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Quantitative and qualitative determination of six xanthones in *Garcinia mangostana* L. by LC–PDA and LC–ESI-MS

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Abstract

A new method was developed for the simultaneous analysis of six naturally occurring xanthones (3-isomangostin, 8-desoxygartanin, gartanin, α-mangostin, 9-hydroxycalabaxanthone and β-mangostin). The quantitative determination was conducted by reversed phase high performance liquid chromatography with photodiode array detector (LC–PDA). Separation was performed on a Phenomenex Luna C18(2) (150 mm × 3.00 mm, 5 μm) column. The xanthones were identified by retention time, ultraviolet (UV) spectra and quantified by LC–PDA at 320 nm. The precision of the method was confirmed by the relative standard deviation (R.S.D.), which was ≤4.6%. The recovery was in the range from 96.58% to 113.45%. A good linear relationship was established in over two orders of magnitude range. The limits of detection (LOD) for six xanthone compounds were ≤0.248 μg/mL. The identity of the peaks was further confirmed by high performance liquid chromatography with time-of-flight mass spectrometry (LC–TOF MS) system coupled with electrospray ionization (ESI) interface. The developed methods were applied to the determination of six xanthones in *Garcinia mangostana* products. The satisfactory results showed that the methods are effective for the analysis of real samples.

Keywords: Xanthone; Mangostin; *Garcinia mangostana*; Mangosteen; LC–PDA; LC–ESI-MS

1. Introduction

The mangosteen (*Garcinia mangostana* L.), belonging to the family Guttiferae, is a tropical evergreen tree. Its origin is in Southeast Asia. It can now be found in Northern Australia, Brazil, Central America, Hawaii, Southern India, Indonesia, Malaysia, Thailand, and other tropical countries. The edible fruit is deep reddish purple when ripe. In Asia, it is known as the "Queen of Fruits" due to its pleasant flavor [1,2].

The fruit hull of mangosteen has been used for hundreds of years in Southeast Asia as a medicine for skin infection, wounds, dysentery and diarrhea [1–3]. Recently other interesting properties of mangosteen are slowly being revealed. Its rind is about the fourth of an inch in thickness. It contains high amounts of xanthones, a class of polyphenolic compounds. Xanthones have antioxidant [4–7], antibacterial [8–10], antifungal [11], antiinflammatory [3,12,13] antitumor [5,14–21], antiplatelet aggregation [22], antithrombotic [23] and vasorelaxant activities [24], prevent oxidative damage of LDL [4], histamine and serotonin receptor blockers [25,26], and also inhibit HIV [27]. However, there is no data from clinical trials to verify these effects in humans. The health benefits of mangosteen need further to be proven scientifically.

Mangosteen has been used as an ingredient in several popular commercially available nutritional supplements, including Vemma and Xango, now. It can be purchased online and in herbal shops. For safety and efficiency, it is important to set up the method to control the quality. There is only one paper published using gas chromatography method to detect trimethylsilyl ethers of xanthones from *G. mangostana* [28]. The purpose of this study is to set up a new method for quality and quantity determination of xanthones in hull of mangosteen.

Herein a simple high performance liquid chromatography with photodiode array detector (LC–PDA) method is reported

