High-Resolution Sampling 2D-LC Analysis of Mangosteen Pericarp Xanthones Using the Agilent 1290 Infinity II 2D-LC Solution

Application Note

Food Testing and Agriculture

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Abstract

A high-resolution UHPLC analysis of xanthones present in the extracts of the pericarp of mangosteen (*Garcinia mangostana* L.) was carried out on an Agilent 1290 Infinity II LC. Excellent resolution and peak capacity (300 calculated at half height) were obtained within a total analysis time, including column reconditioning, of 16.5 minutes. The main xanthone (α-mangostin) was quantified in two extracts.

To ensure and evaluate the purity of target compound peaks, a significant increase in peak capacity is required. Therefore, the analysis was repeated using high-resolution sampling 2D-LC with the Agilent 1290 Infinity II 2D-LC solution. The original separation was coupled online to a second-dimension separation for more resolution on a selection of peaks eluting from the first dimension. Diode array detection (DAD) and mass spectrometry (MS) were used for detection and data analysis.

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Introduction

Xanthones are a group of naturally occurring compounds that exhibit a variety of biological activities and can be of interest as food supplement. Mangosteen xanthones have been reported to inhibit lipoprotein oxidation[1], histamine release, prostaglandin synthesis[2], and the growth of human leukemia cells[3]. The mangosteen fruit rind (pericarp) has been praised in Southeast Asia for its medicinal qualities. It has been used for treatment of a variety of complaints[4], and is even proposed as alternative treatment for Parkinson's disease[5].

The pericarp of mangosteen contains a large diversity of organic molecules, a major portion of which are xanthones and tannins. The xanthones have a tricyclic aromatic core structure on which a variety of chemical groups such as hydroxyl, methoxyl, and isoprenyl are incorporated. This results in a great number of variants. Extracts of the mangosteen pericarp are complex mixtures containing a diversity of organic molecules at different concentration levels. Figure 1 shows the major xanthones present in mangosteen pericarp.

To efficiently analyze the extracts, chromatographic methods with high resolution and peak capacity are required. Coelution of compounds needs to be avoided to enable accurate quantification and evaluate the purity of extracts and fractions. The Agilent 1290 Infinity II LC using Agilent ZORBAX RRHD UHPLC columns proved a valuable tool to meet these requirements. For even higher peak capacity or resolution, the Agilent 1290 Infinity II 2D-LC solution is a suitable addition that enables automated multidimensional LC to be carried out on unresolved peaks or on challenging samples.

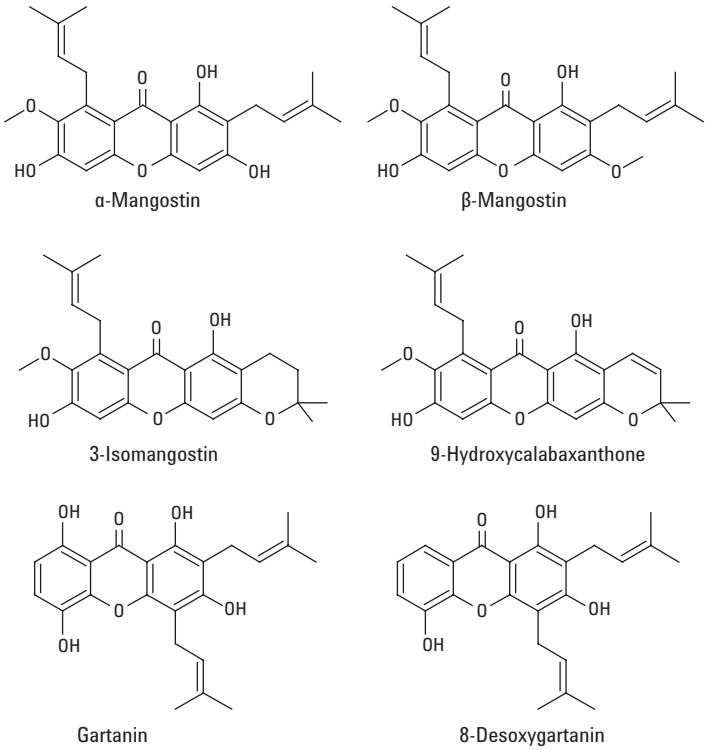


Figure 1. Chemical structures of the major xanthones found in the pericarp of mangosteen.

