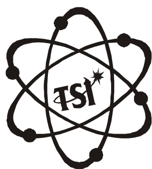

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Stress degradation monitoring of arbidol by HPTLC method

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ABSTRACT

A simple stability-indicting high performance thin layer chromatographic method of analysis of Arbidol as a bulk drug 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 Methanol: Chloroform: Gl. Acetic acid. This system was found to give compact bands for Arbidol (R_f 0.20±0.03). 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 = 0.9973 in the range of 400-2000 ng/band. The limits of detection and quantitation were 35 ng/ band and 106 ng/ band respectively. Stress degradation studies were carried out as per ICH guidelines. There was no interference at the peak for Arbidol as confirmed from the peak purity profile. © 2011 Trade Science Inc. - INDIA

KEYWORDS

Arbidol;
Stability indicating HPTLC.

INTRODUCTION

Arbidol,1-methyl-2-((phenylthio)methyl)-3-carbethoxy-4-((dimethylamino)methyl)-5-hydroxy-6-bromindole hydrochloride, 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]. 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) has been reported 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]. A simple HPTLC method has been reported for routine analysis[8]. To the best of our knowledge there is no stability-indicating HPTLC method reported for determination of Arbidol. Hence the objective of the study was to develop a stability indicating HPTLC method for quantitative estimation of Arbidol as bulk drug.

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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. Glacial acetic acid (AR grade), Methanol (AR grade), Chloroform (AR grade) were purchased from Thomas Baker (chemicals) Pvt Limited, India.

Instrumentation

Chromatographic separation of drug was performed on TLC plates pre-coated with silica gel 60 F_{254} (10 cm ×10 cm with 250 m 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 10×10 cm). Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm and operated by winCATS software (V 1.4.2, Camag).

Chromatographic parameters

(1) **Stationery phase :** Aluminium TLC plates Precoated with Silica gel 60F_{254}
(2) **Mobile phase :** Methanol:Chloroform:Gl. Acetic acid ::1:9:0.5
(3) **Detection wavelength** : 315 nm

Figure 1 shows a representative densitogram.

Stress degradation studies

A stock solution containing 50 mg Arbidol in 25 mL Methanol was prepared. This solution was used for forced degradation.

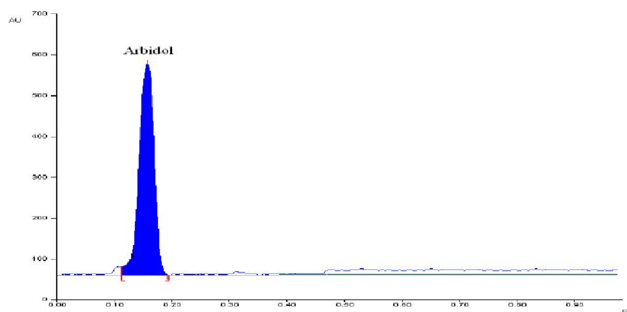

Figure 1 : Representative densitogram of Arbidol 1000 ng/ band

Degradation under acid and alkali catalysed hydrolytic condition

To 5 mL stock solution, 5 mL of 5 N NaOH and 5 N HCl were added separately. The volume was made upto 50 ml with methanol. These mixtures were refluxed for 2 h at 80°C. Appropriate volume of resultant solution (1000 µg per band) was applied on TLC plate and densitograms were developed.

Degradation under neutral hydrolytic condition

To 5 mL stock solution, 5 ml distilled water was added. The volume was made upto 50 ml. The mixture was refluxed for 2 h at 80°C. Appropriate volume of resultant solution (1000 ng per band) was applied on TLC plate and densitograms were developed.

Degradation under oxidative condition

To 5 mL stock solution, 5 mL of 30 % H_2O_2 was added. The volume was made upto 50 ml with methanol. The mixture was refluxed for 2 h at 80°C. Appropriate volume of resultant solution (1000 µg per band) was applied on TLC plate and densitograms were developed.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80°C) for a period of 48 hours. Accurately weighed 50 mg of drug was transferred to the 50 ml of volumetric flask and dissolved in methanol; the volume was made up with methanol. Appropriate volume of resultant solution (1000 µg per band) was applied on TLC plate and densitograms were developed.

