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Research Article

Development and Validation of a Rapid Spectrofluorimetric Method for Quantification of Coal Tar in Topical Formulations

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ABSTRACT

The present research work discusses the development of a spectrofluorimetric method for the estimation of coal tar. The method is simple, accurate and cost efficient and can be routinely employed for the quality control and quantification of coal tar in topical products. The optimum conditions for the analysis of coal tar were established. The relative fluorescence intensity of coal tar was determined in cyclohexane at an excitation wavelength of 384 nm and an emission wavelength of 430 nm. The method validation was accomplished through evaluation of analytical parameters of linearity, range, accuracy, and precision, limit of detection (LOD) and limit of quantification (LOQ) as per ICH guidelines. The linearity range was found to be 1 to 100 µg/mL. The LOD and LOQ were found to be 0.007 µg/mL and 0.022 µg/mL, respectively. The developed method was successfully employed to quantify the coal tar in a commercial topical product and the findings were accurate.

Keywords: Fluorescence spectrophotometry, validation, fluorimetry, coal tar.

1. INTRODUCTION

Pharmaceutical grade coal tar is a viscous liquid mixture of hydrocarbon compounds, derived, along with coke, from the destructive distillation of coal in coking ovens. It is composed of 48% hydrocarbons, 42% carbon, and 10% water [1]. It is "practically insoluble" in water; however "all or almost all" dissolves in benzene, nitrobenzene and cyclohexane [2]. It is frequently prescribed for various dermatological problems like eczema [3], psoriasis [4] and dandruff [5]. It is official in various pharmacopoeias including United State Pharmacopoeia, European Pharmacopoeia, Japanese Pharmacopoeia, British Pharmacopoeia and Indian Pharmacopoeia and is available in various 'Over the Counter' (OTC) topical products [6]. Being crude material, its quantification and quality standards are missing from the pharmacopoeia. There are many efforts to quantify the coal tar by RP-HPLC [7] and gas chromatography [8]. These techniques require sophisticated techniques and instrumentation and have not been applied to the commercial product analysis. Hence, there is an immense need to develop a simple, fast, accurate and economic method for the quantification of coal tar in pharmaceutical products. Herein, the authors have developed and validated spectrofluorimetric technique for the analysis of coal tar and the same has been successfully used for the quantification of coal tar in a marketed product.

2. MATERIAL AND METHODS

A spectrofluorimeter (Hitachi F 2500, Japan), with 1cm matched glass cells was used for the fluorescence measurements. Systronic™ electronic balance (Shimadzu, Japan) was used for weighing the samples. Coal tar was procured from M/s Life Care, Gurgaon, India as a free gift sample. Lipotar S™ gel (Marketed coal tar product) was procured from local chemist shop. All other chemicals were of analytical grade and were used as such without any further purification. Double distilled water was used in the complete study protocols.

2.1. Scan for the excitation and emission wavelengths

The stock solutions were scanned for the wavelengths exhibiting maximum fluorescence for excitation and emission.

2.2. Preparation of Stock Solution

Coal tar, 100 mg was taken in 100 mL volumetric flask and small amount of cyclohexane (around 30 mL) was added to it. Then it was subjected to sonication for 15 minutes. After that the mixture was filtered and filtrate was collected in 100 mL volumetric flasks and volume was made-up to the mark with cyclohexane to get the stock solution of 1000 µg/mL.

2.3. Preparation of calibration plot

Different aliquots were taken from stock solution and diluted with cyclohexane to give series of concentration (1-100 µg/mL).

2.4. Method Validation

The method validation was carried out as per ICH Q2 (R1) guidelines [9, 10]. The following validation parameters; linearity and range, accuracy and precision, limit of detection (LOD) and limit of quantification (LOQ) were studied.

2.4.1. Linearity and Range

To access linearity, ten replicated analysis were carried out separately. Fluorescence versus concentration was plotted to obtained calibration graph. The linearity was calculated by least square regression method. The range of the analytical procedure was given by the interval between the upper and lower concentration of coal tar in the standard solutions.

2.4.2. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the experiment was established by using recovery studies. For this, standard samples (in addition to calibration standards) were prepared in triplicate at different concentration levels (20, 66 and 100µg/mL), covering the entire linearity range. UV fluorescence was noted and % mean recovery and % RSD was calculated.

2.4.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the proposed method was determined for three concentration levels (20, 60, 100µg/mL) covering entire linearity range by inter-analyst and reported as % RSD.

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

The LOD of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value**.** The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy**.** Estimation of LOD and LOQ were based on the standard deviation of the response and the slope of the calibration curve. Equations used for calculation are as follows (Equations 1 and 2):

$$LOD=3.3\sigma/S \quad (1)$$

$$LOQ=10\sigma/S \quad (2)$$

Where, σ is the standard deviation of the absorbance of the sample and, S is the slope of the related calibrations graph.

2.4.5. Extraction and Assay of Coal Tar from Marketed Product

Approximately, 0.5 g of marketed product was taken in a 100 mL volumetric flask. The sample was sonicated for 30 minutes with 50 mL of cyclohexane. The volume was make-up to 100 mL with cyclohexane. The solution was filtered and analyzed using the developed method.

3. RESULTS

3.1. Excitation and Emission Wavelength Scans

The Figure 1 and Figure 2 portray the emission and excitation pattern for the scanned concentrations. Wavelengths 384 nm and 430 nm were selected as emission and excitation wavelengths, respectively.

