Ion mobility as additional Metrological Value – the Invaluable Benefit of another Dimension in Hybrid Mass Spectrometry

Michael McCullagh*[1], Markus Obkircher[2] and Jens Jacobsen[3]

[1]Waters Corporation, Wilmslow, United Kingdom
[2]Sigma-Aldrich Productions, Merck KGaA, Buchs, Switzerland
[3]Waters AG, Baden Dättwil, Switzerland

** Corresponding Author: Mike_McCullagh@waters.com*

“Ion path”

- A small review of Ion Mobility
  - History
  - Robustness, time, space, matrix and platform independence

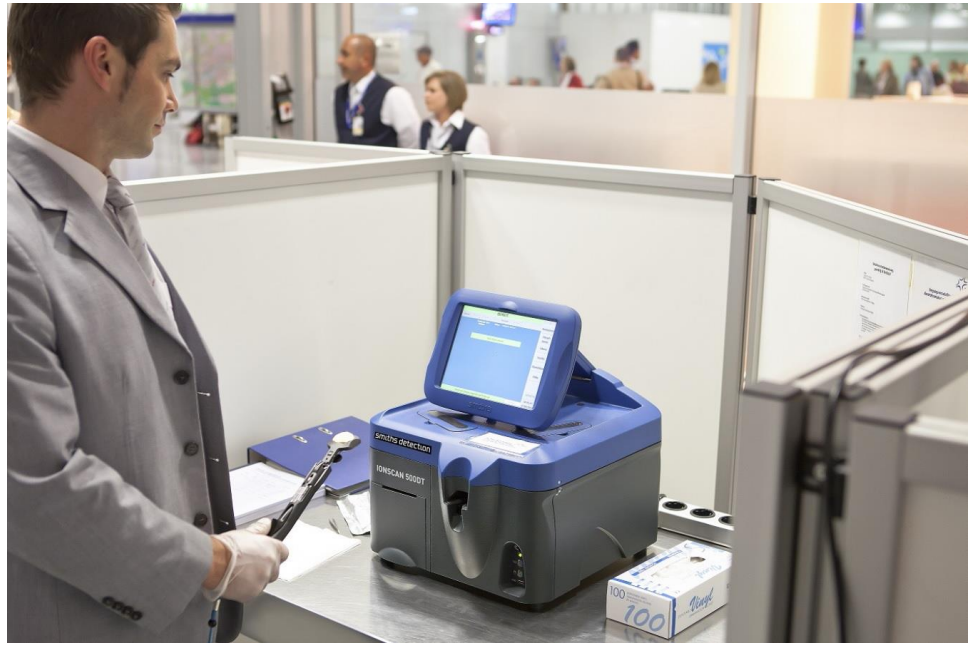
- Examples of IMS-HRMS:

- Summary

Firstly: what on earth has the mutiny on the HMS Bounty (1789) to do with this talk????

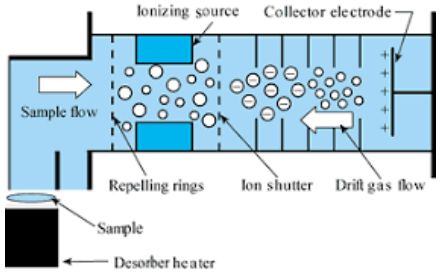
Ion Mobility Spectroscopy Standalone: Screening Instrument for Security/Military uses

Waters™



https://www.asiatraveltips.com/news20/412-AirportSecurity.shtml

The IONSCAN 500DT, certified on the TSA Qualified Products List for security screening, utilizes Ion Mobility Spectroscopy (IMS) to perform trace analysis of explosives in seconds. Easy to use, the operators can detect a wide range of military, commercial and homemade threats.

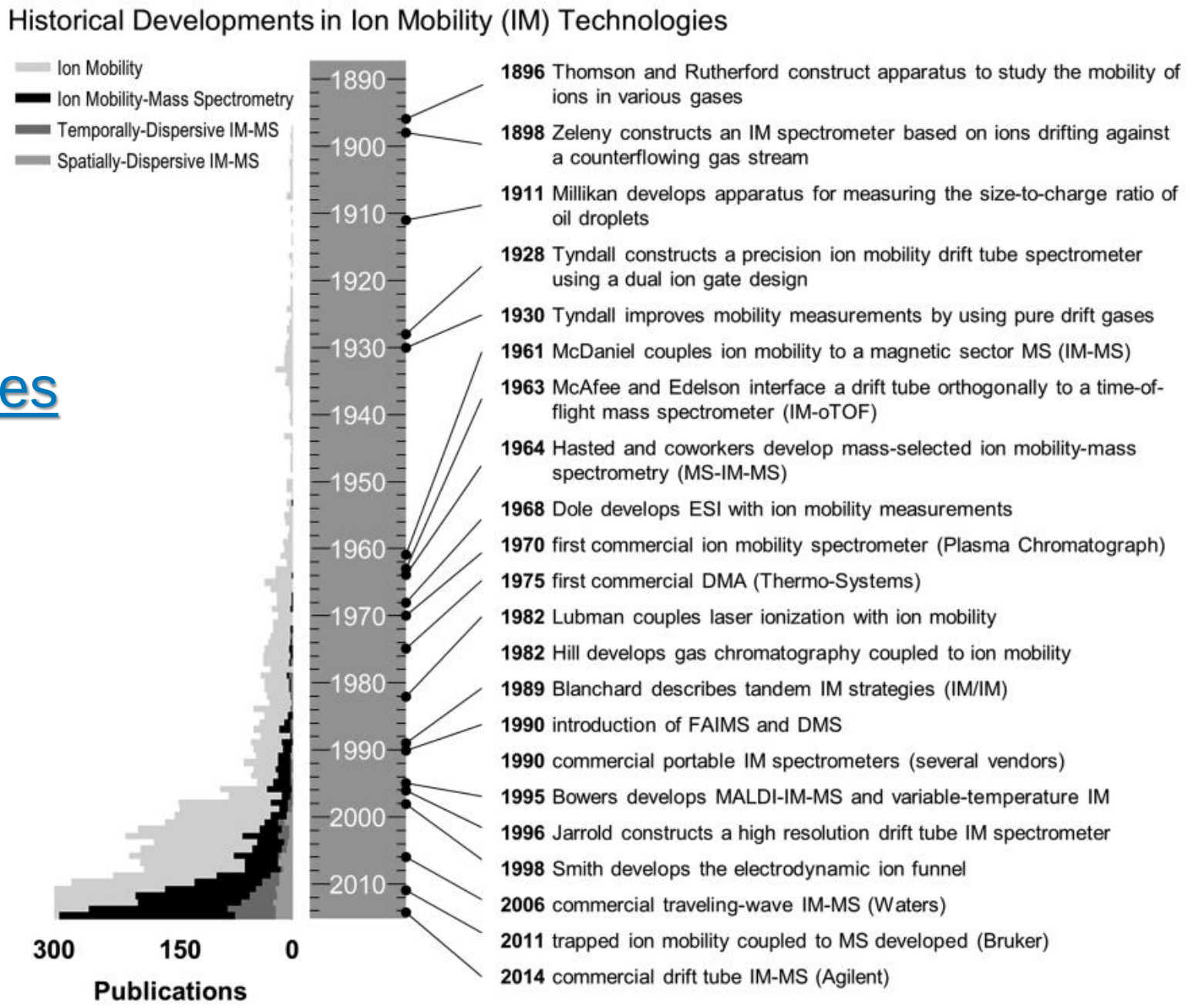


https://www.sas.upenn.edu/~lindamf/spectIMS.html

https://www.bruker.com/en/products-and-solutions/cbrne-detectors/ims.html

https://www.masatech.eu/blog

Historical view on Ion Mobility (IM) Technologies



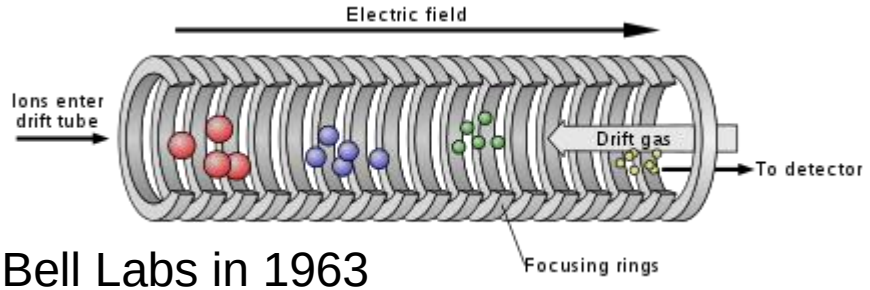
(Jody C. May and John A. McLean; Anal Chem. 2015 Feb 3; 87(3): 1422–1436)*

Ion Mobility Spectroscopy is OLDER than Mass Spectrometry!!

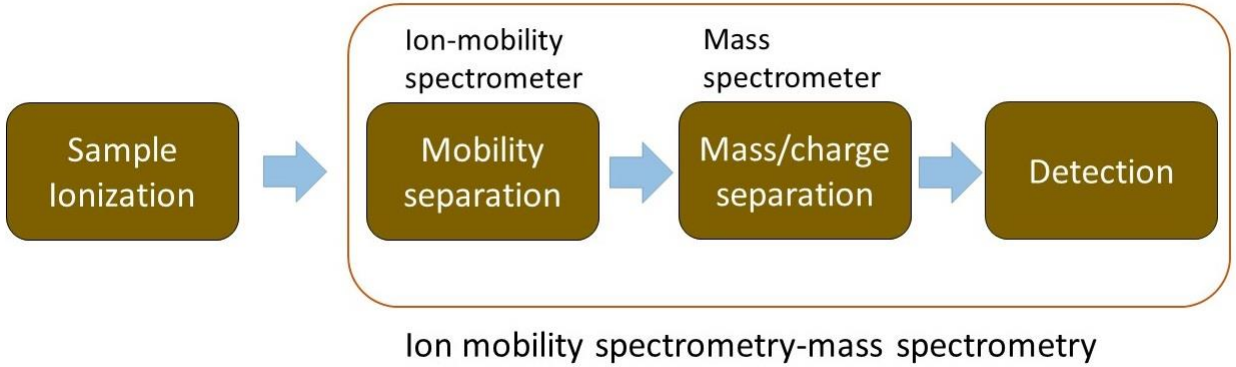
Waters™

Discovered by JJ Thompson and Rutherford 1896, but developed by EW McDaniel in 1950-1960s. First drift tube IMS was designed in 1960s

McDaniel E, Martin DW, Barnes WS (1962). "Drift Tube-Mass Spectrometer for Studies of Low-Energy Ion-Molecule Reactions". *Review of Scientific Instruments*. **33** (1): 2–7. Bibcode:1962RScI...33....2M. doi:10.1063/1.1717656. ISSN 0034-6748.

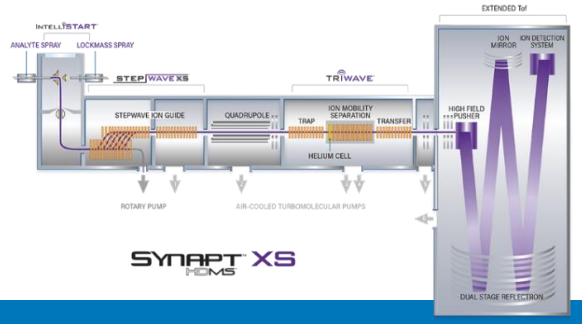


- First Hybrid IMS TOF MS was designed by Bell Labs in 1963



Ion mobility spectrometry-mass spectrometry

- First commercially available IMS QTOF 2006 (Waters)



What is Ion Mobility – Definition:

terminal velocity *v (of an ion) in a gasphase*

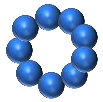
$$v_d = KE$$

E is the electric field strength and K is known as the ion's mobility.

CCS = f{K}, Linear Function of K

(CCS = Collisional Cross Section Size (in Area, $Å^2$))

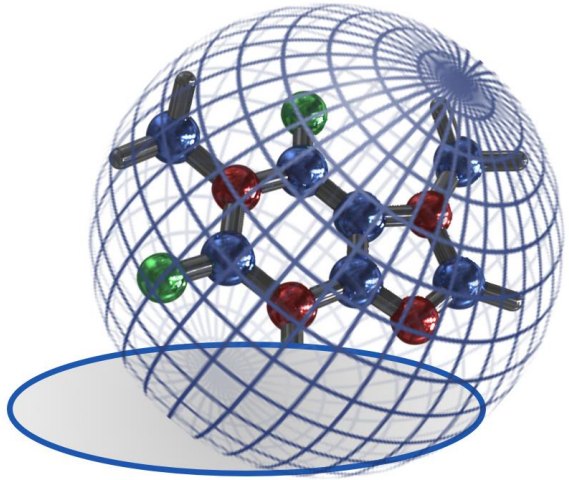
Ion-Mobility principle

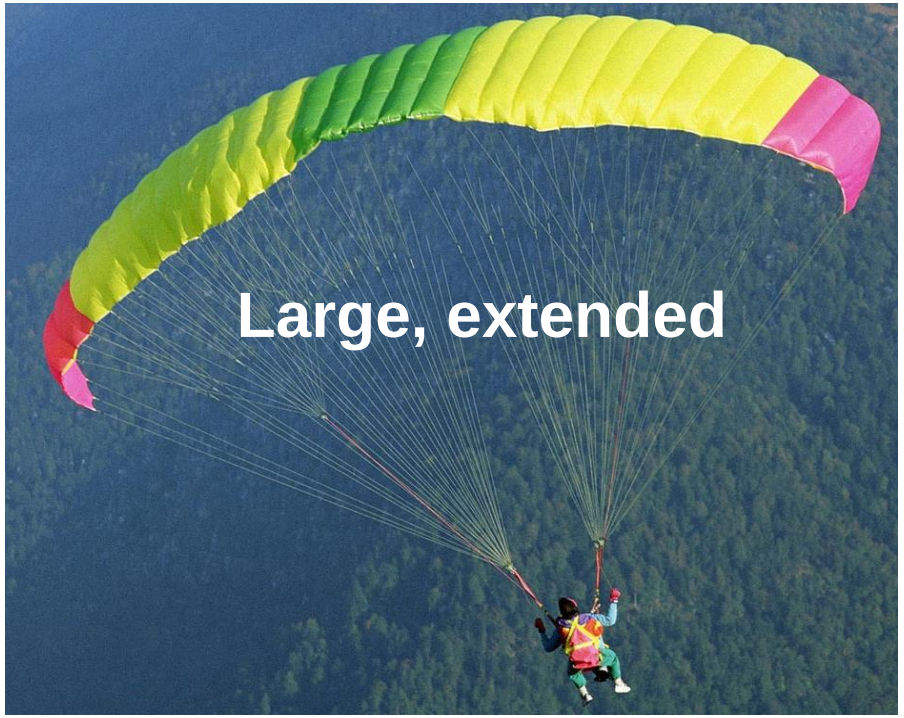


high mobility, more compact, less collisions with gas

low mobility, less compact, more collisions with gas

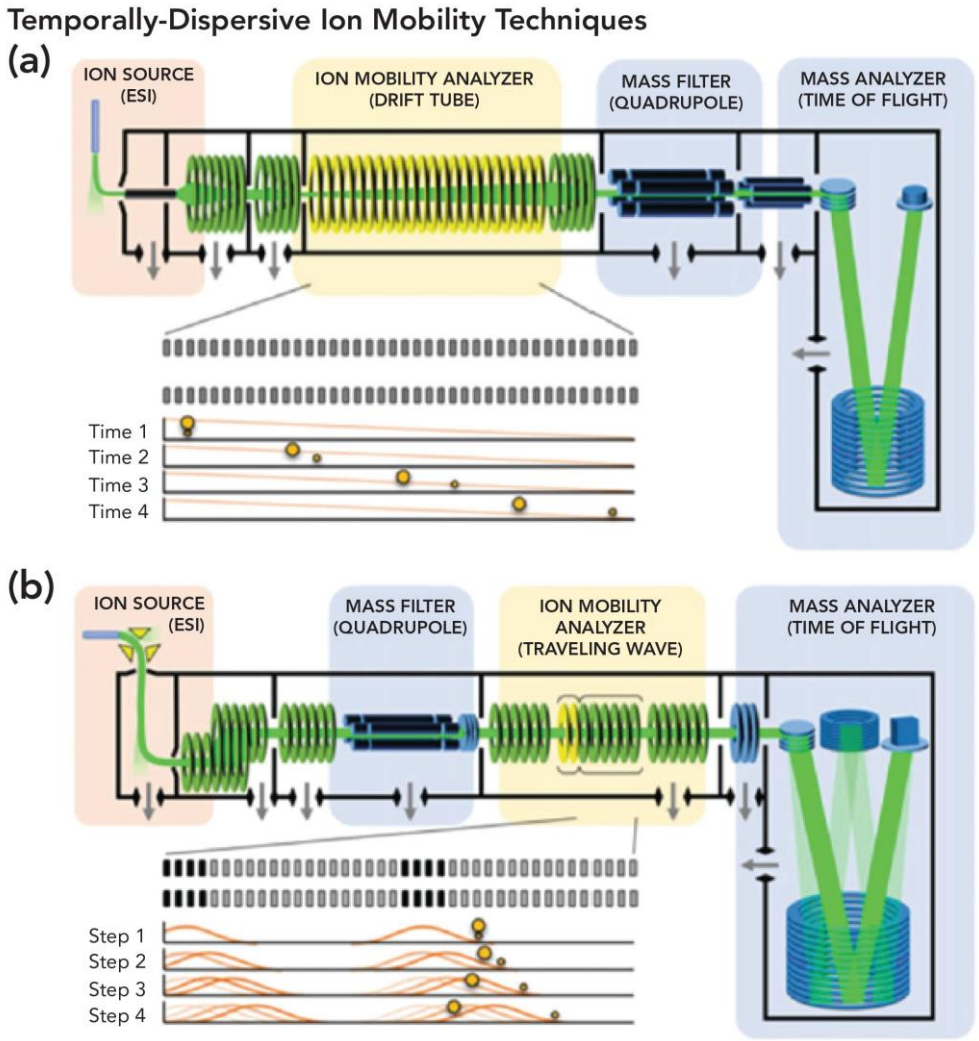
- CCS (~K) is an important distinguishing characteristic of an ion which is related to:
  - chemical structure
  - 3-dimensional conformation
- CCS (~K) is a physicochemical property of an ion.





What is Collision Cross Section (CCS)?

