



Micellar liquid chromatographic determination of arbutin and hydroquinone in medicinal plant extracts and commercial cosmetic products

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Synopsis

A simple micellar liquid chromatographic (MLC) procedure for simultaneous determination of arbutin and hydroquinone in medicinal plant extracts and commercial cosmetic products was proposed. This method was developed and validated. The chromatographic conditions were also optimized. All analyses were performed at room temperature in an isocratic mode, using a mixture of 1% (v/v) acetonitrile and 0.006 mol L⁻¹ Brij 35 (pH 6.0) as a mobile phase. The flow rate was set at 1.0 mL min⁻¹. The analytical column was a 150 × 3.9 mm Nova-Pak C-18 column. The effluent from the analytical column was monitored by UV detection at 280 nm. Under the optimum conditions, arbutin and hydroquinone could be determined within a concentration range of 2–50 µg mL⁻¹ of arbutin, and hydroquinone was obtained with the regression equations; $y = 0.045x + 0.042$ ($r^2 = 0.9923$) and $y = 0.091x + 0.050$ ($r^2 = 0.9930$) respectively. The limits of detection were found to be 0.51 µg mL⁻¹ and 0.37 µg mL⁻¹ for arbutin and hydroquinone respectively. The proposed MLC method was applied for the determination of arbutin and hydroquinone contents in medicinal plant extracts and commercial cosmetic products. This proposed MLC method is thus suitable for routine analysis of arbutin and hydroquinone in the pharmaceutical formulations, cosmetic products and raw medicinal plant extracts.

Résumé

Une procédure simple de chromatographie liquide micellaire (MLC) pour la détermination simultanée de l'arbutine et de l'hydroquinone dans les extraits de plantes médicinales et dans des produits cosmétiques commercialisés est proposée. Cette méthode a été développée et validée. Les conditions chromatographiques ont également été optimisées. Toutes les analyses ont été réalisées à température ambiante dans un mode isocratique, en utilisant un mélange de 1% (v/v) d'acétonitrile et 0.006 mol L⁻¹ Brij 35 (pH 6.0) en tant que phase mobile. Le débit a été fixé à 1.0 mL min⁻¹. La colonne analytique était de 150 × 3.9 mm Nova-Pak colonne C-18. L'effluent de la colonne d'analyse a été suivi par une détection UV à 280 nm. Dans les

conditions optimales, l'arbutine et l'hydroquinone peuvent être déterminés dans un intervalle de concentration de 2 à 50 µg mL⁻¹ d'arbutine et d'hydroquinone; ces valeurs ont été obtenues avec les équations de régression, $y = 0.045x + 0.042$ ($r^2 = 0.9923$) et $y = 0.091x + 0.050$ ($r^2 = 0.9930$), respectivement. Les limites de détection ont été jugées 0.51 µg mL⁻¹ et 0.37 µg mL⁻¹ pour l'arbutine et pour l'hydroquinone, respectivement. La méthode proposée MLC a été appliquée à la détermination du contenu de l'hydroquinone et d'arbutine dans les extraits de plantes médicinales et des produits cosmétiques commercialisés. Cette méthode proposée MLC est donc adaptée à l'analyse de routine de l'arbutine et d'hydroquinone dans les formulations pharmaceutiques, des produits cosmétiques et des matières premières extraits de plantes médicinales.

