

# Screening of Polycyclic Polyprenylated Acylphloroglucinols from *Garcinia* Species Using Precursor Ion Discovery (PID) Scan and Ultra Performance Liquid Chromatography Electrospray Ionization Q-TOF Tandem Mass Spectrometry

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A strategy was newly developed to rapidly screen polycyclic polyprenylated acyl-phloroglucinols (PPAPs) from the plant matrices of nine *Garcinia* species using ultra-performance liquid chromatography (UPLC) coupled with comprehensive mass spectrometric approaches including precursor ion discovery (PID) and tandem mass (MS/MS) scans. The PPAPs share the same diagnostic product ion at  $m/z$  177.02 in positive MS/MS scan, which may be increased as the base peak by ramping the cone voltage from 45 to 100 V. With this ramping cone voltage PID scan, it is feasible to selectively screen the PPAPs from 29 samples of nine *Garcinia* species. This approach has proven to be a powerful, highly selective, and sensitive tool for rapid screening and detection of nontargeted components in natural products before the purification and structural elucidation process. (J Am Soc Mass Spectrom 2009, 20, 1846–1850) © 2009 American Society for Mass Spectrometry

LC-MS is becoming an increasingly important tool in phytochemical analysis due to its potential to circumvent the time-consuming and tedious discovery process on known structures [1, 2]. Among the various LC platforms, UPLC has proven to be an effective separation tool to fractionate complex extracts because of its increased resolution, higher sensitivity, excellent peak shapes, and enhanced reproducibility [3]. Quadrupole TOF-MS techniques, on the other hand, allow the generation of mass information with greater accuracy and precision, and so have been used to determine the molecular formula at low ppm [4].

Recently, some useful approaches for identifying nontarget components based on the use of LC-TOF accurate mass measurement and database searching has been developed and successfully applied to several studies in the fields of pesticide residues and pharmacology/toxicology analyses [5, 6]. However, the methods depend largely on the information contents of the database available. Nevertheless, the database-searching approach failed to detect the isomers with complex structures.

Therefore, comprehensive MS screening methods, which can detect known or unknown structures, are highly desirable. The Q-TOF mass spectrometry precursor ion discovery (PID) approach has been successfully used in selective detection of protein post-translational modifications [7, 8]. So far, PID on a Q-TOF instrument has not been used in natural products compounds identification. The PID product ion scan often failed to detect the characteristic product ion because of the low abundance of the ion. However, characteristic fragmentation product ions could be generated through in source fragmentation by QTOF-MS/MS/MS, [9] making it feasible to selectively screen a typical class of natural products by detecting diagnostic product ions.

Polycyclic polyprenylated acyl-phloroglucinols (PPAPs), featuring a highly oxygenated and densely substituted bicyclo[3.3.1]nonane-2,4,9-trione core decorated with prenyl or geranyl side chains, are a special class of bioactive components mainly from the *Guttiferae* family, in which over 50 PPAPs were identified from different *Garcinia* species [10, 11]. These compounds display a range of biological activities, such as antimicrobial, antidepressant, antibiotic, anti-HIV, antitumor, cytotoxic, and antioxidant activities [11]. Our continuous investigation on *Garcinia* species led to a series of reports on xanthenes, cadged xanthenes, and PPAPs derivatives with anticancer activities [12–14].

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The PPAPs derivatives are hard to separate due to the slight difference in the complicated structures, and most of them exist as isomers. This report described a selective and sensitive method for the detection of the PPAPs by UPLC-QTOF-PID scan. Twenty-nine plant extracts from nine *Garcinia* species were examined. The PPAPs were easily detected using the PID product ion scan by ramping the cone voltage from 45 to 100 V.

## Experimental

### Chemicals

Acetonitrile (HPLC-MS grade) was purchased from Fisher Scientific (Loughborough, UK), and formic acid (spectroscopy grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA); Leucine-enkephalin was obtained from Sigma-Aldrich.

