# Quantitative Determination of Bioactive Constituents in *Noni* Juice by High-performance Liquid Chromatography with Electrospray Ionization Triple Quadrupole Mass Spectrometry

**Yongqiu Yan[1,2], Yu Lu[1], Shiping Jiang[1], Yu Jiang[1], Yingpeng Tong[1], Limin Zuo[1], Jun Yang[1], Feng Gong[3], Ling Zhang[3], Ping Wang[1]**

[1]Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, [2]Ningbo Yufangtang  Biotechnology Co., Ltd., Ningbo, China, [3]Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Fl, USA
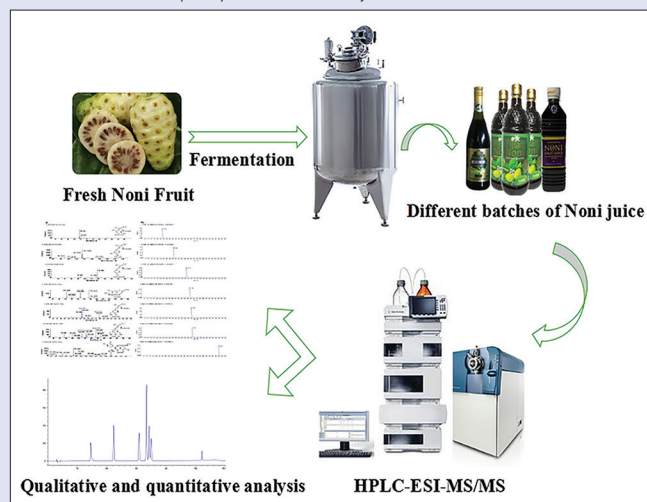
## ABSTRACT

**Background:** *Noni* juice has been extensively used as folk medicine for the treatment of arthritis, infections, analgesic, colds, cancers, and diabetes by Polynesians for many years. Due to the lack of standard scientific evaluation methods, various kinds of commercial *Noni* juice with different quality and price were available on the market. **Objective:** To establish a sensitive, reliable, and accurate high-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (HPLC-ESI-MS/MS) method for separation, identification, and simultaneous quantitative analysis of bioactive constituents in *Noni* juice. **Materials and Methods:** The analytes and eight batches of commercially available samples from different origins were separated and analyzed by the HPLC-ESI-MS/MS method on an Agilent ZORBAX SB-C$_{18}$ (150 mm × 4.6 mm i.d., 5 μm) column using a gradient elution of acetonitrile-methanol-0.05% glacial acetic acid in water (v/v) at a constant flow rate of 0.5 mL/min. **Results:** Seven components were identification and all of the assay parameters were within the required limits. Components were within the correlation coefficient values ($R^2 \geq 0.9993$) at the concentration ranges tested. The precision of the assay method was <0.91% and the repeatability between 1.36% and 3.31%. The accuracy varied from 96.40% to 103.02% and the relative standard deviations of stability were <3.91%. Samples from the same origin showed similar content while different origins showed significant different result. **Conclusions:** The developed methods would provide a reliable basis and be useful in the establishment of a rational quality control standard of *Noni* juice.

**Key words:** Bioactive constituents, high-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry, *Noni* juice, quantitative determination

## SUMMARY

- Separation, identification, and simultaneous quantitative analysis method of seven bioactive constituents in *Noni* juice is originally developed by high-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry
- The presented method was successfully applied to the quality control of eight batches of commercially available samples of *Noni* juice
- This method is simple, sensitive, reliable, accurate, and efficient method

with strong specificity, good precision, and high recovery rate and provides a reliable basis for quality control of *Noni* juice.

