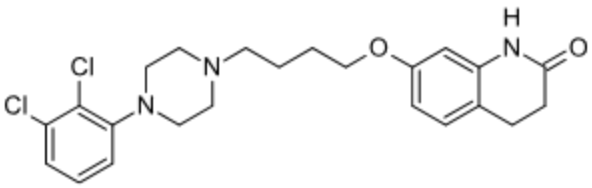
Aripiprazole (USP)



Aripiprazole is an atypical antipsychotic, and it is a partial dopamine agonist.
It is primarily used in the treatment of schizophrenia, bipolar disorder, major depressive disorder, tic disorders, and irritability associated with autism.

First approved by the U.S. Food and Drug Administration (FDA) for schizophrenia in November 2002 and the European Medicines Agency in June 2004; for acute manic and mixed episodes associated with bipolar disorder.

Common commercial brand names: Abilify and Aripiprex

Aripiprazole was developed by Otsuka in Japan, and in the United States, Otsuka America markets it jointly with Bristol-Myers Squibb.

Sales in 2010 were $4.6 billion globally.
Patent expires in 2015

In this compilation, we have followed the methods for:
Identification – FTIR (197K)
Assay – HPLC (gradient method)
Related Substances - HPLC (gradient method)

The HPLC methods are gradient methods, thus non-scalable.
The same chromatographic conditions are used for both assay and related substances methods, and a full validation protocol can be found using USP reference standards; USP Aripiprazole RS and USP Aripiprazole Related Compound F RS.

Aripiprazole (USP)

Definition:

Aripiprazole contains NLT (not less than) 98.0% and NMT (not more than) 102.0% of Aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$), calculated on the dried basis.

Identification:

-A. INFRARED ABSORPTION <197K> **FTIR**

-B. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay. .

Assay: HPLC

-Procedure: (Protect the solutions from light.)
Diluent: Acetonitrile, methanol, water, and acetic acid (30:10:60:1)
Solution A: Acetonitrile and 0.05% trifluoroacetic acid (10:90)
Solution B: Acetonitrile and 0.05% trifluoroacetic acid (90:10)

Gradient: See Table.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 650 µL.
Chromatographic system: (See Chromatography 621, System Suitability.)

Detector: UV 254 nm
Column: 4.6-mm × 10-cm; 3 µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

We have used a Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm (1.50469.0001).
This is a gradient method and can therefore not be changed.

Aripiprazole (USP)

System suitability solution: 1 µg/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound F RS in Diluent
Standard solution: 0.1 mg/mL of USP Aripiprazole RS in Diluent
Sample solution: 0.1 mg/mL of Aripiprazole in Diluent

System suitability
Samples: System suitability solution and Standard solution
The relative retention times (RRT) for aripiprazole and aripiprazole related compound F are 1.0 and 1.1, respectively.

Suitability requirements
Resolution: NLT 2.0 between aripiprazole and aripiprazole related compound F, System suitability solution
Tailing factor: NMT 1.5 for aripiprazole, System suitability solution
Relative standard deviation: NMT 1.0%, Standard solution

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) in the portion of the sample taken:

Result = (rU/rS) × (CS/CU) × 100

rU = peak area from the Sample solution
rS = peak area from the Standard solution
CS = concentration of USP Aripiprazole RS in the Standard solution (mg/mL)
CU = concentration of Aripiprazole in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
A. Residue on Ignition 281: NMT 0.1%
B. Heavy Metals, Method II 231: NMT 10 ppm

Organic Impurities (Protect the solutions from light.)
Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Aripiprazole (USP)

Analysis
Sample: Sample solution
Calculate the percentage of each impurity in the portion of Aripiprazole taken:

Result = (ri/rU) × (1/F) × 100

ri = peak response of each impurity from the Sample solution
rU = peak response of aripiprazole from the Sample solution
F = relative response factor (see Table 2)

Name	RRT	RRF	Acceptance criteria (NMT (%))
Aripiprazole related compound G[a]	0,9	0.72	0.10
Aripiprazole	1.0	-	-
Aripiprazole related compound F[b,c]	1.1	1.0	-
Aripiprazole 4,4-dimer[d]	1.3	1.0	0.10
Any other impurity	-	-	0.10
Total Impurities	-	-	0.50

a) 7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1H)-one.
b) 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin 1-oxide.
c) For system suitability and identification purposes only.
d) 1,1¢-(Ethane-1,1-diyl)bis(2,3-dichloro-4-{4-[3,4-dihydroquinolin-2(1H)-one-7-yloxybutyl]piperazin-1-yl}benzene).

