Ordine dei Tecnologi Alimentari
Regioni Veneto e Trentino Alto Adige

CONFINDUSTRIA VENEZIA
AREA METROPOLITANA DI VENEZIA E ROVIGO

LS analytica

AUTENTICITA E FRODI NEGLI ALIMENTI

Aspetti legali, di gestione e tecniche analitiche

GC-IMS: Tecnica Analitica innovativa per controllo di qualità tramite le sostanze volatili

Dott. Cesare Rossini
Business Development Manager (Lab Service Analytica Srl)

MARGHERA 20 FEBBRAIO 2020

Gas Chromatograhy-Ion Mobility Spectrometer (GC-IMS)

Company:

- Founded in 1997 as spin-off of the ISAS – Leibniz-Institute for Analytical Sciences e.V.
- R&D and manufacturing facilities at Technology Centre Dortmund
- Development, production and distribution of analytical instruments based on Gas Chromatography coupled to Ion Mobility Spectrometry customized to application and customer requirements
- More than 200 installed instruments at global market leaders in food & flavours, process industry, research institutes

VOC Screening Solutions

Gas Chromatography–Ion Mobility Spectrometry

Product Quality Control

- Food & Beverages
- Impartial Flavour Documentation
- Off-Smell Detection

Gas Monitoring

- VOC Monitoring
- Siloxanes in Biogas
- Odorants in Natural Gas
- Filter Loading

Human Breath

- VOCs as diagnostic Markers
- Intoxication
- Pharmacokinetics

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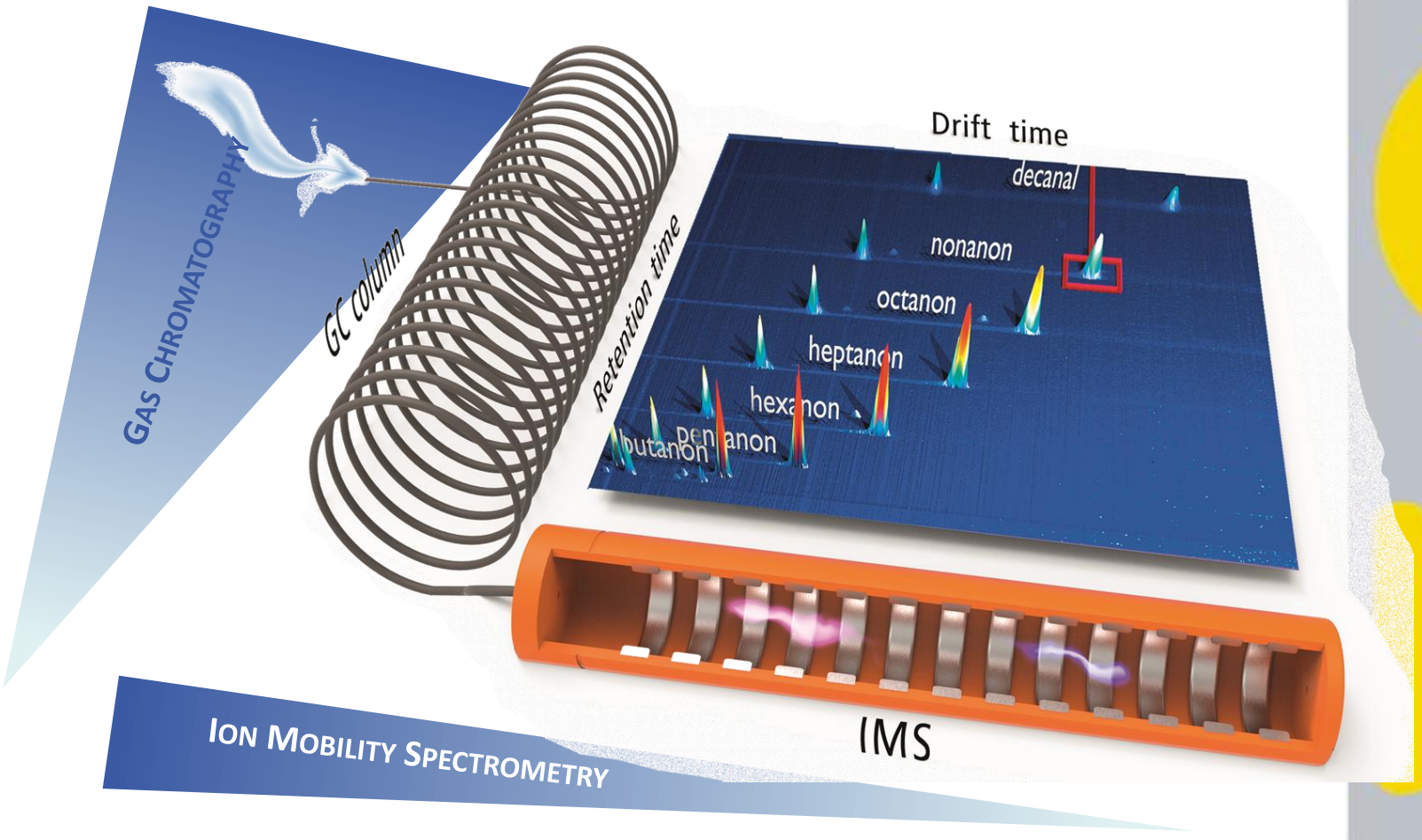


2 DIMENSIONS OF SEPARATION
HIGH SENSITIVITY (LOW PPB RANGE)

FlavourSpec®

Ionisation of the nitrogen of the drift gas by the ß emission:

$$N_2 + ß \rightarrow N_2^+ + ß' + e^-$$

Generation of reaction ions in nitrogen:

$$N_2^+ + 2N_2 \rightarrow N_4^+ + N_2$$

$$N_4^+ + H_2O \rightarrow 2N_2 + H_2O^+$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH$$

$$H_3O^+ + H_2O + N_2 \rightarrow (H_2O)_2H^+ + N_2$$

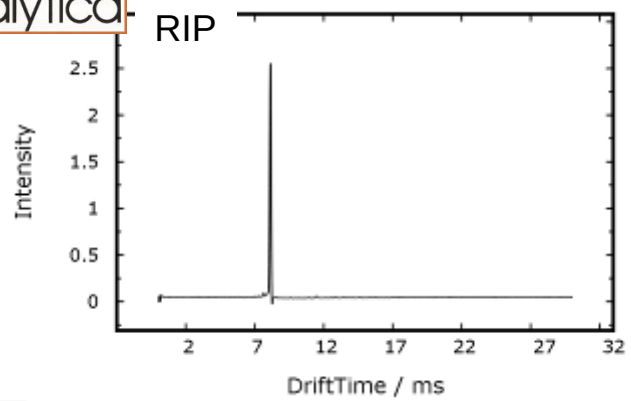
$$(H_2O)_2H^+ + H_2O + N_2 \rightarrow \mathbf{(H_2O)_3H^{+}}\ * + N_2$$

**Number of water molecules vary*

Soft-Ionization no fragmentation – almost 100% output

Ambient pressure – no Dilution!

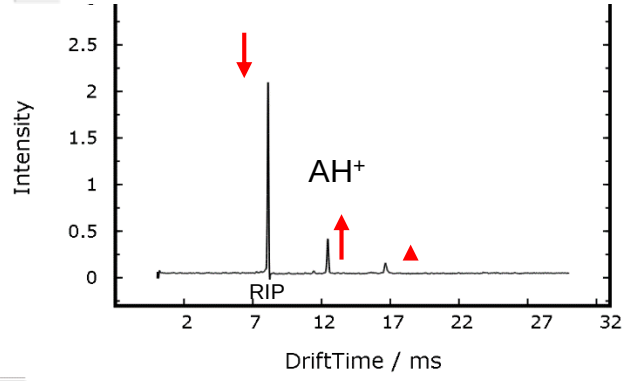
FlavourSpec®



Without analyte:

$$\text{residual water} \xrightarrow{\beta^-} H^+(H_2O)_n$$ | reactive species

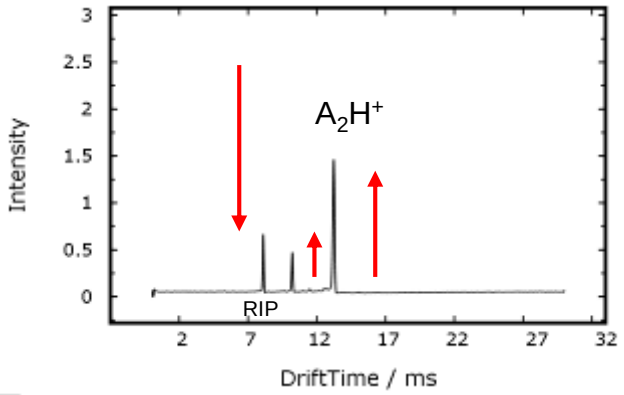
evolution of reactive proton species by interaction with radiation



With analyte:

$$H^+(H_2O)_n + A \xrightarrow{\beta^-} AH^+(H_2O)_n + H_2O$$ | analyte ion

soft chemical ionization by proton transfer



High analyte load:

$$H^+(H_2O)_n + 2A \xrightarrow{\beta^-} A_2H^+(H_2O)_n + H_2O$$ | analyte ion

high analyte concentration due to atmospheric pressure

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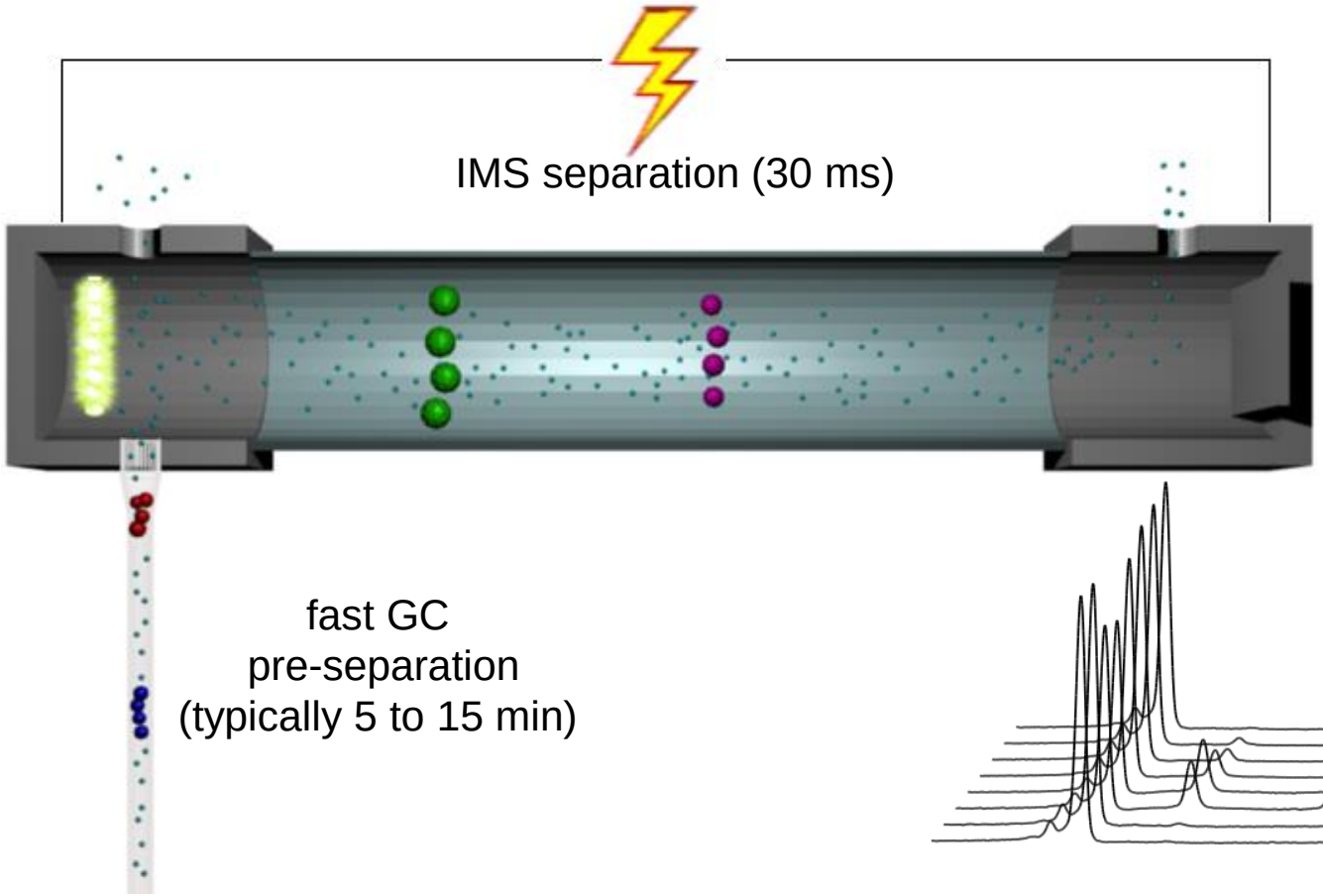

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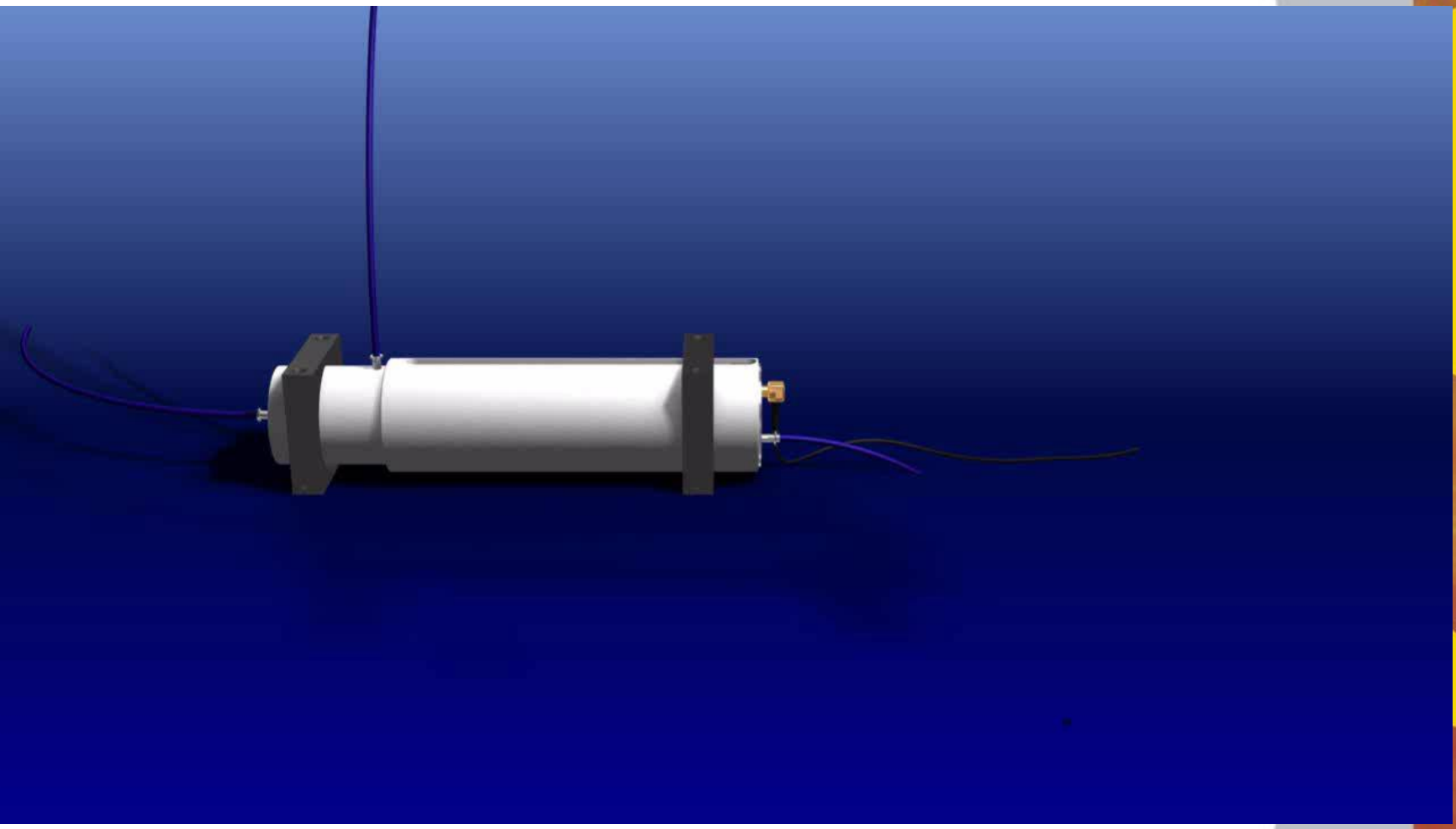

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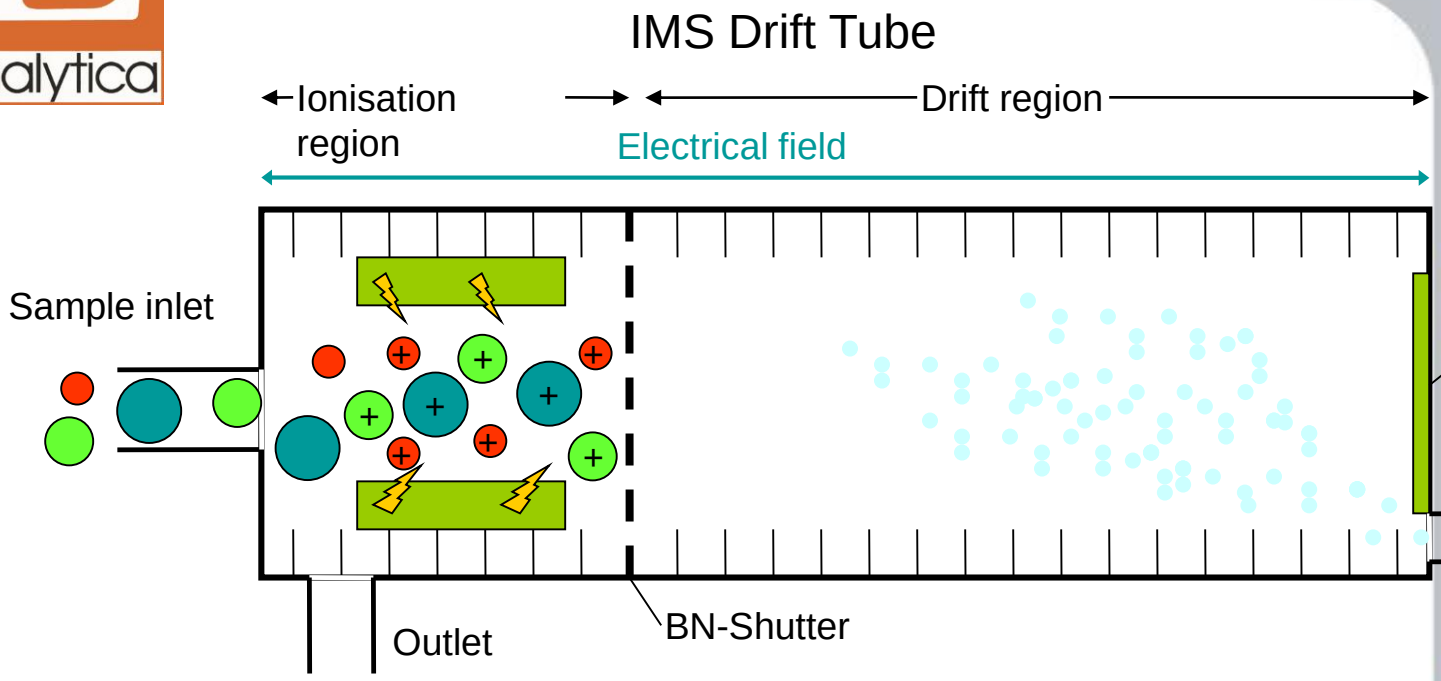

