New way to reliably screen and quantify different chemical compounds in aerosol particles and wastewater effluents

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- Introduction to different data structures
- Datahandling methods: identification
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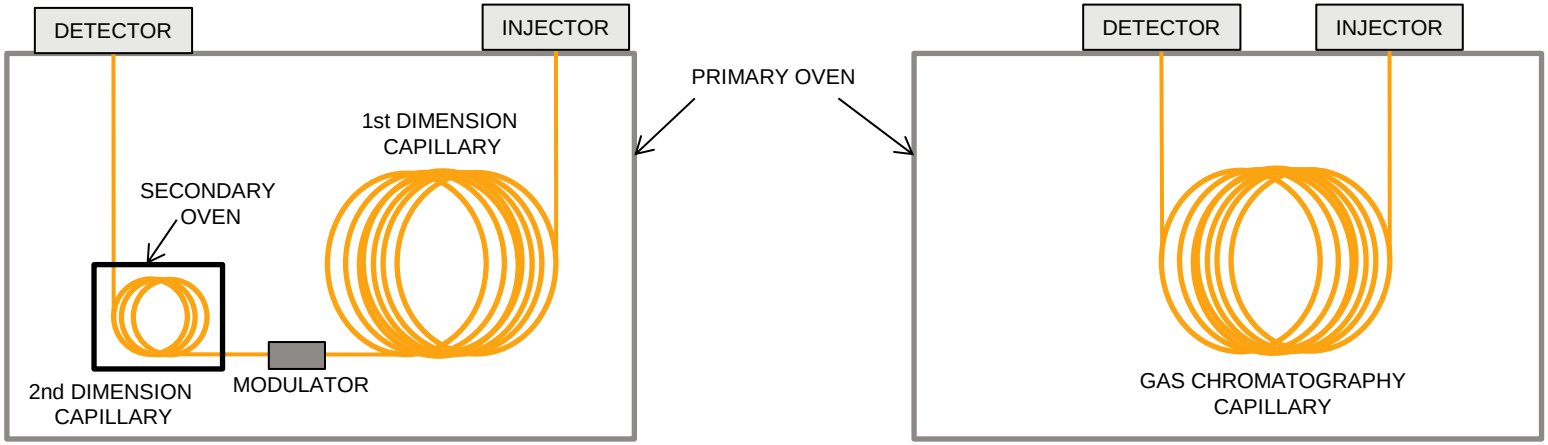
- Overview of our studies
- Methods to improve reliability of identification in our studies
- Quantification in our studies
- Summary

Introduction to Instrumentation

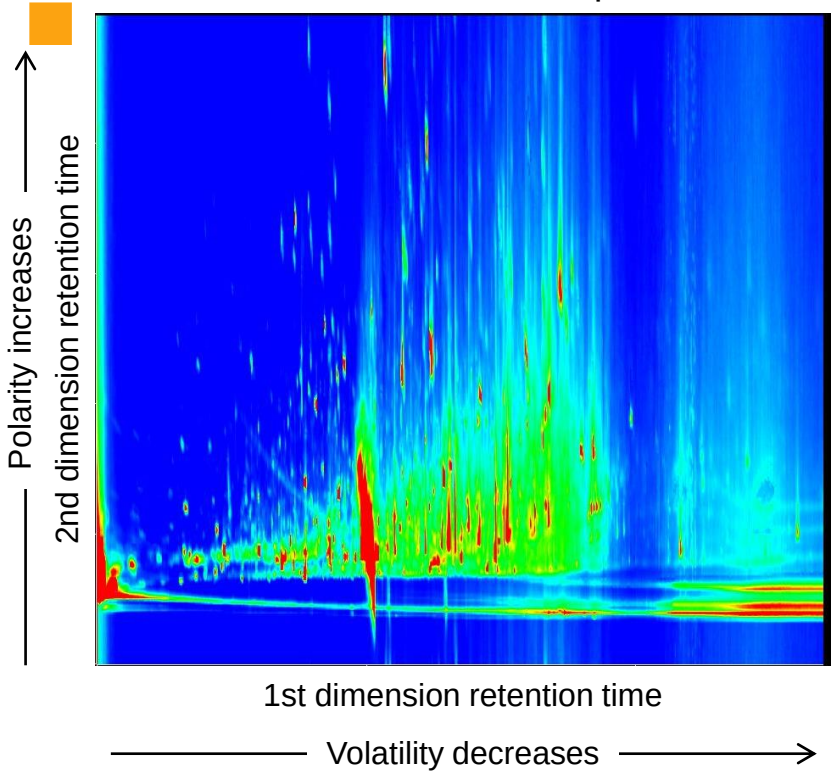


Comprehensive two-dimensional gas chromatography (GCxGC)

