
Abamectin
Acetamiprid
Albendazole
Levamisole
Malaoxon
Mebendazol
Nitrofurantoina
Salbutamol
Tricalbendazol
Malathion
COMPOUND

LOD, LOQ & Linearity

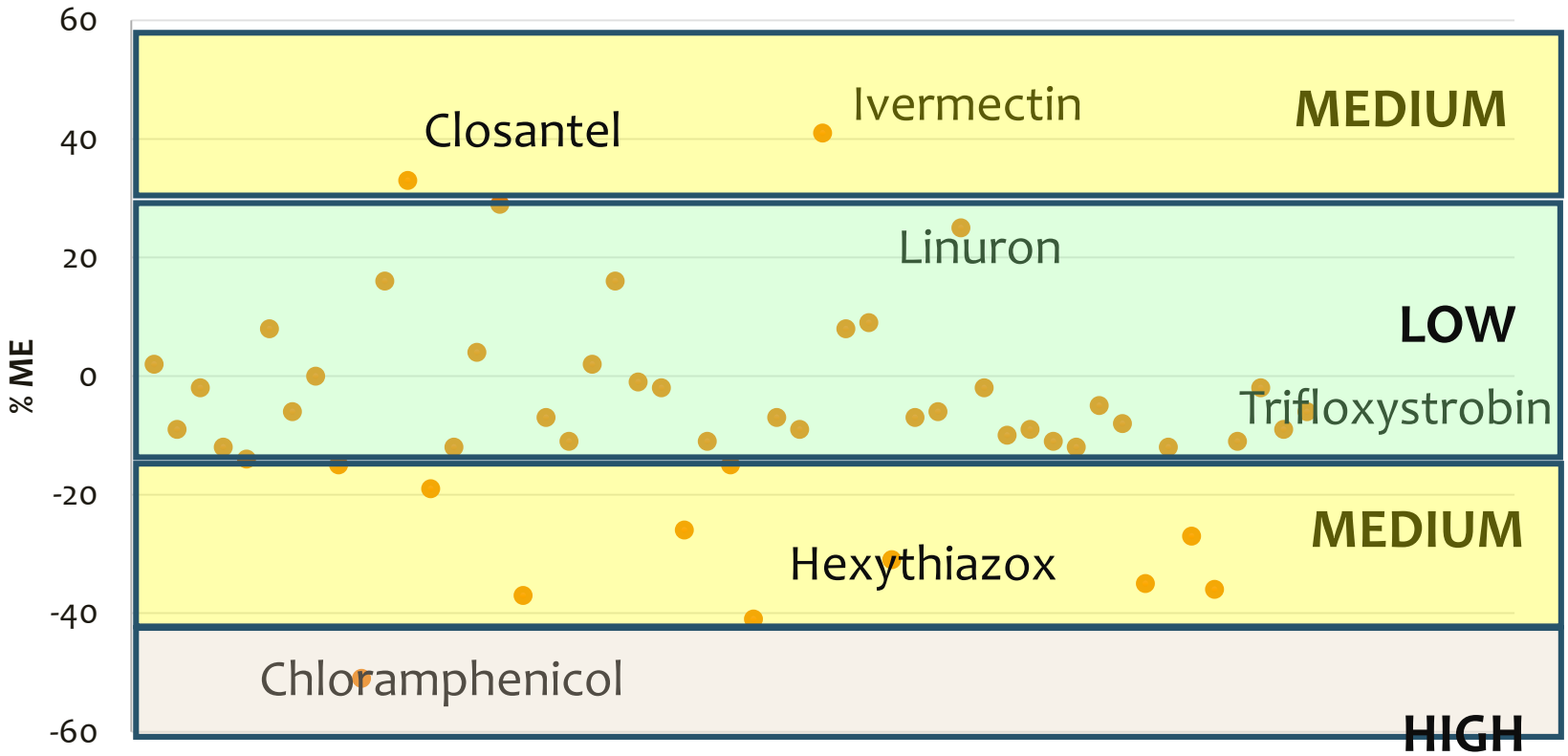
LOD & LOQ

- LOD=LOQ: 2 µg/kg for 51% of the compounds, 10 µg/kg for 43% and 200 µg/kg for 6% of the analytes.

