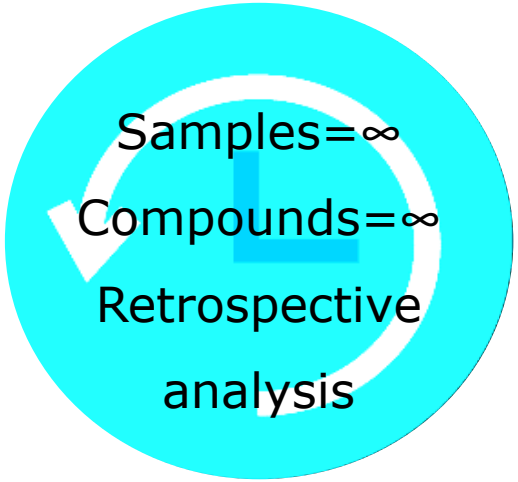
EU analytical performance criteria: time for a revision!

AOAC Annual Meeting, Denver, Colorado, US. 9 October 2019

L.A. van Ginkel, M.H. Blokland and S.S. Sterk



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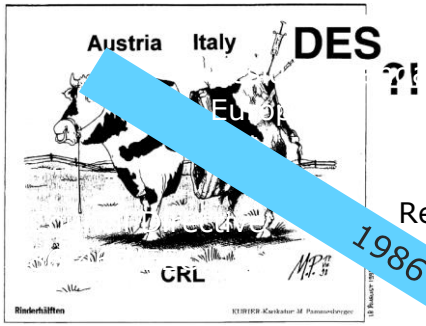
100years
1918 — 2018

European
Union
Reference
Laboratory

The European story in one slide.



1986

Reference Laboratories for residues.

2002

Confirmation and validation criteria CD 2002/657/EC

2004

General Foodlaw 2002
Control regulation No 882/2004

2014

EFSA: Risk-based sampling strategies and analyses

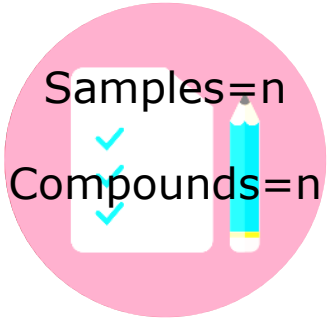
2017

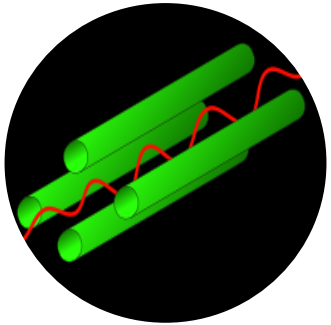
New "all in one" control regulation 2017/625

2020

SANTE 11188-2018 Rev0. To replace CD 2002/657/EC

Traditional routine monitoring for residues and contaminants





MS based screening

LC-MS/MS
LC-Q-Trap-MS
LC-Q-ToF-MS
LC-Orbitrap-MS

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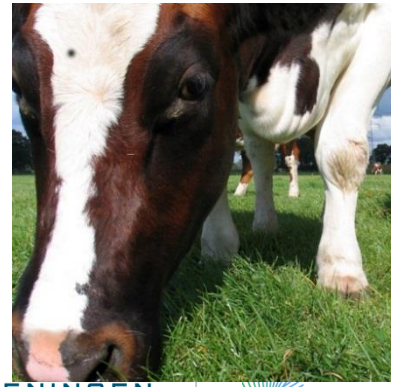
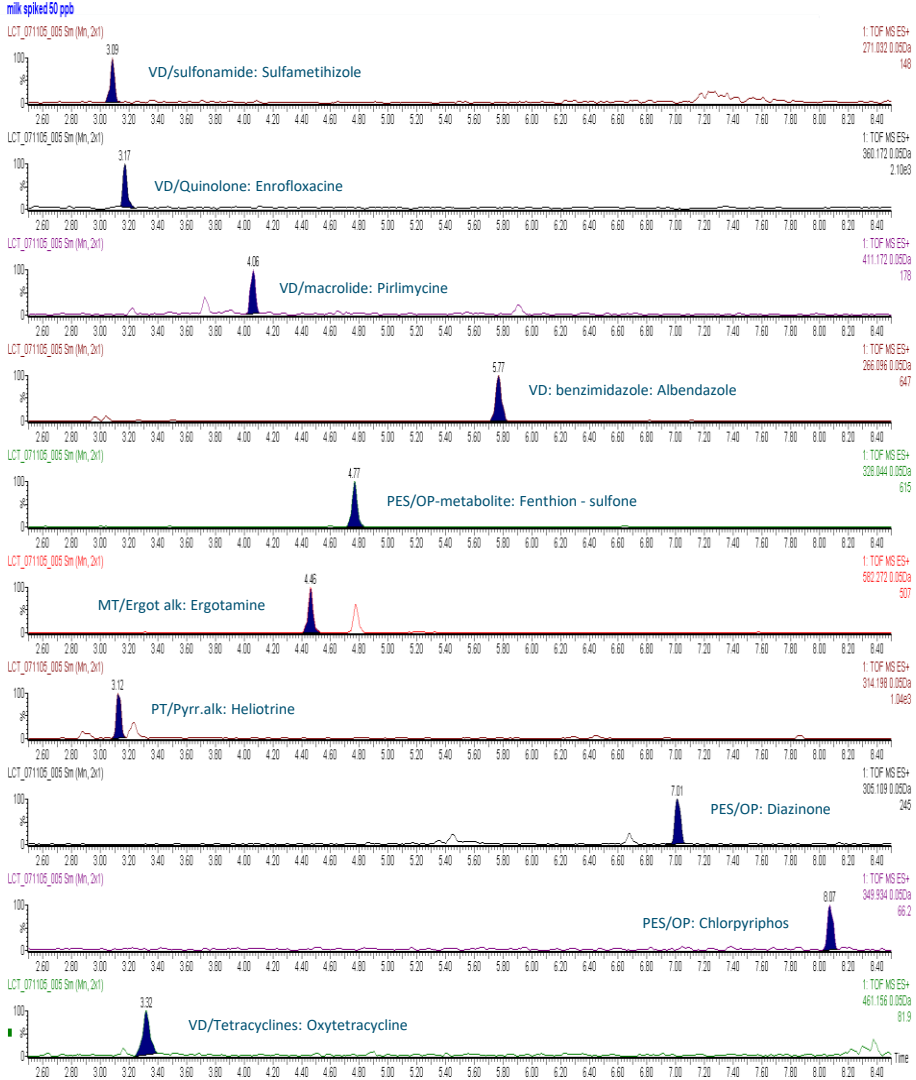
Multi class screening

