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Development And Validation Of High-Performance Thin Layer Chromatography Method For Simultaneous Estimation Of Aripiprazole And Clozapine In Marketed Combined Dose Tablets Formulations

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Article History

***Abstract*:**

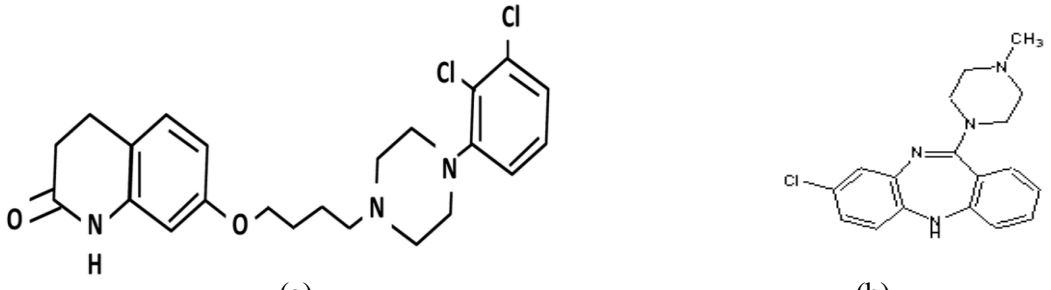
The present work demonstrated the use of a simple validated High-Performance Thin-Layer Chromatography method has been proposed for the simultaneous determination of Aripiprazole (ARI) and Clozapine (CLO) in a tablet dosage form. Materials and methods: The separation was achieved on silica gel 60 F254 coated aluminum sheet as stationary phase using Toluene: Methanol (7.5: 2:5 v/v) as mobile phase which gave compact spots with R_f values 0.51 ± 0.02 for ARI and 0.72 ± 0.03 for CLO. Quantitative densitometric evaluation was done in absorbance-reflectance mode at 258 nm. The developed method was validated with respect to linearity, limits of detection and quantitation, accuracy, precision, specificity and robustness. Results: For proposed method, the response was found to be linear over concentration range of 100-500 ng/spot for ARI and 500-2500 ng/spot for CLO with correlation coefficients 0.997 by peak height and 0.997 by peak area for ARI and 0.996 by peak height and 0.997 by peak area for CLO. The mean percentage recovery of the drug was observed to be 99.79 ± 0.864 by peak height and 100.3 ± 0.699 by peak area for ARI and 99.59 ± 0.578 by peak height and 100.46 ± 0.670 by peak area, respectively. The suitability of method for the quantitative determination of Aripiprazole (ARI) and Clozapine (CLO) was proved by validation. Conclusion: The method was validated for linearity, accuracy, range, precision and robustness according to International Council on Harmonization Q2 (R1) guidelines. The method is simple, accurate, precise and was successfully applied to the assay of drug in tablet formulation. Conclusion: The proposed method and its results had been successfully applied and validated to the simultaneous estimation of Aripiprazole and Clozapine in their combination for quality analysis.

Keywords: Aripiprazole, Clozapine, HPTLC method, Validation, Assay, Quality control

INTRODUCTION

Aripiprazole is a recent Dopamine (D_2) and Serotonin ($5HT_{1A}$) receptors partial agonist used for the treatment of schizophrenia or bipolar disorder. Chemically, itis7-{4-[4(2, 3- dichlorophenyl) piperazin-1-yl]

