Simultaneous Determination of Arbutin and Hydroquinone in Different Herbal Slimming Products by Gas Chromatography-Mass Spectrometry

Arbutin ve Hidrokinonun Farklı Bitkisel Zayıflama Ürünlerinde Gaz Kromatografisi-Kütle Spektrometresi ile Eşzamanlı Belirlenmesi

INTRODUCTION

Arbutin (4-hydroxyphenyl-β-D-glucopyranoside) is a phenolic glucoside that is found mainly in Ericaceae and Saxifragaceae families.[1] It is an inhibitor of tyrosinase which is the essential enzyme for melanin formation. Melanin is important for protection of skin from harmful UVA and UVB radiation. Arbutin is disinfectant in genitourinary diseases and has anti-inflammatory, antioxidant and antitumor effects. It is used in urinary therapeutics, skin-whitening and depigmenting cosmetics.[1-3]

Hydroquinone is 1,4-dihydroxybenzene and the metabolite of arbutin.[4] It has antibacterial, astringent, disinfectant and antioxidant effects. It is used for the treatment of hyperpigmentation and component of topical pharmaceutical agents.[1,5]

Calluna vulgaris (L.) Hull (heather) is a perennial shrub that is member of Ericaceae family. It is distributed throughout most of Europe, Russia, Asia Minor and Atlantic coast of North America.[6,7] Secondary metabolites of *C. vulgaris* are flavonoids, tannins, proanthocyanidins, caffeic acid derivatives, phenols, triterpenes, steroids and hydroquinone glycosides (arbutin). The infusion of aerial parts of *C. vulgaris* is traditionally used in Turkey as urinary tract disenfectant, diuretic and antidiaretic.[8] *C. vulgaris* has diuretic, antimicrobial, antirheumatic, antioxidant, antibacterial and MAO-A inhibitory effects[7,9-11] and is presented in herbal slimming teas due to its diuretic and digestive effects.

Obesity is a serious disease that can due to genetic and environmental reasons, defined as abnormal or excessive fat accumulation that may impair health by World Health Organization (WHO). In addition to the various synthetic medicines used in obesity treatment, there is an increasing trend towards the herbal products in this field.[12] The effectiveness of herbal slimming products and their compliance to standards is a matter to be discussed. Thus, the quality control analysis of these products are important for public health. Arbutin and hydroquinone are among the effective components in herbal slimming products containing *C. vulgaris*.

Several analytical methods have been reported for determination of arbutin and/or hydroquinone including high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), capillary zone electrophoresis, and densitometry.[5,13-22]

However, no method was applied for quantifying the presence of arbutin and hydroquinone simultaneously from different herbal slimming products. Therefore, analytical methods for their separation and simultaneous quantification are required for quality control purpose. Hence, in present research work, a simple, rapid, precise and accurate GC-MS method has been developed and validated using International Conference on Harmonization Guidelines for simultaneous determination and quantification of arbutin and hydroquinone form different herbal slimming products.[23]

MATERIALS AND METHODS

Chemicals

Methanol (France), arbutin (England) and hydroquinone (Switzerland) were purchased from Sigma-Aldrich. Acetonitrile and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were obtained from Sigma (St. Louis, MO, USA).

Plant Material and Pharmaceutics

Nine herbal slimming products containing *Calluna vulgaris* were assured from internet and different pharmacies in Turkey. The herbal slimming products from 1 to 7 are tea mixtures, the product-8 is a slimming tea capsule, the product-9 is *C. vulgaris* tea.

Extraction of the Herbal Slimming Products

Ten grams of each product were extracted with distilled water and methanol (100 mL x 2) separately at 40°C for 30 min. The extracts were filtered. Then, the aqueous extracts were cooled at -80°C and lyophilized. Methanol was evaporated to dryness and the methanol extracts were obtained.

Derivatization Process

Arbutin, hydroquinone and containing extracts were derivatized using MSTFA to increase the performance of the gas chromatographic separation. The hydroxy (-OH)

groups were converted to the corresponding silyl (-O-TMS) groups. After establishing the optimum reaction conditions, the compounds were analyzed.