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# INTRODUCTION

*Morinda citrifolia* L., commonly known as *Noni*, is a Rubiaceous plant widely distributed in many tropical and subtropical regions. *Noni* has been extensively used as food and folk medicine for the prevention or improvement of diversified health problems by Polynesians for more than 2000 years. Hundreds of phytochemical compositions such as flavonoids, anthraquinones, iridoids, and glycosides have been isolated from *Noni*.[1-3] It receives continuous attention for its variety of medicinal value and health-care functions, such as antioxidant, antimicrobial, analgesic, anti-inflammatory, anticancer, antidiabetic, cardiovascular protection, immunoregulation.[4-9]

*Noni* juice, which is traditionally made by fermentation of mature *Noni* fruits in sealed containers for 2–3 months or longer at ambient

temperature, is one of its primary applications.[10] Lots of biological activity were also revealed and reported: antioxidation, anti-inflammation, liver protection, and hypolipidemic effect.[11-15] Despite various of therapeutic effects, reports on the identification of biologically active components of *Noni* juice fermented from the fruit have been less well studied. Previous literatures indicated that phenolic acids and polysaccharides were the major bioactive constituents in *Noni* juice.[13] The volatile compounds were addressed by steam distillation-extraction and solid-phase microextraction coupled with gas chromatography (GC)/atomic emission detection and GC/mass spectrometry, and they made a major contribution to the characteristic flavor of *Noni* juice.[15] The effects of light on total phenolics and antioxidant capacity of *Noni* juice during storage under various conditions were also investigated. Yang's research indicated that illumination affected total phenolics and further influenced antioxidant capacity of *Noni* juice significantly during storage though they did not provide the specific components of phenolics.[16] In addition, other studies identified some of the major components in *Noni* juice as octanoic acid, potassium, Vitamin C, alkaloids, β-sitosterol, carotene, Vitamin A, flavone glycosides, linoleic acid, amino acids, and rutin.[13,17]

Currently, however, markets are loaded with various kinds of commercial *Noni* juice that vary widely in quality and price. Due to the lack of scientific evaluation methods and standards, the quality and curative effect could not be guaranteed for the existence of shoddy or fake juice by unscrupulous producers, which not only undermined the vital interests of consumers and made those who really have the intention to produce good merchandises suffer grievance but also hindered the development of the entire industry seriously. Nevertheless, there were very few reports on the quantitative determination of main ingredients in *Noni* juice to the best of our knowledge.

Hence, the aim of the present work was to establish an analytical method for separation, identification, and simultaneous quantitative determination of potentially bioactive phenolic constituents in *Noni* juice using a validated high-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (HPLC-ESI-MS/MS) method. The method was also applied to analyze the phenolic constituents in eight batches of commercially available *Noni* juice samples from different origins so as to provide the basis for the production process and the quality control of *Noni* juice products. As far as we know, these potentially bioactive phenolic compounds in *Noni* juice were identified, confirmed, and analyzed by HPLC-ESI-MS/MS for the first time. In addition, we are looking forward to that what we do can safeguard the legitimate rights and interests of consumers and promote the balanced, harmonious, and healthy development of the entire industry at the same time.

## MATERIALS AND METHODS

### Materials

Eight different batches of samples have been analyzed, and all of the products were purchased through the Internet during June and July in 2016: Wanzhou *Noni* juice (Hainan Wanzhitang Biotechnology Co., Ltd.), Taiwan *Noni* juice (Guangzhou Takeda Food Co., Ltd), Xisha *Noni* juice (Hainan Grain Source Biological Technology Co., Ltd), and Fijian *Noni* juice (Frezco Beverages Co., Ltd). The voucher specimens, identified by Prof. Ping Wang from Zhejiang University of Technology, were stored for deposition at Key Laboratory for Green Pharmaceutical Technologies and Related Equipment of Ministry of Education, Zhejiang University of Technology, Hangzhou, China.

