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Short communication

Development and validation of a HPLC and a UV derivative spectrophotometric methods for determination of hydroquinone in gel and cream preparations

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Abstract

A high performance liquid chromatographic (HPLC) and a ultraviolet derivative spectrophotometric (UVDS) methods were developed and validated for the quantitative determination of hydroquinone (HQ) in gels and creams containing this compound as a unique active principle. Validation parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantitation (LOQ) were determined. HPLC was carried out by reversed phase technique on a RP-18 column with a mobile phase composed of methanol and water (20:80, v/v). The linearity in the range of 6.0–30.0 μg/mL present a correlation coefficient (*r*) of 0.9999, calculated by least square method. The LOD and LOQ were 0.08 and 0.26 μg/mL, respectively. Based on the preliminary spectrophotometric profile of HQ, a signal at 302.0 nm of the first derivative spectrum ($1D_{302.0}$) was found adequate for validation. The linearity between signal $1D_{302.0}$ and concentration of HQ in the range of 10.0–26.0 μg/mL in sulfuric acid (0.1N) present a correlation coefficient (*r*) of 0.9999. The LOD and LOQ were 0.14 and 0.46 μg/mL, respectively. Statistical analysis by *t*- and *F*-tests, showed no significant difference at 95% confidence level between the two proposed methods.

1. Introduction

Hydroquinone (HQ), 1,4-benzenediol, is a depigmenting agent. It is the most often used compound in skin-toning preparations, which was reported to be effective at 1.5–2.0% [1]. Concentrations higher than 5.0% were liable to cause redness and burning [2]. The depigmenting activity of HQ may partly be related to the ability of the compound to act as an alternate substrate of tyrosinase, competing thereby for tyrosine oxidation in active melanocytes [3–5].

Clinical studies have established the beneficial therapeutic effect of HQ in the treatment of melasma, freckles, lentigines and other skin hyperpigmentary disorders [6–7]. Several analytical methods for the determination of HQ in skin-toning preparations are described, including high performance liquid chromatography (HPLC) [8–11], capillary electrochromatography [12], micellar chromatography [13] and others analytical techniques [14–16]. The aim of this work was to develop and validate two efficient methods using HPLC and UVDS for the quantitative determination of HQ in gel and cream samples where HQ is the only active principle, since preparations containing some other compounds such as kojic acid and arbutin could cause interference in both methods.

2. Experimental

2.1. Chemicals

Hydroquinone (99.8% purity) was kindly supplied by Laboratorios Stiefel S.A. (São Paulo, Brasil). Methanol (HPLC grade) and sulfuric acid (analytical grade) were obtained from Merck®. Ultrapure water was obtained from a Milli-Q® Plus apparatus (Millipore®) and was used to prepare all solutions for the HPLC method. Distilled water was used to prepare

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all solutions for the UVDS method. The gels (samples A–D) and creams (samples I–III) containing either 2.0 or 4.0% of HQ were obtained from a local compounding pharmacy.

2.2. Instrumentation and analytical conditions

The HPLC method was performed on a liquid chromatograph model CG 480 C with an isocratic pump. A UV–vis detector CG 435, a injector fitted with a 20 μL loop and a integrator CG 200 (Instrumentos Cientificos®) were used in the research. The HPLC was carried out at a flow rate of 1.0 mL/min using a mobile phase constituted of methanol and water (20:80, v/v), and detection was made at 289.0 nm. The mobile phase was prepared daily, filtered through a 0.45 μm membrane filter (Millipore®) and sonicated before use. A Lichrospher® 100 RP-18 column (125 mm × 4 mm i.d., 5 μm particle size) (Merck®) was used. The HPLC system was operated at 25 ± 2 °C. UVDS method was performed on a UV–vis spectrophotometer, UV-1601 (Shimadzu®) with the signal at 302.0 nm of the first derivative spectrum and using 1.0 cm quartz cell.

2.3. Preparation of standard solutions

2.3.1. HPLC method

For the calibration curve, accurately weighed 30.0 mg of HQ was transferred to a 10 mL volumetric flask and dissolved in the mobile phase. From this solution, other solutions with concentrations of 6.0, 12.0, 18.0, 24.0 and 30.0 μg/mL were obtained by diluting adequate amounts in triplicate. For the precision determination, accurately weighed 15.0 mg of HQ was transferred to a 25 mL volumetric flask and dissolved in the mobile phase; solutions containing 18.0 μg/mL of HQ were obtained by diluting adequate amounts in triplicate.

