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VALIDATED HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR DETERMINATION OF ARIPIPRAZOLE IN TABLET

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ABSTRACT

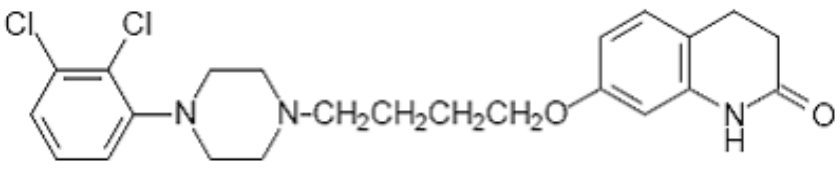
A simple validated high-performance thin-layer chromatography method has been proposed for the determination of Aripiprazole in a tablet dosage form. The separation was achieved on silica gel 60 F_{254} coated aluminum sheet as stationary phase using toluene: methanol (8.5:1.5 v/v) as mobile phase which gave compact spots with R_f value 0.48 ± 0.02. Quantitative densitometric evaluation was done in absorbance-reflectance mode at 254 nm. The response was found to be linear over concentration range of 100-500 ng/spot with correlation coefficients 0.999 and 0.999 and mean percentage recovery of the drug was observed to be 98.18 ± 0.670 and 99.28 ± 0.287 by peak area and peak height, respectively. The method was validated for linearity, accuracy, range, precision and robustness according to ICH Q2 (R1) guidelines. The method is simple, accurate, precise and was successfully applied to the assay of drug in tablet formulation.

KEY WORDS

Aripiprazole, Assay, HPTLC, Validation.

INTRODUCTION

Aripiprazole- 7- [4- [4- (2, 3-dichlorophenyl)-1-piperazinyl] butoxy]- 3, 4-dihydro -2 (1H)- quinolinone (APZ), is a novel atypical antipsychotic drug approved by the United State Food and Drug Administration as the sixth second-generation antipsychotic for the treatment of schizophrenia, schizoaffective disorders, bipolar disorder and adjuvant therapy for major depression [1,2]. Aripiprazole is considered a partial dopamine D2 and D3 receptor agonist, partial 5-HT1A receptor agonist and 5-HT2A receptor antagonist and has already been on the market in the USA, European countries and several other countries. Dehydroaripiprazole, its main active metabolite, has an affinity for dopamine D2 receptors and thus has some pharmacological activities similar to that of its parent compound. It is distinguished from all other antipsychotics by its unique pharmacological profile – i.e. partial agonist activity at dopamine D2 receptors, partial agonist activity at serotonin 5-HT1A receptors, and antagonist activity at serotonin 5-HT2A receptors [1], [3].



(Fig.1) Aripiprazole

A literature survey revealed analytical methods like capillary electrophoresis (CE) [2], [5]. Spectrophotometric [4]. LC–MS–MS [3], [7]. HPLC-DAD [6], [9]. UPLC-electrospray ionization tandem mass spectrometry (ESI-MS/MS) [8]. There is no reported HPTLC method for its estimation in tablet formulation. A HPTLC method for estimation of APZ in tablet formulation is described in the present article.

MATERIALS AND METHODS

Instrumentation:

HPTLC was performed with Camag HPTLC equipment comprising of Linomat IV sample applicator, Linomat Microliter syringe (*Hamilton- Bonaduz Schweiz*) 100 µL, TLC Scanner- III with win CATS software version 1.4.1 for scanning and documentation, High-tech UV cabinet fitted with dual wavelength 254/ 366 nm, 8 volts UV lamps for visual inspection of HPTLC plates. 20 x 20 cm pre-coated Silica Gel 60 F_{254} TLC aluminum plates (E. Merck, Darmstadt, Germany) with layer thickness 0.2 mm were cut to required size (10 x 10 cm) at the time of use. The TLC plates were washed with methanol by over-run technique and activated at 110 ^{0}C for 5 min. The samples were applied with Linomat IV Sample applicator with the settings- band length, 4mm; distance between bands, 3mm; distance from the plate side edge, 10 mm and distance from the bottom of the plate, 10mm. Linear ascending development was performed in a 10 x 10 cm twin trough glass chamber with stainless steel lid, after its saturation with mobile phase vapour for 10 min. The distance traversed for development being about 8 cm. After development, the plates were dried in a current of warm air and densitometric scanning was performed with a TLC Scanner III at 254 nm in absorbance- reflectance mode.

