The Vanquish Platform: Improved Throughput and Resolution of Xanthones in Mangosteen Pericarp

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Overview

Purpose: To develop an improved method to resolve many different analytes within a mangosteen pericarp sample in a finite time period with improved throughput, better resolution and enhanced peak capacity using the Thermo Scientific™ Vanquish™ UHPLC platform.

Methods: A sample of mangosteen pericarp powder was weighed and extracted using the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system. Subsequent analysis of the sample for xanthone content was performed via the Vanquish UHPLC system using a 2.2 μm, 2.1 × 250 mm C18 column.

Results: More than 60 peaks were determined from the mangosteen pericarp sample using the Vanquish system with an average peak width at 50% of 2.21 seconds. This indicates that an approximate peak capacity of 298 in 11 minutes was obtained using Vanquish.

Introduction

There is considerable interest in botanical supplements due to their purported health benefits. Mangosteen (*Garcinia mangostana L)* is a tropical fruit that is indigenous to Southeast Asia, where it has been historically used to treat abdominal pain, diarrhea, dysentery, inflammation, wound infection, suppuration, and chronic ulcer.[1] Recently mangosteen has been proposed as a homeopathic therapy in the treatment of Parkinson's disease.[2] Such therapeutic benefits have been mostly attributed to a unique family of compounds referred to as xanthones that are most abundant in the pericarp of the fruit.[3] The structures of the five major xanthones, including α-mangostin, 3-isomangostin, gartanin, 9-hydroxycalabaxanthone, and 8-desoxygartanin, are presented in Figure 1.

Chromatographic analysis of the xanthones and other important analytes contained in this supplement presents is challenging. Reversed phase HPLC with UV detection is widely used for the analysis of xanthones, but such methods lack analyte resolution and/or require exceedingly long analysis time. [4,5] Improving these issues, by using UHPLC may still require >25 mins to complete since the flow rates required to achieve optimal column efficiency generate exceedingly high back pressures, typically beyond UHPLC system limits. Data is presented illustrating a UHPLC separation using the Thermo Scientific ™ Dionex ™ UltiMate 3000 RSLC system having an upper pressure rating of 1000 bar. This situation is addressed by the new Vanquish UHPLC system, which consists of a binary parallel pump capable of operating at pressures up to 1500 bar, autosampler, thermostated column compartment and diode-array detection as shown in Figure 2.

Consequently, UHPLC columns, containing the smaller particle sizes found in UHPLC columns, can be operated at high flow rates to improve analyte resolution and sample throughput. The analytical power of the Vanquish system is typified by the improvements in analyte resolution and the reduced run time shown for the analysis of mangosteen pericarp.

FIGURE 1. Structures of selected xanthones found in Mangosteen.

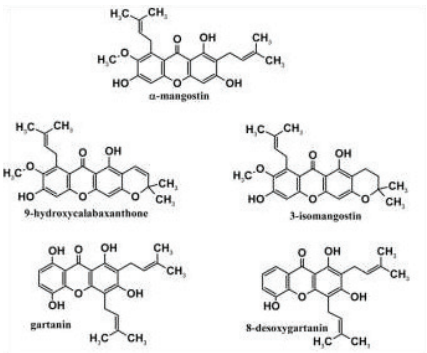


FIGURE 2. The Vanquish UHPLC Platform.

Methods

Sample Preparation
Equipment and Materials:
• ASE 350 Accelerated Solvent Extractor system
• 10 mL stainless extraction cells
• Cellulose filters
• Clear collection vials, 60 mL
• Thermo Scientific™ Dionex™ ASE™ Prep DE Diatomaceous Earth

Accelerated Solvent Extraction Conditions	
Solvent:	95% ethanol
Temperature:	80 °C
Static Time:	5 min
Static Cycle:	4
Flush:	60%
Purge:	90 s

Liquid Chromatography

Method 1: UltiMate 3000 RSLC
• DGP-3600RS pump
• WPS-3000TRS autosampler
• TCC-3000RS column thermostat
• DAD-3000RS diode array detector, 320 nm
Column: Thermo Scientific™ Dionex™ Acclaim™ 120 RSLC C18, 2.2 μm, 2.1 x 100 mm
Temperature: 30 ºC
Mobile Phase A: water
Mobile Phase B: 90% acetonitrile, 10% methanol
Gradient: 0–20 min: 50 -90%B; hold at 90% B for 5min
Flow Rate: 0.5 mL/min
Injection Volume: 10 μL

Method 2: Vanquish UHPLC System
Vanquish binary UHPLC system including:
• Binary Pump H (P/N VH-P10-A)
• Split Sampler HT (P/N VH-A10-A)
• Column Compartment H (P/N VH-C10-A)
• Diode Array Detector HL, 320 nm (P/N VH-D10-A)
Column: Thermo Scientific™ Dionex™ Acclaim™ 120 C18, 2.2 μm, 2.1 x 250 mm
Adiabatic Temperature: 45 ºC
Mobile Phase A: water
Mobile Phase B: acetonitrile
Vanquish Gradient Method