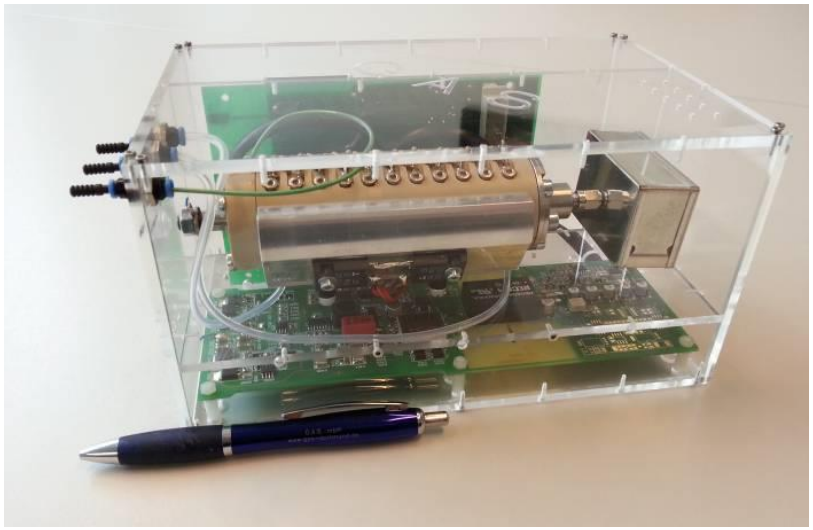

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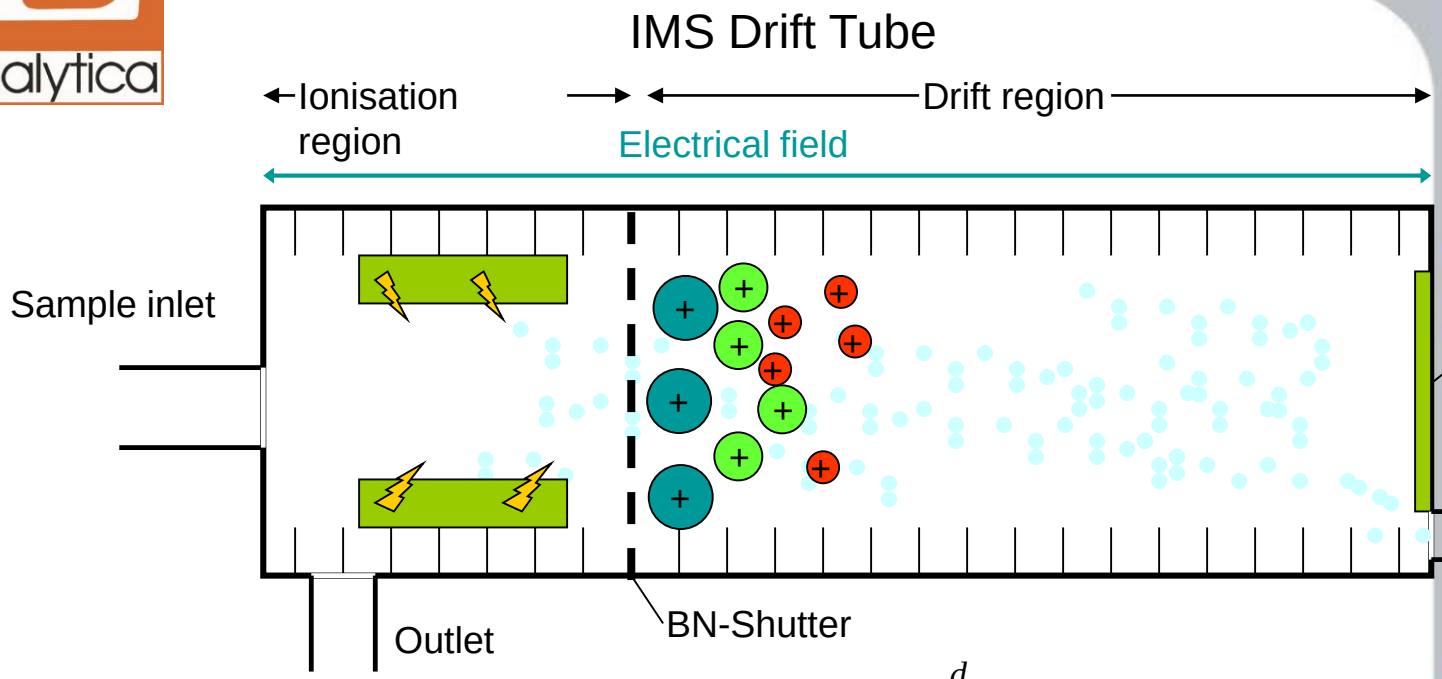

Drift velocity: $v_d = K E$

Mobility: $K = \frac{d}{t_d E}$

$$K = \frac{3}{16}\frac{e}{N}\sqrt{\frac{1}{m}+\frac{1}{M}}\sqrt{\frac{2}{k}\frac{\pi}{T}}\frac{1+\Delta}{\pi r^2 \Omega}$$

E	electric field strength	d	drift path length
t_d	drift time	e	unit charge
m	ion mass (analyte)	M	molecular mass (drift gas)
N	number density (drift gas)	k	Boltzmann-constant
T	temperature	r	minimum in the potential curve
Ω	collision integral 1. order	Δ	correction term for approximations

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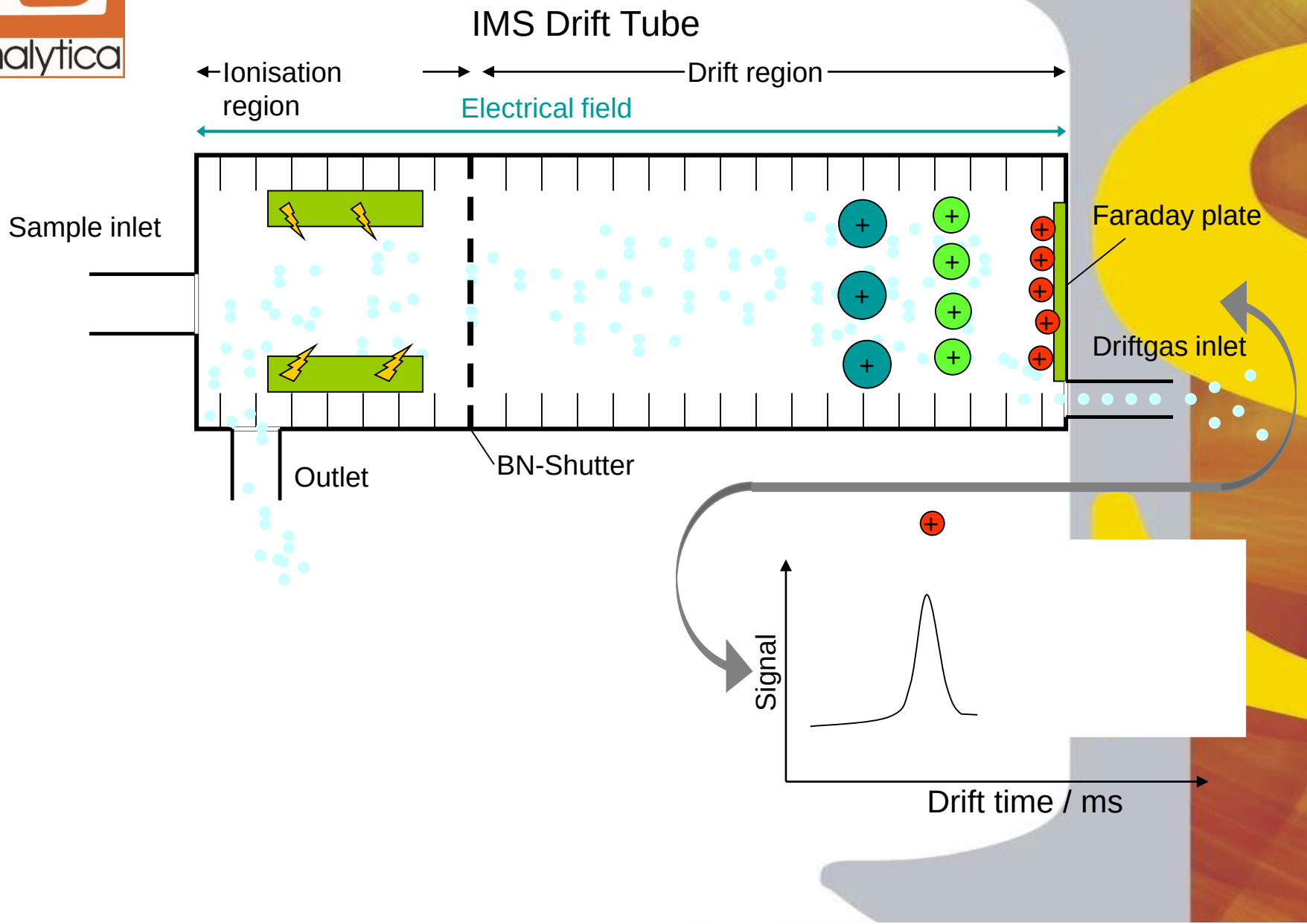

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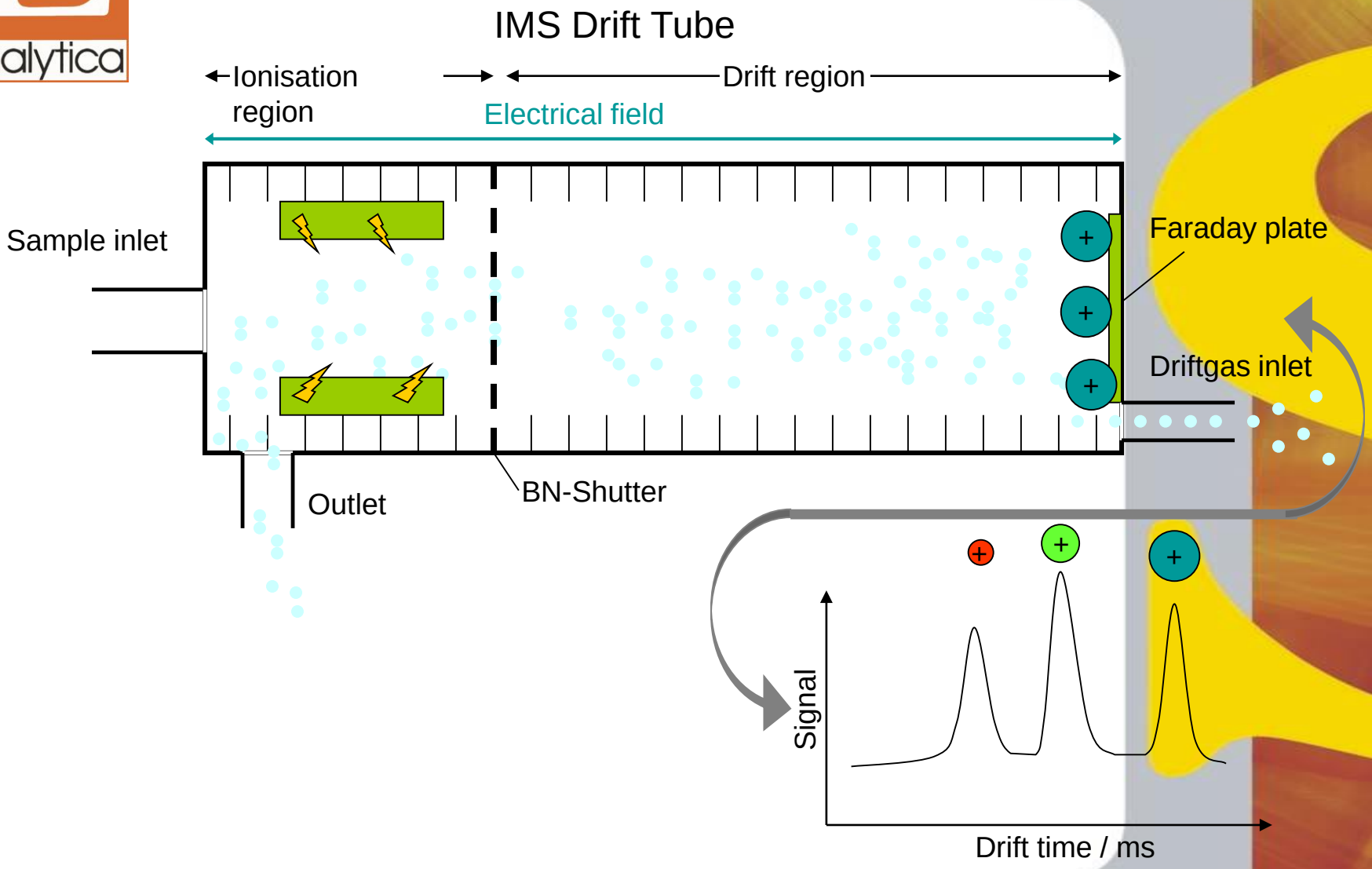

