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Pharmacochemistry

Fractionation of Biologically Active Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid] Preparation from *Symphytum asperum*, Simultaneous Determination of Molecular Weights and Contents of its Fractions Using HPSEC-MALLS-RID

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Fractionation and molecular weight distribution of biologically active poly[3-(3,4-dihydroxyphenyl) glyceric acid] (PDPGA)-preparation from *Symphytum asperum* were carried out. Molecular weights and contents of different fractions for PDPGA-preparation were simultaneously determined using high performance size exclusion chromatography (HPSEC) coupled with multi angle laser light scattering (MALLS) and refractive index detector (RID) with the refractive index increment (*dn/dc*). Results suggested that HPSEC-MALLS-RID with the *dn/dc* method could be used as a routine method for quality evaluation of PDPGA-preparations from the species of genera *Symphytum*, *Anchusa*, *Cynoglossum* and *Borago* of Boraginaceae family on the content of biologically active principle PDPGA, polysaccharides impurities and standardization of PDPGA-preparations. Results may contribute to the rational usage of PDPGA-preparations from several species of Boraginaceae family *S. asperum*, *S. caucasicum*, *S. officinale*, *S. grandiflorum*, *Anchusa italica*, *Cynoglossum officinale* and *Borago officinalis* and are beneficial to improve the quality control of PDPGA-preparations. *© 2021 Bull. Georg. Natl. Acad. Sci.*

Poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], poly[3-(3,4-dihydroxyphenyl)glyceric acid], Symphytum asperum, refractive index increment, high performance size exclusion chromatography, multi angle laser light scattering. molecular weight distribution

In our previous data of liquid-state ^{1}H, ^{13}C NMR, 2D $^{1}H/^{13}C$ HSQC, 2D DOSY and solid-state ^{13}C NMR spectra it was found that the main chemical constituent of crude polysaccharides from *Symphytum asperum, S. caucasicum, S. officinale, S. grandiflorum, Anchusa italica, Cynoglossum officinale* [1] and *Borago officinalis* (unpublished) (Boraginaceae) is biologically active [2] caffeic

acid-derived polyether, namely poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] **(**PDPGA**)** (Fig. 1).

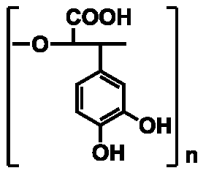

Fig. 1. Poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA).

Recently, a high performance size exclusion chromatography coupled with multi angle laser light scattering and refractive index detector (HPSEC-MALLS-RID) with the universal refractive index increment (*dn/dc*) method has been developed. This method can be utilized for the direct determination of contents based on the concentration-specific refractive index increment equation without reference standard and calibration curves [3]. In this study this method has been applied for quantitative analysis of PDPGA-preparation and its fractions. The molecular masses and contents of fractions for PDPGA-preparation from *S. asperum* were simultaneously determined using HPSEC-MALLS-RID with the *dn/dc*.

Materials and Methods

PDPGA-preparation of *S. asperum* was obtained as discribed in earlier work [4]. The molecular weights (*Mw*), polydispersity (*Mw/Mn*), contents of PDPGA-preparations and their fractions were measured using HPSEC-MALLS-RID with the *dn/dc* method according to previous reported method [3]. In brief, HPSEC-MALLS-RID measurements were carried out on a multi angle laser light scattering detector (MALLS, DAWN HELEOS, Wyatt Technology Co., Santa Barbara, CA, USA) with an Agilent 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA). The separation of PDPGA-preparation was performed on column of TSK-Gel G6000PWXL (300 mm×7.5 mm, i.d.) at 35°C. The MALLS instrument was equipped with a He-Ne laser (λ=658 nm). An Optilab rEX refractometer (RID, DAWN EOS, Wyatt Technology Co., Santa Barbara, CA, USA) was simultaneously connected. The mobile phases were: 1) 0.9% NaCl aqueous solution, 2) 0.9% NaCl aqueous solution and added with Na_2CO_3, 3) 0.9% NaCl aqueous solution and added with NaOH, 4) H_2O, 5) 10 mM NaOH aqueous solution, 6) 100 mM NaOH aqueous solution, at a flow rate of 0.5 mL/min. An injection volume of 50 µL was used. The *Mw* was calculated by the Zimm method of static light scattering based on the basic light scattering equation is as follows [3] (1),

