Acta Poloniae Pharmaceutica – Drug Research, Vol. 63 No. 5 pp. 395–400, 2007 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

DENSITOMETRIC DETERMINATION OF ARBUTIN IN COWBERRY LEAVES (*VACCINIUM VITIS IDAEAE*)

ALINA PYKA[1] , KATARZYNA BOBER[1], and ADAM STOLARCZYK[2]

[1]Department of Analytical Chemistry, [2]Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy, Silesian Academy of Medicine,
4 Jagiellońska Str., 41-200 Sosnowiec, Poland

Abstract: Densitometry was used for quantitative determination of arbutin (*Vaccinium vitis idaeae*) in leaves of cowberry collected from region of Suwalszczyzna, Poland. Arbutin was extracted using methanol. Chromatography was performed on glass TLC plates with layers of silica gel. The quantitative densitometric analysis was performed using internal standard solution method. On the base of densitometric analysis it was shown that the band characteristic for absorption maximum of arbutin is placed at λ_{max} = 285 nm. The second absorption band is at λ = 225 nm. It was stated that contents of arbutin are ca. 35 mg and 47 mg in 1 g of herbs, in cowberry leaves coming from collections in 2005 and 2006 year, respectively. The presented method is accurate, selective, and precise, and can be used for routine quality control analysis and quantitative determination of arbutin in cowberry leaves.

Keywords: Arbutin, densitometry, TLC, cowberry leaves, *VaccinIum vitis idaeae*

Arbutin is a hydroquinone derivative with molecular formula $C_{12}H_{16}O_7$. It shows significant therapeutic activity. Arbutin is, among others, antibacterial agent of urinary tract (1). It is also used in cosmetic preparations as a component causing whitening in melanosis (2). Arbutin is present in many plants, for example in leaves of bergenia (*Bergenia crassiflora, Bergenia cordifolia*) (1), and also in flowers, leaves, peel and pulp of some oriental species of pear (*Pyrus bretschnrideri, Pyrus pyrifolia, Pyrus ussuriensis, Pyrus sinkiangensis*) (3) and also in composition of pear which is present in Poland (*Pyrus communis*) (4), as well as in leaves of *Breynia officinalis,* which originates from *Euphorbiaceae* (5). Arbutin is also present in leaves of bearberry (*Arctostaphylos uva ursi*) and in leaves of cowberry (*Vaccinium vitis idaeae*) (1).

Among others, the spectrophotometric method was used to determine arbutin (6). It was based on the oxidation of arbutin by periodate. The unreacted periodate was determined spectrophotometrically. The identification limit was determined at 25 μg/mL. An alternative method was also proposed, based on spectrophotometric determination of yellowish-green complex which is produced in reaction between arbutin and ferric chloride (6). Arbutin was also determined in whitening cosmetics using HPLC method. The detection limit in that case was 15 μM (7). The HPLC and UV spectrum analyses as well as LC/MS analysis served to determine the oriental species of pear (*Pyrus bretschnrideri*) (3). The leaves, peel and pulp were analyzed. Qualitative and quantitative determination of arbutin was done also in pear species present in Poland (4). Thin layer chromatography was used for qualitative determination whilst the content of arbutin was determined by HPLC. Both leaves (*Folium Vitis idaeae*) and fruits (*Fructus Vitis idaeae*) of cowberry are materials with therapeutic properties (8). According to Polish Pharmacopoeia, only leaves are used as therapeutic agent having exactly determined content of arbutin (4%) (1). In the leaves of cowberry – arbutin, methylarbutin, flavonoids and ursolic acid are present, whilst in fruits there are organic acids, tannins, carbohydrates and vitamins A and C (8). Products prepared from cowberry disinfect urinary bladder and tracks, act diuretically and stiptically (8).

The aim of this study was the use of densitometry for quantitative determination of arbutin in the leaves of cowberry (*Vaccinium vitis idaeae*). Up to now, arbutin was quantitatively determined using spectrophotometric and HPLC methods (3, 4, 6, 9).