Linearity

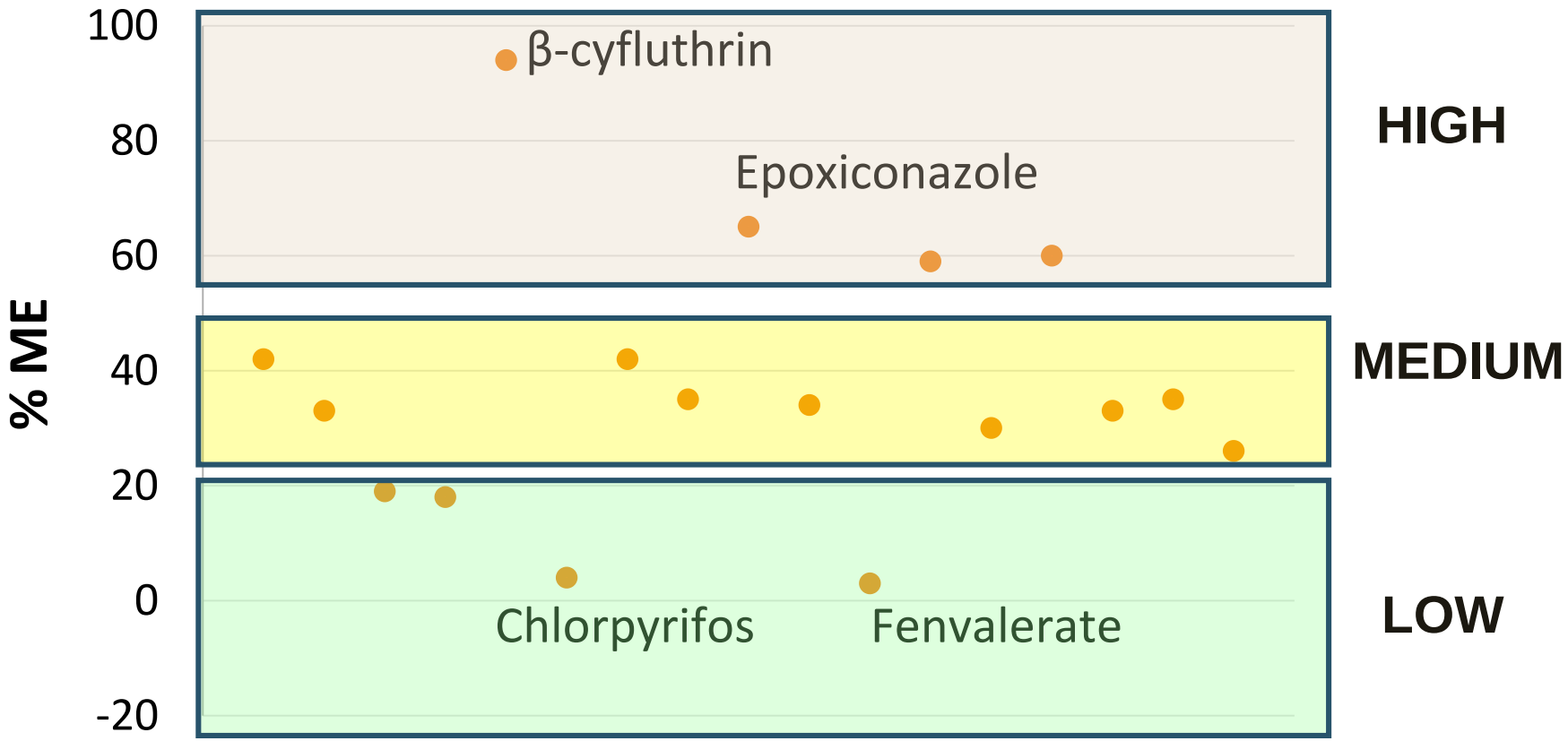
- All the compounds presented good linearity in matrix and solvent (r^2> 0.999) in the range 1-250 µg/L in both instruments.
- The residuals (BCC) were below 20%.