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Protone Affinities

Aromatic Amines	930.0 KJ/mol	Pyridine
Amines	899.0 KJ/mol	Methyl Amine
Phosphorous Compounds	890.6 KJ/mol	Trimethylphosphate
Sulfoxides	884.4 KJ/mol	Dimethyl Sulfoxide
	853.6 KJ/mol	Ammonia
Ketones	832.7 KJ/mol	2-Pentanone
Esters	821.6 KJ/mol	Methyl Acetate
Alkenes	805.2 KJ/mol	1-Hexene
Alcohols	789.2 KJ/mol	Butanol
Aromatics	750.4 KJ/mol	Benzene
	691.0 KJ/mol	**Water**
Alkanes	543.5 KJ/mol	Methane

Source: Gary Eiceman & Zeev Karpas, *Ion Mobility Spectrometry*, CRC Press, 2005, ISBN 0-8493-2247-2

Protone affinities of various VOCs can be found at the NIST chemistry webbook
http://webbook.nist.gov/chemistry/

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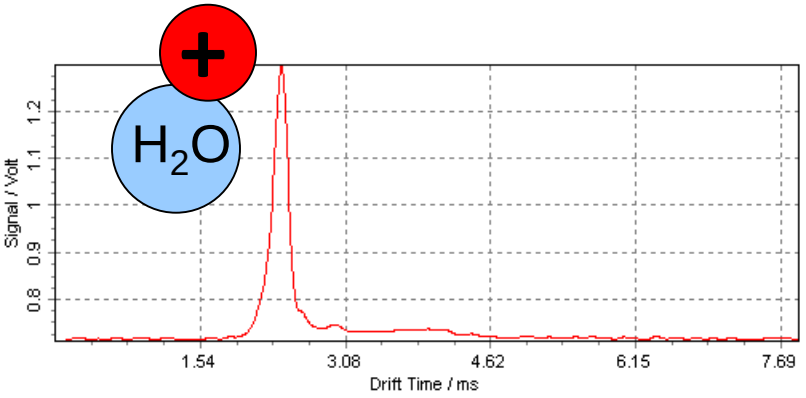
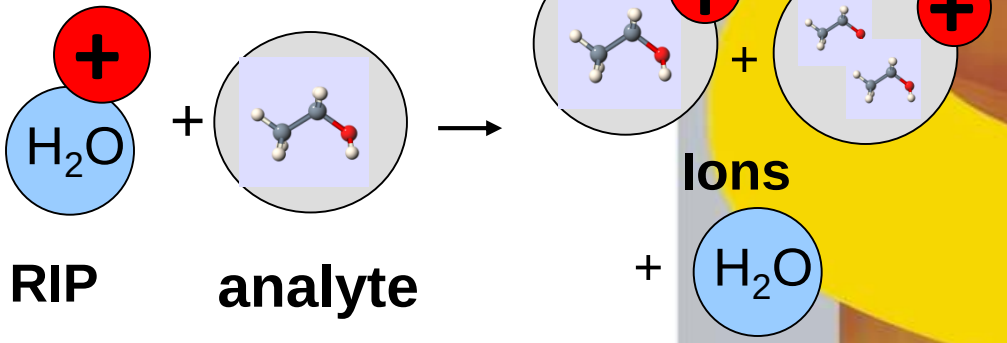
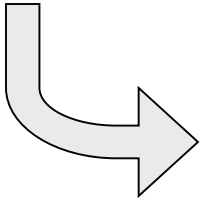
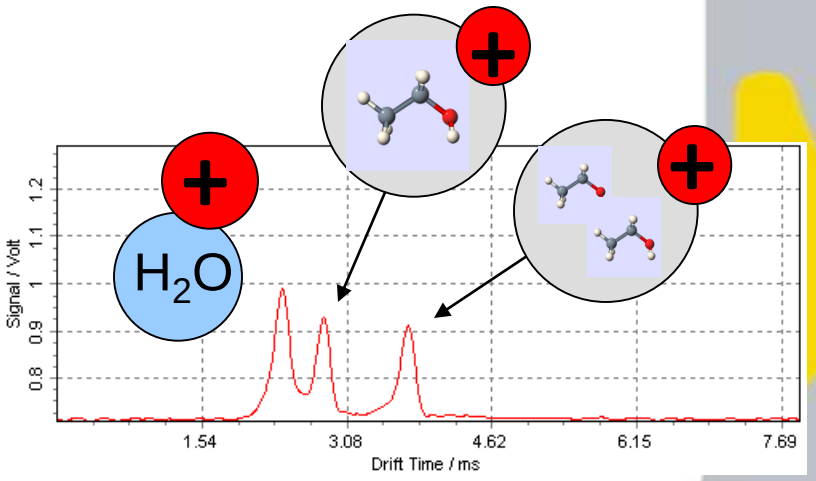




RIP





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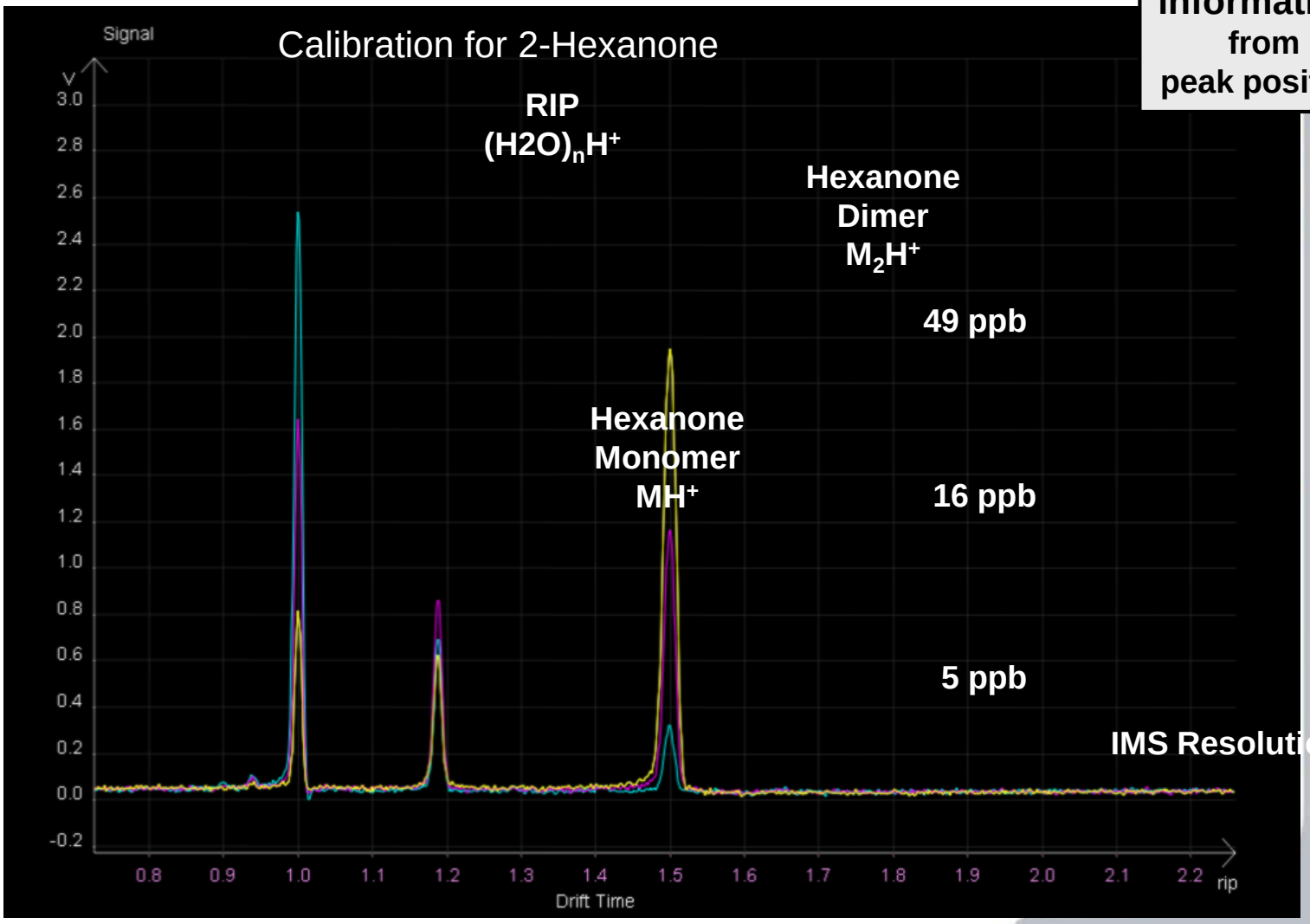


