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Development and Validation of Spectrofluorimetric Method for Estimation Hydroquinone from its Pharmaceutical Dosage Form

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ABSTRACT

A simple, sensitive, rapid, precise, and accurate Spectrofluorimetric method has been developed for estimation of Hydroquinone (HYQ) its pharmaceutical dosage forms.HYQ showed good fluoroscence intensity in double distilled water and so Double distilled water was selected as a solvent for estimation of HYQ. The optimized excitation and emission wavelength were 290 nm and 337 nm respectively with 2.5nm slit width for HYQ determination. The linear regression data for the calibration curves shows a good linear relationship over the concentration range of 50 to 900 ng/mL for HYQ with respect to fluorescence intensity. The LOD and LOQ values were found to be 6.88 ng/mL and 20.85 ng/mL respectively. The developed method was validated in terms of linearity, precision, accuracy, limit of detection and quantification, robustness as per International Conference on Harmonization Q2 (R1) guidelines. The utility of the developed method has been demonstrated by assay of commercially available cream formulations. The developed spectrofluorimetric method can be successfully applied for routine quality control of HYQ from its formulations.

Keywords: Hydroquinone (HYQ), Double distilled water, Spectrofluorimetric method, Validation.

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INTRODUCTION

Hydroquinone (HYQ), chemically 1,4-Benzenediol (Figure. 1) is a depigmenting agent and antioxidant, widely used to fade dark skin spots such as freckles and lentigo. It is official in United States Pharmacopoeia USP-35[1]. Itis a Fine white crystals, or white crystalline powder, which darkens on exposure to light and air, having solubility in water, ethanol, and chloroform[2]. HYQ has effect on the nonfollicular and follicular melanocyte system. It showed decreased formation of melanosomes and destruction of the membranous organelles in the melanocytes. So it is widely used to temporarily fade dark skin spots such as freckles,lentigo,melasma and age spots. Literature review revealed that HYQ showed inherent flouroscence characteristics and many analytical methods have been reported for estimation of HYQ in combination with other drugs [3-9].The reported methods for estimation of HYQ were less economic, less sensitive and complex. Moreover, no method has been reported for estimation of HYQ from its pharmaceutical formulations using flouroscence spectroscopy. So it was endeavoured to develop a very simple, sensitive, cost effective spectrofluorimetric method for estimation of HYQ from its pharmaceutical dosage forms.

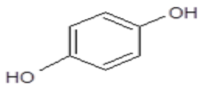

Figure 1: Chemical Structure of HYQ

MATERIALS AND METHOD

Reagents and Chemicals

Reference standard of HYQ was obtained as gift samples from Crystal Quinone ltd; Ahmedabad. Marketed formulations (MELACARE and ESLITE-15) were procured from local market.

Instrumentation

Spectroflurophotometer with model LS 55 Fluroscence Spectroflourometer, manufactured by PERKIN ELMER with xenon discharge lamp (20KW), two automatic monochromators and Photomultiplier tube as detector was used for the study. The results were computed using FL WinLAB software. Analytical balance with model Shimadzu AUX 220, manufactured by SHIMADZU Ltd.; having weighing capacity of 0.01gm to 200gm was used. Distillation Assembly with model no 421/14, manufactured by Jain scientific glass works was utilized for preparing double distilled water.

Preparation of stock solution of HYQ (1000µg/mL) and working standard solution of HYQ (10 µg/mL)

10 mg of HYQ was weighed and transferred to 10 mL volumetric flask and dissolved in double distilled water. The flask was shaken and volume was made up to the mark with Double distilled water to give a final solution containing 1000µg/mL of HYQ. 1 mL of stock solution (1000µg/mL of HYQ) was transferred to a 100 mL volumetric flask and dissolved in Double distilled water. The volume was made up to mark with Double distilled water to obtain final solution containing 10µg/mL of HYQ (10000 ng/mL of HYQ). This solution was further diluted to various concentrations for method validation.

METHOD VALIDATION

The developed spectrofluorimetric method was validated in terms of linearity, accuracy, precision, limit of detection(LOD), limit of quantification (LOQ) , robustness, specificity as per ICH guideline Q2(R1) [10]

Linearity

Appropriate aliquots from HYQ working standard solution were transferred to different volumetric flasks of 10mL capacity. The volume was made up to the mark with Double distilled water to obtain concentration of 50, 100, 300, 500, 700, 900 ng/mL. Calibration curve was constructed by plotting average fluorescence intensity Vs. conc. and regression equation was computed.