$$\frac{K_c}{R_\theta} = \frac{1}{Mw}\left(1 + \frac{16\pi^2 < S^2 > z}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\right) + 2A_2c + ..., \quad (1)$$

where K is an optical constant equal to $[4^2n^2(dn/dc)]^2/(N_A\lambda^4)$; c, the PDPGA-preparation concentration in g/mL; R_θ, the Rayleigh ration; M_w, weight average molecular mass; $<S^2>z$, radius of gyration; λ, the wavelength; n, the refractive index of the solvents. *dn*/*dc*, the refractive index increment of PDPGA-preparation in the solvents. N_A, the Avogadro's number; A_2, the second virial coefficient. The specific refractive index increment (*dn/dc*) of PDPGA-preparation in solvents was measured by RI batch model using an Optilab rEX refractometer at 658 nm and 35°C to be 0.148 mL/g according to a previously reported method [3]. Then the content of fraction of PDPGA-preparation was calculated based on the refractive index difference with *dn/dc* value according to the following equation [3] (2),

$$C_i = \frac{\alpha\left(V_i - V_{i,baseline}\right)}{dn/dc}, \quad (2)$$

where C_i is the concentration of polymer; α is the RID calibration constant (in RI units per volt), which is determined as 3.4756 x 10^{-5} RIU/pixel; V_i and $V_{i,baseline}$ are the RID voltages of sample and baseline, respectively; d_n/d_c is the specific

refractive index increment of PDPGA-preparation, which was determined as 0.148 mL/g by using an Optilab rEX refractometer at 658 nm and 35^{0}C. The Astra software (version 6.0.2, Wyatt Technology Co., Santa Barbara, CA, USA) was utilized for data acquisition and analysis.

All results were expressed as means ± SD.

SEC-MALLS-RID Analysis of PDPGA-Preparation

Sample A:3 mg/mL of sample A was dissolved in mobile phase, 0.9% NaCl aqueous solution, without heating. PDPGA-preparation was poorly dissolved in 0.9% NaCl aqueous solution. Sample B:3 mg/mL of sample B was dissolved in mobile phase, 0.9% NaCl aqueous solution, 60°C for 40 min. Sample C:3 mg/mL sample C was dissolved in 0.9% NaCl aqueous solution and added with Na_2CO_3, 60°C for 40 min. PDPGA-preparation was poorly dissolved in 0.9% NaCl aqueous solution (added with Na_2CO_3). Sample D:3 mg/mL sample D was dissolved in 0.9% NaCl aqueous solution and added with NaOH, 60°C for 40 min.

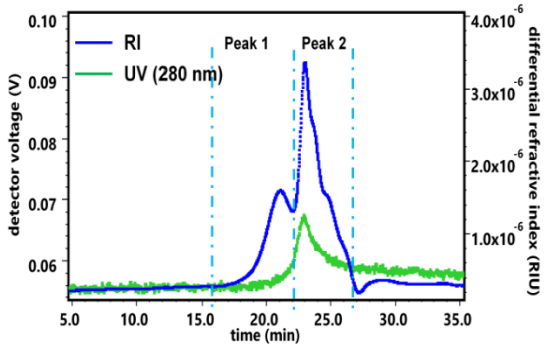

Fig. 2. HPSEC-MALLS-RID analysis of PDPGA-preparation in 0.9% NaCl aqueous solution, without heating (Sample A).

