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Analytical aspects of drugs of natural origin*

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Abstract: The current importance of drugs of natural origin and their different phytotherapeutical preparations are mentioned. Analytical aspects related to vegetable drugs and their extracts are discussed. An overview is given on procedures used for the isolation of reference compounds, sample preparation for high-performance liquid chromatographic analysis, identification of vegetable drugs and detection of adulterants. The different approaches currently employed for the standardization of extracts are presented. The various aspects are discussed with the aid of illustrative examples.

Keywords: *Vegetable drugs; plant extracts; phytotherapeutical preparations; HPLC; sample preparation; standardization.*

Introduction

Within the last 10 years, there has been an increasing interest in drugs of natural origin, and the consumption of medicinal plants has almost doubled in industrialized countries of Western Europe. In these countries, almost 35% of the prescribed drugs contain active principles of plant origin, whereas 80% of the world population use exclusively plants for the treatment of illnesses.

Medicinal plants are used in different forms: (i) isolated pure active constituents (the analytical problems are the same as for synthetic drugs); (ii) complex mixtures of various constituents such as infusions, essential oils, tinctures, extracts or even powdered plant material in different galenical preparations.

Among these forms, extracts are becoming very popular and are sold as prescription drugs or more often "over the counter" (OTC drugs). It is noteworthy that in France and in the FRG, the best-selling drug is a standardized plant extract, namely an alcoholic extract of the "fossil" tree *Ginkgo biloba* (maidenhair tree) which is used for the treatment of cerebral and peripheral ischemia [1]. A French pharmaceutical company utilizes more than 1200 t of dried leaves per year and has recently planted over 15 million *Ginkgo* trees in France and in the USA [2]. This example illustrates the increasing importance of plant extracts in therapy.

Preparation and quality control of drugs of natural origin in the form of crude extracts require special attention. The content of active principles in a plant extract depends on various factors such as growth conditions, climate, soil quality, time of harvesting, drying

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and powdering methods, extraction and stabilization procedures. Phytotherapeutic preparations formed of hundreds of primary and secondary metabolites should contain a constant amount of active constituents. This is achieved by standardization of extracts: quantitative determination of the constituents followed by an adjustment to the amount defined previously [3]. Standardization is required for the registration of drugs of plant origin in numerous countries. The objectives are (i) to guarantee preparations containing therapeutically-effective doses of active principles, (ii) to maintain constant batch composition (process control), and (iii) to check stability of active principles. It should be mentioned that in many phytotherapeutic preparations the active constituents are not known. In this case, standardization is realized by quantitative determination of marker substances (typical constituents of the extract).

All available methods can of course be applied to the analysis of drugs of plant origin. However, high-performance liquid chromatography (HPLC) is most commonly used. In the following pages, some typical examples of HPLC analysis of plant extracts are presented and discussed.

Isolation of Reference Compounds

Any quantitative determination of active principles is unthinkable without pure reference samples of the compounds of interest. While some commonly occurring natural products are commercially available, their degree of purity will be often insufficient for the purpose of quantitative analysis, or the costs may be prohibitive for samples of suitable purity. Less common natural products cannot be obtained from commercial sources. Thus, quantitative analysis begins very often with the isolation of suitable reference compounds. Since the compounds to be analysed are known, the extraction and isolation procedure can be optimized in accordance with their physicochemical characteristics.

Isolation of a plant constituent usually requires several steps of chromatographic purification. Classical methods include enrichment by partitioning or acid–base extraction, followed by open column chromatography. Instrumented preparative techniques, such as low-pressure (LPLC) and medium-pressure liquid chromatography (MPLC) on various stationary phases provide increased flexibility and better separations [4]. Semi-preparative HPLC is used for most difficult separations or final purification. Liquid–liquid partition chromatographic techniques, in particular droplet countercurrent chromatography (DCCC) [5, 6] and the recently developed centrifugal countercurrent methods [7] are particularly suited for very polar or labile compounds and constituents undergoing irreversible adsorption on solid phases. The chromatographic conditions suitable for preparative column chromatography are generally developed by analytical HPLC, which is also used for the final purity check [4].

The transposition from analytical to semi-preparative HPLC can be illustrated by the final purification of antifungal chromenes from the African plant *Hypericum revolutum* (Hypericaceae) [8]. Flash chromatography of a petroleum ether extract, followed by LPLC on silica gel afforded a mixture of the two homologous chromenes **1** and **2**. Analytical separation of the two compounds was achieved on a C_{18} column using methanol–water (80:20%, v/v) with the addition of phosphoric acid to suppress chemical tailing (Fig. 1). For the semi-preparative separation, the proportion of methanol in the eluent was decreased and no acid added, in order to avoid any decomposition during the work-up of the separated compounds.

