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Analytical Methods

Analysis of the monosaccharide composition of water-soluble polysaccharides from *Sargassum fusiforme* by high performance liquid chromatography/electrospray ionisation mass spectrometry

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ABSTRACT

Sargassum fusiforme (hijiki) is the well-known edible algae, whose polysaccharides have been proved to possess interesting bioactivities like antitumor, antioxidant, antimicrobial and immunomodulatory activities. A facile and sensitive method based on high-performance liquid chromatography method of pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) coupled with electrospray ionisation mass spectrometry (HPLC/ESI–MS) has been established for the analysis of the monosaccharide composition of polysaccharides in *S. fusiforme*. Monosaccharides have been converted into PMP-labelled derivatives with aqueous ammonia as a catalyst at 70 °C for 30 min. The optimisation of the pre-column derivatization process was studied. The LODs of the monosaccharides were in the range from 0.01 to 0.02 nmol. PMP-labelled mixture of monosaccharides has been well separated by a reverse-phase HPLC and detected by on-line ESI–MS method under optimised conditions. The mobile phase of elution system was chosen as acetonitrile (solvent A) and 20 mM aqueous ammonium acetate (solvent B) (pH 3.0) with Zorbax XDB-C18 column at 30 °C for the separation of the monosaccharide derivatives. Identification of the monosaccharides composition was carried out by analysis with mass spectral behaviour and chromatography characteristics of 1-phenyl-3-methyl-5-pyrazolone (PMP) labelled monosaccharides. All PMP-labelled derivatives display high chemical stabilities, whose regular MS fragmentation is specific for reducing labelled sugars. The result showed that the *S. fusiforme* polysaccharide consisted of mannose, glucose, galactose, xylose, fucose and glucuronic acid or galacturonic acid, or both uronic acids.

1. Introduction

Sargassum fusiforme (hijiki), one of the well-known brown seaweed and edible algae, has been applied as an important food and therapeutic for thousands of years and widely cultivated in China and Japan (Ji, Wang, Wu, & Ji, 2007; Mao, Li, Gu, Fang, & Xing, 2004; Yan, Chuda, Suzuki, & Nagata, 1999; Zhu, Ooi, Chan, & Ang, 2003). The antioxidant and antimicrobial properties of the seaweeds have been intensively studied (Lu, Yan, Xu, & Nan, 2010; Yan et al., 1999). Recently, studies of polysaccharides from *S. fusiforme* have attracted more attention. Bioactivities including vibriosis resistance, immunity enhancing effect (Huang, Zhou, & Zhang, 2006), antitumor activity against S180 in mice (Ji et al., 2007), inhibitory activity to calcium oxalate (CaOxa) urinary stones (Wu, Ouyang, Deng, & Chen, 2006) and antihyperlipidemia activity (Mao et al., 2004) have been reported. As an important Traditional Chinese Medicine, the water-soluble extract of *S. fusiforme* has been used to treat human disease (Zhang & Wang, 1990), so the water-soluble polysaccharide was focused on in the present study. Monosaccharide composition analysis of polysaccharides is of fundamental importance for the research on polysaccharide structure and its characteristics. A variety of chromatographic systems such as gas chromatography (GC) (Akiyama, Yamazaki, & Tanamoto, 2011; Chen, Xie, Wang, Nie, & Li, 2009), high-performance liquid chromatography (HPLC) (Gomis, Tamayo, & Alonso, 2001; Han, Chen, Sun, Zhan, & Bi, 2009; Ikegami et al., 2008) and high performance liquid chromatography-mass spectrometry (LC–MS) (Dye & Yttri, 2005; Hammad, Derryberry, Jmeian, & Mechref, 2010) have be used to separate and analyse monosaccharides.

Over the past decade, the development of LC/MS methods dedicated to the analysis of sugars and monosaccharides in particular, has led to significant advances in terms of sensitivity and specificity while maintaining speed and simplicity of implementation. However, LC–MS method is limited by loss of sensitivity owing to the low ionisation efficiency of monosaccharides. Therefore, the derivatization of monosaccharides is indispensable to obtain highly sensitive detection. The reagent 1-phenyl-3-

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methyl-5-pyrazolone (PMP), which was first developed in 1989 by the Honda's group (Honda et al., 1989), is one of the popular labels that react with reducing carbohydrate under mild condition, requiring no acid catalyst and causing no desialylation and isomerization (Dai et al., 2010; Zhang, Wang, Xie, Nie, & Huang, 2010). Pre-column derivatization with PMP method was first developed for the analysis of carbohydrates by high-performance liquid chromatography (HPLC) (Shen & Perreault, 1998), and later successfully applied to capillary electrophoresis (CE) (Suzuki et al., 2001), including capillary zone electrophoresis (CZE) (Honda, Suzuki, Nose, Yamamoto, & Kakehi, 1991), micellar electrokinetic chromatography (MEKC) (Janini & Issaq, 1992), and ion-exchange electrokinetic chromatography (IXEKC) (Honda, Toghi, Uegaki, & Honda, 1998).

