Research Article

A Derivative Spectrometric Method for Hydroquinone Determination in the Presence of Kojic Acid, Glycolic Acid, and Ascorbic Acid

Zenovia Moldovan, Dana Elena Popa, Iulia Gabriela David, Mihaela Buleandra, and Irinel Adriana Badea

Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, 4-12 Regina Elisabeta Av., District 3, 030018 Bucharest, Romania

Correspondence should be addressed to Dana Elena Popa; dana_lena1978@yahoo.com

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A new, simple, and sensitive spectrometric method was developed for hydroquinone (HQ) determination in the presence of other depigmenting agents (kojic acid (KA), glycolic acid (GA), and ascorbic acid (AA)), commonly introduced in skin lightening products. The method is based on the oxidation of the depigmenting agents by potassium dichromate in sulfuric acid medium and subsequent measurement of the amplitude of the first-order derivative absorption spectrum at 268 nm. By applying the zero-crossing method, at this wavelength, the oxidation products of KA, AA, and GA do not interfere in the indirect determination of HQ. Beer's law was obeyed in the range of $0.22\text{--}22\ \mu\text{g}\cdot\text{mL}^{-1}$ HQ, with a detection limit of $0.07\ \mu\text{g}\cdot\text{mL}^{-1}$. The developed method was applied with good results for the first time to the rapid determination of HQ in binary, ternary, and quaternary mixtures, thus proving that it could represent an effective tool for various skin lightening products analyses.

1. Introduction

Human skin contains melanocytes (cells located at the base of the epidermis), which produce melanin (a dark macromolecular vital pigment) by a combination of enzymatically catalyzed chemical reactions. This process is named melanogenesis and it intensifies after exposure to UVB radiation, causing the skin to visibly tan. The aim of the melanogenesis is to protect the hypodermis from the DNA photodamage. The first step consists of tyrosine oxidation to dopaquinone catalyzed by tyrosinase [1]. The abnormal accumulation of melanin induces melasma, a chronic skin disorder that results in brownish facial pigmentation. Taking into account that tyrosinase is the enzyme responsible for the melanin synthesis tyrosinase inhibitors are used as whitening or anti-hyperpigment agents due to their ability to suppress dermal-melanin production [2].

Hydroquinone (HQ) is considered to be one of the strongest inhibitors of melanin production and for more than 25 years it has been established as the most effective

ingredient for treating melasma [3]. However, its long-term application has numerous adverse effects, including irritative dermatitis, melanocyte destruction, contact dermatitis, and ochronosis. Matsumoto et al. [4] published recently a study related to the risk of systemic effects of HQ when using skin lightening cosmetics containing it. The adverse effects of HQ are transitory below the 3.0% level (2% HQ is the maximum concentration permitted by the United States Food and Drug Administration (USFDA), whereas 4.0% HQ formulations are only available by prescription [5]). Concentrations of HQ above 5.0% could cause local irritation [6] and even persistent hypopigmentation named leukoderma [7]. Therefore, over the past years HQ has become a controversial skin-care agent for topical use. It should be mentioned that the whitening products containing HQ have been banned in many countries because of concerns related to cancer risk [8]. However, in order to minimize the risk of side effects, many clinical studies reported partial or total replacement of HQ in various cosmetics. The medical literature data also report that combination therapy is more effective than single agent use

[9]. As a result, a series of dermatological creams contain binary, ternary, or quaternary mixtures of HQ and other tyrosinase inhibitors such as glycolic acid (GA), kojic acid (KA), and ascorbic acid (AA). The addition of this last one, which is a well-known antioxidant, enhances the stability of HQ as it can be easily oxidized (even in a tube) and become ineffective [10].

