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Note

Analysis of gallic, digallic and trigallic acids in tannic acids by high-performance liquid chromatography

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Tannic acids belong to the hydrolyzable group of tannins[1]. Four commercially available tannic acids are Alep (from Turkish or Middle East gall nuts, *Quercus infectoria*), Chinese (from Chinese or Korean gall nuts, *Rhus semialata*), Sumac (from the leaves of *Rhus cotynus* or *Rhus coriaria*, Bulgaria or mediterranean region) and Tara (from the pods of *Caesalpinia spinosa*). The first three are polygalloylglucoses and the fourth is a polygalloyl quinic acid. From partial hydrolyzates of Chinese tannic acid we have previously isolated digallic and trigallic acids, and elucidated their structures and some properties[2,3]. With these reference compounds it is now possible to analyse tannic acids by co-chromatography. This analysis is of importance to the brewing industry for which the concentration of gallic acids and its oligomers should be as low as possible.

EXPERIMENTAL

Chromatography

A Varian 5020 liquid chromatograph equipped with a Valco 7000-p.s.i. 10-μl injector and 101 CDS Varian data system were used. The normal phase column (25 × 0.46 cm) was packed with extra-demineralized 5-μm ROSiL (a spherical silica gel from Alltech-RSL) and eluted with a gradient from solvent A (hexane, Burdick & Jackson) to solvent B (methanol–tetrahydrofuran, 75:25; Burdick & Jackson) containing 0.25% citric acid: at t = 0, A:B = 80:20; then in 15 min to A:B = 50:50; then in 30 min to A:B = 35:65. Flow-rate: 1 ml/min. Detector: Varichrom 50 at 280 nm.

The reversed-phase column (15 × 0.46 cm) was packed with 5-μm ROSiL-C_{18}-HL-D (a spherical octadecylated silica gel from Alltech-RSL), and eluted with a gradient from solvent A (water with 0.5% phosphoric acid) to solvent B [methanol (Burdick & Jackson) with 0.5% phosphoric acid]: at t = 0, A:B = 90:10; changed over 30 min to 100% solvent B. Flow-rate: 1 ml/min. Detector: Varichrom 50 at 280 nm.

Quantitation and calibration

Calibration with a constant amount of 2,3,4-trihydroxybenzoic acid and a varying quantity of reference pure gallic acid and its oligomers gave linear calibration

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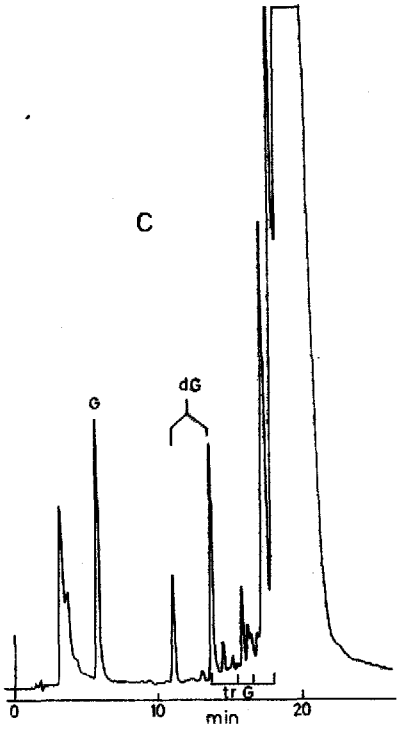

Fig. 1. Reversed-phase HPLC of Chinese tannic acid on a 5-μm ROSiL-C_{18}-HL-D column (15 × 0.46 cm). For details see text. In this and other chromatograms: I.S. = internal standard; G = gallic acid; dG = digallic acid; trG = trigallic acid.

