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RP HPLC method for the quantification of coal tar in topical foam

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ABSTRACT

Coal tar is a brown or black liquid of high viscosity, smells like naphthalene and hydrocarbons. Coal tars are complex and variable mixtures of phenols, polycyclic aromatic hydrocarbons (PAHs), and heterocyclic compounds. A unique stability- indicating HPLC method was developed for the quantitative determination of % coal tar by quantification of marker peaks viz Phenanthrene Anthracene and Pyrene in pharmaceutical dosage forms in the presence of degradation products and excipients. Phenomenex Hypersil BDS 150 mm x 4.6 mm, 3 µm column was used to achieve separation using gradient method. The mobile phase A contains deionised water and the mobile phase B contains acetonitrile. The flow rate was 0.8 mL min^{-1} and the detection wavelength was 240 nm. The retention time of phenathrene, anthracene and pyrene are 21.7, 23.8 and 29.7 minutes respectively. The total run time is 60 minutes within which three marker peaks and degradation products were separated. Calibration showed that the response of phenathrene, anthracene and pyrene was a linear function of concentration over the range 0.25-0.75 µg mL^{-1} ($r \geq 0.999$) and the method was validated over this range for precision, intermediate precision, accuracy, linearity and specificity. The method was developed and validated successfully and applied to the quantitative determination of coal tar marker peaks in coal tar foam product.

KEYWORDS

Coal tar solution;
Method validation.

INTRODUCTION

Coal tar can be used in medicated shampoo, soap and ointment, as a treatment for dandruff and psoriasis, as well as being used to kill and repel head lice. It has been used for decades to help treat the scaling, itching and inflammation of psoriasis, eczema, and other skin disorders. When used as a medication in the U.S., coal tar preparations are considered an OTC (over-the-counter drug) pharmaceutical and are subject to regulation by the United States Food and Drug Administration. The main groups of compounds making up crude coal tar are 48% hydrocarbons, 42% of other carbon compounds and 10% water.

It is supplied in the form of Coal Tar Topical Solution USP, which consists of a 20% w/v solution of coal tar in alcohol, with an additional 5% w/v of polysorbate 80;[2] this must then be diluted in an ointment base such as petrolatum. The gravimetric estimation of coal tar is published in USP. Presently there is no reported method

for the quantification of % coal tar estimation in pharmaceutical dosages. Semi quantification methods were developed for the estimation of coal tar dyes in various food industries by TLC[4,5]. Various other chromatographic methods were available for the estimation of coal tar component in coal tar with hyphenated detectors[6].

A unique stability-indicating HPLC method was developed for the quantitative determination of % coal tar by quantification of marker peaks phenathrene, anthracene and pyrene (PAP) in pharmaceutical dosage forms in the presence of degradation products and excipients.

Coal Tar Foam product is assayed for three known components- PAP using an HPLC method that uses a reverse phase Hypersil BDS, C18 column with UV detection at 240 nm. Samples are quantified using an external standard technique. PAP were chosen as marker peaks as they have UV chromophore and are easily available with high purity.

EXPERIMENTAL

Chemicals and reagents

Deionized water, HPLC grade Acetonitrile (ACN), suitable reference standards of Phenanthrene, Anthracene and Pyrene.

Equipment

HPLC analysis was performed with a Waters HPLC system 2695 equipped with a quaternary solvent manager, sample manager, column-heating compartment, Photodiode Array detector 2996 and UV detector 2487. This system is controlled by Waters Empower software.

Hypersil BDS column, 150 mm × 4.6mm, 3µm (Phenomenex, USA) was employed for chromatographic separation. Class A volumetric glassware, 10-mL Syringes, Transfer tube Harvester, Disposable tubes, Whatman Nylon 0.45 µm Syringe Needle, were used during the experimental work.

Standard and sample preparation

(1) Diluent

A mixture of Acetonitrile and water in the ratio of (65:35) v/v. respectively.

(2) Standard stock solution

Accurately weigh approximately 0.02 g each of the reference standards into separate 200-mL volumetric flasks and add 13 mL ACN. Sonicate for 5 minutes. Then add 7mL deionized water and dilute to volume with diluent and mix by inversion to prepare a solution of concentration 100µg/mL.

Intermediate standard stock solution

Pipette 5.0mL each of the standard stock solutions into a 25mL volumetric flask and dilute to volume with diluent (20µg/mL).

Working standard

Pipette 5.0mL of intermediate standard stock solution into a 200-mL volumetric flask, and dilute to volume with diluent to obtain a solution of 0.5µg/mL.

