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Determination of optimum wavelength and derivative order in spectrophotometry for quantitation of hydroquinone in creams

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UV derivative spectrophotometry was used for quantitative determination of hydroquinone in creams. The aim of this work was to investigate optimum wavelength and order of derivative, and to validate the proposed spectrophotometric method. The results of standard curves were calculated and statistically analyzed through the least squares method in the interval from 10.0 to 26.0 µg/mL, in the first, second, third and fourth order derivatives. The quantitative determination was carried out by using the zero-crossing (Z-C) and zero-peak (Z-P) methods. The proposed method is simple, of low cost and provides reliable results in order to be used in quality control of creams containing hydroquinone as active substance.

Uniterms
- Hydroquinone
- UV derivative spectrophotometry
- Quality control

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INTRODUCTION

The hyperpigmentary disorders of the skin are caused by the over production of melanin, either by a normal or increased number of melanocytes, or by hormonal disorders (Cecil, 1993; Silva, Rezende, 1998). The hydroquinone (HQ) has been used for decades in creams, gels and lotions for the treatment of these disorders. It is the most frequently used compound in skin-toning preparations (Palumbo *et al*., 1991; Katsambas, Antoniou, 1995; Mohamed, Mohamed, 1998). It belongs to the group of phenols and alike most compounds of this group, it easily undergoes oxidative degradation, principally in the presence of metallic ions, high concentration of oxygen, high pH and on exposure to light (Connors *et al*., 1986; Ullmann´s, 1999). The effects of HQ are transitory and concentrations below 3.0% do not cause skin injuries, however concentration above 5.0% could provoke local irritation being this the main reason for the validation of a simple method (Bleehen, 1977; Hardman *et al*., 1996; Zanini and Oga, 1989).

Several analytical methods for the determination of HQ in skin-toning preparations are described, including high performance liquid chromatography (Firth, Rix, 1986; Gagliardi *et al*., 1987; Borremans *et. al*., 1999), capillary electrochromatography (Desiderio *et al*., 2000), micellar chromatography (Sakodinskaya *et al*., 1992) and other analytical techniques (Wang, 1995; Cruz, Fatibello-Filho, 2000; Rueda *et al*., 2003).

The HQ shows a broad band of absorption in the UV region between 190 and 350 nm, whose spectrum can be represented by the curve $A = f(\lambda)$, where A is absorbance and λ the wavelength. From this curve, successive derivatives can be calculated at each point:

$$\frac{\delta A}{\delta \lambda}, \frac{\delta^2 A}{\delta \lambda^2}, \ldots \frac{\delta^n A}{\delta \lambda^n}$$

With the obtained values, the differential curve can be traced $\frac{\delta^n A}{\delta \lambda^n}$ giving rise to derivative spectrum of order n. The derivative spectrophotometry is based on the use of these spectra (Hackmann *et al*., 1991; Hopkala, Kowalczuk, 2000; Karpiñska, 2004).

The aim of this work was to determine most appropriate wavelength, as well as order of the derivative for quantitative determination of HQ in creams preparation (García *et al*., 2005). Such selection contributes to the development and validation of simple and reliable method for the quality control of cream preparations containing this substance.

MATERIAL AND METHODS

Chemicals

Hydroquinone (99.8%), kindly supplied by Laboratórios Stiefel S.A. (São Paulo, Brasil), sulfuric acid (analytical grade) Merck® (São Paulo, Brasil). Distilled water was used to prepare all solutions.

Samples

The cream preparations were obtained from a local compounding pharmacy. Sample I: 2 g HQ/100 g cream; Sample II 4 g HQ/100 g cream and Sample III 4 g HQ/100 g cream.

Instrumentation and analytical conditions

The methods were developed on a UV-Vis spectrophotometer UV-1601 (Shimadzu®) using 1.0 cm quartz cell and data were processed with online UVPC v3.91 Personal Spectroscopy Software (Shimadzu®). The wavelength interval was established between 190 and 350 nm, the delta lambda was 2 nm, the average screening speed was 370 nm/min and the amplitude axis were from 0.000 to 1.200; -1.000 to 0.500; -0.200 to 0.120; -0.050 to 0.040 and -0.010 to 0.010 for zero, first, second, third and fourth orders derivative spectra, respectively.