Introduction

Whitening agents are of major importance because of the negative effect of exposure to UV radiation on the skin. In the past few years skin-whitening products have been increasingly in demand. The main purpose of skin-lightening products is to lighten the skin as well as even out skin tone or to treat pigmentation disorders such as freckles melasma, pregnancy marks and age spots. In the skin, the enzyme tyrosinase biochemically converts the amino acid tyrosine into melanin. Arbutin and hydroquinone (Fig. 1) are tyrosinase inhibitor, which inactivate tyrosinase by chelating with its vital copper ion and suppressing the tautomerization from dopachrome to 5, 6-dihydroxyindole-2-carboxylic acid (DHICA) [1]. Arbutin (hydroquinone-β-D-glucopyranoside) is a naturally occurring glycoside of hydroquinone. It is a natural phenolic glycoside found in various plant species of diverse families such as Ericaceae (*Vaccinium* spp., *Arctostaphylos* spp.), Asteraceae (*Achillea millefolium*), Betulaceae (*Betula alba*) and Rosaceae (*Pyrus communis* L.). Arbutin is commonly used in urinary therapeutics, and as a human skin-whitening agent [2]. Arbutin can prevent serious sunburn caused by an accumulation of melanin in subcutaneous tissue produced via a tyrosinase-catalysed metabolic pathway [3–5]. Tyrosinase is the essential enzyme for melanin formation. The depigmenting effect of arbutin has been reported to reduce cellular tyrosinase activity without changing the cell viability [4]. Arbutin is highly water-soluble and has been used in many skin-whitening, depigmenting cosmetics and antioxidants. Hydroquinone (1, 4-benzenediol) introduced for clinical use in 1961, is the most popular depigmenting agent. It may interfere with

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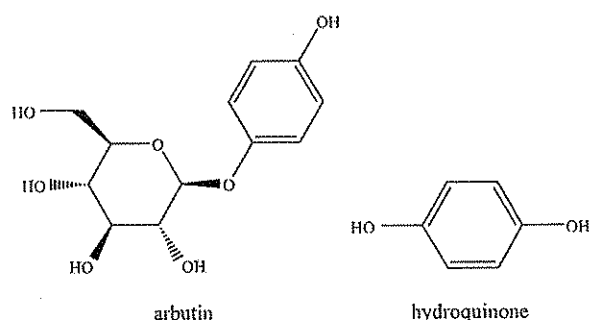


Figure 1. Chemical structures of arbutin and hydroquinone

pigmentation through: the covalent binding to histidine or interaction with coppers at the active site of tyrosinase, the inhibition of DNA and RNA synthesis and the alteration of melanosome formation and melanin. However, because of the hazards of long-term treatments, the European Committee has banned the use of hydroquinone in cosmetics and formulations and it is available only through prescription by physicians and dermatologists [6, 7]. Several methods have been reported for the determination of arbutin and hydroquinone in medicinal plants and commercial whitening cream samples. These include HPLC [8–15, 28], HPTLC densitometry [16], capillary electrophoresis [17] electrochemistry [18] and spectrophotometric methods with different colour reagents [19–22]. Arbutin was also determined by flow injection analysis based on the reaction of arbutin with 4-aminoantipyrine (4-AP) in hexacyanoferrate solution and detected by spectrophotometric method [23]. In recent years, a technique known as micellar liquid chromatography (MLC) has been used as an alternative method to conventional liquid chromatography because of the large number of interactions of solutes with the mobile and stationary phases. Micellar liquid chromatography has several advantages over other chromatographic methods, including low cost, low toxicity, low volatility, the possibility of simultaneous separation of ionic and non-ionic compounds, direct injection of biological fluids and high separation selectivity, because of the involvement of many adjustable conditions [24–31]. The primary objective in the development of MLC separations is to optimize chromatographic performance by adjustment of such experimental conditions as temperature, type and concentration of organic modifier, pH and concentration of surfactant. MLC separations are mainly modified by varying the composition of the mobile phase. An optimization study for the mixture of arbutin and hydroquinone was done. An adequate control of the concentration of the surfactant and modifier can lead to chromatograms presenting a good resolution and sufficient elution strength [32].

In this research work, a rapid and simple micellar liquid chromatographic method for the determination of arbutin and hydroquinone in medicinal plant extracts and pharmaceutical formulations has been developed. The optimized method was applied to the analysis of arbutin and hydroquinone in pear fruits and commercial cosmetic products.

Materials and methods

Apparatus

A Shimadzu model SCL-10A liquid chromatograph (Shimadzu, Japan) was used for the analysis of arbutin and hydroquinone. The

following instruments were also used: spectrophotometer (UV-mini 1240; Shimadzu, Japan); pH-metre (Model pH 900, Precisa, Switzerland); water bath and shaker (Model SB-200-10, Thailand); Ultrasonicator (Model 889, Cole Parmer, U.S.A.); and a rotary evaporator (EYELA N-N series, EYELA, U.S.A.)