butoxy}-3,4-dihydroquinolin-2(1H)-one[1]. A tricyclic dibenzodiazepine, classified as a typical antipsychotic agent. It binds
several types of CNS receptors, and displays a unique pharmacological profile. Clozapine is a serotonin antagonist, with strong binding to 5-HT 2A/2C receptor subtype. It also displays strong affinity to several dopaminergic receptors, but shows only weak antagonism at the dopamine D2 receptor, a receptor commonly thought to modulate neuroleptic activity. It is used in patients with treatment-resistant schizophrenia [2]. Combination of Aripiprazole and Clozapine was studied under clinical trial phase and was proved that the synergistic effect was observed by improving psychotic symptoms and reducing side effects such asagranulocytosis, sedation, weight gain, sialorrhoea and enuresis as compare to Clozapine monotherapy. Chemically, it is 8-chloro-11-(4-methylpiperazin-1-yl) - 5H-dibenzo [b, e]
[1, 4] diazepine. Prasenjit M. et al. [3] have developed and validated rapid, simple RP-HPLC method for analysis of Aripiprazole in bulk and tablet dosage form. Florin S. et al. [4] have developed and validated a reverse phase HPLC-DAD method
for the simultaneous determination of Aripiprazole and five of its chemically related impurities in tablet dosage forms. Ashu M. et al. [5] have developed and validated a simple, selective, rapid and economical RP-HPLC method for the determination of Aripiprazole in pharmaceutical dosage forms. Thakkar R. S. et al. [6] have developed and validated a simple, precise and accurate isocratic stability indicating RP-HPLC assay method for the determination of Aripiprazole in bulk and solid dosage form. Min S. et al. [7] have developed a selective, sensitive and accurate liquid chromatography- tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of Aripiprazole and its active metabolite Dehydro-aripiprazole in human
plasma using Papaverine as internal standard. Matteo C. et al. [8] have developed a sensitive and selective HPLC-MS/MS method to measure serum Aripiprazole and Dehydro-aripiprazole levels in a hospital laboratory, requiring minimum sample preparation compared to previous LC-MS/MS methods. Analytes were separated on reversed phase HPLC. Frederique L. et al. [9] have developed a high-performance liquid chromatography
method with Diode Array Detection (HPLC-DAD) for quantification of Aripiprazole and Dehydro-aripiprazole, in human plasma. Alessandro M. et al. [10] have developed Capillary Electrophoresis (CE) and High-Performance Liquid Chromatography (HPLC) for the analysis of Aripiprazole in human plasma. Yoshihiko et al. [11] have developed and validated a HPLC methods for the determination of Aripiprazole in rat plasma and brain. Chia-jui T. et al. [12] have studied a Capillary electrophoresis method to measure the level of Aripiprazole and its main metabolite, Dehydro-aripiprazole. Masanori K. et al. [13] have developed and validated a liquid LC-MS/MS method for determination of Aripiprazole and its main metabolite, OPC-14857, in human plasma Chromatographic separation was achieved isocratically on a C_{18} column within 7.5 min. The calibration curve ranging from 0.1 to 100 ng/mL was fitted to a $1/y^2$-weighed linear regression model. The assay showed no significant interference. Lower limit of quantitation (LLOQ) for both analytes was 0.1 ng/mL of plasma. The retention times of Aripiprazole and internal standard was found to be 5.37 min and 3.97 min. Xiao-cong Z. et al. [14] have developed and validated a rapid HPLC-MS method, with Estazolam as internal standard, for determination of Aripiprazole in human plasma. Kalaichelvi R. et al. [15] have developed a simple, sensitive and reproducible
spectrophotometric method for the determination of Aripirpazole in pure form and in pharmaceutical formulation. Sevak D. R. et al have developed a simple, accurate and improved HPLC method for the analysis of Clozapine in the tablet form. Ravi Kiran B. et al. [16] have developed and validated a rapid, specific and accurate isocratic RP-HPLC method for the assay of Clozapine in pharmaceutical dosage form. There is no reported HPTLC method for its estimation in tablet formulation. A HPTLC method for estimation of Aripiprazole and Clozapine in tablet formulation is described in the present article.



(a) (b)

Fig. No. 1: Aripiprazole and Clozapine

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile, methanol used were of AR grade, Merck India Ltd, Mumbai (India). A Milli-Q purification system from Merck Company (Darmstadt, Germany) was used to produce ultra-pure water. Standard drug sample of Aripiprazole (99.40% pure, Fig.1) obtained as a gift sample from Watson Pharmaceuticals Limited, Mumbai (India) and Clozapine 99.96% pure, Fig. 1 b) was obtained as a gift sample from Sun Pharmaceuticals Limited, Sikkim (India). The Aripiprazole tablets used in this study with a declared content equivalent to 10 mg Aripiprazole and Clozapine tablets used in this study with a declared content equivalent to 50 mg Clozapine were procured from local market.

Instrumentation and Analytical Conditions

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 F254 TLC alu minum 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, 10 mm. 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 258 nm in absorbance- reflectance mode.

