Fast Analysis of Selected Xanthones in Mangosteen Pericarp Using Accelerated Solvent Extraction and Ultra High Performance Liquid Chromatography

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Overview

Purpose: Development of a fast and robust sample preparation method using accelerated solvent extraction (ASE) and UHPLC separation for quantitative determination of selected xanthones in mangosteen pericarp.

Methods: This poster note describes an accelerated solvent extraction method for extraction of xanthones from mangosteen pericarp and a gradient UHPLC-UV method for quantitative determination

Results: The ASE method presented in this poster takes only 30min with >96% recovery and excellent reproducibility. The ASE and UHPLC-UV method was used to quantitatively determine five selected xanthones in a commercial pericarp powder sample. An alternative method using charged aerosol detection is also discussed.

Introduction

Many tropical plants are important sources of biologically active compounds that have potential therapeutic effects. 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 method presented here for the analysis of xanthones in mangosteen pericarp combines accelerated solvent extraction and UHPLC separation. Conventional extraction methods for mangosteen pericarp, such as soxlet, are time consuming, labor intensive and do not always deliver the desired reproducibility. ASE is an automated extraction technique that rapidly performs solvent extractions using high temperature and pressure. The ASE system has the advantages of short extraction time, low solvent consumption, high extraction efficiency and excellent reproducibility. The ASE extraction described in this poster requires only 30 mins and delivers >96% recovery with excellent reproducibility.

Reverse phase HPLC with UV detection is widely applied for the analysis of xanthones.[4,5] The UHPLC method for xanthone presented in this poster employs a Dionex Acclaim RSLC C18 column and is completed in 25mins. Comparative data using charged aerosol detection s also discussed. Five major xanthones, including α-mangostin, 3-isomangostin, gartanin, 9-hydroxycalabaxanthone, and 8-desoxygartanin, were quantitatively determined in mangosteen pericarp. The structures of the selected xanthones are shown in Figure 1.

Methods

Sample Preparation:

Equipment and Materials:

- Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system
- 10mL stainless extraction cells
- Cellulose filters
- Clear collection vials, 60mL,
- Dionex ASE Prep Diatomaceous Earth

Accelerated Solvent Extraction Conditions:

Solvent:	95% ethanol
Temperature:	80°C
Static time:	5min
Static cycle:	4
Flush:	60%
Purge :	90s

Extraction:

Weigh 0.5g of each mangosteen pericarp powder sample and mix with diatomaceous earth . Transfer the mixture into a separate 10mL 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 25mL volumetric flasks to volume with 95% ethanol. Filter the extract with 0.2 µm filter and dilute 50 fold with 50% acetonitrile before analysis.

Liquid Chromatography:

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system including:

- DPG-3600RS pump
- WPS-3000TRS autosampler
- 3000 TCC-3000RS column thermostat
- DAD-3000RS diode array detector, 320 nm
- Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector, evaporation 50°C, PFV 1.0

Column: Thermo Scientific™ Acclaim™ 120 C18, 2.2µm, 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

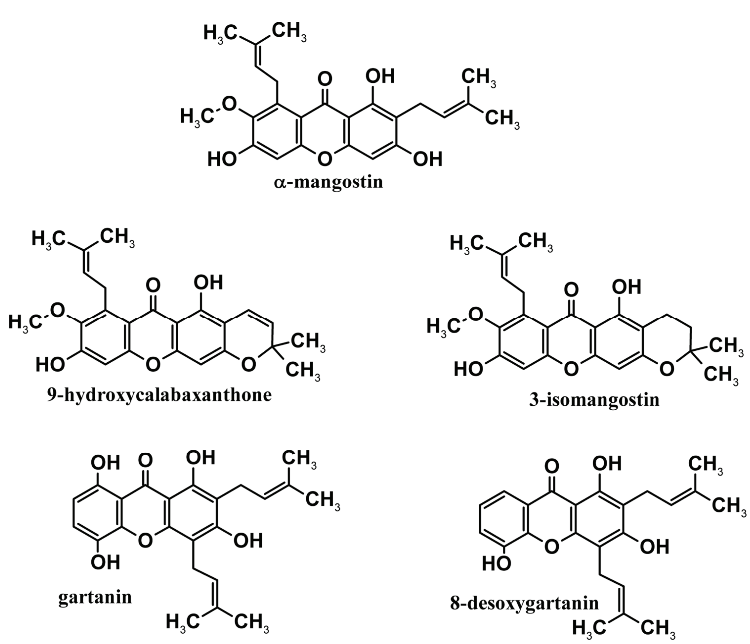
Flow Rate: 0.5 mL/min

Injection Volume: 10 µL

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.1 SR1 L.

Figure 1. Structure of selected xanthones.



Results

Accelerated Solvent Extraction Method

95% ethanol was used as the extraction solvent in this experiment. ASE conditions were optimized in terms of temperature, static time, flush volume and number of cycles. The optimized procedure requires only about 30min and about 20-25 mL solvent.