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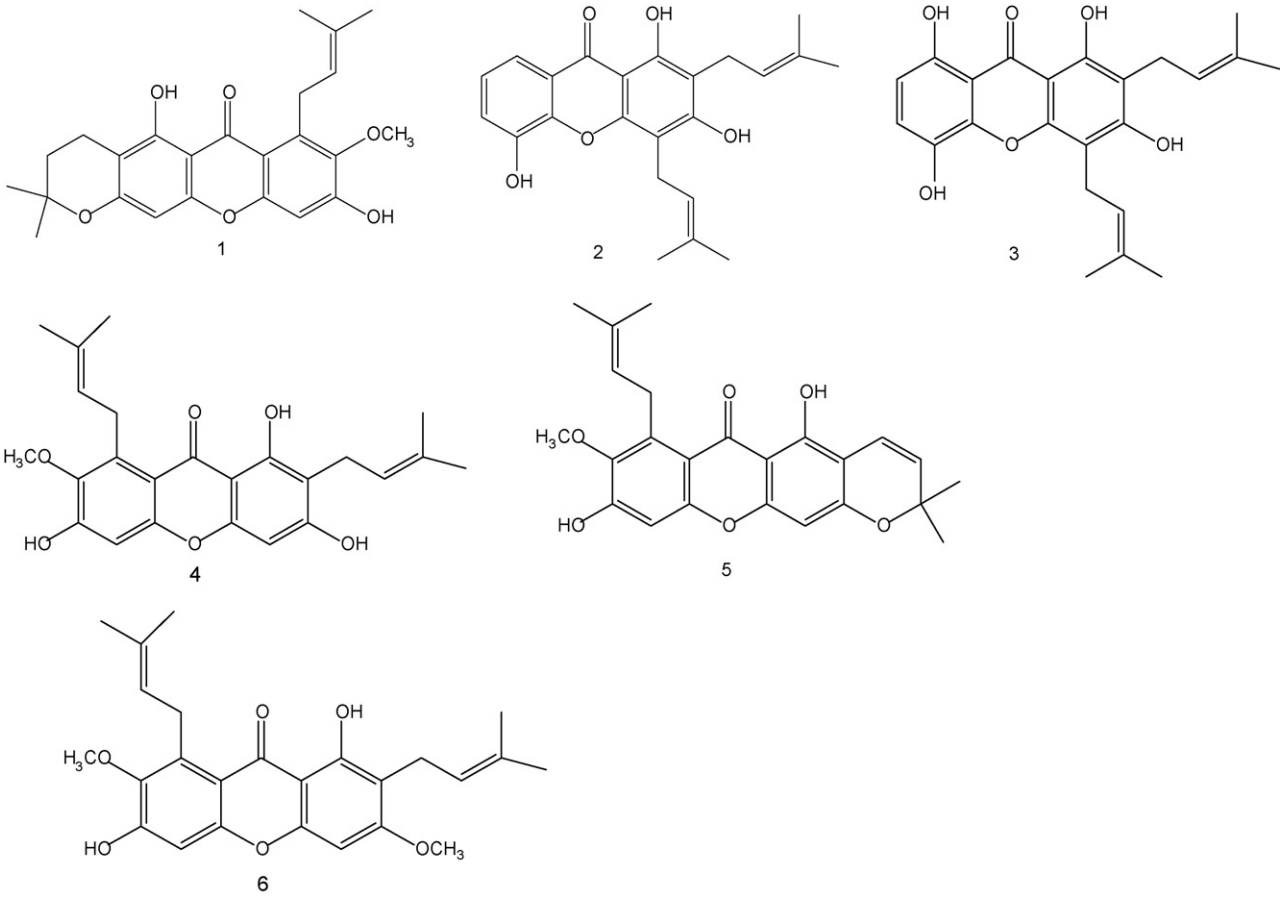

Fig. 1. Structures of xanthones: (**1**) 3-isomangostin; (**2**) 8-desoxygartanin; (**3**) gartanin; (**4**) α-mangostin; (**5**) 9-hydroxycalabaxanthone; (**6**) β-mangostin.

which detects and quantifies six xanthones (3-isomangostin (**1**), 8-desoxygartanin (**2**), gartanin (**3**), α-mangostin (**4**), 9-hydroxycalabaxanthone (**5**) and β-mangostin (**6**)) (Fig. 1). Xanthone peaks were further confirmed by high performance liquid chromatography with mass spectrometry coupled with electrospray ionization interface (LC–ESI-MS). The methods were applied for four products with satisfactory results obtained.

2. Experiment

2.1. Reagents and materials

Methanol, acetonitrile, isopropanol, reagent alcohol, acetone, ethyl acetate, water and acetic acid are HPLC grade (Fisher Scientific, Fairlawn, NJ). Formic acid and trifluoroacetic acid (TFA) are reagent grade from Sigma–Aldrich (Steinheim, Germany).

