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RESEARCH ARTICLE

Development and Validation of Stability-Indicating RP-HPLC and UV Spectrophotometric Methods for Quantitative Determination of Hydroquinone in Pharmaceutical Formulations

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Abstract: This research work focusses on two validated analytical methods for the quantification of hydroquinone in pharmaceutical formulations using reverse-phase high-performance liquid chromatography (RP-HPLC) and UV spectrophotometry. The RP-HPLC method utilized an Agilent C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of methanol and 0.05% orthophosphoric acid (50:50 v/v, pH 3.7) at a flow rate of 0.7 mL/min. Detection was performed at 290 nm with a retention time of 3.859 minutes. The UV spectrophotometric method was developed using methanol as the solvent, with measurements taken at 290 nm. Both methods demonstrated linearity in their respective ranges: 10-50 µg/mL for RP-HPLC and 1-5 µg/mL for UV spectrophotometry, with correlation coefficients exceeding 0.999. Method validation parameters including accuracy, precision, robustness, and system suitability were evaluated according to ICH guidelines. The limits of detection and quantification for the RP-HPLC method were 0.0147 µg/mL and 1.3930 µg/mL, respectively. Recovery studies showed excellent results ranging from 98.56% to 100.24%. Both methods were successfully applied to commercial tablet formulations with high accuracy and precision. The developed methods were found to be simple, rapid, and reliable for routine quality control analysis of hydroquinone in pharmaceutical preparations.

Keywords: Hydroquinone; RP-HPLC; UV spectrophotometry; Method validation; Pharmaceutical analysis.

1. Introduction

Pharmaceutical analysis plays a fundamental role in quality assurance and regulatory compliance of drug products by confirming their identity, strength, quality, and purity [1]. The development of reliable analytical methods is essential for maintaining drug quality throughout the product lifecycle, from development to commercial manufacturing [2]. Hydroquinone (1,4-dihydroxybenzene) is a phenolic compound widely used in pharmaceutical formulations, particularly in dermatological preparations for its skin-lightening properties [3]. It acts by inhibiting tyrosinase, the key enzyme responsible for melanin production, and is clinically effective in treating hyperpigmentation disorders [4]. Given its therapeutic importance and potential for degradation, accurate analytical methods for its quantification are crucial for quality control.

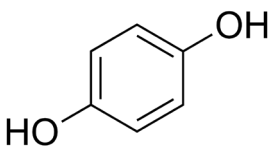

Figure 1. Structure of Hydroquinone

Several analytical techniques have been reported for hydroquinone determination, including spectrophotometry, electrochemical methods, and chromatographic techniques [5]. However, many existing methods have limitations such as complex sample preparation, long analysis times, or use of expensive reagents [6]. High-Performance Liquid Chromatography (HPLC) has emerged as a preferred technique for pharmaceutical analysis due to its superior separation capabilities, precision, and reproducibility [7]. Similarly, UV spectrophotometry offers advantages of simplicity, cost-effectiveness, and rapid analysis, making it suitable for routine quality control [8].

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The present study focuses on developing and validating two complementary analytical methods - RP-HPLC and UV spectrophotometry - for quantitative determination of hydroquinone in pharmaceutical formulations. The methods were validated according to International Conference on Harmonisation (ICH) requirements [9]. The development of dual analytical methods provides flexibility in quality control testing while ensuring method reliability through cross-validation.

2. Materials and Methods

2.1. Chemicals and Standards

Hydroquinone reference standard was obtained from RSITC Jalgaon. HPLC-grade acetonitrile, methanol, and orthophosphoric acid were purchased from Merck Ltd., India. HPLC-grade water was used throughout the study. All other chemicals and reagents were of analytical grade.

2.1.1. Pharmaceutical Formulation

Commercial tablets (Hydroquin) containing 200 mg hydroquinone manufactured by IBN-SINA Pharma Ltd. were procured from the local market.

2.2. Instrumentation

2.2.1. HPLC System

Analysis was performed using an Agilent Technologies Gradient System equipped with an autoinjector, diode array detector (DAD), and Chemstation 10.1 software. Chromatographic separation was achieved using an Agilent C18 column (250 mm × 4.6 mm, 5 µm).

2.2.2. UV Spectrophotometer

UV spectrophotometric measurements were carried out using an Analytical Technologies Limited UV-Visible spectrophotometer with a 1-cm quartz cell.

