UHPLC-TOF-MS REVALIDATION FOR ANTIBIOTIC RESIDUE CONTROL & SURVEILLANCE PLANS

Andreia Freitas

Presentation

National Institute for Agrarian and Veterinary Research

Portuguese Research Center - Network of Chemistry and Technology

Vairão/Vila do Conde

FOOD SAFETY UNIT

- National reference Laboratories
  - Microbiology (Food and Feed)
  - Veterinary medicine Residues & toxicology
  - Pesticides and other contaminants
- Research and inovation
- Oficial Controles
- Forensic toxicology

Goal: Sustainable Chemistry used as a powerful and dynamic tool to tackle the societal, economic, and environmental challenges of modern life, contributing to a World Sustainable Development.

Revalidation: Why?

Commission Decision 2002/657/EC

2002D0657 — EN — 10.01.2004 — 002.001 — 2

▼B

COMMISSION DECISION

of ►C1 14 August 2002 ◄

implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

(notified under document number C(2002) 3044)

(Text with EEA relevance)

(2002/657/EC)

Commission Implementing Regulation 2021/808

L 180/84 EN Official Journal of the European Union 21.5.2021

COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

of 22 March 2021

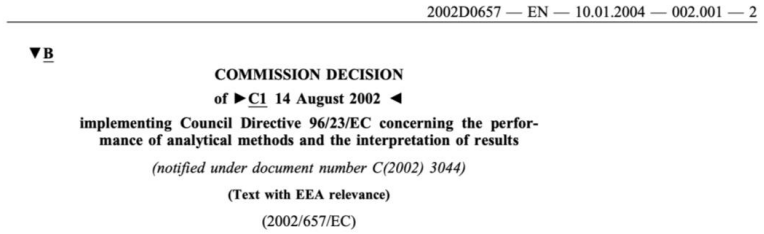
on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

(Text with EEA relevance)

Method performance, identification criteria, validation parameters

What changed…

Commission Decision 2002/657/EC



2002D0657 — EN — 10.01.2004 — 002.001 — 2

▼B

COMMISSION DECISION

of ►C1 14 August 2002 ◄

implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

(notified under document number C(2002) 3044)

(Text with EEA relevance)

(2002/657/EC)

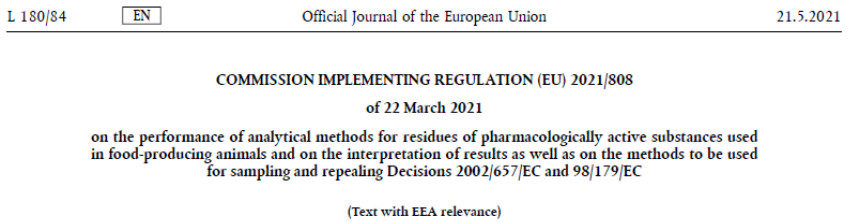
Performance/identification
- ΔRRT < 2.5%
- ΔIon Ratio ⇒ Conc. dependent

Precision and Recovery
(Validation levels)

1, 1.5 & 2 MRPL
0.5, 1 & 1.5 MRL

Monitoring

Commission Implementing Regulation 2021/808



L 180/84 EN Official Journal of the European Union 21.5.2021

COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

of 22 March 2021

on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

(Text with EEA relevance)

Performance/identification
- ΔRRT < 1%
- ΔIon Ratio < 40%
- HRMS Δm<5ppm

Precision and Recovery
(Validation levels)