Chromatographic conditions:

Optimization of mobile phase

Aliquot portions of working standard solution (5 µL) were applied on TLC plates in the form of band. 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 (7.5: 2:5 v/v) was found to be most satisfactory as it gave sharp symmetrical peaks for the drugs Aripiprazole and Clozapine chromatogram with R_f values 0.51± 0.02 for ARI and 0.72± 0.03 for CLO (Fig. 2 a). 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 258 nm was selected as scanning wavelength as it gave maximum absorption for the drug (Fig. 2 b).

Preparation of Standard solutions: Solution A (Stock mixed Standard solution)

Accurately weighed quantities (10.0 mg) of Aripiprazole (ARI) and Clozapine (CLO) (50.0 mg) were mixed and dissolved in 6.0 mL of acetonitrile and volume was made to 10.0 mL (Conc.: 1.0 mg/mL for ARI and 5.0 mg/mL for CLO).

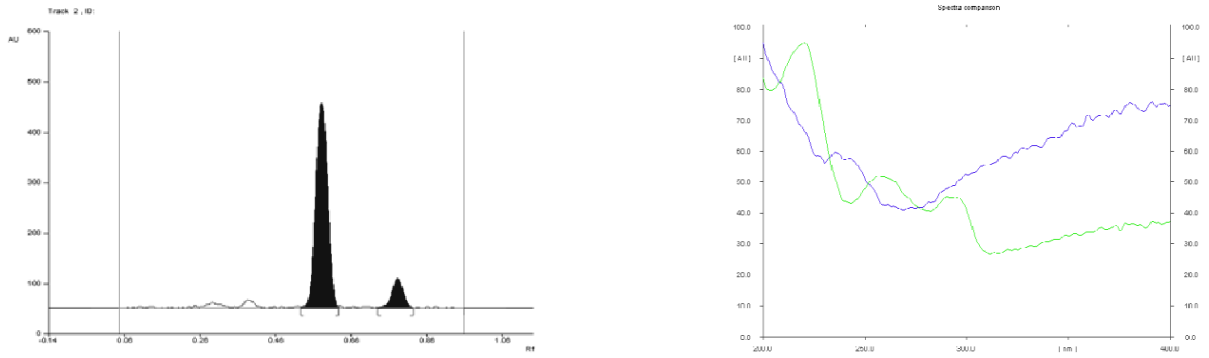
Solution B (Mixed Working standard solution)

Accurately measured 1.0 mL quantity of stock standard solution A was diluted to 10.0 mL with methanol (Conc.:100.0 µg/mL for ARI and 500.0 µg/mL for CLO).

Solution C (Mixed Working standard solution)

Accurately measured 6.0 mL quantity of stock standard solution B was diluted to 10.0 mL with methanol (Conc.: 60.0 µg/mL for ARI for and 300.0 µg/mL for CLO).

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 258 nm. A Densitogram and *in situ* UV spectrum of standard ARI & CLO solution under optimized condition are shown in Fig. 2 (a) & (b).



(a) **(b)**

Fig. No. 2: HPTLC Densitogram of ARI & CLO Standard (a) and *in situ* UV spectrum of ARI & CLO Standard (b)

Assay of ARI & CLO in tablet by proposed method

Working standard solution C was freshly prepared (Conc.: ARI- 60.0 µg/mL and CLO 300.0 µg/mL) as described under preparation of standard solution.

Sample solution: Twenty tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 10 mg (□247 mg) of ARI and (also equivalent to about 50 mg CLO) were ultra-sonicated with about 6 mL of acetonitrile for 10 min and the volume was made up to 10.0 mL with methanol. The solution was filtered to get a clear solution. Aliquot portions (6.0 mL) of each of clear filtrates were diluted to 10.0 mL with methanol to get concentration about 60.0 µg/mL for ARI and 300.0 µg/mL for CLO on labelled claim basis. Six replicate sample solutions were prepared in similar manner.

