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Spectrofluorimetric method for determination of the skin depigmenting substance – 4-hexylresorcinol – in cosmetic products

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This study reports a rapid and sensitive batch spectrofluorimetric method for the quantification of 4-hexylresorcinol in cosmetic formulations. The approach relies on the alkaline oxidative coupling of 4-hexylresorcinol with dopamine to form a highly fluorescent azamonardine derivative ($\lambda_{ex}/\lambda_{em} = 440/470$ nm), enabling selective detection of 4-hexylresorcinol in complex matrices. Sample preparation was optimized for lipophilic creams using a *n*-hexane/isopropanol (90 : 10, v/v) extraction solvent, followed by dilution and 1 min derivatization at ambient temperature. Key derivatization parameters were optimized using a Box–Behnken design, yielding optimal conditions of 0.1 mM dopamine, 100 mM carbonate buffer, pH 11.5, and 1 min reaction time. The method was validated in accordance with ICH guidelines, demonstrating excellent linearity over 5–100 ng mL^{-1} ($r > 0.9994$), satisfactory trueness and precision (recoveries 81.1–120.0%; RSD < 8.4%), and low detection capability (LOD 1.1 ng mL^{-1}; LOQ 3.2 ng mL^{-1}). Robustness, assessed *via* Monte-Carlo simulations and process capability analysis, indicated reliable performance ($C_{pk} = 1.4$). Application to commercial depigmenting creams showed 4-hexylresorcinol levels of 3.09–3.52 mg g^{-1} with results statistically comparable to corroborative HPLC-FLD measurements. A unified multi-color assessment (MA) confirmed the method's balanced sustainability, practicality, analytical performance, and innovation (whiteness score 62.6%). Overall, the proposed assay offers a simple, cost-effective alternative for routine quality control of 4HR in cosmetics while aligning with green analytical chemistry principles.

1. Introduction

Skin lightening agents are widely incorporated into cosmetic formulations to address hyperpigmentation disorders and meet aesthetic preferences. Among these, 4-hexylresorcinol (4HR) was added to the World Health Organization (WHO) drug list in 1999, and it has emerged as a potent depigmenting compound due to its dual inhibition of tyrosinase and peroxidase enzymes involved in melanin biosynthesis.[1] It is an alkylresorcinol that contains both hydrophilic and hydrophobic domains. This amphiphilicity enables 4HR to interact with phospholipid bilayers in biological membranes, while the hydrophilic group allows proton exchange in aqueous environments.[2] In addition, it holds GRAS (Generally Recognized As Safe) status and is considered safe and effective as an anti-browning agent in food applications.[3] However, the growing prevalence of 4HR in commercial formulations necessitates accurate and sensitive analytical methods for its quantification to ensure product quality, regulatory compliance, and consumer safety.

Most published analytical methods aimed at the determination of 4HR in foodstuffs[3–10] while very few approaches have been focused on cosmetics and pharmaceutical products.[9,11,12] These approaches include utilization of liquid chromatography coupled with a tandem mass spectrometric[5,8] or fluorescence detector[4,6] and voltammetry.[10,12] Although LC-MS/MS techniques provide excellent analytical performance, they require expensive instrumentation, underscoring the need for simpler and more cost-effective alternatives. Fluorescence-based HPLC methods typically detect the analyte at low excitation wavelengths (around 280 nm), which generally results in limited selectivity. Electrochemical methods are inherently sensitive; however, their selectivity is limited because many phenolic compounds exhibit overlapping oxidation potentials. As a result, electrode modification with specialized nanomaterials is often required to achieve adequate discrimination.[13]

To address these limitations, fluorescence-based analytical methods have gained considerable attention because they offer high sensitivity, straightforward operation, and suitability for

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rapid measurements. Among them, the dopamine–resorcinol reaction serves as a powerful approach for selective and sensitive determination of resorcinol.[14] The method is based on an oxidative coupling reaction that produces azamonardine, a strongly fluorescent compound. Under alkaline conditions and in the presence of dissolved oxygen, dopamine is oxidized to dopamine quinone, which subsequently undergoes a sequence of nucleophilic substitution and condensation reactions with resorcinol. This pathway leads to the formation of the azamonardine fluorophore, characterized by intense blue-green fluorescence and an emission maximum typically between 450 nm and 480 nm.