Taking into consideration both benefits and risks of using HQ-containing cosmetics, the quantitative determination of the HQ level in bleaching creams is imperative. For this purpose, many studies on HQ determination in different cosmetics are reported. The employed analytical methods are based on the specific properties of HQ exploited by chromatographic (HPLC [11–14]), capillary electrochromatographic [15], voltammetric [16–19], and spectrometric techniques [20–31]. The advantages of the spectrometric techniques consist in the fact that they use accessible and simpler equipment, have shorter analysis time, and are cheaper than the chromatographic techniques. The use of UV-Vis spectrometry has enhanced rapidly over the last few years. Some of the advantages of these methods are precision, short analysis time, and less reagents consumption [20]. The spectrometric determinations of HQ in cosmetic products were based on direct measurement of UV absorbance of HQ [21, 22], UV derivative spectrometry [23], spectrometric ratio difference method [24], the successive ratio subtraction coupled with constant multiplication UV spectrometry [25], fluorescence spectroscopy [26], or the absorbance measurement of the product resulting in a redox reaction between HQ and specific reagents. For instance, trace levels of HQ were determined by UV absorption measurements after its oxidation to p-benzoquinone (BQ) by oxygen in the presence of ammonium meta-vanadate as an oxidizing catalyst [27]. The catalytic oxidation of HQ to BQ by KMnO_4 in alkaline medium was also used for the spectrometric determination of HQ [28]. Ammonium molybdate (Mo(VI)) was used to oxidize HQ in acidic medium and the resulting molybdenum (V) was spectrometrically monitored [29]. The inhibitory effect of HQ on the oxidation of an organic reagent (Rhodamine B) was used for HQ determination by a kinetic spectrometric method [30]. UV spectrometric investigations of the HQ polymerization in the presence of Cr(VI) were also recently reported [31].

In the present work a simple, accurate, and precise first-order derivative spectrometric method was proposed for the first time to quantify HQ in the presence of other depigmenting agents, namely, KA, GA, and AA, commonly present in cosmetic products. The method is based on the oxidation of HQ by $\text{K}_2\text{Cr}_2\text{O}_7$ in sulfuric acid medium and subsequent absorbance measurement of the first-order derivative spectrum of the oxidation product (BQ) at 268 nm.

2. Materials and Methods

2.1. Chemicals. All chemicals were of analytical reagent grade and were purchased from Sigma-Aldrich. Deionized-distilled water was used throughout the experiments.

Aqueous stock solutions of HQ, KA, GA, and AA ($10^{-2} \text{ mol}\cdot\text{L}^{-1}$) were freshly prepared and used to obtain the

working standard solutions. When not used, the solutions were stored in the refrigerator. A $5 \text{ mol}\cdot\text{L}^{-1} \text{ H}_2\text{SO}_4$ solution was obtained by diluting concentrated sulfuric acid (98%, $1.84 \text{ g}\cdot\text{mL}^{-1}$). Working solutions of 10^{-3} and $5 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{Cr}_2\text{O}_7$ resulted after dilution of a $10^{-1} \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{Cr}_2\text{O}_7$ stock solution. Eppendorf vary-pipettes (10–100; 100–1000; and 500–2500 μL) were employed to deliver accurate volumes.

2.1.1. Depigmenting Agents in Acid Medium. An aliquot (1 mL) of $10^{-3} \text{ mol}\cdot\text{L}^{-1}$ solution of HQ, AA, KA, or GA was transferred into a 5 mL volumetric flask. After adding $5 \text{ mol}\cdot\text{L}^{-1} \text{ H}_2\text{SO}_4$ (1 mL), the mixture was brought to the mark with distilled water and homogenized. Then, the absorbance spectrum was recorded against water as reference. The derivative spectra were plotted with a 2 nm interval from the zero-order spectra of the individual analyzed solutions.

2.1.2. Mixtures of Depigmenting Agents and Potassium Dichromate in Acid Medium. An aliquot (1 mL) of $10^{-3} \text{ mol}\cdot\text{L}^{-1}$ solution of HQ, KA, GA, or AA, 1 mL of $5 \text{ mol}\cdot\text{L}^{-1} \text{ H}_2\text{SO}_4$, and a known volume of 10^{-3} or $5 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{Cr}_2\text{O}_7$ (added in small excess, to obtain a weak yellow colored solution) were transferred into a 5 mL volumetric flask and diluted to the mark. For each solution the absorption spectrum was recorded in the range 215–400 nm, against water as reference. This was necessary due to the fact that between 325 and 400 nm only $\text{K}_2\text{Cr}_2\text{O}_7$ is absorbed, thus being mandatory the removal of the $\text{K}_2\text{Cr}_2\text{O}_7$ excess influence. For each mixture, a corresponding blank solution was prepared by transferring into a 5 mL calibrated flask 1 mL of $5 \text{ mol}\cdot\text{L}^{-1} \text{ H}_2\text{SO}_4$ and a known volume of 10^{-3} or $5 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{Cr}_2\text{O}_7$ to obtain a final aqueous solution having the concentration equal to that of the unreacted $\text{K}_2\text{Cr}_2\text{O}_7$ (deduced from its absorbance at 350 nm; see the details given in Section 3.1.). The spectrum of the analyte oxidation product was obtained by subtracting the blank spectrum from the spectrum recorded for the analyte in the presence of a small excess of the oxidant ($\text{K}_2\text{Cr}_2\text{O}_7$).