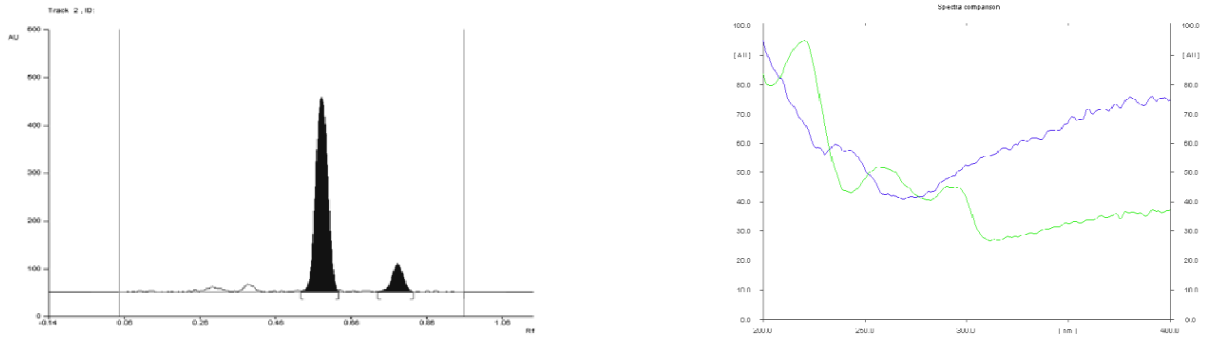


Fig. No. 3: HPTLC Densitogram of ARI & CLO Sample (a) and *in situ* UV spectrum of ARI & CLO Sample (b)

Calculation:

Percent of labelled claim were calculated using following formula-

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

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 of proposed HPTLC method [8]:

Linearity of response

It was followed using stock standard solution B (0.2- 1.2 mL) diluted to 10.0 mL with methanol to get concentration from 20.0-100.0 µg/mL for ARI and 100.0-500.0 µg/mL for CLO. Aliquots portions (5 µL) of

series of standard solutions of six different concentrations 20.0, 40.0, 60.0, 80.0, 100.0 µg/mL of ARI (100-500 ng/spot) and 100.0, 200.0, 300.0, 400.0, 500.0 µg/mL of CLO were applied in duplicate (500-2500 ng/spot) on TLC plate and chromatograms were developed and scanned under optimized chromatographic conditions. The data of linear regression study is given in Table 1.

Table No. 1: Result of Linearity studies of ARI & CLO by Peak height & Peak area

Linearity	**ARI**		**CLO**	
Range (ηg/spot)	**100-500**		**500-2500**	
	By height	**By area**	**By height**	**By area**
Equation for straight line	y = 0.529x - 0.1	y = 16.94x + 50.00	y = 0.416x + 1.399	y = 16.81x + 110
Slope	0.529	16.94	0.416	16.81
Y-intercept	(-) 0.1	50.00	1.399	110
Correlation coefficient	0.997	0.997	0.996	0.997

Table No. 2: Assay of ARI and CLO in combined dose tablet by HPTLC

Sr. No.	**Wt. of tablet powder taken (mg)**	**Scanner response***				**% Labeled claim***			
		ARI		**CLO**		**ARI**		**CLO**	
		By height	**By area**	**By height**	**By Area**	**By height**	**By area**	**By height**	**By Area**
1.	247.33	33.27	1106.7	128.51	5253.1	99.68	101.12	98.75	99.01
2.	247.31	33.19	1098.8	126.29	5198.8	99.43	100.93	98.26	98.51
3.	247.37	34.25	1112.0	121.26	5187.4	100.03	101.49	97.91	98.03
4.	247.29	32.81	1074.2	131.19	5291.6	98.21	98.39	99.48	99.62
5.	247.35	33.56	1083.7	129.44	5239.2	99.07	99.43	99.09	99.16
6.	247.32	34.09	1091.2	125.63	5247.2	100.01	100.36	98.93	99.15
7.	Standard	34.18	1117.6	138.51	5312.4				
*Mean of five observations					**Mean**	**100.01**	**100.28**	**98.93**	**98.97**
					± SD	**0.6889**	**1.1740**	**0.5701**	**0.6243**
					%RSD	**0.6888**	**1.1706**	**0.5762**	**0.6207**

Precision:

Repeatability

Repeatability of results of assay by proposed method was ascertained by replicate analysis (n=6) of ARI and CLO 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.