Reagents and Chemicals:

Methanol, acetonitrile, toluene used were of AR grade, Merck India Ltd, Mumbai (India). Standard drug sample of Aripiprazole (99.70% pure) was obtained as a gift sample from Watson Pharmaceutical Pvt. Ltd, Ambernath (India). The APZ tablets used in this study with a declared content equivalent to 10 mg Aripiprazole were procured from local market.

Chromatographic conditions:

Optimization of chromatographic conditions

Aliquot portions of working standard solution (5 µL) were applied on TLC plates. Various pure solvents with varying polarity and their mixtures were tried for optimum movement of drug with sharp symmetrical peak. After trying several permutations and combinations, the mobile phase containing toluene: methanol (8.5:1.5 v/v) was found to be most satisfactory as it gave sharp symmetrical peaks for the drug with Rf value 0.48 ± 0.02 (Fig. 2 a & b). The migrated band was scanned over the wavelength range 200- 400 nm in an absorbance/reflectance mode and an *in situ* UV-absorption spectrum of drug was obtained. A 254 nm was selected as scanning wavelength as it gave maximum absorption for the drug (Fig. 3 a & b).

Preparation of Aripiprazole stock and working standard solutions

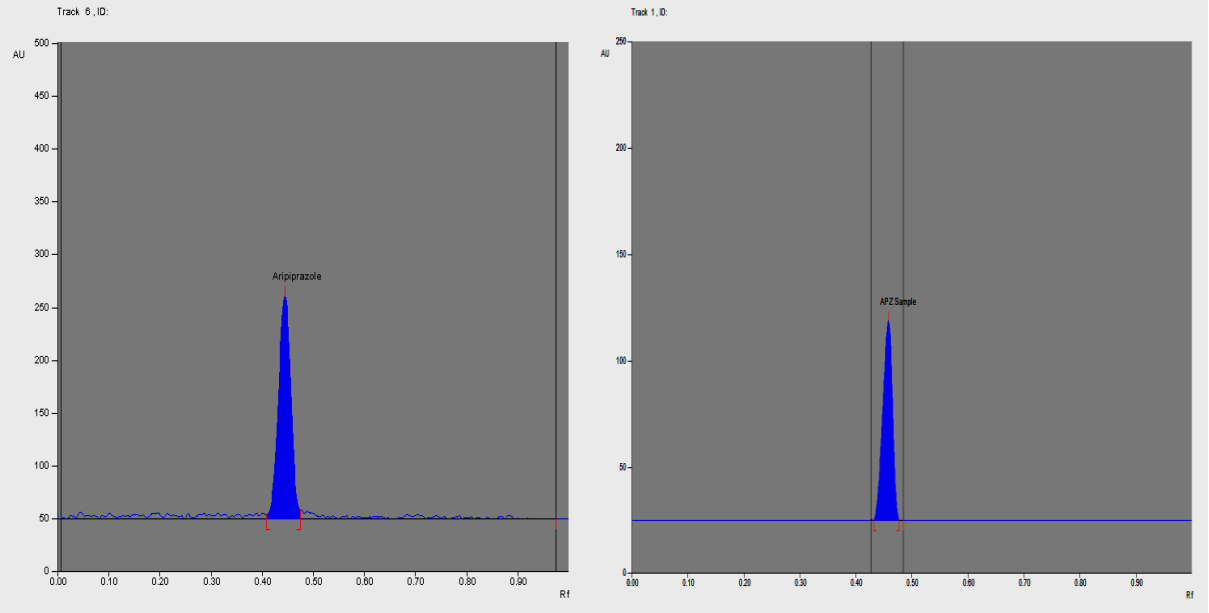
a) **Stock standard solution (Solution A)**
   An accurately weighed quantity of about 10.0 mg of APZ was dissolved in acetonitrile and diluted to 10.0 mL (conc.: 1000.0 µg/mL).

b) **Working standard solution (Solution B)**
   A 0.6 mL of solution A was further diluted to 10.0 mL with methanol to obtain working standard solution (conc.: 60.0 µg/mL).