0.5, 1 & 1.5 RPA
0.1, 1 & 1.5 MRL
1.0, 2 & 3 LCL

Monitoring & Survaillance

Antibiotic Residues

Quantitative Multi-detection Screening Method

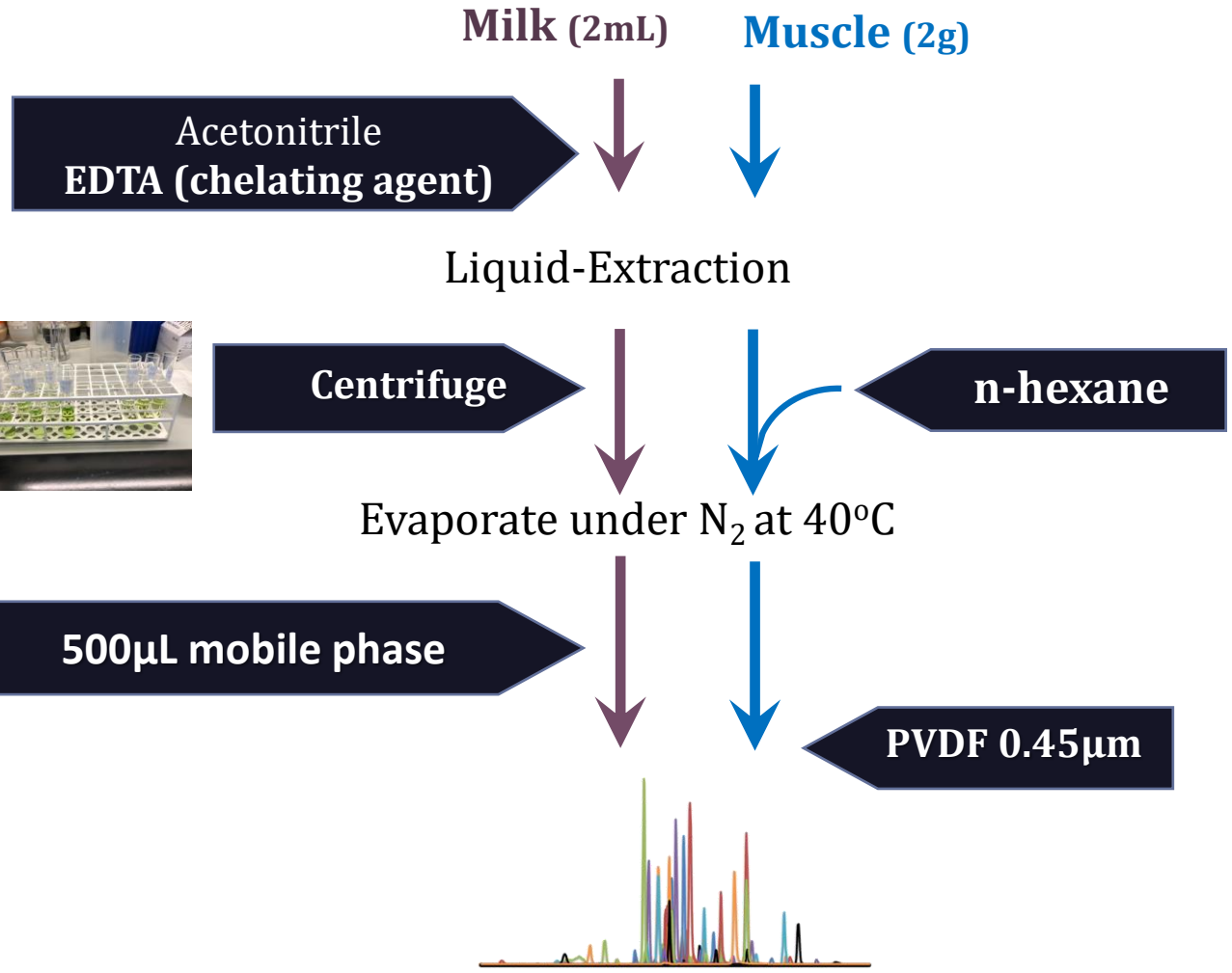
Quantitative Multi-detection Screening Method
Liquid Chromatography coupled with High Resolution Mass Spectrometry (UHPLC-ToF-MS)

Scope of the method: 89 compounds - penicillins, cephalosporins, macrolides, tetracyclines, quinolones, sulfonamides, pleuromutillins, sulfone (dapsone), amphenicols and diaminopyrimidines