Selectivity

An amount of placebo cream equivalent to 20.0 mg of HQ was weighed and transferred to a 100 mL graduated becker. Approximately 50 mL of H_2SO_4 (0.05 M) was added and was shaken during 10 min in a water bath at 40 °C. This solution was transferred to a 100 mL volumetric flask and the volume was completed with the same solvent. The solution was filtered through Whatman® filter paper no.1, discarding first 5 mL. Appropriate aliquots were diluted using sulfuric acid (0.05 M) in 50 mL volumetric flask to obtain solutions with final concentration equivalent to 16.0, 20.0 and 24.0 µg/mL of placebo.

Linearity

To prepare calibration curve, accurately weighed 50.0 mg of HQ was transferred to a 50 mL volumetric flask and dissolved in sulfuric acid (0.05M). The volume was completed with the same solvent. The above solution was systematically diluted in 25 mL volumetric flasks (in triplicate) to obtain final solutions containing 10.0, 14.0, 18.0, 22.0 and 26.0 µg/mL of HQ.

Precision

Accurately weighed amount of cream equivalent to 15.0 mg of HQ was transferred to a 100 mL graduated becker. Approximately 50 mL of H_2SO_4 (0.05 M) were added and were shaken during 10 min in a water bath at 40 ºC. This solution was transferred to a 100 mL volumetric flask and the volume was completed with the same solvent.

The resultant solution was filtered through Whatman filter paper no.1, discarding first 5 mL. A 3 mL aliquot of this solution was diluted in a 25 mL volumetric flask using 0.05 M sulfuric acid to obtain a solution with final concentration of 18.0 µg/mL. Each sample was assayed ten times with each sample solution.

Accuracy

An accurately weighed amount of cream equivalent to 12.0 mg of hydroquinone was transferred to a 100 mL volumetric flask, dissolved in approximately 50 mL of 0.05 M sulfuric acid and was shaken during 10 min in a water bath at 40 °C. After cooling, the volume was completed with the same solvent and aliquots of 10.0 mL of this solution were transferred into 100 mL volumetric flasks containing 1.0, 5.0 and 10.0 mL of a standard hydroquinone solution (100.0 µg/mL) and sulfuric acid (0.05 M) was added to make up the volume to obtain final concentrations of 13.2, 18.0 and 24.0 µg/mL of HQ. All solutions were prepared in triplicate and analyzed. The percentage recovery was calculated using the equation proposed in Official Methods of Analysis of AOAC International (AOAC International, 1990).

Procedure

After homogenization of the solutions and calibration

of the spectrophotometer with 0.05 M sulfuric acid, the absorption spectra of each sample were obtained. The first, second, third and fourth orders derivative spectra were traced from above curves. The quantitative determinations of HQ were made at signal when there was no interfering absorption.

RESULTS AND DISCUSSIONS

The calibration curves were constructed by plotting amplitudes of the first, second, third and fourth derivative spectra versus the concentration of the HQ in 0.05 M sulfuric acid. Table I shows statistically treated data of calibration curves.

Zero-crossing (Z-C) is the method that enables the measurement of the spectrum amplitude where the interference is cancelled till the signal of the HQ. Zero-peak (Z-P) is the method that measures the spectrum amplitude from the base line till the signal of HQ. These two methods were used for quantitative determination of HQ in creams. Figure 1 shows the zero, first, second, third and fourth orders derivative spectra of the calibration curves of HQ in 0.05 M sulfuric acid. It was observed that at designated points, the possible interference from placebo is null. These are the most appropriate wavelengths for quantitative determination of HQ. However, between these points, there is a single wavelength that provides statistically most adequate result and at this point, determination can be made with least relative standard deviation.

After identifying the optimum wavelength and derivative order by statistic treatment (signal at 302.0 nm in the first derivative), the analytical method was validated and applied for quantitative determination of HQ in creams. The results can be observed in Table II. The low relative standard deviation (R.S.D.) values indicates precision of the method. In majority of the determinations it is below 2%, indicating high degree of agreement (repeatability) between experimental values (Bruce *et al*., 1998; ICH Q2A; ICH Q2B; Shabir, 2003). Figure 2 shows UV derivative spectra of HQ standard solution, HQ sample solution and placebo of the cream. No interfering signals and overlaps were observed in placebo at 302.0 nm in the first derivative. A good accuracy of the method was verified with a mean recovery of 99.2% (Table III).