After chromatographic development, bands were scanned over the range 200–400 nm and *in situ* spectrum were recorded and thus inferred that the estimations can be done at the maximum wavelength 254 nm. A representative chromatogram and *in situ* UV spectra are depicted in Fig. 3 (a) & Fig. 3 (b).

Assay Method:

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed quantity of tablet powder equivalent to about 10.0 mg of APZ was taken into 10 mL volumetric flask, shaken with 8.0 mL acetonitrile, sonicated for 5 minutes and the volume was made up to 10.0 mL with acetonitrile and solution was filtered through Whatman Grade I filter paper. A 0.6 mL of the filtrate was diluted to 10.0 mL with methanol to get concentration of 60.0 µg/mL (on labelled claim basis).



a) **b)**

Fig. 2. Chromatogram of 100 ng spot of APZ Standard (a) & Sample (b)

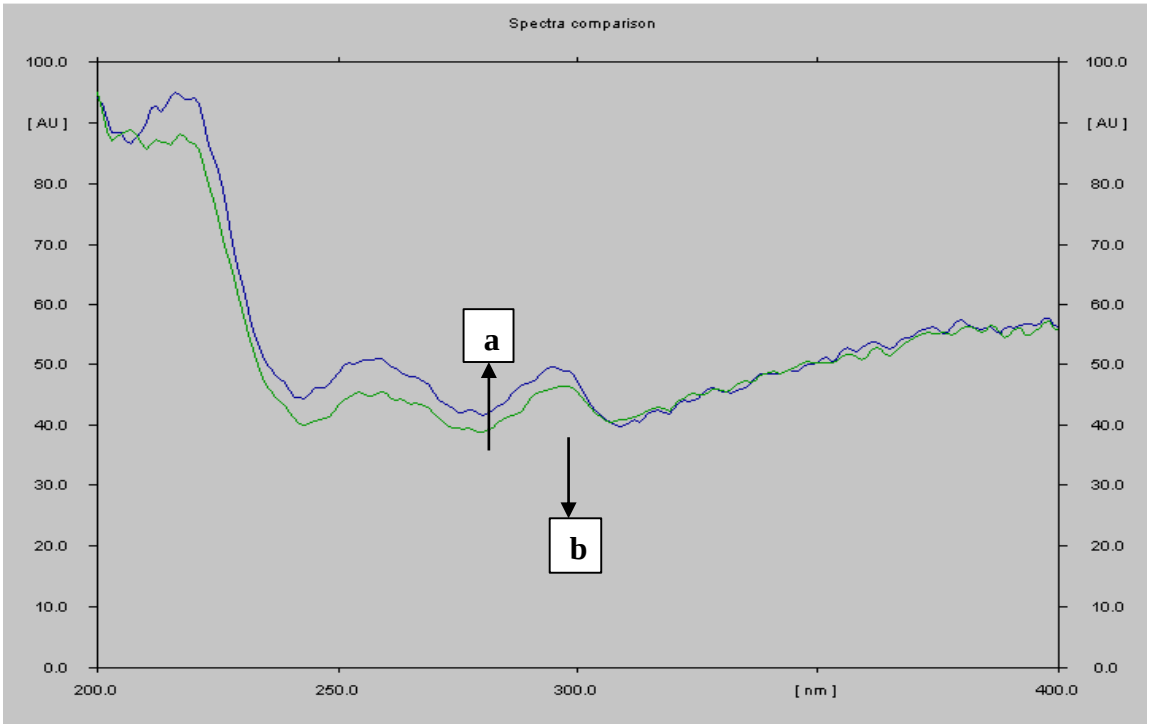


Fig. 3. The overlain UV spectra of 100 ng spot APZ Standard (a) & Sample (b) between 200 and 400 nm