Table No. 3: Results of Intermediate precision study of ARI and CLO

Observations	% Labelled claim			
Intraday Samples	ARI		CLO	
	By height	By area	By height	By area
I	99.92	100.03	99.89	99.99
II	99.95	101.13	98.97	99.94
III	98.99	99.94	99.86	100.09
Mean	**99.62**	**100.36**	**99.57**	**100.00**
±S.D.	**0.5458**	**0.6625**	**0.5227**	**0.0763**
%R.S.D.	**0.5478**	**0.6601**	**0.5249**	**0.0763**

Observations	% Labeled claim			
Interday Samples	ARI		CLO	
	By height	By area	By height	By area
I	98.47	98.93	98.37	98.69
II	99.16	99.87	98.81	99.33
III	98.91	100.01	99.25	99.96
Mean	**98.84**	**99.60**	**98.81**	**99.32**
±S.D.	**0.3493**	**0.5873**	**0.4400**	**0.6350**
%R.S.D.	**0.3533**	**0.5896**	**0.4452**	**0.6393**

Observations	% Labeled claim			
Different analyst	ARI		CLO	
	By height	By area	By height	By area
I	98.99	99.89	98.91	99.78
II	99.97	100.05	99.26	99.93
III	100.02	99.99	99.69	100.01
Mean	**99.66**	**99.97**	**99.28**	**99.90**
±S.D.	**0.5807**	**0.0808**	**0.3906**	**0.1167**
%R.S.D.	**0.5826**	**0.0808**	**0.3934**	**0.1168**

* Each value is mean of five observations.

Accuracy:

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 8mg of ARI (~198 mg) and (also equivalent to about 42 mg CLO) were transferred to five different 10.0 mL volumetric flasks and accurately known amount of standard 2.0 mg ARI and 8.0 mg CLO pure API were added to each flask, followed by addition of about 6 mL of methanol. The flasks were sonicated for 10 min and volumes were made up to the mark with methanol. The solutions were filtered and aliquot portions (6.0 mL) of each of clear filtrates were 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:

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

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 4.

Table No. 4: Results of Recovery study for Simultaneous Estimation of ARI and CLO tablet (Avg. wt. = 247.33 mg, Labelled claim: 10 mg ARI + 50 mg CLO)

Sr. No.	Wt. of tablet powder taken +Std. ARI +Std. CLO (mg)	Amount of drug recovered(mg)				% Labeled claim*			
		ARI		CLO		ARI		CLO	
		By Height	By Area	By Height	By Area	By Height	By Area	By Height	By Area
1.	197.86 + 2.03 + 8.05	2.01	2.02	8.02	8.03	100.05	101.03	99.99	100.01
2.	197.83 + 2.09 + 8.06	2.03	2.06	8.01	8.04	101.02	101.13	100.00	101.05
3.	197.81 + 2.07 + 8.05	2.02	2.04	8.03	8.02	98.99	99.78	98.97	99.95
4.	197.79 + 2.04 + 8.07	2.01	2.03	8.02	8.04	99.97	99.99	100.07	101.33
5.	197.84+ 2.01 + 8.02	1.99	2.01	7.99	8.01	98.93	99.69	98.96	99.98
*Mean of five observations					**Mean**	**99.79**	**100.32**	**99.59**	**100.46**
					± SD	**0.8649**	**0.6995**	**0.5786**	**0.6704**
					%RSD	**0.8667**	**0.6972**	**0.5809**	**0.6673**

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 5).

Table No. 5: Result of Accuracy studies over Range of Method studied tablet (Avg. wt. = 247.33 mg, Labelled claim: 10 mg ARI + 50 mg CLO)

