Identification, Assay and Organic Impurity Profiling Methods for Aripiprazole following the United States Pharmacopeia Monograph

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ARIPIPRAZOLE USP MONOGRAPH METHODS OVERVIEW

Aripiprazole is an atypical antipsychotic and 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. Aripiprazole was first approved by the U.S. Food and DrugAdministration in November 2002 for schizophrenia and by 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. In the United States, Otsuka America markets it jointly with Bristol-Myers Squibb. In 2010, sales were $4.6 billion globally; the patent expired in 2015.

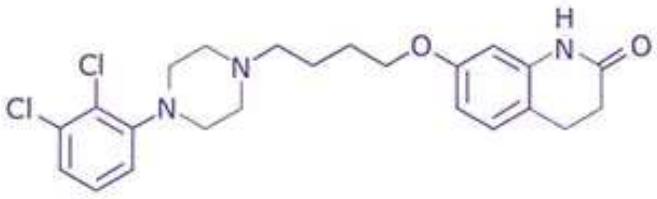


Figure 1. Chemical structure of Aripiprazole

In this compilation, we have used the USP 40–NF 35 experimental conditions for Aripiprazole in the following areas:

- Identification — FTIR
- Assay — HPLC (gradient method)
- Related Substances — HPLC (gradient method)

The HPLC methods are gradient methods, thus they are nonscalable.

The same chromatographic conditions are used for methods in both the assay and related substances, and a full validation protocol can be found using these USP Reference Standards: USP Aripiprazole RS and USP Aripiprazole Related Compound F RS.

IDENTIFICATION AND ASSAY

Definition

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

Identification—FTIR <197K>

A. Infrared absorption
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 (gradient method)

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:** (**Table 1**)
  - Detector: UV 254 nm
  - Column: 4.6 mm × 10 cm (3 µm) packing L1
  - Flow rate: 1.2 mL/min
  - Injection volume: 20 µL

Note: The gradient was established on an HPLC system with a dwell volume of approximately 650 µL.

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

Table 1. Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Product No. **1.50469**). This is a gradient method and can therefore not be changed.

ASSAY SOLUTIONS AND ANALYSIS

- **System suitability solution:** 1 µg/mL each of USP Aripiprazole and USP Aripiprazole Related Compound F in Diluent
- **Standard solution:** 0.1 mg/mL of USP Aripiprazole in Diluent
- **Sample solution:** 0.1 mg/mL of aripiprazole in Diluent
- **System Suitability**
- **Samples:** System suitability solution and Standard solution
  Note: 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 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 ANALYSIS

- **Inorganic Impurities**
  A. Residue on Ignition—USP General Chapter 281: NMT 0.1%
  B. Heavy Metals, Method II—USP General Chapter 231[1]: 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.

Analysis

- **Samples:** 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 (**Table 2**)

USP Reference Standards

- USP Aripiprazole
- USP Aripiprazole Related Compound F

[1]Chapter valid until Jan. 1, 2018.

Name	Relative Retention Time (RRT)	Relative Response Factor (RRF)	Acceptance Criteria (NMT, %)
Aripiprazole related compound G[2]	0.9	0.72	0.10
Aripiprazole	1.0	—	—

Aripiprazole related compound F[3,4]	1.1	1.0	—
Aripiprazole 4,4-dimer[5]	1.3	1.0	0.10
Any other impurity	—	—	0.10
Total impurities	—	—	0.50