Product

Remove and discard the plunger from the Transfer tube Harvester. Attach the clear plastic tube to the tip of an unused foam can. Shake foam can vigorously for at least 15 seconds. Fill a 10-ml plastic syringe with foam, attach plunger and syringe needle. Accurately weigh 0.5 g of sample into a 250-mL volumetric flask. Add 50 mL of diluent, sonicate for 5 minutes and vortex until sample is completely dispersed. Dilute to volume with diluent, vortex and invert to mix well. Filter through 0.45µm Nylon filter.

Raw material (coal tar solution)

Accurately weigh 0.5 g of coal tar solution API into a 100-mL volumetric flask and dilute to volume with diluent, vortex, and invert to mix well. Transfer 4.0 mL of this solution to a 100 mL volumetric flask and dilute to volume with diluent. Filter through a 0.45 µm Nylon filter.

Chromatography

The analytes were separated on an HPLC Hypersil BDS column, 150 mm × 4.6mm, 3µm at column oven temperature of 40°C with a gradient run program at a flow-rate of 0.8 mL min^{-1}. Deionized water and acetonitrile were used as mobile phase A and B respectively which was filtered through a 0.45 µm nylon filter, before use. The separation was achieved by gradient elu-

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TABLE 1A : Force-degradation study coal tar API solution

	Concentration in % w/w							
Marker peaks	Control	H_2O_2	UV	Vis. light	Control	1N HCl	1N NaOH	80°C
Phenanthrene	0.4945	0.4033	0.5990	0.5053	0.4707	0.5154	0.5124	0.5172
Anthracene	0.0966	0.0691	0.0848	0.0684	0.0928	0.0998	0.0992	0.1000
Pyrene	0.2225	0.1828	0.2685	0.2275	0.2151	0.2377	0.2355	0.2418

TABLE 1B : Force-degradation study coal tar foam product

	Concentration in % w/w							
Marker peaks	Control	H_2O_2	UV	Vis. light	Control	1N HCl	1N NaOH	80°C
Phenanthrene	0.0488	0.0473	0.0502	0.0506	0.0486	0.0486	0.0469	0.0486
Anthracene	0.0095	0.0062	0.0056	0.0067	0.0095	0.0094	0.0092	0.0092
Pyrene	0.0221	0.0215	0.0221	0.0222	0.0224	0.0224	0.0215	0.0224

tion starting with isocratic mode for 15 minutes with the mobile phase ratio of A: B as 50:50. Then the ratio was changed linearly for A: B as 37:63 for next 20 minutes, thereafter changing the ratio to 10:90 within 2 minutes. The system was run in the isocratic mode for 20 minutes. The initial ratio of 50:50 was attained in 3 minutes and continued isocratically for 15 minutes. UV detection was performed at 240 nm. The sample injection volume was 50 µL.

Method validation

The method was validated for specificity, precision, accuracy, sensitivity and linear range as per the International Conference on Harmonization (ICH) guidelines[1].

Specificity

The purpose of this study was to examine the degradation products of coal tar foam products using the three components PAP as markers to quantify coal tar content in the products as well as to evaluate the current analytical method used for coal tar quantification for specificity and stability indicating. This was done by subjecting individual reference materials and the coal tar products to acid and base hydrolysis, heat, peroxide oxidation and photo degradation. Control Coal Tar API solution, Foam solution and Placebo solution were used to eliminate any background peaks.

Acid-treated solutions

0.5g each of placebo, foam product and 0.05g of coal tar API solution were treated with 2.5 ml of 1N HCl. The mixture was allowed to stand for 72 hours. It was then neutralized with 2.5 ml of 1N NaOH.

Base-treated solutions

0.5g each of placebo, foam product and 0.05g of coal tar API solution were treated with 2.5 ml of 1N NaOH. The mixture was allowed to stand for 72 hours. It was then neutralized with 2.5 ml of 1N HCl.

Heat-treated solutions

0.5g each of placebo, foam product and 0.05g of coal tar API solution were weighed into a 250 mL volumetric flask and placed in 80°C oven for 72 hours.

Peroxide treated solutions

0.5g each of placebo and foam products and 0.05g of coal tar API solution were weighed into a 250 mL volumetric flask followed by addition of 5.0 mL of 30 % H_20_2. The mixture was allowed to stand for 16 hours.

UV-treated solutions

0.5g each of placebo, foam product and 0.05g of coal tar API solution were weighed into a quartz crucible and placed in UV chamber for 8.9 hours under 441.0 µW/cm^2.

Visible light treated solution

0.5g each of placebo, foam product and 0.05g of coal tar API solution were weighed into a quartz crucible and placed in visible light chamber for 16.0 hours under 842 LUX.

The percentage concentrations of three reference markers in the force degraded samples of coal tar API solution and coal tar foam products were quantified against the reference standard solution of these components. The results of optimized conditions are summarized in TABLE 1A and 1B.