USP Reference Standards
USP Aripiprazole RS
USP Aripiprazole Related Compound F RS

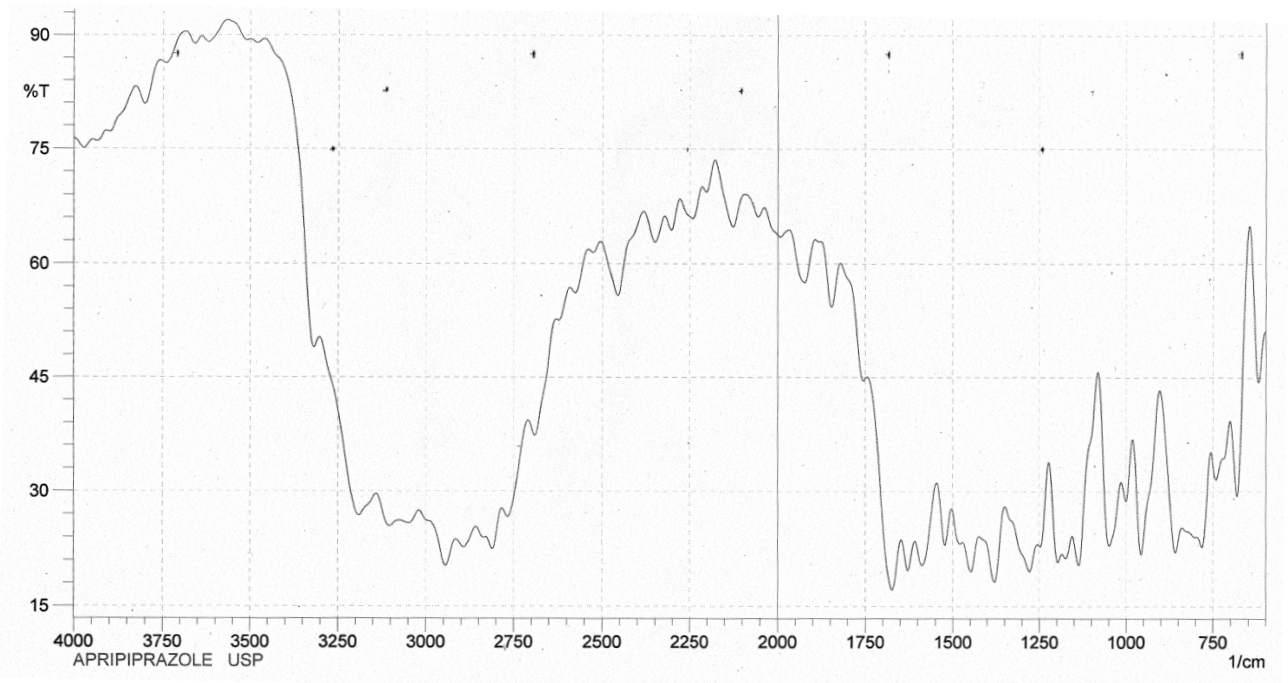
Recommended Merck Millipore products:
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur 1.00063
Acetonitrile (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur 1.00030
Methanol (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur 1.06007
Potassium bromide for for IR spectroscopy Uvasol® (1.04907)
Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm 1.50469
Trifluoroacetic acid for spectroscopy Uvasol® 1.08262
Water for chromatography (LC-MS Grade) LiChrosolv® 1.15333 or fresh water from Milli-Q system.

Aripiprazole Assay and Related Substances

Identification

A. INFRARED ABSORPTION <197K> FTIR

The reference 197K in a monograph signifies that the substance under examination is mixed intimately with potassium bromide. We recommend potassium bromide for IR spectroscopy Uvasol® (1.04907).