Green analytical methods have emerged as a sustainable alternative to conventional analytical techniques by minimizing environmental impact, reducing hazardous chemical consumption, and improving laboratory safety without compromising analytical performance.[15] These methods emphasize the use of eco-friendly solvents, energy-efficient instrumentation, minimal sample preparation, and waste reduction, aligning with the principles of green chemistry. The adoption of green analytical approaches not only decreases the ecological footprint of analytical laboratories but also lowers operational costs and enhances analyst safety. In cosmetic analysis, where routine quality control is essential, green methodologies offer a particularly attractive solution by enabling rapid, sensitive, and reliable determination of active ingredients using non-toxic reagents and simple procedures.[16] Spectrofluorimetric techniques, in particular, represent a powerful green analytical tool due to their high sensitivity, low solvent consumption, minimal sample manipulation, and relatively low energy requirements. Therefore, developing green spectrofluorimetric methods contributes significantly to sustainable pharmaceutical and cosmetic analysis while meeting the growing regulatory and societal demand for environmentally responsible scientific practices.

In this study, we introduce a simple batch spectrofluorimetric method for determining 4HR in skincare formulations. After extraction from the sample matrix, 4HR is derivatized with dopamine under alkaline conditions to form a fluorescent azamonardine derivative. This compound exhibits strong emission, allowing highly sensitive and selective quantification of the 4HR at low ng mL^{-1} levels, even in complex cosmetic samples. The reaction is rapid, proceeds at ambient temperature, and requires no specialized catalysts. Stability testing showed that the fluorescent product remains suitable for accurate analysis for up to 6 hours, thereby enhancing method reliability. Derivatization conditions were optimized through a Box–Behnken design (BBD), and the method was validated following ICH guidelines. Robustness was assessed *via* Monte-Carlo simulation. Moreover, the method's environmental profile, practicality, analytical performance, and degree of innovation were examined using the unified multi-color assessment (MA) tool. The finalized method was successfully applied to commercial cosmetic products with minimal sample preparation, highlighting its practicality and robustness for cosmetic and pharmaceutical analyses.

2. Experimental

2.1. Reagents and solutions

4-Hexylresorcinol (4HR), dopamine, and HPLC-grade acetonitrile (ACN, ≥98.0%), methanol (MeOH), isopropanol (IPA) and *n*-hexane (Hex) were purchased from Merck (Darmstadt, Germany). Formic acid (FA, ≥98.0%), sodium carbonate, sodium hydroxide pellets, and all other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was produced using a B30 purification system (Adrona SIA, Riga, Latvia).

A standard stock solution of 4HR (1000 μg mL^{-1}) was prepared in MeOH, while the dopamine solution (0.1 mM) was prepared in water and both solutions were stored at 4 °C. Working standards of 4HR were prepared in MeOH from the stock solutions by serial dilution. Sodium carbonate buffer (100 mM) was prepared in water, and the pH was adjusted to 11.5 using 1 M NaOH solution.

Skin cream products from international brands were obtained from local cosmetic suppliers. For method validation, a skin rebalancing cream base (Cleoderm™, Fagron Hellas) was utilized. Its main constituents are *Cleome gynandra* L. leaf extract, palmitoyl tripeptide-8, bisabolol, hyaluronic acid, and functional oils. All samples were stored in their original packaging at room temperature until analysis.

2.2. Instrumentation and conditions

A batch spectrofluorimeter, Shimadzu RF-5301PC, operated in low sensitivity mode was utilized throughout this study.

A Shimadzu HPLC-FLD system was used for corroborative analyses. The instrumentation included an LC-20AD four-channel gradient pump, an RF-20A fluorescence detector, a SIL-10AD autosampler, and a CBM-20A system controller, all operated *via* LC Solutions software (version 1.25 SP4). An Elmasonic Easy 30H ultrasonic bath (37 kHz, 80 W) was used throughout the study.

