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### Transition to High-resolution Accurate Mass (HRAM) technology for Quantitative Screening of Antimicrobials in Food of animal origin





### What is High Resolution Accurate Mass (HRAM)?

Resolution is the ability to distinguish between peaks of similar mass. Higher resolution allows for more accurate mass measurements by providing better separation of peaks.

<u>Accuracy</u> describes how close a measured mass is to the true or expected mass. Accuracy is crucial for accurate identification of molecules.





# Why move to HRMS analysis?

Multiple LC-QQQ screening methods based on family class



Multiple methods being used for residue screening of antibiotics, utilising a lot of laboratory resources. Single method with both quantitative and confirmation capability preferred.



Microbiological based screening methods e.g. 6plate

Microbiological tests while fast had limited specificity and sensitivity. Only identify class. Quantitation not possible. False positive and false negatives possible

### All elements have a nominal and an exact mass.

Element	Nominal mass	Exact mass	Mass defect
Carbon <sup>12</sup> C	12	12.000000	0.000000
Hydrogen <sup>1</sup> H	1	1.0078250	0.0078250
Nitrogen <sup>14</sup> N	14	14.0030740	0.0030740
Oxygen <sup>16</sup> O	16	15.9949146	- 0.0050854
Chlorine <sup>35</sup> Cl	35	34.9688527	- 0.0311473
Fluorine <sup>19</sup> F	19	18.9984032	- 0.0015968
Sulfur <sup>32</sup> S	32	31.9720707	- 0.0279293





# **LRMS Versus <u>H</u>RMS**

- Nominal mass
- Low resolution
   <10,000</li>
- Time consuming to tune individual compounds
- Only scans for what you ask



Exact Mass

•High resolution up to 140,000



- Easy set up no need to tune individual compounds
- Full scan and retrospective analysis