How does IMS practically combine into a Mass Spectrometer:

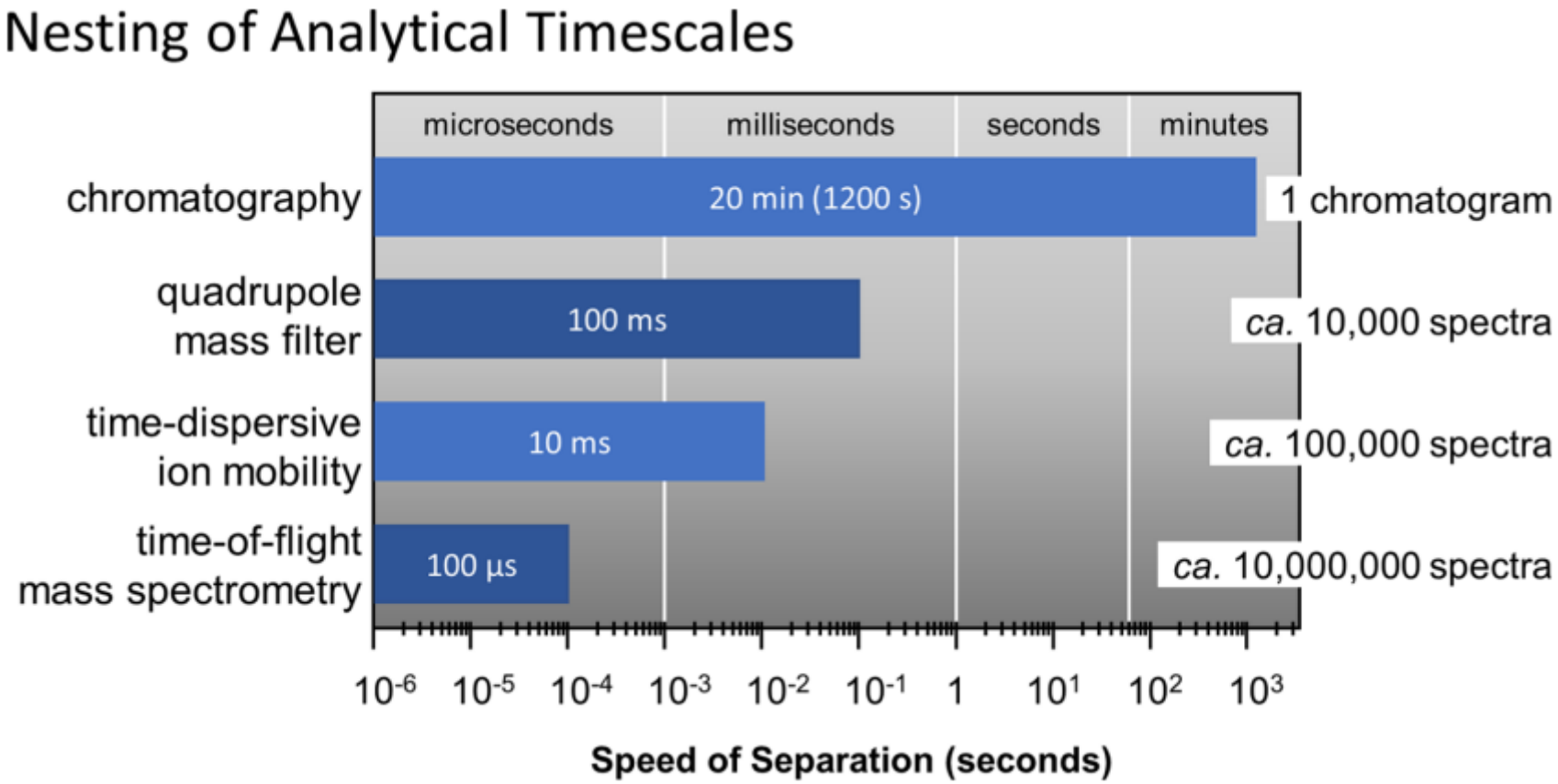


For Targeted methods or for prefiltering there are other techniques – like FAIMS or SelexionTM, but this is not in the scope of this talk

I.S. Krull, A.S. Rathore, Jared Auclair, **LCGC North America**, LCGC-11-01-2020, Volume 38, Issue 11, Pages: 619–624

The reason why IMS data collection fits in between Fast Chromatography (GC, SFC or LC) and (Q)TOF HRMS:

Waters™



(Jody C. May and John A. McLean; Anal Chem. 2015 Feb 3; 87(3): 1422–1436)*

3D resolution: Analysis strategy and sample complexity

Waters™

1: Chromatographic Resolution

2: Ion Mobility Resolution

3: Mass Resolution

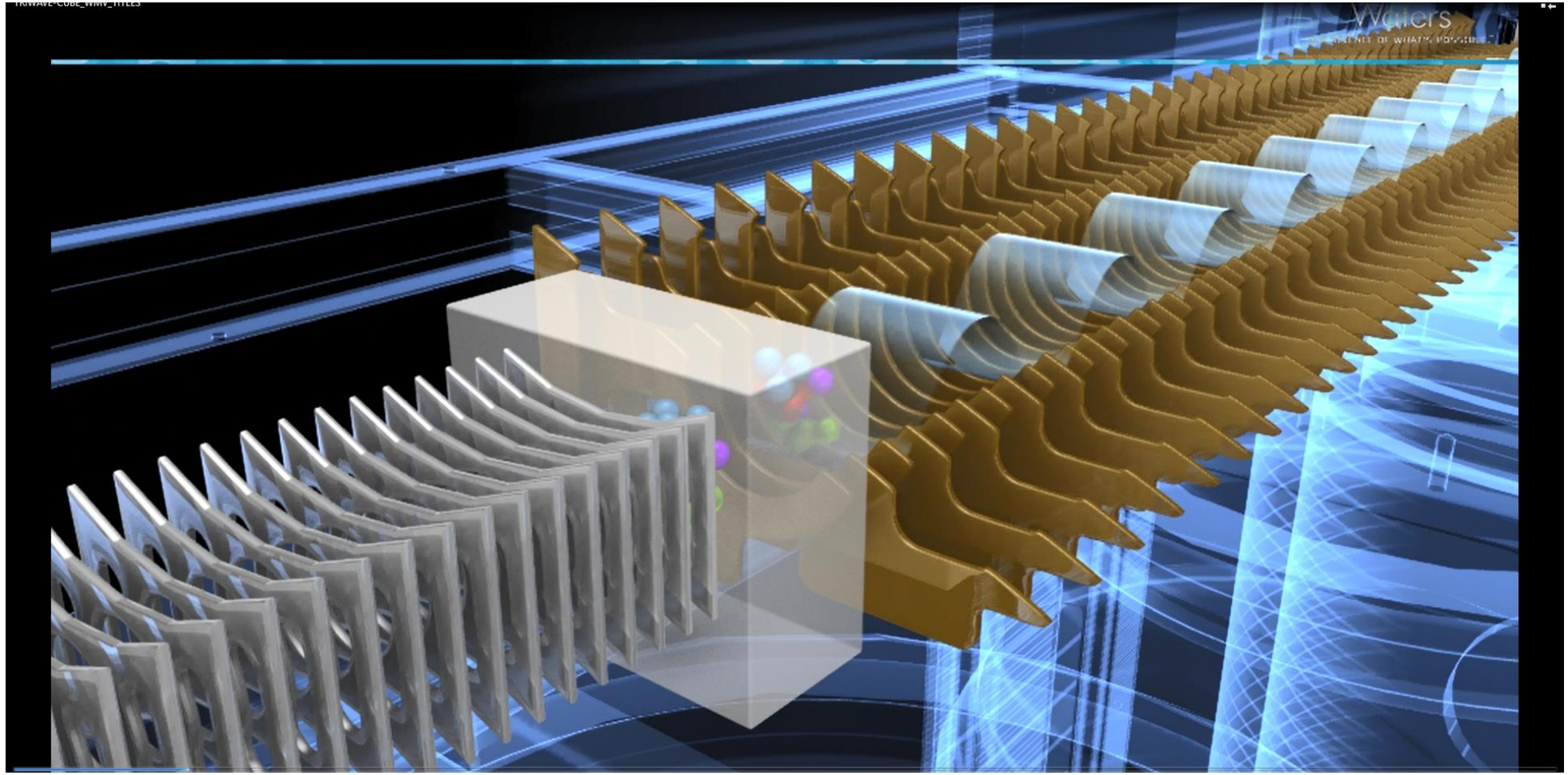
1: UPLC Peak
1-3 secs

2:T-wave
Ion Mobility
10.2 ms

3:TOF MS
~50 µsecs

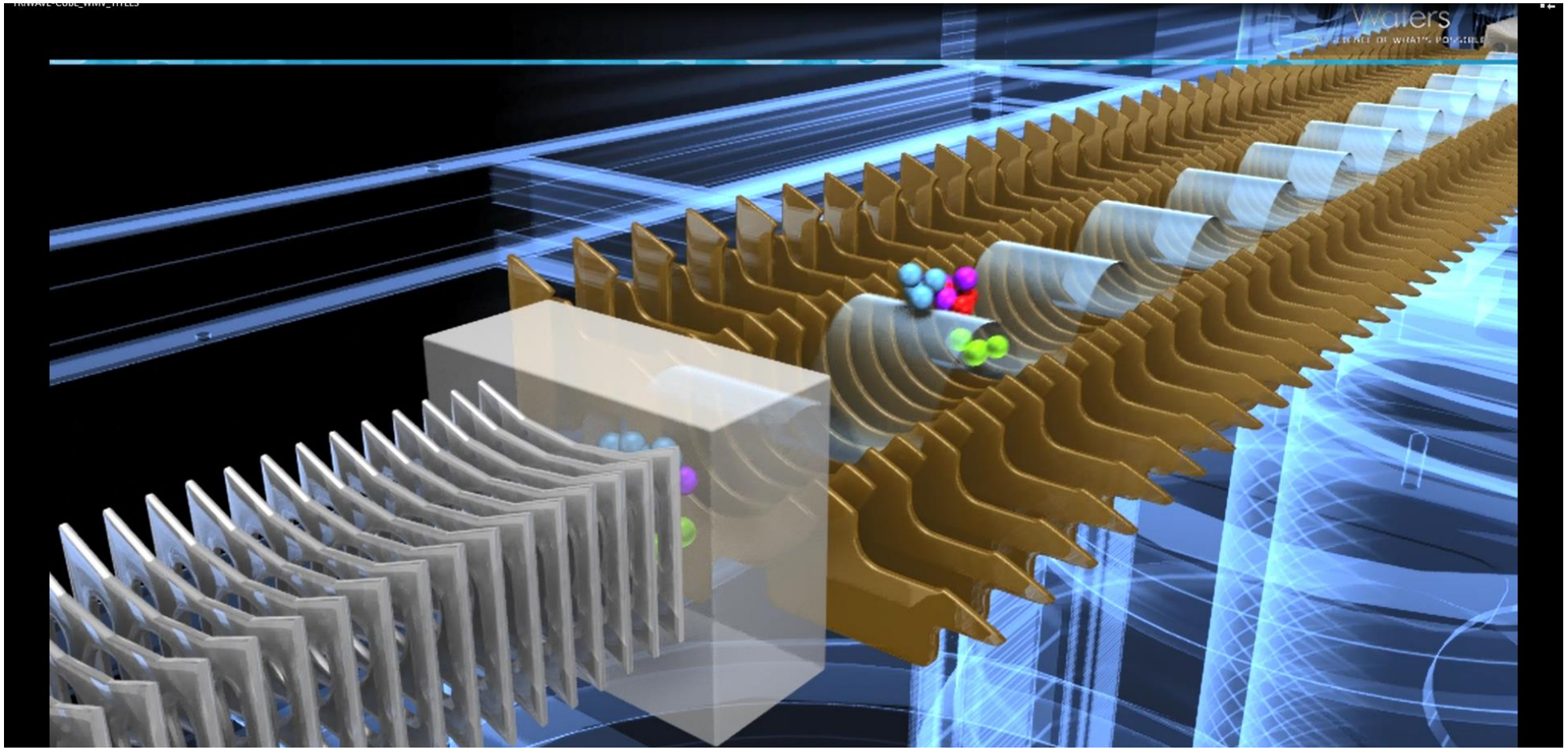
Scan range: m/z 50 -1200

T-wave IMS



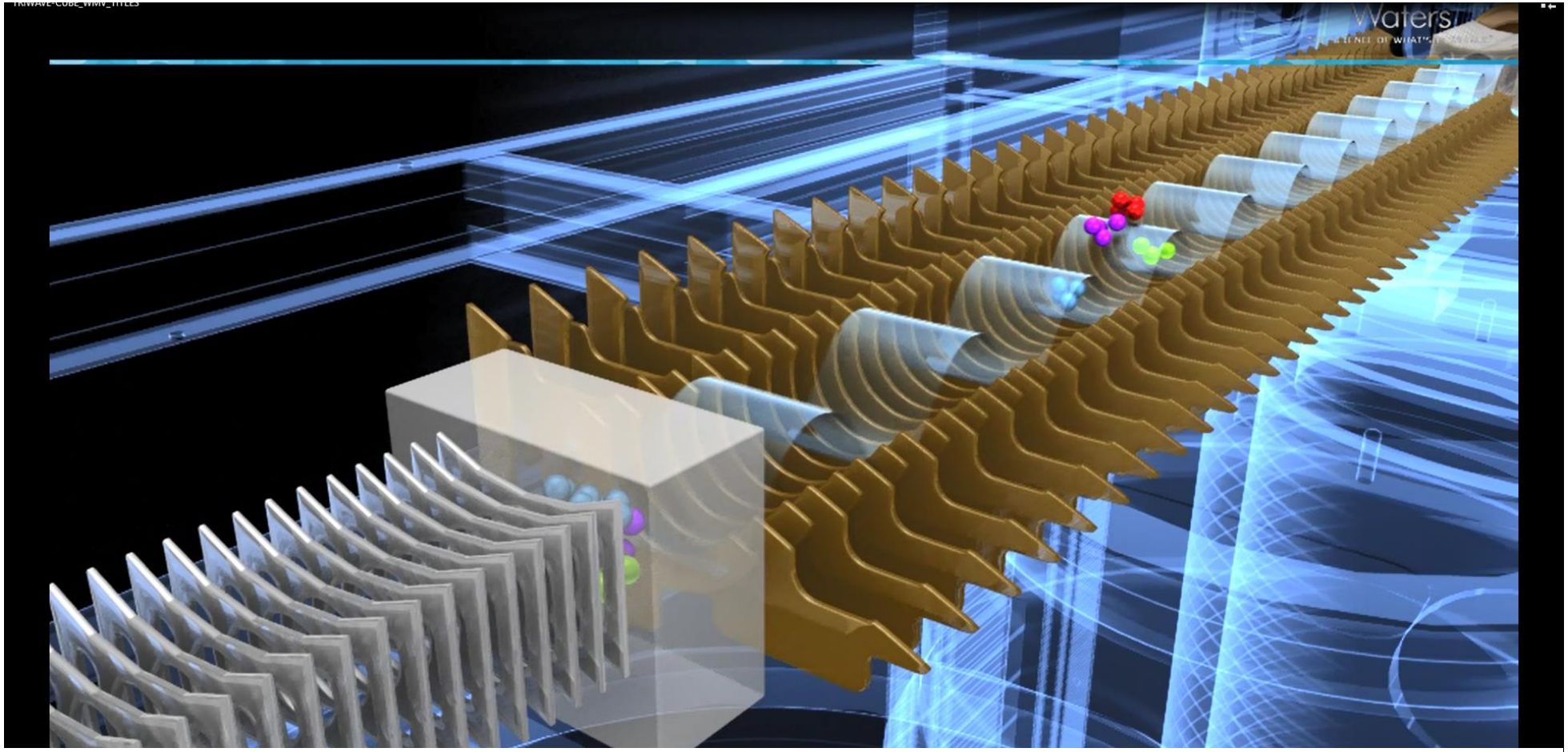
Stacked Ring Ion Guide: Opposite phases of RF voltage are applied to adjacent electrodes to provide radial ion confinement and high transmission.

T-wave IMS



T-Wave DC voltages are applied to electrode pairs in repeating sequence along the device and step to respective adjacent electrode pairs.

T-wave IMS

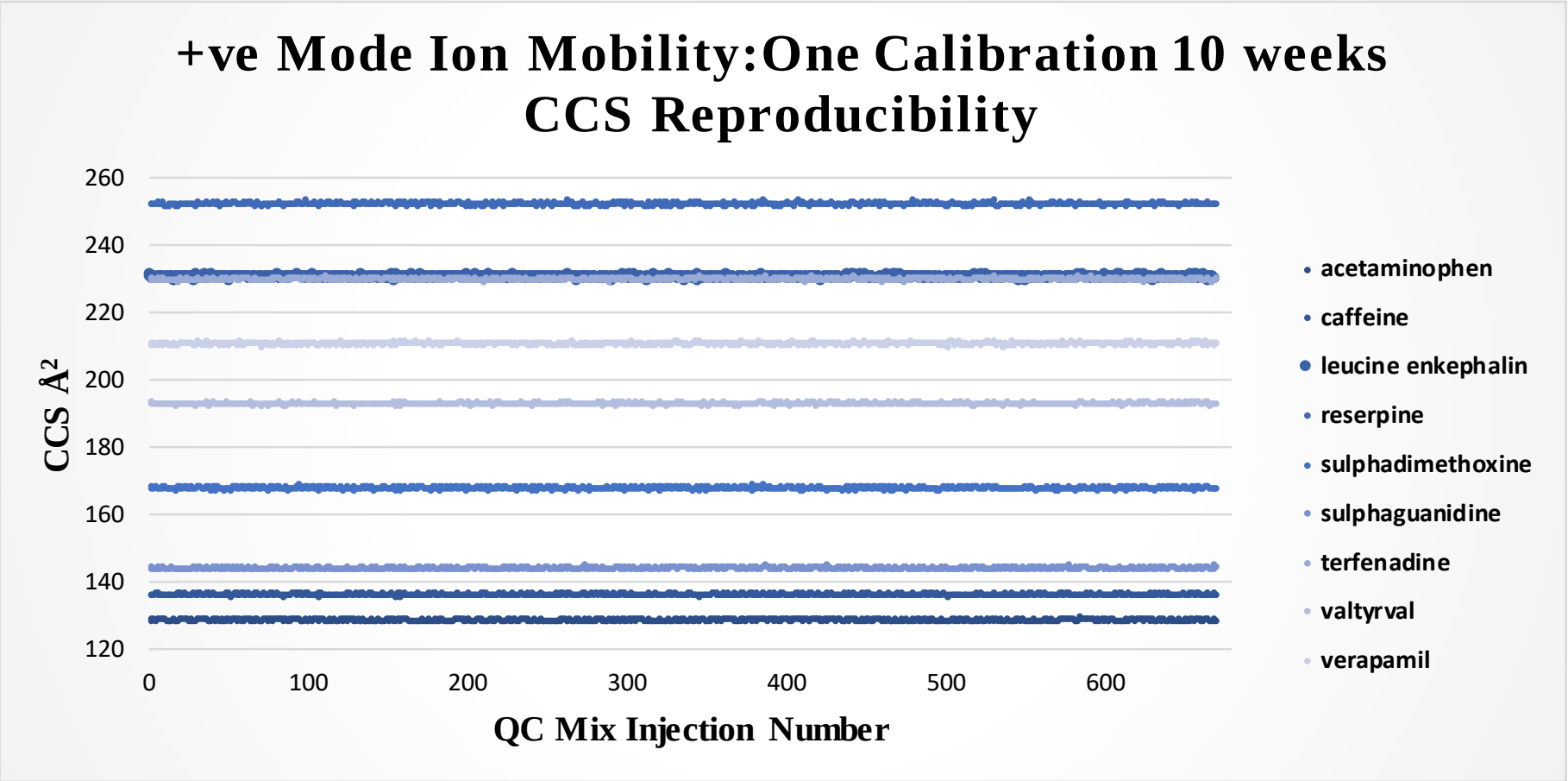


Ions are propelled along the ion mobility separation device.

CCS and Time Robustness

Robustness: CCS as a constant part 1

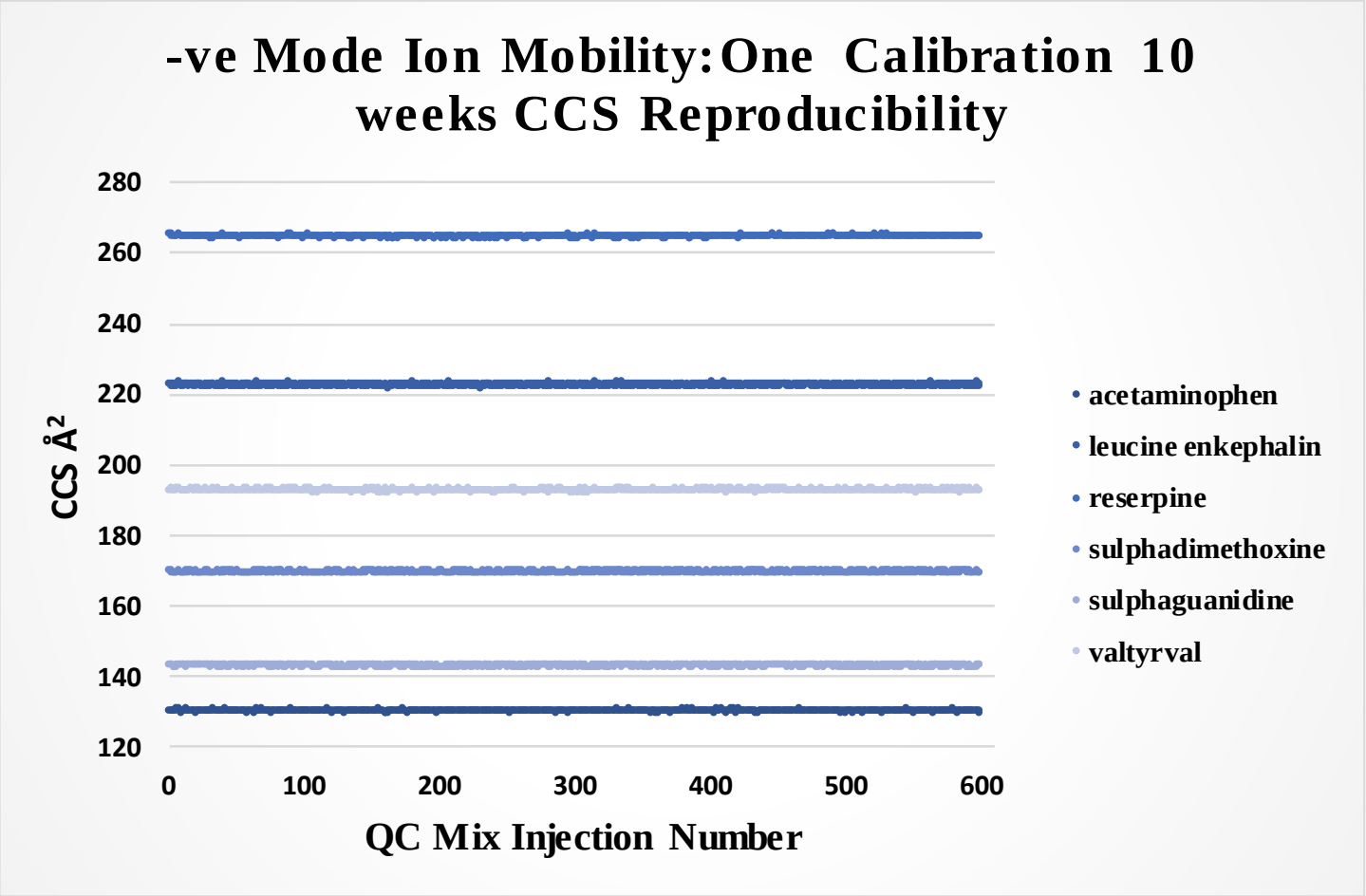
QC mix CCS reproducibility over 10 weeks, 24/7 acquisition ESI+ using a single ion mobility calibration.