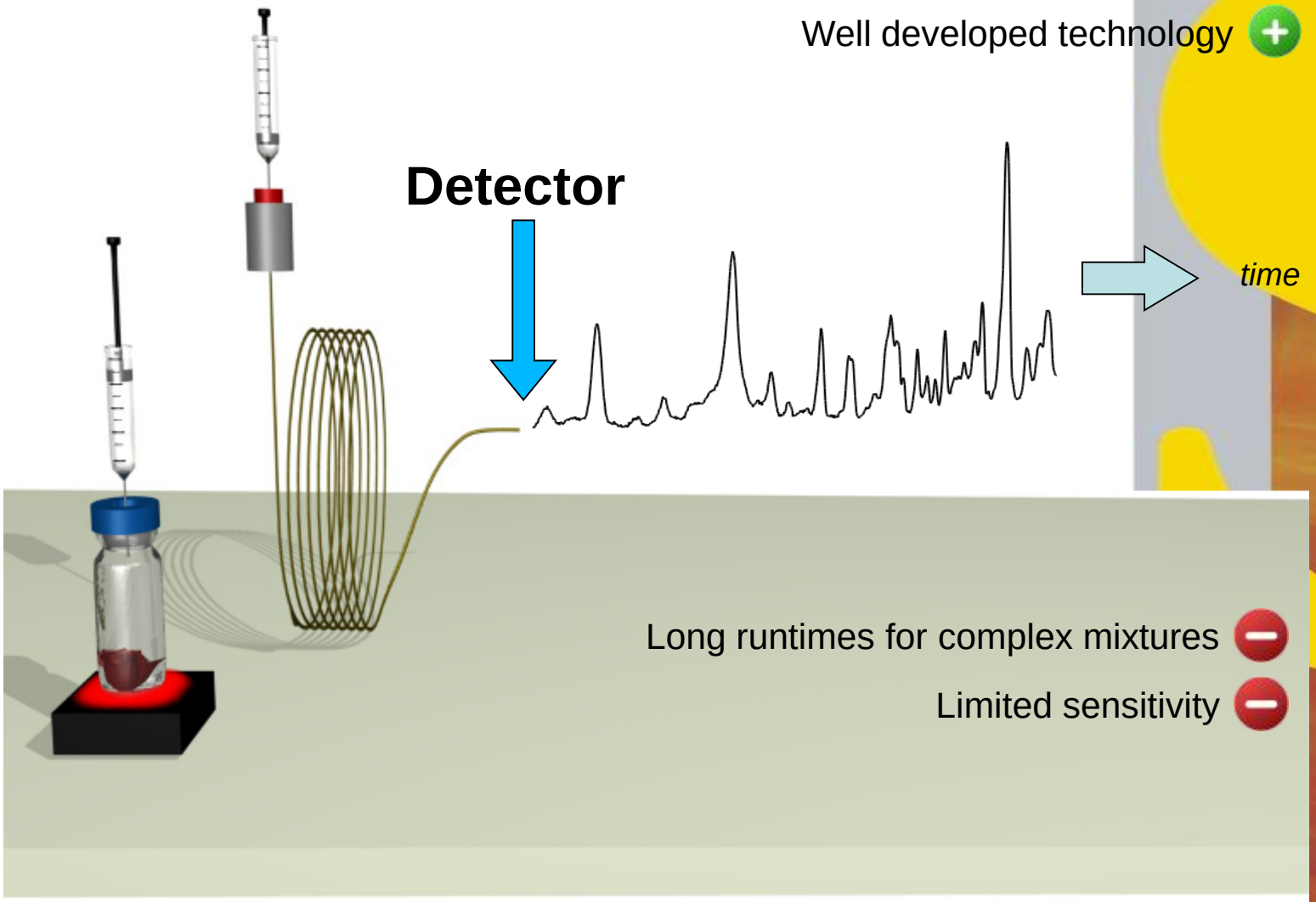
Qualitative information from peak position

