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Analytical Chemistry

Spectrophotomertric Quantitative Determination of Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid]

Vakhtang Barbakadze*, Lali Gogilashvili*, Lela Amiranashvili*, Maia Merlani*, Karen Mulkijanyan*

* *I. Kutateladze Institute of Pharmacochemistry, Tbilisi,*

(Presented by Academy Member Devi Ugrekhelidze)

ABSTRACT. A simple, fast, sensitive, reliable method for the spectrophotometric quantitative determination of poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) based on the measurement of PDPGA at 286 nm has been developed. The method is easy to operate, shows a good reproducibility and thus can be used as a routine detection technique in serial experiments. *© 2010 Bull. Georg. Natl. Acad. Sci.*

Key words: *poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly-[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)-ethylene], spectrophotometric determination*

In our previous studies we isolated crude polysaccharides (CP) from the roots and stems of *Symphytum asperum* (CP-SAR, CP-SAS, respectively) and *S. caucasicum* (CP-SCR, CP-SCS, respectively), from the roots of *S. officinale* (CP-SOR), and from the roots of *Anchusa italica* (CP-AIR) [1-4]). Further fractionation by ultrafiltration on the membrane filters with cut-off values of 1000 kDa or 500 kDa for CP-SAR, CP-SAS, CP-SCR, CP-SCS and CP-AIR led to the elimination of most ballast polysaccharides and obtaining high-molecular (HM) (>1000 kDa or >500 kDa) preparations: HM-SAR (>1000 kDa), HM-SAS (>1000 kDa), HM-SCR (>1000 kDa), HM-SCS (>1000 kDa), HM-SCR (>500 kDa) and HM-AIR (>500 kDa) [3-6]. The main chemical constituent of all these high-molecular fractions is caffeic acid-derived polymer, namely poly[3-(3,4-dihydroxyphenyl) glyceric acid] (PDPGA) or poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] (Fig. 1) [3,4,6-9].

This compound is a representative of a new class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit. This polymer possesses diverse biological activity [9-14].

Due to relative novelty of PDPGA no information was available on the quantitative analysis of such a polyether. Therefore, the aim of the current work was the development of a simple, sensitive and economical spectrophotometric method for quantitative determination of PDPGA, which does not need expensive instruments and lengthy procedure.

Results and Discussion. All the above-mentioned HM preparations contained residual polysaccharide

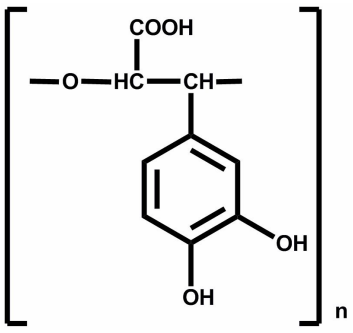

Fig. 1. Poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA)

admixtures and have characteristic absorption maximum at 286 nm both in water and in sodium phosphate buffer, pH 6.8 and veronal-saline buffer, pH 7.35 [5,9]. HM-SAR (>1000 kDa) partially dissolved in barbital buffer, pH 7.35, and after centrifugation got HM-SAR (>1000 kDa supernat.) (60 %) and HM-SAR (>1000 kDa pellet) (27 %) [5]. HM-SAR (>1000 kDa supernat.) was additionally fractionated by gel-filtration chromatography on a Sepharose 2B column. These fractionation procedures did not allow us to remove an admixture of polysaccharides. HM-SAR (>1000 kDa supernat.) still contained some amounts of carbohydrates (25.7 %) and protein (6.68 %) admixtures [5].

In order to develop spectrophotometric quantitative determination of PDPGA we could not use as standard pure synthetic monomer 3-(3,4-dihydroxyphenyl)glyceric acid, as its characteristic bands at 230 and 278 nm are shifted about 7-8 nm towards lower wavelengths than the absorption maxima of natural polymer [15]. That is why we decided to use HM-SAR (>1000 kDa supernat.) as a standard for the plotting of a standard curve on the characteristic absorption maximum at 286 nm. Among the above-listed natural preparations, which contain the PDPGA as a main component, the HM-SAR (>1000 kDa supernat.) is the most pure and soluble. Polysaccharides contents cannot contribute to absorption in UV as sugars have no chromophores. A small amount of protein, which has absorption maximum at 280 nm, can make an insignificant contribution in the total absorption at 286 nm. To prepare a stock standard solution from HM-SAR (>1000 kDa supernat.) we carried out conversion to PDPGA, taking into consideration the contents of carbohydrates and protein admixtures and deducting them from the total weight of HM-SAR (>1000 kDa supernat.).

