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Letter to Editor

The Influence of Solvent Choice on the Recovery of Phytogenic Phenolic Compounds Extracted from Plant Material

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Abstract

Sometimes, the preparation of a plant sample to GC analysis undergoes the stage of drying plant extract from the solvent, and subsequently the repeated extraction of the dry residue in a different solvent. The purpose of this procedure is to separate the studied phenolic compounds from the matrix. This paper presents the results of the research into the effect of the type of solvent used in the proceeding (methanol, water, acetone, diethyl ether or chloroform) on the size of losses in the phenolic compounds during such a procedure. It has been noted that the greatest recovery appears in cases of methanol and acetone, whereas water may be the source of considerable losses in the compounds during the preparation of the plant sample for analysis.

Keywords: phenolic acids, plant material, sample preparation.

Introduction

Among secondary plant metabolites, which can be found in tissues, we can distinguish a large group of compounds and phenol derivatives. These compounds are produced in cells from simple phenols, which become converted into more complex structures afterwards: flavonoids, glycosides, anthocyanins, lignins, etc.

According to the length of the side carbon chain, the basic phenolic compounds are classified as (Fig. 1): **simple phenols** (with $\mathbf{C_6}$ base structure) – for instance compounds I-III, and **phenolic acids:** $\mathbf{C_6C_1}$ (IV-VIII), $\mathbf{C_6C_2}$ (IX-XI) and $\mathbf{C_6C_3}$ (XII-XV) [1]. The phenolic acids C_6C_1 and C_6C_3 are formed on the pathway of shikimic acid (XVI) and this acid can also be found in plant cells.

All these compounds have a functional group (or groups) in their structures, which allow them to enter into reactions leading to the formation of complex structures. It cannot be excluded that such reactions may also occur during the preparation of plant samples for analysis.

While working with pure standard phenolic compounds and with plant extracts, I have observed on frequent occasions that after evaporating the solvent, the thick greasy substance of yellow to brown colour is left.

Therefore, I have decided to discover the course of recovery of phenolic compounds from this greasy residue. It is interesting that separating phenolic compounds from plant material is carried out by means of extraction with methanol/ethanol or with acetone (sometimes with the addition of water) [2,3]. The next stage is often evaporation of the solvent, after which the dry residue is treated with water [3,4], ethanol [5], or another solvent in order to separate the phenolic compounds from the matrix.

The aim of the work presented now was knowledge of the influence of solvent choice: diethyl ether, acetone, methanol, chloroform and water on the recovery of the phenolic compounds during such extraction. The study was carried out on the sample of standard phenolic compounds. In order to make the sample resemble the natural one, a compound from a different chemical group was