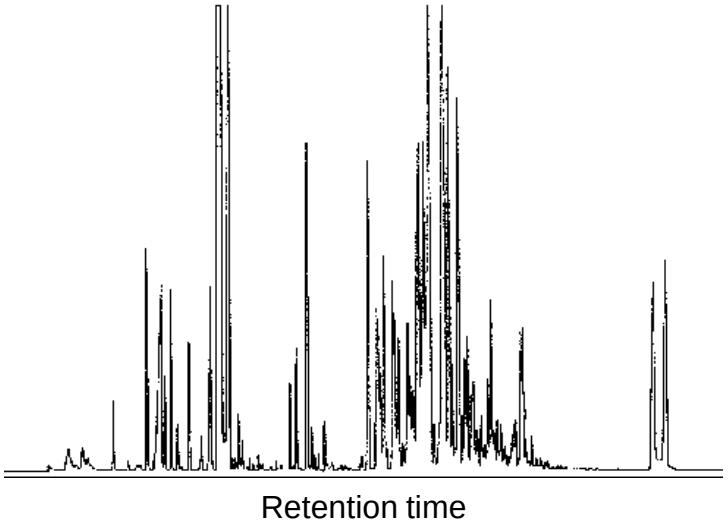
Gas chromatography (GC)

Introduction to Instrumentation

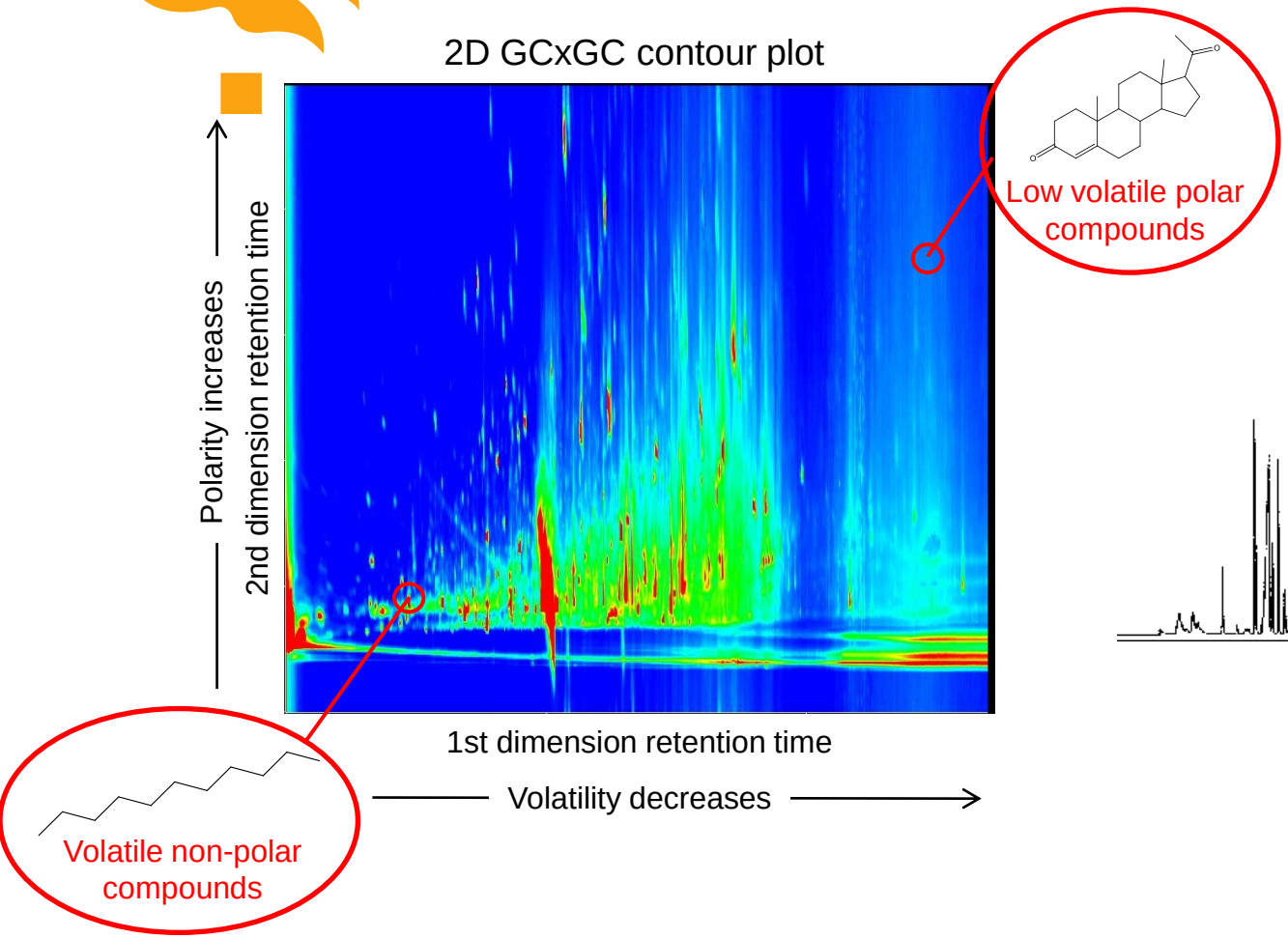
2D GCxGC contour plot

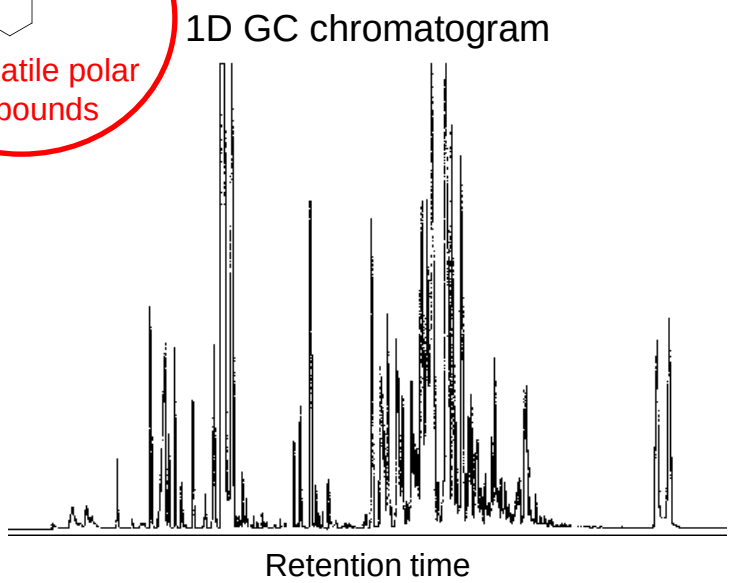


1D GC chromatogram



Introduction to Instrumentation





Introduction to Instrumentation

GCxGC versus GC

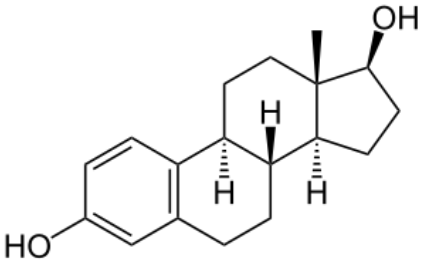
- Optimal for screening studies
- Higher peak capacity = higher quality mass spectra
- Structural patterns
- ”Fingerprinting”
- Less sample preparation

- Fast detector required
- Large datafiles
- More complex optimization
- Expensive

Introduction to Instrumentation

MS
Mass spectrometer
Nominal mass

Estradiol



HRMS
High resolution mass spectrometer
Accurate mass (< 5 ppm)

Calculated nominal mass:
272
Calculated exact (monoisotopic) mass:
272.1776
Measured accurate (monoisotopic) mass:
272.1765
Mass accuracy: $\Delta m/m*10^6 = 0.0011 / 272.1776 *10^6 = 4$ ppm

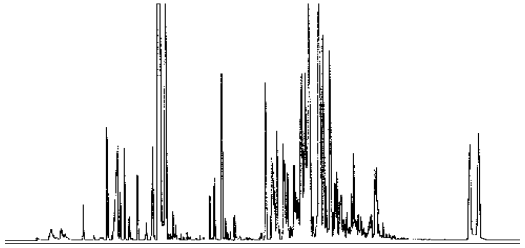
Introduction to Instrumentation

Commonly used criteria for tentative identification with nominal mass and accurate mass

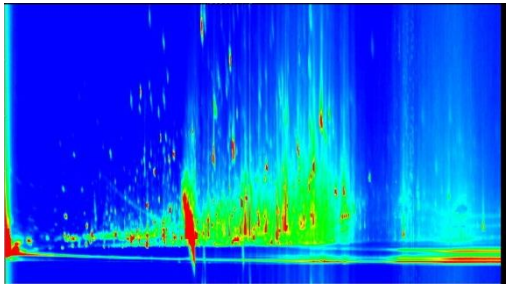
MS	HRMS
Spectral match between unknown compound and library hit > 700	Calculation of possible molecular formula from accurate mass
Difference between first and second library hit >100	Minimize the list by implementing mass difference Δm ±5 mDa
Complementary forward and reversed library search results	At least three matching ions with Δm ±5 mDa
Manual confirmation of ion ratios	Manual confirmation that fragmentation supports the structure