2.3. Sample preparation and derivatization conditions

For each analysis, 10 mg of the sample was accurately weighed and placed into a 10 mL volumetric flask and filled to full volume with a mixture of Hex : IPA, 90 : 10 v/v. The mixture was then sonicated in an ultrasonic bath (80 W, 37 kHz) for 1 min at 25 °C. Prior to derivatization, the resulting extract was diluted 500-fold with MeOH. For method validation, samples were fortified with 1 mL of the 4HR standard solution to reach the desired concentration and processed in the same manner.

An aliquot of 2 mL of the diluted sample was combined with 3 mL of an aqueous dopamine solution (0.1 mM) and 5 mL of sodium carbonate buffer (100 mM, pH 11.5) in a Falcon tube. The mixture was vortexed for 5 seconds, allowed to react for 1 min at room temperature, and subsequently diluted 1 : 1 v/v with MeOH prior to analytical measurement. The derivatization reaction and the fluorescence spectra of the azamonardine derivative are illustrated in Fig. 1.

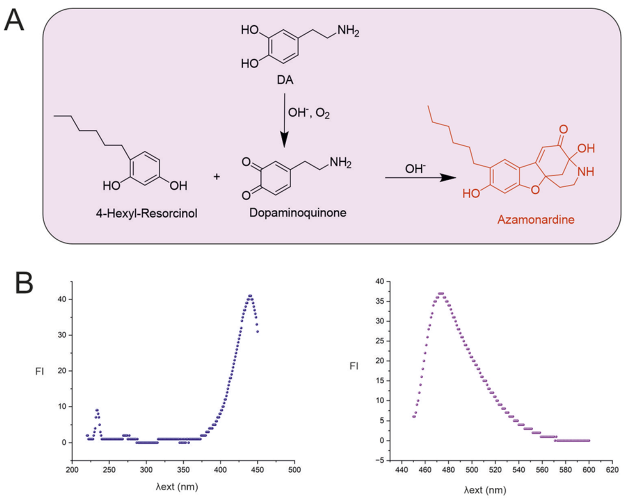

Fig. 1 (A) Reaction scheme of 4HR with dopamine in alkaline medium. (B) Excitation and emission spectra of the azamonardine derivative (50 ng mL^{-1}) in low sensitivity mode.

2.4. Corroborative HPLC-FLD method

The corroborative analysis of cosmetic samples was performed using HPLC-FLD. All separations were performed on a Poroshell 120 C_{18} column (50 × 4.6 mm, 2.7 μm; Agilent Technologies) under isocratic conditions. The mobile phase consisted of 0.1% v/v aqueous FA (phase A) and ACN (phase B) at a ratio of 60 : 40 v/v, delivered at a flow rate of 0.5 mL min^{-1}. The column temperature was maintained at 25 °C, and the injection volume was 5 μL. The azamonardine derivative was detected fluorometrically at $\lambda_{ex}/\lambda_{em} = 440/470$ nm using high sensitivity mode.

3. Results and discussion

3.1. Reaction feasibility and mechanism

The reaction between alkylresorcinols (*e.g.*, resorcinol and 4-butylresorcinol) and dopamine, which leads to the formation of fluorescent compounds such as monardine and azamonardine, has been previously described in the literature.[17–19] In the present study, this established synthetic route was employed to produce azamonardine – a stable, fluorescent derivative – for the analytical detection of 4HR (Fig. 1A). Mechanistically, dopamine undergoes facile oxidation in alkaline media, forming dopaminoquinone. While dopamine does undergo slow self-polymerization at pH 11.5, yielding a pale-yellow product after 30 min, the resulting polymer is largely non-fluorescent.[20] Conversely, 4HR does not oxidize to a quinonoid form under these conditions but undergoes deprotonation of its phenolic groups, resulting in two monoanionic resonance structures that stabilize a reactive phenoxide anion.[21] This nucleophile subsequently attacks the electrophilic dopaminoquinone, generating a tricyclic intermediate with a five-membered oxygen heterocycle. A secondary nucleophilic addition, involving either the hydroxyl or amino group of dopaminoquinone to the intermediate's carbonyl carbon, completes the transformation to azamonardine – a compound with pronounced fluorescence properties.[22]