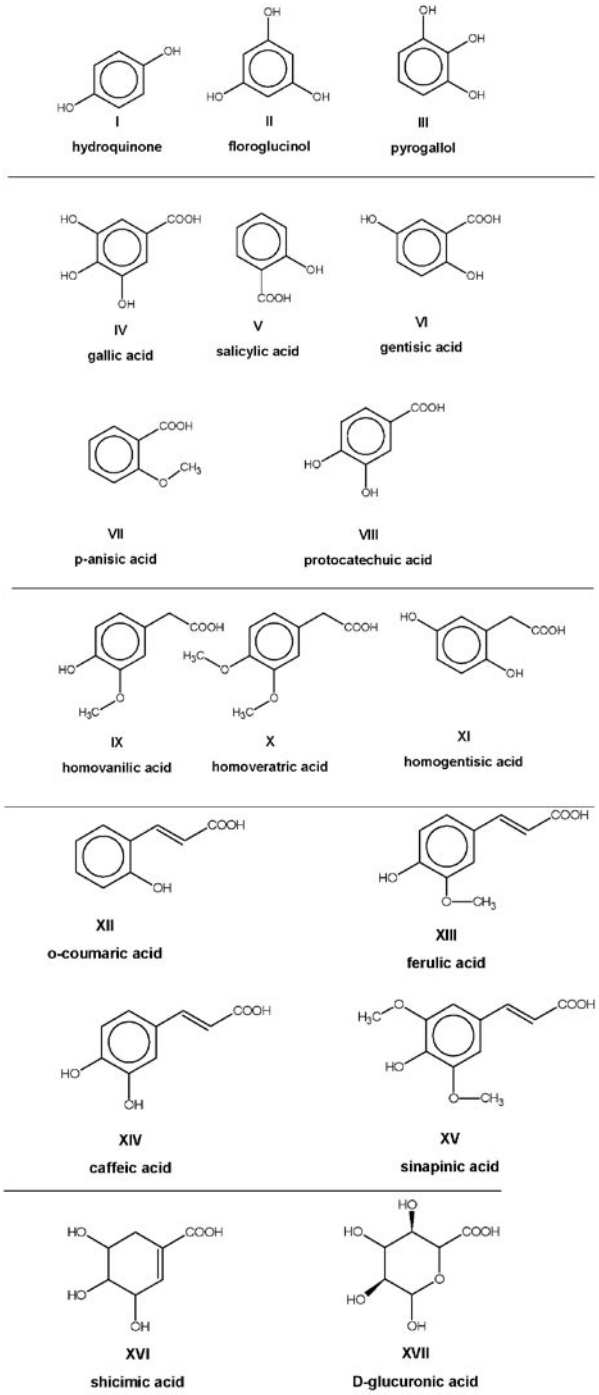

Fig. 1. Structural formulae of the Phenolic compounds (I-XV) and other phytogenic compounds (XVI, XVII).

introduced: D-glucuronic acid (XVII). Derivatives of this compound, e.g. flavonoid glycosides, are also present in extracts of plant materials [6].

Materials and Procedure

The standards of phenolic compounds were specified in Table 1. They all and purified pyridine, bis(trimethylsil yl)trifluoroacetamide BSTFA (with the addition of 1% trimethylchlorosilane TMCS) were purchased from Sigma - Aldrich Co. (Poznań, Poland).

Acetone, methanol, diethyl ether and chloroform obtained from POCh (Gliwice, Poland).

A gas chromatograf HP 4890D with flame ionisation detector FID (Hewlett-Packard Polska sp. z o.o., Warszaw, Poland) and a capillary column 30 m x 0.25 mm x 25 µm with nonpolar phase DB-5 (J&W Scientific, Folsom, CA, USA) were used.

The temperature programme was as follows: T_{init} = 50°C, a rise from 50 to 240°C at 3°C·min^{-1}. Temperatures of the injector and the detector were set at 250°C and 280°C, respectively. Split 1:50. Carrier gas, helium, flow was kept at 1 ml·min^{-1}.

The 7 to 20 mg of compounds listed in Table 1 were dissolved in 25 ml of acetone. The solutions were fivefold diluted of the acetone and 6 samples of 1.2 ml each were taken up from the solution and inserted in 6 vials with caps. The samples were numbered from 1 to 6 and then the acetone was evaporated at 50°C. Afterwards, 1.2 ml of solvent was added to the dry residue according to the scheme: No. 1 – diethyl ether; No. 2 – acetone; No. 3 – methanol; No. 4 – chloroform; No. 5 – water. No solvent was added to vial No. 6 – it is a primary sample.

The samples were shaken for 15 min and left in a dark place [7]. After 20 hours they were shaken again for a moment, after which they were put aside so that the deposit could settle (clearly visible deposit was present in vials No. 1, 2 and 5). Afterwards, 200 µl of clear solutions were measured out and poured into clean vials. The solvents were evaporated at 50°C (the water extract was evaporated at 85 °C using a vacuum rotary evaporator) and the residue was sililated with the addition of 8 µl of pyridine and 42 µl of BSTFA. The dry residue in vial No. 6 was also sililated (40 µl of pyridine + 180 µl of BSTFA).

GC chromatograms were made for all the samples in three repetitions.

Results and Discussion

Exemplary chromatograms made for the primary sample and for the sample extracted in water are shown in Fig. 2. Peak number in this picture corresponds to the number of compounds in Table 1. We can clearly notice the difference in the heights of the peak No. 1 (coming from phenol), No. 5 (pyrogallol), No. 6 (phloroglucinol) and the two peaks (causes from isomers of glucuronic acid) lying between peaks No. 18 and 20. Table 1 shows the content of the compounds in particular solvents. Weights of the compounds contained in the solutions were calculated from the average peak areas on the basis of the standardisation curves determined beforehand [8]. Low values of the relative standard deviations RSD (2.59 – 4.21%) show that contents of the respective compounds with high precision were determined, therefore it is possible to calculate recoveries of the respective compounds in extracts. The recoveries are given in brackets in Tab. 1.

Table 1. Chemical composition, μg, of solutions obtained as a result of extraction by 1.2 ml various solvents. The composition of the extracted sample is given in the "Primary sample" column. Recovery, %, of the substance is given in brackets. The number of compounds in this table corresponds to the number of peaks in Figure 2.