Analyses of polysaccharide from *S. fusiforme* are extensive (Ji et al., 2007; Mao et al., 2004; Zhu et al., 2003). However, the application of analytical methods of PMP precolumn derivatization high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry on *S. fusiforme* have not been reported. In the present study, a precolumn PMP derivatization LC–ESI–MS method for simultaneous determination of six sugars was established. The data on characteristic fragment ions of the six PMP-labelled monosaccharides have been collected. All PMP-labelled derivatives display high chemical stabilities, whose regular MS fragmentation is specific for reducing labelled sugars. The procedure of the pre-column derivatization reaction was optimised by examination of reaction time and neutralization condition of the reaction system for six sugars. At the same time, the optimised method and procedure were successfully applied in sugar compositional analysis of polysaccharide fractions isolated from *S. fusiforme*.

2. Materials and methods

2.1. Materials and reagents

D-Mannose, L-rhamnose, D-glucose, D-xylose, D-galactose, and L-fucose were purchased from the Shanghai Pharmaceutical Holding Co., Ltd. (Shanghai, China). Trifluoroacetic acid (TFA) was obtained from Merck (Darmstadt, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Major Chemicals Co., Ltd. (Hangzhou, China). Acetonitrile (HPLC-grade) was obtained from Merck (E. Merck, Darmstadt, Germany). Water was purified with a Mili-Q academic water purification system (Millipore, Bedford, MA, USA). Other chemicals were of analytical reagent grade. *S. fusiforme* was collected in Dongtou County, Zhejiang Province, People's Republic of China, in September 2009 and identified by Prof. Changxi Zhang (Jinhua Medical College, Jinhua, People's Republic of China). A voucher specimen (Vs71) is maintained at the Jinhua Medical College, Jinhua, People's Republic of China.

2.2. Extraction of the polysaccharide from the Sargassum fusiforme

The polysaccharide was isolated from *Sargassum fusiforme* by hot-water extraction and ethanol precipitation (Wang & Luo, 2007; Yoshizawa, Enomoto, Todoh, Ametani, & Kaminogawa, 1993). The dried *S. fusiforme* (50 g) were extracted with 95% alcohol and dried, then extracted with 100 ml distilled water at 95 °C for 3 h. The water extracts were concentrated to 20 ml under a reduced pressure. Then 60 ml anhydrous alcohol was added slowly by stirring to precipitate the polysaccharide which kept at 4 °C overnight. Then polysaccharide precipitation were obtained by centrifugation at 3000 rpm for 30 min and repeatedly washed sequentially with possibly less amounts of ethanol, acetone and ether, respectively. Residue was dried and the *S. fusiforme* polysaccharide was obtained.

2.3. Hydrolysis of the polysaccharide

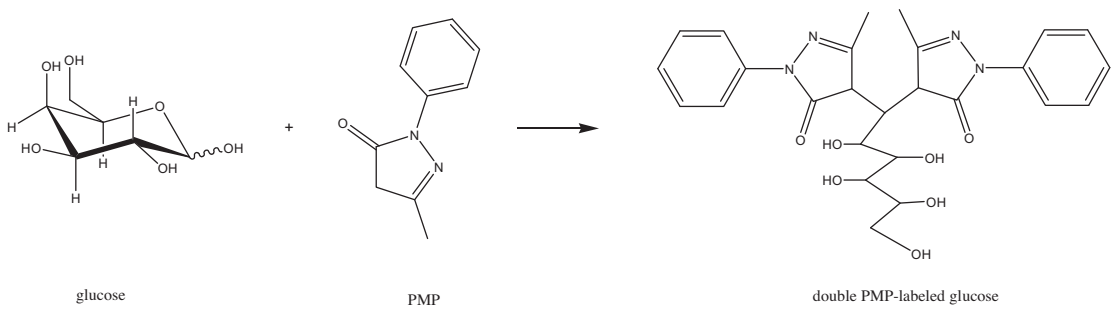
The polysaccharide sample (50 mg) was infiltrated with 4 M TFA (4 ml) (Åman, McNeil, Franzén, & Darvill, 1981) in a sealed flask (10 ml) and kept at ambient temperature for 15 min. Then 1 ml distilled water was added and kept in boiling water bath for 2 h. After that, 2 ml of distilled water was added and kept in boiling water bath for 1 h. After being cooled to room temperature, the reaction mixture was then centrifugated at 3000 rpm for 10 min. The supernatant was collected and dried under a reduced pressure.

2.4. Derivatization procedure

The procedure employed for the derivatization of monosaccharides was carried out according to the method of Daotian, F et al. (Fu & Oneill, 1995) and modified by us. The hydrolysed polysaccharide or monosaccharides were dissolved in 5 ml ammonia. Then, the 100 μl solution was transferred into a clean tube, and 0.5 M methanol solution (100 μl) of PMP was added and mixed. The mixture was allowed to react for 30 min at 70 °C, then cooled to ambient temperature and was neutralized with 20 μl 1% glacial acetic acid. Water and chloroform (1.0 ml each) were added, and the mixture was shaken vigorously. The chloroform layer was discarded, and the extraction process was repeated three times. Then, the supernatant was centrifugated at 10,000 rpm for 10 min, which was collected for analysis directly or stored at −20 °C for later LC/MS analysis. Scheme 1 shows the Glc derivatization reaction.

2.5. Chromatography and mass spectrometry conditions

LC–MS analysis was conducted using an Agilent (Agilent Technologies, Palo Alto, CA, USA) 1100 series HPLC system consisting of a G1312A binary pump, a G1322A degasser and an ALS G1329A auto-sampler interfaced to an Agilent 1100 series



Scheme 1. Derivatization reaction of PMP and glucose.