ME in LC-MS/MS



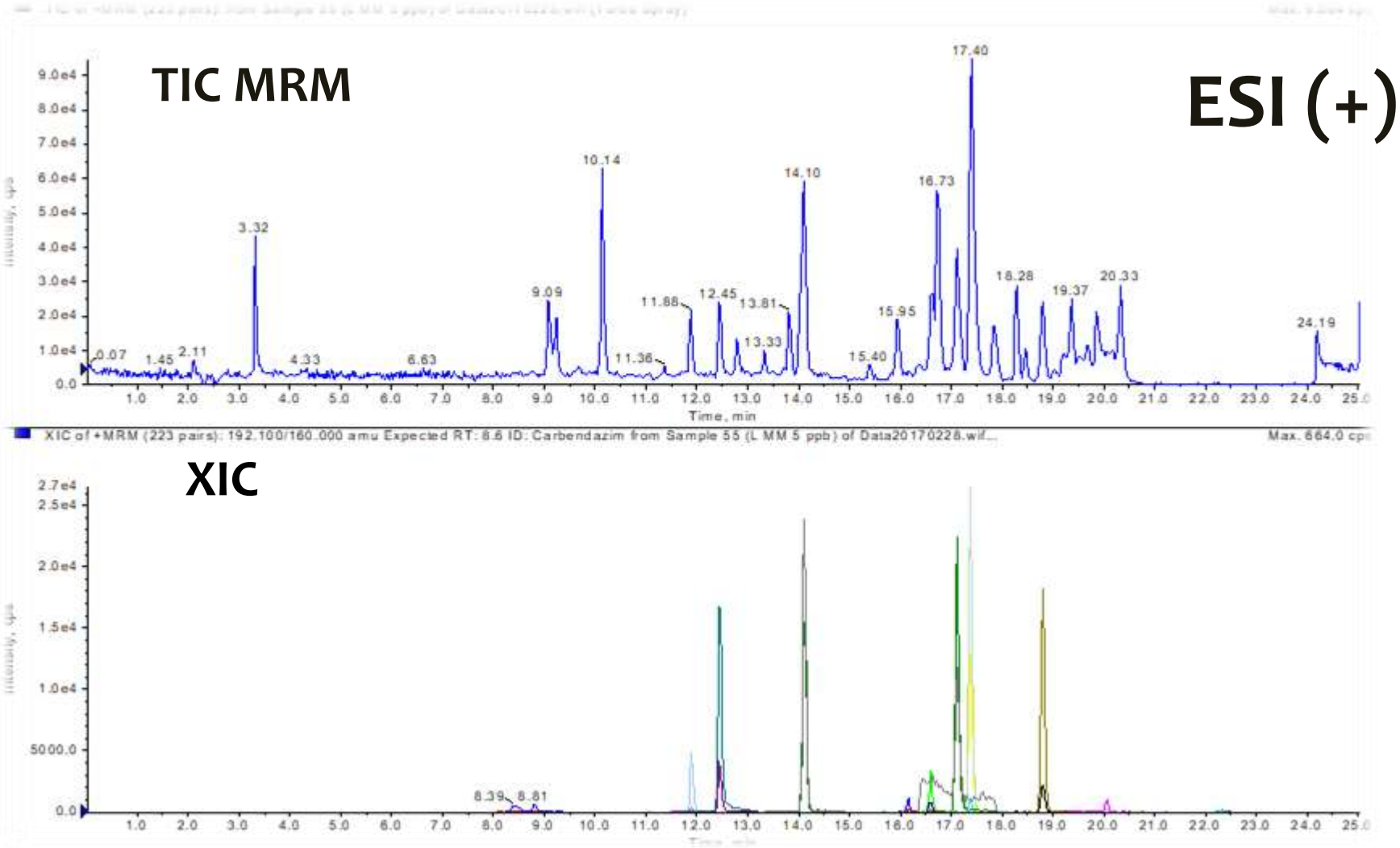
The compounds presented signal enhancement/ suppresion