- Milk fortified with
  - 170 pesticides,
  - mycotoxins,
  - plant toxins +
  - 100 veterinary drugs
- 50 µg/kg for each analyte
- LC-full scan-MS





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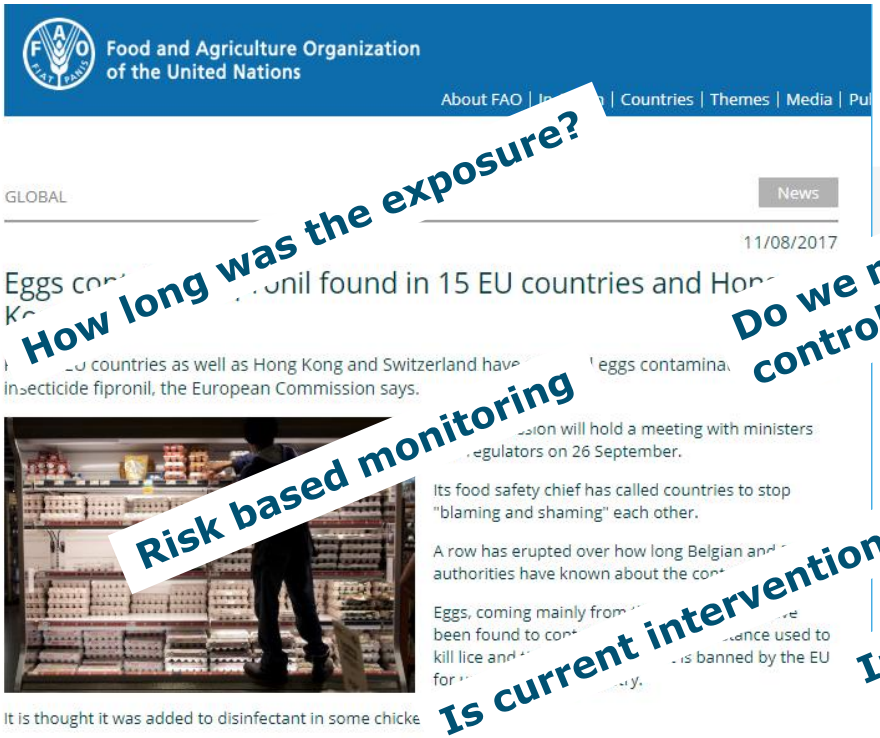
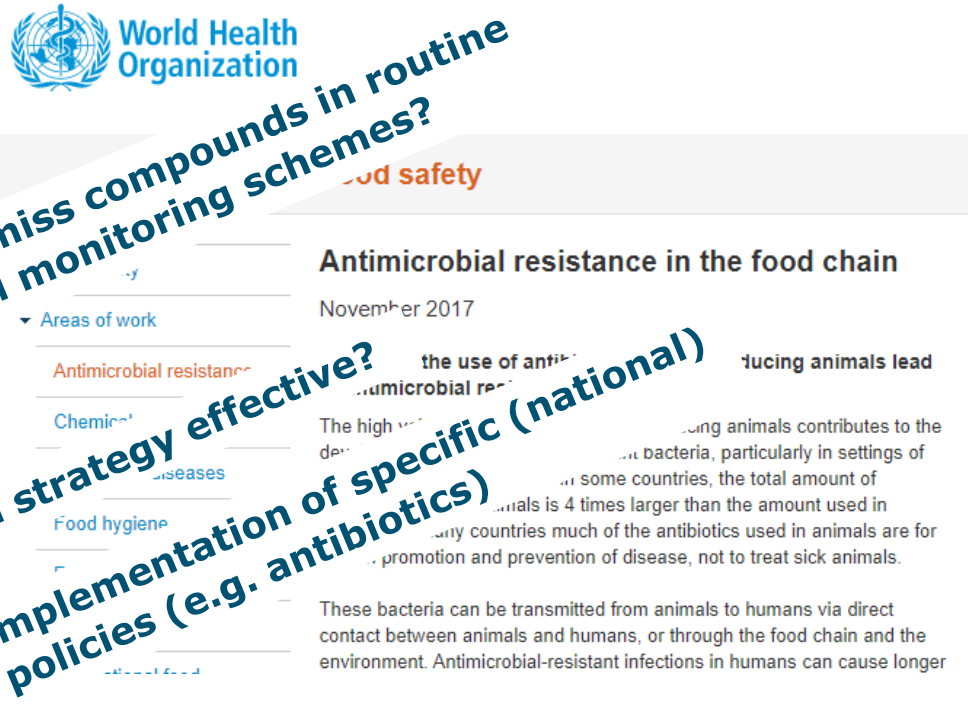
100years
1918 — 2018