# Development of a single HRMS Screening method - TM231





### **Step 1: Preparation of compound database and instrument Methods**





### • • V & +

loxacillin Na loxacillin Na\_1 olistin anofloxacin apsone

### Database - HRMS Dev list

те <b>т</b> ∓ ∓ ×	Pea	k View Pane											
lame	F	Compound Name	Peak Label 🛛 📮	Peak Workflow	ņ	Associated Target Peak	ņ	Chemical Formula	MS Order	+	Precursor m/z	6 F	Produc
	2	4-MAA (4-Methyl An	T1: 218.12879	TargetPeak	-		•	C12H15N3O	ms1	-	218.12879	0	0.000
-WAA (4-Methyl Aminoantipyrine)	3	4-MAA (4-Methyl An	T1F1: 218.12879->56.(	Fragment	•	T1: 218,12879	•	C12H15N3O	ms2	•	218,12879	5	56.0494
cetaminophen (Paracetamol)		4 MAA (4 Mathud An	T1E2, 210 12070 > 07/	Fragmant	_	T1, 210 12070	_	C12H15N2O		_	210 12070		07 0760
cetyltylosin	4	4-IMAA (4-IMEthyl An	11F2: 210.12079-297.1	riagment	•	11: 210.12079	•	CI2HISINSO	msz	•	210.12079	9	11.0700
cyclovir	5	4-MAA (4-Methyl An	T1F3: 218.12879->187	Fragment	•	T1: 218.12879	•	C12H15N3O	ms2	•	218.12879	1	87.086
Ipha-Zearalanol (Zeranol)	6	5-Hydroxyflunixin	T1: 313.07945	TargetPeak	•		•	C14H11F3N2O3	ms1	•	313.07945	0	0.000
ipna-zearaienoi mantadine	7	Acetaminophen (Par	T1: 152.07061	TargetPeak	•		-	C8H9NO2	ms1	•	0.000	0	0.000
moxicillin	8	Acetyltylosin	T1: 958.53699	TargetPeak	•		-	C48H79NO18	ms1	•	0.000	0	0.000
moxicillin Na	0	Acetyltylocia	T1E1, 1040 000 > 772 .	Eragment	-	T1, 050 52600		C49U70NIO19		-	1040.000	7	772 / 177
mpicillin	9	Acetyityiosin	TIF1: 1040.000->772.4	riagment	•	11: 936.33099	•	C40H79N010	msz	•	1040.000	(	12.441
mpicillin D5	10	Acetyltylosin	T1F2: 1040.000->174.	Fragment	•	T1: 958.53699	•	C48H79NO18	ms2	•	1040.000	1	174.1124
rbidol	11	Acetyltylosin	T1F3: 1040.000->109.(	Fragment	•	T1: 958.53699	•	C48H79NO18	ms2	•	1040.000	1	109.064
zithromycin	12	Acyclovir	T1: 248.07541	TargetPeak	•		-	C8H11N5O3	ms1	•	0.000	0	0.000
zithromycin-D5	13	Alpha-Zearalanol (Ze	T1: 345.16725	TargetPeak	•		-		ms1	•	345,16725	0	0.000
acitracin A	14	Alpha-Zearalenel	T1, 220 16220	TargetBeak	-				mc1	-	220 16229	0	000
aquiloprim	14	Alpha-Zearaienoi	11: 520.10250	argereak	•		•		msi	•	520,10250	0	1.000
aquiloprim-do enzoestrol	15	Amantadine	T1: 152.14338	TargetPeak	•		•	C10H17N	ms1	•	0.000	0	).000
enzoestrol_K	16	Amoxicillin	T1: 366.11182	TargetPeak	•		•	C16H19N3O5S	ms1	•	0.000	0	0.000
enzoestrol_Na	17	Amoxicillin	T1F1: 400.000->208.04	Fragment	•	T1: 366.11182	•	C16H19N3O5S	ms2	•	400.000	2	208.0424
eta-Zearalanol (Taleranol)	18	Amoxicillin	T1F2; 400.000->114.0	Fragment	•	T1: 366.11182	-	C16H19N3O5S	ms2	-	400.000	1	114.000
arbadox	10	Amovicillin Na	T1: 288 00276	TargetDeak				C16H10NB05S	mc1	-	0.000	0	000
efacetrile	13	Amoxiciliin Na	T1: 300.09370	argetreak				010119105055	11151		0.000	0	1.000
efacetrile 13C3	20	Amoxicillin Na	11F1: 388.09376->114	Fragment	•	11: 388.09376	•	C16H19N3O5S	ms2	•	388.09376	1	14.000
efadroxil	21	Amoxicillin Na	T1F2: 388.09376->208	Fragment	•	T1: 388.09376	•	C16H19N3O5S	ms2	•	388.09376	2	208.0424
efadroxil Na	22	Ampicillin	T1: 350.1169	TargetPeak	•		•	C16H19N3O4S	ms1	•	0.000	0	0.000
efalenium	23	Ampicillin	T1F1: 400.000->160.04	Fragment	•	T1: 350.1169	-	C16H19N3O4S	ms2	•	400.000	1	160.042
efapirin	24	Ampicillin	T1F2: 400 000-> 174 0	Fragment		T1- 350 1160		C16H10NBOAS	ms?		400.000	1	174.054
efazolin	<												
efoperazone	Con	npound Details Pane											
efquinome													
efquinome ++		Compound Nam	e										
eftiofur		lonizatio											
eftriaxone		1011/28110	None	Ŭ									
efuroxime		Chemical Formul	a			Neutral	Mas	s 0					
efuroxime Na		CASM				C-4	0000						
hioramphenicol D5		CASIN				Cat	egon						
iprofloxacin		Compound Typ	e Analyte	U		Internal Sta	ndaro	ł	~				
larithromycin		Company of Company	-										
lopidol		Compound Group	5										
lopidol-D6													
loxacillin													





### **Thermo Vanquish LC System Parameters TM231**





Thermo Accucore VDX Column 2.1mm x 100mm 2.6um

**Mobile Phase A**: 0.1% Formic acid in Water

Mobile Phase B: Methanol

**Gradient**: t0 1%B, t2 1% B, t10 90%B, t10.1 98%B, t12 98%B

Flowrate: 0.3ml/min

Run time: 14mins

Injection Volume: 5ul (Positive Mode), 3ul (Negative Mode)