Quantitative information from peak intensity

FlavourSpec®

Headspace sampling

Well developed technology



Long runtimes for complex mixtures

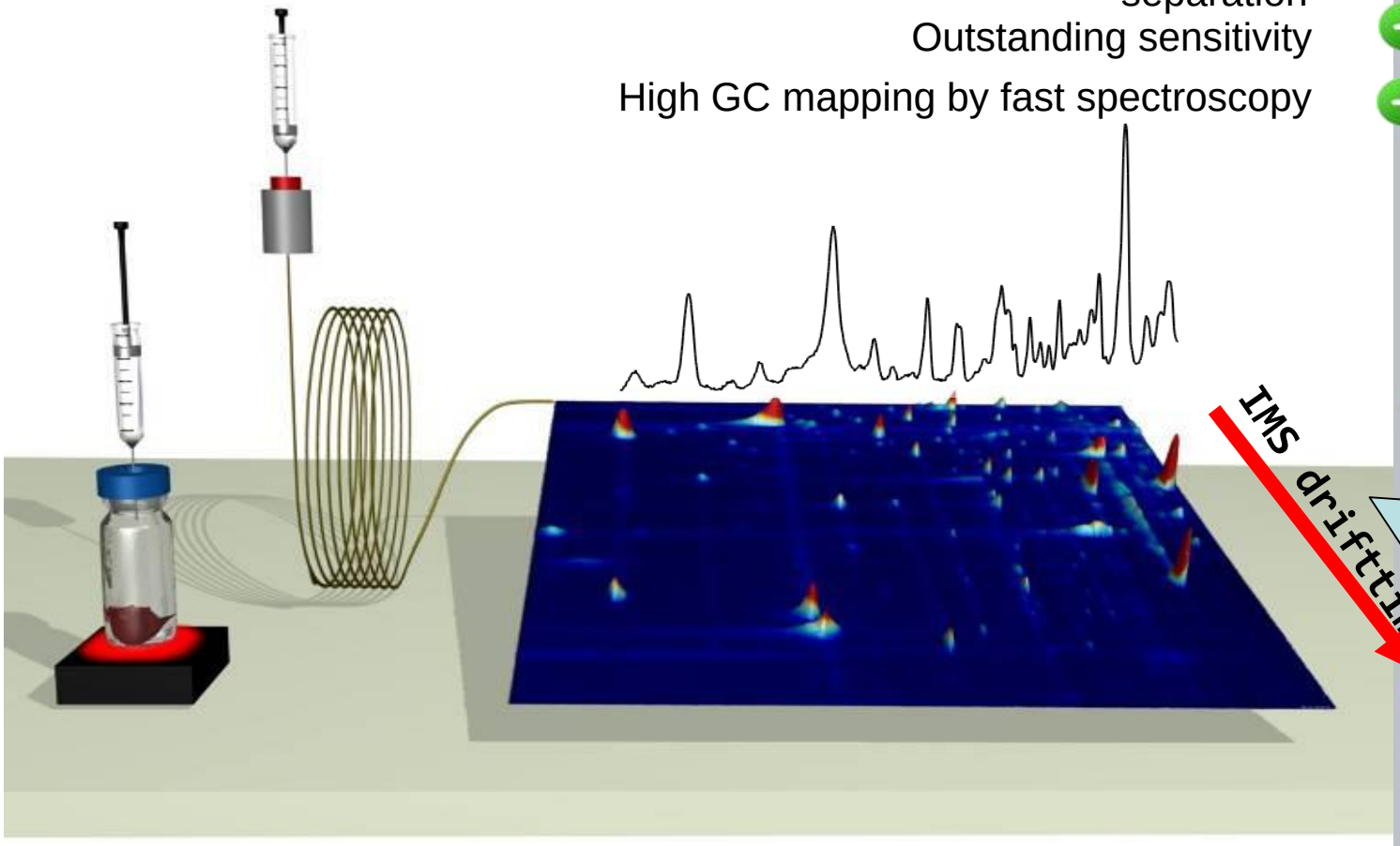
Limited sensitivity

FlavourSpec®

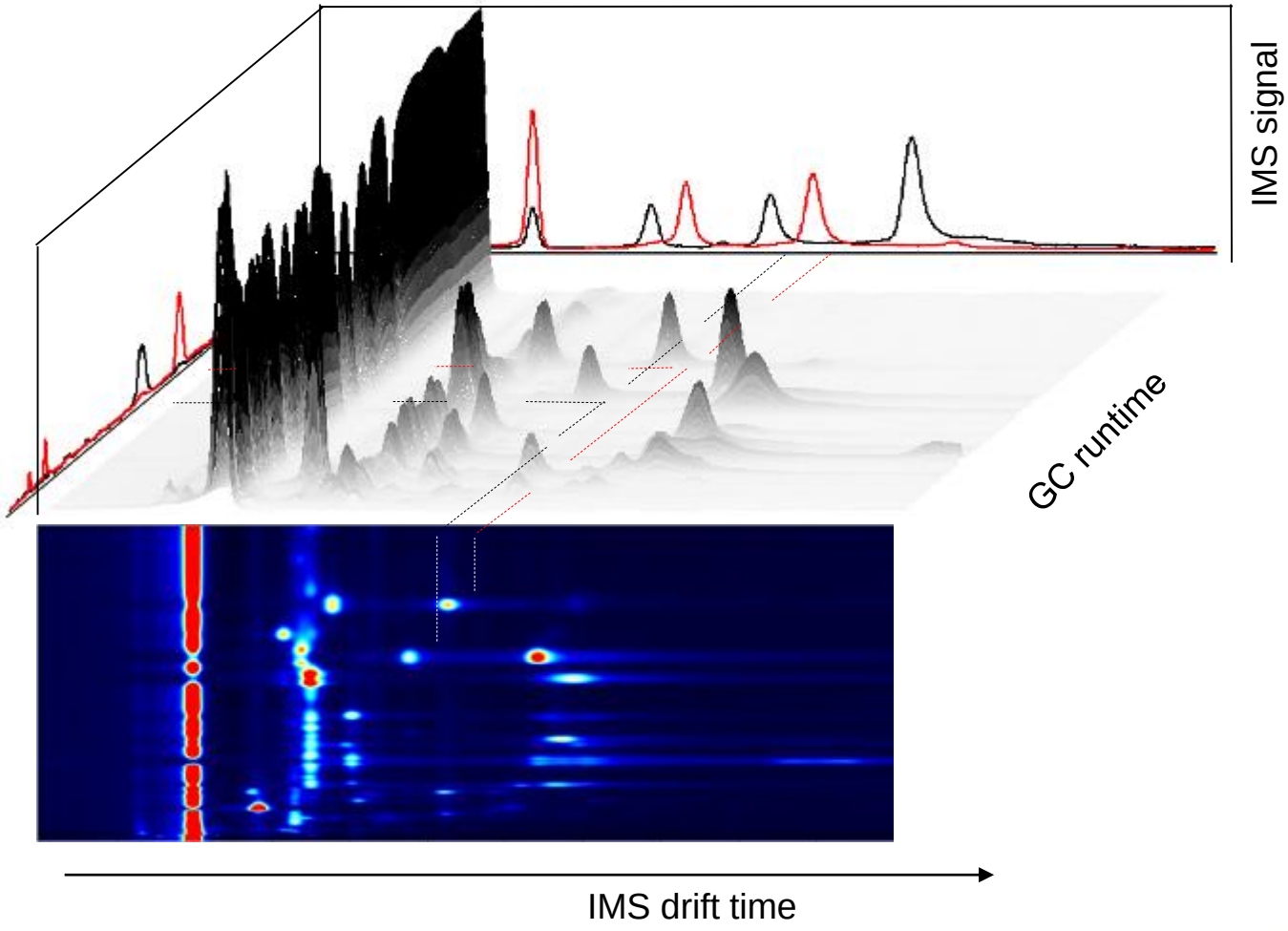
Second physical separation

Outstanding sensitivity

High GC mapping by fast spectroscopy



FlavourSpec®



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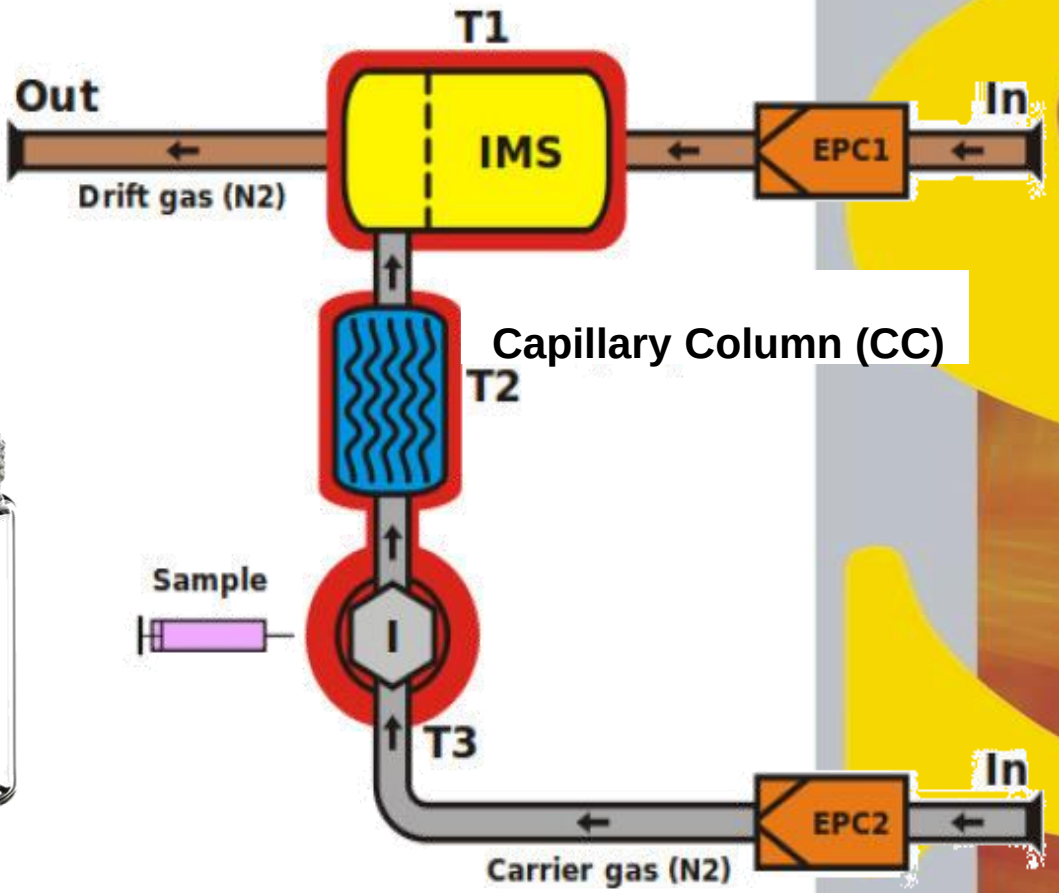

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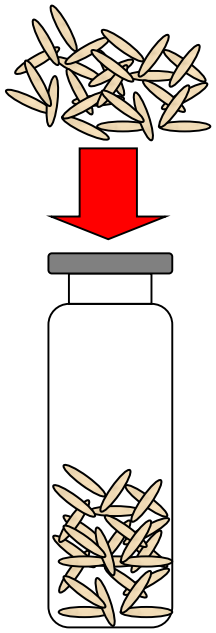
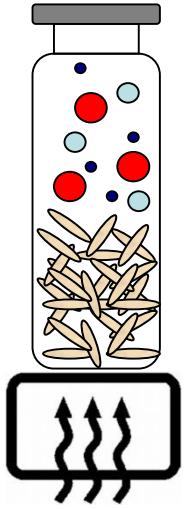
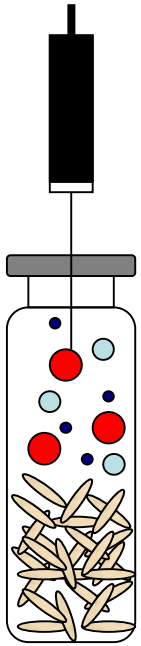


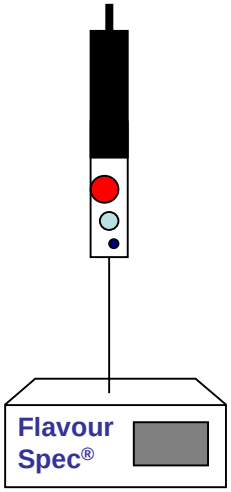
Fill vial



Generate headspace



Sampling



Injection

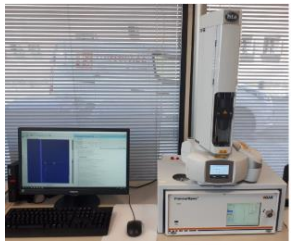
Data Analyis

FlavourSpec®

High-end Mass Spec

- ‘golden standard‘
- approved technology
- R&D
- available data base
- well known

GC-IMS



- easy-to-use
- reliabe (phys. principle)
- QC related
- fingerprint and data base
- attractive value for money
- flexible/portable use