G1946D mass spectrometer equipped with an electrospray ionisation (ESI) interface. The data acquisition software employed was Agilent Chemstation version 10.02. An Agilent Zorbax XDB-C18 column (250 × 4.6 mm, 5 μm) was used and kept at 30 °C. The mobile phase consisted of 20 mM ammonium acetate (adjusted to pH 3.0 with glacial acetic acid)–acetonitrile (85:15, v/v). The flow rate was 1.0 ml/min. The injection volume was 20 μL. The settings of the mass spectrometer were as follows: the drying gas flow rate was 12 L/min and a nebulizer pressure was 50 psi. The drying gas temperature was set at 350 °C. The value of the fragmentor was 100 V with capillary voltage at 4000 V in positive ion scan mode and 3500 V in negative ion scan mode. The scan range was set from *m/z* 100 to 1000 amu.

2.6. Method validation

The analytical method was validated according to the following criteria: linearity, sensitivity, recovery, precision and reproducibility. The linearity of each analyte was evaluated by using a series of standard solutions. The calibration curves were constructed based on peak area versus concentrations of analyte standards. The detection limits (LOQ) of each analyte was obtained by injecting a standard mixture derivatized as mentioned above in the derivatization procedure, followed by the comparison of peak height with baseline noise level and a signal-to-noise ratio (S/N) of three assigned the detection limit. The precisions detection was examined by five repeated injections of a mixed standard solution. In order to examine the recovery of the developed method, the standards of each analyte at three different concentration levels were spiked to samples and then derivatived by the optimised derivatization method. The reproducibilities were examined by six repeated determination of the hydrolysed monosaccharides from *Sargassum fusiforme* polysaccharide.

3. Results and discussion

3.1. Hydrolysis of polysaccharide

H_2SO_4 is the mostly used hydrolysis agent in the studies of polysaccharide, which is difficult to remove and poses negative effect on MS-based measurement. Since polysaccharides in *Sargassum fusiforme* linked mainly by simple carbohydrate chains (Zhou, Hu, Wu, Pan, & Sun, 2008), they could be essentially hydrolysed completely using trifluoroacetic acid (TFA) in mild condition. In the present study, the results showed that TFA hydrolysis was suitable to polysaccharide from *S. fusiforme*, and at least six monosaccharides were obtained. TFA was removed in vacuo before samples running in MS, so the contamination caused by TFA was avoided.

3.2. Optimisation of the derivatization procedure

3.2.1. Effect of a catalyst on derivatization reaction

Although sodium hydroxide is typical catalysts for the derivatization of carbohydrates in alkaline medium, the reaction produces considerable amounts of inorganic salts in the resultant solution. In the present study, ammonia was applied as the catalysts for the derivatization, which can be removed by evaporating the solution to dryness in vacuo, avoiding the production of inorganic salts.

3.2.2. Optimisation of derivatization conditions

The result of the analysis of reaction time effect on derivatization was summarised in Fig. 1(a), which indicated that the labelling

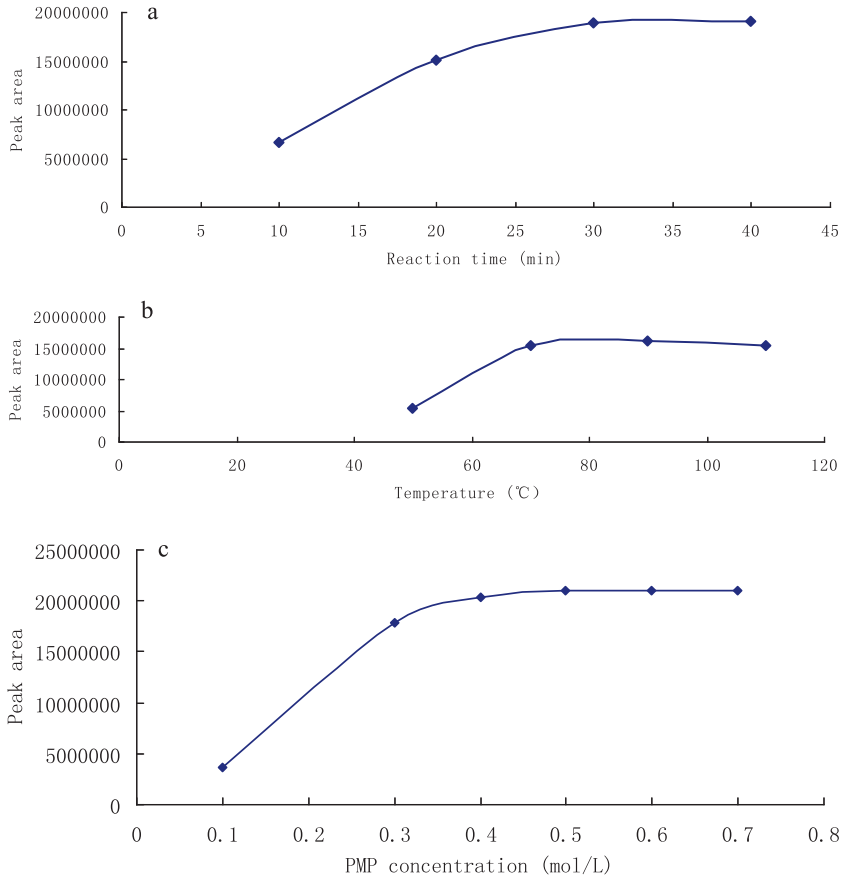


Fig. 1. (a) Effect of reaction time on derivatization of glucose. (b) Effect of reaction temperature on derivatization of glucose. (c) Effect of PMP concentration on derivatization of glucose.