### **Q-Exactive Master Method parameters TM231**

### **Positive Polarity**

### Full Scan MS

- Resolution 70,000
- Scan range • 120-1200 m/z
- AGC target **1e6**

### **DIA** Experiment

- Resolution 35,000
- (N)CE / stepped ce:15, 30, 60







# Q-Exactive Orbitrap

### **Advanced Active** Beam Guide (AABG)

Orbitrap mass analyzer



**RF Lens** 

### **Exploris 120** Master Method Parameters TM231

### **Positive Polarity**

### Full MS

- Resolution 60,000 milk 120,000 eggs
- Scan range 200-1200 m/z
- AGC target standard

### **DIA** Experiment

- Resolution 30,000
- CE: 15, 30, 60



### Negative Polarity

### SIM

Resolution 120,000







# **Exploris 120 Orbitrap**



### Key differences between Q-Exactive and Exploris systems

Q-Exactive resolution 17,500; 35,000, 70,000; & 140,000 (not used) Exploris resolution 15,000; 30,000; 60,000 & 120,000

Exploris scan rate of 22Hz compared to Q-Exactive at 12 Hz.

Exploris has Easy-IC automatic Internal calibration (Flouranthene).

Different tuning solutions used for each instrument.

Updated Tune Software on Exploris.



# **Step 2: Sample Preparation**

Weigh 2g Matrix into a centrifuge tube.

- Add  $100\mu$ l internal standard,  $500\mu$ l acetonitrile
- Vortex all tubes and allow to stand in the dark for 10mins.
- Add130 $\mu$ I 0.1M Na2EDTA and a ceramic mixer.

### **Extraction 1**

- 4mls ACN/Water (80/20)
- Shake for 10mins
- Centrifuge 5mins @ 4000RPM
- Decant extract to glass evaporation tube containing 200ul of DMSO

### **Extraction 2**

- 4mls ACN
- Shake for 10mins
- Centrifuge 5mins @ 4000RPM
- Add extract 2 to extract 1
- Additional QuEChERS extraction for Milk samples

Evaporate under Nitrogen to 200ul mark with Turbovap at 50°C

- Reconstitute with 800ul 80/20 (Ammonium acetate/ACN)
- Centrifuge 5mins @18000RPM and filter with 0.22um PVDF filter into LC vial



# **Step 3: Validation – Design of Experiments**

Chapter 2 of Annex 1 to Commission Implementing Regulation (EU) 2021/808 describes the performance characteristics to be determined for the validation of analytical methods for the determination of pharmacologically active substances in food of animal origin. Qual Scre

> Selo Sta Rugo



litative eening	Semi-Quantitative Screening	Quantitative Screening
ССβ	ССβ	ССβ
lectivity	Selectivity	Selectivity
tability	Stability	Stability
gedness	Ruggedness	Ruggedness
	<b>Precision</b> *	Precision
		Trueness
		Relative matrix effect



# Several performance characteristics were determined simultaneously, and validation experiments were combined to minimise workload.

Validation Series	Muscle Samples	Milk Samples	Honey Samples	Egg Samples
Relative Matrix Effects	24	20	6	21
Specificity	21	21	21	21
Ruggedness	8 aliquots of one sample			
Series 1 Precision	7 5 fortification levels	7 5 fortification levels	7 4 fortification levels	7 3 fortification levels
Series 2 Precision	7 5 fortification levels	7 5 fortification levels	7 4 fortification levels	7 3 fortification levels
Series 3 Precision	7 5 fortification levels	7 5 fortification levels	7 4 fortification levels	7 3 fortification levels



# **Quantitative Analysis Capability**

- Minimum 5-point calibration curve included for all substances
- Detection of authorised Antibiotics at 0.5\*MRL or lower
- Detection of prohibited substances Chloramphenicol & Dapsone at 0.1\*RPA/ MMPR







# Validation/Accreditation Timeline

Jan 2019 Installation of HRAM Instrument LC- Orbitrap Q Exactive

### November 2020

Accreditation of 78 Antimicrobials in **muscle** matrix. Extended to 80 analytes January 2022

Accreditation of 82 Antimicrobials in <u>milk</u> matrix



### 2023

May 2023 Accreditation of chloramphenicol honey matrix

**March 2024** Accreditation of 83 Antimicrobials in egg matrix





### **TM231 Muscle Validation**

Bovine, Ovine, Porcine, Poultry, Cervine and Equine muscle included in validation.



