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A rapid, accurate and sensitive method for determination of monosaccharides in different varieties of *Osmanthus fragrans Lour* by pre-column derivatization with HPLC-MS/MS

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Abstract

A specific HPLC-MS/MS (High-Performance Liquid Chromatography with tandem Mass Spectrometry) method was developed and validated for simultaneous determination of several monosaccharides in three kinds *Osmanthus fragrans Lour*. After extraction, separation, protein removal, pigment removal and hydrolysis, monosaccharides was finally obtained from *Osmanthus fragrans Lour*. Positive ion mode detection and Multiple Reaction Monitoring (MRM) mode were used for quantitative analysis by PMP pre-column derivatization and Electrospray Ionization (ESI). Analysis and content determination of 6 monosaccharide components in 3 kinds of *Osmanthus fragrans Lour*. The HPLC separation was achieved on a Shim-pack VP-ODS6022748 (150 L × 2.0) with gradient elution at a flow rate of 0.2 ml/min in a run time of 40 min, and the mobile phase was acetonitrile-5 mmol/L ammonium acetate. PMP derivatization in HPLC-MS/MS can accurately measure *Osmanthus fragrans Lour*. mannose (Man), ribose (Rib), rhamnose (Rha), galacturonic acid (Gal UA), glucose (Glu), galactose (Gal), xylose (Xyl), fucose (Fuc). The results showed that HPLC-MS/MS pre-column derivatization method was simple and rapid, with small measurement error, but high sensitivity and good repeatability. The analysis of monosaccharide components in polysaccharide components has important practical significance.

Keywords

Osmanthus fragrans Lour.; PMP pre-column derivatization; HPLC-MS/MS

1. Introduction

Osmanthus fragrans Lour belongs to the Oleaceae family and is an ornamental plant used as traditional folk medicine in the southern and central parts of China for the treatment of various diseases [1]. It had been used as a medicinal plant in China for thousands of years

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Author contributions

Conceptualization, B.F. and C.Q.; methodology, B.F., T.L., X.S., C.W.; writing—original draft preparation, B.F.; writing—review and editing, C.Q.; project administration, B.F.; funding acquisition, B.F.

Conflicts of interest

The authors declare no conflict of interest.

and had a wide range of biological effects, including inflammation reduction [2], antioxidation [3], aging prevention [3] and melanogenesis inhibition [4]. In addition, mostly bio-active components in the *Osmanthus fragrans Lour.* extraction are carotenoids and phenolic acids [5]. And there has been extensive study on natural pharmaceutical polysaccharides [6,7]. Beginning in the 1950s till now, hundreds of polysaccharides have been found to be biologically active [8]. Polysaccharides play important biological roles in antiinflammatory, anti-viral, anti-tumor, anti-aging, and immuno-activation processes [9]. Moreover, polysaccharides are a very important class of organic compounds in living organisms. In addition to participating in the structure formation of organisms, they also function as information molecules for energy substances and cell recognition, because they participate in the conversion and metabolism of substances in the body to produce essential amino acids, nucleotides, fatty acids, etc. [10]. Polysaccharide is one of the important components of *Osmanthus fragrans Lour.*, but research on *Osmanthus fragrans Lour.* is mainly focused on essential oil of *Osmanthus fragrans Lour.*, while the monosaccharide content of *Osmanthus fragrans Lour.* polysaccharide is rarely reported.

HPLC-MS/MS is widely used in the determination of active ingredients, including traditional Chinese medicines due to its strong specificity and high sensitivity [11–13]. Over the past decades, there has been significant advances in the development of HPLC-MS/MS methods for the analysis of sugars and monosaccharides with better sensitivity and specificity while maintaining the speed and simplicity of implementation. However, due to the low ionization efficiency of monosaccharides, the HPLC-MS/MS method is limited by sensitivity loss, and therefore, derivatization of monosaccharides is indispensable for obtaining highly sensitive detection. Because 1-phenyl-3-methyl-5-pyrazolone (PMP) pre-column derivatization in high performance liquid chromatography, the product is not stereo isomeric, leading to higher UV detection sensitivity [14–17].

