A chromatography–densitometry method for quantitative analysis of sugars of flavonoid glycosides

Quantitative analysis of the sugars of naturally occurring flavonoid glycosides is often needed in elucidation of the structures of these compounds. An improved method for quantitative determination of the microgram quantities of sugars obtained by hydrolysis of one milligram or less of pure flavonoid glycoside has recently been developed in our laboratory. In this method the sugars are separated by thin-layer chromatography on silica gel and individually quantitated directly on the chromatogram by determination of the density of the spots produced when the plate is sprayed with aniline–oxalic acid reagent[1]. This procedure is both less tedious and more reliable than methods which we have previously employed[2,3]. It appears generally applicable for determination of hexoses; we have employed it mainly for determination of rhamnose and glucose, the sugars occurring most commonly in flavonoid glycosides.

In a typical analysis, 0.25–1.0 mg of flavonoid glycoside is hydrolyzed by refluxing with 5 ml 3 % hydrochloric acid solution for 1–2 h. The liberated sugars are then separated quantitatively from the aglycone by passing the cooled hydrolysate through a 1.2 cm diameter column packed to a depth of 1.5 cm with an aqueous slurry of Polyclar AT polyvinylpyrrolidone (General Aniline and Film Corp., Grasselli, N.J.). The reaction vessel is rinsed 3 times with 5 ml portions of water which are added to the column, and the column is then washed with 30 ml additional water to elute the sugars. The aglycone, which remains on the column during water elution, is then eluted with 75 ml methanol and its quantity determined spectrophotometrically or by fluorescence.

The aqueous eluate containing the sugars from the Polyclar column is fed directly onto a 1.2 × 5 cm column of Amberlite IR-45 anion exchange resin (OH form), which is then washed with 25 ml additional water to insure complete elution of sugars. The resulting 75 ml of deionized sugar solution are evaporated to dryness in a round bottom flask on a rotary evaporator. The residue is transferred, in a total volume of about 15 ml of water, to a 25 ml conical flask; the solvent is again removed by evaporation; and the residue is dissolved in 500 μl of water. A sufficient quantity of this sugar solution to contain 1–10 μg of each individual sugar (usually 10 μl) is spotted in triplicate on a 200 × 200 mm thin-layer plate coated with a 250 μ layer of Adsorbosil 1 silica gel (Applied Sciences Laboratories, Inc., State College, Pa.). A 5 μg standard each of rhamnose and glucose is spotted in triplicate on the same thin-layer plate. The chromatogram is developed to a distance of 10 cm beyond the origin in *n*-propyl alcohol–ethyl acetate–water (7:1:2, v/v) solvent system and dried for 3 h in an air current. It is then sprayed with 10 ml of aniline–oxalic acid reagent, air dried 10 min, and heated at 105° for 20 min to develop the color.

The densities of the brown-yellow spots produced by reaction of the hexoses with the aniline–oxalic acid reagent are determined by scanning the thin-layer chromatogram with a Photovolt densitometer, Model 530, equipped with an Integraph integrator, Model 49 (Photovolt Corp., New York, N.Y.). A Wratten 47B filter is employed for the phototube to enhance sensitivity to the sugar-reagent spots, and the response selector switch of the recorder is set at position 6. Under these conditions, the density of the sugar-reagent spots on the chromatogram is a linear function of sugar concentration in the range 1–10 μg of rhamnose or glucose. The quan-

tities of these sugars in the sample are thus determined by comparing densities of standard and sample spots. Duplicate thin-layer chromatograms are usually run for each sample to minimize errors due to individual plate variation.

TABLE I

DETERMINATION OF SUGARS IN NARINGIN SAMPLES BY THE CHROMATOGRAPHY–DENSITOMETRY METHOD

Sample	*Quantities (μg) determined by chromatography-densitometry method*		*Quantities (μg) calculated from sample weight*		*Ratio of determined to calculated values*	
	Glucose	*Rhamnose*	*Glucose*	*Rhamnose*	*Glucose*	*Rhamnose*
1	263	267	292	269	0.90	0.99
2	296	288	300	273	0.99	1.05
3	288	245	299	272	0.96	0.90

Table I shows results obtained when this method was employed for determination of rhamnose and glucose in three samples consisting of approximately 1 mg each of authentic naringin. The method has been successfully applied in our laboratory for quantitative determination of sugars in previously unidentified flavanone triglycosides which were available only in very small quantities. The thin-layer chromatography–densitometry procedure employed in this method should be applicable for quantitative determination of microgram quantities of free hexoses occurring in small samples of biological materials as well as for quantitative determination of hexoses constituting the sugar moieties of other compounds in addition to the flavonoid glycosides.

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1 S. M. PARTRIDGE, *Biochem. Soc. Symp. (Cambridge, Engl.)*, 3 (1950) 52.
2 C. W. NYSTROM, W. L. HOWARD AND S. H. WENDER, *J. Org. Chem.*, 22 (1957) 1272.
3 C. PRATT AND S. H. WENDER, *J. Am. Oil Chemists' Soc.*, 38 (1961) 403.

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