Photo-degradation studies

The photochemical stability of the drug was also studied by exposing the drug sample to UV light up to illumination of 200 watt hr/m^2 followed by visible light up to illumination of 1.2 million Lux hr. Accurately weighed 50 mg of drug was transferred to the 50 ml of volumetric flask, the volume was made up with methanol. Appropriate volume of resultant solution (1000 ng per band) was applied on TLC plate and densitograms were developed.

Method validation

The method was validated as per ICH guidelines[9].

Linearity

TABLE 1 : Method validation parameters

S.N.	Validation parameter	Arbidol
1	Linearity Equation (r^2) range	y = 273.4 x + 2390 (0.9973) 400 – 2000 ng per band
2	Precision (% RSD) intraday interday	NMT 0.85 % NMT 1.41 %
3	Accuracy (% mean recovery)	100.23 %
4	LOD	35 ng / band
5	LOQ	106 ng/ band
6	Specificity peak purity	Specific r(s,m) = 0.9955 r(m,e) = 0.9958

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.

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 band. The % RSD values were determined for intra-day and inter-day variation.

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.

Limit of detection and limit of quantitation

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

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.

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 μg/μL). The solution was suitably diluted. Appropriate volume of solution was applied on TLC plate followed by development and scanning.

RESULTS AND DISCUSSION

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 Methanol: Chloroform: Gl. Acetic acid (9:1:0.5 v/v/v) gave sharp and symmetrical peak with R_f 0.20 ± 0.03. Well-defined bands were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

Validation of the method

Linearity

The response for the drugs was found to be linear in the concentration range 400–2000 μg / band with correlation co-efficient of 0.9973.

Precision

The % RSD value for intra-day and inter-day variation study was found to be not more than 0.85 % and 1.41 % respectively, thus confirming precision of the method.

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 100.23%.

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 35 ng / band and 106 ng/ band respectively.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s, m) 0.9955 and r(m, e) 0.9958 for arbidol, indicating the non interference of any other peak of degradation product, impurity or matrix. The validation results are listed in TABLE 1.

Analysis of marketed formulation

There was no interference from the excipients present in the suspension. The drug content was found to be 99.05 %.

Stress degradation results

Arbidol was found to be prone to acid as well as base catalysed hydrolysis. There was an unusual observation upon sample exposure to heat or light. The drug peak area increased without affecting the peak purity value. Methanolic drug solutions were found to darken upon exposure to UV light and peaks for the products of degradation appeared at higher R_f values.

CONCLUSION

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

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REFERENCES

[1] www.curevents.com/vb/showthread.php?p=161013 Accessed on 13/12/09
[2] Y.S.Boriskin, I.A.Leneva, E.I.Pecheur, J.Polyak; Current Medicinal Chemistry, **15(10)**, 997-1005 **(2008)**.
[3] L.Shi, H.Xiong, J.He, H.Deng, Q.Li, Q.Zhong, W.Hou, L.Cheng, H.Xiao, Z.Yang; Archives of Virology, **152(8)**, 1447-55 **(2007)**.
[4] http://en.wikipedia.org/wiki/Arbidol Accessed on 13/12/2009
[5] X.Liu, X.H.Chen, Y.Y.Zhang, W.T.Liu, K.S.Bi; Journal of Chromatography B., **856(1-2)**, 273-7 **(2007)**.
[6] X.Liu, Y.W.Huang, J.Li, X.B.Li, K.S.Bi, X.H.Chen; Journal of Pharmaceutical and Biomedical Analysis, **1**, 371-375 **(2007)**.
[7] O.S.Anisimova, L.V.Frolova, V.V.Chistyakov, I.A.Ermachenkov, I.V.Golovanova, S.A.Zotova, A.P.Pleshkova, V.A.Yadrovskaya, Y.N.Sheinker; Pharmaceutical Chemistry Journal, **29**, 78-82 **(1995)**.
[8] M.C.Damle, S.A.Phadtare, H.D.Raskar, K.G.Gadge, Ratnakar Mehendre, K.G.Bothara; International Journal of Chem.Tech.Research, **2(2)**, 1042-1046 **(2010)**.
[9] Validation of Analytical Procedures, Text and Methodology Q1A (R2), ICH Harmonized Tripartite Guideline, 1 **(2003)**.