ESI + Ion Source
ToF Mass range:
100 - 1400 Da
(More Data can be reviewed **afterwards)**

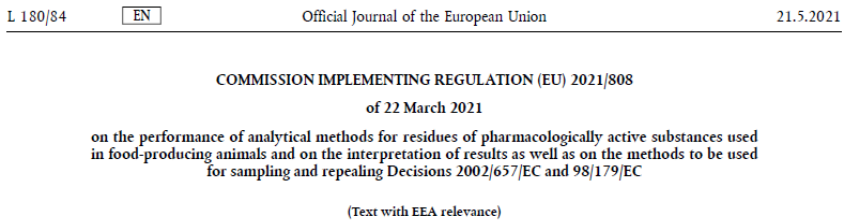
Confirmation Method - targeting detected compound, UHPLC-MS/MS

ESI + Ion Source
MRM acquisition mode
(**2 MRMs** per compound)



Antibiotic Residues

Commission Implementing Regulation 2021/808



L 180/84 EN Official Journal of the European Union 21.5.2021

COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

of 22 March 2021

on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

(Text with EEA relevance)

Revalidation parameters for a quantitative screening method:

CCβ, trueness (or recovery), precision, selectivity/specificity and linearity of calibration curves;

Levels tested: 0.1MRL to 3MRL

Identification criteria mandatory: exact mass (with maximum acceptable error Δppm = 5) and the variation of the relative retention time (not exceeding 1%).

Initial sets of experiments: example for sulfonamides

Levels of spiking concentrations									
MRL/10	MRL/8	MRL/5	MRL/4	MRL/2	MRL	1,5*MRL	2*MRL	3*MRL	ppb
10	12,5	20	25	50	100	150	200	300	

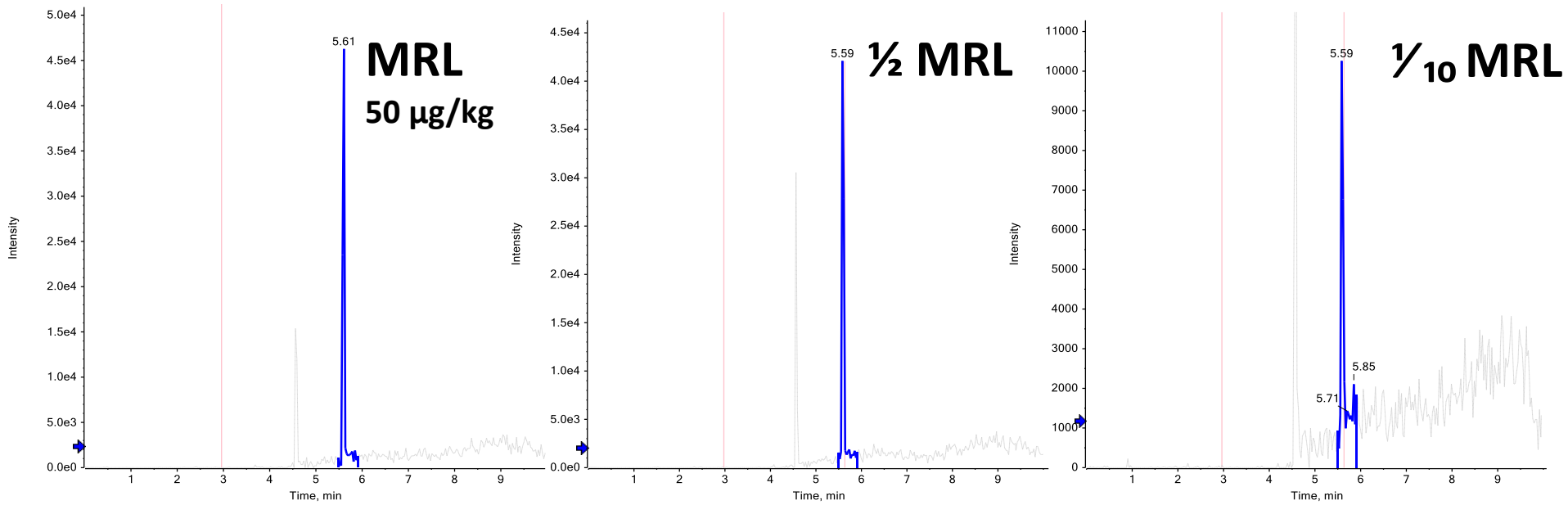
CCß ??

