Development and Validation of LC–ESI–MS/MS Method for Simultaneous Determination of Four Coumarin Derivatives and an Alkaloid from Root and Stem Bark of Aegle marmelos Correa

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Summary. Aegle marmelos Correa (Bael tree) is a medicinal fruit tree, widely used for healing purposes in various systems of medicines. Coumarins and alkaloids present in various parts of bael tree including roots and fruit pulp are the primary active constituents implicated for its biological activities. An efficient liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS) method was developed for identification and simultaneous determination of four coumarin derivatives, namely, umbelliferone, psoralene, marmin, and imperatorin, and an alkaloid, skimmianine, in root and stem bark of A. marmelos. The chromatographic separation of analytes was performed on Altima C18 (50 × 4.6 mm, 3 μm) column using methanol and 0.1% acetic acid in water (54:46, v/v) as the mobile phase under isocratic conditions. The LC–MS/MS parameters were optimized in the positive ionization mode using electrospray ionization source. The quantification of the analytes was performed using multiple reaction monitoring (MRM) transitions, umbelliferone (m/z 163.1 → 107.1), psoralene (m/z 187.2 → 131.1), marmin (m/z 333.5 → 163.2), imperatorin (m/z 271.1 → 203.1), and skimmianine (m/z 260.1 → 227.0). The extraction method was standardized for optimum yield of coumarin derivatives and the alkaloid in different extraction solvents. Higher yield of the analytes was found in methanolic extracts in comparisons to petroleum ether, chloroform, ethyl acetate, ethanol, and water. The method was validated for linear range, intra- and inter-batch precision and accuracy. The distribution of coumarin derivatives and an alkaloid was found to vary significantly in different plant samples, and their concentration was much higher in roots as compared to stem bark.

Key Words: Aegle marmelos, root and stem bark, coumarins, alkaloid, LC–ESI–MS/MS, multiple reaction monitoring

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Introduction

*Aegle marmelos* Correa, a flowering plant, is known for its medicinal importance and belongs to the family of *Rutaceae*. The common name for this plant is “Bael tree,” a native of India and is also found in various other parts of Asia such as Burma, Pakistan, Bangladesh, Sri Lanka, and Thailand [1, 2]. Bael fruit pulp is consumed directly or in preparations such as drinks and *murrabba*, mainly as tonic or for healing purposes. Bael tree is extensively used in medicinal preparations in various systems of medicines in India and neighboring countries.

Every part of this plant, like fruits, leaves, stem, and root bark, possess some medicinal property and is known to contain several important phytochemicals such as marmin, marmenol, marmelosin, marmelide, marmelin, marmesin, anhydromarmelin, psoralene, imperatorin, alloimperatorin, rutaretin, scopeletin, aegelin, fagarine, limonene, betulinic acid, luvangentin, auroptene, and many others [3–5]. These compounds have shown potential pharmacological activity against various diseases, and their extracts are used to cure fever, dysentery, diabetes, asthma, heart problems, ophthal-mia, and urinary problems in humans [6]. Additionally, they are also used as anticancer, antiulcer, antihyperlipidemic, anti-inflammatory, and antispermatogenic agents as demonstrated through animal models [7–11]. Monographs on stem bark and root of this plant are also presented in the Ayurvedic Pharmacopoeia of India [6]. Based on its extensive medicinal value, chemical standardization of an herbal formulation from this plant for major active constituents is essential.