* Corresponding autor: e-mail: alinapyka@wp.pl

EXPERIMENTAL

Apparatus

Densitometer: Camag (Muttenz, Switzerland) TLC Scanner 3 with witCats 1.4.2 software. Computer: PC Pentium MMX, 128 MB RAM, Hewlett-Packard DeskJet 930 printer (Microsoft Office 2000 Premium, Statistica 7.0)

TLC plates: 20 × 20 cm (glass plates precoated with silica gel $60F_{254}$; Art. 1.05715; Merck Darmstadt, Germany). The 5 μL Camag micropipettes (Muttenz, Switzerland) were used to apply the solution to the plates.

Chromatographic chamber: twin-trough chamber (Camag, Muttenz, Switzerland).

Chemicals

Arbutin: Cat. No. A 4256 (Sigma – Aldrich) was used as a standard. All chemicals and reagents used for TLC were of analytical grade and were purchased from POCh, Gliwice, Poland.

Plant material

Leaves of cowberry were collected in 2005 and 2006 year from the region of Suwalszczyzna (Poland). They were dried to constant mass in a form of thin layer in permeable to air and shaded place.

Preparation of extract of cowberry leaves

To 1 g of powdered material 5 mL of water-methanol solution (1:1, v/v) was added and then the solution was refluxed for 15 min. The hot solution was filtered and washed with 5 mL of methanol. Then it was cooled down and 0.5 g of lead(IV) hydroxyacetate was added (10). The final volume of extract was 10 mL.

Preparation of standard solution of arbutin

Standard solution of arbutin was prepared by dissolving 60 mg of arbutin in 10 mL of methanol.

Quantitative determination of arbutin in extract of cowberry leaves

The internal standard solution method was used. The following solutions were prepared:
Solution **A**: 1 mL of extract + 1 mL of standard solution of arbutin;
Solution **B**: 1 mL of extract + 0.5 mL of standard solution of arbutin;
Solution **C**: 1 mL of extract + 1 mL of methanol;
Solution **D**: 1 mL of extract + 0.5 mL of methanol.

Thin-layer chromatography

The plates were prewashed with methanol and dried for 24 h at room temperature. Before use they were activated at 120°C for 30 min. The activated plates were manually spotted with 5 μL aliquots of the solutions. The mobile phase was ethyl acetate-methanol-water, (40+5.4+4, v/v) (10); 50 mL of the mobile phase was used per development. Plates were developed to a distance of 14 cm in chromatographic chamber previously saturated with the mobile phase for 30 min at room temperature (20±1°C). After development, the plates were dried in a current of air by means of an air dryer. Densitometric scanning was then performed at λ_{max} =285 nm. The radiation source was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. The slit dimensions were 10.00 × 0.40 mm, Macro; the optimized optical system was light; the scanning speed was 20 mm × s^{-1}; the data resolution was 100 mm × $step^{-1}$; the measurement type was remission; the measurement mode was absorption; the optical filter was second order. Each analysis was repeated five times, whilst each track was scanned three times, and baseline correction (lowest slope) was used.

The spectrum scan was also performed using Camag Scanner TLC 3. The radiation source was a deuterium lamp. The start wavelength was 200 nm and the end wavelength was 350 nm. The slit dimensions were 10.00 × 0.40 mm, Macro; the optimized optical system was resolution; the scanning speed was 20 nm × s^{-1}; the data resolution was 1 nm × $step^{-1}$.

Validation of the Method

Linearity of detector response

The linearity of the TLC method was evaluated by analysis of eleven standard solution of arbutin of concentrations 0.2, 0.4, 0.6, 1, 1.5, 2, 3, 6, 8, 12, and 15 mg × mL^{-1}. The solutions (5 mL) were applied on the same plate. The plate was developed using the above-mentioned mobile phase.