In this study, by collecting data on the characteristic fragment ions of six PMP-derived monosaccharides, we optimized the detection and analysis time, polysaccharide hydrolysis conditions, and pre-column pre-derivative reaction conditions. A new method for the simultaneous determination of six monosaccharides in *Osmanthus fragrans Lour.* by precolumn PMP derivatization with HPLC-ESI-MS/MS method was established. The method was applied to analyze the monosaccharide content of three different varieties of *Osmanthus fragrans Lour.*, providing a basis for the development and utilization of *Osmanthus fragrans Lour.* resources.

2. Results

2.1. Mass spectrometry

All analytes were monitored in the positive mode electrospray ionization (ESI) and quantified by the multiple reaction monitoring (MRM) mode. MS parameters were as follows: heating block temperature, 400 °C; DL temperature, 250 °C; electrospray voltage, 3.5 KV; collision gas pressure, 230 KPa; nebulizer gas flow rate, 3.0 L/min; dry gas flow rate, 15.0 L/min. Nitrogen was used in all cases. The analytes were quantified by scanning from m/z 100–1000 at a scan rate of 0.1 s/cycle. Figs. 1 and 2 shows Q3 scan and Product Ion Scan of six monosaccharide standards.

2.2. Preparation of standard solutions

An accurately weighed amount (10 mg) of mannose, ribose, glucose, galactose, xylose, fucose was transferred into a 10-ml volumetric flask and dissolved in ammonia solution (5 ml) to produce mixed monosaccharide standard solutions of 2 mg/ml, according to the method for PMP derivatization. Fig. 3 shows PMP derivatization process of glucose standards and MS fragmentation pathway of standards for PMP-labeled glucose derivatives in positive ion mode.

2.3. Linearity

The standard curve consisted of six concentration levels, and each level of solution was prepared and assayed during the following days. The concentrations of standard solution were 2 µg/ml, 200 ng/ml, 100 ng/ml, 50 ng/ml, 20 ng/ml, 2 ng/ml. According to the PMP derivatization of monosaccharides, the samples were transferred to a 1.5 ml autosampler vial for HPLC-MS/MS analysis within 7 days, to record the Chromatographic peak areas of the corresponding concentration. The standard curves were shown in Table 1, The HPLC-MS/MS method showed excellent linearity over the entire concentration range (2–2000 ng/mL) for monosaccharides, with correlation coefficient (R^2) >0.9990.

2.4. Precision (n = 6)

The precision of the method was assessed by analyzing the 200 ng/ml of mixed monosaccharide standard solution, according to the PMP derivatization of monosaccharides. The intra-day precision was evaluated by six replicate standard solutions on the same day. After the injection, the chromatographic peak areas were measured, the precision was expressed as relative standard deviation (RSD). The results were: mannose 1.98%, ribose 1.48%, glucose 3.33%, galactose 2.75%, xylose 3.81%, and fucose 0.82%. The precision of the method was evaluated by repeatability studies, and RSD values calculated were lower than 8% indicating a good repeatability of the method, indicating that the instrument had good precision.

2.5. Stability (n =6)

To facilitate derivatization reaction of the analytes, the stability of the method was assessed by analyzing the 200 ng/ml of mixed monosaccharides standard solutions, samples were placed at 4 °C for 24 h overnight. 2 µl of solutions were injected in sequence at 0,4, 6,10,12,24 h, and continuously injected 6 times, and recorded the peak areas values. The relative standard deviations were calculated and the results were: mannose 1.50%, ribose 3.00%, rhamnose 1.81%, galacturonic acid 3.09%, glucose 1.99%, galactose 4.94%, xylose 0.99%, fucose 4.21%. The RSD is in the range of 0.99%–4.94%, the stability of this method was evaluated with analytical determination repeated at different intervals, revealing that the RSD of the saccharides was <5% within 12 h. The stability of the analytes in this situation is important for accurate quantification.