Method **validated** according to Commission Implementing Regulation (EU) 2021/808.

80 compounds from 10 family classes passed validation

59 meet Quantitative criteria.

21 meet Semi-quantitative criteria.



Accredited by the Irish National Accreditation Board in 2022.



### **TM231 Milk Validation**



Bovine, Ovine, Caprine Milk included

QuEChERS extract was used as an additional sample clean-up. This improved responses for low responding analytes.

EUR-Lex

Method **validated** as a screening method according to Commission Implementing Regulation (EU) 2021/808.



82 compounds from 10 family classes passed validation



30 meet Quantitative criteria

52 meet **Semi-quantitative** criteria



Accredited by the Irish National Accreditation Board in 2023



### TM231 Egg Validation





Method validated as a screening method according to Commission Implementing Regulation (EU)



83 Compounds from 11 family classes passed validation.



**31** meet **Semi-quantitative** criteria.



Accredited by the Irish National Accreditation Board in 2024.





### **Positive Mode XIC of analytes (Egg Matrix)** Cal 3



22

![](_page_21_Picture_3.jpeg)

![](_page_21_Picture_4.jpeg)

# **Routine use of method in Official Controls**

### TM231 has been in routine use since 2022.

### LC-HRMS analysis is used for all our antibiotic screening.

### 4 LC-Orbitrap Instruments are used in the lab. 2 Q-Exactive instruments and 2 Exploris 120 instruments

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![](_page_22_Picture_5.jpeg)

# **Positive Sample detection**

![](_page_23_Figure_2.jpeg)

![](_page_23_Picture_3.jpeg)

![](_page_23_Picture_5.jpeg)

### Target Muscle - Antibiotics Detected

![](_page_23_Picture_7.jpeg)

![](_page_23_Picture_8.jpeg)

![](_page_23_Picture_9.jpeg)

## **Positive Sample detection**

![](_page_24_Figure_2.jpeg)

![](_page_24_Picture_4.jpeg)

![](_page_24_Picture_5.jpeg)

### **Suspect Muscle - Antibiotics detected**

![](_page_24_Picture_7.jpeg)

# **Positive Sample detection**

![](_page_25_Figure_1.jpeg)

**Milk - Antibiotics Detected** 

![](_page_25_Picture_4.jpeg)

### Positive Sample detection Egg - Antibiotics Detected

![](_page_26_Figure_1.jpeg)

0

![](_page_26_Picture_3.jpeg)

	4	
	1	

### Oxytetracyline

2022 2023 2024

![](_page_26_Picture_7.jpeg)