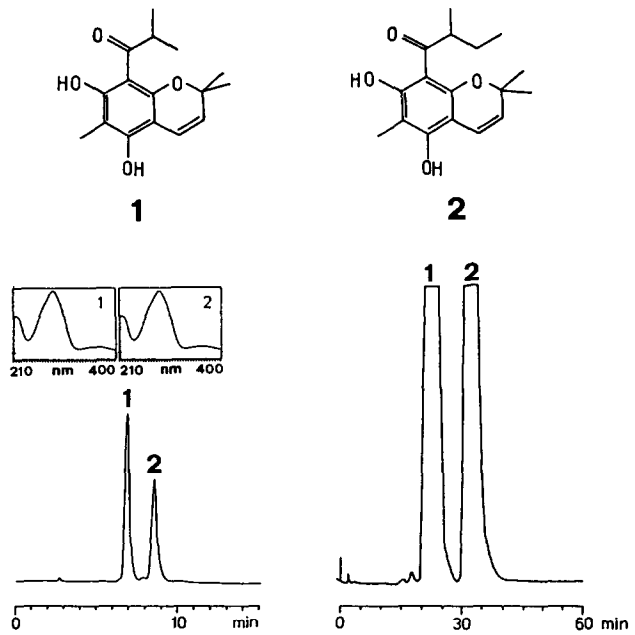


Figure 1
Left: analytical HPLC separation of antifungal chromenes **1** and **2** from *Hypericum revolutum* showing spectra acquired by photodiode array. Column, Knauer LiChrosorb RP-18 (250 × 4.6 mm, i.d.); eluent methanol–water (80:20%, v/v), with phosphoric acid (5 ml l^{-1}); flow-rate, 1.5 ml min^{-1}; detection, 254 nm. *Right*: semi-preparative HPLC of compounds **1** and **2**. Column: μ-Bondapak (300 × 7.8 mm, i.d.); eluent: methanol–water (63:37); detection, 254 nm; flow-rate, 5 ml min^{-1}; sample, 5 mg/injection. (Reprinted with permission from ref. 8.)

Sample Preparation for HPLC Analysis

Plant extracts are complex mixtures of hundreds of primary and secondary metabolites. The analysis is generally hampered by two factors, that is the rather low concentration of active principles to be determined, and the presence of interfering compounds that may be difficult to eliminate. Baseline separation of the peaks of interest cannot always be achieved. The large polarity differences of the constituents in a plant extract usually require gradient elution. These obstacles can, in part, be overcome by selective detection, judicious sample work-up or pre-column derivatization of the compounds to be quantified.

The quantitative determination of tabersonine in the seeds of the African medicinal plant *Voacanga africana* is an example of selective extraction and detection. This lipophilic indole alkaloid, after extraction with choroform, was quantified by HPLC on a C_{18} column (Fig. 2). Detection at the absorption maximum of tabersonine (330 nm) eliminated any interfering peaks originating from other lipophilic constituents (sterols, waxes), as well as minor indole alkaloids possessing a simpler chromophore and thus absorbing at lower wavelength. The quinoline alkaloid quinine was used as internal standard [9].

The goals of a sample work-up of extracts prior to HPLC analysis are (i) to remove compounds interfering with the detection of the active principles, and (ii) to increase the

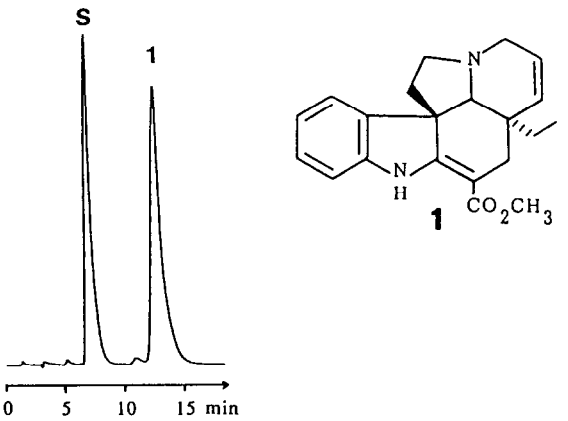


Figure 2
Quantitative determination of tabersonine (**1**) in *Voacanga africana* seeds. Column, μ-Bondapak C_{18} (300 × 3.9 mm, i.d.); eluent, methanol–water–triethylamine (68:32:0.5%, v/v/v), flow-rate, 1.5 ml min^{-1}; detection, 330 nm; internal standard (S), quinine.

lifespan of the column. Judicious work-up procedures are especially important, if the compounds of interest are minor constituents or absorb at short wavelength. The most currently used methods include: (i) partition between non-miscible solvents, (ii) solid-phase extraction, (iii) irreversible adsorption or precipitation of undesirable compounds, and (iv) acid–base extraction.

Partition between *n*-butanol and water is very often employed for extracts containing terpenoid glycosides, such as saponins in order to remove interfering water-soluble compounds such as polysaccharides [10]. Partition with less polar organic solvents such as ethyl acetate or chloroform has been used for terpenoid aglycones [11]. Solid-phase extraction with cartridges packed with chemically modified silica gel is also useful for the pre-purification of terpenoid containing extracts [11, 12]. Unwanted phenolic compounds can be removed by filtration over aluminium oxide or precipitation with lead salts. These methods however entail generally a partial elimination of other constituents, and are therefore not suitable for quantitative analysis. Better methods are filtration over Sephadex LH-20 or polyamide [13]. Acid–base extraction has been most frequently used for alkaloid containing extracts [14]. If a sample work-up procedure should be used in quantitative analysis, the recovery rate has to be determined for the compounds of interest.

The absence of a chromophore suitable for UV–vis detection is quite common, especially in the case of terpenoid compounds. Triterpenoid saponins, for example, are the active principles in many vegetable drugs. Pre-column derivatization is one possibility to improve the detectability of such compounds, but has not been very frequently applied to plant extracts. In the saponin field, the derivatization with 4-bromophenacyl bromide has been employed for the quantitative analysis of plant extracts active against the aquatic snails serving as intermediate vectors for the transmission of the tropical disease schistosomiasis (bilharzia). Among the most promising plants for the local treatment of infested water sites in Third-World countries are the saponin containing plants *Phytolacca dodecandra* (Phytolaccaceae) and *Swartzia madagascariensis* (Leguminosae) [15]. While triterpene saponins with a free carboxylic group at C-28 show potent molluscicidal properties, the corresponding bidesmosidic

saponins bearing a second sugar chain esterified at C-28 are inactive [16]. The saponins of *P. dodecandra* and *S. madagascarienis* were initially analysed without derivatization [17]. The absence of a chromophore required detection at 206 nm, but severe problems with baseline drifts and interfering compounds were observed. In an aqueous extract of *P. dodecandra*, the inactive bidesmosidic saponins **1–3** are well separated, whereas the slower running molluscicidal saponins are hidden by interfering compounds (Fig. 3). Derivatization yields selectively the 4-bromophenacyl derivatives of the molluscicidal saponins bearing a free carboxyl, thus enabling their detection at 254 nm [18] (Fig. 4).

