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Analysis and Comparisons of the Natural Product Contents of Herbal Supplements Using a Drift Tube Ion Mobility Mass Spectrometer

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Introduction

For generations herbal supplements have been the foundations of folk and traditional medicines with rich bioactive natural product profiles. In modern times there is resurgent interests in dietary herbal supplements often being perceived as a natural alternative to western medicine. However, regulatory oversight of these products varies widely and many supplements are complex mixtures of natural products that may vary widely by manufacturer. The identity various of the supplements may contain many unknowns and identifying them in complex mixtures can be an analytical challenge. Many of the natural products are small molecules that may have complex 3D shapes making them amenable to analysis using an ion mobility mass spectrometer..

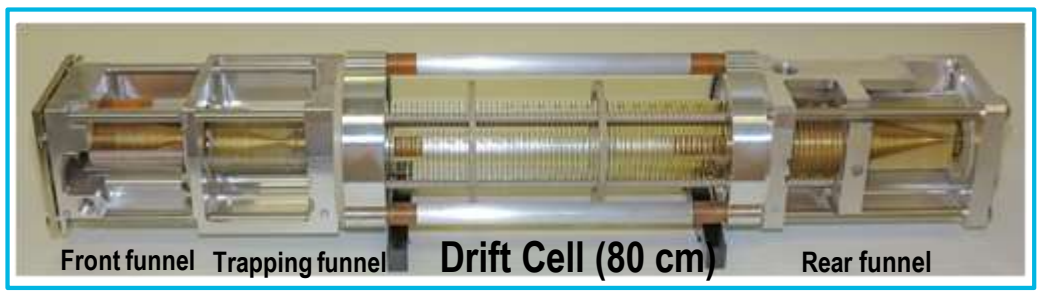

Figure 1: An overview of the uniform field drift tube used in the Agilent 6560 QTOF.

The mobility of an ion depends on the drag force of an ion through an inert drift gas. The mobility though the drift cell depends on the molecules collisional cross section which is a function of the molecules size, shape and charge. Additionally the mobility constant of is influenced on the following characteristics of the drift cell:

$$K_0 = \frac{L}{t_d E} \frac{P}{760} \frac{273.2}{T}$$

- T is temperature of the drift gas
- P is the pressure of the gas in the drift cell
- E is the electric field across the drift cell
- L is the length of the drift cell
- t_d is the corrected drift time

Additionally the use of a uniform field drift cell gives the additional advantage that RF is not applied in the drift cell. This reduces the amount of ion heating leading to a reduction the amount of fragmentation and ion heating related conformer induction in the drift cell. This "gentle" drift cell design reduces the complexity of data analysis when studying natural products.

Experimental

Figure 2: Agilent 6560 Ion Mobility QTOF LC/MS

Herbal supplements from three different manufacturers for Gingko biloba (standardized to 24% flavonoid glycosides) were decapsulated and then extracted in 100% methanol and then filtered for analysis. Samples were then analyzed by LCMS in flow injection mode or by reverse phase LC analysis coupled to a drift tube ion mobility mass spectrometer. When in ion mobility mode the nitrogen was used as the drift gas in a uniform drift field to separate compounds in the second dimension. 4D Feature finding was done in Mass Hunter IM Browser B.08.00 and Mass Profiler B.08.00. Univariate and Multivariate analysis including hierarchical clustering analysis was done in Mass Profiler Professional 14.9.1.

Jetstream Source Settings			
Dry Gas Temp	325°C	Fragmentor	400 V
Dry Gas Flow	8 l/min	Nozzle Voltage	1000 V
Sheath Gas Temp	275°C	Skimmer	30 V
Sheath Gas Flow	8 l/min	Vcap	4000 V
Nebulizer Pressure	20 psi	Octopole RF	750
Ion Mobility MS Settings			
Mass Range	100-100 m/z	Trap Fill Time	20000 µs
Frame Rate	1 frame/sec	Trap Release Time	200 µs
Max Drift Time	60 ms	Trap Funnel Delta	180
Trap Funnel RF	150	Trap Funnel Exit	10
LC Gradient (when used)			
Column: Agilent SB-Aq 2.1mm X 100 mm, 1.8µm			
Solvent A	100% H_2O/0.1% Formic Acid	Solvent B	90% ACN 5mM NH_4OAc
Gradient:	A (%)		B(%)
Initial	97		3
3.00 min	97		3
12.00 min	0		100
15.00 min	0		100
Post Time:	4 minutes		