Matrix Effect GC-MS

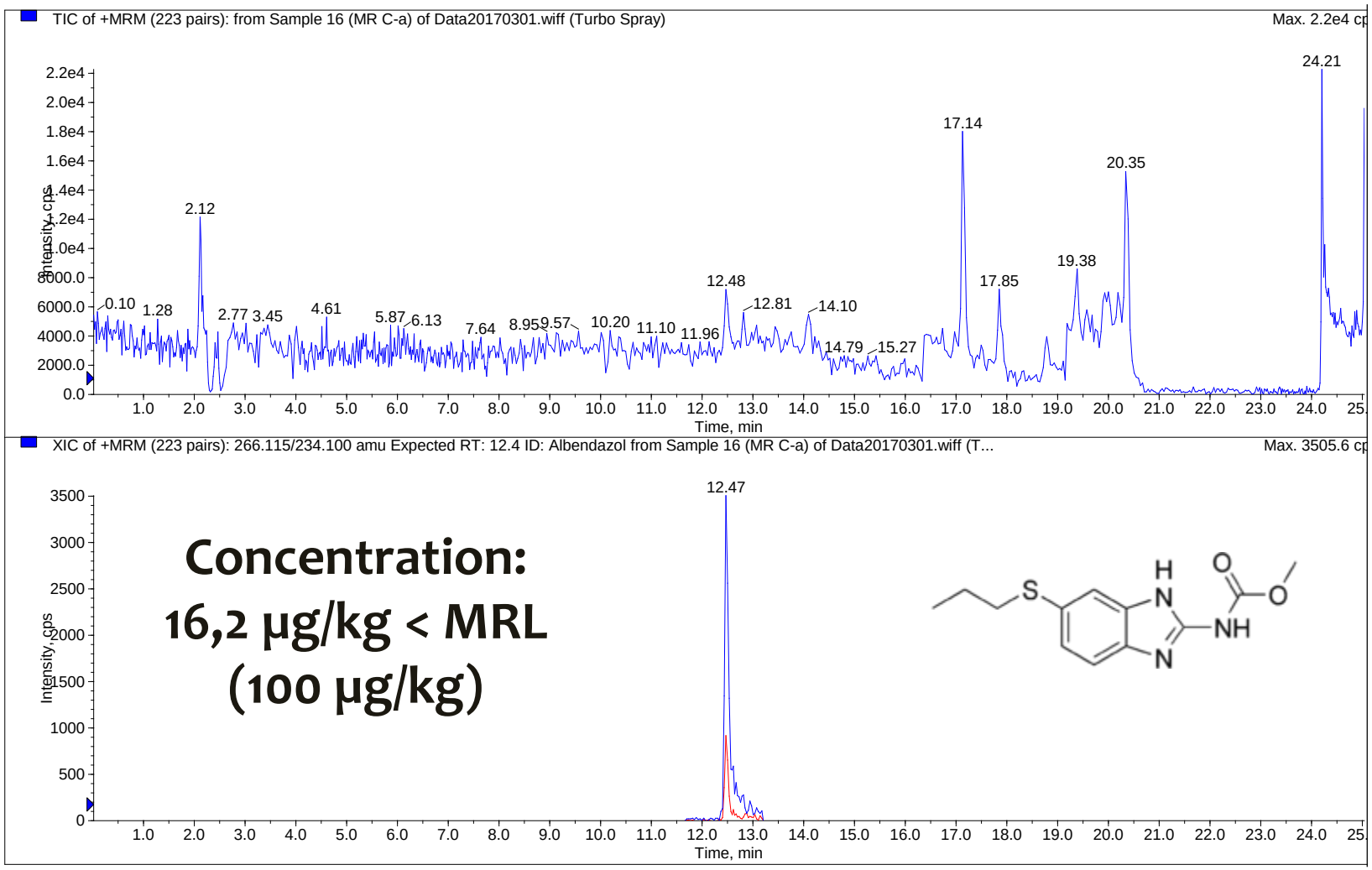


All the compounds presented signal enhancement

Milk extract spiked at 5 µg/kg



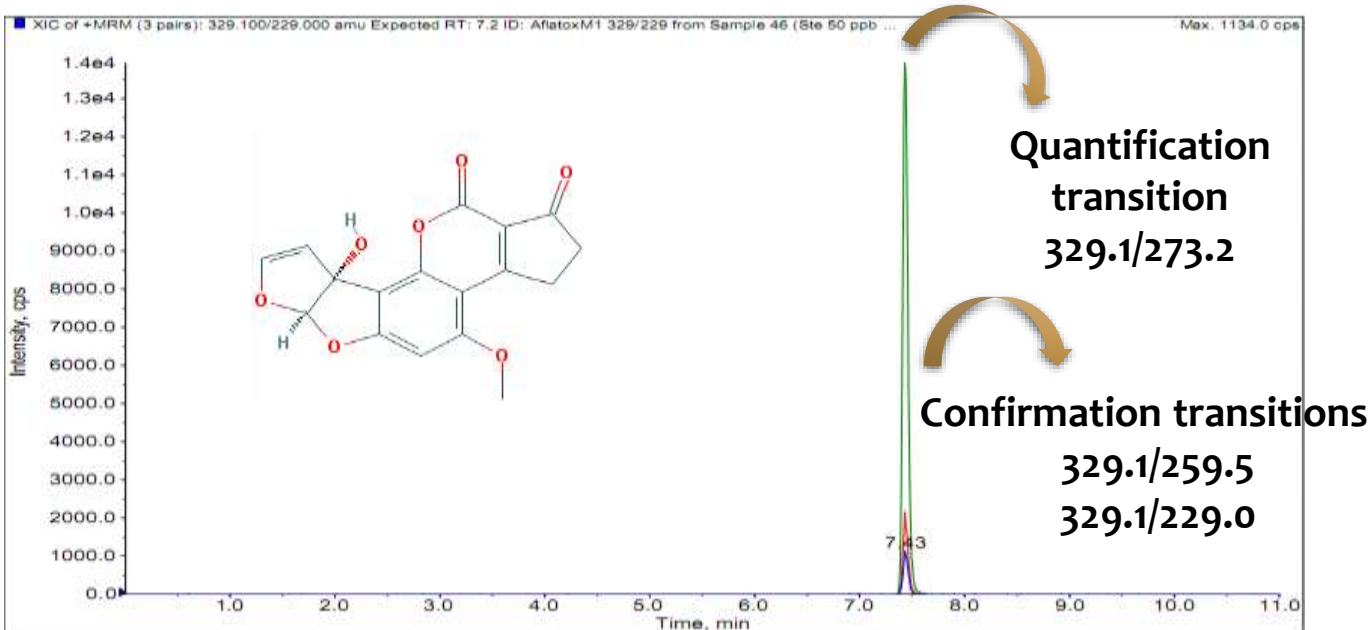
Albendazole in a milk sample



Aflatoxin M1

MS/MS Parameters

Transition	DP (V)	EP (V)	CE (V)	CXP (V)
329.1/273.2	93	13	42	10
329.1/259.5	93	13	35	10