Chemicals

The arbutin and hydroquinone standards were obtained from Sigma (Saint Louis, U.S.A.). Cetyl trimethylammonium bromide (CTAB), Polyethylene dodecyl ether (Brij 35) and Sodium dodecyl sulphate (SDS) were purchased from Fluka (Buchs, Switzerland). Acetonitrile, potassium dihydrogen phosphate and 2-propanol were obtained from Carlo-Erba (Italy), disodium hydrogen phosphate from BDH (Poole, U.K.) and orthophosphoric acid 85% (w/v) from Fisher (U.K.). Deionized water was obtained from a Millipore Milli-Q system (Millipore, Bedford, U.S.A.). Other chemicals were analytical reagent grade.

Standard and Mobile Phase Preparation

Stock standard solutions of arbutin and hydroquinone were prepared in water to provide a concentration of 1 mg mL^{-1} . These stock solutions were freshly prepared each time and stored below 4°C and protected from light. The solutions were diluted with 0.006 mol L^{-1} Brij 35 to the desired concentration levels just before performing the analysis. A 0.006 mol L^{-1} Brij 35 stock solution was prepared in phosphate buffer solution. Organic solvents were added to the mobile phases where necessary. All mobile phases and standard solutions were filtered through $0.45 \mu\text{m}$ Nylon membranes (Millipore, Bedford, U.S.A.).

Instrumentation and Chromatographic Conditions

The MLC analyses were carried out with a Shimadzu Model SCL-10A liquid chromatograph, thermostatic column compartment, online degasser with an UV-visible detector model SPD-10A (Shimadzu, Japan) chromatographic separations were performed on a Nova-Pak C_{18} column ($150 \times 3.9 \text{ mm}$, $5.0 \mu\text{m}$ Water Associates, Harrow, U.K.) at room temperature. A mixture of 1% (v/v) acetonitrile and 0.006 mol L^{-1} Brij 35 (pH 6.0) was used as a mobile phase with a flow rate of 1.0 mL min^{-1} . Each sample and/or standard aliquots of $20 \mu\text{L}$ were injected by auto sample injector onto the analytical column. The effluent from the analytical column was monitored by UV detection at 280 nm . The quantification was achieved based on peak area of arbutin and hydroquinone.

Under the optimum conditions used, the time taken for complete separation and determination of arbutin and hydroquinone was within 6.0 min. A representative chromatogram of a standard mixture of arbutin and hydroquinone is shown in Fig. 2 (C).

Sample preparation

Commercial Whitening cream

The skin-whitening products (Samples; A, B and C) were purchased from local retail markets in Phitsanulok, Thailand. The skin-whitening samples were stored at 4°C before use; about 0.5 g of each whitening cream sample was accurately weighted and transferred into three separated 25 mL volumetric flasks and dissolved in micellar mobile phase. To each flask, 10 mg L^{-1} of resorcinol was added as internal standard. The sample solution was sonicated vig-

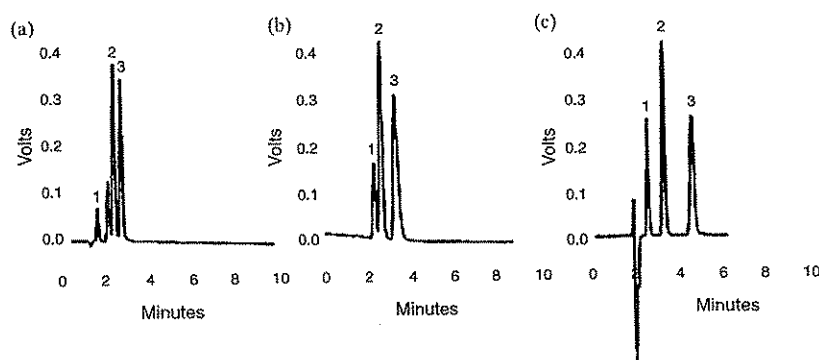


Figure 2 Chromatograms of the (1) arbutin, (2) hydroquinone and (3) resorcinol as internal standard using Nova-Pak C₁₈, 150 × 3.9 mm, 5.0 μm utilizing surfactants mobile phase of (a) SDS, (b) CTAB and (c) Brij 35 respectively

orously for 30 min, centrifuged at 1,252 *g* for 30 min and filtered through millipore membrane (0.45 μm). The supernatant liquid of each sample was kept for further MLC analysis.