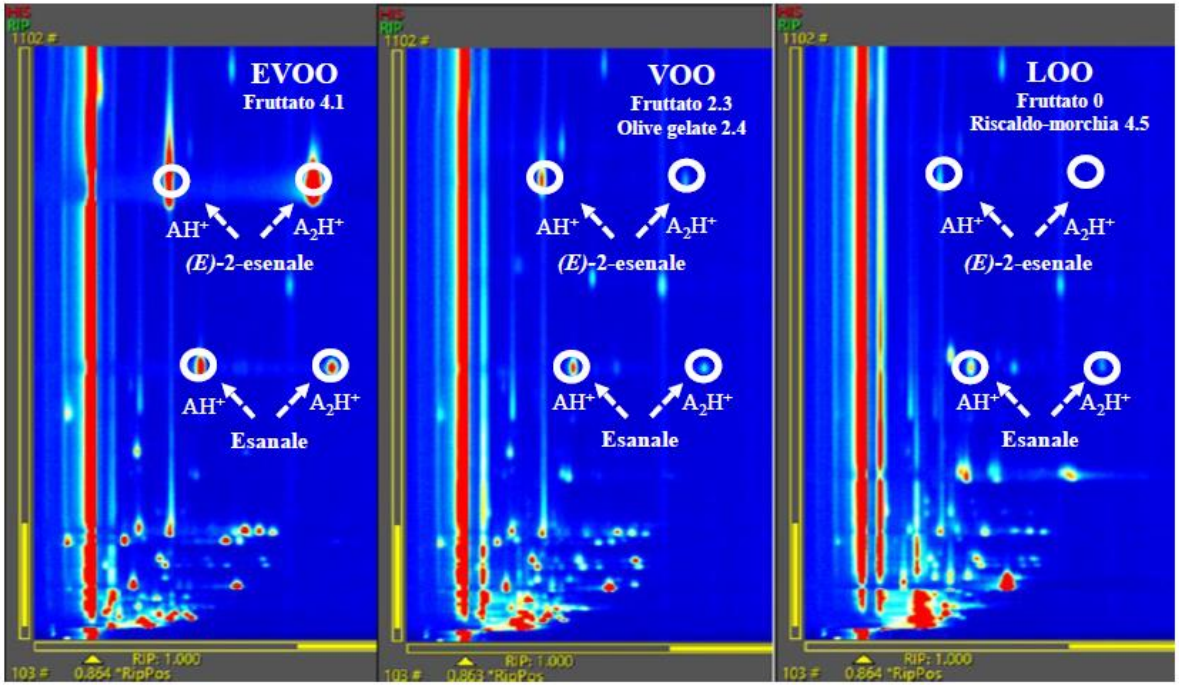
Hedonistic Evaluation

- most decisive Criteria
- availability critical
- subjectivity problematic

FlavourSpec®

Olio di OLIVA

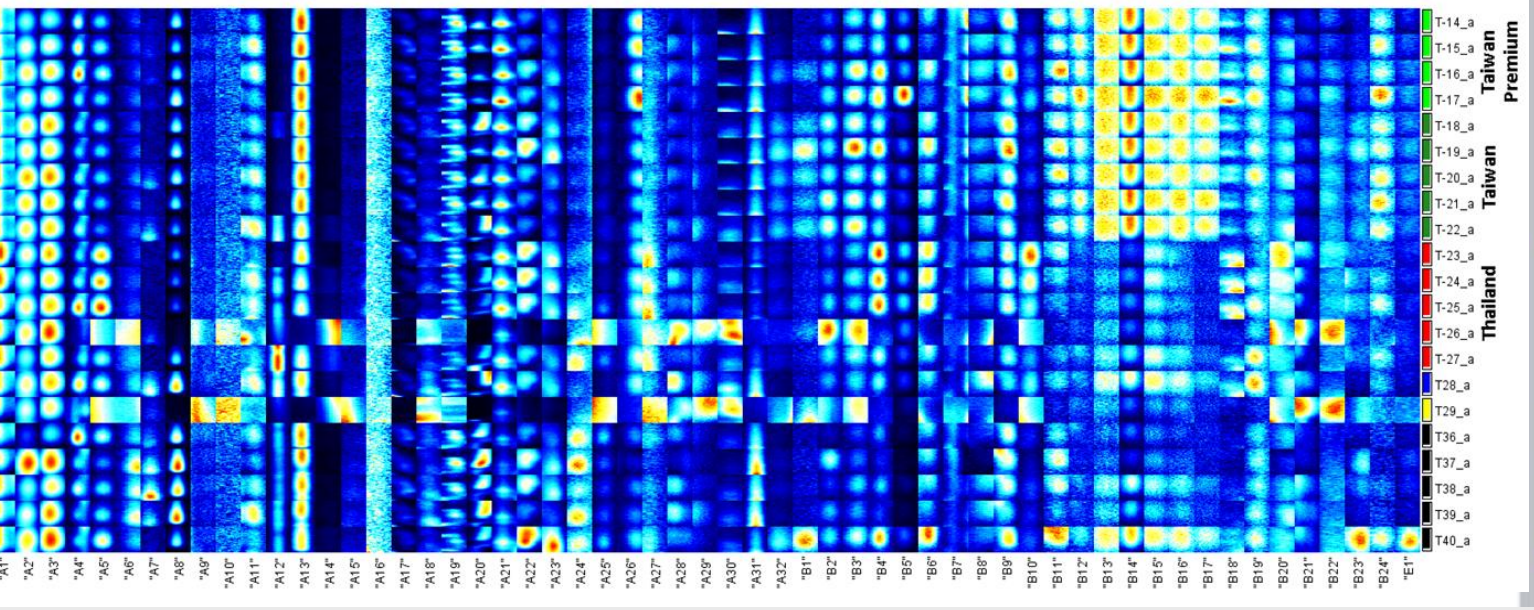
Analisi *Targeted*



FlavourSpec®

Miele

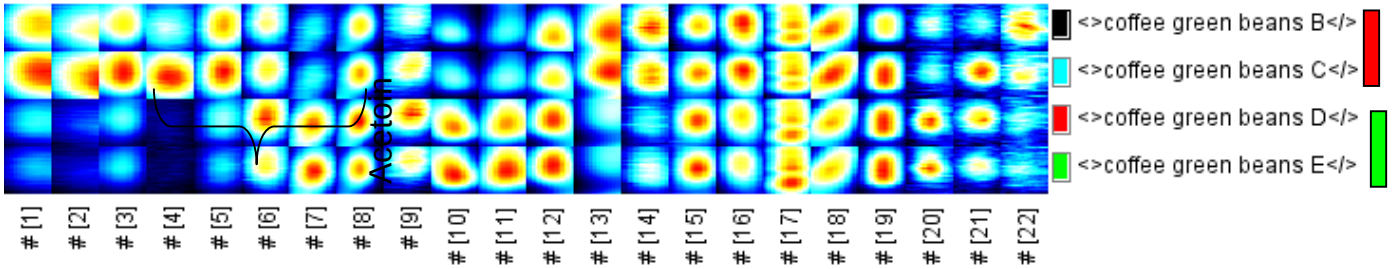
Analytical Task D: Comparison of honey from different countries



Premium taiwanese honey exhibits unique VOC pattern

FlavourSpec®

Caffè



unwanted odor

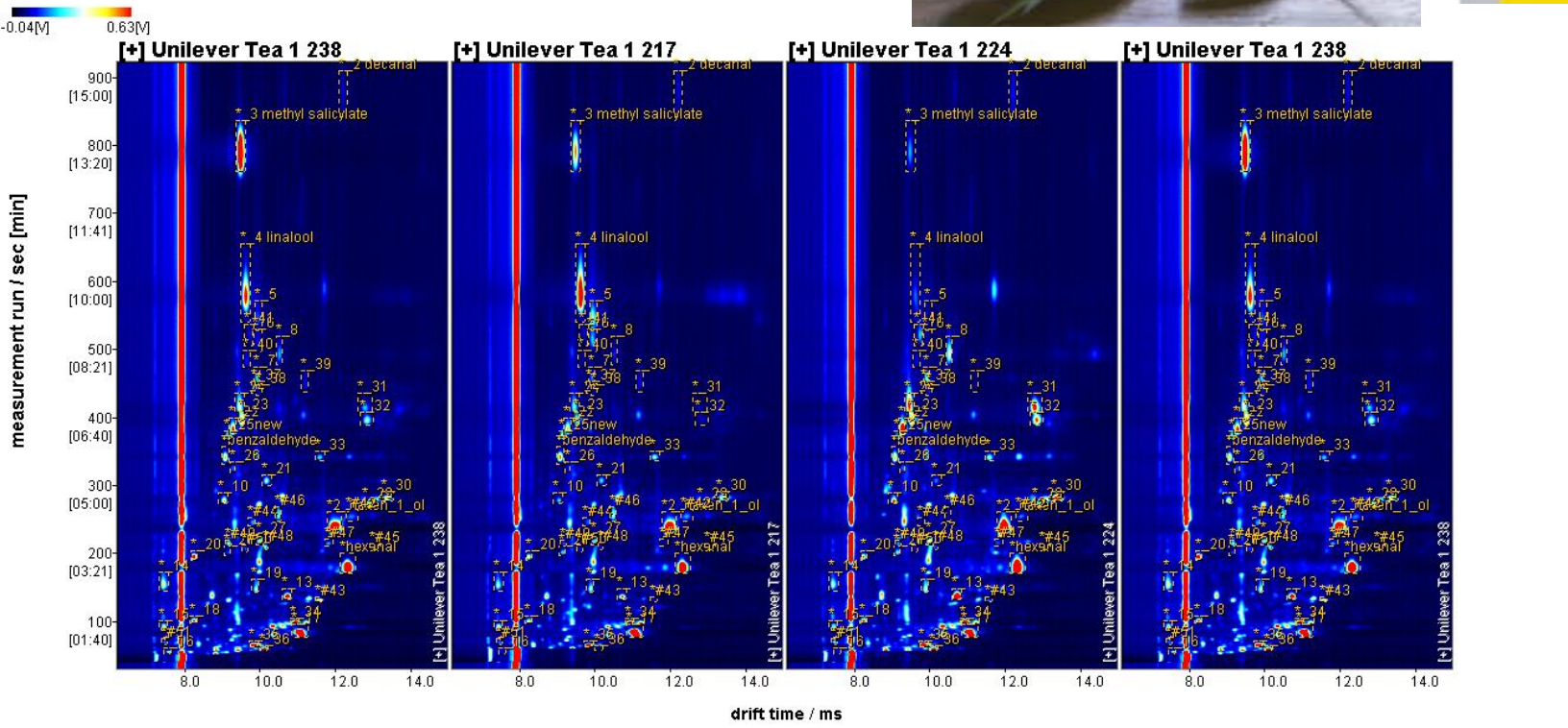
normal odor

thiazole	C288471	C3H3NS	85,1
(E)-3-penten-2-one	C3102338	C5H8O	84,1
Methyl isothiocyanate	C556616	C2H3NS	73,1
3-Methylbutanenitrile	C625285	C5H9N	83,1
Methanedithiol	C6725640	CH4S2	80,2
3-Methyl-3-buten-1-ol	C763326	C5H10O	86,1
3-methylbutan-1-ol	C123513	C5H12O	88,1
2-methylbutan-1-ol	C137326	C5H12O	88,1
2-methylbutan-1-ol	C137326	C5H12O	88,1

Off-Smell Detection: Series of compounds found to be unique and other elevated in concentration

FlavourSpec®

Tea



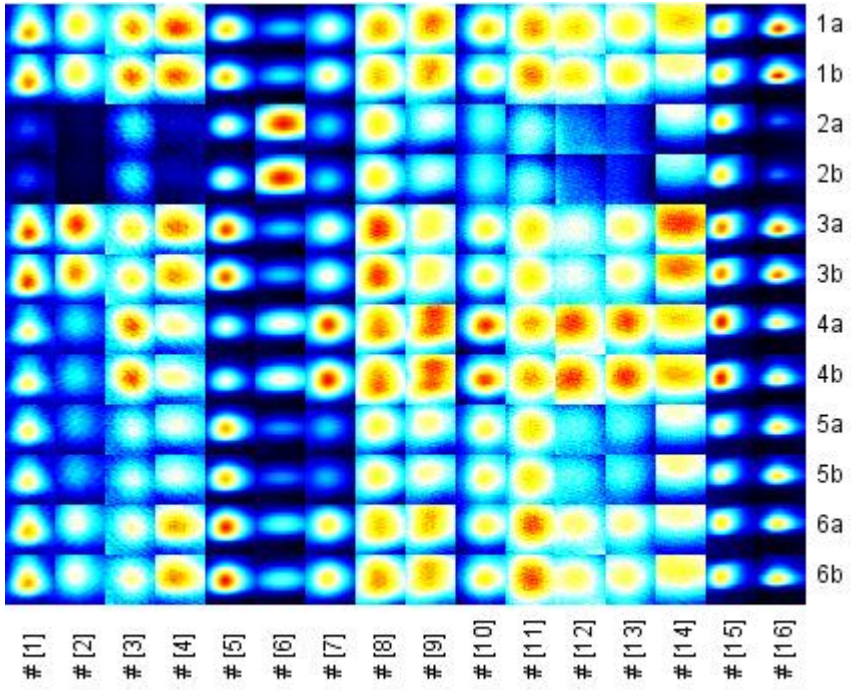
Complex fingerprint of tea

Signals are first marked by drawing rectangles around the signals

FlavourSpec®

Vino

Analysis of the aroma composition to validate origin



One sample exhibits different Pattern -> produced in other Chinese Province

FlavourSpec®

Freschezza pesce

Storage of raw material / finished products degrade the quality and affect the flavour

Applications:

- Monitoring aroma during shelf life testing
- Determination of ‘best-before’ data
- Monitoring of product ripening

Provenienza pesce

Ensuring the Integrity of the European food chain

GC-IMS screening to assess Salmon authenticity

Linda Monaci[1**], Simone Guidotti[2], Antonio Fornaro[2], Cesare Rossini[2*]

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[2] LabService Analytica s.r.l, Anzola Emilia (Bologna), Italy
*cesare.rossini@labservice.com
**linda.monaci@ispa.cnr.it

OVERVIEW

Gas chromatography coupled to ion mobility spectrometry is a potential tool exploited if food authenticity assessment as documented by several investigations[1,2,3]. In this work, the analysis of the volatile fraction of salmon harvested under different living conditions was investigated by exploiting the coupling between gas chromatography and ion mobility spectrometer by using FlavourSPec (GAS) instrument equipped with a tritium source. The method allowed to discriminate wild-type from farmed salmon collected in Canada without requiring an extraction step.