Upon reacting 4HR with dopamine, the solution exhibited a pale-yellow hue and strong blue fluorescence under 440 nm excitation, as shown in the fluorescence spectra (Fig. 1B). These results are consistent with previous reports.[23]

3.2. Optimization of sample preparation conditions

Sample preparation for cosmetic creams is a critical step that ensures accurate and reliable analysis of active pharmaceutical ingredients (APIs) and other components. The procedures vary depending on the cream's composition, the analyte of interest, and the analytical technique to be employed. In our case, we selected a lipophilic skin rebalancing cream base (Cleoderm™) to study the extraction conditions as the worst-case scenario. It is a multifunctional formulation made with carefully selected ingredients, making it an excellent base for compounding topical therapies for compromised and sensitive skin. Its key components include *Cleome gynandra* L. leaf extract, palmitoyl tripeptide-8, bisabolol, hyaluronic acid, and functional oils. Together, these ingredients give Cleoderm™ strong anti-inflammatory, moisturizing, and sebum-regulating properties.

Our intention was to identify a solvent capable of completely solubilizing the sample in order to avoid potential matrix effects on the determination of the analyte. Initial experiments were conducted to dissolve 10 mg of cream in 10 mL of solvent. Owing to the lipophilic nature of the cream, solvents such as MeOH and IPA proved insufficient to solubilize the sample, even when sonication for up to 15 min was applied. In these cases, emulsions were formed, resulting in poor method reproducibility (RSD > 30%) and low trueness (recovery < 50%), due to the binding of the hydrophobic analyte to the lipophilic microdroplets.

This observation prompted us to use *n*-Hex as the solvent. However, pure *n*-Hex also led to turbid solutions (emulsions), probably due to the dispersion of water (content of the cream) into the hexane phase. To overcome this problem, we employed a mixture of *n*-Hex/IPA (90/10, v/v), which produced clear solutions and provided almost quantitative extraction recovery (% ER) of the spiked amount of 4HR. Higher IPA contents yielded comparable % ER values up to 30% (v/v); however, further increases led to cream insolubility issues. Thus, a mixture of *n*-Hex/IPA, 90/10 v/v was finally selected.

3.3. Optimization of the derivatization conditions using BBD

A BBD under the response surface methodology approach was applied to evaluate and optimize the derivatization yield by studying the effects of dopamine concentration (factor A), buffer concentration (factor B), buffer pH (factor C), and reaction time (factor D). The experimental design comprised 30 randomized trials, including six center points (Table S1), allowing reliable estimation of both linear and quadratic effects. Data analysis was performed using Design Expert® 13 software (Stat-Ease Inc., Minneapolis, MN, USA). ANOVA results (Table S2) revealed that dopamine concentration and buffer pH had the most pronounced impact on the derivative peak area, emphasizing their critical role in the derivatization process. In contrast, buffer concentration within the investigated range (10–100 mM) showed no significant effect. The model exhibited satisfactory predictive capability, as indicated by R^2 and adjusted R^2 values of 0.7270 and 0.6526, respectively, along with a non-significant lack-of-fit ($p > 0.05$), confirming its adequacy.

Three-dimensional response surface plots (Fig. 2) demonstrated the interactive influence of the studied variables on the fluorescence intensity (FI) of the derivative. As shown, higher FI of the derivative was obtained at elevated dopamine and buffer concentration levels while the reaction time remained unaffected in the range of 1–10 min. The model's reliability was further supported by an adequate precision value of 11.3, exceeding the recommended minimum of 4. Diagnostic plots, including the normal probability plot of residuals and the residuals *versus* predicted values (Fig. S1), showed random residual distribution, indicating good agreement between experimental and predicted results. Additionally, the lack-of-fit test was non-significant ($p = 0.9557$), reinforcing the suitability of the model.

