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Simultaneous Quantification of Para-Phenylenediamine and Resorcinol in Hair Dyes Using Franz Diffusion Technique Coupled With HPLC

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Abstract

Hair dyes commonly contain para-phenylenediamine (PPD) and resorcinol, which are widely used as oxidative coloring agents. Due to their potential skin penetration and associated health risks, it is crucial to assess their permeation through the skin and quantify their levels in cosmetic formulations. This study aims to determine the diffusion and quantification of PPD and resorcinol using the Franz diffusion cell technique coupled with high-performance liquid chromatography (HPLC).

The experimental setup involves applying commercially available hair dye formulations onto a synthetic membrane in a Franz diffusion cell to simulate dermal absorption. Samples from the receptor compartment are collected at predetermined intervals and analysed using an optimized HPLC method. The chromatographic separation is performed on a C18 column with a suitable mobile phase, ensuring efficient resolution and detection of both compounds.Method validation follows ICH guidelines, assessing parameters such as linearity, accuracy,precision, and sensitivity.

This study provides critical insights into the skin permeability of PPD and resorcinol, contributing to the safety assessment of hair dye formulations. The developed analytical method ensures precise quantification, supporting regulatory compliance and consumer safety in cosmetic product development.

Keywords: Para-phenylenediamine, Resorcinol, Franz diffusion cell, HPLC, Skin permeation, Cosmetic safety, Dermal absorption, Hair dye formulations

Introduction

The term 'cosmetic' is derived from the Greek 'kosmētikos,' meaning 'adornments-skilled.' Nowadays, cosmetics can be termed widely as those substances or items used for their application on the exterior parts of the human body for cleaning, beautifying, altering appearance, or improving appearance without changing the body's function or structure [1]. The application of cosmetics is generally considered to be safe if the products are applied within their stated intent. However, many case reports, epidemiological studies, and surveillance programs have documented adverse reactions attributed to the use of cosmetics. The most commonly reported effects include allergic contact dermatitis, irritant dermatitis, urticaria, photosensitivity reactions, pigmentary diseases, and acneiform eruptions [2]. For these reasons, the safety of cosmetic products is a continuing focus of evaluation by regulatory agencies such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Scientific Committee on Consumer Safety (SCCS) of the European Union [3].

Hair dyes are among the most popular cosmetic products in the world. They are predominantly applied for purposes of beauty enhancement, covering up gray hair, or for fashion purposes. Natural dyes such as henna, indigo, and herbal extracts were previously used to color hair. This is unlike the modern era, where synthetic dyes have dominated the market based on their durability, brightness, and the possibility of tailoring colors[4]. The chemical makeup of hair dyes includes Primary intermediates: e.g., para-phenylenediamine (PPD), Couplers: e.g., resorcinol, m-aminophenol, Oxidizing agents: e.g., hydrogen peroxides, Conditioning agents, emulsifiers, pH stabilizers. When hair dye products are applied to the scalp, they come in direct contact with the skin and hair follicles, and thus percutaneous absorption may be possible. Although most dyes are meant for topical application and are washed off after a certain period of time, there can be partial absorption through the skin, particularly in people with impaired or sensitive skin [5].

Para-phenylenediamine (PPD) is an aromatic amine with the molecular formula C6H4 (NH2)2. PPD is a diamine derivative, often used as intermediate for oxidative hair dyes. These chemicals react with oxidizing agents, usually hydrogen peroxide, incorporated in the hair dye formulation to form coloured products [6]. PPD itself is not coloured and, when mixed with other chemicals, undergoes a chemical reaction that forms a visible pigment inside the hair shaft.The PPD molecule is oxidized by peroxide to form a reactive species. This intermediate then acts with a coupler, for example, resorcinol, to form a dye complex that binds to the keratin matrix of the hair. It is this complex that is accountable for depositing the new colour on the hair [7]. PPD is a potent sensitizer and has been linked to many cases of allergic contact dermatitis.The toxicity of PPD is not limited to skin reactions; it can also lead to systemic absorption, especially when used on broken or damaged skin. In this case, PPD can enter the blood and affect organs like the liver and kidneys [8].Long-term use of hair dyes that contain PPD an increased risk of bladder cancer and lymphoma. However, the data linking PPD to these cancers is not conclusive, and research is needed to clarify these risks. Some aromatic amines, including PPD, have been classified by the International Agency for Research on Cancer (IARC) as potentially carcinogenic to humans (Group 2B) based on data from animal studies and limited human data [9].

