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Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 414–419
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Determination of hydroquinone in cosmetic emulsion using microdialysis sampling coupled with high-performance liquid chromatography

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Received 5 August 2003; received in revised form 23 December 2004; accepted 4 January 2005
Available online 7 March 2005

Abstract

A method based on microdialysis sampling combined with high-performance liquid chromatography (HPLC) has been developed for the determination of hydroquinone in cosmetic emulsion. In microdialysis sampling, using deionized water as perfusate, a probe length of 10 mm and dialysis flow-rate of 5 μl min^{-1} were found to be the optimum conditions for the analysis. The accuracy (% bias) for intra-day ($n = 3$) and inter-day ($n = 12$, four consecutive days) ranged from −9.5 to 13.0% with a precision less than 7.55% relative standard deviation (R.S.D.). Recovery obtained between 89 and 112% by adding hydroquinone standards into medicated cosmetics and the coefficients of variance between 0.5 and 3.3%. The linearity of calibration curve is in the range of 2 μM–2 mM with an R^2 value for the linear regression of 0.9981. The detection limit is 0.2 μM derived from the three times of signal-to-noise ratio. This method has been applied to determine the hydroquinone content in medicated and non-medicated cosmetics. The contents of hydroquinone in the medicated cosmetics are 5.36% (0.12% R.S.D., $n = 3$) and 3.85% (0.26% R.S.D., $n = 3$), respectively; in contrast to the percentages given by the manufacturer are 5 and 4%, the accuracy (% bias) are 7.20 and 3.75%, respectively. No hydroquinone was detected in non-medicated cosmetics. The proposed method has the advantages of easy sample pretreatment, rapid isolation and lower organic solvent consumption than in other methods for the determination of hydroquinone in cosmetics.

Keywords: Microdialysis; Hydroquinone; Cosmetics; HPLC

1. Introduction

Hydroquinone and products containing hydroquinone have been widely used as depigmenting agents to lighten skin. Its mechanism of action is based on the inhibition of melanin formation [1]. However, the use of skin lightening creams and gels is not without risk. Exposure to hydroquinone can cause skin irritation and sensitisation, nail discoloration, and hyperpigmentation [2]. In addition, hydroquinone has been shown to be mutagenic in animals [3]. Hydroquinone-containing products are available for medication and non-medication purposes; low concentrations are found in non-medicated cosmetics, while the high concentrations are often found in prescription drugs for medical treatment.

Several analytical techniques have been used for the determination of hydroquinone in samples of differing matrices. These include spectrophotometry, high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), micellar electrokinetic chromatography (MEKC), and capillary electrochromatography (CEC) [4–8]. In order to lower the matrix effect in real samples, conventional extraction techniques (e.g. liquid–liquid extraction (LLE), solid-phase extraction (SPE), solid-phase micro-extraction (SPME), and supercritical fluid extraction (SFE)) for organic species in aqueous samples, are frequently used as sample pretreatment

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doi:10.1016/j.jpba.2005.01.031

methods [9–11]. However, the use of large amounts of organic solvents or carbon dioxide and /or large sample volumes might limit the application of these methods.

Dialysis has proven very efficient in removing low molecular weight salts and other contaminants present in various samples. However, conventional extraction methods are usually time-consuming and tedious. Microdialysis is a sampling technique whereby chemical information is obtained by implanting a probe consisting of a hollow fiber of semi-permeable membrane into the liquid of interest [12]. Microdialysis sampling has been shown to be a powerful technique in pharmacokinetic and neurochemical studies [13–17]. Recently, in addition to being a sampling technique, microdialysis has also been shown to be an effective sample cleanup technique in biomedical and environmental investigations [18–21].

Hydroquinone is permitted at a concentration of up to 2% by mass under the Cosmetic Safety Regulation of EU, however it has been shown that some available products contain more than the permitted level of hydroquinone [22]. Thus, the measurement of hydroquinone in cosmetics is very important for human health protection and consumer safeguarding [23]. Because of the characteristics of viscous emulsion samples, it is difficult to isolate the analytes of interest by using conventional separation techniques. In this study, using microdialysis sampling coupled with HPLC, we aimed to optimize a simple and sensitive method for the determination of hydroquinone in cosmetic products.