Optimization was carried out using Derringer's desirability function, aiming to maximize the FI while minimizing dopamine concentration. Lowering dopamine consumption is consistent with the principles of Green Analytical Chemistry (GAC), which emphasize reducing reagent usage.[24] The optimization process yielded a high overall desirability value of 0.971 and suggested optimal conditions of 0.1 mM dopamine concentration, 1 min reaction time, 100 mM buffer concentration, and a buffer pH of 11.5 (rounded) (Fig. S2 and S3). This high desirability reflects an effective compromise between enhancing analytical performance and reducing reagent consumption and reaction duration. The optimized method was validated using six replicate experiments at 25 ng mL^{-1}, showing excellent agreement between experimental and predicted values, with peak area recoveries ranging from 96% to 107% at a 95% confidence level.

3.4. Analytical method validation

The developed spectrofluorimetric method was validated using the ICH guidelines[25] by investigating the parameters including specificity, linearity, precision, trueness, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

A skin rebalancing cream base (Cleoderm™, Fagron, Greece) presented as 4HR-free was used to assess the selectivity of the proposed method. No fluorescence was obtained corresponding to other formulation excipients. Moreover, a variety of possible interfering compounds commonly used as ingredients in skincare formulations (including $MgCl_2$, $ZnSO_4$, KCl, Na_2CO_3, $CaCl_2$, EDTA, propanetriol, α-bisabolol, phenoxyethanol, polysorbate 80, ascorbic acid, hyaluronic acid, hydroquinone and quercetin) were evaluated. These substances were tested at concentrations 200-fold higher than that of the target 4HR concentration. As shown in Fig. 3, none of these compounds produced significant fluorescence signals under the optimized conditions (<1.8%), mainly because they are unable to participate in the specific cyclization reaction with dopamine. The slightly higher fluorescence intensity observed for quercetin relative to the other interfering compounds may be attributed to its intrinsic fluorescence. Owing to its high selectivity, sensitivity, simplicity, and rapid response, the proposed assay demonstrates strong potential for the quantitative determination of 4HR in cosmetic products.

Linearity was assessed using analyte standard solutions in the concentration range from 5 to 100 ng mL^{-1} analyzing six different concentration levels in solvent and in Cleoderm™ cream base. Calibration parameters including slope, intercept, and the correlation coefficient (r) were determined by plotting the derivative's peak area against 4HR concentration using least-squares linear regression. In order to minimize the residuals a double logarithmic transformation was applied. Matrix-effect assessment was performed by comparing the slope ratio of the aqueous to matrix-matched calibration curve. The ratio was found to be 0.81 corresponding to approximately 19%

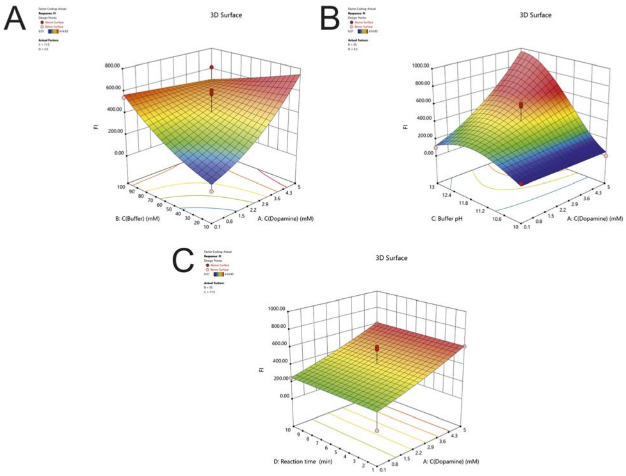

Fig. 2 3D plots of the effect of (A) buffer and dopamine concentrations, (B) buffer pH and dopamine concentration and (C) reaction time and dopamine concentration on the 4HR derivative fluorescence intensity.

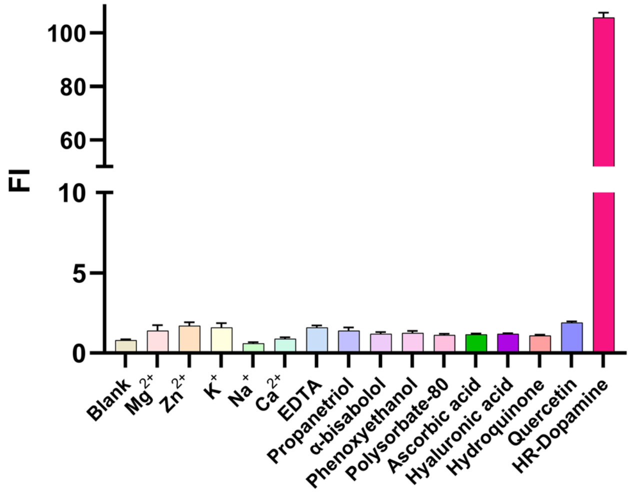

Fig. 3 Selectivity study against different substances on the fluorescence intensity of 4HR.