Table 1
The calibration ranges, LODs and precision for five monosaccharides.

Analyte	Calibration curve	Range (nmol)	Correlation coefficient	LODs (nmol)	Precision (RSD,%)
Mannose	$Y = 383X-5.86$	0.1–10	0.9999	0.01	0.21
Glucose	$Y = 188X-4.6$	0.1–10	0.9999	0.01	1.76
Galactose	$Y = 256X-1.14$	0.1–10	0.9999	0.02	1.40
Xylose	$Y = 250X-15$	0.1–10	0.9999	0.02	0.56
Fucose	$Y = 218.7X-5.79$	0.1–10	0.9999	0.02	1.34

Table 2
Recovery analysis of five monosaccharides in the sample of *Sargassum fusiforme* polysaccharide ($n = 5$).

Analyte	Content in sample (nmol)	Spiked amount (nmol)	Found amount (nmol)	Recovery (%)	RSD (%)
Mannose	0.44	0.25	0.65	85.1	2.29
		2.5	2.62	87.3	0.99
		10	9.33	88.9	0.91
Glucose	0.47	0.25	0.69	86.1	3.64
		2.5	3.02	101.9	2.83
		10	11.59	111.2	2.56
Galactose	1.20	0.25	1.42	87.4	2.91
		2.5	3.60	96.0	1.90
		10	11.77	105.7	1.10
Xylose	0.14	0.25	0.36	88.6	1.98
		2.5	2.48	93.6	1.07
		10	9.58	94.40	0.78
Fucose	1.19	0.25	1.42	89.2	2.56
		2.5	3.88	107.1	2.15
		10	12.48	112.8	0.63

Table 3
Reproducibility of five monosaccharides in the sample of *Sargassum fusiforme* polysaccharide ($n = 6$).

	Content in sample (nmol)	Mean value (nmol)	Reproducibility RSD (%)
Mannose	0.88	0.87	1.27
	0.87		
	0.85		
	0.87		
	0.87		
	0.87		
Glucose	0.95	0.94	1.39
	0.95		
	0.95		
	0.93		
	0.94		
	0.92		
Galactose	2.46	2.46	0.75
	2.47		
	2.43		
	2.44		
	2.49		
	2.46		
Xylose	0.28	0.29	1.99
	0.28		
	0.30		
	0.29		
	0.29		
	0.28		
Fucose	2.39	2.45	1.61
	2.41		
	2.47		
	2.49		
	2.46		
	2.47		

reaction completes in 30 min. To investigate the temperature effect on derivatization, a series of test reactions with glucose as a model saccharide were performed. The result of the effect of reaction temperature of 50, 70, 90 and 110 °C on the peak area (proportional to the yield of the derivative) is presented in Fig. 1(b). The yield of labelled glucose increases with the increase of the temperature within a range from 50 to 90 °C. Performing the reaction at higher temperatures from 70 to 110 °C gives only little effect. Therefore, performing of the labelling routine at 70 °C should be recommended. To define the optimal amount of PMP on derivatization reaction, a series of derivatization experiments with different molar ratios of PMP to Glc were carried out. The results were presented in Fig. 1(c). The yield of the reaction is very low at PMP concentration of 0.1 mol/L and dramatically increases with the PMP concentration of 0.3 mol/L. Then, the growth of the peak area slows down, and the graph reaches the saturation state at 0.5 mol/L PMP. In the present work, 0.5 mol/L PMP was chosen in the derivatization reaction.

3.3. Results of method validation

According to Section 2.6, the calibration curves were constructed based on peak area versus concentrations of analyte standards. Table 1 is the summary of calibration curves, linear ranges and limit of detection. Good linearities were found in the ranges of 0.1–10 nmol for each analyte. The results of detection limits analysis showed that the LODs of the monosaccharides were in the range from 0.01 to 0.02 nmol (Table 1), indicating that the sensitivity of the method was satisfactory. The results of precisions detection were summarised in Table 1, showing that the relative standard deviations (RSDs) of peak areas were all under 2.0% for each analyte, which indicated that the method precision was satisfactory. The recoveries are summarised in Table 2. Good recoveries from 85% to 113% were obtained. The result of reproducibility analysis was summarised in Table 3. The relative standard deviations (RSDs) values fell within 0.75–1.99%.

3.4. HPLC separation of PMP-monosaccharides derivatives

The HPLC separation conditions were adjusted and tested for a mixture of 6 PMP-labelled monosaccharides (mannose, rhamnose, glucose, galactose, xylose and fucose). Since no nonvolatile inorganic salts are allowed in the MS-based measurement methods, the mobilephase of elution system was chosen as acetonitrile (solvent A) and 20 mM aqueous ammonium acetate (solvent B) (pH 3.0). Six PMP-labelled monosaccharides were separated successfully in the order of peak 1 for mannose, peak 2 for rhamnose, peak 3 for glucose, peak 4 for galactose, peak 5 for xylose and peak 6 for fucose. In this study, the good separation of the six monosaccharide derivatives was achieved within 45 min (see Fig. 2). In the process of mobilephase evaluation, we found that the concentration of ammonium acetate affect the separation of PMP-labelled monosaccharides. The mobilephase of elution

