# Notes

CHROM. 4166

## The silylation of substances occurring in natural products and detectable by gas chromatography

The importance of silylation, *i.e.* substitution of the active hydrogen in HO, HS and HN-functions by the trimethylsilyl group–$Si(CH_3)_3$ in the gas chromatography of important natural substances has increased over the past few years[1–3]. Normally, the process can only be carried out in solvents which themselves do not contain active hydrogen. Sometimes this results in unfavorable solubility conditions particularly in the case of some important natural substances suitable for silylation, such as carbohydrates, hydroxy carbonic acids and amino acids which are strong hydrophilic substances. This does not entail any difficulty as long as the substances to be silylated are present in their pure form when the silylation reaction occurs quantitatively despite the very low solubility of the initial compounds. However, if the same substances are to be determined in natural products, the initial extraction required often results in so many impurities being co-extracted that it is almost impossible to carry out a reproducible silylation and gas chromatographic determination of the components of interest because of the incomplete solubility of the extract residues.

We were interested in a gas chromatographic determination of the soluble substances occurring in potatoes. However, co-extraction of higher molecular fractions such as soluble starch, pectins, and dextrins could not be avoided when the tubers were extracted with methanol or methanol–water mixtures. In addition, after evaporation of the extracts we obtained hard, glassy residues which showed only superficial solubility in pyridine which was used as solvent for the silylation, in our case, and which could not be purified satisfactorily afterwards.

Thus the possibility of direct silylation of crystallized sugars in a heterogenous phase, mentioned by SWEELEY[4], was tried out on the evaporation residues of the potato extracts. Our experiments indicated that the initially closely packed residues could be homogenized successively by intensive stirring in the presence of the silylation agent. The insoluble fractions which could not be silylated were precipitated together with the crystallized salts as precipitates of such a uniform finely powdered quality that a largely quantitative silylation—as far as this is possible in the light of the activity of the silylation agent—could be assumed for the components of the extracts lending themselves to chromatographic determination. Probably, the homogenizing effect of the stirring is greatly enhanced by the silylation process which helps to disintegrate the solid when the initially polar components are changed into non-polar silyl compounds which are more soluble in the reaction medium.

Subsequent experiments showed that the method of silylation described can very probably be applied to any evaporation residue irrespective of its chemical composition and physical condition. Thus, *e.g.*, it is possible to dissolve any additives in the

extracts before evaporation as long as there is a sufficient excess of silylation agent. For instance in potato extracts, silylated citric acid and fructose can only be separated incompletely on packed columns (Fig. 1). However, citric acid can easily be removed from the extracts in advance by precipitation as sparingly soluble barium citrate, without the excess of barium salt in the evaporation residues interfering with the subsequent silylation.

The special advantage of silylation together with simultaneous homogenization of the products to be silylated mainly lies in the fact that in this way heating of the silylation mixtures, and thus the danger of secondary reactions, can be avoided even with reactants of very low solubility. If necessary, the stirring time must be increased to allow for complete homogenization of the initial products.

The method outlined below has proved successful, especially for silylations with hexamethyldisilazane and trimethylchlorosilane in pyridine. Since the conditions established are most advantageous for a gentle and uniform silylation at room temperature irrespective of the initial conditions, it may also be used for silylations with other reagents, such as N-trimethylsilylamides.

*Procedure*

The extract, after addition of an internal standard if necessary, is evaporated directly in the vessel to be used for the silylation and the drying is completed over phosphorus pentoxide in a vacuum at room temperature. A teflon stirrer is introduced into the vessel and the silylation agent is then added. The vessel is closed tightly, briefly shaken and the contents are stirred at room temperature until the evaporation residue has changed completely into a uniform precipitate of fine powder. The solution can then be injected directly into the gas chromatograph in the usual way. In the case of a complete conversion there is no danger of the injection needle being blocked by the precipitate. It is even advisable to shake the silylation mixture again for 1–2 min before each injection.

Sometimes, for example, after the addition of barium acetate to potato extracts, unconverted remains of the evaporation residue stick firmly to the bottom of the vessel. Quantitative silylation can be achieved subsequently by scratching these remains off with a microspatula and by renewed intensive stirring.

In our case, a special way of executing the method turned out to be successful. 2 ml aliquots of the extract were gradually dried in micro-erlenmeyer flasks* in a vacuum desiccator over phosphorus pentoxide at room temperature, with a stepwise increase in the vacuum. Although drying in this case takes about 2 days, it is possible by this means to dry and silylate a larger number of samples at the same time with a minimum of manual effort.