Identification of Crude Vegetable Drugs

The identity of vegetable drugs and the absence of adulterants has to be ascertained prior to extraction and further processing towards pharmaceutical preparations. While

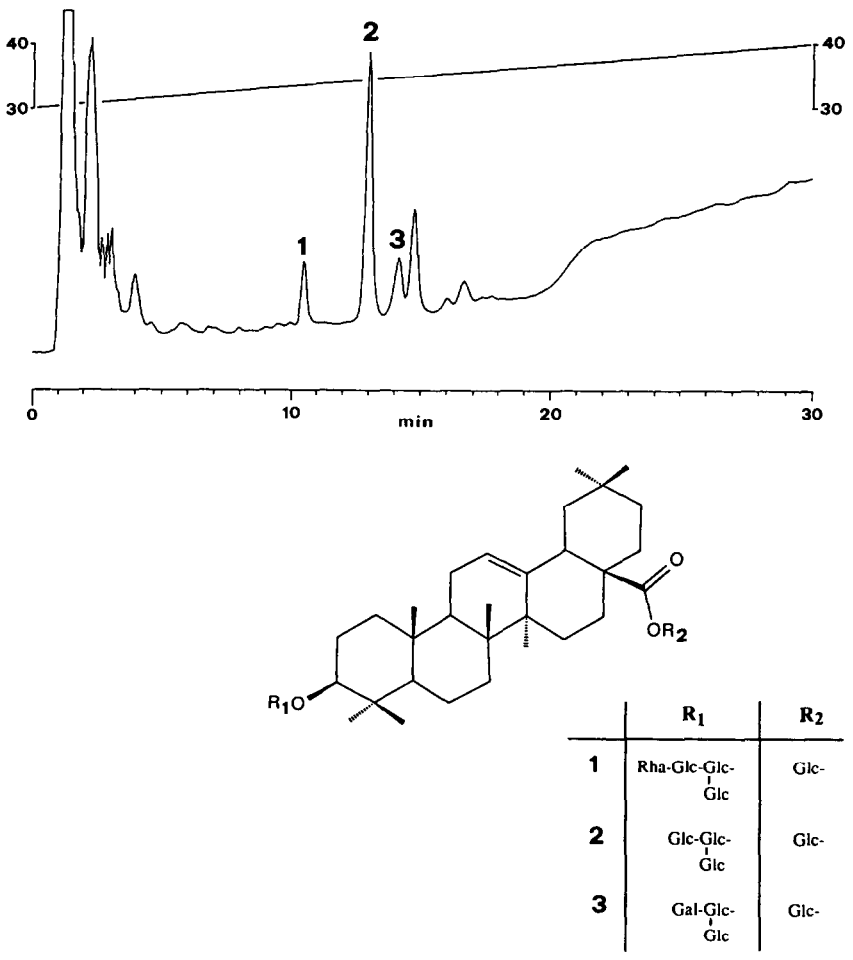


Figure 3
HPLC analysis of an aqueous extract of *Phytolacca dodecandra* berries illustrating the detection of inactive bidesmosidic saponins **1–3**. Column, Knauer LiChrosorb RP-8 5 μm (250 × 4.6 mm, i.d.); eluent, 30–40% acetonitrile over 30 min; flow-rate, 1.5 ml min^{-1}; detection, 206 nm. (Reprinted with permission from ref. 18.)

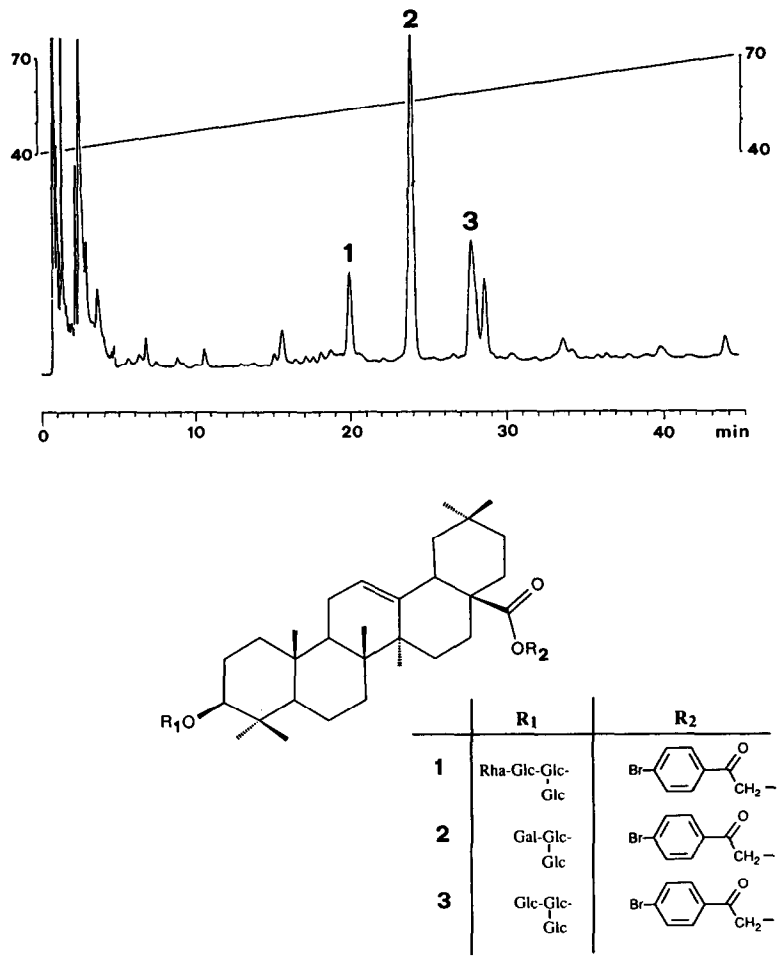


Figure 4
HPLC analysis of a derivatized aqueous extract from *Phytolacca dodecandra* berries illustrating the detection of molluscicidal saponins **1–3**. Column, NovaPak C_{18} 4 μm (150 × 3.9 mm, i.d.); eluent, 40–70% acetonitrile over 45 min; flow-rate, 1 ml min^{-1}; detection, 254 nm. (Reprinted with permission from ref. 18.)

macroscopic and microscopic methods are still very important, the analysis by HPLC produces a characteristic "fingerprint" of the constituents, and allows rapid detection of adulteration by other plants.