Several chromatographic methods based on high-performance liquid chromatography (HPLC), LC–MS, gas chromatography (GC), and high-performance thin-layer chromatography (HPTLC) have been utilized to determine bioactive components from different parts of the plant. Reversed-phase HPLC was used to determine the amount of tannin, riboflavin, and various organic acids like oxalic acid, tartaric acid, ascorbic acid, and malic acid from the fruits of the plant [12]. A normal-phase HPLC method with refractive index detector has been reported to determine several sugars from fruit pulp and studied the effect of ripening on their contents [13]. Another RP-HPLC method is described for the quantification of umbelliferone in the roots [14]. Similarly, Prakash et al. [15] have characterized different phenolic compounds such as chlorogenic acid, ellagic acid, ferulic acid, gallic acid, protocatechuic acid, and quercetin in fruits by LC–MS analysis. A solid-phase microextraction (SPME)–GC–MS method was used to analyze 28 volatile compounds from the fruit pulp of this plant which included...
monoterpenes and sesquiterpenes. The major component found was limonene (~32%), which is chiefly responsible for the characteristic flavor of the fruit, together with p-cymene (27.19%) [5]. A HPTLC has been used to standardize the plant material for marmelosin in ripe fruits [16]. Another validated HPTLC method described simultaneous quantification of umbelliferone, psoralene, and eugenol in fruit pulp [17]. We had reported a simple and reliable HPTLC method for the simultaneous determination of four coumarin derivatives, namely, umbelliferone, psoralene, marmin, and imperatorin, and an alkaloid, skimmianine, in root bark of *A. marmelos* [18].

![Chemical structures of four coumarin derivatives and an alkaloid](image)

*Fig. 1. Chemical structures of four coumarin derivatives and an alkaloid*
Determination of coumarin derivatives like marmelosin, umbelliferone, scopoletin, and psoralene from bael fruit using HPLC has been reported [19, 20]. Mass spectrometry provides rapid and sensitive quantification of biomolecules, and LC–MS has become ubiquitous as the technique of choice for many quantitative analysis applications involving pharmaceuticals and phytochemicals. We report here a validated LC–MS/MS method which is rapid, sensitive, and accurate with multiple reaction monitoring for the simultaneous identification and determination of these five bioactive compounds from roots and stem bark of A. marmelos as per standard guidelines (ICH Q2, 2005). The chemical structures of four coumarin derivatives and an alkaloid are shown in Fig. 1.

Experimental

Chemicals and Materials

Reference standards of umbelliferone (99%), psoralene (99%), and imperatorin (98%) were purchased from Sigma-Aldrich (Bangalore, India). Marmin (98%) and skimmianine (98%) were isolated from root of A. marmelos and identified by spectral analysis and followed by comparison of spectral data [21, 22]. The HPLC grade methanol, ethanol, petroleum ether, chloroform, and ethyl acetate were purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). Ultra-pure distilled water with resistively greater than 18 MΩ was used. The Laborota 4010 rotary evaporator from Heidolph, Germany was used for solvent evaporation. Samples and solutions were filtered with 0.45-μm membrane filters, while solvents were degassed prior to use. The root and stem bark samples of A. marmelos were collected from five plants (7 to 10 years old) from the experimental field of Directorate of Medicinal and Aromatic Plant Research, Boriavi, Gujarat. The plant materials were identified by A.S. Reddy, Ph.D., Reader (Botany & Plant taxonomy), Department of Bioscience, Sardar Patel University, Vallabhbh Vidhyaganagar, Gujarat, India, and voucher sample (DMAPR 10-11/AM-1) was submitted to DMAPR, Anand, Gujarat. The root and stem bark materials were air-dried and powdered in an electric grinder and the powdered material was used for the extraction purpose.
Extraction of Plant Material

For quantitative determination of bioactive compounds, the powdered samples of root and stem barks (2.0 g) of five experimental *A. marmelos* plants were extracted with different solvents, i.e., petroleum ether, chloroform, ethyl acetate, methanol, ethanol, and distilled water (3 × 75 mL), for 30 min under reflux, in a thermostatically controlled water-bath maintained at 90 °C. The extracts were combined and filtered, and the solvents were evaporated under reduced pressure on a rotary evaporator. After the removal of solvents, the residual weight was recorded. The residue was quantitatively transferred to a 100-mL volumetric flask with methanol as the solvent. All the samples were filtered through 0.45-μm membrane filter before injection into the chromatographic system.