2. Experimental

2.1. Equipment

A schematic diagram of the microdialysis–HPLC system for determination of hydroquinone in cosmetic samples is shown in Fig. 1. The microdialysis sampling system was purchased from the CMA (Stockholm, Sweden). The sampling system used consists of a microinjection pump (CMA/100), a microdialysis probe (CMA/20, 14/10) made of polycarbonate fiber (o.d. 500 μm, length 10 mm and molecular weight cut-off 20 kDa) and a CMA 1.0 ml syringe. The syringe containing perfusate was connected to the inlet of the probe with PE tubing. The outlet of the microdialysis probe was connected to a collection vial with PE tubing (i.d. 380 μm, o.d. 1090 μm) for chromatographic detection.

The HPLC system used consists of a JASCO 880PU pump (JASCO, Tokyo, Japan), a Rheodyne 7725 injector (Rheodyne, Cotati, USA) with a 20 μl sample loop and a JASCO 975 UV–vis detector (JASCO, Tokyo, Japan). To ascertain the optimal wavelength for hydroquinone analysis, an ultraviolet scan was performed from 190 to 390 nm with a U-2000 UV–vis spectrophotometer (Hitachi, Tokyo, Japan). Separation was achieved on a 5 μm, 250 mm × 4.6 mm i.d. Hypersil Fluophase PFP column (Thermo, Hypersil-Keystone, Cheshire, England). A pH meter (pHM210; Radiometer, Villeurbanne Cedex, France) equipped with a glass electrode was used for pH control of the eluents. Integration of peak areas and retention times was performed using a SISC chromatography Data station v.1.0 software (SISC, Taipei, Taiwan). This software was also used to obtain the chromatograms and to perform the necessary calculations.

2.2. Chemicals and reagents

Methanol, potassium dihydrogen phosphate, phosphoric acid and sodium hydroxide (Merck, Darmstadt, Germany) were used to prepare eluents and adjust the pH value. High-purity water (18.3 MΩ cm) was prepared with a Barnstead Nanopure water system (Dobuque, Iowa, USA) for all aqueous solutions. Standard stock solutions (20 mM) of hydroquinone (Sigma, Steinhein, Germany) were prepared by dissolving hydroquinone (0.2202 g) in water (50 ml), then adding water to adjust the volume to 100 ml. This solution was stored in a brown glass bottle and kept at 4 °C. Under these conditions it was stable for at least 7 days. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. The HPLC eluent was a solution of methanol (40%, v/v) in phosphate buffer (pH 5.50, 0.020 M). The eluent was filtered through 0.20 μm mixed cellulose ester polymer membrane filter (Advantec, Pleasanton, USA) and degassed by reducing the pressure for 20 min, with vigorous magnetic mixing.

2.3. Method validation

All calibration curves of hydroquinone were determined prior to the experiments with correlation values of at least 0.995. The intra- and inter-day variability for hydroquinone added to a non-medicated cosmetic serum not containing hydroquinone was assayed in triplicate at concentrations of 0.1, 1, and 2 mM on the same day and on four consecutive days, respectively. The accuracy (% bias) was calculated from the added concentration (C_{added}) and the mean value of concentration found (C_{found}) as follows: bias (%) = $[(C_{added} - C_{found})/(C_{added})] \times 100$. The precision (relative standard deviation, R.S.D.) was calculated from the concentrations found as follows:

$$\%\,\mathrm{RSD} = \left[\frac{\text{standard deviation(S.D.)}}{\text{found}}\right] \times 100$$

The accuracy (% bias) and precision (R.S.D.) values of within ±15% covering the range of actual experimental concentrations were considered acceptable [24]. The spike recoveries for hydroquinone standards, with four different levels (0.25, 0.50, 0.75, and 1.00 mM), added to a medicated cosmetic lotion containing hydroquinone were assayed in triplicate. The recovery was calculated from the amount detected and the mean value of the amount added as follows:

$$\text{recovery}\,(\%) = \left[\frac{C_{\text{detected 2}} - C_{\text{detected 1}}}{C_{\text{added}}}\right] \times 100$$