Sr. No.	Wt. of tablet powder taken + Std. ARI +Std. CLO (mg)	Amount of drug recovered (mg)				% Labeled claim*			
		ARI		CLO		ARI		CLO	
		By Height	By Area	By Height	By Area	By Height	By Area	By Height	By Area
1.	173.13 + 0.0	-	-	-	-	99.23	99.89	99.37	99.68
2.	173.11 +1.51 +7.52	1.46	1.49	7.47	7.49	99.46	100.05	98.91	99.93
3.	173.16+3.02+15.04	2.89	2.99	15.01	15.03	98.74	99.66	100.01	100.03
4.	173.09+4.51+22.51	4.46	4.48	22.45	22.46	100.01	101.09	99.95	99.96
5.	172.99+6.09+30.01	6.03	6.05	29.97	29.98	99.38	100.03	99.87	99.89
*Mean of five observations					**Mean**	**99.36**	**100.14**	**99.62**	**99.89**
					± SD	**0.4566**	**0.5512**	**0.4717**	**0.1321**
					%RSD	**0.4595**	**0.5504**	**0.4734**	**0.1322**

Table No.6: Results of Range of method by HPTLC

Parameters	Result			
Range	70-130% of Labelled claim			
	ARI		CLO	
	By height	By area	By height	By area
Equation for straight line	y = 0.312x + 1.579	y = 11.13x - 8.684	y = 1.305x - 2.407	y = 53.19x - 86.68
Slope	0.312	11.13	1.305	53.19
Y-intercept	1.579	8.684	2.407	86.68
Correlation coefficient	0.998	0.999	0.998	0.998

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 [17].
LOD = 3.3 × σ/S
LOQ = 10 × σ/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 for six times and S is the slope of calibration curve (obtained from calibration curve).
The results of study are given in Table 7.

Table No. 7: LOD and LOQ values of ARI and CLO

Sr. No.	Parameters	ARI		CLO	
		By height	By area	By height	By area
1.	LOD (ηg/spot)	9.694	1.657	10.67	2.481
2.	LOQ (ηg/spot)	29.37	5.022	32.35	7.519

Robustness:
The samples were analyzed using proposed method by deliberate small change in the scanning wavelength (258 ± 2 nm) and mobile phases with different compositions (± 0.2 mL) of n-Hexane: Ethyl acetate (7.3 mL: 2.7 mL v/v, 7.5 mL : 2.5 mL v/v, 7.7 mL : 2.3 mL v/v).
The results of study are given in Table 8.

Table No. 8: Results of Robustness study

Sr. No.	Parameters	% Labelled claim ± SD (n = 3)			
		ARI		CLO	
		By height	By area	By height	By area
1.	Change in wavelength				
	256.0 nm	98.63±0.237	98.75±0.225	98.42±0.252	98.83±0.217
	258.0 nm	99.97±0.103	100.03±0.104	99.89±0.111	99.98±0.121
	260.0 nm	99.11±0.190	99.29±0.171	99.04±0.196	99.17±0.183
2.	Change in composition of mobile phase				

	7.3 mL : 2.7 mL v/v	98.52±0.248	98.64±0.234	98.33±0.267	98.75±0.225
	7.5 mL : 2.5 mL v/v	99.98±0.101	100.02±0.101	99.91±0.119	100.01±0.101
	7.7 mL : 2.3 mL v/v	99.13±0.171	99.35±0.165	99.09±0.101	99.23±0.173
3.	Change in saturation time				
	10 min	98.47±0.253	98.59±0.241	98.26±0.274	98.63±0.237
	15 min	99.99±0.113	100.01±0.101	99.94±0.106	99.99±0.103
	20 min	98.97±0.203	98.99±0.211	99.03±0.197	99.17±0.183

Specificity:

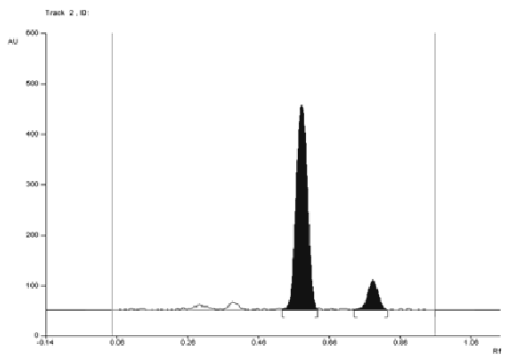
The specificity studies were carried out by attempting deliberated degradation of the tablet sample with exposure to stress conditions like acidic (0.1 M HCl), basic (0.1 M NaOH), normal, oxidizing (3% H_2O_2), dry heat (80 ^{0}C) and direct sunlight.