Each sample was extracted for a second time to evaluate the extraction efficiency. At 80°C with four extraction cycles, extraction efficiency was above 96% for all three samples with excellent reproducibility, as shown in Table 1. Figure 2 shows comparison of HPLC chromatograms of first extraction and second extraction with optimized conditions. 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.

HPLC –UV Method

The extracted sample was directly analyzed by HPLC after simple filtration and dilution. A gradient UHPLC-UV method was developed for quantitative determination of the five selected xanthones including, α-mangostin, 3-isomangostin, gartanin, 9-hydroxycalabaxanthone, and 8-desoxygartanin. This method uses a Dionex C18 column and the analysis time is 25min. Figures 3 and 4 show chromatograms for the standard of five selected xanthones and a 50-fold diluted mangosteen pericarp extract sample. Figure 5 shows the calibration cures for the standards in the concentration range of 1 to 50 μg/mL. Table 2 summarizes quantitave results for the selected xanthones as extraction yield of the original sample dry weight and total UV area. Alpha-mangosteen is the major component of the extract, and accounts for over 60% of total UV response. The amount of gartanin was not significant compared to the other four xanthones and was not quantitatively determined with this method.

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%

Figure 2. Comparison of chromatogram of first and ASE extract of mangosteen pericarp powder sample.

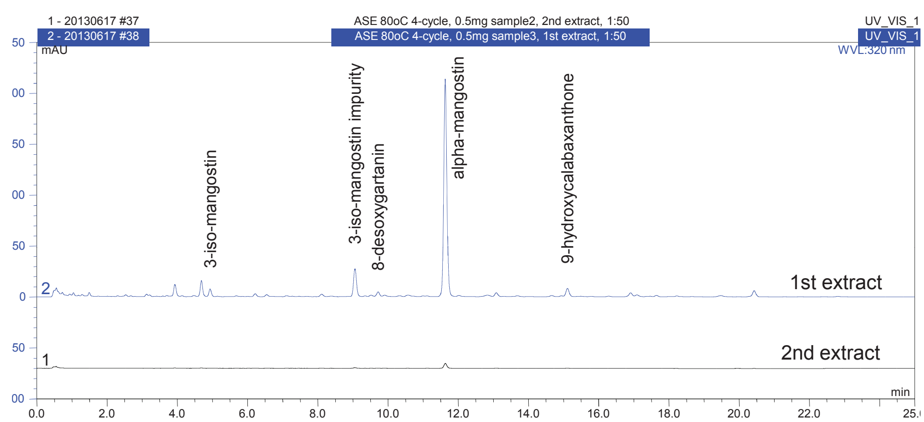


Table 2. Summary of quantitative results for four selected xanthones in mangosteen pericarp powder.

	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 3. UV chromatogram showing separation of five selected xanthone standards.

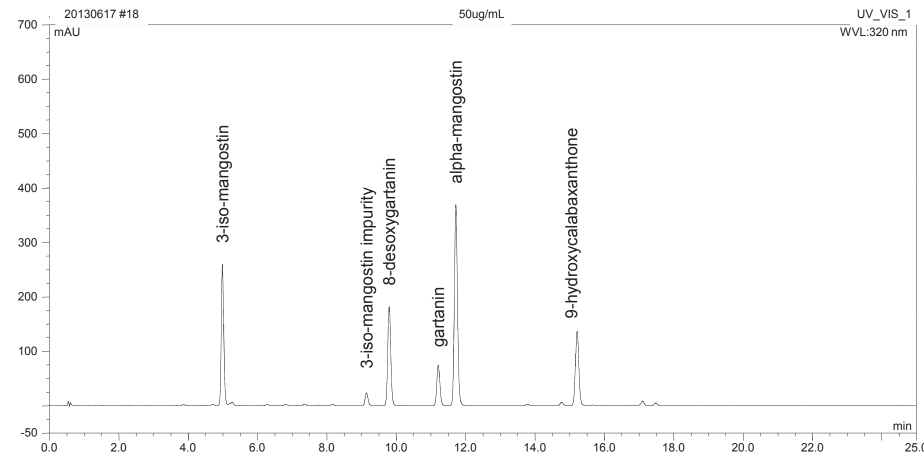


Figure 4. UV chromatogram showing separation of the extract of mangosteen pericarp powder sample and detection of four xanthones.