2.3. Method Development

2.3.1. RP-HPLC Method

The chromatographic conditions were optimized through systematic trials of various parameters. The selection of mobile phase composition was based on peak parameters, resolution, and analysis time [10]. Different ratios of methanol and 0.05% orthophosphoric acid (OPA) were evaluated (Table 1). The optimal conditions were established using methanol:0.05% OPA (50:50 v/v) at pH 3.7, with a flow rate of 0.7 mL/min and detection at 290 nm.

Table 1. Optimization of Mobile Phase Composition for RP-HPLC Method

Mobile Phase Composition	Retention Time (min)	Theoretical Plates	Peak Area RSD (%)
Methanol:Water (40:60)	5.24	5234	1.85
Methanol:Water (50:50)	4.56	6123	1.34
Methanol:0.05% OPA (40:60)	4.12	6892	0.98
Methanol:0.05% OPA (50:50)*	3.859	7245	0.42
Methanol:0.05% OPA (60:40)	3.12	6543	0.76

*Optimized condition

2.3.2. UV Spectrophotometric Method

The selection of analytical wavelength was performed by scanning standard hydroquinone solution (10 µg/mL) in the range of 200-400 nm using methanol as blank [11]. The wavelength of maximum absorption (λmax) was identified at 290 nm, which was selected for all subsequent measurements.

2.4. Preparation of Solutions

2.4.1. Standard Stock Solutions

For HPLC method, standard stock solution (1000 µg/mL) was prepared by dissolving 10 mg of hydroquinone reference standard in methanol in a 10 mL volumetric flask [12]. Working standards (10-50 µg/mL) were prepared by appropriate dilution with mobile phase. For UV Spectroscopy, stock solution was similarly prepared, with working standards (1-5 µg/mL) prepared by dilution with methanol.

2.4.2. Sample Preparation

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 11.75 mg of hydroquinone was accurately weighed and transferred to a 10 mL volumetric flask containing methanol [14]. The solution was sonicated for 15 minutes and filtered through a 0.45 µm membrane filter. Appropriate dilutions were made to achieve final concentrations within the calibration range.

2.5. Method Validation

Method validation was performed according to ICH Q2(R1) guidelines [15], evaluating the following parameters:

2.5.1. System Suitability

System suitability was assessed by injecting six replicate injections of standard solution (40 µg/mL). Parameters including retention time, theoretical plates, tailing factor, and relative standard deviation (RSD) of peak area were evaluated.

2.5.2. Linearity

Calibration curves were constructed by plotting peak areas versus concentration for HPLC (10-50 µg/mL) and absorbance versus concentration for UV method (1-5 µg/mL) [16]. Linear regression analysis was performed to calculate correlation coefficients and regression equations.

2.5.3. Accuracy

Recovery studies were conducted by standard addition method at three concentration levels (80%, 100%, and 120%) [17]. Known amounts of standard were added to pre-analyzed samples, and percentage recovery was calculated.

2.5.4. Precision

Precision was evaluated at repeatability, intra-day, and inter-day levels [18]. For repeatability, six replicate analyses of same homogeneous sample were performed. Intra-day precision was assessed by analyzing samples at three different times within the same day, while inter-day precision was evaluated over three consecutive days.

2.5.5. Robustness

Robustness was evaluated by deliberately varying the chromatographic conditions within a realistic range [19]. The parameters modified included:

- Mobile phase composition (±1% v/v)
- Flow rate (±0.1 mL/min)
- Detection wavelength (±1 nm)
- pH of mobile phase (±0.2 units)
- The effects on retention time, peak area, and system suitability parameters were documented.

2.5.6. Detection and Quantification Limits

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard deviation of response and slope method [20]:

- $LOD = 3.3\sigma/S$

- LOQ = 10σ/S

where σ is the standard deviation of y-intercepts and S is the slope of calibration curve.

3. Results and Discussion

3.1. Method Development and Optimization

3.1.1. RP-HPLC Method

The chromatographic conditions were optimized to achieve good peak shape and resolution. Initial trials with various mobile phase compositions revealed that methanol:0.05% OPA (50:50 v/v) at pH 3.7 provided optimal separation [21]. The retention time of hydroquinone was 3.859 minutes (Figure 1), indicating efficient separation with minimal analysis time. The system suitability parameters met the acceptance criteria, with theoretical plates >7000 and tailing factor <1.0, as shown in Table 2.