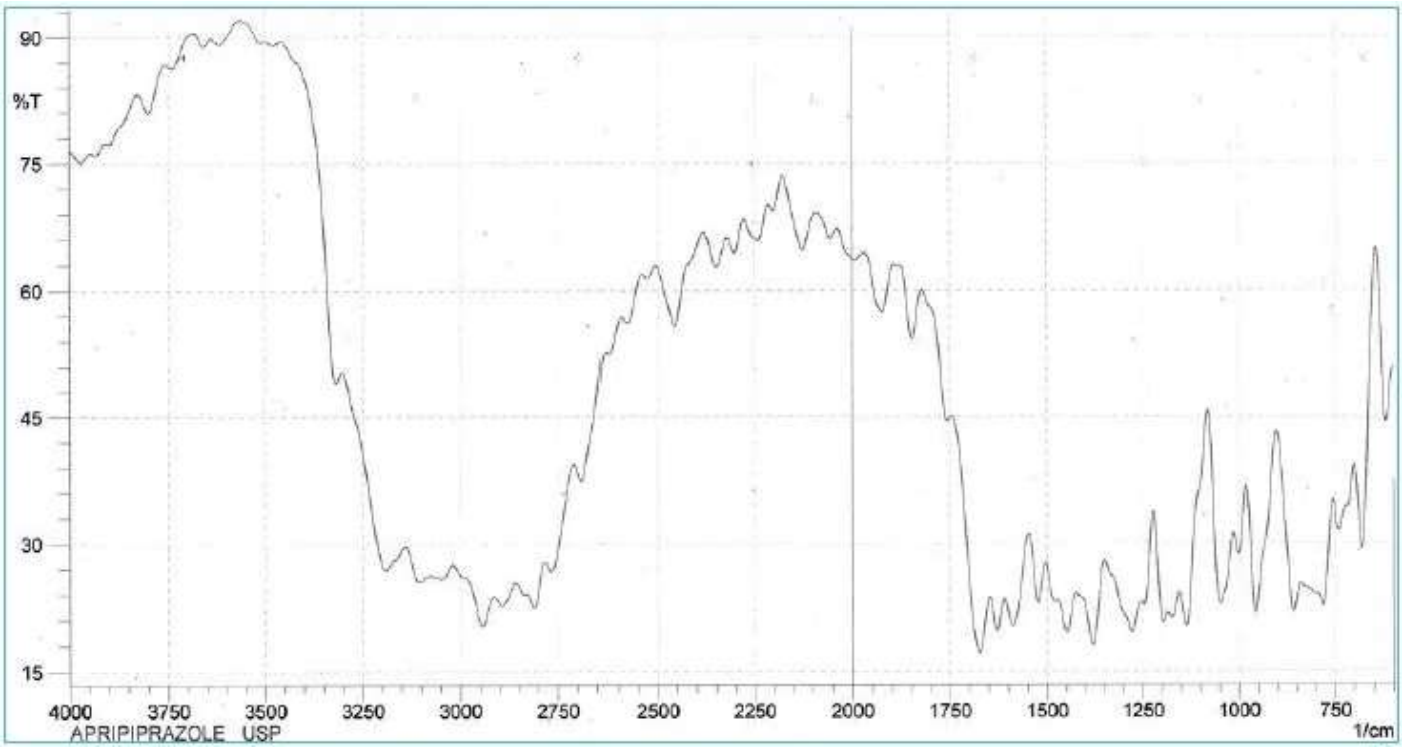
Table 2. Related Substances (Organic Impurities)

[2] 7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1H)-one
[3] 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin 1-oxide
[4] For system suitability and identification purposes only
[5] 1,1¢-(Ethane-1,1-diyl)bis(2,3-dichloro-4-{4-[3,4-dihydroquinolin-2(1H)-one-7-yloxybutyl]piperazin-1-yl}benzene)

IDENTIFICATION DATA

Infrared Absorption

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® (Product No. **1.04907**).



ASSAY AND RELATED SUBSTANCES DATA

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Product No. **1.50469**)
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, and acetic acid (30:10:60:1 v/v)
Sample: 1 µg/mL (1 ppm) each of aripiprazole and aripiprazole related compound F in Diluent
Pressure drop: 95–170 Bar (1,377–2,465 psi)