Recovery

The recovery was computed as a ratio of constituent concentration determined in the model product to its weighed amount.

Precision

The precision of the method was determined as the degree of consistency between the peak areas recorded for individual arbutin spots. For this purpose 5 mL of solution A was applied onto the plates.

Specificity

The specificity of the method was ascertained by comparing the R_F values and the spectrum of

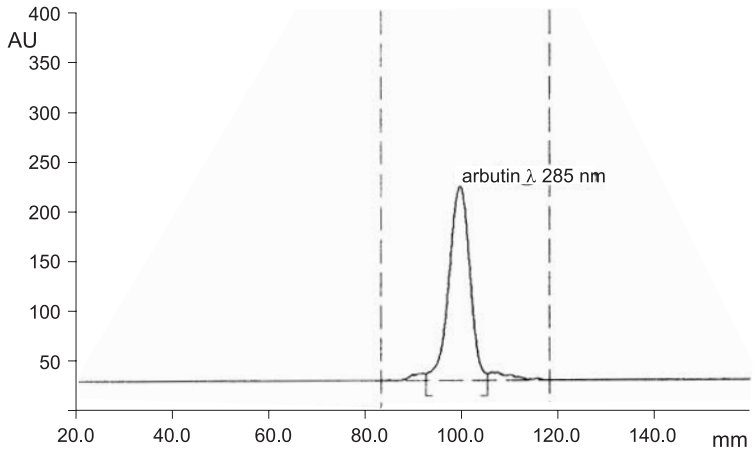

Figure 1. Densitogram of arbutin coming from the cowberry leaves extract without addition of the internal standard solution (solution D).

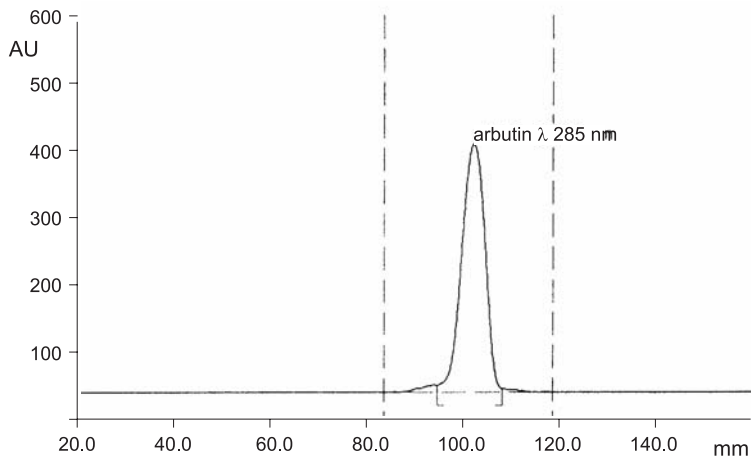

Figure 2. Densitogram of arbutin coming from the cowberry leaves extract with addition of the internal standard solution (solution B).

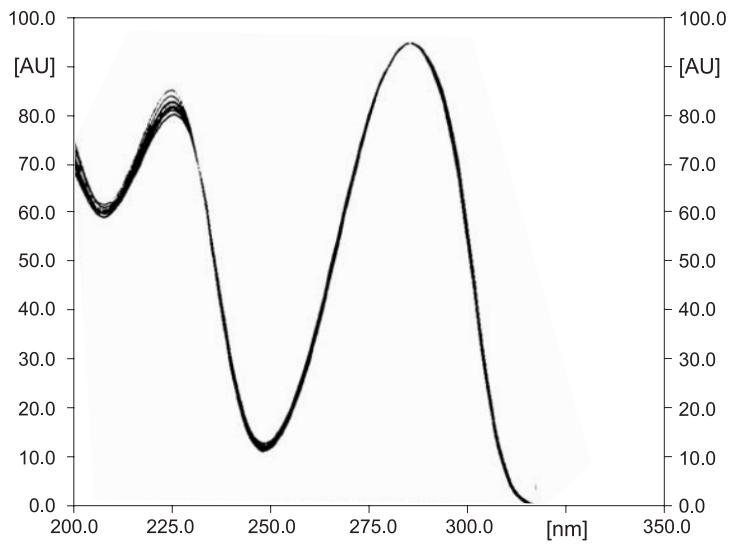

Figure 3. Spectrodensitograms of arbutin coming from the solutions of cowberry leaves extract with and without addition of the internal standard solution, as well as arbutin standard.