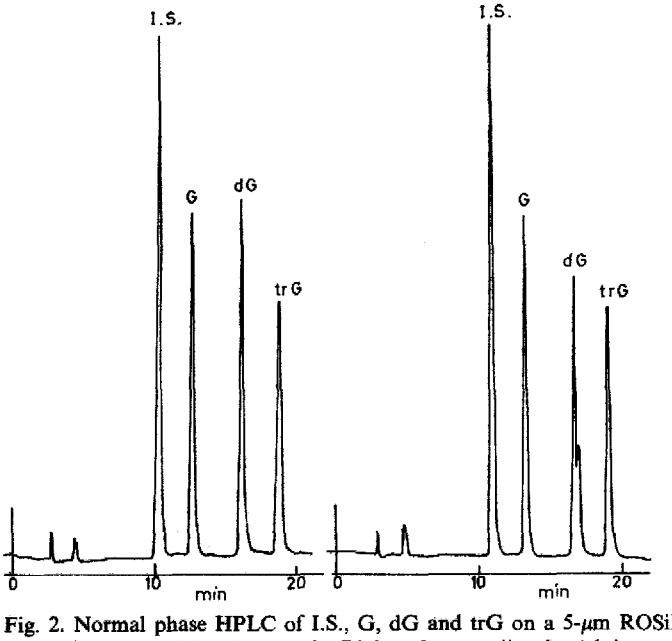

Fig. 2. Normal phase HPLC of I.S., G, dG and trG on a 5-μm ROSiL column (25 × 0.46 cm). Left: directly after dissolving the sample. Right: after standing for 1 h in methanol.