Waters Application Note March 2020 720006769EN AG-PDF: Small molecule ion mobility investigations into cross-platform and long-term robustness of a CCS metric Mike McCullagh[1]; Michelle Wood[2]; Nayan Mistry[2]; Severine Goscinny[3] and Petur Dalsgaard[4]
[1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium; [3]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

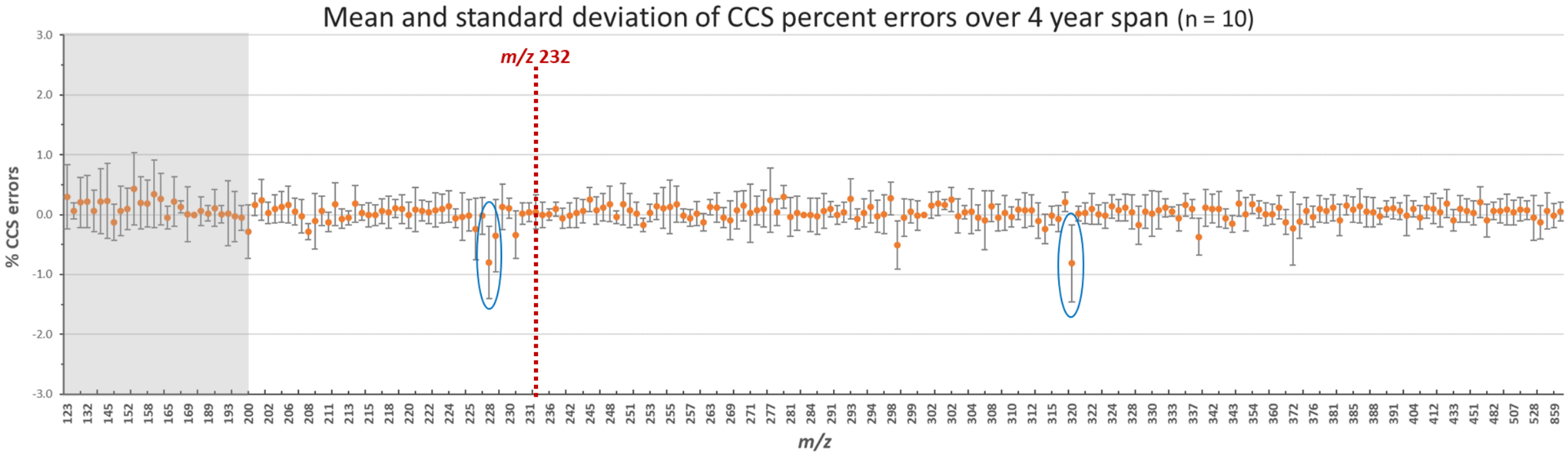
Robustness: CCS as a constant part 2

QC mix CCS reproducibility over 10 weeks, 24/7 acquisition ESI- using a single ion mobility calibration.



Waters Application Note March 2020 720006769EN AG-PDF: Small molecule ion mobility investigations into cross-platform and long-term robustness of a CCS metric Mike McCullagh[1]; Michelle Wood[2]; Nayan Mistry[2]; Severine Goscinny[3] and Petur Dalsgaard[4]
[1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium; [3]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

Synapt mean CCS errors (±SD) for the 200 pesticides over 4 years. Zone in grey highlights the compounds with m/z below 200

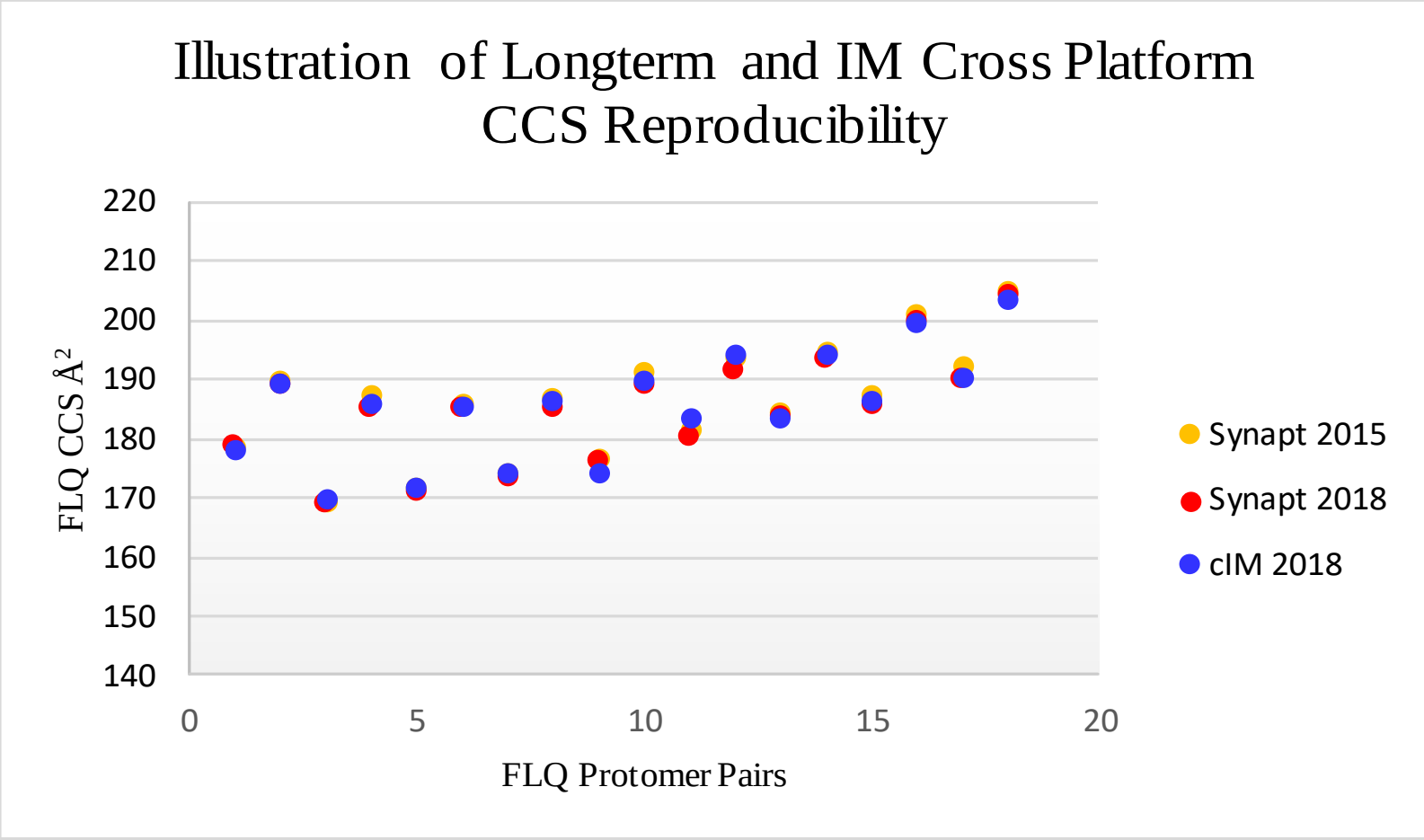


Towards the use of ion mobility mass spectrometry-derived collision cross section as a screening approach for unambiguous identification of targeted pesticides in food. Séverine Goscinny, Michael McCullagh, Johann Far, Edwin De Pauw and Gauthier Eppe. *Rapid Commun Mass Spectrom*. 2019;1–15.

CCS and Platform Robustness

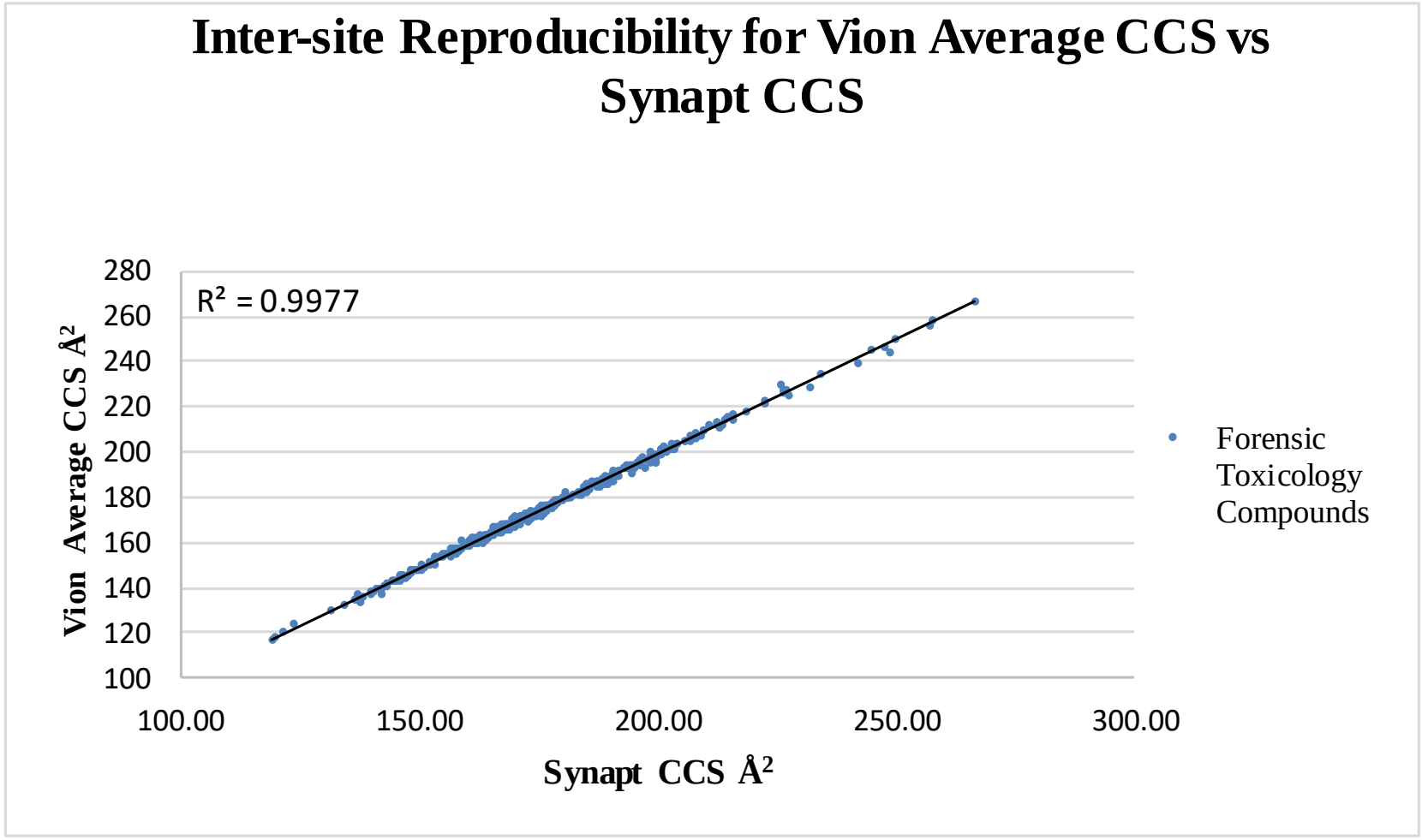
Robustness between platforms (Waters): CCS as a constant part 1

Synapt linear IM (compared over a period of 3 years) and cIM for fluoroquinolone protomer CCS reproducibility.



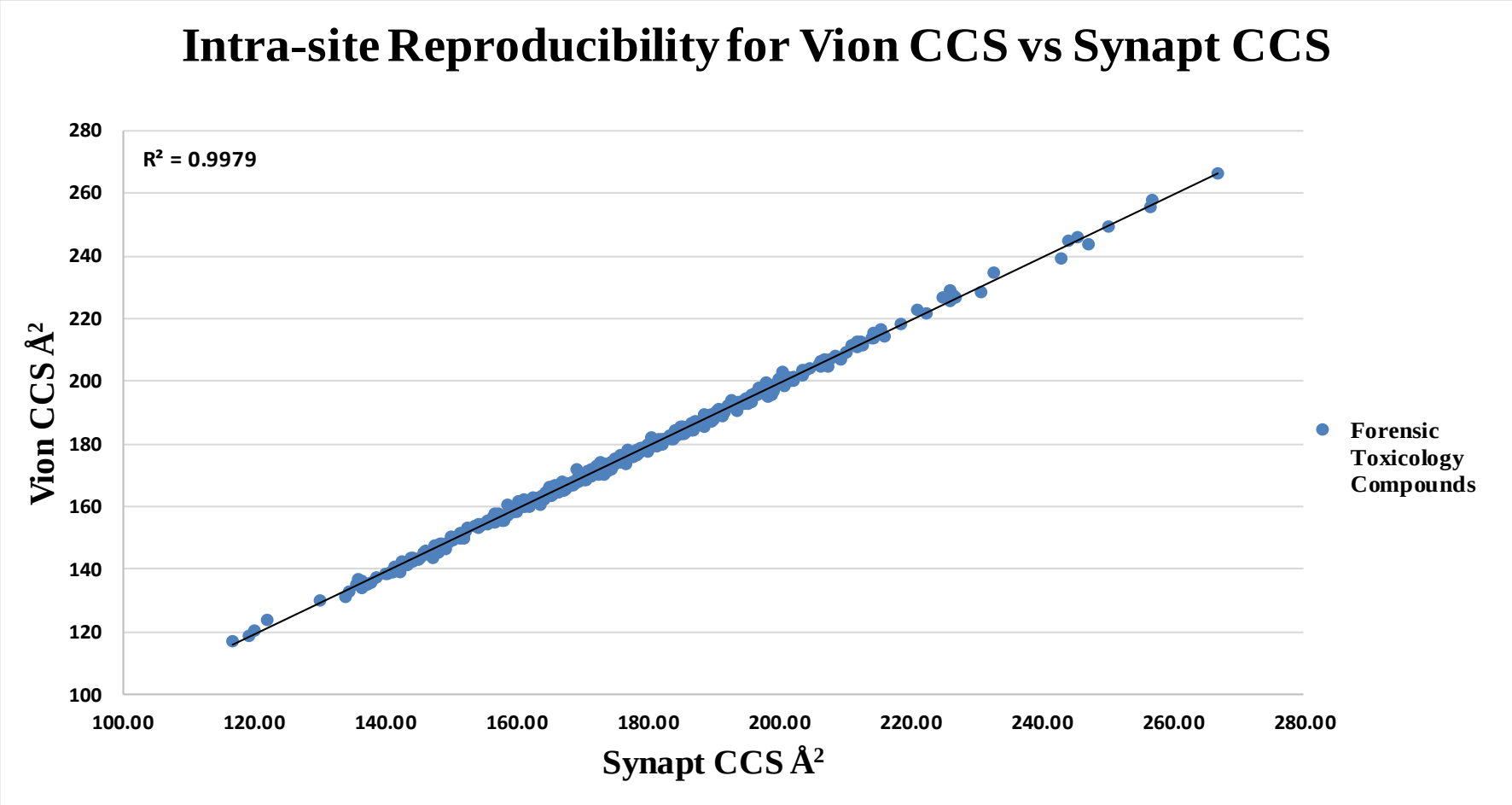
Investigations into the performance of travelling wave enabled conventional and cyclic ion mobility systems to characterise protomers of fluoroquinolone antibiotic residues Michael McCullagh, Kevin Gile Keith Richardson, Sara Stead and Martin Palmer. *Rapid Commun Mass Spectrom*. 2019;1–11

Vion inter-site CCS versus SYNAPT CCS and corresponding frequency distribution for inter-site Vion library ΔCCS.



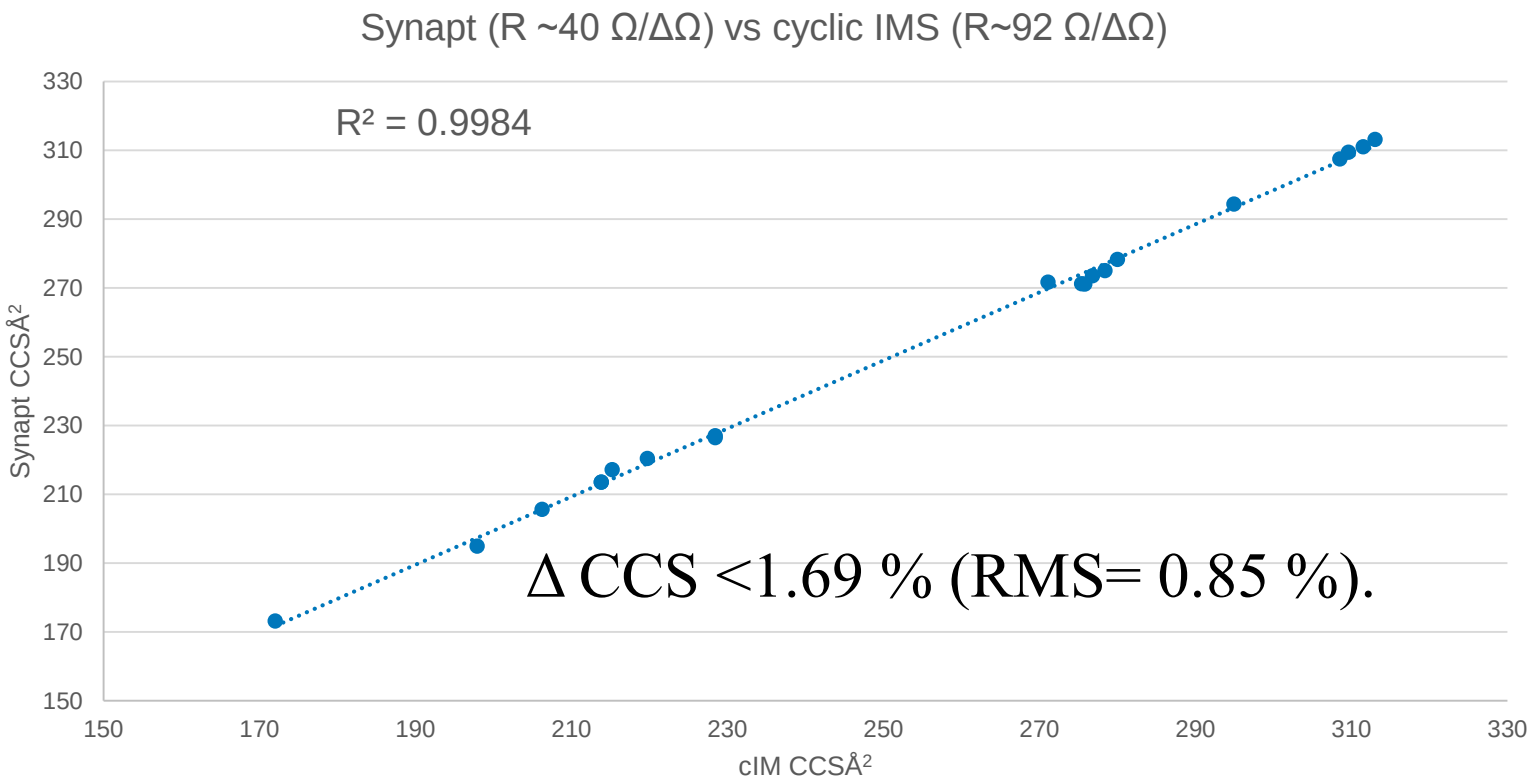
Waters Application Note March 2020 720006769EN AG-PDF: Small molecule ion mobility investigations into cross-platform and long-term robustness of a CCS metric Mike McCullagh[1]; Michelle Wood[2]; Nayan Mistry[2]; Severine Goscinny[3] and Petur Dalsgaard[4]
[1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium; [3]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

Vion intra-site CCS versus SYNAPT CCS and corresponding frequency distribution for intra-site Vion library ΔCCS.



