Workshop on ion chemistry and plasmas
Bratislav-2022

Plant IMS

Fast and Sensitive Detection of Plant Hormones by Ion Mobility Spectrometry

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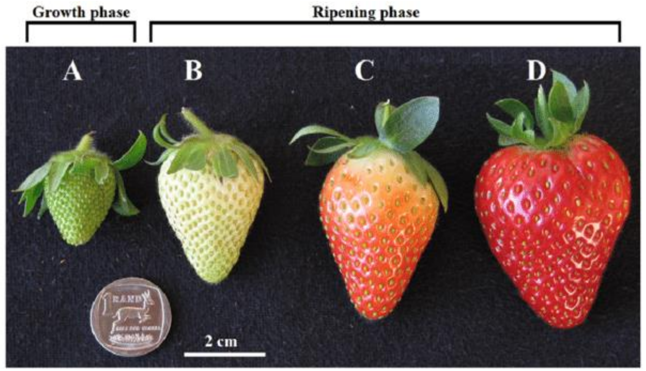
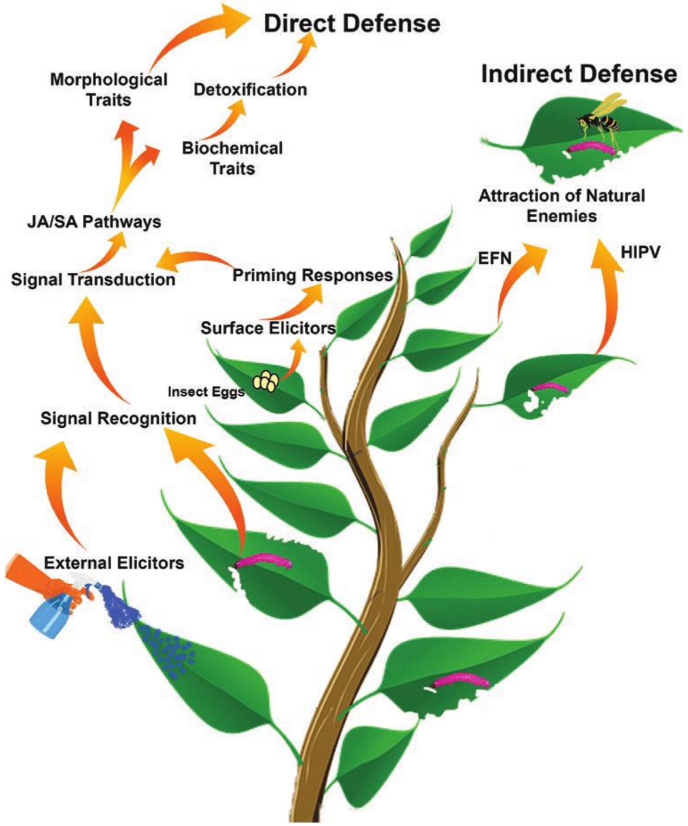
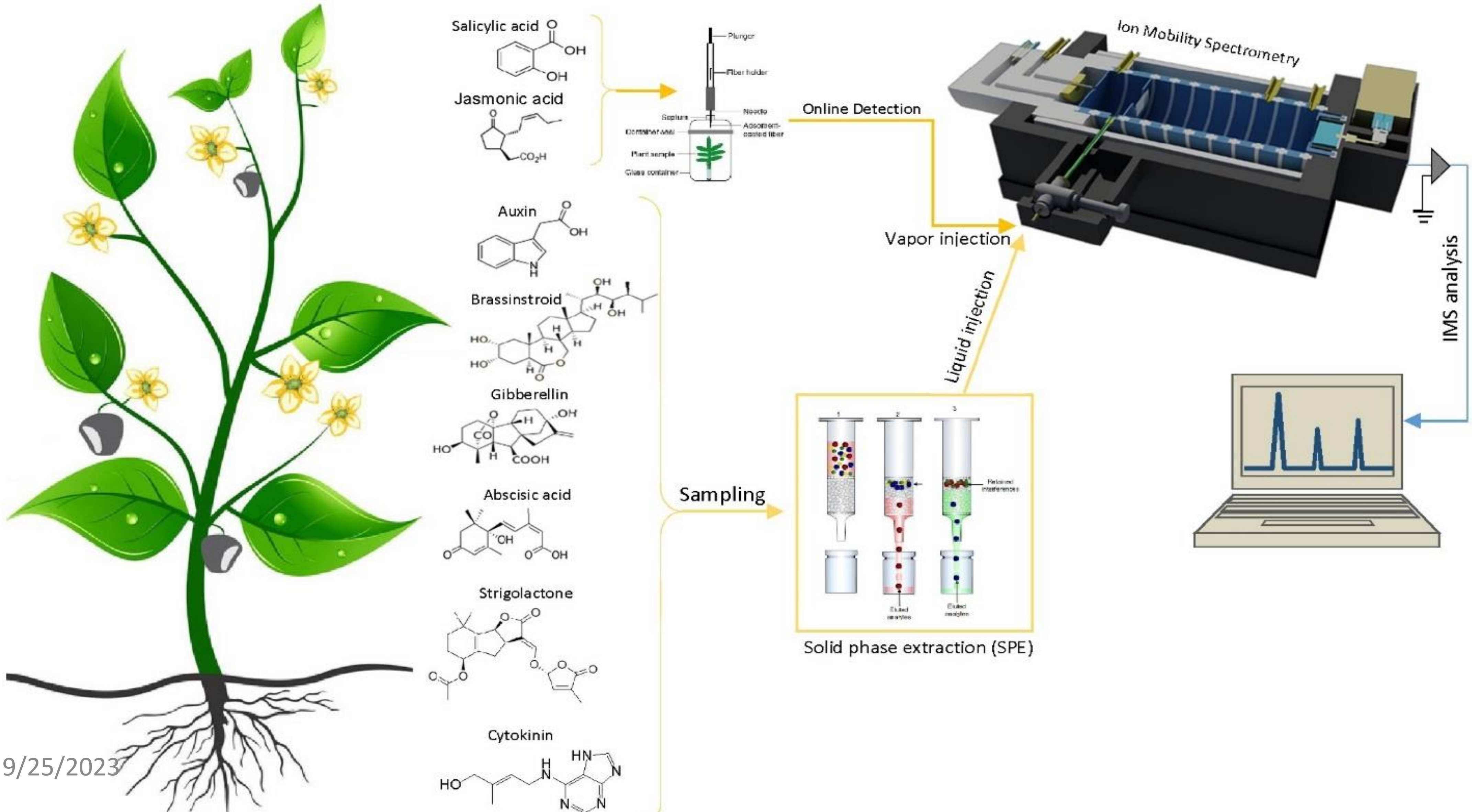
Plant Hormones

- The plant hormones (PHs), are signal molecules produced within plants that are at extremely low concentration.
- PHs influence the plant growth, seed germination, fruit maturation and fruit ripening.
- PHs play important roles in plant response to a wide range of biotic and abiotic stress.