Table 1. The molecular masses (Mw) and polydispersity (Mw/Mn) of fractions (Peak 1, 2) for Sample A (Fig. 2)

	Peak 1	Peak 2
Mw(Da)	2.154×10^5 (±7.028%)	1.457×10^5 (±17.845%)
Mw/Mn	1.063 (±9.655%)	11.214 (±10.865%)

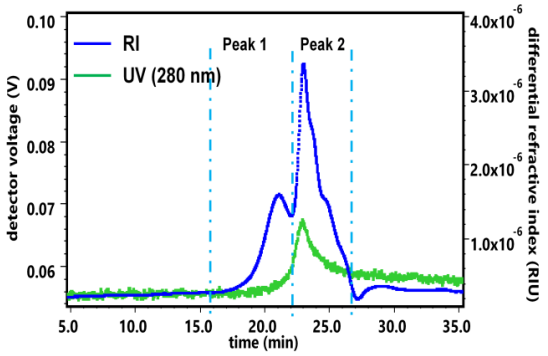

Fig. 3. HPSEC-MALLS-RID analysis of PDPGA-preparation in 0.9% NaCl aqueous solution, 60°C for 40 min. (Sample B).

Table 2. The molecular masses (*Mw*) and polydispersity (*Mw/Mn*) of fractions (Peaks 1, 2) for Sample B (Fig. 3)

	Peak 1	Peak 2
Mw(Da)	8.045×10^4 (±8.173%)	2.089×10^4 (±18.685%)
Mw/Mn	1.219 (±10.463%)	1.269 (±27.551%)

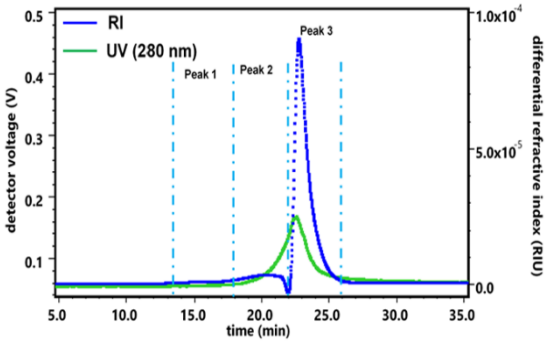

Fig. 4. HPSEC-MALLS-RID analysis of PDPGA-preparation in 0.9% NaCl aqueous solution and added with Na_2CO_3, 60°C for 40 min. (Sample C).

Table 3. The molecular masses (*Mw*) and polydispersity (*Mw/Mn*) of fractions (Peaks 1, 2, 3) for Sample C (Fig. 4)

	Peak 1	Peak 2	Peak 3
Mw (Da)	3.908×10^6 (±0.626%)	3.313×10^5 (±1.04%)	9.749×10^3 (±6.172%)
Mw/Mn	1.107 (±0.901%)	1.518 (±1.505%)	1.564 (±11.090%)

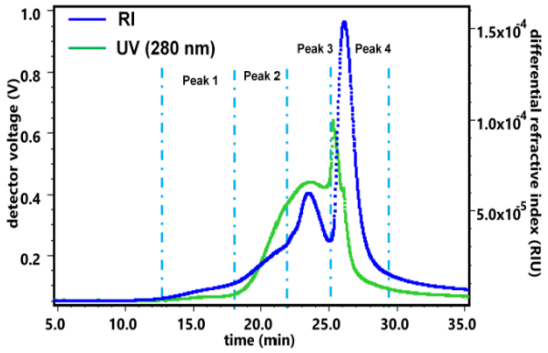

Fig. 5. HPSEC-MALLS-RID analysis of PDPGA-preparation in 0.9% NaCl aqueous solution and added with NaOH, 60°C for 40 min. (Sample D).

Then we tried to increase the solubility of PDPGA-preparation from *S. asperum*. Solvent selection: The PDPGA-preparation could be partially dissolved in water (solution A) and almost completely dissolved in 0.1 M NaOH solution (solution B). The PDPGA-preparation could not be dissolved in organic solvents. Solutions A and B were centrifuged at 6000 g for 10 min. Precipitate in solution B is less than that in solution A. To compare the characters of solution A and B the following analysis was carried out.

