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Highly Sensitive and Ecologically Sustainable Reversed-Phase HPTLC Method for the Determination of Hydroquinone in Commercial Whitening Creams

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Abstract: Hydroquinone (HDQ) is a natural depigmenting agent, which is commonly used in skin-toning preparations. The safety and greenness of analytical methods of HDQ quantification were not considered in previous literature. Therefore, a highly sensitive and ecologically greener reversed-phase high-performance thin-layer chromatography (RP-HPTLC)-based assay was established for HDQ estimation in four different commercial whitening creams (CWCs). The binary ethanol–water (60:40, $v{\cdot}v^{-1}$) mixture was utilized as the green solvent system. The estimation of HDQ was carried out at 291 nm. The present RP-HPTLC-based assay was linear in the 20–2400 ng band^{-1} range. The present analytical method was highly sensitive based on the detection and quantification data. The other validation parameters, such as accuracy, precision, and robustness, were also suitable for the determination of HDQ. Maximum HDQ quantities were obtained in CWC A (1.23% $w{\cdot}w^{-1}$) followed by CWC C (0.81% $w{\cdot}w^{-1}$), CWC D (0.43% $w{\cdot}w^{-1}$), and CWC B (0.37% $w{\cdot}w^{-1}$). The analytical GREEnness (AGREE) score for the present analytical method was estimated as 0.91, indicating the excellent greener characteristics of the present RP-HPTLC assay. These results suggest that the present analytical method is highly sensitive and ecologically sustainable for the quantitation of HDQ in its commercial formulations.

Keywords: agree; hydroquinone; ecologically sustainable RP-HPTLC; validation

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1. Introduction

Hydroquinone (HDQ) is a natural compound, which is present in several skin-toning commercial formulations for the treatment of melasma (a disease caused by the over accumulation of melanin in human skin) [1,2]. It is a potent depigmenting agent and used as an alternative to tyrosinase [3]. It is one of the most commonly used agents in the treatment of human skin hyperpigmentation [4,5]. The effective concentration of HDQ in commercial skin-toning formulations varies from 1.5 to 2.0% $w{\cdot}w^{-1}$ [6]. The high concentration of HDQ (above 5% $w{\cdot}w^{-1}$) causes local irritation and leukoderma to human skin [5,6]. Due to its controversial side effects, many countries have banned HDQ as a whitening agent in topical formulations [7]. Nevertheless, several clinical investigations have suggested various protective effects of HDQ in the management of different skin hyperpigmentary disorders such as melasma, freckles, lentigines, etc. [8,9]. By considering both the benefits and risks of HDQ, its quantitative analysis in different commercial skin-toning formulations is necessary.

Different pharmaceutical assays are utilized for the quantification of HDQ either alone or in combination with other whitening agents in marketed whitening creams (CWCs). A variety of ultra-violet (UV) spectrometry-based assays have been documented for the

quantitative analysis of HDQ in commercial whitening products (CWPs) and pharmaceutical preparations [10–13]. A wide range of high-performance liquid chromatography (HPLC)-based assays have been documented for the determination of HDQ along with its ethers in a variety of CWCs and CWPs [14–23]. Various voltametric methods have also been established for the simultaneous determination of HDQ and its ether derivatives in CWPs [24–29]. Some other analytical assays such as flow-injection electrochemical [30], micellar electrokinetic chromatography [31], capillary electrochromatography [32], and nanocomposite [33] based assays have also been established for the HDQ analysis along with its ether derivatives and other whitening agents in CWPs. Some electrochemical-based nanosensors have also been reported for HDQ analysis [34,35]. A single normal-phase high-performance thin-layer chromatography (HPTLC)-based method was also applied for the qualitative and quantitative analysis of HDQ in CWCs by our research group [1].

After exhaustive analysis of reported assays on HDQ analysis, it was observed that the safety and ecological sustainability of the pharmaceutical methods in the literature have not been assessed or considered for evaluation. In addition, the green/ecologically sustainable reversed-phase HPTLC (RP-HPTLC)-based assays have not yet been utilized for the estimation of HDQ in its CWCs. Ecologically sustainable/green HPTLC-based assays offer many advantages such as simplicity, economicity, low operation cost, short analysis time, parallel analysis of multiple samples, detection clarity, and reduction in environmental toxicity over HPLC and other analytical methods [36–39]. Accordingly, an RP-HPTLC method for the determination of HDQ was selected for this study. Different approaches are used for the assessment of the greenness profile of the pharmaceutical assays [38–43]. Nevertheless, only the analytical GREEnness (AGREE) metric approach applies all 12 principles of green analytical chemistry (GAC) for the greenness assessment [42].

