Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation — Nantes Atlantique
Santé et alimentation au cœur de la vie

EuroResidue VIII
Conference on Residues of Veterinary Drugs in Food

PRE-CONFERENCE WORKSHOP

RESIDUE ANALYSIS FOR DUMMIES

Egmond aan Zee, The Netherlands, 23-25 May 2016

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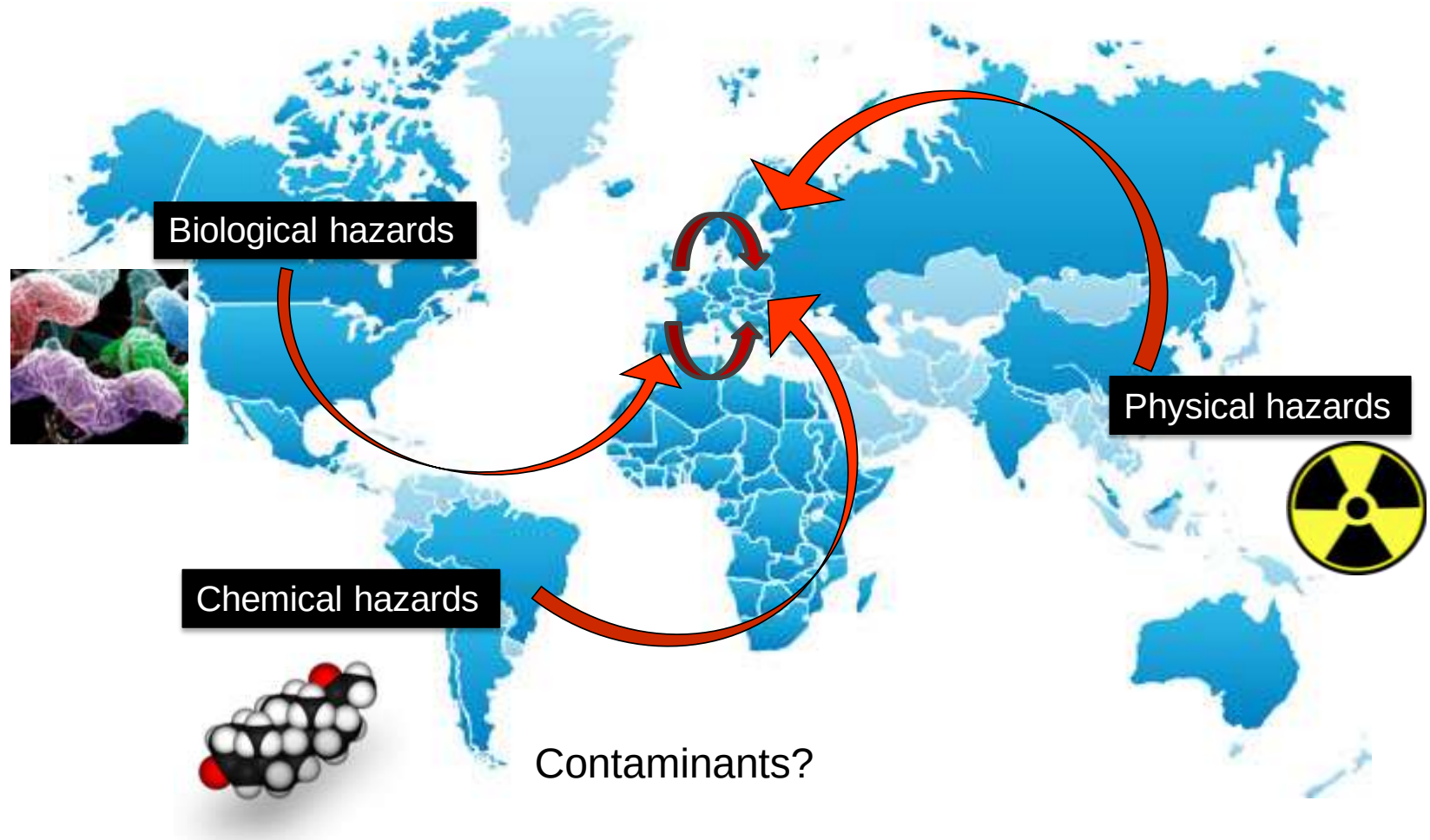
RIKILT WAGENINGEN UR
SARAF School for Advanced Residue Analysis in Food
LABERCA

1. INTRODUCTION

1.1 Different categories of hazards: natures and origins



Residues?

→ substances that can occur in foodstuffs as a side effect of using veterinary medicines or phytosanitary products.

Contaminants?

→ substances that can non-intentionally enter food during its production or marketing. These can include environmental pollutants.

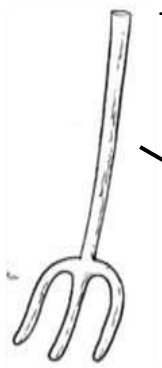
1. INTRODUCTION

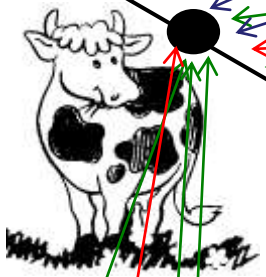
1.1 Substances entering in the food chain

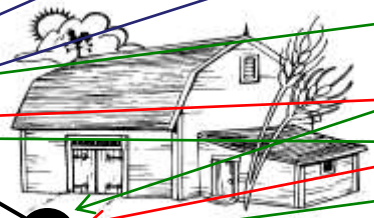
HORMONES

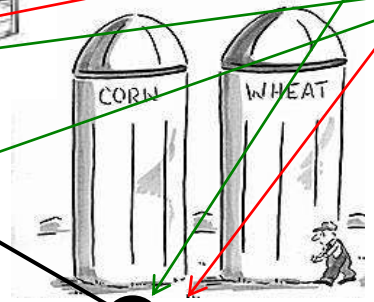
VET DRUGS

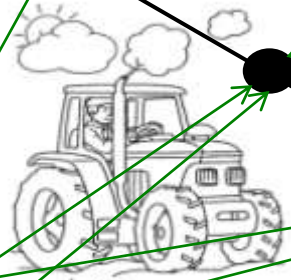
Phytosanitary products
Mycotoxins
POPs

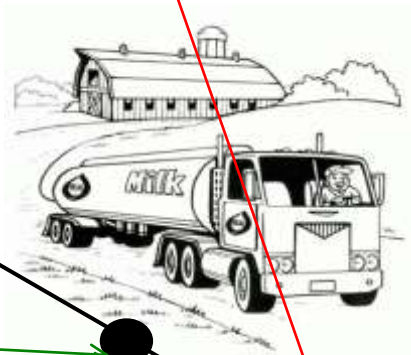


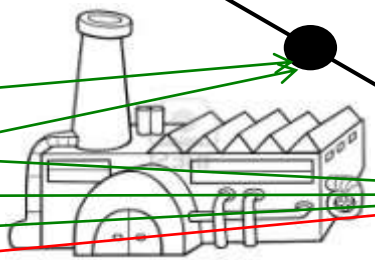




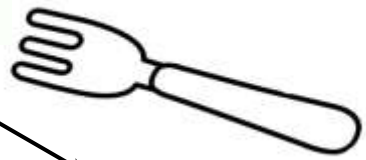








Additives
Disinfectants
Heavy metals
Heatox
FCM



1. INTRODUCTION

2. REGULATION

3. COMPOUNDS/MATRICES TO MONITOR

4. ANALYTICAL METHODS

5. IP, CCα, MRPL & RPA

2. REGULATION

Article 3 Regulation (CE) n° 178/2002

2.1 Risk analysis, a short introduction

POLITIC
(GENERAL DIRECTORATES)

ASSESSMENT

Hazard Identification

Hazard characterization

Exposure assessment

Risk characterization

MANAGEMENT

Risk assessment

Assessment of management options

Application of management options

Monitoring and review

Scientific advices,

Opinions, recommandations

Request

SCIENCE
(INDEPENDENCE, COMPETENCY, TRANSPARENCY…)

COMMUNICATION

RISK MANAGER + RISK ASSESSOR

STAKEHOLDERS
(PRODUCERS, TRANSFORMATORS, DISTRIBUTORS, CONSUMERS, ACADEMIA, MEDIAS..)

2. REGULATION

2.1 Risk analysis, a short introduction

	ASSESSMENT	MANAGEMENT
WORLD	JECFA — Comité mixte FAO/OMS d'experts des additifs alimentaires — Joint Expert Committee on Food Additives Joint Meeting on Pesticide Residues — Joint FAO/WHO Meeting on Pesticide Residues	CODEX ALIMENTARIUS — CODEX COMMITTEES CCFAC CCPR CCRVDF
EU	EUROPEAN MEDICINES AGENCY — SCIENCE MEDICINES HEALTH EFSA — European Food Safety Authority	DG SANTE Commission DG Trade
COUNTRIES	anses — agence nationale de sécurité sanitaire alimentation, environnement, travail ATSDR — AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY rivm BfR — Risiken erkennen – Gesundheit schützen US Environmental Protection Agency	Liberté • Égalité • Fraternité — RÉPUBLIQUE FRANÇAISE MINISTÈRE DE L'AGRICULTURE DE L'ALIMENTATION DE LA PÊCHE DE LA RURALITÉ ET DE L'AMÉNAGEMENT DU TERRITOIRE DGAl

2. REGULATION

2.2 General Scheme

FOOD & FEED SAFETY — Reg 2002/178/EC *Food Law*

OFFICIAL CONTROLS ON PRODUCTS OF ANIMAL ORIGIN INTENDED FOR HUMAN CONSUMPTION — (Reg 2004/854/EC) (Reg 2004/882/EC) *Official controls*

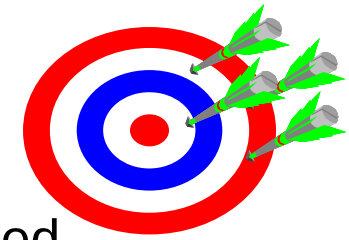
CHEMICAL FOOD SAFETY

- Contaminants
- Food contact materials
- Residues of **veterinary** medicine
- Contamination *via* substances having **hormonal** effects
- Pesticides

Monitoring substances having a hormonal action and other substances in animals and animal products	**Residues** of veterinary medicinal products in foodstuffs of animal origin	**Prohibition** on administering hormones and other substances to farm animals
Dir 1996/23 *Framework residue monitoring*	**Dec 2002/657** *Analyticals*	**Reg 470/2009** *Establishing MRL principles*
		Reg 37/2010 *Listing MRLs values*
		Dir 1996/22 *Hormonal substances*
		Dir 2003/74 Dir 2008/97 *Ban E_2*

2. REGULATION

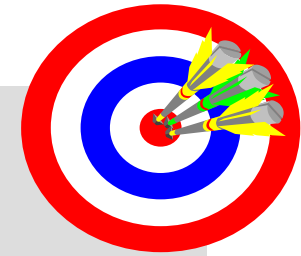
2.3 Directive 96/23/EC: scope of monitoring plan



Targeted samples

- Samples must be targeted
- with the aim of detecting illegal treatment or controlling compliance with MRLs

Testing at farms and slaughterhouses

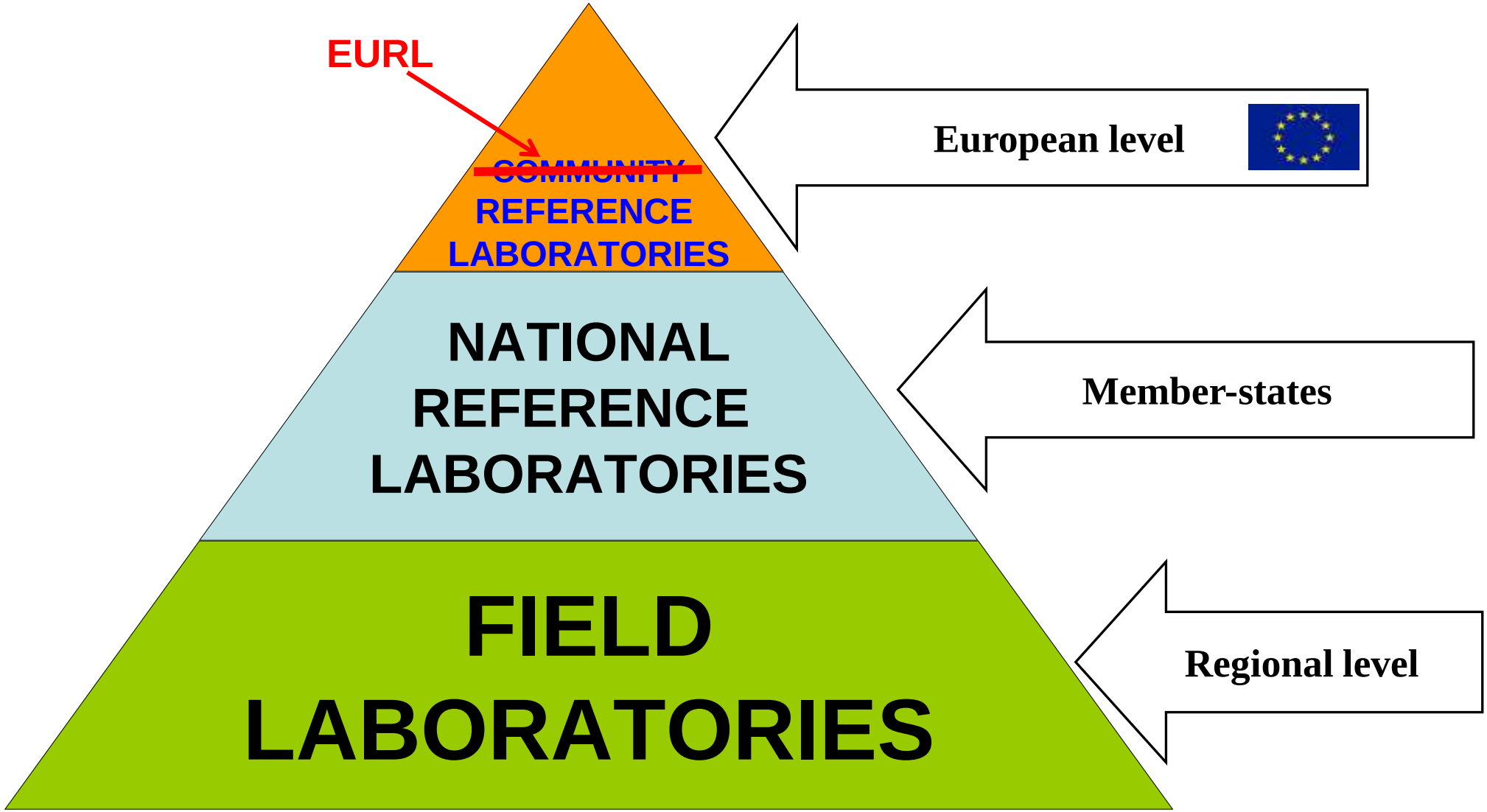


Suspect samples

- Consequence of non-compliant results
- presence of prohibited substances
- Evidences of illegal treatment or non-compliance withdrawal period

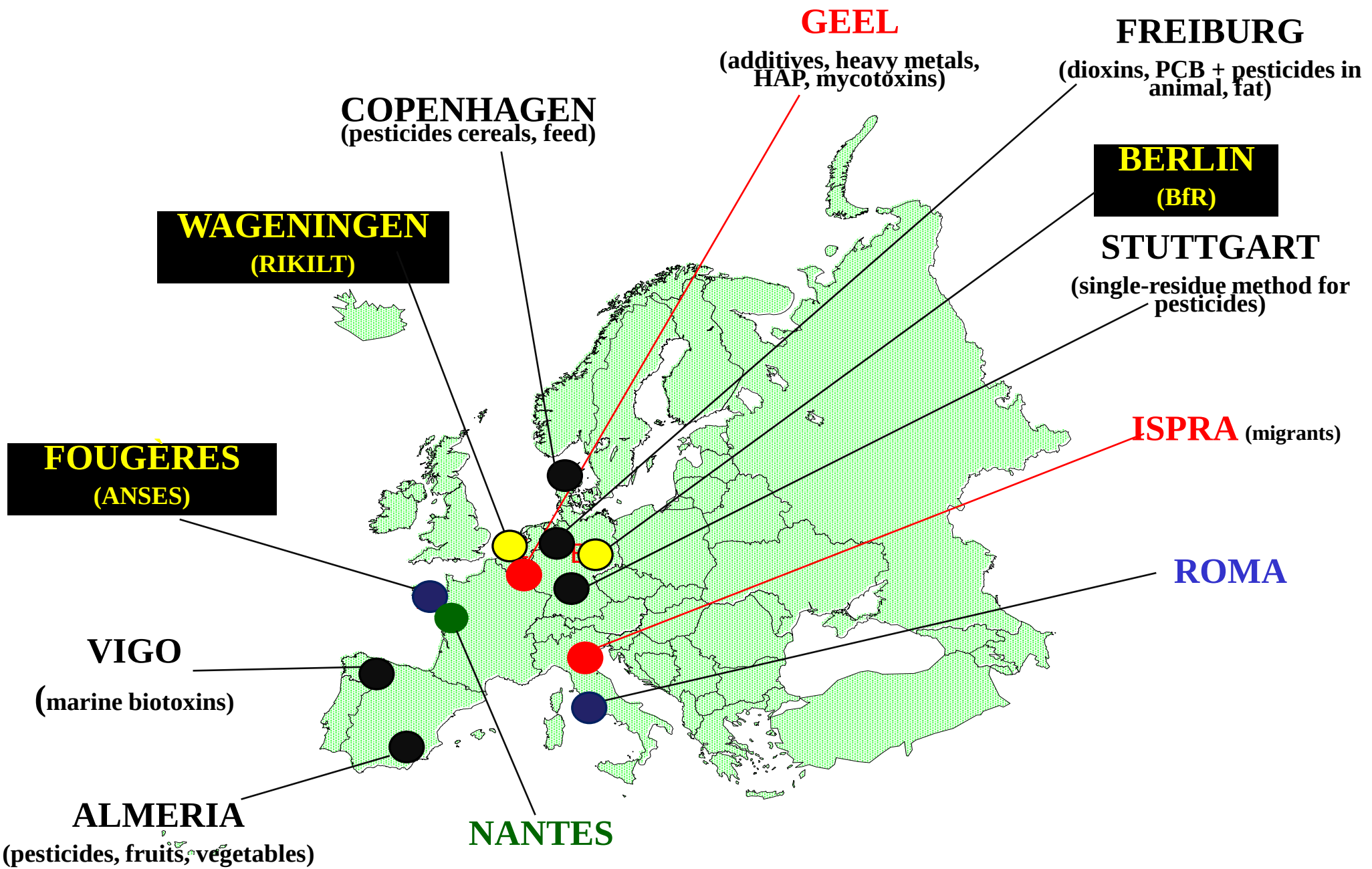
2. REGULATION

2.4 Laboratories Network



2. REGULATION

2.4 Laboratories Network



1. INTRODUCTION

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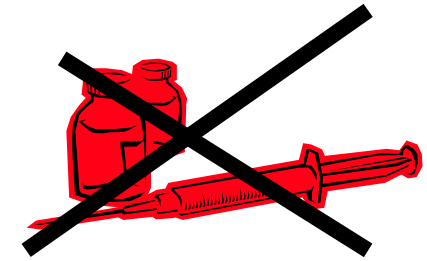
5. IP, CCα, MRPL & RPA

3. COMPOUNDS AND MATRICES

3.1 Monitored substances



- Forbidden substances (Group A)
  - Hormones, Beta agonists, etc.
  - Forbidden veterinary products

Dir. 96/22 or

37/2010: Table 2 → no MRL

- Veterinary medicines (Groups B1, B2) with LMR
  - Antibiotics,
  - Anthelminthics, etc.

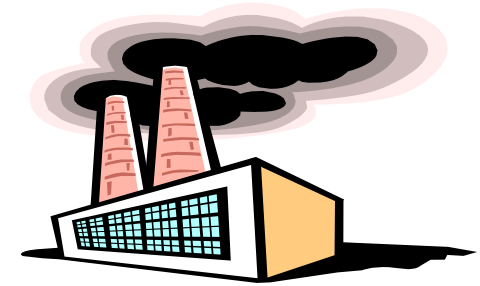
470/2009 +

37/2010: Table 1 → MRL

- Contaminants (Group B3)
  - Pesticides, dioxins,
  - Heavy metals...

Reg. Contaminants

Reg. Pesticides

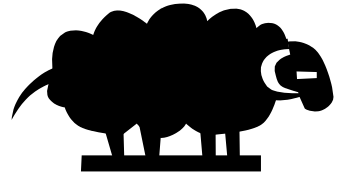


3. COMPOUNDS AND MATRICES

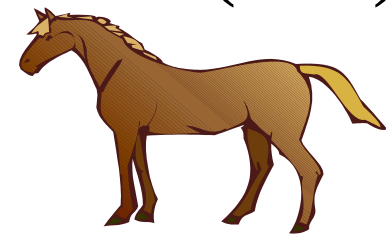
3.2 Species and Food matrices

Bovines

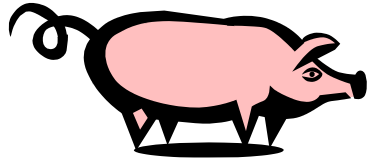
Sheep/goats



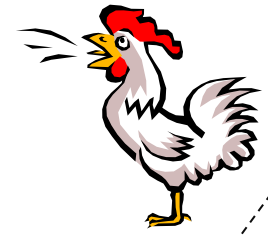
Horses (food)

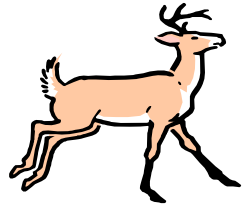


Pigs



Poultry





Farmed game
Wild game

Aquaculture

Milk

Honey

Eggs

3. COMPOUNDS AND MATRICES

3.3 Sampling

Table 2 SUBSTANCES OR GROUP OF SUBSTANCES TO BE MONITORED FOR THE RELEVANT COMMODITY

Animal species or food covered by the plan		bovine	ovine/ caprine	swine	equine	poultry	Aquaculture	milk	eggs	rabbit	wild game	farmed game	honey
Substances and group of substances to be monitored													
A1[1]	**Stilbenes**	E[2]	E	E	E	E	E			E		E	
A2	**Thyrostats**	E	E	E	E					E		E	
A3	**Synthetic steroids** (with prostagen, gestagenic, or estrogenic activity)	E	E	E	E	E	E			E		E	
A4	**Resorcylic acid lactones**	E	E	E	E	E				E		E	
A5	**Beta agonists**	E	E	E	E	E				E		E	
A6	**Compounds included in Annex IV to Council Regulation 2377/90/EEC**	E	E	E	E	E	E	E	E	E		E	E
	Chloramphenicol	E	E	E	E	E	E	E	E	E		E	E
	Nitrofurans			E		E	E		E	E			E
	Nitroimidazoles			E		E	E		E	HD		HD	
B1	**Antibacterial substances**	E	E	E	E	E	E	E	E	E		E	E
	Streptomycin												E
	Sulphonamides												E
	Tetracyclines												E
	Tylosin												E
B2a	**Anthelmintics**	HD[1]	HD	HD	HD	HD	HD	HD		HD		HD	
B2b	**Anticoccidials**	HD	HD	HD	HD	HD			HD	HD		HD	
B2c	**Carbamates and pyrethroids**	HD	HD	HD	HD	HD				HD		HD	HD
B2d	**Sedatives**	HD	HD	HD	HD								
B2e	**Non steroidal anti-inflammatory drugs**	HD	HD	HD	HD	HD		HD		HD		HD	
	Phenilbutazone	HD		HD	HD	HD		HD		HD		HD	
B3a	**Organochlorine compounds including PcBs**	HD	HD	HD	HD	HD	HD	HD	HD	HD		HD	HD
B3b	**Organophosphorus compounds**	HD	HD	HD	HD			HD					HD
B3c	**Chemical elements**	HD	HD	HD	HD	HD	HD	HD		HD	E	HD	HD
B3d	**Mycotoxins**	HD	HD	HD	HD	HD	HD	HD					
B3e	**Dyes (malachite green, leucomalachite green)**						E						

[1] Groups defined in Annex I of Directive 96/23/EC

[2] E, the monitoring of these substances or group of substances is mandatory; [2] HD, other groups of substances that should be monitored.

LEGISLATION

COUNCIL DIRECTIVE 96/23/EC ON MEASURES TO MONITOR CERTAIN SUBSTANCES AND RESIDUES THEREOF IN LIVE ANIMALS AND ANIMAL PRODUCTS OF 29 APRIL 1996

ANNEX I

GROUP A — **Substances having anabolic effect and unauthorized substances**

(1) Stilbenes, stilbene derivatives, and their salts and esters

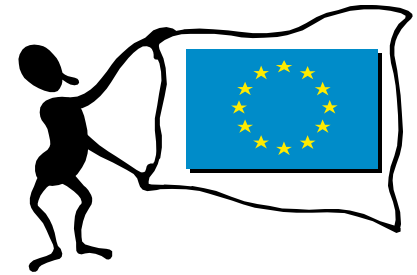
(2) Antithyroid agents

(3) Steroids

(4) Resorcylic acid lactones including zeranol

(5) Beta-agonists

(6) Compounds included in Annex IV to Council Regulation (EEC) No 2377/90 of 26 June 1990



Commission Regulation (EU) No 37/2010 of 22/12/2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

STILBENS (A1)

- 1st synthesis and use, USA, 1938
- Synthetic estrogens
- … but non steroid-like structure

Recommended for routine prophylaxis in all pregnancies
… No side effects with DES… at high or low dosage

- → Extensively used in human (1938 to 1971)
- → In 1971, proved to be teratogen, carcinogenic (adenocarcinoma of the vagina), and to cause infertility

- → Used as feed additive or as implant in the
- ear to promote growth (>1947 in poultry)
- → 1980: DES banned in cattle feed (USA).