Sample solution: Accurately weighed quantities of tablet powdered equivalent to about 10.0 mg ARI (~ 247 mg) and (also equivalent to about 50 mg CLO) were transferred to six different 10.0 mL volumetric flasks. After stipulated time of each stress conditions the samples were dissolved in methanol 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.

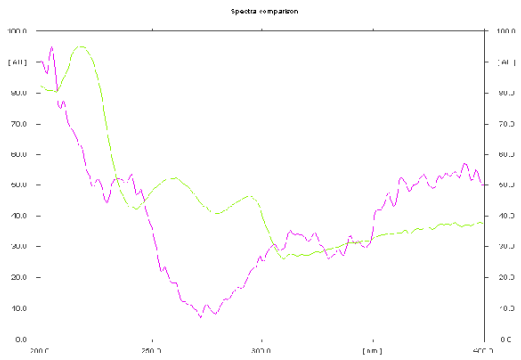
The samples were then exposed to stress conditions as follows:

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

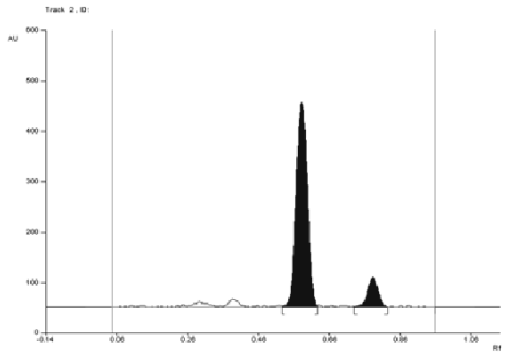
The typical densitograms and *in situ* spectra of principle spots (analyte) of sample exposed to stress conditions are shown in Fig. No. 4 and results of specificity study are shown in Table No. 9.



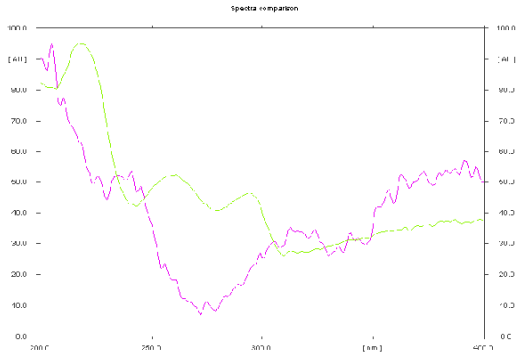
(a)