The AGREE metric approach was applied for the greenness evaluation of the present RP-HPTLC method [42]. Therefore, the present work was carried out to develop a highly sensitive and green/ecologically sustainable RP-HPTLC method for the estimation of HDQ in four different CWCs. The greenness profile of the present RP-HPTLC method was obtained by AGREE: The Analytical Greenness Calculator. The present analytical assay for HDQ analysis was validated according to the International Council for Harmonization (ICH) Q2 (R1) guidelines [44].

2. Materials and Methods

2.1. Materials

The reference standard of HDQ (purity: 99%) was procured from Fluka Chemica (Darmstadt, Germany). HPLC-grade methanol (MeOH) and ethanol (EtOH) were procured from Alfa Aesar (Tewksbury, MA, USA). HPLC-grade water (H_2O) was collected from a Milli-Q water purifier system (E-Merck, Darmstadt, Germany). Other solvents and reagents used were of analytical grade. Four different CWCs of HDQ were obtained from a pharmaceutical market in Al-Kharj, Saudi Arabia, in the month of June 2021. The CWCs of HDQ were stored in a cool and dark place at 22 °C before the beginning of the experiments. The CWCs were stored for about one month before the beginning of experiments.

2.2. Chromatography

The RP-HPTLC densitometry quantification of HDQ in its reference standard and four different CWCs was conducted using an HPTLC instrument (CAMAG, Muttenz, Switzerland). The quantitative analysis of HDQ was carried out on 10 × 20 cm^2 glass-backed plates pre-coated with RP silica gel 60 F254S plates (E-Merck, Darmstadt, Germany). The samples on the TLC plates were spotted as the 6 mm bands utilizing an automatic sampler 4 (ATS4) applicator (CAMAG, Geneva, Switzerland). The sample applicator was fitted with a CAMAG microliter syringe (Hamilton, Bonaduz, Switzerland). The application rate for the quantitative analysis of HDQ was kept constant at 150 nL s^{-1}. The plates were developed in an automatic developing chamber 2 (CAMAG, Muttenz, Switzerland) at 80 mm distance. The green solvent system for HDQ analysis was EtOH-

H_2O (60:40, $v{\cdot}v^{-1}$). The developing chamber was saturated previously with the vapors of mobile phase for 30 min at 22 °C. The HDQ was detected at 291 nm. The slit dimensions were 4 × 0.45 mm^2 and the scanning rate was 20 mm s^{-1}. Each experiment was carried out in triplicate. The software used for the data processing was WinCATs (v. 1.4.3.6336, CAMAG, Muttenz, Switzerland).

2.3. HDQ Calibration Curve and Preparation of Quality Control Samples

The specified quantity of HDQ (10 mg) was dispensed in 100 mL of EtOH-H_2O (60:40, $v{\cdot}v^{-1}$) green solvent systems to achieve the stock solution with the concentration of 100 µg mL^{-1}. The different volumes of stock solutions were diluted further using EtOH-H_2O (60:40, $v{\cdot}v^{-1}$) systems to achieve HDQ concentrations in the 20–2400 ng $band^{-1}$ range. The obtained solutions of HDQ containing different concentrations were spotted to HPTLC plates. The HPTLC peak area for HDQ was obtained for each HDQ solution utilizing the present pharmaceutical assay. The calibration curve of HDQ was generated by plotting HDQ concentrations against its HPTLC area. In addition, three different quality control (QC) samples, such as low QC (LQC; 20 ng $band^{-1}$), middle QC (MQC; 600 ng $band^{-1}$), and high QC (HQC; 2400 ng $band^{-1}$) samples, were obtained separately in order to determine different validation parameters for the present pharmaceutical assay.

2.4. Sample Processing for HDQ Determination in CWCs

The HDQ was extracted from four different CWCs by adopting the procedure reported in the literature [1]. The accurately weighed (5.0 g) amounts of four different CWCs, including A, B, C, and D, were transferred to the separating funnel separately. Each CWC was shaken in the separating funnel with MeOH (3 × 70 mL) for a period of 30 min at 22 °C. The MeOH extracts from each CWC were combined and evaporated separately to dryness under reduced pressure using a rotary vacuum evaporator. The residues obtained were reconstituted with 10 mL of MeOH and stored in a refrigerator until further evaluation. The obtained samples were subjected for HDQ analysis utilizing the present analytical method at 291 nm.