Table 1. Method-validation data for the quantitation of arbutin by TLC[a]

Method characteristic	Results
Wavelength	285 nm
R_F	0.58
Linearity range [mg × spot^{-1}]	7¸60
Correlation coefficient (r)	0.998
Limit of detection (LOD) [mg × spot^{-1}]	2
Limit of quantitation (LOQ) [mg × spot^{-1}]	7
Precision n = 8	$\bar{x}$ =23031.3 [AU] SD=185.91 RSD=0.81% μ = ± 65.73
Recovery	$\bar{x}$ = 98.06 % SD = 1.20 RSD=1.22%

[a] SD – standard deviation, RSD – relative standard deviation

Table 2. Statistical data concerning results of quantitative determination of arbutin in extract of cowberry leaves from collection in 2005 and 2006 years.

Statistical data	Quantitative determination of arbutin in the extract of cowberry leaves from the collection in	
	year 2005	year 2006
Number of analyses	10	10
Average contents of arbutin [mg] in 1g of herbs	34.92	46.78
Minimum contents of arbutin [mg] in 1g of herbs	34.35	46.10
Maximum contents of arbutin [mg] in 1g of herbs	35.35	47.45
Variance	0.112	0.234
Standard deviation	0.34	0.47
Relative standard deviation [%]	0.97	1.00
Standardized skewness	– 0.394	– 0.594
Standardized kurtosis	– 0.264	– 0.721

arbutin standard with the spectra obtained from a sample from an arbutin extract, at three different positions on the bands, i.e. peak start (S), peak apex (M), and peak end (E).

RESULTS AND DISCUSSION

On the base on spectrodensitometric analysis it was shown that band characterized by absorption maximum of arbutin is placed at λ_{max} = 285 nm. The second absorption band is at λ = 225 nm. In our earlier work we obtained similar results (11). Therefore, in this work quantitative determination of arbutin was performed at 285 nm.

In the present work the contents of arbutin in extract of cowberry leaves was calculated from difference area of chromatographic bands obtained for extract of cowberry leaves with and without adding the internal standard. Typical densitograms of arbutin coming from the extract of cowberry leaves without (solution D) and with addition of internal standard solution (solution B) as an example are presented in Figure 1 and 2, respectively.

Validation data for the presented method are summarized in Table 1. The statistical data shown in Table 1 indicate that linear relationships exist between the area of peaks [AU] and the concentration of arbutin [mg per spot]. The plot was linear in the

range 7 to 60 mg per spot and correlation coefficient was 0.998. A graph of residuals against the concentration of arbutin was also plotted. It was observed that the residuals were distributed both above and below the zero residuals line. The limits of detection (LOD) and limit of quantitation (LOQ), defined as signal-to-noise ratios of 3 and 10, respectively, were 2 and 7 mg per spot, respectively (Table 1).

The precision of the determination is very good; the relative standard deviation (RSD) value for arbutin from extract is 0.81%. Because RSD was less than 2% (Table 1) the proposed method is precise (12).

The accuracy of the method, as determined by using the model product, was very high and was equal to 98.06%.

