Determination of Limonin in Citrus Flour by Time of Flight (TOF) LC/MS

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Introduction

Limonin is a bitter, white, crystalline substance found in citrus and other plants. Limonin is enriched in citrus fruits and is often found in higher concentrations in seeds, pulp and peel of oranges, grapefruit, lemons, limes, pomelos, bergamots and mandarins.[*1]

The recent rise in popularity of meat and dairy plant-based substitutes has necessitated the use of citrus fiber or flour to replace starches, gums, chemical emulsifiers and/or stabilizers. Citrus four holds water, emulsifies, binds oil, and is vegan. This natural citrus fiber improves texture and stability over time and is particularly useful in bakery, beverages, dairy products, dressings, meats, sauces, frozen foods, pet foods, dairy alternatives & plant-based meats. Citrus fiber or flour is made from leftover dried fibrous material after the fruit is juiced and can have high concentrations of limonin and other limonoids that contribute to the delayed bitter taste.[*2]

The method discussed here is useful in the quantitation of limonin in foods, food additives, juices and plants. The use of the Agilent LC/ToFMS allows for excellent quantitation of limonin as well as the characterization of other congeners.

Limonin
$C_{26}H_{30}O_8$
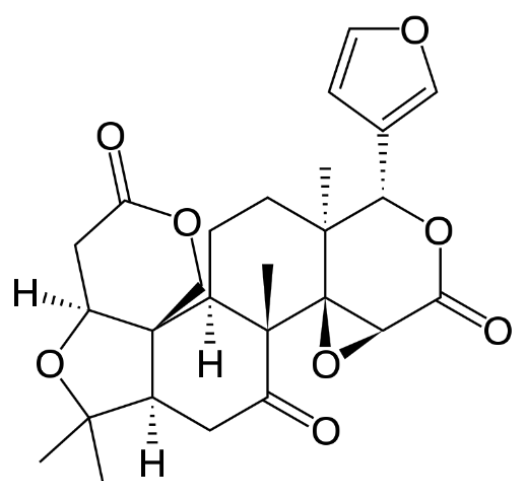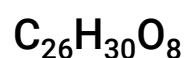
(2aR,4aR,4bR,5aS,8S,8aS,10aR,10bR,14aS)-8-(Furan-3-yl)-2,2,4a,8a-tetramethyldecahydro-11H,13H-oxireno[2,3-c]pyrano[4'',3'':2',3']furo[3',4':5,6]naphtho[1,2-d]pyran-4,6,13(2H,5aH)-trione[*3]

Experimental

Equipment

All experiments in this study were performed using an Agilent 1290 Infinity II LC consisting of an Agilent 1290 Infinity II multisampler (G7129B), an Agilent 1290 Infinity Flexible pump (G7104A), and an Agilent 1260 Infinity II multicolumn thermostat (G7116A) coupled to an Agilent Time of Flight (G6230B) mass spectrometer. The system was controlled by Agilent Mass Hunter software, version 11. Data processing was performed using the same Agilent Mass Hunter software

Samples, Standards and Consumables

Chromatographic Conditions

Parameter	Setting
Analytical Column	Agilent EC-C18 3x100mm 2.7 Part number: 695975-302
Column Oven	30.0ºC
Injection Volume	0.1 μl
Run Time	15.00 min.
Post-run Time	5.00 min.
Flow Rate	0.6 ml/min

Channel	Solvent	%A	%B
A	H2O + 0.1% Formic Acid	0 Min	95
B	Methanol	10 Min	5

Time of Flight Conditions

Parameter	Value
Ion Source & Polarity	Dual ESI positive
Gas Temperature (ºC)	350
Drying Gas Flow (l/min)	8
Nebulizer (psi)	60
Capillary Voltage	3500
Fragmentor Voltage	380
Skimmer Voltage	140
Mass Range (m/z)	100-1700
Acquisition Rate (spectra/sec)	1.0