3. COMPOUNDS AND MATRICES

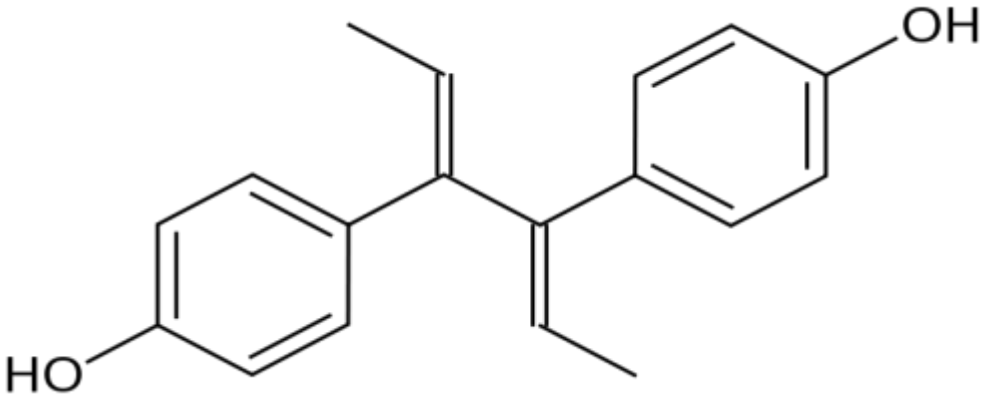
3.4 Compounds

STILBENS (A1)

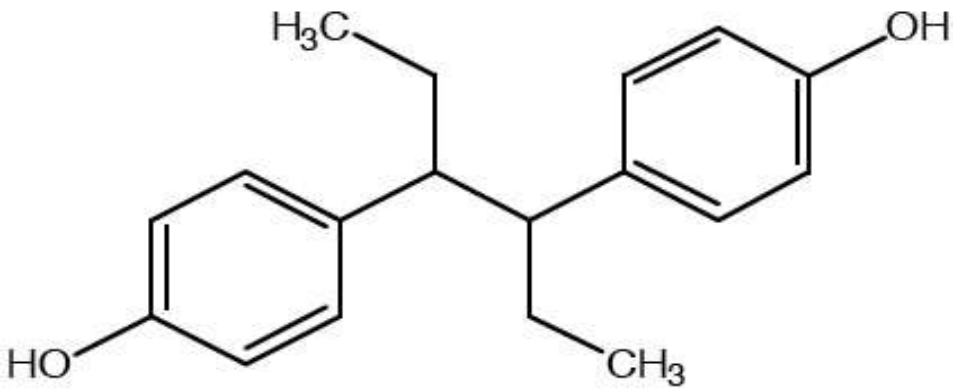
0.86 nm

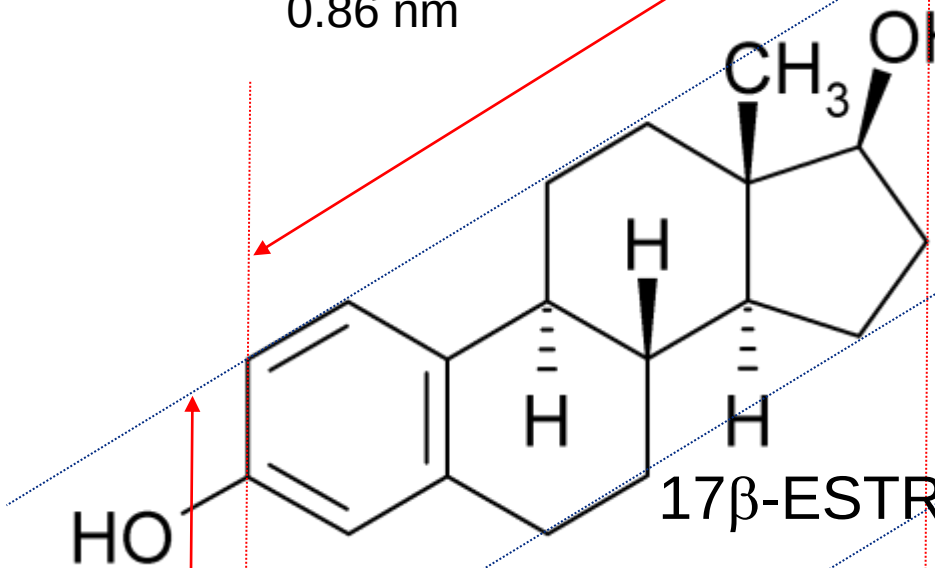
0.39 nm

DIENESTROL

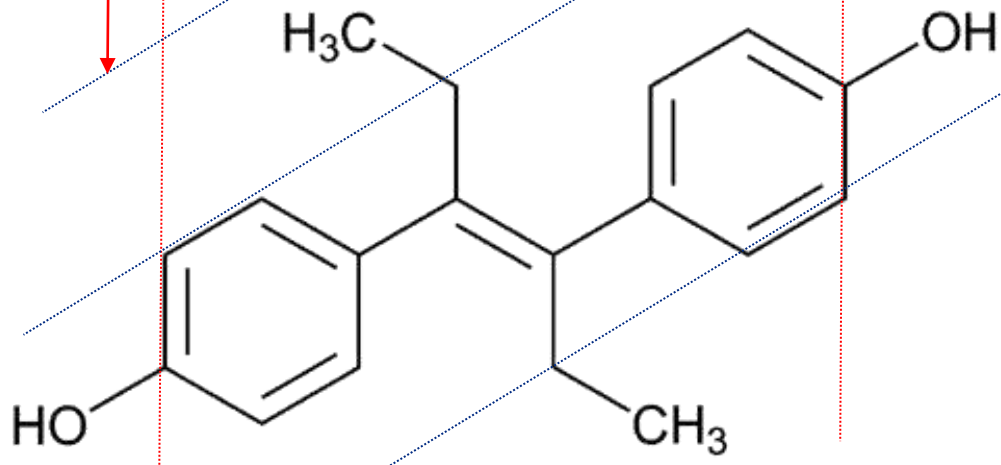


HEXESTROL





17β-ESTRADIOL



DIETHYLSTILBESTROL

THYROSTATS (A2)

- **Action**

  Inhibition of the thyroid gland

- **Performance in animal**

  Increased filling of gastro-intestinal tract

  Higher water retention (sell “water” for the price of meat)

  Global increase of the body weight of living animals

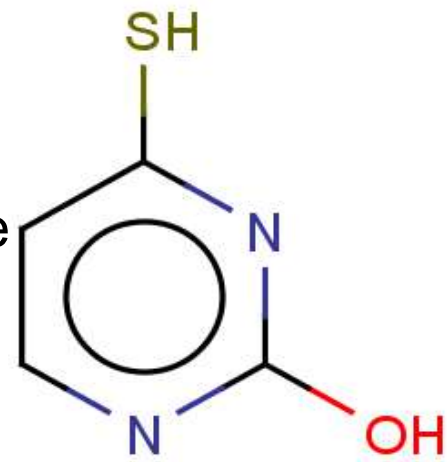
- **Toxicological aspects**

  Carcinogenic and teratogenic

International Agency for Research on Cancer
Centre International de Recherche sur le Cancer

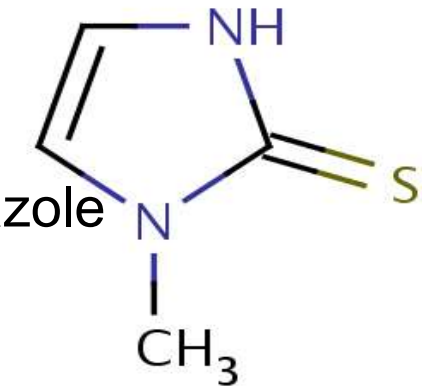
THIOURACILE

Methylthiouracile
Ethylthiouracile
Dimethylthiouracile
Propylthiouracile
Benzylthiouracile
Phenylthiouracile



TAPAZOLE

Carbimazole
Mercaptobenzimidazole



3. COMPOUNDS / MATRICES TO MONITOR

STEROIDS (A3)

Directorate - General for Health and Consumers **SANCO**

FEI
Fédération Equestre Internationale

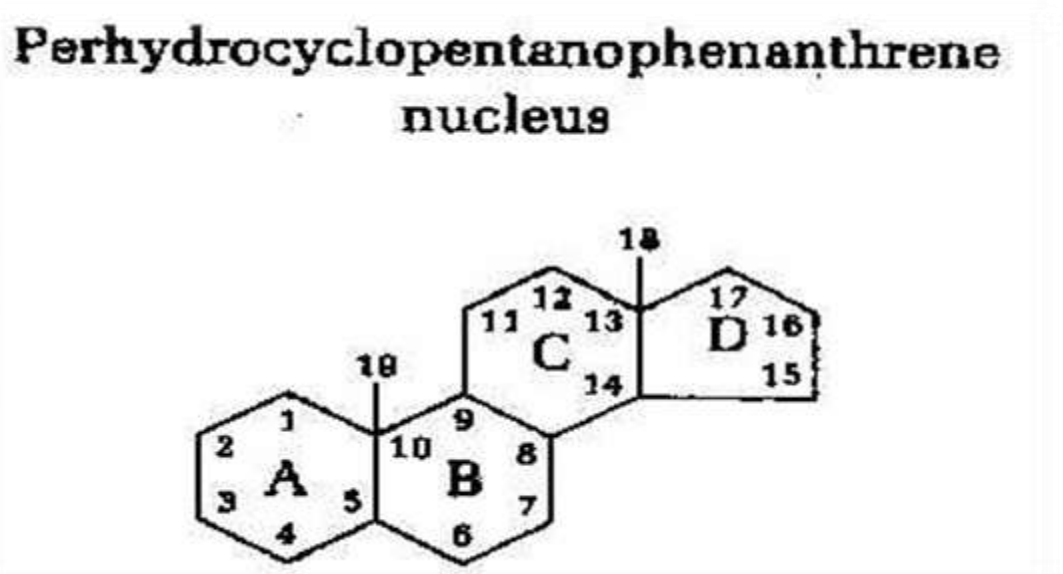
WORLD ANTI-DOPING AGENCY

3. COMPOUNDS / MATRICES TO MONITOR

STEROIDS (A3)

- **Stimulate growth**
  - gain in protein deposition
  - improved food conversion rate

- **Chemical structure**
  - 3 hexane rings (A, B, C)
  - 1 pentane ring (D) with linear alkane chain or different substituent's



3. COMPOUNDS / MATRICES TO MONITOR

STEROIDS (A3)

Marque	Composition	Utilisation
[illegible]oid	10 mg d'œstradiol + 100 mg de progestérone	veaux
[illegible]ponent C	10 mg d'œstradiol + 100 mg de progestérone	veaux
Component H	20 mg d'œstradiol + 200 mg de testostérone	génisses
Component S	20 mg d'œstradiol + 200 mg de progestérone	bouvillons
Component TH	200 mg d'acétate de trenbolone	génisses
Compudose	25,7 mg d'œstradiol	bouvillons et génisses
Encore	43,9 mg d'œstradiol	bouvillons et génisses
Finaplix-H	200 mg de testostérone	génisses
Finaplix-S	140 mg de testostérone	bouvillons
Forplix	36 mg de zéranol + 140 mg d'acétate de trenbolone	
Heifer-oid	20 mg d'œstradiol + 200 mg de testostérone	génisses
Implix	20 mg d'œstradiol + 200 mg de testostérone	
Implus-C	10 mg d'œstradiol + 100 mg de progestérone	veaux
Implus-H	20 mg d'œstradiol + 200 mg de testostérone	génisses
Implus-S	20 mg d'œstradiol + 200 mg de progestérone	bouvillons
MGA	acétate de mélengestrol	génisses
Ralgro	36 mg de zéranol	
Revalor	20 mg d'œstradiol + 140 mg d'acétate de trenbolone	
Revalor-G	8 mg d'œstradiol + 40 mg d'acétate de trenbolone	bouvillons et génisses
Steer-oid	20 mg d'œstradiol + 200 mg de progestérone	bouvillons
Synovex C	10 mg d'œstradiol + 100 mg de progestérone	veaux
Synovex H	20 mg d'œstradiol + 200 mg de testostérone	génisses
Synovex S	20 mg d'œstradiol + 200 mg de progestérone	bouvillon
Torelor	40 mg d'œstradiol + 200 mg d'acétate de trenbolone	
Torevex-S	20 mg d'œstradiol + 200 de progestérone	

3. COMPOUNDS / MATRICES TO MONITOR

STEROIDS (A3)

- *Natural*
  - **Testosterone**
- *Synthetic*
  - Nandrolone, methyltestosterone, 17β-boldenone, methylboldenone, trenbolone, stanozolol,…
- *Matrix and method*
  - Urine, muscle, liver, hair, feed
  - GC-MS/MS and LC-MS/MS

- *Natural*
  - **Estradiol**
- *Synthetic*
  - derivatives of estradiol: EE_2, VE, and mestranol
- *Matrix and method*
  - Urine, muscle, liver, hair, feed
  - GC-MS/MS and LC-MS/MS

- *Natural*
  - **Progesterone**
- *Synthetic*
  - Mainly acetates forms: MPA, MLGA, CMA, DMA, MGA
- *Matrix and method*
  - Feces, fat, feed
  - LC-MS/MS

3. COMPOUNDS / MATRICES TO MONITOR

β-AGONISTS (A5)

ILLEGAL USE OF CLENBUTEROL IN FOOD ANIMALS 9

By Dr. William C. Keller

As we mentioned in the last issue of the *FDA Veterinarian*, FDA is investigating the illegal use of the drug, clenbuterol, in animals used for food, particularly animals being prepared for livestock show competition. The purpose of this article is to illustrate the potential consequences of illegal drug use in food animals by describing an outbreak of clenbuterol-related drug residue poisoning, and to explain the scientific basis for the Center for Veterinary Medicine's (CVM) particular concern for illegal use of clenbuterol in food producing animals. The following description of an outbreak of clenbuterol residue toxicity demonstrates the potential public health consequences of illegal use of drugs in animals used for food.

Numerous cases of illness, which appeared to be due to food poisoning, or in feed to animals, and of apparent therapeutic or production value in animal husbandry. Based on these criteria, it was suspected that illegal use of a β agonist in cattle was responsible for the poisoning outbreak. Prompt follow-up on a number of patients had allowed the investigators to collect samples of the suspected food, as well as urine samples from the individuals. Analysis of these samples revealed that a β agonist, clenbuterol, was present at levels of 2-4 ppb in patients' urine and 160-291 ppb in beef liver samples. This confirmed the investigators' suspicions that an illegal animal drug residue present in liver had produced the outbreak of food-borne poisoning.

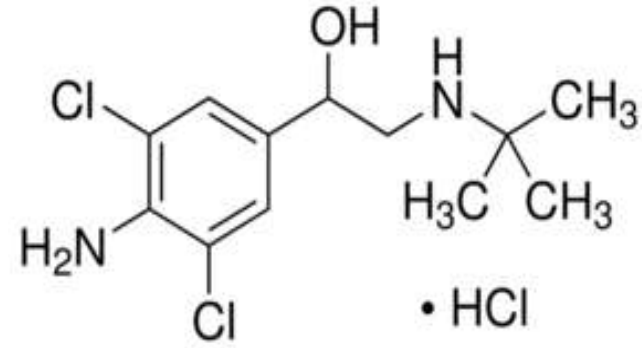
"Analysis of these samples ... confirmed the investiga-

WITHOUT β-AGO

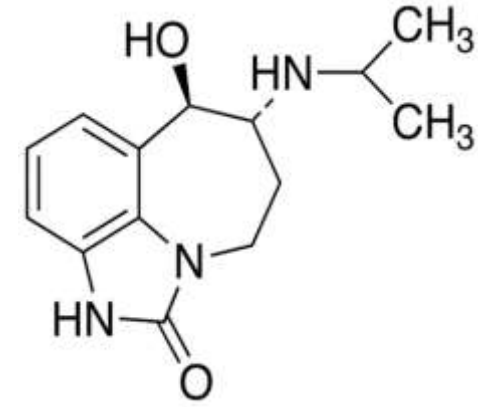
WITH β-AGO

3. COMPOUNDS / MATRICES TO MONITOR

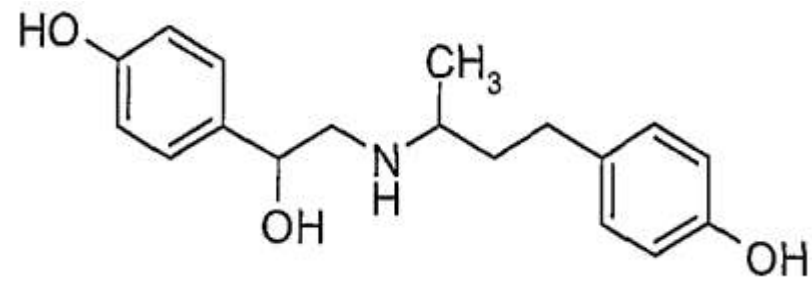
β-AGONISTS (A5)



- **Action**
  - bronchodilators
  - better feed conversion
  - repartitioners: fat ↓ and muscle ↑
- **Dose, performance**
  - 1-10 μg/kg bw/d in the feed for 2 – 3 weeks
  - Growth increases → 20 %
  - Carcass quality improves → 10 %
  - Carcasses contain 1/3 less fat



- **Matrices, methods**
  - Urine, hair, liver, lung, feed
  - ELISA, LC-MS/MS, GC-MS/MS



3. COMPOUNDS / MATRICES TO MONITOR

UNAUTHORIZED SUBSTANCES (A6)

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)

Pharmacologically active substance	MRL
Aristolochia spp. and preparations thereof	MRL cannot be established
Chloramphenicol	MRL cannot be established
Chloroform	MRL cannot be established
Chlorpromazine	MRL cannot be established
Colchicine	MRL cannot be established
Dapsone	MRL cannot be established
Dimetridazole	MRL cannot be established
Metronidazole	MRL cannot be established
Nitrofurans (including furazolidone)	MRL cannot be established
Ronidazole	MRL cannot be established

Table 2

Prohibited substances

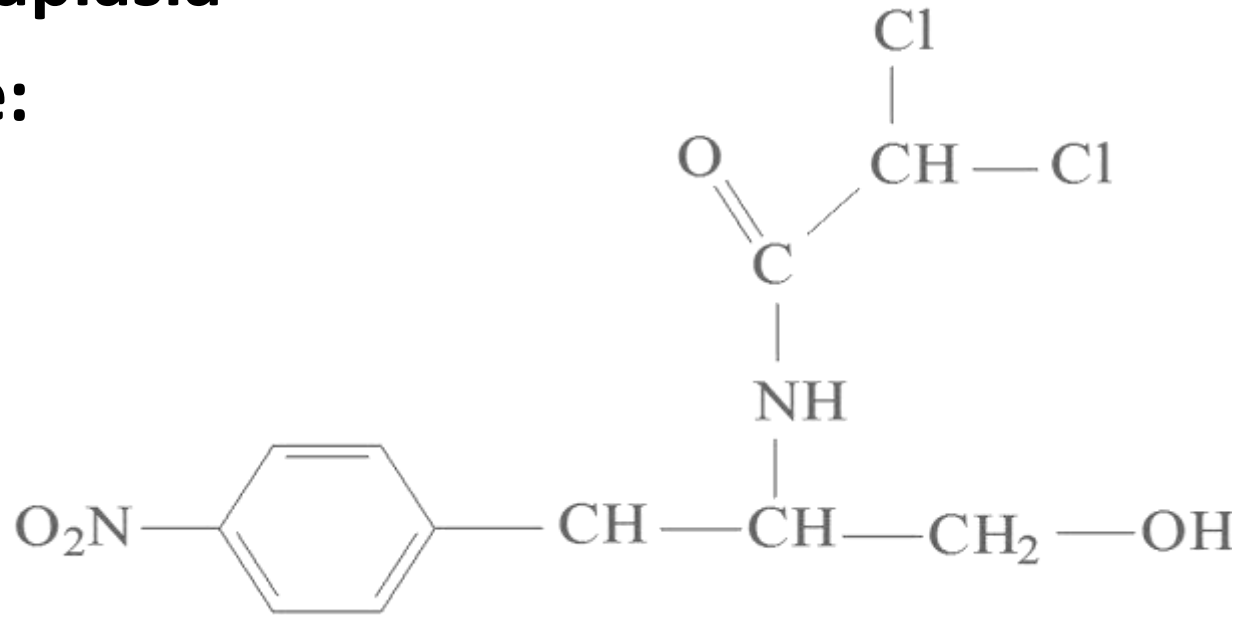
For these pharmacologically active substances no maximum residue limit could be established because residues of those substances, at whatever limit, constitute a hazard to human health.

25

UNAUTHORIZED SUBSTANCES (A6)

Chloramphenicol

- **Broad spectrum antibiotic**
- **Against pathogenic G^- bacteria**
- **Action: inhibition of bacterial protein biosynthesis**
- **Toxicity: medullar aplasia**
- **Chemical structure:**



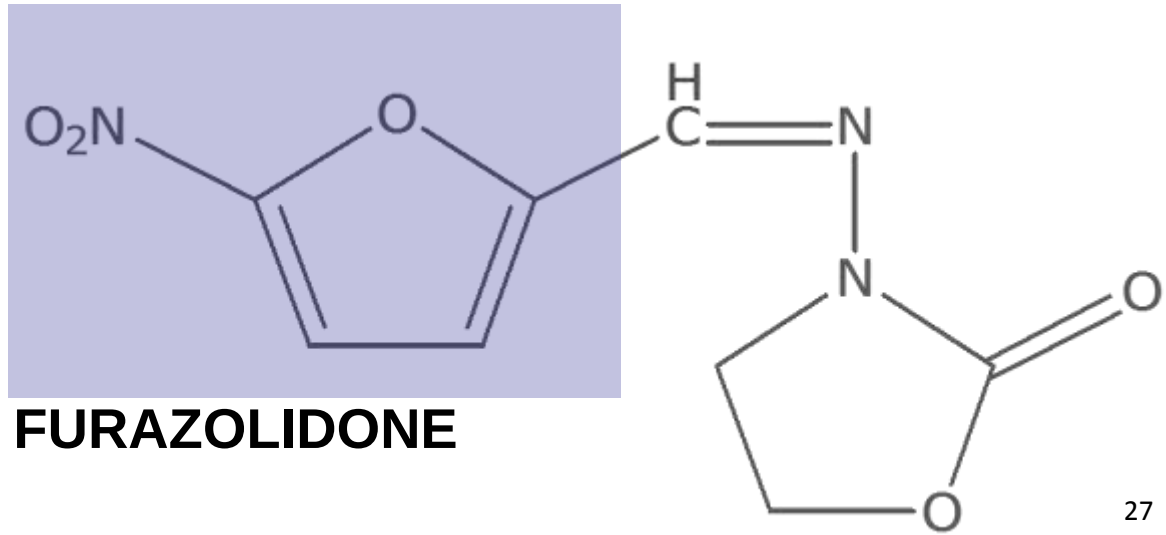
→ MRPL for meat, eggs, milk, urine, aquaculture products and honey of 0.3 µg/kg (Decision 2003/181/EC)

UNAUTHORIZED SUBSTANCES (A6)

Nitrofurans

- **Synthetic antibacterial compounds**
- **Treat: salmonellas and E. coli infections**
- **Mutagenic**
- **Prohibited for use in food-producing animals**

Banned 1993



FURAZOLIDONE

3. COMPOUNDS / MATRICES TO MONITOR

GROUPS B1 and B2

(1) <u>ANTIBACTERIAL SUBSTANCES</u>, including sulphonamides, quinolones

(2) <u>OTHER VETERINARY DRUGS</u>

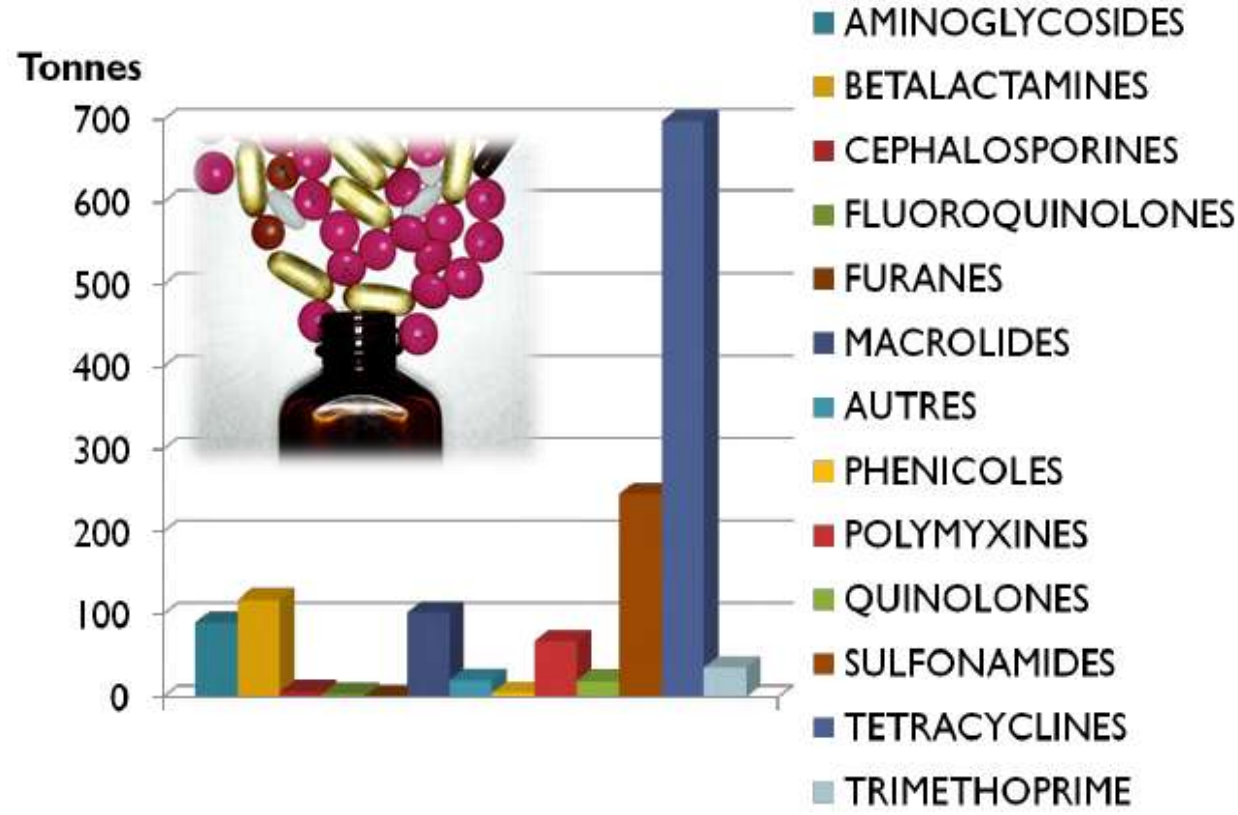
- (a) Anthelmintics
- (b) Anticoccidials, including nitroimidazoles
- (c) Carbamates and pyrethroids
- (d) Sedatives
- (e) Non-steroidal anti-inflammatory drugs (NSAIDs)
- (f) Other pharmacologically active substances

3. COMPOUNDS / MATRICES TO MONITOR

B1 ANTIBACTERIAL SUBSTANCES

- The term "antibiotics" is reserved for agents derived from living organisms or for synthetic or semi-synthetic analogues of such compounds. The antibiotics fall into the following classes:
  - ß-lactams
  - tetracyclines
  - macrolides
  - Aminoglycosides (very polar)
  - amphenicols
  - polypeptides

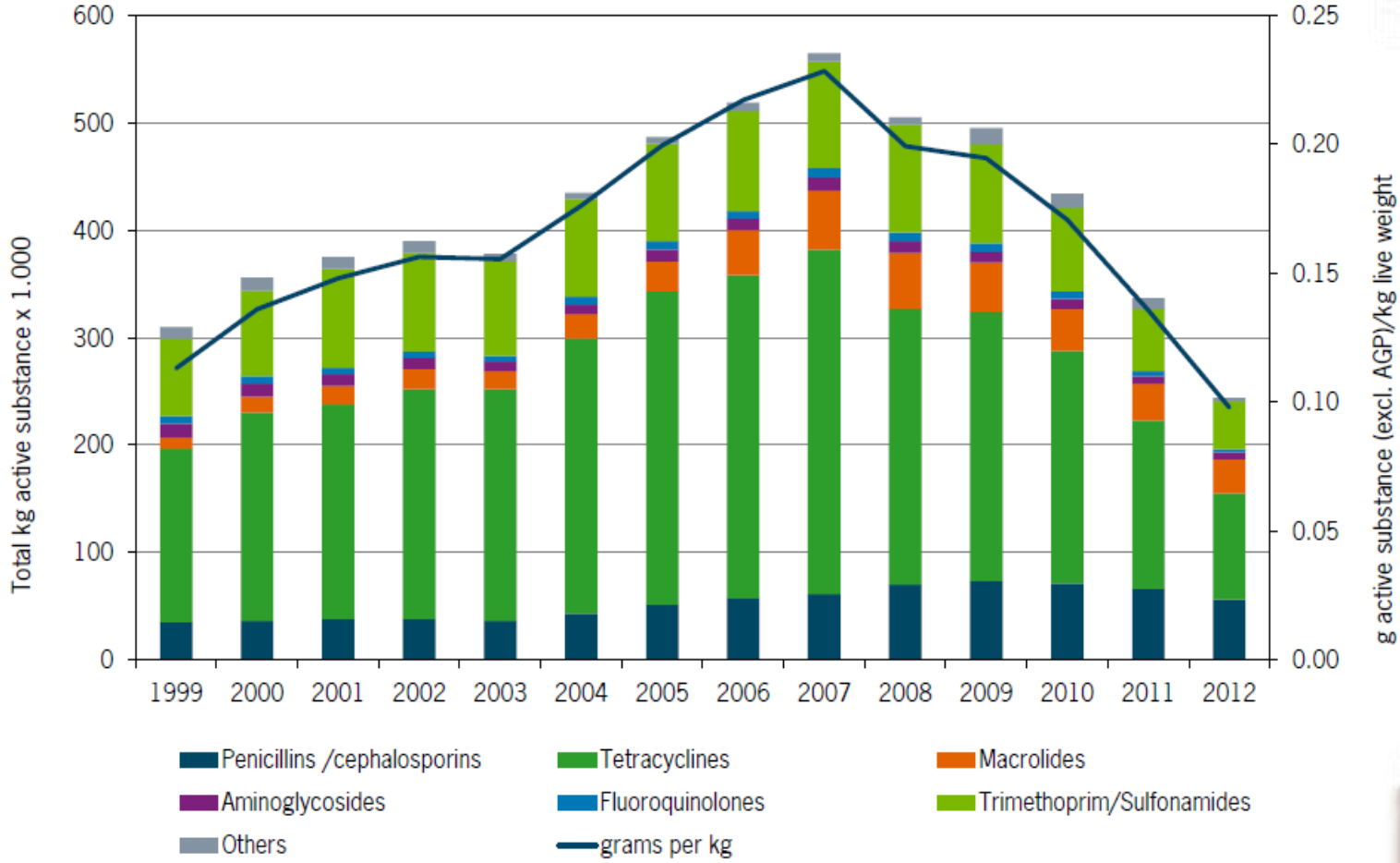
- Chemotherapeutics (synthetic)
  - sulfonamides
  - Quinolones



3. COMPOUNDS / MATRICES TO MONITOR

B1 ANTIBACTERIAL SUBSTANCES

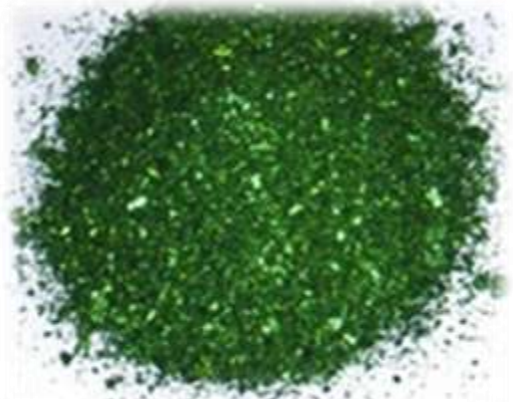
Figure 3.1 Therapeutic antibiotic sales, 1999-2012 a)



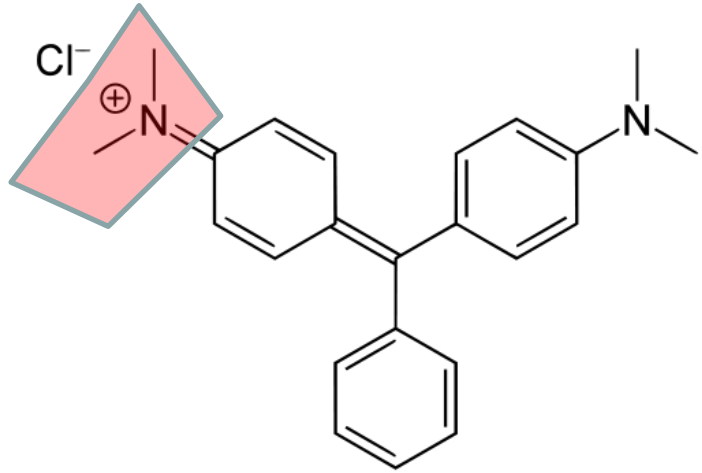
a) Sales for 2012 are estimates, based on preliminary data of the first half year.
Source: FIDIN (2012).

