Asian Journal of Chemistry Vol. 18, No. 2 (2006), 1534–1536

NOTE

Quantification of Oligomeric Proanthocyanidins in Grapes Seed Extract by Spectrophotometric Method

H.N. SHIVAPRASAD* and SALMA KHANAM†
Department of Pharmacognosy and Phytochemistry, PES College of Pharmacy
Hanumanthanagar, Bangalore-560 050, India
Fax: (91)(80)26506928; Tel.: 09448517387 [M], (91)(80)26507428 [O]
E-mail: shivaprasad_hn@rediffmail.com

Oligomeric proanthocyanidin complexes are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers and bark. Proanthocyanidins belong to the category known as condensed tannins, one of the two main categories of plant tannins. In the present communication we have developed a simple UV spectrophotometric method for the analysis of proanthocyanidins in grape seed extract.

Key Words: Spectrophotometric method, Oligomeric proanthocyanidins, Grape seeds.

Proanthocyanidins are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers and bark[1]. Proanthocyanidins belong to the category known as condensed tannins, one of the two main categories of plant tannins. These are high molecular weight polymers comprised of the monomeric unit flavan-3-ol {(+) catechin and (–) epicatechin} that are linked through C_4-C_6 or C_4-C_8 units[2].

Proanthocyanidins, being short chains of catechin subunits, are also called oligomeric condensed tannins and are occasionally referred to as oligomeric proanthocyanidin complexes (OPCs).

Synonyms for proanthocyanidins are leucocyanidins, pycnogenols, procyanidins, oligomeric proanthocyanidins (OPCs) and proanthocyanidins (PACs). Grape seeds are obtained from the fruits of *Vitis vinifera* belonging to the family Vitaceae. Grape skin contains oligomers of polymers of catechin and epicatechin in abundant amount. In seeds these are called as oligomeric proanthocyanidin complex (OPCs).

These oligomeric proanthocyanidins (OPCs) are not easily detected by

†Department of Pharmacognosy, Al-Ameen College of Pharmacy, Hosur Road, Bangalore-560 027, India.

reversed-phase HPLC[3]. The review of literature reveals that although reversed-phase C_{18} columns have the ability to separate oligomers of equivalent molecular mass into their isomers, analysis of higher oligomeric proanthocyanidins is not feasible due to the fact that, with increasing degrees of polymerization, the number of isomers also increases[4]. This effect results in a retention time overlap of isomers containing differing degrees of polymerization, causing the oligomers to co-elute as a large unresolved peak[5]. Hence in the present communication we report a simple, UV spectrophotometric method for the analysis of pro-anthocyanidin in grapes seed extract.

Reagents: Butanol-HCl reagent

0.7 g of iron(II) sulfate heptahydrate was dissolved in 25 mL of HCl and 500 mL of *n*-butanol was added and sonicated till all the iron(II) sulfate heptahydrate dissolved. Finally volume was made up to 1000 mL with *n*-butanol.

Standard preparation: 50 mg of standard grape seed extract containing 95% of proanthocyanidins (obtained from Sami Labs, Bangalore) was dissolved in 100 mL of methanol and sonicated till it dissolved. From this solution 5 mL was further diluted to 50 mL with methanol.

Sample preparation: 50 mg of grape seed extract (GSE, obtained from a local herbal extract agency) was dissolved in 100 mL of methanol and sonicated till it dissolved. From this solution 5 mL was further diluted to 50 mL with methanol.

Procedure: Hydrolysis of standard and sample solutions: To 2 mL of standard and sample solutions, 10 mL of *n*-butanol HCl reagent was added and heated at 105°C on an oil bath for 1 h, cooled and volume was made up to 25 mL with *n*-butanol HCl reagent.

The absorbance maxima of the standard and sample were first determined. The spectra are shown in Figs. 1 and 2.

Absorbances of standard and sample solutions were measured spectrophotometically at 550 nm using *n*-butanol HCl reagent as blank.