Binary, ternary, and quaternary mixtures of HQ and other dermatological active agents (KA, GA, and AA) in acidic medium and in the presence of $\text{K}_2\text{Cr}_2\text{O}_7$ were prepared in the same manner as the individual depigmenting agents. The differences between absorbencies of the mixtures and the corresponding blank solutions were also obtained.

2.2. Apparatus. Absorbance measurements were performed in the 215–400 nm wavelength range on a UV-VIS spectrometer (V-530 Jasco-Japan), with fully integrated PC running Spectra Manager software, equipped with quartz cells of 1.00 cm. Suitable settings were slit width 1 cm and scan speed $100 \text{ nm}\cdot\text{min}^{-1}$.

3. Results and Discussions

3.1. Zero- and First-Order Derivative UV Spectrometric Studies. HQ, KA, GA, and AA absorb UV radiation having char-

FIGURE 1: UV absorption spectra of HQ, KA, AA, and GA; every compound has the same concentration ($2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$).

acteristic absorption spectra (Figure 1). As it can be observed, the spectra of HQ and KA overlap significantly. Moreover, in the given experimental conditions, the calculated molar absorptivity ($\epsilon_{\text{HQ}} = 2600 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ at $\lambda_{\text{max,HQ}} = 289 \text{ nm}$) of HQ is smaller than that of AA ($\epsilon_{\text{AA}} = 7350 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ at $\lambda_{\text{max,AA}} = 244 \text{ nm}$) and of KA ($\epsilon_{\text{KA}} = 7500 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ at $\lambda_{\text{max,KA}} = 269 \text{ nm}$).

By applying the zero-crossing method, the first- and second-order derivative spectra of HQ, KA, and AA do not permit the determination of HQ in ternary mixtures due to the fact that there is no wavelength where only HQ presents a measurable signal (Figures 2(a) and 2(b)).

It was observed that HQ presents an analytical signal at 303 nm in its third-order derivative spectrum, while the same order derivative spectra of the other tested compounds intersect the abscissa (Figure 2(c)). However, the analytical signal of HQ is very small, even at a concentration of $2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ($22 \mu\text{g}\cdot\text{mL}^{-1}$), which leads to a very low sensitivity.

Under these circumstances, a new methodology was established. This one considers the capacity to be oxidized of the above-mentioned compounds when $\text{K}_2\text{Cr}_2\text{O}_7$ in acidic medium is used as oxidizing agent. Thus, another series of UV spectra were recorded for the individual active compounds in the presence of $\text{K}_2\text{Cr}_2\text{O}_7$ in sulfuric acid medium. As depicted in Figure 3 the oxidation product of HQ in presence of the $\text{K}_2\text{Cr}_2\text{O}_7$ excess (spectrum (1)) exhibits an absorption band in the same wavelength region (325–400 nm) as the $\text{K}_2\text{Cr}_2\text{O}_7$ solution (spectrum (2)). It must be mentioned that the UV absorption spectrum of BQ (not shown) presents a characteristic absorption band in the range 225–250 nm similar to the spectrum of the HQ oxidation product resulting from the reaction of HQ with $\text{K}_2\text{Cr}_2\text{O}_7$ in sulfuric acid medium (spectrum (2)). In order to obtain only the spectrum of the HQ oxidation product (spectrum (3)), the spectral subtraction of spectrum (2) from spectrum (1) was performed. Each analyzed mixture was prepared in the presence of a known $\text{K}_2\text{Cr}_2\text{O}_7$ excess.