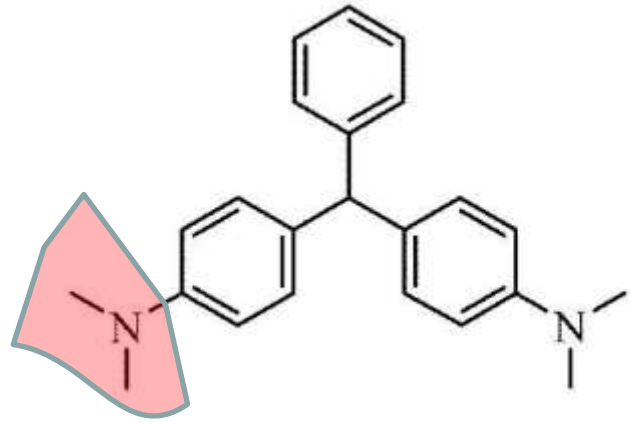
3. COMPOUNDS / MATRICES TO MONITOR

B3e DYES



- Used for a long time in the aquaculture industry as fungicide, parasiticide and disinfectant because of low cost and effectiveness



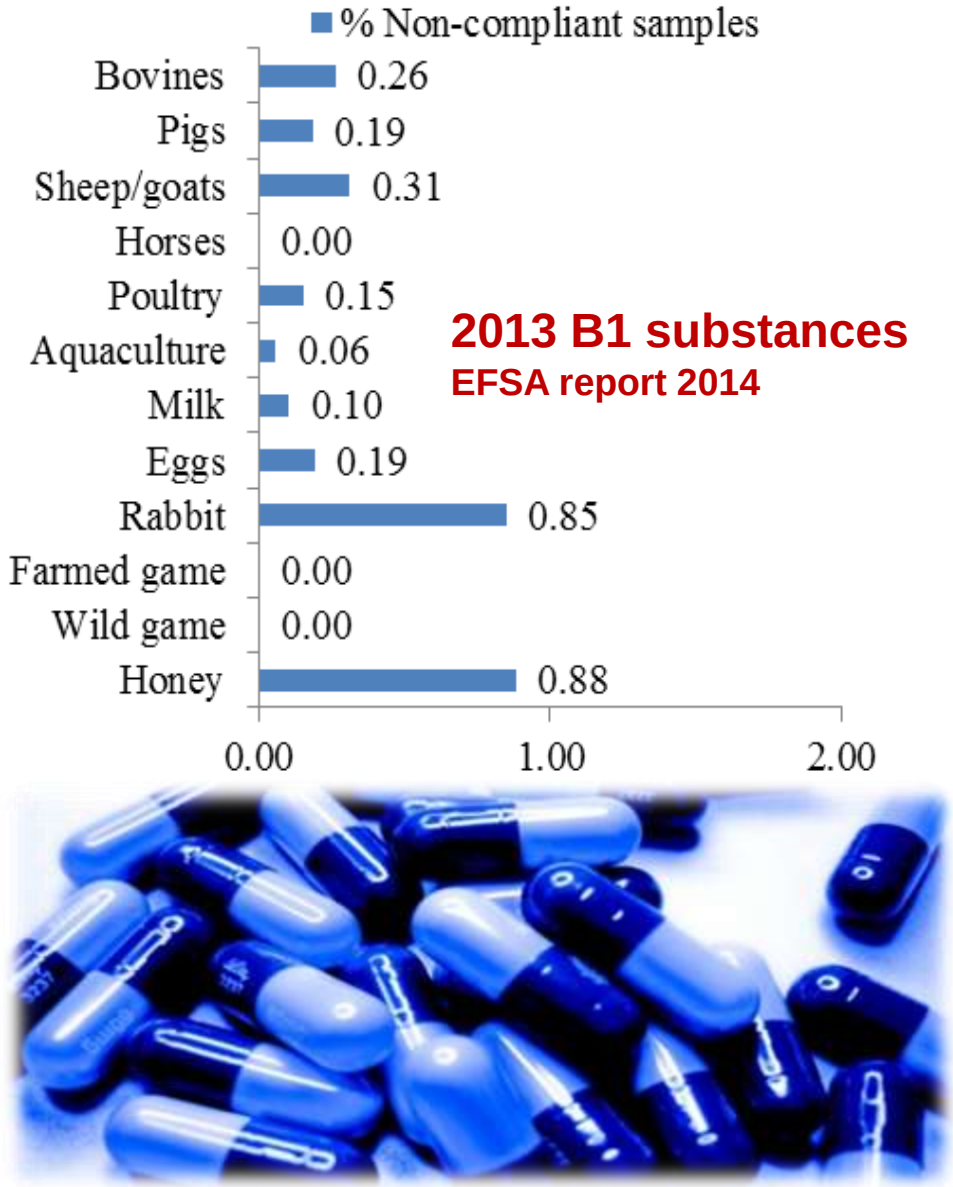


- Similarity in structure to carcinogenic triphenylmethane dyes
- Potential human health hazard
- MG metabolized to the more lipophilic leucoMG (retains longer in edible fish tissues than MG)
- MRPL in meat of aquaculture products of 2 µg/kg for MG + LeucoMG (Decision 2004/25/EC)

3. COMPOUNDS / MATRICES TO MONITOR

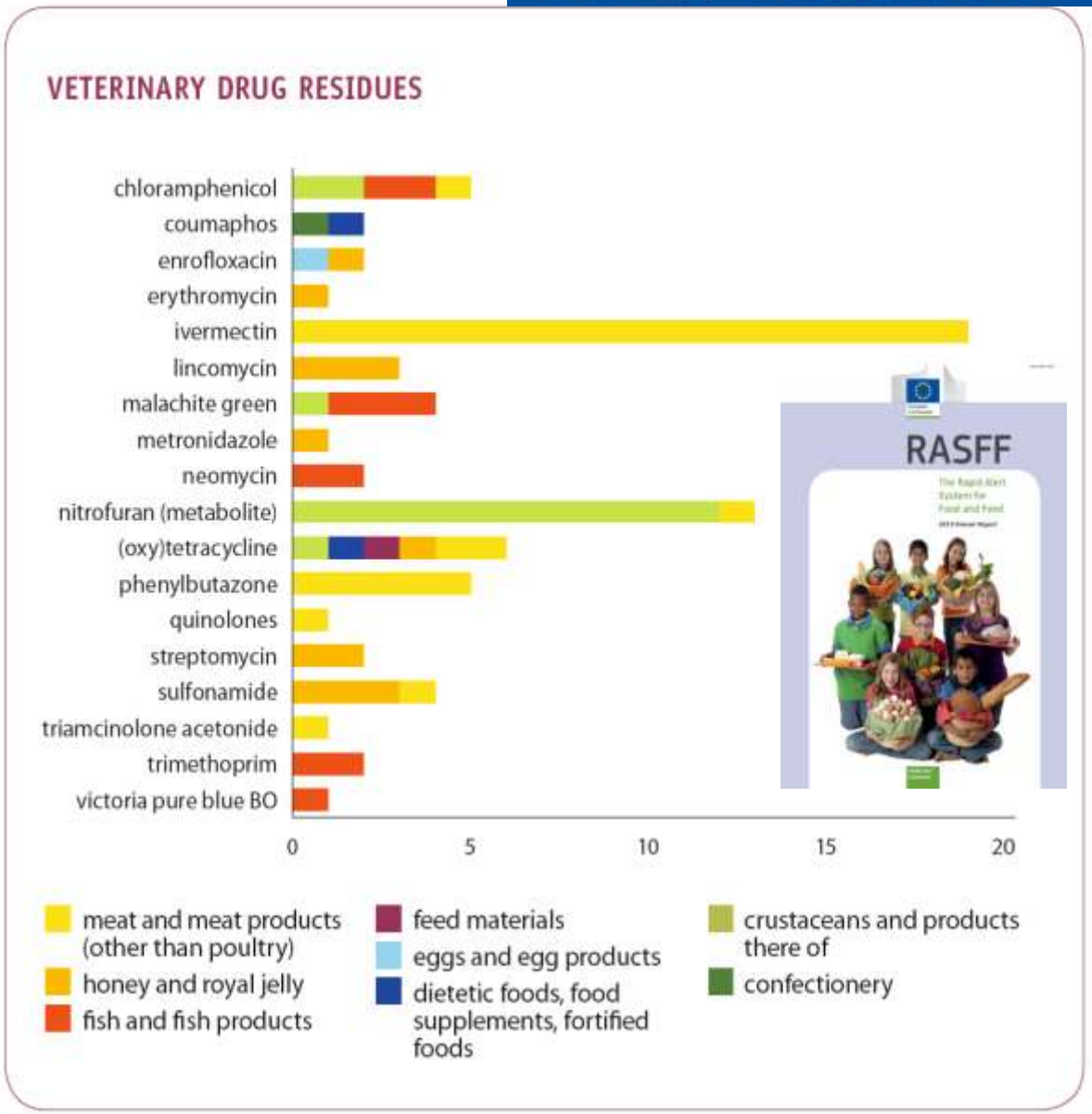
B1- B2 - B3e

RASFF



2013 B1 substances

EFSA report 2014



1. INTRODUCTION

2. REGULATION

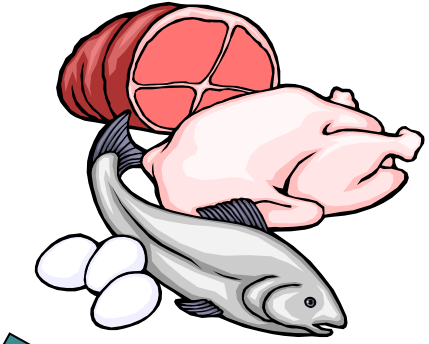
3. COMPOUNDS/MATRICES TO MONITOR

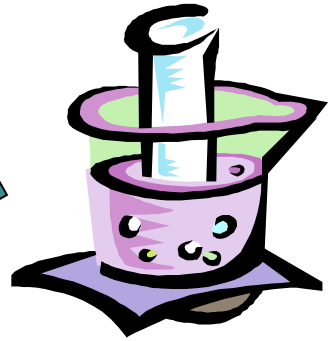
4. ANALYTICAL METHODS

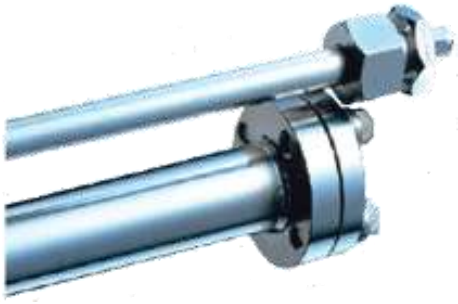
5. IP, CCα, MRPL & RPA

4. ANALYTICAL METHODS

4.1 Different steps of the workflow… before the signal





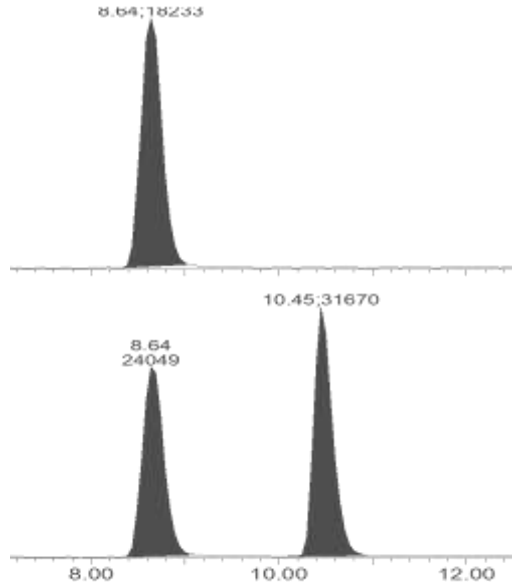


CHROMATOGRAPHY

MOLECULE SEPARATION

MASS SPECTROMETRY

SIGNAL CHARACTERISATION



SIGNALS

4. ANALYTICAL METHODS

4.1 Different steps of the workflow… before the signal

Sample pre-treatment

Sample

Sample extraction

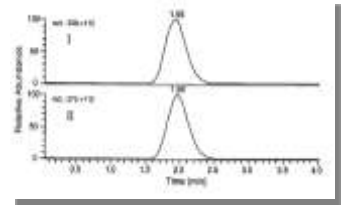
Analytical sample

Sample clean-up, concentration, derivatisation

Crude extract

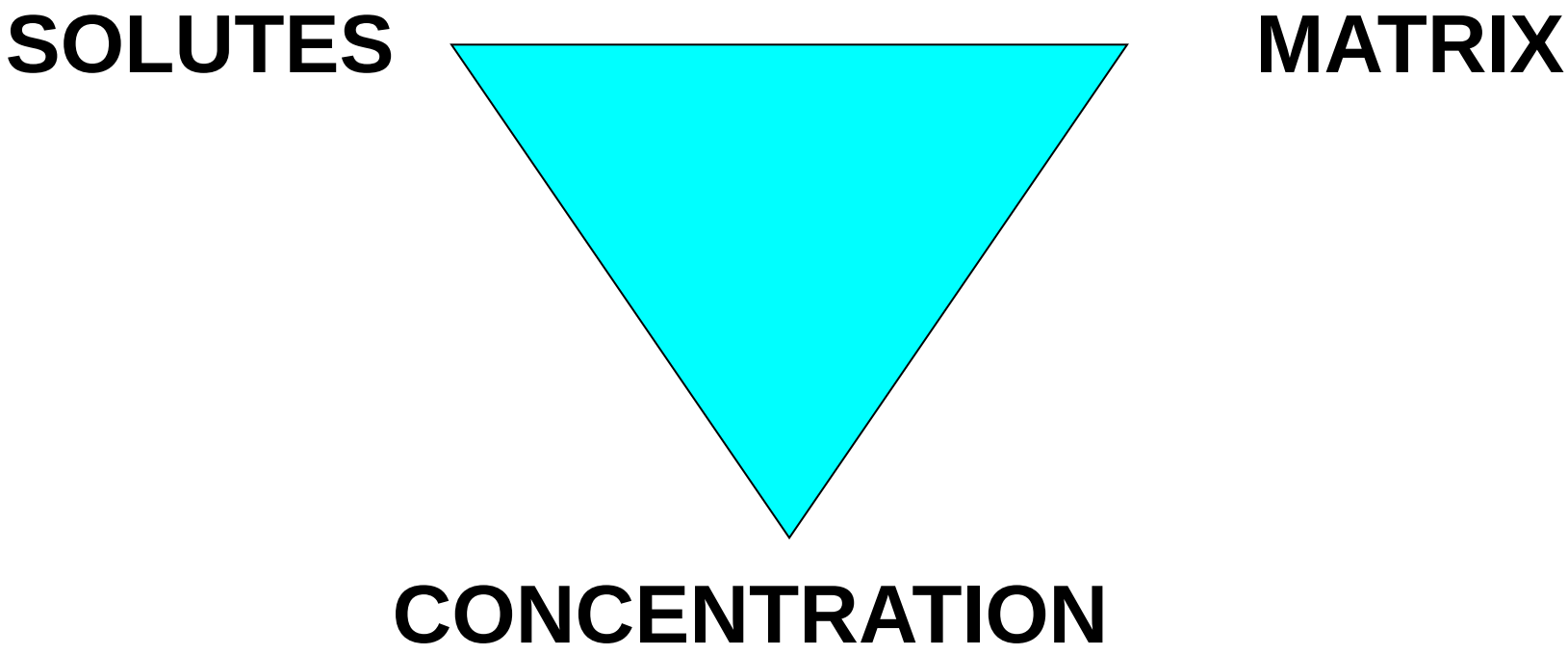
Sample analysis

Purified extract



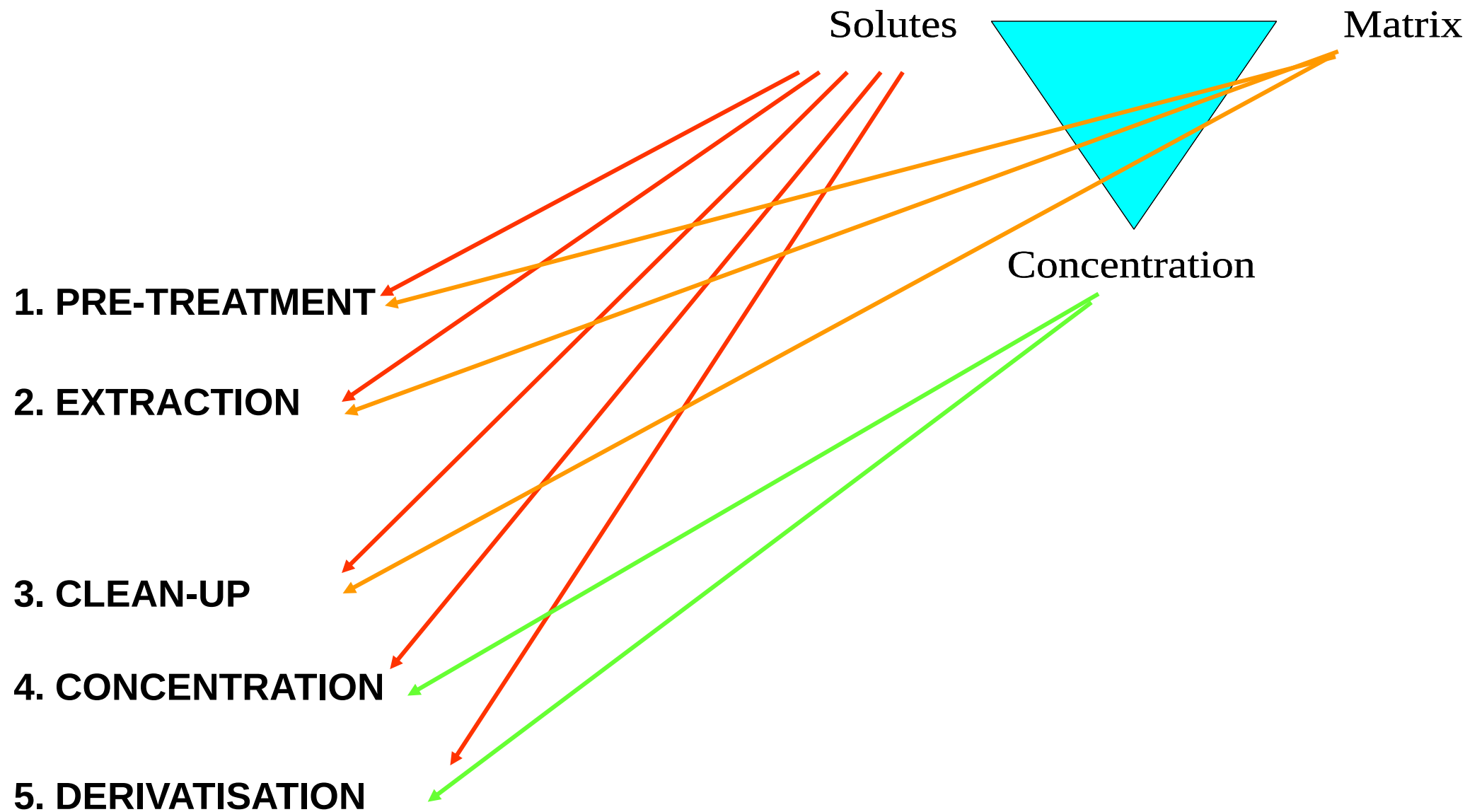
4.1 Different steps of the workflow… before the signal

The key parameters



4. ANALYTICAL METHODS

4.1 Different steps of the workflow… before the signal



4. ANALYTICAL METHODS

4.1 Different steps of the workflow… before the signal

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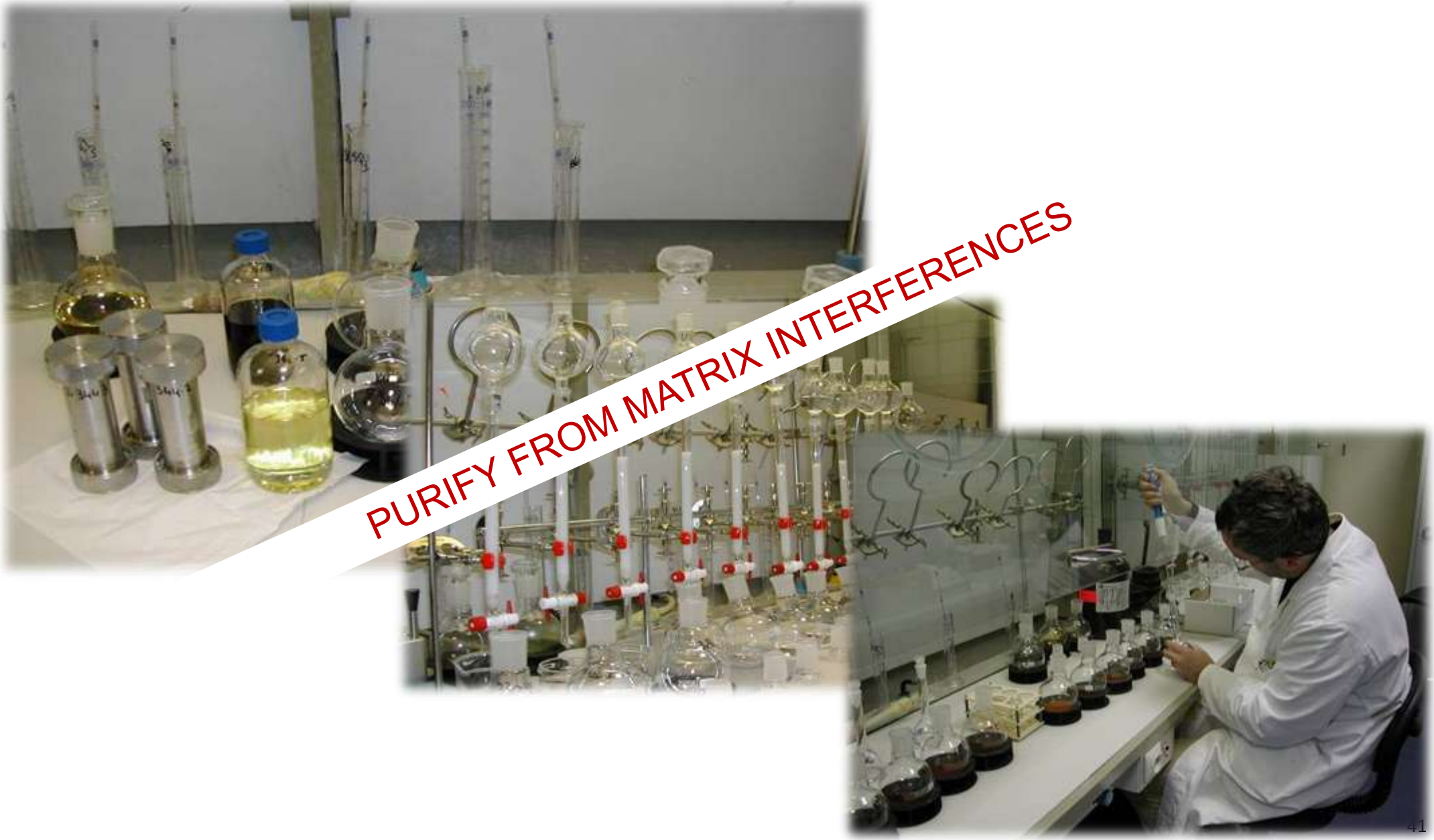
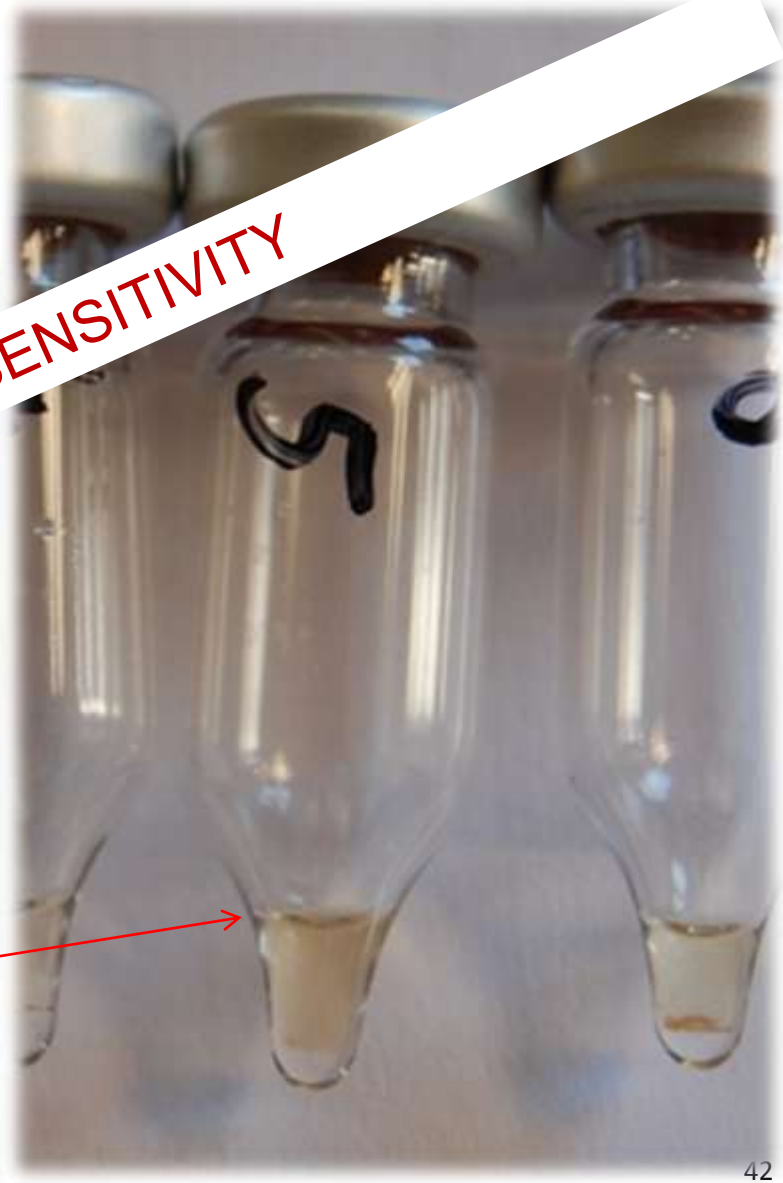