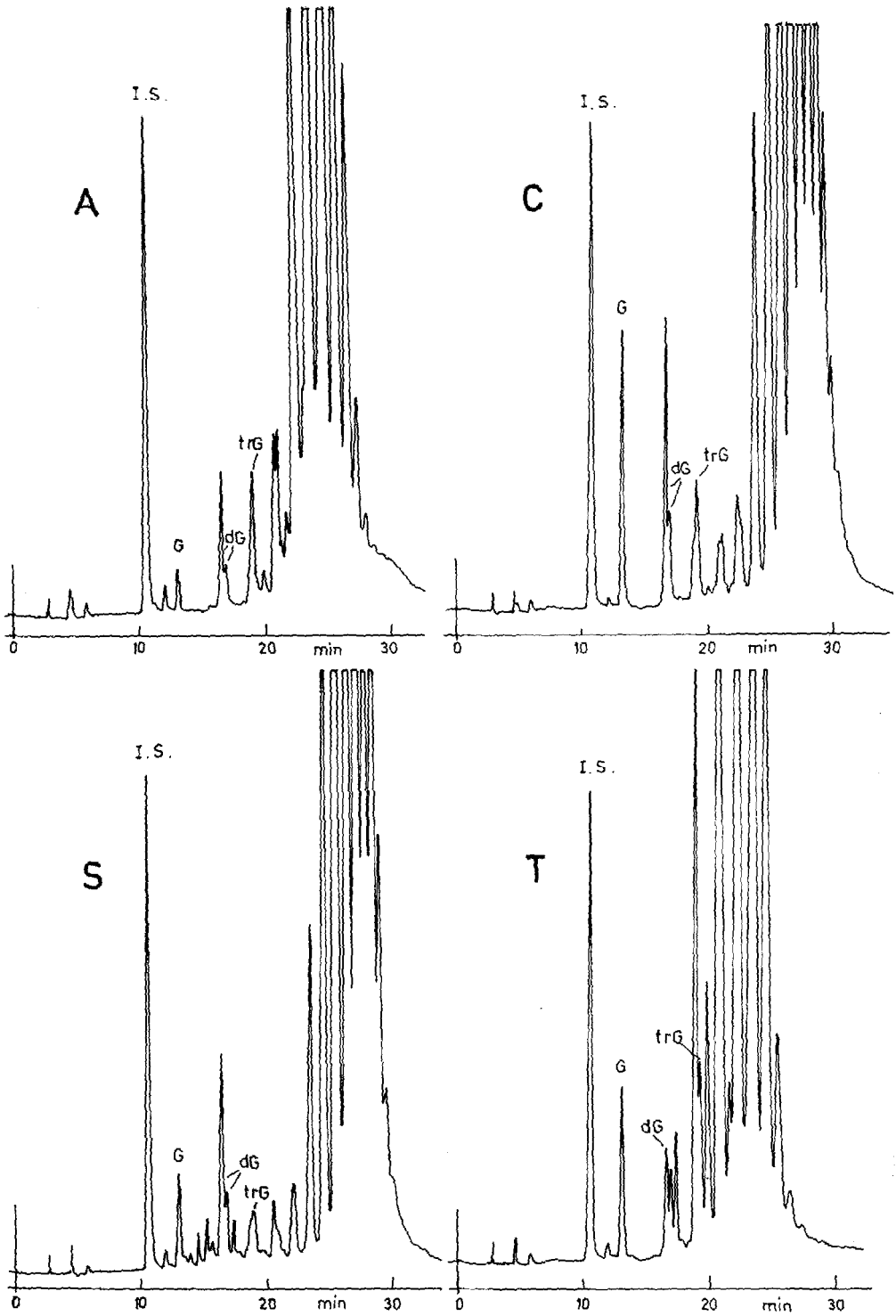

Fig. 3. Normal-phase HPLC as in Fig. 2 of four commercial tannic acids with high sample loading and sensitive detector setting: A, Alep; C, Chinese; S, Sumac; T, Tara.

graphs. The corresponding equations are

gallic acid $y = 0.88\ x$
digallic acid $y = 0.93\ x$
trigallic acid $y = 0.89\ x$

where y is the ratio of the chromatographic peak areas of the sample and internal standard and x is the ratio of the corresponding amounts.

RESULTS

Reversed-phase high-performance liquid chromatography (HPLC) of the reference compounds produces two and four peaks respectively[2,3]. The position of these peaks is indicated in Fig. 1 for a Chinese tannic acid. A high sample loading is needed because gallic, digallic and trigallic acids are only present in very small quantities (around 1 %) in the tannic acids investigated. While the gallic acid and the two digallic acid peaks are well separated from the main peak of tannic acids, this is not the case for the four peaks of trigallic acid. Reversed-phase HPLC is therefore not the method of choice for this purpose.

In normal-phase HPLC the acids in question produce only one peak each at least when analyzed immediately after dissolving the samples in methanol. After standing in this solvent for 1 h, digallic acid shows a close doublet (Fig. 2). Shifting of

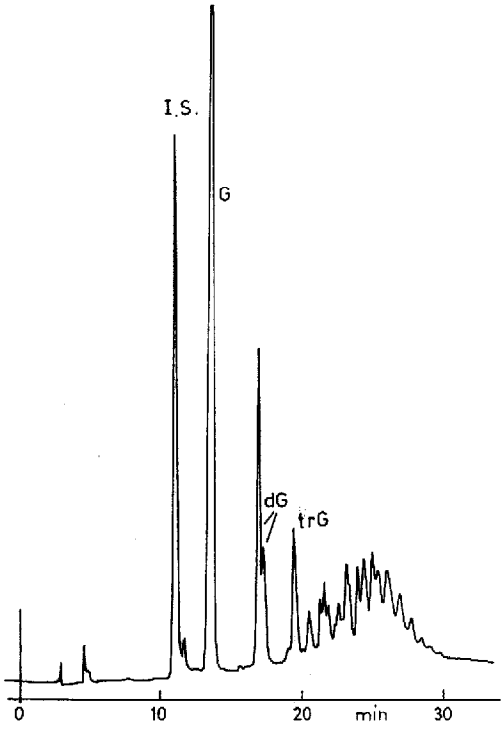

Fig. 4. Normal-phase HPLC as in Fig. 2 of a partial hydrolyzate of Chinese tannic acid. Further details in legends of other figures and in the text.

TABLE I

HPLC ANALYSIS (%) OF TANNIC ACIDS

	Gallic acid	*Digallic acid*	*Trigallic acid*
Alep	0.3	1.0	1.2
Chinese	1.7	1.8	1.1
Sumac	0.6	1.6	0.7
Tara	1.2	0.6	1.3
Partially hydrolyzed sample of tannic acid	27.0	11.0	5.0

the *meta* to the *para* position of the ester function is indeed solvent dependent as discussed[2,3]. The internal standard is 2,3,4-trihydroxybenzoic acid.

The appearance of analytical chromatograms for the four commercial tannic acids is illustrated in Fig. 3. Again the sample loading is very high in order to be able to see the small peaks of interest. There is interference from other peaks, especially for the Tara tannic acid. Still, the data system was able to cope with the situation and to produce acceptable results. These are summarized in Table I for the four commercial tannic acids mentioned above. The composition of a partially hydrolyzed tannic acid is also mentioned in Table I, and its chromatogram is shown in Fig. 4.

The concentrations in Table I are probably typical. Lower concentrations of the gallic acids and their oligomers can be achieved by passage over an ion exchanger[4]. Higher concentrations can be expected for preparations which were not processed like the ones investigated here.

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