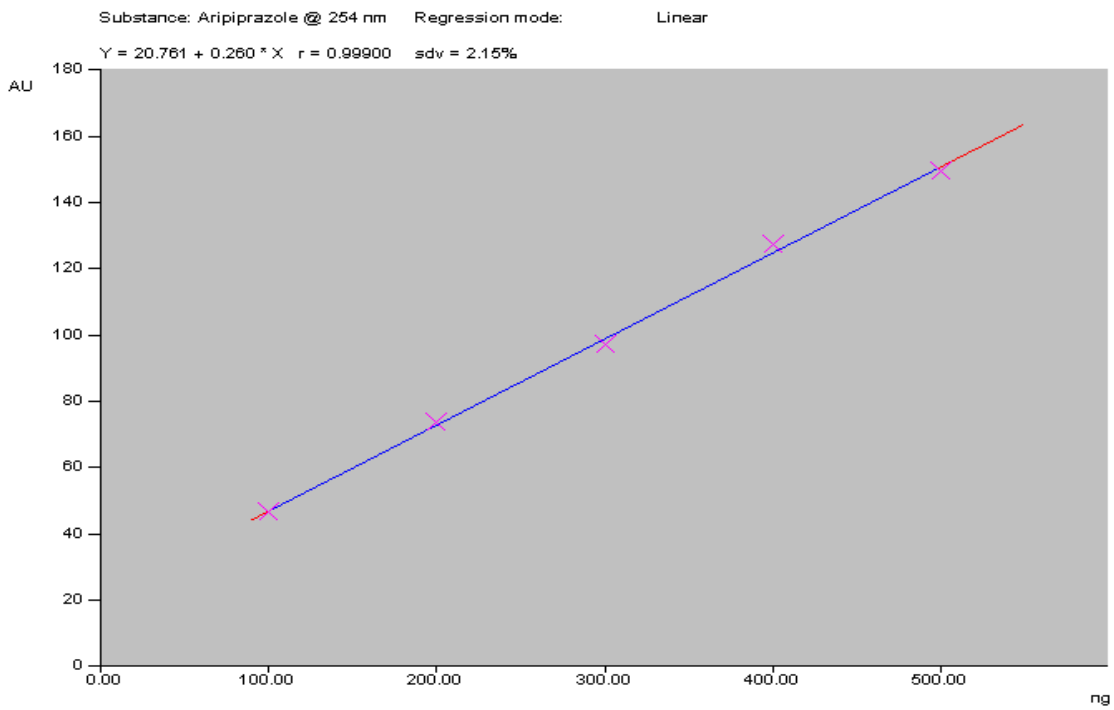


Fig. 4 (a) Linearity study of ARPZ by Peak height