No	Compounds	Primary sample	Solvent				
			Diethyl ether	Acetone	Methanol	Chloroform	Water
1	Phenol	812±13	245±13 (30.2)	142±19 (17.5)	430±12 (52.9)	trace	85±4 (10.5)
2	Hydroquinone	992±10	936±18 (94.3)	1016±38 (102.4)	998±18 (100.6)	959±28 (96.7)	964±32 (97.2)
3	Salicylic acid	400±17	384±10 (96.0)	367±18 (91.7)	398±9 (99.5)	393±13 (98.2)	313±11 (78.2)
4	p-Anisic acid	370±12	339±12 (91.6)	375±19 (101.3)	370±10 (100.0)	350±11 (94.6)	338±10 (91.3)
5	Pyrogallol	492±15	451±13 (95.7)	507±13 (103.0)	489±8 (99.4)	455±11 (92.5)	204±8 (41.5)
6	Phloroglucinol	435±18	471±11 (103.7)	438±11 (100.7)	420±12 (96.5)	320±9 (73.6)	14±6 (3.2)
7	Homoveratric acid	359±9	324±11 (90.2)	353±13 (98.3)	352±9 (98.0)	346±11 (96.4)	346±9 (96.4)
8	Vanillic acid	238±10	217±6 (91.2)	230±19 (96.6)	238±8 (100.0)	233±7 (97.9)	230±3 (96.6)
9	Homovanilic acid	185±8	174±4 (94.0)	181±11 (97.8)	180±3 (97.3)	178±6 (96.2)	172±3 (93.0)
10	Gentisic acid	253±9	250±8 (98.8)	246±19 (97.2)	248±5 (98.0)	250±2 (98.8)	228±8 (90.1)
11	o-Coumaric acid	313±11	282±9 (90.1)	313±17 (100.0)	310±9 (99.0)	294±8 (93.9)	295±13 (94.2)
12	Protocatechuic acid	378±15	341±10 (90.2)	381±10 (100.8)	375±6 (99.2)	377±5 (99.7)	380±11 (100.5)
13	Shikimic acid	184±5	152±3 (82.6)	182±8 (98.9)	186±7 (101.1)	74±2 (40.2)	188±4 (102.2)
14	Homogentisic acid	750±18	663±18 (88.4)	710±30 (94.6)	750±18 (100.0)	717±18 (95.6)	592±18 (78.9)
15	m-Coumaric acid	310±15	271±10 (87.4)	300±27 (96.8)	307±12 (99.0)	291±9 (93.9)	315±17 (101.6)
16	Syringic acid	322±14	282±11) (87.6	307±12 (95.3)	317±12 (98.4)	306±9 (95.0)	316±14 (98.1)
17	p-Coumaric acid	786±22	689±18 (87.6)	780±16 (99.2)	780±20 (99.2)	714±22 (90.8)	526±26 (66.9)
18	Gallic acid	262±11	230±9 (87.8)	254±8 (96.9)	260±6 (99.2)	102±3 (38.9)	249±9 (95.0)
19	Glucuronic acid	342±15	-	-	107±7 (31.2)	-	-
20	Ferulic acid	173±2	157±10 (90.7)	169±9 (97.7)	178±7 (102.9)	178±6 (102.9)	138±6 (79.8)
21	Caffeic acid	309±9	256±11 (82.8)	282±10 (91.2)	301±9 (97.4)	275±8 (89.0)	136±8 (44.0)
22	Sinapinic acid	480±15	379±10 (82.7)	487±11 (101.4)	498±13 (103.7)	446±13 (92.9)	352±20 (73.3)
	Σ, μg s, μg RSD, %	9145 273 2.98	7493 235 3.14	8020 338 4.21	8492 220 2.59	7258 201 2.77	6381 240 3.76

Comparison of the qualitative composition of the respective extracts (Tab. 1) showed in four extracts (ether ethyl, acetone, chloroform and water) confirmed absence of glucuronic acid. Qualitative composition of methanol extract is the same as the composition of the primary sample, yet the recovery of glucuronic acid and phenol were only 31.2 % and 52.9 %, respectively.

While using water, a slight decrease of recoveries were observed in relation to four compounds: salicylic, homogentisic, ferulic and sinapinic acids, whereas the value of