### Sample Preparation

The twigs of *G. xanthochymus*, *G. xipshuanbannaensis*, *G. lancilimba*, and *G. cowa* were collected in Xishuangbanna Prefecture, Yunnan Province. *G. yunnanensis* and *G. esculenta* were collected from Dehong, Yunnan Province. *G. multiflora*, *G. subelliptica*, and *G. oblongifolia* were collected in Guanxi, Shenzhen, and Hainan, respectively. The plant materials were identified by Professor Wang Hong, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. All voucher specimens (CMED-0468~0478) were deposited in the Hong Kong Jockey Club Institute of Chinese Medicine. Nine reference compounds, namely (–) 30-epicambogin (1), (–) cambogin (2), guttiferones K (3), (–) guttiferone F (4), (+) oblongifolin B (5), (+) oblongifolin A (6), (+) oblongifolin C (7), (+) guttiferone B (8), (+) oblongifolin D (9) were isolated from *G. yunnanensis* and *G. oblongifolia* in our laboratory and were identified based on IR, UV, NMR spectroscopy analysis. They were dissolved in acetonitrile to give a concentration of 0.1–0.5 µg/mL.

### Chromatography

UPLC was performed using a Waters ACQUITY UPLC system (Waters Corp., Milford, MA, USA), equipped with a binary solvent delivery system, autosampler, and a PDA detector. The chromatography was performed on a Waters ACQUITY BEH C<sub>18</sub> column (100 × 2.1 mm, 1.7 µm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile containing 0.1% formic acid. The UPLC eluting conditions were optimized as follows: isocratic at 75% B (0–3 min), linear gradient from 75% to 85% B (3–4 min), isocratic at 85% B (4–6 min), linear gradient from 85% to 95% B (6–7 min), isocratic at 95% B (7–8 min), and linear gradient from 95% to 75% B (8–12 min). The flow rate was 0.6 mL/min. The column and autosampler were maintained at 35 °C and 10 °C, respectively.

### Mass Spectrometry

Mass spectrometry was performed using a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) operating in positive mode. The nebulization gas was set to 600 L/h at temperature of 300 °C, the cone gas was set to 50 L/h, and the source temperature was set to 100 °C. The capillary voltage was set to 3 KV. Argon was employed as the collision gas. The masses are accurately determined with reference compound leucine-enkephalin in the LockSpray mode ( $m/z$  556.2771). The data were collected into two separate data channels, with the instrument spending 0.2 s on data acquisition for each channel and a 0.02 s inter-channel delay. Mass accuracy was set less than 3 ppm for all the ions detected in MS and MS/MS scan.

### PID Experiments

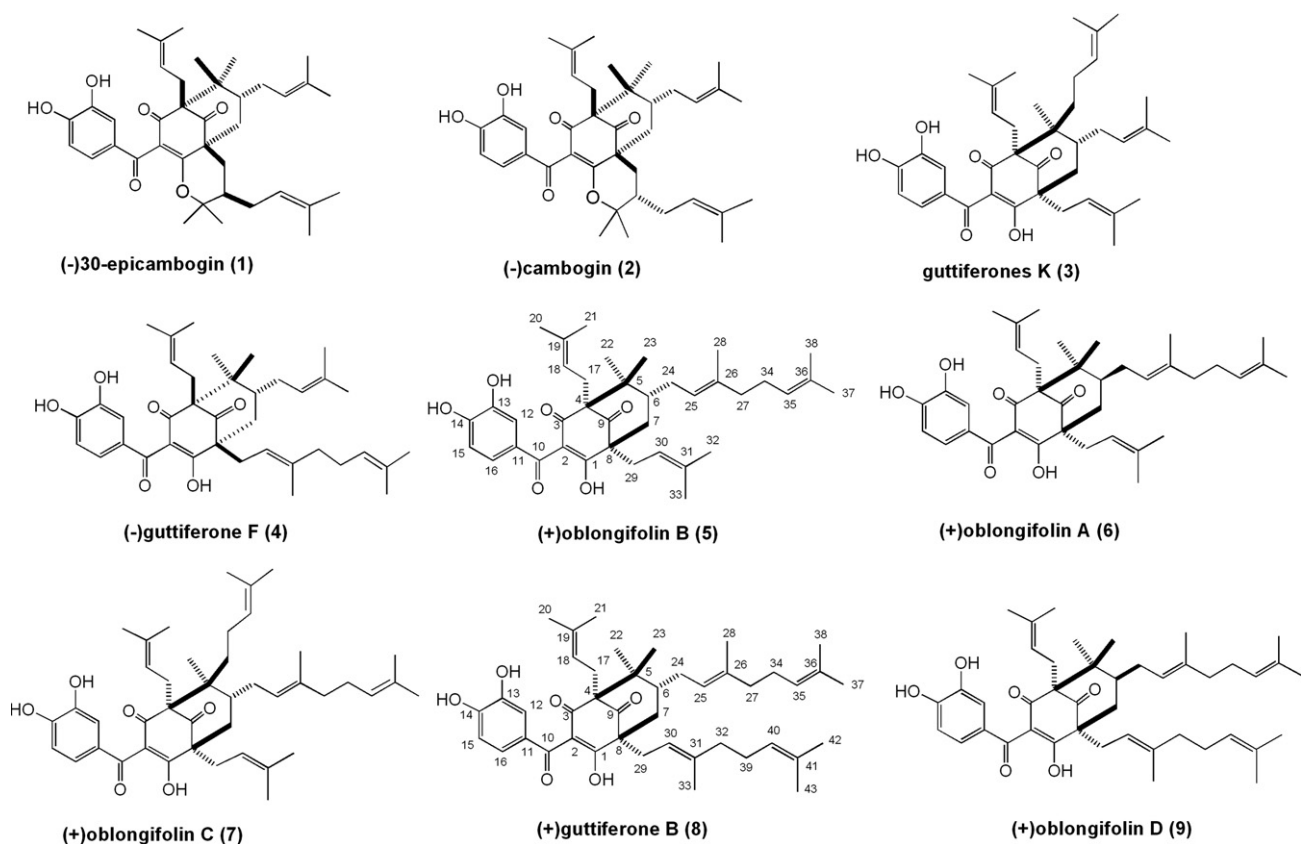
In PID mode, during UPLC gradient, the mass spectrometer switched back and forth continuously between MS and MS/MS survey conditions with low and high collision energies until certain criteria, indicating the presence of a given product ion, were met. The mass spectrometer, when operating in the survey MS mode (quadrupole in nonresolving mode), alternates between: (1) the low (collision) energy mode (for which the cone voltage is 45 V and gas cell collision energy voltage is set to 5 V), and (2) the high (collision) energy mode (for which the cone voltage is ramped from 45 to 100 V and the gas cell collision energy voltage is set to 30 V). On detection of a candidate precursor ion the mass spectrometer switches to the MS/MS mode (quadrupole in nonresolving mode, the cone voltage ramped from 45 to 100 V, and the gas cell collision energy voltage is 30 V).