CONCLUSIONS

The proposed UV derivative spectrophotometric method proved to be an excellent option for HQ determination in creams. The interference from excipients was eliminated by selecting the most adequate wavelength. The first derivative with zero-peak method at 302.0 nm was successfully applied for quantitative determination of HQ. The proposed method can be used for analysis in routine quality control of cosmetic preparations with HQ as a unique active substance. The method is simple, rapid, precise and of low cost.

TABLE I - Statistics evaluation of the calibration curves of HQ in 0.05 M sulfuric acid

Derivative	λ (nm)	Method	Regression equation (interval 10.0 - 26.0 µg/mL)	Correlation coefficient	R.S.D. (%) intercept	Test "a"
1 D	225.8	Z – C	y=-2.64x10^{-2}x + 1.03x10^{-2}	-0.9923	1.6272	0.9171
1 D	302.0	Z – P	y=-1.76x10^{-2}x + 1.41x10^{-3}	-0.9993	1.5051	0.1985
2 D	242.8	Z – C	y=1.04x10^{-3}x – 1.28x10^{-3}	0.9989	2.0334	2.4237
2 D	267.8	Z – C	y=5.28x10^{-4}x + 6.50x10^{-5}	0.9976	2.7741	0.1643
2 D	306.4	Z – P	y=1.97x10^{-3}x + 4.50x10^{-5}	0.9990	1.7709	0.048
3 D	221.8	Z – C	y=-7.48x10^{-4}x – 6.50x10^{-5}	-0.9992	1.5218	0.2118
3 D	232.0	Z – P	y=8.15x10^{-4}x + 3.10x10^{-4}	0.9991	1.5984	0.8679
3 D	302.4	Z – P	y=4.70x10^{-4}x + 2.60x10^{-4}	0.9988	1.9189	1.0417
3 D	311.2	Z – P	y=-2.30x10^{-4}x + 4.00x10^{-5}	-0.9990	1.7812	0.3672
4 D	217.4	Z – C	y=-3.30x10^{-4}x – 1.40x10^{-4}	-0.9981	2.4023	0.6426
4 D	235.4	Z – C	y=-1.32x10^{-4}x – 2.15x10^{-4}	-0.9841	6.6986	0.8276
4 D	305.6	Z – P	y=-1.15x10^{-4}x – 9.00x10^{-5}	-0.9962	3.3810	0.8262
4 D	313.4	Z – P	y=3.25x10^{-5}x – 2.50x10^{-5}	0.9912	5.6469	0.521

(Standard solutions n = 5); R.S.D. = Relative Standard Deviation; Z-C = Zero-Crossing; Z-P = Zero-Peak

