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Development of RP-HPLC Method for Estimation of Dithranol in Hydrogel based Lipid Nanoparticle Formulation

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Abstract — A simple, specific, accurate, precise and reproducible method has been developed and validated for the estimation of Dithranol in lipid nanoparticle by RP-HPLC method. RP-HPLC estimation of dithranol in prepared formulation was performed using Phenomenex ODS 5µ, C18 column and Acetonitrile: Methanol: Buffer (20:20:60) as mobile phase which had shown sharp peak when detected at 258 nm. The linearity range was found to be in concentration range of 20-100 µg/mL for dithranol. The retention time, correlation coefficient and mean percentage recovery was 9.3 minutes, 0.9989 and 98.90 respectively. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. Proposed method was validated for its precision, specificity, ruggedness and accuracy according to ICH guidelines. This method can be applied successfully in routine work for the determination of dithranol in prepared hydrogel based formulations.

Keywords — Psoriasis, Dithranol, RP-HPLC method, chromatogram, hydrogel.

1. INTRODUCTION

Dithranol belongs to the family of hydoxyanthrones, which have been used in the treatment of psoriasis for more than a century[1]. The therapeutic action of dithranol has been linked to its ability to generate free radicals[2]. Dithranol has been shown to accumulate in the mitochondria where it induces morphological and functional changes. This affects supply of cellular energy which, in turn, results in inhibition of energy dependent process such as DNA replication which slows down excessive cell division as seen in plaque type psoriasis. Cyclic nucleosides play an important role in the regulation of epidermal cell division. The psoriatic hyperproliferative epidermis contains elevated levels of cyclic guanosine monophosphate. It has been found that dithranol reduces the elevated level of cGMP back to normal level; it could be an additional mechanism of action for the dithranol. Dithranol was used successfully by Ingram in 1953[3] to treat chronic plaque psoriasis with the introduction of short contact therapy[4]. Literature survey revealed that the methods reported earlier were only for the analysis of dithranol like Reverse Phase HPLC in Pharmaceutical Ointment Forms[5], HPLC in hydro gel[6], HPLC and MASS in Dithranol: polyvinylpyrrolidone dispersion[7], Mass spectrometric HPLC and photostability of dithranol[8]. This paper presents easy, accurate, reproducible, rapid HPLC method for the analysis of dithranol in hydrogel formulations.

2. MATERIALS AND METHODS

Material:

Dithranol standard (Standardized known purity of Dithranol Himedia Ltd, Banglore) Acetonitrile (HPLC Grade Rankem), Methanol (HPLC Grade Rankem), water (Milli-Q, Millipore), Hydrochloric acid (GR grade, Merck), Sodium hydroxide (Pure, Merck), Hydrogen peroxide (Pure, Merck) and Potassium dihydrogen phosphate (HPLC Grade Rankem)

Standard Stock solution

Stock solution containing dithranol was prepared in methanol having concentration 1000 µg/ mL. Aliquot of the standard solution was appropriately diluted with the mobile phase containing Acetonitrile, methanol and buffer pH 2.2 in the ratio 20:20:60 v/v to get the concentration of 40µg/mL

Instrument

Shimadzu HPLC 1100 series chromatograph equipped with quaternary gradient mode and photo diode array (PDA) detector, Rheodyne Manual injector with 20µL loop and a reversed phase 5µ Phenomenex OSD C18 column with pore size of 100A^{o} was used for the chromatographic studies. Citizen digital balance was used for weighing the samples. Data acquisition was performed by LC solution software. Analysis was carried out at 258nm with a reversed phase phenomenex C18 column at 25°C temperature with mobile phase consisting of Acetonitrile, methanol and buffer pH 2.2 in the ratio 20:20:60 v/v. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system. Injection volume was set at 20 µl, detection at 258 nm, and run time 30 mins. All the chemicals and solvents used were of HPLC grade. Double distilled water and filter paper (Whatmann no.41) were used throughout the experimental work.

Procedure

The chromatographic condition mentioned here under was kept constant throughout the experimentation and

mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Column - Phenomenex ODS 5 µ C18 column
Detection Wavelength - 258 nm
Flow rate - 1.0 mL/min
Temperature: Ambient - (28-30^{0}C)
pH - 2.2
Buffer - Potassium dihydrogen phosphate

A 20 µL solution of above standard was injected through manual injector and chromatogram was recorded using mobile phase containing acetonitrile, methanol and buffer pH 2.2 (20:20:60). Dithranol had shown sharp peak with reasonable retention time in the above selected mobile phase. A chromatogram for dithranol so recorded is in shown in **Fig.1**.