4. ANALYTICAL METHODS

4.1 Different steps of the workflow… before the signal

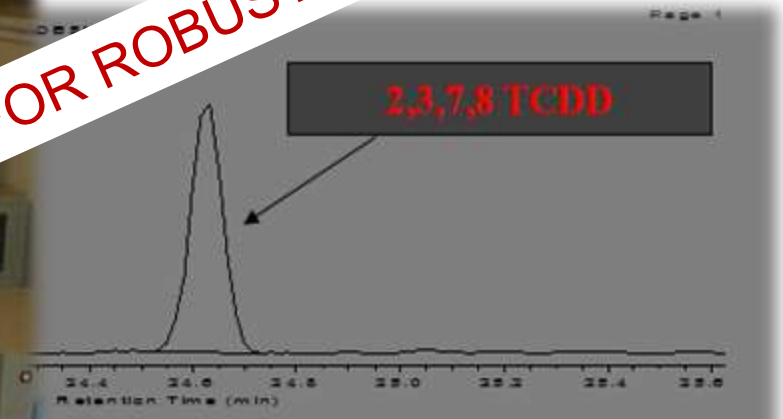


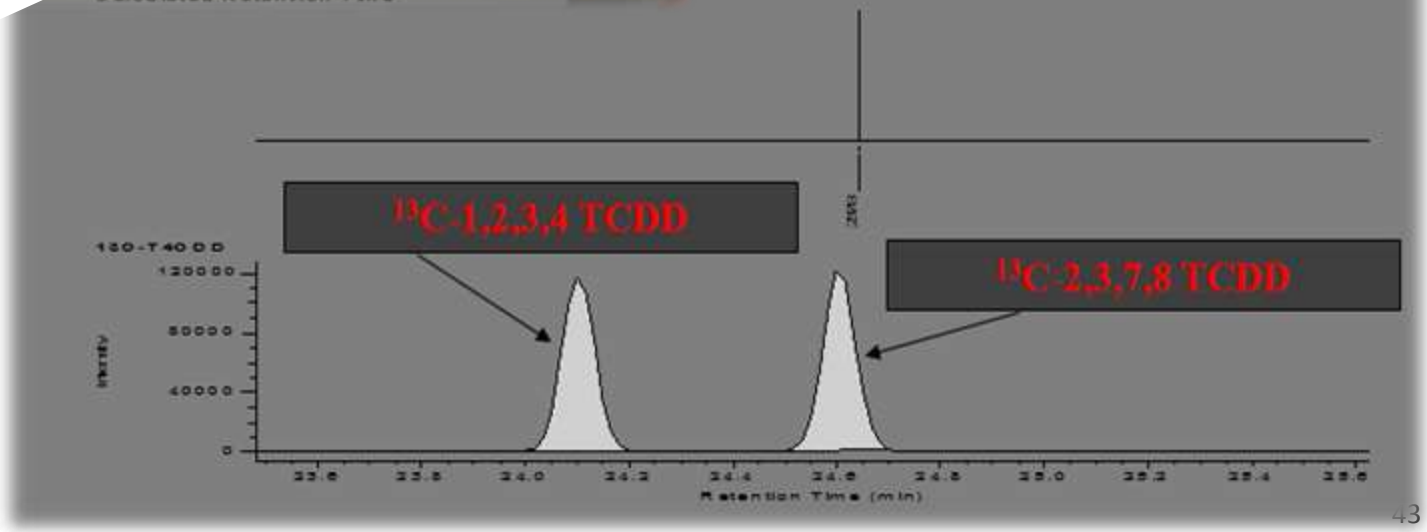
CONCENTRATE FOR MAXIMUM SENSITIVITY

4. ANALYTICAL METHODS

4.1 Different steps of the workflow… before the signal

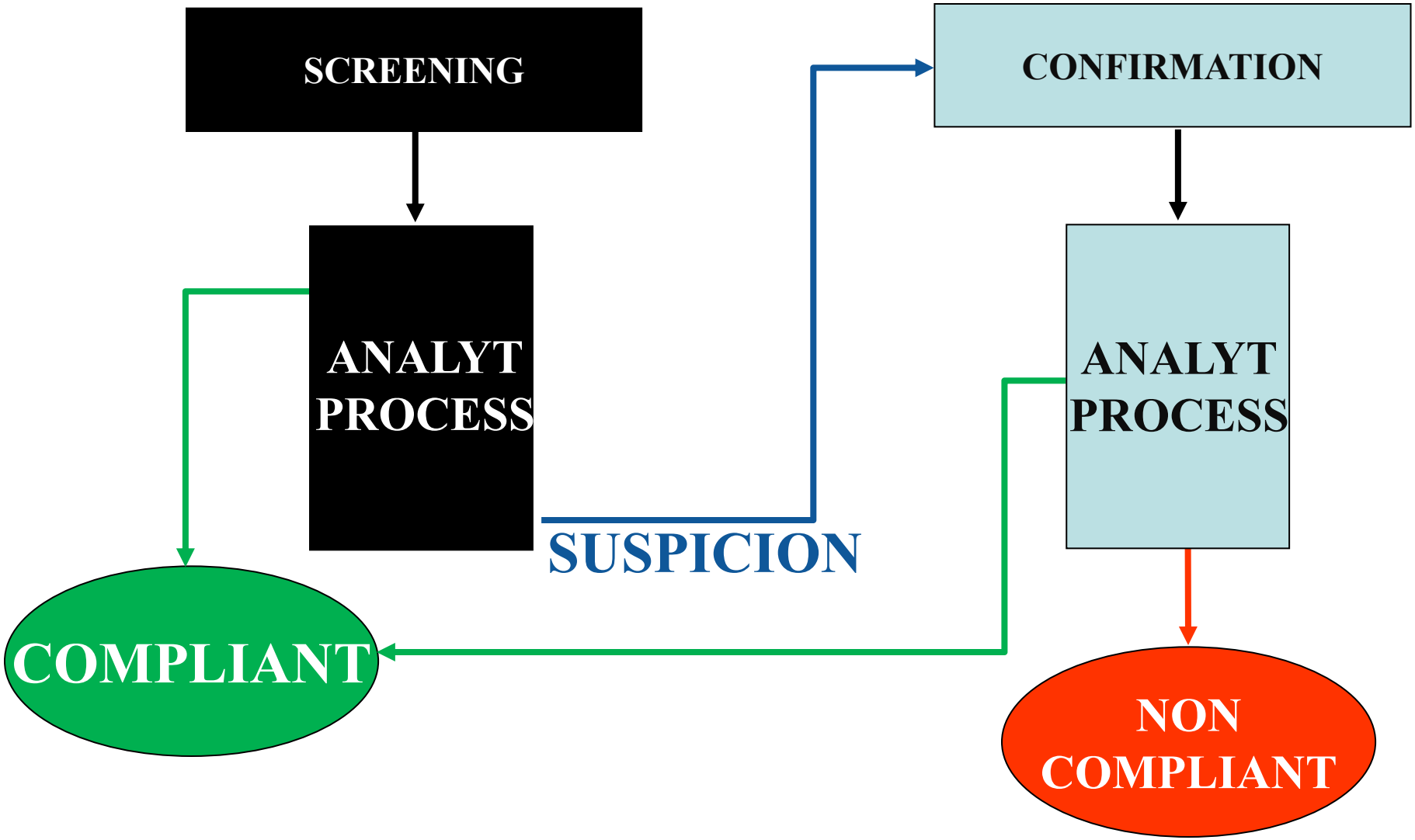
SPECIFIC AND SENSITIVE TECHNIQUE FOR ROBUST DATA





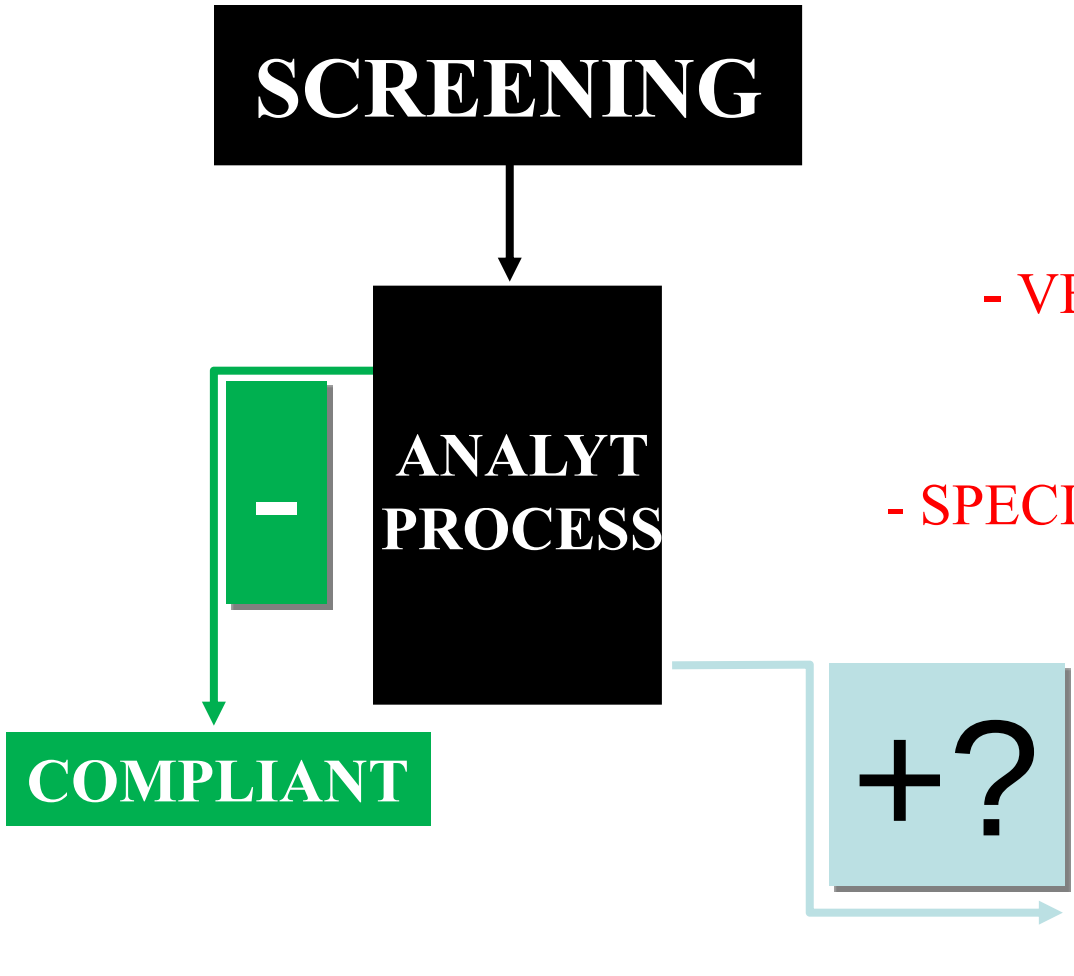
4. ANALYTICAL METHODS

4.2 SCREENING vs CONFIRMATION



4.2 SCREENING vs CONFIRMATION

SHOULD BE VERY FAST AND COST EFFECTIVE



REQUIREMENTS

- VERY SENSITIVE to **guarantee no false negative** results

+

- SPECIFIC ENOUGH to limit false positive results

IMMUNOTECHNIQUES
'OMIC TECHNIQUES
CHROMATOGRAPHY

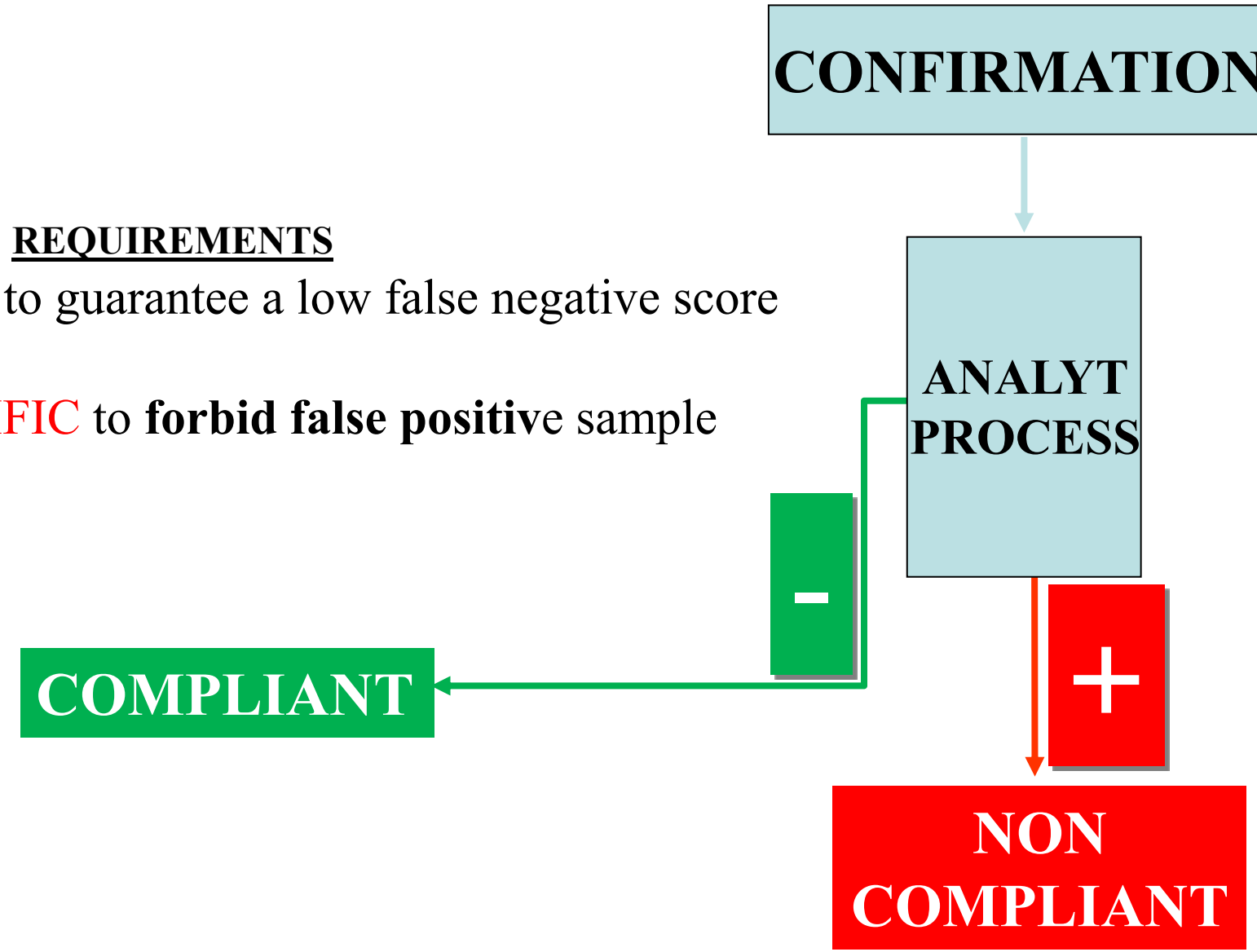
4. ANALYTICAL METHODS

4.2 SCREENING vs CONFIRMATION

REQUIREMENTS

- VERY SENSITIVE to guarantee a low false negative score

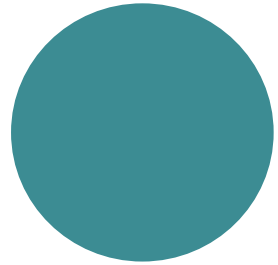
- HIGHLY SPECIFIC to **forbid false positive** sample