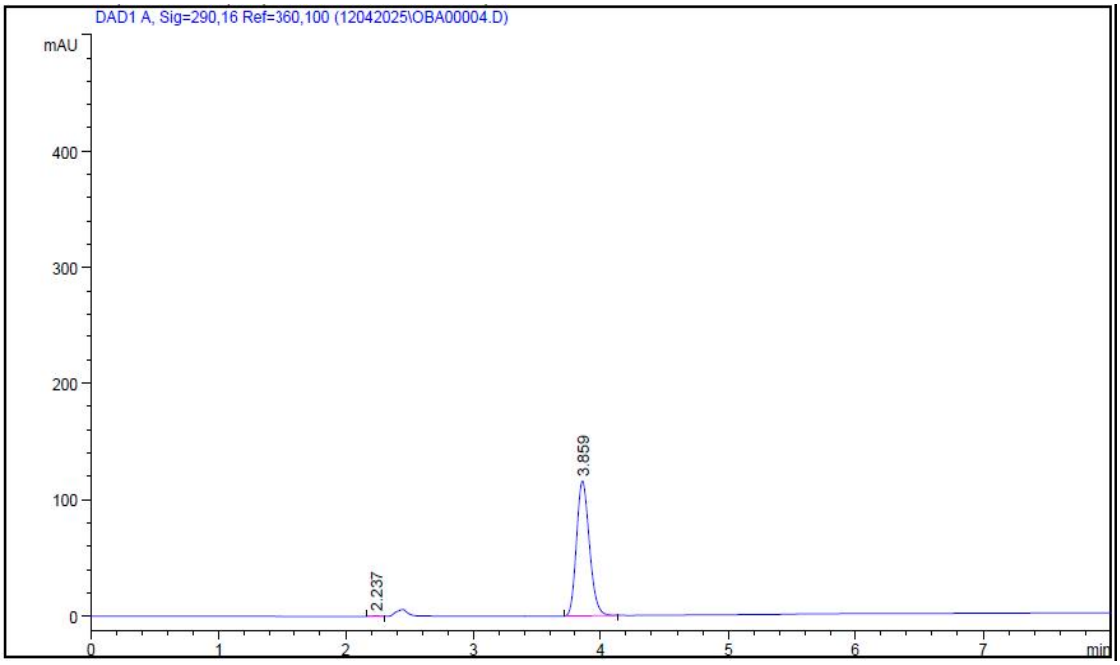

Figure 1. Chromatogram of Optimized Trial (Trial 6)

Table 2. System Suitability Parameters for RP-HPLC Method

Parameter	Observed Value	Acceptance Criteria	Status
Retention Time	3.859 ± 0.012 min	RSD ≤ 2%	Compliant
Theoretical Plates	7245	> 2000	Compliant
Tailing Factor	0.98	≤ 2	Compliant
Peak Area RSD	0.42%	≤ 2%	Compliant

3.1.2. UV Spectrophotometric Method

The UV spectrum of hydroquinone showed maximum absorption at 290 nm (Figure 2), with no interference from excipients present in the tablet formulation [22]. The method demonstrated good sensitivity and reproducibility within the selected concentration range.

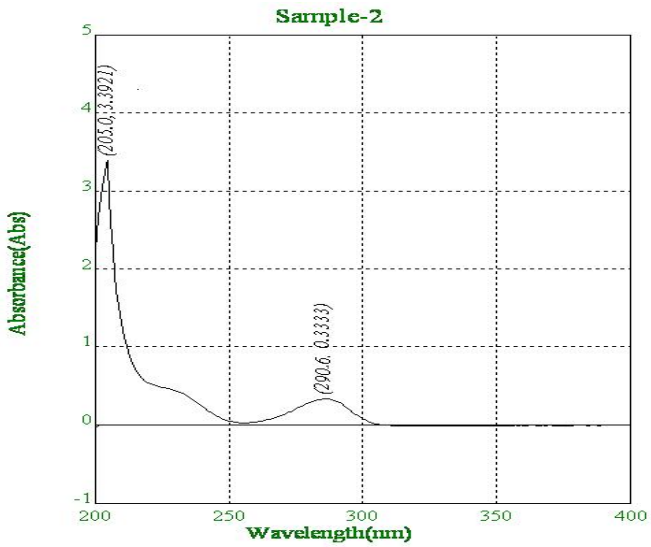

Figure 2. UV Spectrum of Hydroquinone showing absorption maxima at 290 nm

3.2. Method Validation

3.2.1. Linearity and Range

The RP-HPLC method showed excellent linearity over 10-50 µg/mL range with correlation coefficient (r^2) of 0.999 (Figure 3a). The regression equation was y = 14.07x + 4.764. Linearity Data for RP-HPLC Method. The UV method demonstrated linearity from 1-5 µg/mL (r^2 = 0.999) with regression equation y = 0.085x + 0.012 (Figure 3b).