System precision and method precision

Six assay specimens of product as foam were prepared and analyzed according to the method. The relative standard deviation of each marker peak PAP was 0.1%, 0.2% and 0.1% respectively. Method precisions the relative standard deviation for the recoveries for the sum of coal tar peaks in foam was 1.40% which was within the limit of 2% RSD.

Accuracy

To confirm the accuracy, product placebo was pre-

TABLE 2 : Robustness

Conditions		Phenanthrene			Anthracene				Pyrene				%w/w coal tar
Flow rate	Column Temperature	P^1/T^2	SP^3		P^1/T^2	R^4	SP^3		P^1/T^2	R^5	SP^3		
			PT^6	PA^7			PT^6	PA^7			PT^6	PA^7	
0.8	40	0.6/1.1	1.89	0.54	0.4/1.1	2.4	6.67	0.02	1.3/1.1	6.5	11.1	1.04	9.06
0.7	40	0.9/1.1	2.06	0.48	1.1/1.2	2.2	5.62	0.94	0.4/1.2	6.1	9.86	0.86	8.91
0.9	40	0.7/1.2	1.92	0.57	0.3/1.2	2.5	3.41	1.04	0.5/1.2	6.7	11.0	1.16	8.85
0.8	35	0.2/1.2	1.96	0.55	0.2/1.2	2.2	3.47	0.98	0.4/1.3	6.2	10.8	1.01	8.77
0.8	45	0.4/1.2	1.62	0.60	0.2/1.2	2.6	3.82	1.2	0.8/1.3	7.0	9.62	1.22	8.96

P^1:Precision, T^2: Tailing, SP^3: Spectral purity, R^4: Resolution between Phenanthrene and Anthracene, R^5: Resolution between Anthracene and Pyrene, PT^6: Purity Threshold, PA^7: Purity angle

pared by omitting PAP. All other ingredients were added at the normal formulation ratios. Triplicate vehicles were spiked at 80%, 100% and 120% of the method concentration level (0.5µg/ml PAP). About 0.5 gram of the vehicle was accurately weighed out into ten 250-mL volumetric flasks. Known concentrations of PAP were spiked into each of the 250-mL volumetric flasks. Each volumetric flask was diluted to volume with diluents and prepared as per method. A vehicle without PAP was also prepared as per method and used as a control. The recovery of each marker peak was about 99.0% and the % RSD was > 1.0%.

Linearity of detector response

Linearity studies were performed using PAP reference standard at concentrations corresponding to 50%, 75%, 100%, 125% and 150% of the method target levels (0.5 µg mL of PAP). The data shows that all the marker peak response was linear. The correlation coefficient (r) for each peak was 0.9999.

Filter study and solution stability

One set of six replicate samples of Coal Tar Solution raw material were prepared and analyzed as per the method. The same samples were re-analyzed after storage at room temperature for 24 and 48 hours. This was done to simulate unexpected instrument delays. The sum of % w/w Coal Tar Solution peaks was calculated at each test point and was compared to the initial results. A filter study was conducted on a sample solution of the Coal Tar Solution raw material. The sample solution was passed through a Whatman 0.45 µm nylon filter, before dispensing the filtrate into an HPLC vial. An unfiltered raw material sample solution was also vailed. The filtered and unfiltered solutions were assayed as per the method.

Robustness

Robustness evaluations were conducted for PAP in Coal Tar Solution raw material by varying the following method conditions: Flow Rate 0.8mL/minute ± 10%. Column Temperature 40°C ± 10%. The results of robustness are summarized in TABLE 2.

Ruggedness

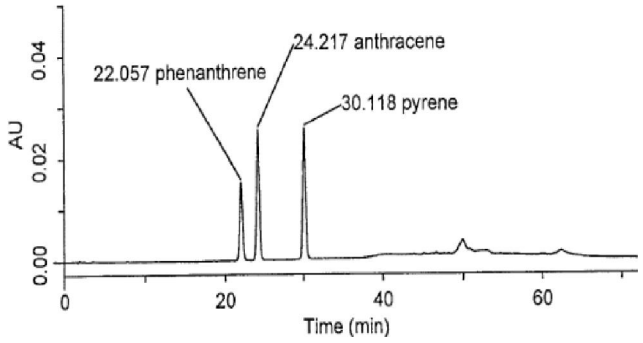
Intermediate precision was also studied using different column and performing analysis on different day. The mean of n=18 determination of three analyst on three different day was 8.15% w/w with the %RSD of 1.68%.