Isolation of Marmin and Skimmianine

The dry root bark of *A. marmelos*, 1.0 kg, was extracted by reflux in methanol (3X3L) at 80 ± 5 °C on water bath. The pooled extract was combined and evaporated to dryness by rotary evaporator (M/s Heidolph, Germany). The residue dissolved in methanol and mixed with silica gel (100–200 mesh) and subjected to flash chromatography (M/s Teledyne Isco, NE). The column was eluted by gradual increasing percentage of ethyl acetate in petroleum ether. The enriched fraction was reloaded on flash chromatograph and recrystallized in methanol. The recrystallized compounds were tested by TLC and a single spot for each compound was detected. These compounds were identified based on molecular weight and its fragmentation pattern and related reference [18, 21, 22].

Chromatographic Conditions

A modular HPLC (Shimadzu Corporation, Kyoto, Japan), LC system consisting of two LC-20AD pumps, DGU-20A3 degasser, SIL-20AC HT auto sampler, a CTO-10ASvp column oven, and CBM-20 communications bus module, was used for chromatographic separation of analytes on a Grace Alltima (50 × 4.6 mm, 3 μm) analytical column (Grace Davison Discovery Sciences, UK). The mobile phase consisted of methanol and 0.1% acetic acid in water (54:46, v/v ratio) and was delivered at a flow rate of 1.0 mL min⁻¹. The total eluant from the column was split in 80:20 ratio; flow directed to
the ion spray interface was equivalent to 200 μL min⁻¹. The column temperature was maintained at 40 °C for better resolution, and the sample injection volume was kept at 10 μL.

**Mass Spectrometric Conditions**

An Applied Biosystems/MDS SCIEX (Toronto, Canada) API-2000 triple-quadrupole mass spectrometer equipped with turbo ion spray source and operating in the positive ionization mode was used in the study. Purified air was used as a nebulizing gas, and nitrogen generated from nitrogen generator (Peak Scientific, Model NM 20Z, USA) was used as turbo, curtain, and collision gas, respectively. The MS/MS parameters were optimized by constant infusion of known concentration (1000 ng mL⁻¹) of each analyte. Quantitation was performed using multiple reactions monitoring (MRM) mode to monitor protonated precursor → product ion transitions. Collision energy (CE) and collision activated dissociation (CAD) gas were suitably optimized to yield consistent fragments in the experiments. The optimized source dependent mass parameters were as follows: ion spray voltage, 5500 V; curtain gas, 10 L min⁻¹; ion source temperature, 350 °C; nebulizer gas flow (GS1), 45 L min⁻¹; heater gas flow (GS2), 50 L min⁻¹; focusing potential, 400 V; and CAD gas flow, 6 L min⁻¹. Compound dependent parameters and MRM transitions for all the analytes are given in Table I. Analyst software version 1.4.2 was used to control all parameters of LC and MS for data acquisition and analysis.

*Table I. Optimized MS/MS parameter for the analysis of five bioactive compounds*

<table>
<thead>
<tr>
<th>Analytes</th>
<th>MRM transitions (m/z)</th>
<th>Dwell time (ms)</th>
<th>DP (V)</th>
<th>CE (V)</th>
<th>EP (V)</th>
<th>CXP (V)</th>
<th>CEP (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbelliferone</td>
<td>163.1/107.1</td>
<td>200</td>
<td>32.0</td>
<td>32.0</td>
<td>9.0</td>
<td>4.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Psoralene</td>
<td>187.2/131.1</td>
<td>200</td>
<td>44.0</td>
<td>35.0</td>
<td>9.0</td>
<td>4.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Marmin</td>
<td>333.5/163.2</td>
<td>200</td>
<td>40.0</td>
<td>15.0</td>
<td>9.0</td>
<td>8.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Imperatorin</td>
<td>271.1/203.1</td>
<td>200</td>
<td>24.0</td>
<td>16.0</td>
<td>10.0</td>
<td>8.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Skimmianine</td>
<td>260.1/227.0</td>
<td>200</td>
<td>50.0</td>
<td>30.0</td>
<td>10.0</td>
<td>8.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

DP: declustering potential; CE: collision energy; EP: entrance potential; CXP: collision cell exit potential; CEP: collision cell entrance potential.
Calibration Standards and Quality Control Samples