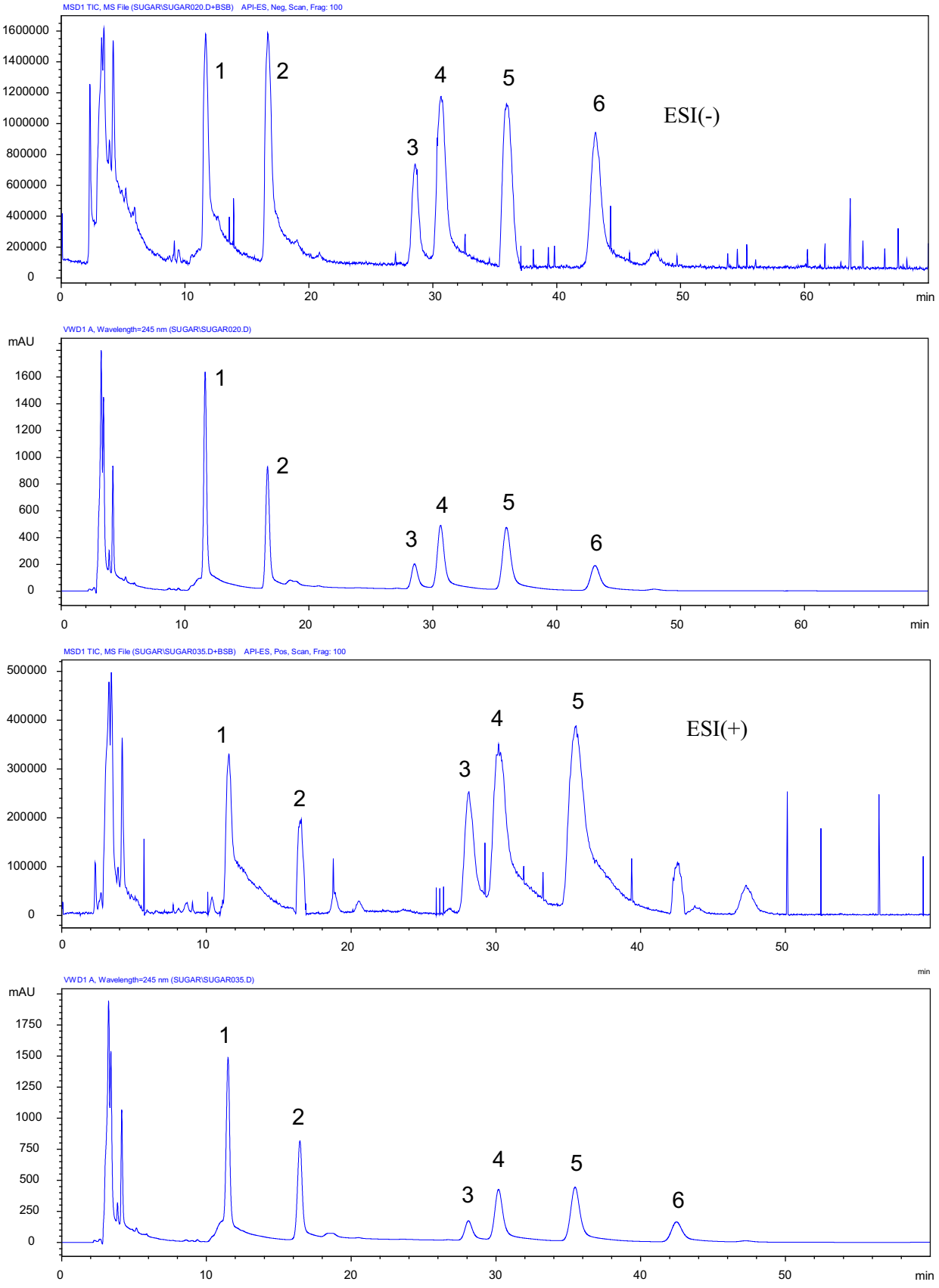


Fig. 2. Positive and negative total ion current chromatogram (TIC) and HPLC chromatogram of six PMP-labelled monosaccharides.

Table 4
MS data of double PMP-labelled saccharide.

Saccharides	tR(min)	$[M-H]^-$	$[M+Cl]^-$	Fragment ions (*m/z*)
Mannose	11.6	509.2	545.2	180
Rhamnose	16.7	493.2	529.2	194
Glucose	28.5	509.2	545.2	208
Galactose	30.6	509.2	545.5	180, 176
Xylose	35.9	479.2	515.2	180
Fucose	43.1	493.2	529.2	164

system, acetonitrile and 20 mM aqueous ammonium acetate, is the best condition for separating samples, especially for separating Glu and Gal. According to the results of our experiments and other reports (Abdel-Hamid, Novotny, & Hamza, 2001; Kim, Yoo, Han, Lee, & Lee, 2003), MS can tolerate MS-friendly mobile phases consisting of ammonium acetate of 20 mM concentration.

3.5. LC–ESI–MS characterisation

All PMP-labelled monosaccharides were characterised by LC–ESI–MS method (positive-ion and negative-ion mode, see Fig. 2). Although both ion modes has high response, negative-ion mode was chosen, since more fragments were obtained by negative-ion mode. For example, PMP-labelled glucose comprises peaks of *m/z* 514, 509, 371, 335, 299 and 263 correspondent to $[M+Cl]^-$, $[M-H]^-$, $[M-PMP+Cl]^-$, $[M-PMP-H]^-$, $[M-2PMP+Cl]^-$ and $[M-2PMP-H]^-$ by negative-ion mode, whereas, only *m/z* 511 $[M+H]^+$ was detected by positive-ion mode. The numerical data are collected in Table 4, which allow suggesting the fragmentation mode for PMP-labelled monosaccharides.