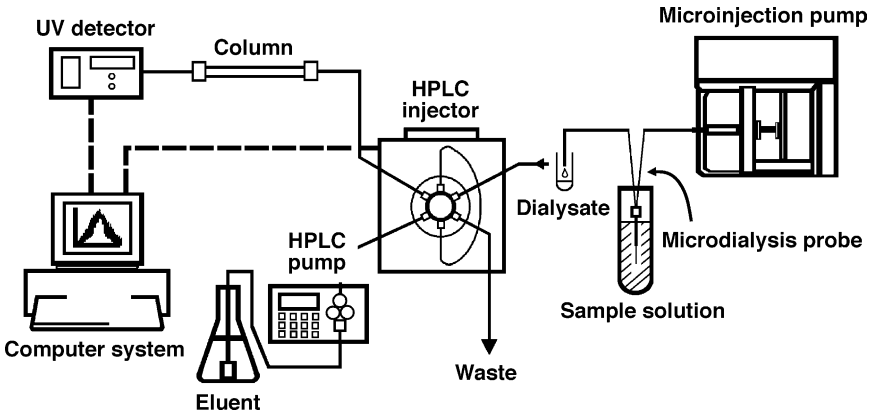

Fig. 1. Schematic diagram of the microdialysis–HPLC system for the analysis of cosmetic samples.

$C_{detected2}$ represents the amount of hydroquinone detected in cosmetics with added hydroquinone standards, $C_{detected1}$ represents the amount detected in cosmetics without added hydroquinone and C_{added} represents the amount of hydroquinone standard added.

2.4. Analysis of real samples

To examine the applicability of the proposed method, four real samples: (i) a medicated skin cosmetic cream; (ii) a medicated skin cosmetic lotion; (iii) a non-medicated cosmetic cream; (iv) a non-medicated cosmetic serum sample, purchased from local drugstores were measured. Aliquots of the cosmetic products were weighed, and then diluted to 100 ml with deionized water in a measuring flask. After mixed thoroughly with a vortex mixer, the sample solution was poured into a 10 ml dialysis-vial. The dialysate was collected for HPLC analysis.

3. Results and discussion

In order to verify the applicability of the proposed method, factors that may affect the chromatographic conditions and the dialysis recovery such as the flow-rate of perfusion were studied thoroughly to optimize the sampling and analytical procedure.

3.1. Optimization of chromatographic conditions

In order to ascertain the values providing high sensitivity and good resolution with the shortest analysis time, the detection wavelength, the composition of the mobile phase, the eluent pH and flow-rate were optimized prior to investigation of microdialysis conditions. The UV spectrum of hydroquinone dissolved in the mobile phase was obtained by using an UV–vis spectrophotometer. Because of the auxochromic substituents of hydroquinone, the maximum absorbance is at 290 nm, which is longer than other cosmetic whitening agents [25–28].

The effect of the amount of methanol on the hydroquinone retention time is shown in Fig. 2a. It is clear that the higher the methanol concentration the shorter the hydroquinone retention time. For a reversed-phase chromatograph, the retention time would decrease when the percentage of non-polar mobile phase increases. Considering the solubility of potassium dihydrogen phosphate in methanol, 40% (v/v) of methanol in 0.020 M phosphate buffer solutions as eluent is optimal. Because, on the one hand, the oxidation of hydroquinone is rapid in the presence of alkali and on the other, the tolerance of the hypersil fluophase PFP column

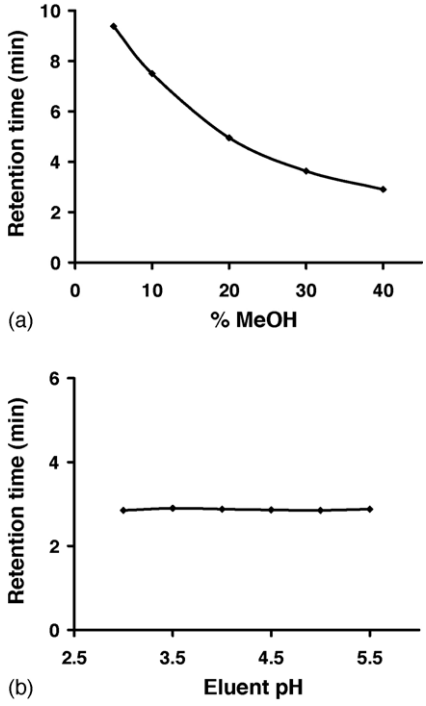

Fig. 2. Effect of chromatographic variables on the retention time of hydroquinone: (a) effect of percentage (v/v) of MeOH in the mobile phase. Mobile phase: MeOH—0.020 M potassium dihydrogenphosphate buffer of pH 5.50, at 1.50 ml min^{-1} flow-rate. (b) Effect of mobile phase pH. Mobile phase: MeOH—0.020 M potassium dihydrogenphosphate buffer (40:60, v/v) of variable pH at 1.5 ml min^{-1} flow-rate.

