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Development and validation of liquid chromatographic method for aripiperazole

Nandini R. Pai * and Deeptaunshu Atul Pusalkar

Department of Chemistry, D.G. Ruparel College, Mahim, Mumbai 400016, Maharashtra, INDIA

ABSTRACT

A simple isocratic, rapid and sensitive high performance liquid chromatographic method has been developed for quantitative determination of Aripiperazole and its four process related impurities. The method has been validated for determination of related substance in aripiperazole using C8 ODS (150X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 0.5.The elution is carried out by using mobile phase of 500ml aq.buffer(0.2%TFA),400ml acetonitrile and 100 ml methanol. pH is adjusted to 3 bt TEA. The detection is carried out at 254nm with injection volume of 10 microlitre. Specificity, system suitability, linearity, precision, ruggedness, robustness has been carried out for aripiperazole. Limit of quantification and limit of detection has been carried out for the impurities of aripiperazole. Forced degradation study of aripiperazole has been carried out .Impurity profiling is also been carried out.

Keywords: HPLC, Aripiperazole, related impurities, validation

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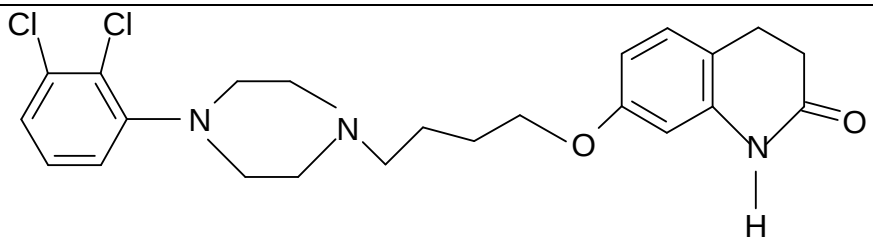
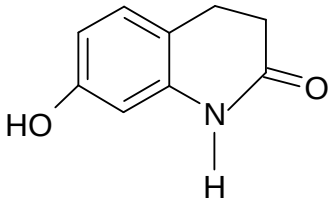
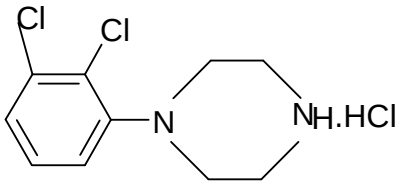
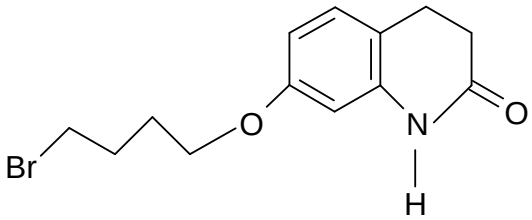
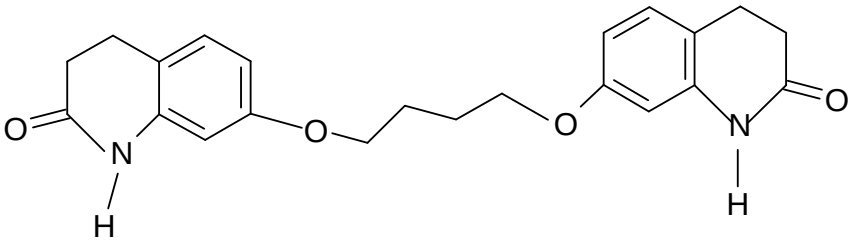
INTRODUCTION

Aripiperazole is an atypical antidepressant used in the treatment of bipolar disorder, schizophrenia, and clinical depression. It's a off-white crystalline powder with chemical name 7-[4-[4-(2,3-dichlorophenyl)-1- piperazinyl] butoxy]-3,4-dihydrocarbostyril. Several liquid chromatographic methods are reported for estimation of aripiperazole in various matrix systems.