The concentration of the unreacted $\text{K}_2\text{Cr}_2\text{O}_7$ was deduced from the absorbance of the mixture at 350 nm, where only dichromate ion is absorbed. To achieve this aim a calibration curve was accomplished using $\text{K}_2\text{Cr}_2\text{O}_7$ solutions with different concentrations (2×10^{-5} – $2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$) in $1 \text{ mol}\cdot\text{L}^{-1} \text{ H}_2\text{SO}_4$. Using the equation of the regression line ($A_{350 \text{ nm}} = 0.4875c - 0.0002$; $R^2 = 0.9994$; c is the dichromate concentration) the blank concentration (having the same absorbance at 350 nm as the mixture of the analytes in presence of dichromate excess) is calculated.

The UV spectra of the investigated compounds in the absence and in the presence of $\text{K}_2\text{Cr}_2\text{O}_7$ differ significantly in the case of HQ and AA (Figure 4), whereas for KA only a small decrease of the absorption band intensity is observed in the presence of $\text{K}_2\text{Cr}_2\text{O}_7$ in sulfuric acid medium, suggesting that KA is not oxidized in these conditions.

The redox reactions between HQ, GA [32], AA [33], and $\text{K}_2\text{Cr}_2\text{O}_7$ in sulfuric acid medium are shown in Scheme 1.

By overlaying the spectra of the reaction products of HQ, KA, GA, AA, and $\text{K}_2\text{Cr}_2\text{O}_7$, the first one cannot be determined in presence of the others (Figure 5(a)). Although at the first sight one may consider that HQ can be determined in the presence of GA and AA, it must be mentioned that in real samples the GA and AA concentrations are even five times higher than the HQ concentration, their contribution becoming significant.

Applying the first-order derivative, the analytical signal attributed to the oxidation product of HQ (benzoquinone (BQ)) could be used for the indirect determination of HQ in the presence of the other ingredients, at the zero-crossing of KA oxidation product (268 nm), where the amplitudes of the first-order derivative spectra of AA and GA oxidation products are also zero (Figure 5(b)).

3.2. Optimization of the Working Parameters. In order to optimize the working conditions of the proposed method, the influence of the sulfuric acid concentration at constant HQ and dichromate contents was studied. It was observed that the analytical signal increases with increasing the concentration of H_2SO_4 up to $1 \text{ mol}\cdot\text{L}^{-1}$; then it remains almost constant. Further experiments were made on samples prepared in $1 \text{ mol}\cdot\text{L}^{-1} \text{ H}_2\text{SO}_4$. The stability of the reaction product between HQ and dichromate was monitored by spectrometry in the time range 0–1800 sec. The measurements made at 240 nm (the wavelength corresponding to the maximum absorbance of the HQ oxidation product in the presence of dichromate) indicated that the absorbance was stable within the tested period.

3.3. Analytical Parameters of the Indirect Spectrometric Method Developed for HQ Quantitative Determination. Using the above optimized spectrophotometric method developed for the indirect determination of HQ (via its oxidation in the presence of dichromate) a linear relationship was obtained between $dA/d\lambda$ (at 268 nm) and the HQ concentration. The parameters of the calibration curve, obtained by linear square regression of the results, are given in Table 1.

FIGURE 2: (a) First-order, (b) second-order, and (c) third-order derivative spectra of HQ, KA, AA, and GA; every compound has the same concentration ($2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$).

FIGURE 3: UV spectra of (1) $2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ HQ in the presence of $2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{Cr}_2\text{O}_7$ (in excess), in $1 \text{ mol}\cdot\text{L}^{-1}$ sulfuric acid; (2) $\text{K}_2\text{Cr}_2\text{O}_7$ at a level of concentration corresponding to those unreacted in solution (1); (3) = (1) - (2).

The limit of quantification (LOQ) was determined by the analysis of samples with known concentration of HQ and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. The limit

of detection (LOD) was considered as the signal to noise ratio of 3:1 [34].

A comparison with other reported methods shows that the proposed spectrometric method is more sensitive, with

FIGURE 4: Overlaid spectra of the studied compounds in the absence (solid lines) and in the presence (dashed line) of $K_2Cr_2O_7$ in $1\text{ mol}\cdot\text{L}^{-1}$ sulfuric acid; every compound has the same concentration ($2 \times 10^{-4}\text{ mol}\cdot\text{L}^{-1}$).

TABLE 1: Analytical parameters of the first-order derivative spectrometric method for the indirect HQ determination.