Introduction to data structures

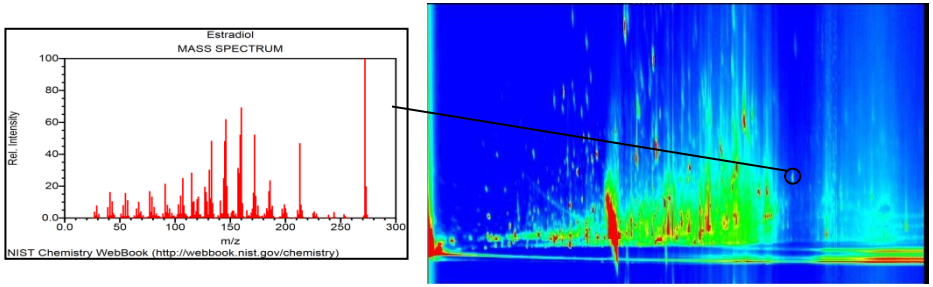
- First-order, linear 1D data (GC-FID or GC-MS-SIM)



- Second-order, bilinear 2D matrix (GCxGC-FID or GC-MS)



- Third-order, trilinear 3D cube (GCxGC-MS)



Introduction to data structures

- Peak defining = peak finding, determination of retention times, identification, integration of peak area

- High order data often unfolded to 1D-data -> peak finding must include the combination of multiple modulated peaks generated from a single first-dimension peak

- If high order data is not unfolded, contour plots can be used for peak finding and integration

- Chemometrics with GCxGC data:
  - Qualitative analysis by PCA (Principal Component Analysis) and ANOVA (ANalysis Of VAriance)
  - Quantitative analysis by PARAFAC (PARAllel FACtor analysis) and GRAM (Generalized Rank Annihilation Method)

Datahandling methods: identification

- First-order, 1D linear data (GC-FID):
  Identification by retention time
- Second order, 2D matrix data (GC-MS):
  Identification by retention time and mass spectral information
- Second order, 2D matrix data (GCxGC-FID):
  Identification by a pair of retention time coordinates
- Third-order, 3D cubic data (GCxGC-MS):
  Identification by a pair of retention time coordinates and mass spectral information
- Identification can be improved in GCxGC by chemical group type patterns in the 2D contour plot

Datahandling methods: quantification

- First-order, 1D linear data:

  - Quantification by peak area or height

- Second order and third-order data:

  - **First-order approach**: Quantification by combining peak areas of multiple modulated peaks. Accurate peak identification vital for reliable quantification.

  - **Second-order approach**: Quantification by measuring pixel intensity in the 2d contour plot

Overview of our studies on aerosols and wastewater

- Screening (identification / classification) and semi-quantitation of semivolatile organic compounds in aerosol particles (30 and 50 nm particles)

- Samples collected to filters in SMEARII-station (Hyytiälä, Finland)

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- Ultrasound-assisted extraction from filters into organic solvent