329.1/229.0	93	13	41	10



Validaton results AFM1

Milk´s method	Assayed level (µg/kg)	% Rec	%RSD
With freeze-out	0.1	85	6
	0.5	89	3
	10.0	89	6
w/o freeze-out	0.1	81	9
	0.5	82	7
	10.0	104	5

LOD & LOQ

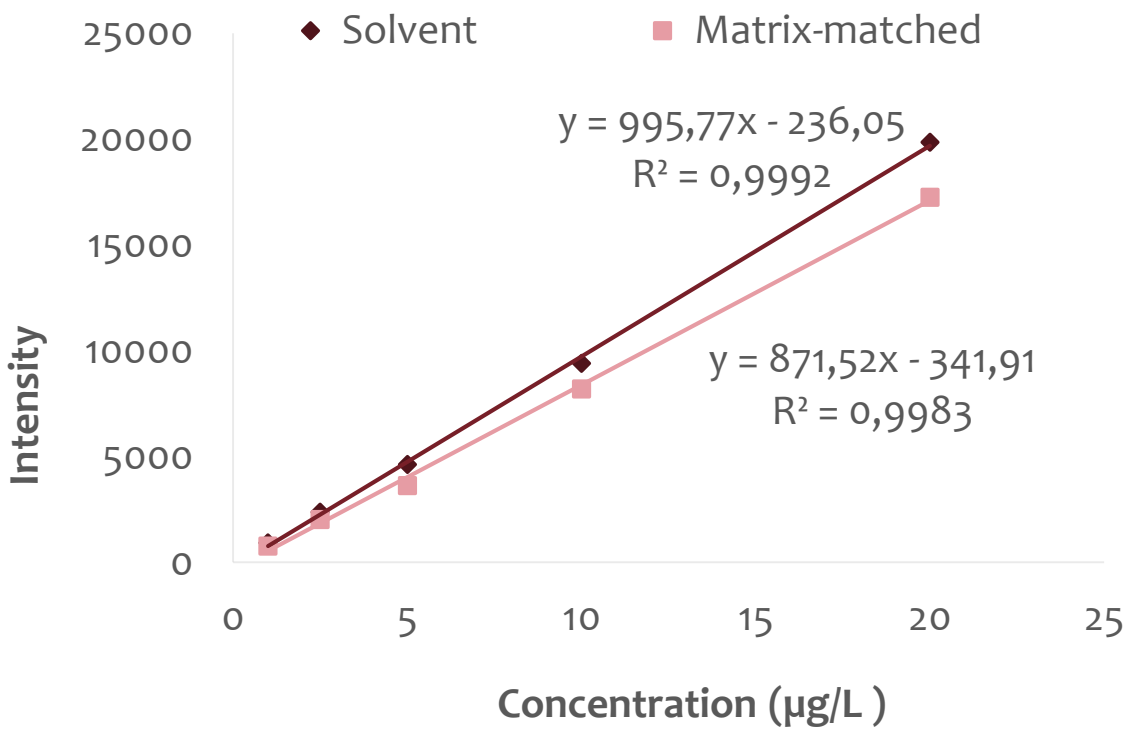
Milk´s method	Assayed level (µg/kg)	% Rec	%RSD
	0.05	112	7
w/o freeze-out	0.10	89	19
	0.50	75	9