XTerra RP18 (150 mm × 4.6 mm, 5 μm, Waters), Gemini C18 (150 mm × 3.00 mm, 5 μm, Phenomenex), Luna 18(2) (150 mm × 3.0 mm, 5 μm and 250 mm × 4.6 mm, 5 μm, Phenomenex), and Luna C(8) (150 mm × 3.00 mm, 3 μm, Phenomenex) were used.

The standard compounds **1**–**6** were purchased from ChromaDex Inc. (Santa Ana, CA). The purity of these standard compounds was calculated (88.08%, 99.31%, 99.98%, 97.03%, 87.94%, 98.96%, respectively) by relative area percentage with chromatogram at Max plot using the same LC condition as sample detection. Four products were obtained from commercial source, which claim to be dried powder extract of pericarp of *G. mangostana* with different content of α-mangostin.

2.2. Sample preparations

In order to perform the determinations, about 0.1–0.2 g powdered products were weighed separately and then transferred to the separate centrifuge tubes, filled by 3 mL of acetone as extraction solvent. All the samples were vortexed and then sonicated for 20 min at room temperature. After centrifugation of the sonicated sample, the supernatant was transferred to a 10-mL volumetric flask. This procedure was repeated twice with the corresponding supernatants transferred to the corresponding 10-mL volumetric flask. The samples were then diluted to the final volume with acetone. Prior to injection, each sample was filtered through a 0.45 μm nylon membrane filter.

2.3. LC–PDA analysis

A Waters 2695 Alliance Separations Module equipped with a 996 PDA detector (Waters, Milford, MA) was used. Separation was achieved on a 150 mm × 3.0 mm, 5 μm Luna C18(2) column (Phenomenex, Torrance, CA). The mobile phase consisted of water with 0.1% TFA (A), methanol with 0.1% TFA (B) and isopropanol (C), which were applied in the following gradient

Table 1
Data of calibration curve, LOD and concentration in products (wt.%)

Compound	LOD (μg/mL)	Linear range (μg/mL)	R^2	GMP-1	GMP-2	GMP-3	GMP-4
1	0.132	264.24–0.26	0.9998	0.10	0.84	1.55	2.17
2	0.221	554.15–0.55	0.9999	0.07	0.54	0.80	1.53
3	0.248	496.00–4.96	0.9999	0.11	0.76	1.56	2.18
4	0.133	796.42–1.33	0.9999	1.27 (1.02)[a]	11.13 (11.92)	21.71 (20.72)	35.18 (42.84)
5	0.131	262.06–0.26	1.0000	0.06	0.51	0.83	1.71
6	0.104	520.53–0.52	0.9999	0.02	0.24	0.43	0.83

[a] In parenthesis is the label claim of α-mangostin in products.

elution: 50% A/30% B/20% C (v/v/v) to 10% A/70% B/20% C (v/v/v) in 45min; finally in the next 2 min to 80% A/20% C (v/v) and held at that composition for another 5 min. Each run was followed by equilibration time of 15 min. The flow rate was adjusted to 0.5 mL/min. The temperature was held constant at 40 °C. The injection volume was 10 μL. Ultraviolet (UV) spectra were recorded in the 210–400 nm range and the chromatograms were acquired at 320 nm. The data was collected and analyzed by Waters Millennium^{32} software.