Medicinal plant material

Arbutin was presented in many plants, for example in flowers, leaves, peel and pulp of some oriental species of pear (*Pyrus bretschneideri*, *Pyrus pyrifolia*, *Pyrus ussuriensis*, *Pyrus sinkiangensis*) [33]. In this study, attempt will be made to determine arbutin in pear which is present in Thailand (*Pyrus communis*). The medicinal plant samples used in this study are Ya pear, Fragrant pear, Early-Matured pear and Chinese pear (commercially available from markets in Thailand). The kernel of each sample was peeled, dried at 60°C for 12 h and then powdered. About 2.0 g of each plant powder was weighed accurately, sonicated for 30 min with 30 mL of deionized water and 0.10 g calcium carbonate and filtered through membrane filter paper (Whatman®, No. 42). The residue was washed thoroughly with deionized water. Then 5 mL of 4% lead(II) acetate solution was added to the filtrate, cooled and diluted to 50 mL with deionized water and filtered. Then 0.20 g of sodium phosphate was added to the filtrate, mixed and filtered through a millipore membrane again. This solution was kept for further MLC analysis.

Results and Discussion

Retention Behaviour of Arbutin and Hydroquinone with Brij 35 Micellar Mobile phase

To select the nature of surfactant for preparing micellar mobile phases it is important to take into account the interactions that take place in the chromatographic system between the solutes and the surfactants. To achieve a high sensitivity for determination of studied compounds, the suitable detection wavelength was investigated. The UV spectra obtained from each drug showed the absorption maxima at approximately 280–290 nm. For this reason, a detection of 280 nm was initially selected.

Selection of Surfactant

In this study, three different surfactants, including SDS, CTAB and Brij 35 were tested. CTAB was used at the concentration of 0.0018 mol L⁻¹ (two times of its CMC; 0.00 083 mol L⁻¹) as mobile phase at pH 2.5; arbutin, hydroquinone and resorcinol were

Table 1 Retention factor (*k*) and efficiency (*N*) of the compounds in different surfactant mobile phases

Compounds	Retention factor (<i>k</i>); Efficiency (<i>N</i>)		
	SDS	CTAB	Brij 35
Arbutin	0.25; 1106	0.48; 1959	0.50; 1530
Hydroquinone	0.38; 1358	0.66; 484	0.96; 1770

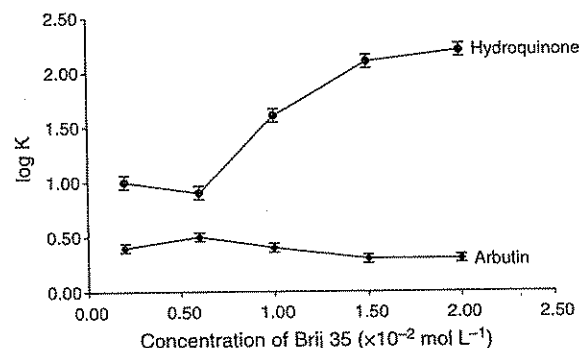


Figure 3 The effect of Brij 35 concentration for determining arbutin and hydroquinone in the concentration of 0.002–0.02 mol L⁻¹

eluted in the void volume because of their expected electrostatic repulsion between the protonated form of the analyte and the surfactant-modified stationary phase (arbutin; p*K*_a = 12.2 and hydroquinone; p*K*_a = 9.9). SDS micellar mobile phase at pH 2.5 was used at a concentration of 0.0164 mol L⁻¹ (CMC = 0.0082 mol L⁻¹); all analytes were retained in the column and not separated. In contrast, the mobile phase of Brij 35 at pH 5.0 was used at the concentration of 0.0018 mol L⁻¹ (CMC = 0.0009 mol L⁻¹); arbutin, hydroquinone and resorcinol were retained and good separated peaks. A mobile phase of Brij 35 was selected as a compromise among the retention factor, resolution and acceptable analysis time as shown in Table 1 and Fig. 3.