2.3.2. UVDS method

For the calibration curve, accurately weighed 50.0 mg of HQ was transferred to a 50 mL volumetric flask and dissolved in sulfuric acid (0.1N); from this solution, other solutions containing 10.0, 14.0, 18.0, 22.0 and 26.0 μg/mL were obtained in 25 mL volumetric flasks (triplicate). For the precision determination, accurately weighed 45.0 mg of HQ was transferred to a 50 mL volumetric flask and volume completed with sulfuric acid (0.1N). This solution was diluted in a 50 mL volumetric flask in order to obtain a final concentration of 18.0 μg/mL (triplicate).

2.4. Preparation of sample solutions

2.4.1. HPLC method

Accurately weighed amount of gel or cream equivalent to 4.5 mg of HQ was transferred to 25 mL volumetric flask and dissolved in the mobile phase to obtain a concentration of 180.0 μg/mL. An aliquot of this solution was diluted in mobile phase to obtain a solution with final concentration of 18.0 μg/mL.

2.4.2. UVDS method

Accurately weighed amount of gel or cream equivalent to 15.0 mg of HQ was transferred to a 100 mL volumetric flask and dissolved in sulfuric acid (0.1N) to obtain a concentration of 150.0 μg/mL. An aliquot of this solution was diluted in sulfuric acid (0.1N) to obtain a solution with final concentration of 18.0 μg/mL.

2.5. Method validation

The methods were validated according to the International Conference on Harmonization [17] and AOAC International [18] guidelines for validation of analytical methods. Tests of *t* and *F* were used to compare the proposed methods [19].

2.5.1. Linearity

The calibration curve was obtained at five concentrations levels of HQ solutions (6.0–30.0 μg/mL for HPLC method and 10.0–26.0 μg/mL for UVDS method). The linearity was evaluated by the least square regression method with triplicate determinations at each concentration level.

2.5.2. Precision

The precisions of the methods were determined by intra-day repeatability, which was evaluated by analysing all samples, at the same concentration and on the same day. Ten sample solutions (18.0 μg/mL for HPLC and UVDS methods) were prepared and assayed.

2.5.3. Accuracy

The accuracy was determined by recovery test. Known amounts of HQ were added to the samples and analysed by the proposed methods [18]. For the HPLC method, an accurately weighed amount of gel or cream equivalent to 2.5 mg of HQ was transferred to a 25 mL volumetric flask and dissolved in the mobile phase (100.0 μg/mL). Aliquots of 1.0 mL of this solution were transferred into 10 mL volumetric flasks containing 1.0, 4.0 and 5.0 mL of a standard HQ solution (20.0 μg/mL). Mobile phase was added to make up the volume to give final concentrations of 12.0, 18.0 and 20.0 μg/mL. For the UVDS method, an accurately weighed amount of gel or cream equivalent to 12.0 mg of HQ was transferred to a 100 mL volumetric flask and dissolved in sulfuric acid (0.1N) (120.0 μg/mL). 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 HQ solution (100.0 μg/mL) and sulfuric acid (0.1N) was added to make up the volume to give final concentrations of 13.2, 18.0 and 24.0 μg/mL. All solutions were prepared in triplicate and analysed.

2.5.4. Specificity

The specificity was determined for both HPLC and UVDS methods. The specificity was evaluated by analysing placebos, wherein the sample matrix without the analyte was analyzed. The system response was examined

for the presence of interference or overlaps with the HQ responses.

2.5.5. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for both HPLC and UVDS methods were determined based on standard deviation amongst responses and slope of the regression equation of a curve constructed at lower concentration levels (2.0, 4.0, 6.0, 8.0 and 10.0 μg/mL) [20].

