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A Validated HPTLC Method for Determination of Arbidol from Pharmaceutical Formulation

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ABSTRACT: A simple, specific and precise high performance thin layer chromatographic method of analysis of Arbidol, both as a bulk drug and in formulation was developed and validated. The method employed TLC (Thin Layer Chromatography) aluminum plates pre-coated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of dichloromethane: methanol (9:1, v/v). This system was found to give compact bands for Arbidol (R_f 0.70±0.02). Densitometric analysis of Arbidol was carried out in the absorbance mode at 254 nm and 315nm. Linear regression analysis data for the calibration spots showed good relationship with regression coefficient $r^2 = 0.9917$ in the range of 400-2000 ng/ band. The limits of detection and quantitation were 32 ng/ band and 98 ng/ band respectively.
KEYWORDS : Arbidol, HPTLC.

1. INTRODUCTION

Arbidol,1-methyl-2-((phenylthio)methyl)-3-carbethoxy-4-((dimethylamino)methyl)-5-hydroxy-6-bromindole hydrochloride (Fig. I) is an antiviral active chemical entity.[1] It has been in use in Russia for several years for the treatment of influenza. Arbidol is a Russian-made potent broad-spectrum antiviral with demonstrated activity against a number of enveloped and non-enveloped viruses.[2] Orally administered arbidol at 50 or 100 mg/kg/day beginning 24 h pre-virus exposure for 6 days significantly reduced mean pulmonary virus yields and the rate of mortality in mice infected with FLU-A (A/PR/8/34 H1N1).[3] The compound exerts its effect by activation upon phagocytic activity of the macrophages, and also stimulates some forms of cellular and humoral immunity. Arbidol inhibits viruses of influenza type A and type B and has the capacity to induce serum interferon. Metabolic and pharmacokinetic studies in animals have shown that orally administered arbidol is rapidly absorbed and distributed quickly into tissues and organs.[4] A method based on cloud-point extraction (CPE) was developed to determine arbidol in rat plasma by high performance liquid chromatography separation and ultraviolet detection (HPLC-UV).[5] The use of LC–ESI-MS method for determination in human plasma has been reported.[6,7] To the best of our knowledge there is no HPTLC (High Performance Thin Layer Chromatography) method for determination of Arbidol. Hence the objective of the study was to develop a simple, accurate and precise HPTLC method for quantitative estimation of Arbidol as bulk drug as well as in formulation.

2. MATERIALS AND METHODS

Arbidol (purity 99.78%) was provided as a gift sample by Alkem Laboratories Ltd. Mumbai, India and was used without further purification. All the other reagents used were of analytical grade. Dichloromethane (AR grade), Methanol (AR grade), Chloroform (AR grade), Acetone (AR grade) were purchased from Thomas Baker (chemicals) Pvt Limited, India.

2.1 Instrumentation

Chromatographic separation of drug was performed on Merck TLC plate pre-coated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a band with 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-2000 ng per band and operated by winCATS software (V 1.4.2, Camag).

2.2 Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that the drug showed considerable absorbance at 254 nm and 315 nm. (Figure II). So, 254 nm and 315 nm were selected as the wavelength for detection

2.3. Method validation

2.3.1 Linearity

A stock solution of Arbidol (2000 µg /ml) was prepared in methanol and diluted suitably to obtain concentration of 200 µg /ml. Different volumes of the dilution, 2, 4, 6, 8, 10 µl were spotted on TLC plate to obtain concentration of 400-2000 ng/ band of Arbidol, respectively. The data of peak area v/s drug amount were treated by linear least-square regression analysis.

2.3.2. Precision

The intra and inter-day variation for the determination of arbidol was carried out at three different concentration levels of 800, 1200 and 1600 ng per spot. The % RSD values were determined for intra-day and inter-day variation.

2.3.3. Accuracy

The analysed samples were spiked with 80, 100 and 120 % of the standard Arbidol and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug by standard addition method.

