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Note

Determination of arbutin in uvae-ursi folium (bearberry leaves) by capillary zone electrophoresis

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Uvae-ursi folium are the dried leaves of *Arctostaphylos uva-ursi* (L.) Sprengel (bearberry). The crude drug consists mainly of three groups of pharmaceutically relevant compounds: phenols, tanning agents and flavonoids[1,2]. The main phenolic constituent is arbutin (hydroquinone β-D-monoglucopyranoside), with a content of 6–15% (w/w) relative to the dry plant material. Other phenolic compounds found are methylarbutin (up to 2.5%) and hydroquinone (in low concentration). Tanning agents are present in uvae-ursi folium at concentrations of 15–20%, mainly as gallotannins but also as catechotannins, as derivatives of ellagic acid and as galloyl arbutin esters. Free gallic acid is also found in the leaves, and is used with other compounds as an indicator substance to prove the identity of the crude drug[1,2]. The main flavonoid found is quercetin-3-O-galactoside (hyperoside).

The aqueous extract of the crude drug acts as a urinary disinfectant[1,3]. The pharmacologically active compound is hydroquinone, originating from arbutin by *in vivo* glucoside cleavage. Free hydroquinone formed in this way is then conjugated, leading to (non-active) glucuronides and sulphates, which are cleaved in alkaline urine to free hydroquinone, a process supported by simultaneous application of sodium carbonate.

The determination of arbutin and the proof of the identity of the crude drug, as specified in DAB 9[1], are carried out not by a single but by at least two different methods. The identity of the drug is controlled either by microsublimation and determination of hydroquinone, formed by the procedure described, or by the qualitative analysis of arbutin, hydroquinone and gallic acid by thin-layer chromatographic separation followed by derivatization.

The determination of arbutin according to DAB 9 is carried out photometrically, based on the derivative formed by the so-called Emerson reaction[4] after treatment with 4-aminoantipyrine, ammonia and potassium hexacyanoferrate(III). By this procedure an overall parameter for phenolic compounds in general is determined, although the result is related to arbutin.

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In addition to the procedures specified by DAB 9, arbutin has been determined in crude drugs by high-performance liquid chromatography (HPLC) in the presence of other hydroquinone derivatives[5,6]. The identity of the drug, however, was not proved by this method in accordance with DAB 9, because gallic acid, specified as an indicator substance besides arbutin and hydroquinone, was not determined.

In this paper, a simple procedure based on capillary zone electrophoresis is introduced, which simultaneously provides proof of the identity of the drug and the determination of arbutin.

EXPERIMENTAL

All chemicals and solvents used for the preparation of the buffering electrolytes were of analytical-reagent grade (E. Merck, Darmstadt, F.R.G.). Water was distilled twice from a quartz apparatus before use. Resorcinol, used as the internal standard, hydroquinone and gallic acid were also of analytical-reagent grade (E. Merck). Arbutin was isolated on a semipreparative scale from uvae-ursi folium[7].

The aqueous extracts of the crude drugs were prepared as follows: 4 g of sliced dried bearberry leaves were suspended in 100 ml of water, the solution was boiled for 30 min and filtered twice and the resulting filtrate was centrifuged at 15000 rpm, representing a maximum of 15850 *g* at the bottom of the centrifugation tubes, for 5 min (Microfuge E; Beckman, Palo Alto, CA, U.S.A.). A 5-ml volume of the supernatant solution was diluted to an appropriate volume, the internal standard was added and an aliquot of a few nanolitres was injected directly.

Zone electrophoresis was performed on a P/ACE System 2000 instrument (Beckman) equipped with a separation capillary made from fused silica (75 μm I.D., 50 cm lenght to the detector). The field strength was about 350 V/cm at an electric current of about 70 μA. Hydrodynamic injection was carried out pneumatically for 1 s. Between separations, two washing steps were carried out automatically: rinsing with sodium hydroxide solution (0.1 mol/l) for 2 min was followed by washing the capillary with working buffer (sodium borate of different pH values, borate concentration 0.1 mol/l) for 2 min.

Detection by UV absorbance was carried out at 214 nm. Recording and processing of the data were performed with a computerized system (System Gold, Beckman).

RESULTS AND DISCUSSION

Qualitative analysis

Important characteristics of an analytical procedure are sufficiently high precision and accuracy and also the time of analysis. Therefore, often it is not relevant in method developement to maximize the resolution of the sample components without fulfilling the demands on analysis time.