2.5. Validation Parameters

The present RP-HPTLC assay for HDQ analysis was validated for different validation parameters by following the ICH-Q2 (R1) guidelines [44]. The HDQ linearity was evaluated by plotting HDQ concentrations against its measured peak area. The HDQ linearity was evaluated at 11 different QC samples of 20, 40, 60, 100, 200, 300, 400, 500, 600, 1200, and 2400 ng $band^{-1}$ for the present pharmaceutical assay. The system efficiency parameters for the present analytical method were evaluated in terms of the retardation factor (R_f), asymmetry factor (As), and number of theoretical plates per meter (N m^{-1}). The R_f, As, and N m^{-1} were obtained at MCQ (600 ng $band^{-1}$), as reported previously in the literature [45].

The accuracy for the present RP-HPTLC method was determined as the % recovery. The % recovery was obtained at LQC (20 ng $band^{-1}$), MQC (600 ng $band^{-1}$), and HQC (2400 ng $band^{-1}$) for the present analytical method.

The precision for the present analytical method was evaluated as intra/interday precision. Intraday precision was determined by the analysis of HDQ at LQC, MQC, and HQC on the same day for the present analytical assay. Interday precision was determined by the analysis of HDQ at LQC, MQC, and HQC on three different days for the present analytical assay [44]. Each precision was measured six times (n = 6).

The robustness was evaluated by introducing some small changes in the green solvent systems for the present RP-HPTLC method. For robustness evaluation, the original EtOH-H_2O (60:40, $v{\cdot}v^{-1}$) solvent system was changed to EtOH-H_2O (62:38, $v{\cdot}v^{-1}$) and EtOH-H_2O (58:42, $v{\cdot}v^{-1}$) solvent systems, and the specific HPTLC response and R_f values were recorded and interpreted [44].

The sensitivity for the present analytical method was evaluated as detection (LOD) and quantification (LOQ) limits using a standard deviation method. The LOD and LOQ of HDQ for the present analytical method was calculated, as reported in the literature [44,45].

The peak purity/specificity was evaluated by comparing the R_f values and UV spectra of HDQ in CWCs A, B, C, and D with that of standard HDQ for the present pharmaceutical assay.

2.6. Quantitative Analysis of HDQ in CWCs

The obtained samples of CWC A, B, C, and D were spotted to HPTLC plates, and their TLC responses were noted. The peak area for HDQ in CWCs was recorded. The HDQ contents in CWCs were calculated utilizing the calibration curve of HDQ for the present analytical method.

2.7. Greenness Evaluation

The greenness characteristics for the present analytical method were obtained utilizing the AGREE metric approach [42]. The AGREE scores (0.0–1.0) of the present analytical method were recorded utilizing the AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020).

3. Results and Discussion

3.1. Method Development

Based on literature analytical methods, it has been found that the ecologically sustainable/green RP-HPTLC method for the analysis of HDQ in commercial cosmetics is lacking. Therefore, the present study was carried out to develop the rapid, highly sensitive, and ecologically sustainable RP-HPTLC method for HDQ analysis in CWCs.

For the RP-HPTLC analysis of HDQ, different proportions of EtOH and H_2O, including EtOH-H_2O (50:50, $v{\cdot}v^{-1}$), EtOH-H_2O (60:40, $v{\cdot}v^{-1}$), EtOH-H_2O (70:30, $v{\cdot}v^{-1}$), EtOH-H_2O (80:20, $v{\cdot}v^{-1}$), and EtOH-H_2O (90:10, $v{\cdot}v^{-1}$), were evaluated as the green solvent combinations for the development of a reliable band for HDQ analysis. The solvent mixtures were developed under chamber saturation conditions. From the data recorded, it was noticed that EtOH-H_2O (50:50, $v{\cdot}v^{-1}$), EtOH-H_2O (70:30, $v{\cdot}v^{-1}$), EtOH-H_2O (80:20, $v{\cdot}v^{-1}$), and EtOH-H_2O (90:10, $v{\cdot}v^{-1}$) green solvent mixtures offered a poor chromatogram of HDQ with an unacceptable As value (As = 1.29). However, the EtOH-H_2O (60:40, $v{\cdot}v^{-1}$) green solvent combination had shown to offer a well-resolved chromatogram of HDQ at R_f = 0.83 ± 0.02 with an acceptable As value (As = 1.03) (Figure 1). Therefore, the EtOH-H_2O (60:40, $v{\cdot}v^{-1}$) was optimized as the green solvent mixtures for HDQ analysis in the CWCs. The UV-spectral bands for the present RP-HPTLC method were recorded densitometrically, and the maximum HPTLC response was found at 291 nm for the present RP-HPTLC method. Therefore, the whole analysis of HDQ was performed at 291 nm.