Experimental

Chemicals, samples, and sample preparation

All solvents used were LC grade from Biosolve (Valkenswaard, The Netherlands). Formic acid was from Sigma-Aldrich (Bornem, Belgium).

Two mangosteen pericarp samples were analyzed. The first one was a commercially available powder (Fruit Rind VBRM, p/n 00030992, Chromadex, Irvine, CA, USA), and extraction was performed on the sample as such. The second extract was prepared from fresh mangosteen fruits purchased at a local market. The pericarp was separated from the fruit, cut into small (about 0.5 cm) portions, dried by lyophilization, and ground before extraction.

The samples were extracted based on the procedure described by Walker[6] with water/acetone 20/80 v/v at a concentration of 10 mg/mL. The samples were shaken and sonicated for 10 minutes. An aliquot was filtered through a 0.45 µm syringe filter (regenerated cellulose), and injected for analysis.

The α-mangostin standard was purchased from Sigma (p/n M3824, Bornem, Belgium). A stock solution containing 2,000 µg/mL was prepared in water/acetone 20/80 v/v, and was diluted in the same solvent to the appropriate concentration.

Instrumentation

An Agilent 1290 Infinity II 2D-LC solution was used. The configuration is described below.

UHPLC configuration used for one-dimensional analyses:

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) equipped with a 10 mm flow cell
- Agilent 1290 Infinity II Low Dispersion Kit (5067-5963)

Additional modules used for multidimensional analyses:

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) equipped with a 10 mm flow cell
- Agilent 1290 Infinity Valve Drives (3x G1170A)
- Agilent 2D-LC Valve (2-position/4-port duo-valve) (G4236A)
- Agilent Multiple Heart-Cutting Valves (2x G4242-64000) equipped with 40 µL loops

Figure 2 shows the configuration of the valves for multiple heart-cutting and high-resolution sampling 2D-LC with the 1290 Infinity II 2D-LC solution.

An Agilent 6130 Single Quadrupole LC/MS system was installed after the second dimension for the determination of the molecular weights. The flow from the second-dimension column was split between the DAD and MS. In this manner, the flow rate entering the MS was optimal, and both DAD and MS could be carried out simultaneously. Splitting was done by a T-piece from which a 0.12 × 270 mm stainless steel capillary was connected to the DAD inlet (DAD outlet goes to waste), and a 0.075 × 340 mm stainless steel capillary (p/n 5067-4783) was connected on the MS ESI source.

Software

Agilent OpenLab CDS ChemStation Edition software, version C.01.07 [27] with Agilent 1290 Infinity 2D-LC acquisition software, version A.01.02 [24].