2.6. Repeatability (n = 6)

The *Osmanthus fragrans* Lour. polysaccharide hydrolysate was derived, the samples were analyzed by HPLC-MS/MS within 7 days, this operation was repeated six times. and the

results were: mannose 2.88%, Ribose 2.72%, rhamnose 2.36%, galacturonic acid 2.77%, glucose 1.80%, galactose 4.58%, xylose 1.02%, fucose 3.86%. It could be seen that the RSD was in the range of 1.02%–4.58%, which demonstrated that the derivative reaction of the sample was very reproducible.

2.7. Recovery (n = 6)

To determine the accuracy of the proposed method, the analytical recoveries were determined after spiking of known amount of monosaccharide standard. The recoveries were evaluated by analyzing samples (low, medium, high concentration), the blank samples of this study were prepared from mixed monosaccharide standard solution. The effects were evaluated by comparing the peak areas of the analytes in the *Osmanthus fragrans Lour.* polysaccharide hydrolysate samples with those of pure standard solutions. Each monosaccharide RSD, as shown in Table 2.

2.8. Sample content determination

Three kinds of *Osmanthus fragrans Lour.* samples were taken for polysaccharides extraction, hydrolysis (n = 6), and the sample solutions were prepared by PMP derivatization of monosaccharides, the samples were analyzed by HPLC-MS/MS, the peak areas were recorded and the content of each monosaccharide was calculated.

2.9. Data processing

The concentration of monosaccharides in sweet-scented *Osmanthus fragrans Lour.* polysaccharides:

$$Cx(\mu\text{g/ml}) = (Ax - b)/a$$

Among them: Cx is the concentration of each monosaccharide in the polysaccharide sample (ug/ml), Ax is the peak area of the monosaccharide in the polysaccharide sample (mAU.s), a is slope of the standard curve, and b is the intercept of the standard curve.

Monosaccharide content in *Osmanthus fragrans Lour.* polysaccharide:

$$(\text{mg/g}) = Cx \times V \times 0.001/Mx$$

Among them: V is the total volume of the polysaccharide sample solution (ml) and Mx is the mass of the samples (mg).

According to the existing study, it can be concluded that all three kinds of *Osmanthus fragrans Lour.* contain mannose, ribose, glucose, galactose, xylose and fucose. Based on the references [19–23], the retention time (polar order) of each monosaccharide, the mass spectrometry data and the abundance ratio of the pyrolysis fragments in the mass spectrometer, it can be determined that the *Osmanthus fragrans Lour.* contain rhamnose and galacturonic acid. According to the MRM map (Fig. 4), it can be concluded that rhamnose has large response value in *Osmanthus fragrans var. semperflorens*. According to the specific content of each monosaccharide in *Osmanthus fragrans Lour.*, it could be seen at a glance

that the content of monosaccharides in *Osmanthus fragrans Lour.* was relatively stable (Figs. 4, 5, 6 and Table 3), with little change in error, and could more accurately reflect monosaccharides content in *Osmanthus fragrans Lour.* The identified contents in *Osmanthus fragrans var. semperflorens* are: galactose glucose > xylose > mannose > ribose > fucose; *Osmanthus fragrans scv. latifolius* and *Osmanthus fragrans var. thunbergii*: galactose > xylose glucose > mannose > ribose > fucose. The ratio of monosaccharide content in *Osmanthus fragrans var. semperflorens* was mannose: ribose: glucose:galactose:xylose:fucose 0.9:0.7:5.2:5.8:4.8:0.1; the ratio of monosaccharides in *Osmanthus fragrans scv. latifolius* was mannose: ribose: Glucose:Galactose:Xylose:Fucose 0.5:0.3:2.3:3.6:3.1:0.2; the ratio of monosaccharide content in *Osmanthus fragrans var. thunbergii* was mannose: ribose: glucose: galactose: xylose: fucose was 0.5:0.3:1.9:3.2:3.0:0.2.