For PPAPs detection, the PID was triggered by the detection of the product ion at  $m/z$  177.02 as initially determined using all the PPAPs standards. MS/MS spectra on all PPAPs standards were analyzed over a series of collision energies and cone voltages to determine optimal conditions for tandem fragmentation. The product ion window was set at  $m/z$  177.02 with an acceptance mass window of 100 mDa. Cone voltage ramped from 45 to 100 V, and CE was 30 V under MS/MS acquisitions. MS to MS/MS switch criteria was dependent on the reporter ion intensity (10 counts/s) and charge state peak selection (1<sup>+</sup> charge state). MS/MS mode was switched back to MS if the expected product ion was absent or below 10 counts/s.

## Results and Discussion

### Mass Spectrometry Analysis of Known PPAPs

Reference standards of nine PPAPs, including six isomers with molecular weight (MW) at 602.3607 and three isomers of MW at 670.4233 (Figure 1) were studied by means of QTOF-MS in positive mode. All reference Compounds 1–9 produced abundant  $[M + H]^+$  ions as



**Figure 1.** Polycyclic polyprenylated acylphloroglucinols (PPAPs) isolated from *Garcinia* species.

the base peak, and all yielded similar fragment ions in the positive MS/MS spectra (as shown in MS/MS spectra of Compounds 1–9). In this study, the Q-TOF MS/MS experiments was optimized and developed through raising in-source fragmentation to induce more fragment ions. Among the MS parameters, such as rf lens value, sampling cone voltage, nitrogen gas flow rate, collision energy, and cell entrance, cone voltage plays the key role in influencing the in-source dissociation. The first stage of fragmentation of the precursor ions was accomplished by in-source collision-induced dissociation (CID) by ramping the cone voltage from 45 to 100 V to achieve optimal fragmentation of the different precursor ions. (The cone voltage fragmentation of  $[M + H]^+$  of Compound 8 is shown in the Supplementary Figures, which can be found in the electronic version of this article.) Interesting findings indicated that the PPAPs analogs showed similar fragmentation pathways in positive mode. The product ion spectra of reference compounds at low and high cone voltage with the same collision energy showed different fragmentation pathways depending on their structures. Under the conventional MS/MS approach, where fragmentation is induced in the collision cell only (CE at 30 V), fragmentation mainly occurred at the peripheral substituents. In contrast, using the two-stage fragmentation approach (in-source CID and further fragmentation in the collision cell), the characteristic fragment ion  $m/z$  177.02

representing the fragmentation of PPAPs of cone structure was produced as the base peak. An example of the MS/MS spectra of Compound 5 at low and high cone voltage with the same CE at 30 V are shown in the Supplementary Figures, which clearly illustrate the fragmentation behavior observed for the PPAPs in the two conditions.