Aripiprazole is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives whose major therapeutic role is to treat the symptoms of schizophrenia. Aripiprazole is a selective monoaminergic antagonist with high affinity for the serotonin Type 2 (5HT2), dopamine Type 2 (D2), 1 and 2 adrenergic and H1 histaminergic receptors. Aripiprazole acts as an antagonist at other receptors, but with lower potency. Antagonism at receptors other than dopamine and 5HT2 with similar receptor affinities may explain some of the other therapeutic and side effects of aripiprazole. Aripiprazole's antagonism of histamine H1 receptors may explain the somnolence observed with this drug. Aripiprazole's antagonism of adrenergic a1 receptors may explain the orthostatic hypotension observed with this drug.

For the development and validation for liquid chromatographic method of aripiperazole following parameters were evaluated: Specificity, system suitability, accuracy, linearity, precision, ruggedness, robustness, limit of Quantification and Detection according to USP and ICH guidelines. Also forced degradation study has been done for this API drug molecule.

The impurities or unreacted precursors in aripiperazole are following:

Compound	Structure
Aripiperazole	
(Imp A)	
(ImpB)	
(Imp C)	
(Imp D)	

MATERIALS AND METHODS

Reagents and Chemicals

Aripiperazole drug substance was prepared and well characterized with the help of various spectroscopic and chromatographic techniques. This was used as reference standard for further work. The reference standard samples of impurity A, B, C and D which are intermediates are obtained from respective lab experiments after characterization using various spectroscopic and chromatographic techniques and are taken as standards for further experiment. Analytical reagent grade Trifluroacetic acid was purchased from merck Chemicals and HPLC grade methanol, Acetonitrile, from S.D Fine chemicals. Imp A i.e 7-Hydroxy carbostyril is purchased from spectrochem.

A chromatographic system is Agilent 1100 series equipped with a quaternary gradient pump, photodiode array detector. All the data was acquired using Chemstation data acquisition and integration software. A Bruker 300MHz NMR spectrometer was used for recording the ^1H spectrum. A Shimadzu UV spectrophotometer was used for recording the UV spectrum. An FTIR Spectrum One from Perkin Elmer was used for Infra Red analysis. Spectroscopic Data of aripiperazole and related impurities are given in table no.1 and 2.

Preparation of Solutions, Chromatographic Conditions and System Suitability Parameter-
Chromatographic Conditions-
The elution is carried out by using mobile phase of 500ml aq.buffer(0.2%TFA),400ml acetonitrile and 100 ml methanol. pH is adjusted to 3 bt TEA. The detection is carried out at 254nm with injection volume of 10 microlitre.

Standard solution Preparation-
About 10 mg of Aripiperazole Reference standard, accurately weighed was transferred in 50 mL volumetric flask, dissolved in sufficient mobile phase and diluted to the mark. This solution was further diluted with mobile phase to obtain required ppm solutions.

Method Validation:
The proposed method for estimation of related substances of Aripiperazole is validated as per the guideline of United States Pharmacopoeia and ICH guidelines.

(1) Specificity-
By injecting diluent & individual components into the chromatograph Diluent, Aripiperazole and related impurities namely Impurity A, Impurity B, Impurity C ,and impurity D100 ppm each are injected individually and in combination into the chromatograph.

Retention times of all the components are given in table no.4. From retention time, it can be seen that, all the components have different retention time. Diluent, Aripiperazole, and its impurities show different retention times. Thus all the components are well separated from each other indicating specificity of the analytical method.

(2) Linearity
The linearity of an analytical method is its ability to elicit test results that are directly, or by well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. A graph of concentration (on X-axis) Vs Area (on Y-axis) is plotted.

About 10 mg of accurately weighed sample is taken in 50ml volumetric flask and dissolved in sufficient amount of mobile phase and diluted up to the mark. This is taken as stock solution. From stock solution serial dilutions are made of different concentration level and injected for aripiperazole and its process related impurities.

Linearity is mentioned in table no 5.

The graph of concentration (on X-axis) Vs Area (on Y-Axis) is linear in nature passing through origin.