Waters Application Note March 2020 720006769EN AG-PDF: Small molecule ion mobility investigations into cross-platform and long-term robustness of a CCS metric Mike McCullagh[1]; Michelle Wood[2]; Nayan Mistry[2]; Severine Goscinny[3] and Petur Dalsgaard[4]
[1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium; [3]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

Cross platform comparison of charged isomer species

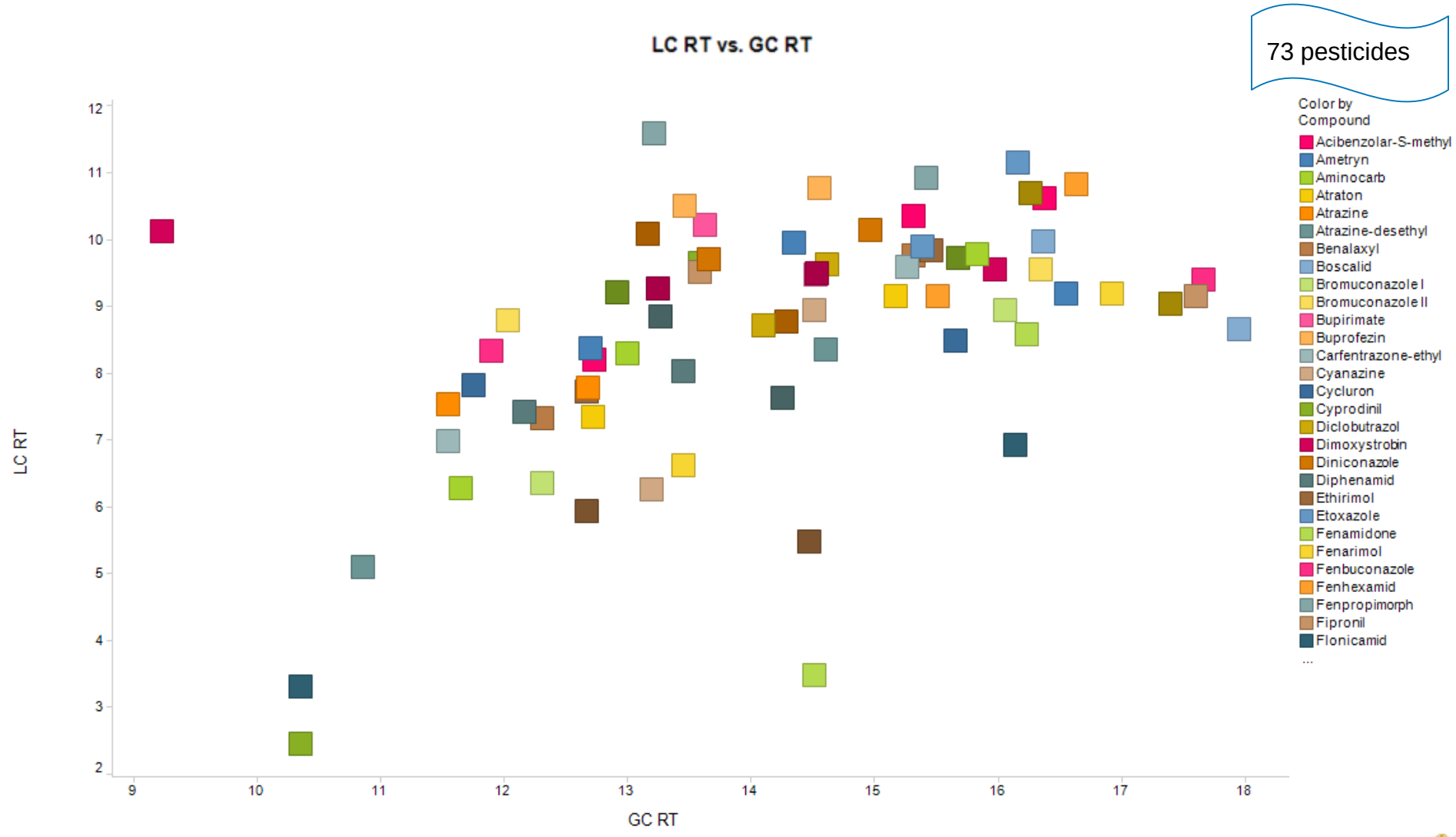


Metaflumizone (Z)-Isomer Species I
Metaflumizone (Z)- Isomer Species II
Metaflumizone (E)-Isomer Species I
Metaflumizone (E)-Isomer Species II
Spinosyn D [M+H]+
Spinosyn D Sodimer I
Spinosyn D Sodimer II
Spinosyn D Potassimer I
Spinosyn D Potassimer II
Spinosyn A [M+H]+
Spinosyn A Sodimer I
Spinosyn A Sodimer II
Spinosyn A Potassimer I
Spinosyn A Potassimer II
Epoxiconazole
Spinosyn D
Indoxacarb Protomer I
Indoxacarb Protomer II
Fenpyroximate Protomer I
Fenpyroximate Protomer II
Avermectin B1a

Investigations into pesticide charge site isomers using conventional IM and cIM systems. Michael McCullagh, Séverine Goscinny, Martin Palmer, Jakub Ujma, Talanta, Volume 234, 2021, 122604.

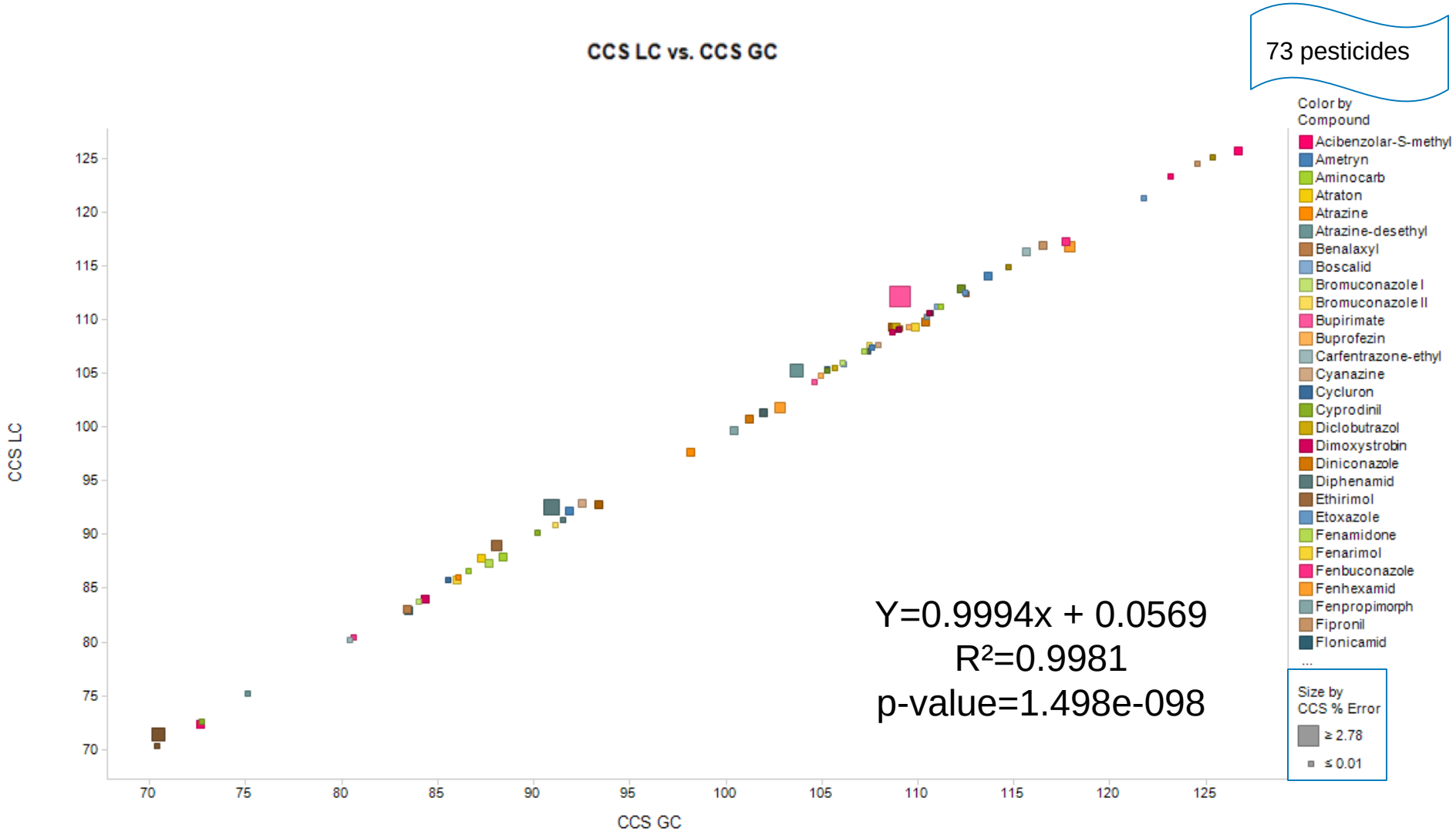
GC and LC Retention Times

Waters™



GC and LC CCS Values : Independent of Inlet/Chromatography

Waters™



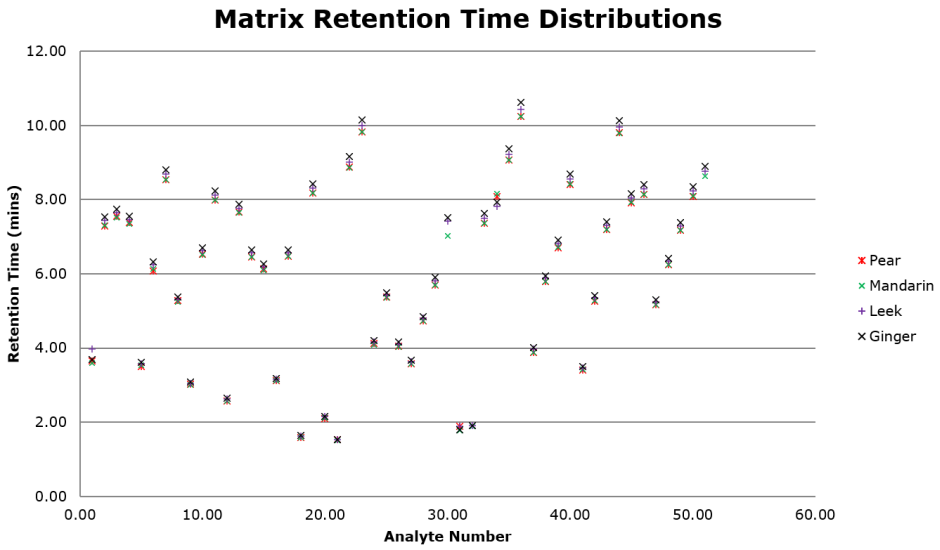
Time, Space and Platform Robustness and Indepence:

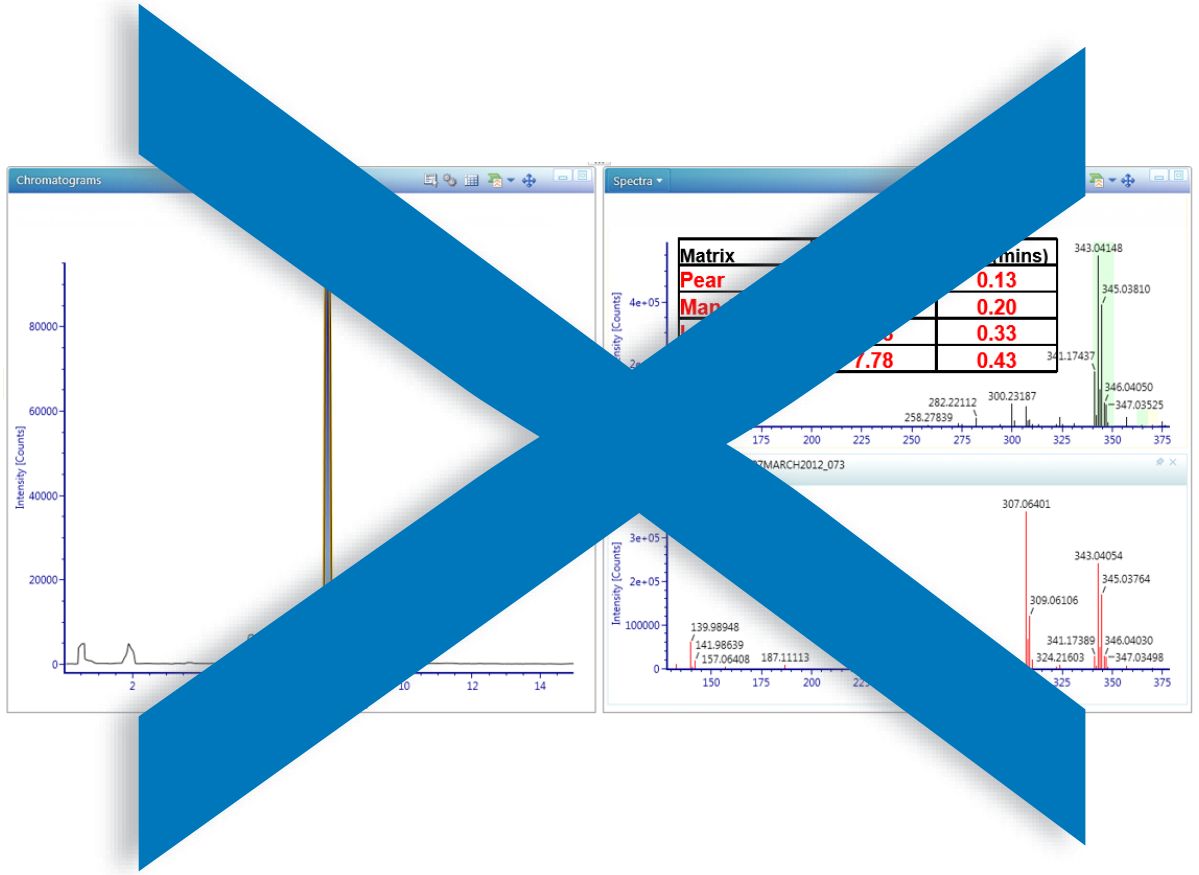
- Within Waters portfolio : Yes
- Between Instrument Manufacturers? Maybe if ...$v_d = KE$

CCS and MATRIX ROBUSTNESS

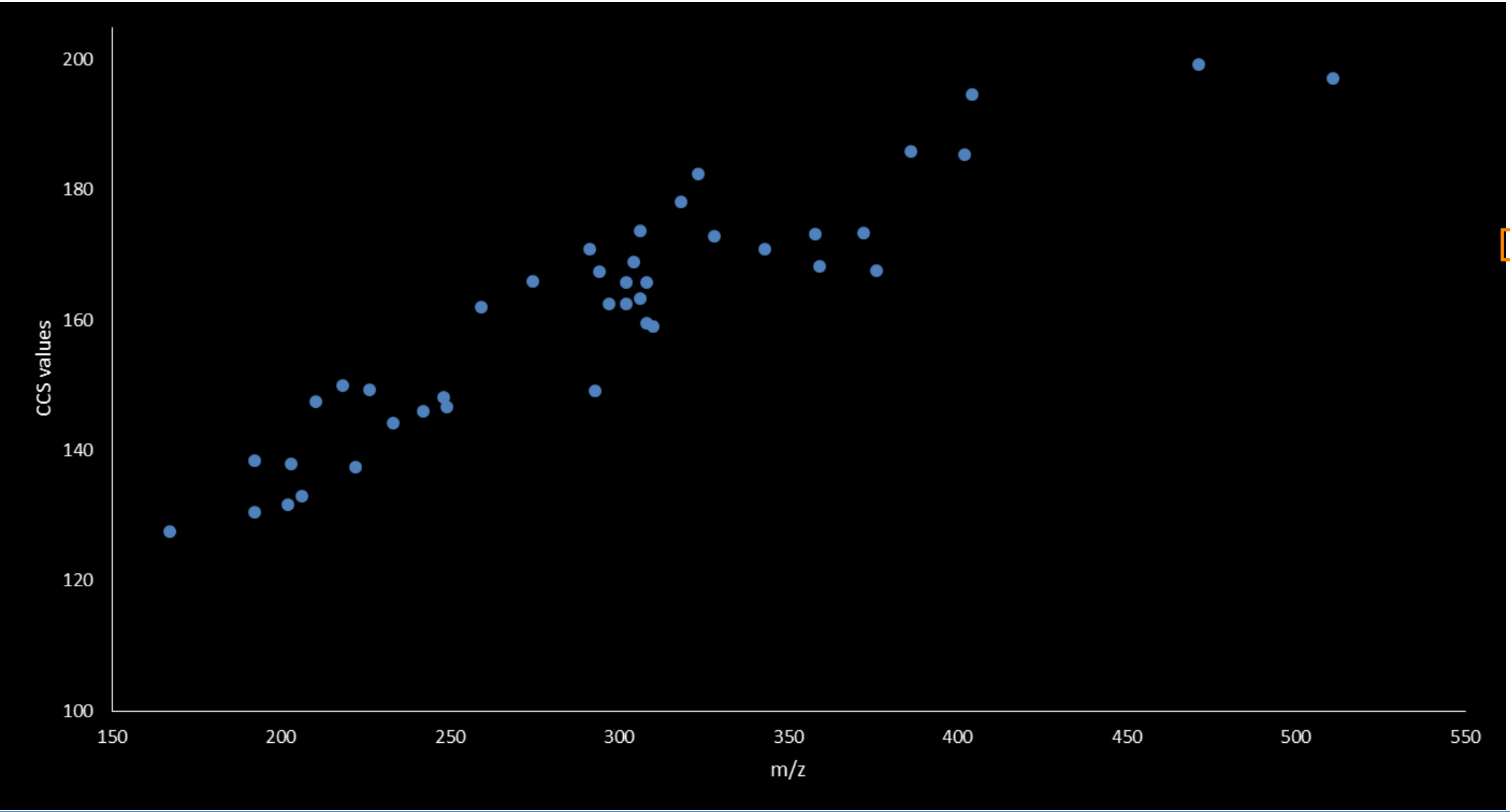
Matrix and Chromatography (UHPLC)