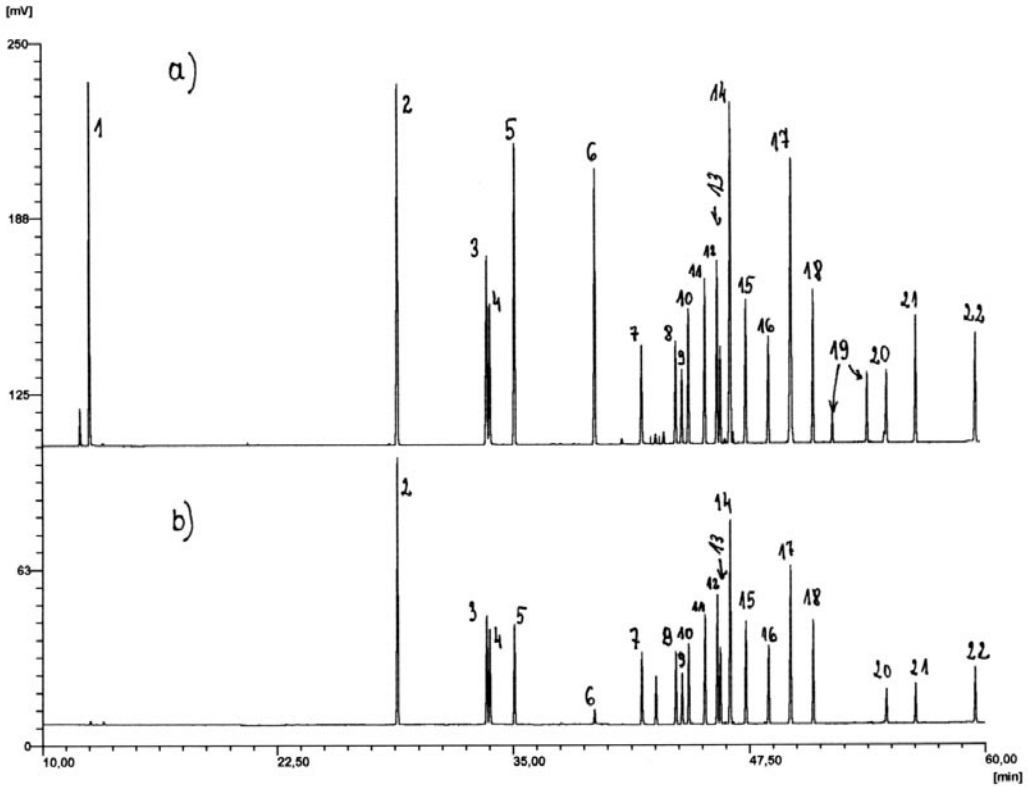

Fig. 2. Fragment of chromatograms obtained for (a) - primary sample; (b) - water extract. Number of peaks corresponds to number in Table 1.

the recovery of five other compounds (phloroglucinol, phenol, pyrogallol, caffeic and p-coumaric acids) was significantly lower: 3.2, 10.5, 41.5, 44.0 and 66.0%, respectively.

Lower recovery of the phloroglucinol (73.6%), shikimic (40.2%) and gallic (38.9%) acids were also observed in the chloroform extract comparison to primary sample. Likewise, the use of diethyl ether resulted in losses in the content of phenol (recovery only 30.2%) and in the content shikimic, caffeic and sinapinic acids (recoveries about 82%).

Study of extraction of phenolic acids from plant samples was performed also by Sotillo et al. They on the basis of the chlorogenic acid, gallic acid, protocatechnic acid, and caffeic acid proved that water at room temperature is not a very effective solvent and that better effects can be obtained using methanol [7].

The quantitative and qualitative composition of the acetone extract is also worth noticing. It is different from the composition of the primary sample. Is it a signal that during the process of drying of the plant extract from the solvent some reactions changing the composition of the sample may proceed? Is the thick greasy residue a consequence of these reactions?

Research shows that when the plant extract is evaporated to dryness and then the solution again in the same volume of a different solvent, considerable losses in the content of some simple compounds that are present in the studied sample may appear.

References

1. RYAN D., ROBARDS K., PRENZLER P., ANTOLOVICH M. Aplications of mass spectrometry to plant phenols. Trends in Anal. Chem. **18** (5), 362, **1999**.
2. KEINÄNEN M., JULKUNEN-TIITTO R. High-performance liguid chromatographic determination of flavonoids in *Betula pendula* and *Betula pubescens* leaves. J. Chromatogr. A. **793**, 370, **1998**.
3. CONDE E., CADAHÍA E., GARCÍA-VALLEJO M. C. Low molecular weight polyphenols in leaves of *Eucalyptus camaldulensis, E. globulus and E. rudis*. Phytochem. Anal. **8**, 186, **1997**.
4. SIEROTA Z. H., GAYNY B., ŁUCZKO A. Variability of some phenolic acids in phloem of 1-year-old shoots of Scots pine trees growing with *Heterobasidion annosum* (Fr.) Bref. Trees. **12**, 230, **1998**.
5. SHAHRZAD S., BITSCH I. Determination of some pharmacologically active phenolic acids in juices by high-performance liquid chromatography. J. Chromatogr. A. **741**, 223, **1996**
6. HATANO T., TAKAGI M., ITO H., YOSHIDA T. Acylated flavonoid glycosides and accompanying phenolics from licorice. Phytochemistry, **47** (2), 287, **1998**.
7. DE SOTILLO D. R., HADLEY M., HOLM E. T. Phenolics in Aqueous Potato Peel Extract: Extraction, identification and degradation. J. Food Science. **59** (2), 649, **1994.**
8. JAROSZYŃSKA J. A Method of extracting phenol compounds from arboreal leaves by means of the Florisil/C_{18} system. In preparation.