2.10. MRM detection chromatogram and pyrolysis analysis of monosaccharide standards

The PMP-derived monosaccharide standards were diluted to a concentration of 200 ng/ml and directly subjected to HPLC-MS/MS analysis. Fig. 1 showed the mass spectrum of ion peaks obtained by qualitative analysis of each monosaccharide in positive ion mode, in the preliminary experiment, the response values of monosaccharides in ESI positive ion mode and negative ion mode were compared, it was found that when ammonium acetate (5 mmol/L)-acetonitrile was used as the mobile phase, the response of monosaccharides in negative ion mode was very low. In positive ion mode, the response value reached 10^6 . Considering the ESI positive ion mode, 5 mmol/L of ammonium acetate-acetonitrile was used as the mobile phase for detection and analysis.

By observing Q3 scan and product ion scan, and the main MS parameters of the PMP-labeled monosaccharide standard in positive ion mode (Fig. 1, Table 4), it was found that monosaccharides PMP derivative signal had the strongest excimer ion peaks $[M + 2PMP - H_2O + H]^+$, while monosaccharides derivative secondary fragment ion peaks cracked the $m/z 175[PMP + H]^+$ child ion. It was anticipated that the fragment ions of $m/z 175$ may be the result of breaking a PMP and a monomolecular monosaccharide, with a uniform cleavage pattern. The specific cleavage process is exemplified by a glucose derivative as shown in Fig. 2.

In this experiment, the chromatographic peaks were confirmed by comparing the retention time of the standard monosaccharides, literature data and mass spectrometry fragment information. As shown in Fig. 7, it could be seen from the figure that the chromatographic peaks of the standard derivatives were symmetrical with each other, and there was no tailing phenomenon, indicating that the monosaccharides reacted exclusively with the PMP derivative, with no by-products formed, and that the mixed polysaccharides were well separated. It was anticipated that the PMP derivatization process would greatly change the polarity of the sugars. Because the hydrophobicity of the derivative products were enhanced, it is easier to separate the sugars on the column.

2.11. MRM detection chromatogram and pyrolysis analysis of *Osmanthus fragrans* Lour. polysaccharide

After MRM analysis of the derivatized products of *Osmanthus fragrans* Lour. polysaccharides by water extraction and acid hydrolysis, there were eight ion peaks derived from the same rule of monosaccharides cleavage (Figs. 8, 9 and 10), which was consistent with the standards. Following the cleavage law of monosaccharides derivative that was optimized for the mass spectrometry conditions, standards for selecting the precursors and daughter ions were optimized for mass spectrometry conditions in MRM mode, as shown in Table 5. The monosaccharides composition of three kinds of *Osmanthus fragrans* Lour. are mannose, ribose, rhamnose, galacturonic acid, glucose, galactose, xylose, fucose.

2.12. Selection of pre-treatment methods of osmanthus

The selection of optimal parameters for HPLC and MS played an important role in quantitative analysis. For example, impurities in the samples might inhibit or enhance the ion signal, which affects the precision and repeatability of HPLC-MS/MS. In this study, the time, temperature and hydrochloric acid concentration of the acid hydrolysis in *Osmanthus fragrans* Lour. polysaccharides were carefully selected: the approximate range of factors affecting hydrolysis was obtained through preliminary experiments, the selected hydrochloric acid concentration of 1 mol/L, 2 mol/L, 3 mol/L, hydrolysis temperature at 80 °C, 90 °C, 100 °C, hydrolysis time of 1 h, 3 h, 5 h, were performed in three-factor and three-level orthogonal experiments. The total sugars peak areas and those of the derivative were analyzed shown in Table 6.