4. ANALYTICAL METHODS

4.3 Mass spectrometry: principles (simplified ☺)

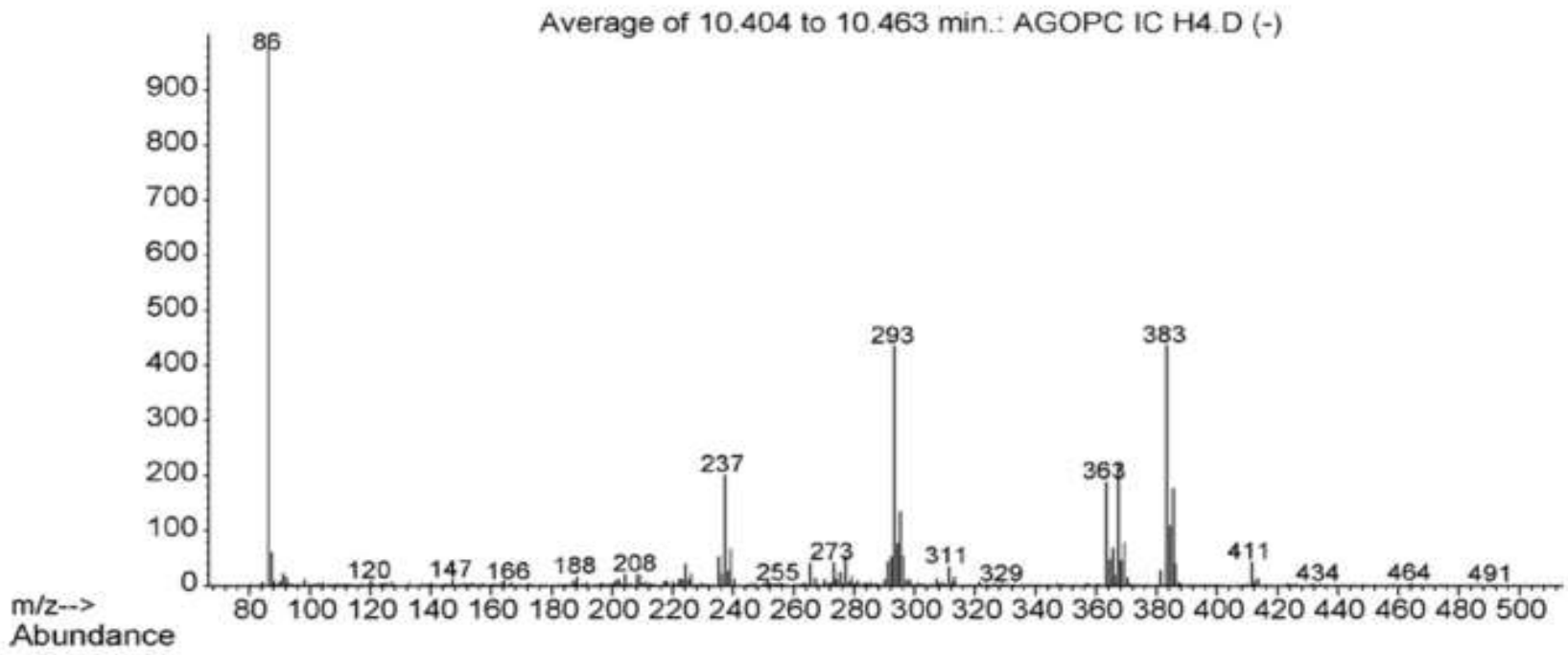
SAMPLE INTRODUCTION



IONIZATION

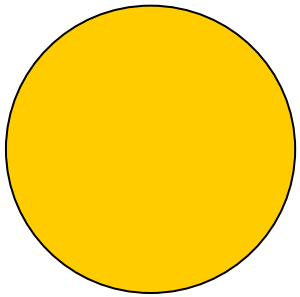
ION CHARACTERIZATION

ION DETECTION



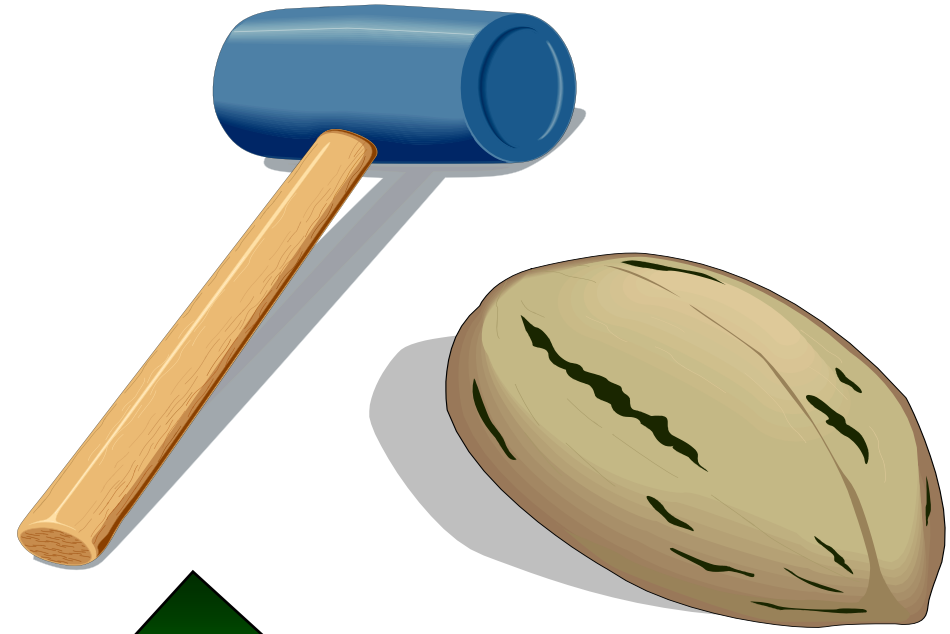
4. ANALYTICAL METHODS

4.3 Ionisation (simplified ☺)



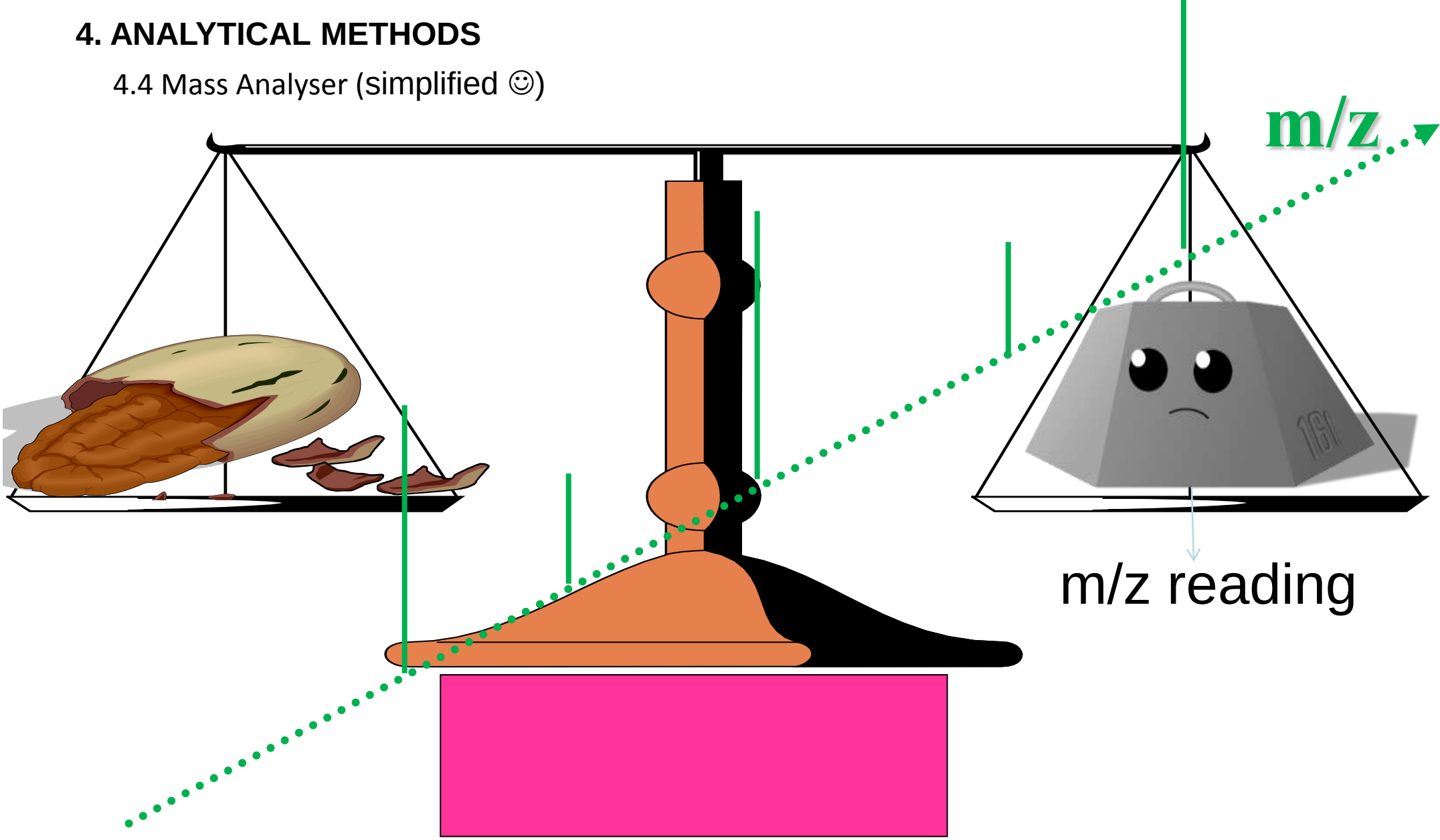
ION SOURCE

Production of ions



4. ANALYTICAL METHODS

4.4 Mass Analyser (simplified ☺)

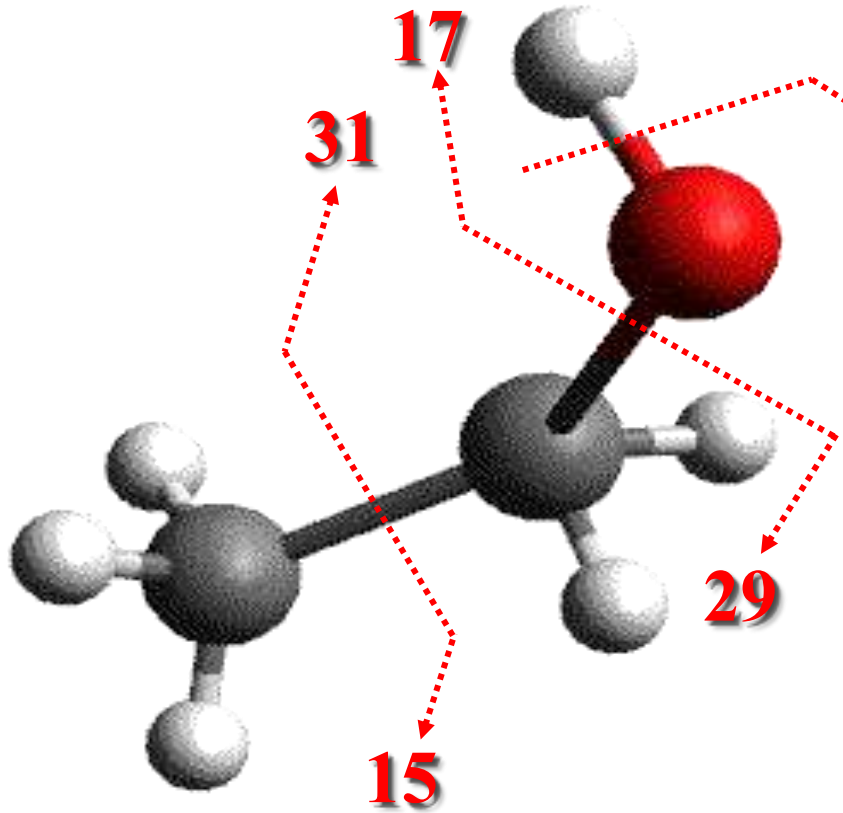


THE SPECTRUM

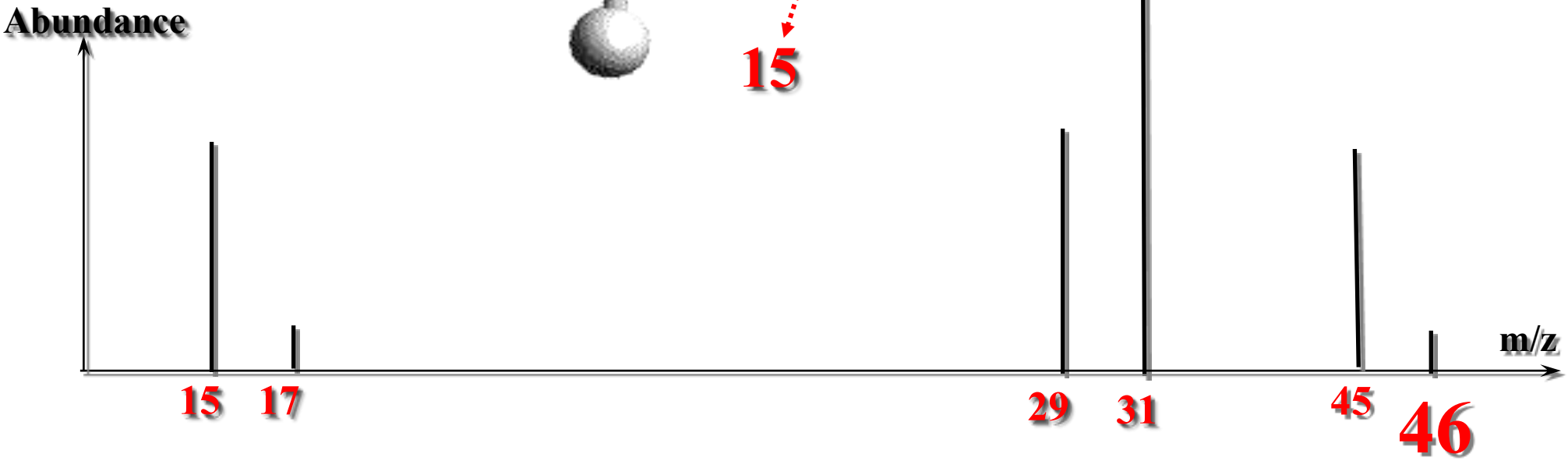
H = 1 u

C = 12 u

O = 16 u



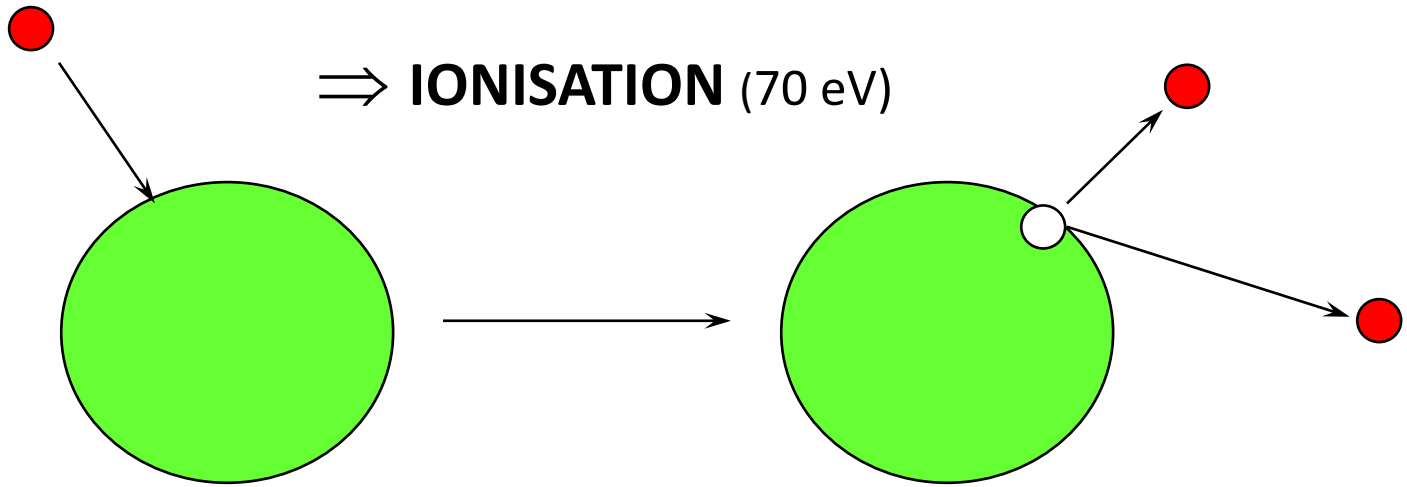
$M^{+\bullet}$ = 46 u



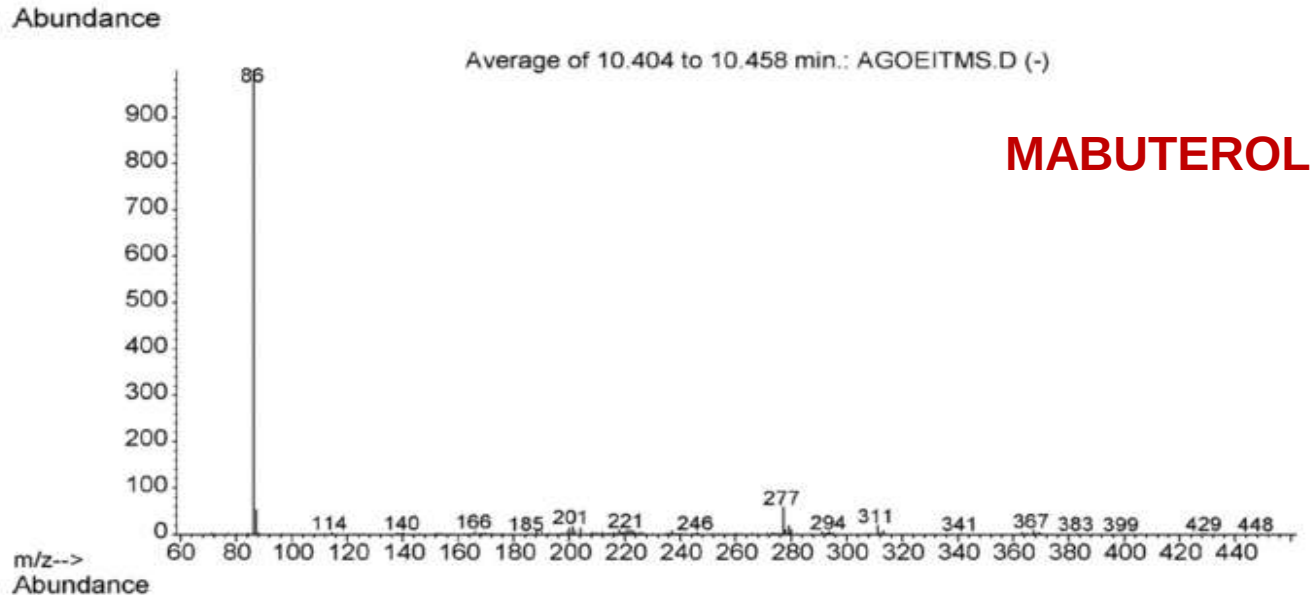
4. ANALYTICAL METHODS

4.4 Mass spectrometry

IONISATION



$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$

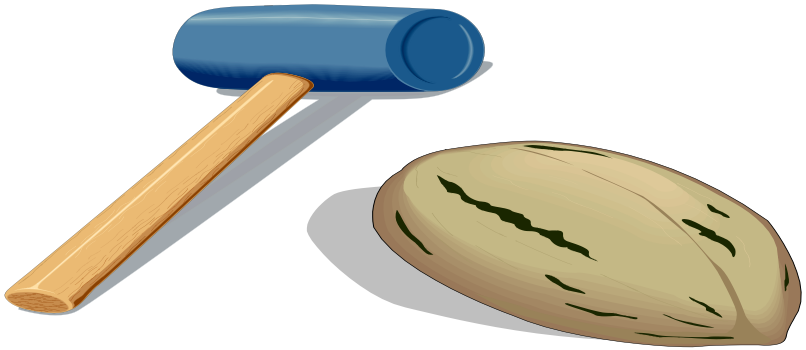


MABUTEROL

4. ANALYTICAL METHODS

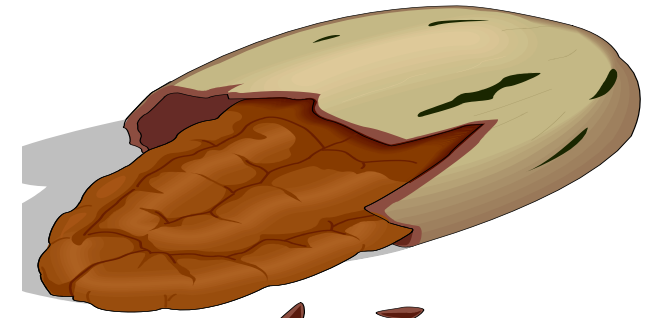
4.4 Mass spectrometry

FOR LABILE COMPOUNDS



EI

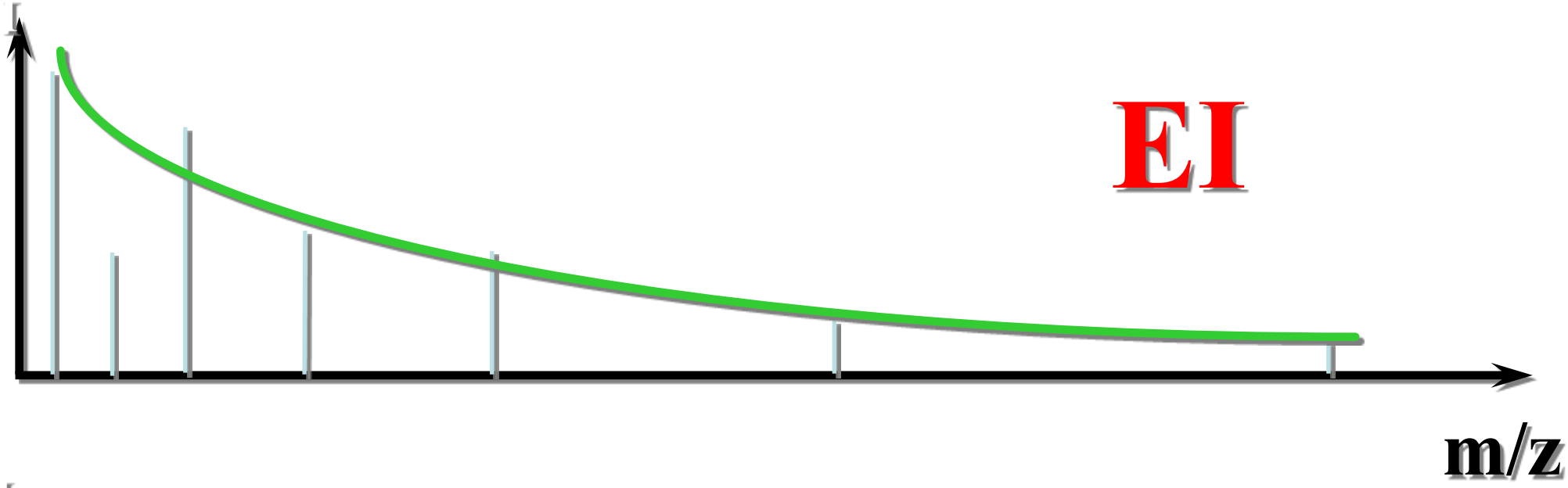
PCI

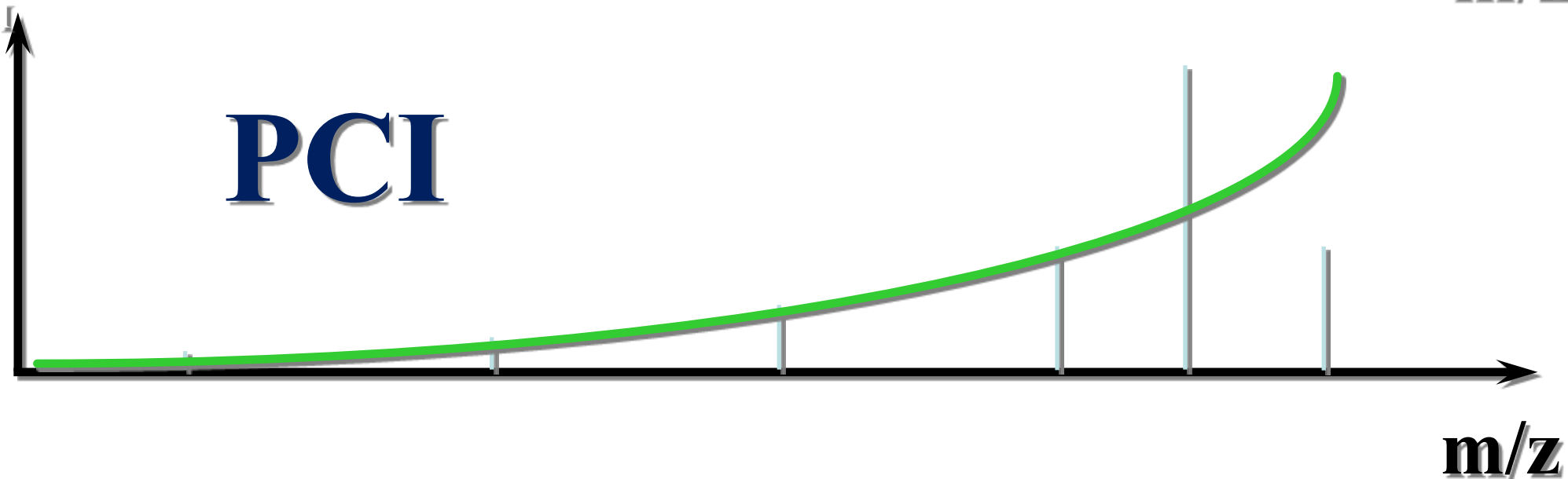


4. ANALYTICAL METHODS

4.4 Mass spectrometry

FOR LABILE COMPOUNDS

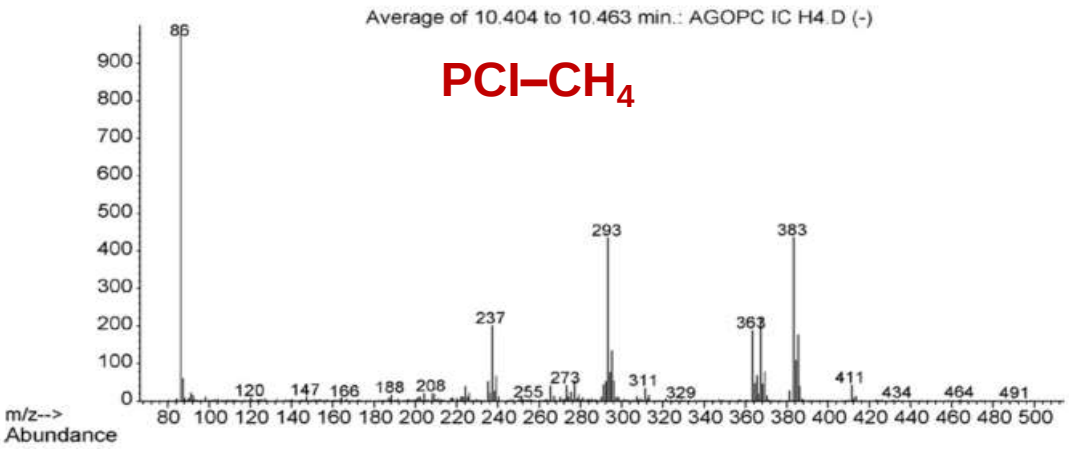




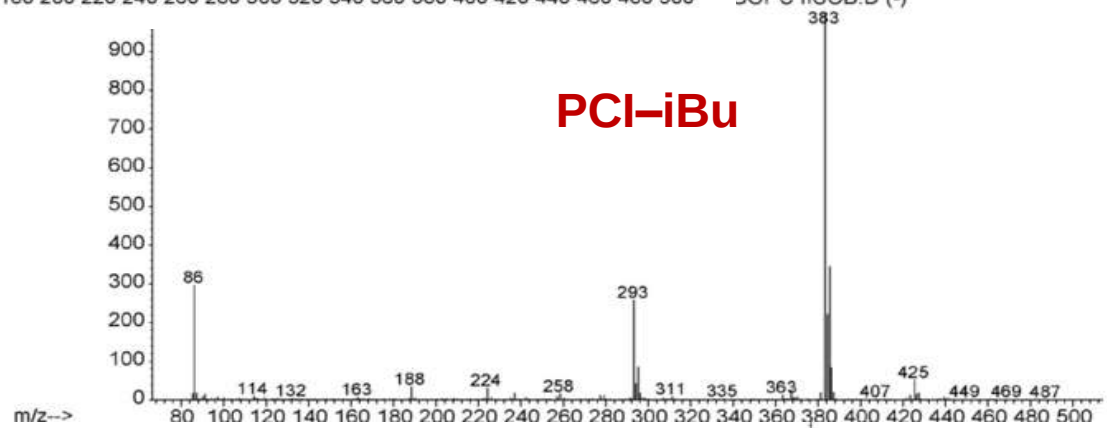
4. ANALYTICAL METHODS

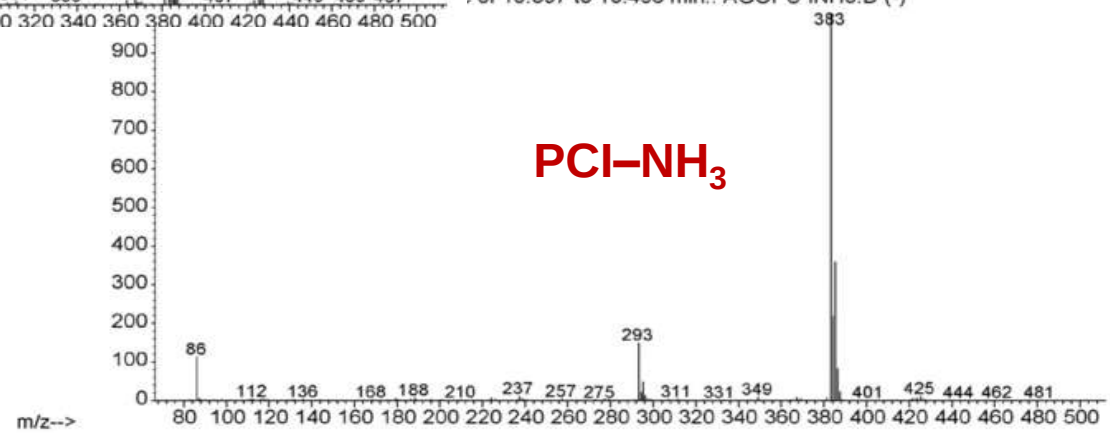
FOR LABILE COMPOUNDS

4.4 Mass spectrometry



MABUTEROL





ATMSPHERIC PRESSURE IONISATION

LC-MS

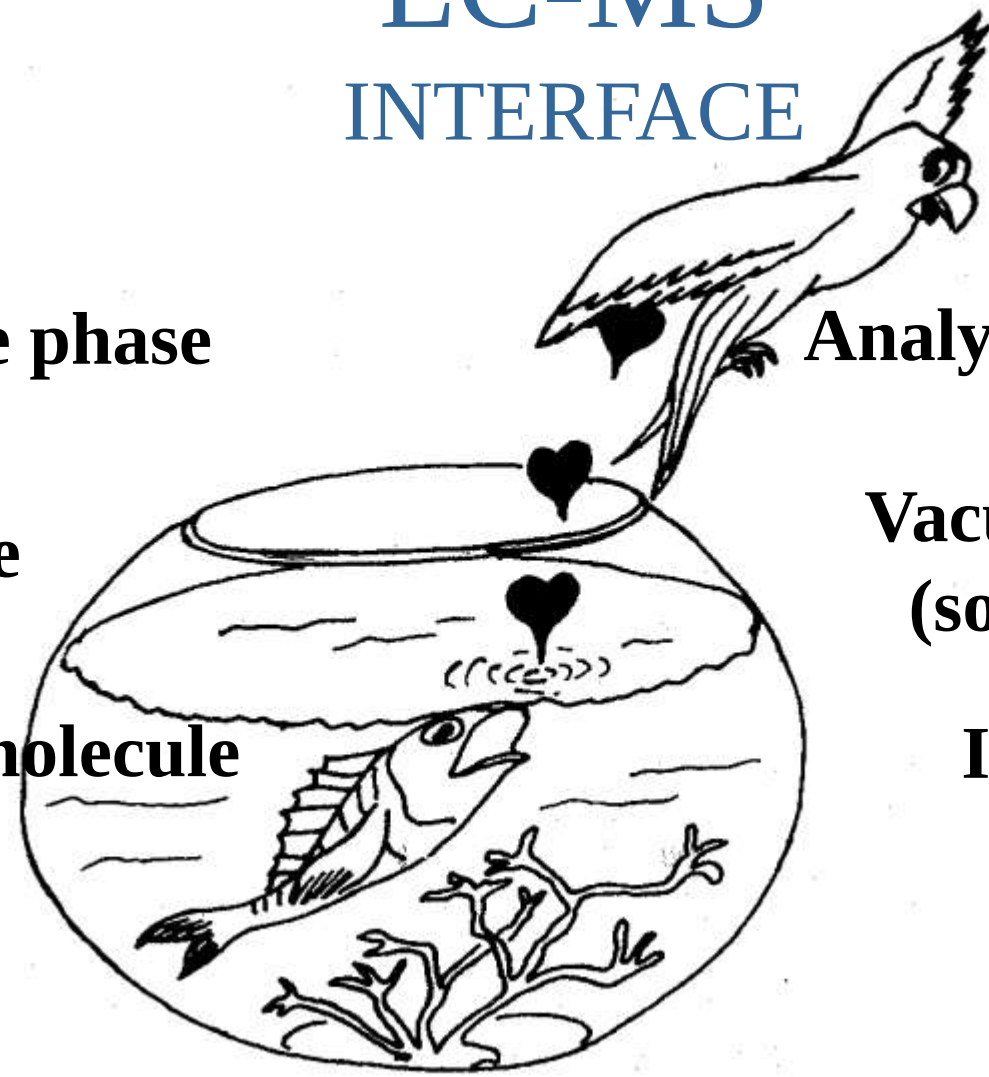
INTERFACE

LC

Liquid mobile phase

High flow rate

Non ionized molecule



MS

Analyte in gaseous phase

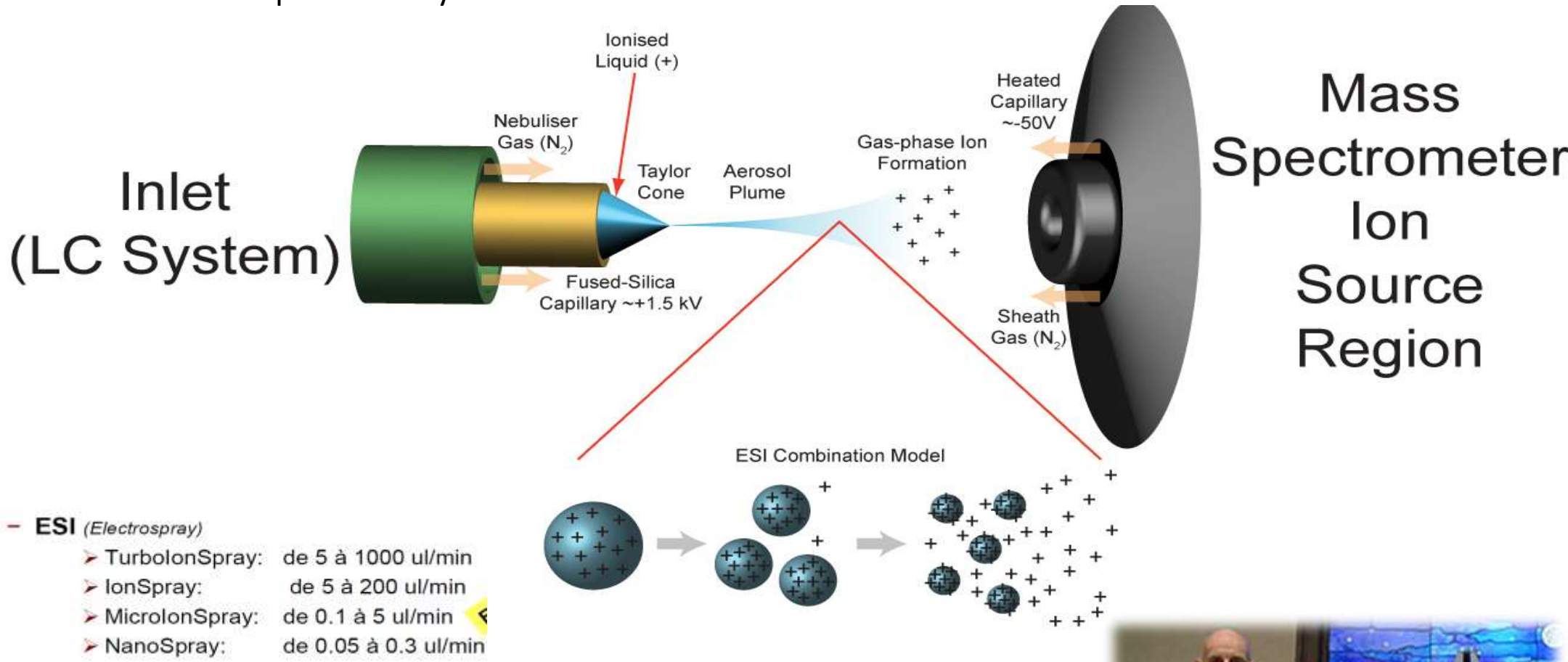
Vacuum of second order (source and analyzer)

Ionized molecules

4. ANALYTICAL METHODS

4.4 Mass spectrometry

Nguyen, S.; Fenn, J.B.; *PNAS*, **2007**, 104 (4), 1111.