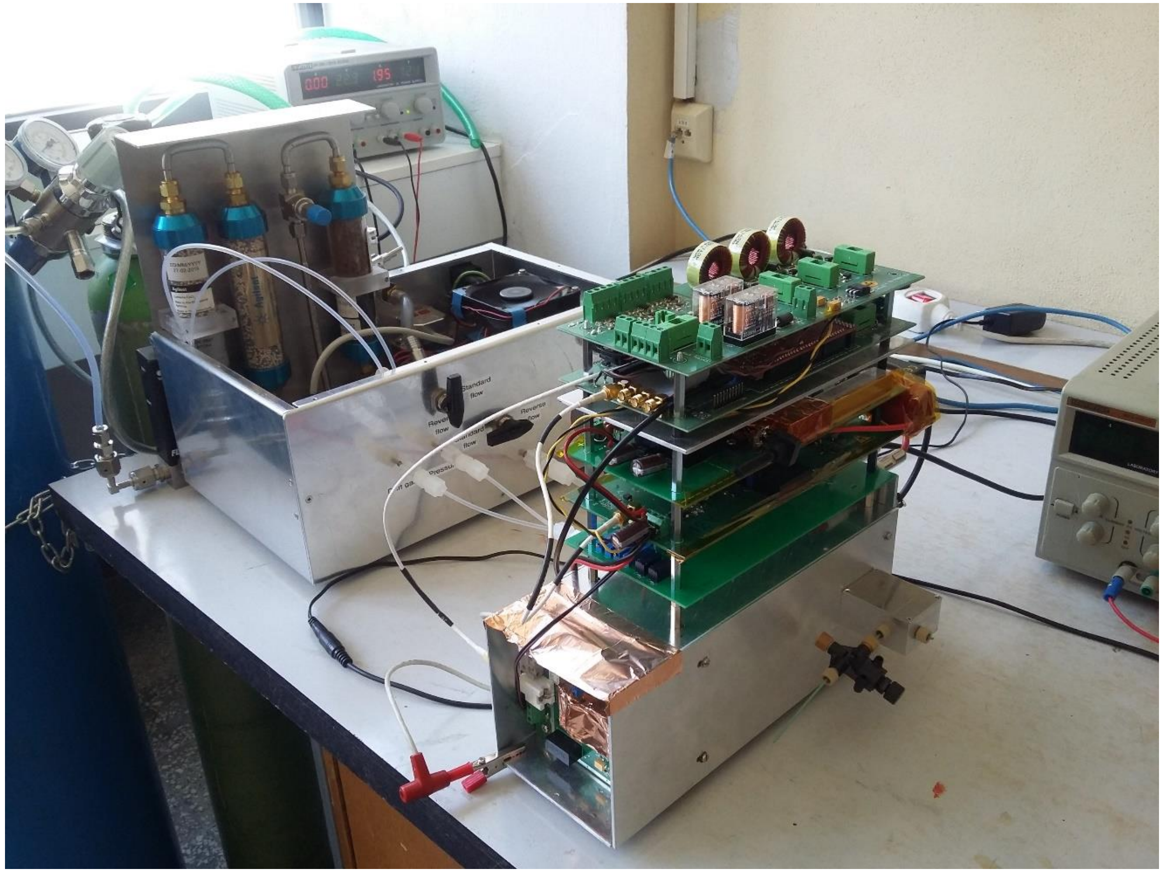




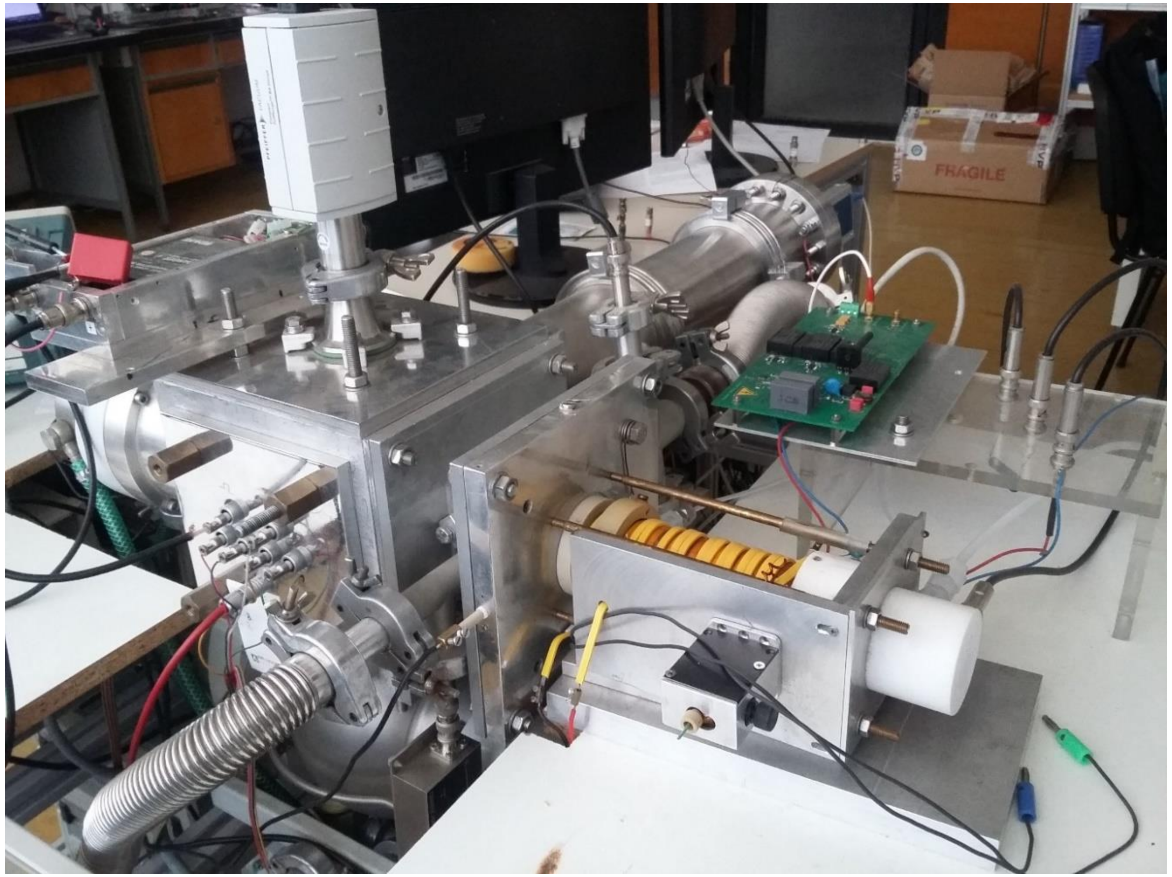
Aim of the project: Detection and quantification of PH by coupling MCC and SPME techniques with IMS-MS to achieve high sensitivity, selectivity and fast analysis



IMS & IMS-MS in our Lab



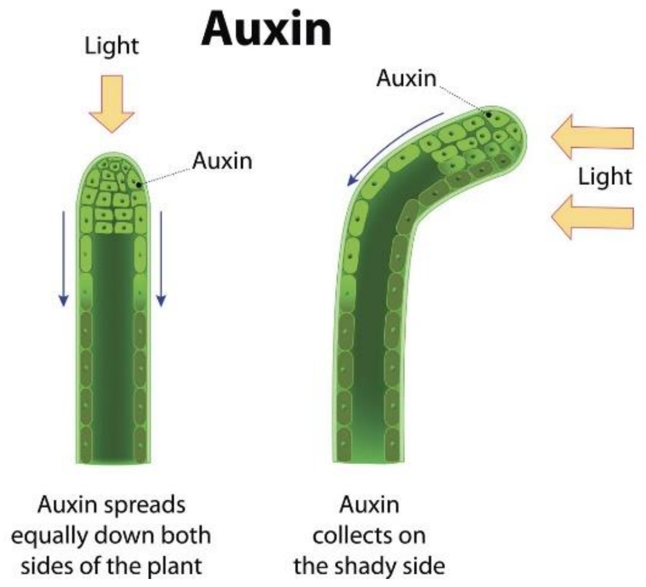
IMS



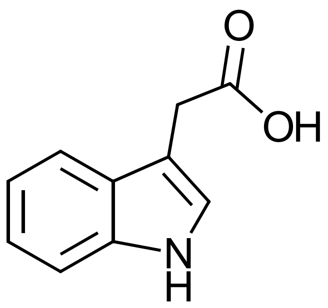
IMS-MS

Project 1: Auxins

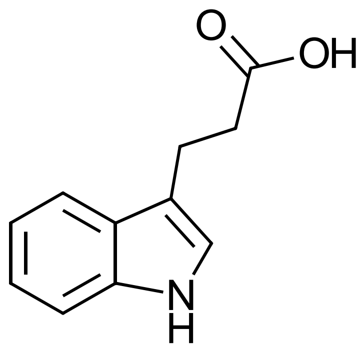
- Auxins are an important class of Plant Hormones
- Effects in plants: cell enlargement and stem growth, cell division, response to light, fruit ripening



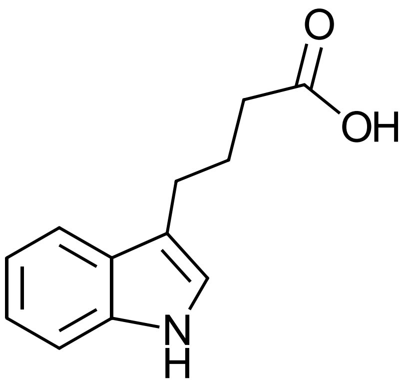
Structures of three auxins studied in this work:



IAA



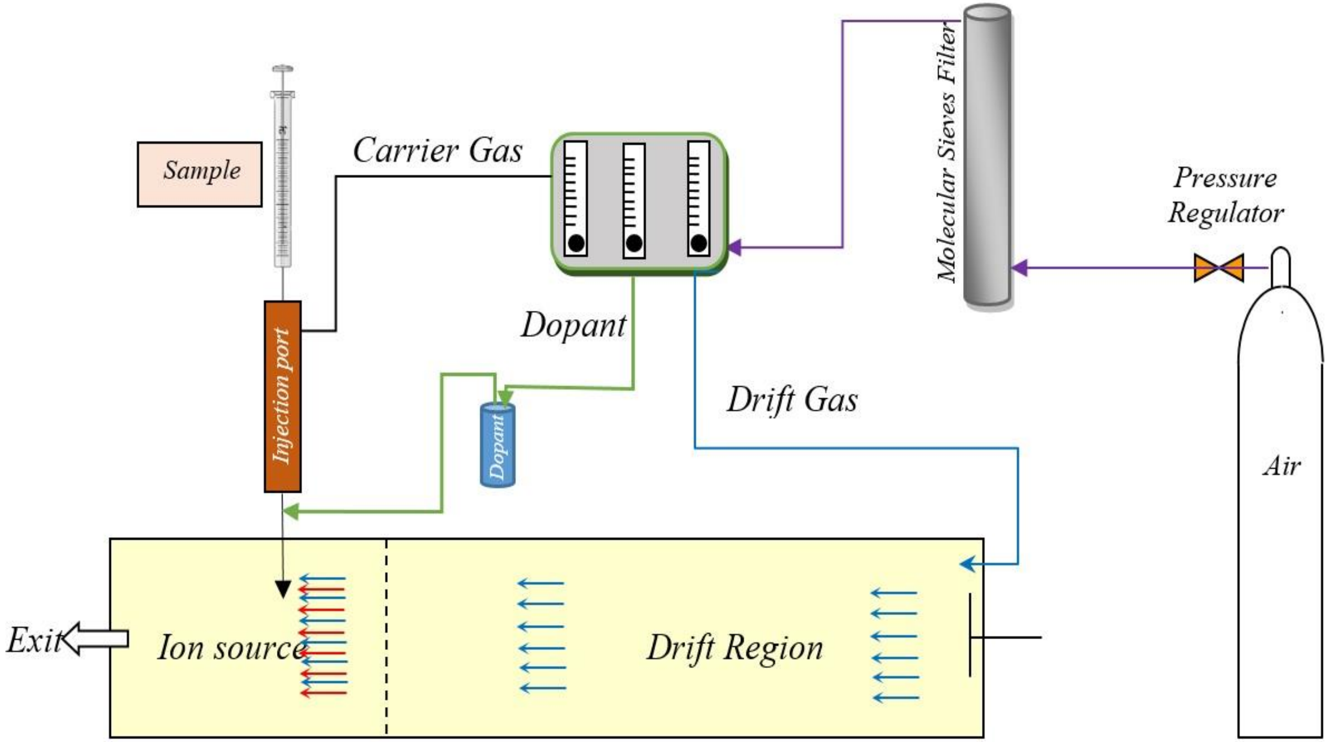
IPA



IBA

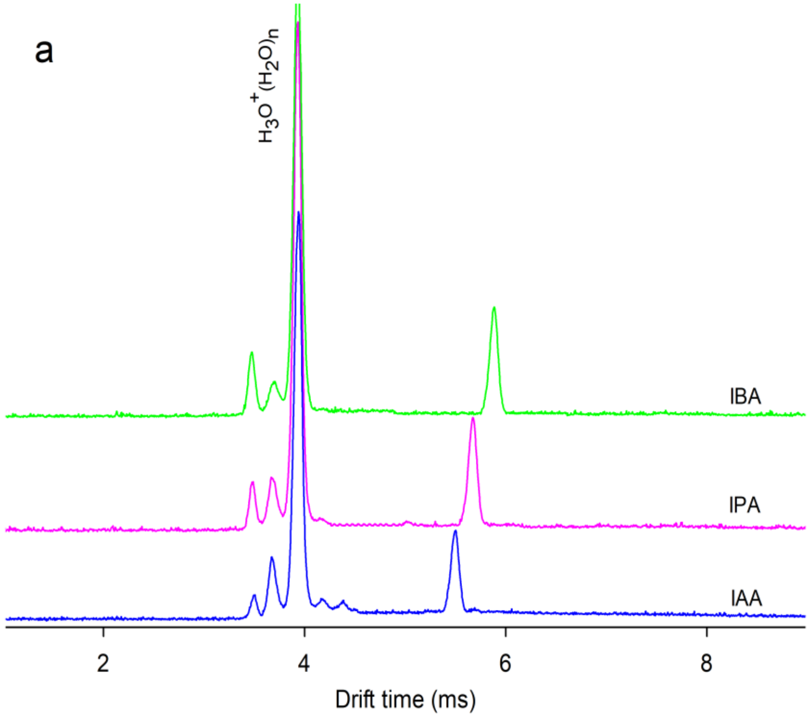
Chemical structures of indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), and indole-3-butyric acid (IBA)

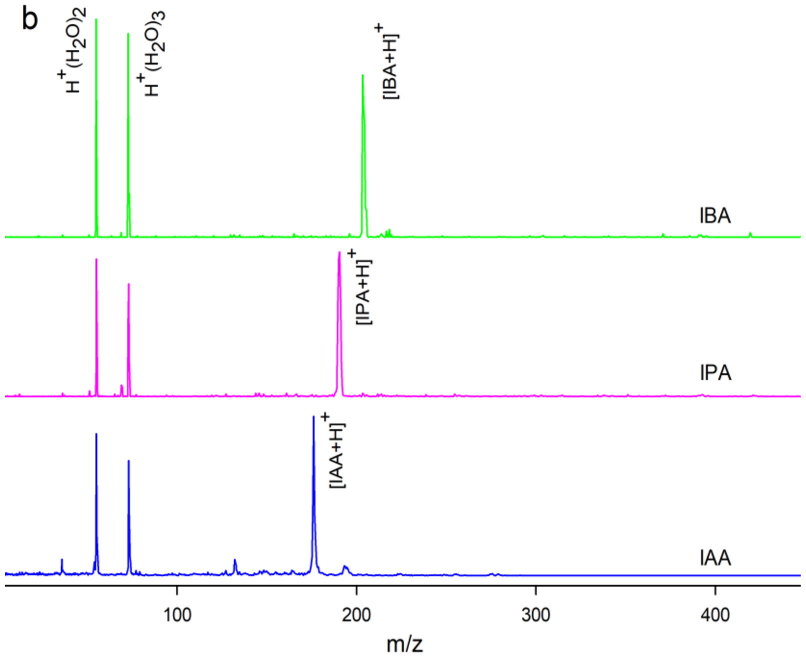
Experimental set up for injection of Auxin samples and dopants