Complex matrix dependent retention time shift: Boscalid as example



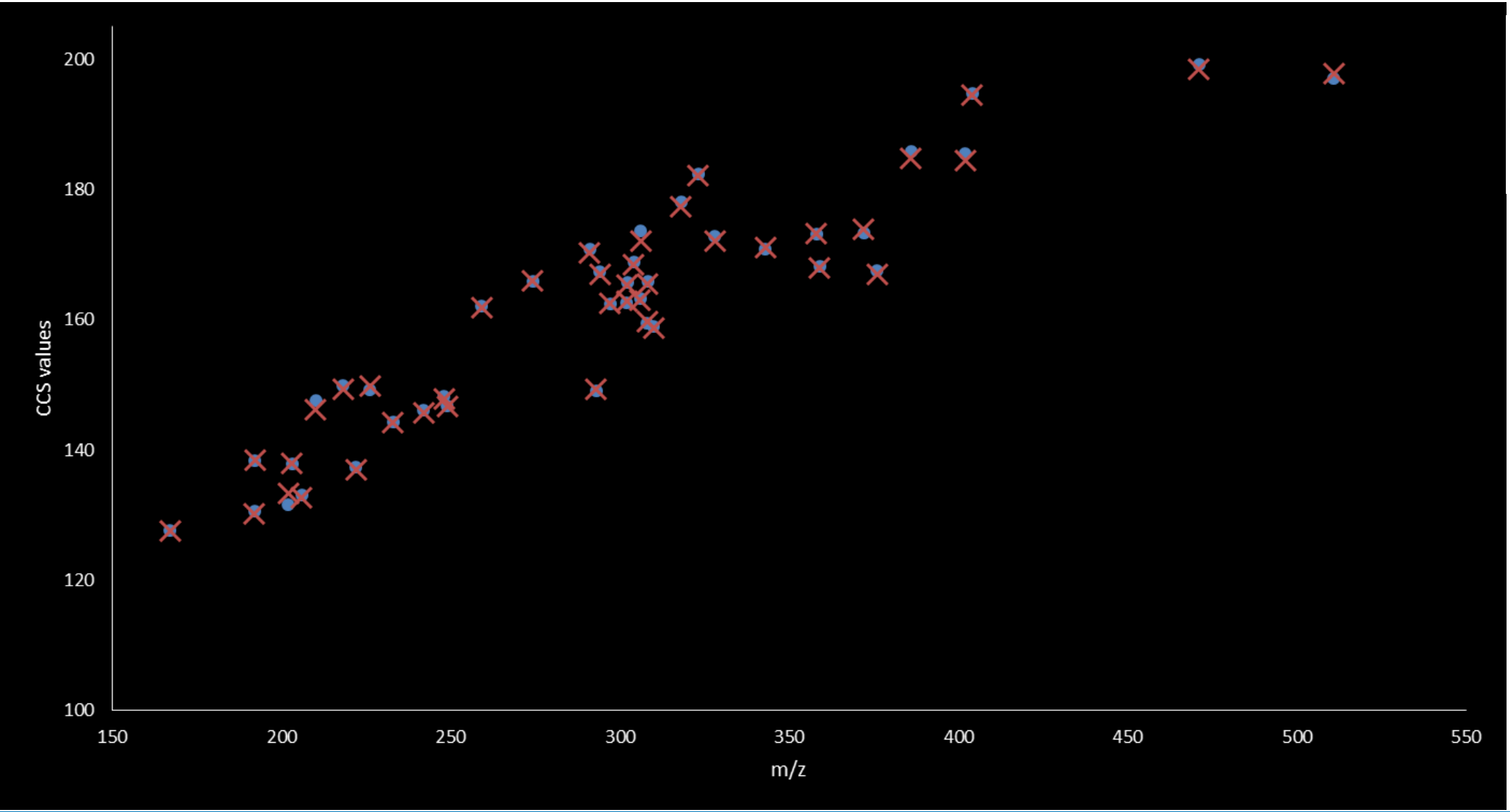


Impact of Matrix Type Concentration on CCS Reproducibility



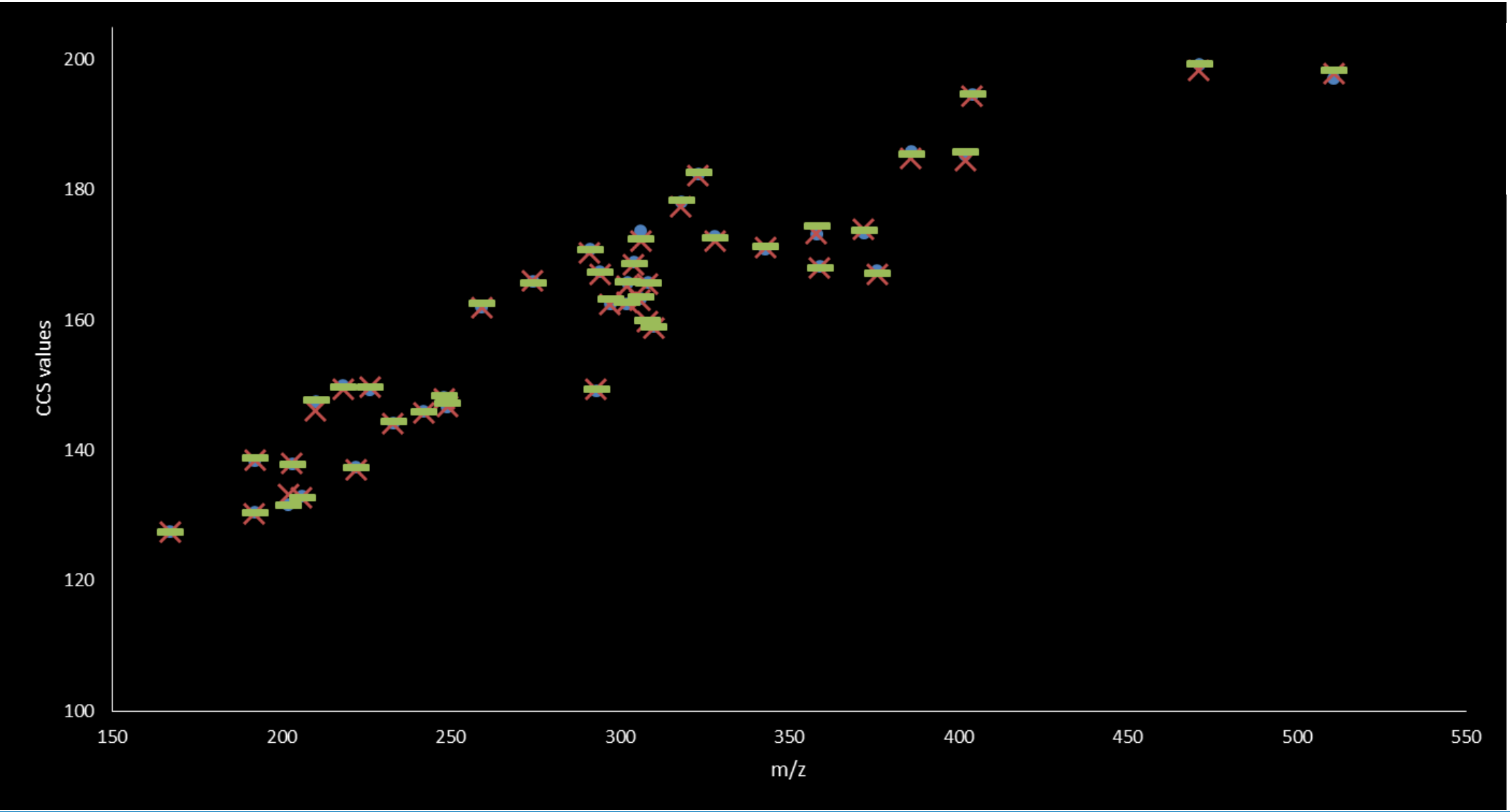
Dried cumin

Impact of Matrix Type Concentration on CCS Reproducibility



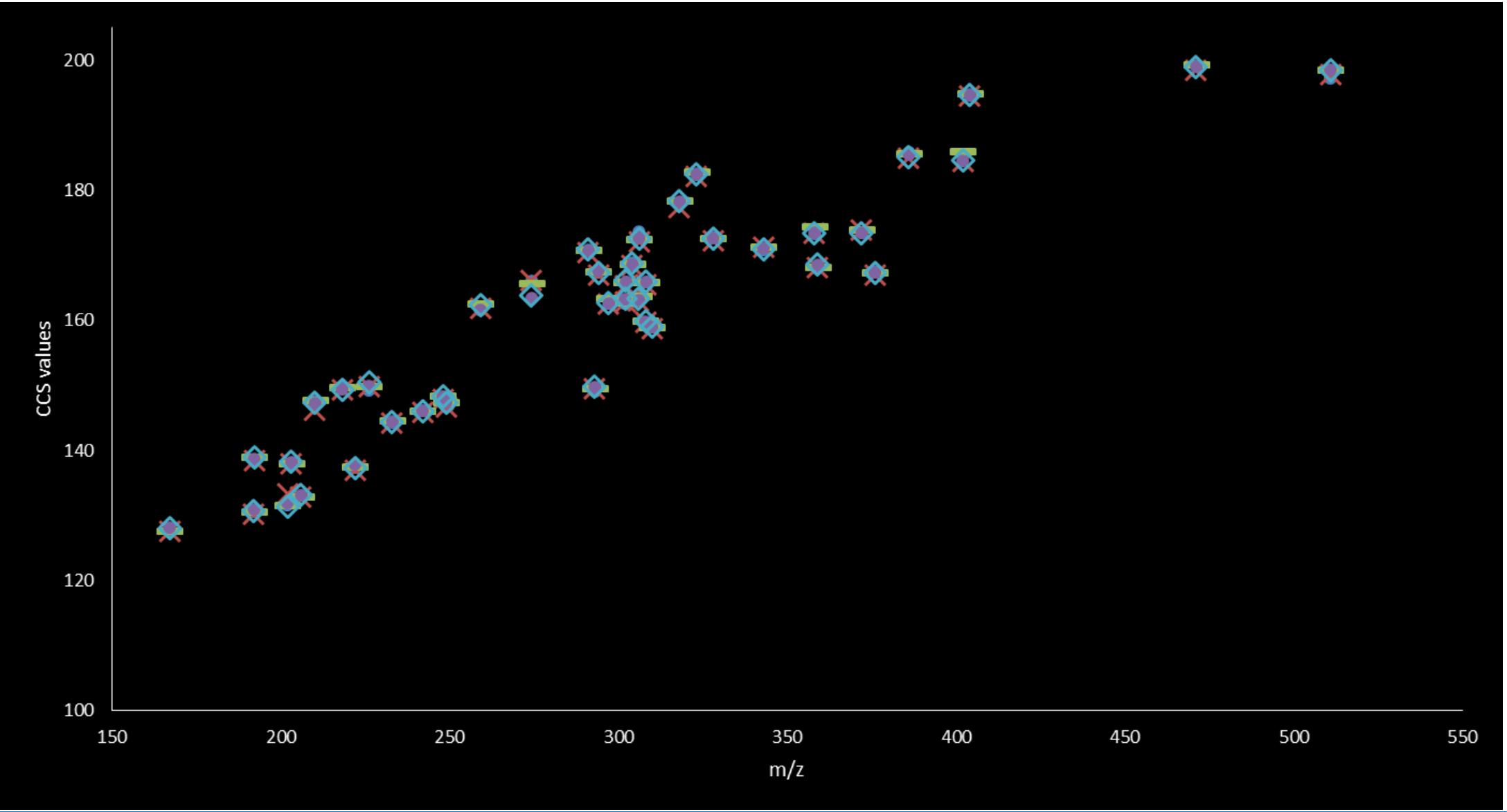
Black tea

Impact of Matrix Type Concentration on CCS Reproducibility



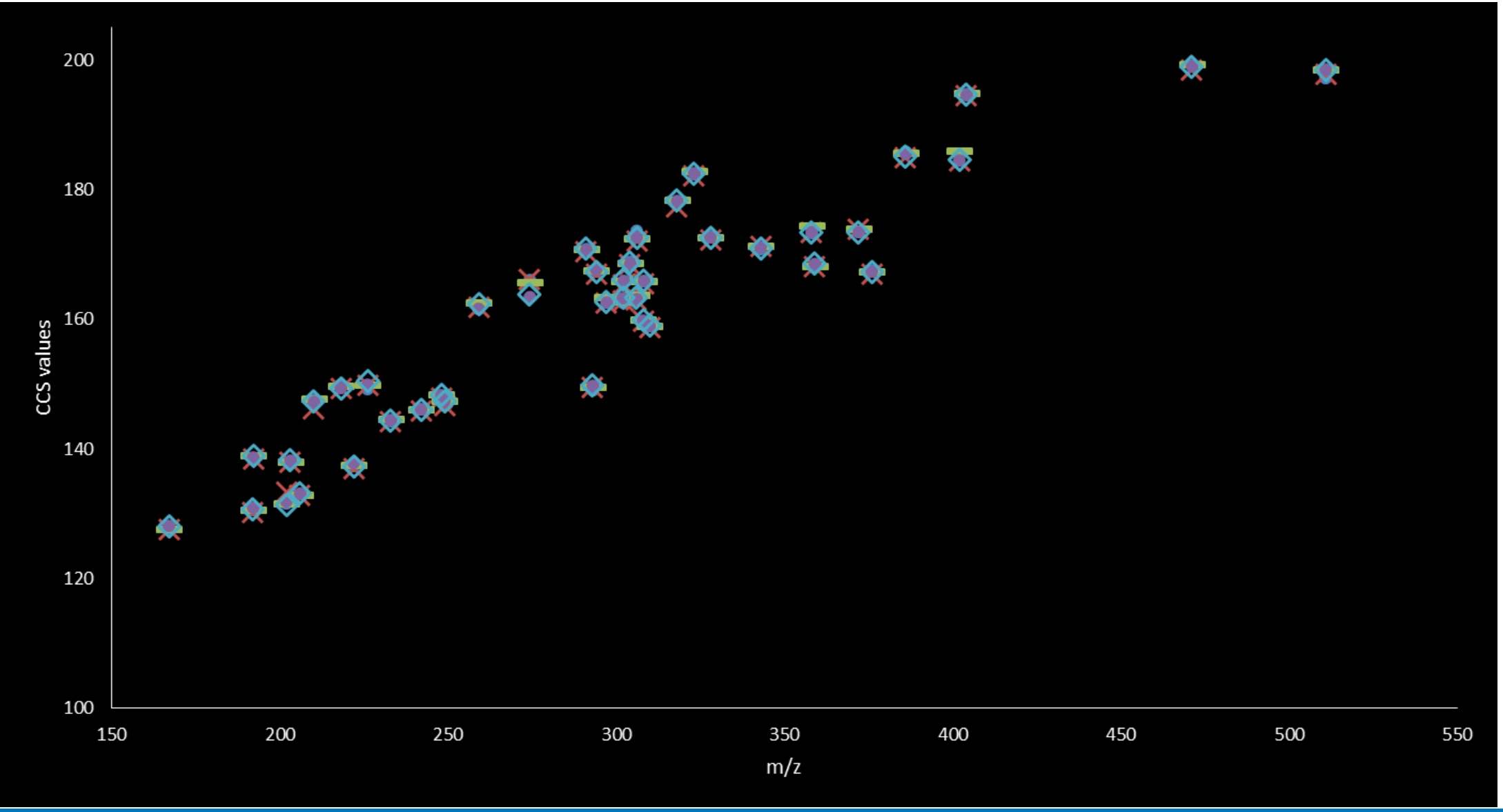
Mixed rice

Impact of Matrix Type Concentration on CCS Reproducibility



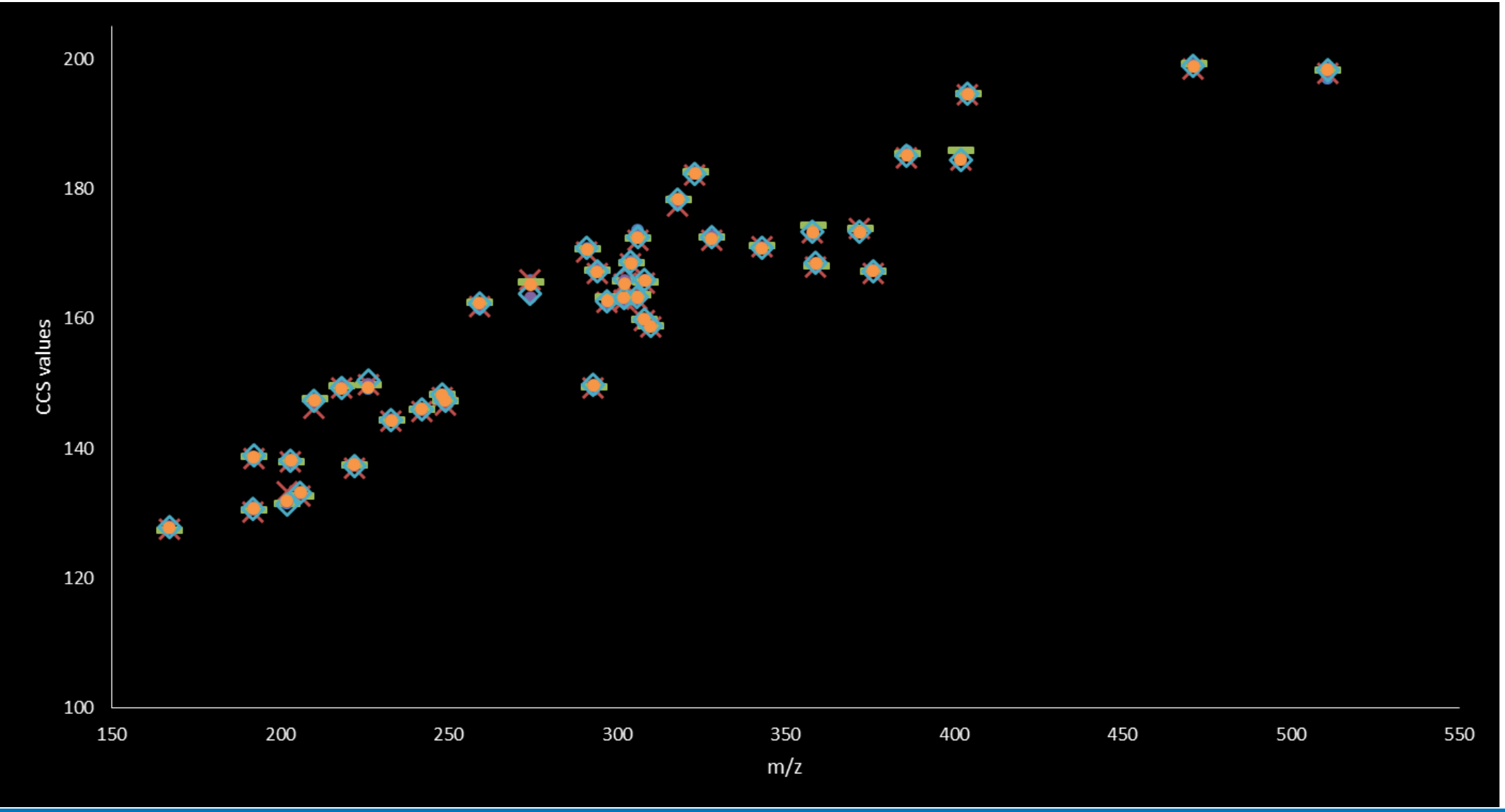
Honey

Impact of Matrix Type Concentration on CCS Reproducibility



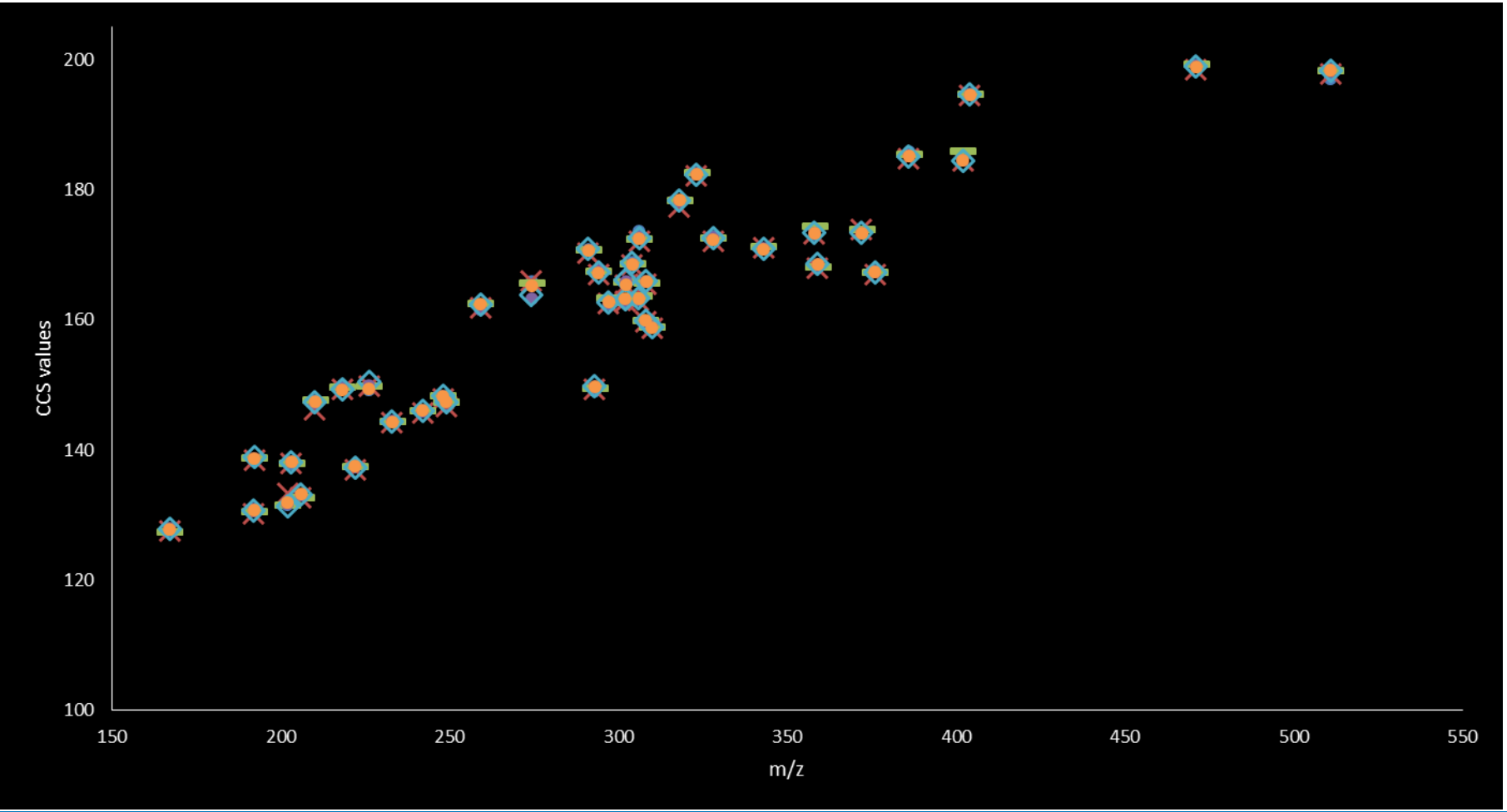
Courgette

Impact of Matrix Type Concentration on CCS Reproducibility



Apple

Impact of matrix type concentration on CCS reproducibility



Dried cumin

Apple

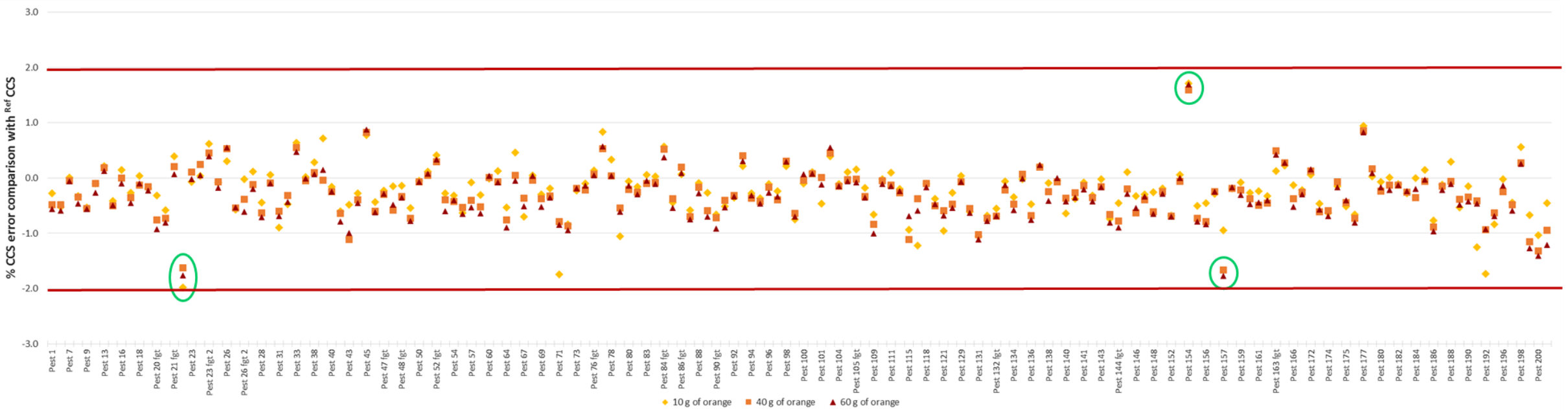
Black tea

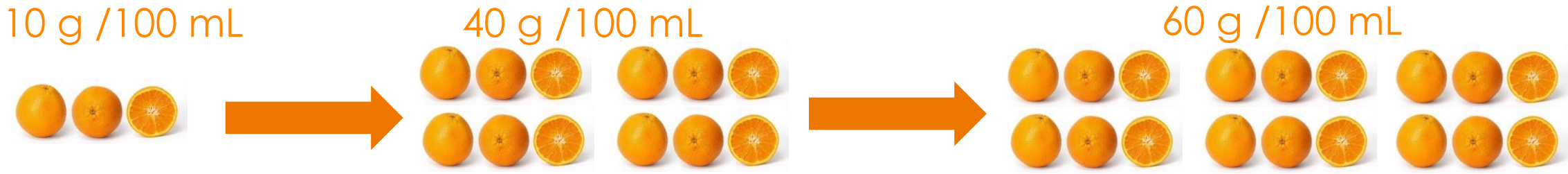
Mixed rice

Honey

Courgette

Impact of matrix load on CCS measurement for pesticides at constant concentration (100 ng.mL^{-1}). Comparison shown as percent difference with ^{Ref}CCS