The orthogonal $L_9 (3^3)$ experiment was performed further to investigate the appropriate hydrolytic conditions. The hydrolysis temperature and concentration of HCl were the major factors to affect the release degree, while hydrolysis time was the minor factor. The appropriate hydrolytic conditions of *Osmanthus fragrans* Lour. polysaccharides were 90 °C, 3 h, 2 mol/L HCl (namely, $A_2B_2C_2$ in the orthogonal experiment). During the experiment, when the concentration of hydrochloric acid was too high, the phenomenon of sugar carbonization occurred after hydrolysis. When the acid concentration was 1 mol/L, hydrolysis of the polysaccharide was insufficient. As a result, the peak areas of Monosaccharides derivatives decreased when the concentration of HCl is increased.

2.13. Derivative concentration selection

The dosages of the derivative are the key to derivatization. In this study, the effects of different PMP concentrations of 0.1 mol/L, 0.5 mol/L, and 0.7 mol/L on the peak areas of monosaccharide derivatives were investigated. The results were shown in Fig. 10. The approximate PMP concentration range was obtained by pre-experiment, and then the results showed that almost no monosaccharide was detected at a PMP concentration of 0.1 mol/L, and the peak areas were quite small. When the concentration of PMP was too large, the excess PMP in the derivatization process was not completely removed, the signal of PMP was detected to be large, which caused errors in the analysis results by HPLC-MS/MS. A comprehensive selection of 0.5 mol/L was the optimal PMP derivative concentration (Fig. 11).

2.14. Optimization of separation conditions

Since ribose and xylose are isomeric, the excimer ion peak $[M + 2\text{PMP-H}_2\text{O} + \text{H}]^+$ at 481.36 is the same; Mannose, glucose and galactose are isomeric, and the excimer ion peak $[M + 2\text{PMP-H}_2\text{O} + \text{H}]^+$ at 511.40 is the same; Rhamnose and fucose are isomeric, and the excimer ion peak $[M + 2\text{PMP-H}_2\text{O} + \text{H}]^+$ at 495.20 is the same. The fragment ions $[\text{PMP} + \text{H}]^+$ at 175.10 of all monosaccharides are also the same, and a single monosaccharide standard for liquid chromatographic separation is required to characterize the retention time of the reference substance to avoid errors. Gradient elution could obtain better separation, improve the detection sensitivity of sugar in mass spectrometry, shorten the separation time and improve the peak shape (Table 7).

2.15. Polysaccharides component detection methods

3. Discussion

In this study, the HPLC-MS/MS method was used to simultaneously determine the monosaccharide content of three different varieties of *Osmanthus fragrans* Lour., the method was simple, precise, highly-selective to achieve accurate quantification of compounds, thus provided scientific basis for sugars analysis.

4. Materials and methods

Osmanthus fragrans var. *semperflorens*, *Osmanthus fragrans* var. *thunbergii*, *Osmanthus fragrans* scv. *latifolius* (Guangxi) were purchased from Hubei Jishantang Chinese Herbal Pieces Co.; Ltd., and authenticated by Prof. Hongguo Chen, Hubei University of Science and Technology. Acetonitrile (HPLC grade), methanol (HPLC grade) were obtained from Tianjin Oubokai Chemical Co.; Ltd.. Ammonium acetate (HPLC grade) was obtained from Tianjin Komio Chemical Reagent Co.; Ltd.. PMP, D-Man (99.0%(GC)) were purchased Sinopharm Chemical Reagent Co., Ltd.; D-Glu, D-Gal, D-Xyl (99.0%(GC)) were purchased Aladdin Industrial Corporation; D-Rib, L-Fuc (99.0%(GC)) were purchased from Cool Chemical Technology Co., Ltd.; Watsons water was from Guangzhou Watsons Food & Beverage Co., Ltd.; And all other reagents were of analytical grade.