Resorcinol (1, 3-dihydroxybenzene) is a phenolic compound with the molecular formula C6H4(OH)2. It is used as a coupler in hair dye products, which is commonly used in combination with PPD in the manufacture of permanent hair colorants. In these preparations, the role of resorcinol is to bond with oxidized PPD to form a stable, initially colourless substance that then develops into a pigment as it bonds to the hair shaft. This bonding process is responsible for the final colour and allows for a great variety of shades, making resorcinol an essential ingredient in many commercial hair dyes [10]. Although resorcinol is relatively less allergenic than PPD, it does have a sensitizing potential on repeated exposure. Sensitivity to resorcinol can manifest in the form of allergic contact dermatitis (ACD), presenting with features like redness, itching, swelling, and blistering. In a small percentage of individuals, systemic allergic reactions such as respiratory distress have been reported on exposure to resorcinol-containing hair dyes [11]. Allergic contact dermatitis to resorcinol is common in persons who work with hair dyes regularly, highlighting the importance of carrying out patch tests to identify possible allergic sensitivities before engaging in widespread use of hair dye preparations. Moreover, repeated exposure to resorcinol has also been found to increase the risk of developing contact urticaria (hives) or other hypersensitivity responses [12].

Franz diffusion cell

The Franz diffusion cell is a widely used vertical permeation apparatus designed to study drug diffusion across biological or synthetic membranes under controlled laboratory conditions. It consists primarily of a donor chamber, receptor chamber, membrane interface, sample port, magnetic stirring system, and a temperature control mechanism.

The donor chamber, situated at the top of the system, is a small cylindrical reservoir designed to hold the test formulation. It is typically made of borosilicate glass or quartz and has an inner diameter ranging from 9 to 15 mm, which defines the effective diffusion area—commonly between 0.64 and 5 cm² depending on the cell design [13]. The donor compartment is open to the environment or sealed with materials such as Para film or glass cover slips to prevent evaporation during the experiment. Semi-solid, gel, liquid, or cream formulations are applied here, directly in contact with the membrane.

Beneath the donor compartment is the receptor chamber, which holds a receptor fluid designed to maintain sink conditions for the drug. This compartment is cylindrical and contains a precisely known volume of solution, usually between 5 and 25 mL The receptor fluid is typically a buffered aqueous medium, saline, or ethanol-water mixtures, depending on the solubility and stability of the test compound. The receptor chamber is maintained at a constant temperature of 32 ± 1 °C to simulate the human skin surface temperature [14].

Separation between the donor and receptor compartments is achieved using a membrane, which can be synthetic (e.g., cellulose acetate, silicone rubber, Strat-M®) or biological (e.g., human epidermis, porcine ear skin). The membrane is clamped between the two chambers using clips or threaded systems to ensure tight sealing and eliminate leakage or lateral diffusion. Membrane hydration and pre-conditioning are necessary for reproducibility and to maintain biological relevance, especially in skin studies [15].

Materials and Methods

Standard Preparation

One gram of para-phenylenediamine and resorcinolworking standard was accurately weighed and transferred into a 100ml volumetric flask separately and about 25ml of acetonitrile and 75ml phosphate buffer at PH 6 added as a diluent and sonicated to dissolve drug completely and volume was made up to the mark with the same solvent - Stock solution of 1000µg/ml.

Sample preparation

Three different kinds of sample were procured from the local market. One gram of sample was weighed dissolved with 100 ml of diluent which contain 25ml of acetonitrile and 75ml phosphate buffer at PH 6. Kept in sonicator for 15 mins and filtered it with Whatman filter paper. Same solution was injected in the system.

Method validation of HPLC:

1. Linearity:

HPLC was used to assess ten standard solution concentrations in the 1-60 µg/ml range. Plotting the average peak areas against the concentrations allowed calibration curves to be created.