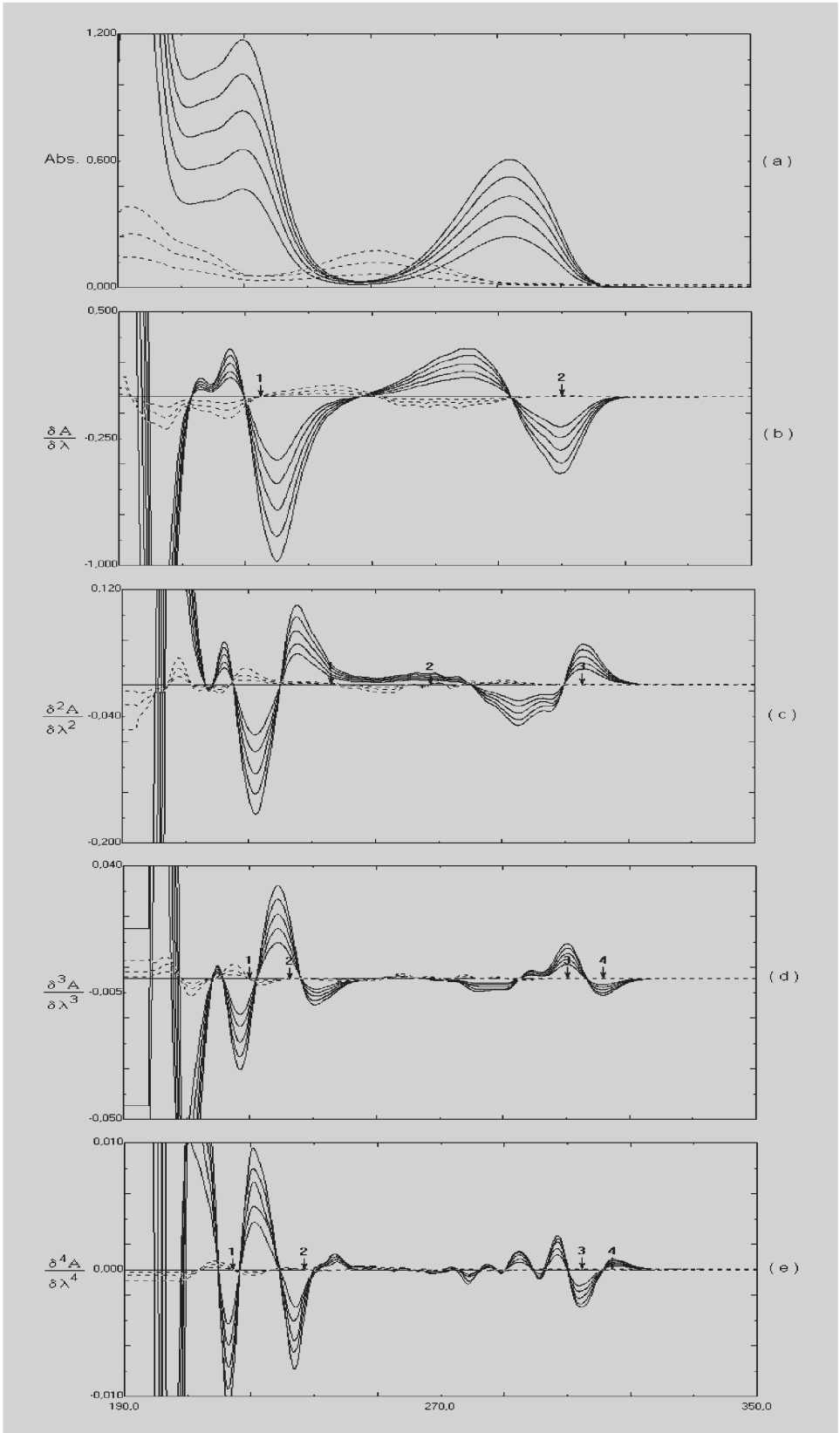

FIGURE 1 - Absorption spectra of zero (a), first (b), second (c), third (d) and fourth (e) derivative of HQ solutions (———) and placebos (-----) in 0.05 M sulfuric acid. Concentrations of 10.0, 14.0, 18.0, 22.0 and 26.0 µg/mL of HQ and 16.0, 20.0 and 24.0 µg/mL of placebo.