Parameter	Value
Linear range, $\mu\text{g}\cdot\text{mL}^{-1}$	0.22–22
Intercept, a	0.0018
Intercept standard deviation, s_a	0.00032
Slope, b	0.0031
Slope standard deviation, s_b	0.00003
Determination coefficient, R^2	0.9994
LOD, $\mu\text{g}\cdot\text{mL}^{-1}$	0.07
LOQ, $\mu\text{g}\cdot\text{mL}^{-1}$	0.22

a larger linear range of $0.22\text{--}22\text{ }\mu\text{g}\cdot\text{mL}^{-1}$. At the same time, the proposed method is less time consuming, no heating is required, and it is eco-friendly (no use of organic solvents) and inexpensive (Table 2).

3.4. Precision and Accuracy. The precision and the accuracy of the proposed spectrometric method were obtained using solutions containing HQ at three different concentration levels, within the established linear range. The results presented

in Table 3 show high accuracy (estimated by the percent recovery ($R\%$), between 99.39% and 100.15%) and precision (estimated by the means of relative standard deviation (RSD%), between 1.34% and 2.60%) of the obtained results.

The obtained percent recovery values lie within the accepted limits for these concentration levels [35].

3.5. Application of the Developed Method to the Determination of HQ in Binary, Ternary, and Quaternary Mixtures. The literature data report various combinations of different topical agents for melasma treatment [36]. Hydroquinone is generally the main component of the formulations [10]. It is usually combined with glycolic acid, kojic acid, and ascorbic acid resulting in binary (4% HQ + 2% GA; 4% HQ + 10% GA; 2% HQ + 2% KA) [37, 38], (4% HQ + 10% AA) [39]; ternary (2% HQ + 10% GA + 2–4% KA) [40], (4% HQ + 2.5% AA + 0.75% KA) [41]; or quaternary (2% HQ + GA + KA + AA) [42] mixtures.

As it can be seen, the four ingredients are found in the dermatological formulations in variable mixtures and concentrations ($\text{HQ} \leq 4\%$; $\text{AA} \leq 10\%$; $\text{KA} \leq 2\%$; $\text{GA} \leq 10\%$). In order to determine how the possible interfering compounds affect the HQ quantitation, binary mixtures were prepared, the concentration of the other active compounds

FIGURE 5: Overlaid (a) zero-order and (b) first-order derivative spectra of HQ, KA, GA, and AA in the presence of $K_2Cr_2O_7$ in $1 \text{ mol} \cdot L^{-1}$ sulfuric acid; every compound has the same concentration ($2 \times 10^{-4} \text{ mol} \cdot L^{-1}$).

TABLE 2: Comparison of the proposed method with the reported spectrometric methods for HQ determination.

Analytical signal/working conditions	Linear range, $\mu\text{g} \cdot \text{mL}^{-1}$	Application	Ref.
$A_{302 \text{ nm}}/\text{HQ}$ in H_2SO_4	2–12	Body lotions	[22]
$A_{293 \text{ nm}}/\text{HQ}$ in methanolic solution	10–50	Cosmetic creams	[21]
$A_{293 \text{ nm}}/\text{HQ} + \text{O}_2 + \text{NH}_4\text{VO}_3$ in 2-propanol : water 1 : 1	0.025–2	Cosmetic creams	[27]
$dA/d\lambda_{302 \text{ nm}}/\text{HQ}$ in H_2SO_4	10–26	Cosmetic creams	[23]
$A_{580 \text{ nm}}/\text{HQ} + (\text{NH}_4)_2\text{MoO}_4 + 10\% \text{H}_2\text{SO}_4$; 20-minute reaction time, 100°C	10–100	Skin whitening formulations	[29]
$A_{610 \text{ nm}}/\text{HQ} + \text{KMnO}_4$ in alkaline medium; 30–35-minute reaction time	1–26	Pharmaceuticals	[28]
$dA/d\lambda_{302 \text{ nm}}/\text{HQ}$ in the presence of KA, AA, and GA + $K_2Cr_2O_7$ in H_2SO_4 , instantaneous reaction; room temperature	0.22–22	Synthetic mixtures	Present work

being smaller than, equal to, and higher than the HQ concentration.