GC-MS Conditions

Chromatographic analysis was carried out on an Agilent 7820A gas chromatography system equipped with 5977 series mass selective detector, 7673 series autosampler and Agilent chemstation (Agilent Technologies, Palo Alto, CA). HP-5 MS column with 0.25 µm film thickness (30 m × 0.25 mm I.D., USA) was used for separation. Splitless injection was used and the carrier gas was helium at a flow rate of 1.0 mL/min. The injector volume was 1 µl. The MS detector parameters were transfer line temperature 230˚C, solvent delay 3 min and electron energy 70 eV. The GC temperature gradient program was as follows: initial temperature was 100˚C, held for 2 min, increased to 220˚C at a rate of 30˚C/min, held for 1 min, and finally to 300˚C at a rate of 20˚C/min and held for 2.0 min. The MS detector parameters were: transfer line temperature 280°C; solvent delay 3 min; electron energy 70 eV; the MS was run in electron impact mode with selected ion monitoring (SIM) for quantitative analysis [m/z 254 for arbutin, m/z 239 for hydroquinone].

Standards and Quality Control Samples

Stock solutions of arbutin and hydroquinone were prepared by dissolving the accurately weighed reference compounds in acetonitrile to give a final concentration of 100 µg/mL of both. The solution was then serially diluted with chloroform to achieve standard working solutions at concentrations of 5, 10, 25, 50, 100, 250 and 500 ng/mL for arbutin and hydroquinone, respectively. Structural formula and derivatization of arbutin and hydroquinone are shown in Figure 1.

All solutions were stored at 4˚C and were brought to room temperature before use. The quality control solutions were prepared by adding aliquots of standard working solution of final concentrations of 7.5, 75 and 375 ng/mL for arbutin and hydroquinone, respectively.

RESULTS

Method development and optimization

Arbutin and hydroquinone are polar molecules so a capillary column coated with 5% phenyl and 95% dimethylpolysiloxane were used for separation. During GC-MS

method development, the injection port and detector temperatures were set to 250°C and 290°C, respectively. Different temperature programs were investigated to give an optimum temperature program as follows; initial temperature was 100˚C, held for 2 min, increased to 220˚C at 30˚C/min held for 1 min, and finally to 300˚C at 20˚C/min with a final hold of 2.0 min. The injector volume was 1 μL in split less mode.

MSTFA is an effective trimethylsilyl (TMS) donor. MSTFA reacts to replace labile hydrogens on a wide range of polar compounds with a TMS group and is used to prepare volatile and thermally stable derivatives for GC-MS.[24]

The effects of time and temperature on the reaction were investigated. Therefore, arbutin and hydroquinone were dissolved in acetonitrile. To 50 µL of 200 ng/mL arbutin and hydroquinone solution and 50 µL of MSTFA solution were added and reacted at room temperature, 50 and 75˚C for 5, 15, 30 and 45 min. The resulting samples were quantitated by GC-MS system. The optimised conditions for derivatisation are 50˚C and 30 min.

Then, dry residue of the herbal slimming products were dissolved in 100 µL of a mixture of acetonitrile and MSTFA (50:50, v/v). The mixture was vigorously shaken and then delayed at 50˚C temperature for 30 min. 1 µL sample was injected into the GC-MS system.

Validation of Method

To evaluate the validation of the present method, parameters as selectivity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were investigated according to ICH validation guidelines.

Selectivity

The selectivity of the GC-MS method was investigated by observing interferences between arbutin and hydroquinone. For GC-MS, electron impact mode with selected ion monitoring (SIM) was used for quantitative analysis [m/z 254 for arbutin and m/z 239 for hydroquinone]. The mass spectra of the arbutin and hydroquinone are shown in Figure 2.

The retention times of arbutin and hydroquinone in GC-MS method were approximately 11.32 and 5.44 min with good peak shape (Figure 3).