### Chemicals and reagents

Methanol (MeOH) and acetonitrile (MeCN) were HPLC grade (Tedia, USA), and the deionized water was prepared from a Millipore

water purification system (Milford, USA). Formic acid (FA), glacial acetic acid (HAc), phosphoric acid (PA), and ethyl acetate (EA) of analytical grade were obtained from Sigma-Aldrich (St. Louis, USA). Among the seven chemical standards, gentisic acid (1), caffeic acid (2), p-coumaric acid (3), ferulic acid (5), sinapic acid (6), and quercetin (7) were purchased from Beijing Lark Technology Co., LTD (Beijing, China) and whose purities were >98%. Scopoletin (4) was isolated from authentic *Noni* fruit in our laboratory and its identification was determined by HPLC, mass spectrometry, and nuclear magnetic resonance (purity >99%), by comparison with the literature.[18]

### Apparatus and conditions

Chromatographic analysis was performed on an Agilent 1260 HPLC system consisting of quaternary pumps, a degasser, and an autosampler coupled with an Empower chromatography workstation (Agilent Technologies, Palo Alto, CA, USA). The pumps were connected to three mobile phases: (a) MeCN (A), (b) MeOH (B), and (c) 0.1% HAc in water (C). The mobile phases were programmed consecutively in linear gradients as follows: 0 min, 4% A, 4% B, and 92% C; 12 min, 8% A, 8% B, and 84% C; 37 min, 15% A, 15% B, and 70% C; 50 min, 25% A, 25% B, and 50% C; and 55 min, 45% A, 45% B, and 10% C. Separations were carried out on an Agilent ZORBAX SB-C$_{18}$ (150 mm × 4.6 mm i.d., 5 μm) column. The column was maintained at 30 and 10 μL of samples that were injected into the chromatograph and the flow rate was 0.5 mL/min. Taking into consideration, their maximum ultraviolet absorbance and separation efficiency, 330 nm, were chosen for the detection.

MS/MS experiments were conducted on an AB SCIEX TripleTOF™ 5600$^+$ System equipped with an ESI in the negative ionization mode (AB SCIEX, Framingham, USA). The optimal source conditions were as follow: ion spray voltage was −4.5 kV and the source temperature was 550; curtain gas (N$_2$), gas 1 (air), and gas 2 (air) were each set at 30, 50, and 50 psi, and the collision gas was set at medium. The exact mass calibration was performed automatically before each analysis employing the automated calibration delivery system. The scan range of mass spectra of precursor ion and product ion was set as 100–2000 Da and 50–2000 Da, respectively.

### Preparation of standard solutions

The reference standard solutions were diluted to a series of appropriate concentrations with methanol-H$_2$O (1:1, v/v): gentisic acid - 2.176, 5.440, 10.880, 21.760, 54.400 μg/mL; caffeic acid - 0.432, 1.440, 4.320, 8.640, 21.600 μg/mL; *p*-coumaric acid - 0.515, 2.575, 5.150, 10.300, 25.750 μg/mL; scopoletin - 1.650, 6.600, 33.000, 66.000, 330.000 μg/mL; ferulic acid - 0.452, 2.260, 4.520, 9.040, 22.600 μg/mL; sinapic acid - 0.544, 1.360, 2.720, 5.440, 13.600 μg/mL; and quercetin - 3.600, 4.800, 7.200, 14.400, 36.000 μg/mL. All samples were filtered through a nylon microfilter (0.45 μm pore size) and stored at 4 before HPLC experiments. The injection volume was 10 μL.

### Sample preparation

Accurately measured 10 mL of *Noni* juice sample was extracted with triple volume of EA for three times, and then, the organic phase was combined and concentrated on a rotary evaporator under 35. The residue of the extracts was dissolved in 2 mL of methanol-H$_2$O (1:1, v/v) and filtered through a nylon microfilter of 0.45 μm pore size and stored at 4 until analysis.

### Validation of the developed method

The proposed HPLC-ESI-MS/MS method validation of quantitative analysis was evaluated by determining linearity, limit of detection (LOD),

limit of quantitation (LOQ), precision, repeatability, stability, and recovery test.[19] Statistical analysis was carried on Microsoft Excel Software 2007.