Table 1: IM QTOF and LC Analysis Conditions

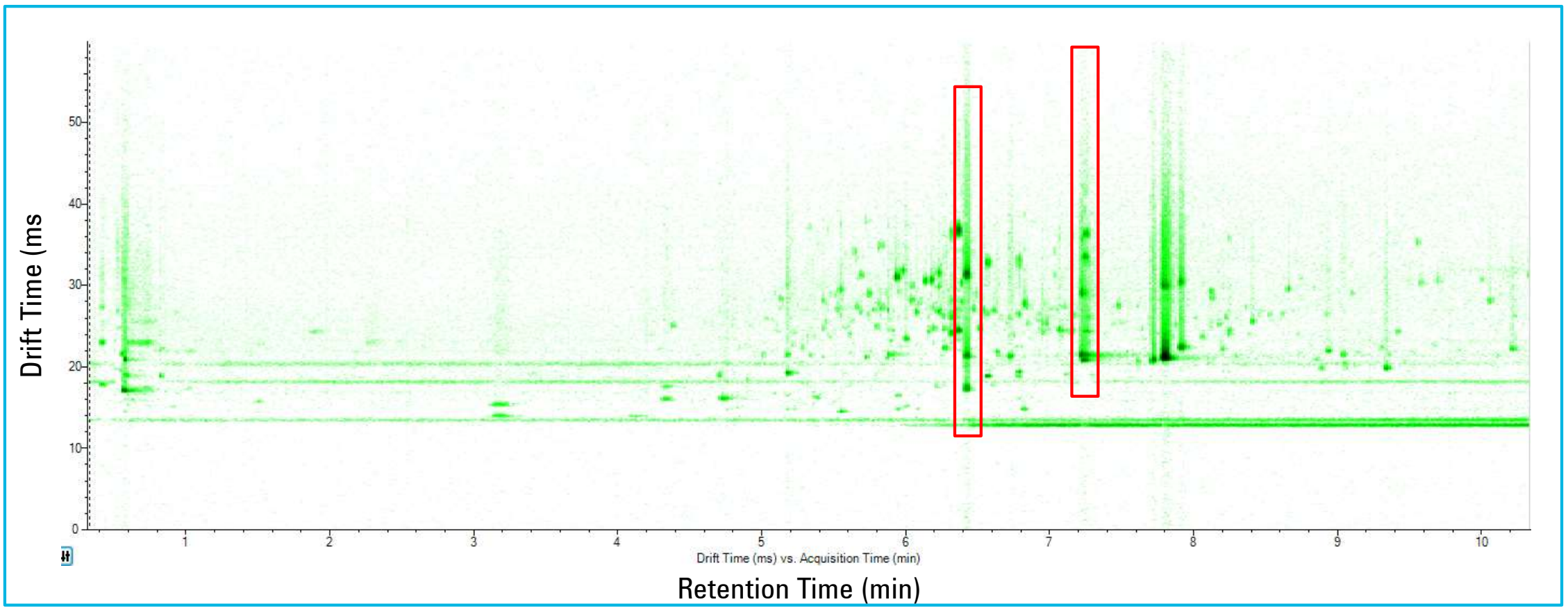

Figure 3: Drift Time Vs Retention Time Plot generated in MassHunter IM-MS Browser B.08.00 SP1 for a selected Gingko biloba supplement from Manufacturer 3 in positive ion polarity. Deep green colors indicate higher intensity. The plot easily shows IM separations of supplement components that had the same retention times at 6.4 minutes and 7.2 minutes. These may be biologically relevant conformers or isomers.