The HPLC-MS/MS was a Shimadzu CBM-20A system with an SPD- M30A detector coupled to a mass spectrometer (triple quadrupole analyzer) equipped with an ESI interface and column oven CTO-20 AC type. The HPLC system consisted of a SIL-20 AC autosampler, two LC-20ADXR pumps, and a column oven CTO-20 AC. Separation was performed on a column type Shim-pack VP-ODS6022748 (150 Lx 2.0), the mobile phase consisted of 5 mmol/L aqueous ammonium acetate solution (solvent A) and pure acetonitrile (solvent B). Chromatographic separation was achieved with the following gradient: 0–5 min, 17% B; 5–10 min, 18% B; 10–15 min, 19% B; 15–19 min, 21% B; 19–25 min, 18% B; and 25–40 min, 15% B. The flow rate 0.2 ml/min. 2 µl of each sample were injected. The column was maintained at 30 °C.

The sample (10 g *Osmanthus fragrans* Lour.) and ethanol absolute (200 ml) were spiked into a 500-ml round-bottomed flask, they were attached to reflux condensation for 1 h in a constant temperature water bath at 80 °C. After filtration was removed, the operation was

repeated once again, filtered and dried again. Then, the filter residue and water (400 ml) were placed in a round bottom flask (1000 ml), Crude polysaccharides were extracted by 400 ml water (two times, 90 min each) in constant temperature water bath at 90 °C. After combining the filtrate obtained by suction filtration for two times, the filtrate was concentrated to 150 ml by rotary evaporator. Ethanol absolute (600 ml) was added to the concentrated polysaccharide solution, followed by filtering, precipitating and drying to obtain the crude polysaccharide of *Osmanthus fragrans* Lour. [18]. the crude polysaccharide was re-dissolved in 50 ml of water, and then added by Sevage reagent (chloroform: n-butanol = 4:1, 10 ml) before being shaken vigorously. The digested mixture was centrifuged (3500 r/min, 5 min) and the supernatant was precipitated with ethanol absolute (200 ml). After standing at 4 °C for 12 h, the precipitates were washed with ethanol and acetone, and dried under vacuum, before *Osmanthus fragrans* Lour. polysaccharide was finally obtained.

The *Osmanthus fragrans* Lour. polysaccharide (10 mg) was dissolved with 5 ml of 2 mol/L hydrochloric acid solution at 90 °C for 3 h. after the samples were cooled down to room temperature, centrifuged for 5 min, obtained monosaccharide sample hydrolysate solution, monosaccharide hydrolysate was stored at 4 °C for further use.

Osmanthus fragrans Lour. polysaccharide hydrolysate was measured (100 µl), PMP methanolic solution (100 µl, 0.5 mol/L) and ammonia solution (100 µl) were mixed in a 7 ml centrifuge tube, shaken well, the mixture was heated at 70 °C for 30 min, allowed to cool. Subsequently, 10% glacial acetic acid aqueous solution and CHCl₃ (1.0 mL each) were added. The mixture was shaken vigorously, before CHCl₃ was removed to extract unincorporated PMP, following a second CHCl₃ wash, the mixture was centrifuged for 10 min (8000 r/min). The upper aqueous phase was filtered through a 0.22-µm membrane for further analysis.

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Fig. 1.
Q3 Scan of PMP-labeled monosaccharide standard. a-Man, b-Rib, c-Glu, d-Gal, e-Xyl, f-Fuc

Fig. 2. Product Ion Scan of PMP-labeled monosaccharide standard. a-Man, b-Rib, c-Glu, d-Gal, e-Xyl, f-Fuc.

Fig. 3. PMP derivatization of glucose standards and mass spectrometry fragmentation of PMP-labeled glucose standard derivatives in positive ion mode.

Fig. 4.

The SD of the content of each monosaccharide in *Osmanthus fragrans var. semperflorens* is indicated by error bars (n = 6).

Fig. 5.
The SD of the content of each monosaccharide in *Osmanthus fragrans* scv. *Latifolius* is indicated by error bars (n = 6).