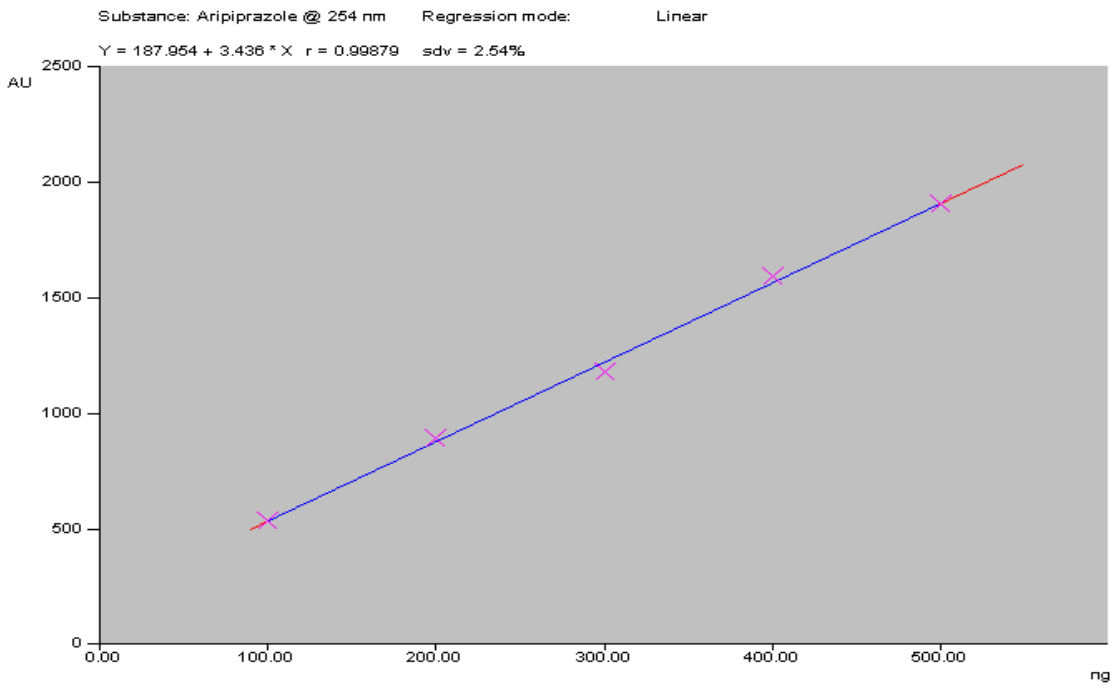
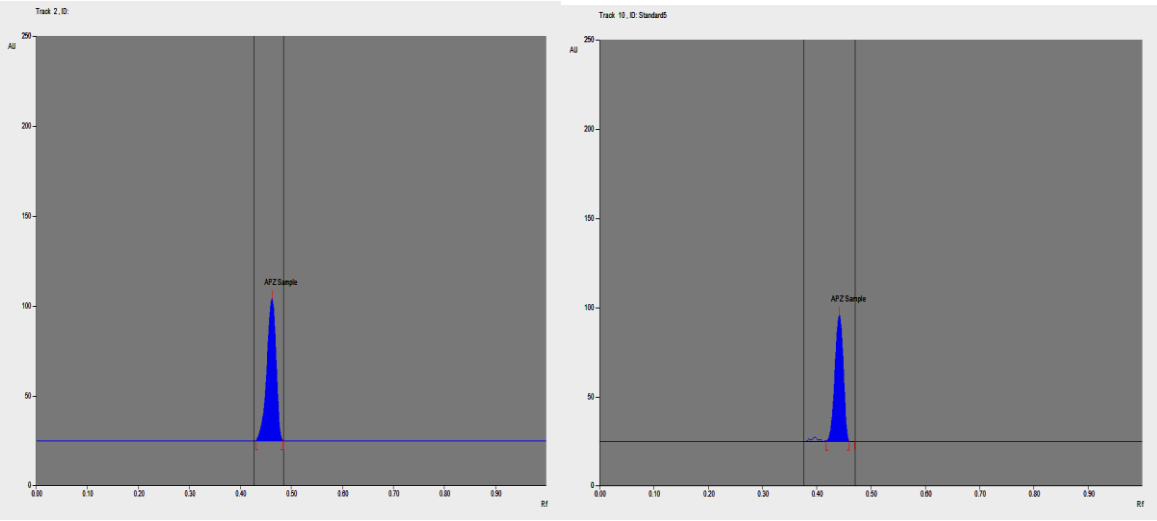
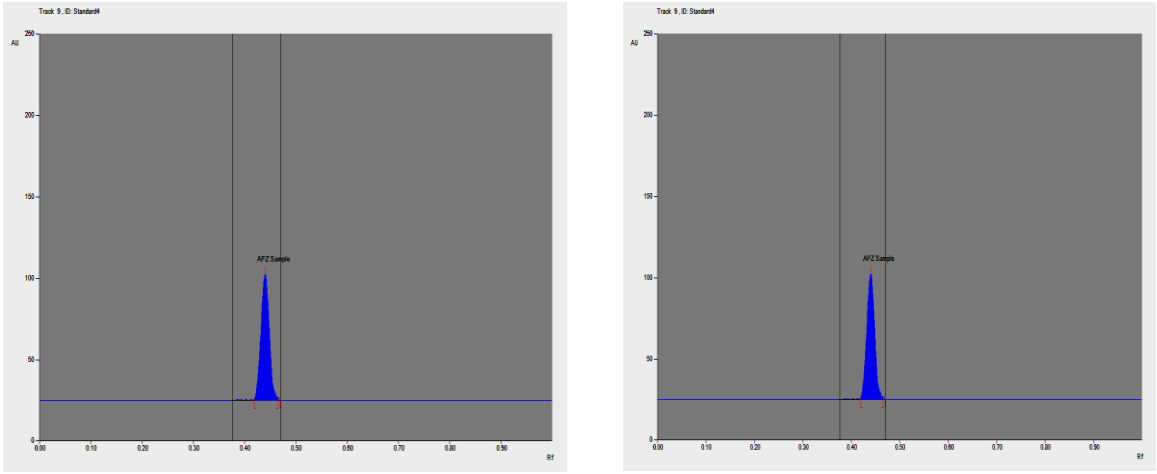