2.4. LC–ESI-MS analysis

The system was an Agilent MSD TOF coupled to an Agilent 1100 series HPLC. For the LC condition, the column and operating temperature were same as those for LC–PDA analysis. The mobile phase consisted of water with 0.5% acetic acid (A), acetonitrile with 0.5% acetic acid (B) and isopropanol (C), which were applied in the following gradient elution: 40% A/40% B/20% C (v/v/v) to 10% A/70% B/20% C (v/v/v) in 45 min; finally in the next 5 min to 100% B at a flow rate of 0.3 mL/min. For the MS, mass spectra were acquired using an Agilent ESI-MSD TOF. Drying gas (N_2) flow was 13 L/min; nebulizer pressure was 40 psig; drying gas temperature was 325 °C. For positive ESI analysis, the parameters were: capillary voltage, 4000 V; fragmentor, 100 V; skimmer, 60 V; Oct RF V 250 V. For negative ESI analysis, the parameters were: capillary voltage, 3500 V; fragmentor, 175 V; skimmer, 40 V; Oct RF V, 250 V. The mass range was from 200 to 900 *m/z*. The data acquire was at one scan per second; with each scan composed of 10,000 transients. Data acquisition and processing was done with the software Analyst QS.

2.5. Preparation of standard solutions and method validation

2.5.1. Standard solutions and calibration curves

For quantitation, an external standard method was utilized. About 5–10 mg of a standard weighed accurately was dissolved into a 10-mL volumetric flask with acetone to obtain stock solutions and stored in freezer. Working standard solutions were diluted to series of concentrations with acetone. Six standards, at the concentration ranges shown in Table 1 with five concentration levels, were injected in triplicate to obtain calibration curves, respectively. Peak areas from the LC chromatogram were plotted against the known corrected concentrations of standard solutions with purity at varying concentrations to establish calibration equations. The equation of linear regression was calculated by the method of least squares. A $1/x$ weighting factor was applied.

2.5.2. Detection limits

For the evaluation of detection limits, a concentration sequence of the standards was obtained by diluting standard solutions. Limit of detection (LOD) was based on three times of signal-to-noise ratio.

2.5.3. Repeatability

The solution of one product was used to achieve repeatability testing for six compounds. Repeatability experiments were conducted for intraday and interday. The data used to calculate R.S.D. % of interday repeatability was the areas of twelve injections in three days (four injections in succession each day). The data of intraday repeatability was the areas of six injections separately in the same day.

2.5.4. Recovery

A standard additional method was utilized to assess recovery behavior. Six standard stock solutions were added into a product, which contain the highest matrix. After evaporation of solvent at room temperature with nitrogen gas, the sample was extracted using the method described in sample preparations. Four replicates were performed. The percent ratio between the observed and expected amount was calculated.

2.6. Identification

The peaks in products were assigned by comparison of the retention time and the UV spectra with the standard compounds with LC–PDA method. Identified peaks were then further confirmed by spiking sample with standard mixtures using LC–PDA method and by mass spectra in LC–ESI-MS method.

3. Results and discussion

All of the structures of xanthone were shown in Fig. 1. The UV spectra from 210 to 400 nm of the six compounds are shown in Fig. 2. As all the six compounds have good absorption at 320 nm, this wavelength was used for quantity. A typical HPLC chromatogram of six xanthone standards and a product is presented in Fig. 3, which showed all the six compounds were eluted within 40 min with satisfactory resolution;