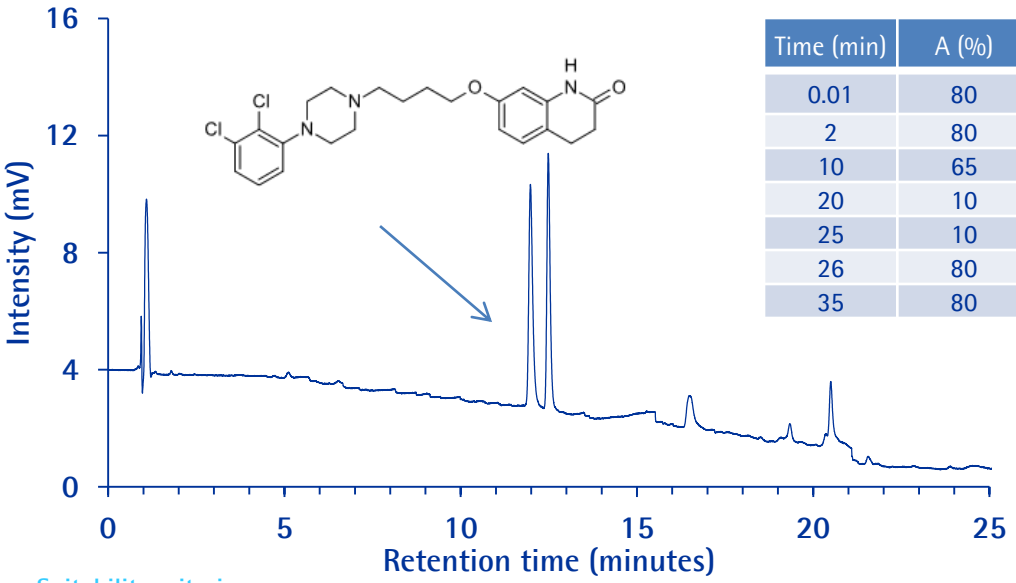


Aripiprazole Assay and Related Substances

Purospher STAR® RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm	1.50469.0001
Injection:	20 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.2 mL/min	
Mobile Phase A:	Acetonitrile and 0.05% trifluoroacetic acid (10:90 v/v)	
Mobile Phase B:	Acetonitrile and 0.05% trifluoroacetic acid (90:10 v/v)	
Gradient:	See table	
Temperature:	40°C	
Diluent:	Acetonitrile:Methanol:Water:Acetic acid (30:10:60:1 v/v)	
Sample:	1 µg/mL (1ppm) each of Aripiprazole and Aripiprazole Related Compound F in diluent	
Pressure Drop:	95-170 Bar (1377-2465 psi)	



Time (min)	A (%)	B (%)
0.01	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

System Suitability criteria:
Resolution: NLT 2.0 between aripiprazole and aripiprazole related compound F
Tailing factor: NMT 1.5 for aripiprazole
RRT is 1.0 and 1.1 for aripiprazole and aripiprazole related compound F, respectively