Accuracy

To check the accuracy of proposed method, recovery studies were carried out from pre analyzed samples at three different levels of standard addition 80%, 100% and 120% of label claim. The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The recovery values between 98-102% indicated that the method is free from interference of excipients and the drugs can be recovered accurately.

Precision

For intraday precision, lower, middle and higher level concentrations of linearity range were selected and solutions were analyzed under the optimized conditions for three times in a same day and fluorescence intensity was recorded. For inter day precision, lower, middle and higher level concentrations of linearity range were selected and solutions were analyzed under the optimized conditions for three consecutive days and fluorescence intensity was recorded. Repeatability was established by performing the experiment for six times consecutively and measuring the fluorescence intensity of the concentration nearer to assay concentration. The %RSD values for intra day, inter day and repeatability were found to be less than 2%, indicating the method was precise.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD & LOQ were calculated by using standard deviation and slope value obtained from calibration curve.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the developed method was tested by varying emission wavelength (±2 nm) of optimized conditions. The %RSD values of less than 2% indicated that the method is robust for changes in ±2 nm emission wavelength of estimation.

Specificity

The specificity of developed method to analyze HYQ from its formulation was checked by comparing the spectra of Blank, Standard solution of HYQ (500ng/mL) and Sample solution of HYQ (500ng/mL).

Analysis of Marketed Formulation

Marketed formulations containing HYQ was analyzed. Cream equivalent to 20 mg of HYQ was taken and dissolved in 20 mL of Double distilled water. The cream was triturated for 10-15 min and filtered through whatman filter paper no 41. The volume was made up to 100mL with double distilled water. From this aliquot of 0.5 mL solution was taken and transferred to 10 mL volumetric flask and volume was made up to the mark The final solution obtained has concentration of 10 µg/mL of HYQ. From this 0.4mL was pipetted in 10mL volumetric flask and volume was made up to mark with double distilled water to obtain concentration of 400ng/mL.This solution was analyzed using spectrofluorometer under optimized conditions.

RESULTS AND DISCUSSION

Optimization of Specrofluorimetry method

Selection of solvent was done by preparing solution of HYQ (500 ng/mL) in different solvents like double distilled water, methanol, Acetonitrile, 0.1 M NaOH, 0.1 M H_2SO_4. The spectra were scanned in range of 200-600 nm and flouroscence intensities were measured. HYQ showed flouroscence in double distilled water, methanol, ACN and 0.1 M H_2SO_4, but of varying intensities. It was observed that Double distilled water showed good and reproducible flouroscence intensity of HYQ when compared to other solvents. Moreover, an emission maximum of HYQ was free from interference of solvent peak. Because of its simplicity, easy of availability, cost effectiveness and lack of interference of other components, Double distilled water was selected as a solvent for estimation of HYQ.The emission spectra of HYQ show maximum emission at 337 nm after excitation at 290 nm. So, 290 nm and 337 nm were selected as excitation wavelength and emission wavelength respectively for the analysis of HYQ (Fig 2-3). It has been observed that if emission slit width was increased, it lead to increase in sensitivity but decrease in range of detection and peak broadening. If excitation slit width was increased then peak intensity at emission maxima reduced by 4 times, leading to decreased sensitivity. At 2.5nm slit width of excitation and emission, reproducible and desirable results were obtained. So, 2.5nm was optimized slit width of Excitation and emission for HYQ determination. The optimized parameters for estimation of HYQ are reported in table 1.