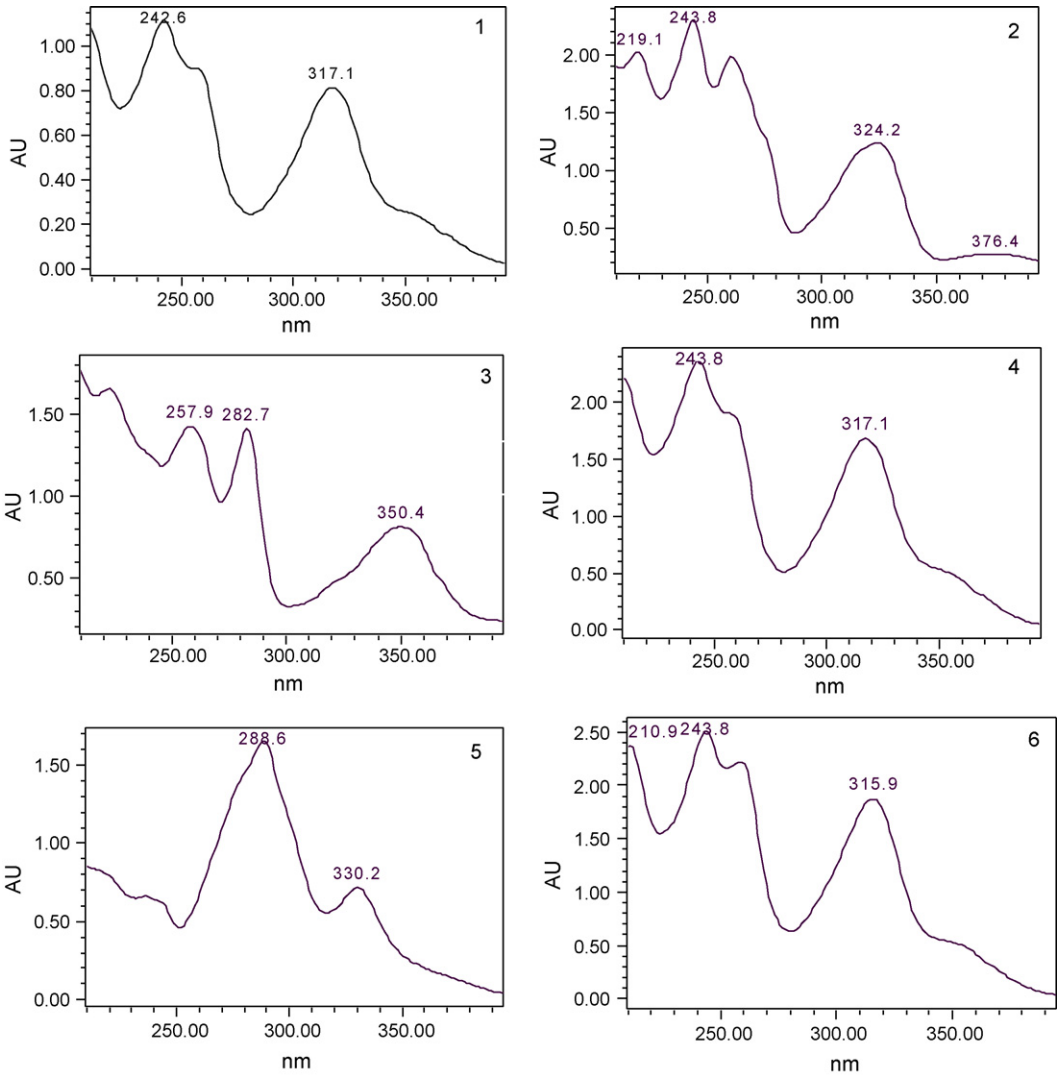

Fig. 2. UV spectra of compounds **1**–**6**.

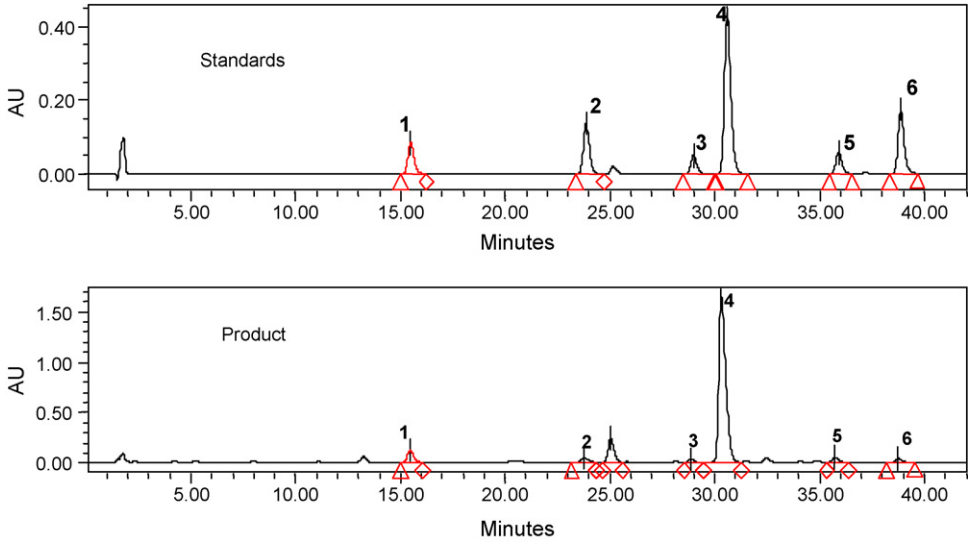

Fig. 3. Typical LC–PDA chromatograms of standard compounds (**1**–**6**) and one product at 320 nm.