(3) Precision-
The precision of analytical method is the degree of agreement among the individual test results when method is applied repeatedly to multiply sampling of homogenous sample. To ensure analytical system is working satisfactorily and giving precise results, 100ppm solution (from stock solution) of aripiperazole and its impurities were injected 5 times. RSD for retention time and area are calculated and tabulated in table no. 6. Limit RSD: +/- 2.0% [98.0% to 102.0%]. The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

(4) Ruggedness-
Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Its a degree of exactness of a measurement to its true value. Rugdness of aripiperazole and its impurities are given in table no 7. The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

(5) Accuracy-
The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. From stock solution of 200 ppm further dilutions are for the analysis. Accuracy of aripiperazole and its impurities are given in table no 8.

(6) Robustness-
Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results. It was carried out by change in flow rate, change in mobile phase composition, change in wavelength and change in pH. It is observed

that method is unaffected by small changes in experimental conditions complises the robustness. Results are mentioned in table no 9.

(7) limit of quantification-

Limit of quantification is lowest amount of analyte present in sample that can be determined with acceptable precision and accuracy under stated experimental conditions. Limit of quantification is calculated from signal to noise ratio. To determine limit of quantification, sample blank is injected first and noise is integrated at different intervals at different retention time near the peak of interest. Results are mentioned in table no 10.

(8) limit of detection-

The detection limit is characteristic of limit test. It is lowest amount of analyte present in sample that can be detected but not necessarily quantities, under stated condition. Limit of detection is calculated from signal to noise ratio. To determine limit of detection, sample blank is injected and noise is integrated at different retention time near the peak of interest.. It was observed that signal to noise ratio must be 3:1 as given in ICH guideline. Results are mentioned in table no 10.

(9) Forced Degradation Studies results-

To demonstrate the specificity and stability indicating characteristics of the method, samples of aripiprazole were subjected to various stress conditions such as 0.1M HCl acid, 0.5 NaOH base, 10% v/v H2O2, Heat (105 0C), UV light (254 nm, 24 h). Results are mentioned in table no 11.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions, the main objective of the chromatographic method is to separate aripiprazole from Imp-A, Imp-B, Imp-C and Imp- D Impurities were coeluted using different stationary phases such as C18, C8 and cyano as well as different mobile phases. Effective chromatographic separation was achieved with using C8 ODS (150X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 0.5.The elution is carried out by using mobile phase of 500ml aq.buffer(0.2%TFA),400ml acetonitrile and 100 ml methanol. pH is adjusted to 3 bt TEA. The detection is carried out at 254nm with injection volume of 10 microlitre.

Table 1-I.R spectra of Aripiperazole and its process related impurities

Compound	I.R spectra KBr cm^{-1}
Aripiperazole	678(C-Cl str),1589(aromatic C=C str), 3101(aromatic C-H str),2947(sp3 C-H str), 1272(C-O str), 3471(N-H str), 1674(C=O str)
Imp B	786(C-Cl str), 1581(aromatic C=C str), 3062(aromatic C-H str), 2121(C-N str)
Imp C	1589(aromatic C=C str), 3109 (aromatic C-H str), 1272(C-O str),2931(sp3 C-H str), 3332(N-H str), 1674(C=O str),
Imp D	1596 (aromatic C=C str), 3055 (aromatic C-H str), 1272 (C-O str), 1689 (C=O str), 2954 (sp3 C-H str), 3201(N-H str)

Table 2-^{1}HNMR of Aripiperazole and its process related impurities.