An illustrative example is the analysis of *Echinacea* roots. Root extracts of *E. purpurea* and *E. angustifolia* (Compositae) are very popular as immunostimulants for the prevention of infections. In the FRG alone, about 230 different pharmaceutical preparations containing *Echinacea* extracts are commercially available. The increasing demand led to massive intentional adulteration of the drug. All the *Echinacea* root imported from the USA was found to be in fact 100% adulterated by the much cheaper *Parthenium integrifolium* root. HPLC analysis of the chloroform extracts allows a rapid and unambigous distinction of the two drugs (Fig. 5) [19]. While the major peaks of the *Parthenium* extract are due to sequiterpene esters, they are absent in *E. purpurea*. The peaks A–D in the chromatogram of *E. purpurea* are isobutylamides.

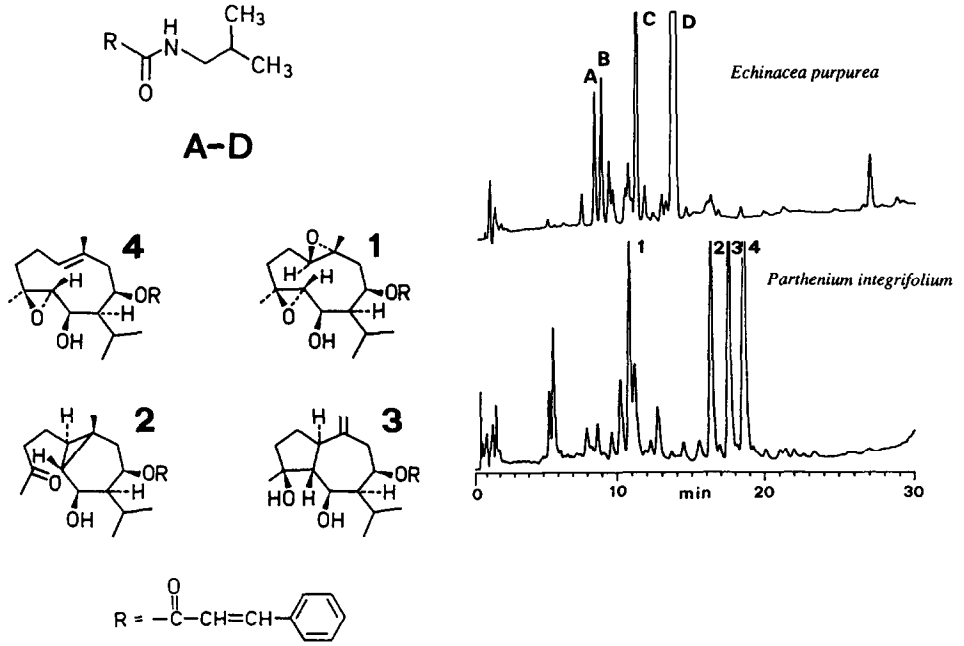


Figure 5
HPLC analysis of the chloroform extracts from the roots of *Echinacea purpurea* and *Parthenium integrifolium*. Column, Hibar LiChrospher RP-18 5 μm (125 × 4 mm, i.d.); eluent, 40–80% acetonitrile over 30 min; flow-rate, 1 ml min^{-1}; detection, 210 nm. (Reprinted with permission from ref. 19.)

Simultaneous measurement of the UV spectra with a photodiode array detector increases the usefulness of the chromatographic "fingerprints", but cannot always exclude ambiguities in case of extracts containing structurally related compounds with similar retention times. For extracts containing polyphenols such as flavonoids or xanthones, additional structural information can be obtained via post-column derivatization with UV–vis shift reagents. Characteristic shifts in the UV–vis spectrum can be induced by chelating agents ($AlCl_3$, H_3BO_3) and deprotonation of phenolic groups in basic solution. This technique has been applied so far to the analysis of extracts from various gentians, which are medicinally used for their bitter principles [20].

Standardization of Extracts

As already mentioned in the Introduction, the standardization of extracts is essential to ensure the quality of phytotherapeutical preparations. The need for stability control and standardization of plant extracts is not only limited to phytotherapeutical preparations in a restricted sense. The use of plant extracts for the control of schistosomiasis may serve as an illustration.