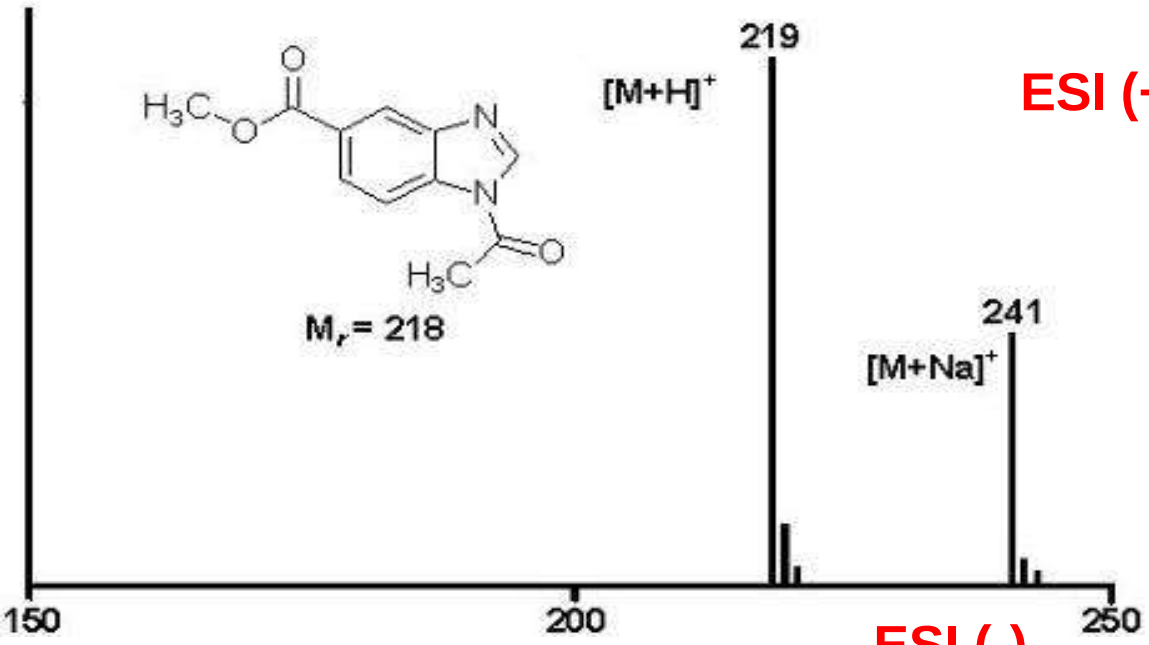
- **ESI** *(Electrospray)*
  - TurboIonSpray: de 5 à 1000 ul/min
  - IonSpray: de 5 à 200 ul/min
  - MicroIonSpray: de 0.1 à 5 ul/min
  - NanoSpray: de 0.05 à 0.3 ul/min

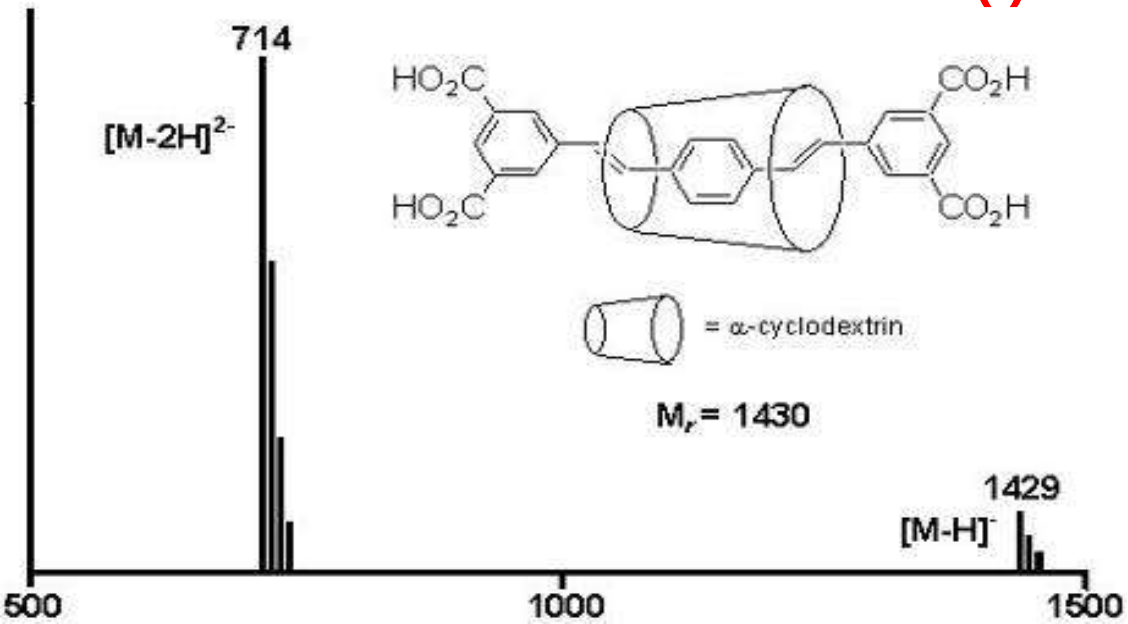
Positive mode: [M+H]+ protonated molecule
[M+Na] +, [M+K] + … adducts
[M+CH3CN+H] + protonated, + solvent adducts

Negative mode: [M-H] - deprotonated molecule
[M+HCOO -] -, … adducts

4. ANALYTICAL METHODS

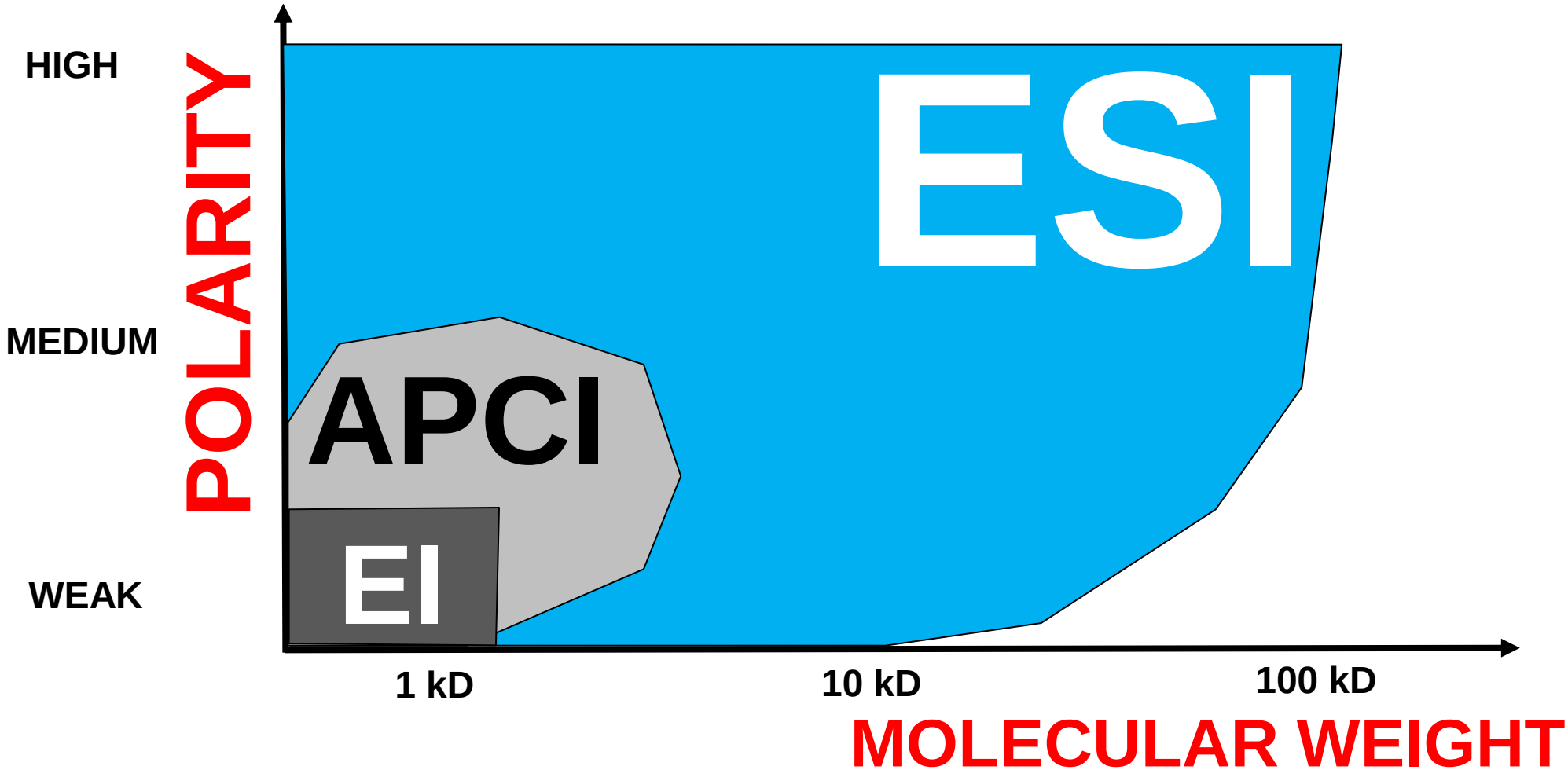
4.4 Mass spectrometry





4. ANALYTICAL METHODS

4.4 Mass spectrometry

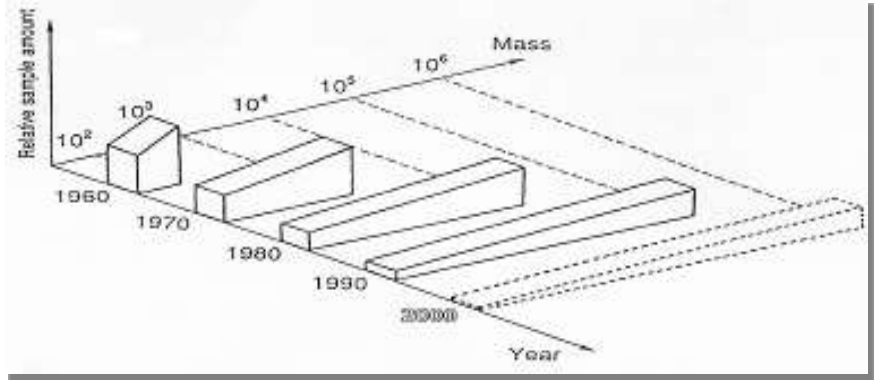


4. ANALYTICAL METHODS

4.4 Mass spectrometry

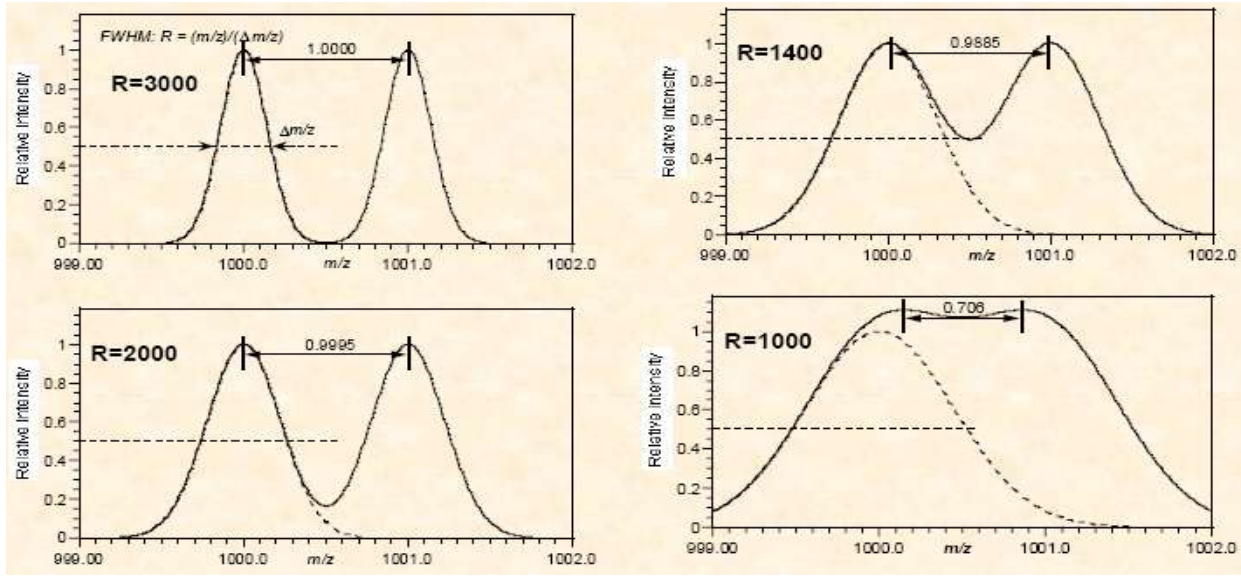
QUALIFICATION PARAMETERS

- **MASS RANGE:** lowest to highest mass that can be characterized by the mass analyzer



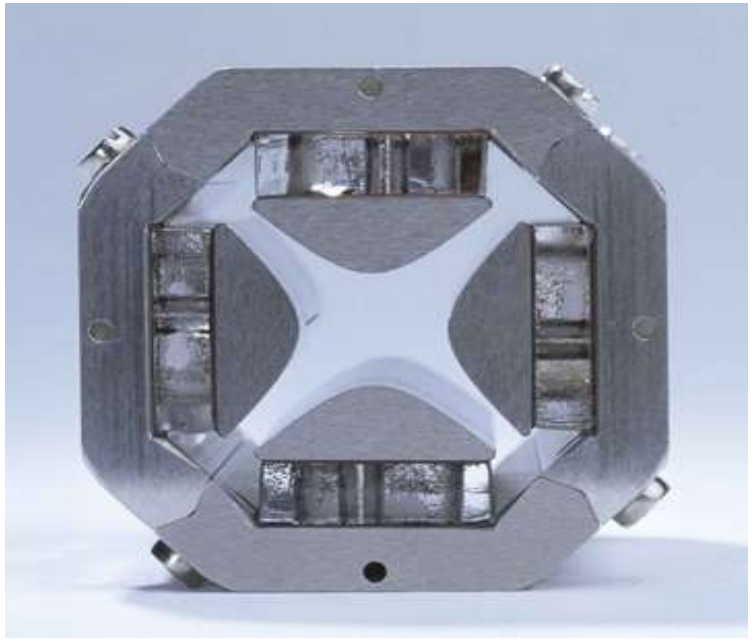
- **RESOLUTION:** the capacity of the mass analyser to distinguish two ions with very close m/z

$$R = M/\Delta M$$

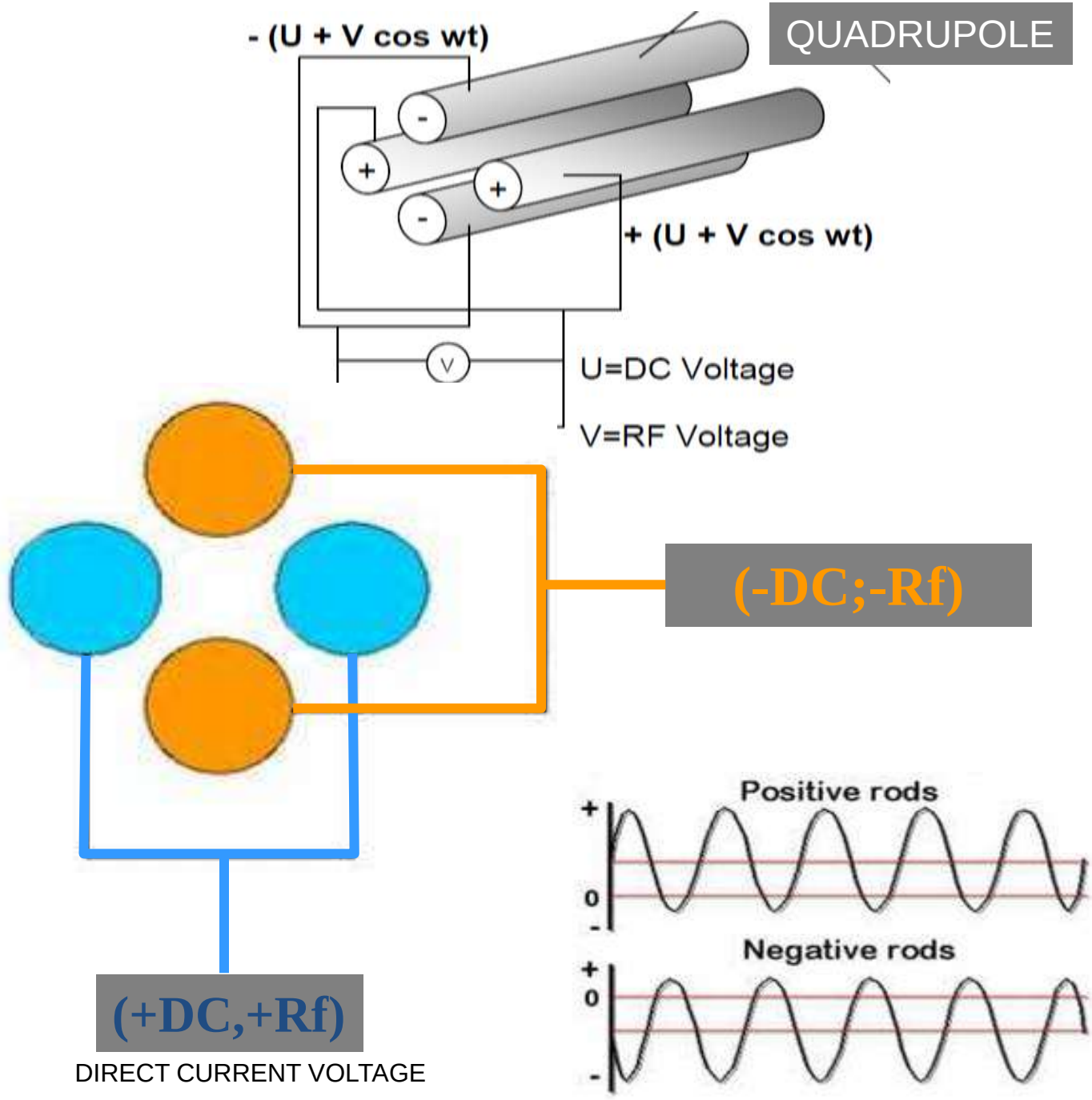


4. ANALYTICAL METHODS

4.4 Mass spectrometry

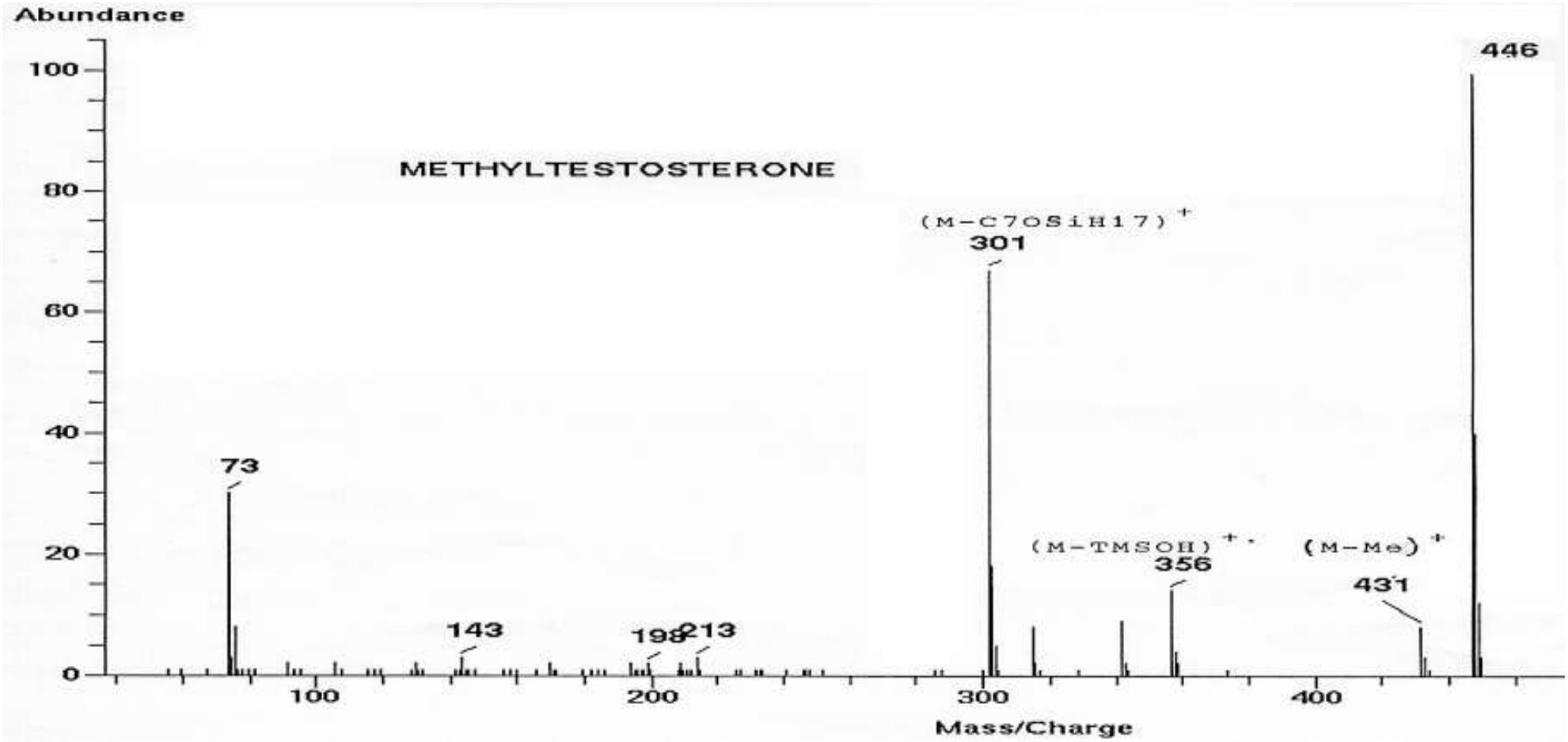


QUADRUPOLE



MS1 - SCAN mode

- All ions recorded in-between two masses (e.g. m/z 50 → 500)
- + very selective / - sensitivity

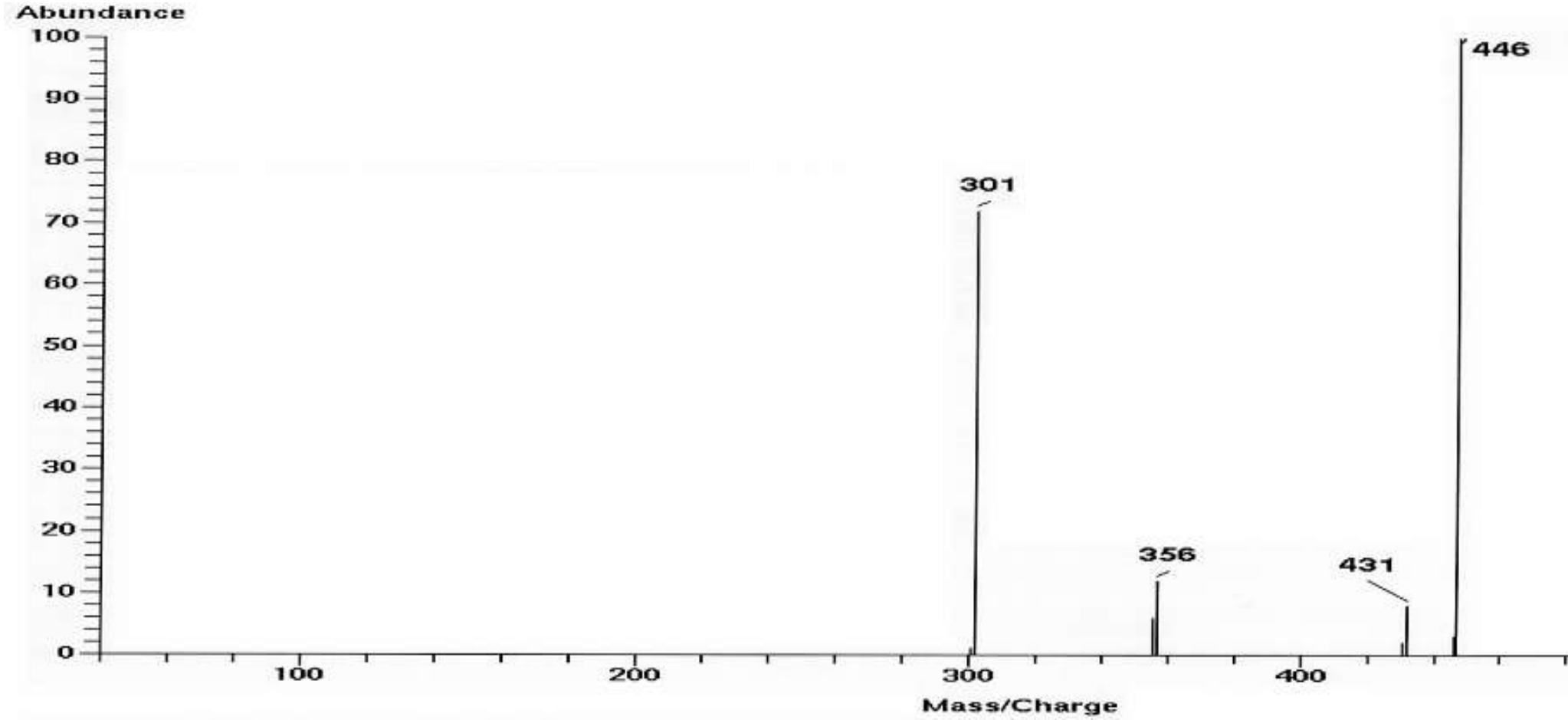


4. ANALYTICAL METHODS

4.4 Mass spectrometry

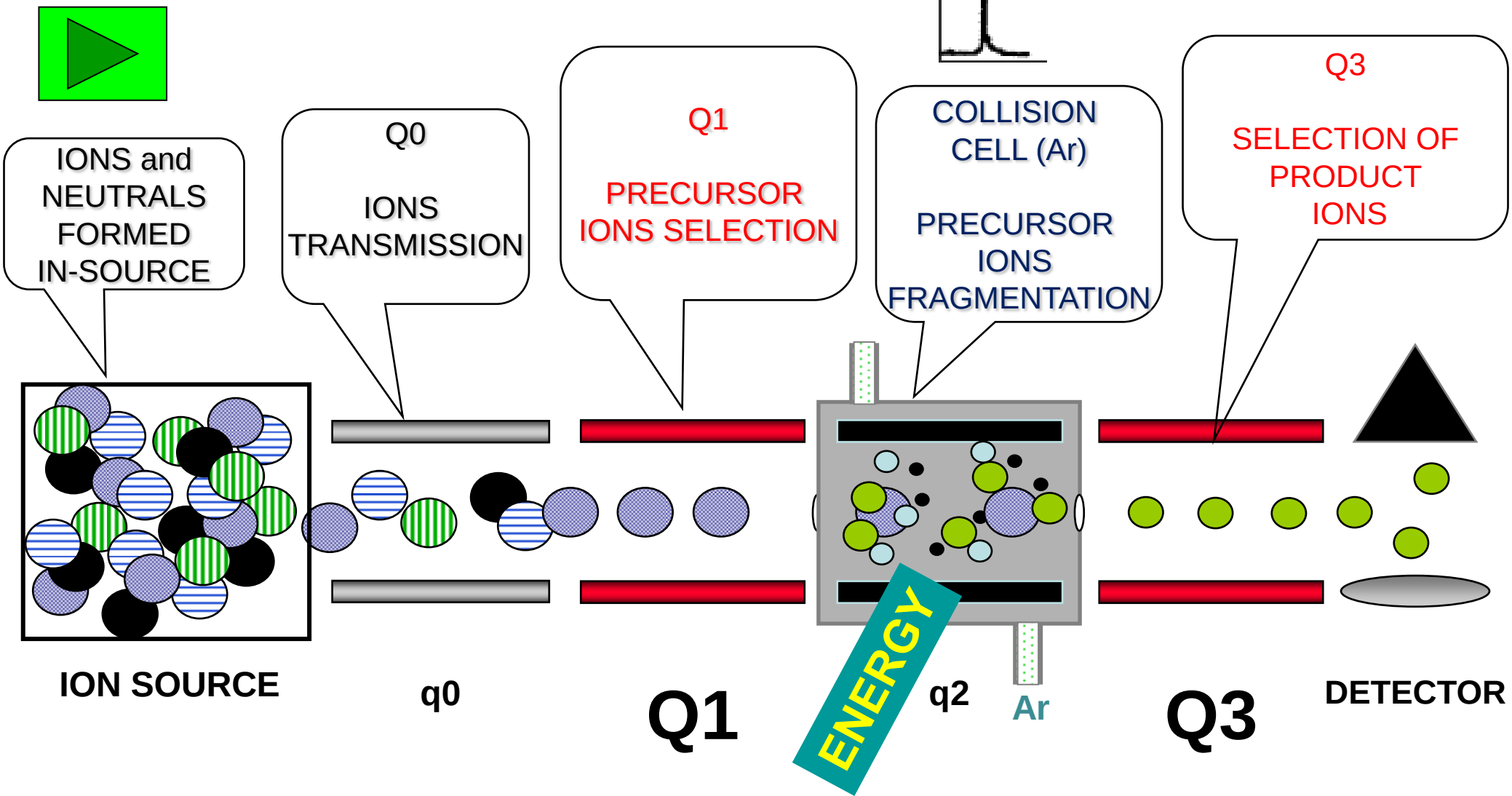
MS1 - SIM mode

- Recording of pre-defined ions (e.g. m/z 446, 431, 356, 301)
- + medium/high selectivity, excellent sensitivity, trace quantitation