Sample Extraction

1. Mix 1g matrix powder with 10 ml water in a 50 ml centrifuge tube.

2. Add 10 ml acetonitrile.

3. Add the contents of Agilent QuEChERS Extract Pouch P/N 5982-0650 and vortex or shake manually for 5 minutes.

4. Centrifuge sample at >3200 rcf for 5 minutes.

Sample Clean-up

1. Transfer 1 ml of supernatant to an Agilent Dispersive 2 ml Universal Kit P/N 5982-0028

2. Vortex samples for 30 seconds.

3. Centrifuge sample at >3000 rcf for 5 minutes.

4. Transfer 500-600ul of purified supernatant into an autosampler vial for analysis.

Limonin Calibration Curves

The instrumental portion of this method was evaluated using standard solutions of limonin in acetonitrile. The calibration curves were generated utilizing the load-on-column method to minimize propagation of pipetting errors. The in-matrix calibration range was chosen to be 12.5 – 1250 ppm.
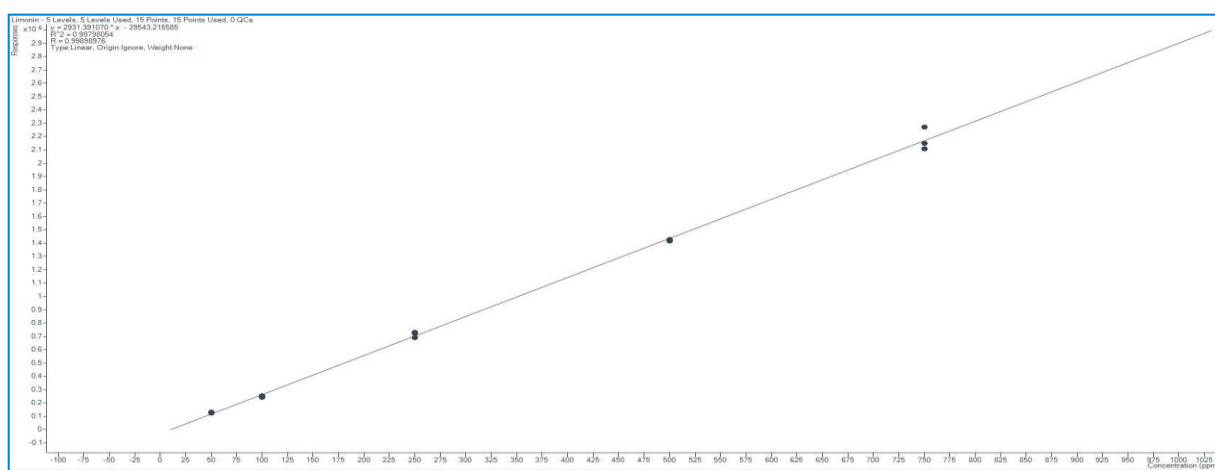
Calibration Curve – Limonin in ACN
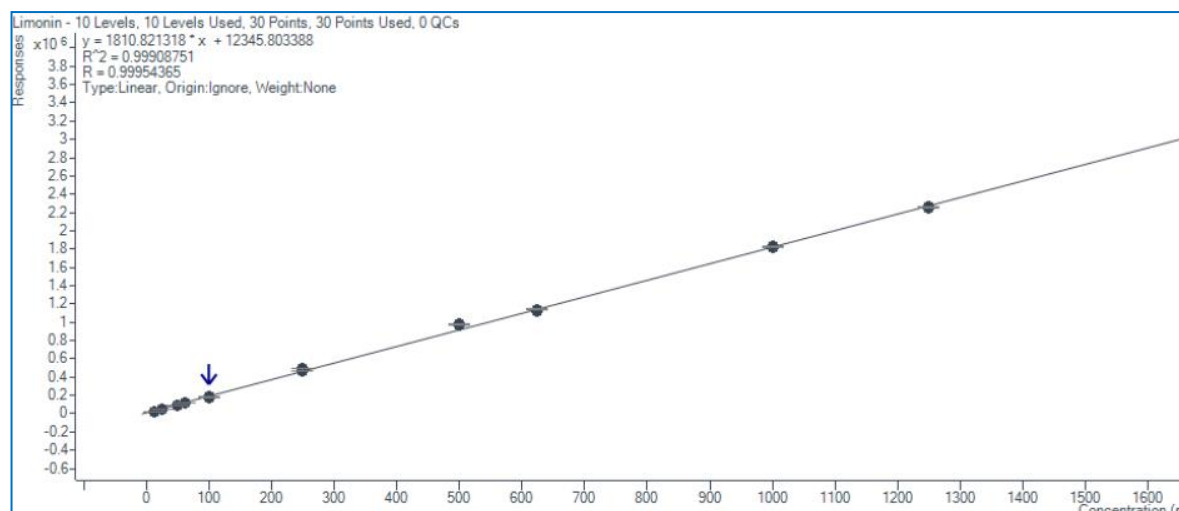
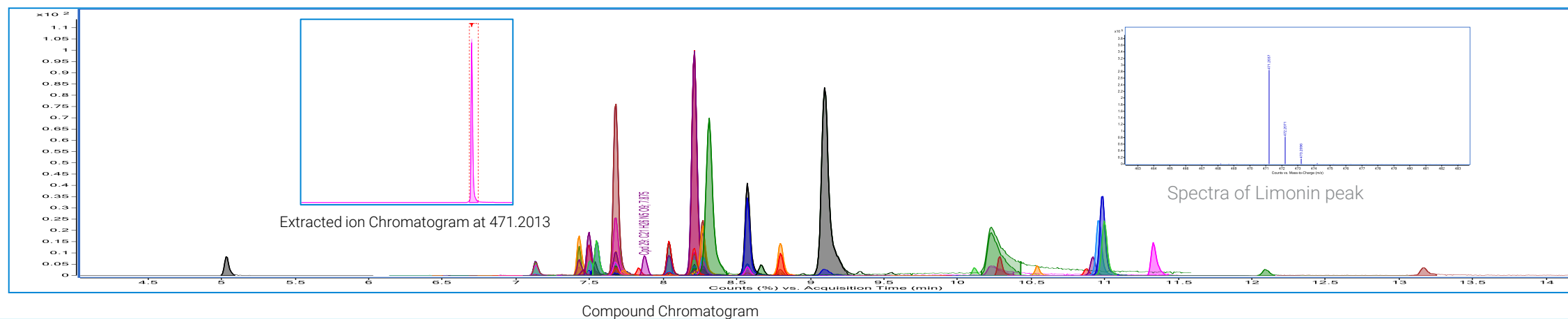| Parameter	Value
Slope	1810
R	0.9995
R^2	0.9991
Range (ppm)	12.5 – 1250