Linearity

Linearity was determined for arbutin and hydroquinone in the range of 5-500 ng/mL. The calibration curves constructed were evaluated by their correlation coefficients. The calibration equations from three replicate experiments demonstrated the linearity of the method. Standard deviations of the slope and intercept for the calibration curves were given in Table 1.

Precision and Accuracy

The precision of the GC-MS method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by analyzing quality control samples six times per day, at three different concentrations which were quality control samples. The intermediate precision was evaluated by analyzing the same samples once daily for three days. The relative standard deviation (RSD) of the predicted concentrations from the regression equation was taken as precision. The accuracy of this analytic method was assessed as the percentage relative error. For all the concentrations studied, intra- and inter-day relative standard deviation values were ≤2.73% and for all concentrations of arbutin and hydroquinone the relative errors were ≤2.56%.

Limits of Detection (LOD) and Quantification (LOQ)

The limit of detection (LOD) is the lowest amount of arbutin and hydroquinone in a sample which can be detected but not necessarily quantitated as an exact value. The limit of quantification (LOQ) is the lowest amount of arbutin and hydroquinone which can be quantitatively determined with suitable precision. The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution under the chromatographic conditions. The lowest concentrations assayed where the signal/noise ratio was at least 10:1, this concentration was regarded as LOQ. The LOD was defined as a signal/noise ratio of 3:1. The results are shown in Table 1.

Application of Method

The developed GC-MS method was used for simultaneous determination of arbutin and hydroquinone from different herbal slimming products**.** The sample working solutions (1 µL) were injected and the height of both arbutin and

hydroquinone peak were measured. From the calibration curve, the amount of arbutin and hydroquinone in different herbal slimming products were calculated. The retention time of arbutin and hydroquinone in sample solutions were 11.32 and 5.44, respectively (Figure 4). The mean amounts and percent values of arbutin and hydroquinone found in different herbal slimming products were given in Table 2.

DISCUSSION

Comparison of Methods

Today, GC-MS is a powerful technique for highly specific and quantitative measurements of low levels of analytes in samples. As compared to HPLC, high-resolution capillary GC has been less frequently used.[25]

During method development, it became evident that arbutin and hydroquinone were very sensitive to matrix effects during the derivatization process in different herbal slimming products. Sample preparation techniques such as extraction and derivatization were used in order to minimise matrix suppression effects.

GC-MS method sensitivity is not enough for the determination of arbutin and hydroquinone in different herbal slimming products. For this reason, MSTFA was chosen as a chromagenic derivatization reagent. In this study, the purpose of the derivatization reaction is the raise of sensitivity thus the possibility of working in low concentrations has been occurred.

Literature survey revealed that some of the related methods were reviewed. A GC-MS method was reported for separating and determining arbutin and hydroquinone from strawberry tree leaf extracts.[4] In a reported method, the calibration curve of GC-MS method was linear for arbutin and hydroquinone in the range 0.5-200 µg/mL. Intra- and inter-day precision, expressed as the relative standard deviation (RSD) were less than 5.0%, and accuracy (relative error) was beter than 3.80%. In statistical comparison ($P<0.05$) with other method in the literature the proposed method has indicated high accuracy, precision and sensitivity.[4] The minimum determinable concentration is 9 ng/mL. The present method has the following advantage over the reported method. The LOQ of the reported method was 29 ng/mL whereas the present method LOQ was 0.093 ng/mL.

When this method is applied to different herbal slimming products samples, its sensitivity was found to be adequate for analysis studies. The present method has the following advantages over the reported method. The sensitivity was evaluated by

the LOQ, which was determined to be 0.093 ng/mL. This method is as good as or superior to that reported in the other papers.[4,16,19,20]

Calibration curves of arbutin and hydroquinone were linear over the concentration range of 5-500 ng/mL for the study, which is as good as or superior to that reported in other papers.[15-20]

CONCLUSION

In the present work, a new, simple and sensitive GC-MS method has been developed for the simultaneous quantitation of arbutin and hydroquinone in whole plant powder of different herbal slimming products. The method was validated to track the active principles in the complex mixture of herbal ingredients. The method could be extended for the marker-based standardization of other herbal products containing arbutin and hydroquinone. The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and for the quantification of these compounds in plant materials.