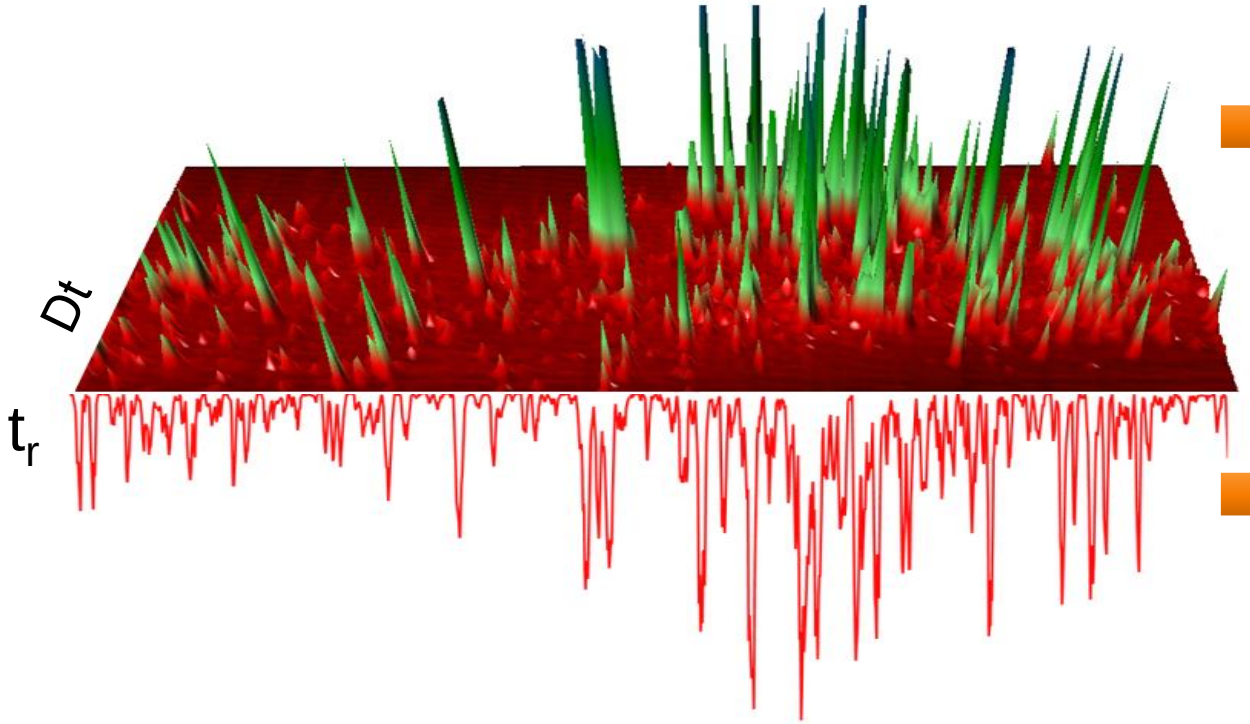
Towards the use of ion mobility mass spectrometry-derived collision cross section as a screening approach for unambiguous identification of targeted pesticides in food. Séverine Goscinny, Michael McCullagh, Johann Far, Edwin De Pauw and Gauthier Eppe. *Rapid Commun Mass Spectrom*. 2019;1–15.

UPLC Ion mobility mass spectrometry 3D resolution: enhanced peak capacity and selectivity

- Unique analyte visibility in complex sample analysis
- Unique Scientific Outcomes

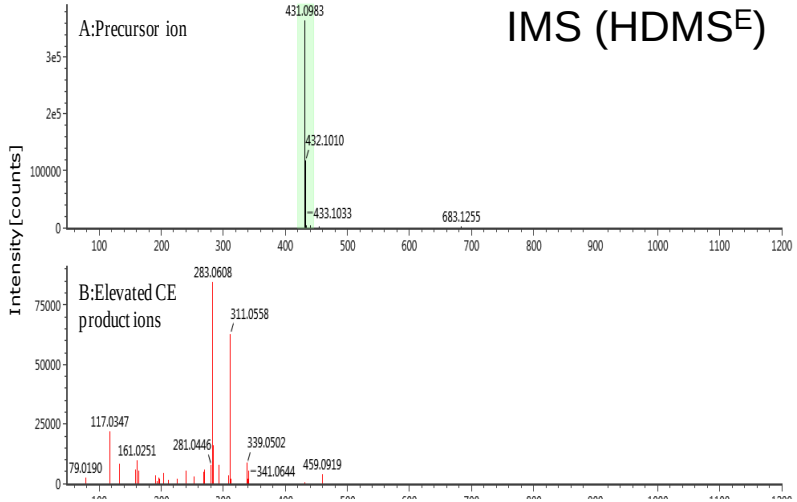
I

Ion Mobility Separation



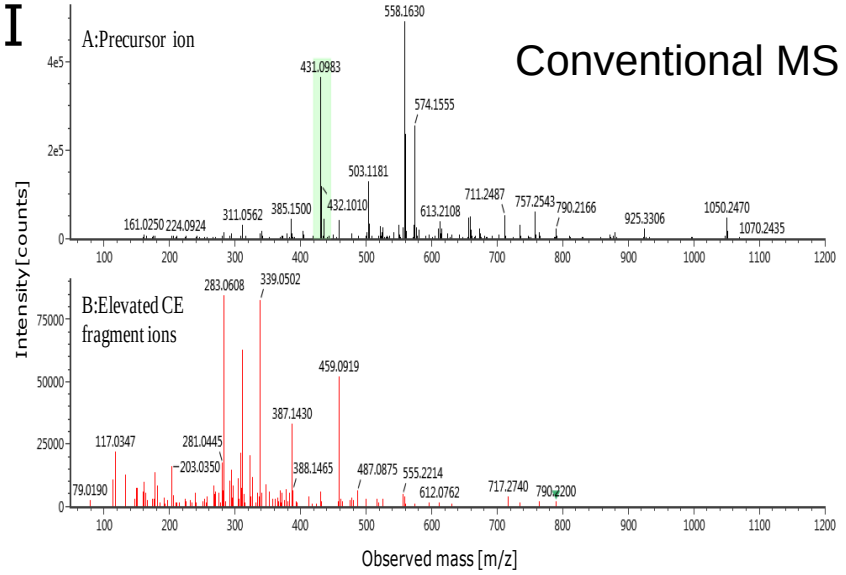
Total Ion Chromatogram

II



IMS (HDMS^E)

III



Conventional MS (MS^E)

Ion Mobility Examples: LC-IMS-HRMS

Steviol glycosides Analysis: Authentication Profiling Using CCS Libraries

- What are Steviol glycosides.
- Where/Why Are They Used.
- Why is Analysis Required.
- Why IMS

analytical chemistry
Article
Cite This: Anal. Chem. 2018, 90, 4585−4595
pubs.acs.org/ac

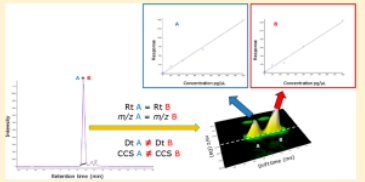
Exploring the Complexity of Steviol Glycosides Analysis Using Ion Mobility Mass Spectrometry

Michael McCullagh,*[†] David Douce,[†] Els Van Hoeck,[‡] and Severine Goscinny*[‡]

[†]Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX, U.K.
[‡]Scientific Institute of Public Health, Rue Juliette Wytsmans 14, 1050 Brussels, Belgium

Supporting Information

ABSTRACT: A proof of principle method using ion mobility-mass spectrometry (IM-MS) and collision induced dissociation (CID) coupled with micro flow ultra high-performance chromatography (UHPLC-IM-MS) has been developed to screen for steviol glycosides. Traveling wave ion mobility was used to determine rotationally averaged collision cross sections in nitrogen buffer gas ($^{TW}CCSN_2$). To explore the evolving applicability of ion mobility screening, the analytical approach was initially developed and applied to the analysis of a steviol/steviol glycoside spiked chocolate spread extract. Subsequently 55 food commodities were screened using a steviol glycoside $^{TW}CCSN_2$ library. IM analyses produced $^{TW}CCSN_2$ values, enabling the unequivocal identification of the steviol glycosides and isomeric pairs (negating the reliance on product ions). In addition, coeluting isomeric species, comprising (labile fragment ions, doubly charged dimers, and multiply charged species) have been identified and resolved. Isomeric false detections were avoided, with the coeluting isomeric species quantified. A quantitative assessment of $^{TW}CCSN_2$ in the analysis of steviol glycosides was performed.



Production of food commodities containing sweeteners has increased; a driving force being to reduce global incidence of obesity and the associated health impact (such as diabetes type II).[1] Sweeteners can provide reduced or zero calorific intake. They are a diverse group of chemical compounds derived from plants or chemical synthesis. As a food additive, safety evaluation of sweeteners is a basic requirement. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established regulations for steviol glycosides, requiring a purity level of at least 95% for seven chemically defined steviol glycosides.[2] In Europe, this food additive (E 960) is authorized in specific food at defined rates (maximum permitted level) laid down in Commission Regulation No. 1131/2011,[3] with acceptable purity criteria defined in Regulation (EU) No. 231/2012.[4] This later legislation established for the pristine additive, a minimum of 95% content of 10 steviol glycosides with at least 75% of stevioside and/or rebaudioside A within the mixture.

For consumers, the European Food Safety Authority (EFSA), perform risk assessments, and investigate health claims/beneficial effects related to sweeteners. A revised dietary exposure assessment of adults and children to steviol glycosides, through their use as a (food additive) was carried out. The variety of food products containing steviol glycosides as sweeteners in Europe is extensive. Smoked/dried fish, fruit based drinks, cocoa based confectionary, sweet/sour preserves, breakfast cereals, beers, ciders, sweeteners, and reduced sugar products are examples of where steviol glycosides exposure may occur in a European diet. Health effects of food additives/sweeteners on consumers will be impacted by the level of dietary exposure, e.g., by work life balance, socioeconomics, lifestyle choice, and typical national cuisine. The mean dietary exposure to steviol glycosides is expressed in terms of steviol equivalents.[5] The EFSA report details the revisions of acceptable use levels in a wide variety of food commodities and also the acceptable daily intake (ADI) of 4 mg/kg body weight (bw)/day for toddlers, which still exceeds the 95th percentile in a number of European Union countries.[6]

Reasons to explore a new approach for steviol glycoside analysis include meeting legislative requirements, (determination of ADI), ensuring all steviol glycosides (including isomers) are identified/detected and the true steviol equivalent determined. Also authentication profiling to determine origin in food commodities, optimization of stevia processing methodology, and breeding of *Stevia rebaudiana* Bertoni to produce more favorable flavor characteristics.[7] In addition for product quality, where new information on steviol glycoside makeup could help characterize minor novel steviol glycosides, which may contribute to/reduce bitter aftertaste or have more potent sweetness intensity and can be characteristic of products containing steviol glycosides.[8−10] Extracts of *Stevia rebaudiana* Bertoni leaves may contain isomers, which can have different

Received: December 1, 2017
Accepted: March 14, 2018
Published: March 14, 2018

DOI: 10.1021/acs.analchem.7b05002
Anal. Chem. 2018, 90, 4585−4595

SCIENTIFIC OPINION
EFSA Journal
ADOPTED: 24 March 2020
doi: 10.2903/j.efsa.2020.6106

Safety of a proposed amendment of the specifications for steviol glycosides (E 960) as a food additive: to expand the list of steviol glycosides to all those identified in the leaves of *Stevia Rebaudiana Bertoni*

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Ursula Gundert-Remy, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright, Gisela Degen, Alessandra Giarola, Ana M Rincon and Laurence Castle

Abstract

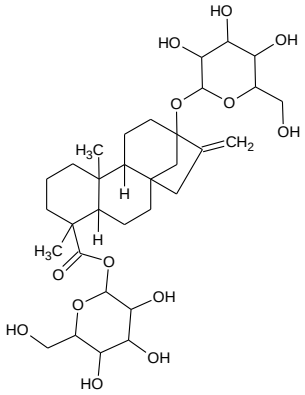
The EFSA Panel on Food Additives and Flavourings (FAF) provides a scientific opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive, in particular to expand the list of steviol glycosides to 60 steviol glycosides identified in the leaves of *Stevia Rebaudiana* Bertoni. With the existing specifications, the food additive must be comprised of not less than 95% of the 11 named steviol glycosides. The proposed change is to include all 60 steviol glycosides in the same limit value of 95% and this would allow the presence of up to 5% of impurities. FAF Panel considered that all steviol glycosides share the same metabolic fate, and therefore, the safety of 60 identified steviol glycosides can be based on read-across from toxicological data previously evaluated by EFSA and the acceptable daily intake (ADI) of 4 mg/kg body weight (bw) per day will apply to all those steviol glycosides. However, according to the proposed change in specifications, there remains a small but not insignificant fraction of the additive that would be undefined and therefore cannot be evaluated by the Panel. The Panel concluded that the inclusion of the 60 steviol glycosides in the proposed specifications for steviol glycoside (E960) would not be of safety concern. However, the Panel cannot conclude on the safety of the proposed amendment to the specifications of steviol glycosides (E 960) as food additive if the purity assay value of not less than 95% for the total content of steviol glycosides is maintained.

Keywords: Steviol glycosides, E960, food additive

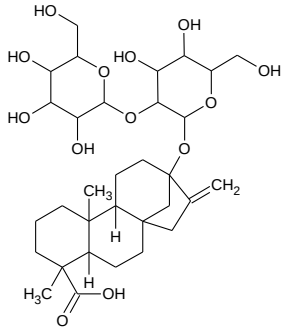
Requestor: European Commission
Question number: EFSA-Q-2019-00063
Correspondence: fip@efsa.europa.eu

Steviol glycosides profiled using ion mobility

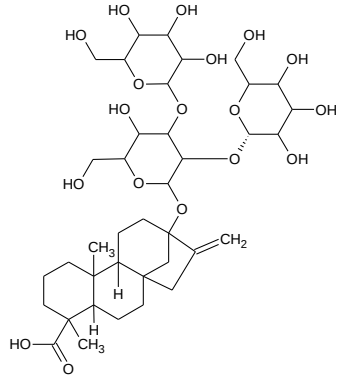
Waters™



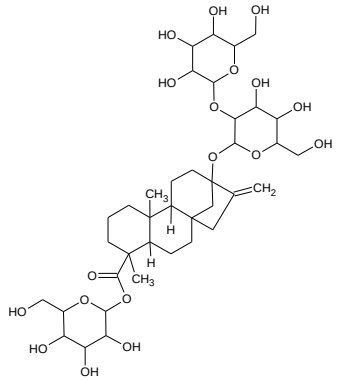
Rubusoside
M-H=641.3179 Da



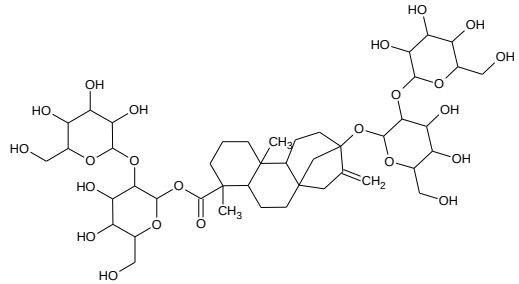
Steviolbioside
M-H=641.3179 Da



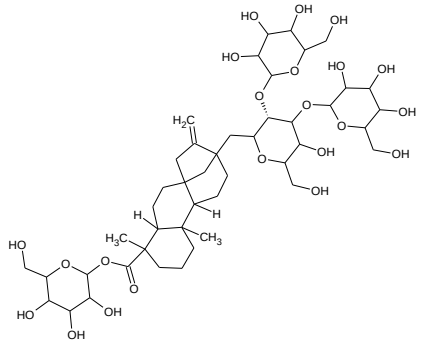
Rebaudioside B
M-H=803.3707 Da



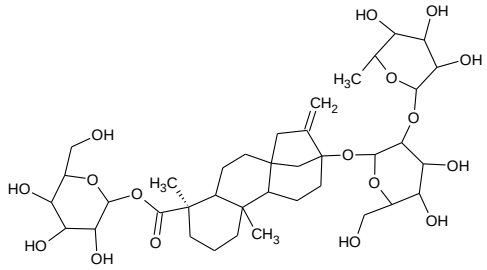
Stevioside
M-H=803.3707 Da*



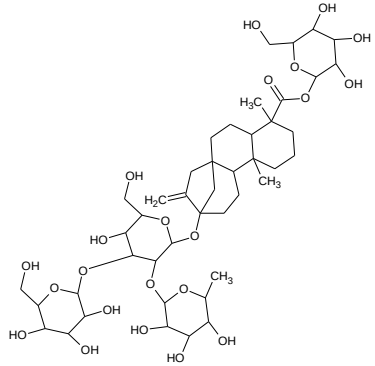
Rebaudioside E
M-H=965.4235 Da



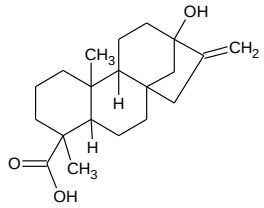
Rebaudioside A
M-H=965.4235 Da*



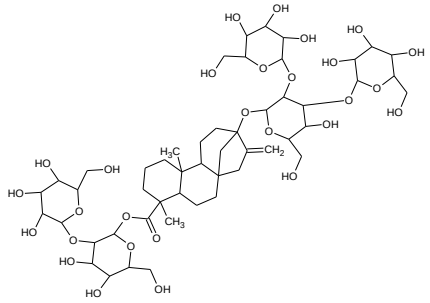
Dulcoside A
$[M-H]^-$=787.3758 Da



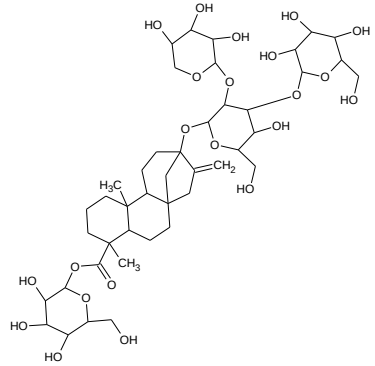
Rebaudioside C
$[M-H]^-$=949.4286 Da



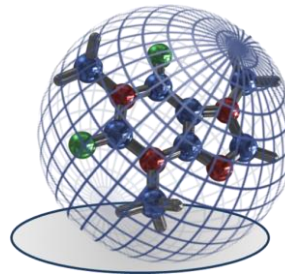
Steviol
$[M-H]^-$=317.2122 Da



Rebaudioside D
$[M-H]^-$=1127.4763 Da



Rebaudioside F
$[M-H]^-$=935.4129 Da

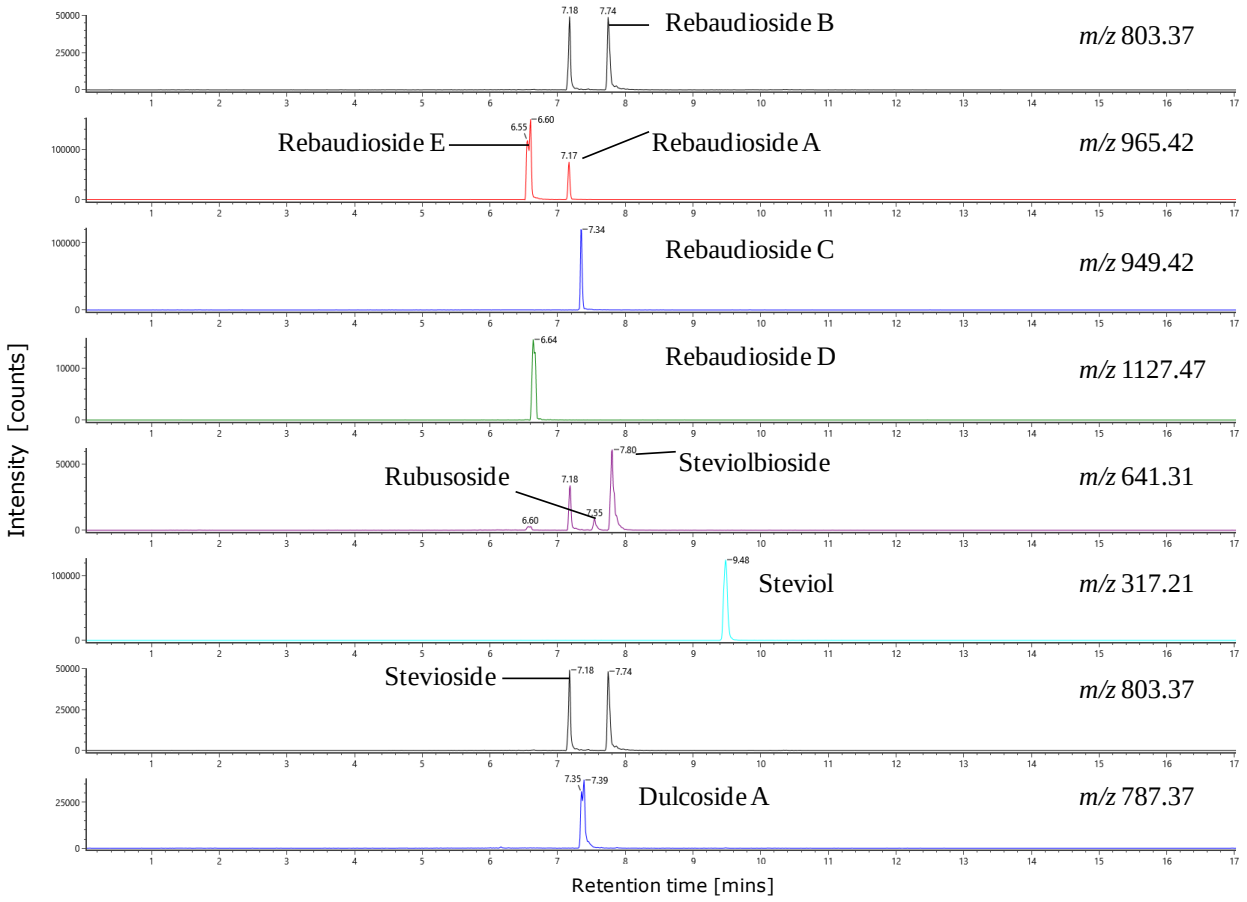


Determined micro-flow UPLC retention times and $^{TW}CCSN_2$ values

Compound	Formula	$[M-H]^-$ m/z	Expected Rt (mins)	Reference $^{TW}CCSN_2$ ($Å^2$)	Reference $[M - H + HCO_2H]^-$ $^{TW}CCSN_2$ ($Å^2$)
rebaudioside E	$C_{44}H_{70}O_{23}$	965.4230	6.61	289.2	298.7 (5%).
rebaudioside D	$C_{50}H_{80}O_{28}$	1127.4758	6.68	321.8	324.5 (11%)
rebaudioside A	$C_{44}H_{70}O_{23}$	965.4230	7.17	298.9	311.3 (19%)
stevioside	$C_{38}H_{60}O_{18}$	803.3701	7.20	269.6	278.1 (46%),
rebaudioside F	$C_{43}H_{68}O_{22}$	935.4124	7.32	293.2	306.5 (9%),
rebaudioside C	$C_{44}H_{70}O_{22}$	949.4280	7.37	299.5	308.1 (10%),
dulcoside A	$C_{38}H_{60}O_{17}$	787.3752	7.40	270.6	
rubusoside	$C_{32}H_{50}O_{13}$	641.3173	7.56	241.3	250.2 (284%)
rebaudioside B	$C_{38}H_{60}O_{18}$	803.3701	7.77	261.2	
steviolbioside	$C_{32}H_{50}O_{13}$	641.3173	7.81	235.8	
steviol	$C_{20}H_{30}O_3$	317.2117	9.48	173.4	