Food safety challenges in the past years





How long was the exposure?

Risk based monitoring

Do we miss compounds in routine control monitoring schemes?

Is current intervention strategy effective?

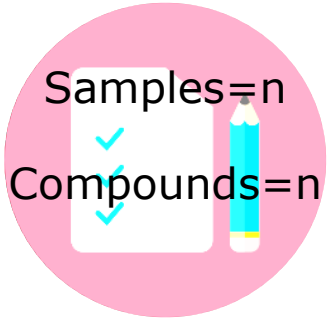
Implementation of specific (national) policies (e.g. antibiotics)

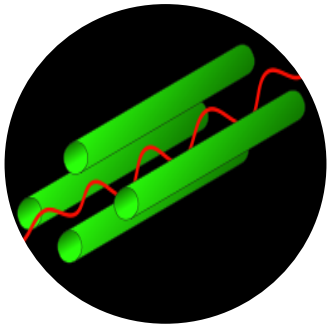
Collecting early signals indicating possible new food safety Risks

Collecting early signals indicating possible new food safety Risks

- Early warning systems based on ”Big data” analyses and “machine learning”
- Signals from food producers
- Effect assays such as ERA, RAA etc
- Untargeted chemical screening with smart dataprocessing

Traditional routine monitoring for residues and contaminants





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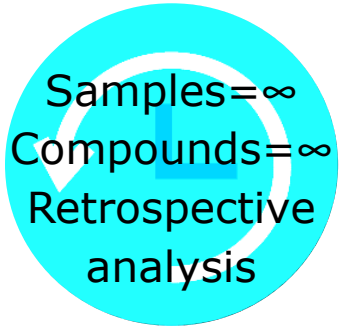
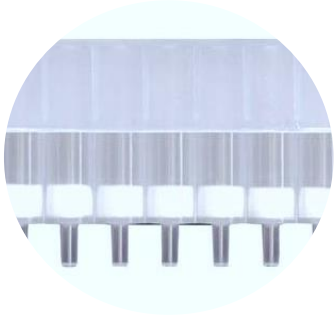
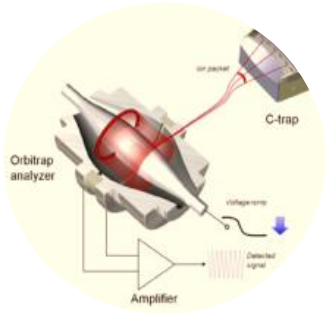
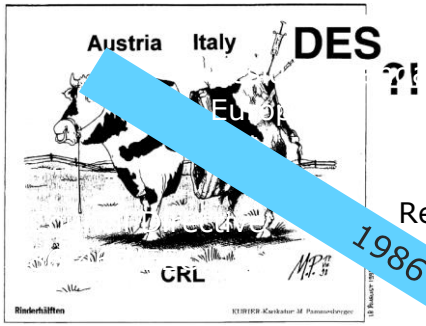
New approach to answer new questions, need new criteria







The European story in one slide.



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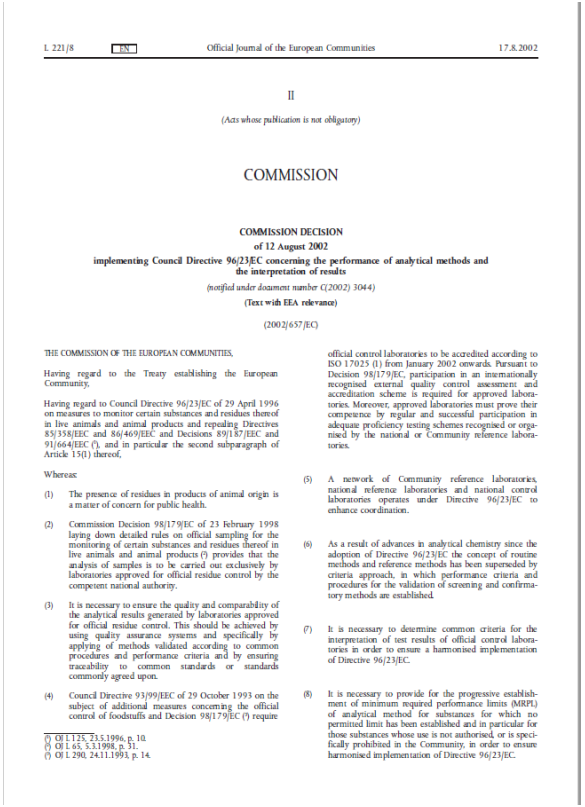
EU analytical performance criteria: time for a revision!