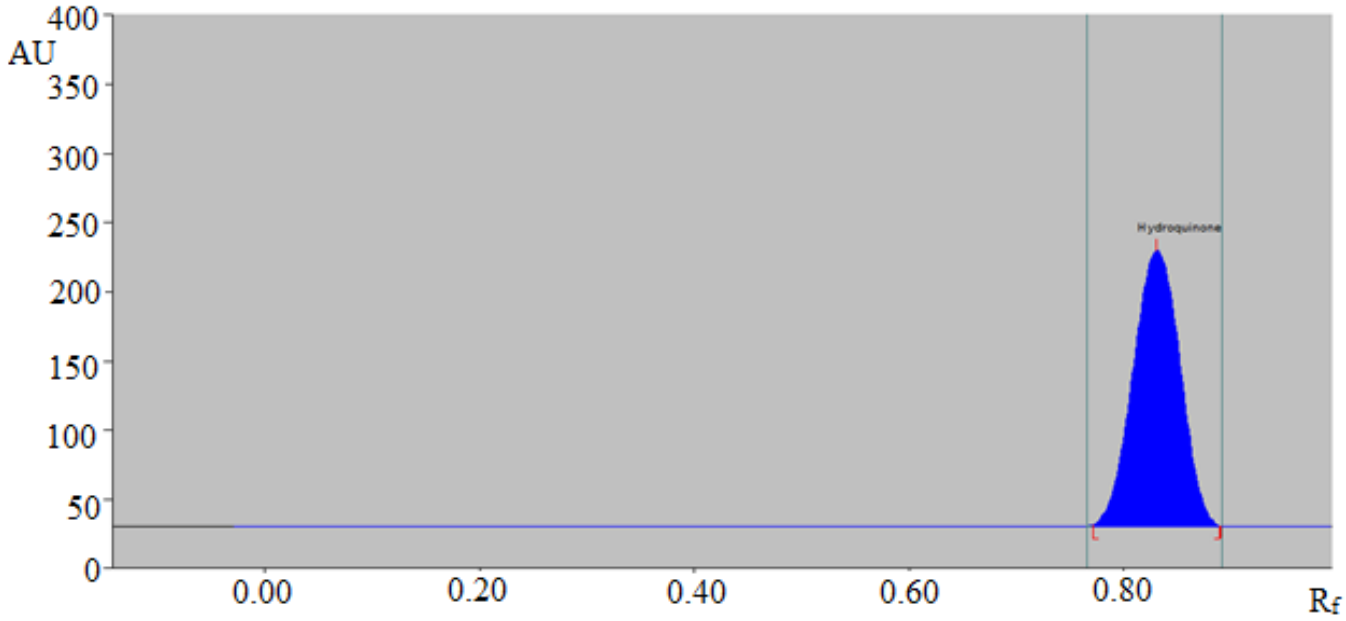

Figure 1. Representative chromatogram of 600 ng band^{-1} concentration of standard hydroquinone (HDQ) for the green/ecologically sustainable high-performance thin-layer chromatography (HPTLC) method.

3.2. Validation Parameters

The present pharmaceutical assay for HDQ quantitation was validated for linearity range, system efficiency parameters, accuracy, precision, robustness, sensitivity, and peak purity/specificity by following the ICH recommendations [44]. The results for the least-squares regression analysis of the calibration curve of HDQ for the present RP-HPTLC method are presented in Table 1. The HDQ calibration curve was linear in the range of 20–2400 ng band^{-1} with determination coefficient (R^2) of 0.9997 for the present analytical method. These data suggested good linearity between the HDQ concentration and its peak response.

Table 1. Results for least-squares regression analysis for the determination of hydroquinone (HDQ) using an ecologically sustainable high-performance thin-layer chromatography (HPTLC) method (mean ± SD; *n* = 6).