### **Tulathromycin detected in bovine muscle sample**

### 👧 Thermo TraceFinder EFSLC

File View Teels Hele

File view tools Help																				Real time status	User: COMPUTER	I 🕜 🌣
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Analysis 🗸 🗸 🗸	Data Review - WS 36-24	4 POSITIVE	[Quan]																			
Ratch View	Compounds 🛛 🔻 🕂 🗙	Sample Re	esults																			🚽 म 🗙
· baten new	🚰 Compound RT	<b>₽</b> ⊡€	Sele 📮	џ Sta	atus 🏨	Sample ID	џ Area ц	ISTD Respo	onse 📮 RT	🐥 🛛 Actual Rī	Г 📮 RT Delta	Theoretica	📮 Calculate	🏨 m/z (Delta)	φ S/N φ	Exclude 📮	PK	џ IR 4	IP	џ Isotopic џ	F	ilename 🔿
Samples	<u>A</u> a - <u>A</u> a -		Aa	= <u>A</u> a	•	<u>A</u> a -	<u>A</u> a 👻	<u>A</u> a	<b>-</b> <u>A</u> a -	<u>A</u> a -	Aa 🗸	<u>A</u> a -	<u>A</u> a ▪	<u>A</u> a 🗸	<u>A</u> a ▼		= -	= -	= -	= -		
Auto Samples	49 Oxytetracyclir 6.13	± 4	~	4	•	Cal O	N/F	41249618	6.07	N/F	N/F	0.000	N/F	N/F	N/A					N/A	24_4_29_004	
Reference Sample	50 Penicillin V D! 9.03	<b>⊕</b> 5	$\checkmark$	5	•	Cal 1	14380466	41309329	6.07	6.08	0.01	10.000	10.128	.2967 (ppm)	INF		•	•	•	100	24_4_29_005	
	51 Penicillin-G 8.63	± 6	✓	б	•	Cal 2	40831665	48551994	6.07	6.08	0.00	25.000	24.492	.1835 (ppm)	INF		•	•	•	100	24_4_29_006	
Threshold Samples	52 Penicillin-G N 8.63	± 7	✓	7	•	Cal 3	80132386	48969186	6.07	6.07	0.00	50.000	47.671	.2967 (ppm)	INF		•	•	•	100	24_4_29_007	
🔻 Data Review 🔷 🔪	53 Penicillin-V 9.05	<b>⊕</b> 8	$\checkmark$	8	•	Cal 4	165940522	46442323	6.07	6.08	0.00	100.000	104.110	2694 (ppm)	INF		•	•	•	100	24_4_29_008	
	54 Penicillin-VIN 9.05	± 9	$\checkmark$	9	•	Cal 5	390710174	44957436	6.07	6.07	0.00	250.000	253.249	3826 (ppm)	INF		•	•	•	100	24_4_29_009	
Sample View	55 Pirlimycin 7.83	⊕ 10	✓	10	•	Wash_3	N/F	N/F	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_010	
Compound View	57 Rifaximin-d6 9.99	⊕ 11	~	11	•	Reagent Blank	N/F	63045274	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_011	
Comparative View	58 Sarafloxacin 6.49		~	12	•	Compliant Control	N/F	50125661	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_012	
	59 Spiramycin 7.03	⊞ 13	~	13	•	LC24-1367	N/F	43093561	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_013	
Report View	60 Sulfacetamid 3.79	⊞ 14	~	14	•	LC24-1372	N/F	51194340	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_014	
	61 Sulfachloropy 6.35	⊕ 15	~	15	•	LC24-1373	N/F	48411547	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_015	
Local Method	62 Sulfaclozine 7.32	⊞ 16	✓	16	•	LC24-1383	6450693564	44897677	6.07	6.06	-0.02	N/A	4187. <b>00</b> 5	3826 (ppm)	35734. <b>0</b> 3		•	•	•	100	24_4_29_016	
Acquisition	63 Sulfadiazine 4.76	⊕ 17		17	-	LC24-1384	N/F	37911432	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24_4_29_017	
Our at het in a	64 Sulfadimetho 7.46	± 18		18	2	LC24-1573	N/F	51706179	6.07	N/F	N/F	N/A	N/F	N/F	N/A		- 21			N/A	24_4_29_018	
Quantitation	65 Sulfadoxine 6.67	± 19		19	2	LC24-1592	N/F	54286200	6.07	N/F	N/F	N/A	N/F	N/F	N/A		- 21			N/A	24_4_29_019	
Processing	66 Sulfamerazin∈ 5.43	< 20	<b>V</b>	20	-	LC24-1613	N/F	54694713	6.07	N/F	N/F	N/A	N/F	N/F	N/A					N/A	24 4 29 020	>
Compounds	67 Sulfamethazir 5.95	Compoun	nd Details																			<b>→</b> ₽ ×
OAOC	68 Sulfamethizol 5.95	Ouan Peal	ık	~				₹×	Calibration Cu	rve 🗸				<b>▼</b> ×	Confirmina lo	ns V						<del>,</del> x
Groups	59 Sulfamethoxa 6.49										Tulathro	mvcin			<i>-</i>							
Groups	70 Sulfamethox 611	LC24-13	383 Tulaf	thromycin	m/z: 269.	5294			)	' = 3.431e-2X + 5	5.727e-4; R^2: 0.99	982; Origin: Ignore	; W: 1/X^2; Area	a 🛛	All Peaks		L	.C24-1383 Tul	LC	C24-1383 Tul	LC24-1383 Ti	al
Intel Seq	72 Sulfamonomi 6.57					RT 6 06			10						🔵 M+H (400	0.0000->158.11	76)					
Reports	73 Sulfaphenazo 7.21					AA: 6450693564			9-						M+H (40)	0.0000->116.10	70)					
	74 Sulfapyridine 5.25	400				AH: 1864782665 SN: 35734.03							/		• M. H. (40)	0000 - 400.00		400		400	400	
	75 Sulfaquinoxal 7.62	100	ĹŢ			Ť			8-						🕛 IVI+H (400	1.0000->420.29	56)	100		100	100-	
	76 Sulfasalazine 9.05	90				1			7-									90-		90-	90-	
	77 Sulfathiazole 5.13	Usity 80	1						-			/							nsitv		-08 Isity	
	78 Sulfisoxazole 6.75	Inte 10	1						6-								1	ae 70-	Inte		- <sup>07</sup> Ite	
	79 Tetracycline 6.03	e 60	1			A A			Harris S-									a <sup>60</sup> −	tive	a 60-	-00 ti	
	80 Tiamulin 8.44	l leg 50	1						- Area									50- -	Blac	50-	-05 ge	
	81 Tildipirosin 5.13	- 40	님						4-									40-	-	40-	40-	
	82 Tilmicosin 7.66	30	勹						3-		_							30-		30-	30-	
	83 Trimethoprin 5.71	20	4						-	/								20-		20	20-	
	84 Tulathromyci 6.07	10	님						2-									10-		10-	10-	
Acquisition	85 Tylosin 8.57	0	᠈᠋᠆᠇᠆ᠬ			····		7.0		/								᠐᠆┹ᡣᠯᡨᢇ		0-4	0-6	1
Analysis	87 Valnemulin 0.27			0.0		RT(min)	0.0	1.0		l												
	88 Virginiamycir 9.25	m/z: 269	9.5294					^	0	50	100	150	200	250			n	n/z: 158.1176	~ m	n/z: 116.1070 🔥	m/z: 420.2956	^
Method Development	<	Apex RT	: 6.06 MEACODEC/	Left RT: 6	0.00 R	ight RI: 6.20	E010E 07	~	, , , , , , , , , , , , , , , , , , ,	50	 เ	ug/kg					0	).00% - 22.08%	·	.00% - 22.44% 1	0.00% - 20.21%	°4 ~ ~