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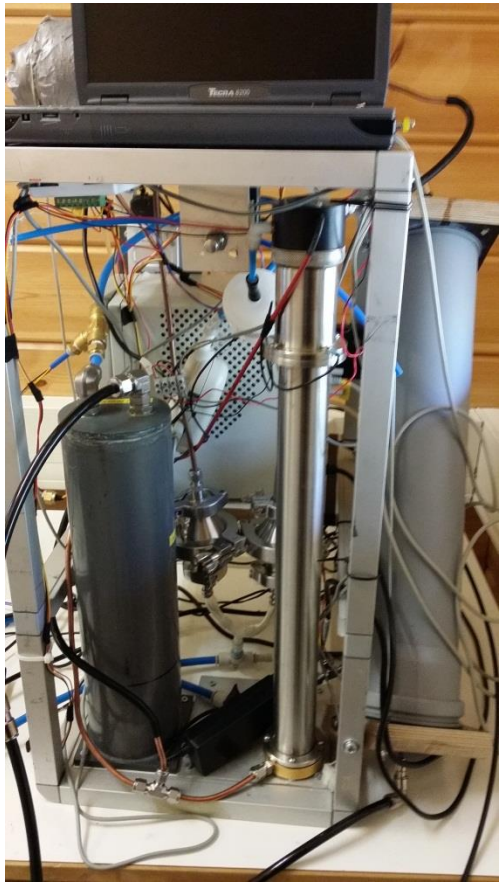
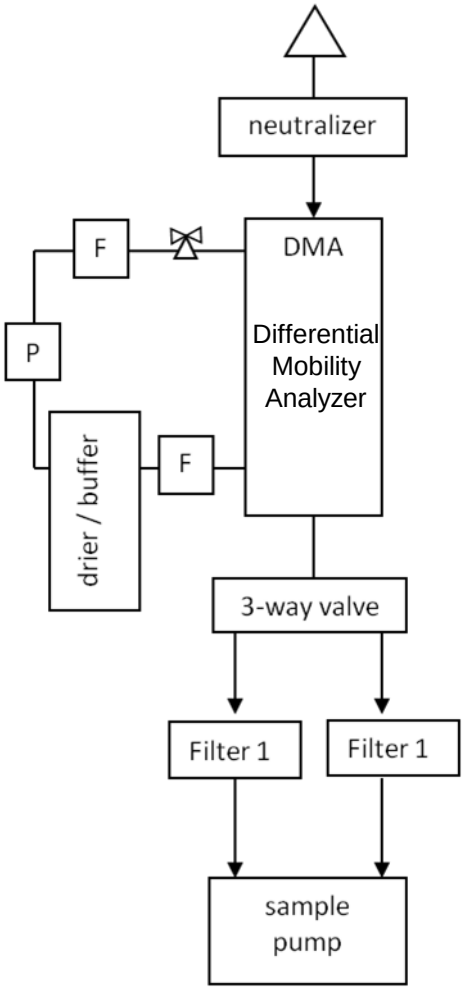
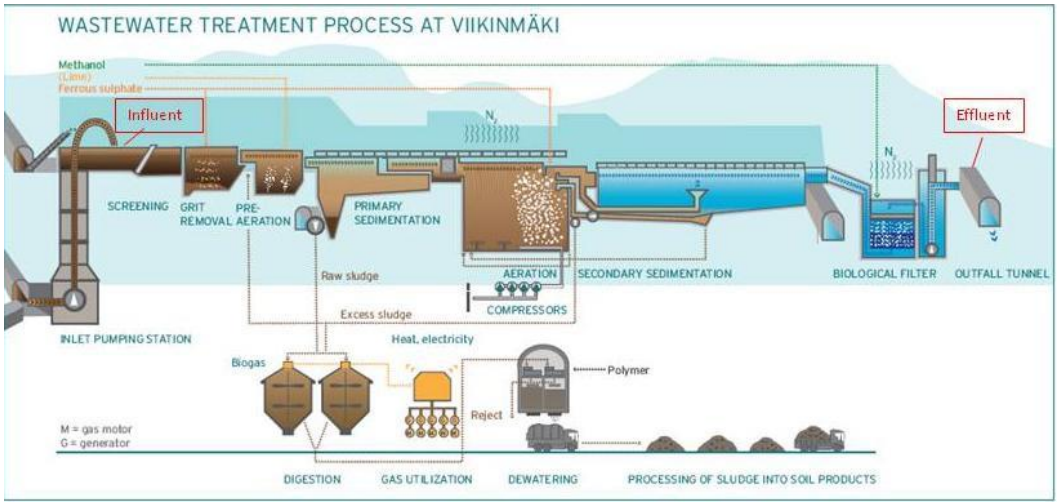
- Derivatization and GCxGC-TOFMS analysis





Overview of our studies on aerosols and wastewater



(http://www.hsy.fi/vesi/Documents/J%C3%A4tevedenpuhdistus/Wastewater_treatment_process_Viikinmaki.pdf

- Screening and quantitation of steroidal compounds in wastewater and suspended particles
- Samples collected from influent and effluent wastewater (Helsinki, Finland)

↓

- Ultrasound-assisted extraction of suspended particles, solid-phase extraction of wastewater

↓

- Derivatization and GCxGC-TOFMS analysis

↗

- Identification of steroidal compounds with two mass spectral libraries and retention indices
- Quantification with a chemometric model

Methods to improve reliability of identification in our studies

- Identification criteria in **NIST**:
  - S/N >10
  - peak area > 1000
  - minimum fits were fixed at 850 and 750 for the reverse and normal search algorithms, respectively. Both methods should give same results for identified compounds.
  - Kovats retention index (*I*) difference < 200:

$$I = 100 \times \left[\mathrm{n} + (\mathrm{N} - \mathrm{n}) \frac{\mathrm{t_{r(unknown)}} - \mathrm{t_{r(n)}}}{\mathrm{t_{r(N)}} - \mathrm{t_{r(n)}}} \right]$$

$$\mathrm{n} = \text{carbons in the smaller } n\text{–alkane}$$
$$\mathrm{N} = \text{carbons in the larger } n\text{-alkane}$$

- compounds outside these limits were put to **GOLM**-database:
  - search by mass spectrum and retention index

