



Simultaneous Determination of Arbutin and Hydroquinone in Different Herbal Slimming Products Using Gas Chromatography-mass Spectrometry

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

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ABSTRACT

Objectives: The aim of our study was to develop a simple, precise, sensitive and specific method for the simultaneous determination of arbutin and hydroquinone in different herbal slimming products using GC-MS.

Materials and Methods: The methanol and aqueous extracts of nine herbal slimming products in Turkey were evaluated for analysis of arbutin and hydroquinone using GC-MS method.

Results: The retention times of arbutin and hydroquinone were found as 11.32 and 5.44 min, respectively. The linear ranges in this method were 5-500 ng/mL for arbutin and hydroquinone, respectively. The intra- and inter-day precisions, expressed as the relative standard deviation, were less than 1.94 and 2.73%, determined from quality control samples for arbutin and hydroquinone, and accuracy was within 1.13 and 2.56% in terms of relative error, respectively. The limit of detection and quantification were 0.555 and 1.665 ng/mL for arbutin, and 0.031 and 0.093 ng/mL for hydroquinone, respectively.

Conclusion: The developed method can be used for routine quality control analysis of arbutin and hydroquinone in different herbal slimming products.

Key words: Arbutin, hydroquinone, GC-MS, herbal slimming products, *Calluna vulgaris*

ÖZ

Amaç: Bu çalışmanın amacı, farklı bitkisel zayıflama ürünlerinde arbutin ve hidrokinonun GC-MS yöntemiyle, eşzamanlı olarak belirlenmesi için basit, hassas ve spesifik bir yöntem geliştirmektir.

Gereç ve Yöntemler: Türkiye'de bulunan dokuz bitkisel zayıflama ürününün sulu ve metanolik ekstratları, arbutin ve hidrokinon analizi için GC-MS yöntemi ile değerlendirilmiştir.

Bulgular: Arbutin ve hidrokinon için alıkonma zamanları sırasıyla 11.32 ve 5.44 dakika olarak bulunmuştur. Geliştirilen yöntemin doğrusallık aralığı arbutin ve hidrokinon için 5-500 ng/mL'dir. Kalite kontrol numunelerinden belirlenen ve bağıl standart sapma olarak ifade edilen gün-ichi ve günler-arası kesinlik, arbutin ve hidrokinon için sırasıyla %1.94 ve %2.73'ten düşüktür. Bağıl hataya göre doğruluk değerleri arbutin ve hidrokinon için sırasıyla %1.13 ve %2.56 arasındadır. Gözlenebilme ve miktar tayin sınırı arbutin için sırasıyla 0.555 ve 1.665 ng/mL, hidrokinon için 0.031 ve 0.093 ng/mL'dir.

Sonuç: Geliştirilen yöntem, farklı bitkisel zayıflama ürünlerinde arbutin ve hidrokinonun rutin kalite kontrol analizleri için kullanılabilir.

Anahtar kelimeler: Arbutin, hidrokinon, GC-MS, bitkisel zayıflama ürünleri, *Calluna vulgaris*

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INTRODUCTION

Arbutin (4-hydroxyphenyl- β -D-glucopyranoside) is a phenolic glucoside that is found mainly in the Ericaceae and Saxifragaceae families.¹ It is an inhibitor of tyrosinase, which is the essential enzyme for melanin formation. Melanin is important for the protection of skin from harmful ultraviolet (UV)-A 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 a metabolite of arbutin.⁴ It has antibacterial, astringent, disinfectant, and antioxidant effects. It is used for the treatment of hyperpigmentation and is a component of topical pharmaceutical agents.^{1,5}

Calluna vulgaris (*C. vulgaris*) (L.) Hull (heather) is a perennial shrub that is a member of the Ericaceae family. It is distributed throughout most of Europe, Russia, Asia Minor, and the 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 a urinary tract disinfectant, diuretic, and antidiabetic.⁸ *C. vulgaris* has diuretic, antimicrobial, antirheumatic, antioxidant, antibacterial, and monoamine oxidase-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 be due to genetic and environmental reasons, defined as abnormal or excessive fat accumulation that may impair health by the World Health Organization. In addition to the various synthetic medicines used in obesity treatment, there is an increasing trend towards herbal products in this field.¹² The effectiveness of herbal slimming products and their adherence to standards is a matter of debate. Thus, the quality control analysis of these products is 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 the 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 has been used to quantify 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 the present research, a simple, rapid, precise and accurate GC-MS method was developed and validated using International Conference on Harmonization Guidelines for the simultaneous determination and quantification of arbutin and hydroquinone from 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) (TMS) trifluoroacetamide (MSTFA) were obtained from Sigma (St. Louis, MO, USA).