Fig. 6.
The SD of the content of each monosaccharide in *Osmanthus fragrans* var. *thunbergii*. is indicated by error bars (n = 6).

Fig. 7. Multiple reaction detection chromatogram of PMP-labeled mixed monosaccharide standard in positive ion mode. 1-mannose, 2-ribose, 3-glucose, 4-galactose, 5-xylose, 6-fucose.

Fig. 8. MRM of PMP-labeled *Osmanthus fragrans* var. *sempervlorens* in positive ion mode. 1-mannose, 2-ribose, 3-rhamnose, 4-galacturonic acid, 5-glucose, 6-galactose, 7-xylose, 8-fucose.

Fig. 9.
MRM of PMP-labeled *Osmanthus fragrans* scv. *latifolius* in positive ion mode. mannose, 2-ribose, 3-rhamnose, 4-galacturonic acid, 5-glucose, 6-galactose, 7-xylose, 8-fucose.

Fig. 10.

MRM of PMP-labeled *Osmanthus fragrans* var. *thunbergii* in positive ion mode. 1-mannose, 2-ribose, 3-rhamnose, 4-galacturonic acid, 5-glucose, 6-galactose, 7-xylose, 8-fucose.

Fig. 11.
Effect of PMP concentration on derivative results.

Table 1

Standard curve of mixed monosaccharide standards.

Analyte	Regression equation	R ²	Linear range (ng/ml)	LOD (ug/ml)	LOQ (ug/ml)
Man	$Y = 25.693x + 1099$	0.9996	2–2000.0 ng/ml	0.06	0.18
Rib	$Y = 50.486x + 13.571$	0.9992	2–2000.0 ng/ml	0.06	0.20
Glu	$Y = 25.14x + 2756.7$	0.9999	2–2000.0 ng/ml	0.17	0.50
Gal	$Y = 33.63x + 2074.1$	0.9997	2–2000.0 ng/ml	0.08	0.20
Xyl	$Y = 75.164x + 20.942$	0.9992	2–2000.0 ng/ml	0.15	0.45
Fuc	$Y = 47.213x + 12.139$	0.9994	2–2000.0 ng/ml	0.20	0.60

Table 2Recovery rate of each monosaccharide reference substance in *Osmanthus fragrans* Lour.

Analyte	Original quantity (mg/g)	Amount added (mg/g)	Measured amount (mg/g)	Recovery rate (%)	The average recovery rate (%)	RSD (%)
Man	1.3446	15.3159	16.7829	100.80	104.53	4.28
	0.7180	15.3159	16.4978	103.29		
	0.6208	15.3159	17.3907	109.49		
Rib	1.2725	15.5708	16.6808	98.96	101.36	4.66
	0.7060	15.5708	17.3352	106.80		
	0.4550	15.5708	15.7632	98.31		
Glu	5.3383	16.1272	22.4894	106.35	105.37	1.04
	4.6002	16.1272	21.6465	105.57		
	4.1715	16.1272	20.9750	104.19		
Gal	5.0956	13.8081	18.5045	97.11	96.81	0.67
	4.3390	13.8081	17.7675	97.25		
	3.9596	13.8081	17.2250	96.07		
Xyl	4.2522	14.1550	18.4164	100.06	100.87	0.71
	4.6142	14.1550	18.9303	101.14		
	3.8275	14.1550	18.1834	101.42		
Fuc	0.5447	16.0358	16.3473	98.55	98.63	1.14
	0.2258	16.0358	15.8670	97.54		
	0.0721	16.0358	16.0757	99.79		

Table 3SD and RSD of monosaccharides content in three *Osmanthus fragrans* Lour. samples.