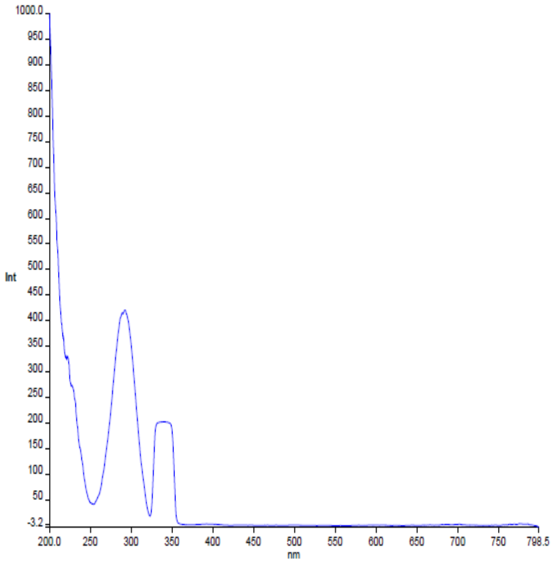

Figure 2: Excitation Spectra of HYQ in Water at 337nm emission

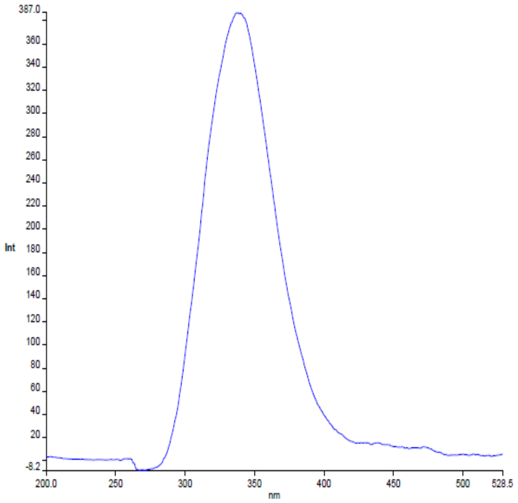

Figure 3: Emission Spectra of HYQ in Water at 290nm excitation

Table 1: Optimized Spectroflourimetric parameters

Solvent	Double distilled water
Excitation wavelength	290 nm
Emission wavelength	337 nm
Slit width	2.5 nm
Scanning speed	1200 nm/min

Validation of developed method

The linearity was observed in the range of 50-900ng/mL for HYQ as shown in figure 4-5 and table 2. The developed method was found to be accurate, indicated by mean % recoveries ranging from 99.47 to 100.68% as mentioned in table 3. The summary of validation parameters is mentioned in table 4. The method is found to be specific as shown in fig 6. The results of assay of marketed formulations by the developed method are shown in table 5. The assay result between 98-102% of label claim indicates that the method can be applied to routine analysis of HYQ from its formulations.

Table 2: Linearity data for HYQ by developed method

Conc. (ng/mL)	Mean ± SD*	%RSD
50	66.99143 ± 1.148	1.714
100	113.9307 ± 1.865	1.637
300	314.3596 ± 4.583	1.458
500	523.6449 ± 7.007	1.338
700	725.371 ± 8.795	1.212
900	935.4616 ± 4.617	0.493
Regression equation	Y=1.0227x + 11.987	
r^2	0.9999	

***n=6**

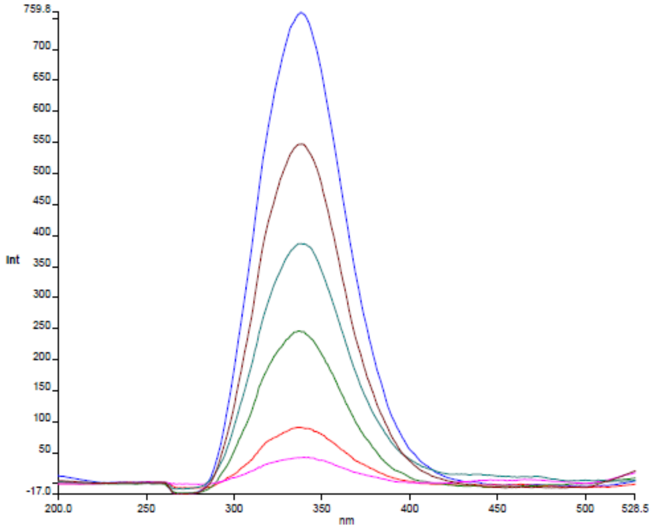

Figure 4: Overlay spectra of HYQ in water at 337nm emission (50-900ng/mL)