Methods to improve reliability of identification in our studies

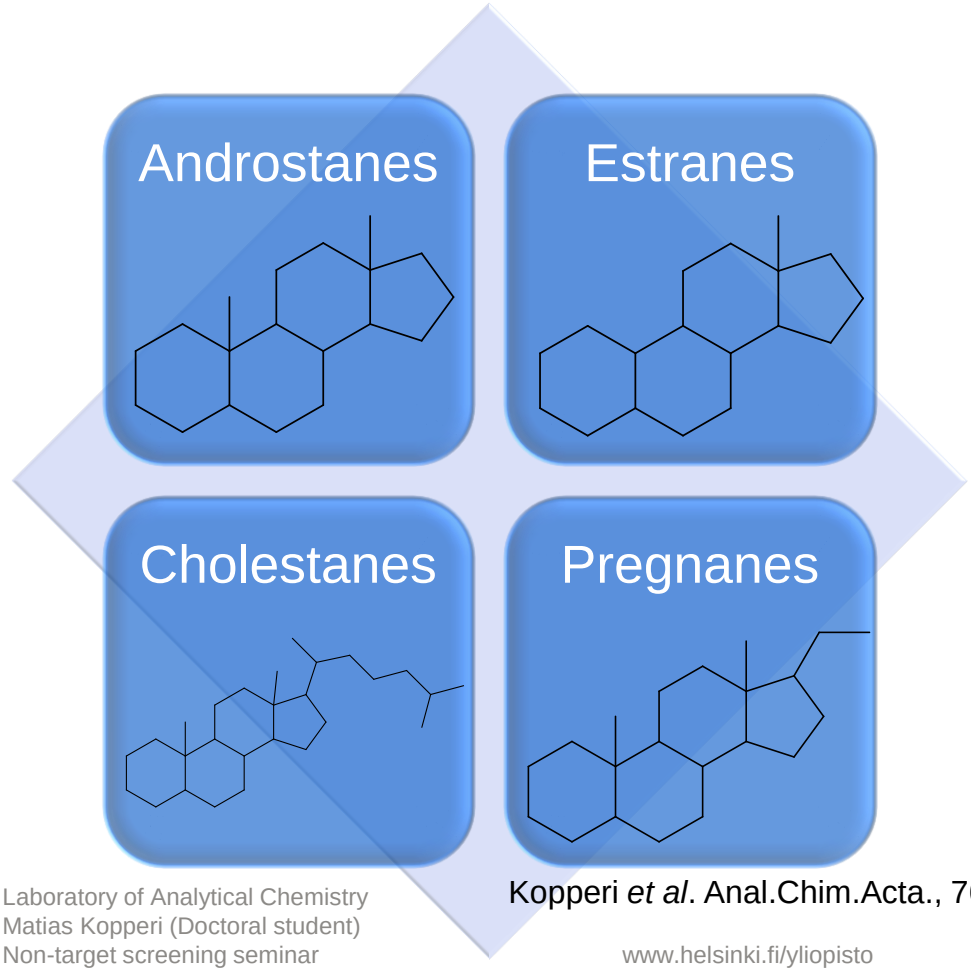
Classification of aerosol compounds:

Hydrocarbons	Halogenated compounds	Nitrogen compounds	Sulfur compounds	Carboxyl compounds	Hydroxyl compounds	Carbonyl compounds
•Alkanes •Alkenes •Cyclic hydrocarbons •Aromatic hydrocarbons	•Fluorinated compounds •Chlorinated compouds •Brominated compounds •Iodinated compounds	•Amino acids •Nitros •Amines and amides •Nitriles •Imides •Urea derivatives •Amino acids-N-derivatives •Glycosamines •Heterocyclics	•Sulfonamides •Mercapto compounds •Sulfonic acid derivatives •Thio compounds	•Free acids •Hydroxy acids •Keto acids •Esters •Anhydrides	•Alcohols •Polyols •Ethers	•Aldehydes •Ketones

Ruiz-Jiménez *et al*. J. Environ. Monit., 13 (2011) 2994-3003

Methods to improve reliability of identification in our studies

Classification of steroids in wastewater:



Kopperi *et al*. Anal.Chim.Acta., 761 (2013) 217-226

Quantification in our studies

- Reliable quantitation of a compound requires a calibration curve made by dilutions of the standard of the compound in question.
- Non-target screening analysis might have hundreds of identified compounds, which require quantification

→

It is expensive or even impossible to purchase reference materials for the reliable quantitation of all identified compounds

Quantification in our studies

Quantification without multiple reference materials?
Option 1:

<u>Surrogate approach (TIC)</u>

Calibration curve made with a single standard compound

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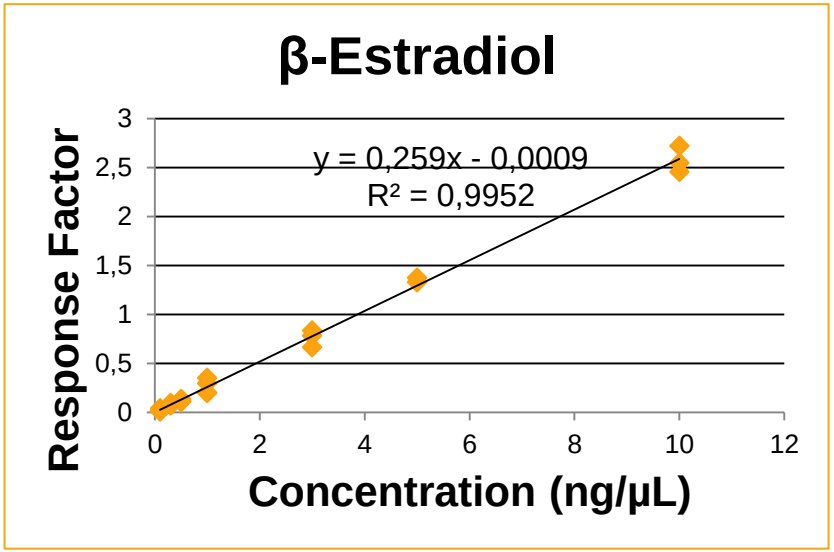