Arbutin and hydroquinone (one of the components used to prove the identity of uvae-ursi folium) are weakly acidic phenols with pK_a values of about 10. A high pH of the buffering electrolyte is therefore required in order to separate these analytes via the different effective mobilities of the anions formed. It can be seen from Fig. 1 that at pH 9, both analytes exhibit about equal migration times, which are very close to that of an

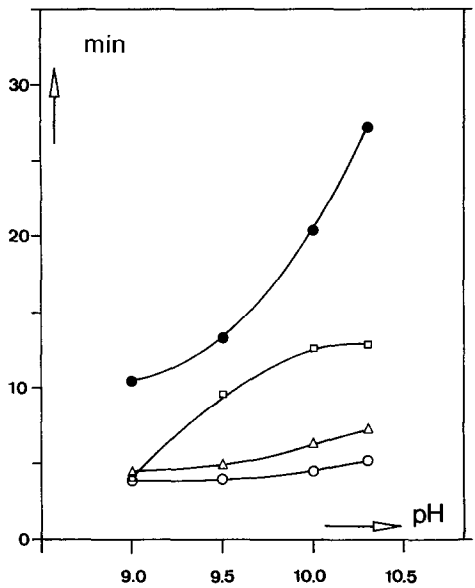

Fig. 1. Dependence of the migration times of arbutin, hydroquinone and gallic acid on the pH of the buffering electrolyte. The migration time of neutral compounds, transported by electroosmosis, was measured from the occurrence of a negative signal on the UV detector due to water injected as the solvent of the standards. ○, Neutral compounds; △, arbutin; □, hydroquinone; ●, gallic acid. Buffers: sodium borate of different pH (0.1 mol/l).

uncharged species. Migration is caused mainly by the electroosmotic flow of the bulk solvent, directed towards the cathode, which originates from the electric double layer between the surface of the fused-silica material and the buffering electrolyte. Increasing the pH of the buffer leads to an increase in the degree of dissociation of these phenols, and the times of migration are enhanced in this way. In fact, it can be seen from Fig. 1 that the difference in the migration times is drastically affected by changing the pH from 9.0 to 9.5. This difference is not influenced significantly by a further increase in pH. On the other hand, the time of migration of gallic acid, which is also an important marker substance for proving the identity of the crude drug under consideration, increases steeply from about 10.5 min at pH 9 to more than 27 min at pH 10.3.

Based on the dependence of the migration times on pH (Fig. 1), it can be concluded that the most favourable electrolyte system is that with pH 9.5. Therefore, this system was applied for the determination of arbutin, and also for monitoring hydroquinone and gallic acid in the extracts of the leaves. At pH 9.5 it was found that arbutin is sufficiently separated from non-ionic compounds, which migrate due to electroosmosis and are detected at about 4 min, arbutin is well separated from hydroquinone and the time of analysis, given by the migration time of the last-eluting component of interest (gallic acid), is only about 13 min, which can be considered to be acceptable.

A typical electropherogram obtained from an aqueous extract of uvae-ursi folium with a buffering electrolyte of pH 9.5 is shown in Fig. 2. Arbutin, hydroquinone