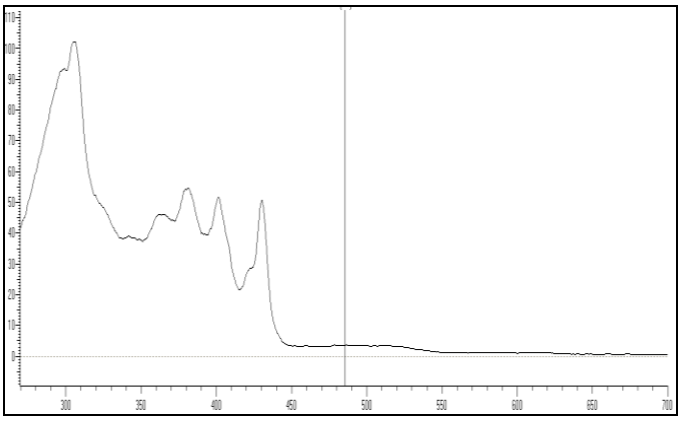

Figure1: Wavelengths scan for emission spectra of coal tar solution

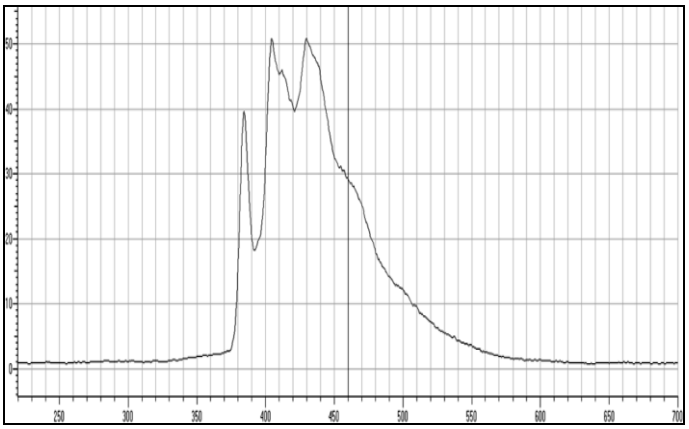

Figure2: Wavelengths scan for excitation spectra of coal tar solution

3.2. Calibration Plot

Fluorescence range was found to be 8.23 to 76.79 for the concentration range of 1-100 µg/mL (Figure 3).

3.3. Linearity and Range

Linearity was found to be in the range of 1-100 µg/mL with significant high value of correlation coefficient,

R^2=0.999; the representative equation was y=7.649x + 1.7123 (Figure 3).

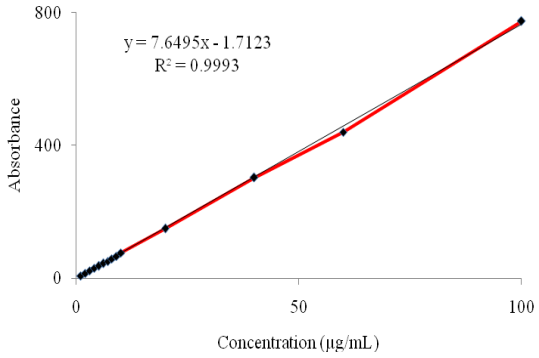

Figure3: Calibration plot of coal tar

3.4. Accuracy and Precision: The accuracy and precision parameters have been shown in Table 1 and Table 2, respectively.

Table 1: Accuracy parameters of coal tar spectroflourimetry*

Conc. of coal tar taken (µg/mL)	Conc. of coal tar observed (µg/mL)±S.D.	% Mean recovery	% RSD	% Bias
20	20.06 ± 0.009	100.3	0.44	3.02
60	59.88 ± 0.006	99.8	0.1	-1.90
100	100.26 ± 0.017	102.6	0.17	2.69

**Each value is average of three determinations, S.D. = Standard Deviation, RSD = Relative Standard Error*

Table 2: Precision parameters of coal tar spectroflourimetry

Concentration of coal tar (µg/mL)	Analyst	Day 1	Day 2	Day 3	Average Concentration	% Mean Recovery	% Bias
20	1	19.8	18.9	19.5	19.4	97.0	-2.88
20	2	18.8	19.6	19.9	19.4	97.0	-2.61
60	1	60.0	59.0	59.4	59.5	99.16	-0.83
60	2	59.1	58.9	59.8	59.3	98.88	-1.11
100	1	99.5	99.0	98.7	99.0	99.09	-0.90
100	2	99.1	99.7	99.5	99.4	99.47	-0.52

3.5. LOD and LOQ

The LOD and LOQ of coal tar were found to be 0.007µg/mL and 0.022µg/mL, respectively.

3.6. Extraction of coal tar from marketed product

The percent drug assay from the marketed product containing 2% w/w coal tar was found to be 99.8 ± 0.98. Hence, the method was found to be suitable for the assay of coal tar in the marketed dermatological products.

4. DISCUSSION

A spectrofluorimetric method for quantifying coal tar in bulk and pharmaceutical samples has been developed and validated. Beer-Lambert's law obeyed in the concentration range of 1-100µg/mL, with coefficient of correlation, slope and intercept as 0.999, 7.649 and 1.712, respectively. The results of recovery studies reflected that method is free from interference of the impurities during the estimation of coal tar. The low relative standard deviations values for all parameters confirmed the validity and reliability of method. All the above results manifested that developed method is selective, precise, accurate and linear over the concentration range of 1-100µg/mL. The method was found to be appropriate for the marketed products as the assay was very close to the labeled claim.

In summary, the proposed method can be adopted for the routine estimation of coal tar in bulk and pharmaceuticals.

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