Separate stock solutions (2.0 mg mL$^{-1}$) of all the analytes were prepared by dissolving accurately known weighted amounts in methanol. A mixed intermediate solution was prepared from stock solutions by appropriate dilution with methanol. This was then serially diluted with methanol to establish six point calibration curves over the range of 50–1000 ng mL$^{-1}$ for umbelliferone and skimmianine, 20–400 ng mL$^{-1}$ for psoralene, 10–200 ng mL$^{-1}$ for imperatorin, and 50–2000 ng mL$^{-1}$ for marmin, respectively. The quality control (QC) samples of analytes were prepared at low (100, 40, 100, 20, and 100 ng mL$^{-1}$), medium (500, 200, 1000, 100, and 500 ng mL$^{-1}$), and high (1000, 400, 2000, 200, and 1000 ng mL$^{-1}$) levels for umbelliferone, psoralene, marmin, imperatorin, and skimmianine, respectively.

Method Validation

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is defined as the lowest concentration of an analyte that can be reliably detected but not necessarily quantified, while LOQ is taken as the lowest concentration that can be determined with acceptable accuracy and precision, under the optimized experimental conditions. The LOD and LOQ of the method for all five analytes were estimated by measuring the signal to noise ratio corresponding to 3 and 10, respectively, by loading series of diluted solutions of analytes.

Specificity, accuracy, linearity, and precision

The specificity of the method was assessed for any possible interference at the retention time of the analytes under the established MRM conditions by loading blank samples in six replicates. The accuracy of method was determined in terms of percentage recovery. Recoveries of all the analytes were determined by spiking the plant sample with QC samples of low, medium, and high concentrations. Three calibration lines containing six nonzero concentrations were used to determine the linearity of the method for all the analytes. The linear equation ($y = mx + c$), established between the concentration of the standard injected ($x$) and the peak area ($y$), was then used to calculate the predicted concentrations within the analytical runs. The preci-
sion of method expressed in terms of relative standard deviation (RSD %) was calculated by six replicate injections of three different concentrations (low, medium, and high) for all the five analytes.

**Results and Discussions**

**Optimization of Mass Spectrometry Conditions**

During method development, ionization of analytes was tested in positive as well as negative ionizations modes. However, better sensitivity with higher response was achieved in positive electro spray ionization (ESI) and, hence, was selected for further study. The Q1 spectra showed predominant protonated precursor ions [M+H]+ at \( m/z \) 163.1 for umbelliferone, \( m/z \) 187.2 for psoralene, \( m/z \) 333.5 for marmin, \( m/z \) 271.1 for imperatorin, and \( m/z \) 260.1 for skimmianine by infusion of 1000 ng mL\(^{-1}\) solution of analytes through a Hamilton syringe pump. In the Q3 spectra, the most intense and consistent product ions were found at \( m/z \) 107.1, 131.1, 163.2, 203.1, and 227.0 for umbelliferone, psoralene, marmin, imperatorin, and skimmianine, respectively (Supplementary Fig. 1a–e).

**Optimization of Liquid Chromatography**

For chromatographic separation of analytes, Grace Altima RP-18 analytical column of different dimensions (50/100 mm × 4.6 mm, 3 µm) was tested by changing the mobile phase composition (methanol and water) and the concentration of additives like formic acid and acetic acid (0.01–0.1%). Although both columns provided adequate separation of the analytes, the total analysis time was much higher for 100 mm column length (14.0 min) compared to 50 mm (8.0 min). Moreover, an increase in organic modifier content (≥60%) resulted in shorter analysis time but significantly compromised resolution. Further, it was observed that the peak shape and analyte response were dependent on the type and concentration of additives in the mobile phase. Comparatively, higher response was found with acetic acid and was selected for further optimization of peak shapes. The best chromatographic conditions in terms of peak shape, adequate response, and analysis time were obtained on Altima RP 18 (50 × 4.6 mm, 3 µm) using methanol and 0.1% acetic acid in water (54:46, v/v) as the mobile phase at a flow rate of 1.0 mL min\(^{-1}\). All the analytes were baseline resolved with retention
times of 0.91, 1.76, 3.31, 6.67, and 2.71 for umbelliferone, psoralene, marmin, imperatorin, and skimmianine, respectively, within 8.0 min (*Supplementary Fig. 2a–e*).