Parameters	Values
Linearity range (ng band^{-1})	20–2400
Regression equation	y = 12.133x + 316.50
R2	0.9997
Slope ± SD	12.133 ± 0.870
Intercept ± SD	316.50 ± 8.14
Standard error of slope	0.35
Standard error of intercept	3.32
95% confidence interval of slope	10.60–13.66
95% confidence interval of intercept	302.19–330.80
LOD ± SD (ng band^{-1})	6.91 ± 0.23
LOQ ± SD (ng band^{-1})	20.73 ± 0.69

The system efficiency parameters of the present pharmaceutical method were studied at MQC (600 ng band^{-1}), and results are included in Table 2. The R_f, As, and N m^{-1} values for the present analytical method were predicted as 0.83 ± 0.02, 1.03 ± 0.03, and 4987 ± 2.87, respectively. These results indicated that the present analytical method was reliable for HDQ analysis in the CWCs.

Table 2. System efficiency parameters including retardation factor (R_f), asymmetry/tailing factor (As), and number of theoretical plates per meter (N m^{-1}) of HDQ determined at MCQ (600 ng $band^{-1}$) for ecologically sustainable HPTLC method (mean ± SD; *n* = 3).

Conc. (ng $Band^{-1}$)	Parameters	Value
600	R_f	0.83 ± 0.02
	As	1.03 ± 0.03
	N m^{-1}	4987 ± 2.86

The results for the accuracy analysis for the present analytical method are listed in Table 3. The % recovery of HDQ for the present RP-HPTLC method was determined as 101.80%, 98.16%, and 99.38% at LQC, MQC, and HQC, respectively. The high values of % recoveries indicated the accuracy of the present RP-HPTLC method for HDQ analysis in the CWCs.

Table 3. Measurement of accuracy of HDQ for ecologically sustainable HPTLC method (mean ± SD; *n* = 6).

Conc. (ng $Band^{-1}$)	Conc. Found (ng $Band^{-1}$) ± SD	Recovery (%)	CV (%)
20	20.36 ± 0.13	101.80	0.63
600	588.98 ± 3.34	98.16	0.56
2400	2385.32 ± 6.58	99.38	0.28

The precision was determined as the percent of the coefficient of variation (% CV), and results are shown in Table 4. The % CVs of HDQ for the present analytical method were predicted as 0.91, 0.59, and 0.26% at LQC, MQC, and HQC, respectively, for the intraday precision. The % CVs of HDQ for the present RP-HPTLC method were predicted as 0.98, 0.69, and 0.32% at LQC, MQC and HQC, respectively, for the interday precision. The low values of % CV indicated the precision of the present RP-HPTLC method for HDQ analysis in CWCs.

Table 4. Measurement of intra/interday precision of HDQ for ecologically sustainable HPTLC method (mean ± SD; *n* = 6).

Conc. (ng $Band^{-1}$)	Intraday Precision			Interday Precision		
	Conc. (ng $Band^{-1}$) ± SD	Standard Error	CV (%)	Conc. (ng $Band^{-1}$) ± SD	Standard Error	CV (%)
20	19.67 ± 0.18	0.07	0.91	20.28 ± 0.20	0.08	0.98
600	603.65 ± 3.61	1.47	0.59	590.45 ± 4.12	1.68	0.69
2400	2567.54 ± 6.72	2.74	0.26	2391.23 ± 7.78	3.17	0.32

The results of the robustness analysis for the present analytical method are shown in Table 5. The % CVs for the robustness analysis were predicted as 0.59–0.66% for the present analytical method. The R_f values of HDQ were found in the 0.82–0.84 range for the present analytical method. The narrow changes in the R_f values of HDQ and lower % CVs showed the robustness of the present analytical method for HDQ quantification in CWCs.

Table 5. Results of robustness analysis for HDQ for ecologically sustainable HPTLC method (mean ± SD; *n* = 6).

Conc. (ng Band^{-1})	Mobile Phase Composition (Ethanol/Water)			Results		
	Original	Used	Level	Conc. (ng Band^{-1}) ± SD	% CV	R_f
600	60:40	62:38	+2.0	604.74 ± 3.60	0.59	0.82
		60:40	0.0	610.24 ± 3.80	0.62	0.83
		58:42	−2.0	615.63 ± 4.10	0.66	0.84

The sensitivity for the present analytical method was recorded as "LOD and LOQ", and their physical values are shown in Table 1. The "LOD and LOQ" for the present analytical method were predicted as 6.91 ± 0.23 and 20.73 ± 0.68 ng band^{-1}, respectively, for HDQ quantification. These physical values of "LOD and LOQ" for the present analytical method indicated the sensitivity for HDQ analysis in CWCs.