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![](_page_27_Picture_7.jpeg)

# **Customised Reports**

### Screening Report TM 231

Lab Name:	Veterinary Residues		
Instrument:	ChromLC 142.0	Method Name:	WS 38-25 POSITIVE BATCH_TM231_Muscle_Positive Mode_001
Master Method:	TM231_Muscle_Positive Mode_001		
Batch:	WS 38-25 POSITIVE BATCH		

Vial Pos	Sample ID	File Name	Level	Sample Name	File Date	Comment
B:D3	LC25-1837 (PT)	2025_05_21_033	N/A	LC25-1837 (PT)	5/21/2025 10:53:53 PM	
Internal Oten deads						
Internal Standards						
Compound Name	Retention Time	Expected RT	RT Delta	m/z Delta		
Ampicillin D5	6.34	6.33	0.00	1.8174 (ppm)		
Azithromycin	7.14	7.14	0.00	2.1110 (ppm)		
Penicillin V D5	8.98	8.97	0.02	-2.3314 (ppm)		
Oxolinic Acid D5	7.47	7.47	0.00	7755 (ppm)		
Rifaximin-d6	9.92	9.91	0.01	-1.2604 (ppm)		
Sulfamethoxypyrodazine-d3	6.03	6.02	0.01	.0769 (ppm)		
Baquiloprim-d6	3.23	3.44	-0.21	1963 (ppm)		
The following compounds were	o found in the comm	la				