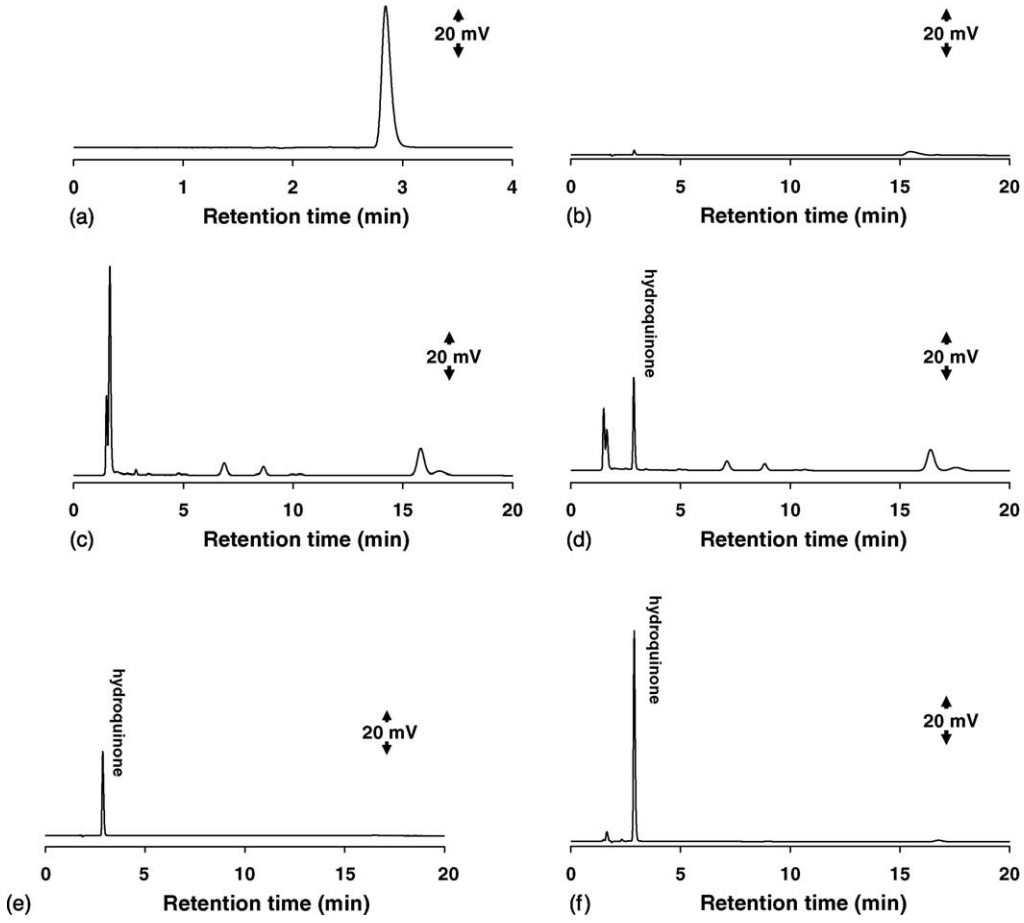

Fig. 3. Typical chromatograms for (a) hydroquinone standards. (b) A non-medicated cosmetic cream. (c) A non-medicated cosmetic serum. (d) A non-medicated cosmetic serum added 1 mM hydroquinone standard. (e) A medicated cosmetic cream. (f) A medicated cosmetic lotion.

to acid is lowest at pH 2.0, the pH was studied over the range 3.0–5.5. No difference in retention time was found in the range selected (Fig. 2b). The pK_{a_1} and pK_{a_2} values of hydroquinone are 8.72 and 11.35, respectively, [5] and hence it is largely in the undissociated form in the pH range 3.0–5.5, and the retention time remains constant. For convenient adjustment of the eluent, pH 5.5 was chosen for the subsequent analyses. The eluent flow-rate was studied over the range 0.5–1.5 ml min^{-1}. A rate of 1.5 ml min^{-1} provided the best results. Under these conditions, a typical chromatogram for a hydroquinone standard is shown in Fig. 3a; the retention time is 2.88 min and the reproducibility of quantitative detection for 1 mM hydroquinoine was 2.15% R.S.D. for three determinations.