3. Results and discussion

3.1. HPLC method

A reversed phase HPLC method was proposed as a suitable method for quantitative determination of HQ in gels and creams at concentrations of 2.0 and 4.0%. The chromatographic conditions were adjusted in order to obtain efficient and simple routine analysis. Mobile phase selection was based on the time, ease of preparation and cost. Fig. 1 show typical chromatograms obtained in the analysis of a standard and sample solution of HQ using the proposed method. As shown in Fig. 1, HQ was eluted well separated from the solvent front. The retention time observed (2.14 min) allows a rapid determination.

The calibration curve for HQ was constructed by plotting concentration versus corresponding mean peak area. Good linearity was observed in the 6.0–30.0 μg/mL range. The least square regression date showed excellent correlation coefficient $r = 0.9999$, highly significant for the method (Table 1). The precision of the method was determined by repeatability (intra-day) and was expressed as relative standard deviation (R.S.D.) of a series of measurements. The experimental values obtained in the determination of HQ in the samples are presented in Table 2. The accuracy of the method was determined by the recovery test, indicating a good agreement between "true value" and found value (Table 3). The proposed HPLC method is specific, since no interfering peaks were observed with placebo samples. The LOD and LOQ were found to be 0.08 and 0.26 μg/mL, respectively, indicating a high sensitivity of the method (Table 1).

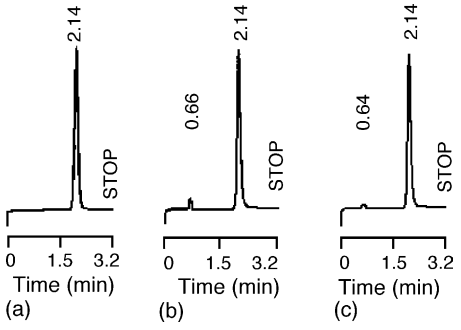

Fig. 1. Chromatograms of hydroquinone standard solution (18.0 μg/mL) (a), sample gel solution (18.0 μg/mL) (b) and sample cream solution (18.0 μg/mL) (c). *Conditions*: mobile phase methanol:water 20:80 (v/v), flow rate 1.0 mL/min, column Lichrospher® 100 RP-18, UV detector at 289.0 nm, injection volume of 20 μL and room temperature 25 ± 2 °C.

Table 1
Results of regression analysis of data for the quantitative determination of hydroquinone by the proposed methods

Statistical parameters	HPLC	UVDS
Concentration range (μg/mL)	6–30	10–26
Regression equation	$y = 639.19x + 87.87$	$y = 0.01756x + 0.00142$
Correlation coefficient (r)	0.9999	0.9999
LOD (μg/mL)	0.08	0.14
LOQ (μg/mL)	0.26	0.46

3.2. UVDS method

The UVDS method allows a rapid and low-cost quantitative determination of HQ in gels and creams without any time-consuming sample preparation. The first derivative spectrum shows an intense negative maximum at 302.0 nm with evidently useful characteristics in the analytical determinations (Fig. 2). For more accurate analysis, Ringbom curve was constructed and the linear range was observed. The calibration curve was constructed in the range of 10.0–26.0 μg/mL in sulfuric acid (0.1N). The least square regression date showed excellent linearity with correlation coefficient $r = 0.9999$ (Table 1).

The experimental values obtained in the determination of HQ in samples, indicated a satisfactory intra-day variability expressed by R.S.D. (Table 2). A good accuracy of the method was verified through recovery test (Table 3). No interfering signals and overlaps were observed in placebo samples at 302.0 nm (Fig. 2). The described UVDS method is specific for HQ determination in gel and cream preparations. The LOD and LOQ were found to be 0.14 and 0.46 μg/mL, respectively (Table 1).

Table 2
Results of the determination of hydroquinone in gel and cream by the proposed methods

Sample (g/100 g)	HPLC[a]		UVDS[a]	
	(g/100 g)	R.S.D. (%)	(g/100 g)	R.S.D. (%)
Sample A (gel 2%)	2.05 ± 0.12	0.92	2.06 ± 0.11	0.85
Sample B (gel 4%)	4.12 ± 0.11	0.83	4.05 ± 0.11	0.83
Sample C (gel 2%)	2.02 ± 0.11	0.84	2.01 ± 0.11	0.83
Sample D (gel 4%)	4.08 ± 0.12	0.93	4.08 ± 0.11	0.85
Sample I (cream 2%)	1.91 ± 0.17	1.33	1.87 ± 0.12	0.97
Sample II (cream 4%)	3.93 ± 0.17	1.31	3.92 ± 0.11	0.85
Sample III (cream 4%)	4.04 ± 0.14	1.12	4.06 ± 0.11	0.81

[a] Mean of 10 determinations.