The peak purity/specificity for the present analytical method was evaluated by comparing the overlaid UV spectra of HDQ in four different CWCs with those of standard HDQ. The overlaid UV spectra of standard HDQ and HDQ in four different CWCs are shown in Figure 2. The highest chromatographic response for HDQ in standard HDQ and studied CWCs was observed at 291 nm for the present analytical method. The identical UV spectra, R_f values, and wavelength of HDQ in standard HDQ and CWCs indicated the peak purity/specificity for the present analytical method.

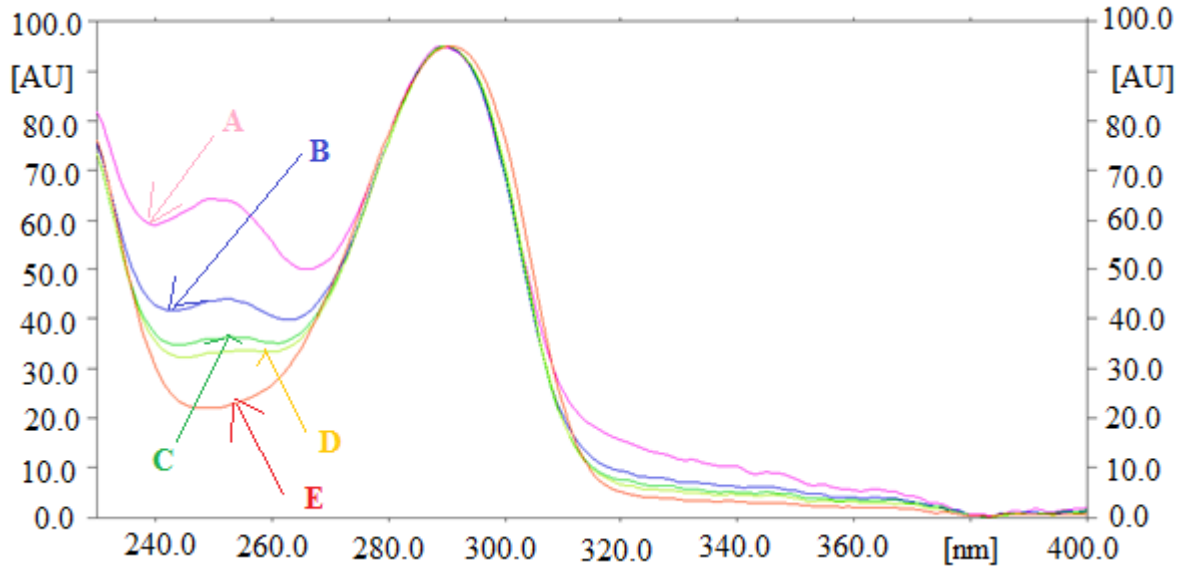

Figure 2. Overlaid ultraviolet (UV) absorption spectra of (A) standard HDQ, (B) commercial whitening cream (CWC) A, (C) CWC B, (D) CWC C, and (E) CWC D.

3.3. Analysis of HDQ Contents in CWCs

The applicability of the present analytical assay was verified in the quantitative estimation of HDQ in CWCs. The chromatogram of HDQ from CWCs was identified by comparing its TLC spot at R_f = 0.83 ± 0.02 with those of standard HDQ for the present analytical method. The chromatograms of HDQ in CWCs A and B for the present analytical assay are summarized in Figure 3. The HPTLC chromatograms of HDQ in CWCs were identical with those of pure HDQ. Some extra peaks also appeared in the chromatograms of CWCs, which might be associated with different excipients present in CWCs. The ecologically sustainable HPTLC method was selective for HDQ analysis at R_f = 0.83 without interference from the other ingredients of the CWCs. The R_f value (0.83) of HDQ in CWCs was found to be identical with that of standard HDQ (0.83), indicating that there was no interaction between HDQ and CWC ingredients. Hence, there was no influence of formulation ingredients on the quality of HDQ chromatogram, LOD, and