Table II Retention factors (*k*) and efficiencies (*N*) of the compounds in Brij 35 mobile phase

Compound	Retention factors (<i>k</i>) and efficiencies (<i>N</i>)									
	0.0006 mol L ⁻¹ Brij 35					0.0006 mol L ⁻¹ Brij 35 + organic modifier				
	2-Propanol					Acetonitrile				
	pH 4.0	pH 5.0	pH 6.0	pH 7.0		1%	2%	3%	4%	5%
Arbutin	0.29; 1448	0.33; 1407	0.35; 1432	0.40; 1324	0.25; 253	0.26; 227	0.27; 211	0.28; 235	0.29; 207	0.30; 209
Hydroquinone	2.07; 1204	1.48; 1366	1.09; 1275	0.85; 1304	1.60; 200	1.72; 273	1.89; 257	2.01; 266	2.12; 289	2.27; 236

Effect of pH

The influence of pH of the mobile phase on the retention of arbutin, hydroquinone and resorcinol was studied. A series of measurements were carried using pure Brij 35 as micellar eluent (without modifiers). In this study, increase in pH value of the mobile phase in the range of 4.0–7.0 showed an increase in the retention factors of the analytes (Table II). A surfactant mobile phase of pH 6 was selected, because its resolution was good and also acceptable analysis time.

Selection of the Organic Modifier and Concentration

The organic modifier and its concentration for the separation of arbutin and hydroquinone were investigated. Acetonitrile and 2-propanol were examined as modifiers (Table II). Addition of organic modifiers to the micellar mobile phase reduces the film thickness of the surfactant molecules covering the stationary phase and thus, produces an enhancement in efficiency [34]. Different compounds including 2-propanol and acetonitrile were examined as modifiers. As can be seen in Table II, addition of modifiers into mobile phases improved peak efficiencies in all cases. Compared with a purely micellar mobile phase comprising 0.006 mol L⁻¹ Brij 35 at pH 6.0, the retention factors are higher with addition of 1% (v/v) 2-propanol or acetonitrile. An organic modifier (acetonitrile) imparts interesting changes in retention time and resolution. Acetonitrile increases the retention times in all cases and difference in retention time is seen at small organic modifier content but resolution is better at lower concentration. This study indicated that retention behaviour in MLC changes at 1% acetonitrile with non-ionic Brij 35 surfactant when hybrid micellar mobile phase in HPLC with C-18 column is used. Better separation of arbutin and hydroquinone is observed at higher acetonitrile concentration in most cases studied here at relatively sharp peaks appeared as compared to those at low acetonitrile content. This may be because of decreased amount of adsorbed surfactant on C-18 stationary phase, which help better mass transfer between stationary and mobile phase hence reduce diffusion of analyte.

Effect of Brij 35 Concentration

The effect of Brij 35 concentration for determining arbutin and hydroquinone was studied by varying at higher concentration than the critical micellar concentration (0.002–0.02 mol L⁻¹). For arbutin, the lower retention factors were obtained as Brij 35 concentration increased. For hydroquinone, the higher retention factors were obtained as Brij 35 concentration increased but also the retention times were long. A 0.006 mol L⁻¹ Brij 35–1% (v/v) acetonitrile eluent was finally selected as a compromise between resolution and run-time analysis.

Method Validation

Range and Linearity

Under the selected micellar chromatographic conditions, the linear range of the signal response for arbutin and hydroquinone was studied over the concentration range of 2.0–500.0 mg L⁻¹. The linearity of calibration graph was determined using the optimal experimental parameters. Nine standard solutions ranging from 2.0–50.0 mg L⁻¹ in concentration, in three replicates each.

were injected into the MLC system. The calibration graph was obtained by plotting the absorbance of the solutions against the standard concentrations. Linear calibration graph over the concentration range 2.0–50.0 $\mu\text{g mL}^{-1}$ of arbutin and hydroquinone was obtained with the regression equations; $y = 0.045x + 0.042$ ($r^2 = 0.9923$) and $y = 0.091x + 0.050$ ($r^2 = 0.9930$) respectively.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) of arbutin and hydroquinone were estimated from the calibration curves using the expression $3.3 \text{ SD}/S$ where SD is standard deviation of the response and S is the slope of calibration curve and limit of quantification (LOQ) = $10 \text{ SD}/S$.