3.2. Effect of dialysis flow-rate

It is well known that the detection sensitivity decreases with a higher dialysis flow-rate due to the dilution effect. In order to obtain an acceptable dialysis recovery (D_R) in a reasonable operating time, the influences of dialysis flow-rate on the D_R of hydroquinone was examined. As shown in Fig. 4, the higher the dialysis flow-rate, the lower the recovery. Although a low dialysis flow-rate increases the D_R, it takes time to collect sufficient dialysate to conduct an analysis. Hence, the sampling time and detection sensitivity have to be compromised in an analytical protocol including microdialysis. The chromatograms for a non-medicated cosmetic cream and a non-medicated cosmetic serum show that no UV absorption peaks (Fig. 3b and c). The chromatograms for a non-medicated cosmetic

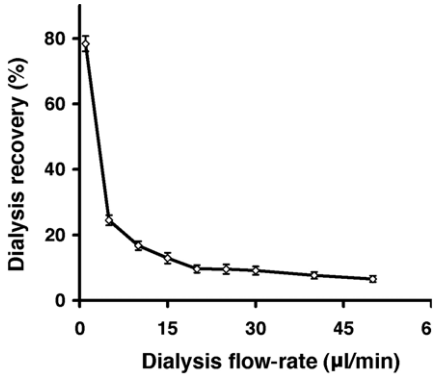

Fig. 4. Effect of dialysis flow-rate on the dialysis recovery of hydroquinone.

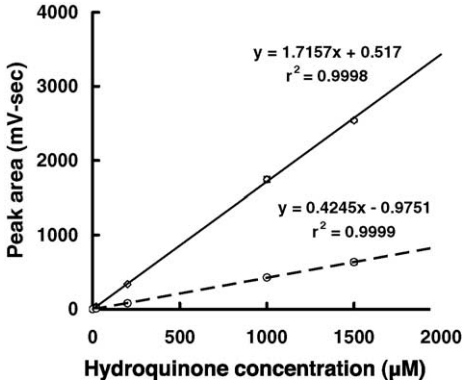

Fig. 5. Calibration plots of hydroquinone: (—) direct injection; (- - -) posterior to dialysis.

serum with added hydroquinoine standard, a medicated cosmetic cream and a medicated cosmetic lotion are shown as Fig. 3d–f, respectively. No absorption peaks were found after four minutes in the Fig. 3b, e and f. Four minutes is adequate for a single chromatographic determination of non-medicated cosmetic cream and medicated cosmetic samples.

In order to evaluate the robustness of the proposed method for dialysis flow-rate, a 5% experimental variation with respect to its nominal level [29] was performed. Our result indicated that when compared with the use of 5 μl min^{-1} as dialysis flow-rate, a decrease of 1.5% and an increase of 13.8% in D_R of 5.25 and 4.75 μl min^{-1} were found, respectively. As the performance of the microdialysis probe mainly depends on the type of probe and the dialysis flow-rate, we used the commercial probe described above and selected a 5 μl min^{-1} flow-rate for subsequent detection.

3.3. Calibration plots of hydroquinone

The D_R is the proportion of the analyte in the sample solution, compared to those that are detected after sampling with a microdialysis probe. The D_R obtained for the analytes of interest normally determines the feasibility of applying microdialysis sampling. In order to apply the quantitative determination, calibration plots were established by injecting hydroquinone standard solutions directly or the dialysates collected from standard solutions. The calibration plot was linear with the defining equation: $Y = 1.715X + 0.517$ in the range of 20 μM to 2.0 mM for direct injection, and $Y = 0.425X - 0.975$ in the same range for spiked samples measured after dialysis (Fig. 5), both linear correlation coefficients were 0.999. The detection limit is 0.2 μM derived from 3*S/N. From the ratio of slopes for both linear regression equations, the average D_R of hydroquinone in the concentration range under the optimal dialysis conditions is 24.50%. As the useful life of the microdialysis probe's polycarbonate fiber may be limited by the clogging of matrix for a complex viscous matrix sample; it was rinsed with deionized water after each dialysis run. To evaluate the reproducibility of D_R, a 0.2 mM solution of hydroquinone was dialyzed repeatedly after analyses of 10 real samples. The measured D_R was 23.68 ± 0.32% (n = 3). The result indicates that the same probe can be used for prolonged periods without losing its D_R.