The annual herbaceous plant *Ambrosia maritima* is widely distributed throughout the Mediterranean region. This herb has been found to be an efficient natural molluscicide and has been subsequently used successfully in large-scale field trials for the control of schistosomiasis transmitting snails in water courses [21]. The sesquiterpene lactones ambrosin **2** and damsin **3** (Figs 6 and 7) have been isolated and found to have

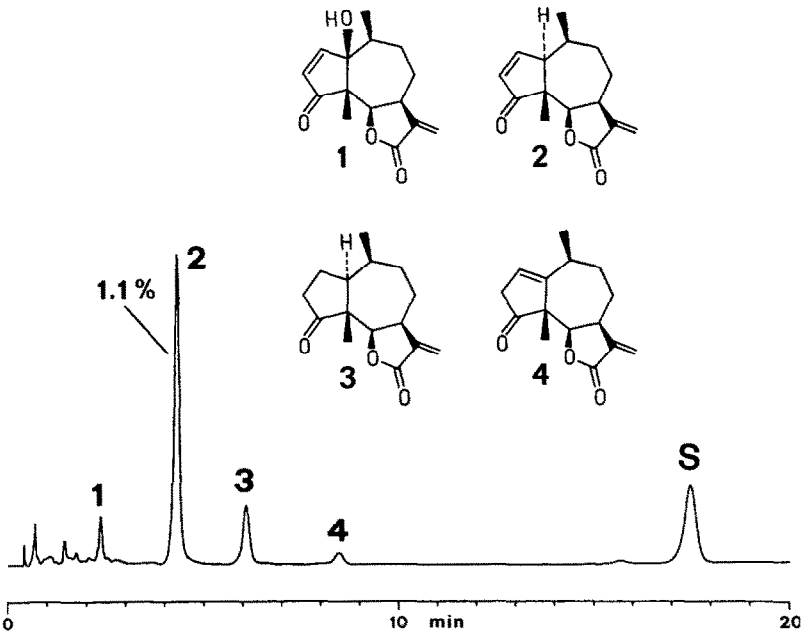


Figure 6
Analysis of sesquiterpene lactones in a chloroform extract of *Ambrosia maritima*. Column: Nucleosil 120-5 C_8 (125 × 4 mm, i.d.); eluent, 35% acetonitrile for 10 min, increasing to 40% acetonitrile over 10 min; flow-rate, 1 ml min^{-1}; detection, 220 nm; internal standard (S), naphthalene. (Reprinted with permission from ref. 22.)

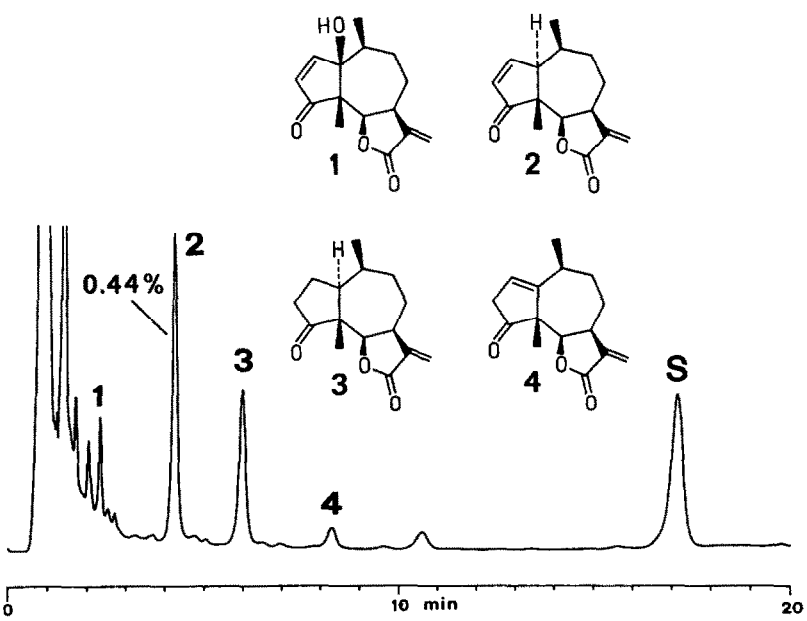


Figure 7
HPLC analysis of a water extract (12 h) of *Ambrosia maritima*. Conditions as in Fig. 6. (Reprinted with permission from ref. 22.)

molluscicidal activity. A HPLC method for the determination of the major sesquiterpene lactones became necessary to compare the plant material from different strains and geographical locations, to optimize extraction methods, and to investigate stability and biodegradation.

Quantitative analysis of the four major compounds **1–4** was carried out with naphthalene as internal standard, because its absorption maximum is identical with that of the sesquiterpene lactones. Aqueous extracts obtained after varying extraction time (6 h–7 days) were analysed and compared with chloroform extracts (exhaustive extraction of the sesquiterpene lactones) (Figs 6 and 7). Aqueous extraction for 12 h was found to produce the highest yields in molluscicidal compounds, while the sesquiterpene lactones were completely degraded after 7 days. No exhaustive extraction of the rather lipophilic compounds could be obtained with water [22].

For many medicinal plants, the active principles are still not known with certainty. While, for example, the immunostimulating effect of *E. purpurea* roots has been shown *in vivo* on animals and in humans [23], the nature of the active principles are still under debate. Water soluble polysaccharides [24] and liphophilic constituents [25] have shown activity in model systems *in vitro* and *in vivo*, but have not been tested in clinical trials. Therefore, *Echinacea* extracts are usually standardized with the aid of a marker substance, that is a characteristic constituent of the plant. Although this approach obviously cannot provide a direct correlation with the pharmacological activity, it nevertheless allows a process control which, by extrapolation, should guarantee a constant activity from one batch to another. In the case of *Echinacea* preparations, the phenylpropanoid ester echinacoside is generally used as marker [3]. A chromatogram of an ethanolic extract of *E. angustifolia* is shown in Fig. 8. At 330 nm, echinacoside produces the major peak in the chromatogram and can therefore be easily determined [26].