2.3.4. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard formula as per the ICH guidelines, where σ is the standard deviation of response.

Formula :-

LOD = 3.3 × Standard Deviation ÷ slope

LOQ =10 × Standard Deviation ÷ slope

2.3.5. Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peaks was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on winCATS software.

2.4. Analysis of the marketed formulation

To determine the content of Arbidol in suspension (label claim: each 5ml of suspension contains 90.90 mg of Arbidol). 1.2gm (Wt per ml = 1.2 gm) of suspension was weighed and transfered to a 100 ml of beaker. Methanol was added gradually upto 10 ml. After each addition, the solution was stirred with the help of magnetic stirrer. This beaker was kept covered with aluminum foil. This solution was centrifuged for 10min. and filtered through whatmann filter paper No. 41. Finally volume was made upto 10ml with methanol to get stock solution of (1800 ng/µL). The solution was suitably diluted. Appropriate volume of solution was applied on TLC plate followed by development and scanning.

3. RESULTS AND DISCUSSION

3.1. Development of the optimum mobile phase

TLC procedure was optimized with a view to develop an accurate assay method. The drug reference standard was spotted on the TLC plate and developed in different solvent systems. The mobile phase Dichloromethane: Methanol (9:1 v/v) gave sharp and symmetrical peak with R_f 0.55. Well-defined bands were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature. The representative densitogram is given in (Figure III).

3.2 Validation of the method

3.2.1 Linearity

The response for the drugs was found to be linear in the concentration range 400–2000 ng / band with correlation co-efficient of 0.9917

3.2.2 Precision

The % RSD value for intra-day and inter-day variation study was found to be not more than 0.725 % and 1.21 % respectively, thus confirming precision of the method.

3.2.3 Recovery

Acceptable recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80 %, 100 %, 120 % level) indicated the mean recovery 101.21%

3.2.4 Limit of Detection and limit of Quantitation

The limit of detection and limit of quantitation as calculated by standard formula as given in ICH guidelines was found to be 32 ng / band and 98-ng/ band respectively.

3.2.5 Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s, m) 0.9978 and r(m, e) 0.9959 for arbidol, indicating the non interference of any other peak of degradation product, impurity or matrix. The validation results are listed in Table I.

3.3Analysis of marketed formulation

There was no interference from the excipients present in the suspension. The drug content was found to be 101.05 %.

4. CONCLUSION

The developed method was found to be simple, precise, and sensitive. High throughput ability of HPTLC makes it a very useful method for routine analysis of bulk drug as well as formulation.

Table I: Validation Parameters.

S.N.	Validation Parameter	Arbidol
1	Linearity Equation (r^2) Range	y = 521.82x + 59.93 (0.9917) 400 – 2000 ng per band
2	Precision (% RSD) Intraday Interday	 NMT 0.725 % NMT 1.21 %
3	Accuracy (% mean recovery)	101.21 %
4	LOD	32 ng / band
5	LOQ	98 ng/ band
6	Specificity Peak Purity	Specific r(s,m) = 0.9978 r(m,e) = 0.9959

Fig. I: Structure of Arbidol.

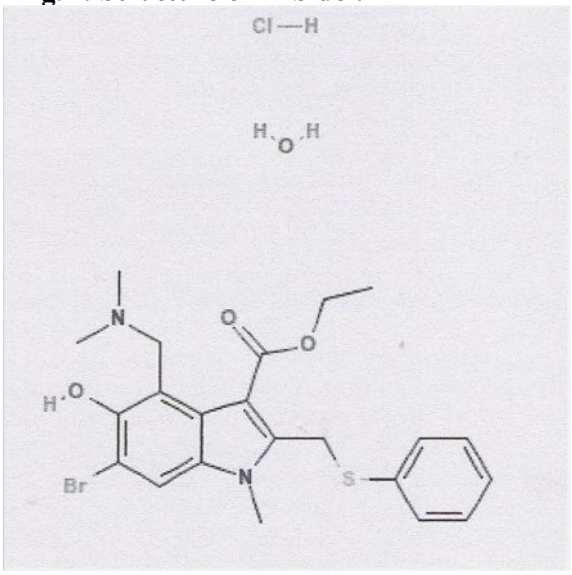


Fig. II: Spectrum of Arbidol.