2. Accuracy:

To measure the closeness of the test results obtained by the method to the true value. Analyse samples with known amounts of analyte (typically spiked samples). Calculate the recovery percentage at 80%, 100% and 120%.

3. Robustness:

Three duplicates of the sample were used to examine the robustness techniques at concentration levels of 20 µg/ml for HPLC.

4. Precision:

Intraday precision:

Intraday precision, also known as repeatability, measures the consistency of the method within the same day. It assesses the variation in results when multiple samples are analysed by the same analyst using the same equipment over a short period.

Inter-day Precision:

Inter day precision (also known as intermediate precision or ruggedness) measures the consistency of the method when repeated measurements are taken under different conditions over multiple days.

System precision:

System precision in High-Performance Liquid Chromatography (HPLC) refers to the consistency and reliability of the HPLC system itself. It measures the repeatability of the chromatographic system and ensures that the system is functioning properly. This is usually assessed by multiple injections of a standard solution to evaluate the precision of the system

6. Assay method:

One gram of sample was weighed dissolved with 100 ml of diluent which contain 25ml of acetonitrile and 75ml phosphate buffer at PH 6. Kept in sonicator for 15 mins and filtered it with Whatman filter paper. Same solution was injected in the system. Lastly, the sample and standard's percentages purity were examined using the HPLC methods.

7. Diffusion study:

The receptor compartment fluid of 38 ml consists of 25 % of acetonitrile and 75% phosphate buffer at ph 6 as diluent used in the HPLC method validation. Sampling is done by weighing 5gram of hair dye mixed with water. Normal cellophane membrane used for transdermal patch diffusion rate calculation was also used in this experiment. Sample 1 ml volume withdrawn each 5-minute time and replaced with receptor fluid. Study was carried out for 30 minutes and sampling is done at 5, 10, 15,20,25,30 minutes.

Optimized ChromatographicConditions:

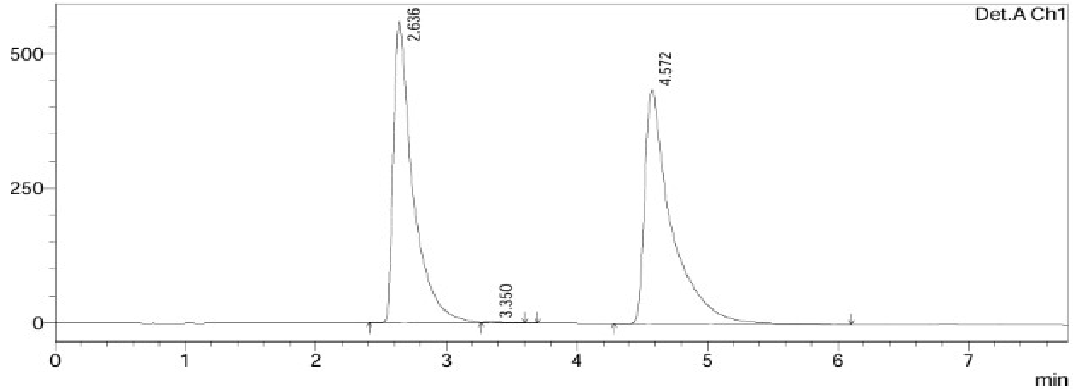
Chromatographic Condition	
Column	C18 250 X 4.6 mm 5 microns
Flow rate	1.0ml/min
Temperature	40^{0}C
Run time	10mins
Mobile phase	Phosphate buffer(pH-6) : acetonitrile
Mobile phase composition	75:25
Injection volume	20 µL
Wavelength	223 nm

Chromatographic separation was achieved using mobile phase Phosphate buffer (pH-6): acetonitrile in the ratio of 75:25. A flow rate of 1.0 ml/min was maintained throughout the separation process. Run time pressure of 2300 psi was maintained. For each trial, 20 µl samples were i njected manually, and a total run time of 10 min was maintained. The eluent was detected at 223 nm.