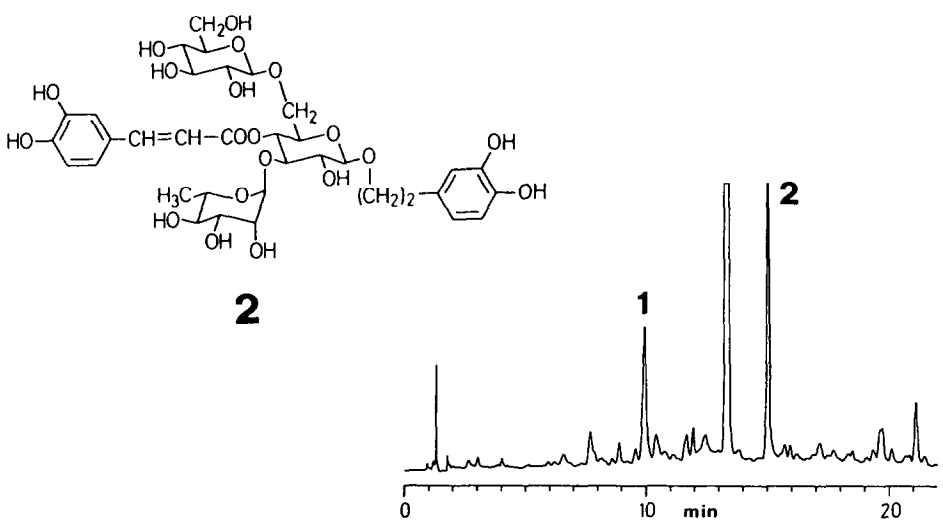
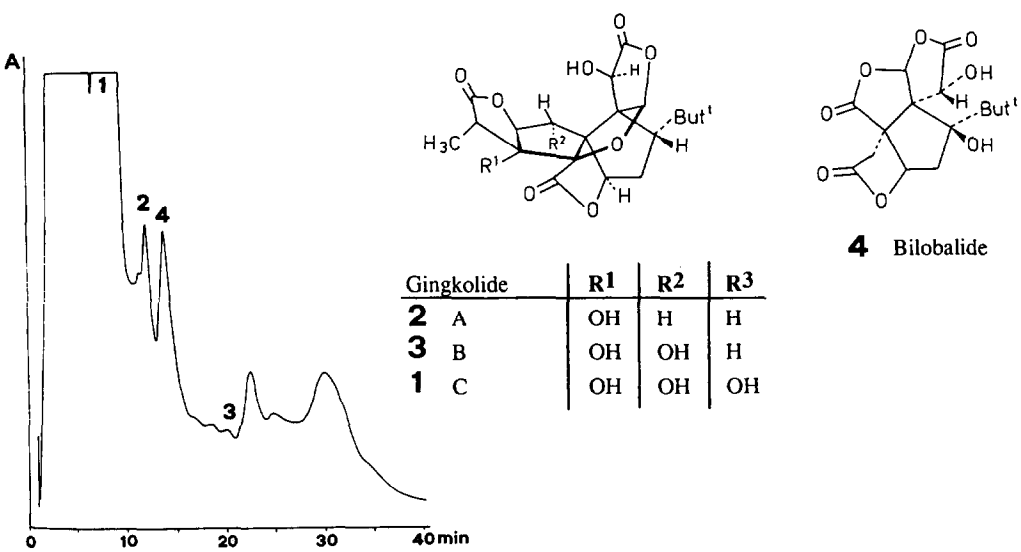


Figure 8
HPLC separation of the phenolic constituents of *Echinacea angustifolia* roots: (**1**) cynarin, (**2**) echinacoside. Column, Hibar LiChrospher C_{18} 5 μm (125 × 4 mm, i.d.); eluent, 5–25% acetonitrile over 20 min; mobile phase with 1% (v/v) 0.1 N phosphoric acid; flow-rate, 1 ml min^{-1}; detection, 330 nm. (Reprinted with permission from ref. 26.)

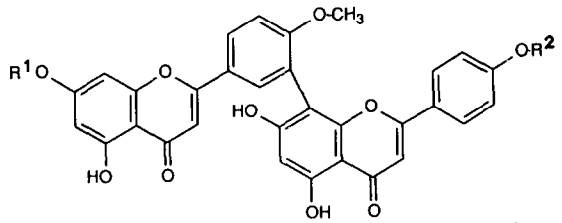
Even when the active principles of a medicinal plant are known, the extracts may have to be standardized with the aid of marker compounds. This is the case for *G. biloba* extracts. Their pharmacological properties can be ascribed to the ginkgolides, diterpenoids with a unique skeleton that has not been found in other plants. Ginkgolides are potent inhibitors of the platelet activating factor [1]. The absence of a chromophore renders their quantitative analysis in a crude extract impossible. Figure 9 shows a HPLC chromatogram of a prepurified ethanolic extract. Only ginkgolides A, B and bilobalide can be detected, while the faster eluting ginkgolide C is hidden under interfering peaks [27]. Thus, even partially purified extracts do not allow standardization on the diterpenoids. *Ginkgo* leaves, however, contain many flavonoids, among others several biflavones. A quantitative determination of biflavones is possible in an ethanolic extract without any sample work-up. Detection at 330 nm significantly reduces the number of peaks in the chromatogram (Fig. 10) [28]. Thus, the commercialized *Ginkgo* preparations are all standardized on the flavonoids, either biflavones or simple flavonol aglycones obtained after acid hydrolysis of the extracts.

Unlike synthetic drugs, plant extracts contain usually several active principles. If they are structurally related and exhibit identical or similar pharmacological properties, the method employed for the standardization should determine the active principles as a whole. Chestnuts (seeds of *Aesculus hippocastanum*, Hippocastanaceae) are the source of escin, used for its antioedematous properties in a multitude of phytotherapeutical preparations to counteract capillary fragility. Although obtained in crystalline form, escin is in fact a complex mixture of closely related triterpenoid saponins which differ only in the nature of the acids attached to the hydroxyl groups at C-21, C-22 and C-28 [29]. Basic hydrolysis (0.1 N KOH, reflux for 15 min), followed by a purification over a C_{18} cartridge yields a simplified chromatogram which shows one major saponin peak



Gingkolide	R1	R2	R3
2 A	OH	H	H
3 B	OH	OH	H
1 C	OH	OH	OH

Figure 9
Analysis of diterpenoids from *Ginkgo biloba* leaves. Chromatogram of a partially purified ethanolic extract from the leaves. Column, Hibar LiChrosorb RP-18 10 μm (250 × 4 mm, i.d.); eluent, water–methanol–tetrahydrofuran (75:5:15%, v/v/v); flow-rate, 1.5 ml min^{-1}; detection, 220 nm. (Reprinted with permission from ref. 27.)