Table 3
Results obtained in the recovery of a hydroquinone standard solution added to gel and cream and analysed by the proposed methods

Sample	HPLC			UVDS		
	Amount added (μg)		Recovery (%)[a]	Amount added (μg)		Recovery (%)[a]
	Added	Found		Added	Found	
A	20.0	19.7	98.50 ± 0.30	100.0	100.8	100.80 ± 0.60
	80.0	79.4	99.20 ± 0.39	500.0	496.7	99.34 ± 0.66
	100.0	99.1	99.10 ± 0.31	1000.0	981.7	98.17 ± 0.56
B	20.0	19.9	99.50 ± 0.74	100.0	99.17	99.17 ± 0.40
	80.0	80.5	100.60 ± 0.70	500.0	496.7	99.34 ± 0.42
	100.0	98.8	98.80 ± 0.78	1000.0	984.2	98.42 ± 0.47
C	20.0	20.4	102.00 ± 0.30	100.0	100.8	100.80 ± 0.30
	80.0	79.4	99.20 ± 0.32	500.0	495.8	99.16 ± 0.33
	100.0	99.4	99.40 ± 0.37	1000.0	983.3	98.33 ± 0.38
D	20.0	19.8	99.00 ± 0.67	100.0	100.0	100.00 ± 0.60
	80.0	80.2	100.20 ± 0.60	500.0	495.8	99.16 ± 0.62
	100.0	98.6	98.60 ± 0.69	1000.0	985.0	98.50 ± 0.68
I	20.0	19.6	98.00 ± 0.41	100.0	98.3	98.30 ± 0.80
	80.0	79.0	98.80 ± 0.42	500.0	491.7	98.34 ± 0.81
	100.0	99.0	99.00 ± 0.43	1000.0	1000.0	100.00 ± 0.80
II	20.0	19.9	99.50 ± 0.62	100.0	100.0	100.00 ± 0.70
	80.0	80.4	100.50 ± 0.60	500.0	495.8	99.16 ± 0.78
	100.0	99.0	99.00 ± 0.63	1000.0	983.3	98.33 ± 0.78
III	20.0	19.8	99.00 ± 0.70	100.0	100.0	100.00 ± 0.70
	80.0	79.7	99.60 ± 0.73	500.0	494.2	98.84 ± 0.67
	100.0	100.7	100.70 ± 0.60	1000.0	984.2	98.42 ± 0.69

[a] Mean of three determinations.

3.3. Comparison between HPLC method and UVDS method

The proposed analytical methods were compared using statistical analysis. The *F*-test was applied to determine whether one population is more variable than another in R.S.D. (repeatability). The results are shown in Table 4. The *t*-test was applied to determine whether or not there is a statistically significant difference between the means of two proposed methods. The results are shown in Table 4. The