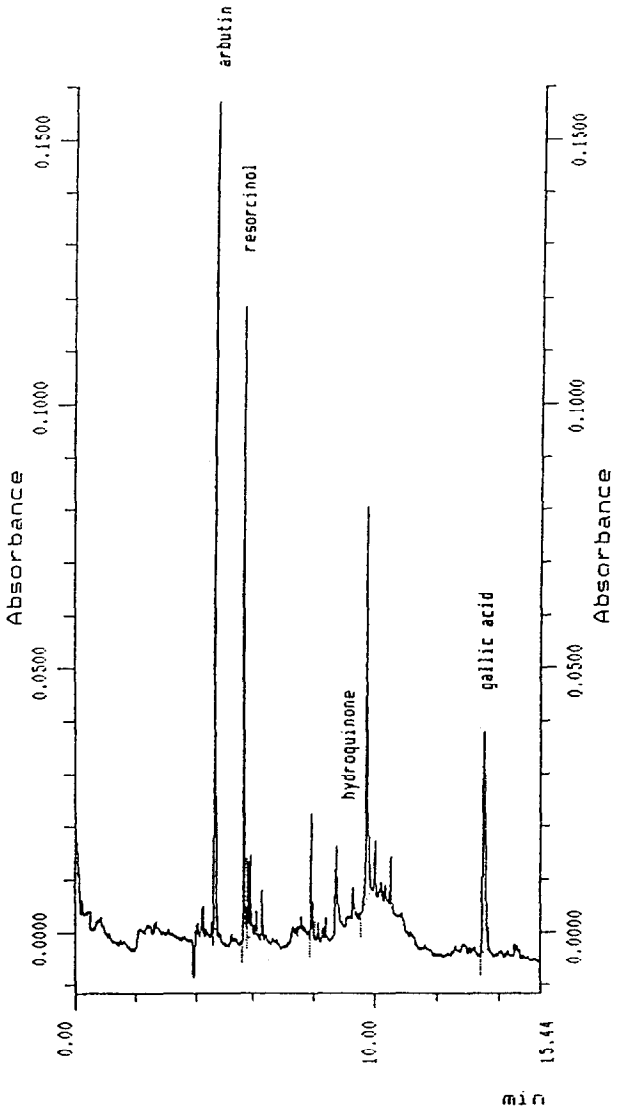

Fig. 2. Typical electropherogram of an aqueous extract of uvae-ursi folium. The electropherogram was obtained from sample 1 in Table I. Conditions: separation capillary, fused silica, 75 μm I.D., 50 cm length to detector; 350 V/cm; 70 μA; buffer, pH 9.5 (sodium borate, 0.1 mol/l); detection, UV, 214 nm.

and gallic acid are clearly distinguished; therefore, the identity of the drug is easily proved. Further, arbutin is well separated from other components under the given conditons permitting an appropriate determination of this compound. The main fraction of anionic components (probably flavonoids), which are detected between 7 and 9 min, do not interfere with this determination.

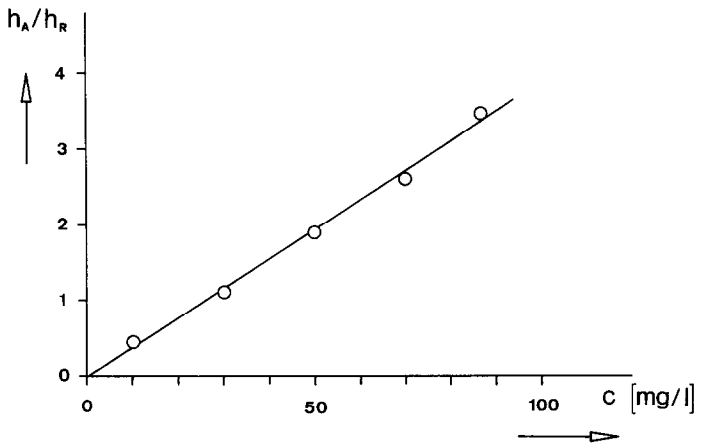

Fig. 3. Calibration graph for the determination of arbutin in crude drugs. h_A/h_R = ratio of the peak heights of arbutin and resorcinol (internal standard); c = concentration of arbutin (mg/l) in the solution injected.

Determination of arbutin

In addition to the proof of identity of the plant drug, arbutin was determined in the extracts of crude drugs using an internal standard. Resorcinol was selected for this purpose, because it does not occur naturally in uvae-ursi folium and it has similar chemical properties to the phenolic analyte. Peak heights of the analyte and the internal standard were used because they are not as strongly affected by variations in the electroosmotic flow as peak areas. The calibration graph is shown in Fig. 3. The linearity was good (linear correlation coefficient 0.998).

The detection limit for arbutin, defined for a signal-to-noise ratio of 3, is about 10 fmol. It is of minor importance for the samples of interest.

The results of the determination of arbutin in four different commercially available crude drugs, obtained from different sources, are given in Table I. The values were calculated from duplicate measurements. In all instances the content of arbutin is within the range specified by DAB 9 (6–15%).

The precision, expressed as the standard deviation, determined, *e.g.*, from eight measurements on extract 4, (Table I), was 0.135%, related to a concentration of 6.4% of arbutin. The corresponding relative confidence interval (for $P=95\%$) is 1.8%.

TABLE I

RESULTS OF THE DETERMINATION OF ARBUTIN IN UVAE-URSI FOLIUM

Sample No.	*Arbutin content (%, w/w)*[a]
1	7.0
2	7.2
3	7.6
4	6.4

[a] The content is relative to the dry leaves.

Hence it can be concluded that the precision of the measurement meets the demands for this type of analysis.

ACKNOWLEDGEMENTS

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