Time (min)	Flow (mL/min)	%B	Curve
-2.5	1.0	50	5
0.1	1.0	50	5
9.0	1.4	90	5
11.0	1.4	90	5
12.0	1.0	50	5

Injection Volume: 3.0 μL
Flow Cell: LightPipe™, 10 mm

Data Analysis
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 6.8

Results

Accelerated Solvent Extraction Method

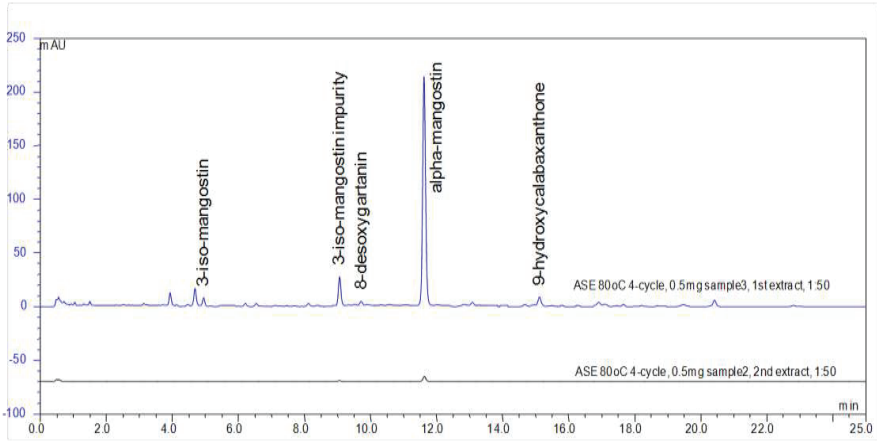
Ethanol (95%) was used as the extraction solvent ASE conditions were optimized for temperature, static time, flush volume, and number of cycles. The optimized procedure required only about 30 minutes and about 20-25 mL solvent.
Each sample was extracted for a second time to evaluate the extraction efficiency. At 80°C and with four extraction cycles, the extraction efficiency was above 96% for all three samples with excellent reproducibility, as shown in Table 1.

TABLE 1: Extraction efficiency of ASE method for Mangosteen pericarp powder.

	Sample 1	Sample 2	Sample 3
80°C, 3 cycles	90.0%	91.3%	90.6%
80°C, 4 cycles	96.0%	98.4%	96.5%

A comparison of HPLC chromatograms of first and second extractions with optimized conditions is shown in Figure 3. There was essentially no xanthones recovered in the second extraction. Equal or higher extraction efficiency can be achieved at higher temperature of 100-120°C without loss of xanthones, but the extract may contain material that precipitates when the extract cools. Also precipitates can form when diluting filtered extract in 50% acetonitrile for direct analysis on HPLC. The extracted sample was analyzed by HPLC after simple filtration and dilution.

FIGURE 3. Comparison of chromatogram of first and second ASE extract of mangosteen pericarp powder sample.



UltiMate 3000 – UV Method
A gradient UHPLC-UV method was developed for quantitative determination of the five selected xanthones (Figure 1). Chromatograms for a standard of five selected xanthones and a 50-fold diluted sample of mangosteen pericarp extract, respectively, are shown in Figure 4. The method took 25 minutes to complete, and a total of 70 peaks were resolved with an average peak width of 5.802 sec at half height. Quantitative data is shown in Table 2 for the Xanthone content found in extracted samples.

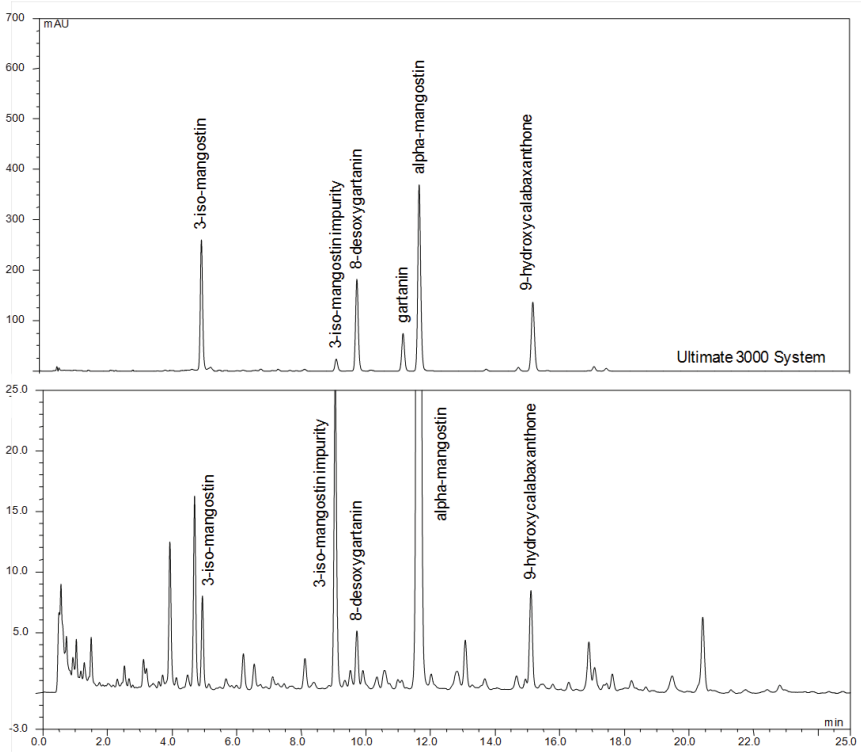
Table 2: Summary of quantitative results for four selected xanthones in Mangosteen pericarp powder.