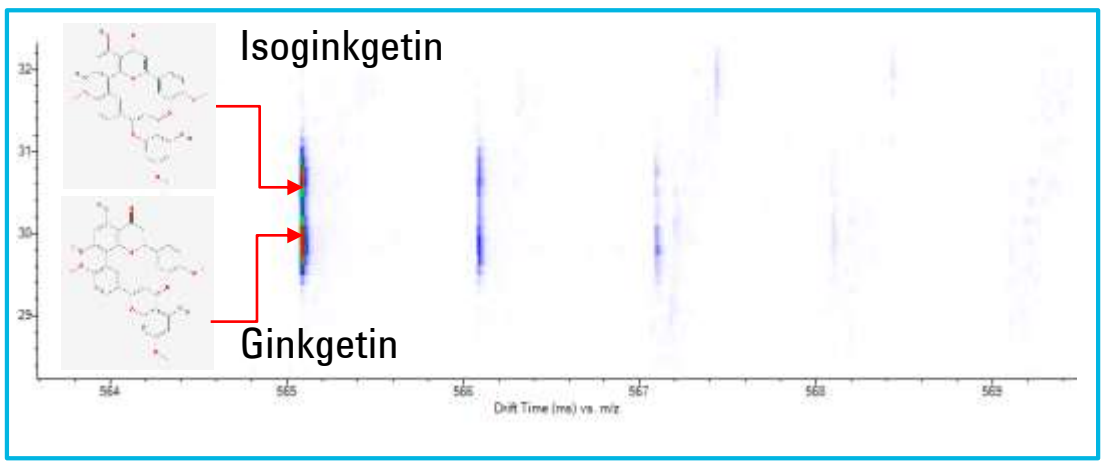

Figure 4: The Agilent 6560 was able to separate two Ginkgo biloba specific biflavones that were unable to be separated by standard LCMS. The two biflavones differ only in the position of a methoxy group, enough perhaps to change the shapes of the molecules to allow for IM separation.

	Manufacturer 1	Manufacturer 2	Manufacturer 3
FIA-MS	261	424	436
FIA-IMS-MS	172	199	212
LC-QTOF	407	910	827
LC-IMS-QTOF	1412	1814	1582

Table 2: A comparison of features detected in the various modes of the Agilent 6560 for Gingko biloba supplements. Flow injection had lower numbers of features detected with ion mobility mode on, possibly due to trap capacity. However, when orthogonal separation techniques like LC and IMS are done together feature numbers increase greatly.