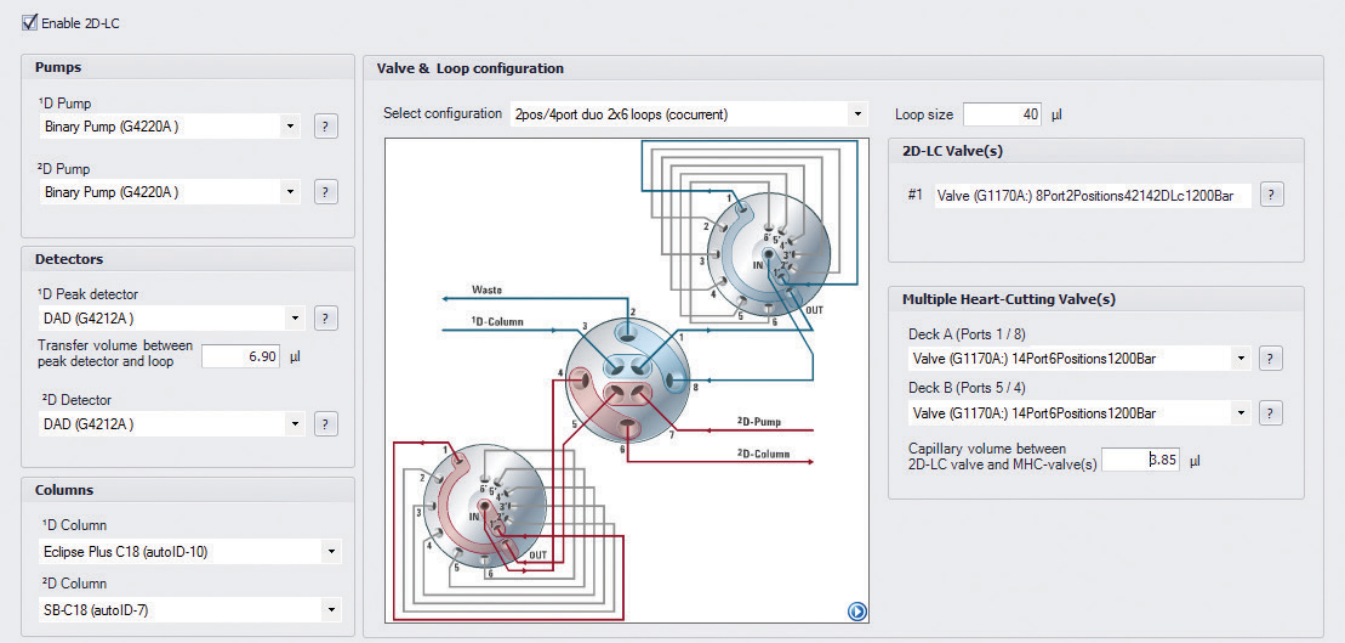


Figure 2. Valve configuration for multiple heart-cutting and high-resolution sampling 2D-LC analysis.

Method one-dimensional and first-dimension analyses

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Mobile phase A	0.1 % Formic acid in water
Mobile phase B	0.1 % Formic acid in acetonitrile
Flow rate	0.6 mL/min
Gradient	0 to 10 minutes – 45 to 100 %B 10 to 13.5 minutes – 100 %B Post time 3 minutes at 45 %B
Column temperature	40 °C
Detection DAD	320/8 nm (Reference off) Peak width > 0.025 min (10 Hz)
Injection	1 µL (with needle wash, flush port, 3 seconds, methanol)
Injector temperature	10 °C

Second-dimension analysis

Parameter	Value
2D-LC mode	High-resolution sampling
Column	Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Mobile phase A	0.1 % Formic acid in water
Mobile phase B	0.1 % Formic acid in methanol
Flow rate	1.2 mL/min
Gradient	0 to 1.5 minutes – 65 to 95 %B 1.5 to 1.6 minutes – 95 %B 1.6 to 2 minutes – 65 %B
Cycle time	2 minutes
Column temperature	50 °C
Detection DAD	320/8 nm (Reference off) Peak width > 0.025 minutes (10 Hz)

MS conditions

Parameter	Value
Ionization	Electrospray, positive ionization, fast scan mode
Scan range	*m/z* 150–550
Fragmentor	100 V
Drying gas temperature	340 °C at 10 L/min
Nebulizer pressure	45 psig
Capillary voltage	3,000 V

Results and Discussion

One-dimensional analysis

Figure 3 shows the results obtained for the analyses of the extracts with the one-dimensional UHPLC setup. Both samples are highly complex. The peaks are nicely distributed over the gradient, and the obtained peak capacity was calculated. Therefore, the peak width at half height of a selection of peaks was determined. The peak capacity was then calculated as the ratio between gradient or elution window time and the average peak width. In this example, the average peak width was 0.036 minutes (2.16 seconds), and an elution window of 11 minutes was considered. As a result, the peak capacity at half height is over 300 in a total analysis time (including column re-equilibration) of 16.5 minutes. For a separation with unit resolution (Rs = 1, that is, 13.4 % peak height) the peak capacity was approximately 170. This is a good result, considering the relatively short analysis time.