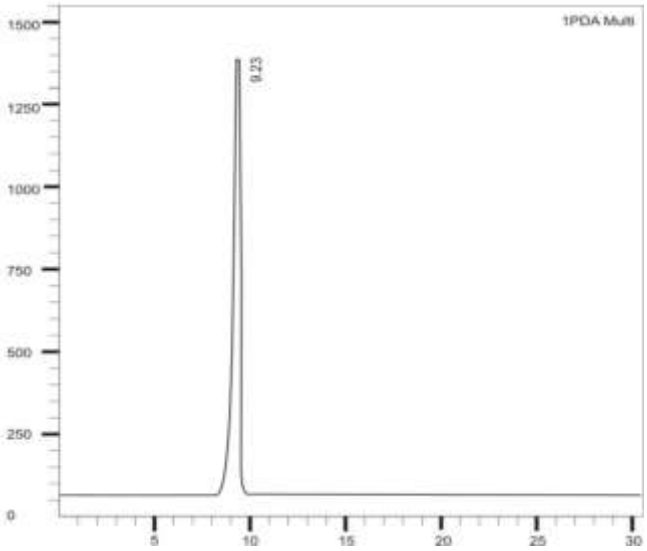

Fig.1. Chromatogram for dithranol

Study of System Suitability Parameters:

After equilibration of column with mobile phase reached, seven replicate injections of 20 µL solution of standard solution was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in **Table 1.**

Table1: System suitability parameter

S. No.	Parameter	Values
1	Mobile phase	Acetonitrile, Methanol and Phosphate Buffer pH 2.2 (20:20:60)
2	Retention time	9.23
3	Asymmetry	1.115
4	Efficiency	65580

Study of Linearity Range

Aliquots of standard stock solution were diluted in range 1.0 mL to 5.0 mL in 50 mL volumetric flask with mobile phase, volume was made up to mark with mobile phase to obtain concentration 20µg/mL to 100µg/mL for dithranol. The graphs of concentration of drug vs. area under curve were plotted. The correlation coefficient was found to be 0.9989.

Assay in prepared Formulation

An accurately weighed quantity of formulated hydrogel equivalent to 10.0 mg of dithranol was transferred to 25.0 mL volumetric flask, sonicated (Lark lab., Chennai, India) for 30 minutes with sufficient quantity of methanol and volume was made up to mark with methanol. The contents of the flask were filtered through filter paper (Whatmann no.41). A 5.0 mL portion of the filtrate was further diluted to 50.0 mL with a mobile phase. The sample solution was injected and the chromatogram was recorded. The content of dithranol was calculated by comparison of the standard area and sample area and results are shown in **Table 2.**

Table 2: Results of prepared formulation and recovery study

Drug	Mean of % label claim* ± S.D.	Mean Recovery* ± CV
Dithranol	101.31 ±1.80	100.62 ± 1.01

*Mean of five observations, S.D. = Standard Deviation, CV= Coefficient of variance

3. VALIDATION

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalyzed by proposed method and the mean % recovery was found to be 100.62.

Stability Studies

The forced degradation studies were carried out at 500C using 1ml of 1N NaOH, 1N HCL, 6% H2O2 and the chromatograms recorded are shown in fig 2, 3, 4 respectively. Volumes were made up to the mark with methanol, further aliquots were diluted with mobile phase and sample solutions were injected separately and chromatograms under stress conditions were recorded. The results showed slight difference in the percent label claim as compared with normal condition. In all the stress condition dithranol was found to be more sensitive to hydrolysis and oxidation. The results are shown in **Table 3.**

Table 3: Forced degradation study

Con-dition	% label claim			
	No treatment	1N NaOH	1N HCl	6% H_2O_2
%estimation	97.92	96.23	95.20	81.91

Precision and Intermediate Precision:

Intra day and Inter day parameter shows the % Label claim values within limits (% R.S.D. not more than 2). The method was found to be précised. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision and ruggedness parameter are shown in **Table 4**.