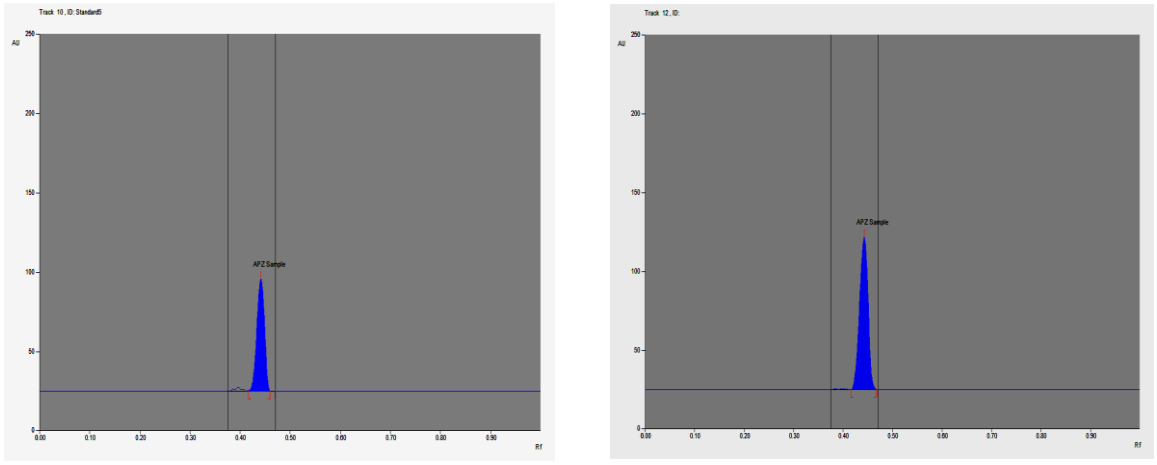
Fig. 4 (b) Linearity study of ARPZ by Peak area



A)Normal, 24 h **B) 0.1M HCl, 24**



C) 0.1 M NaOH, 24 h **D) 3% H_2O_2, 24 h**



E) Thermal, 24 h **F) Sunlight, 24 h**

Fig. 5 (A-F) Chromatogram of specificity studies of APZ tablets

Procedure

Two bands of standard solution and six bands of sample solution of equal volume (5 µL) were applied on TLC plate and the plate was developed and scanned as per optimized chromatographic conditions.

Calculation

Percent of labelled claim were calculated using following formula-

$$\%\ \text{of Labeled claim} = \frac{\text{Au} \times \text{Wstd} \times \text{Wav}}{\text{Astd} \times \text{Ws} \times \text{Lc}} \times 100$$

where,
Au = area/height of sample peak
Astd = area/height of standard peak
Wstd = standard weight (mg)
Ws = sample weight (mg)
Wav = average weight of tablet (mg)
Lc = labelled claim (mg/tablet)

METHOD VALIDATION [10]

Linearity of response:

Aliquots portions (5 µL) of series of standard solutions of five different concentrations of APZ 20.0, 40.0, 60.0, 80.0, 100.0 µg/mL were applied in duplicate (100-500 ng/spot) on TLC plate and chromatograms were developed and scanned under optimized chromatographic conditions.

The linear regression curves are depicted along with correlation coefficient; slope and y-intercept by peak height and area are shown in Fig. 4 (a) & (b). The data of linear regression study is given in Table 1.

Table 1. Results of Linearity studies of APZ by Peak height & Peak area

Concentration range, 100-500 ng/spot		
Parameter	**Peak height**	**Peak area**
Regression equation	**y = 20.761 + 0.260x**	**y = 187.954 + 3.436x**
Slope	**0.260**	**3.436**
Y- intercept	**20.761**	**187.954**
Correlation coefficient (R^2)	**0.99900**	**0.99879**

Table 2. Results of Precision and Accuracy studies of APZ by Peak height & Peak area

Parameter	**Mean ± SD, % RSD**	**Peak height**	**Peak area**
Precision:			
i. Repeatability (n=6)	99.48 ± 0.307,	0.309%	99.26 ± 0.801, 0.807%
ii.Intermediate precision			
a) Intra-day (n=3)	98.29 ± 0.404, 0.411%	98.81 ± 0.281, 0.284%	
b) Inter-day (n=3)	98.03 ± 1.002, 1.020%	99.92± 1.260, 1.261%	
c) Different analyst (n=3)	98.15 ± 1.299, 1.323%	99.81 ± 0.434, 0.434%	
iii. Accuracy at 70-130 % of labelled claim (n=5)	99.28 ± 0.287, 0.289%	98.18 ± 0.670, 0.683%	