#### *Precursor Ion Discovery Product Ion Approach*

For PPAPs detection, the precursor ion discovery (PID) product ion method was set up by searching for the characteristic fragment ion at  $m/z$  177.02 from the survey modes. In the first survey mode, where a cone voltage of 45 and CE of 5 V were applied to the collision cell, only molecular precursors were shown. When the CE was 30 V and the cone voltage was ramped from 45 to 100 V for the second survey mode, the fragment ions could be generated from the first survey mode spectrum. The high-energy survey mode spectra were examined for the presence of the predefined product ions at  $m/z$  177.02. When this species was detected, the instrument automatically switched to record the product ion spectrum of the candidate precursor ions to verify that the chosen precursor generated the observed product ion. If confirmed, the QTOF could remain in MS/MS mode to acquire more sequence data before reverting to acquisition of alternate spectra in the two survey modes.

The identification specificity of PPAPs by means of the above described PID method based on detection of product ion of  $m/z$  177.02 was then applied to 29 samples from 10 *Garcinia* species derived from different parts of the plants, including the twig, leaves, peels and fruits.

Figure 2 shows a representative PID chromatogram of the bark of *G. lancilimba* obtained with UPLC-QTOF. The total ion chromatograms for the lower and higher CE data are shown in Figure 2a and b, respectively. The MS/MS chromatograms triggered by the characteristic fragment ion at  $m/z$  177.02 (Figure 2c) clearly shows, as expected, that only tandem mass spectrum acquired during the chromatographic separation was registered for the single charged ion of PPAPs at  $m/z$  177.02 (theoretical 177.0188) at the retention time of 2.8, 3.2, 6.3, 6.5, 6.8, and 8.9 min, which correspond to Compounds 1, 2, 4, 5, 6, and 7, respectively. In contrast, when conventional LC-MS/MS approach was performed on the same samples, large amounts of xanthenes in the crude extracts interfered with the identification of PPAPs from the complicated profile. Hence, PID ap-

proaches simplify the identification of known PPAPs from the LC-MS/MS chromatograms.

To investigate whether PPAPs are in the 29 samples from the nine *Garcinia* species, combined techniques of  $m/z$  177.02 precursor ion discovery (PID) and LC-MS/MS scans were carried out. As a result, PPAPs were found abundantly in *G. lancilimba*, *G. yunnanensis*, *G. multiflora*, and *G. oblongifolia*. Some PPAPs were found in *G. cowa*, *G. xipshuanbannanensis*, *G. xanthochymus*, and *G. subelliptica*, but few, if any, PPAPs were detected in *G. esculenta*.

This detection specificity was verified by analysis of nine standards at different concentration levels. Serial dilutions were prepared to inject decreasing amounts of the PPAPs standards (100  $\mu\text{g/mL}$ , 10  $\mu\text{g/mL}$ , 1  $\mu\text{g/mL}$ , and 0.1  $\mu\text{g/mL}$ ). All analytes were detected below concentrations 5 ng, demonstrating the broad applicability range of this method. However, it should be pointed out the increase in cone voltage would lead to increased base line noise/background and, thus, resulting in decreased signal to noise ratio towards the LLQ.

