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ANALYSIS OF TANNIC ACIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A method is described for the quantitative analysis of tannic acids by high-performance liquid chromatography (HPLC). Four tannic acids of different origin, Alep, Chinese, Sumac and Tara, were purified to serve as reference materials. HPLC calibration data in the normal- and reversed-phase modes were established against 2,3,4-trihydroxybenzoic acid as internal standard. Similarly, and using the same standard, calibration data for gallic acid were also determined. With the results it is possible to analyse tannic acid preparations for purity and gallic acid content. This application of HPLC is primarily of importance for the brewing industry.

INTRODUCTION

Tannic acid is an important commercial chemical. Its many applications, either as the acid itself or in chemically modified form, reflect this. The application of interest to us in the present paper is its use as a stabilizing agent for beer. Tannic acids precipitate potential haze-forming proteinic material and thus it is important to have appropriate analytical techniques for evaluation of quality and eventually for the determination of residual traces of the tannic acids in beer along with other possible impurities such as gallic, digallic and trigallic acid. For the latter compounds we have recently published a high-performance liquid chromatographic (HPLC) method[1]. For the evaluation of the quality of tannic acids, the most important contribution is the straight-phase HPLC work of Beasley *et al.*[2] whose chromatographic results we confirm. We have improved the resolution of their methodology and our approach for the quantitation is different. We also have used reversed-phase HPLC and this leads to some contradictory conclusions which will be discussed later.

There are many types of tannic acids. We shall only discuss the commercial tannic acids extracted from Chinese or Korean gall nuts (*Rhus semialata*, which we will call "Chinese"), from Turkish or Middle East gall nuts (*Quercus infectoria*, "Alep"), from the leaves of *Rhus cotynus* or *R. coriaria* (Bulgaria or Mediterranean region, "Sumac") and from the pods of *Caesalpinia spinosa* ("Tara"). The first three are polygalloylglucose tannic acids, the fourth is a polygalloylquinic acid mixture.

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Tannic acids contain from five to twelve gallic acids per sugar residue. When more than five gallic acid residues are bonded in one tannic acid molecule, some must be present as digallic acid or even as trigallic acid. In recent papers we have described the isolation and general properties of digallic acid[3] and trigallic acid[4]. In these compounds the ester function shows a phenomenon of shifting, in a thermodynamically controlled equilibrium, from the *meta* to the *para* position and *vice versa*. Digallic acid therefore occurs in two forms and trigallic acid in four. This complication, together with the possible positional isomerism of the galloy groups on the sugar nucleus, explains the great complexity of tannic acids. Until only recently no single pure compound occurring in tannic acids had been isolated. In a recent publication, however, Nishizawa *et al.*[5] describe this quite extensively for Chinese tannic acid. They used various forms of chromatographic separation and characterised many of the galloylglucose components by ^{13}C nuclear magnetic resonance (NMR) spectroscopy. They also described the above mentioned positional isomerism in digallic acid.