Analytes	<u><i>Osmanthus fragrans</i> var. <i>semperflorens</i></u>		<u><i>Osmanthus fragrans</i> scv. <i>latifolius</i></u>		<u><i>Osmanthus fragrans</i> var. <i>thunbergii</i></u>	
	SD (%)	RSD (%)	SD (%)	RSD (%)	SD (%)	RSD (%)
Man	7.34	7.89	5.10	11.45	0.305	8.632
Rib	8.10	10.99	6.66	23.57	0.279	12.476
Glu	5.93	11.51	1.51	7.13	2.223	10.794
Gal	3.87	6.87	1.91	5.58	2.845	8.391
Xyl	4.20	9.24	2.02	6.45	0.588	2.234
Fuc	0.53	27.05	1.87	10.30	0.432	8.011

Table 4

Main MS parameters of monosaccharide standard derivatization products in positive ion mode.

Analyte	keep time (min)	Molecular weight	Precursorion (m/z)	Production (m/z)	CE/V
D-Man	12.172	180	511.40 [M + H] ⁺	175.10	−35
D-Rib	15.220	150	481.36 [M + H] ⁺	175.10	−35
D-Glu	22.297	180	511.40 [M + H] ⁺	175.10	−35
D-Gal	23.968	180	511.40 [M + H] ⁺	175.10	−35
D-Xyl	24.775	150	481.35 [M + H] ⁺	175.10	−35
L-Fuc	29.090	164	495.20 [M + H] ⁺	175.10	−35

Main mass spectrometry parameters of monosaccharide PMP derivatization products in *Osmanthus fragrans Lour.* polysaccharides in positive ion mode.

Table 5

Monosaccharide	<i>Osmanthus fragrans</i> var. <i>semperflorens</i> keep time (min)	<i>Osmanthus fragrans</i> sev. <i>latifolius</i> keep time (min)	<i>Osmanthus fragrans</i> var. <i>thunbergii</i> keep time (min)	Molecular weight	Precursor ion (<i>m/z</i>)	Production (<i>m/z</i>)	CE/V
D-Man	12.568	12.996	13.282	180	511.40 [M + H] ⁺	175.10	-35
D-Rib	15.487	16.565	16.583	150	481.36 [M + H] ⁺	175.10	-35
D-Rha	16.024	16.843	17.065	164	495.20 [M + H] ⁺	175.10	-35
GalUA	20.264	21.992	21.890	194	524.18 [M + H] ⁺	175.10	-35
D-Glu	22.297	23.604	23.854	180	511.40 [M + H] ⁺	175.10	-35
D-Gal	24.235	25.898	26.425	180	511.40 [M + H] ⁺	175.10	-35
D-Xyl	25.581	28.039	28.979	150	481.36 [M + H] ⁺	175.10	-35
L-Fuc	29.410	35.053	35.794	164	495.20 [M + H] ⁺	175.10	-35

Table 6

Factors and levels.

Levels	Hydrochloric acid (mol/L)-A	Temperature (°C)-B	Time (h)-C
1	1	80	1
2	2	90	3
3	2.5	100	5

Table 7

Method comparison.

	Method	Composition	Analysis time	LOD	LOQ	References
<i>Morus nigra</i> Linn polysaccharides	HPLC	Glc, Xyl, Man, Gal, GalUA	35 min	–	–	[24]
Hazelnut polysaccharides	GC-MS	Glu, Fru, Man, Gal, Xyl, Rha, etc.	32 min	0.1–1.0 µg/ml	0.5–5.0 µg/ml	[25]
Honey fungus polysaccharides	UPLC-MS	Man, Gal, Xyl, Glu, Fuc, etc.	7.5 min	0.01–0.1 u/ml	0.03–0.3 µg/ml	[26]
Sea cucumbers	HPLC-MS	Fucosylated chondroitin sulfate, fucoidan	14 min	0.06–0.07 µg/ml	0.20–0.24 µg/ml	[27]
<i>Osmanthus</i> polysaccharides	HPLC-MS	Man, Rib, Glu, Gal, Xyl, Fuc	30 min	0.06–0.20 µg/ml	0.18–0.60 µg/ml	*

* This study.