Table 1
Intra-day and inter-day accuracy and precision of hydroquinone added in a non-medicated cosmetic serum

Concentration added (mM)	Concentration found (mM)	Accuracy (% bias)	Precision (% R.S.D.)
0.1			
day 1 (n = 3)	0.09	13.0	7.14
day 2 (n = 3)	0.10	−3.0	7.55
day 3 (n = 3)	0.11	−9.0	5.31
day 4 (n = 3)	0.09	8.0	2.17
Inter-assay (n = 12)	0.10	2.3	5.10
1.0			
day 1 (n = 3)	1.02	−2.0	1.25
day 2 (n = 3)	0.89	11.0	5.51
day 3 (n = 3)	0.93	7.0	5.48
day 4 (n = 3)	1.08	−8.0	2.13
Inter-assay (n = 12)	0.98	2.0	4.41
2.0			
day 1 (n = 3)	2.19	−9.5	5.32
day 2 (n = 3)	1.84	6.0	2.87
day 3 (n = 3)	2.07	−7.0	3.09
day 4 (n = 3)	1.95	5.0	1.85
Inter-assay (n = 12)	2.01	−1.4	3.29

3.4. Accuracy, precision and spike recovery

To determine the intra- and inter-day accuracy and precision of the method, hydroquinone standards added to a cosmetic serum not containing hydroquinone, were analyzed in triplicate on four different days. The accuracy (% bias) ranged from −9.5 to 13.0% with a precision of less than 7.55% R.S.D. (Table 1). The recovery of the method was tested by spiking the medicated cosmetic lotions with four different levels of hydroquinone standards and analyzing the mixture in triplicate. The resulting recovery values in the range 89–112% and the coefficients of variance between 0.5 and 3.3% (Table 2), within ± 15% covering the actual

Table 2
Spike recovery of hydroquinone in a medicated cosmetic lotion by the proposed method

Amount added (mM)	Amount detected[a] (mM)	Amount expected (mM)	Recovery (%)	CV (%)
0.00	0.62			3.3
0.25	0.90	0.87	112	2.8
0.50	1.16	1.12	108	1.9
0.75	1.34	1.37	96	0.5
1.00	1.51	1.62	89	1.3

[a] Each value is the mean of triplicate.

Table 3
Assay results for determination of the hydroquinone in four commercial cosmetic products using the proposed method

Sample	Label claim (%, w/w)	Found[a] (%, w/w)	Percentage of label (%)	CV (%)
Medicated cosmetic cream	4	3.85	96.3	0.26
Medicated cosmetic lotion	5	5.36	107.2	0.12
Non-medicated cosmetic cream	–	N.D.	–	–
Non-medicated cosmetic serum	–	N.D.	–	–

N.D.: not detectable (detection limit of this propose method is 0.2 μM).
[a] Each value is the mean of triplicate.

range of experimental concentrations, were considered acceptable.

3.5. Applications

This method has been applied to determine the levels of hydroquinone in four commercial cosmetics including two medicated and two non-medicated cosmetics. From the results and calculations, the hydroquinone contents in the medicated cosmetics are 5.36% (0.12% R.S.D., $n = 3$), and 3.85% (0.26% R.S.D., $n = 3$), respectively; in contrast with the percentages declared by the manufacturers are 5 and 4%, the accuracy (% bias) are 7.20 and 3.75%, respectively. No detectable amounts of hydroquinone were observed in the two non-medicated cosmetics in which hydroquinone is strictly forbidden (Table 3).

4. Conclusions

A microdialysis clean-up process coupled with HPLC/UV has been developed and validated for the determination of hydroquinone in cosmetic products. Good clean-up, accuracy, precision and recovery were obtained for all the studied samples. The method was applied to determine hydroquinone in four commercial cosmetic samples. The results agreed well with the compositions provided by the manufacturers. The results indicate that the proposed method is simple, reliable, rapid and does not require extensive preliminary sample treatment. Compared to conventional extraction methods, the proposed microdialysis clean-up method offers some advantages such as less tedious sample pretreatment and lower consumption of organic solvents.

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