Table 2
Retention time, intraday and interday precision of xanthones with LC–PDA method

Compound	Retention time (min) (R.S.D. %, $n=12$)	R.S.D. % (interday, $n=12$)	R.S.D. % (intraday, $n=6$)
1	15.54 (0.2)	2.1	1.0
2	23.85 (0.2)	4.6	1.4
3	28.99 (0.1)	2.7	0.8
4	30.45 (0.1)	2.5	2.2
5	35.81 (0.1)	2.2	0.7
6	38.84 (0.1)	2.4	0.3

Table 3
Recovery of xanthones **1**–**6** in a product ($n=4$)

Compound	Added amount (μg)	Detected amount (μg)	Recovery (%)	R.S.D. (%)
1	264.24	277.17	104.89	0.3
2	55.80	61.96	111.04	1.3
3	248.00	281.36	113.45	0.2
4	2654.74	2580.48	97.20	0.3
5	131.03	130.60	99.67	0.4
6	263.00	254.01	96.58	0.1

no indication of impurities were found. The retention time is very consistent with R.S.D. no more than 0.2%, showed in Table 2.

3.1. Extraction

A few different extract solvents (methanol, acetone, acetonitrile and ethyl acetate) were tried. Their chromatogram and peak area for six compounds are almost same with the same weight of material using the method described in Section 2.3. Using the Millennium[32] software for peak purity test, the purity test only for methanol and acetone is passed for all six compounds. That means each peak is only one compound, no coelution for methanol and acetone extraction. The color of methanol solution is black; this means it contains more color impure compounds, so we chose the acetone as extraction solvent. No cleanup was necessary because no interfering peaks were present using acetone. The recovery data for the six compounds are shown in Table 3. Recoveries ranged from 96.58% to 113.45%, with R.S.D. between 0.1% and 1.3%. This indicates that acetone can be acceptable as extract solvent.

3.2. LC–PDA method

Three kinds of C18 and one kind of C8 columns described in Section 2.1 were utilized for the initial attempt. During some tentative isocratic (methanol/water with 0.1% acetic acid at 1:2, 1:1, 2:1) and linear gradient elution procedures (methanol/water with 0.1% acetic acid from 10/90 to 100), considering the trade-off between separation efficiency and time, the column described in Section 2.3 was chosen for succeeding optimization as this column has shorter retention time and better separation. For the separation, it was difficult to separate compounds **3** and **4**, and at same time to get pure peak compound **2** with methanol/water system as mobile phase, so we tried methanol, acetonitrile, isopropanol, reagent alcohol and water with acids in different combinations and at mobile flow rate from 0.5 to 1.5 ml/min, with column temperature at 30, 35 and 40 °C. For acid additives in the mobile phase can suppress ionization of xanthones, they can make the peak shape of xanthone symmetric and sharp. Acetic acid, formic acid and TFA were tried. With the TFA, the peak shape was narrower and sharper, so we chose 0.1% TFA in mobile phase for LC–PDA. Finally, the mobile phase described in Section 2.3 was used for analysis.

Utilizing the PDA makes it possible to obtain the UV spectra (Fig. 2). The compounds in products were confirmed by overlapping their spectra with those of the standards at same retention time. Spiking sample with reference compounds performed a further confirmation assay.

The precision under conditions of repeatability was determined by performing six injections of product extract on the same day or 12 injections of the same solution in three different days, respectively. The R.S.D. of intraday was ≤2.2% and R.S.D. of interday was ≤4.6%, indicating repeatability is acceptable. The data were shown in Table 2.