Schematic representation of the experimental setup and the gas flow paths. The flow rates of drift, carrier and dopant gases are 700, 50, and 5 mL min^{-1}, respectively

<u>Positive mode</u>: (a) IMS and (b) MS spectra of IAA, IBA, and IPA



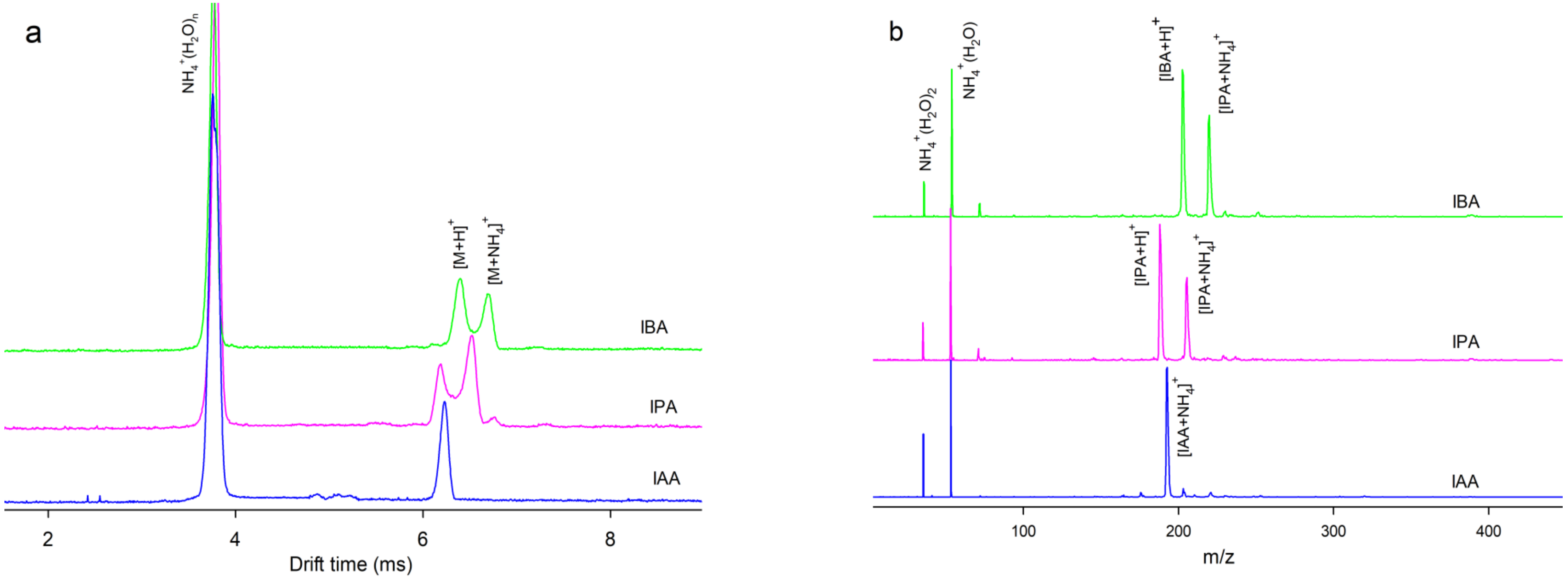


Compound	PA (kJ mol^{-1})	GB (kJ mol^{-1})
H_2O	687.8	659.5
NH_3	852.8	824.5
IAA	850.6	816.3
IPA	867.8	828.4
IBA	865.0	828.5

B3LYP-calculated proton affinities (PA) and gas phase basicities (GB) of the auxins

- Auxins are ionized by protonation
- Protonation of Auxins by H_3O^+ is thermodynamically possible

Positive mode: (a) IMS and (b) MS spectra with NH_3 dopant

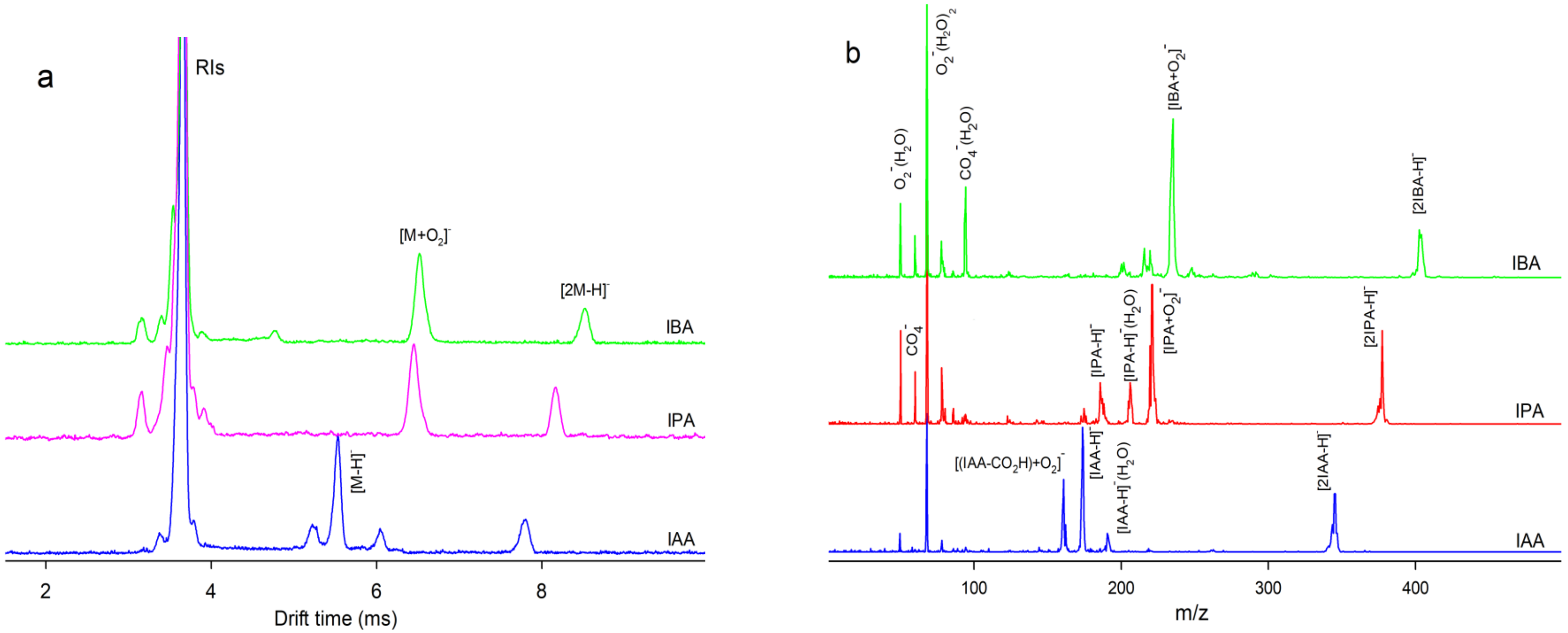


Protonation	ΔH	ΔG	NH_4^+ attachment	ΔH	ΔG
IAA + NH_4^+ → $[IAA+H]^+$ + NH_3	2.2	8.2	IAA + NH_4^+ → $[IAA+NH_4]^+$	-150.5	-103.6
IPA + NH_4^+ → $[IPA+H]^+$ + NH_3	-15.0	-3.9	IPA + NH_4^+ → $[IPA+NH_4]^+$	-112.4	-78.5
IBA + NH_4^+ → $[IBA+H]^+$ + NH_3	-12.2	-4.0	IBA + NH_4^+ → $[IBA+NH_4]^+$	-151.5	-102.8

B3LYP-calculated ΔH and ΔG for protonation of the auxins by NH_4^+ and their NH_4^+ attachment. The energies are in kJ mol^{-1}

- IAA is ionized only by NH_4^+ attachment→ one peak
- IBA and IPA: by protonation and NH_4^+ attachment