Application of developed method

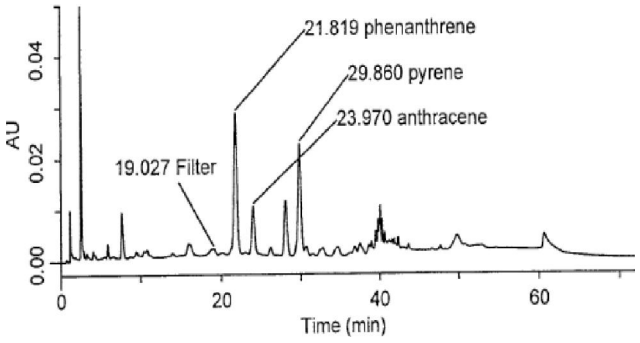
The Developed method is stability indicating and can be used for the quantitative determination of the % of coal tar in any formulated product with the help of sum of areas of three major marker peaks (PAP) in presence of degradation products in stability by the industry. On the same concept other pharmaceutical formulations containing the natural products can also be quantitated in initial release and stability monitoring.

RESULTS AND DISCUSSION

Developing a method for natural product is a great challenge and is tedious. Coal tar solution is the liquid carbonis detergent composed of coal tar (Coal tar or crude coal tar is obtained by the destructive distillation of bituminous coal at very high temperatures). It is believed that over 10,000 different compounds make up coal tar but only 400 or so have been identified. Devel-



Typical chromatograms of standard



Typical chromatogram of sample

opment of HPLC method was carried out with the test for solubility of various components in a mixture of organic solvent and aqueous solvent in different ratio. The ratio of water: acetonitrile at 65:35 was selected. Chromatographic separations of individual peaks including unknown peaks were established on reversed-phase at 240nm. Phenanthrene, Anthracene and Pyrene were the automatic choice as marker peaks because of their maximum quantity in the Coal Tar solution with respect to the other small peaks. Apart from these three marker peaks additional peak was also observed in the sample preparations, which was well separated from the peak of interest and did not undergo any major degradation in all the specificity conditions. The fourth peak was not considered for the quantifications as the available standards at the time of development matches with only three marker peaks PAP. However as already stated that the fourth peak doesn't impact the estimation of coal tar in specificity experiments. The coal tar assay reflects the sum of three marker peaks in the coal tar solution used in manufacturing of foam.The formulation product was prepared by addition of known amount of coal tar in the formulation. The sum of the three marker peaks in the coal tar formulation reflects the amount (%) of coal tar in the compounded foam.

$$\text{Coal tar solution } \% = \frac{\text{\% sum of coal tar peaks in foam}}{\text{\% sum of coal tar peaks in API}} \times 100$$

After satisfactory method development, it was subjected to method validation as per ICH guidelines[1]. The method was validated to demonstrate that it is suitable for its intended purpose by standard procedure to evaluate adequate validation characteristics. The result of system suitability parameter was found to be complying with acceptance criteria: relative standard deviation of six replicate injections was not more than 2.0% and resolution between three marker peaks phenanthrene, anthracene and pyrene were 2.4 and 6.5. The result of specificity study ascertained the separation of degradation peaks from three marker peaks and the spectral purity of all exposed samples were found spectrally pure and data of degradation studies are shown in TABLE 1A and 1B. Out of the three marker peaks anthracene shows significant degradation in H_20_2, UV and visible light. Under UV and Visible light degradation different time exposure has been optimized to achieve the justified % degradation for method validation. Phenanthrene and pyrene does not undergo any degradation in any conditions[3]. The peak purity of these analytes were detected by comparing the UV spectra of the peaks in the formulation against those in the standard. It shows that the peaks are pure indicating that these three markers can be used to track changes on self life stability. In conclusion, the HPLC method could be used as stability indicating assay method for assaying three known components of the coal tar from drug products.

The accuracy studies were performed by spiking PAP in to the placebo in triplicate at 80%, 100% and 120% of the method target concentration levels.The percent recovery of the sum of PAP was found to be between 97% to 103%.The %RSD of the percent recovery for replicate determination was less than 3.0%. The calibration curve of PAP was obtained by plotting the peak area of individual marker versus concentration over the range of about 0.25-0.75 µg/mL and were found to be linear (r = 0.999). The standard and sample solution was found to be stable in diluents up to 48 hrs and there is no filter interference. The variation in flow rate and temperature had no significant impact on the resolution, tailing and purity of PAP. The applicability

of the method was verified by the determination of PAP in Foam stability sample of formulation (40°C/75%RH, 3Month). The % assay of PAP formulations was found to be satisfactory in all stability conditions and there was no significant change with respect to assay values.

CONCLUSION

The Developed method is stability indicating and can be used for the quantitative determination of the % of coal tar in formulated product with the help of sum of areas of three major marker peaks in presence of degradation. The developed method can only be used in a quality control environment to monitor the amount of coal tar in a manufactured product. Coal tars are not consistent in their composition and this method is only useful when the particular coal tar used in a formulation is available to prepare the standard.

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