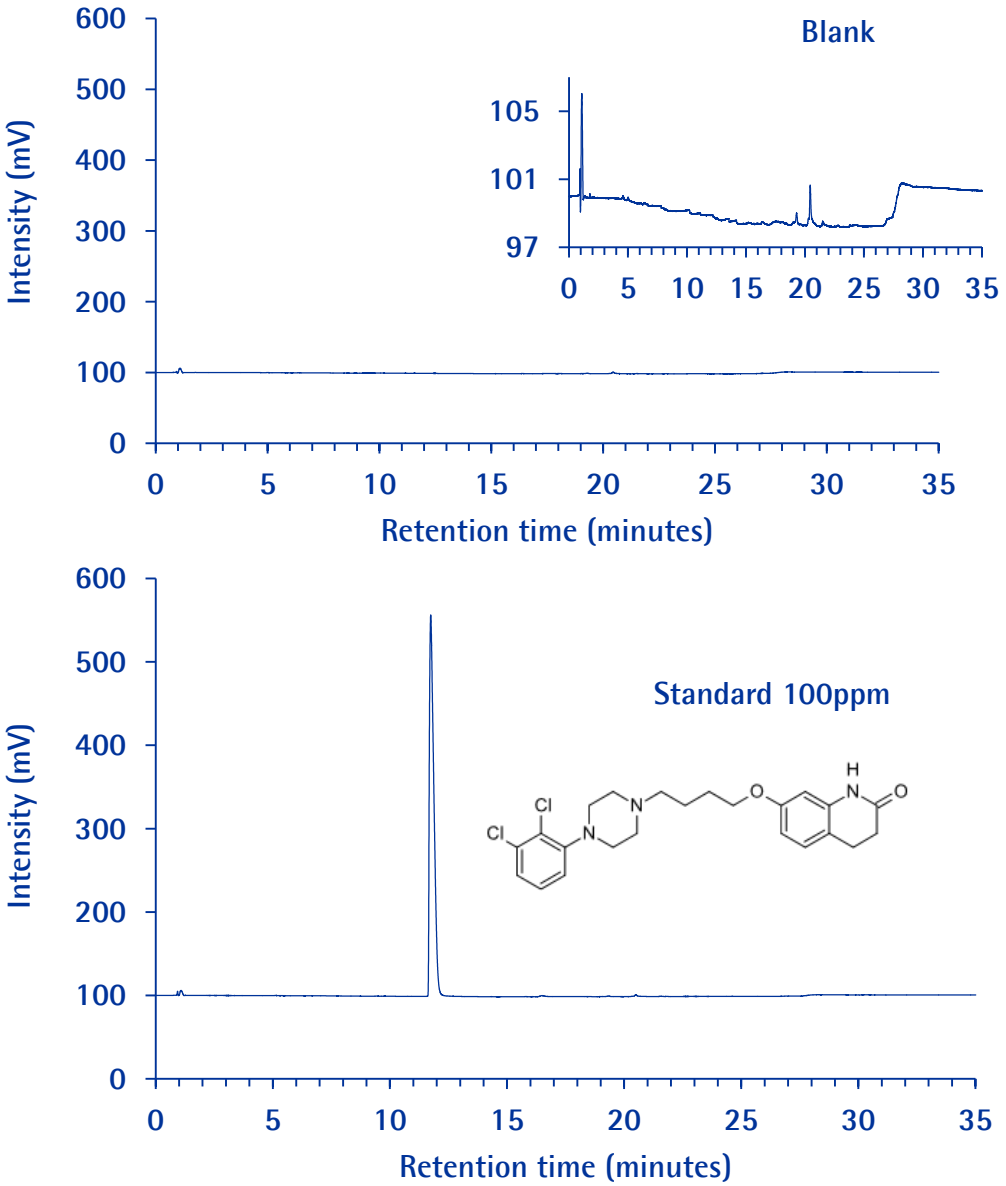
Chromatographic Data: SST solution

No.	Compound	Retention Time (min)	RRT	Tailing factor	Resolution
1	Aripiprazole	12.0	1.0	1.4	-
2	Aripiprazole RS F	12.5	1.05	1.3	2.8

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/perfect-solution

Aripiprazole Assay and Related Substances

Purospher STAR® RP-18 endcapped



Chromatographic Data: Standard 100ppm

No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Aripiprazole	12.0	1.4	16118

Aripiprazole Assay and Related Substances

Validation and Verification Data

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Aripiprazole RS and Aripiprazole Related Compound F

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Aripiprazole	12.0	-	1.4	-
Aripiprazole RS F	12.5	1.05	1.3	2.8

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 100 ppm Aripiprazole and 1 ppm of Aripiprazole Related Compound F

	Aripiprazole (Area units)	Aripiprazole RS F (Area units)
Standard 1	60114	6047450
Standard 2	60363	6080108
Standard 3	60308	6086256
Standard 4	60174	6081386
Standard 5	60316	6089267
Mean	60255	6076893
Stdev	105.6	16868.6
RSD (%)	0.18	0.28

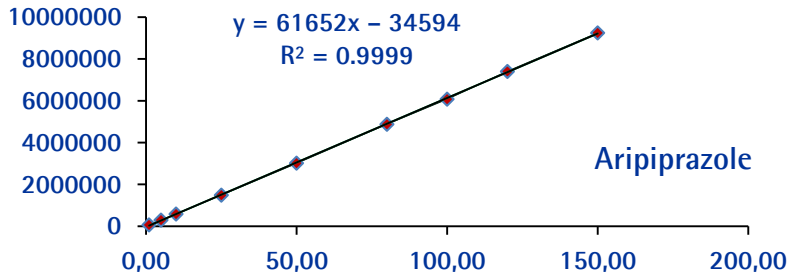
3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 0.1-1.5 ppm of Aripiprazole Related Compound F, and nine (9) concentration levels ranging from 1-150 ppm of Aripiprazole

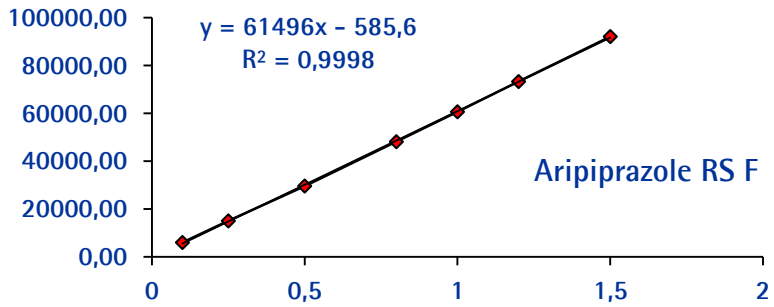
	[Aripiprazole RS F] (ppm)	Area	[Aripiprazole] (ppm)	Area
	0.1	6020	1.0	63167
	0.25	15062	5.0	292127
	0.5	29604	10.0	586674
	0.8	48154	25.0	1487549
	1	60630	50.0	3013178
	1.2	73335	80.0	4878836
	1.5	92098	100.0	6076893
			120.0	7401807
			150.0	9241987
STEYX				35391.1
SLOPE				61651.7
LOD				0.57
LOQ				1.7

Aripiprazole Assay and Related Substances

Validation and Verification Data

3. **Lineratity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 0.1-1.5 ppm of Aripiprazole Related Compound F, and nine (9) concentration levels ranging from 1-150 ppm of Aripiprazole





4. **Limit of Quantitation (LOQ) Accuracy**
   Determined by injecting ten (10) standard solutions at LOQ level of Aripiprazole.

	Area Units
1	152629
2	151490
3	152632
4	151973
5	151697
6	151808
7	151794
8	151659
9	152285
10	151524
Mean	151949
STD DEV	424.9
RSD	0.28