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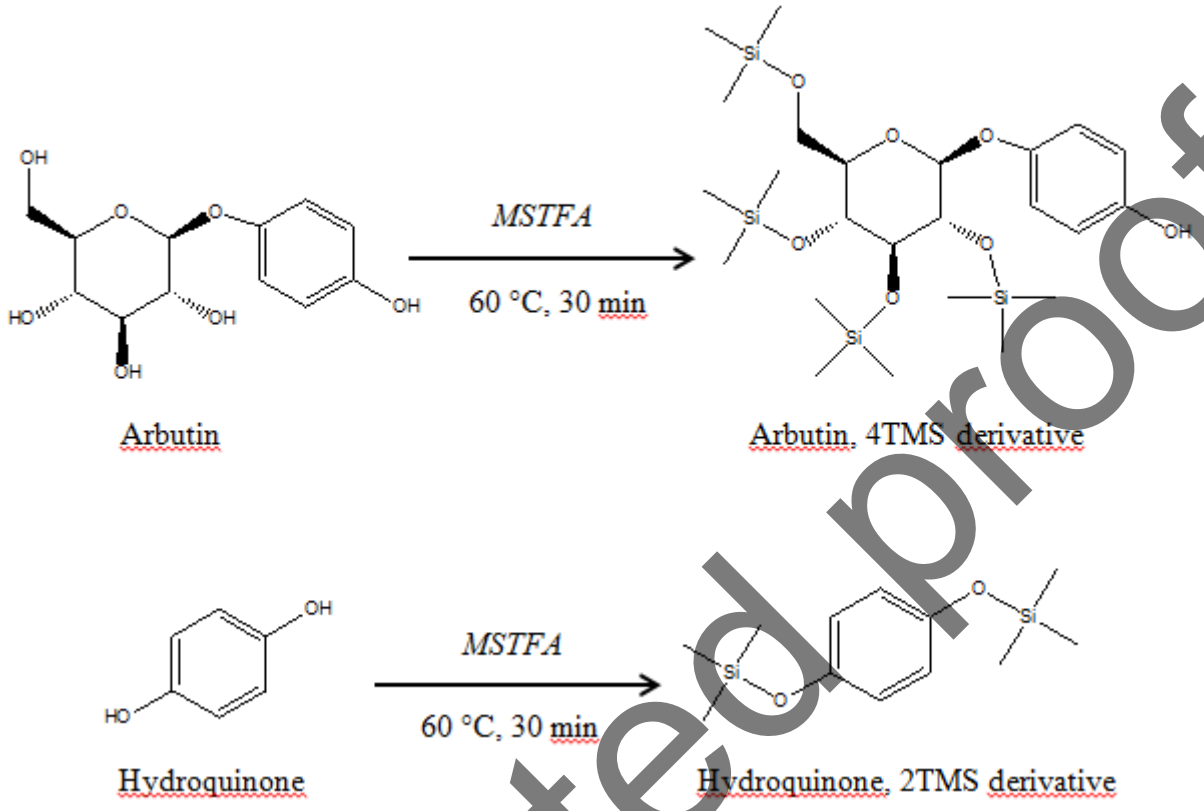
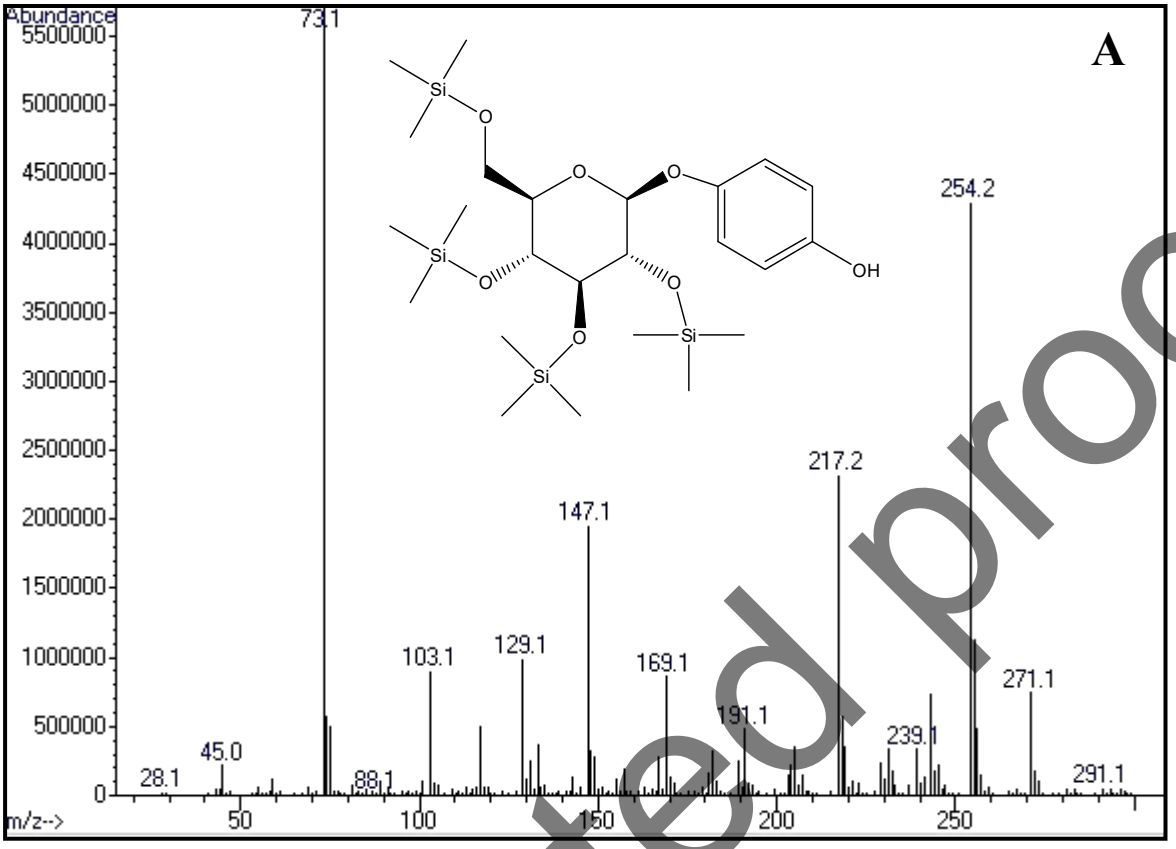