Method 2: by investigation of fortified blank material at concentration levels below the permitted limit.

Antibiotic Residues

Sulfachlorpyridazine

$[C_{10}H_{10}ClN_4O_2S]^+$= 285,02075



ΔRRT = 0.0%
Δm= -1.4 ppm

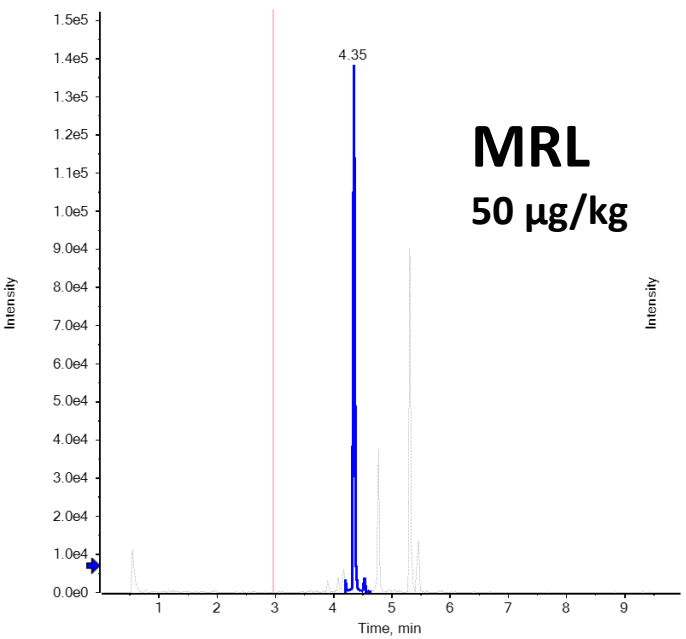
ΔRRT = 0.0%
Δm= -0.8 ppm

ΔRRT = 0.0%
Δm= -2.1 ppm

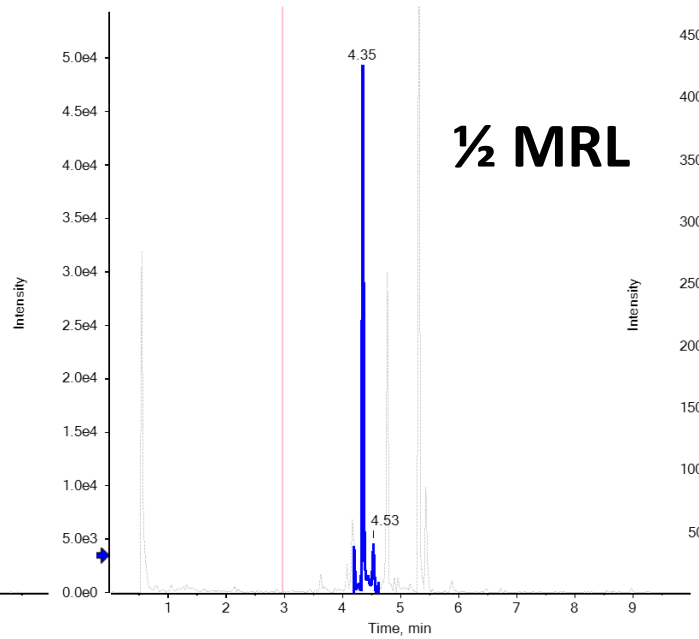
Antibiotic Residues

Sulfisoxazole

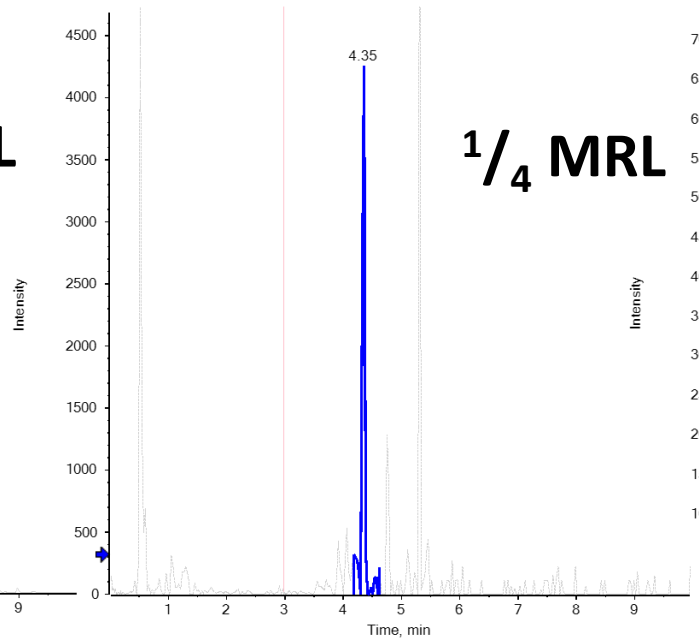
$C_{11}H_{14}N_3O_3S]^+$= 268,07504



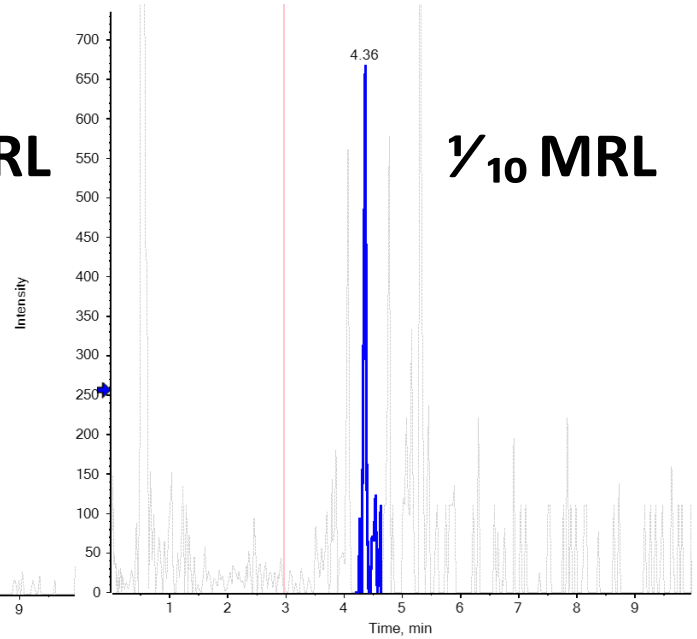
MRL
50 µg/kg



½ MRL



1/4 MRL



1/10 MRL

ΔRRT = 0.0%
Δm= 0.4 ppm

ΔRRT = 0.0%
Δm= 0.3 ppm

ΔRRT = 0.0%
Δm= 5.0 ppm

ΔRRT = 0.0%
Δm= 8.7 ppm

Antibiotic Residues

Class of antibiotics	Compound	Group (A/B)	MRL (µg.kg^{-1})	CCβ (µg.kg^{-1})	Work range (µg.kg^{-1})	Linearity	RSD_r (CV%)	RSD_R (CV%)	Recovery (%)	CCα (µg.kg^{-1}) LC-MS/MS
Sulfonamides	Sulfacetamide	B	100	50	50 to 300	0.999	7.3	8.6	99.1	115.4
	Sulfachlorpyridazine	B	100	10	10 to 150	0.999	7.2	9.8	100.3	119.0
	Sulfaclozine	B	100	10	10 to 150	0.996	7.8	10.8	108.2	133.0