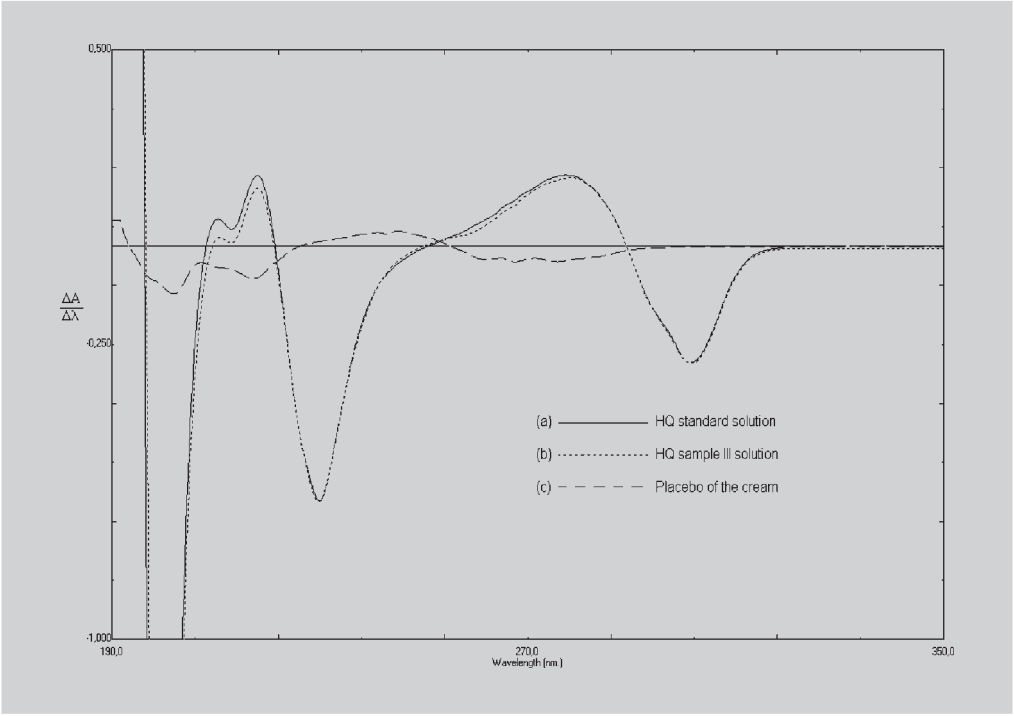

FIGURE 2 - UV derivative spectra of (a) HQ standard solution, (b) HQ sample III solution and (c) placebo of the cream in 0.05 M sulfuric acid. Concentration 18.0 µg of HQ/mL.

TABLE II - Determination of the HQ content in cream utilizing the first, second, third and fourth derivative orders in 0.05 M sulfuric acid

Sample	Declared amount HQ (g/100 g)	Found amount * HQ (g/100 g)	Amount (%)	R. S. D. (%)	C. I. P = 95 %
		First derivative 302.0 nm			
I	2	1.870	93.5	0.968	0.12
II	4	3.916	97.9	0.854	0.11
III	4	4.057	101.4	0.812	0.11
		Second derivative 306.4 nm			
I	2	1.870	93.5	1.174	0.14
II	4	3.876	96.9	1.463	0.18
III	4	4.012	100.3	1.121	0.14
		Third derivative 221.8 nm			
I	2	1.873	93.6	2.363	0.28
II	4	3.873	96.8	1.331	0.16
III	4	3.988	99.7	1.432	0.18
		Fourth derivative 217.4 nm			
I	2	1.717	85.8	1.994	0.22
II	4	3.664	91.6	1.292	0.15
III	4	3.678	91.9	0.869	0.12

* n = 10; R.S.D. = Relative Standard Deviation; C.I. = Confidence Interval

TABLE III - Recovery of a standard HQ content added to cream and determined by first derivative at 302.0 nm in 0.05 M sulfuric acid

Sample	Concentration added (μg/mL)	Concentration found (μg/mL)	Recovery (%)[a]
I	1.20	1.18	98.30 ± 0.80
II	6.00	5.90	98.30 ± 0.81
III	12.00	12.00	100.00 ± 0.80
I	1.20	1.20	100.00 ± 0.70
II	6.00	5.95	99.20 ± 0.78
III	12.00	11.80	98.30 ± 0.78
I	1.20	1.20	100.00 ± 0.70
II	6.00	5.93	98.80 ± 0.67
III	12.00	11.81	98.40 ± 0.69

[a] = mean of three determination

RESUMO

Determinação do comprimento de onda ótimo e da ordem da derivada em espectrofotometria para quantificação de hidroquinona em cremes

A espectrofotometria derivada no UV foi usada para a determinação quantitativa de hidroquinona em cremes. O objetivo desta pesquisa foi investigar o melhor comprimento de onda e a ordem da derivada, bem como validar o método proposto. Os resultados das curvas analíticas foram analisados estatisticamente pelo método dos mínimos quadrados no intervalo de 10,0 a 26,0 μg/mL, na primeira, segunda, terceira e quarta ordens da derivada. As determinações quantitativas foram realizadas utilizando os métodos "zero-crossing (Z-C)" e zero-pico (Z-P). O método proposto é simples, de baixo custo e fornece resultados confiáveis podendo ser usado no controle de qualidade de cremes contendo hidroquinona como substância ativa.

UNITERMOS: Hidroquinona. Espectrofotometria derivada no UV. Controle de qualidade

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