Plant material and pharmaceuticals

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

Extraction of the herbal slimming products

Ten grams of each product were extracted with distilled water and methanol (100 mLx2) 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 contained 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 performed on an Agilent 7820A GC system equipped with a 5977 series mass selective detector, 7673 series autosampler, and Agilent chemstation (Agilent Technologies, Palo Alto, CA). An HP-5 MS column with 0.25- μ m film thickness (30 m x 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 mL. 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: the initial temperature was 100°C, held for 2 min, which was 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 μ g/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.

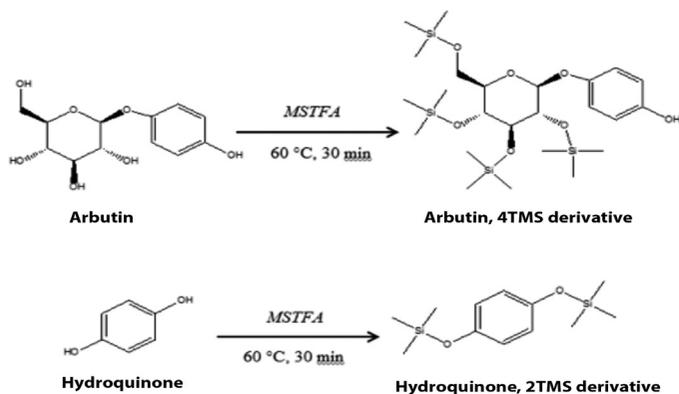


Figure 1. Structural formula and derivatization mechanism of arbutin and hydroquinone

All solutions were stored at 4°C and were brought to room temperature before use. 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.

There were no experimental animal or clinical studies in our study. Thus, we did not need ethics committee approval for the study.

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 mL in splitless mode.

MSTFA is an effective 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. 50 µL of MSTFA solution was added to 50 µL of 200 ng/mL arbutin and hydroquinone solution and reacted at room temperature, 50 and 75°C for 5, 15, 30 and 45 min. The resulting samples were quantitated using the GC-MS system. The optimized conditions for derivatization were 50°C and 30 min.

Then, dry residue of the herbal slimming products was 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 for 30 min. A 1-µL sample was injected into the GC-MS system.

Method validation

To evaluate the validation of the present method, parameters such 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, the electron impact mode with 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).

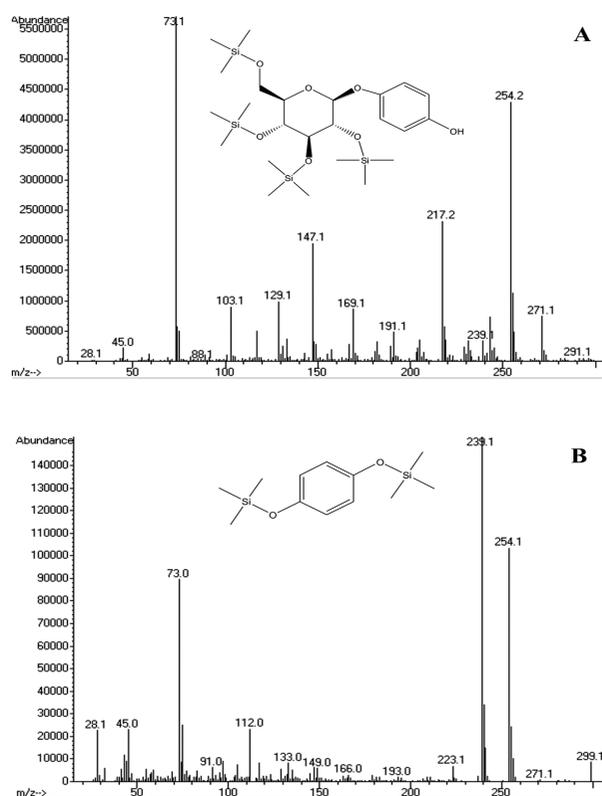


Figure 2. Mass spectrometry spectra of arbutin (A) and hydroquinone (B)

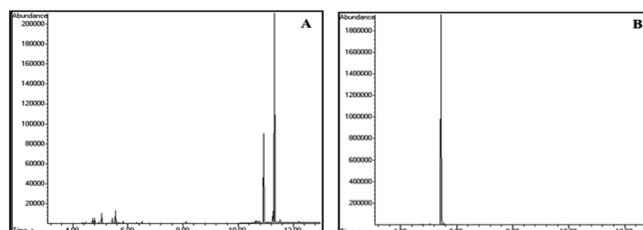


Figure 3. Gas chromatography-mass spectrometry chromatograms of arbutin (A) and hydroquinone (B) (500 ng/mL)

Linearity

Linearity was determined for arbutin and hydroquinone in the range of 5-500 ng/mL. The calibration curves constructed were evaluated using 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 are given in Table 1.

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 coefficient | 0.9933 | 0.9915 |
| Standard deviation of correlation coefficient | 5.0×10^{-3} | 2.9×10^{-3} |
| Limit of detection (ng/mL) | 0.555 | 0.031 |
| Limit of quantification (ng/mL) | 1.665 | 0.093 |

^aBased on three calibration curves, y: peak-height, x: arbutin and hydroquinone concentration

Precision and accuracy

The precision of the GC-MS method was determined through 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 RSD values were $\leq 2.73\%$ and for all concentrations of arbutin and hydroquinone the relative errors were $\leq 2.56\%$.