Compound	Solvent	^{1}HNMR
Aripiperazole	DMSO	1.62-1.52(m,2H,CH2), 1.76-1.69(m,2H,CH2), 2.35-2.27(t,4H,piperazine), 2.50(t,2H,CH2-C=O), 2.85-2.70(m,2H,Ar-CH2), 3.28(t,4H,piperazine), 3.41(t,2H,NCH2), 3.93-3.89(t,2H,O-CH2), 6.55-6.42(m,2H,Ar-H), 7.05-7.02(d,1H,Ar-H), 7.27-7.10(m,1H,Ar-H), 7.35-7.33(d,2H,Ar-H),10.00(s,1H,N-H)
Imp B	DMSO	7.30(m,8H,piperazine), 9.5(s,3H,Ar-H)
Imp C	DMSO	1.82-1.79(t,2H,CH_2), 1.96-1.91(m,2H,CH_2), 2.50-2.37(t,2H,CH_2-C=O), 2.79-2.74(t,2H,Ar-CH_2), 3.61-3.56(t,2H,Br-CH_2), 3.93-3.89(t,2H,O-CH_2),6.49-6.42(m,2H,Ar-H), 7.04-7.02(d,1H,Ar-H), 9.99(s,1H,N-H)
Imp D	DMSO	1.9(s,4H,CH_2), 2.45(m,4H,N-H,C=O-CH_2), 2.86-2.81(t,4H,Ar-CH_2), 4.00(s,4H,O-CH_2), 6.56-6.49(m,4H,Ar-H), 7.11-7.08(d,2H,Ar-H), 10.00(s,2H,N-H)

Table 3- Relative retention time of the Aripiperazole and its Process related impurities.

Concentration	Sample	Retention time(min)
100 ppm	Aripiperazole	3.87
100 ppm	Imp A	1.96
100 ppm	Imp B	2.46
100 ppm	Imp C	7.17
100 ppm	Imp D	4.98

Table 4- Specificity of aripiperazole.

Sr. No	Component	Retention Time
1	diluent	1.49, 1.82,1.94
2	Aripiperazole	3.87
3	Imp A	1.96
4	Imp B	2.46
5	Imp C	7.17
6	Imp D	4.98

Table no 5- Linearity of Aripiperazole and its process related impurities.

Sr no	Concentration (ppm)	aripiperazole	Imp A	Imp B	Imp C	Imp D
1	80	5745.602	7186.626	4107.642	4143.289	6835.975
2	90	6503.272	8078.996	4667.252	4668.789	7736.540
3	100	7215.625	8989.420	5170.461	5186.443	8532.712
4	110	7940.626	9852.666	5683.222	5705.000	9355.976
5	120	8637.031	10753.145	6139.661	6216.974	10047.444

Table 6-Precision for Aripiperazole and process related impurities.

Sr no	Concentration(ppm)	aripiperazole	Imp A	Imp B	Imp C	Imp D
1	100	7215.625	8989.420	5170.461	5186.443	8532.712
2	100	7230.813	8985.643	5203.792	5178.147	8543.962
3	100	7218.168	8974.594	5179.614	5183.378	8528.980
4	100	7227.438	8997.230	5190.612	5186.071	8529.157
5	100	7232.977	8985.381	5181.142	5175.000	8535.083
	Mean	7225.004	8986.381	5185.124	5181.807	8533.978

Table 7-Rugdness for Aripiperazole and process related impurities.

Sr no	Concentration(ppm)	aripiperazole	Imp A	Imp B	Imp C	Imp D
1	120	8637.031	10753.145	6139.661	6216.974	10047.444
2	120	8650.282	10744.690	6122.976	6225.447	10024.609
3	120	8694.012	10806.496	6127.509	6212.746	10017.560
4	120	8691.051	10724.507	6162.598	6239.990	10035.884
5	120	8666.441	10724.915	6138.274	6241.848	10004.712
	Mean	8667.763	10750.750	6138.203	6227.601	10026.041

Table 8-Accuracy for Aripiperazole and process related impurities.

(8.1) Aripiperazole

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	5745.602	79.52	99.27
100%	100	7225.004	_	_
120%	120	8667.763	119.96	99.96

(8.2) Imp A

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	7186.626	79.97	99.96
100%	100	8986.381	_	_
120%	120	10750.750	119.63	99.69

(8.3) Imp B

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	4107.642	79.21	99.01
100%	100	5185.124	_	_
120%	120	6138.203	118.38	98.65

(8.4) Imp C

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	4143.289	79.95	99.93
100%	100	5181.807	_	_
120%	120	6227.601	120.18	100.15

(8.5) Imp D

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	6835.975	80.10	100.125
100%	100	8533.978	_	_
120%	120	10026.041	117.48	97.9

Table 9-Robustness for Aripiperazole and process related impurities.