<u>Negative mode</u>: (a) IMS and (b) MS spectra of IAA, IBA, and IPA

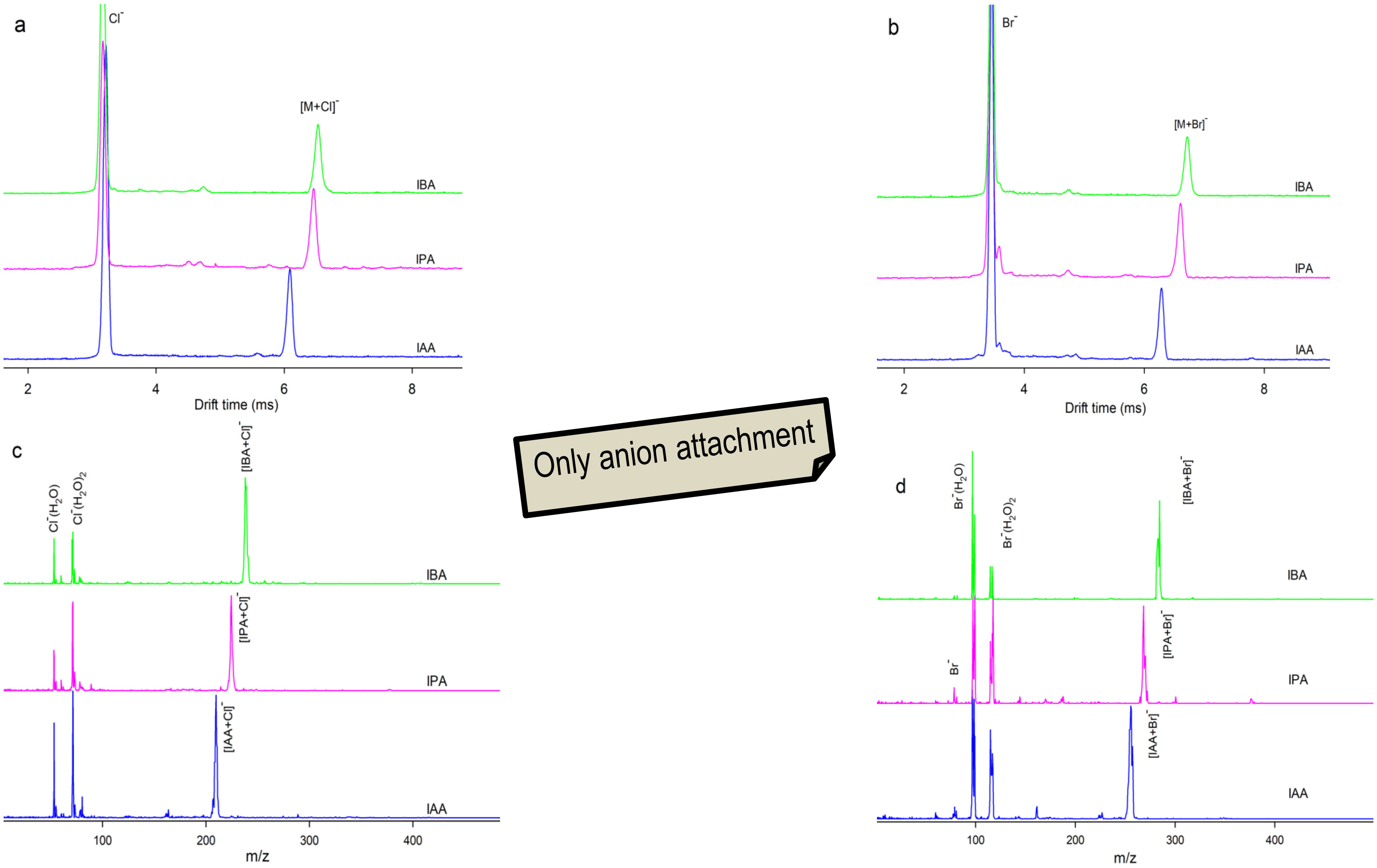


	Reaction	ΔH	ΔG
Deprotonation	IAA + O_2^- → $[IAA\text{-}H]^-$ + HO_2	-42.1	-50.7
	IPA + O_2^- → $[IPA\text{-}H]^-$ + HO_2	-40.9	-44.2
	IBA + O_2^- → $[IBA\text{-}H]^-$ + HO_2	-40.5	-42.5
Anion Attachment	IAA + O_2^- → $[IAA+O_2]^-$	-159.9	-130.5
	IPA + O_2^- → $[IPA+O_2]^-$	-165.2	-125.4
	IBA + O_2^- → $[IBA+O_2]^-$	-162.0	-121.6
Dimer formation	IAA + $[IAA\text{-}H]^+$ → $[2IAA\text{-}H]^+$	-114.0	-66.3
	IPA + $[IPA\text{-}H]^+$ → $[2IPA\text{-}H]^+$	-119.7	-72.4
	IBA + $[IBA\text{-}H]^+$ → $[2IBA\text{-}H]^+$	-123.8	-74.9

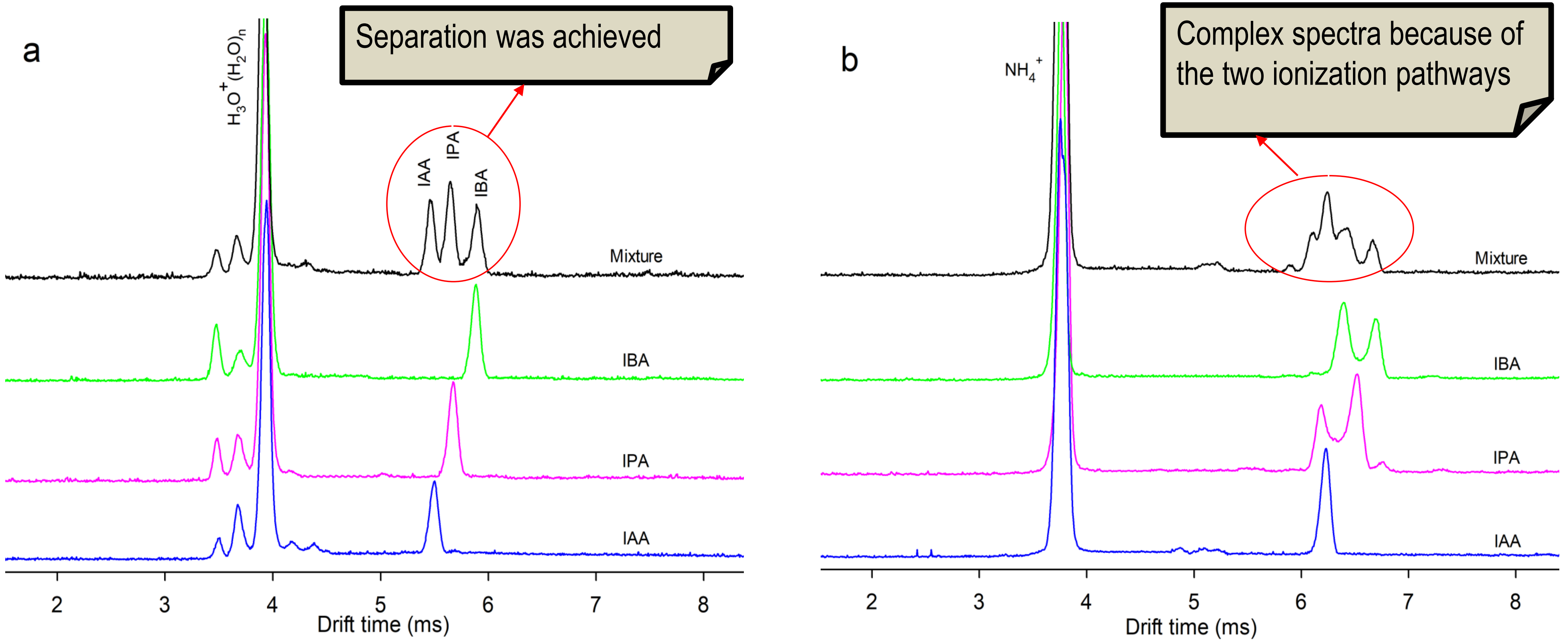
B3LYP-calculated ΔH and ΔG for ionization of auxins by O_2^-

- <u>IAA</u> is ionized mainly by deprotonation
- <u>IPA</u> is ionized by O_2^- attachment and deprotonation
- <u>IBA</u> is ionized mainly by O_2^- attachment
- <u>Dimer formation</u> is possible for all auxins

Negative mode: (a) , (b) IMS and (c), (d) MS spectra with CCl_4 and $CHBr_3$ dopants

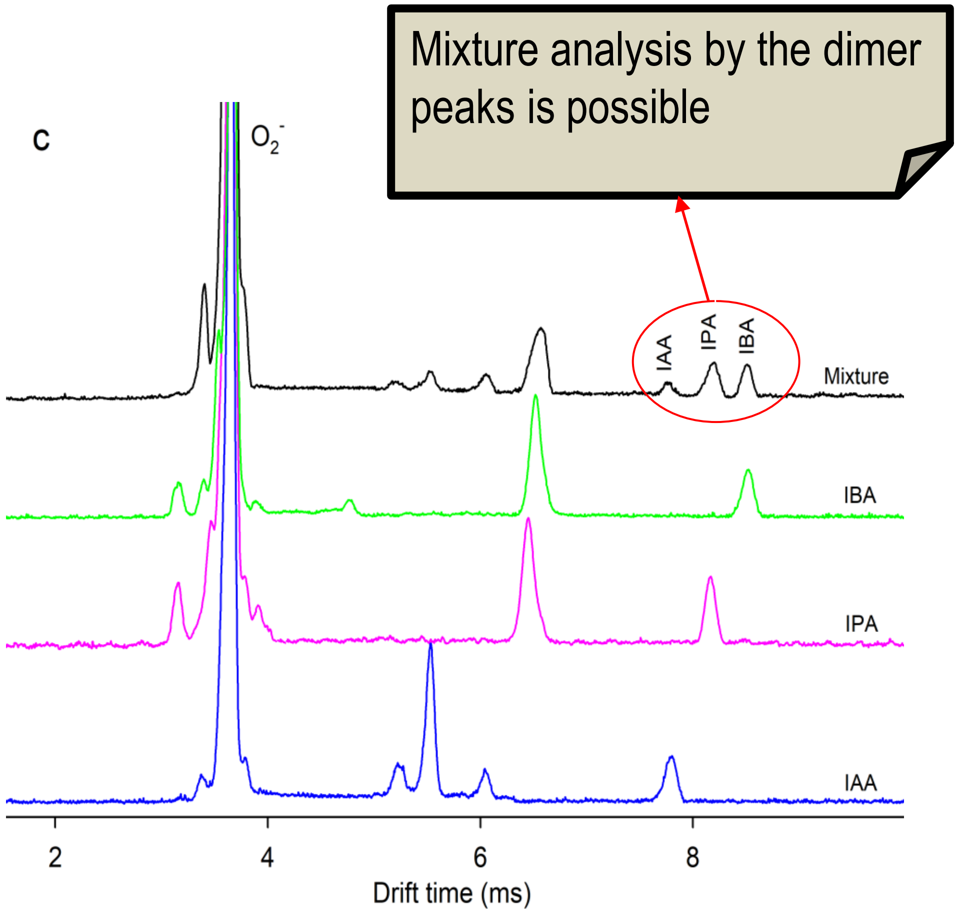


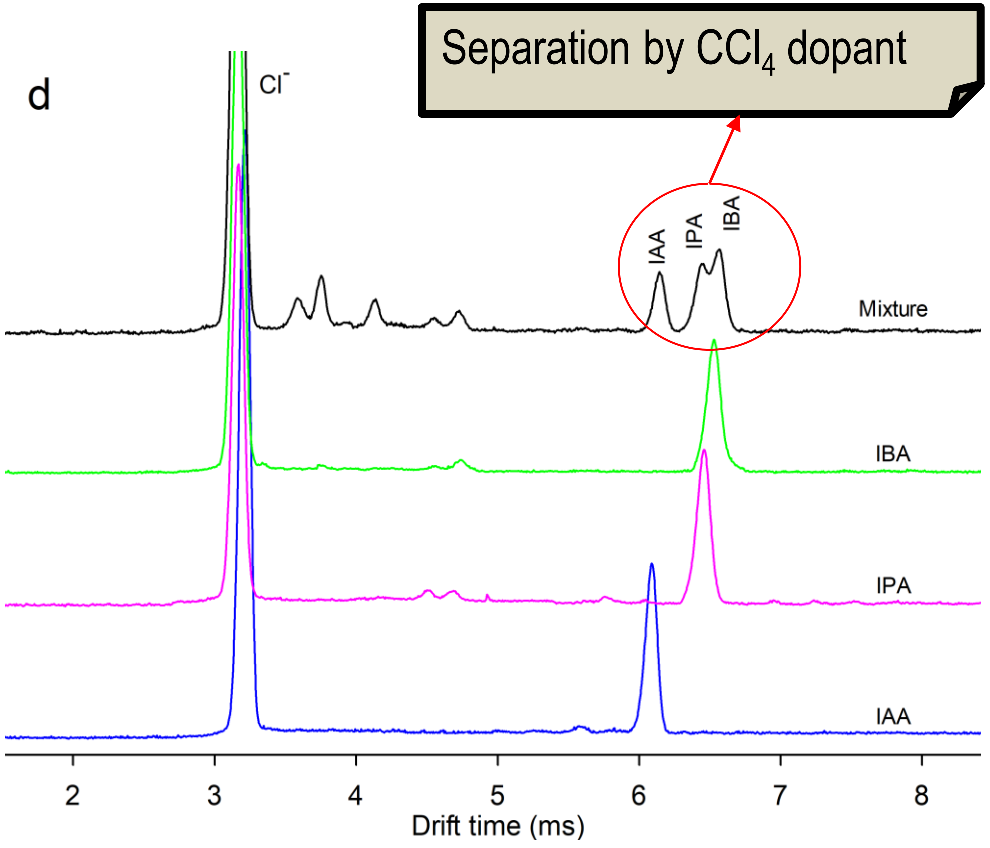
Auxin Mixture in Positive mode: IMS spectra (a) without and (b) with NH_3 dopant



IMS spectra for a mixture of IAA, IPA, and IBA in positive mode with and without NH_3 dopant

Auxin Mixture in Negative mode: IMS spectra (a) without and (b) with CCl_4 dopant





IMS spectra for a mixture of IAA, IPA, and IBA in negative mode with and without CCl_4 dopant

Quantitative Analysis:

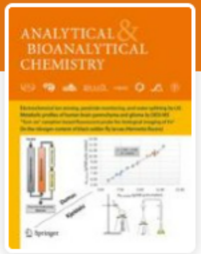
The LODs and linear ranges (in ng) for IAA, IPA, and IBA in the positive and negative polarities with different dopants

	IAA		**IPA**		**IBA**	
Polarity/dopant	Linear range	LOD	Linear range	LOD	Linear range	LOD
positive	15-60	5	10-60	4	15-60	6
Positive + NH_3	10-60	4	15-60	6	12-50	4
Negative	25-80	12	8-50	3	25-70	10
Negative + CCl_4	8-80	3	10-100	4	10-100	4
Negative + $CHBr_3$	15-60	5	15-90	5	12-100	4

- IMS showed high sensitivity toward auxins in ng
- The LODs are comparable with those reported for HPLC-FL

Dobrev PI, Havlicek L, Vanger M, Malbeck J, Kaminek M. *J. Chromatogr. A*. **2005**, 1075,159-166.