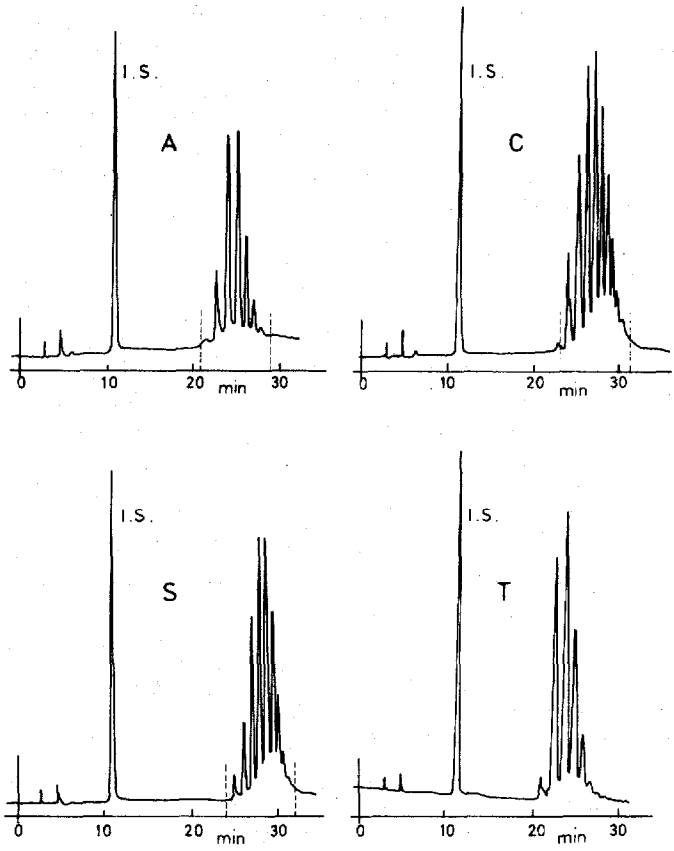

Fig. 1. Four tannic acids (purified references; A = Alep, C = Chinese, S = Sumac, T = Tara) in normal-phase gradient HPLC mode on 5-μm ROSiL as detailled in the text. Collection for quantitation purposes was between dotted lines. I.S. = internal standard, G = gallic acid.

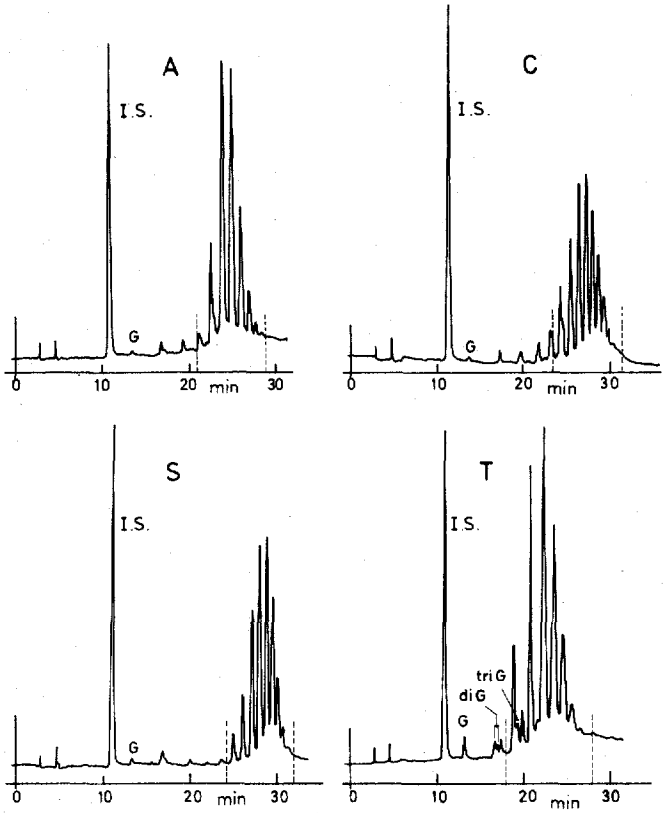

Fig. 2. As in Fig. 1 for the four commercial tannic acids (Alep, Chinese, Sumac and Tara). Note the great change in the Tara pattern before and after ion-exchange purification.

EXPERIMENTAL, RESULTS AND DISCUSSION

Purification of tannic acids

Commercial preparations of the tannic acids Alep, Chinese, Sumac and Tara were provided by Omnichem, Wetteren, Belgium. These tannic acids are off-white and produce a clear solution in water. All the tannic acids contained traces of gallic acid, digallic acids and possibly trigallic acids. These were removed by filtration over an anion exchanger as follows. The anion exchanger (40 g; Dowex 1-x 8 minus 400 mesh) in chloride form was rinsed with 500 ml of 2 *N* sodium hydroxide followed by 500 ml of water. A solution of tannic acid in water (2 g per 10 ml) was introduced on to the column and eluted with water (50 ml). The collected filtrate was freeze dried.