The limit of detection (LOD) and limit of quantification (LOQ) values were found to be 0.51, 1.70 mg L^{-1} and 0.37, 1.25 mg L^{-1} for arbutin and hydroquinone respectively.

Precision

The precision of the method was determined by measuring the repeatability (intraday precision) and the intermediate precision (inter-day precision), both expressed as relative standard deviation (R.S.D.). The precision was evaluated by assaying six replicate injections of 5, 10, 15 and 20 $\mu\text{g mL}^{-1}$ of arbutin and hydroquinone. The results are summarized in Table III.

Table III Repeatability and intermediate precision studied for arbutin and hydroquinone

Compounds	concentration ($\mu\text{g mL}^{-1}$)	Intra-day precision Concentration found ($\mu\text{g mL}^{-1}$) $\pm$ % R.S.D	Inter-day precision Concentration found ($\mu\text{g mL}^{-1}$) $\pm$ % R.S.D
Arbutin	5	5.9 $\pm$ 0.6	5.8 $\pm$ 1.0
	10	10.6 $\pm$ 0.4	10.1 $\pm$ 0.3
	15	15.0 $\pm$ 0.4	15.4 $\pm$ 1.0
	20	19.9 $\pm$ 2.9	19.8 $\pm$ 1.3
Hydroquinone	5	6.9 $\pm$ 2.0	5.4 $\pm$ 0.6
	10	11.0 $\pm$ 0.8	10.9 $\pm$ 0.8
	15	15.2 $\pm$ 0.6	15.7 $\pm$ 1.0
	20	20.4 $\pm$ 0.3	20.2 $\pm$ 1.2

Table IV Accuracy of the proposed MLC method ($n = 5$)

Arbutin concentration; $\mu\text{g/mL}$			Hydroquinone concentration; $\mu\text{g/mL}$		
Added	Found (mean $\pm$ SD)	% Recovery (mean $\pm$ SD)	Added	Found (mean $\pm$ SD)	% Recovery (mean $\pm$ SD)
5	4.9 $\pm$ 0.1	98.2 $\pm$ 2.5	5	4.5 $\pm$ 0.4	96.9 $\pm$ 2.8
10	9.8 $\pm$ 0.4	98.6 $\pm$ 3.4	10	9.3 $\pm$ 0.3	96.6 $\pm$ 1.6
15	4.7 $\pm$ 0.2	98.1 $\pm$ 1.7	15	5.0 $\pm$ 0.2	99.0 $\pm$ 3.6
20	9.2 $\pm$ 0.2	96.0 $\pm$ 1.2	20	9.6 $\pm$ 0.3	95.5 $\pm$ 3.4

Accuracy

The accuracy of the proposed MLC method was assessed with recovery using the addition of four known concentration levels (5, 10, 15 and 20 $\mu\text{g mL}^{-1}$) of arbutin and hydroquinone. All samples were injected in three replicates for each concentration. The concentration found was calculated against the concentration added. The recoveries of analyte were in the ranges of 96.04–98.58% and

Table V Robustness of the MLC method

Chromatographic changes	Level	Arbutin t_R (min)	Hydroquinone t_R (min)
A: Brij 35 concentration (mol L^{-1})			
0.005	–0.001	2.80	4.08
0.006	0	2.77	4.03
0.007	0.001	2.74	3.98
Mean $\pm$ SD		2.77 $\pm$ 0.03	4.03 $\pm$ 0.05
R.S.D (%)		0.4	0.5
B: Percentage of acetonitrile (v/v)			
0.8	–0.2	2.94	4.03
1	0	2.77	4.03
1.2	0.2	2.79	4.05
Mean $\pm$ SD		2.83 $\pm$ 0.09	4.03 $\pm$ 0.01
R.S.D (%)		1.01	0.2
C: pH of mobile phase			
5.8	–0.2	2.76	4.03
6	0	2.77	4.03
6.2	0.2	2.77	4.08
Mean $\pm$ SD		2.76 $\pm$ 0.01	4.04 $\pm$ 0.01
R.S.D (%)		0.2	0.2
D: Flow rate (mL min^{-1})			
0.9	–0.1	2.87	4.23
1	0	2.77	4.03
1.1	0.1	1.87	3.88
Mean $\pm$ SD		2.50 $\pm$ 0.7	4.04 $\pm$ 0.3
R.S.D (%)		9.7	4.8

