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

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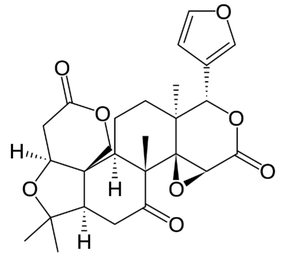
Introduction

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

Experimental

Samples, Standards and Consumables

Biorbyt Limonin Reference Standard, P/N orb322562
Agilent QuEChERS Extract Pouch P/N 5982-0650
Agilent Dispersive 2 ml Universal Kit P/N 5982-0028
OmniSolv Acetonitrile LCMS Grade P/N AX0156-6

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.

Chromatographic Conditions

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

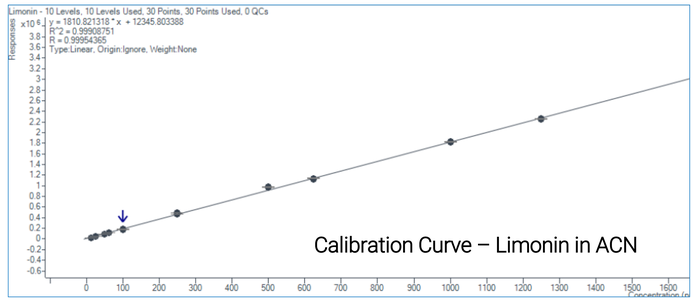
Channel	Solvent	%A	%B
A	H2O + 0.1% Formic Acid	0 Min	95
B	Methanol	10 Min	5

Parameter	Value
Ion Source & Polarity	Dual ESI positive
Gas Temperature (°C)	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

Results and Discussion

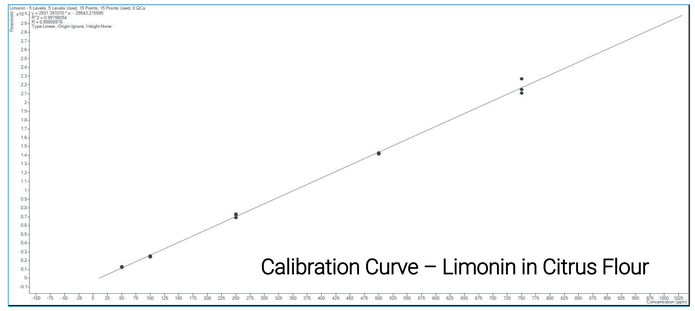
Limonin Calibration Curves

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



Calibration Curve – Limonin in Citrus Flour

62% Difference in slopes indicates significant matrix effects.

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

Limonin in Acetonitrile Results

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 Flower Results

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

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.