The tannic acids obtained were nearly colourless. For Alep, Chinese and Sumac, HPLC of the references and the commercial materials as such showed practically no difference in the major chromatographic patterns (Figs. 1–4). Only gallic acid and its oligomers were removed by the ion-exchange process. This is not so for Tara, which showed great differences and changes in relative peak intensity of the HPLC pattern before and after the ion-exchange process. For this reason, calibration

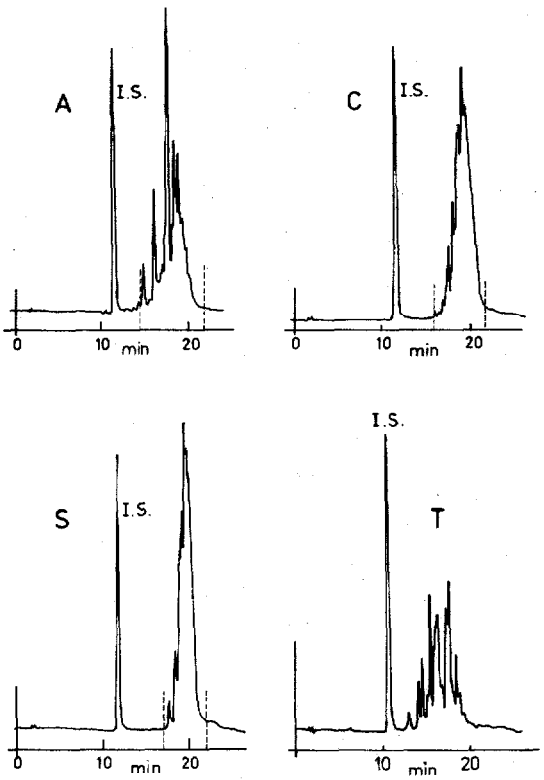

Fig. 3. The same purified reference materials as in Fig. 1 but in the reversed-phase gradient HPLC mode on 5-μm ROSiL-C_{18}-D as detailed in the text. See Fig. 1 for details.

curves against the internal standard were established for the "purified" Alep, Chinese and Sumac tannic acids (see below), but for Tara we used the unmodified dried commercial material. At the start of our efforts on this project we attempted "purification" of the tannic acids by various, and often repeated, solvent extraction steps. Some lower- and higher-molecular-weight peaks can be removed from the tannic acids in this way. It might be argued that removing lower-molecular-weight material is a step in the right direction towards an acceptable reference material. This, however cannot be said of the higher-molecular-weight tannic acids compounds which are also removed and which must have important tanning properties. We believe therefore that intensive "purification by extraction", as we have attempted, has no sense in the context of the desired analytical methodology. The best way seems to be to accept a suitable commercial sample and just to remove the lower-molecular-weight gallic acid and its oligomers. Beasly *et al.*[2] also used commercial tannic acids as reference materials, but these workers mixed two different tannic acids (Alep and Chinese) and proposed this as *external* standard. We consider that it is better to establish (if possible) by HPLC what sort of tannic acid is under investigation and to use an internal standard, calibrated against a suitable sample of that particular tannic acid. It is, indeed, mostly possible to differentiate tannic acids by the "total profile" or "peak pattern" of the chromatogram. The chromatograms of Figs. 1 and

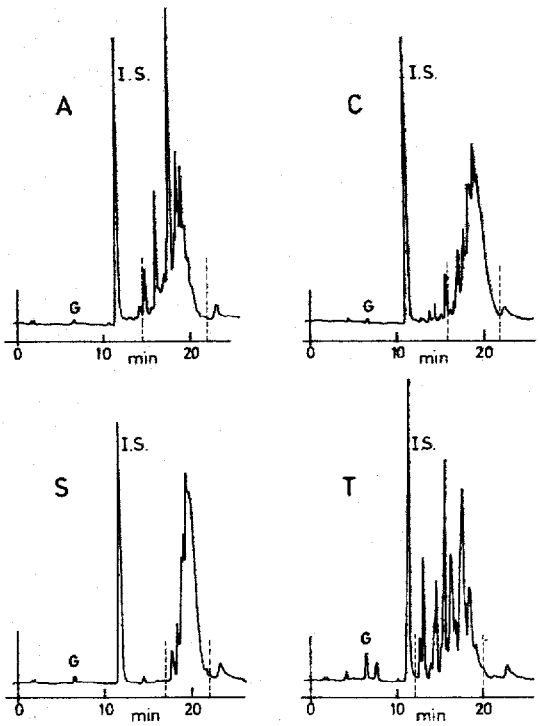

Fig. 4. As in Fig. 2 but in the reversed-phase gradient HPLC mode for the four commercial tannic acids.