### The following compounds were found in the sample

Compound Name	<b>Retention Time</b>	Expected RT	RT Delta	Calculated Amount (µg/Kg)	Sum Compounds	>ССβ
Flumequine	8.47	8.47	0.00	144.16		TRUE
Baquiloprim	3.74	3.88	-0.15	0.54		FALSE
Tilmicosin	7.56	7.55	0.02	0.49		FALSE
Valnemulin	9.17	9.14	0.02	0.14		FALSE
Difloxacin	6.26	6.25	0.01	0.12		FALSE
Erythromycin	8.38	8.51	-0.13	0.09		FALSE
Enrofloxacin	6.05	6.04	0.01	0.01		FALSE

m/z Delta

-.4568 (ppm)

1.0872 (ppm)

.8728 (ppm)

-1.0187 (ppm)

3.6058 (ppm)

4.6344 (ppm)

-.5543 (ppm)

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![](_page_28_Picture_7.jpeg)

### TM231 Positive Screening Report

![](_page_28_Figure_9.jpeg)

545488	
14	

t	- 24	 -	

# **Routine maintenance**

Poor peak resolution and loss of sensitivity caused by hydrophobic contamination. Column cleaned with "Chromacare" solution (45% IPA, 45% ACN and 10% Acetone)

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![](_page_29_Picture_5.jpeg)

- Systems perform best when in continuous use. Monthly Full calibration Mass calibration on day of use.
- Ion transfer tube and Ion sweep cone cleaned weekly S-Lense cleaned quarterly

# **Excellent Platform for Further Expansion**

![](_page_30_Figure_1.jpeg)

![](_page_30_Figure_3.jpeg)

![](_page_30_Picture_4.jpeg)

### **Exploris 120 Muscle Development Method Positive**

![](_page_31_Figure_1.jpeg)

60000	
100-1500	
70	
Standard	
Custom	
100	
1	
Profile	
Positive	

Time Range (min) 0-15 CLEAR 前 Data-Dependent MS<sup>2</sup> Scan Properties 1.5 Isolation Window (m/z) Full Scan Isolation Offset Off Intensity 1 Collision Energy Type Normalized scans Dynamic Exclusion HCD Collision Energies (%) 30,50,150 ddMS<sup>2</sup> Orbitrap Resolution 15000 Scan Range Mode Auto AGC Target Standard Maximum Injection Time Mode Custom 22 Maximum Injection Time (ms) Microscans

Data Type

Profile

### **Muscle Development Method Positive XIC (Cal 2 Level)** 风 XIC Overlay

Show Peaks: O Top 20 O Selected 
All

![](_page_32_Figure_2.jpeg)

	RT: 8.25						
RT: 7.47							
			10,65				
DT: 7.6	5 RT	8 95					
	J						
			DT	0.67			
		RT: 9.08	-RI.	0.07			
26	RT: 8.46						
RT:	7.87	RT: 9.10 RT: 9.	85				
RT: 7.44	DT						
	RI:	5.95 PT: 0.50		RT: 11.35			
-RT: 7.	52	KI. 9.59		RT: 11.4	8		
	RT: 8.5			RT: 11.7	3		
RT 7.43	RI: 8.12	RT: 9.32					
7.63	8.49	RT: 8199 RT: 9.6	9 RT: 10.42	RT. 11.5	53		
VIME	-8	d 45	04 10 30 10	99 17 32 MA 58	12.26 12.46	13,13 13,87	7
	8	9 10	0 1	1 1	2 1	3 1	4 15
KI (min)							

![](_page_32_Picture_4.jpeg)