Table 1 Linearity, LOD, LOQ, LLOQ data of the developed spectrofluorimetric method for the determination of 4HR

Parameter	4HR
Linear range (ng mL^{-1})	5–100
Slope$_{\text{aqueous}}$ ± SD	0.8925 ± 0.007
Intercept$_{\text{aqueous}}$ ± SD	0.2249 ± 0.012
Coefficient of determination (r_{aqueous})	0.9994
Slope$_{\text{matrix-matched}}$ ± SD	0.7266 ± 0.019
Intercept$_{\text{matrix-matched}}$ ± SD	0.517 ± 0.029
Coefficient of determination ($r_{\text{matrix-matched}}$)	0.9941
LOD (ng mL^{-1})	1.1
LOQ (ng mL^{-1})	3.2
LLOQ (ng mL^{-1})	5

signal suppression. Although this indicates the presence of matrix effects, the value remains within the commonly accepted ±20% range (0.8–1.2), suggesting that the effect is low and acceptable.[26] Therefore, the external standard calibration was deemed appropriate for the quantitation of the samples. The resulting calibration data are presented in Table 1. Excellent linearity was achieved for the analyte, with the *r* value exceeding 0.9994.

The intra- and inter-day trueness and precision of the analytes were evaluated using a blank Cleoderm™ sample spiked at three concentration levels at 5, 50 and 100 ng mL^{-1} corresponding to 2.5, 5 and 10 mg g^{-1}. Intra-day precision was determined by three consecutive analyses of each concentration within a single day. Inter-day precision was calculated from the average of three intra-day measurements collected on three non-consecutive days.

Trueness was expressed as the recovery, defined as the closeness between the measured mean concentration and the spiked true value, whereas precision was reported as the relative standard deviation (RSD) of the measured concentrations. The results, summarized in Table 2, showed satisfactory recoveries (81.1–120.0%) and precision (<8.4%), confirming the reliability of the spectrofluorimetric method for quantifying 4HR in the lipophilic cream base.[27] The LOD and LOQ were determined using the following equations:

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

where σ and S are the standard deviations of the intercept and the slope, respectively, of the external standard calibration curve. The LOD and LOQ were estimated to be 1.1 and 3.2 ng mL^{-1}, respectively, while the LLOQ was set as 5 ng mL^{-1}.

Table 2 Precision and trueness of the method for determination of 4HR in the Cleoderm™ 4HR-free cream base

	Intra-day ($n = 3$)		Inter-day ($n = 3$)	
Nominal concentration (ng mL^{-1})	Precision (% RSD)	Trueness (*R*%)	Precision (% RSD)	Trueness (*R*%)
5	1.9	114.3	8.4	120.0
50	1.7	92.2	2.4	93.5
100	3.3	83.1	4.9	81.1

The robustness of the derivatization conditions was assessed through Monte-Carlo simulations and process capability analysis. A total of 100 000 simulated iterations were generated, and the resulting data were used to calculate C_{pk} values. An acceptance range of ±5% relative to the predicted FI from the optimization stage was defined for the 4HR signal. Simulations were performed using mean values of 0.1 mM dopamine, 100 mM buffer concentration, pH 11.5, and a reaction time of 1 min, with corresponding standard deviations of 0.01, 1, 0.15, and 0.5, respectively. The capability analysis yielded a C_{pk} of 1.4 indicating that only 0.01% of outcomes would fall outside the specification limits which meet the commonly accepted threshold (≥1.33). The histogram representing the capability analysis of the derivative FI is shown in Fig. S4.