3.6. LC–ESI–MS analysis of monosaccharide composition of polysaccharides in Sargassum fusiforme

When HPLC and MS are combined on-line, the total ion current chromatogram (TIC) of the molecular ion peaks and HPLC spectrum (see Fig. 3) of the separated PMP-labelled monosaccharides are obtained simultaneously. In the present study, seven PMP-labelled compounds were separated, among which six PMP-labelled monosaccharides were identified under conditions as described in Section 2.5 by means of HPLC–ESI–MS/MS combination technique.

Peaks 1, 4, 5, 6 and 7 could be unambiguously identified as PMP-labelled mannose, glucose, galactose, xylose and fucose based on the comparison of retention times and MS data with those of authentic PMP-labelled monosaccharides. Peak 2 gave negative ions at *m/z* 523.1 $[M-H]^-$ and 349.2 $[M-PMP-H]^-$. The mass difference between the fragments of the compound and those of authentic PMP-labelled glucose was 14 Da, and the molecular weight was 194 Da, which indicated that peak 2 might be a PMP-labelled GlcUA or GalUA. Similar ions at *m/z* 509.2, 505.2, 335.1, 331.1 as those of PMP-labelled authentic glucose were observed at peak 4 based on the above analyses for the reference standards in the HPLC–ESI–MS experiments. Two negative ions at *m/z* 509.2 and 335.1 were consistent with fragments of $[M-H]^-$ and $[M-PMP-H]^-$ of PMP-labelled glucose. However, peak 4 also gave negative ions at *m/z* 505 $[M-H]^-$ and 331 $[M-PMP-H]^-$. Peak 4 was obviously the mixture of a PMP-labelled glucose and another unknown compound. The mass difference between the unknown compound and the authentic PMP-labelled glucose was 4 Da, which indicated that peak 4 might exist another byproduct derivative from glucose lactone. From analysis of the fragmentation pattern and the molecular weight of 176 Da, this compound could be tentatively identified as ascorbic acid. Although, ascorbic acid widely

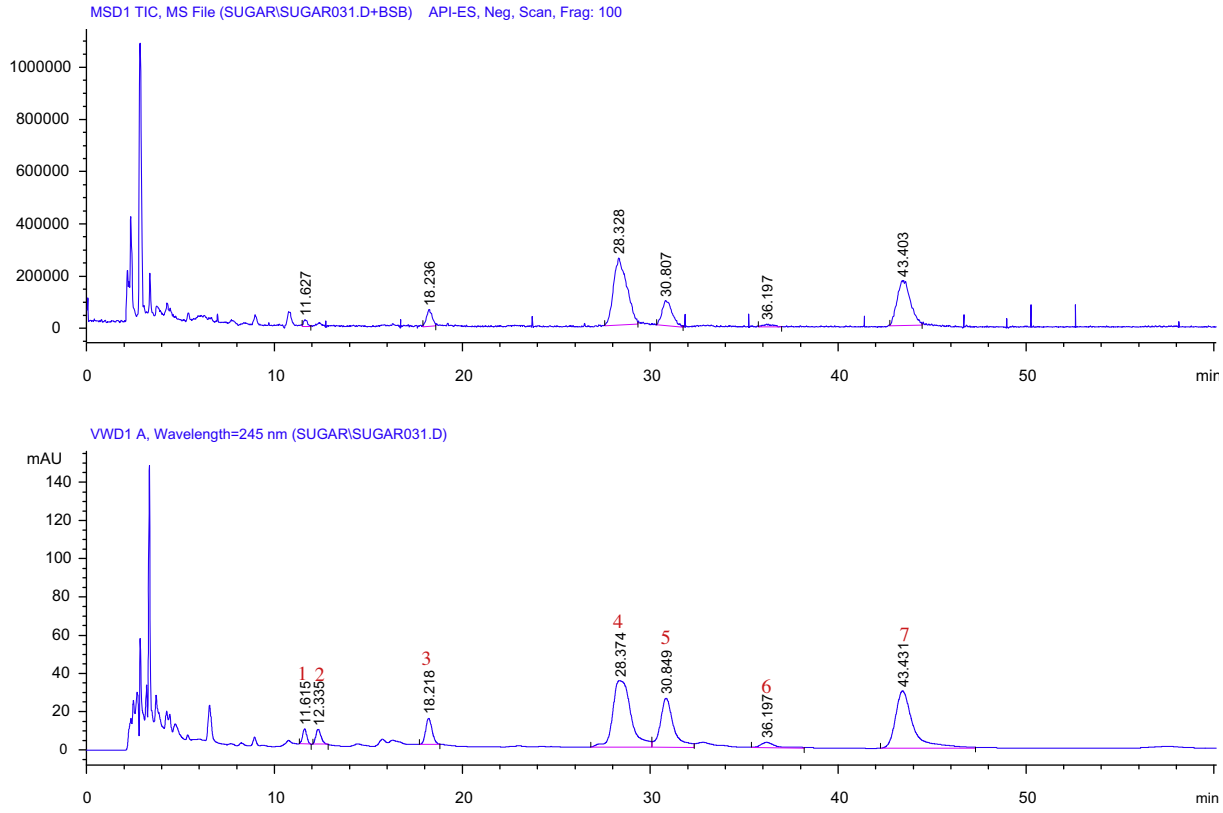


Fig. 3. The total ion current chromatogram and HPLC spectrum of PMP-labelled monosaccharide composition of polysaccharides.