LOD and LOQ

The LOD is the lowest amount of arbutin and hydroquinone in a sample that can be detected but not necessarily quantitated as an exact value. The LOQ is the lowest amount of arbutin and hydroquinone that can be quantitatively determined with suitable precision. The LOD and LOQ of the developed method were determined by injecting progressively lower concentrations of the standard solution under the chromatographic conditions. The lowest concentrations assayed where the signal/noise ratio was at least 10:1 was regarded as the LOQ. The LOD was

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 | 15.4885 | 0.0015 |
| 2 | Aqueous extract | n.d. | 8.6724 | 0.0008 |
| | Methanol extract | 33.9755 | 26.2841 | 0.0026 |
| 3 | Aqueous extract | n.d. | 7.2009 | 0.0007 |
| | Methanol extract | 63.4331 | 18.0860 | 0.0018 |
| 4 | Aqueous extract | n.d. | 9.2036 | 0.0009 |
| | Methanol extract | 2.7145 | 23.9543 | 0.0024 |
| 5 | Aqueous extract | n.d. | 7.6376 | 0.0007 |
| | Methanol extract | 34.4716 | 41.1235 | 0.0041 |
| 6 | Aqueous extract | n.d. | 7.7472 | 0.0007 |
| | Methanol extract | 36.2843 | 16.9257 | 0.0017 |
| 7 | Aqueous extract | n.d. | 8.1354 | 0.0008 |
| | Methanol extract | 18.8898 | 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 | 15.7178 | 0.0016 |
| | Methanol extract | 35.2968 | 11.6379 | 0.0012 |

n.d.: not determined

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 the simultaneous determination of arbutin and hydroquinone from different herbal slimming products. The sample working solutions (1 μ L) were injected and the height of both arbutin and hydroquinone peaks were measured. From the calibration curve, the amounts 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 min, respectively (Figure 4). The mean amounts and percent values of arbutin and hydroquinone found in different herbal slimming products are presented 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 with HPLC, high-resolution capillary GC has been less frequently used.²⁵

During the development of the method, 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 minimize matrix suppression effects.

GC-MS 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 to raise the sensitivity, thus the possibility of working at low concentrations was able to be realized.

A literature survey revealed that some of the related methods have been 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 μ g/mL. Intra- and inter-day precision, expressed as the RSD were less than 5.0%, and accuracy (relative error) was better than 3.80%. In statistical comparison ($p < 0.05$) with the other method in the literature, the proposed method indicated high accuracy, precision, and sensitivity.⁴ The minimum determinable concentration was 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 LOQ of the present method was 0.093 ng/mL.

When this method is applied to different herbal slimming product 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 using the LOQ, which was determined as 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

CONCLUSIONS

In the present work, a new, simple and sensitive GC-MS method was 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 can 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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Conflict of Interest: No conflict of interest was declared by the authors.

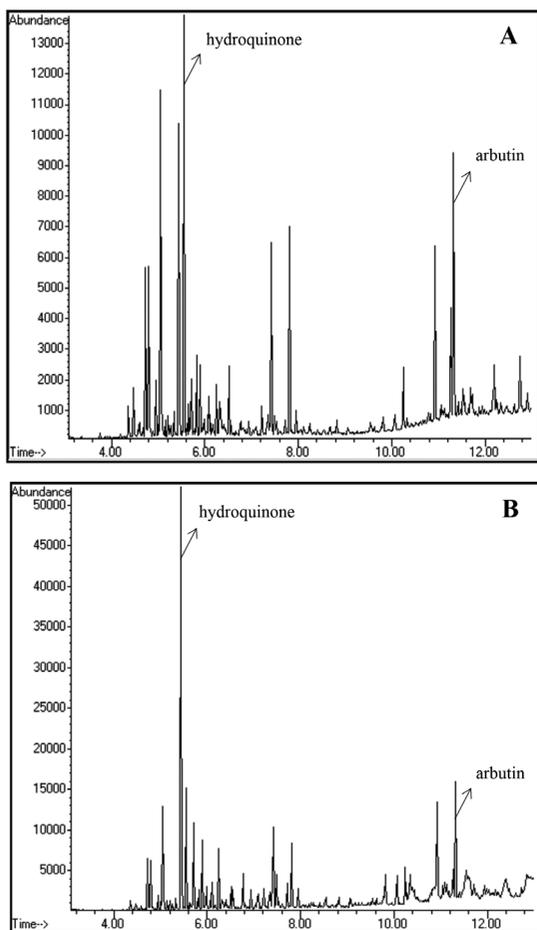


Figure 4. Typical chromatograms of methanol (A) and aqueous (B) extracts of product 9 (1.0 mg/mL)

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