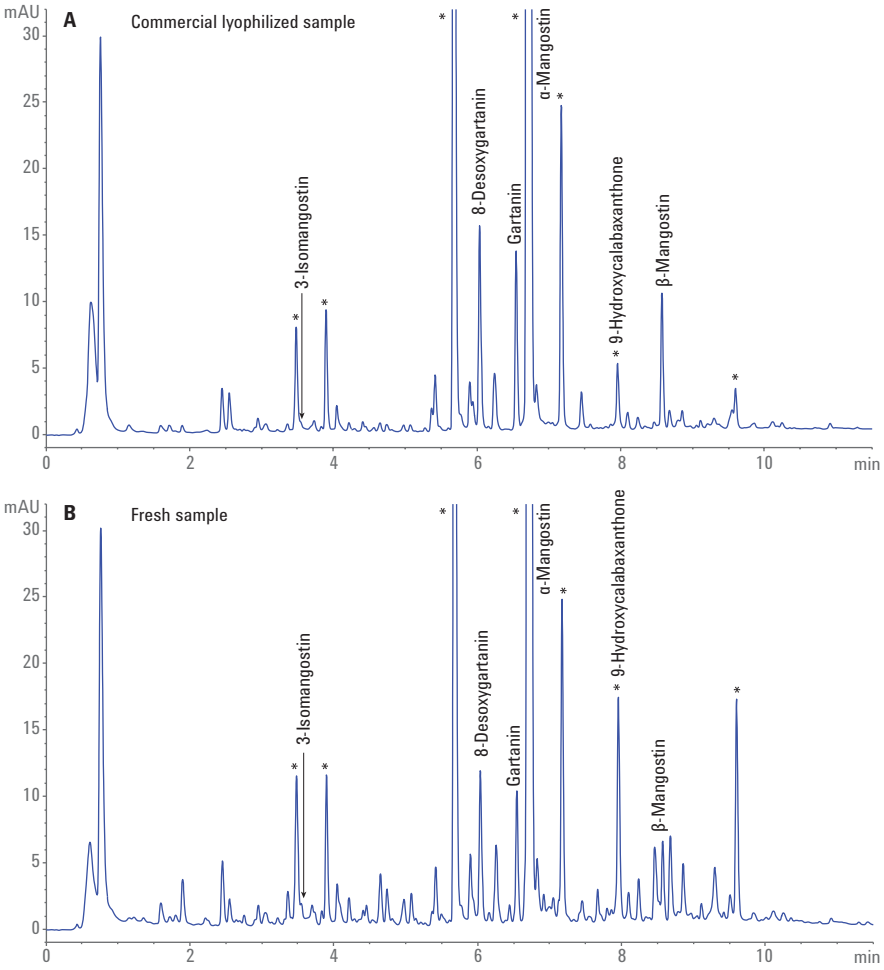


Figure 3. UHPLC analysis of the mangosteen pericarp extracts. Peaks marked with * were used to calculate peak capacity.

The method was applied to a set of α-mangostin standard solutions to evaluate repeatability of injection and linearity of the method. Six replicates of a 100 µg/mL standard solutions were injected consecutively to calculate the precision, while the linearity was determined by single injections of standard solutions between 50 and 750 µg/mL. Figure 4 summarizes the excellent results.