Table 5
Identification of monosaccharide composition of Polysaccharides in *Sargassum fusiforme*.

Peak No.	tR(min)	$[M-H]^-$	Fragment ions (*m/z*)	Mw	Saccharides
1	11.6	509.2	335.1	180	Mannose
2	12.5	523.2	349.1	194	Glucuronic acid or Galacturonic acid
3	18.2	537.2	363.2	208	Unknown
4	28.3	509.2, 505.2	335.1, 331.1	180, 176	Glucose, L-ascorbic acid
5	30.8	509.2	335.1	180	Galactose
6	36.2	479.2	341.1	150	Xylose
7	43.4	493.2	319.1	164	Fucose

distribute in nature, its existence in this case might be a byproduct from PMP label reaction. Peak 3 afforded negative ions at *m/z* 537.2 $[M-H]^-$, 363.2 $[M-PMP-H]^-$ revealing that the molecular weight could be 208 Da. However, its exact structure needs to be confirmed in future work. A total of six peaks were identified or tentatively identified. From above evidence, polysaccharides from *Sargassum fusiforme* mainly compose of mannose, glucose, galactose, xylose, fucose, and glucuronic acid or galacturonic acid, or both uronic acids (Table 5).

4. Conclusion

Studies on polysaccharide from *S. fusiforme* are extensive (Ji et al., 2007; Mao et al., 2004; Zhu et al., 2003). However, analytical methods of PMP precolumn derivatization high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry have not been reported. In the present study, a facile and sensitive method based on precolumn derivatization HPLC/ESI–MS has been established for the analysis of the monosaccharide composition of water-soluble polysaccharides in *S. fusiforme*. All PMP-labelled derivatives display high chemical stability, their regular MS fragmentation is specific for reducing labelled sugars. The suggested method for monosaccharide composition analysis with PMP pre-column derivatization by on-line HPLC–ESI–MS was tested for a monosaccharide composition prepared from natural *S. fusiforme* water-soluble polysaccharide, which provides a rapid, reproducible, accurate, and economic alternative for the identification of monosaccharides from *S. fusiforme*. Capillary electrophoresis (CE) is the well studied method for PMP-labelled monosaccharides, which has an advantage over HPLC in the separation of PMP-labelled monosaccharides since there is no packing material nor metal plumbing involved which cause metal oxidation of PMP-labelled monosaccharides and sample adsorption or degradation. Relatively low sensitivity and reproducibility are two major factors limiting the application of CE in the analysis of monosaccharides, which could be overcome by on-line HPLC–ESI–MS method.

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References

Abdel-Hamid, M. E., Novotny, L., & Hamza, H. (2001). Determination of diclofenac sodium, flufenamic acid, indomethacin and ketoprofen by LC–APCI-MS. *Journal of Pharmaceutical and Biomedical Analysis, 24*, 587–594.

Akiyama, T., Yamazaki, T., & Tanamoto, K. (2011). Analysis of thickening polysaccharides by the improved diethyldithioacetal derivatization method. *Shokuhin Eiseigaku Zasshi, 52*, 40–46.

Åman, P., McNeil, M., Franzén, L.-E., & Darvill, A. G. (1981). Structural elucidation, using h.p.l.c.-m.s. and g.l.c.-m.s., of the acidic polysaccharide secreted by rhizobium meliloti strain 1021. *Carbohydrate Research, 95*, 263–282.

Chen, Y., Xie, M., Wang, Y., Nie, S., & Li, C. (2009). Analysis of the monosaccharide composition of purified polysaccharides in Ganoderma atrum by capillary gas chromatography. *Phytochemical Analysis, 20*, 503–510.

Dai, J., Wu, Y., Chen, S., Zhu, S., Yin, H., Wang, M., et al. (2010). Sugar compositional determination of polysaccharides from Dunaliella salina by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. *Carbohydrate Polymers, 82*, 629–635.

Dye, C., & Yttri, K. E. (2005). Determination of monosaccharide anhydrides in atmospheric aerosols by use of high-performance liquid chromatography combined with high-resolution mass spectrometry. *Analytical Chemistry, 77*, 853–1858.

Fu, D. T., & Oneill, R. A. (1995). Monosaccharide composition analysis of oligosaccharides and glycoproteins by high-performance liquid chromatography. *Analytical Biochemistry, 227*, 377–384.

Gomis, D. B., Tamayo, D. M., & Alonso, J. M. (2001). Determination of monosaccharides in cider by reversed-phase liquid chromatography. *Analytica Chimica Acta, 436*, 173–180.

Hammad, L. A., Derryberry, D. Z., Jmeian, Y. R., & Mechref, Y. (2010). Quantification of monosaccharides through multiple-reaction monitoring liquid chromatography/mass spectrometry using an aminopropyl column. *Rapid Communications in Mass Spectrometry, 24*, 1565–1574.

Han, J., Chen, X. H., Sun, L. X., Zhan, Z. H., & Bi, K. S. (2009). Analysis of monosaccharide compositions in Heterosmilax japonica polysaccharide by precolumn derivation HPLC. *Journal of Chinese medicinal materials, 32*, 893–895.