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

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)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC (1), and in particular the second subparagraph of Article 15(1) thereof,

Whereas:

(1) The presence of residues in products of animal origin is a matter of concern for public health.

(2) Commission Decision 98/179/EC of 23 February 1998 laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products (2) provides that the analysis of samples is to be carried out exclusively by laboratories approved for official residue control by the competent national authority.

(3) It is necessary to ensure the quality and comparability of the analytical results generated by laboratories approved for official residue control. This should be achieved by using quality assurance systems and specifically by applying of methods validated according to common procedures and performance criteria and by ensuring traceability to common standards or standards commonly agreed upon.

(4) Council Directive 93/99/EEC of 29 October 1993 on the subject of additional measures concerning the official control of foodstuffs and Decision 98/179/EC (3) require official control laboratories to be accredited according to ISO 17025 (1) from January 2002 onwards. Pursuant to Decision 98/179/EC, participation in an internationally recognised external quality control assessment and accreditation scheme is required for approved laboratories. Moreover, approved laboratories must prove their competence by regular and successful participation in adequate proficiency testing schemes recognised or organised by the national or Community reference laboratories.

(5) A network of Community reference laboratories, national reference laboratories and national control laboratories operates under Directive 96/23/EC to enhance coordination.

(6) As a result of advances in analytical chemistry since the adoption of Directive 96/23/EC the concept of routine methods and reference methods has been superseded by criteria approach, in which performance criteria and procedures for the validation of screening and confirmatory methods are established.

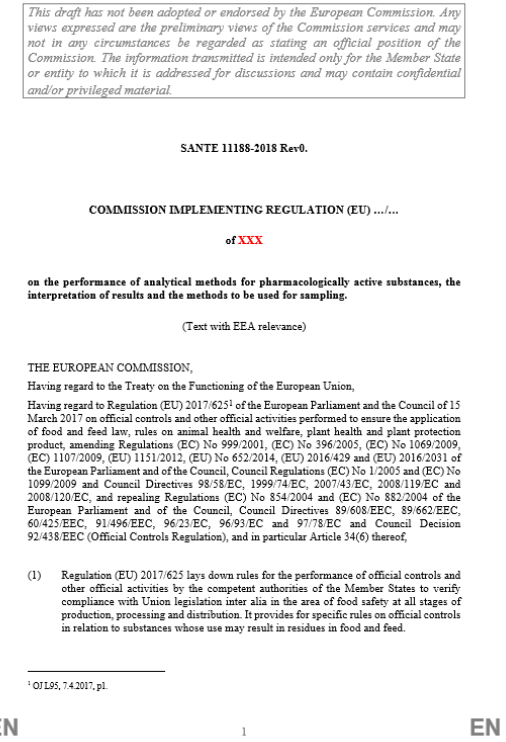
(7) It is necessary to determine common criteria for the interpretation of test results of official control laboratories in order to ensure a harmonised implementation of Directive 96/23/EC.

(8) It is necessary to provide for the progressive establishment of minimum required performance limits (MRPL) of analytical method for substances for which no permitted limit has been established and in particular for those substances whose use is not authorised, or is specifically prohibited in the Community, in order to ensure harmonised implementation of Directive 96/23/EC.

(1) OJ L 125, 23.5.1996, p. 10.
(2) OJ L 65, 5.3.1998, p. 31.
(3) OJ L 290, 24.11.1993, p. 14.

Revision should:

- Be more science based
- Reflect technical progress
- Include the lessons learned



This draft has not been adopted or endorsed by the European Commission. Any views expressed are the preliminary views of the Commission services and may not in any circumstances be regarded as stating an official position of the Commission. The information transmitted is intended only for the Member State or entity to which it is addressed for discussions and may contain confidential and/or privileged material.

SANTE 11188-2018 Rev0.

COMMISSION IMPLEMENTING REGULATION (EU) …/…

of XXX

on the performance of analytical methods for pharmacologically active substances, the interpretation of results and the methods to be used for sampling.

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) 2017/625[1] of the European Parliament and the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection product, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) 1107/2009, (EU) 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 60/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation), and in particular Article 34(6) thereof,

(1) Regulation (EU) 2017/625 lays down rules for the performance of official controls and other official activities by the competent authorities of the Member States to verify compliance with Union legislation inter alia in the area of food safety at all stages of production, processing and distribution. It provides for specific rules on official controls in relation to substances whose use may result in residues in food and feed.

[1] OJ L95, 7.4.2017, p1.