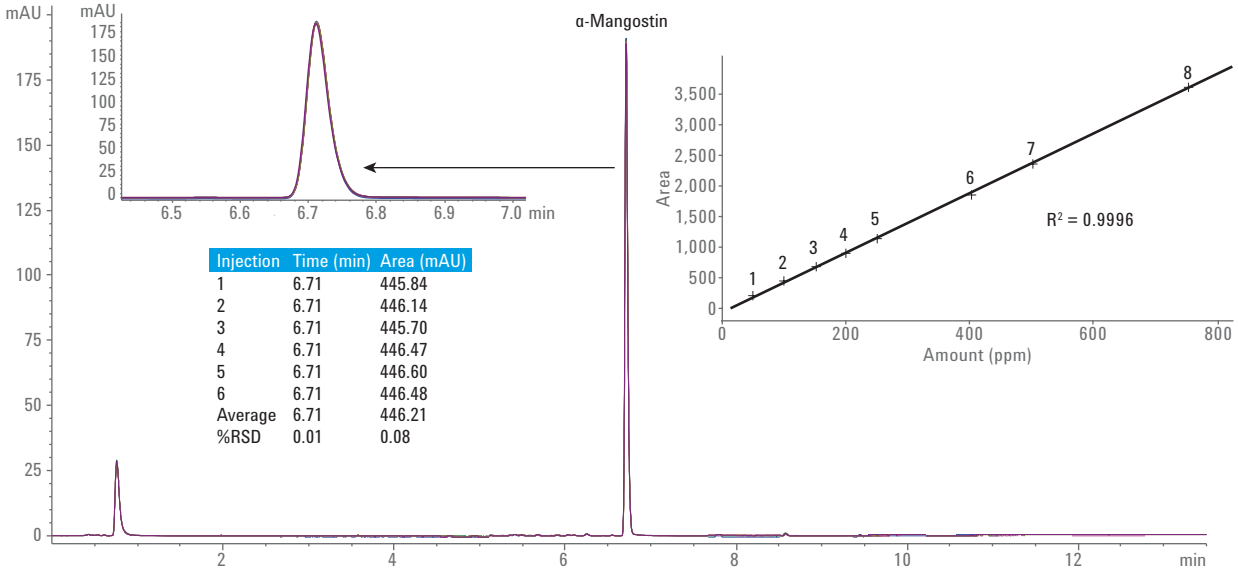
The concentration of α-mangostin in the pericarp extracts was determined using the calibration curve. The concentration was 376 µg/mL in the commercial sample and 335 µg/mL in the fresh sample, corresponding to 37.6 and 33.5 mg/g dried weight, respectively. Both samples are similar in terms of α-mangostin content, however the profiles differ significantly when a closer look is taken over the entire run (Figure 3).

Multidimensional analyses

It is possible that many of the detected peaks are not pure, and that one or more interfering compounds are coeluting with the detected compound. Even though the one-dimensional method performs excellently in terms of peak capacity, we have no guarantee of complete separation. Peak capacity calculations give an idea of the overall method performance but do not estimate the true number of compounds separated. Since peaks are randomly distributed along the chromatogram, the number of compounds that will be separated are only a fraction of the calculated peak capacity, as described in the statistical theory of component overlap[7]. The peak capacity must significantly exceed the number of compounds that need to be separated, and for complex samples such as those studied in this application note, is extremely challenging.

The increase in peak capacity with a one-dimensional UHPLC setup is actually small and requires long analysis times. A more effective approach is to use multidimensional LC, as we have described in a previous report[8].

The Agilent 1290 Infinity II 2D-LC solution enables comprehensive 2D-LC (LC×LC) and heart-cutting 2D-LC (LC-LC). In LC×LC, the complete separation from the first column is on-line subjected in small fractions to a second separation (dimension), whereas in LC-LC, one or a limited number of fractions is transferred to the second dimension. Using these techniques, the peak capacity generated in the first dimension is significantly increased by the separation carried out in the second dimension. The principles and practical implementation of 2D-LC can be found in a recently published primer about the technique[9].



Injection	Time (min)	Area (mAU)
1	6.71	445.84
2	6.71	446.14
3	6.71	445.70
4	6.71	446.47
5	6.71	446.60
6	6.71	446.48
Average	6.71	446.21
%RSD	0.01	0.08

Figure 4. Results of method evaluation. Overlay of six replicate injections of a 100 µg/mL α-mangostin (and a detail in the insert) together with the calculated RSD% on retention time and area. The inset shows the calibration curve after single injections of standard solutions of 50 to 750 µg/mL.