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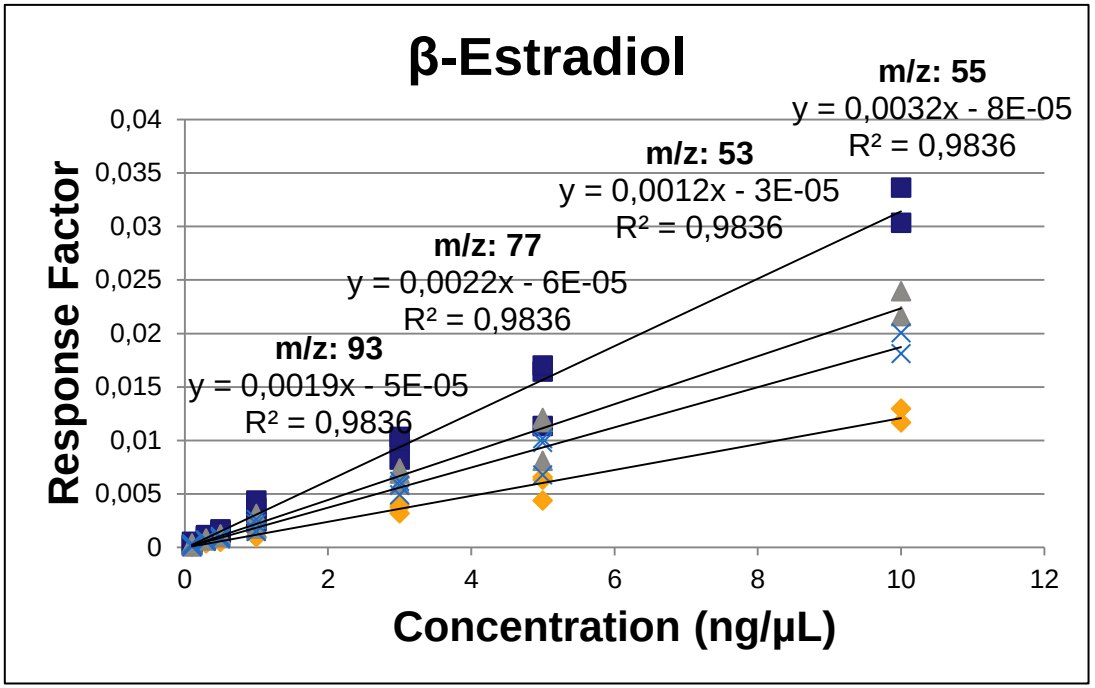
Quantification of all `similar` compounds using this one surrogate

Quantification in our studies

Quantification without multiple reference materials?
Option 2:

<u>Surrogate approach (selected ions)</u>

→ Similar to option 1, but selective ions are used instead of TIC



→ Quantification of these ions in similar compounds

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Concentration calculated from the average of these ions

Quantification in our studies

Quantification without multiple reference materials?
Option 3:

Partial-least-square equations (m/z-range 50-600)