**Selection of Solvent for Optimum Yield of Analytes**

As all the five analytes have different physicochemical properties, the extraction trials were carried out in solvents with different polarities. The extraction efficiency (based on dry residue after solvent evaporation) from root bark in different solvents by the reflux method was 11.16, 12.75, 13.37, 21.71, 18.58, and 30.56% in petroleum ether, chloroform, ethyl acetate, methanol, ethanol, and water, respectively. Similarly, in stem bark, the extraction yield was 2.73, 2.11, 3.24, 14.89, 10.62, and 24.77% in petroleum ether, chloroform, ethyl acetate, methanol, ethanol, and water, respectively. The maximum yield of the dry residue was obtained in water for stem as well as root bark samples. However, the combined yield of the analytes was recorded highest in methanol and lowest in petroleum ether as shown in Fig. 2. Hence, methanol was selected as the extraction solvent for quantitative extraction of all five analytes.

![Fig. 2. Extraction efficiency of four coumarin derivatives and an alkaloid in different solvents](image-url)
Determination of Analytes in Root and Stem Bark of 
*A. marmelos*

The identification and quantification of five bioactive compounds in the root and stem bark extracts of *A. marmelos* were based on MRM analysis and chromatographic retention. *Figure 3* shows MRM chromatograms for all the analytes in a standard mixture. The content of umbelliferone, psoralene, marmin, imperatorin, and skimmianine was estimated in root and stem bark extract of five individual plants of *A. marmelos* by the proposed method. The MRM chromatograms showing the presence of these bioactive components in root and stem bark are presented in *Figs. 4* and *5*, respectively. The distribution of coumarin derivatives and an alkaloid was found to vary significantly in the five plant samples studied. The concentration of bioactive compounds was much higher in root bark as compared to stem bark. A similar study conducted on *Xylopia aethiopica* also showed that the

![Fig. 3. Representative MRM chromatograms of standard mixture of umbelliferone (100 ng mL⁻¹), psoralene (200 ng mL⁻¹), marmin (1000 ng mL⁻¹), imperatorin (100 ng mL⁻¹) and skimmianine (500 ng mL⁻¹)](image-url)
Fig. 4. MRM chromatograms of extract of root bark (1000 ng mL\(^{-1}\)), based on extracted residue in methanol

Fig. 5. MRM chromatograms of extract of stem bark (1000 ng mL\(^{-1}\)), based on extracted residue in methanol
Fig. 6. Concentration of four coumarin derivatives and an alkaloid found in the root bark of *Aegle marmelos* from five different plants.

Fig. 7. Concentration of four coumarin derivatives and an alkaloid found in the stem bark of *Aegle marmelos* from five different plants.
quantity of alkaloids in the roots was significantly higher compared to stem bark [23]. In the root bark, the marmin content ranged from 3.39 to 5.46 mg g⁻¹, umbelliferone 0.21–0.49 mg g⁻¹, psoralene 0.01 to 0.002 mg g⁻¹, imperatorin 0.07–0.25 mg g⁻¹, and skimmianine 0.50–1.59 mg g⁻¹. The marmin content recorded was higher compared to other four bioactive compounds in the root bark and stem bark. The yield of all five components isolated from root and stem bark is presented in Figs. 6 and 7, respectively.