SD- Standard Deviation, % RSD- Percent Relative Standard Deviation

Table 3: Results of Range of Method by Peak height & Peak area

Concentration range	**70 – 130 % Labelled claim**	
Parameter	**Peak height**	**Peak area**
Regression equation	**y = 1.942x - 102.22**	**y = 21.11x-1010**
Slope	**1.942**	**21.11**
Y- intercept	**(-) 102.22**	**(-) 1010**
Correlation coefficient (R^2)	**0.9994**	**0.9996**

Table 4: Results of Robustness studies by Peak height & Peak area

Parameter	Mean ± SD, % RSD	
	Peak height	Peak area
Robustness:		
i. Change in wavelength (254 ± 2 nm)	98.67 ± 0.100, 0.101%	98.41± 0.070, 0.071%
ii. Change in ratio of mobile phase	98.35 ± 0.320, 0.323%	98.17 ± 0.159, 0.160%
	98.67 ± 0.100, 0.101%	
	98.35 ± 0.320, 0.323%	
	98.41± 0.070, 0.071%	
	98.17 ± 0.159, 0.160%	

SD- Standard Deviation, % RSD- Percent Relative Standard Deviation

Table 5: Results of Specificity studies by Peak height & Peak area

Sample	% of Labelled Claim*	
Normal	100.07	100.13
Acid		99.34
		99.85
Alkali		99.62
		99.97
Oxide	99.49	99.71
Heat	100.21	101.14
Sunlight	100.15	100.98

Table 6: Results of LOD & LOQ studies by Peak height & Peak area

LOD & LOQ values	Peak height	Peak area	Peak height	Peak area
	LOD (ng/spot)	4.731	0.789	
	LOQ (ng/spot)	14.33	2.391	

Precision:

Repeatability

Repeatability of results of assay by proposed method was ascertained by replicate analysis (n=6) of homogeneous sample of tablet powder. The results are shown in Table 2.

Intermediate precision

The samples were analysed by proposed method on same day in quick succession (intra-day), on different days (inter-day), and by different analyst. The results of study are given in Table 3.

Accuracy (% Recovery):

To check the accuracy of the method, recovery was measured by addition of standard drug at five different levels (70, 85, 100, 115 and 130% of labelled claim) to pre-analyzed sample. Accurately weighed quantities of pre-analyzed tablet powder equivalent to about 7.0 mg of APZ were transferred to five different 10.0 mL volumetric flasks and accurately weighed 1.5, 3.0, 4.5 and 6.0 mg of standard DVS were added to 2nd, 3rd, 4th, 5th flask respectively (representing 70-130 % of labelled claim). This was followed by addition of about 8.0 mL of acetonitrile in each flask and the contents were shaken and sonicated for 15 minutes. Sufficient acetonitrile was added to each flask to adjust the volume to 10.0 mL and filtered. A 0.6 mL of each of the filtrate was diluted to 10.0 mL with methanol. Resultant sample solutions were analyzed as described under assay method.

The percent recovery was then calculated at different levels of sample concentration using the formula:

$$\text{Recovery \%} = \frac{T}{B + C} \times 100$$

where,
T = total drug estimated (mg)
B = amount of drug contributed by pre-analyzed tablet powder (mg)
C = weight of pure drug added (mg)
The results of study are given in Table 2.

Range of method:

A graph was plotted as densitometric response (peak height or area) vs. percent of labelled claim on the basis of accuracy studies data (Table 3).

Robustness:

The samples were analyzed using proposed method by deliberate small change in the scanning wavelength (254 ± 2 nm) and mobile phases with different compositions (± 0.2 mL) of toluene: methanol (**8.3: 1.7** v/v, 8.5: 1.5 v/v, **8.7: 1.3** v/v). The results of study are given in Table 4.

Specificity:

The specificity studies were carried out by attempting deliberated degradation of the tablet sample with exposure to stress conditions like acidic (0.1 M HCl), basic (0.1 M NaOH), normal, oxidizing (3% H_2O_2), dry heat (80 ^{0}C) and direct sunlight.