EN 1 EN

New performance criteria should provide

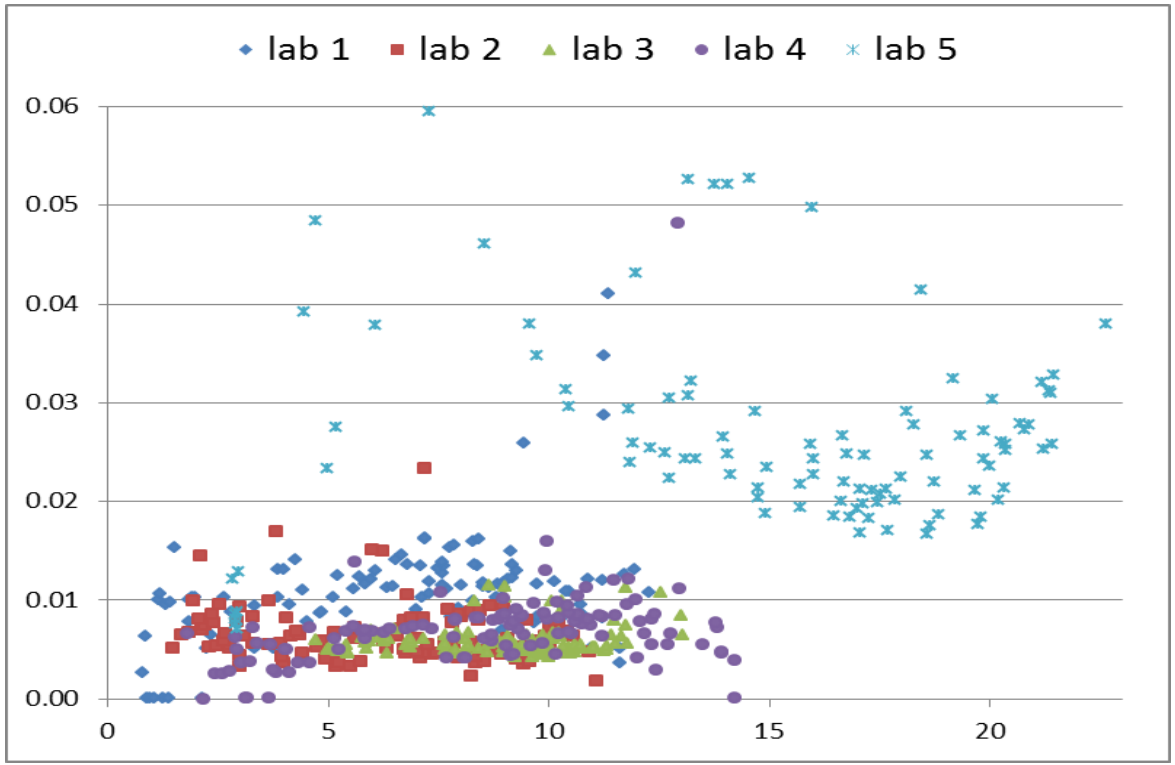
- Updated (science based) criteria for screening and confirmation
    - Retention time (Screening and confirmation)
    - Detection of screening ion (Screening)
    - Detection of multiple (fragment)ions (Confirmation)
        - Identification points
- Guidelines for validation as screening method (either qualitative or quantitative)
    - CCα ad CCß
    - False negative rate

Revision: Retention times

- Almost all current guidelines have a relative requirement (e.g. 2.5%) for the maximum deviation of the Rt between sample and standard

- Using gradient elution, empirical studies (Mol et al. Berendsen et al.) show deviation is absolute over the whole retention time range

Revision: Retention times



From: Mol et al: Analytica Chimica Acta, 2015, *873*, 1-13

Revision: Retention times

Proposal for the retention time criteria

- Use an absolute criterion instead of relative
- Twice the retention time corresponding to the void volume of the column
- Absolute retention time criterium of ±0.1 min
- In case fast chromatography is used <5% in case the retention time is below 1 minute

Mol et al: Analytica Chimica Acta, 2015, *873*, 1-13
Berendsen et al: Drug Testing and Analysis, 2016, *8*,477-90

MSMS analysis using hrMS

"MSMS" acquisition modes

Data Depended	one precursor	most specific
Data Independed	Considered as full-scan hrMS techniques in SANTE 11188-2018	
All Ion Fragmentation	all precursors	not specific

Proposal: full scan hrMS SANTE 11188-2018

- High-resolution mass spectrometry (HRMS), including e.g. double sectors, Time of Flight (TOF) and Orbitrap instruments are appropriate

- In high-resolution mass spectrometry (HRMS), the resolution shall typically be greater than 10,000 for the entire mass range at 10 % valley or 20,000 at full width at half maximum (FHWM)

- The mass deviation of all diagnostic ions should be below 5 ppm (or in case of m/z < 200 below 1 mDa).

Additional criteria’s

- Full scan and SIM (both LRMS and HRMS):
  - When mass spectrometric determination is performed by the recording of full scan spectra, only diagnostic ions with a relative intensity of more than 10 % in the reference spectrum of the calibration standard or MMS are suitable.
  - Adducts and isotopes of selected diagnostic ions are excluded.
  - In case the precursor selection in MSMS has a mass selection window of more than one Dalton (e.g. in case of Data Independent Acquisition) the technique is considered as full-scan confirmatory analysis.

