

# The Vanquish Platform: Major Improvement in Throughput and Resolution of Xanthenes in Mangosteen Pericarp

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## Key Words

Botanical supplements, Gradient method, Acclaim RSLC column, Natural products, UHPLC, Xanthenes, Vanquish UHPLC

## Goal

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

## 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.<sup>1</sup> Recently mangosteen has been proposed as a homeopathic therapy in the treatment of Parkinson's disease.<sup>2</sup> Such therapeutic benefits have been mostly attributed to a unique family of compounds referred to as xanthenes that are most abundant in the pericarp of the fruit.<sup>3</sup> The structures of the five major xanthenes, including  $\alpha$ -mangostin, 3-isomangostin, gartanin, 9-hydroxycalabaxanthone, and 8-desoxygartanin, are presented in Figure 1.

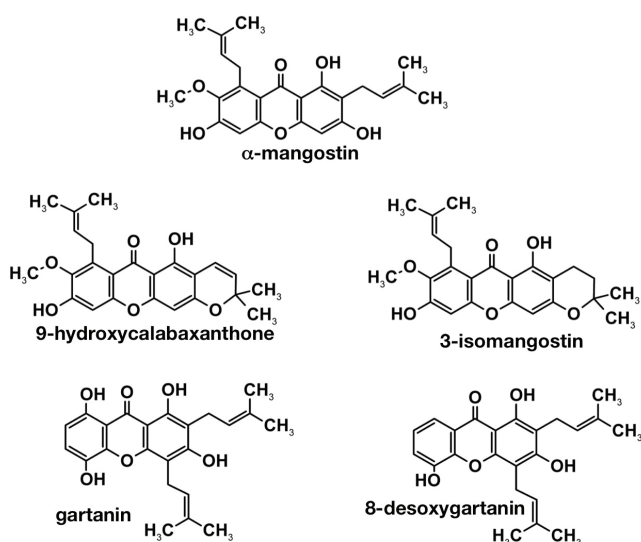


Figure 1. Structures of selected xanthenes found in mangosteen.



The chromatographic analysis of the xanthenes and other potentially important analytes contained in this supplement presents a huge challenge. Although reversed phase HPLC with UV detection is widely used for the analysis of xanthenes, such methods lack analyte resolution and/or require exceedingly long analysis time.<sup>4,5</sup> These issues can be improved, somewhat, by using UHPLC but even then such methods still require >25 mins to complete since the flow rates required to achieve optimal column efficiency generate exceedingly high back pressures, well beyond those that can be used with the typical UHPLC system. In order to address this situation the new Vanquish UHPLC system, was developed. The Vanquish platform consists of a binary parallel pump capable of operating at pressures up to 1500 bar, autosampler and diode-array detection. Consequently, UHPLC columns containing smaller particle size 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 shortened analysis time shown for the analysis of mangosteen pericarp.

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## Methods

### Sample Preparation

#### Equipment and Materials:

- Thermo Scientific™ Dionex™ 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

#### Extraction:

Weigh 0.5 g of each mangosteen pericarp powder sample and mix with diatomaceous earth. Transfer the mixture into a separate 10 mL stainless steel cell (equipped with two cellulose filters on the bottom) to nearly fill the cell. Extract the loaded cells with the above conditions and transfer the extracts into separate 25 mL volumetric flasks to volume with 95% ethanol. Filter the extract with a 0.2 µm filter and dilute 50-fold with 50% acetonitrile before analysis.

## Liquid Chromatography

### Vanquish UHPLC Method

Vanquish 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)

#### Conditions

Column:	Thermo Scientific™ Acclaim™ 120 C18, 2.2 µm, 2.1 × 250 mm
Still Air Temperature:	45 °C
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Injection Volume:	3.0 µL
Flow Cell:	LightPipe™, 10 mm

#### 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

#### Data Analysis

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

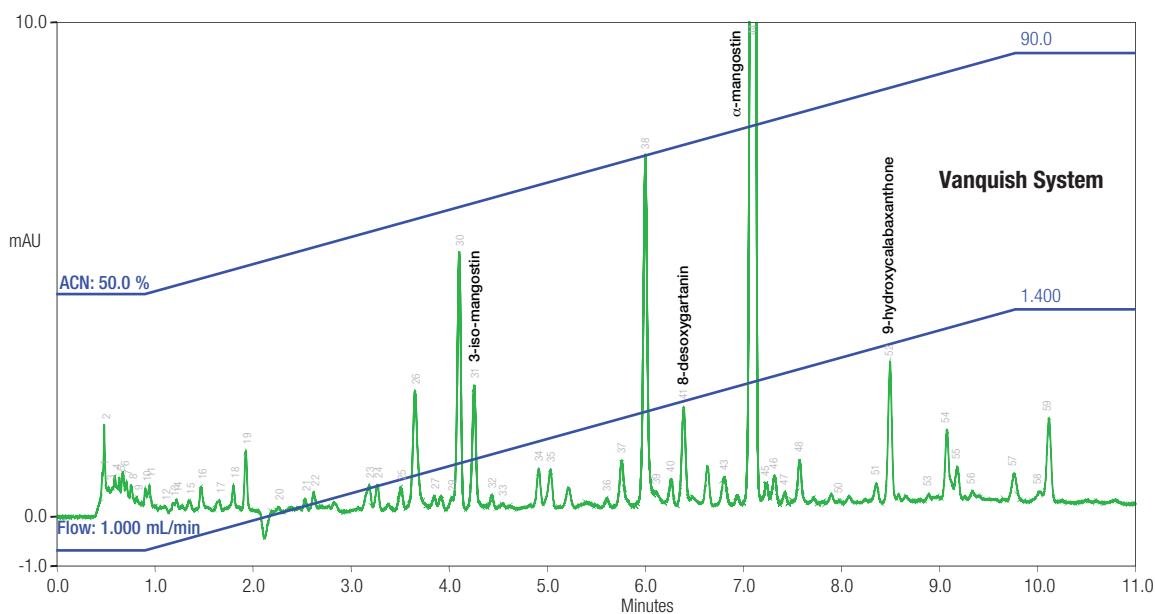


Figure 2. UV chromatogram showing separation of the extract of mangosteen pericarp powder sample and detection of xanthenes using the Vanquish system with an Acclaim RSLC C18 column.

## Results and Discussion

### Vanquish UHPLC – UV Method

The extracted sample was separated using an Acclaim 120 C18, 2.2  $\mu\text{m}$ , 2.1  $\times$  250 mm column on the Vanquish platform. 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 2. As the viscosity of the solvents decreased the flow rate could be increased constantly to gain additional speed for the analysis without a significant impact on resolution. The pressure trace observed using these conditions exceeded 1100 bar during the run. More than 60 peaks were determined with an average peak width at half height of 2.21 seconds. This indicates that an approximate peak capacity of 300 was obtained using the Vanquish system with the Acclaim 2.2 micron fully porous UHPLC column.

### Conclusions

This application describes an improved method achieved on the Vanquish platform that provides enhanced throughput with better resolution and peak capacity. The analysis time was completed in 11 minutes on the Vanquish platform using gradient conditions with a flow ramp from 1.0 mL/min to 1.4 mL/min over the course of the analysis.

- 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 an approximate peak capacity of 300 is achieved in 11 minutes.
- 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 the ones reported in this application note provide a quick way to verify product quality of complex samples.

### Acknowledgements

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