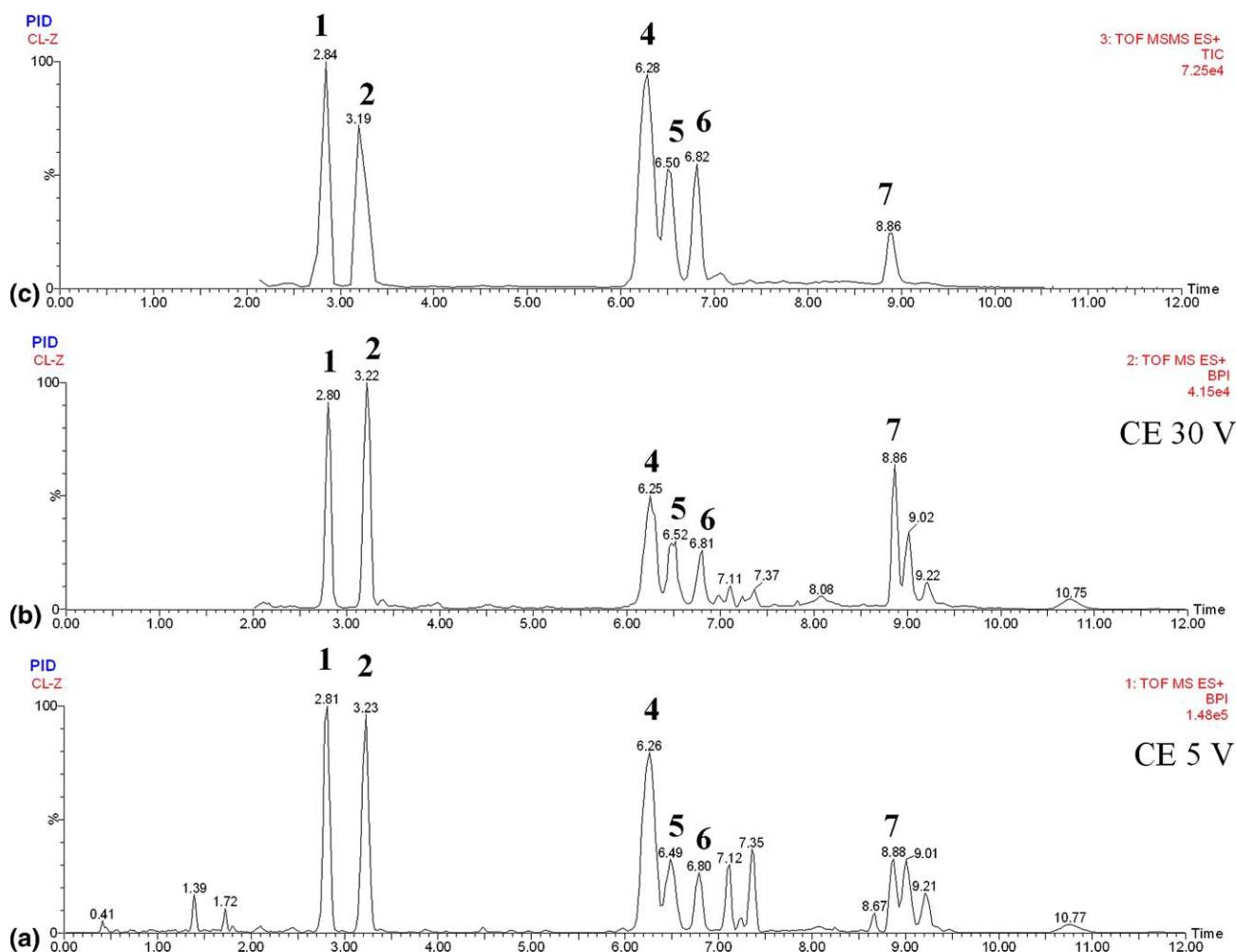


Figure 2. (a) and (b) BPI chromatogram for the low (a) and high (b) CE data derived from LC-MS acquisition in PID mode of the bark of *G. lancilimba*. (c) MS/MS trace triggered by product ion at  $m/z$  177.02 corresponding to PPAPs.

## Conclusions

This manuscript describes and validates a comprehensive UPLC-Q-TOF tandem mass spectrometric precursor ion discovery (PID) method for nontargeted discovery of the polycyclic polyprenylated acylphloroglucinols (PPAPs) from the plant matrices of nine *Garcinia* species. This approach employs a cone voltage gradient while performing PID scan with MS/MS and, thus, characteristic fragmentation product ions could be generated abundantly through in source fragmentation. However, as the increase in cone voltage might lead to increase in base line noise/background and, thus, resulting in decreased signal to noise ratio towards the LLQ. This is the first report of the use of PID on a Q-TOF instrument in the discovery and identification of natural product small molecules. As a result, isomeric PPAPs analogs could be separately screened in the UPLC-MS-PID run without matrix interference as that in the UPLC-MS run, so that the trace level PPAPs could be identified. This strategy also has the potential to screen and identify other types of compounds from complex matrices in the fields of natural products, metabolomics, synthetic chemistry, and so on.

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## Appendix A Supplementary Material

Supplementary material associated with this article may be found in the online version at [doi:10.1016/j.jasms.2009.06.008](https://doi.org/10.1016/j.jasms.2009.06.008).

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