Results and Discussion

Linearity

Linearity of proposed para-phenylenediamine and resorcinolemploying HPLC method was constructed by considering concentration (µg/ml) on X–axis and peak area on Y-axis. The regression coefficient r2 of para-phenylenediamine was considered to be 0.9991 and for resorcinol is 0.9992 over a concentration range of 1–



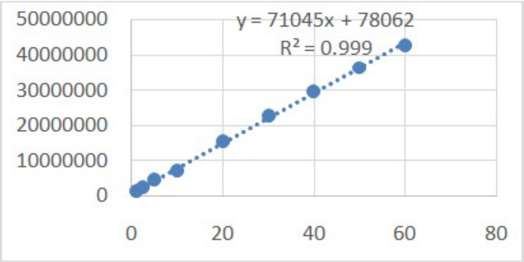


Fig. 1: Linearity graph of para-phenylenediamine

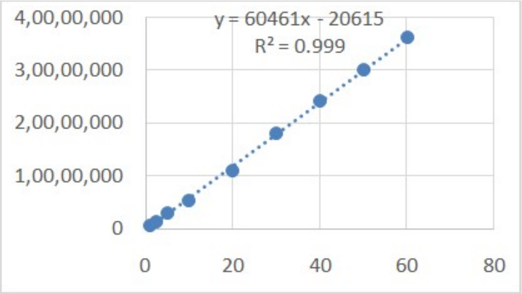


Fig. 2: Linearity graph of resorcinol

Table 1: Linearity of para-phenylenediamine and resorcinol by HPLC method

Concentration	Para-phenylenediamine	Resorcinol
	Peak area	Peak area
1	761651	1279612
2.5	1555412	2327196
5	3027032	4615420
10	5369186	7273740
20	11013983	15410912
30	18176151	22572859
40	24132384	29630495
50	30093087	36390738
60	36124836	42759198
(r^2)	0.9991	0.9992

60µg/ml. The representative linearity equation of resorcinoly = 710456x + 780624 and for para-phenylenediamine was found to be y = 604618x - 206154 and is as showed in Figs. 1 & 2 and the corresponding data were shown in Table 1.

Recovery and Accuracy:

The chromatograms for the recovery of para-phenylenediamine and resorcinol by HPLC method are shown in Figs. 3, 4 and 5. The results of recovery of para-phenylenediamine by HPLC method are shown in Table 2 and the recovery of resorcinolare shown in Table 3.

The % recovery values range from 96.96% to 102.5%, indicating good accuracy of the method across different sample concentrations. Average % recovery values are within a close range of their respective concentrations, demonstrating consistency in the method's accuracy.

ROBUSTNESS

The results of robustness are given in Table 4.

1. Mobile phasevariation:

Parameter Variation: Solvent B (+2% and -2%)

Impact on Results:

Peak Shape: Maintained symmetrical shape across variations.

Retention Time (RT): Varied slightly from 5.175 to 5.199 minutes and 3.425 to 3.443, indicating minor shifts.

Peak Area: Ranged from 11012848 to 12002020 and 14756785 to 16353563 showing a moderate impact on peak area with ±2% variation.

2. Temperature Variation:

Parameter Variation: Testing temperature changes by ±2°C.

Impact on Results:

Peak Shape: Consistently symmetrical shape observed.

Retention Time (RT): Slight variation within 5.175 to 5.199 minutes and 3.398 to 3.443 indicating minimal impact.

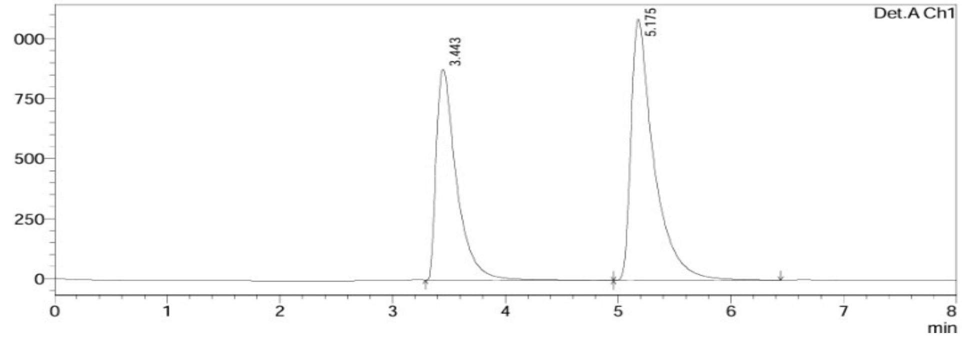


Fig. 3: Chromatogram of para-phenylenediamine and resorcinol sample (80% recovery)