	3-iso-mangostin	8-desoxygartanin	alpha-mangostin	9-hydroxy-calabaxanthone
extraction yield* (%db)	0.32 ± 0.01	0.31 ± 0.01	7.33 ± 0.20	0.57 ± 0.02
Relative UV area (%)	1.76 ± 0.01	1.27 ± 0.01	64.05 ± 0.11	2.35 ±0.02

•Dry weight basis of original sample of pericarp powder.

Values are the mean ± std deviation (n=3)

FIGURE 4. UV chromatogram of selected xanthones in standards and extract of mangosteen pericarp powder sample using the UltiMate 3000 system.



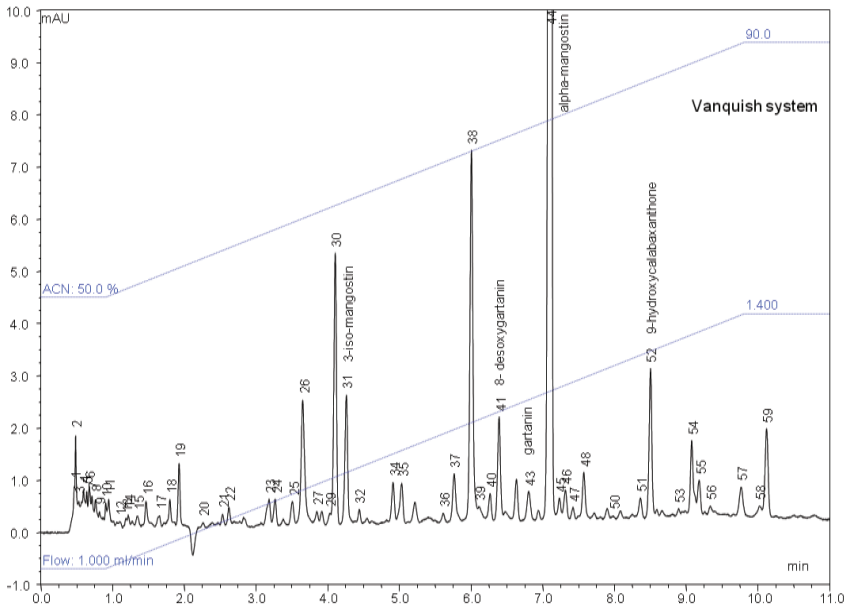
Vanquish UHPLC – UV Method

One advantage of the 1500 bar upper pressure limit offered by the Vanquish platform is that higher flows can be delivered even when longer UHPLC columns are employed to assist with rapid elution of complex samples. The extracted sample was separated using an Acclaim 120 C18, 2.2 µm, 2.1 × 250 mm column on the Vanquish platform. As the viscosity of the solvents decreased the flow rate could be increased to gain additional speed for the analysis without a significant impact on resolution. The analysis was now completed in 11 minutes using gradient conditions with a flow ramp from 1.0 mL/min to 1.4 mL/min over the course of the chromatogram as illustrated in Figure 5. The pressure trace observed using these conditions exceeded 1100 bar during the analysis. The ultra-wide dynamic range of the DAD detector is ideal for simultaneous detection of highly concentrated main compounds and minor sample components down to trace levels. More than 60 peaks were determined with an average peak width at half height of 2.21 seconds. This indicates that a peak capacity of 298 was obtained using the Vanquish system with the Acclaim 2.2 micron fully porous UHPLC column.

Conclusion

- A 30 min ASE method for extraction of xanthones from mangosteen pericarp powder was developed . Extraction efficiency was above 96% with excellent reproducibility
- The productivity enhancement of the Vanquish UHPLC over the UltiMate 3000 UHPLC was significant. The analysis time was reduced 2.5-fold using the Vanquish system with an enhancement of resolution of 53%
- When using the Acclaim 120 C18 column with the Vanquish platform more than 60 peaks were determined with an average peak width at half height of 2.21 seconds. This indicates that a peak capacity of 298 is achieved during the 11 minute run
- The DAD, fitted with a 10 mm LightPipe™ flow cell, provided excellent sensitivity and sufficient dynamic range to detect 60 peaks from the a small 3.0 µL aliquot of mangosteen pericarp extract that was injected
- With the increased focus on the quality of analytical data and the need for valid authentication of raw materials and ingredients, methods that offer high resolution and fast throughput are extremely important. Important, actionable decisions concerning product quality can be made sooner so that unacceptable products never leave the factory. Newer chromatographic tools, such as these, provide a quick way to verify product quality of complex samples

FIGURE 5. UHPLC-UV chromatogram showing separation of the extract of mangosteen pericarp powder sample and detection of xanthones using the Vanquish system using an Acclaim RSLC C18 column.



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