Table 4. The molecular masses (*M*w) and polydispersity (*Mw/Mn*) of fractions (Peaks 1, 2, 3, 4) for Sample D (Fig. 5)

	Peak 1	Peak 2	Peak 3	Peak 4
Mw (Da)	3.613×10^{6} (±1.094%)	3.587×10^{6} (±0.710%)	3.436×10^{6} (±1.335%)	1.520×10^{6} (±9.486%)
Mw/Mn	1.048(±1.517%)	1.011(±1.015%)	1.147 (±1.609%)	2.162 (±13.095%)

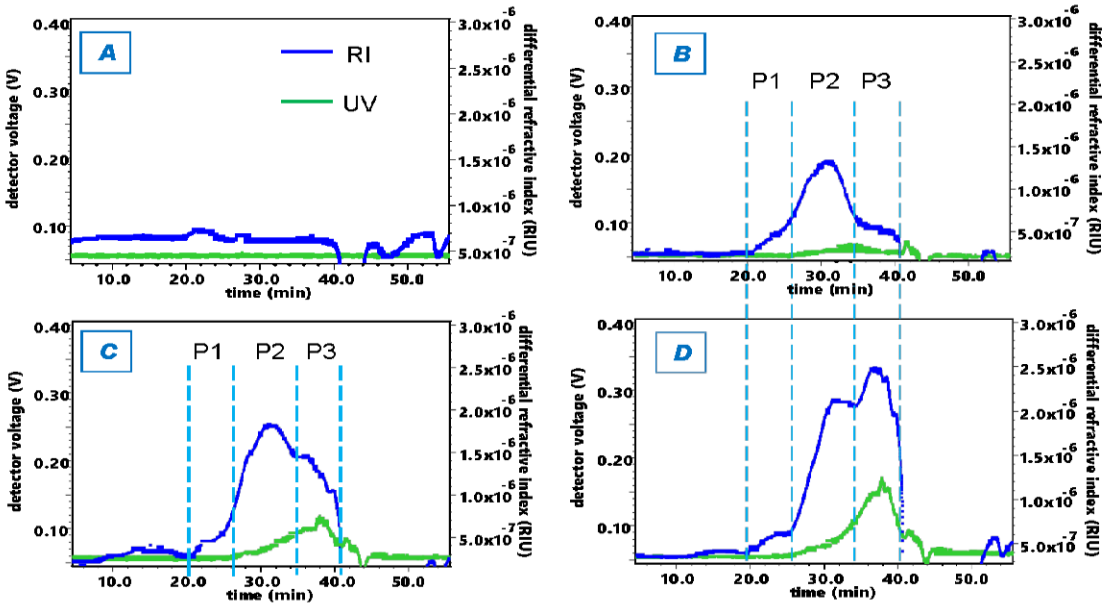

Fig. 6. HPSEC-MALLS-RID analysis of A, B, C and D.

Table 5. The molecular masses (*M*w) of fractions (Peaks 1, 2, 3) for Samples B, C, D (Fig. 6) and contents of Samples B, C, D

	Mw (Da)			Mass (μg)	Soluble mass(%)
	P1	P2	P3		
B	9.177×10^{5} (±6.99%)	1.289×10^{5} (±6.37%)	1.644×10^{5} (±17.42%)	30.0	10
C	1.303×10^{6} (±9.88%)	1.456×10^{5} (±5.07%)	8.883×10^{4} (±17.23%)	56.2	18.7
D	2.950×10^{6} (±2.58%)	2.751×10^{5} (±2.41%)	8.049×10^{4} (±6.76%)	74.9	24.9

HPSEC-MALLS-RID Analysis

A: Blank, 10 mM NaOH; B:3.0 mg/mL PDPGA-preparation in H_2O, 50^0C, 4 h; C:3.0 mg/mL PDPGA-preparation in 10 mM NaOH, 50^0C, 4 h; D:3.0 mg/mL PDPGA-preparation in 100 mM NaOH, 50^0C, 4 h. UV spectra of B,C and D samples had absorption maxima at 212, 236, 286 nm.