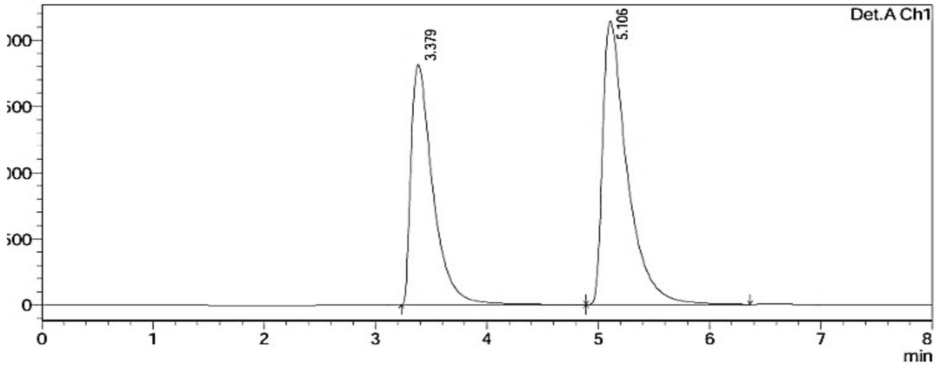


Fig. 4: Chromatogram of para-phenylenediamine and resorcinol sample (100% recovery)

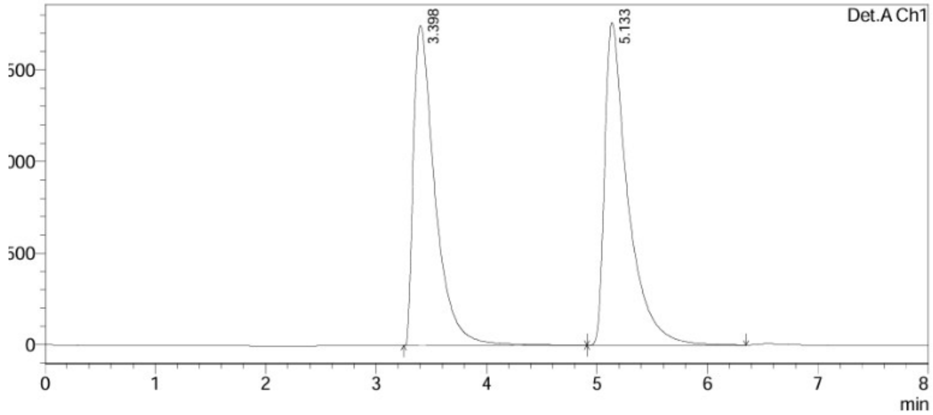


Fig. 5: Chromatogram of para-phenylenediamine and resorcinol sample (120% recovery)

Peak Area: Ranged from 11102102 to 12047477 and 14568353 to 15364747 showing a moderate impact on peak area with ±2% variation.

The robustness study indicates that the HPLC method is reasonably robust under the tested variations in mobile phase composition and temperature

Table 2: Recovery of para-phenylenediamine by HPLC method

	Peak Area	Recovery	% Recovery
	14568353	105.2	
80%	16353563	94.2	102.467
	14254648	108	
	23564963	102.4	
100%	24398567	98.9	99.3
	24975679	96.6	
	41564346	102.8	
120%	41849256	10.1	100.5
	43654655	97.9	

Table 3: Recovery of resorcinol by HPLC method

	Peak Area	Rec	% Recovery
	11102102	99.2	
80%	11021844	99.92	96.96
	120020203	91.76	
	24230495	99.5	
100%	24432384	98.7	98.3
	25225299	95.6	
	35628798	101.3	
120%	3662880	98.6	101.4
	34628821	104.3	

SYSTEM PRECISION (Table 5):

Table 5: System precision of para-phenylenediamine by HPLC method

Peak area	Rsd	%RSD
15288293	0.001814	0.1814
15177592	0.009041	0.9041
15245677	0.004596	0.4596
15234556	0.005323	0.5323
15267865	0.003137	0.3147
15234567	0.005322	0.5322

INTERDAY PRECISION:

The Inter-day and intra-day precision of para-phenylenediamine by HPLC method are given in Tables 6 and 7. The Inter-day and intra-day precision of resorcinol by HPLC method are given in Tables 8 and 9.