1978
Triple quad
Yost, Enke

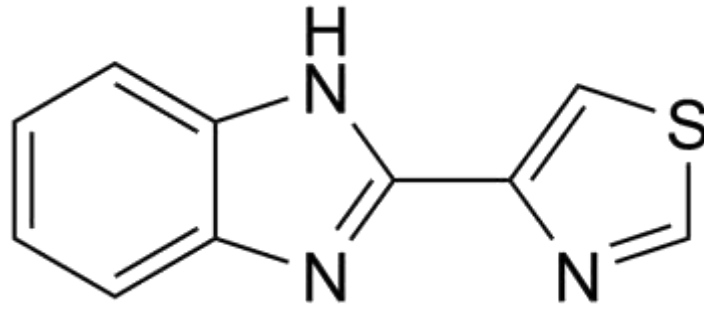
QqQ



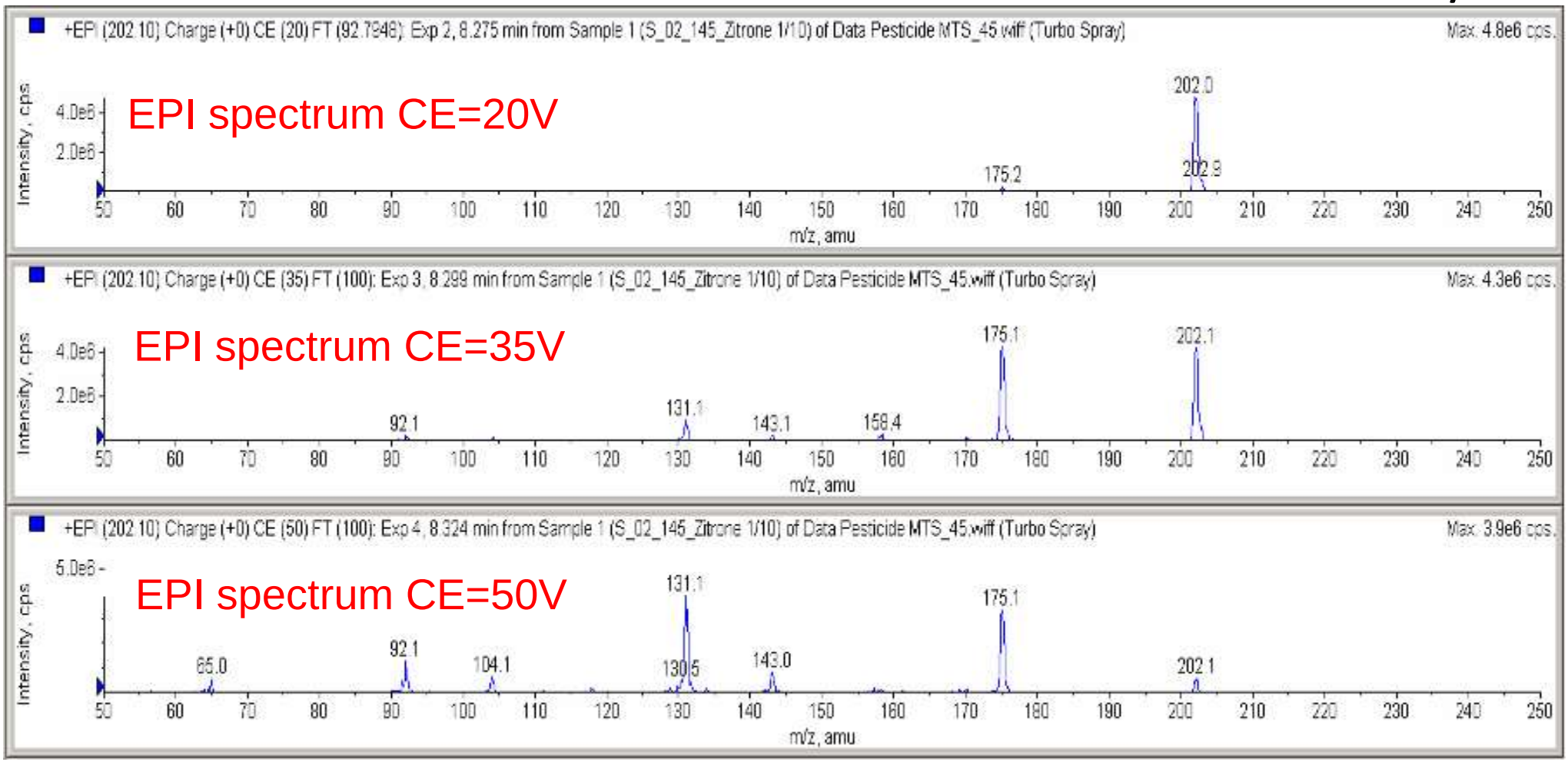
4. ANALYTICAL METHODS

4.4 Mass spectrometry

ENERGY



Thiabendazole - SRM 202.1/175.1



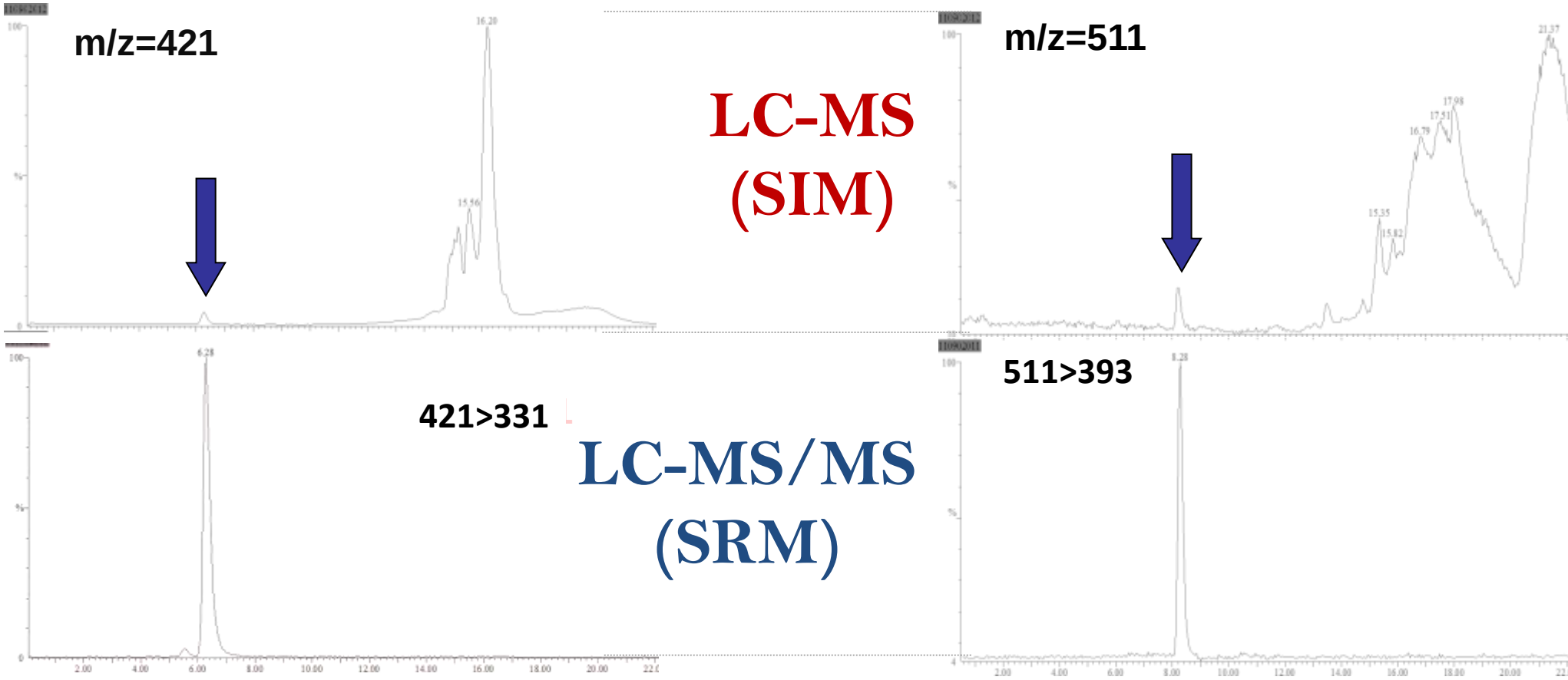
4. ANALYTICAL METHODS

4.4 Mass spectrometry

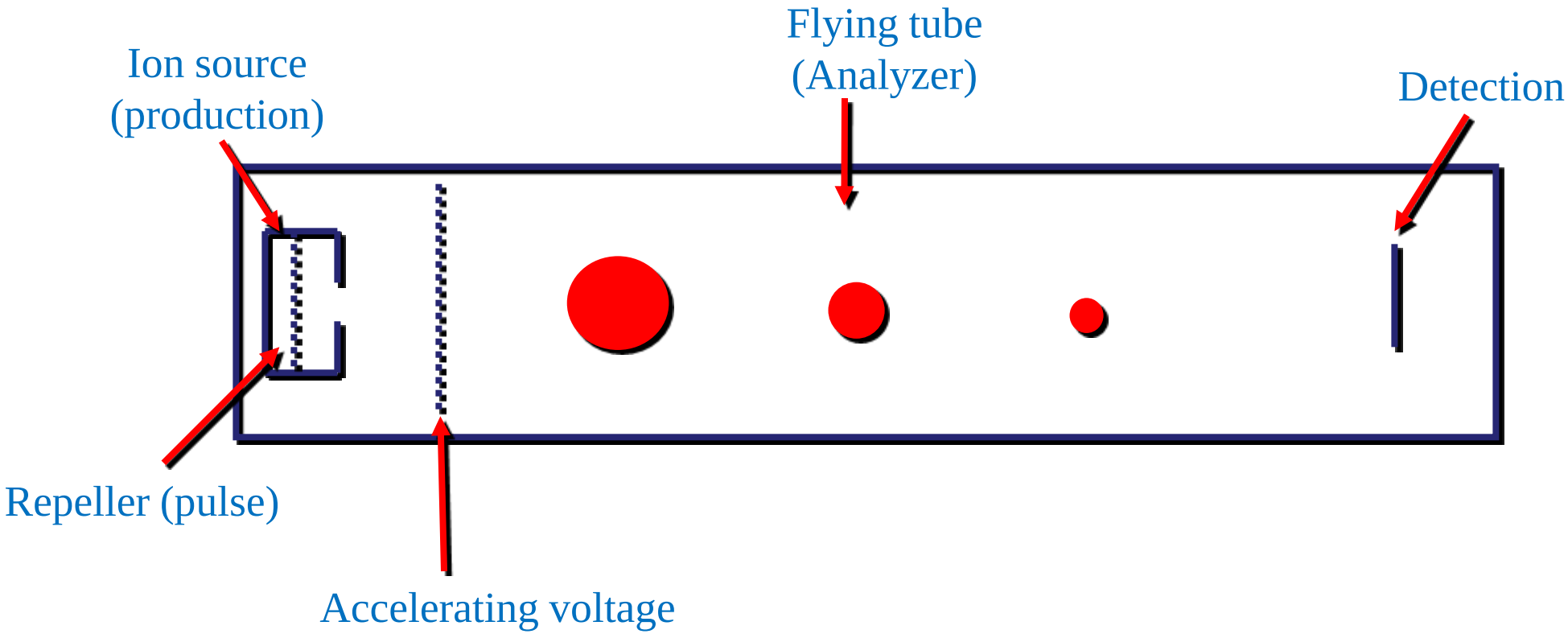
MS^1 *versus* MS^2

ENDOGENOUS CORTISOL IN LIVER

FLUOCINOLONE ACETONIDE IN LIVER (1.5 ng.g^{-1})



TIME OF FLIGHT (TOF)

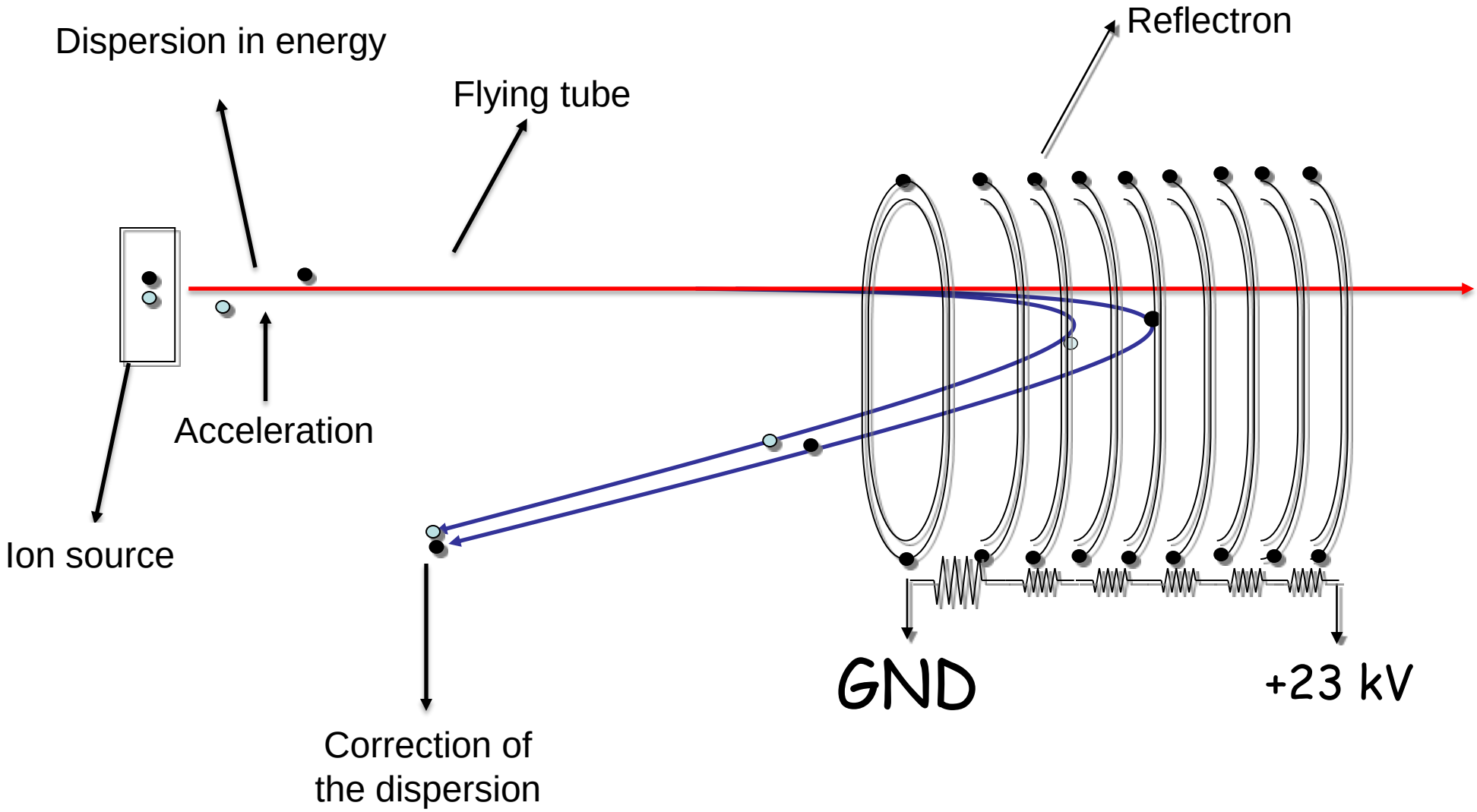


Kinetic energy: $qV = mv^2 / 2$ (with $q = ze$)

Flying time: $t = d / v$

$$\mathbf{m/z = t^2 (2Ve / d^2)}$$

TIME OF FLIGHT (TOF)



TIME OF FLIGHT (TOF)

Element	Nuclide	Nominal Mass	Exact Mass	Mass Defect	Isotopic Abundance
Hydrogen	H	1	1.0078	0.00783	100.00%
	D	2	2.0141	0.0141	0.02%
Carbon	C^{12}	12	12.0000	0	100.00%
	C^{13}	13	13.0034	0.00336	1.10%
Nitrogen	N^{14}	14	14.0031	0.003074	100.00%
	N^{15}	15	15.0001	0.0001	0.37%
Oxygen	O^{16}	16	15.9949	-0.0051	100.00%
	O^{17}	17	16.9991	-0.0009	0.04%
	O^{18}	18	17.9992	-0.0008	0.20%

- $N_2 = 2 \times 14.0031 = 28.0061$
- $CO = 12.0000 + 15.9949 = 27.9949$
- $C_2H_4 = 2 \times 12.0000 + 4 \times 1.0078 = 28.0312$
- $CH_2N = 12.0000 + 2 \times 1.0078 + 14.0031 = 28.0187$

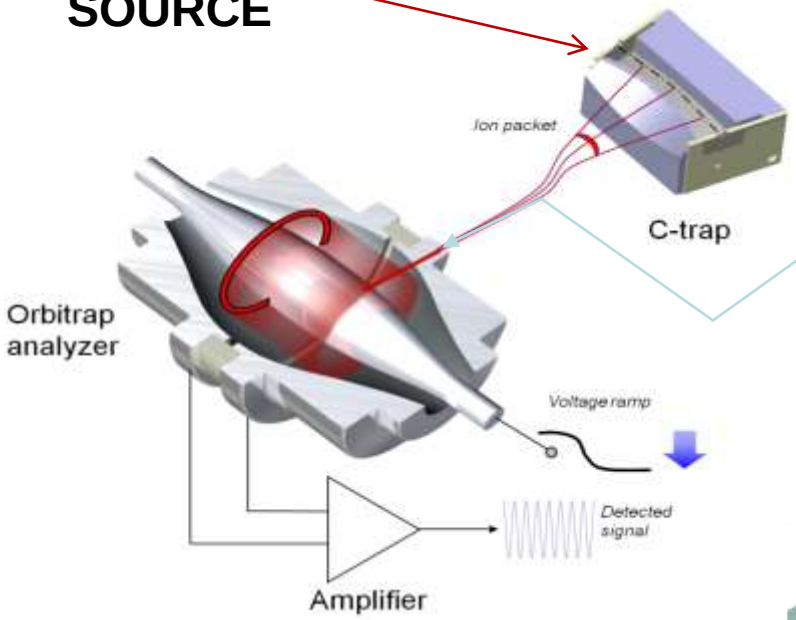
4. ANALYTICAL METHODS

4.4 Mass spectrometry

Orbitrap®

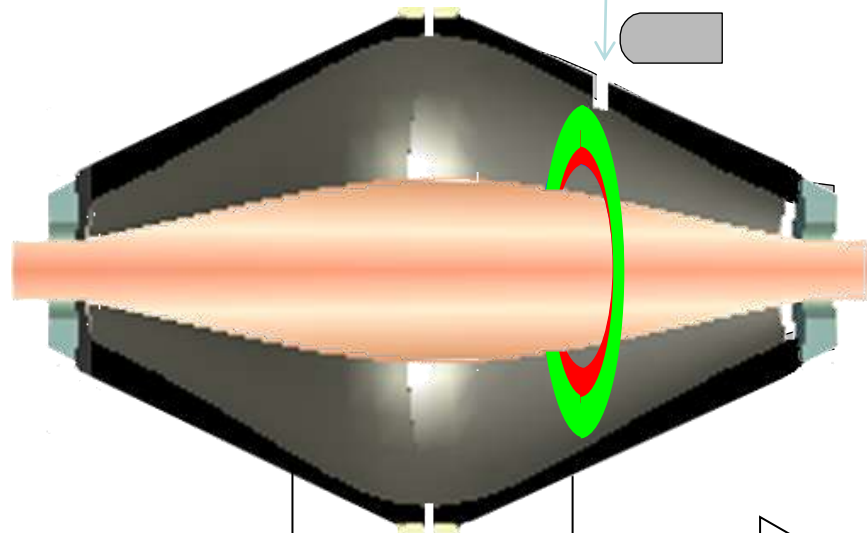
ION SOURCE

FAST INJECTIONS OF IONS

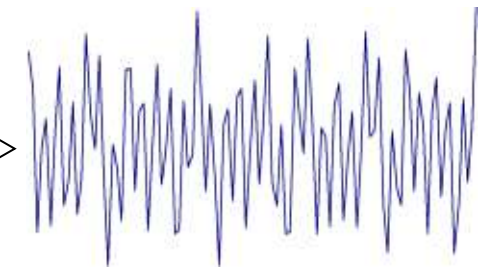


MEASUREMENT OF THE FREQUENCE

→ FOURIER TRANSFORM



$$\omega = \sqrt{\frac{k}{m/z}}$$

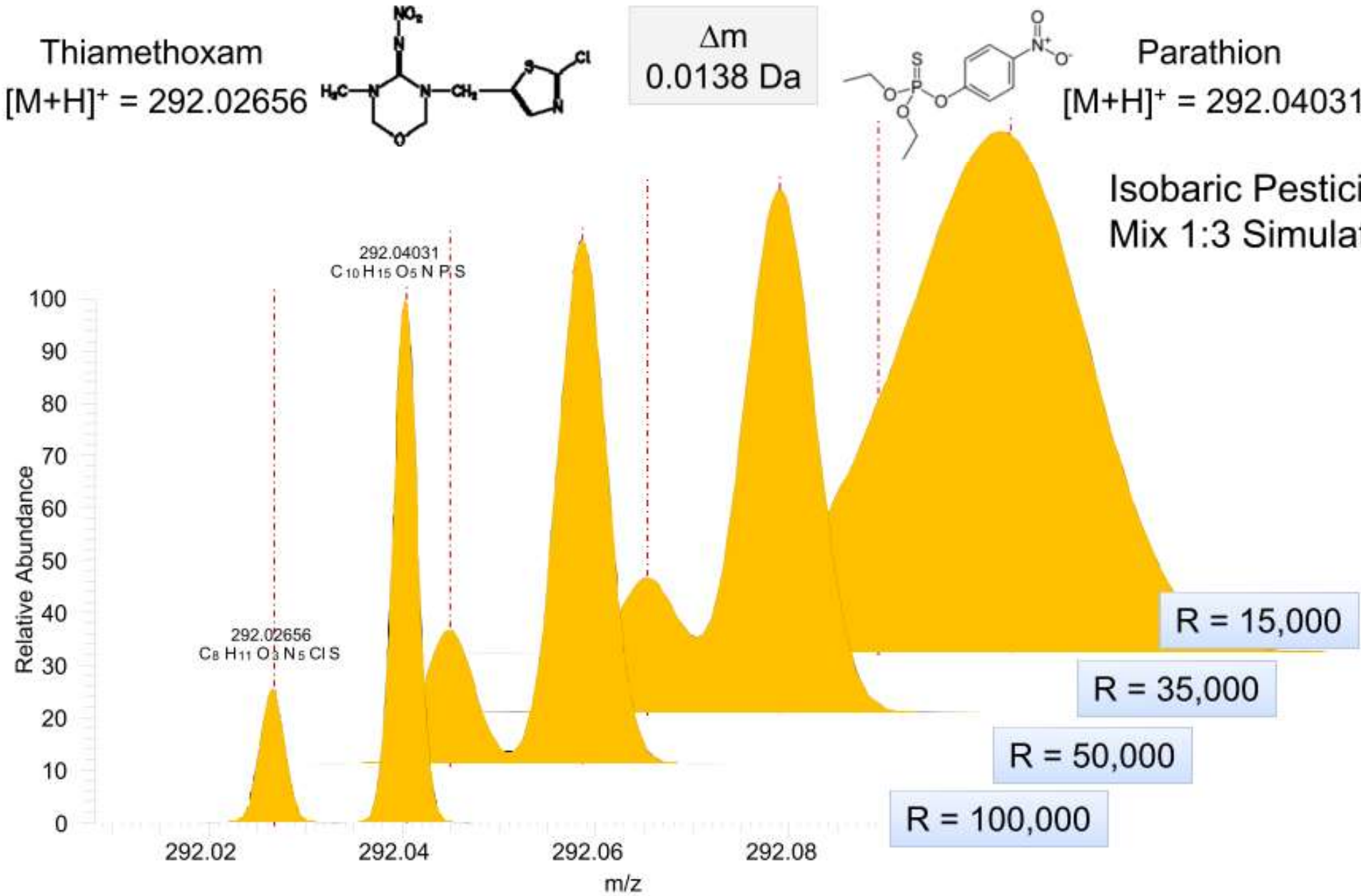


A.A. Makarov, Anal. Chem., v.72 (2000), No.6, p.1156-1162.
A.A. Makarov, US Pat. 5,886,346, 1999.

4. ANALYTICAL METHODS

4.4 Mass spectrometry

Orbitrap®



1. INTRODUCTION

2. REGULATION

3. COMPOUNDS/MATRICES TO MONITOR

4. ANALYTICAL METHODS

5. IP, CCα, MRPL & RPA

5. IP, CCα, MRPL and RPA

5.1 Decision 2002/657/EC

L 221/8 EN Official Journal of the European Communities 17.8.2002

2002/657/EC

II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DECISION

of 12 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)

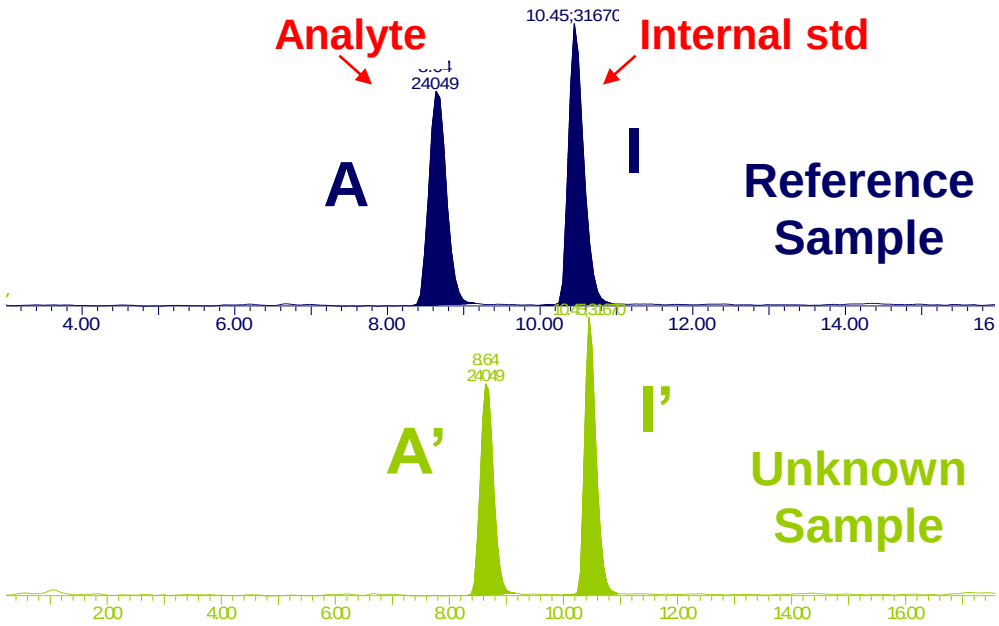
(2002/657/EC)

5. IP, CCα, MRPL and RPA

5.2 Chromatographic criteria

2.3.3.1. *Chromatographic separation*

For GC-MS procedures, the gas chromatographic separation shall be carried out using capillary columns. For LC-MS procedures, the chromatographic separation shall be carried out using suitable LC columns. In any case, the minimum acceptable retention time for the analyte under examination is twice the retention time corresponding to the void volume of the column. The retention time (or relative retention time) of the analyte in the test portion shall match that of the calibration standard within a specified retention time window. The retention time window shall be commensurate with the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 0,5 % for GC and ± 2,5 % for LC.



$$\frac{(A'/I') - (A/I)}{(A/I)} \times 100$$

LC → 2.5%

GC → 0.5%

TOLERANCE

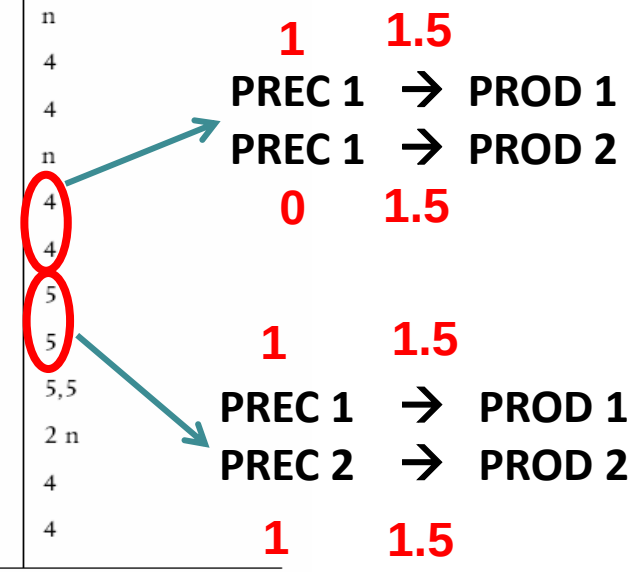
5. IP, CCα, MRPL and RPA

5.3 Spectrometric Criteria

- Presence (S/N > 3)

Examples of the number of identification points earned for a range of techniques and combinations thereof (n = an integer)

Technique(s)	Number of ions	Identification points
GC-MS (EI or CI)	N	n
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS (EI or CI) 2 derivatives	2 (Derivative A) + 2 (Derivative B)	4
LC-MS	N	n
GC-MS-MS	1 precursor and 2 daughters	4
LC-MS-MS	1 precursor and 2 daughters	4
GC-MS-MS	2 precursor ions, each with 1 daughter	5
LC-MS-MS	2 precursor ions, each with 1 daughter	5
LC-MS-MS-MS	1 precursor, 1 daughter and 2 granddaughters	5,5
HRMS	N	2 n
GC-MS and LC-MS	2 + 2	4
GC-MS and HRMS	2 + 1	4



- Matching

Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS^n LC-MS, LC-MS^n (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

5.4 What are α- and ß-errors ?

α-error = probability of a false non-compliant result

False Positive

β-error = probability of a false compliant result

False Negative

Critical Concentrations

where something happens …

CCα : controls false non-compliant rate (false positive)

CCβ : controls false compliant rate (false negative)

…and in effect, reflects the MU …a*t the level of interest : zero or MRL*

Definition

Decision Limit (CCα)

« Limit at and above which it can be concluded with an error probability of α that a sample is non-compliant »