3.5. Application to commercial skin-depigmenting creams

The applicability of the proposed spectrofluorimetric method was evaluated by analyzing commercially branded skin-depigmenting creams. The sample preparation procedure is described in detail in Section 2.3. Each sample was analyzed in triplicate, and the results are presented in Fig. 4. 4HR was detected in all products at concentrations ranging from 3.09 to 3.52 mg g^{-1}. The results obtained using the proposed method were statistically comparable to those obtained with the corroborative HPLC-FLD method using GraphPad software (trial version). As can be seen in Fig. 4, all *p*-values derived by Welch's test were higher than 0.05. Representative HPLC-FLD chromatograms from the analysis of the samples are illustrated in Fig. S5.

Spiked recovery tests were also performed by fortifying the real samples with concentration levels of 2.5, and 5 mg g^{-1} of 4HR standard solution. The obtained recoveries ranged from 73.2% to 122.0% (Table S3). Although some variability was observed, particularly at lower concentration levels, these values fall within generally accepted limits for complex matrices. The variability can be attributed to matrix complexity and differences in extraction efficiency, which may lead to signal suppression or enhancement effects. Despite this, the method demonstrated acceptable trueness and good reproducibility,

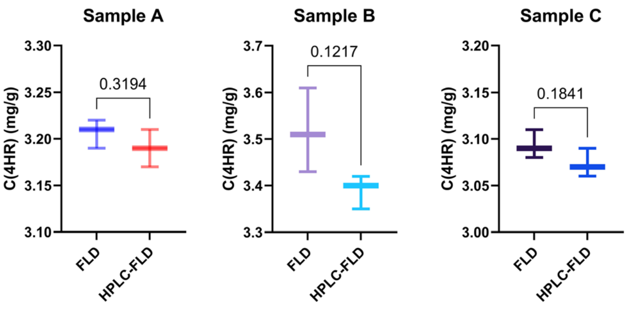

Fig. 4 Graphical comparison of the results obtained from the analysis of a branded face cream samples by the developed and the reference HPLC-FLD methods. The values present the *p*-values of Welch's test.

Table 3 Comparison of the proposed method with previously published approaches for the determination of 4HR in cosmetics

Sample	Sample pretreatment	Analytical technique	LOQ (μg mL^{-1})	RSD (%)	RR (%)	Ref.
Pharmaceutical formulation, shrimp	Ultrasound-assisted solvent extraction using H_2O/ACN (70/30, v/v)	HPLC-UV UHPLC-MS/MS	0.38 (HPLC) 0.013 (UHPLC-MS/MS)	<1.56 (HPLC-UV)	—	9
Antiseptic solution	Liquid–liquid extraction using CH_2Cl_2 and HCl solution (1 M)	HPLC-UV	250	<4.8	97–107	11
Branded face creams	**Dissolution in Hex : IPA, 90/10 v/v followed by derivatization**	**Batch spectrofluorimetry**	**0.005**	**<3.3 (intra-day)** **<8.4 (inter-day)**	**81.1–120.0**	**Proposed method**

supporting its suitability for the quantitative determination of 4HR in real samples.

3.6. Comparison with other published methods

Table 3 summarizes the analytical performance of the proposed method in comparison with previously reported methods for 4HR determination in cosmetics. The proposed method clearly outperforms all others in terms of sensitivity, achieving the lowest LOQ (0.005 μg mL^{-1}), surpassing even UHPLC-MS/MS (0.013 μg mL^{-1}), which is typically considered a highly sensitive technique.[9] This highlights the effectiveness of derivatization in enhancing detectability. In terms of precision, the proposed method is acceptable but not superior compared to the significantly lower variability (<1.56%) observed in the HPLC-UV approach.[9] Chromatographic methods—particularly UHPLC-MS/MS—offer a better balance between sensitivity and precision, albeit with higher instrumental cost. Overall, the proposed method stands out for its exceptional detection capability.