Published Paper for Project 1



Analytical and Bioanalytical Chemistry

Analytical and Bioanalytical Chemistry (2022) 414:6259–6269
https://doi.org/10.1007/s00216-022-04198-x

RESEARCH PAPER

Check for updates

Effect of ion source polarity and dopants on the detection of auxin plant hormones by ion mobility-mass spectrometry

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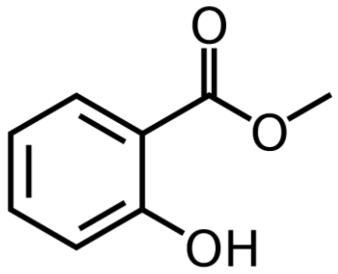
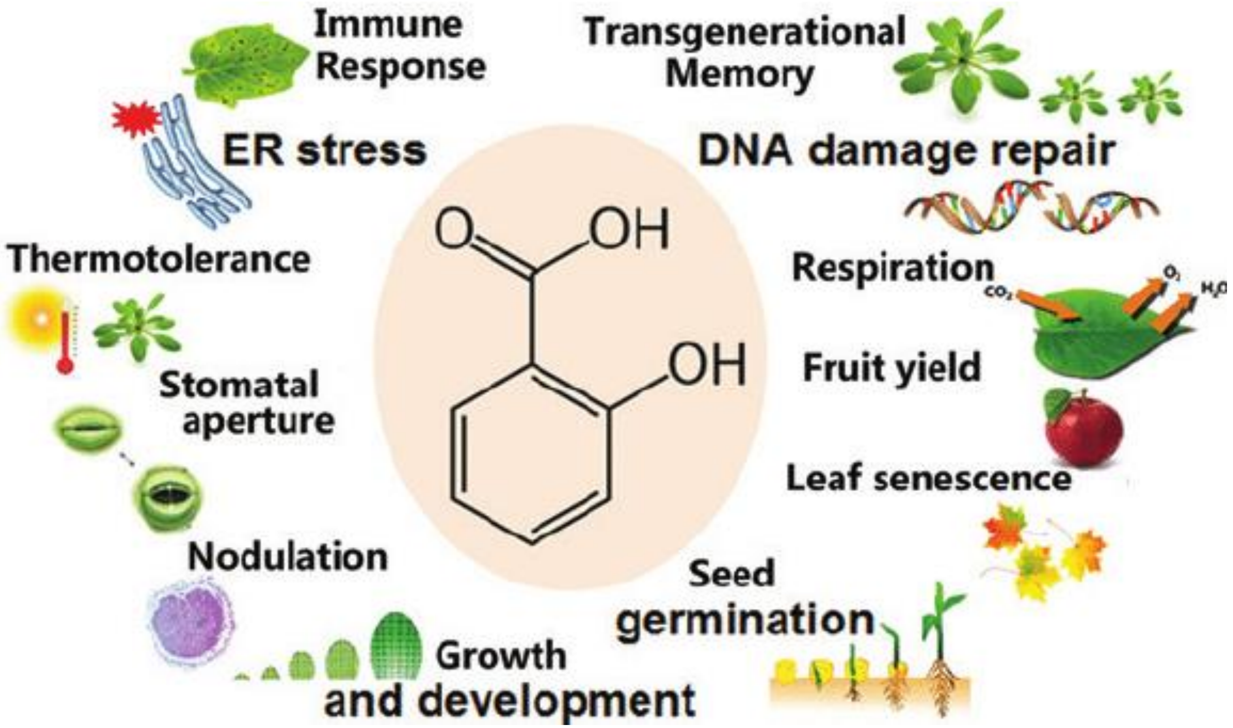
Project 2: Salicylates (SAs)

Methyl salicylate (MeSA) is synthesized from **salicylic acid (SA)** in plant

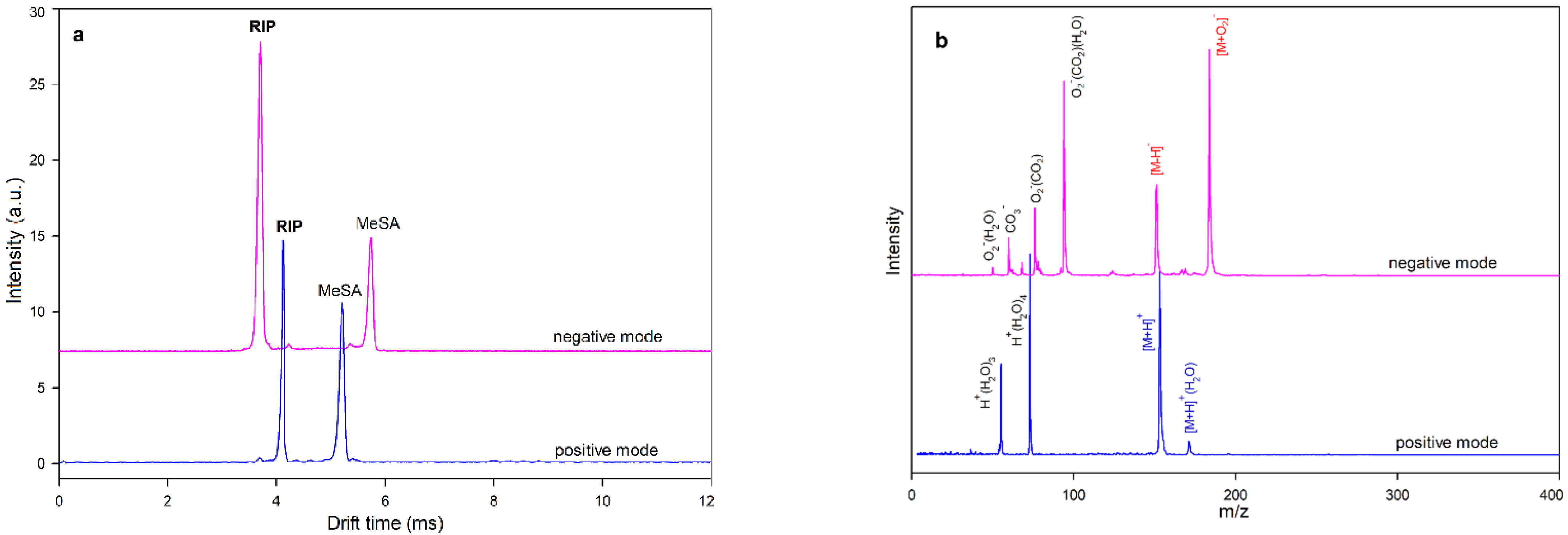


Methyl salicylate

SAs roles in plants



<u>Standard MeSA</u>: (a) IMS and (b) MS spectra in positive and Negative Polarities

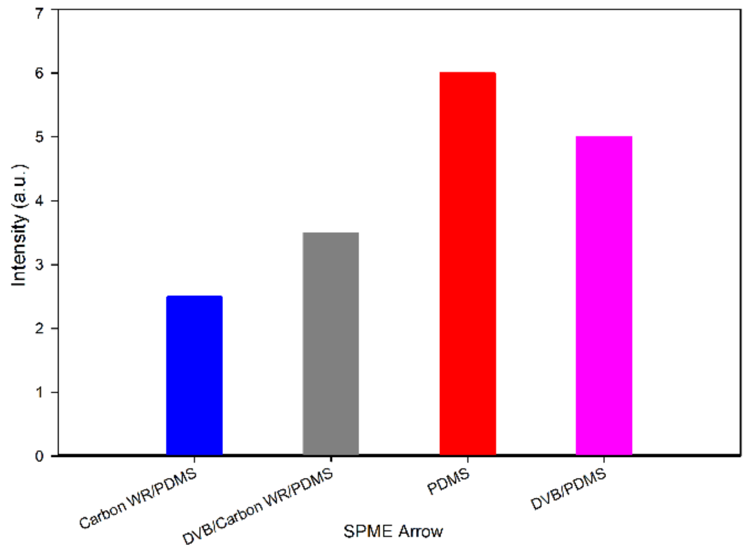


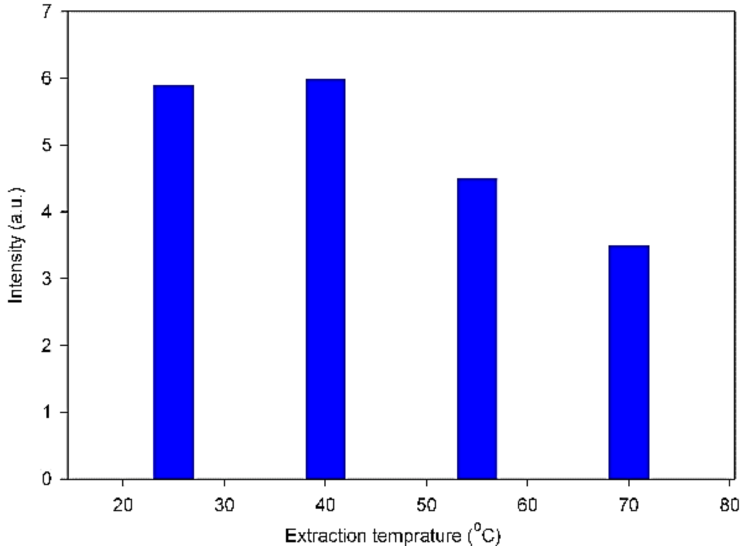
- <u>Positive Mode:</u> Ionization by protonation
- <u>Negative mode</u>: Ionization by deprotonation and O_2^- attachment