efficiency of the present HPTLC method of HDQ analysis. The presence of extra peaks in the chromatograms of CWCs indicated that present RP-HPTLC method was reliable for the HDQ estimation in the presence of formulation ingredients. The HDQ contents of CWCs were determined from the calibration curve of HDQ, and results are included in Table 6. Table 6 also summarizes the labelled amount of HDQ and its formulation ingredients. The HDQ contents were highest in CWC A (1.23% $w{\cdot}w^{-1}$) followed by CWC C (0.81% $w{\cdot}w^{-1}$), CWC D (0.43% $w{\cdot}w^{-1}$), and CWC B (0.37% $w{\cdot}w^{-1}$). The recorded contents of HDQ were much lower than the labelled amount (2.00% $w{\cdot}w^{-1}$) of HDQ in studied CWCs. The HDQ contents in two different CWCs (A and B) were recorded as 0.69% $w{\cdot}w^{-1}$ and 0.34% $w{\cdot}w^{-1}$, respectively, using the normal-phase HPTLC method in the literature [1]. The reported contents of HDQ were also much lower than the labelled amount (2.00% $w{\cdot}w^{-1}$) of HDQ in the literature [1]. Several CWCs or CWPs are marketed under the claim of being all-natural. However, it is common to find some synthetic chemicals as adulterants with the same effects found in such CWCs or CWPs as a fraud. The amount of HDQ recorded in this work and those recorded in the literature indicated that the studied CWCs have a low amount of HDQ and did not match label claims [1]. Hence, it is expected that the studied CWCs contain some synthetic chemicals as adulterants. Overall, the present analytical assay can be used for HDQ analysis in cosmetic and pharmaceutical preparations.

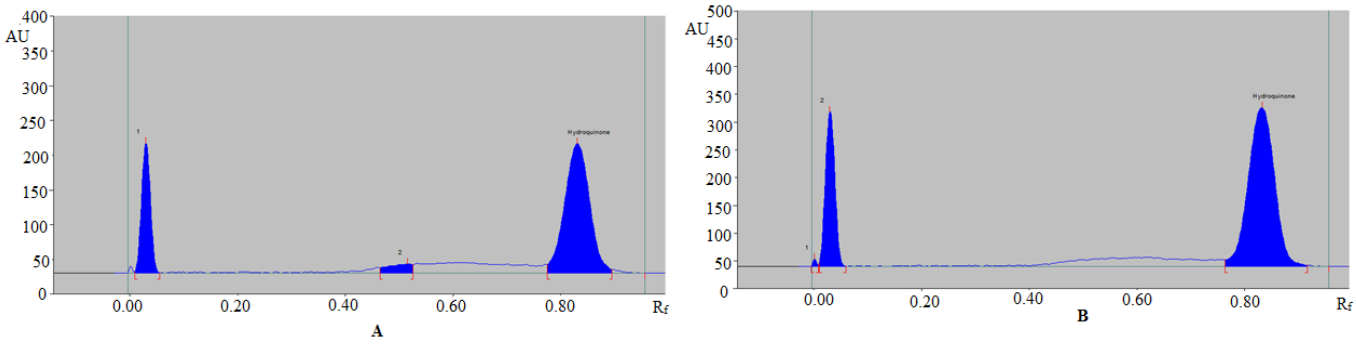

Figure 3. HPTLC chromatogram of HDQ in (**A**) CWC A and (**B**) CWC B.

Table 6. Determination of HDQ in four different commercial whitening creams (CWCs) using an ecologically sustainable HPTLC method (mean ± SD; n = 3).

Formulation	Formulation Ingredients	Label Amount of HDQ (%$w{\cdot}w^{-1}$)	Amount Found (%$w{\cdot}w^{-1}$)
CWC A	Parafinnum liquidum, methylparaben, polyoxyl-40-steaarte, propylene glycol, propylparaben, sodium lauryl sulphate, sodium metabisulphate, stearic acid, stearyl alcohol, and purified water	2.00	1.23 ± 0.03
CWC B	Emulgin B2, cetostearyl alcohol, paraffin oil, benzoic acid, glycerin, vitamin E, vitamin C, propylene glycol, citric acid, sodium lauryl sulphate, sodium metabisulphate, octyl methoxycinnamate, and purified water	2.00	0.37 ± 0.01
CWC C	Propylparaben, glyceryl monostearate, mineral oil, PEG-25 propylene glycol stearate, polyoxyl-40-steaarte, sodium metabisulphate, squalene, stearic acid, propylene glycol, and purified water	2.00	0.81 ± 0.02
CWC D	Ethanol, capryloyl glycine, C-13-14 isoparaffin, glycolic acid, kojic acid, laureth-7, lecithin, polyacrylamide, sodium hydroxide, squalene, xanthan gum, and purified water	2.00	0.43 ± 0.02