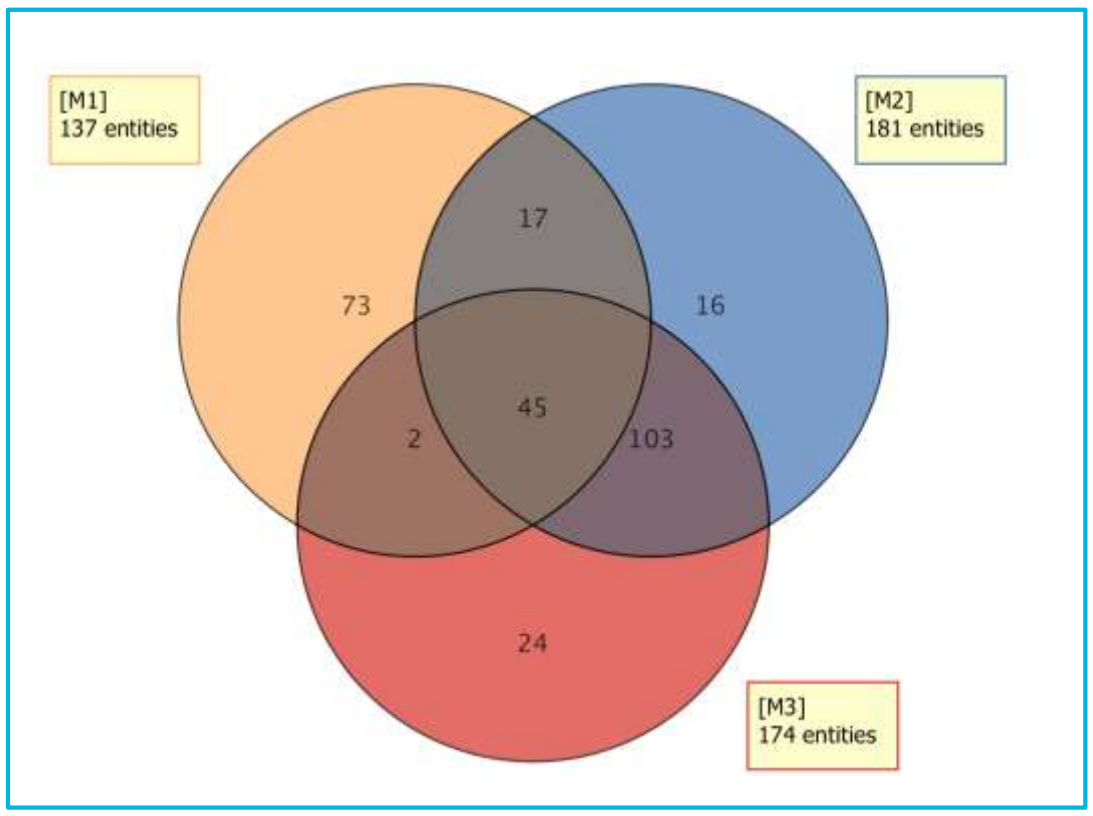

Figure 5: Find Unique Entities analysis determined the shared and unique compositions of the Gingko supplements as detected by FIA-IM-MS. The analysis shows a high degree of heterogenicity in the samples from different manufacturers despite being from the sample plant species and same "standardization" to 20% flavonoid glycosides. This likely is a result of different manufacturing processes. M1 = Manufacturer 1 M2 = Manufacturer 2 M3= Manufacturer 3