Figure 1. Structural formula and derivatization mechanism of arbutin and hydroquinone



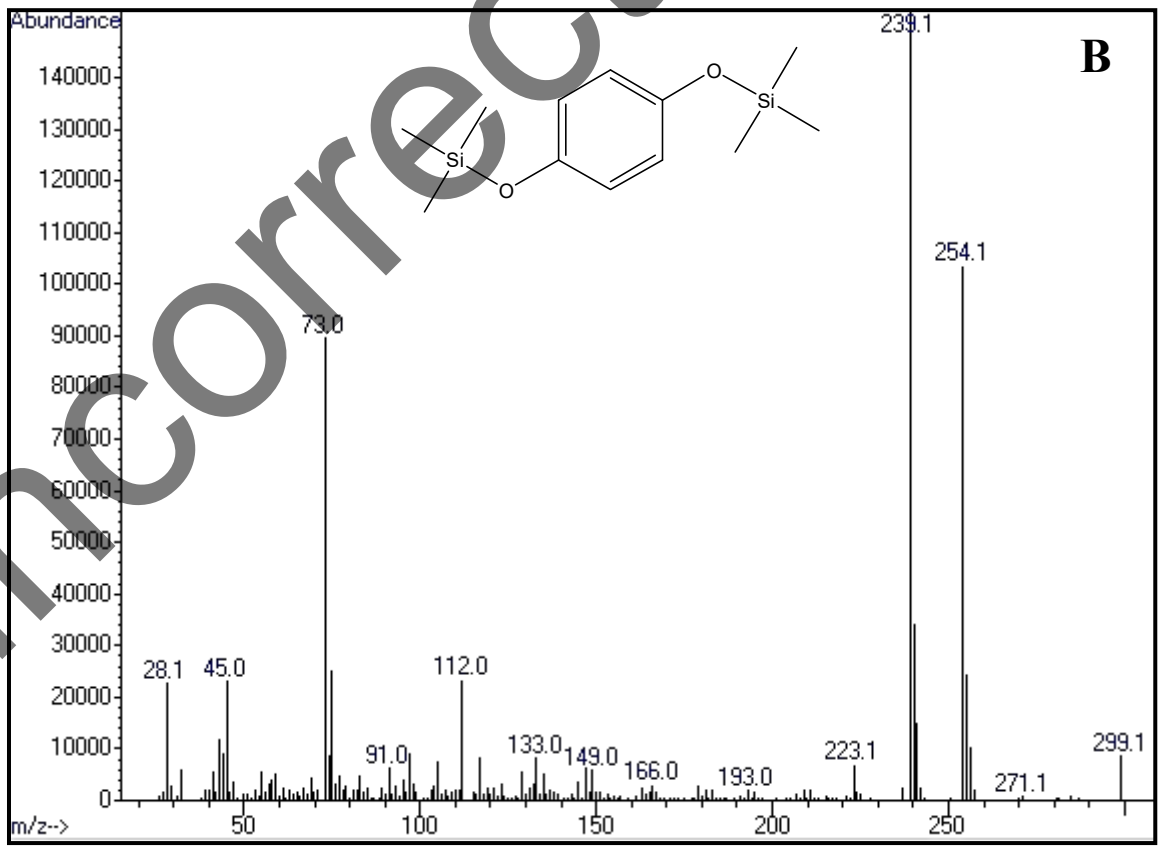


Figure 2. MS spectra of arbutin (A) and hydroquinone (B)