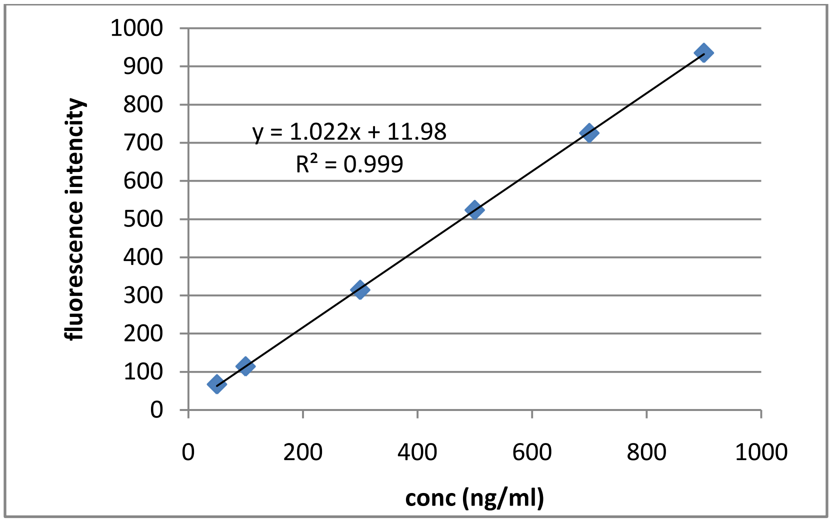

Figure 5: Calibration curve for HYQ (50 to 900 ng/mL)

Table 3: Accuracy (Recovery) data for HYQ by developed method

Level of recovery	Sample Conc. (ng/mL)	Conc. Of std added (ng/mL)	Total Conc. (ng/mL)	F.I.	Amt Recovered (ng/mL)	% Recovery	Mean %recovery
80%	400	320	720	744.795	716.542	99.519	99.473
				752.816	724.385	100.609	
				735.748	707.696	98.291	
100%	400	400	800	836.189	805.907	100.738	100.680
				825.746	795.696	99.462	
				845.205	814.723	101.840	
120%	400	480	880	901.641	869.907	98.853	
				901.423	869.693	98.828	
				921.787	889.605	101.091	99.591

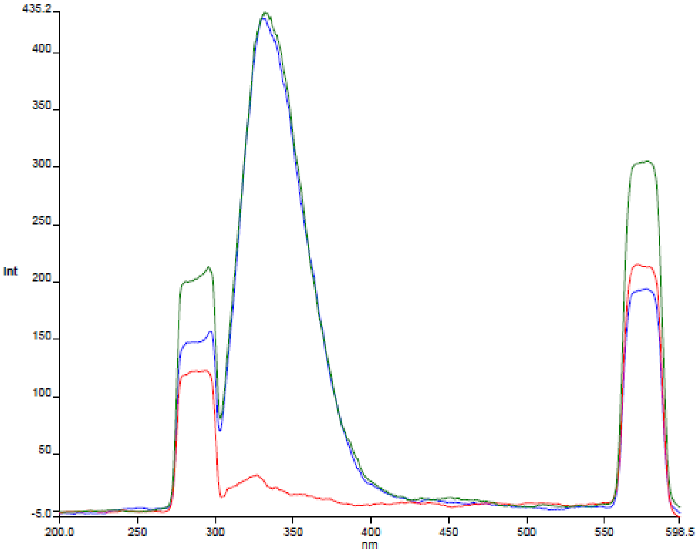

Figure 6: Fluorescence spectra of blank, HYQ (500 ng/mL) and sample (500 ng/mL) to check specificity of developed method.

Table 4: Summary of Validation Parameters for developed method

Parameters	HYQ
Range	25 ng/mL to 900 ng/mL
Linearity	50 ng/mL to 900 ng/mL
r^2	> 0.995
Regression Equation	**y = 1.0227x + 11.987**
LOD (ng/mL)	6.882953
LOQ(ng/mL)	20.85743
Intra day Precision (%RSD) (n=3)	0.788 to 1.823%
Inter day Precision (%RSD) (n=3)	0.903 to 1.982063%
Repeatability (%RSD) (n=6)	1.995%
%Recovery	99.473 -100.680%
Robustness (%RSD)	1.063(+2nm) 0.983(-2nm)

Table 5: Analysis of marketed formulations

BRAND	DRUG	Label Claim (%w/w)	Mean F.I	% Assay ± SD*	%RSD
MELACARE	HYQ	2	423.851	100.680±8.329	1.965
	MOM	0.1	---	-----	----
	TRE	0.025	---	-----	----
ESLITE	HYQ	2	417.247	99.066±6.416	1.537

***n=6**

CONCLUSION

The developed method is accurate, precise, fast, inexpensive, and sensitive. The most important advantage of this method is its simplicity and free of organic solvent usage. The developed method can be used for quality control of HYQ from its pharmaceutical formulations.

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