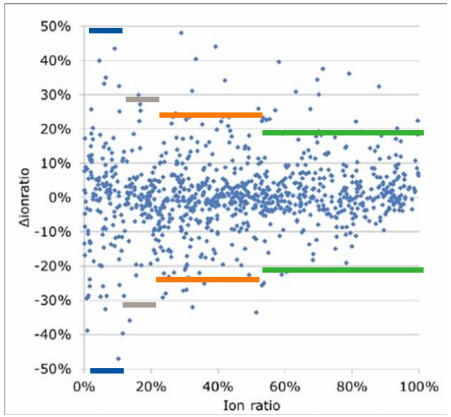
LC-MS/MS ion ratio's

- Requirements for MS/MS confirmatory analysis are often based on the requirements from CD 2002/657.
- In CD 2002/657 they were based on expert opinion; at that time no scientific data available
- Experimental data is now available

Mol et al: Analytica Chimica Acta, 2015, *873*, 1-13
Berendsen et al: Drug Testing and Analysis, 2016, *8*,477-90

LC-MS/MS ion ratio’s

- CD 657/2002 allows a deviation based on the ratio in the reference standards

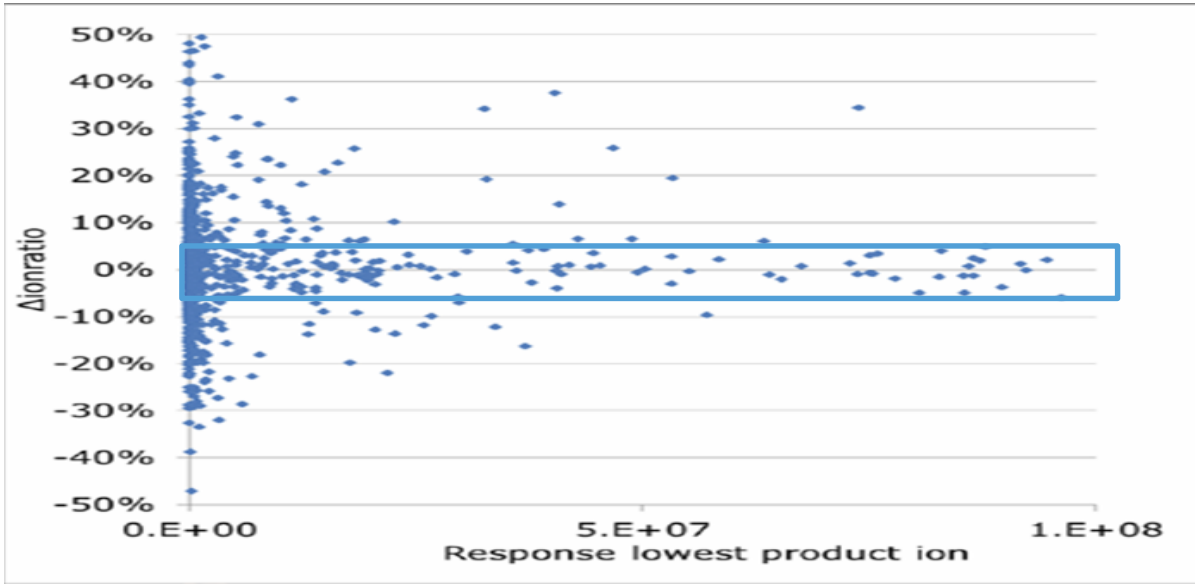


Relative intensities	Allowed Dev. [rel]
>50 %	± 20 %
>20 % to 50 %	± 25 %
>10 % to 20 %	± 30 %
<10 %	± 50%

From: Berendsen et al: Drug Testing and Analysis 2016, *8*, 477-490

LC-MS/MS ion ratio’s

- Deviations depend on the intensity of the less abundant ion



From: Berendsen et al: Drug Testing and Analysis 2016, *8*, 477-490

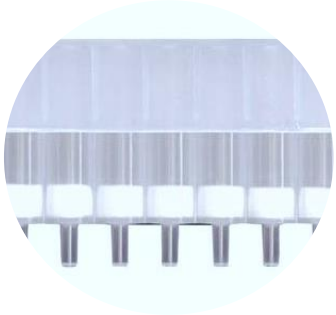
LC-MS/MS ion ratio's

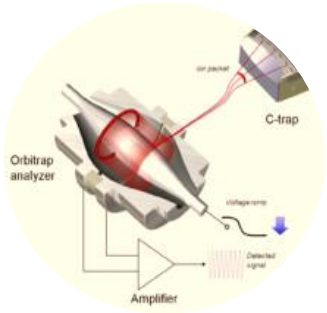
- Diagnostic ions shall include the molecular ion if present at ≥10% intensity of the base peak and characteristic fragment or product ions
- When mass spectrometric determination is performed by fragmentation after precursor ion selection, precursor ion selection is carried out at unit mass resolution or better.
- The selected precursor ion should be the molecular ion, characteristic adducts of the molecular ion, characteristic product ions or one of their isotope ions.
- Maximum allowed deviation a compromise between the false positive and false negative rate
    - New guideline proposed ± 30 % (relative deviation)

Why focus on hrMS?