Table VI Determination of studied arbutin and hydroquinone in medicinal plant extracts and commercial pharmaceuticals ($n = 5$)

Samples	Compounds	MLC method average (mg g^{-1})	Published method [35] average (mg g^{-1})
Chinese pear	Arbutin	0.38	0.35
	Hydroquinone	ND	ND
Fragrant pear	Arbutin	0.59	0.55
	Hydroquinone	ND	ND
Early-matured pear	Arbutin	0.52	0.51
	Hydroquinone	ND	ND
Cream No.1	Arbutin	0.93	0.89
	Hydroquinone	ND	ND
Cream No.2	Arbutin	1.72	1.69
	Hydroquinone	ND	ND
Cream No.3	Arbutin	0.53	0.49
	Hydroquinone	ND	ND

*Chromatographic conditions: an Inertsil-ODS3 (C_{18}) column (250 mm $\times$ 4.6 mm i.d.; 5- μm particles), (89:10:1, v/v/v) water-methanol-0.1 mol L^{-1} acetic acid, 1.0 mL min^{-1} , 222 nm.

95.49–99.03% for arbutin and hydroquinone respectively (Table IV). Additives and excipients did not interfere in the determination of those active ingredients as the samples used to evaluate recovery were prepared with those additives and excipients.

Robustness of the Method

The robustness of the method was evaluated in terms of Brij 35 concentration, percentage of acetonitrile (v/v), pH and the flow rate of the mobile phase by six replicated injections of the standard solution at $10 \mu\text{g mL}^{-1}$. The R.S.D (%) of the retention times calculated from these variations is shown in Table VI and was lower than 9.7%. Variation of the flow rate values had a stronger influence on the retention of the studied compounds than other parameters. However, the variations in all the parameters had no significant effect on resolution, peak area and peak shape (Table V).

Method Application

The proposed MLC method was applied for the determination of arbutin and hydroquinone in pear extracts and skin-whitening creams. The samples were extracted and prepared according to sample preparation section. The content of arbutin and hydroquinone in each sample solution was determined using the MLC optimum conditions. The samples gave well-defined peaks. There is no interference peak present in each sample. The average contents of arbutin and hydroquinone in each sample were presented in Table VI.

The results were compared favourably with those obtained by the published HPLC method [35]. Statistical analysis of the results by using a *t*-test showed that the calculated *t*-values were less than

the table list *t*-value at 95% confidence limit [36]. Therefore, there two methods are not significantly different in any of the samples.

Conclusion

The proposed MLC method can be used for the determination of arbutin and hydroquinone in commercial cosmetic products and medicinal plant extracts. Good sensitivity, linearity and robustness were obtained. R.S.D values were lower than 2.9% and 1.3% for intra- and inter-day analyses respectively. The recoveries in the sample were within a range of 95.5–99.0% and no interference from excipients was observed. This proposed method has proven to be selective, fast, simple, relatively inexpensive, precise and accurate. The speed of analysis and the precision make this method suitable for quality control of arbutin and hydroquinone in commercial cosmetic products and medicinal plant extracts. In addition, the selected mobile phase is cheaper and less toxic than those used in the published HPLC method [35]. The sensitivity of the proposed MLC method is compared with the published analytical methods. It is found that the MLC method is as sensitive as the HPLC method [12]. It is also more sensitive than the HPLC method [14], capillary electrophoresis [17] and indirect spectrophotometry [22] for determining arbutin and hydroquinone as skin-whitening cosmetics.

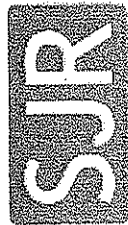
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