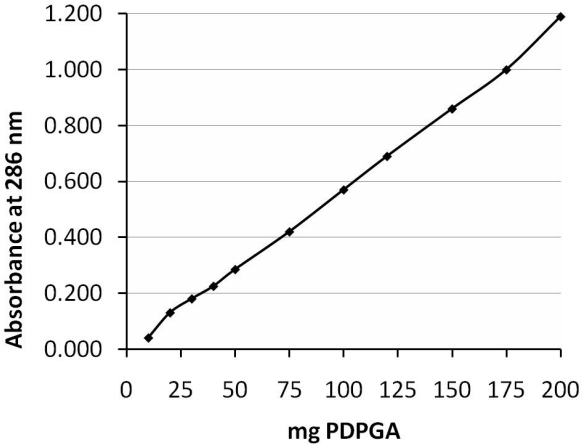

Fig. 2. Calibration graph for PDPGA

There is a linear relation between the absorptivity and the PDPGA content at the characteristic maximum absorption (286 nm). The standard curve obeys the Lambert-Beer law in the range of 30 - 150 μg/ml (Fig. 2).

The calibration curve of PDPGA was prepared by varying the amount of PDPGA in the range of 10-200 ìg/ml and is shown in Fig. 2. It is clear from the standard curve that absorbance is directly proportional to the PDPGA concentration. This method is very simple, fast, sensitive, reliable, easy to operate and less time consuming, allowing to analyze much more samples. It shows a good reproducibility and can be used as a routine detection technique in serial experiments.

The concentration of PDPGA in CP and HM preparations of *S. asperum, S. caucasicum, S. officinale* and *Anchusa italica* was determined. PDPGA content of samples is given in Table.

Table.

The concentration of PDPGA in different preparations of *S. asperum, S. caucasicum, S. officinale* and *Anchusa italica*.

Sample	Concentration, mean±SD (standard deviation, n=8), %	Coefficient of variation, %
1. CP-SAR	25.30 ± 1.06	4.19
2. CP-SAS	36.31 ± 1.51	4.16
3. CP-SCR	26.13 ± 1.21	4.64
4. CP-SCS	34.88 ± 2.08	5.96
5. CP-SOR	31.38 ± 1.92	6.13
6. CP-AIR	44.48 ± 0.95	2.13
7. HM-SAR (>1000 kDa, pellet)	68.25 ± 2.79	4.09
8. HM-SAS (>1000 kDa)	46.75 ± 3.62	7.73
9. HM-SCR (>1000 kDa)	49.13 ± 4.45	9.07
10. HM-SCR (>500 kDa)	45.25 ± 4.21	9.30
11. HM-SCS (>1000 kDa)	37.88 ± 2.59	6.83
12. HM-AIR (>500 kDa)	57.18 ± 3.36	5.88

Evaluation of the relative precision of the method is given in Table 1. Coefficients of variations in all cases are less than 10% and, therefore the proposed method can be considered as quite precise.

Materials and Methods. *Apparatus.* A DU 520 UV/Vis spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) was used for the measurement of absorbance at 286 nm.

Preparation of standard stock solution and calibration graph

To prepare stock standard solution containing 1 mg/ml PDPGA we took 36.97 mg HM-SAR (>1000 kDa supernat.) which contained 67.62 % PDPGA and dissolved it in 25 ml of distilled water, using a volumetric flask. 2.5 ml of stock standard solution was further diluted to 25 ml with water which contained 100 μg/ml of PDPGA. A calibration graph for the determination of PDPGA was prepared by varying the amount of PDPGA in the range 10 – 200 μg (Fig. 2). In order to obtain solutions with 10, 20, 30, 40 etc 200 ìg/ml PDPGA we took 0.1, 0.2, 0.3, 0.4 etc. 2 ml of 100 ìg/ml PDPGA and added 2.9, 2.8, 2.7, 2.6 etc 1 ml water, respectively. The regression equation was $y = 0.0058x - 0.0073$, where y is the absorbance and x is the amount of PDPGA in micrograms. The linear correlation coefficient $r = 0.9993$, the coefficient of determination $r^2 = 0.9986$.

Preparation of samples. Each sample used for determination of PDPGA at 286 nm was dissolved in distilled water prepared solution with concentration 1 mg/ml.

Conclusion. The spectrophotometric method based on the measurement of PDPGA at 286 nm could be applied for PDPGA determination in various samples of CPs and HM preparations. The information gained from these measurements will help in establishing a baseline analysis of PDPGA of plant HM preparations. This method is simple, rapid, economical as well as sensitive and does not need expensive instrumentation.