New approach to answer new questions, need new criteria

Samples=∞
Compounds=∞
Retrospective analysis



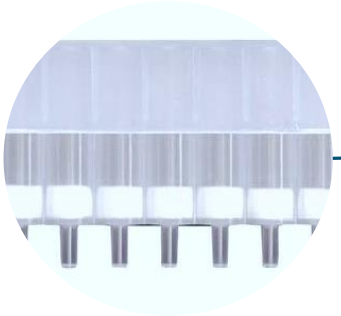


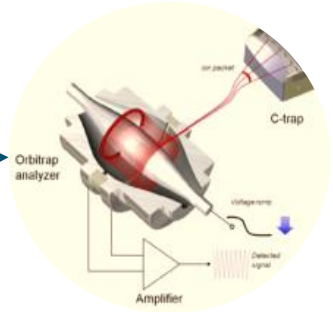
Why focus on hrMS

- Advantages of hrMS coupled to LC (and now also GC) techniques:
  - Easier to expand/maintain methods with more compounds
  - Possibility to do a retrospective search
  - Higher resolving powers
  - Better mass accuracy
  - More stability of the systems
  - Use of profiles / fingerprints instead of targeted to one compound
- Guidelines / Regulations need to catch up

Complete workflow of screening analysis

Samples=∞
Compounds=∞
Retrospective analysis





Data stored and compressed using MetAlign

Targeted data-procesing using list of knowns

Data mining using advanced statistic tools

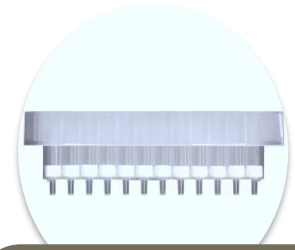
Retrospective analysis

Generic (untargeted) hrMS screening for urine metabolites

Urine

- 500µl
- 1:1 buffer pH 6.8
- Isotope labelled IS

- Bovine urines
- Human urines
- Collected in the past/present and also in the future



96 wells SPE

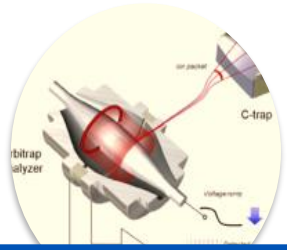
- Wash 200µl H_20
- Elution 90% ACN
- Final volume 100µl

- Generic
- High throughput

LC BEH C_{18}

- T=30 min
- Acid run
- Alkaline run

- Stable chromatography over the years
- Optimal LC conditions for most compounds



hrMS

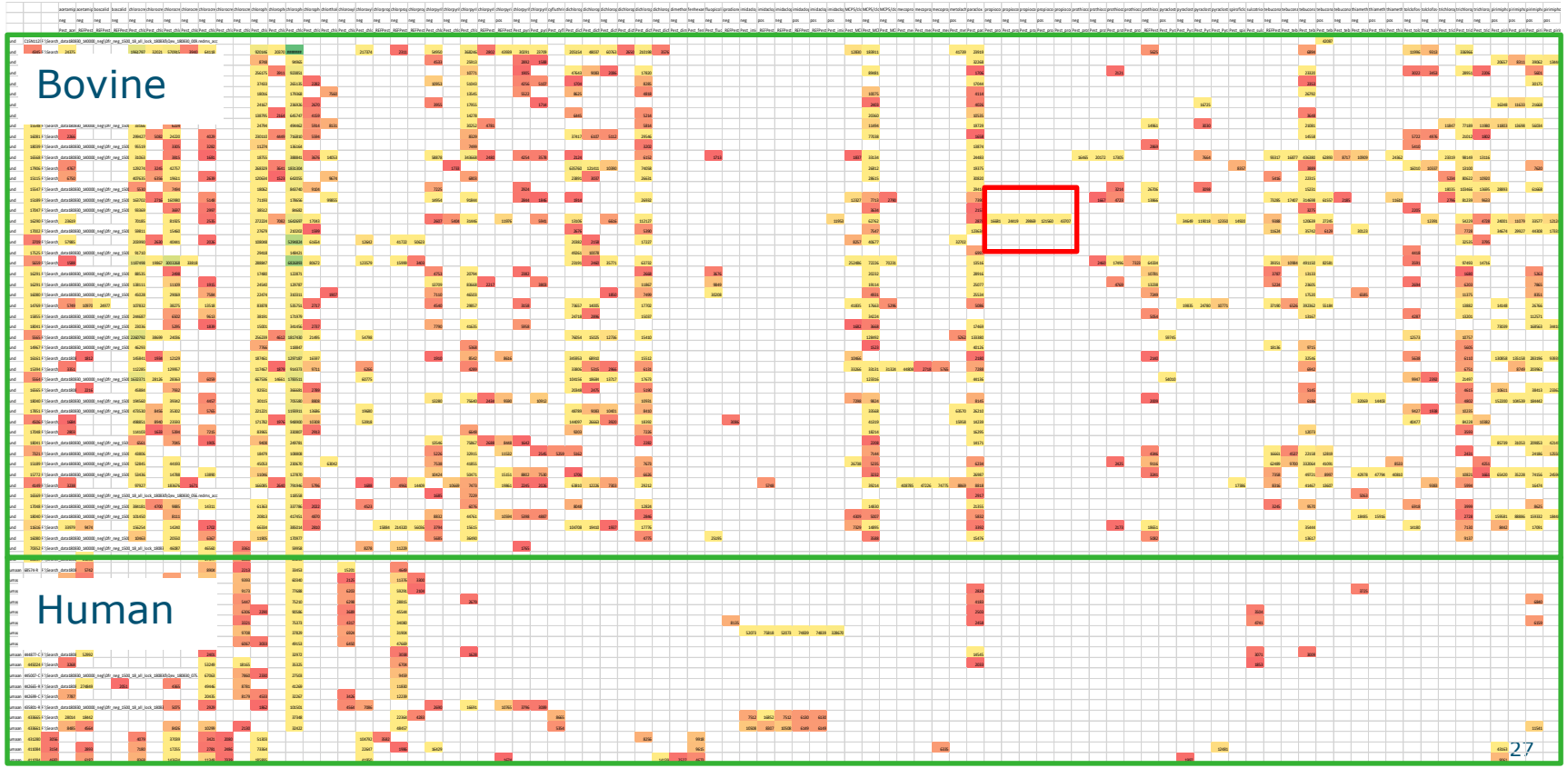
- R=140000
- Positive mode
- Negative mode