Selection and Optimization of SPME

MeSA concentration: 2 µg mL^{-1}
Extraction time: 30 min

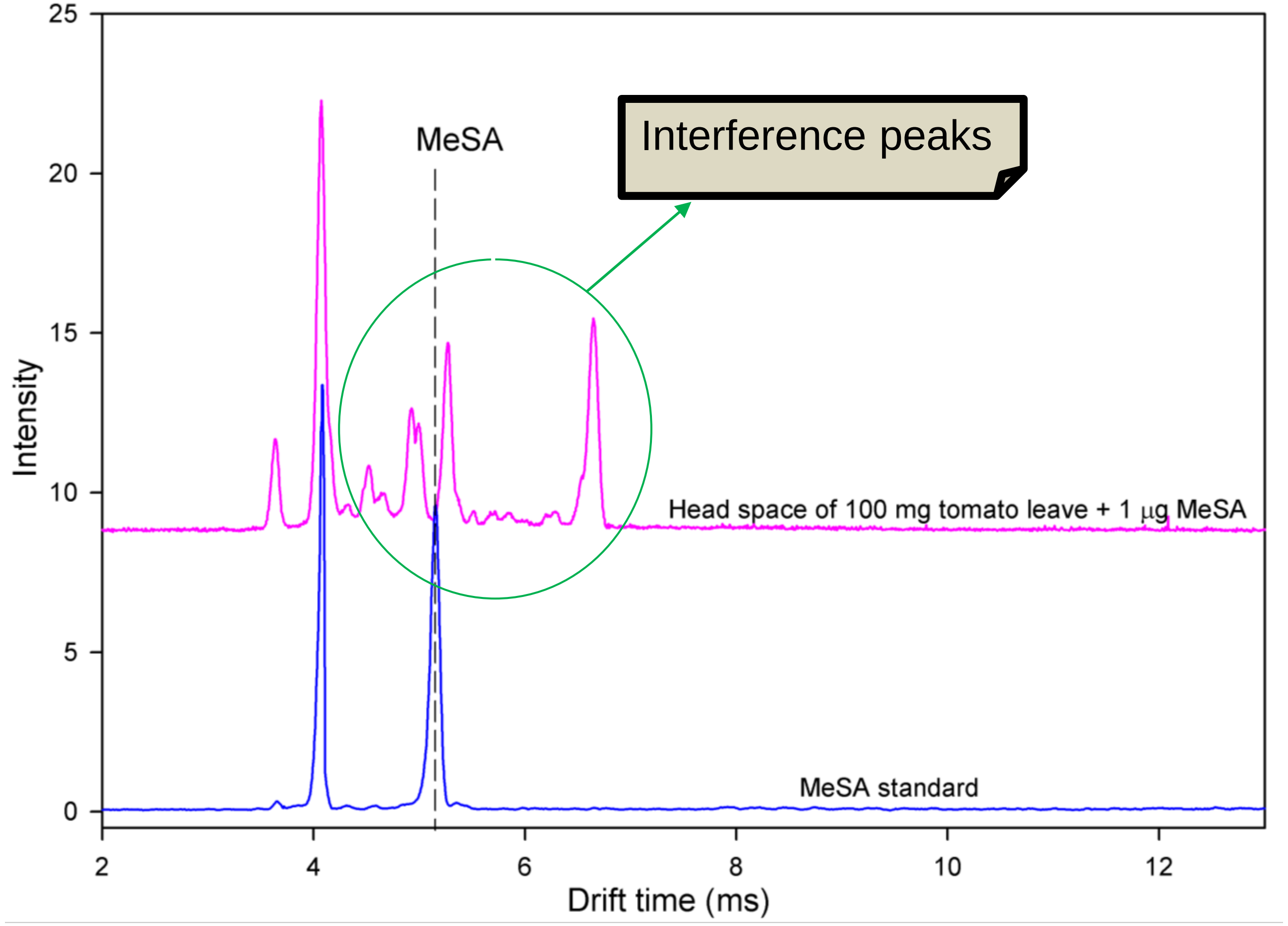
SPME Arrow	Diameter	material
	1.1 mm	100 µm Polydimethylsiloxane (PDMS)
	1.1 mm	120 µm Divinylbenzene (DVB)/PDMS
	1.1 mm	120 µm Carbon Wide Range (WR)/PDMS
	1.1 mm	120 µm DVB/Carbon WR/PDMS





- PDMS fiber was selected for SPME
- Optimum Extraction time was 20 min

Real Sample Analysis by SPME-IMS: Detection of MeSA in Tomato Leaves

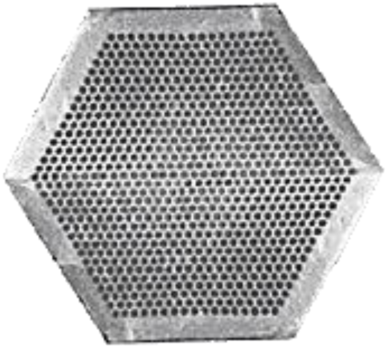


Positive mode

SPME-IMS measurement: MeSA detection is not possible in tomato leaf

Interference removal by MCC separation is necessary

Multicapillary column (MCC): Four commercial types

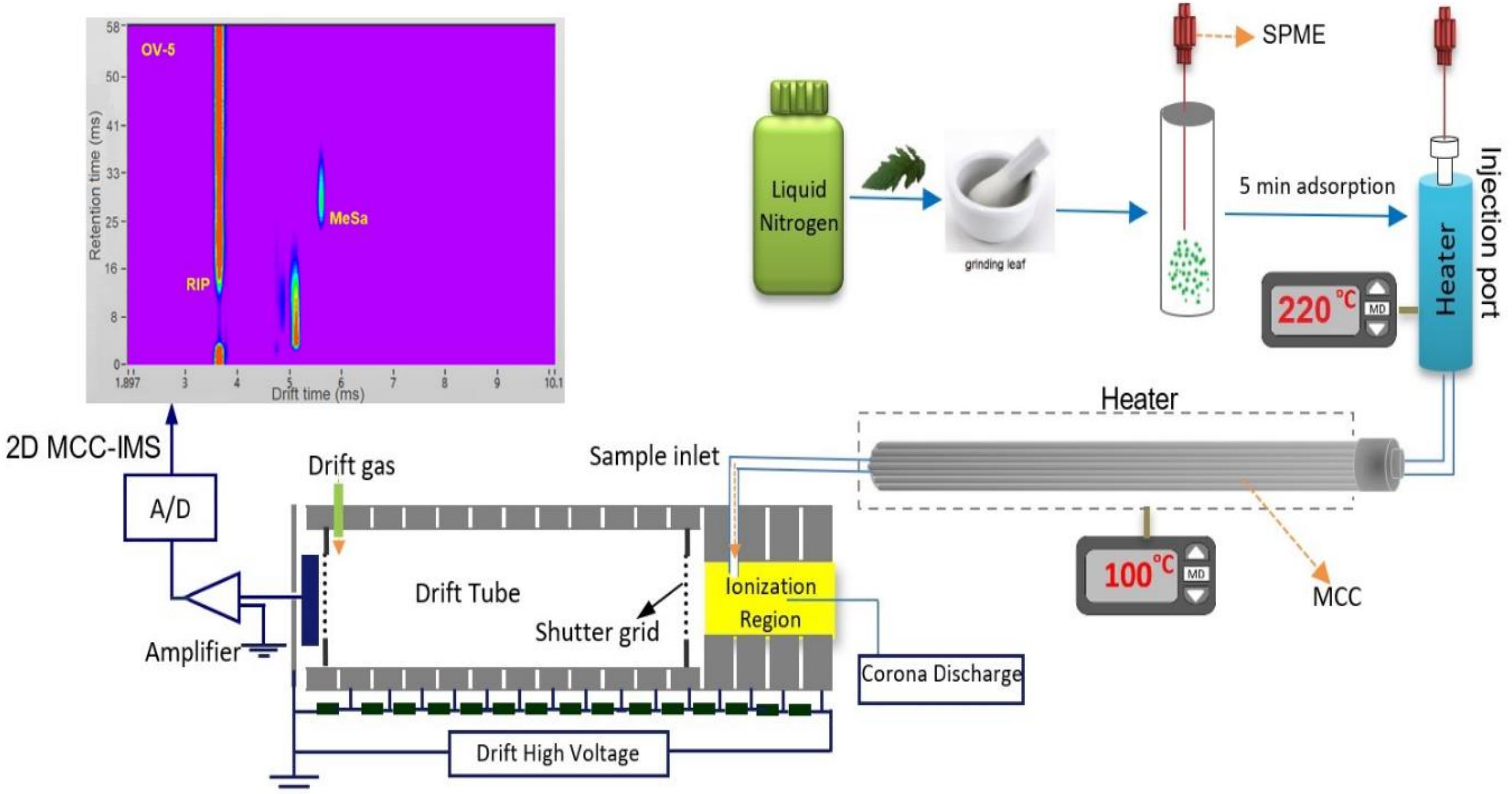


Cross-section of a MCC

	Stationary Phase Description
OV-1	100% - polydimethylsiloxane, non-polar
OV-5	5% - diphenyl, 95% - dimethylpolysiloxane, non-polar
OV-17	50% - diphenyl, 50% - dimethylpolysiloxane, weak polar
OV-20	20% - diphenyl, 80% - dimethylpolysiloxane, weak polar

Multichrom Ltd. Russia

Experimental setup for Real Sample Analysis: SPME-MCC-IMS



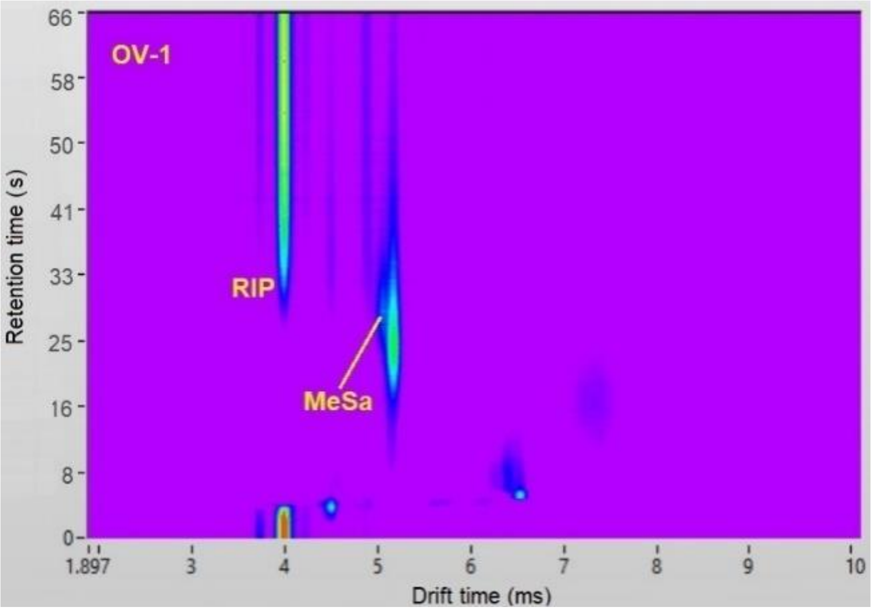
SPME: Solid Phase Micro-Extraction

MCC: Multi-Capillary Column

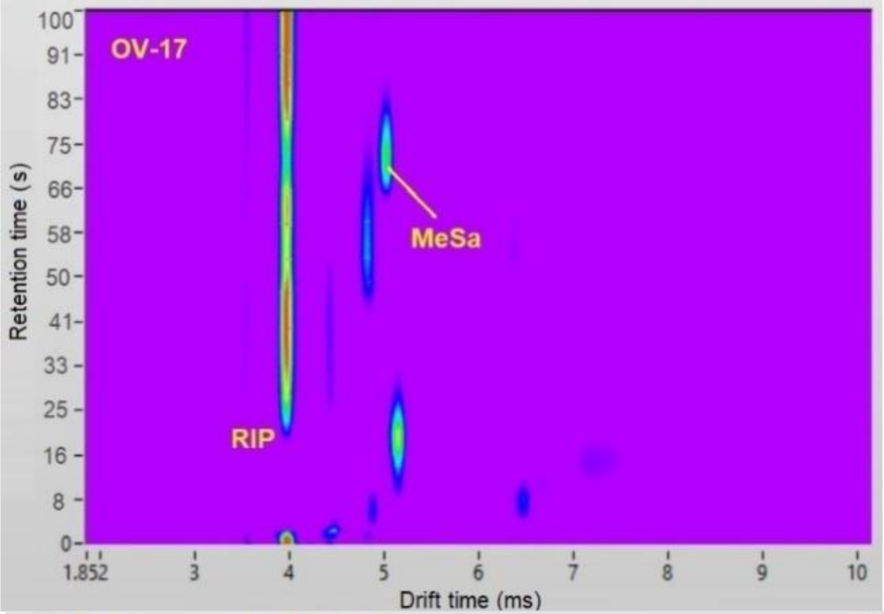
Schematic presentation of the method and instrumentation