NL:	1	00E7
RT:	8.	48
NL:	1	87E6
RT:	9.	45
NL:	1	12E5
RT:	6.	79
NL:	9	22E5
RT:	1.	24
NL:	3	05E6
RT:	6.	41
NL:	1	.02E5
RT:	2.	70
NL:	1	.02E5
RT:	2.	70
NL:	1	25E6
RI:	3.	46
NL:	5	30E5
RI:	3.	40
NL:	3.	29E6
RI.	э. 4	2055
	1.	39E5
кі. ын.	4	7457
DT-	1. 2	65
NI -	4	0456
RT.	4	00
NI -	2	78E6
RT:	4	12
NI ·	1	28E6
RT:	4	23
NL:	3	75E5
RT:	4.	33
NL:	1	22E7
RT:	4.	44
NL:	2	03E6
RT:	4.	54
NL:	1	.09E5
RT:	4.	55
NL:	1	23E6
RT:	4.	55
NL:	9	05E6
RT:	4.	76
NL:	1	94E7
RT:	4.	89
NL:	1	95E7
RT:	4.	.92
NL:	1	.59E7
RT:	4.	94
NL:	8	43E5
RI:	э.	03
NL: DT:	1. E	56E7
NI -	э. Л	6555
RT:	5	19

# **Exploris 120 Muscle Development Method Negative**

### Time Range (min) 0-15 CLEAR 🍿 Full Scan Properties Full Scan Orbitrap Resolution Scan Range (m/z) Intensity 4 RF Lens (%) scans Dynamic Exclusion AGC Target ddMS<sup>2</sup> Maximum Injection Time Mode Maximum Injection Time (ms) Microscans Data Type Polarity Source Fragmentation

120000
100-1500
70
Standard
Custom
100
5
Profile
Negative

![](_page_33_Picture_4.jpeg)

![](_page_33_Picture_5.jpeg)

# **Muscle Development Method Negative XIC (Cal 2 Level)**

![](_page_34_Figure_1.jpeg)

	_

	RT:	8.40												
			RT	9.61										
				RT: 9.88	RT:	10.43								
			PT: 0.19	RT:	10.06	RT: 10.7	79							
RT: 7.39	8 10				RT: 10.2	26	0.91	DT: 11 7	7					
	8		9 9	1	0		11	ייידי וו. <i>ו</i> דייןייידילידיי 1	  2	 1	  1	  4	• • • • • • • • • • • • • • • • • • • •	+ 15
RT(min)														

![](_page_34_Picture_4.jpeg)

NL: 9.29E3
RT: 4.84
NL: 1.72E4
RT: 0.35
NL: 2.83E4 RT: 10.53
NI - E 24E7
RT: 8.40
NL: 3.91E6
RT: 10.79
NI : 0
NL: 0
NL: 0
NL: 0
NL: 0
NL: 4 07E5
RT: 10.26
KI. 10.20
NL: 2.04E6
RT: 6.40
NL: 1.54E6
RT: 6.10
NL: 8.18E4
RT: 7.39
NU : 4 CEEC
NL: 1.05E0
RT: 10.20
NL: 2.47E5
RT: 6.33
NL: 1.66E7
RT: 6.35
NL: 4.55E6
RT: 10.06
NI · 9 48E5
RT: 10.86
NIL 0 4750
NL: 2.4/E6
RT: 9.18
NL: 2.86E6
RT: 9.88
NL: 5.47E6
RT: 10.43
NL: 2.61E6
RT: 9.15
NU - 2 04EC
NL. 2.04E0
RT: 10.01
NL: 3.55E4
RT: 11.34
NL: 4.70E7
RT: 9.61
NL: 1.39E7
RT: 6.51

### Limitations

- Legislation has not kept up to pace with use of HRMS techniques.
- Guidelines are heavily based on QQQ methods.
- No criteria for Isotopic pattern and Library matches
- Revision of HRMS guidelines are underway, this will hopefully allow additional identification and confirmatory criteria specific to HRMS.
- Difficult to obtain reference standards and to keep solutions within their expiry dates.

![](_page_35_Picture_7.jpeg)

![](_page_35_Picture_8.jpeg)

### Conclusions

- Thank you for your attention.
- Any questions?
- Email. lan.Kelleher@agriculture.gov.ie

![](_page_36_Picture_5.jpeg)

![](_page_36_Picture_7.jpeg)

![](_page_36_Picture_8.jpeg)