3.7. Greenness assessment using the unified MA tool

Several green metric tools have been developed in the last few years.[28,29] In our case, the environmental performance of the proposed method was assessed using the unified MA tool,[30] a comprehensive online platform that integrates four well-established evaluation frameworks into a single harmonized system. This tool combines the Green Evaluation Metric for Analytical Methods (GEMAM), the Blue Applicability Grade Index (BAGI), the Red Analytical Performance Index (RAPI), and the Violet Innovation Grade Index (VIGI) through an extensive 51-item questionnaire, enabling a holistic appraisal of sustainability, practicality, analytical efficiency, and innovation. The above metric tools have been also utilized individually.[31,32]

The developed method achieved a GEMAM score of 70.6%, reflecting a moderate level of environmental sustainability. This performance is mainly associated with the simplified sample preparation and reduced solvent consumption; however, it should be noted that the use of organic solvents such as Hex and MeOH limits the overall greenness of the method (Fig. 5). In addition, the method demonstrated excellent practical applicability, as reflected by a BAGI score of 70.0%, indicating operational convenience and suitability for routine analysis. Its innovative potential was also evident, with a VIGI score of 55.0%, highlighting the originality and advancement offered by the proposed strategy, along with good alignment with the principles of White Analytical Chemistry (WAC). Furthermore, the analytical performance was found to be satisfactory, supported by a RAPI score of 55.0%.

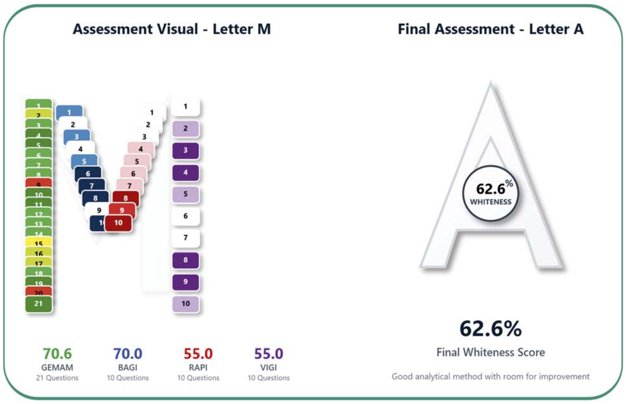

Fig. 5 MA whiteness assessment score of the proposed spectrofluorimetric method for the determination of 4HR.

Overall, the method attained an MA whiteness score of 62.6%, indicating a balanced profile in terms of sustainability, analytical performance, and applicability. These results suggest that, while the method incorporates certain elements aligned with green analytical chemistry principles, it should be regarded as relatively greener compared to conventional approaches rather than fully green.

4. Conclusions

A simple, sensitive, and cost-effective batch spectrofluorimetric method was developed for the determination of 4HR in cosmetic formulations. It is rapid, operates at room temperature, and does not require sophisticated instrumentation. Optimization using a Box–Behnken design, validation according to ICH guidelines, and robustness testing *via* Monte-Carlo simulation confirmed the reliability and analytical performance of the method. Its favourable, practical, and innovative features, as demonstrated by the MA tool, together with its successful application to commercial face cream products, highlight its suitability as an efficient alternative to more expensive and complex chromatographic or electrochemical techniques for routine quality control and regulatory compliance.

Author contributions

Marianna Ntorkou: investigation, formal analysis, data curation, validation. Emmanouela Katzouraki: formal analysis, validation. Vasileios D. Alampanos: methodology, writing – review and editing. Constantinos K. Zacharis: conceptualization, investigation, methodology, supervision, visualization, writing – original draft, writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

4HR	4-Hexylresorcinol
ACN	Acetonitrile
APIs	Active pharmaceutical ingredients
BAGI	Blue Applicability Grade Index
BBD	Box–Behnken design
FA	Formic acid
FI	Fluorescence intensity
FLD	Fluorescence detector
GAC	Green Analytical Chemistry
GEMAM	Green Evaluation Metric for Analytical Methods
GRAS	Generally Recognized as Safe
Hex	*n*-Hexane
IPA	Isopropanol
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LOQ	Limit of quantitation
MA	Multi-color assessment
MeOH	Methanol
RAPI	Red Analytical Performance Index
RSD	Relative standard deviation
VIGI	Violet Innovation Grade Index
WAC	White Analytical Chemistry

Data availability

The data supporting (DoE raw data, Monte-Carlo simulation graph, HPLC chromatograms, and ANOVA table) this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d6ay01006g.

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