	Sulfadiazine	B	100	10	10 to 150	0.999	4.7	6.0	100.4	117.3
	Sulfadimethoxine	B	100	10	10 to 150	0.993	8.5	12.3	114.2	132.8
	Sulfadoxine	B	100	10	10 to 150	0.997	3.9	6.4	106.1	123.9
	Sulfaguanidine	B	100	25	25 to 250	0.999	4.3	5.9	100.0	109.0
	Sulfamerazine	B	100	25	25 to 250	0.999	4.9	5.5	99.6	109.5
	Sulfamethazine	B	100	10	10 to 150	0.999	5.5	7.1	99.6	116.0
	Sulfametiazol	B	100	10	10 to 150	0.999	6.2	7.0	100.6	113.0
	Sulfamethoxazole	B	100	10	10 to 150	0.999	9.7	13.5	96.6	114.8
	Sulfamethoxypyridazine	B	100	10	10 to 150	0.999	6.3	8.3	104.2	118.7
	Sulfamonomethoxine	B	100	25	25 to 250	0.999	3.5	4.4	99.8	113.0
	Sulfamoxol	B	100	10	10 to 150	0.985	6.6	6.9	118.9	122.2
	Sulfanilamide	B	100	25	25 to 250	0.998	13.3	14.3	102.6	137.3
	Sulfapyridine	B	100	10	10 to 150	0.999	5.7	7.6	98.6	111.7
	Sulfaquinoxaline	B	100	10	10 to 150	0.995	8.1	11.4	105.4	129.4
	Sulfasalazine	B	100	25	25 to 250	0.991	18.2	21.1	96.3	155.1
	Sulfathiazole	B	100	25	25 to 250	0.999	4.0	4.9	101.7	108.1
	Sulfisomidine	B	100	10	10 to 150	0.997	4.7	6.9	102.6	117.3
	Sulfisoxazole	B	100	25	25 to 250	0.997	3.8	5.5	100.4	116.8