Linearity AFM1

Linearity : 1-20 µg/L

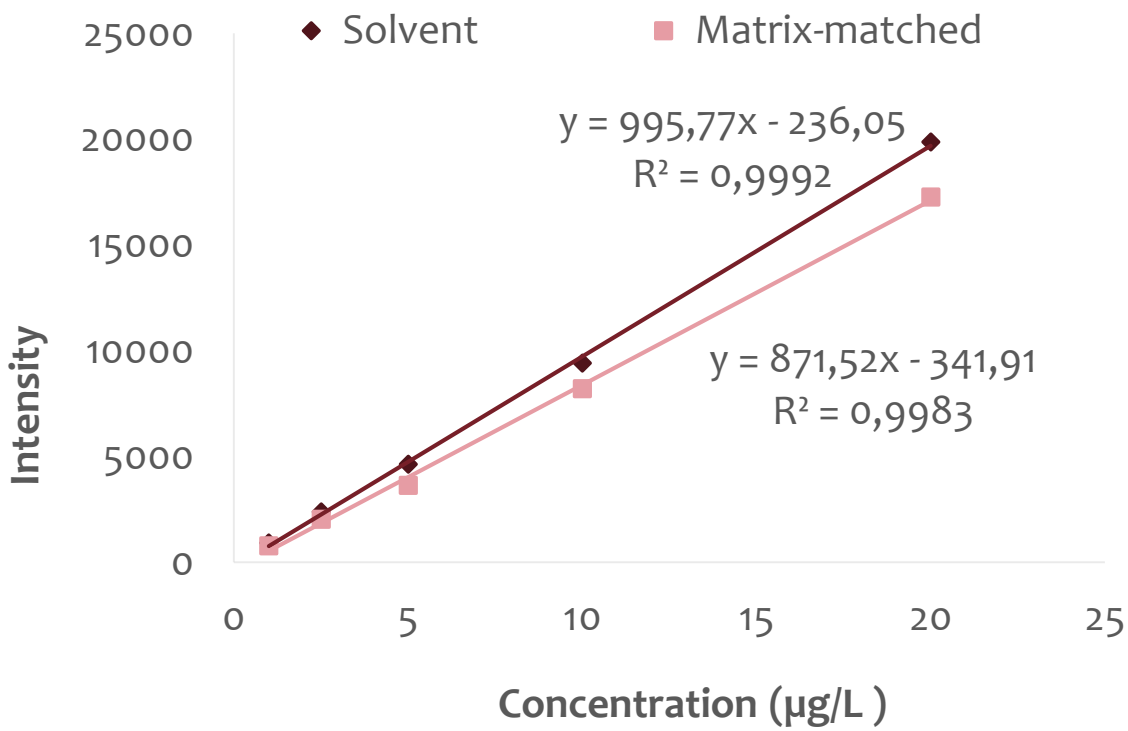
Level (µg/L)	%Residuals
1.0	-11
2.5	-8
5.0	9
10.0	2
20.0	-1