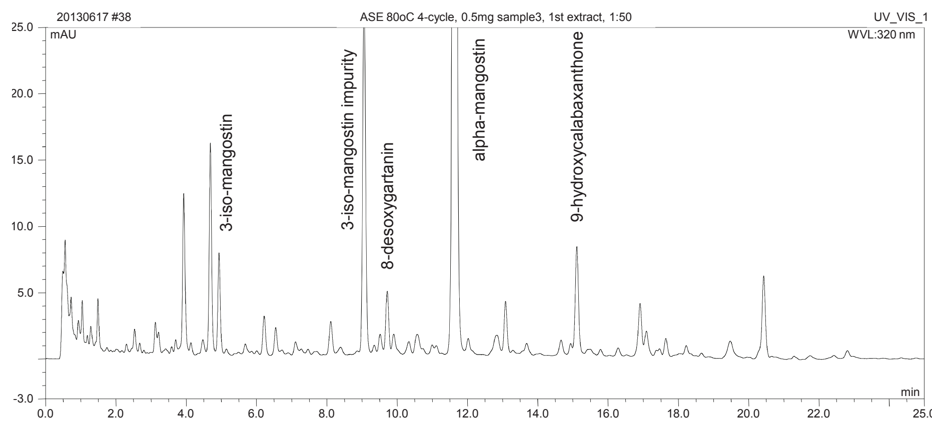
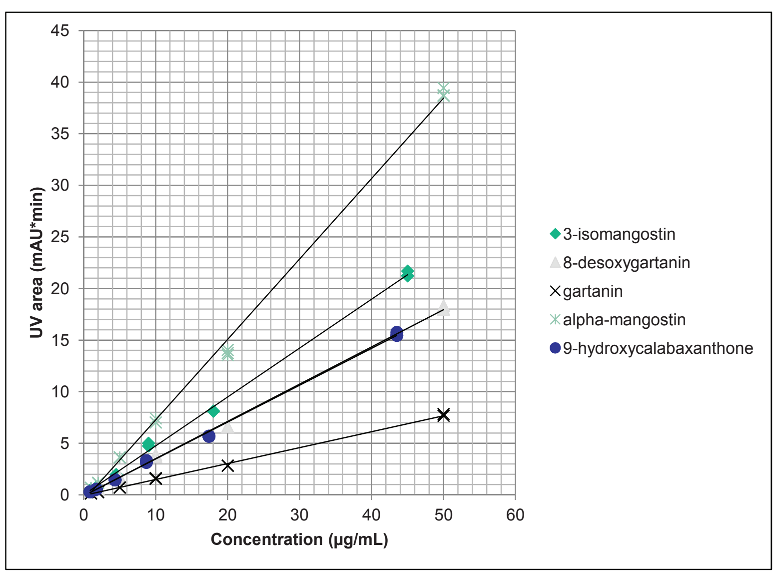


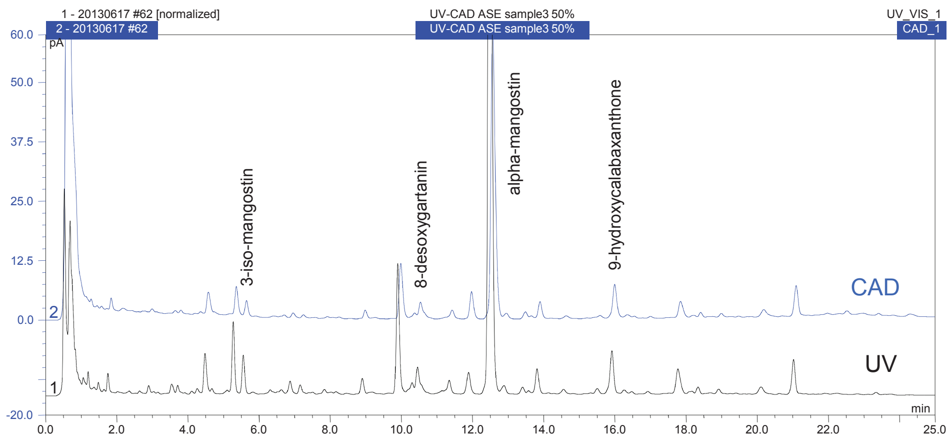
Figure 5. Calibration curves for UV response of five selected xanthones.



HPLC-Charged Aerosol Detection

The use of the Corona Veo, a charged aerosol detector, was evaluated for detection of xanthones in mangosteen pericarp. Figure 5 shows the comparison of UV and Corona Veo detector chromatograms for this sample. The two detectors give almost identical results except for the difference in relative response between alpha-mangosteen and other peaks. Although in these experiments there is no appreciable advantage over UV, charged aerosol detection with its ability to measure all non-volatile analytes with similar response independent of chemical structure, can be used to measure levels of unknown analytes in mangosteen pericarp, even those that lack a suitable chromophore.

FIGURE 6. Comparison of UV and Corona Veo detection for xanthones in the same mangosteen pericarp extract sample.



Conclusion

- A 30 min ASE method for the extraction pf xanthones from mangosteen pericarp powder was developed . Extraction efficiency was above 96% with excellent reproducibility.
- A 25 min gradient UHPLC-UV method was developed for the analysis of extracted samples and the yield of five selected xanthones was quantitatively determined.

References

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