*Results*

Fig. 1 shows an initial chromatogram of a potato extract (variety Hansa, stored at +5°).

Fig. 2 shows the results of quantitative investigations in which the completeness of the silylation was investigated on the basis of a series of concentrations of four characteristic substances occurring in potato extracts.

The series of concentrations were prepared by dissolving trehalose as an internal

* Total volume 2.8–3 ml, with NS 7/16 and teflon stirrers of 5 × 10 mm.

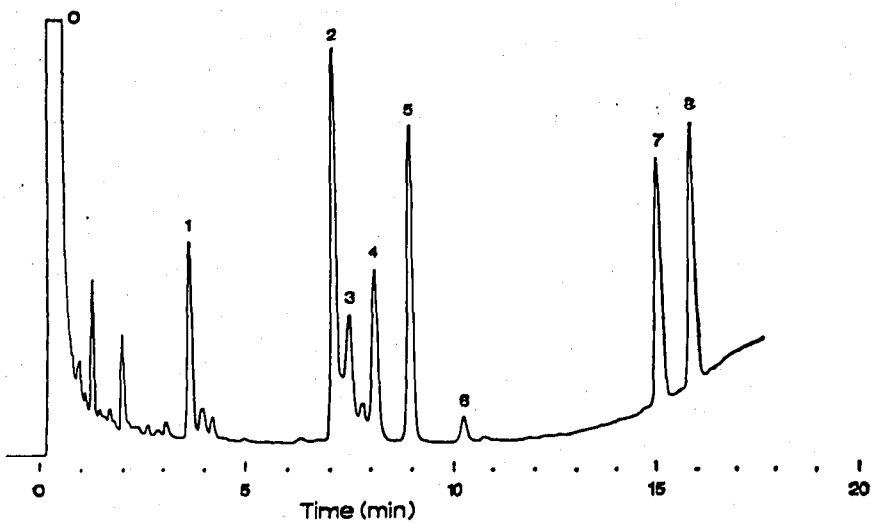


Fig. 1. Gas chromatogram of potato extract. Equipment: Hewlett Packard model 810 with flame ionization detector; column, stainless steel 4 ft., 3/16 in. O.D. packed with 10% silicone gum rubber UCC-W-982 (methyl vinyl) on Diatoport S, 80–100 mesh. Temperatures: injection port about 250°, detector about 340°. Oven programmed linearly with 10°/min from 150 to 310°. Carrier gas: helium, about 80 ml/min. 0 = solvent (pyridine); 1 = malic acid; 2 = citric acid; 3 = fructose; 4 = α-glucose; 5 = β-glucose; 6 = myo-inositol; 7 = sucrose; 8 = trehalose (added as an internal standard).