Forbidden substances

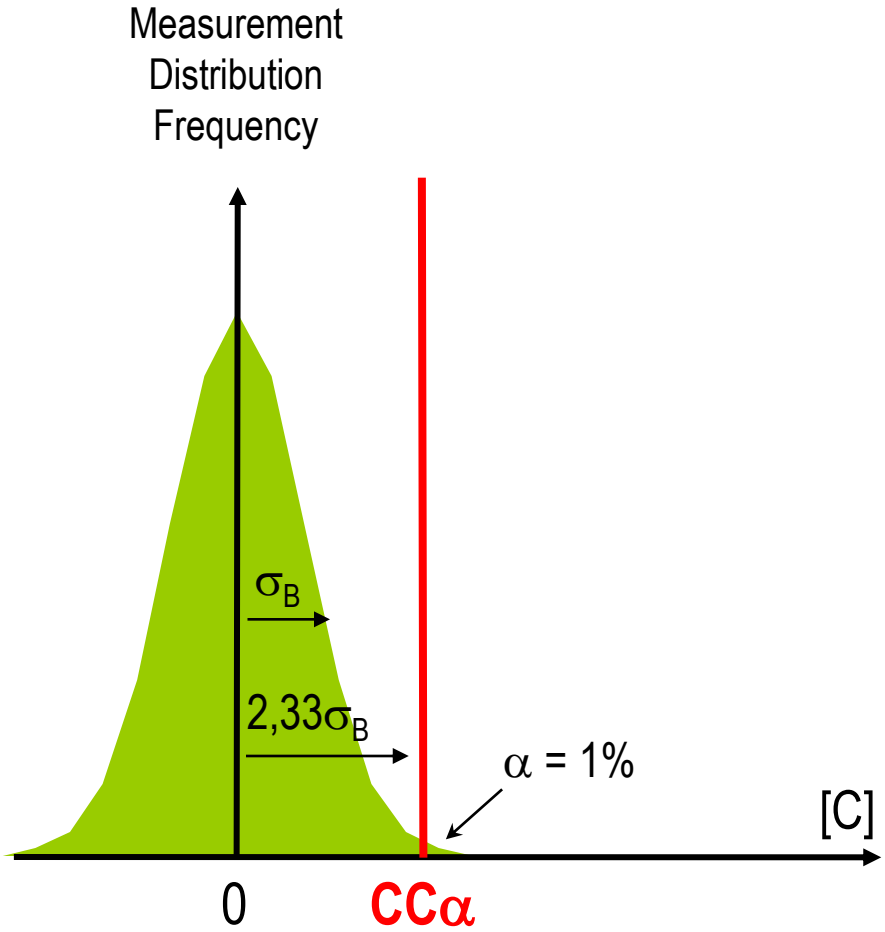
Smallest measurement result from which it can be concluded, with a **99% confidence level**, that the target analyte is present **(α=1%).**

MRL substances

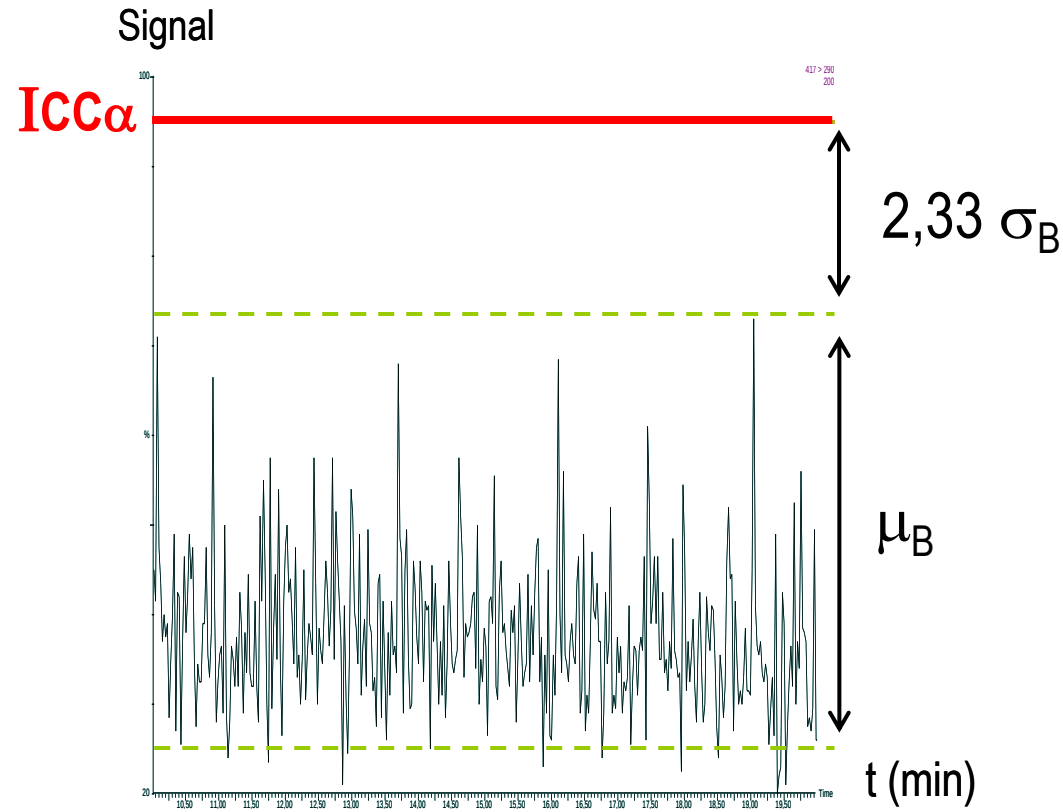
Smallest measurement result from which it can be concluded, with a **95% confidence level**, that the target analyte is present at a concentration higher than the MRL **(α=5%).**

5.5 What are CCα and CCβ ?

Decision Limit (CCα)
Signification : forbidden substances



Chromatogram representative of an « average » blank sample



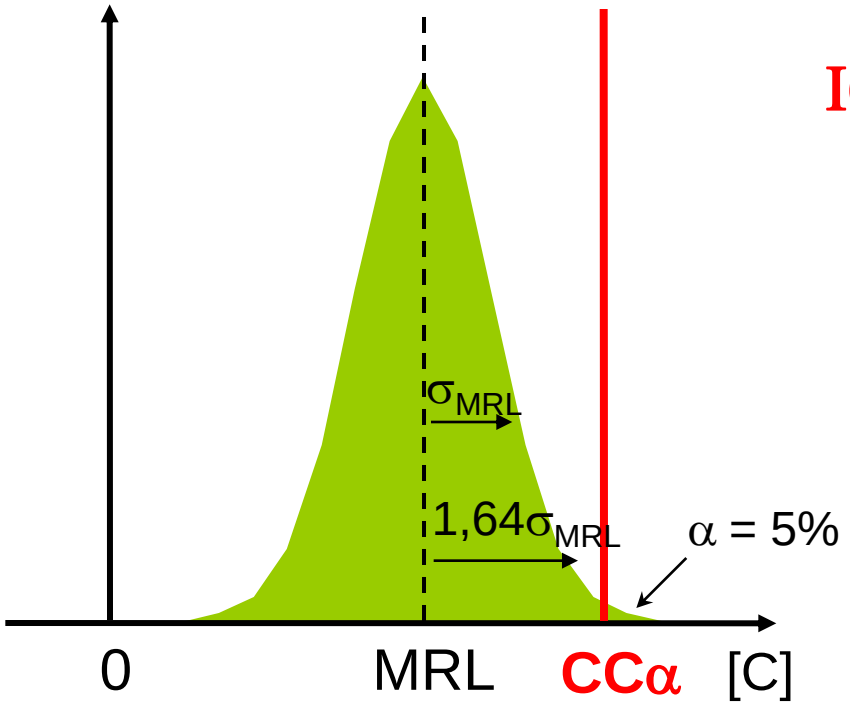
$$ICC\alpha = \mu_B + 2{,}33\ \sigma_B$$

5.5 What are CCα and CCβ ?

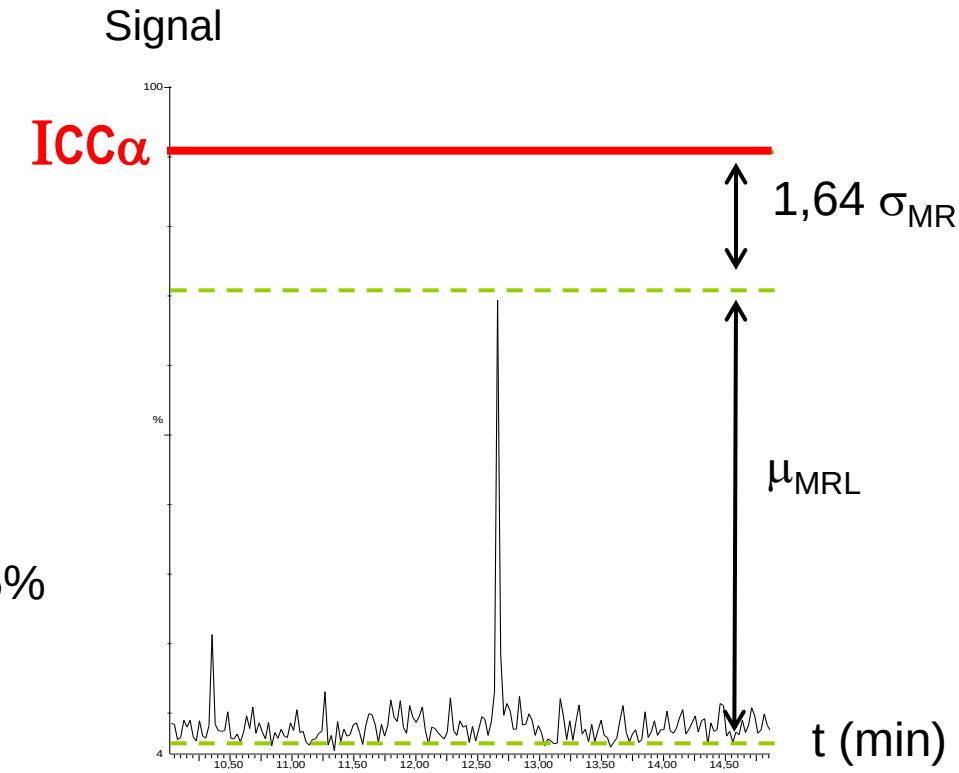
Decision Limit (CCα)

Signification: MRL substances

Measurement Distribution Frequency



« Average » chromatogram containing the analyte at a concentration equal to the MRL



$$ICC\alpha = \mu_{MRL} + 1{,}64\ \sigma_{MRL}$$

5. IP, CCα, MRPL and RPA

5.6 MRPL

1.18. Minimum required performance limit (MRPL) means minimum content of an analyte in a sample, which at least has to be detected and confirmed. It is intended to harmonise the analytical performance of methods for substances for which no permitted limited has been established.

- Decision 2002/657/EC sets minimum required performance limits (MRPL)
  - *MRPL : minimum content of an analyte in a sample, which at least has to be detected and confirmed*
  - Fixed by regulatory instance
  - *To harmonize analytical performance between laboratories within EU*
  - *To harmonize control*

Minimum required performance limits

Substance and/or metabolite	Matrixes	MRPL
Chloramphenicol	Meat Eggs Milk Urine Aquaculture products Honey	0,3 µg/kg
Medroxyprogesterone acetate	Pig kidney fat	1 µg/kg
Nitrofuran metabolites: — furazolidone — furaltadone — nitrofurantoin — nitrofurazone	Poultry meat Aquaculture products	1 µg/kg for all
Sum of malachite green and leuco-malachite green	Meat of aquaculture products	2 µg/kg

5. IP, CCα, MRPL and RPA

5.7 Recommended Concentrations

CRL GUIDANCE PAPER (7 December 2007)

CRLs VIEW ON STATE OF THE ART ANALYTICAL METHODS FOR NATIONAL RESIDUE CONTROL PLANS

methods in residue control. The recommended concentrations in this document have no legal force. MRPLs, on the other hand, serve according to Commission Decision 2005/34/EC as

Substances	Matrix	Recommended concentration*
Diethylstilbestrol (DES) Dienestrol (DE) Hexestrol (HEX)	Urine	1 ppb for DES 2 ppb for DE 2 ppb for HEX
	Liver	2ppb (for all substances)
	Meat (including fish)	1 ppb (for all substances)

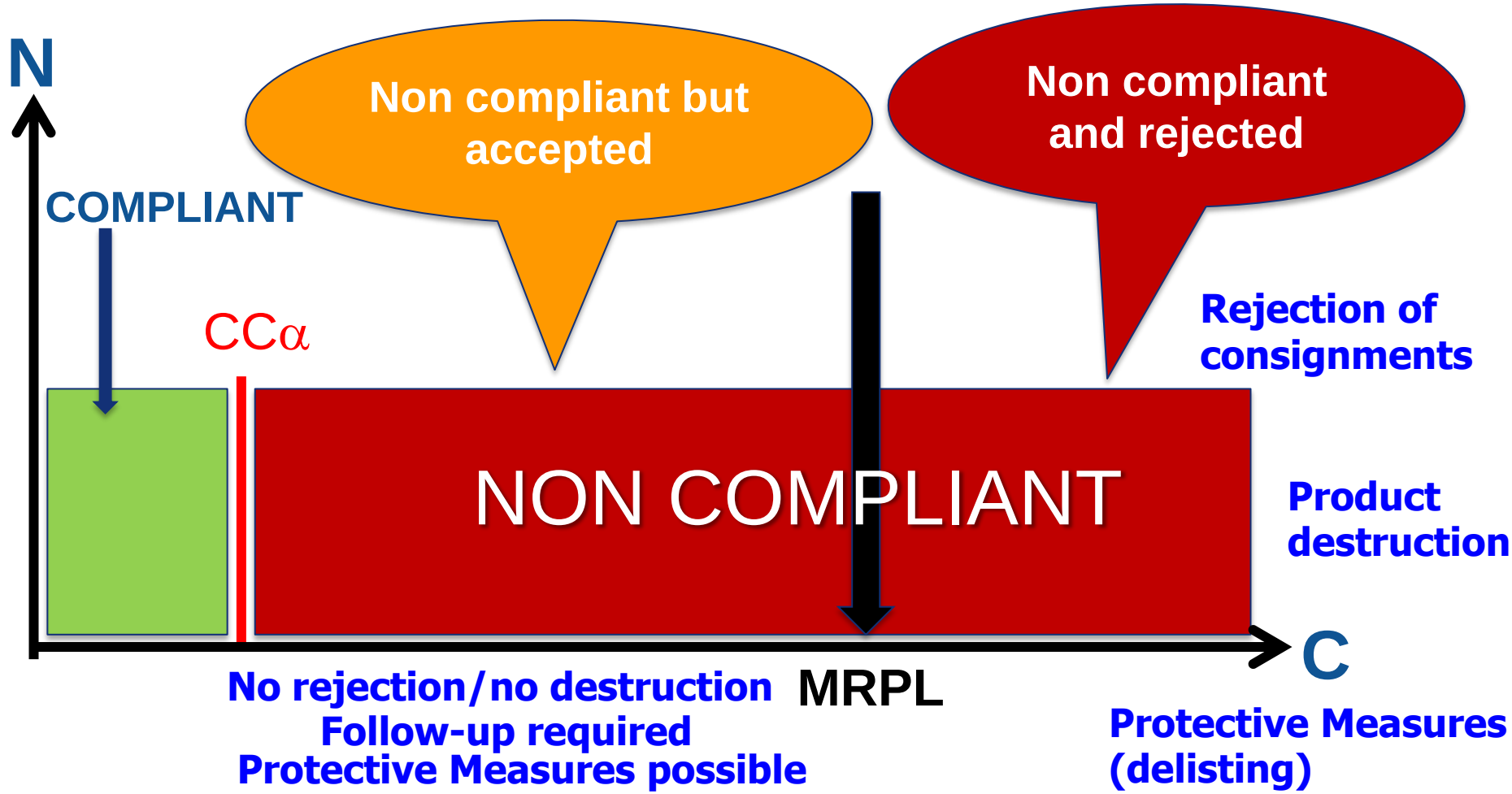
** CCbeta for screening methods or CCalpha for confirmatory methods should be lower than the value expressed in this column*

Substances	Marker residue-metabolite	Matrix	Recommended concentration*
Zeranol[1]	Taleranol	Urine	2 ppb
		Liver	2 ppb
		Muscle	1 ppb
Zearalanone		Urine	2 ppb
		Liver	2 ppb

**CCbeta for screening methods or CCalpha for confirmatory methods should be lower than the value expressed in this column*

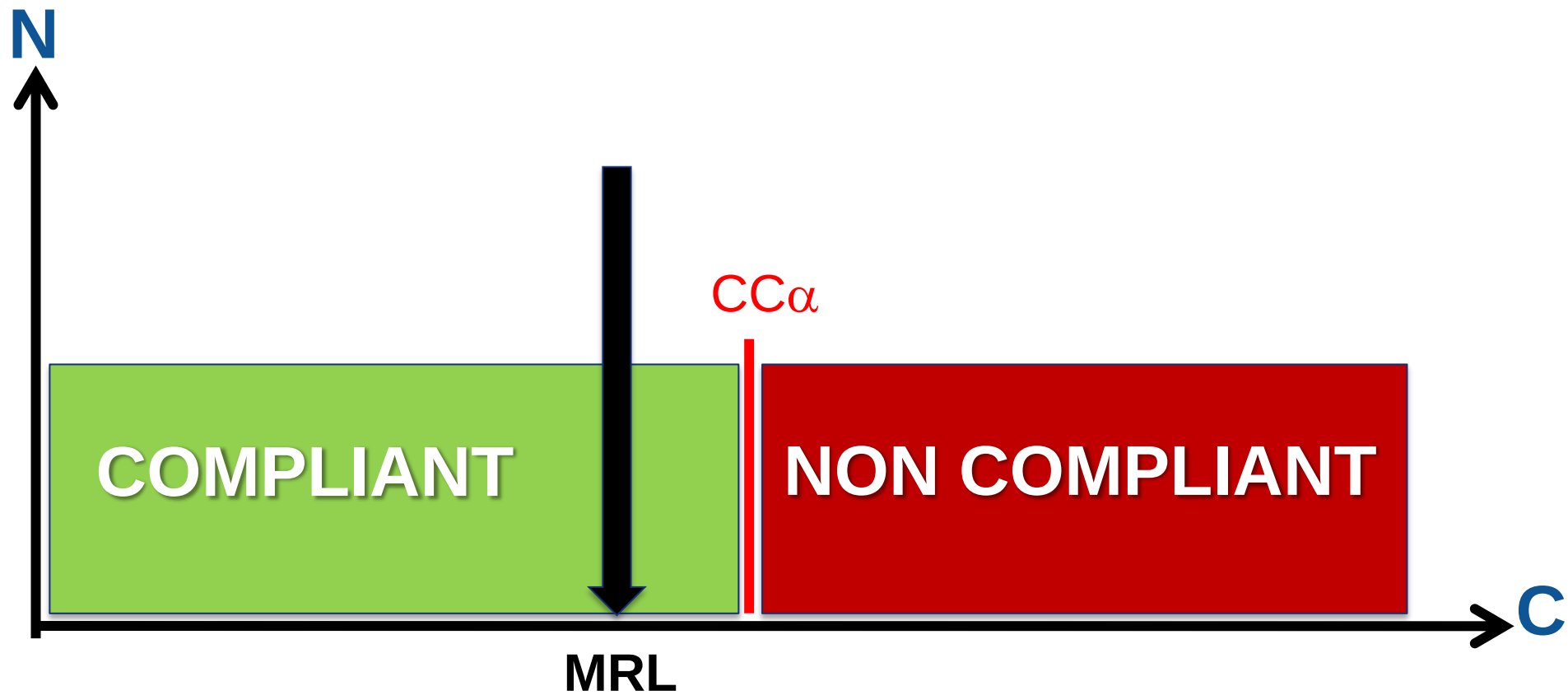
Control of forbidden and unauthorised compounds

Interpretation of the results



Authorised substances

Interpretation of the results



RPA: Reference Point for Action

5.9 RPA

RPAs may be established for non-allowed pharmacologically active substances when it is deemed necessary to ensure the functioning of controls for food of animal origin that is imported or placed on the market.

- **Decision 2005/34/EC (imported products)**
  - A1 Lays down RPA for substances having a MRPL
  - A2 MRPL shall be used as RPA (regardless the matrix)

- **Regulation EC N° 470/2009, Tittle III**
  - A 19.1: *RPAs shall be based on Analytical Possibilities* (lowest residue concentration that can be quantified with a validated analytical method)
  - *but,* A 19.2:*where appropriate, the Commission shall request for a Risk Assessment to check if RPAs are appropriate to protect human health.*

- EFSA *Guidance on methodological principles and scientific methods to be taken into account when establishing RPAs for non-allowed pharmacologically active substances present in food of animal origin* EFSA Journal 2013;11(4):3195

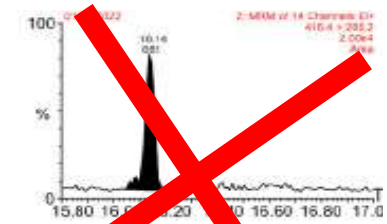
PERSPECTIVES & CONCLUSION

6. PERPECTIVES

6.1 New challenges

DESIGNER DRUGS

CLASSICAL APPROACH



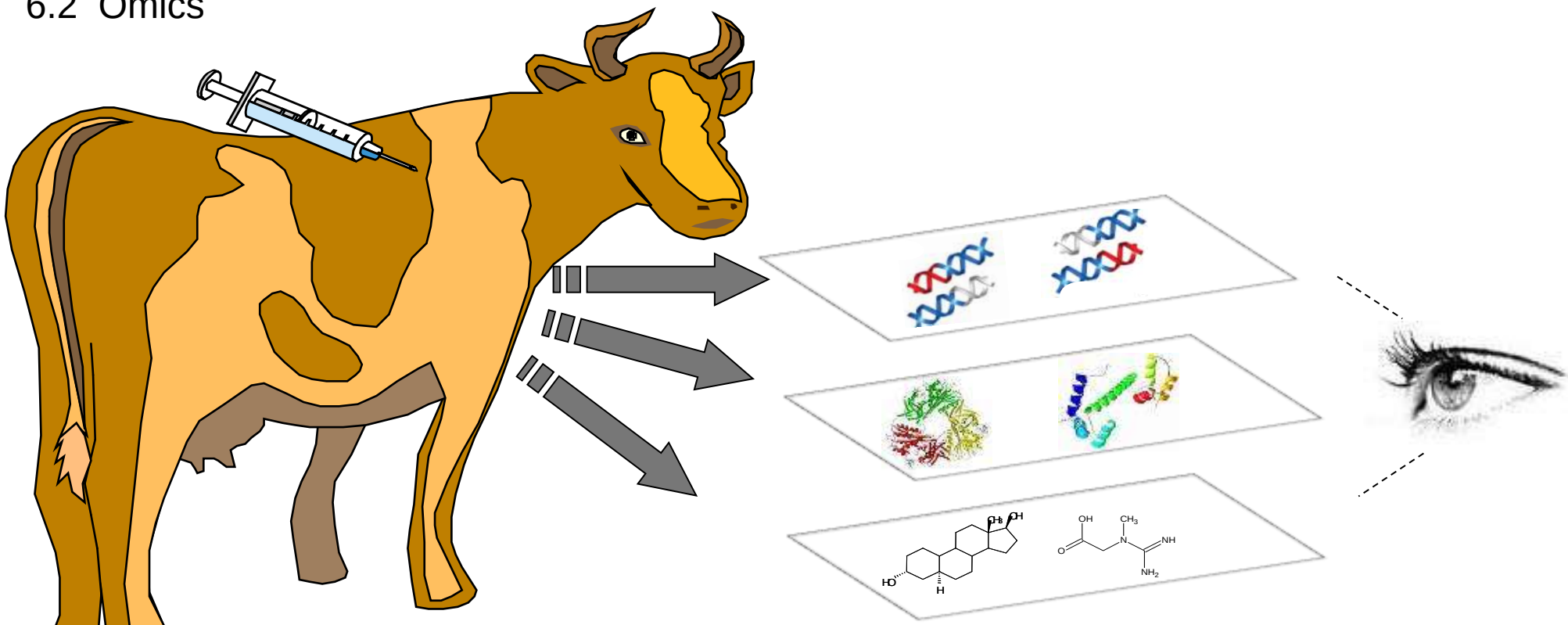
~~TARGETED MEASUREMENTS~~

EMERGING STRATEGIES

EFFECT BASED MEASUREMENTS

6. PERPECTIVES

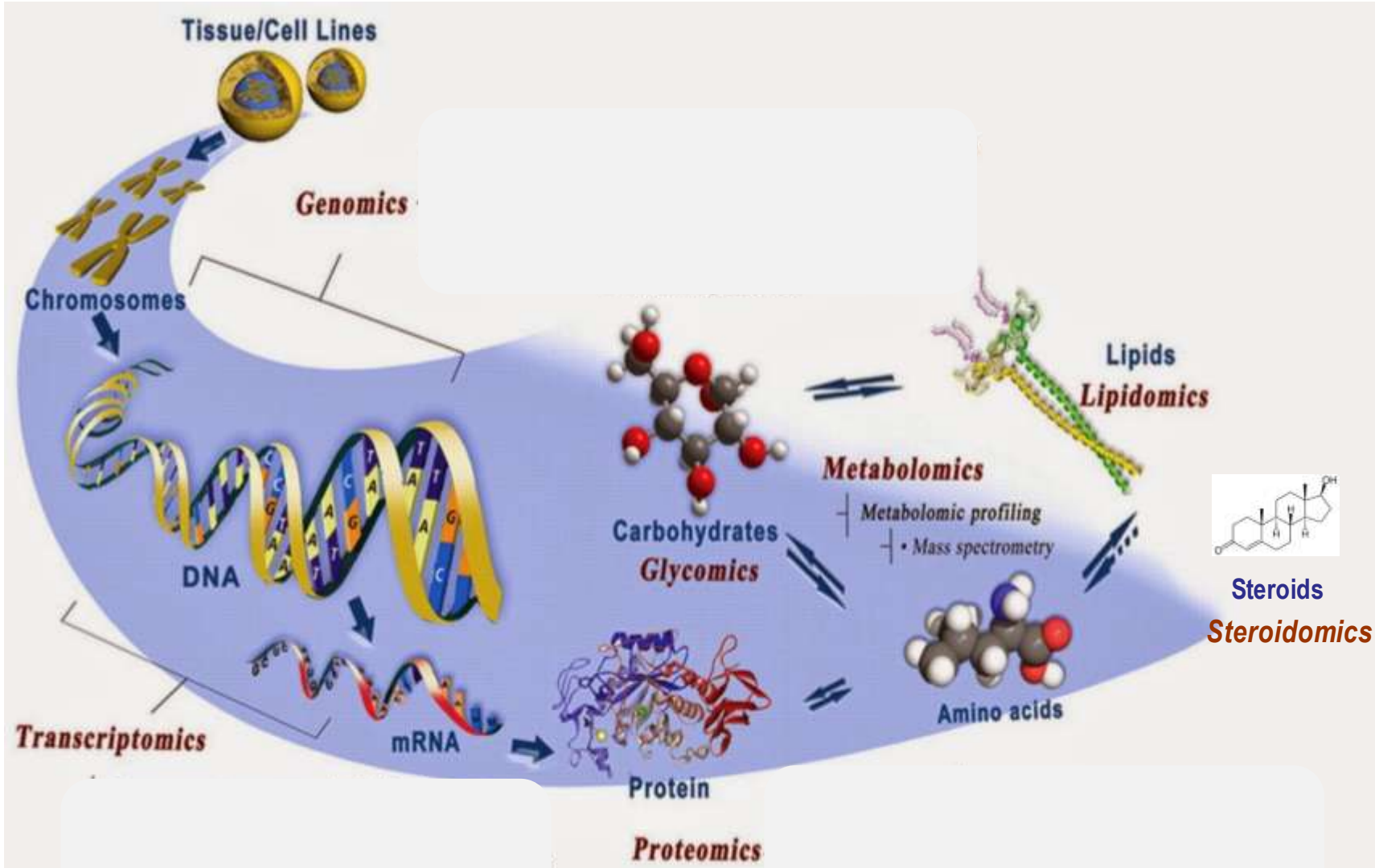
6.2 Omics



≠ LEVELS OF INVESTIGATION
SIMILAR OBJECTIVES

6. PERPECTIVES

6.2 Omics



6. CONCLUSIONS

6.3 Conclusions and remarks

Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation — Nantes Atlantique
Santé et alimentation au cœur de la vie

EuroResidue VIII
Conference on Residues of Veterinary Drugs in Food

PRE-CONFERENCE WORKSHOP

RESIDUE ANALYSIS FOR DUMMIES

Egmond aan Zee, The Netherlands, 23-25 May 2016

Bruno LE BIZEC,
Gaud DERVILLY-PINEL

Laboratoire d'Étude des Résidus et Contaminants dans les Aliments
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SARAF School for Advanced Residue Analysis in Food
LABERCA