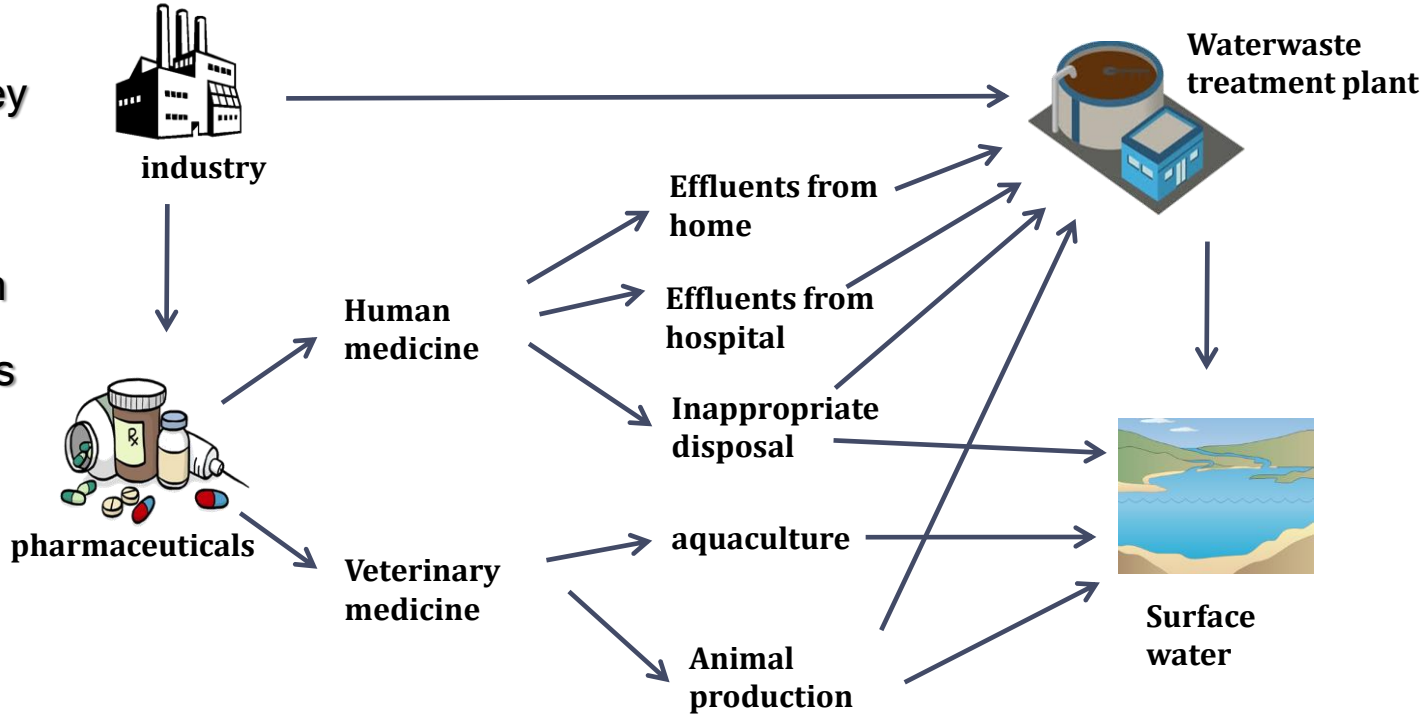
Monitoring & Surveillance

1. **High demand** for **food** leads to intensive animal production - **excessive use of veterinary medicines** - flow of contaminants along the chain (manure, waste water...)
2. Main Focus on Food Products - however, they may occur in the **ecosystem** and became persistent
3. Cross Contamination & Circular contamination
4. Mapping persistant/constant low residual levels

Routine analysis and official monitoring plans

Research data

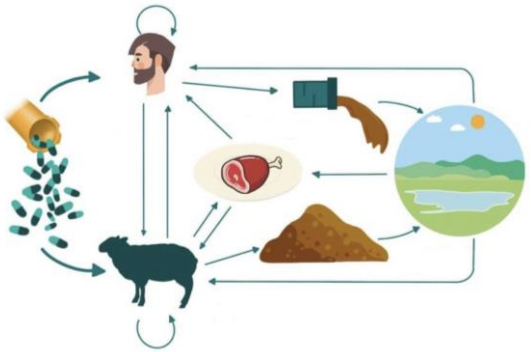
Human and veterinary drugs widely used ⟷ Potential environmental contaminants are overlooked or not monitored



Surveillance though Research

RUMIRES

Epidemiological Surveillance and Awareness of Antimicrobial Resistance and Drug Residues in Small Ruminants of the Centre Region

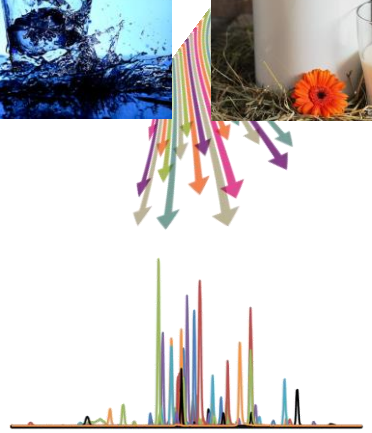


"ONE HEALTH"

SMARTgNOSTICS

Global Testing & Diagnostics Solutions for antimicrobial resistances -

Monitoring of antibiotic resistant pathogens and antibiotic residues



BeSafe BeeHoney

BEekeeping products valorization and biomonitoring for the SAFEty of BEEs and HONEY (BeSafeBeeHoney)

1. Nutritional & medicinal properties
2. **Abiotic stressors and anthropogenic contaminants in the environment using hive products as indicators.**
3. Prevalent diseases and biotic stressors
4. Pollinators in agriculture
5. Policy & market analysis

One of the main assumptions is that the highest impacts on the honey production decline are environmental contaminants such as pharmaceuticals, which are used both for animals and humans, a global concern about the spread of antimicrobial resistance.

Thank you for your attention

and keep in mind:

Ensuring the monitoring and surveillance of veterinary is essential not only for public health protection, but also for promoting sustainable animal production and guaranteeing consumers' confidence in a safe food supply chain

andreia.freitas@iniav.pt