DG-SANTE Criteria



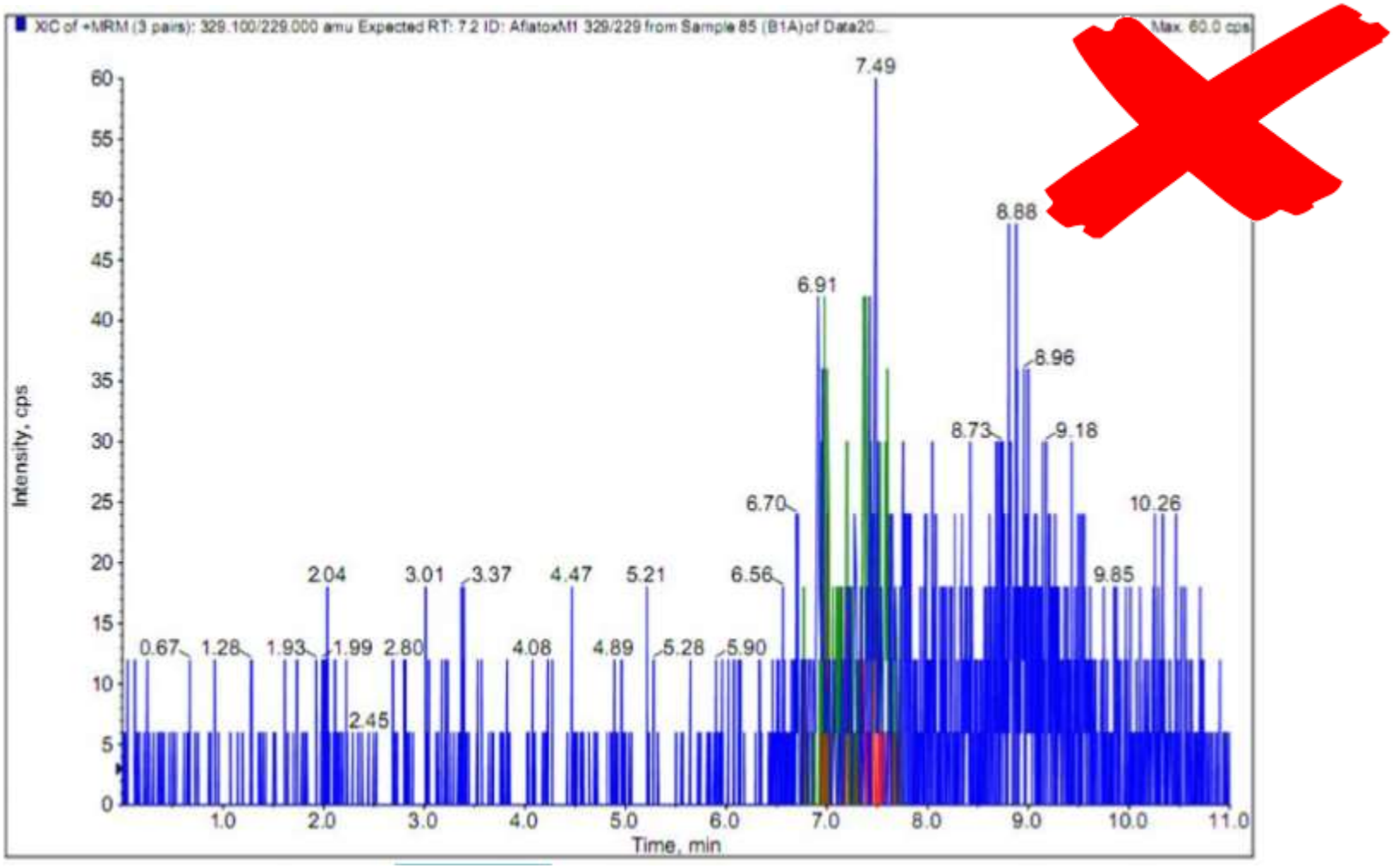
Matrix Effect AFM1

$$ME\,(\%) = \left(\left(\frac{\text{signal in matrix}}{\text{signal in solvent}}\right) - 1\right) \times 100$$