INTRADAY PRECISION (Tables 6 and 7)

Table 6: Inter-day precision of para-phenylenediamine by HPLC method

Peak area	Rsd	%RSD
15234556	0.000728	0.0728
15267865	0.001457	0.1457
15234567	0.000728	0.0728

Table 7: Intraday precision of para-phenylenediamine by HPLC method

Peak area	Rsd	%RSD
15288293	0.0036	0.36
15177592	0.0037	0.37
15234558	0.00007	0.007

Table-4: Robustness of Atorvastatin by HPLC method

Exp	Parameter variation	Peak shape	Rt	Peak area	Rt	Peak area
			Mobile phase			
1	Mobile solvent B	Symmetrical shape	5.175	11102102	3.443	14568353
2	+2%	Symmetrical	5.199	11021844	3.428	16353563
3	-2%	Symmetrical	5.194	12002020	3.425	14254648
			Temperature			
1	Temp 40^{0} C	Symmetrical	5.175	11236845	3.443	15364747
2	Temp 42^{0} C	Symmetrical	5.194	12047477	3.425	14756785
3	Temp 38^{0} C	Symmetrical	5.199	11012848	3.398	14858986

INTRADAY PRECISION (Tables 8 and 9)

Table 8: Inter-day precision of resorcinol by HPLC method

Peak area	Rsd	%RSD
23244974	0.000306	0.0585
23238485	0.000585	0.0359
23248584	0.000150	0.0150

Table 9: Intra-day precision of resorcinol by HPLC method

Peak area	Rsd	%RSD
23271964	0.000855	0.0855
23264745	0.000545	0.0545
23243746	0.000359	0.0306

Intra-day precision and Inter-day precision

Acceptance Criteria: Relative Standard Deviation (%RSD) should be ≤ 2%.

Observation:

Intra-day precision: %RSD for PPD = 0.007–0.37%, for resorcinol = 0.015–0.0855%

Inter-day precision: %RSD for PPD = 0.0728–0.1457%, for resorcinol = 0.015–0.0585%

All values are well within acceptable limits, confirming excellent reproduciblity.

SYSTEM PRECISION (Table 10):

Table 10: System precision of resorcinol by HPLC method

Peak area	Rsd	%RSD
23271964	0.000855	0.0855
23264745	0.000545	0.0545
23243746	0.000359	0.0306
23244974	0.000306	0.0585
23238485	0.000585	0.0359
23248584	0.000150	0.0150

System Precision

Acceptance Criteria: %RSD of peak area should be ≤ 1% for multiple injections of the same standard.

Observation:

PPD: %RSD = 0.1814–0.9041%, Resorcinol: %RSD = 0.015–0.0855%

These values indicate high system precision and reliability.

Assay (Table 11):

Table 11: Assay of hair dye samples

Sample	para-phenylene diamine		resorcinol	
	concentration	% assay	concentration	% assay
Sample A	22.34 μg/mL	0.2234% w/w	Not present	Not present
Sample B	37.39 μg/mL	0.3739% w/w	Not present	Not present
Sample c	44.45 μg/mL	0.4445% w/w	3.84 μg/ml	0.00384 %

DIFFUSION STUDY (Table 12):

Table-12: Diffused concentration of hair dye samples

TIME (minutes)	Sample A(PPD)	Sample B(PPD)	Sample C(PPD)	Sample C (Resorcinol)
5	11.06	20.18	2.59	2.09
10	35.80	47.01	31.45	0.73
15	55.74	72.74	47.56	3.23
20	66.34	85.83	51.90	4.49
25	66.16	101.26	85.60	5.95
30	63.13	110.71	101.37	6.94

DIFFUSION PARAMETERS

The Diffusion rate of para-phenylenediamine and resorcinol are shown in Figs. 6 and 7 (Tables 13)

Observations:

1. **Sample A**
- Shows strong and linear permeation.
- Highest flux (212.4 μg/cm²/h).s
- Suggests very efficient release and skin penetration of PPD.
- Suitable for rapid action.