## RESULTS AND DISCUSSION

### Optimization of the chromatographic conditions

Due to the complexity of the chemical constituent in *Noni* juice, a number of mobile phase compositions were tested to identify the optimal separation conditions. Based on the analysis of the structures and characteristics of the seven active ingredients, we considered that all of them contained phenolic hydroxyl groups while components with phenolic hydroxyl and carboxyl groups were easily ionized in aqueous solution, causing the polarity enhancement. As a result, a dual retention was formed on the surface of the stationary phase, which could cause the chromatographic peaks tailing seriously and large error in quantitative analysis. To address this concern, the addition of a small quantity of acidity regulator was taken into account, which could inhibit the ionization of polyphenols and weaken its polarity, thus enhance the retention on the stationary phase, and improve the effect of separation and shape of peaks. In our study, HAc had the same effect as trifluoroacetic acid and both of them were better than FA and PA. Considering the safety of apparatuses and convenience, we chose HAc as mobile phase acidity regulator and different concentrations ranging from 0.01% to 1% were investigated. What's more, mobile phases at different flow rates (0.1, 0.3, 0.5, 0.7, and 0.9 mL/min) as well as column temperatures (25, 30, 35, and 40) were examined for better chromatographic behavior. Accordingly, a MeCN-MeOH-0.05% of HAc solution at a flow rate of 0.5 mL/min with the column temperature of 30 was selected as the best condition to separate the seven active ingredients.

### Optimization of the mass spectrometry conditions

For the sake of developing an accurate and sensitive quantitative method, the MS/MS fragmentation patterns for seven constituents were investigated in both positive and negative modes. It was intriguing that all analytes showed maximum signal sensitivity response in the negative ion mode due to the phenolic hydroxyl group and carboxyl group in their chemical structures.[20] The parameters of declustering potential (DP) and collision energy (CE) were optimized so as to achieve the most abundant, specific, and stable multiple reaction monitoring (MRM) conditions for each compound investigated. The retention time (RT), molecular formula, molecular weight, and MS information for analytes including [M−H]−, DP, and CE were shown in Table 1.

The MS/MS spectra, fragmentation scheme, and the MRM extracted ion chromatogram of the seven bioactive ingredients were shown in Figure 1. As shown for peak 1, the [M−H]− ion in the ESI-MS spectrum was observed at m/z 153.0201, which was identified as gentisic acid, while fragment ion at m/z 109.0297 was attributed to the loss of a $CO_2$ molecule, providing an anion of [M−H−$CO_2$]−. In peak 2, the [M−H]−

ion was found at m/z 179.0356, presented fragments at m/z 135.0459, which also due to the loss of a $CO_2$ molecule. Thus, this ingredient was identified as caffeic acid. Peak 3, 5, and 6 were identified as *p*-coumaric acid, m/z 163.0409 [M−H]−, ferulic acid, m/z 193.0512 [M−H]−, and sinapic acid, m/z 223.0615 [M−H]−, respectively. Corresponding to elimination of $CO_2$ from the carboxylic acid function,[21] the three compounds yielded an anion of [M−H−$CO_2$]− at m/z 119.0507, m/z 149.0611, and m/z 179.0715 (too weak to be obvious but could be detected), respectively. Ferulic acid generated the principal fragment ions at m/z 134.0377 and m/z 178.0272, corresponding to [M−H−$CO_2$−$CH_3$]− and [M−H−$CH_3$]−. Sinapic acid, containing two methoxyl groups, yielded more stable fragment ions due to the loss of two or at least one methyl radical from a fragment ion at m/z 179.0715 or m/z 223.0615. Peak 4 showed a protonated molecule at m/z 191.0338 [M−H]−, a major fragment ion at m/z 176.0110 attributed to the loss of a methyl radical for [M−H−$CH_3$]−. Besides, fragment ions were also observed at m/z 148.0167 and 120.0224 due to elimination of one CO and two CO from ion at m/z 176.0110, such fragments suggestive of scopoletin. Peak **7** was identified as quercetin, m/z 301.0345 [M−H]−, yielded a major fragment ion at m/z 151.0036 due to the retro-Diels–Alder reaction.[20]