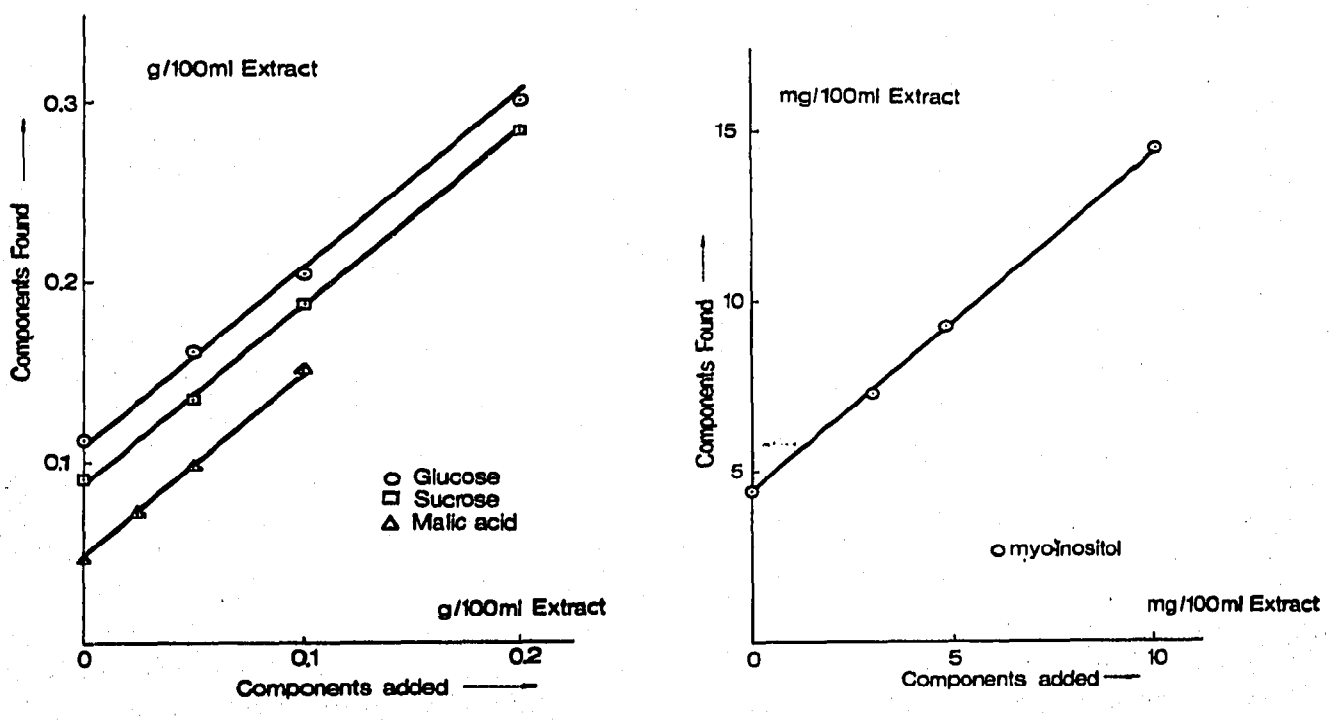


Fig. 2. Results of four series of concentrations. Abscissae: quantities of pure components added to the samples of potato extracts. Ordinates: concentrations of the same components found in the samples of extracts. Conditions of chromatography as indicated under Fig. 1. Internal standard (trehalose): 100 mg/100 ml extract.

standard and increasing quantities of glucose, sucrose, malic acid, and myo-inositol in the extract samples; the same extract was always used for a series*.

Of each of the solutions 2 ml were dried in the micro-erlenmeyer flasks described above and the residues were silylated with a mixture of 1.25 ml pyridine, 0.5 ml hexamethyldisilazane, and 0.25 ml trimethylchlorosilane, with stirring for some hours at room temperature. 1.5–2 $\mu$l of silylation mixture were injected into the gas chromatograph. The peak areas were determined from height × half width. Prior to this, the calibration curves had been established under the same conditions of silylation and chromatography.

The solid lines in Fig. 2 represent the fitted straight lines with the angle of 45° given by taking the scale into account. The diagrams show that in potato extracts a quantitative silylation of all four components can be anticipated.

*Institute of Radiation Technology, Federal Research Centre for Food Preservation, 75 Karlsruhe (G.F.R.)* GÜNTHER RUMPF

1 C. C. SWEELEY, R. BENTLEY, M. MAKITA AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497.
2 J. F. KLEBE, H. FINKBEINER AND D. M. WHITE, *J. Am. Chem. Soc.*, 88 (1966) 3390.
3 L. BIRKOFER AND M. DONIKE, *J. Chromatog.*, 26 (1967) 270.
4 C. C. SWEELEY, paper published as a private reprint by the F & M Scientific Europa N.V., Amsterdam.

Received May 2nd, 1969

* The extracts were each prepared by homogenizing in a Waring blendor 100 g of fresh potatoes with a mixture of 100 ml water, 125 ml methanol, and 25 ml pyridine (as an enzyme inhibitor) and by centrifuging the homogenates.



CHROM. 4156

## A microanalytical method for the determination of dihydroquercetin in wood

Dihydroquercetin (3,5,7,3′,4′-pentahydroxyflavanone) is a major constituent of the alcohol soluble materials in the wood of Larch species and the wood and bark of Douglas-fir. A sensitive analytical method is needed to enable rapid assessment of amounts of dihydroquercetin (DHQ) when processing commercial materials and for studies of biochemical aspects of DHQ formation. Ability to analyse very small wood samples is a most important criterion from a biochemical standpoint.

Most quantitative estimates of the amount of DHQ in wood have employed the method of BARTON AND GARDNER[1,2]. This method suffers from limitations in that it is specific for 3-hydroxyflavanones not only DHQ, has a relatively low sensitivity, and is extremely dependent upon reagent impurities.

SQUIRE *et al.*[3], using a quantitative paper chromatographic (PC) technique found