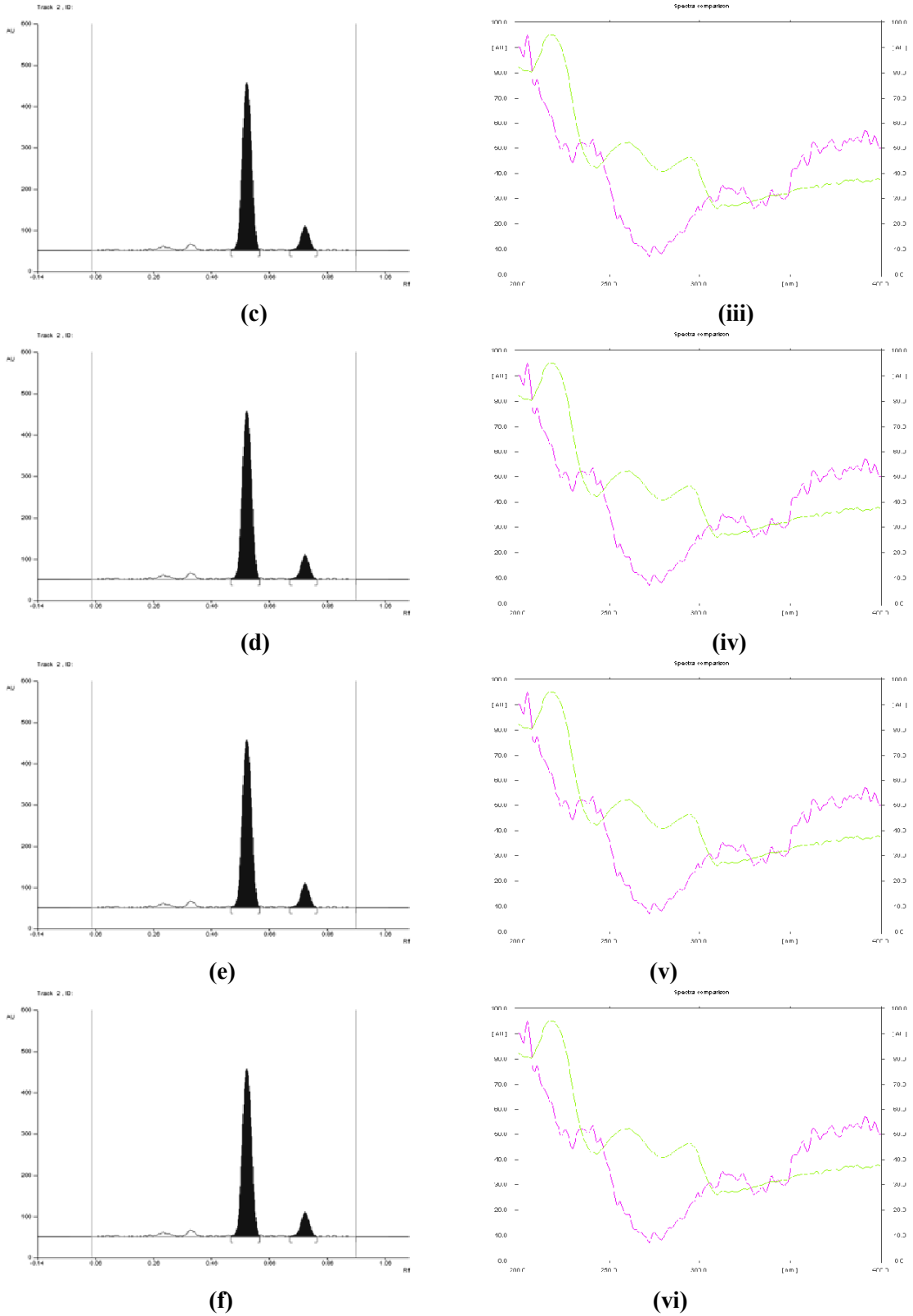
(i)



(b)



(ii)



(c) **(iii)**

(d) **(iv)**

(e) **(v)**

(f) **(vi)**

Fig. No. 4: HPTLC Densitograms of Specificity studies in (a) acidic, (b) basic, (c) oxidative, (d) thermal (dry heat), (e) photolytic (sunlight) (f) and normal conditions, 24 and corresponding *in situ* UV spectra (i-vi) of ARI and CLO

Table No. 9: Results of Specificity study

Sr. No.	Stress conditions	% Labelled claim*			
		ARI		CLO	
		By height	By area	By height	By area
1.	Acidic	98.33	98.57	98.42	98.68
2.	Basic	98.41	98.69	98.54	98.72
3.	Oxidative	98.22	98.37	98.43	98.59
4.	Dry Heat	99.38	99.42	99.56	99.65
5.	Sunlight	98.65	98.73	98.42	98.91
6.	Normal sample	99.77	99.81	99.67	99.89

*Mean of five observations

RESULTS

Optimization of chromatographic conditions:
The mobile phase comprising of mixture of Toluene: Methanol in the ratio 7.5:2.5 v/v have repeatedly yielded sharp symmetrical peaks with Rf values 0.51 ± 0.02 for ARI and 0.72 ± 0.03 for CLO for standard and sample (Fig. 2 a & 3 a). The in-situ UV spectra of developed standard and sample spots indicated 258 nm as suitable wavelength for quantitation of drug (Fig. 2 b & 3 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 of ARI and 500-2500 ng/spot for CLO (Table 1).

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, Table 3 and Table 5).

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 6).

Robustness:
The deliberate minor changes in optimized chromatographic conditions did not have any significant effect on the results (Table 8).

Specificity:
Under the mild stress conditions of the sample, the assay results were not affected and were close to normal sample (Table 9). Moreover, no additional peaks were observed in the chromatograms of stress samples indicating the stability of ARI and CLO 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 7.

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 and Table 3). 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 non-interference of excipients (Table 4 and Table 5). 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 8). 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 9 & 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 7). 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 and Clozapine in tablet.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

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