### Method validation

Calibration curves of the seven chemical standards were obtained based on the linear relationship between peak areas (y) and concentration of standard solution (x) ranging at five concentrations in duplicate. The regression equation and correlation coefficients ($R^2$) were listed in Table 2, and the high correlation coefficient values ($R^2 \geq 0.9993$) showed a suitable linearity at a relatively wide range of concentration for all standards. The LODs and LOQs for the seven analytes under the present chromatographic conditions were determined at a signal-to-noise ratio of about 3 and 10, respectively.

The precision of the assay method was carried out by intraday and interday variation determination. The intraday precisions were investigated by determining levels of each reference standard at one concentration level in six replicates during a single day while the interday precisions were performed by duplicating measurements on three consecutive days. All of the variations were expressed by relative standard deviations (RSDs) and results were shown in Table 3. Either intraday or interday precision was <0.91%.

To evaluate the repeatability of the assay, six samples of Wanzhou *Noni* juice (NJ-1) were prepared as described in 2.5 and the contents of the seven ingredients were calculated according to the calibration curves. The RSDs were used as means of evaluation of repeatability and varied from 1.36% to 3.31%. The stability test was examined by testing the same sample (NJ-1) used in the repeatability evaluation over a period of 0, 2, 4, 6, 8, and 12 h. Stability measurements were also represented by the RSDs and were <3.91% and were shown in Table 3.

**Table 1:** Retention time and main mass parameters of seven constituents in *Noni* juice by high-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry/mass spectrometry analysis

| Peak | RT (min) | Analyte | Molecular formula | Molecular weight (Da) | [M−H]− (m/z) | DP (V) | CE (eV) |
|---|---|---|---|---|---|---|---|
| 1 | 13.32 | Gentisic acid | $C_7H_6O_4$ | 154.0272 | 153.0201 | −76 | −35 |
| 2 | 20.41 | Caffeic acid | $C_9H_8O_4$ | 180.0423 | 179.0356 | −50 | −21 |
| 3 | 28.77 | *p*-coumaric acid | $C_9H_8O_3$ | 164.0468 | 163.0409 | −93 | −28 |
| 4 | 31.24 | Scopoletin | $C_{10}H_8O_4$ | 192.0423 | 191.0338 | −29 | −18 |
| 5 | 32.05 | Ferulic acid | $C_{10}H_{10}O_4$ | 194.0579 | 193.0512 | −58 | −23 |
| 6 | 32.62 | Sinapic acid | $C_{11}H_{12}O_5$ | 224.0679 | 223.0615 | −55 | −27 |
| 7 | 49.93 | Quercetin | $C_{15}H_{10}O_7$ | 302.0426 | 301.0346 | −95 | −31 |