2 reveal the great similarity between Chinese and Sumac tannic acids. Alep and Tara are obviously quite different and of lower molecular weight than the first two. The highest peaks of Chinese tannic acid in Fig. 1 are octa- and nonagalloylglucose mixtures. The same applies obviously to Sumac tannic acid. On the basis of retention times, the main peaks of the Alep tannic acid could be hexa- and heptagalloylglucose. Tara is completely different.

Chromatography and calibration data

Normal-phase HPLC of tannic acid produces a regular pattern of about six peaks showing a general Gaussian-shaped pattern (Fig. 1). It is possible to suppose that these peaks represent respectively monogalloylglucose, digalloylglucose, trigalloylglucose, etc.[2]. The reversed-phase chromatograms of these collected single peaks, however show, that this is not the case and that each peak in the normal-phase chromatograms still represents a complex mixture (see later). The total area of the complex peak pattern of Figs. 1 and 2 can be used as a quantitative measure for tannic acid content. Calibration can be against an external standard of a similar tannic acid as explained above, but the "internal standard" approach is also possible. For this, 2,3,4-trihydroxybenzoic acid can be used*. Calibration was achieved against

* This internal standard was suggested by J. Strating and L. Verhagen of Heineken Breweries (The Netherlands).

the reference tannic acids run through an ion-exchange purification step for Alep, Chinese and Sumac and against dried commercial Tara, as explained above.

Chromatographic conditions

A Varian 5020 liquid chromatograph with a 10-μl Valco 7000 p.s.i. sample loop injector, a Varian 50 detector, a Varian 25 recorder and a Varian CDS-101 integrator were used.

Normal phase

A 25 × 0.46 cm column packed with 5-μm ROSiL (a spherical silica gel from Alltech-RSL) was used. The solvent employed were (A) hexane (Burdick & Jackson Labs.) and (B) methanol–tetrahydrofuran (75:25) (both from Burdick & Jackson Labs.). To solvent B, 0.25% of pure citric acid was added. The gradient used was 80% A and 20% B at 0 min changing over 15 min to 50% A and 50% B and changing further over the next 15 min to 35%A and 65%B. The flow-rate was 1 ml/min. Detection was at 280 nm. It is essential when using this method to use solvents of high purity.

Reversed phase

Conditions were as above but with a 15 × 0.46 cm column packed with extra demineralized 5-μm ROSiL-C_{18}-D (an octadecylated spherical silica gel from Alltech-RSL). The solvents were (A) water containing 0.5% phosphoric acid and (B) methanol (Burdick & Jackson Labs.) containing 0.5% phosphoric acid. A linear gradient of 90% A and 10% B changing over 30 min to 0% A and 100% B was used. The concentration of the internal standard was 9.99 mg per 100 ml for all measurements. The amount of the other compounds was varied between 1 and 10 mg for gallic acid and between 10 and 100 mg for the tannic acids. The point at which integration of the composite tannic acid peak was started is indicated on some of the chromatograms shown. At least four points on the calibration graph were determined for all calibrations: the linearity and fit for all points was very good. The results are summarized in Table I.

The linearity and good fit of the points leading to the data in Table I reflect

TABLE I

CALIBRATION EQUATIONS FOR THE HPLC QUANTITATION OF THE MENTIONED COMPOUNDS

y = Ratio of the chromatographic surface area of the analyte to that of the internal standard; x = ratio of the weight of the sample to that of the internal standard. The tannic acids were purified reference materials as explained in the text. Drying of all materials was achieved in a drying pistol.

Compound	*Normal phase*	*Reversed phase*
Gallic acid	$y = 0.88x$	$y = 0.92x$
Alep*	$y = 0.78x$	$y = 0.91x$
Chinese*	$y = 0.945x$	$y = 1.10x$
Sumac*	$y = 0.95x$	$y = 1.06x$
Tara*	$y = 0.82x$	$y = 0.69x$

* Tannic acids were purified; Tara was dried only.

TABLE II

TANNIC ACID (TA) AND GALLIC ACID (GA) CONTENTS OF FOUR COMMERCIAL TANNIC ACIDS

Values were determined by the methods described in the text. TA (%) is the result on the undried materials. H_2O (%) is the weight loss on drying.