Sample solution: Accurately weighed quantities of tablet powdered equivalent to about 10.0 mg of APZ were transferred to six different 10.0 mL volumetric flasks. The samples were then exposed to stress conditions as follows:

1) Normal (control) for 24 h at room temperature
2) Acidic: At room temperature for 24 h on addition of 1.0 mL of 0.1 M HCl
3) Basic: At room temperature for 24 h on addition of 1.0 mL of 0.1 M NaOH
4) Oxidative: At room temperature in dark for 24 h on addition of 1.0 mL of 3 % H_2O_2
5) Dry heat: At 80^{0}C for 24 h
6) Sunlight: For 24 h in sunlight on three consecutive days

After stipulated time of each stress conditions the samples were dissolved in acetonitrile and volume was made to 10.0 mL and sonicated for 15 minutes. The solutions were filtered, and 0.6 mL of each filtrate was diluted to 10.0 mL with methanol and analyzed in similar manner as described under assay method (Table 5 & Fig. 5 A-F).

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ were determined by the method based on standard deviation of the response and slope of calibration curve as per ICH guidelines [10].

$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$

Where σ is the standard deviation of the response (estimated by measuring the response in term of peak height or peak area of standard solution of conc. 100 ng/spot for six times and S is the slope of calibration curve (obtained from calibration curve). The results of study are given in Table 6.

RESULTS

Optimization of chromatographic conditions:

The mobile phase comprising of mixture of toluene and methanol in the ratio 8.5:1.5 v/v have repeatedly yielded sharp symmetrical peaks with Rf value 0.48 ± 0.02 for standard and sample (Fig. 2 a & b). The *in-situ* UV spectra of developed standard and sample spots indicated 254 nm as suitable wavelength for quantitation of drug (Fig. 3 a & b).

Linearity of response:

A graph plotted as peak height or peak area as a function of concentration of standard was found to be linear over the concentration range of 100-500 ng/spot (Table 1) and Fig. 4 a & b.

Precision and Accuracy:

The assay results of repeatability and intermediate precision studies were found to be quite precise. Accuracy studies over the range of 70-130 % of labelled claim had shown the recoveries of the drug from sample matrix close to about 99 % (Table 2).

Range of the method:

A graph plotted on the basis of accuracy studies as response of analyte in sample solution (peak height or peak area) vs. % labelled claim was found to be linear over the range of 70-130 % of labelled claim (Table 3).

Robustness:

The deliberate minor changes in optimized chromatographic conditions did not have any significant effect on the results (Table 4).

Specificity:

Under the mild stress conditions of the sample, the assay results were not affected and were close to normal sample (Table 5). Moreover, no additional peaks were observed in the chromatograms of stress samples

indicating the stability of APZ against the stress conditions studied (Fig. 5 A-F).

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD & LOQ values of proposed method are given in Table 6.

DISCUSSION

The results of the repeatability and the intermediate precision were quite reproducible with % RSD value well below 1.0 indicates high level of precision of the proposed method under the conditions studied (Table 2). The recovery studies performed by standard addition method over range of 70-130 % of labeled claim yielded the recovery close to 100 % indicating the capability of the method to accurately measure the drug contents free of interference of excipients. The linear response of the analyte concentration in sample matrix as a function of labeled claim indicates the wide range of accurate measurement of drug content over 70-130 % of labeled claim indicating noninterference of excipients (Table 3). The deliberate small changes in experimental conditions with respect to scanning wavelength and mobile phase composition have no significant effect on the results by the proposed method indicates reasonable robustness of the method (Table 4). Specificity of estimation with respect to degradation product does not appear to be big problem as drug appears to be stable to likely stress it may have to withstand during its shelf life as evident from results of specificity studies (Table 5 & Fig. 5. A-F). The LOD and LOQ values are indicative of sensitivity of the method to detect and to determine the drug content down to few nanograms (Table 6). The proposed HPTLC method is comparable with the reported HPLC methods [6], [9]. Moreover, the proposed HPTLC is more sensitive, simpler and suitable alternative for other reported methods with the advantage such as several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

CONCLUSION

The results of the various validation parameters indicate that the method is quite simple, precise, accurate, sensitive and rapid which may be used for routine assay of Aripiprazole in tablet.

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