Calibration curves were constructed by plotting analyte corrected concentrations with purity against peak areas. A good linearity was achieved in the range 264.24–0.26 μg/mL for compound **1** with the determination coefficient (R^2) 0.9998; 554.15–0.55 μg/mL for compound **2** with $R^2=0.9999$; 496.00–4.96 μg/mL for compound **3** with $R^2=0.9999$; 796.42–1.33 μg/mL for compound **4** with $R^2=0.9999$; 262.06–0.26 μg/mL for compound **5** with $R^2=1.000$; 520.53–0.52 μg/mL for compound **6** with $R^2=0.9999$. The LODs for all six xanthone compounds were ≤0.248 μg/mL (Table 1).

Table 4
Mass spectra of compounds (**1**–**6**) with LC–ESI-MS method

Compound	Retention time (min)	Negative mass spectra (*m*/*z*)	Positive mass spectra (*m*/*z*)
1	17.8	427.18 ($[M+H_2O–H]^-$)	411.17 ($[M+H]^+$), 429.19 ($[M+H_2O+H]^+$)[a], 451.17 ($[M+H_2O+Na]^+$)
2	26.9	379.16 ($[M-H]^-$)	381.17 ($[M+H]^+$)[a], 403.14 ($[M+Na]^+$)
3	32.9	395.15 ($[M-H]^-$)	397.16 ($[M+H]^+$)[a], 419.14 ($[M+Na]^+$)
4	34.6	409.18 ($[M-H]^-$)	411.18 ($[M+H]^+$)[a], 433.16 ($[M+Na]^+$)
5	41.5	407.17 ($[M-H]^-$)	409.16 ($[M+H]^+$)[a], 431.14 ($[M+Na]^+$)
6	44.6	423.19 ($[M-H]^-$)	425.20 ($[M+H]^+$)[a], 447.17 ($[M+Na]^+$)

[a] Base peaks.

Four commercial products were analyzed using the LC–PDA method. The contents of six xanthone were calculated and the results were shown in Table 1 with the mean values of three replicate injections. The most abundant of xanthone was α-mangostin. The content of α-mangostin in products, which is the only xanthone ingredient marked in label, is almost consistent with our results.

3.3. LC–ESI-MS method

LC–ESI-MS experiments were performed to further confirm the identity of peaks 1–6 using the conditions described in Section 2.4. TFA was reported to suppress the ion form in ESI spray [29], so 0.5% acetic acid was used for our experiment. In our experience the HPLC flow rate had to be decreased