(9.1) change in flow rate

(I) Aripiperazloe (200 ppm)

flow	R.T(min)	Area
1 ml / min	4.13	13545.488
	4.13	13506.490
	4.14	13563.449
0.8ml/min	5.15	17079.195
	5.14	17067.674
	5.14	17069.439
1.2 ml/min	3.43	11412.643
	3.44	11332.201
	3.45	11407.248

(II) Imp A (80 ppm)

flow	R.T(min)	Area
1 ml / min	1.93	8460.870
	1.93	8430.327
	1.93	8333.321
0.8ml/min	2.41	10526.836
	2.42	10535.024
	2.41	10528.375
1.2 ml/min	1.60	6844.344
	1.61	6867.657
	1.60	6898.046

(III) Imp B (200ppm)

flow	R.T(min)	Area
1 ml / min	2.61	10957.957
	2.61	10883.178
	2.59	10950.234
0.8ml/min	3.27	13579.043
	3.24	13703.145
	3.24	13779.508
1.2 ml/min	2.16	9137.189
	2.17	9167.236
	2.17	9169.177

(IV) Imp C (200 ppm)

flow	R.T(min)	Area
1 ml / min	7.46	15440.7520
	7.51	15363.9145
	7.52	15489.0118
0.8ml/min	9.09	19242.2676
	8.97	19323.0896
	8.96	19359.2958
1.2 ml/min	6.29	12994.7462
	6.21	13108.5892
	6.32	12980.6099

(V) Imp D (200 ppm)

flow	R.T(min)	Area
1 ml / min	4.99	25647.357
	4.94	15433.248
	4.96	15293.239
0.8ml/min	6.24	18617.984
	6.23	18573.574
	6.23	18524.364
1.2 ml/min	4.13	12672.760
	4.13	12571.582
	4.14	12460.848

(9.2) change in wavelength-

(I)Aripiperazole (200 ppm)

Wavelength(nm)	R.T(min)	Area
254	4.79	22686.672
	4.87	22837.228
	4.71	22271.361
256	4.90	22437.451
	4.87	21718.889
	4.88	22776.893
252	4.89	23243.751
	4.99	22749.619
	4.84	22941.690

(II)Imp A(80 ppm)

Wavelength(nm)	R.T(min)	Area
254	2.22	11509.564
	2.24	11597.724
	2.27	11590.860
256	2.21	11354.484
	2.28	11419.162
	2.26	11579.206
252	2.28	11380.025
	2.18	11157.846
	2.29	11352.834

(III)Imp B(200 ppm)

Wavelength(nm)	R.T(min)	Area
254	3.14	17415.193
	3.03	17029.615
	3.09	17335.023
256	3.08	16042.039
	3.08	16625.433
	3.09	16680.578
252	3.12	17637.135
	3.12	17787.427
	3.16	17949.523

(IV) ImpC(200 ppm)

Wavelength(nm)	R.T(min)	Area
254	7.63	15602.446
	7.59	15578.537
	7.53	15805.202
256	8.68	18506.976
	8.42	18376.766
	8.41	18258.920
252	8.35	18124.652
	8.58	18061.378
	8.48	18128.436

(V) Imp D (200 ppm)

Wavelength(nm)	R.T(min)	Area
254	5.40	16874.185
	5.38	16755.603
	5.36	16766.412
256	5.40	16459.718
	5.40	16304.281
	5.42	16668.256
252	5.47	16084.015
	5.02	15693.216
	5.39	15949.506

(9.3) change in pH of mobile phase-

(I) Aripiperazole (200 ppm)

pH	R.T(min)	Area
3.00	4.57	19043.125
	4.64	19431.598
	4.57	19488.155
3.20	4.26	20395.717
	4.27	20510.242
	4.30	20491.771
2.80	4.30	20560.277
	4.26	20538.266
	4.26	20627.889