- Obtain high quality MS data
- Enormous amount of data

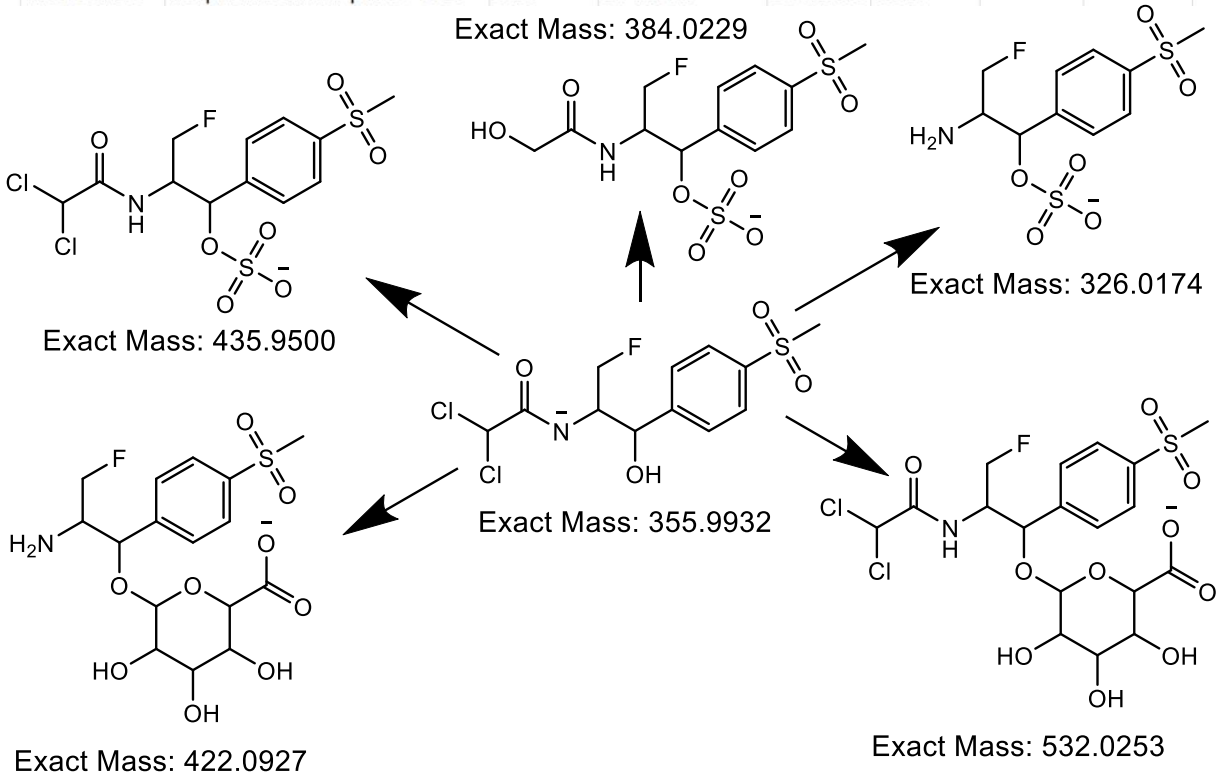
Examples

- Hundreds of urine samples from 2014 – 2019
- Analysis performed in negative and positive ionization mode
- Total 386 urine samples x2 (pos and neg)
  - 194 GB reduced to 1.1 GB
- Subsets created
- Parent and metabolites detected
- Confirmatory analysis

Subsets, example Cl-containing compounds



	A	B	C	D	E	F	G	H	I	J	K
1	sample ID	max value		Retention	Quantifier Mass	Mass1	Intensity1	Mass2	Intensity2	Mass3	Intensity3
2	14967	308603584	florphenicol	8.00	355.9932	355.9932	1000	357.9902	640	359.9873	100
3	14967	2918788.75	florphenicol>C6H8O6	6.43	532.0253	532.0253	1000	534.0223	640	536.0194	100
4	14967	358278	florphenicol>SO3	6.57	435.9500	435.9500	1000	437.9470	640	439.9441	100
5	14967	148892.453	florphenicol#nC2Cl2O>SO3	4.06	326.0174	326.0174	1000				
6	14967	200822.828	florphenicol#nC2Cl2O>C6H8O6	3.95	422.0927	422.0927	1000				
7	14967	471660.438	florphenicol#nCl2pH2O>SO3	5.06	384.0229	384.0229	1000				



Conclusions

- When finalized, SANTE 11188-2018 will provide an updated and science based revision of CD 2002/657 on method performance criteria. (2002 > 2020)
- Broad and untargeted screening will replace current multi-analyte and multi-class analytical methods.
- Updated guidelines for the validation of screening methods, focussing on avoidance of false compliant results, are under preparation by EURLs “Berlin” and “Fougieres”.


Thank
you!