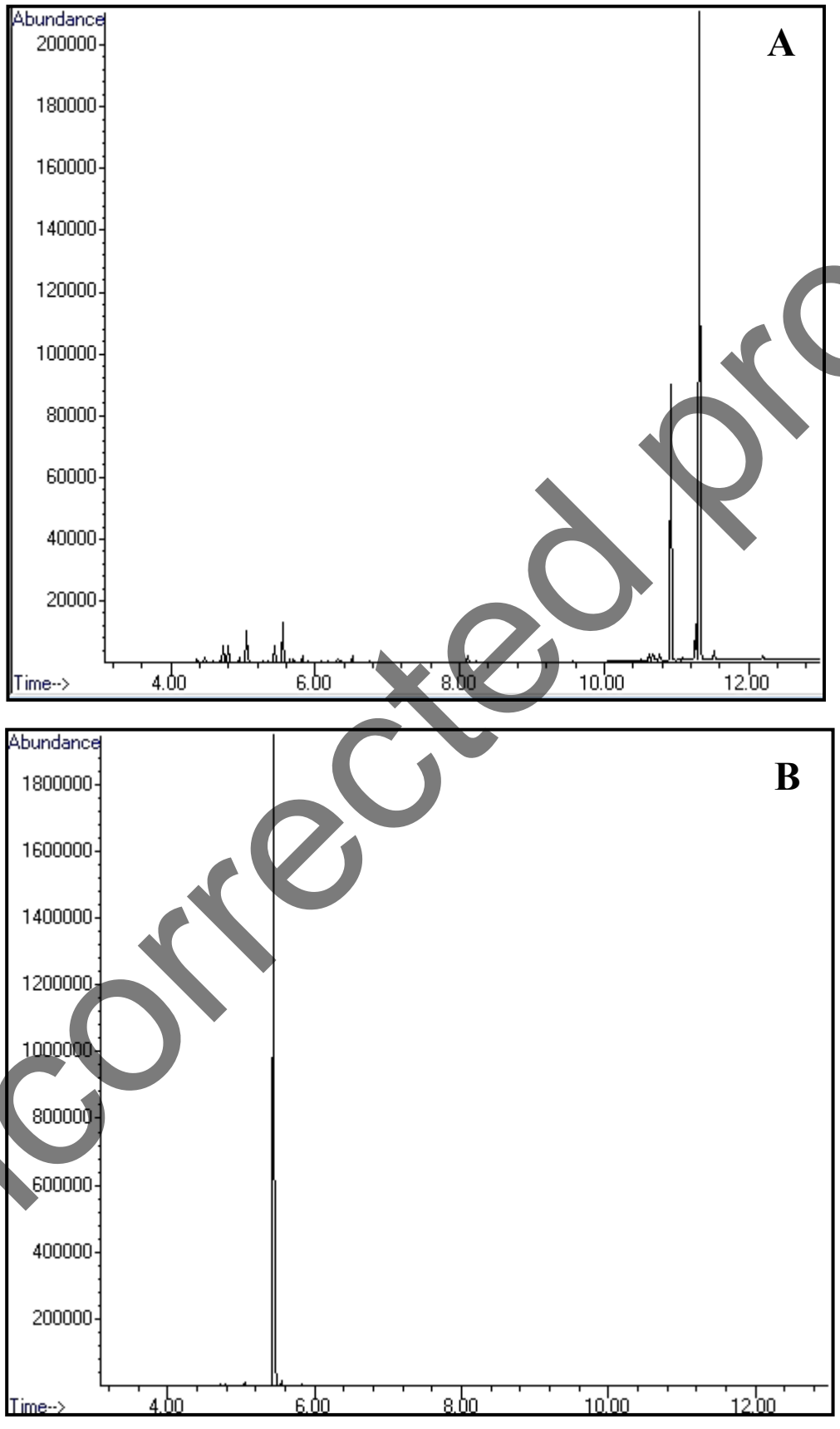


Figure 3. GC-MS chromatograms of arbutin (A) and hydroquinone (B) (500 ng/mL)