2. **Sample B**
- Highest total permeation (Qmax ~109.36 μg/cm²).
- Flux slightly lower than A but still very strong.
- Most likely the best candidate in terms of both quantity and rate of delivery.

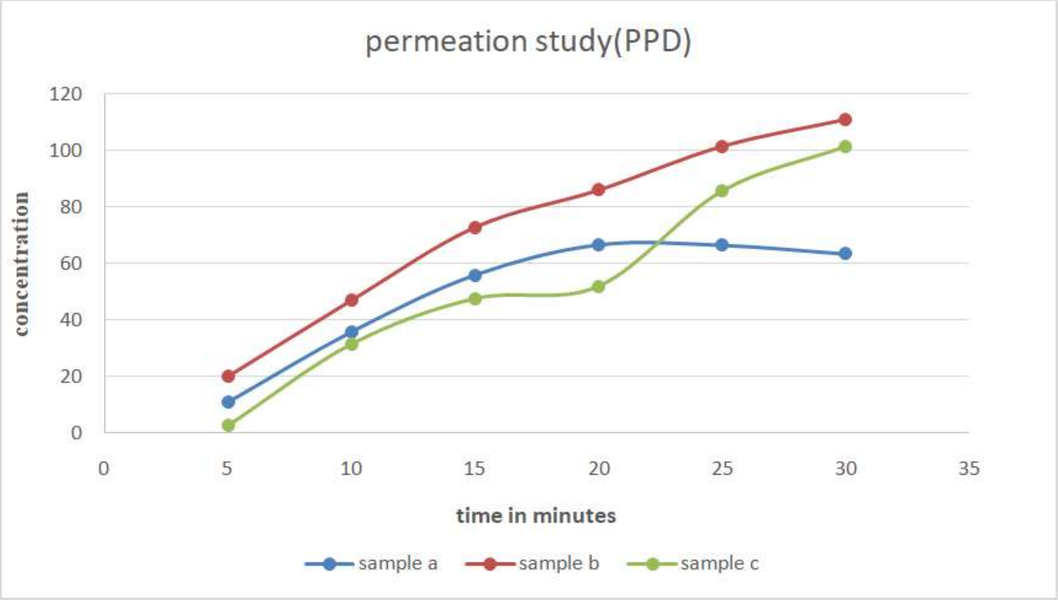


Fig. 6: Diffusion rate of para-phenylenediamine

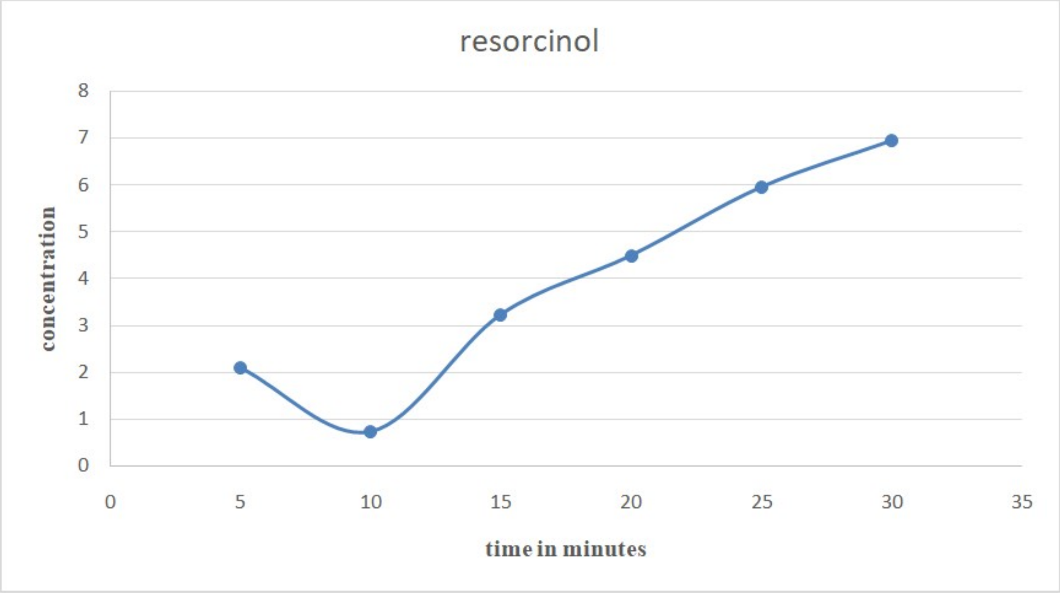


Fig. 7: Diffusion rate of resorcinol

Table 13: Parameters of hair dye samples

Parameter	Sample A(PPD)	Sample B(PPD)	Sample C(PPD)	Sample C (Resorcinol)
Max Permeation (Qmax)	~101.37 μg/cm²	~109.36 μg/cm²	~101.37 μg/cm²	~6.94 μg/cm²
Steady-State Flux (J)	212.4 μg/cm²/h	208.6 μg/cm²/h	197.2 μg/cm²/h	14.84 μg/cm²/h
Lag Time (Tlag)	4.0 minutes	4.1 minutes	4.2 minutes	1.9 minutes
Overall Trend	Linear, consistent	Strong flux, consistent	Clear release, minor early fluctuation	Slow permeation, lower flux

3. Sample C

- Consistent and steady permeation, similar Qmax to A.
- Slightly lower flux, suggesting slower but steady release.
- May be suited for controlled or extended release formulations.

4. Resorcinol compound

- Significantly lower flux and permeation than PPD-based samples.
- J = 14.84 μg/cm²/h vs. ~200+ for PPD.
- Suggests much slower skin penetration, possibly due to lower lipophilicity, molecular size, or formulation factors.
- Might be safer but less potent in transdermal action.

All PPD-containing samples (A, B, C) show efficient transdermal delivery with high flux and fast lag time. Resorcinol demonstrates lower penetration, which may relate to its physicochemical properties. Among PPD samples, Sample B is the most effective overall. Lag times for all PPD samples are similar (~4 min), showing comparable membrane interaction. These findings support Franz diffusion cell + HPLC as a valid quantitative method for evaluating permeation performance in cosmetic or pharmaceutical formulations.