The specificity of the methods was ascertained by comparing the R_F values of arbutin standard, and arbutin from extract without and with the internal standard. In each case, the R_F of arbutin was equal to 0.58. The peak purity of arbutin was assessed by comparing the spectra obtained from a standard at the peak start, peak apex, and peak end of a spot. It was found that r(S,M) = 0.9989, and r(M,E) = 0.9981. Good correlations (r = 0.9999) were also found between spectra of arbutin standard and spectra of arbutin from the extract. The spectrodensitograms of arbutin coming from the solution of extract of cowberry leaves with (solutions A and B) and without adding internal standard solution (solutions C and D) and standard solution of arbutin are presented in Figure 3. The very good correspondence of spectrodensitograms was stated. In all cases the absorption maximum (λ_{max}) was equal to 285 nm, whilst the second absorption band was at λ = 225 nm.

The five average values of difference in area of chromatographic bands of arbutin for the extract of cowberry leaves with and without addition of internal standard solution were obtained for solutions A and C as well as B and D. On the basis of these values the contents of arbutin in the extract of cowberry leaves were calculated. The results are presented in Table 2 along with respective statistical data. Of particular interest here are the standardized skewness and standardized kurtosis, which can be used to determine whether the sample comes from a normal distribution. The values of these statistics outside the range of –2 to +2 indicate significant departures from normality, which would tend to indicate many of the statistical procedures normally applied to this data. In this case, the variables do not show standardized skewness and standardized kurtosis values outside the expected range.

From the data obtained, it follows that the medium contents of arbutin in 1g of herbs is equal to 34.92 mg and 46.78 mg, respectively, in cowberry leaves coming from collections in 2005 and 2006 year.

CONCLUSIONS

A new way of determination of arbutin is presented in this work. It was stated that the contents of arbutin are ca. 35 mg and 47 mg in 1 g of herbs, respectively, in cowberry leaves coming from the collections in 2005 and 2006 year. The presented method is accurate, selective, and precise, and can be used for routine quality control analysis and quantitative determination of arbutin in cowberry leaves. The proposed method seems to be more reliable for evaluation of arbutin content in plant material than the visual method proposed and described in Polish Pharmacopoeia VI. The conditions of chromatographic analysis worked out can be also useful for stability estimation as well as chemical changes of arbutin during its storage.

Acknowledgment

This research was financed by the Ministry of Science and Higher Education as the research project No. 3 T09A 155 29.

REFERENCES

1. Kohlmünzer S.: Pharmacognosy (in Polish). PZWL, Warszawa: 1993.
2. Gallarate M., Carlotti M. E., Trotta M., Grande A. E., Talarico C. J.: Cosmet. Sci. 55, 139 (2004).
3. Cui T., Nakamura K., Ma L., Li J. Z., Kayahara H.: J. Agric. Food Chem. 53, 3882 (2005).
4. Rychlinska I. Gudej J.: Acta Pol. Pharm.- Drug Res. 60, 309 (2003).
5. Morikawa H., Kasai R., Otsuka H., Hirata E., Shinzato T., Aramoto M. Takeda Y.: Chem. Pharm. Bull. 52, 1086 (2004).
6. Barsoom B. N., Abdelsamad A. M., Adib N. M.: Spectrochim. Acta A 64, 844 (2006).
7. Lin C. H., Wu H. L., Huang Y. L.: J. Chromatogr. B 829, 149 (2005).
8. Kuznicka B., Dziak M: Herbs and their application. History and present-day (in Polish). PZWL, Warszawa: 1992.
9. Polish Farmakopea VI; PTFarm. Warszawa, 2002.
10. Wagner H., Bladt S.: Plant Drug Analysis, Thin Layer Chromatography Atlas, Springer-Verlag, Berlin, Heidelberg 1996.

11. Pyka A.: Phenolic Drugs, New Visualizing Reagents for Detection in TLC, in: Encyclopedia of Chromatography, 2nd ed., Cazes J., Ed., Taylor & Francis, Boca Raton, FL: 1 (2007).

12. ICH Guidance on Analytical Method Validation, in: Proceedings of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, September 2002.

Received: 13.12.2006