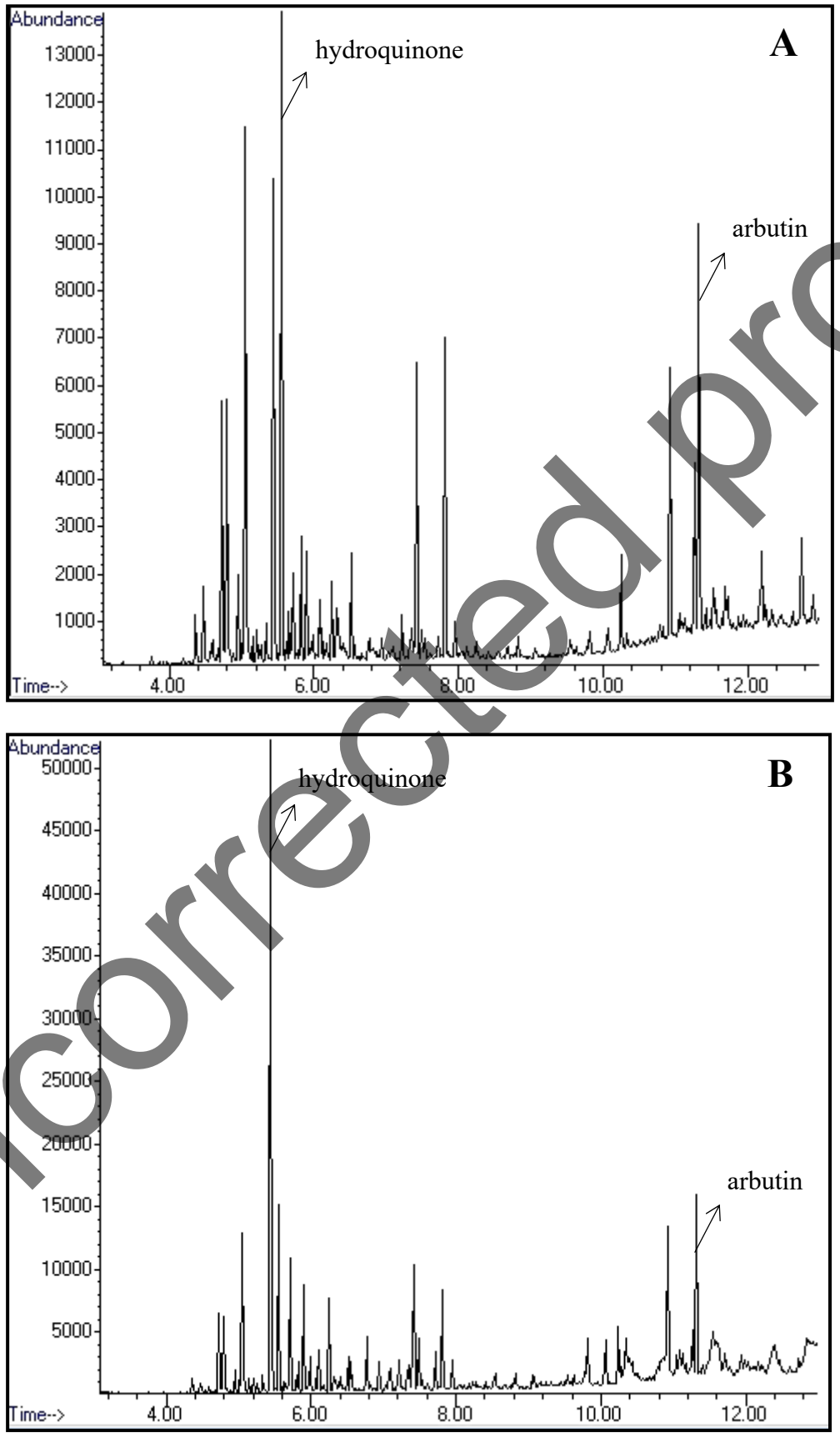


Figure 4. Typical chromatograms of methanol (A) and aqueous (B) extracts of the product-9 (1.0 mg/mL)

Table 1. Features of the calibration curves of arbutin and hydroquinone

Parameters	**Arbutin**	**Hydroquinone**
Linearity (ng/mL)	5-500	5-500
Regression equation[a]	y=1215.7x+810.48	y=1195.4x+4902
Correlation coefficent	0.9933	0.9915
Standard deviation of correlation coefficent	$5.0x10^{-3}$	$2.9x0^{-3}$
Limit of detection (ng/mL)	0.555	0.031
Limit of quantification (ng/mL)	1.665	0.093

[a]Based on three calibration curves, y: peak-height, x: arbutin and hydroquinone concentration

Table 2. Application of arbutin and hydroquinone in different herbal slimming products (1.0 mg/mL)

Sample Name (1 mg/ml)		Arbutin (ng/mL)	% Concentration	Hydroquinone (ng/mL)	% Concentration
1	Aqueous extract	n.d.	-	8.4867	0.0008
	Methanol extract	20.0256	0.0020	15.4885	0.0015
2	Aqueous extract	n.d.	-	8.6724	0.0008
	Methanol extract	33.9755	0.0034	26.2841	0.0026
3	Aqueous extract	n.d.	-	7.2009	0.0007
	Methanol extract	63.4331	0.0063	18.0860	0.0018
4	Aqueous extract	n.d.	-	9.2036	0.0009
	Methanol extract	2.7145	0.0002	23.9543	0.0024
5	Aqueous extract	n.d.	-	7.6376	0.0007
	Methanol extract	34.4716	0.0034	41.1235	0.0041
6	Aqueous extract	n.d.	-	7.7472	0.0007
	Methanol extract	36.2843	0.0036	16.9257	0.0017
7	Aqueous extract	n.d.	-	8.1354	0.0008
	Methanol extract	18.8898	0.0019	16.3477	0.0016
8	Aqueous extract	n.d.	-	7.4452	0.0007
	Methanol extract	n.d.	-	25.7495	0.0026
9	Aqueous extract	60.6236	0.0060	15.7178	0.0016
	Methanol extract	35.2968	0.0035	11.6379	0.0012

n.d.: not determined