An LC-LC variant, namely high-resolution sampling 2D-LC, was performed on the α-mangostin peak of the one-dimensional separation of the mangosteen pericarp extract. The importance of this approach lies in an additional control of the purity of peaks detected with the developed method. This control can be done before purification, for example, using preparative LC with fraction collection, and enables the analyst to select suitable samples or conditions.

In high-resolution sampling 2D-LC, up to 10 consecutive cuts can be defined for the first dimension, and they are stored in the 40 µL loops multiple heart-cutting valves. Each of these cuts is then automatically analyzed in series with the second-dimension method. High-resolution sampling 2D-LC enables the analysis of larger areas of interest or broad unresolved peaks in the first dimension. Thus, the entire target peak can be sampled, and each of the snips can be analyzed to get a detailed view of its purity. This allows the determination of the amount and molecular weight (if MS is used) of any impurities present. Figure 5 shows an example of such an analysis on the α-mangostin peak in the commercial pericarp extract. The complete peak was sampled in four small fractions of 3.2 seconds (each resulting in 32 µL that was stored in the 40 µL loops), which were all analyzed on the second dimension. It is clear that the peak is not completely pure, and that some impurities are well separated from the α-mangostin in the second dimension. Particularly in the tail of the first-dimension peak, there is an impurity present with *m/z* of 397 $[M+H]^+$, as detected by the MS. This is the same mass as gartanin, which elutes before the main peak, and is visible as a small peak in the analysis of the first cut. Although the relative area of the impurity compared to the α-mangostin is low (below 0.01 %), the result shows the potential of high-resolution sampling 2D-LC for obtaining a better insight into coeluting peaks. Two isomers were separated from each other and the main peak using this approach.

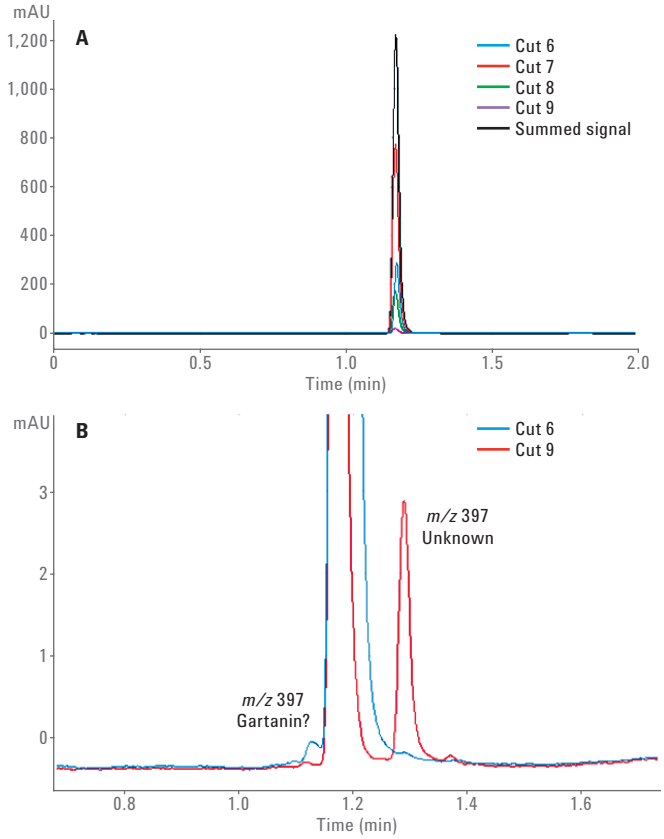


Figure 5. Second dimension results after high-resolution sampling 2D-LC of the α-mangostin peak in the commercial sample. A) The result for all four cuts and the summed signal. B) Detail of the analysis of the first (blue) and last (red) fraction.

Conclusion

The Agilent 1290 Infinity II LC system provides good peak capacity in a relatively short time for the analysis of xanthones in mangosteen pericarp extracts. The injection precision and linearity method are excellent, and the method can be used to determine the α-mangostin content. Due to the complexity of the samples, it is highly probable that the detected peaks will contain coeluting interferences. The Agilent 1290 Infinity II 2D-LC solution can be used as an additional tool to evaluate the purity of the detected peaks.

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