3.4. Greenness Assessment

Different methods are utilized for the greenness evaluation of the pharmaceutical assays [38–43]. However, only the AGREE approach utilizes all 12 principles of GAC for the evaluation of greenness [42]. Therefore, the greenness profile of the present analytical method was obtained using the AGREE Calculator. The predicted AGREE score utilizing 12 different principles of GAC for the present analytical assay is presented in Figure 4. The AGREE score for different principles of GAC was recorded as follows:

Sample treatment: 0.61
Positioning of analytical device: 1.00
Steps for sample preparation: 1.00
Degree of automation: 0.80
Derivatization: 1.00
Amount of waste: 1.00
Analysis throughput: 1.00
Energy consumption: 1.00
Sample treatment: 0.51
Source of reagent: 1.00
Toxicity of solvents: 1.00
Operator's safety: 1.00

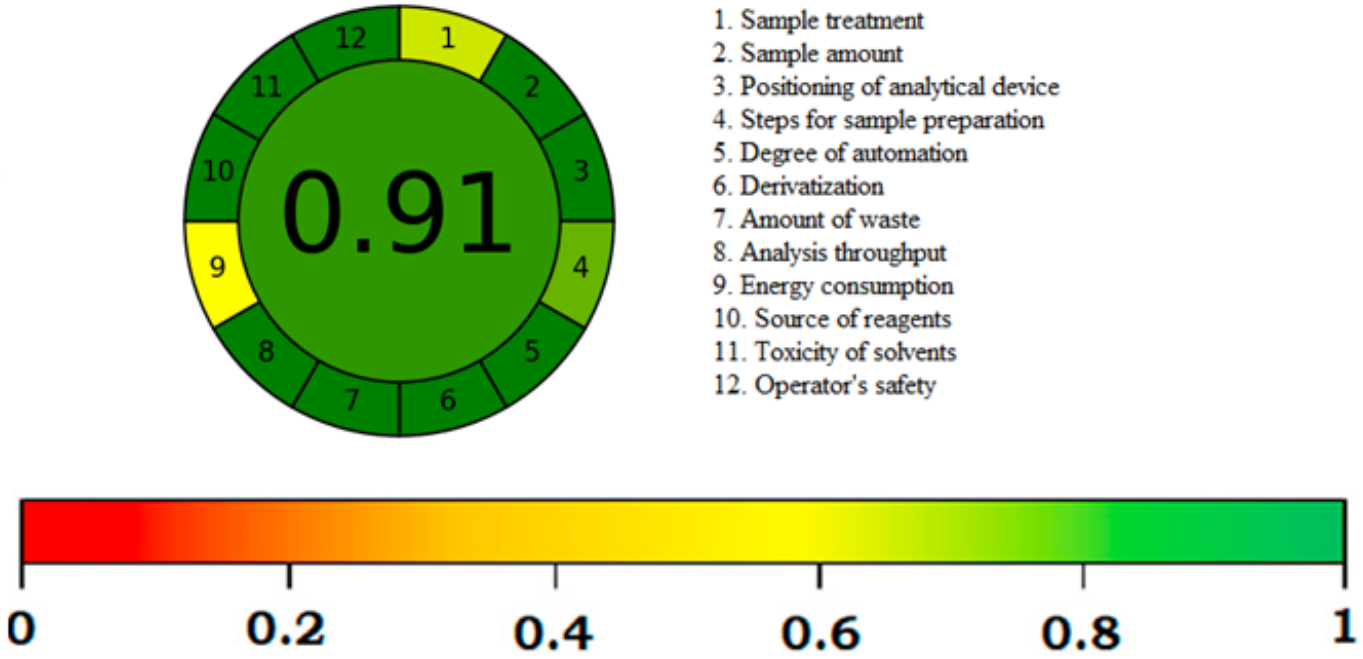

Figure 4. Analytical GREEnness (AGREE) score for the ecologically sustainable HPTLC method.

The overall AGREE score for the present analytical method was recorded as 0.91, indicating the excellent green analytical method for HDQ quantification.

4. Conclusions

The RP-HPTLC-densitometry method was developed for HDQ analysis in four different CWCs of HDQ. The present RP-HPTLC assay was validated for different validation parameters. The present analytical method was highly sensitive, rapid, and ecologically sustainable for HDQ analysis. The AGREE score for the present analytical method suggested the excellent analytical assay for HDQ quantification. The present RP-HPTLC method was suitable for HDQ analysis in four different CWCs. These results indicated

that the present analytical assay can be applied for HDQ analysis in different cosmetic and pharmaceutical preparations.

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