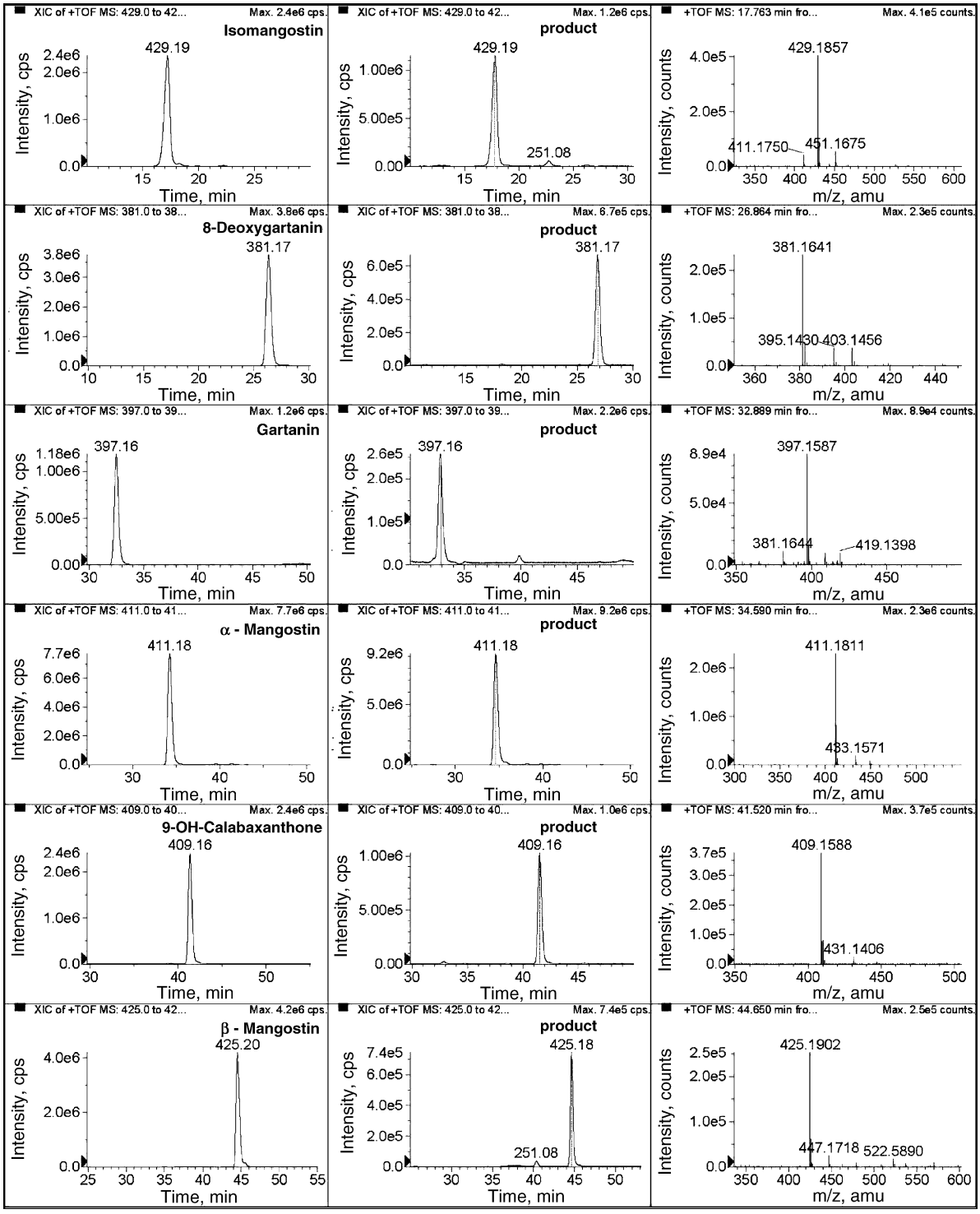

Fig. 4. Extracted ion chromatogram of standard compounds (**1**–**6**) and compounds of a product, and their mass spectra in positive ion mode.

to 0.3 mL/min. However, with this low flow rate, while keeping same solvent system and gradient as those for LC–PDA method, the retention time will be too long. To make the retention time shorter, the solvent system and gradient were slightly modified, which was described in Section 2.4. We used acetonitrile instead of methanol and the initial condition contains more organic solvent. For ESI-MS analysis, positive and negative ionization mode was used. Positive ESI mode had more adduct peaks, while the negative ESI had more abundance and showed only one peak. Positive MS spectra were dominated by the $[M+H]^+$ ion except compound **1** with water adduct. Negative MS spectra were dominated by the $[M-H]^-$ ions except compound **1** with water adduct (Table 4). Fig. 4 shows extracted ion chromatograms (XIC) in positive ion mode for standards and a product. Standards and products were detected, respectively. The retention times and mass spectra of products exactly matched with the corresponding standard compounds, which were shown in Fig. 4 and Table 4.

4. Conclusion

This was the first report of simultaneous determination of six major xanthones in *G. mangostana* by LC. A simple and accurate LC–PDA approach for quantity was presented. The extraction method is simple with acetone as an extract solvent by sonication. No cleanup was necessary. The LC–PDA method showed a good linearity, precision and accuracy, so it was suitable for quality control the products of mangosteen. The LC–ESI-MS experiment was performed to further confirm the identity of the peaks of product. The methods were successful in the qualitative and quantitative evaluation of four *G. mangostana* products in this paper.

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