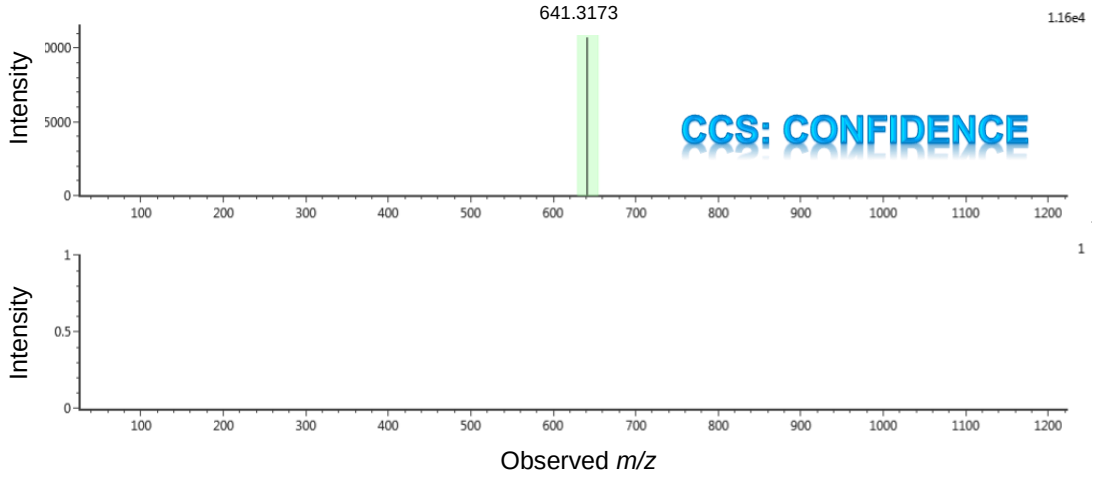
Steviol glycoside characterisation (spiked chocolate spread extract matrix)

Extracted mass chromatograms for steviol and profiled steviol glycosides ≤100pg/µL spiked into chocolate spread extract.



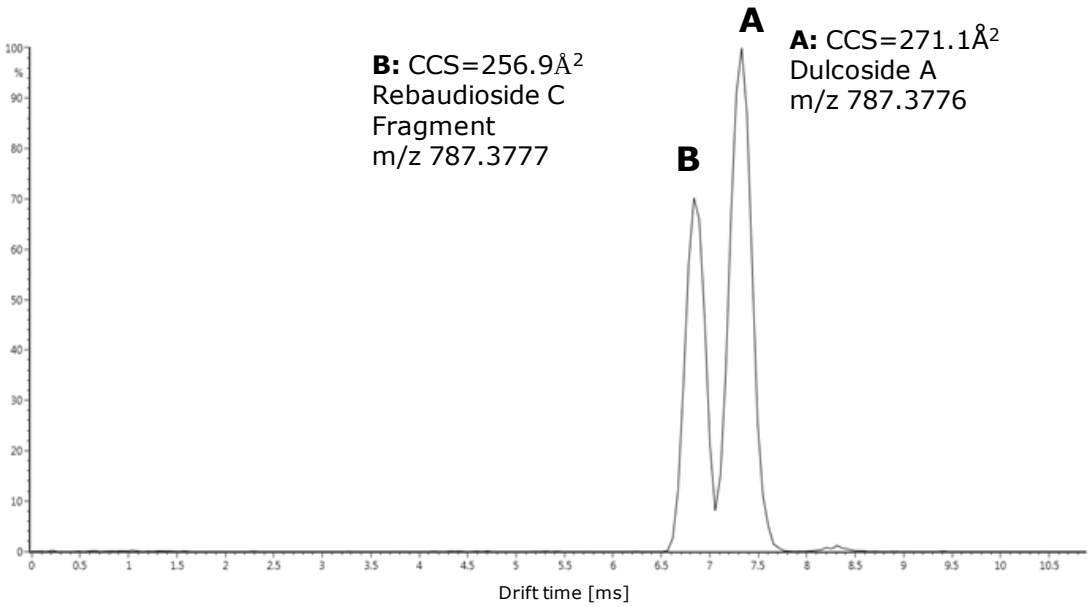
- Chromatographically coeluting isomeric species observed

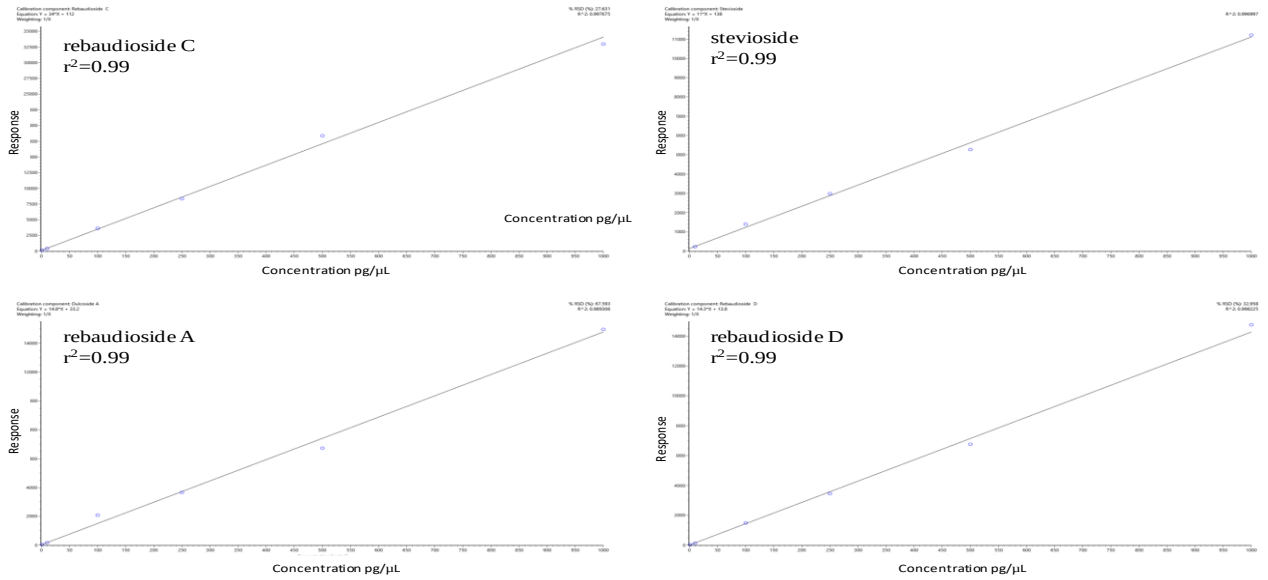
Rebaudioside A 1pg
Dulcoside A 1pg
Rebaudioside F 0.76pg
Rebaudioside E 0.92pg
Rebaudioside B 0.7pg
Rebaudioside C 1pg
Rebaudioside D 0.80pg
Stevioside 1pg
Rubusoside 0.68pg
Steviolbioside 0.72pg



- Waters ACQUITY UPLC M-Class® System
- Miroflow reverse phase gradient: iKey @ 2.0µL/min
- Injection Volume: 1µl (full loop)

Ion mobility ATD drift time plot showing two mobility separated species at *m/z* 787.37 for Reb C and Dulcoside A





Ion Mobility convoluted isomeric quantitation of steviol glycosides

	Calculated Concentration pg/µL			
	HRMS^E	HDMS^E	HRMS^E	HDMS^E
Sample code	dulcoside A $[M-H]^-$	dulcoside A $[M-H]^-$	stevioside $[M-H]^-$	stevioside $[M-H]^-$
Sample 1	386.10	98.39*	1843.24	1374.0
Sample 2	31.33	**Not observed**	1823.76	**Not observed**
Sample 3	18.97	**Not observed**	1505.52	**Not observed**
Sample 4	10.14	**Not observed**	1337.54	**Not observed**
Sample 5	37.24	**Not observed**	1840.37	**Not observed**
Sample 6	577.18	105.93	2315.34	2196.0
Sample 7	128.54	**Not observed**	1620.93	1306.62
Sample BE22	61.54	**Not observed**	1370.90	190.79
Sample T9	503.15	262.58	2578.72	2285.08
Sample TB6	152.90	**Not observed**	1730.29	1610.28
Sample BB1	549.45	**Not observed**	1682.17	1404.13

Soft drink analysis 2020: And still working in 2023!

- Robust, reproducible CCS values: Standing the test of time
- Methodology: UPLC-IM-MS
- Steviol glycosides identified using CCS: Retention times determined using 10 min retention time window

Component Summary

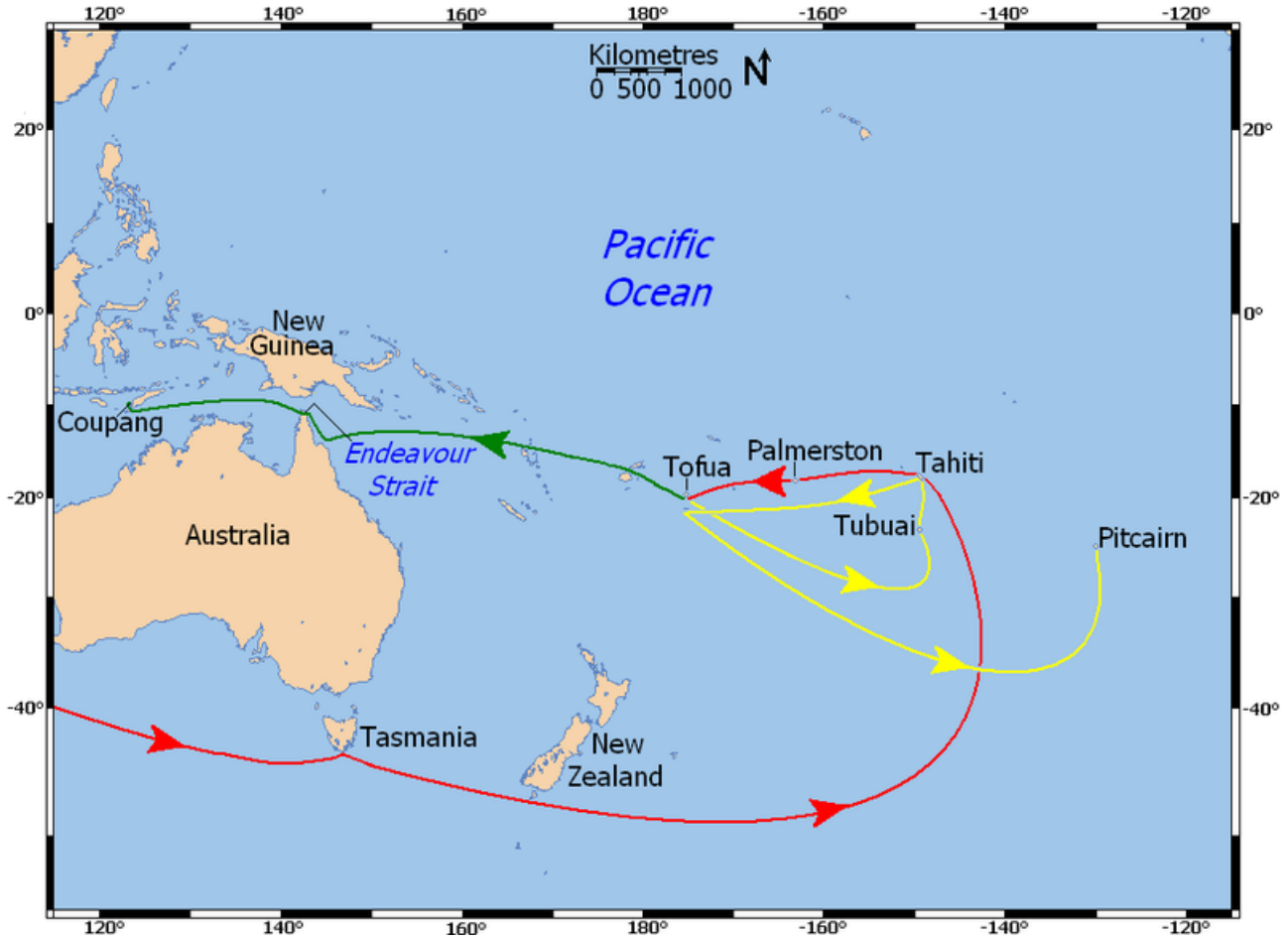
	Component name	Identification status	Observed m/z	Mass error (ppm)	Expected RT (min)	Observed RT (min)	Observed CCS (Å²)	Expected CCS (Å²)	CCS delta (%)	Response	Expected Fragments Found	Adducts
1	Citric acid	Identified	191.0193		-2.1	0.79	0.76	127.41	127.30	0.08	54912	0 -H
2	Dulcoside A	Identified	787.3749		-1.2	5.06	5.30	269.53	270.05	-0.19	282	0 -H
3	Dulcoside A Adduct	Identified	833.3828		1.9	5.06	5.06	277.10	275.23	0.68	3078	0 +HCOO
4	Hesperidin 1	Identified	609.1818		-1.1	3.44	3.48	231.22	231.15	0.03	5988	1 -H
5	Hesperidin 2	Identified	609.1818		-1.1	3.44	3.48	242.90	242.96	-0.03	2010	1 -H
6	Rebaudioside A	Identified	965.4240		0.5	4.81	4.81	296.21	298.90	-0.90	134205	1 -H
7	Rebaudioside B	Identified	803.3725		2.3	5.38	5.39	262.08	261.20	0.34	31245	0 -H
8	Rebaudioside C	Identified	949.4308		2.3	5.01	5.01	300.12	299.50	0.21	74692	0 -H
9	Rebaudioside D	Identified	1127.4765		0.2	4.17	4.17	323.68	321.80	0.58	2264	0 -H
10	Rebaudioside F	Identified	935.4145		1.7	4.95	4.96	293.77	293.20	0.19	32357	1 -H
11	Rebaudioside A Fragm...	Identified	803.3690		-2.1	4.81	4.81	261.15	261.20	-0.02	123587	0 -H
12	Rebaudioside C Fragm...	Identified	787.3754		-0.5	5.01	5.01	257.80	256.95	0.33	40854	0 -H
13	Rebaudioside E	Identified	965.4243		0.8	4.09	4.09	290.46	289.20	0.44	830	0 -H
14	Rubusoside	Identified	641.3165		-2.1	5.25	5.25	242.69	241.30	0.57	2862	0 -H
15	Rubusoside adduct	Identified	687.3237		0.5	5.25	5.25	251.08	249.63	0.58	7761	0 +HCOO
16	Stevioside	Identified	803.3692		-1.9	4.85	4.85	270.86	269.60	0.47	99541	0 -H

Column temp 45 °C

C18 HSS T3 2.1x 100 mm

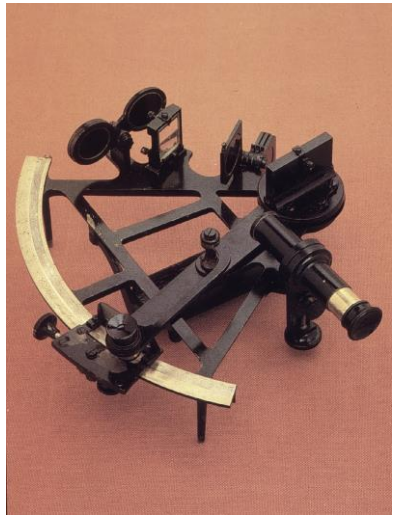
Time	Flow	%A	%B	Curve
0	0.4	95	5	6
0.5	0.4	95	5	6
6	0.4	0	100	6
9	0.4	0	100	6
9.5	0.4	95	5	6
11	0.4	95	5	6

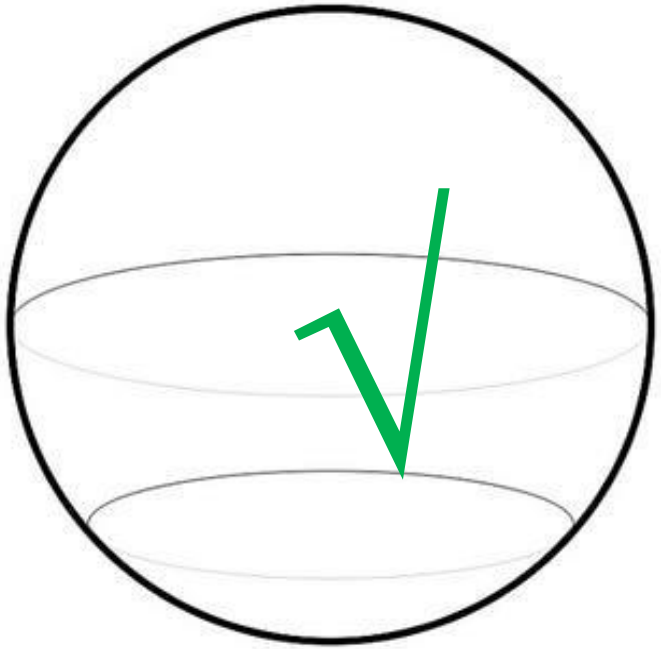
The “HMS Bounty Rule”
Never Trade Dimensions for Accuracy (Bleiner *et al.* (*in Prep*))