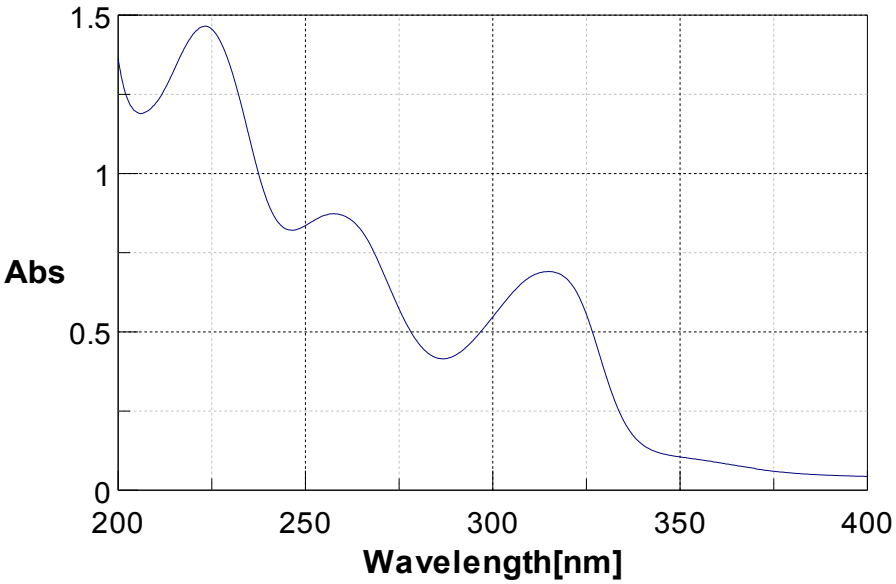
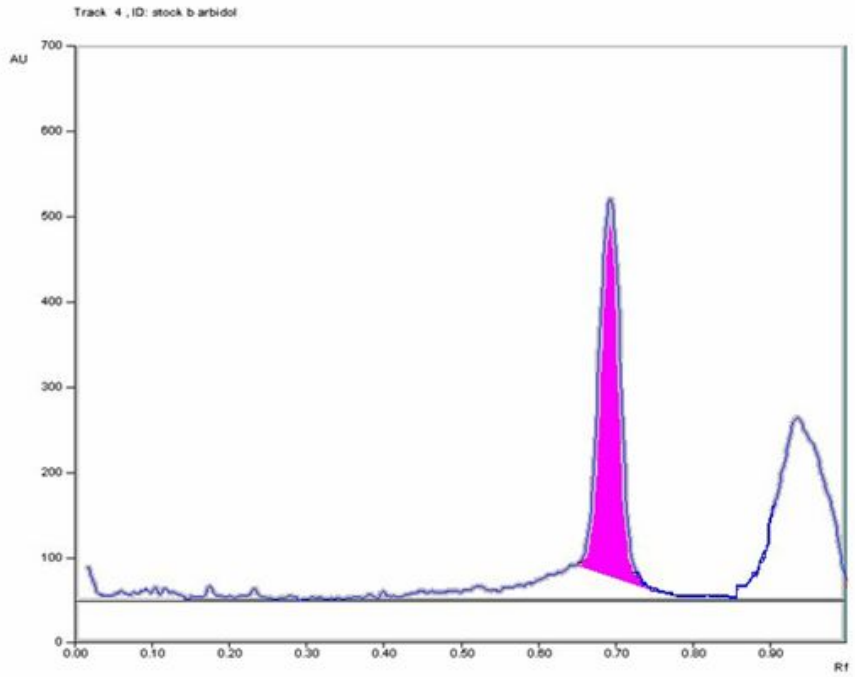


Fig. III: Representative Densitogram of Arbidol



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