%ME= -14%

Commercial samples



New scope

Pesticides & veterinary drugs

2, 4 Dimetilamine, Abamectine, Amitraz, Carbaryl, Cypermethrin, Cyromazine, Chlorpyrifos, Clorsulon, Closantel, Cyalothrin Lambda, Diazinon, Dichlorvos, Doramectin, Eprinomectin, Ethion, Fipronil, Fipronil carboxamide, Fipronil desulfinyl, Fipronil sulfide, Fipronil sulfone, Fluazuron, Flumethrin, Ivermectin, Pirimiphos methyl, Propoxur

Antibiotics: Amoxycilin, Ampicillin, Ceftiofur, Chlortetracyciline, Cloxacilin, Enrofloxacin, Spiramycin, Monensin, Oxytetracycline, Penicillin G, Rifaxymin, Tetracycline, Tilmycosin, Tylosin

Mycotoxins: aflatoxin B1, B2, G1, G2, M1, M2

Preliminary results LC-MS/MS-ESI (+)

	M1		M1 prima		M2		M2 prima		M3		M5	
	% REC	% RSD	% REC	% RSD	% REC	% RSD	% REC	% RSD	% REC	% RSD	% REC	% RSD
Abamectin	112	49	86	20	181	25	123	16	5	3	7	33
Amoxycillin	94	2	239	3	885	42	-	-	-	-	-	-
Ampycilin	76	24	204	5	182	5	-	-	-	-	-	-
Carbaryl	267	4	104	10	230	4	-	-	-	-	-	-
Ceftiofour	248	13	146	3	83	11	35	6	96	3	362	18
Chlorpirifos	112	54	80	28	109	7	42	24	3	34	2	45
Ciromazine	80	8	186	3	134	2	94	4	234	2	249	12
Chlortetracicline	-	-	-	-	-	-	48	8	163	60	186	11
Cloxacilin	321	19	135	5	149	4	75	5	61	2	138	21
Cyhalothrin lambda	-	-	-	-	-	-	32	27	13	7	24	2
Diazinon	199	13	82	17	128	24	86	5	7	15	38	9
Dichlorvos	225	89	311	3	342	4	-	-	-	-	-	-
Doramectin	104	43	92	15	179	2	88	11	4	8	2	45
Enrofloxacin	176	6	153	6	166	7	90	6	62	1	304	14
Eprinomectin	120	53	89	39	190	13	107	5	6	19	13	34
Spyramicin	47	102	158	9	123	22	106	10	120	13	13	88
Ethion	148	35	73	19	103	29	47	23	3	18	2	41
Fluazuron	199	21	72	19	83	44	30	36	3	13	1	53
Flumethrin	120	24	59	26	7	105	19	89	9	90	111	149
Ivermectin	96	42	88	14	151	4	95	16	4	13	2	71
Monensin	104	16	76	8	83	5	100	11	6	17	73	11
Oxytetracicline	69	20	149	7	113	8	61	9	86	4	154	17
Penicilin G	265	22	168	5	27	3	61	9	108	4	5	47
Pirimifos Methyl	195	17	78	22	168	30	82	5	5	7	13	19
Propoxur	251	9	113	10	207	2	108	1	16	11	294	12
Rifaxymin	259	13	83	22	452	37	87	4	8	8	136	30
Tetracicline	67	13	132	1	100	3	61	4	71	8	122	30
Tylmicosin	80	67	168	2	149	4	97	4	87	2	254	23
Tylosin	193	3	140	8	190	5	103	4	43	9	39	27

Preliminary results LC-MS/MS-ESI (-)

	M1		M1´		M2		M2´		M3		M5	
	% REC	% RSD	% REC	% RSD	% REC	% RSD	% REC	% RSD	% REC	% RSD	% REC	% RSD
Clorsulon	236	7	62	14	86	6	69	10	44	7	254	30
Closantel	6153	7	15	17	10	9	--	--	11	14	1	84
Fipronil	155	10	40	15	170	8	107	9	18	10	54	30
Fipronil desulfinyl	209	6	32	18	117	3	82	7	13	11	21	41
Fipronil sulfide	179	6	32	20	141	4	89	9	14	16	8	27
Fipronil sulfone	197	5	30	19	130	2	80	10	13	13	5	20

Acknowledgements

RALACA
Red Analítica de Latinoamérica y el Caribe

IAEA

PEDECIBA

CSIC
COMISIÓN SECTORIAL DE INVESTIGACIÓN CIENTÍFICA

Thank you for your attention!

lpareja@fq.edu.uy