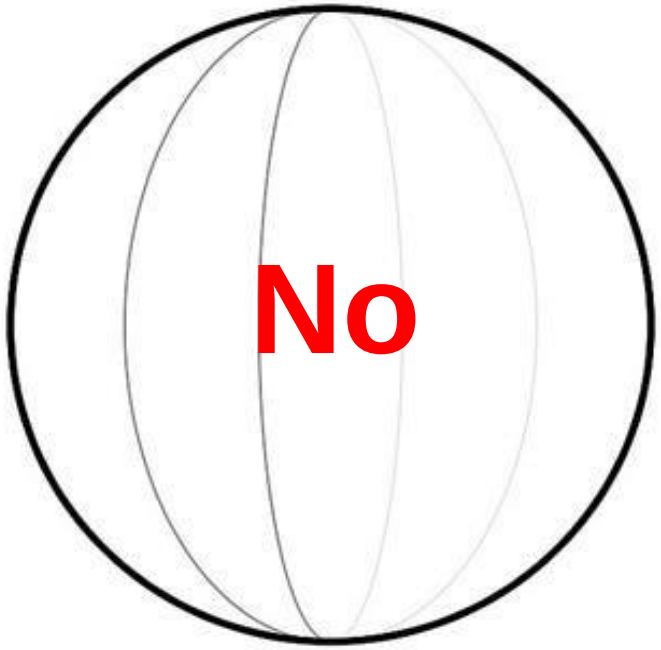
Wikipedia 2023

Before accurate chronometers: No accurate determination of Longitude

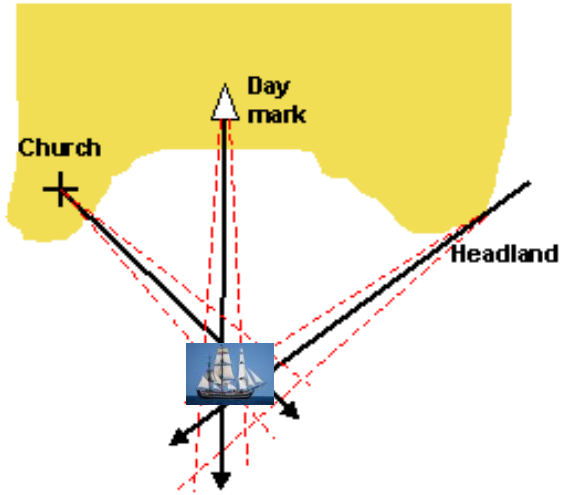




Latitude



Longitude



“The HMS Bounty Rule” applied to (LC) HRMS

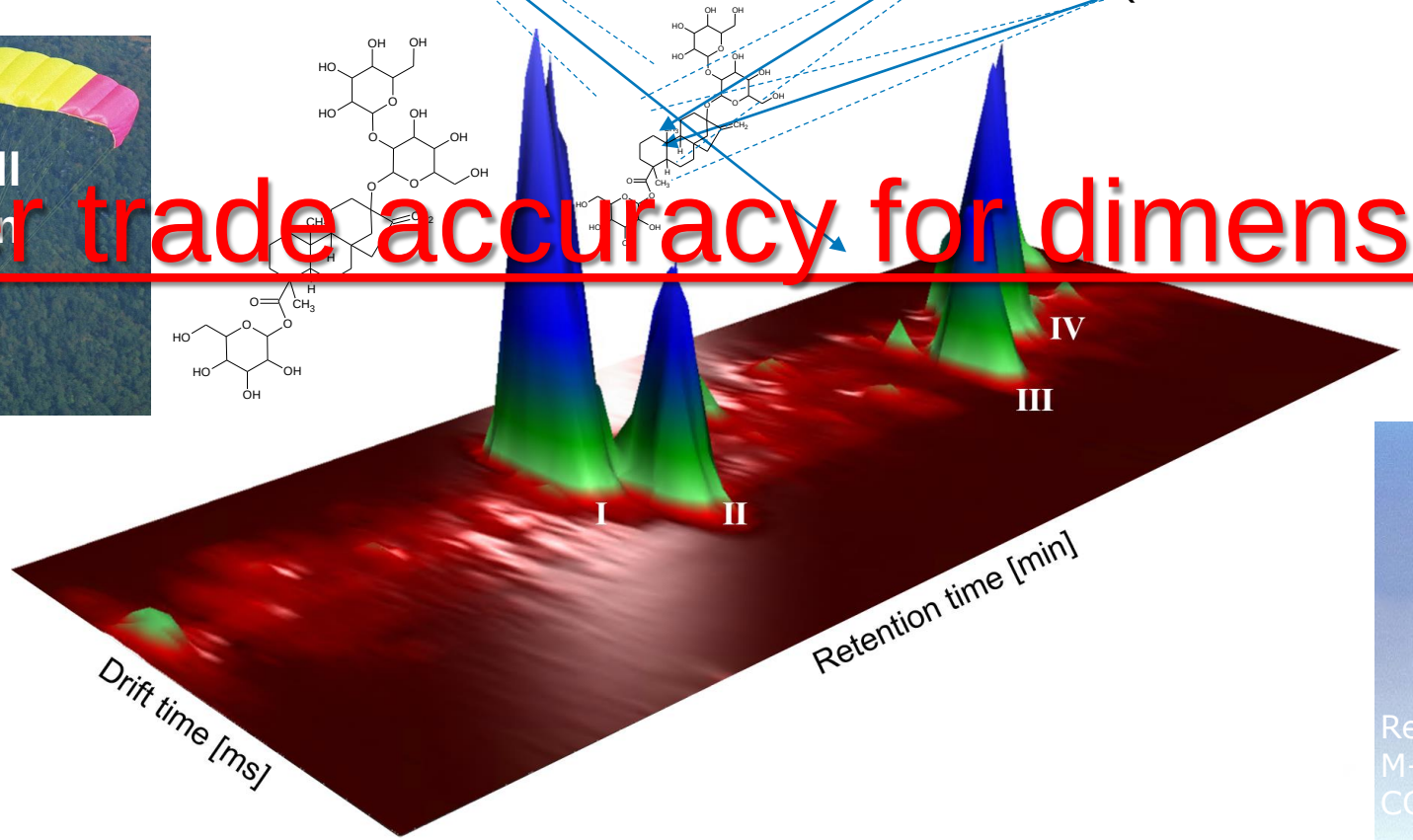
Waters™

Collisional Cross-Section (IMS)

Accurate Mass (HRMS)

Retention Time (xC; x = G, L, SF)

Never trade accuracy for dimensions!



Adapted from M. McCullagh *et al* 2021

Collaboration between Merck KGaA and Waters

Home › Press Releases › Merck and Waters to Collaborate on Extractables and Leachables Reference Library

Merck and Waters to Collaborate on Extractables and Leachables Reference Library

- **Library will enable analytical labs to identify potential E&L compounds in their samples using instruments from Waters and confirm the identity using Merck's reference materials**
- **Collaboration to provide testing labs with unrivalled confidence in their results and enhance consumer safety**

Darmstadt, Germany, February 15, 2022 – Merck, a leading science and technology company, today announced that its Life Science business sector has entered into a collaboration with **Waters Corporation** to build and expand an Extractables and Leachables (E&L) Reference Library to include ion mobility measurements. The library will enable analytical labs to identify potential extractables and leachable compounds in their samples by using Waters' ion mobility-enabled Liquid Chromatography Mass Spectrometry (LC-MS) instruments and then confirming the identity and quantity using Merck reference materials.

"Accurate screening for extractables and leachables is imperative to ensuring consumer safety, especially in pharmaceuticals, food packaging, or medical devices," said Heike Petri, Head of Advanced Analytical and Industrial & Testing. "This collaboration will provide manufacturers with unrivalled confidence in their results, help improve workflow efficiency for labs, and ultimately contribute to consumer safety."

Under the agreement, Waters will use high-quality analytical standards and Reference Materials from Merck to build and expand an E&L library of collision cross-section (CCS) values for Waters' LC-MS instruments. The library, which will be available for download from the **Waters Marketplace** (login required), will help to identify E&L compounds, with each addition to the library carefully selected to ensure maximum relevance to users. The library is cross-linked to the Merck online product catalogue to provide users access to reference materials to confirm their results.

About Merck

Merck, a leading science and technology company, operates across healthcare, life science, and electronics. Around 58,000 employees work to make a positive difference to millions of people's lives every day by creating more joyful and sustainable ways to live. From advancing gene-editing technologies and discovering unique ways to treat the most challenging diseases to enabling the intelligence of devices – the company is everywhere. In 2020, Merck generated sales of € 17.5 billion in 66 countries.

Scientific exploration and responsible entrepreneurship have been key to Merck's technological and scientific advances. This is how Merck has thrived since its founding in 1668. The founding family remains the majority owner of the publicly listed company. Merck holds the global rights to the Merck name and brand. The only exceptions are the United States and Canada, where the business sectors of Merck operate as EMD Serono in healthcare, MilliporeSigma in life science, and EMD Electronics.

All Merck news releases are distributed by email at the same time they become available on the Merck website. Please go to **www.merckgroup.com/subscribe** to register online, change your selection or discontinue this service.

Contact

Timo Breiner
Phone: +49 151 14542512
Email:
timo.breiner@merckgroup.com

LATEST PRESS RELEASES

07 Feb 2022 - Merck Announces Organizational Transformation to Strengthen CDMO Offering...

26 Jan 2022 - Merck Announces MoU with ID Pharma to Accelerate Development and...

20 Jan 2022 - Merck Announces Elevian as Winner of its North American Advance Biotech...

11 Jan 2022 - Merck Announces Expansion of Stable Isotope Center of Excellence in...

More Press Releases| ›

Collaborating with MilliporeSigma to incorporate CCS values

Accurate screening for extractables and leachables (E&Ls) is imperative for consumer safety, no matter the product. Be it a pharmaceutical, an item of food packaging, or a medical device, consumers place their trust in manufacturers to protect them from the thousands of potentially harmful chemicals that can leach into their products.

Through an exciting collaboration between Waters Corporation and the Life Science Business of MilliporeSigma, cross-collision (CCS) values are referenced on Sigma-Aldrich.com for LC/MS amendable E&L Certified Reference Materials (CRM) and analytical standards. As ion mobility spectrometry (IMS) becomes more routine, CCS values provide an additional identification point, strengthening results for labs and enhancing safety for consumers.

E&L Reference Materials that you can trust

As part of the collaboration, Waters and MilliporeSigma have jointly constructed a comprehensive UPLC-IMS-MS E&L library based on high-quality reference materials. The library can help users screen for a wide array of E&L compounds, with each addition to the library carefully selected to ensure maximum relevance to users. Each reference material also provides users with major identification points including *m/z* values for product ions, fragment ions and CCS.

Through application of IMS, the rotationally-averaged collision cross-section (CCS) of a given ion can be determined. It is a distinguishing characteristic and provides users with an additional descriptor that can improve confidence in their results.

We sat down with some of the minds that helped drive this collaboration forward so they could share their insights into how it will support analytical labs and enable greater throughput efficiency. Speaking to us from the Waters team were Mike McCullagh, [Consultant Scientist], Ben MacCreath, [Chemical Materials Business Operations], and Jens Jacobsen, [Sales Specialist] at Waters. From the MilliporeSigma side, we spoke to Coralie Leonard, [Business development, licensing and innovation manager], Dr Matthias Nold, [Product Manager for Reference Materials], and Markus Obkircher, [Director of R&D].

Share this article

IMS Newsletter

Stay updated with new IMS posts & articles

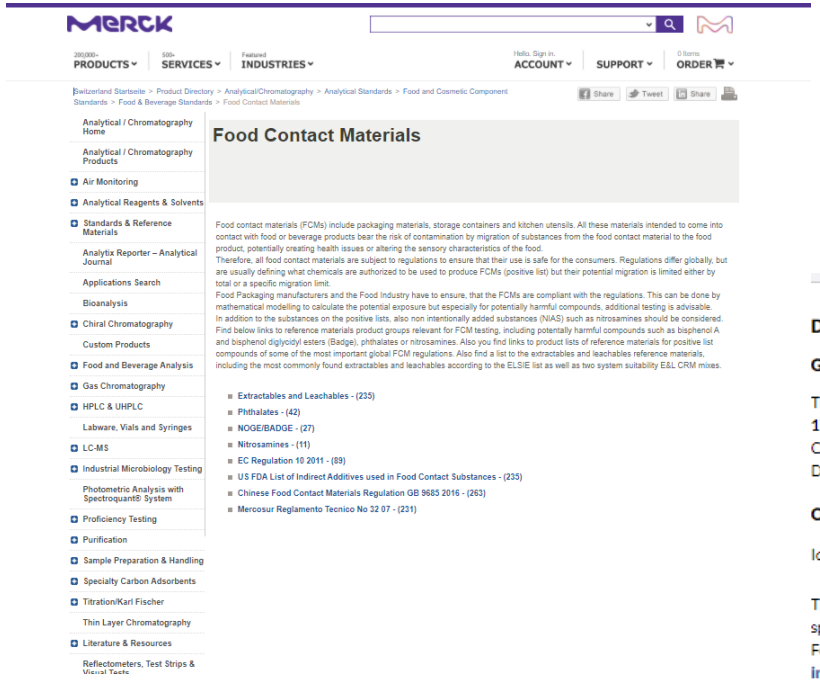
First Name*
Last Name*
Organization*
Email*
Country*
City*
Province/Region*
Zip/Postal Code*

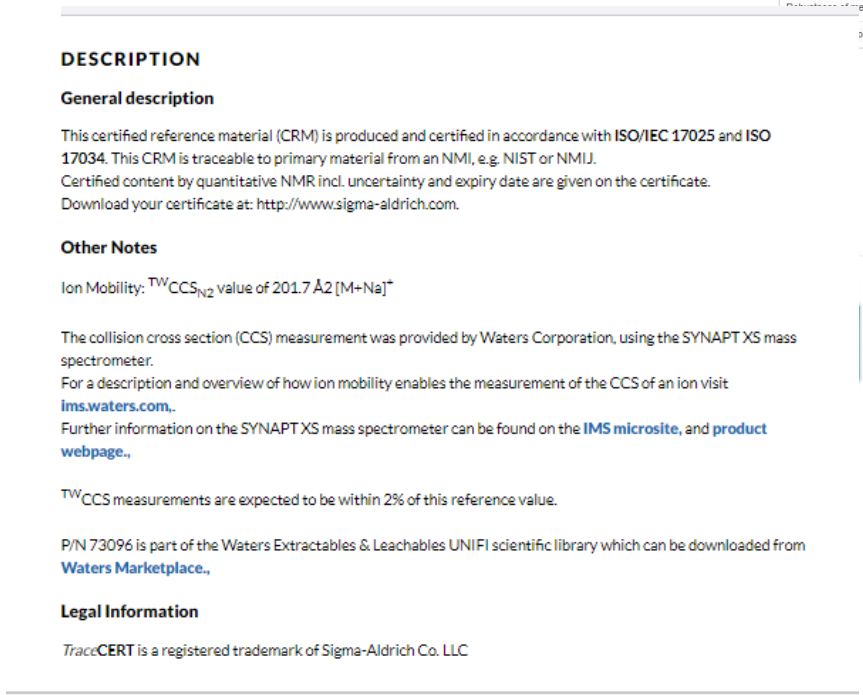
Please have a Waters representative contact me about (Please select one)

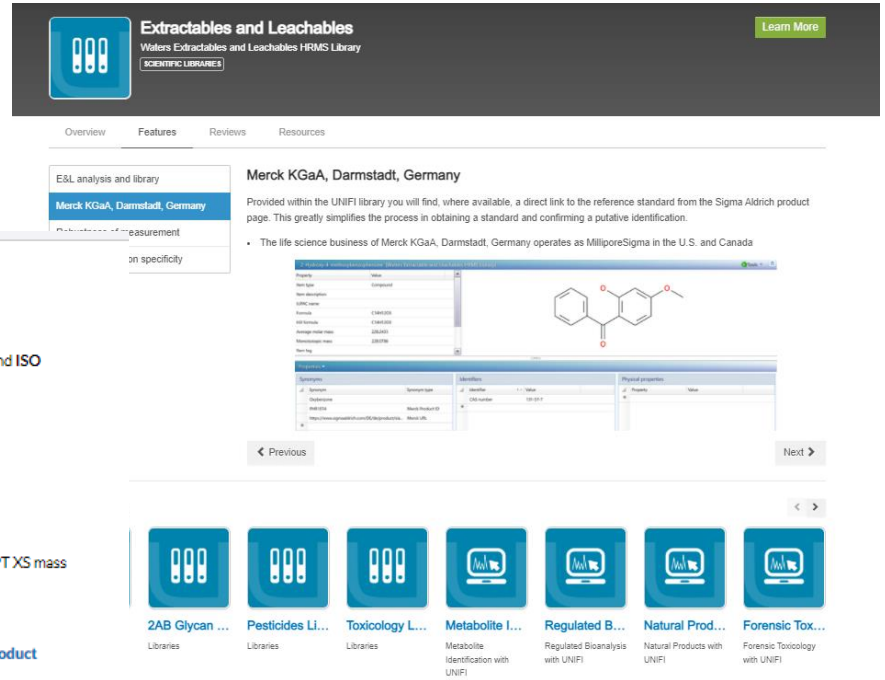
☐ SELECT SERIES Cyclic IMS

Collaboration with Sigma-Aldrich (Merck KGaA) part 2:

- Cross referencing Merck (Sigma-Aldrich) and Waters







https://www.sigmaaldrich.com/DE/en/product/sial/73096?context=product

Summary

- CCS is complementary to mass and retention time (3 dimensions of resolution) √
- CCS robustness√
- Non-targeted screening in complex matrices√
- Reduced false detection rates√
- Differentiated isomers using CCS√
- Low intensity monoisotopic peak/ no product ions: CCS additional metric √
- Non labile compounds/ no product ions: CCS additional metric √
- Unique isomeric quantitation: CCS additional metric √
- True calculated concentration determined √
- Adducts provide an additional descriptor √
- Ion mobility product ions √

Acknowledgements

- Eurachem for inviting ☺
- Mike McCullagh Waters & Waters Corp Wilmslow
- Markus Obkircher, Sigma-Aldrich (Merck) Switzerland
- Matthias Nold & Hanspeter Sprecher Sigma-Aldrich (Merck) Switzerland
- Coralie Leonard, Sigma-Aldrich (Merck), Europe

THANK YOU !

Waters™

Merci viu mau!

Recent Publications

- **Use of ion mobility mass spectrometry to enhance cumulative analytical specificity and separation to profile 6-*C*/8-*C*glycosylflavone critical isomer pairs and known–unknowns in medicinal plants**. Michael McCullagh, Cintia Alessandra Matiucci Pereira and Janete Harumi Yariwake. *Phytochemical Analysis*. 2019;1–13

- **Exploring the Complexity of Steviol Glycosides Analysis Using Ion Mobility Mass Spectrometry.** Michael McCullagh, David Douce, Els Van Hoeck and Severine Goscinny. Anal. Chem. 2018, 90, 4585−4595.

- **Investigations into the performance of travelling wave enabled conventional and cyclic ion mobility systems to characterise protomers of fluoroquinolone antibiotic residues**. Michael McCullagh, Kevin Giles, Keith Richardson, Sara Stead and Martin Palmer. *Rapid Commun Mass Spectrom*. 2019;1–11.

- **Towards the use of ion mobility mass spectrometry-derived collision cross section as a screening approach for unambiguous identification of targeted pesticides in food**. Séverine Goscinny, Michael McCullagh, Johann Far, Edwin De Pauw and Gauthier Eppe. *Rapid Commun Mass Spectrom*. 2019;1–15.

- **A Comparison of Collision Cross Section Values Obtained via Ion Mobility Spectrometry Following Direct Infusion and an Evaluation of U(H)PLC-IM-MS for the Characterisation of Metabolites in Rat Urine.** Leanne C. Nye, Jonathan P. Williams, Nyasha C. Munjoma, Marine P.M. Letertre, Muireann Coen, Robbin Bouwmeester, Jeremy K. Nicholson, Robert S. Plumb, Michael McCullagh, Lee A, Gethings, Steven Lai, James I. Langridge, Johannes P.C. Vissers, Ian D. Wilson. J Chromatogr A2019 Sep 27;1602:386-396.

Recent Publications

- **Profiling of the known-unknown *Passiflora* variant complement by liquid chromatography - Ion mobility - Mass spectrometry.** Michael McCullagh, Jeff Goshawk, David Eatough, Russell J. Mortishire-Smith, Cintia AM. Pereira, Janete H. Yariwake, Johannes PC. Vissers. Talanta 221 (2021) 121311

- **Travelling Wave Ion Mobility-Derived Collision Cross Section for Mycotoxins: investigating interlaboratory and interplatform reproducibility.** Laura Righetti, Nicola Dreolin, Alberto Celma, Mike McCullagh, Gitte Barknowitz, Juan V. Sancho, Chiara Dall'Asta. *J. Agric. Food Chem.* 2020, Publication Date:September 1, 2020.

- **APPLICATION NOTES**
- **Collision Cross Section- A New Identification Point for a "Catch All" Non-Targeted Screening Approach.** Michael McCullagh[1] and Severine Goscinny[2]. [1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium. Waters Application Note June 2014 720005055EN.
- **Discovery of pesticide protomers using routine ion mobility screening**. Michael McCullagh and Severine Goscinny[2]. [1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium. *Note* 2014, *720005028E*.
- **Ion Mobility in a Routine Workflow to Understand the Challenge of Analyzing Fluoroquinolone Antibiotic Residues**. Michael McCullagh and Sara Stead. Waters Corporation, Wilmslow, United Kingdom *Note June* 2014, *720005078E*.
- **Cleanup and Collision Cross Section Values toIncrease Confidence and Efficiency in Pesticide Residues Screening Strategies**. Michael McCullagh and Severine Goscinny[2]. [1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium. *Note* 2014, *720005080E*.

- **Enhancing analysis specificity and deconvolution of natural products using a positive mode ion mobility mass spectrometry library.** Michael McCullagh; Jeff Goshawk; Russell Mortishire-Smith. **Waters Application Note** September 2019 720006650EN

- **Un-paralleled multi-factor authentication mass spectrometry of complex natural products utilising UPLC and ion mobility.** Michael McCullagh; Russell Mortishire-Smith and Jeff Goshawk. **Waters Application Note March** 2020 720006791EN

- **Small molecule ion mobility investigations into cross-platform and long-term robustness of a CCS metric.** Mike McCullagh[1]; Michelle Wood[2]; Nayan Mistry[2]; Severine Goscinny[3] and Petur Dalsgaard[4]. [1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium; [3]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark. **Waters Application Note** March 2020 720006769EN.

- **Use of ion mobility $^{TW}CCSN_2$ values in non-targeted food additives screening** Michael McCullagh[1]; Jeff Goshawk[1], and Severine Goscinny[2]. [1]Waters Corporation, Wilmslow, United Kingdom; [2]Sciensano, Brussels, Belgium. **Waters Application Note** February 2020 720006768EN.

- **A Workflow for Automatic MS Library Creation from Time-of-Flight Full-Spectra Data Processed in UNIFI.** Jeff Goshawk, Gitte Barknowitz, and Michael McCullagh February 2020 720006783EN