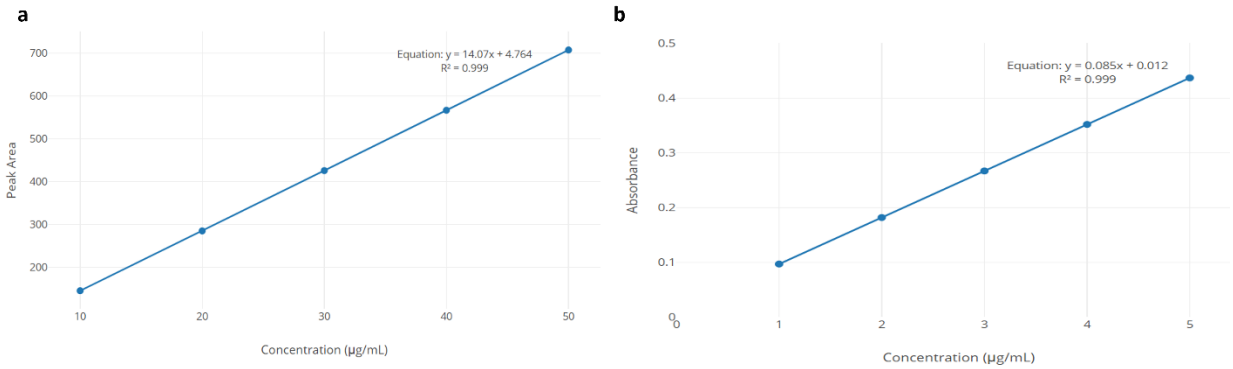

Figure 3. Calibration curves of Hydroquinone for a. RP-HPLC method b. UV method

3.2.2. Accuracy

Recovery studies for both methods showed excellent results [23]. The mean recovery percentages for RP-HPLC method ranged from 98.56% to 99.83%, while the UV method showed recovery values from 99.71% to 100.24%. These values indicate high accuracy of both methods, as shown in Table 3.

Table 3. Recovery Studies for Both Analytical Methods

Method	Level (%)	Amount Added (µg/mL)	Amount Found (µg/mL) ± SD	Recovery (%) ± SD*	RSD (%)
RP-HPLC	80	16	15.77 ± 0.071	98.56 ± 0.44	0.45
RP-HPLC	100	20	19.89 ± 0.076	99.45 ± 0.38	0.38
RP-HPLC	120	24	23.96 ± 0.101	99.83 ± 0.42	0.42
UV	80	1.6	1.60 ± 0.006	100.24 ± 0.36	0.36
UV	100	2.0	1.99 ± 0.008	99.71 ± 0.41	0.41
UV	120	2.4	2.40 ± 0.009	100.12 ± 0.38	0.38

(*Mean ± SD, n = 3 observations)

3.2.3. Precision

Both methods demonstrated high precision with relative standard deviation values well within acceptance limits [24]. For the RP-HPLC method, repeatability showed RSD of 0.17%, intra-day precision ranged from 0.18% to 0.44%, and inter-day precision from 0.18% to 0.46%. The UV method demonstrated similar precision with repeatability RSD of 0.18%, intra-day precision between 0.10% to 0.28%, and inter-day precision from 0.17% to 0.28%. The results of method precision is presented in Table 4.

Table 4. Results of Precision for Both Methods

Method	Concentration (µg/mL)	Mean Found (µg/mL) ± SD*	RSD (%)
RP-HPLC	20	19.95 ± 0.034	0.17
Intra-day	20	19.92 ± 0.036	0.18
Inter-day	20	19.88 ± 0.091	0.46
UV	2.0	1.99 ± 0.004	0.18
Intra-day	2.0	1.98 ± 0.006	0.28
Inter-day	2.0	1.97 ± 0.006	0.28

(*Mean ± SD, n = 3 observations)

3.2.4. Robustness

The RP-HPLC method demonstrated good robustness when subjected to minor deliberate variations in analytical conditions [25]. Changes in mobile phase composition (49:51 and 51:49 v/v) resulted in RSD values of 0.16% and 0.12% respectively. Flow rate variations (0.6 and 0.8 mL/min) showed RSD values of 0.12% and 0.06%. Wavelength modifications (289 and 291 nm) produced RSD values of 0.04% and 0.38%. The robustness values are presented in Table 5.