The recipes of the dermatological formulations that contain all the four active ingredients do not contain information about the KA, GA, and AA concentrations. Therefore, in the present study, the ternary mixtures contain all the four components at the lowest concentration level (percent ratio, HQ:KA:GA:AA = 1:1:1:1) and at the highest accepted concentration level (percent ratio, HQ:KA:GA:AA = 2:1:5:5).

The determination of HQ in combination with the mentioned active ingredients was studied by applying the proposed indirect derivative spectrometric method. Different combinations of HQ and the other depigmenting compounds and the analytical results for HQ determination are given in Tables 4 and 5. The percent recovery values ($R\%$) summarized in Table 3 are very close to 100% (between 99.20 and 101.60%), a fact that reveals the accuracy of the proposed method in the determination of HQ in binary mixtures.

TABLE 3: The precision and accuracy of the results obtained by the proposed spectrometric method; n -number of independent measurements; $n = 5$; SD: standard deviation.

HQ, $\mu\text{g} \cdot \text{mL}^{-1}$		RSD, %	R, %
Considered	Found $\pm$ SD		
2.2	2.19 ± 0.05	2.31	99.39
5.5	5.51 ± 0.07	1.34	100.15
11	10.99 ± 0.29	2.60	99.88

In the case of ternary and quaternary mixtures, HQ can be determined by the proposed spectrometric method with good recovery values, the standard deviations supporting also the fact that the results are reliable and comparable.

All the experimental data obtained during this study led to the results presented throughout this paper and these revealed that the developed spectrometric method is a useful

SCHEME 1

TABLE 4: Results of the HQ determination in synthetic binary mixtures by applying the proposed spectrometric method; $n = 3$.

Mixtures	Percent ratio, HQ : KA/AA/GA	HQ concentration, $\mu\text{g}\cdot\text{mL}^{-1}$		R%
		Considered	Found $\pm$ SD	
HQ + KA	4 : 1	4	4.04 ± 0.08	101.06
	2 : 1	4	4.04 ± 0.05	101.06
	1 : 1	4	4.06 ± 0.07	101.33
	1 : 1.5	4	3.96 ± 0.05	99.47
	1 : 2	4	3.97 ± 0.04	99.20
HQ + AA	2 : 1	4	3.99 ± 0.06	99.73
	1 : 1	4	3.98 ± 0.05	99.47
	1 : 2	4	4.02 ± 0.04	100.53
	1 : 2.5	4	4.00 ± 0.06	100.00
	1 : 5	4	4.04 ± 0.07	101.06
HQ + GA	1 : 1	4	4.04 ± 0.05	101.06
	1 : 2	4	4.05 ± 0.06	101.17
	1 : 3	4	4.03 ± 0.07	100.80
	1 : 4	4	4.02 ± 0.06	100.53
	1 : 5	4	4.06 ± 0.08	101.60

analytical tool for HQ quantitation in the presence of GA, KA, and AA from real samples containing varied mixtures of the mentioned compounds.

4. Conclusions

The new proposed method for the hydroquinone determination in the presence of other dermatologic active ingredients (kojic acid, ascorbic acid, and glycolic acid) is cheap and

simple, and it is not time consuming. The used reaction system contains an oxidant ($\text{K}_2\text{Cr}_2\text{O}_7$) that does not necessitate any additional steps. Moreover, the method does not contain variables which influence the reliability of the results. The accuracy, the precision, and the results obtained analyzing the synthetic mixtures recommend the spectrometric procedure for skin depigmenting products analysis and control by means of hydroquinone level monitoring, in binary, ternary, or quaternary mixtures with kojic acid, glycolic acid, and ascorbic acid. The application of the spectrometric method

TABLE 5: Results of the HQ determination in ternary and quaternary synthetic mixtures by applying the proposed spectrometric method; $n = 3$.

Percent ratio, HQ : GA : KA : AA	HQ concentration, $\mu\text{g}\cdot\text{mL}^{-1}$		R%
	Considered	Found $\pm$ SD	
1:5:1:0	2	1.85 ± 0.08	92.55
1:5:2:0	2	1.87 ± 0.06	93.62
4:0.75:0:2.5	8	8.34 ± 0.35	104.26
1:1:1:1	2	1.83 ± 0.05	91.49
2:5:1:5	4	4.19 ± 0.05	104.79

on synthetic mixtures represents the first step for further researches on real samples.

Competing Interests

The authors declare that they have no competing interests.

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