	R_1	R_2
1	CH_3	CH_3
2	CH_3	H
3	H	CH_3
4	H	H

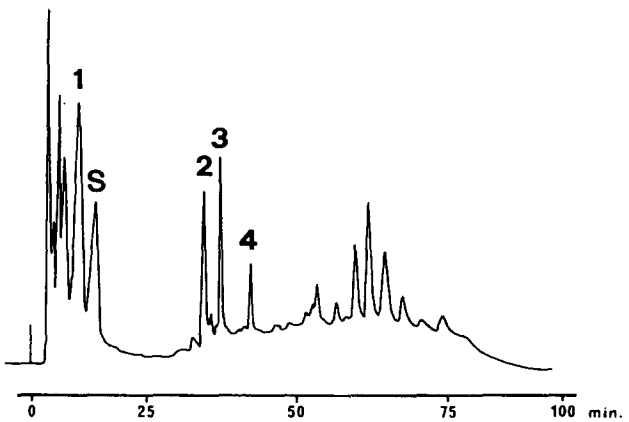


Figure 10
HPLC analysis of a crude ethanolic extract from the leaves of *Ginkgo biloba*. Column, Hibar LiChrosorb DIOL 5 μm (250 × 4 mm, i.d.); eluents, A = hexane–chloroform (25:75%, v/v), B = tetrahydrofuran gradient profile, 0.1% A for 15 min, 0.1–50% A from 15 to 45 min (concave gradient), 50–75% A from 45 to 65 min (linear gradient), 75% A from 65 to 100 min; flow-rate, 1 ml min^{-1}; detection 330 nm. (Reprinted with permission from ref. 28.)

corresponding to glycosides of protoescigenin (Fig. 11) [30]. *p*-Hydroxybenzoic acid methyl ester has been used as internal standard (S). With this method, the total saponin content of chestnut extracts can be reliably determined after basic hydrolysis. The values obtained are generally lower than those determined with the older colorimetric methods.

Conclusion

Modern analytical methods, in particular HPLC, allow rapid and reliable analysis of complex mixtures such as plant extracts. HPLC is used in all domains related to drugs from natural origin, from the isolation of reference compounds to the detection of adulterants and finally to the standardization of plant extracts to be incorporated into pharmaceutical preparations. Other important fields of application include notably the selection of high-producing strains and stability control. It is obvious that modern HPLC contributes in a significant way to the safety and quality of phytotherapeutical preparations.