<u>2D MCC-IMS plots</u>: SPME-MCC-IMS analysis of tomato leaves in Positive mode

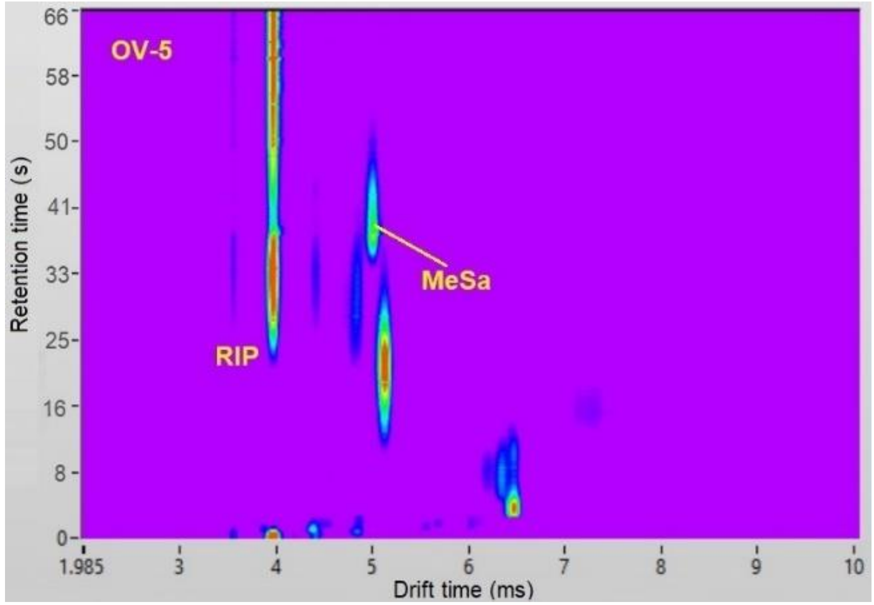
OV-1



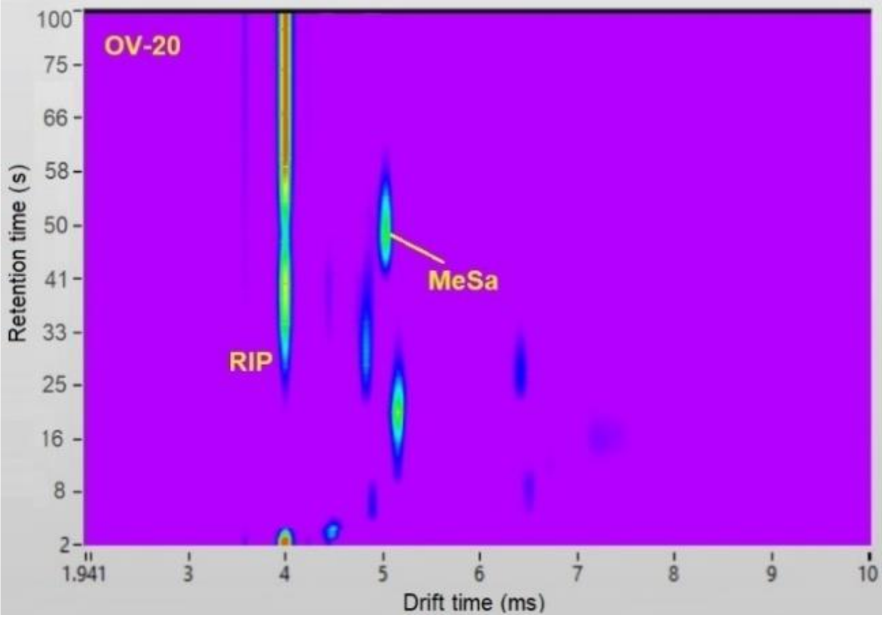
OV-17



OV-5

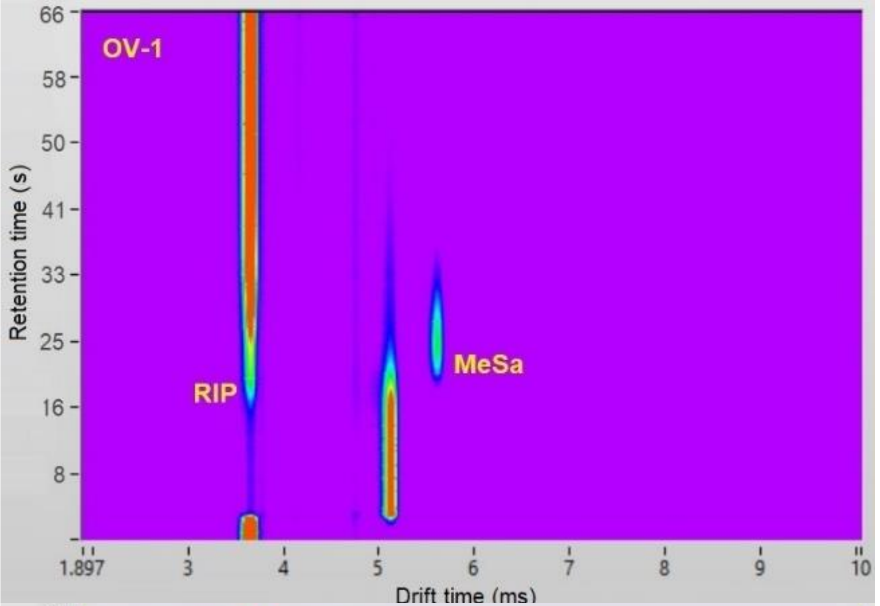


OV-20

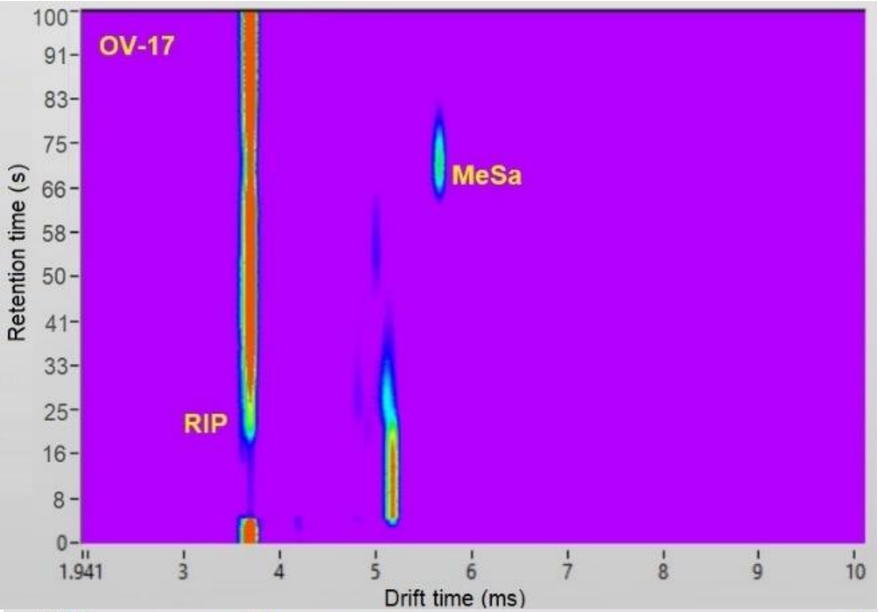


2D MCC-IMS plots: SPME-MCC-IMS analysis of tomato leaves in Negative mode

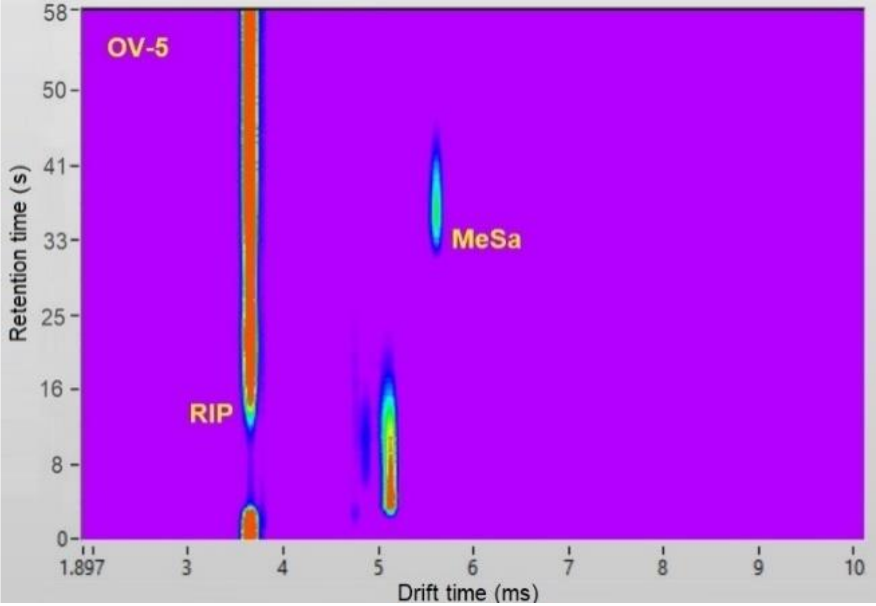
OV-1



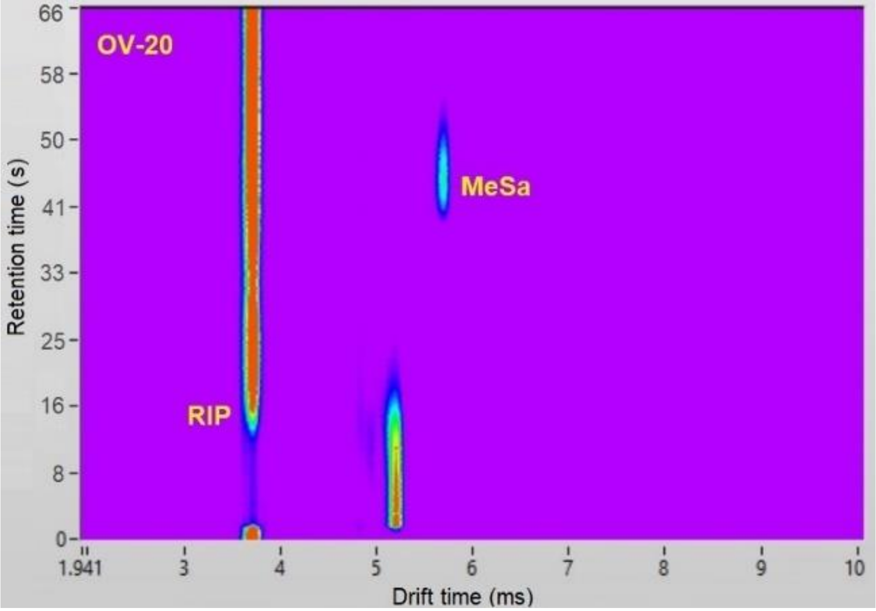
OV-17



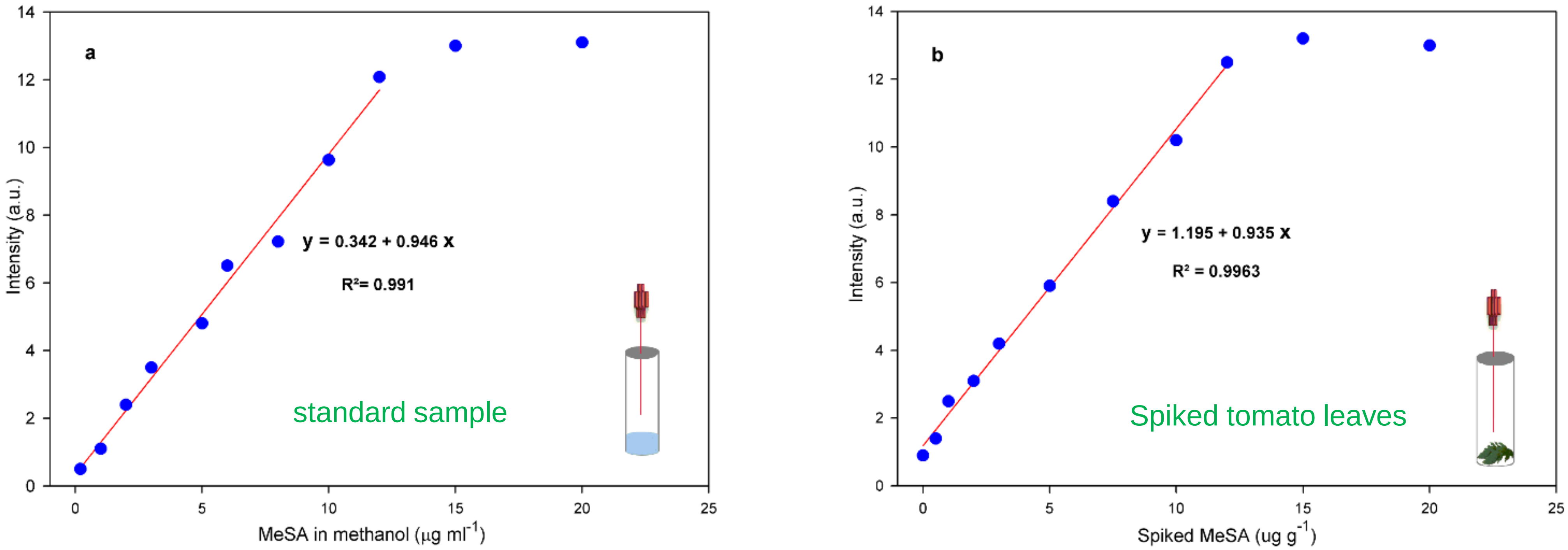
OV-5



OV-20



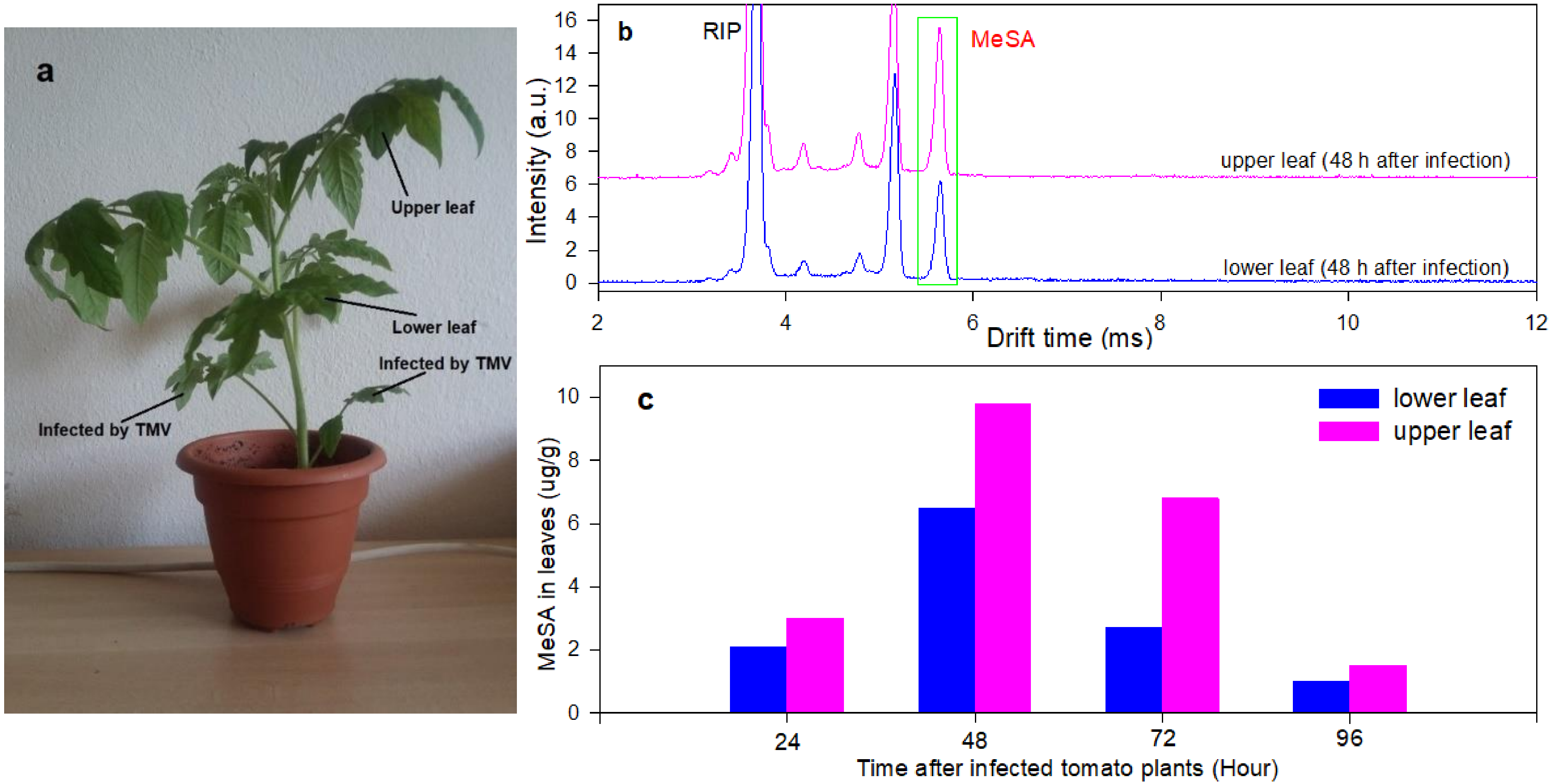
Quantitative Analysis by head space SPME-MCC-IMS



Linear range: 0.25-14 µg ml^{-1}

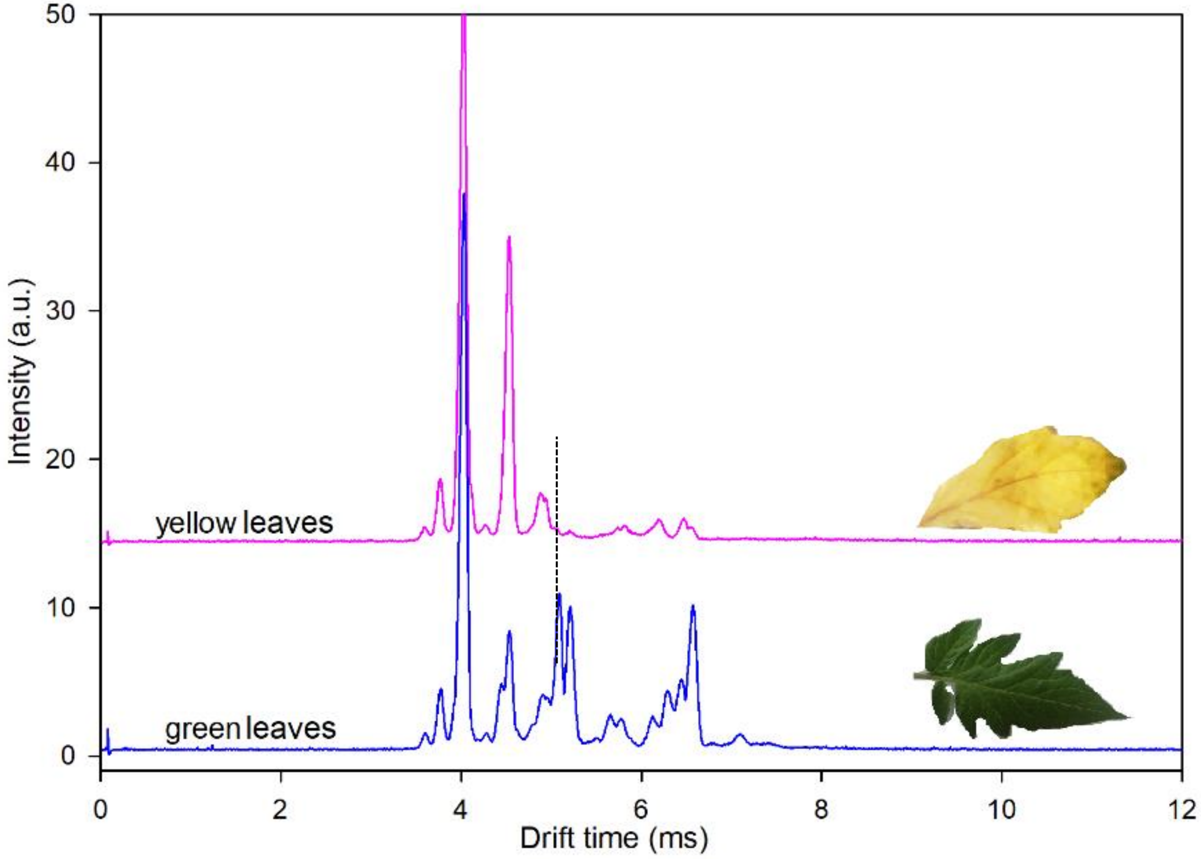
LOD: 0.1 µg ml^{-1}

Application of SPME-MCC-IMS: Monitoring MeSA in tomato leaves with time



The ToRSV inoculated, lower and upper leaves in a typical tomato plant. (b) The MCC-separated IMS spectra obtained 48 h after inoculation by ToRSV. (c) The measured MeSA content of upper and lower leaves 24 to 96 hours after inoculation.

Application of SPME-MCC-IMS: Comparison of MeSA content of different leaves



No MeSA was detected

Contains 10 μg g^{-1} MeSA

Conclusion: Advantages of the SPME-MCC-IMS method

- In this work, a new Ion Mobility Spectrometry (IMS) based method of MeSA detection in tomato leaves is presented. The method couples the Solid Phase Micro Extraction (SPME) sampling method, Multi Column Capillary Gas Chromatography (MCC GC) pre-separation and Ion Mobility Spectrometry (IMS) detection of MeSa from complex matrices.

- The developed method provides 2D-separation of the real sample ingredients resulting in fast analysis (<100s) and high sensitivity (0.1 µg g^{-1}) of MeSA detection in different parts of tomato leaves.

- The fast analysis of real samples allows time dependent measurements of MeSa after inoculation of plants by pathogens.

Project 2 Paper: Under review

Journal of Agricultural and Food Chemistry

Solid Phase Microextraction-Multi Capillary Column-Ion Mobility Spectrometry (SPME-MCC-IMS) for Detection of Methyl Salicylate in Tomato Leaves

Manuscript ID: jf-2022-05570p
Status: Under Review
Submitted on 12 August 2022

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