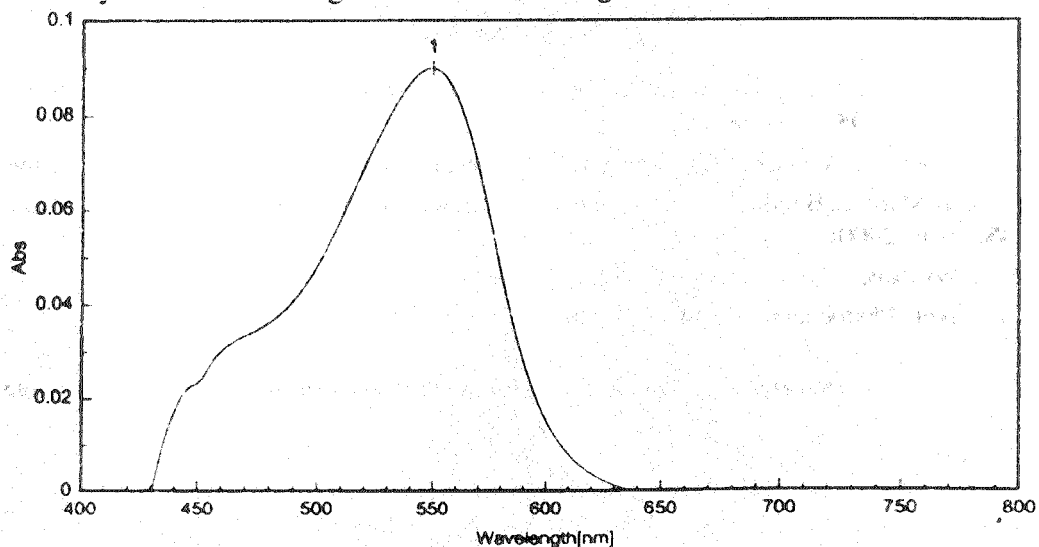

Fig. 1. Chromatogram of standard grape seed extract (GSE)

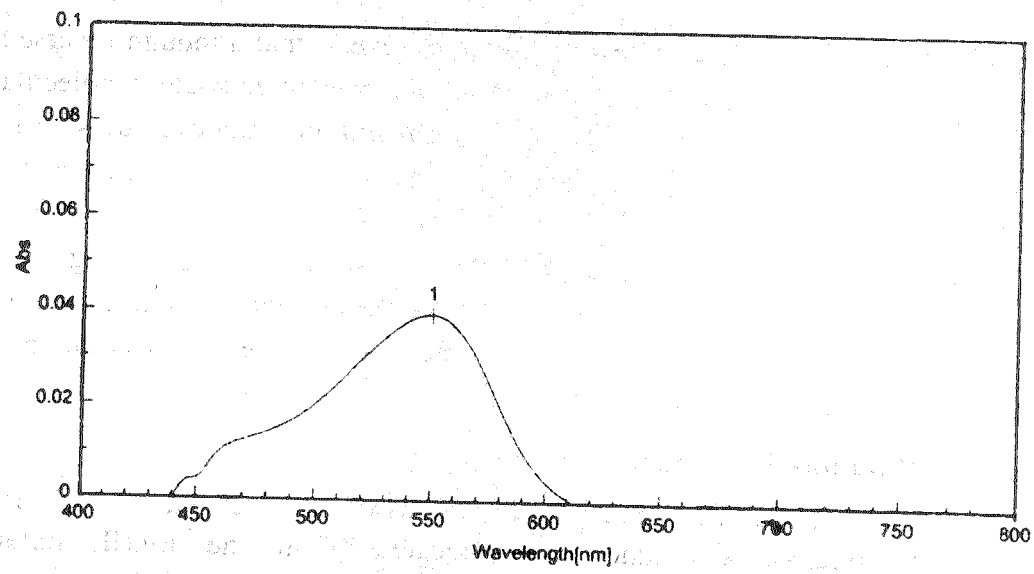

Fig. 2. Chromatogram of sample grape seed extract [GS-I]

% of proanthocyanidins can be calculated by using the following formula:

$$\frac{\text{Abs of sample} \times \text{Concentration of standard} \times \text{Assay of standard}}{\text{Abs of standard} \times \text{Concentration of sample} \times (100 - \text{LOD})} \times 100$$

In our study sample grape seed extract [G.S-I] showed 35.8% of OPCs compared to that of standard grape seed extract [GSE-94.5%] (Table-1) procured form Sami Labs, Bangalore.

TABLE-1
PERCENTAGE OF OPCs IN GRAPE SEED EXTRACTS
BY SPECTROPHOTOMETRIC METHOD

Extracts	% OPCs
GSE [Standard]	94.5
G.S-I [Sample]	35.8

REFERENCES

1. D. Bagchi, A. Garg, R.L. Krohn, M.X. Tran and S.J. Stohs, *Res. Commun. Mol. Pathol. Pharmacol.*, **95**, 179 (1997).
2. A.L. Sheryl, E.A. Garry, F.H. John and H.S. Harold, *J. Agri. Food Chem.*, **43**, 3693 (1999).
3. S. Jean-Marc, L. Benoit, L.G. Christine, C. Veronique and M. Michel, *J. Agric. Food Chem.*, **48**, 1076 (2000).
4. E.L. Willson, *J. Sci. Food Agric.*, **32**, 257 (1981).
5. S. Guyot, *Phytochemistry*, **44**, 351 (1997).

(*Received*: 31 January; *Accepted*: 31 December 2005) AJC-4609