Method Validation Results

The quality of a developed analytical method is characterized in terms of its suitability for the intended purpose. LOD and LOQ are two important performance characteristics for method validation. Based on the signal to noise ratio of 3 and 10, the LOD/LOQ values found were 10.0/50.0 ng mL⁻¹ for umbelliferone, 5.0/20.0 ng mL⁻¹ for psoralene, 5.0/50.0 ng mL⁻¹ for marmin, 2.5/10 ng mL⁻¹ for imperatorin, and 2.5/50 ng mL⁻¹ for skimmianine, respectively. The calibration curve established over the range of 50–1000 ng mL⁻¹ for umbelliferone and skimmianine, 20–400 ng mL⁻¹ for psoralene, 10–200 ng mL⁻¹ for imperatorin, and 50–2000 ng mL⁻¹ for marmin showed good linearity with coefficient correlations ($r^2$) ≥ 0.9947 for all the analytes (Table II). The accuracy and precision (%RSD) for the calibration standards (CSs) varied from 96.5 to 103.7% and 0.27 to 5.63%, respectively, for all the analytes.

Table II. Response characteristics of five bioactive compounds using LC–MS/MS

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Retention time (min)</th>
<th>Calibration curve ($y$ is the peak area, and $x$ represents concentration)</th>
<th>$r^2$</th>
<th>Linear range (ng mL⁻¹)</th>
<th>Detection limit (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>CV</td>
<td>$y$ = 150$x$ + 0.00232</td>
<td>0.9947</td>
<td>50–1000</td>
<td>10.0</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>0.91</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psoralene</td>
<td>1.76</td>
<td>0.58</td>
<td>$y$ = 526$x$ + 0.597</td>
<td>0.9969</td>
<td>20–400</td>
</tr>
<tr>
<td>Marmin</td>
<td>3.31</td>
<td>0.61</td>
<td>$y$ = 60.8$x$ + 0.608</td>
<td>0.9980</td>
<td>50–2000</td>
</tr>
<tr>
<td>Imperatorin</td>
<td>6.67</td>
<td>0.45</td>
<td>$y$ = 350$x$ + 99.5</td>
<td>0.9968</td>
<td>10–200</td>
</tr>
<tr>
<td>Skimmianine</td>
<td>2.71</td>
<td>0.38</td>
<td>$y$ = 57.3$x$ + 0.00108</td>
<td>0.9981</td>
<td>50–1000</td>
</tr>
</tbody>
</table>
The intra-day and inter-day precision and accuracy (in terms of recovery) across three QC (low, medium, and high) levels are shown in Table III. The intra-day precision (%RSD) and accuracy (recovery) for the analytes ranged from 0.7 to 9.78% and 94.92 to 109.75%, respectively. For inter-day experiments, the precision varied from 2.19 to 9.57%, and the recovery was within 93.27–106.67%.

Table III. Accuracy (recovery) and precision (% CV) of the method at three quality control levels

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Amount added (ng mL⁻¹)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>100</td>
<td>96.83</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>95.43</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>99.83</td>
<td>3.42</td>
</tr>
<tr>
<td>Psoralene</td>
<td>40</td>
<td>109.75</td>
<td>4.66</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>94.92</td>
<td>5.07</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>100.88</td>
<td>4.42</td>
</tr>
<tr>
<td>Marmin</td>
<td>100</td>
<td>100.08</td>
<td>7.62</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>100.18</td>
<td>9.15</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>97.33</td>
<td>7.58</td>
</tr>
<tr>
<td>Imperatorin</td>
<td>20</td>
<td>100.80</td>
<td>5.19</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98.08</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>99.50</td>
<td>1.11</td>
</tr>
<tr>
<td>Skimmianine</td>
<td>100</td>
<td>96.07</td>
<td>8.62</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>103.23</td>
<td>7.26</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>99.05</td>
<td>9.78</td>
</tr>
</tbody>
</table>

Conclusions

The present study describes a validated LC–MS/MS method for the identification and simultaneous quantification of five bioactive compounds, umbelliferone, psoralene, marmin imperatorin, and skimmianine from the
roots and stem bark of *A. marmelos* as per the ICH-Q2 (R1) guidelines. The method was found to be simple, rapid, and sensitive to quantify these analytes up to nanogram level. The results showed that the distribution of these analytes varied considerably in different plant samples studied. Further, the content of bioactive compounds was more in roots than stem bark. The standardized method can be readily applied for rapid screening and quantification of these compounds in different plant extracts.

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**References**