62% Difference in slopes indicates significant matrix effects.

Calibration Curve – Limonin in Citrus Flour

Parameter	Value
Slope	2931
R	0.9989
R^2	0.9979
Range (ppm)	50 – 750

Components in **Citrus** Flour

Extracted ion Chromatogram at 471.2013

Spectra of Limonin peak

Compound Chromatogram

Limonin in Acetonitrile Results

Precision

Sample	RT	Concentration
1	7.624	126.313
2	7.626	125.750
3	7.621	125.352
4	7.624	126.015
5	7.628	125.843
6	7.626	125.087
7	7.625	125.874
8	7.623	125.981
9	7.627	124.986
10	7.625	126.078
11	7.624	124.990
12	7.626	124.412
13	7.623	126.102
14	7.623	125.047
15	7.627	124.862
Mean	7.625	125.580
STD	0.0019	0.494
RSD (%)	0.0245	0.393

Accuracy

Reference Std. Conc (ppm)	Measured amount (ppm)	Accuracy %
125	125.107	100.085
250	233.062	92.225
375	373.274	99.540
500	493.597	98.719
625	628.115	100.498
875	899.789	102.833
1250	1247.536	99.8029
Mean Accuracy		99.243
STD		2.946
RSD (%)		2.968

Limonin in Citrus Flour Results

Precision

Sample	RT	Conc-ppm		Sample	RT	Conc-ppm
1	8.454	245.95		11	8.454	268.31
2	8.454	263.39		12	8.454	265.76
3	8.454	258.74		13	8.454	263.86
4	8.454	256.97		14	8.437	259.77
5	8.454	263.30		15	8.454	269.66
6	8.454	257.71		16	8.454	268.98
7	8.437	263.69		17	8.437	255.30
8	8.454	251.05		18	8.454	258.75
9	8.454	259.32		19	8.437	249.96
10	8.454	249.17		20	8.454	262.61
		Average	8.4489	259.61		
		STD	0.00821	6.7519		
		RSD	0.097%	2.601%		

Accuracy

Reference Standard Conc (ppm)	Measured amount (ppm)	Accuracy %
40	40.541	101.35
60	59.012	98.35
80	77.189	96.49
200	215.055	107.53
400	400.486	100.12
600	593.019	98.84
800	812.998	101.62
Mean Accuracy		100.61
STD		3.53
RSD (%)		3.51

Conclusions

This method for the determination and quantitation of Limonin in citrus flour by the Agilent Time of Flight LC/MS has been shown to be linear, accurate and precise over typical industrial concentrations. The utilization of the Time of Flight LC/MS technology also allows for the characterization of congeners and other matrix components

https://www.agilent.com/en/promotions/asms

References

1. Gualdani R, Cavalluzzi MM, Lentini G, Habtemariam S. The Chemistry and Pharmacology of Citrus Limonoids. Molecules. 2016 Nov 13;21(11):1530. doi: 10.3390/molecules21111530. PMID: 27845763; PMCID: PMC6273274.

2. https://agresearchmag.ars.usda.gov/2005/feb/citrus

3. PubChem, US National Library of Medicine. 17 December 2022.

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