Table 4: Intermediate precision and Ruggedness study

S. No.	Parameters (n=3)	Mean % label claim ±S.D.
1	Different Analyst	100.38 ±0.54
2	Intraday Variation	99.95±0.32
3	Inter day Variation	99.25 ±0.85

S.D. = Standard Deviation

Linearity and Range

Accurately weighed quantities of hydrogel content equivalent to about 80, 90, 100, 110 and 120% of label claim of dithranol were taken and dilutions were made as described under assay in prepared formulation. The chromatograms of the resulting solutions were recorded. The plot of AUC Vs Percent label claim was found to be linear with correlation coefficient of 0.9976.

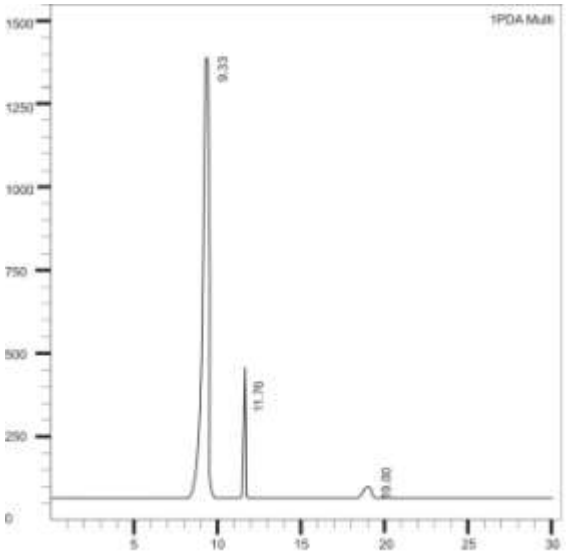

Fig.2. Chromatogram of dithranol (1N NaOH)

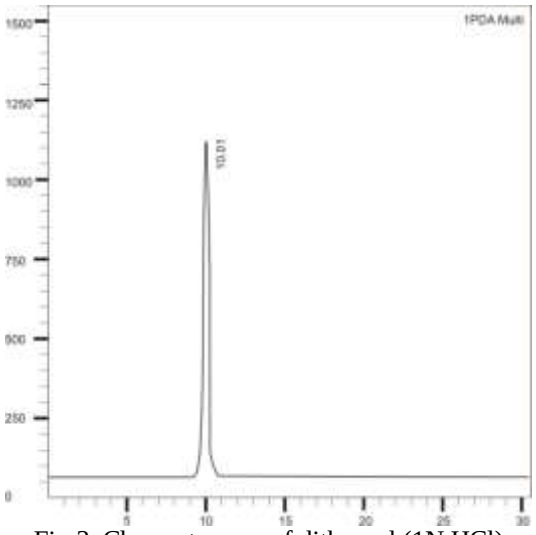

Fig.3. Chromatogram of dithranol (1N HCl)

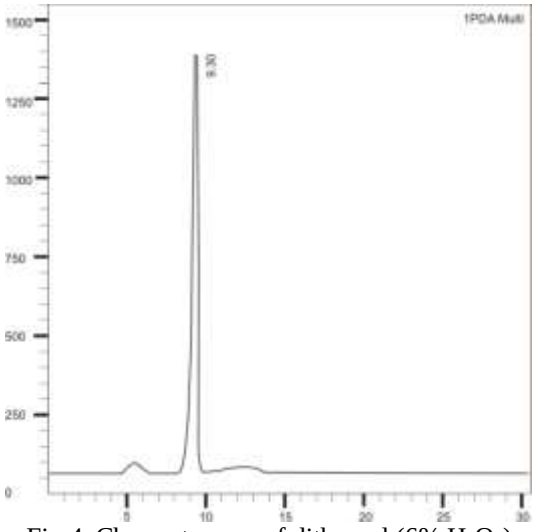

Fig.4. Chromatogram of dithranol (6% H_2O_2)

4. Conclusion

Topical therapy whether it is conventional or novel is always choice of delivery system for pharmaceutical technocrats. Estimation of drug in such delivery vehicles is always needed. A simple, specific, accurate, precise and reproducible method has been developed and validated for the estimation of Dithranol in lipid nanoparticle by RP-HPLC method. To ensure the reliability and accuracy of the method recovery studies were carried out and found to be 100.62. Dithranol had shown sharp peak with retention time of 9.23 in the above selected mobile phase while correlation coefficient was found to be 0.9989.

5. Reference

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6. CONFLICT OF INTEREST:

None declared