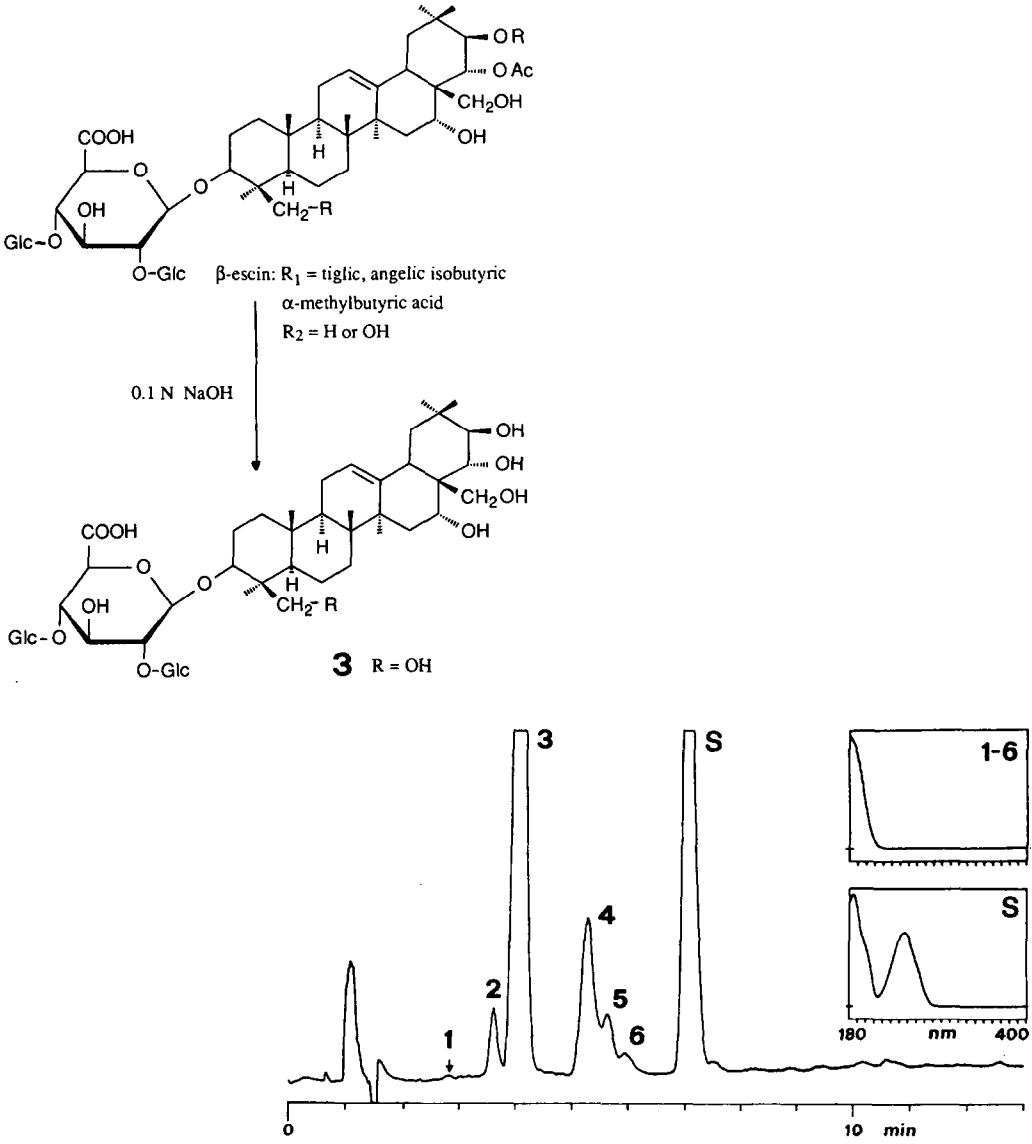


Figure 11
HPLC chromatogram of β-escin after basic hydrolysis showing spectra acquired by photodiode array. Column, Hibar LiChrospher RP-18 (125 × 4 mm, i.d.); eluent, 22–28% acetonitrile in 10 min; mobile phase with 1% 0.1 N phosphoric acid; flow-rate, 1.0 ml min^{-1}; detection, 200 nm. (Reprinted with permission from ref. 30.)

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References

[1] P. Braquet, *Drugs Future* **12**, 643–699 (1987).
[2] J. P. Balz, in *Proceedings of Sixième colloque international consacré aux plantes médicinales et substances d'origine naturelle*, pp. 21–30. Editions P. Fabre, Angers (1988).

[3] G. Harnischfeger (Ed.), *Qualitätskontrolle von Phytopharmaka*, G. Thieme, Stuttgart (1985).
[4] K. Hostettmann, M. Hostettmann and A. Marston, *Preparative Chromatography Techniques — Applications in Natural Products Isolation*. Springer, Berlin (1986).
[5] K. Hostettmann, *Advances in Chromatography*, (J. C. Giddings, E. Grushka, J. Cazes and P. R. Brown, Eds), Vol. 21, pp. 165–186. Marcel Dekker, New York (1983).
[6] K. Hostettmann, M. Hostettmann and A. Marston, *Natural Products Reports* **1**, 471–481 (1984).
[7] A. Marston, C. Borel and K. Hostettmann, *J. Chromatogr.* **450**, 91–99 (1988).
[8] L. A. Decosterd, A.-C. Dorsaz and K. Hostettmann, *J. Chromatogr.* **406**, 367–373 (1987).
[9] O. Potterat and K. Hostettmann, unpublished results.
[10] O. Sticher and F. Soldati, *Planta Med.* **36**, 30–42 (1979).
[11] X. Dong, M. Hamburger, G. A. Cordell and H. H. S. Fong, *Planta Med.* **55**, 185–187 (1989).
[12] H. Wagner, H. Reger and R. Bauer, *Dtsch. Apoth. Ztg.* **125**, 1513–1518 (1985).
[13] D. Schaufelberger and K. Hostettmann, *J. Chromatogr.* **389**, 450–455 (1987).
[14] R. Verpoorte and A. Baerheim Svendsen, *Chromatography of Alkaloids, Part B: Gas liquid Chromatography and High-Performance Liquid Chromatography*. Elsevier, Amsterdam (1984).
[15] K. Mott (Ed.), *Plant Molluscicides*. Wiley, Chichester (1987).
[16] K. Hostettmann, H. Kizu and T. Tomomori, *Planta Med.* **44**, 34–35 (1982).
[17] B. Domon, A.-C. Dorsaz and K. Hostettmann, *J. Chromatogr.* **315**, 441–446 (1984).
[18] I. Slacanin, A. Marston and K. Hostettmann, *J. Chromatogr.* **448**, 265–274 (1988).
[19] R. Bauer, *Dtsch. Apoth. Ztg.* **127**, 1325–1330 (1987).
[20] K. Hostettmann, B. Domon, D. Schaufelberger and M. Hostettmann, *J. Chromatogr.* **283**, 137–147 (1984).
[21] M. F. El-Sawy, J. Duncan, T. F. de C. Marshall, M. A. R. Shehata and N. Brown, *Tropenmed. Parasitol.* **35**, 100–104 (1984).
[22] I. Slacanin, D. Vargas, A. Marston and K. Hostettmann, *J. Chromatogr.* **457**, 325–331 (1988).
[23] G. Harnischfeger and H. Stolze, *Bewährte Arzneipflanzen in Wissenschaft und Medizin*. Notamed, Bad Homburg/Melsungen (1983).
[24] H. Wagner, A. Proksch, I. Riess-Maurer, A. Vollmar, S. Odenthal, H. Stuppner, K. Jurcic, M. le Turdu and J. N. Fang, *Arzneim. Forsch.* **35**, 1069–1075 (1985).
[25] R. Bauer, K. Jurcic, J. Puhlmann and H. Wagner, *Arzneim. Forsch.* **38**, 276–281 (1988).
[26] R. Bauer, I. A. Khan and H. Wagner, *Planta Med.* **54**, 426–430 (1988).
[27] A. Lobstein-Guth, F. Briançon-Scheid and R. Anton, *J. Chromatogr.* **267**, 431–438 (1983).
[28] F. Briançon-Scheid, A. Lobstein-Guth and R. Anton, *Planta Med.* **49**, 204–207 (1983).
[29] H. Wagner, *Pharmazeutische Biologie*, Drogen und Ihre Inhaltsstoffe, 3rd edn, Vol. 2, pp. 149–150. G. Fischer, Stuttgart (1985).
[30] H. Wagner, H. Reger and R. Bauer, *Dtsch. Apoth. Ztg.* **125**, 1513–1518 (1985).

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