Tannic acid	*Normal phase*			*Reversed phase*	
	H_2O (%)	*TA (%)*	*GA (%)*	*TA (%)*	*GA (%)*
Alep	6.3	92.5	0.6	91.0	0.2
Chinese	6.5	91.0	0.3	89.0	0.2
Sumac	4.3	91.0	0.4	88.0	0.7
Tara*	4.7		1.2		1.2

good procedures. This should not be interpreted in the sense that the equations can be trusted blindly for unknown samples. If the light absorption by the tannic acids is due only to the galloyl residues, their correlation coefficients should be higher than that in the gallic acid equation. This is so for only two tannic acids. Other remarks like this could be made. For the moment, however, there is no alternative and the value of the proposed methods will have to be established through practice.

The tannic acids, as provided by Omnichem, were analysed using the above equations. The results are summarised in Table II. No values for Tara can be given since the material as such was used for calibration. The equation in Table I for Tara can be used to compare other Tara preparations with the one used in the present investigation. In the other tannic acids, the very small amount of material that is not accounted for is probably di- and trigallic acid.

As is shown by Table II, it is essential to dry the tannic acids (drying pistol) before analysis or to determine the water content.

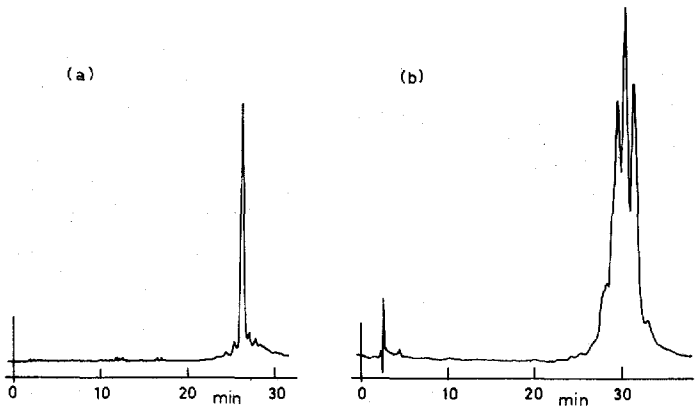

Fig. 5. HPLC of the fourth peak in the normal-phase separation of Chinese tannic acid. (a) Normal-phase HPLC of the collected fourth-peak fraction of Chinese tannic acid; conditions as in Figs. 1 and 2. (b) Reversed-phase HPLC of the single peak of (a) on the 15 × 0.46 cm column of Figs. 3 and 4 but with a different gradient. Solvents: A = water containing 0.5% phosphoric acid; B = methanol (Burdick & Jackson Labs.) containing 0.5% phosphoric acid. Gradient: 75% A and 25% B changing linearly over 40 min to 35% A and 65% B.

Chromatograms of tannic acids

The normal-phase chromatograms of the four reference tannic acids are shown in Fig. 1. The normal-phase chromatograms of the four commercial tannic acids are shown in Fig. 2. The similarity of Sumac and Chinese is obvious as they are practically identical. Alep and Tara also look similar although they are, in fact, very different. There is a qualitative-identifying value in the greater simplicity and shorter retention times for Alep–Tara than for Sumac–Chinese.

The reversed-phase chromatograms of the four reference tannic acids are shown in Fig. 3 and that of the commercial materials in Fig. 4. The differences between the tannic acids are clearer in these reversed-phase chromatograms than in the normal-phase ones. The main pattern for Chinese and Sumac is the same and the difference between Alep and Tara is now clearer. In the normal-phase mode, gallic acid is eluted after the internal standard, this order being reversed in the reversed-phase mode. For the chromatograms shown, the gradients were designed so as to bunch the tannic acid peaks together. It is, of course, possible to obtain higher resolution of the different peaks. The peaks of the highest intensity of the Chinese and Sumac tannic acids are octa- and nonagalloylglucose. The highest-intensity or fourth peak in the normal-phase HPLC run of Chinese tannic acid was collected by highly efficient preparative HPLC[6] and reanalysed on a reversed-phase column with the gradient indicated in Fig. 5. The complexity of the single peak in the normal-phase mode is now obvious.

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