Results and Discussion

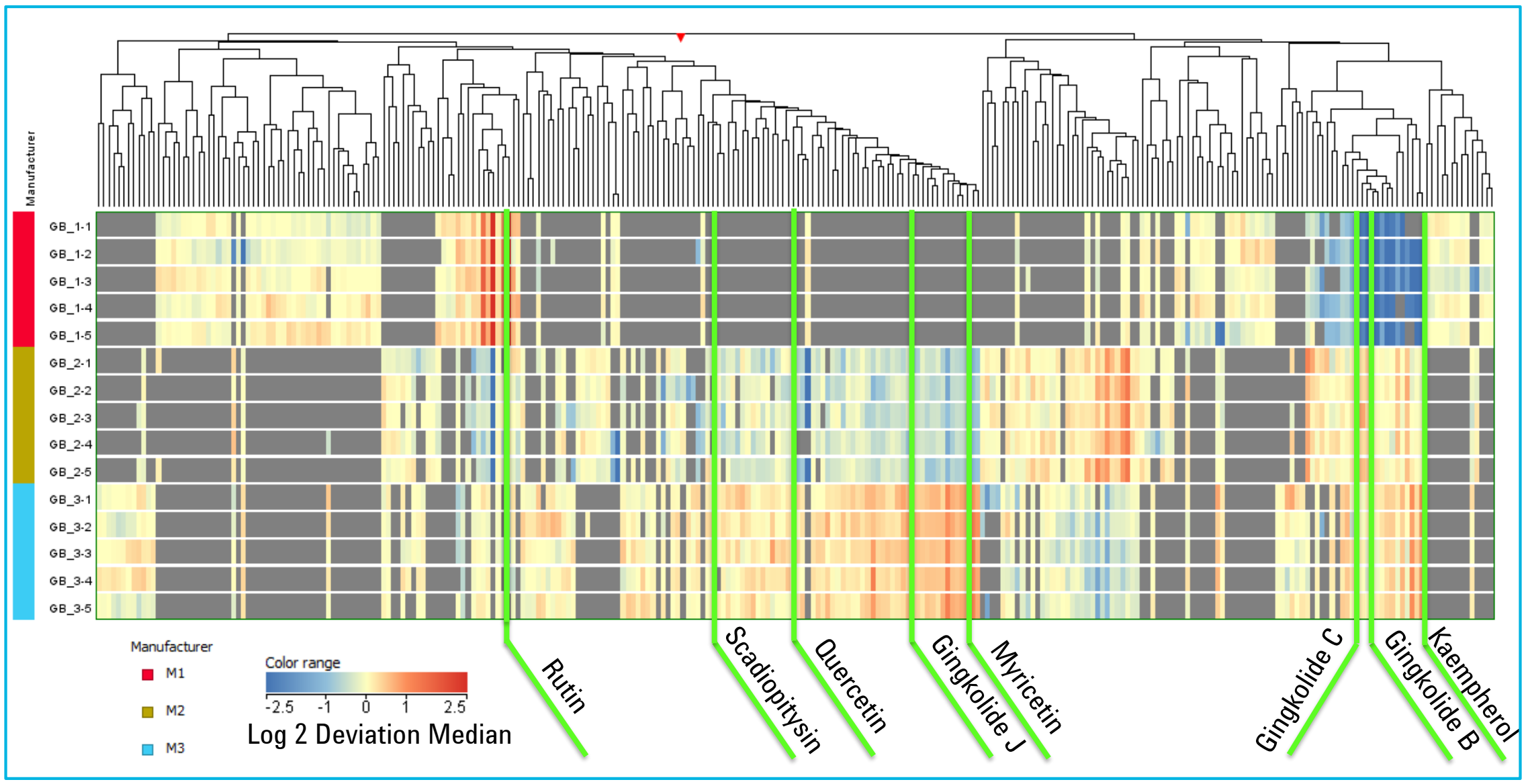

Figure 6: Hierarchical Cluster Analysis of Gingko supplements of with statistically different features. Grey indicates missing from the analysis. Manufacturer 1 shows a high degree of flavonoid glycosides in form of Rutin and related compounds but is low intensity for terpene lactones. Manufacturers 2 and 3 have a much higher Terpene lactone concentrations, a much larger diversity in the flavonoid glycoside profile and lower concentrations of Rutin.

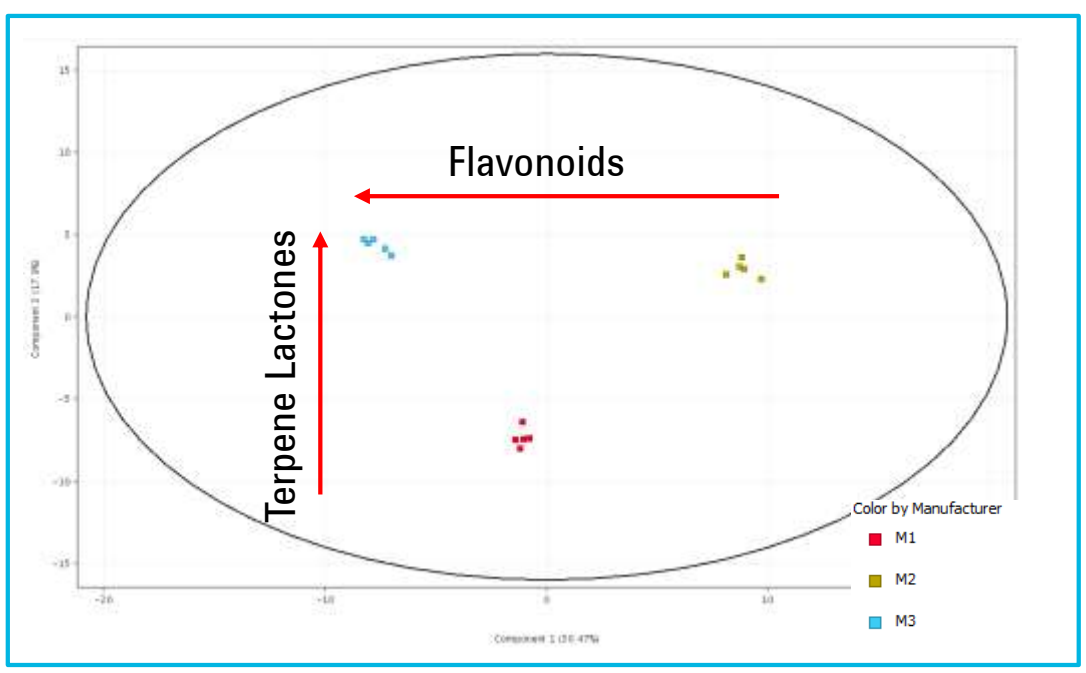

Figure 7: Principal Component Analysis of the Gingko biloba supplements . PC1 (30.47%) related to the concentration of flavonoid glycosides in the sample Manufacturer 3 (M3) was most abundant for these compounds. PC2 (17.9%) related to the concentration of terpene lactones where Manufacturer 2 (M2) and Manufacturer 3 were the highest, where Manufacturer 1 (M1) was the lowest.

Conclusions

Feature extraction from flow injection, chromatographic, ion mobility and ion mobility data of the selected herbal supplements indicated wide variability in the contents of supplements from each manufacturer. Ion mobility seems to be especially amenable to separating flavanoid isomers including ginkgetin and isoginkgetin. Feature finding seemed especially enhanced by orthogonal LC-IM-MS techniques, in part because the ion mobility separation was able to bring many flavonoids out of the underlying matrix, removing interferences and make them much more easily visible and quantifiable to the automated feature finding algorithm. Ultimately, the combination of LC, IM and MS has proven to be powerful in natural products and herbal supplement research as it is uniquely able to take advantage of complex molecular shapes for enhanced separations. This new technique may enhance our understanding of what's in the supplements we take and perhaps eventually their biological significance when ingested.

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