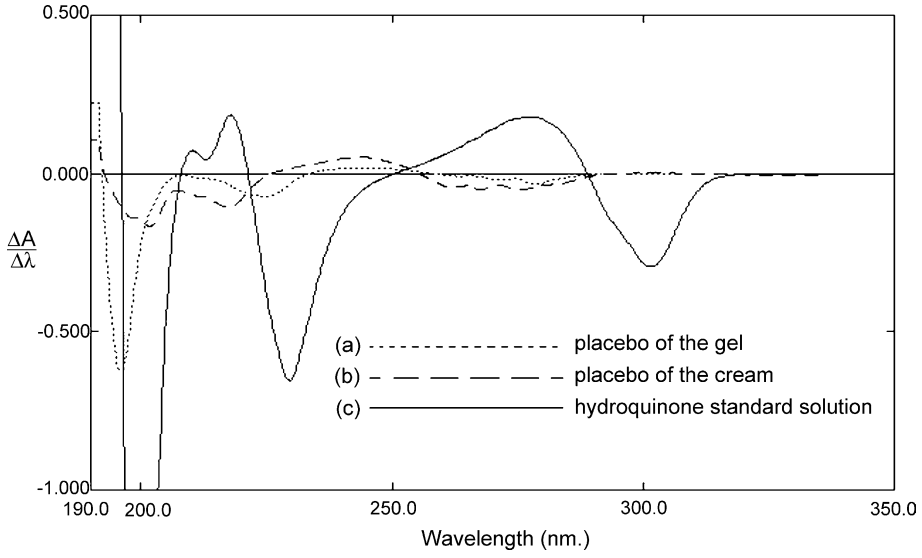

Fig. 2. UVD spectra of the placebo of the gel (a), placebo of the cream (b) and hydroquinone standard solution (18.0 μg/mL) in H_2SO_4 (0.1N) (c).

Table 4
Results obtained in the comparision of HPLC and UV derivative methods

Sample	*F*-test[a]	*t*-Test[b]
A	1.21	0.05
B	1.08	0.97
C	1.08	0.20
D	1.20	0.04
I	2.04	0.48
II	2.50	0.21
III	1.88	0.21

[a] Value at 95% confidence 3.18.
[b] Value at 95% confidence 2.10.

calculated *F*-values and *t*-values were found to be less than the critical values at 95% confidence level (3.18 and 2.10, respectively).

4. Conclusions

The proposed methods can be used for analysis in routine quality control and the quantitative determination of HQ in gels and creams containing this compound as a unique active principle, since HQ together with some other compounds such as kojic acid and arbutin could cause interference in both methods. The described methods were found to be simple, rapid, precise, accurate and sensitive. Its advantages over other existing methods are its low-cost and non-polluting conditions.

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References

[1] M.C. Spencer, Arch. Dermatol. 84 (1961) 131–134.
[2] M.C. Spencer, S.W. Becker, Clin. Med. 70 (1963) 1111–1114.
[3] K. Jimbow, H. Obata, A. Pathak, T.B. Fitzpatrick, J. Invest. Dermatol. 62 (1974) 436–449.
[4] K.B. Penney, C.J. Smith, C.J. Allen, J. Invest. Dermatol. 82 (1984) 308–310.
[5] A. Palumbo, M. d'Ischia, G. Misuraca, G. Prota, Biochem. Biophys. Acta 1073 (1991) 85–90.
[6] A. Katsambas, Ch. Antoniou, J. Eur. Acad. Dermatol. Venereol. 4 (1995) 217–223.
[7] A. Mohamed, M. Mohamed, Int. J. Dermatol. 37 (1998) 449–450.
[8] J. Firth, I. Rix, Analyst 111 (1986) 129–132.
[9] L. Gagliardi, A. Amato, G. Cavazzutti, F. Chimenti, A. Bolasco, D. Tonelli, J. Chromatogr. 404 (1987) 267–272.
[10] M. Borremans, J. De Beer, L. Goeyens, Chromatographia 50 (1999) 346–352.
[11] United States Pharmacopeia, 28th ed., Unites States Pharmacopeial Convention, Rockville, 2005, pp. 974–975.
[12] C. Desiderio, L. Ossicini, S. Fanali, J. Chromatogr. A 887 (2000) 489–496.
[13] I.K. Sakodinskaya, C. Desiderio, A. Nardi, S. Fanali, J. Chromatogr. 596 (1992) 95–100.
[14] L. Wang, Analyst 120 (1995) 2241–2244.
[15] V.I. Cruz, O. Fatibello-Filho, Talanta 52 (2000) 681–689.
[16] M.E. Rueda, L.A. Sarabia, A. Herrero, M.C. Ortiz, Anal. Chim. Acta 479 (2003) 173–184.
[17] International Conference on Harmonization (ICH), Validation of Analytical Procedures: Methodology, Q2B, 1995.
[18] Association of Official Analytical Chemists International, Official Methods of Analytical Chemists of AOAC International, 15th ed., XVII, 1990.
[19] J.C. Miller, J.M. Miller, H.R. Izquierdo, J.C. Mate, Estadística para química analítica, second ed., Addison-Wesley Iberoamerican, Wilmington, 1993, pp. 20–63.
[20] M.E. Swartz, I.S. Krull, Pharm. Technol., Ed. Bras. 2 (1998) 12–20.