Conclusion

In the present study,we successfully developed and validated a robust and reliable HPLC method for the quantitative analysis of para-phenylenediamine (PPD) and resorcinol from semi-solid formulations. The calibration curves for both compounds demonstrated excellent linearity ($R^2 > 0.99$) across the concentration ranges tested. The method showed good precision, accuracy, sensitivity, and reproducibility, confirming its suitability for use in permeation studies and routine analytical evaluation.

The Franz diffusion cell technique was employed to investigate the transdermal permeation behaviour of PPD from different sample formulations (Samples A, B, and C) and compared with resorcinol. The experimental setup provided effective simulation of dermal exposure using synthetic membranes and receptor media under controlled conditions.

All PPD samples demonstrated efficient permeation profiles, with rapid onset (lag times ~4 minutes), high cumulative permeation values (Qmax > 100 μg/cm²), and elevated flux rates (197–212 μg/cm²/h). Among them, Sample B exhibited the highest cumulative release and flux, indicating superior formulation characteristics for dermal delivery. In contrast, the resorcinol compound showed significantly lower permeation (Qmax = 6.94 μg/cm², flux = 14.84 μg/cm²/h), suggesting slower skin penetration possibly due to its molecular properties.

In conclusion, the combination of Franz diffusion cell methodology and validated HPLC analysis provides a reliable and sensitive approach for assessing dermal drug delivery of cosmetic and pharmaceutical compounds. The findings highlight formulation-dependent differences in PPD release and permeation, supporting the importance of in vitro diffusion studies in formulation screening and safety assessment.

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