(II) Imp A(80 ppm)

pH	R.T(min)	Area
3.00	2.00	11679.357
	2.00	11640.602
	2.01	11740.488
3.20	2.16	13795.167
	2.09	13667.017
	2.14	13613.774
2.80	2.14	13646.818
	2.09	13913.7611
	2.10	13872.766

(III) Imp B (200 ppm)

pH	R.T(min)	Area
3.00	2.74	12988.073
	2.76	12914.646
	2.76	12942.886
3.20	2.63	13608.312
	2.63	13582.780
	2.71	13578.088
2.80	2.65	13511.391
	2.63	13474.660
	2.61	13520.524

(IV) Imp C (200ppm)

pH	R.T(min)	Area
3.00	7.96	14646.989
	8.03	14609.266
	7.83	14593.462
3.20	8.34	15104.293
	8.50	15205.292
	8.53	15182.031
2.80	8.87	15225.296
	8.87	15263.792
	8.73	15249.969

(V) Imp D (200 ppm)

pH	R.T(min)	Area
3.00	5.38	16641.556
	5.40	16410.099
	5.38	16452.081
3.20	5.75	19595.899
	5.84	19333.272
	5.74	19095.758
2.80	6.09	19304.260
	6.14	20500.589
	6.15	20589.271

(9.4) change in mobile phase composition-

(i) **Mobile phase 1-** 550ml aq. buffer (0.2% TFA)+350ml Acetonotrile+100ml Methanol pH adjusted to 3 by TEA.

Compound	R.T (min)	Area
Aripiperazole (200ppm)	7.49	19112.819
	7.24	18917.429
	7.39	18902.538
Imp A(80 ppm)	1.96	9528.566
	1.99	9412.960
	2.02	9461.772
Imp B (200 ppm)	3.18	15066.116
	3.18	14986.635
	3.18	14946.517
Imp C (200 ppm)	13.15	11432.900
	13.32	11499.872
	13.09	11574.699
Imp D(200ppm)	9.49	8177.135
	9.42	7718.503
	9.42	7753.078

(ii) **Mobile phase 2-** 500 ml aq buffer (0.2% TFA)+ 440 Acetonitrile +60 ml Methanol pH adjusted to 3 by TEA

Compound	R.T (min)	Area
Aripiperazole (200ppm)	3.78	19106.009
	3.84	19156.301
	3.81	19218.271
Imp A(80 ppm)	1.96	14785.112
	1.95	14831.135
	1.99	14888.383
Imp B (200 ppm)	2.44	14838.245
	2.48	14704.647
	2.49	14902.114
Imp C (200 ppm)	6.68	16728.208
	6.68	16778.577
	6.68	16847.640
Imp D(200ppm)	4.29	17356.370
	4.27	16847.129
	4.34	16746.032

Table 10- LOQ and LOD for impurities of Aripiperazole.

Compound	LOQ (ppm)	LOD (ppm)
Imp A	0.025	0.008
Imp B	0.075	0.025
Imp C	0.1	0.00625
Imp D	0.03	0.0125

Table 11- Forced degredation study of Aripiperazole.

Condition	% degredation
Temperature 105^{O} C	51.81
UV	11.09
moisture	7.02
0.1M HCl	5.12
0.1 M NaOH	31.82
10% H_2O_2	12.13

CONCLUSION

A. Analytical method is found to be specific as proved by injecting known amount of component into the chromatogram.
B. Limit of quantification and limit of detection for Aripiperazole and process related
Impurities has been established and it is found to be within the range.
C. Analytical method is found to be linear over a specific range.
D. Analytical method is found to be précised and accurate.
E. Analytical method is found to be robust.
F. Sample prepared in analytical solution is found to be for at least 24 hrs.

The above mentioned isocratic method for the analysis of Aripiperazole and it's related impurities is found to be Simple, rapid and sensitive.

The method is completely evaluated for its specificity linearity, precision, accuracy, robustness, ruggdness, limit of quantification and detection

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