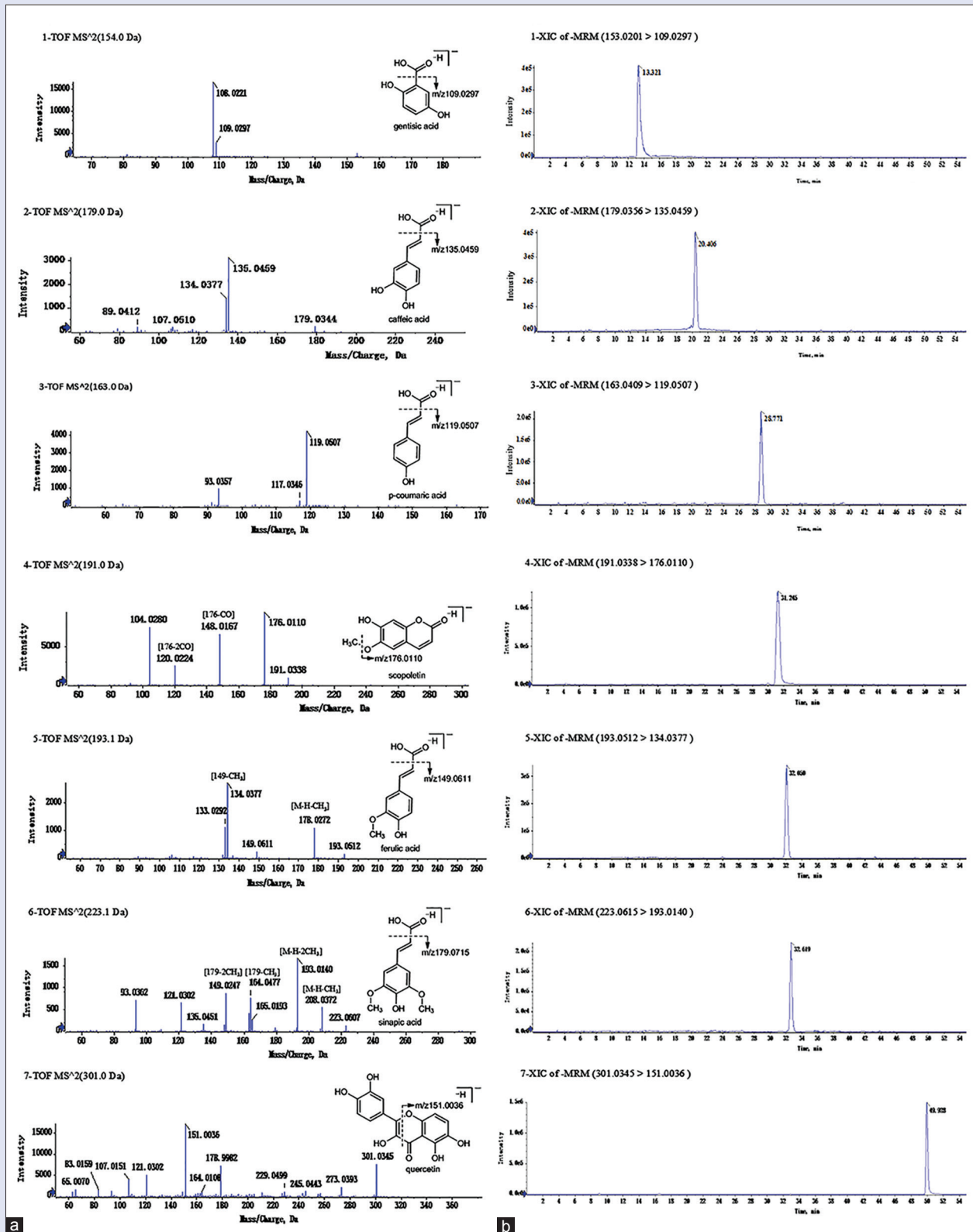RT: Retention time; DP: Declustering potential; CE: Collision energy

**Figure 1:** (a) The mass spectrometry spectra and the fragmentation scheme and (b) the multiple reaction monitoring extracted ion chromatogram of the seven bioactive ingredients

The recovery test was applied to examine the accuracy of the method evaluation. Three different concentration levels, namely, 80%, 100%, and 120% of known amounts of the seven standards were added into the well-analyzed *Noni* juice sample NJ-1 by thoroughly incorporating in triplicate, and the average recovery rate of the seven analytes was from 96.40% to 103.02%, with RSD values varying between 0.23% and 2.74%, demonstrating the reliability and accuracy of the measurement of these constituents and was presented in Table 4.

## Sample analysis

The validated method was applied to analyze the seven components in the eight batches of commercially available samples and was randomly referred to as NJ-1 ~ NJ-8. The origins of the samples and the results were summarized in Table 5. By comparing all of the samples, many significant differences were revealed on the amount of each ingredient.