ანალიზური ქიმია

პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] სპექტროფოტომეტრული რაოდენობრივი განსაზღვრის მეთოდი

ვ. ბარბაქაძე*, ლ. გოგილაშვილი*, ლ. ამირანაშვილი*, მ. მერლანი*, კ. მულკიჯანიანი*

** ი. ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი*

(წარმოდგენილია აკადემიის წევრის დ. უგრეხელიძის მიერ)

დამუშავებულია პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] (პდფგმ) სპექტროფოტომეტრიული რაოდენობრივი განსაზღვრის მეთოდი, რომელიც დაფუძნებულია პდფგმ-ის გაზომვაზე 286 ნმ-ზე. მეთოდი არის მარტივი, სწრაფი, მგრძნობიარე, საიმედო, ადვილად შესრულებადი; აჩვენებს კარგ განმეორებადობას და შესაძლებელია გამოყენებული იქნას როგორც რუტინული დეტექტირების მეთოდი სერიულ ექსპერიმენტებში.

REFERENCES

1. *V.V.Barbakadze, R.A.Gakhokidze Z.S.Shengelia, A.I.Usov* (1989), Chem. Nat. Compds., **25,** 3: 281-286.
2. *V.V.Barbakadze, E.P.Kemertelidze, G.E.Dekanosidze, T.G.Beruchashvili, A.I.Usov* (1992), Bioorg. Khim., **18,** 5: 671-679 (in Russian).
3. *V. Barbakadze, A.J.J. van den Berg, C.J. Beukelman, J. Kemmink, H.C. Quarles van Ufford* (2009), Chem. Nat. Compds., **45,** 1: 6-10.
4. *V.Barbakadze, L.Gogilashvili, L.Amiranashvili, M.Merlani, K.Mulkijanyan, M. Churadze, A. Salgado, B. Chankvetadze* (2010), Natur. Prod. Communs., **5**, 7: 1091-1095.
5. *V. Barbakadze, E. Kemertelidze, A.I. Usov, B.H. Kroes, H.C. Quarles van Ufford, E. van den Worm, C.J. Beukelman, A.A.J. van den Berg, R.P. Labadie* (1999), Proc. Georg. Acad. Sci., Biol. Ser., **25,** 3: 207-216.
6. *V.Barbakadze, E.P.Kemertelidze, I.Targamadze, K.Mulkijanyan, J.Kemmink, A.J.J. van den Berg, C.J.Beukelman, A.I.Usov* (2005), Chem. Nat. Compds., **41,** 4: 374-377.
7. *V.V.Barbakadze, E.P.Kemertelidze, A.S.Shashkov, A.I.Usov* (2000), Mendel. Communs., **10,** 4: 148-149.
8. *V.V.Barbakadze, E.P.Kemertelidze, I.L.Targamadze, A.S.Shashkov, A.I.Usov* (2002), Rus. J. Bioorg. Chem., **28,** 4: 326-330.
9. *V.Barbakadze, E.Kemertelidze, I.Targamadze, K.Mulkijanyan, A.S.Shashkov, A. I.Usov* (2005), Molecules, **10,** 9: 1135-1144.
10. *C.M.Barthomeuf, E.Debiton, V.V.Barbakadze, E.P.Kemertelidze* (2001), J. Agric. Food Chem., **49,** 8: 3942-3946.
11. *V.V.Barbakadze, E.P.Kemertelidze, K.G.Mulkijanyan, A.J.J.van den Berg, C.J.Beukelman, E.van den Worm, H.C.Quarles van Ufford, A.I.Usov* (2007), Pharm. Chem. J., **41,** 1: 14-16.
12. *L.Kardava, Kh.Gabunia, M.Tevzadze, V.Barbakadze, D.Ghirdaladze, G.Iosava N.Porakishvili* (2000), Bull. Georg. Acad. Sci., **162,** 4: 47-50.
13. *L.Kardava, N.Kulikova, M.Tevzadze, Kh.Gabunia, V.Barbakadze, D.Ghirdaladze, G.Iosava, N.Porakishvili* (2001), Proc. Georg. Acad. Sci., **27,** 4-6: 465-470.
14. *V.Barbakadze, K.Mulkijanyan, M.Merlani, L.Gogilashvili, L.Amiranashvili, F.Vidal-Vanaclocha* (2008), Bull. Georg. Natl. Acad. Sci., **2,** 3: 108-112.
15. *M.Merlani, V.Barbakadze, L.Amiranashvili, L.Gogilashvili, E.Yannakopoulou, K.Papadopoulos, B.Chankvetadze* (2010), Chirality, **22,** 8: 717-725.

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