Thus, soluble mass of the sample increased with increasing alkaline concentration (Table 5, Samples B, C and D). UV absorption was mainly derived from fraction of P3 (Fig. 6, Samples B,C and D).

Conclusion

Molecular weights and contents of biologically active poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA)-preparation fractions from *Symphytum asperum* (Boraginaceae family) were simultaneously determined and compared by using HPSEC-MALLS-RID with the *dn/dc* method. This method is one of the efficient and powerful techniques for analysis of the molecular weight and molecular weight distribution of polymers from natural resources. Thus, obtained results suggested that HPSEC-MALLS-RID with the *dn/dc* method could be used as a routine method for quality evaluation of PDPGA-preparations from natural resources on the content of biologically active principle PDPGA, polysaccharides impurities and standardization of PDPGA-preparations. Results may contribute to the rational usage of PDPGA-preparations from several species of genera *Symphytum, Anchusa, Cynoglossum* and *Borago* (Boraginaceae family) and are beneficial to improve the quality control of preparations.

ფარმაკოქიმია

Symphytum asperum-ის ბიოლოგიურად აქტიური პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] პრეპარატის ფრაქციონირება, ფრაქციების შემცველობის და მოლეკულური წონების ერთდროული განსაზღვრა – HPSEC-MALLS-RID-ის გამოყენებით

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**ჩინური მედიცინის ხარისხის კვლევის სახელმწიფო საკვანძო ლაბორატორია, ჩინეთის მედიცინის მეცნიერებათა ინსტიტუტი, მაკაუს უნივერსიტეტი, მაკაო, ჩინეთი

§აკადემიის წევრი, ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ფიზიკური და ანალიზური ქიმიის ინსტიტუტი, ზუსტი და საბუნებისმეტყველო მეცნიერებების სკოლა, თბილისი, საქართველო

განხორციელდა Symphytum asperum–ის ბიოლოგიურად აქტიური პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] (პდფგმ) პრეპარატის ფრაქციონირება და მოლეკულურ-წონითი განაწილება. პდფგმ-ის პრეპარატის სხვადასხვა ფრაქციის შემცველობა და მოლეკულური წონის ერთდროული განსაზღვრა განხორციელდა მაღალეფექტური ზომის ექსკლუზიური ქრომატოგრაფიის (მეზექ) გამოყენებით, რომელიც შეწყვილებული იყო მრავალკუთხოვანი ლაზერული სინათლის გაფანტვის (მკლსგ) და რეფრაქციის ინდექსის დეტექტორთან (რიდ) რეფრაქციის ინდექსის ზრდით (*dn/dc*). მიღებული შედეგების თანახმად მეზექ-მკლსგ-რიდ *dn/dc* შეიძლება გამოყენებულ იქნეს, როგორც რუტინული მეთოდი Boraginaceae-ს ოჯახის *Symphytum*-ის, *Anchusa*-ს, *Cynoglossum*-ის და *Borago*-ს გვარების სახეობების პდფგმ–ის პრეპარატების ხარისხის შეფასებისას ბიოლოგიურად აქტიური მოქმედი საწყისის შემცველობაზე, ნარჩენი პოლისაქარიდების არსებობაზე და პდფგმ-ის პრეპარატების სტანდარტიზაციისათვის. მიღებულმა შედეგებმა შეიძლება ხელი შეუწყოს Boraginaceae ოჯახის სხვადასხვა სახეობის *S. asperum*-ის, *S. caucasicum*-ის, *S. officinale*-ის, *S. grandiflorum*-ის, *Anchusa italica*-ს, *Cynoglossum officinale*-ის და *Borago officinalis*-ის პდფგმ-ის პრეპარატების რაციონალურ გამოყენებას და მნიშვნელოვანი წვლილი შეიტანოს აღნიშნული პრეპარატების ხარისხის კონტროლის გაუმჯობესებაში.

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