Phenolic constituents contributed to the major pharmacological effects of antioxidant, antibacterial, and inhibition of cancer cell development.[22] In light of the results, the total content of the seven analytes in samples from Wanzhou (NJ-1, NJ-2) and Taiwan (NJ-3, NJ-4) was generally much higher than that from Xisha (NJ-5, NJ-6) and Fijian (NJ-7, NJ-8). While samples from the same origin showed similar content

of each component. Data mining was further performed and it was noteworthy that the content of scopoletin in samples NJ-5 and NJ-6 from Xisha was 2.31 and 2.01 mg/mL, dramatically lower than that in any other samples. Scopoletin is a 6-methoxy-7-hydroxycoumarin and is one of the most important compositions in *Noni* juice, which could influence its pharmacological activities to some extent. It possesses potent hepatoprotective,[23] anti-inflammatory,[24] antioxidant,[25] and antiangiogenic activity.[26] The quality and phytochemical components of commercial *Noni* products may vary obviously, mainly due to different geographical conditions and postgrowth factors, such as the difference between soil, sunlight, precipitation, air and the harvesting time, the way of storage, transportation, and manufacturing processes.[27]

**Table 2:** Linearity of the standard curves, limit of detection, and limit of quantitation data of the seven analytes

| Analyte | Regression equation | Linear range (µg/mL) | $R^2$ | LOD (ng/mL) | LOQ (ng/mL) |
|---|---|---|---|---|---|
| Gentisic acid | y=23.16x−28.52 | 2.176-54.400 | 0.9993 | 2.45 | 8.17 |
| Caffeic acid | y=92.854x−9.2316 | 0.432-21.600 | 1.0000 | 0.42 | 1.42 |
| *p*-coumaric acid | y=61.839x−5.454 | 0.515-25.750 | 1.0000 | 0.75 | 2.50 |
| Scopoletin | y=62.356x−13.513 | 1.650-330.000 | 1.0000 | 0.73 | 2.44 |
| Ferulic acid | y=88.582x−10.837 | 0.452-22.600 | 0.9999 | 0.52 | 1.72 |
| Sinapic acid | y=89.796x−7.4007 | 0.544-13.600 | 0.9999 | 0.49 | 1.65 |
| Quercetin | y=20.689x−66.393 | 3.600-36.000 | 1.0000 | 2.49 | 8.30 |

LOD: Limit of detection; LOQ: Limit of quantitation

**Table 3:** Results of precision parameters, repeatability, and stability

| Analyte | Intraday precision | | Interday precision | | Repeatability (*n*=6) | | Stability RSD (%) |
|---|---|---|---|---|---|---|---|
| | Mean±SD (µg/mL) | RSD (%) | Mean±SD (µg/mL) | RSD (%) | Mean±SD (µg/mL) | RSD (%) | |
| Gentisic acid | 20.346±0.062 | 0.31 | 20.456±0.158 | 0.77 | 21.526±0.439 | 2.04 | 2.89 |
| Caffeic acid | 7.528±0.033 | 0.44 | 7.529±0.029 | 0.38 | 8.300±0.275 | 3.31 | 2.55 |
| *p*-coumaric acid | 10.481±0.028 | 0.26 | 10.475±0.030 | 0.29 | 9.334±0.260 | 2.79 | 2.15 |
| Scopoletin | 28.031±0.033 | 0.12 | 28.029±0.035 | 0.13 | 142.531±3.334 | 2.34 | 2.18 |
| Ferulic acid | 9.079±0.022 | 0.24 | 9.075±0.030 | 0.32 | 6.226±0.185 | 2.97 | 2.49 |
| Sinapic acid | 5.602±0.024 | 0.42 | 5.597±0.046 | 0.83 | 2.018±0.060 | 2.99 | 3.63 |
| Quercetin | 9.616±0.026 | 0.27 | 9.625±0.088 | 0.91 | 8.188±0.112 | 1.36 | 3.91 |