MATERIALS AND METHODS

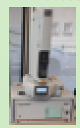
Samples

26 Canadian wild type salmon (CND WT) — 25 Canadian farmed salmon (CND F)

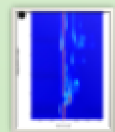
NO sample preparation

- 2 g of each sample
- injected by a headspace device
- conditions: T = 40°C for 5 min

GC-IMS analysis and data processing



Gas Chromatography - IMS SpectralFlavour (Gas Dortmund, Germany)



RESULTS

A total of 51 salmon samples distinguished in farmed and wild-type samples (collected in Canada) already analysed by DART-HRMS method[4] were analysed for the volatile fraction by Gas Chromatography and Ion Mobility Spectrometry in order to find spots able to discriminate *farmed* from *wild-type* samples. The spectral data collected from the spots detected (after exposing samples at 40° C for 20 minutes), were analysed by PCA as shown in Figure 1, after normalizing the area intensities of a total of five selected spots. Results showed a good separation of three different clusters relative to wild-type, farmed and blank samples and approximately 95% of variance was explained by the first two components (PC1 and PC2).

As a second approach, a supervised method was applied to the whole dataset. In this case it was used Linear Discriminant Analysis (LDA) by using Statistica v. 7 software, on the normalized intensities of five spots detected.

According to the results obtained one discriminant analysis function was obtained (root 1) whose values for each sample are reported in Figure 2. As appearing from the graphical representation a clear separation of two groups (*farmed vs wild-type*) was highlighted accounting for 88% of variance explained.

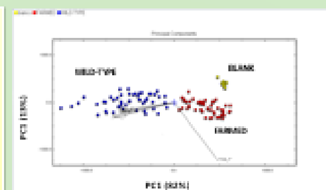


Figure 1. Score plot obtained from PCA of data arising from the GC-IMS analysis of 51 salmon Canadian samples in colored circles (blue = WT, red = F, yellow = B) Canadian salmon samples. Analysis were run in ... three replicates for each salmon sample.

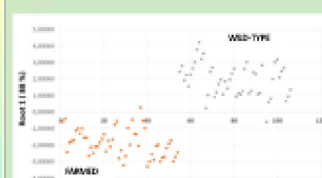


Figure 2. Graphical representation of the values of Root 1 obtained after applying Linear Discriminant Analysis to normalized intensities of 5 selected spots detected by GC-IMS.

CONCLUSIONS

The method developed based on the analysis of the volatile compounds by gas chromatography coupled to ion mobility spectrometry appears very promising for its application to food authentication. In particular, in this note we demonstrated the feasibility of such method to the discrimination of living conditions of Canadian salmon samples also opening for marker discovery able to trace the leaving conditions of wild type salmon samples.

REFERENCES

1. Volatile Compound Fingerprinting by Headspace Gas Chromatography Ion Mobility Spectrometry (HS-GC-IMS) as alternative to ... Profiling for Assessment of the Authenticity of Honey, Analytical Chemistry ...
2. ... Food Chemistry, 2016 ...
3. ... Ion mobility spectrometry coupled to gas chromatography ... Food Chemistry, 2017 ...
4. ... Monaci L., Assessing fish authenticity by direct analysis in real time high resolution mass spectrometry and multivariate analysis: discrimination between wild-type and farmed salmon, 2016, Food Research International ...

www.foodintegrity.eu

The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

Freschezza uova

ION MOBILITY SPECTROMETRY: A RAPID TOOL TO ASSESS EGGS FRESHNESS

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OVERVIEW

PURPOSE: develop an easy and rapid method able to assess eggs freshness, easy to use also in the production plants

METHOD: GC coupled with an Ion Mobility Spectrometer. High sensitive and reproducible volatile fingerprints. Marker compounds identified with SPME-GC-MS technique

RESULTS: several spots highlighted as freshness markers. The chemometric data evaluation identifies clear samples clustering according to the time spent at room temperature. Subsequent compounds identification and GCxIMS spot correlation.
Total analysis time from sampling to the result: approx. **45 minutes.**

INTRODUCTION

In the last decade, the attention on the authenticity and quality of food commodities increased for commercial and safety reasons and that is why, from both industries and research institutes, the demand for the development of rapid methods able to detect frauds increased as well.

Eggs, mostly in the egg products form, are largely used for the creation of different industrial products (egg pasta, bakery etc.) and their freshness is a crucial step for the production of safe and high quality commodities; however, there are not several rapid ways able to assess if egg products are as fresh as declared.

In this study, a rapid GC-IMS method is presented as a possible way to quickly solve this problem.

METHODS

SAMPLE PREPARATION: 10 drops of fresh eggproduct directly transferred in a headspace vial. Samples were stored at room temperature and the same sample preparation was repeated after 24h, 48h, 72h and 144h from the receipt of the batch.
For each time point, three sample preparations were executed.

INSTRUMENT: FlavourSpec® (Gas Dortmund – Germany)

ESSENTIAL PARAMETERS

GC
Column: SF-54-CB-1 15m x 0.53 mm d_f 1 µm @ 40 °C
Incubation time: 20 min @ 40 °C
Injection volume: 0.5 ml
Carrier gas: Nitrogen
Flow gradient: 2 ml/min for 5 mins and then increased to 70 ml/min in 20 min
GC Runtime: 30 min

IMS
Drift gas: Nitrogen
Drift time: 30 ms
Drift gas flow rate: 150 ml/min
Drift tube length: 6.8 cm
Drift Voltage: 5kV
IMS Temperature: 45°C
Ionization mode: positive

COMPOUNDS ID

SPME-GC-MS

SAMPLE PREP.: 2 g of sample weighed directly in the Headspace vials

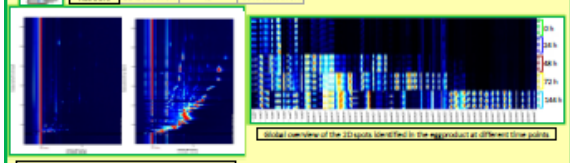
INSTRUMENT: Trace GC Ultra with DSQ II detector (Thermo Fisher Scientific-USA)

ESSENTIAL PARAMETERS

Fiber type: DVB/CAR/PDMS
Incubation time: 60 min
Extract time: 180 min
Column: Restek RXI-5ms 30m x 0.25 mm d_f 0.5 µm
Ionization mode: EI (+)

RESULTS

GC X IMS RESULTS | STATISTIC ELABORATION | COMPOUNDS ID | STANDARD CONFIRMATION



2D GC-IMS graph of fresh egg (left) and after 144h at RT (right)

Global overview of the 2D spots identified in the eggproduct at different time points

Specific marker spots can be identified in the final 2D graphs that change their intensity or that can be detected or not, according to the time and storage conditions of the egg products.

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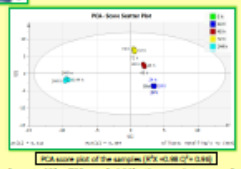

PCA score plot of the samples (R²X =0.88 Cum = 0.88)

Samples at 48h, 72h and 144h time points are clearly clustered; no significative difference can be detected between the fresh and the 24 hours samples

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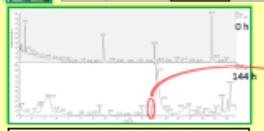
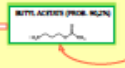
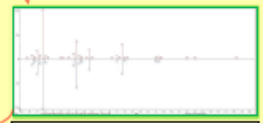


TIC chromatogram of fresh egg (top) and after 144h at RT (bottom)

Each egg sample was injected in the GC-MS system, following the same experimental design used for the GC-IMS approach. Several time point marker compounds can be identified thanks to the GC-MS chromatograms and the NIST libraries

More than 20 marker compounds identified





Comparison between the experimental (top) and the library recorded (bottom) mass spectra of peak at RT 13.76 min

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The available reference standards of the compounds identified with the GC-MS approach were separately spiked into the fresh eggproduct samples and analyzed with the GC-IMS system, with the aim to find a correlation between the marker spots and the target compunds.

Comparison of the most relevant 2D spots after standards addition

Some of the spots previously detected are uniquely correlated with the presence of specific compounds that can be identified as freshness markers.
Due to their similar structure, the sulfur compounds are not separated but in general we can assume that the increase in epeak 1x intensity can be considered a freshness issue

CONCLUSIONS

This method is able to clearly discriminate the eggproducts according to the time spent at room temperature. Some of the marker compounds were identified; thanks to this technique, fraudulent freshness declarations can be rapidly detected

The 24 hours samples present the same volatile profile of the fresh ones: this shows that, within this short period of time, eggproducts can be exploited into the production chain without significantly affect the quality and the safety of the corresponding finished products

After a complete method validation, this GC-IMS technique could be implemented in the production sites

REFERENCES

- M.Suman et al- MOS based artificial olfactory system for the assessment of egg products freshness- Sensors and Actuators B 125, 2007: 40-47
- Liu, Y. et al- Measurement of internal quality in chicken eggs using visible transmittance spectroscopy technology- Food Control 18 (1), 2007: 18-22
- A.Castanio et al- The colour of sun: sun-related differences in volatile compound composition among bare swallow eggs carrying and eyes of either sex- PLoS ONE 11(11), 2016

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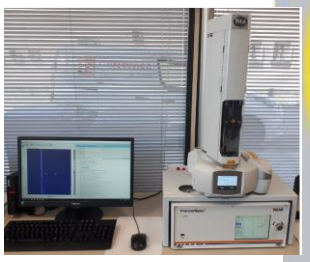
Summary

UV-IMS



- **”Fingerprint”** for fast classification (good, bad, fresh etc.) Single substances can be identified and quantified using
- **Sensitive** - Detection limits for VOCs in the low ppb_v / µg/L range

- **static headspace analysis**
- **automated sample handling**
- **solid / liquid / gaseous samples (headspace analysis)**
- **no sample pretreatment necessary**
- **No vacuum pump**
- **Only N_2 and power supply needed**