Honda, S., Akao, E., Suzuki, S., Okuda, M., Kakehi, K., & Nakamura, J. (1989). High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3methyl-5-pyrazolone derivatives. *Analytical Biochemistry, 180*, 351–357.

Honda, S., Suzuki, S., Nose, A., Yamamoto, K., & Kakehi, K. (1991). Capillary zone electrophoresis of reducing mono-and oligo-saccharides as the borate complexes of their 3-methyl-1-phenyl-2-pyrazolin-5-one derivatives. *Carbohydrate Research, 215*, 193–198.

Honda, S., Toghi, K., Uegaki, K., & Honda, S. (1998). Enhancement of the zone electrophoretic separation of 1-phenyl-3-methyl-5-pyrazolone derivatives of aldoses as borate complexes by concerted ion-interaction electrokinetic chromatography with Polybrene. *Journal of Chromatography A, 805*, 277–284.

Huang, X., Zhou, H., & Zhang, H. (2006). The effect of Sargassum fusiforme polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, Fenneropenaeus chinensis. *Fish and Shellfish Immunology, 20*, 750–757.

Ikegami, T., Horie, K., Saad, N., Hosoya, K., Fiehn, O., & Tanaka, N. (2008). Highly efficient analysis of underivatized carbohydrates using monolithic-silica-based capillary hydrophilic interaction (HILIC) HPLC. *Analytical and Bioanalytical Chemistry, 391*, 2533–2542.

Janini, G. M., & Issaq, H. J. (1992). Micellar electrokinetic capillary chromatography: basic considerations and current trends. *Journal of Liquid Chromatography, 15*, 6–7.

Ji, Y., Wang, C., Wu, T., & Ji, C. (2007). Effect of Sargassum fusiforme polysaccharides on the complex mobility of erythrocytes in tumor-bearing organisms using high performance capillary electrophoresis. *Chinese Journal of Chromatography, 25*(3), 322–325.

Kim, H., Yoo, J.-Y., Han, S. B., Lee, H. J., & Lee, K. R. (2003). Determination of ambroxol in human plasma using LC–MS/MS. *Journal of Pharmaceutical and Biomedical Analysis, 32*, 209–216.

Lu, Y., Yan, M.-C., Xu, H.-X., & Nan, C.-R. (2010). Antimicrobial activities of extracts from Sargassum fusiforme and Enteromorpha clathrata against aquatic animal pathogen. *Marine Sciences, 10*, 6.

Mao, W. J., Li, B. F., Gu, Q. Q., Fang, Y. C., & Xing, H. T. (2004). Preliminary studies on the chemical characterization and antihyperlipidemic activity of polysaccharide from the brown alga Sargassum fusiforme. *Hydrobiologia, 512*, 263–266.

Shen, X. D., & Perreault, H. (1998). Characterization of carbohydrates using a combination of derivatization, high-performance liquid chromatography and mass spectrometry. *Journal of Chromatography A, 811*, 47–59.

Suzuki, S., Tanaka, R., Takada, K., Inoue, N., Yashima, Y., Honda, A., et al. (2001). Analysis of sialo-N-glycans in glycoproteins as 1-phenyl-3-methyl-5-pyrazolone derivatives by capillary electrophoresis. *Journal of Chromatography A, 910*, 319–329.

Wang, Z., & Luo, D. (2007). Antioxidant activities of different fractions of polysaccharide purified from Gynostemma pentaphyllum Makino. *Carbohydrate Polymers, 68*, 54–58.

Wu, X. M., Ouyang, J. M., Deng, S., & Chen, Y. (2006). Inhibition of the crystal growth and aggregation of calcium oxalate by algae sulfated polysaccharide in-vitro. *Chinese Chemical Letters, 17*, 97–100.

Yan, X., Chuda, Y., Suzuki, M., & Nagata, T. (1999). Fucoxanthin as the Major Antioxidant in Hijikia fusiformis, a Common Edible Seaweed. *Bioscience, Biotechnology, and Biochemistry, 63*, 605–607.

Yoshizawa, Y., Enomoto, A., Todoh, H., Ametani, A., & Kaminogawa, S. (1993). Activation of murine macrophages by polysaccharide fractions from marine algae (Porphyra yezoensis). *Bioscience, Biotechnology, and Biochemistry, 157*, 1862–1866.

Zhang, J., & Wang, Z. (1990). Review on Chinese Traditional Medicine from the sea. *Zhongguo Haiyang Yaowu, 9*, 21–33.

Zhang, P., Wang, Z., Xie, M., Nie, W., & Huang, L. (2010). Detection of carbohydrates using a pre-column derivatization reagent 1-(4-isopropyl) phenyl-3-methyl-5-pyrazolone by high-performance liquid chromatography coupled with electrospray ionization mass spectrometry. *Journal of Chromatography B, 878*, 1135–1144.

Zhou, J., Hu, N., Wu, Y., Pan, Y., & Sun, C. (2008). Preliminary studies on the chemical characterization and antioxidant properties of acidic polysaccharides from Sargassum fusiforme. *Journal of Zhejiang University-Sience B, 9*, 721–727.

Zhu, W., Ooi, W., Chan, P., & Ang, P. (2003). Isolation and characterization of a sulfated polysaccharide from the brown alga Sargassum patens and determination of its anti-herpes activity. *Biochemistry and Cell Biology, 81*, 25–33.