Six standard compounds are analyzed in different concentrations

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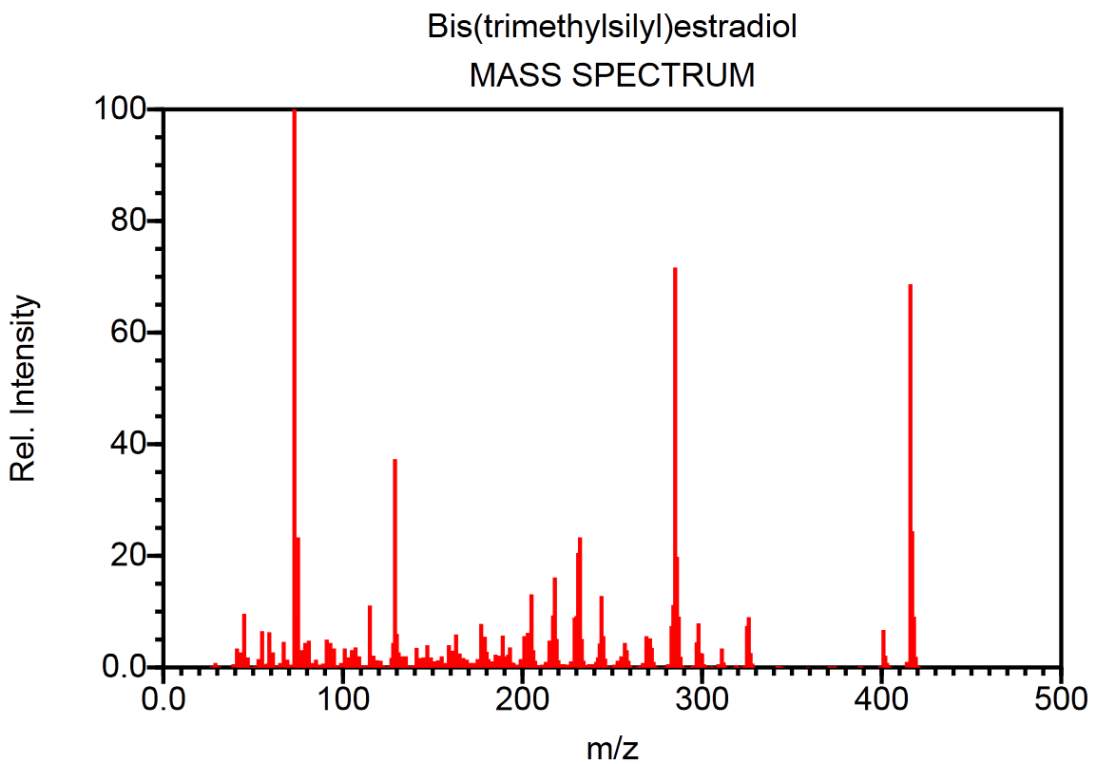
Response factors (A_{std}/A_{istd}) are converted into ion intensities ($RF_{TIC}=\sum RF_{50\text{-}600}$)

↓

Quantification in our studies



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Estradiol			
Mass spectrum			Response Factor
m/z	Relative ion intensity	Relative ion intensity (%)	1 ppm
50	4	0,0022	0,0009
51	7	0,0038	0,0015
52	2	0,0011	0,0004
53	18	0,0097	0,0039
54	3	0,0016	0,0006
55	51	0,0275	0,0110
56	3	0,0016	0,0006
57	9	0,0049	0,0019
58	14	0,0076	0,0030
59	74	0,0399	0,0159
60	10	0,0054	0,0022
61	36	0,0194	0,0077
62	5	0,0027	0,0011
63	8	0,0043	0,0017
64	2	0,0011	0,0004
65	11	0,0059	0,0024
66	3	0,0016	0,0006
67	40	0,0216	0,0086
68	4	0,0022	0,0009
69	10	0,0054	0,0022
70	4	0,0022	0,0009
71	6	0,0032	0,0013
72	13	0,0070	0,0028
73	999	0,5388	0,2149
74	94	0,0507	0,0202
75	303	0,1634	0,0652
76	23	0,0124	0,0049
77	44	0,0237	0,0095
78	7	0,0038	0,0015
79	47	0,0254	0,0101
		.....	
600	0	0	0
Total	**1854**	**1**	**0,9542**

Quantification in our studies

↓

All ion fragments of all standard compounds are used to build up a chemometric model based on PLSR-equations

↓

Validation of the model by leaving one standard out of the model and predicting its concentration with the model

Quantification in our studies

	External compounds prediction error (EPE) / %		
	Calibration curve (TIC)	Calibration curves (m/z: 53; 55; 77; 93)	PLSR (m/z: 50-600)
trans-Androsterone	40	50	17
Estrone	188	47	16
β-Estradiol	123	67	14
Testosterone	60	51	15
17α-ethynylestradiol	107	51	15
Estriol	53	70	17
Mean EPE	**95**	**56**	**16**

Kopperi *et al*. Anal.Chim.Acta., 761 (2013) 217-226

Summary

- GCxGC-TOFMS can be a very valuable tool for the screening of different compounds in environmental samples
- Powerful statistical tools are required to utilize the full potential of the technique
- Strict criteria must be met in order to increase the reliability of identification and to reduce the amount of false positives
- A chemometric model can predict the concentrations of homologous compounds in a sample with good accuracy utilizing just a few selected reference compounds

References

Kopperi, M., Ruiz-Jiménez, J., Hukkinen, J.I., Riekkola, M.-L. ”*New way to quantify multiple steroidal compounds in wastewater by comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry*” Anal.Chim.Acta., 761 (2013) 217-226

Ruiz-Jiménez, J., Parshintsev, J., Laitinen, T., Hartonen, K., Riekkola, M.-L., Petäjä, T. and Kulmala, M. ”*Comprehensive two-dimensional gas chromatography, a valuable technique for screening and semiquantitation of different chemical compounds in ultrafine 30 nm and 50 nm aerosol particles*” J. Environ. Monit., 13 (2011) 2994-3003

Thank you
for
your attention!