SD: Standard deviations; RSD: Relative standard deviations

**Table 4:** Results of recovery test

| Analyte | Recovery (80%) | | Recovery (100%) | | Recovery (120%) | |
|---|---|---|---|---|---|---|
| | Mean±SD (%) | RSD (%) | Mean±SD (%) | RSD (%) | Mean±SD (%) | RSD (%) |
| Gentisic acid | 101.34±2.06 | 2.03 | 103.02±1.86 | 1.81 | 100.81±2.29 | 2.27 |
| Caffeic acid | 98.15±1.90 | 1.93 | 98.55±2.16 | 2.19 | 102.59±0.77 | 0.75 |
| *p*-coumaric acid | 96.40±2.67 | 2.72 | 97.74±1.84 | 1.88 | 101.26±1.46 | 1.44 |
| Scopoletin | 101.06±0.23 | 0.23 | 101.73±0.29 | 0.28 | 99.40±0.94 | 0.95 |
| Ferulic acid | 97.62±0.61 | 0.62 | 97.44±2.17 | 2.22 | 98.06±1.23 | 1.26 |
| Sinapic acid | 96.88±2.63 | 2.71 | 100.23±0.54 | 0.54 | 98.83±0.65 | 0.66 |
| Quercetin | 99.06±2.52 | 2.55 | 97.94±2.69 | 2.74 | 97.37±2.39 | 2.46 |

SD: Standard deviations; RSD: Relative standard deviations

**Table 5:** Quantitative determination of seven constituents in *Noni* juice

| Sample | Origin | Content (µg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gentisic acid | Caffeic acid | *p*-coumaric acid | Scopoletin | Ferulic acid | Sinapic acid | Quercetin |
| NJ-1 | Wanzhou | 26.17 | 8.67 | 8.88 | 146.25 | 6.10 | 2.14 | 12.13 |
| NJ-2 | Wanzhou | 25.83 | 9.14 | 9.26 | 143.78 | 6.37 | 2.30 | 9.43 |
| NJ-3 | Taiwan | 45.83 | 1.33 | 9.09 | 171.21 | 0.94 | 0.26 | 5.52 |
| NJ-4 | Taiwan | 41.55 | 1.46 | 9.26 | 155.19 | 0.97 | 0.33 | 5.08 |
| NJ-5 | Xisha | 9.19 | 2.63 | 5.59 | 2.31 | 0.77 | 0.23 | 7.80 |
| NJ-6 | Xisha | 7.94 | 2.43 | 5.12 | 2.01 | 0.89 | 0.69 | 6.54 |
| NJ-7 | Fijian | 19.29 | 5.01 | 5.16 | 99.04 | 4.82 | 2.78 | 11.81 |
| NJ-8 | Fijian | 21.50 | 6.10 | 6.80 | 131.13 | 3.93 | 3.02 | 9.92 |

NJ: *Noni* juice

Undoubtedly, the actual content of active ingredients would inevitably affect the efficacy, highlighting that the quality evaluation criteria were deeply warranted.

## CONCLUSIONS

In this study, a sensitive, reliable, accurate, and efficient HPLC-ESI-MS/MS method operating in negative scanning modes in a single run for the simultaneous quantification of seven phenolic constituents in *Noni* juice was designed and fully validated. The established methodology revealed acceptable analytical parameters of linearity, LOD, LOQ, precision, repeatability, stability, and accuracy. Our assay was successfully applied to analyze eight batches of commercially available samples of *Noni* juice. The results indicated that the chemical compositions of *Noni* juice from different origins were significantly different. Considering the excellent validation results obtained, the proposed method could be employed for quality evaluation criteria and control assay of *Noni* juice. The results obtained from the study would be useful in the establishment of a rational quality control standard.

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## Conflicts of interest

There are no conflicts of interest.

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