Table 5. Results of Robustness for RP-HPLC Method

Parameter	Modification	Retention Time (min) ± SD*	Peak Area RSD (%)
Mobile Phase Composition	49:51	3.892 ± 0.006	0.16
	51:49	3.825 ± 0.005	0.12
Flow Rate (mL/min)	0.6	4.125 ± 0.005	0.12
	0.8	3.654 ± 0.002	0.06
Detection Wavelength (nm)	289	3.859 ± 0.002	0.04
	291	3.859 ± 0.015	0.38

(*Mean ± SD, n = 3 observations)

3.2.5. Detection and Quantification Limits

For the RP-HPLC method, the LOD and LOQ values were calculated based on the standard deviation of response and slope of calibration curve [26]. The LOD was found to be 0.0147 µg/mL and LOQ was 1.3930 µg/mL, indicating adequate sensitivity for the intended application. These parameters are summarized in Table 6.

Table 6. Results of Method Validation

Parameter	RP-HPLC Method	UV Spectrophotometric Method
Linearity Range	10-50 µg/mL	1-5 µg/mL
Regression Equation	y = 14.07x + 4.764	y = 0.085x + 0.012
Correlation Coefficient (r^2)	0.999	0.999
LOD	0.0147 µg/mL	0.124 µg/mL
LOQ	1.3930 µg/mL	0.376 µg/mL
Precision (%RSD)	< 0.46	< 0.28
Accuracy (% Recovery)	98.56-99.83	99.71-100.24

3.3. Analysis of Marketed Formulation

The developed methods were successfully applied to the analysis of commercial tablet formulations containing hydroquinone [27]. Twenty tablets were weighed and the average weight was calculated as 0.235 g. The tablet powder equivalent to 11.75 mg of hydroquinone was extracted and analyzed using both methods. The results of the marketed formulation analysis are presented in Table 7

Table 7. Analysis of Commercial Tablet Formulation

Method	Amount Found (mg) ± SD*	% Label Claim ± SD*	RSD (%)
RP-HPLC	203.30 ± 0.894	101.65 ± 0.45	0.44
RP-HPLC	202.00 ± 0.909	101.00 ± 0.45	0.45
UV	201.88 ± 0.081	100.94 ± 0.040	0.040
UV	201.76 ± 0.083	100.88 ± 0.041	0.041

(*Mean ± SD, n = 3 observations)

The RP-HPLC method showed assay values of 101.65% and 101.00% with RSD values of 0.44% and 0.45% respectively. The UV spectrophotometric method demonstrated assay values of 100.94% and 100.88% with RSD values of 0.040% and 0.041%. These results confirm that both methods are suitable for routine quality control analysis of hydroquinone in pharmaceutical formulations.

Table 8. Stability Studies Data for RP-HPLC Method

Storage Condition	Time Period	% Drug Remaining ± SD	RSD (%)
Room Temperature (25°C)	24 h	99.45 ± 0.378	0.38
	48 h	99.12 ± 0.416	0.42
	72 h	98.86 ± 0.445	0.45
Refrigerated (4°C)	24 h	99.89 ± 0.320	0.32
	48 h	99.76 ± 0.359	0.36
	72 h	99.54 ± 0.398	0.40

(*Mean ± SD, n = 3 observations)

4. Conclusion

The research successfully established two analytical methods for the quantitative determination of hydroquinone in pharmaceutical formulations. The RP-HPLC method utilizing methanol:0.05% OPA (50:50 v/v) mobile phase and UV spectrophotometric method at 290 nm were developed and thoroughly validated. Both methods demonstrated excellent linearity, precision, accuracy, and robustness within their respective working ranges. The RP-HPLC method showed high sensitivity with LOD of 0.0147 µg/mL and LOQ of 1.3930 µg/mL, while maintaining short analysis time with hydroquinone eluting at 3.859 minutes. The UV spectrophotometric method proved to be simple, rapid, and cost-effective, making it suitable for routine analysis. The methods were successfully applied to pharmaceutical formulations with high reproducibility and recovery values ranging from 98.56% to 100.24%.

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