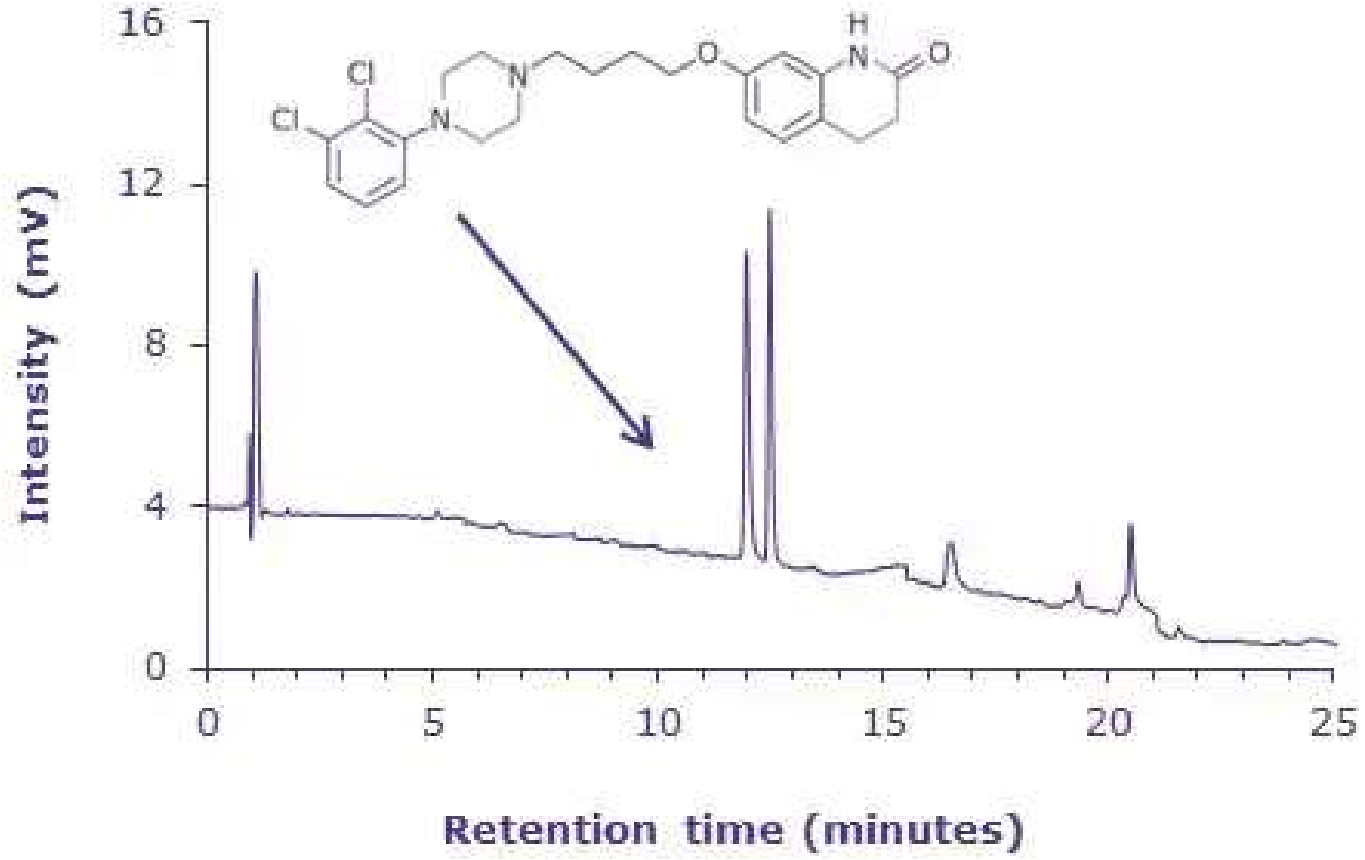
Time (min)	A (%)	B (%)

0.01	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

Table 3. Chromatographic Conditions

Suitability Requirements

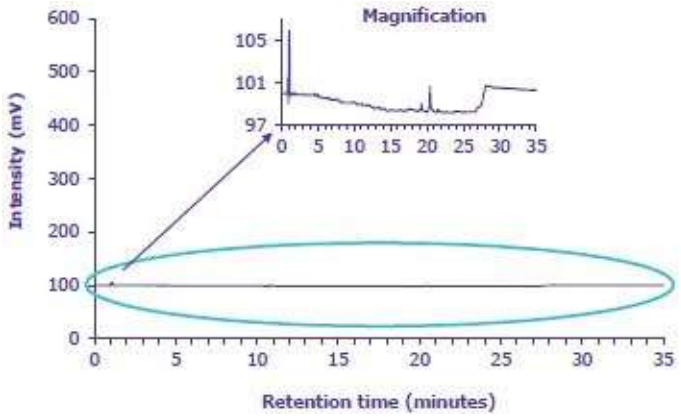
- **Resolution:** NLT 2.0 between aripiprazole and aripiprazole related compound F
- **Tailing Factor:** NMT 1.5 for aripiprazole
- **Relative Retention Time (RRT):**
  1.0 for aripiprazole and
  1.1 for aripiprazole related compound F

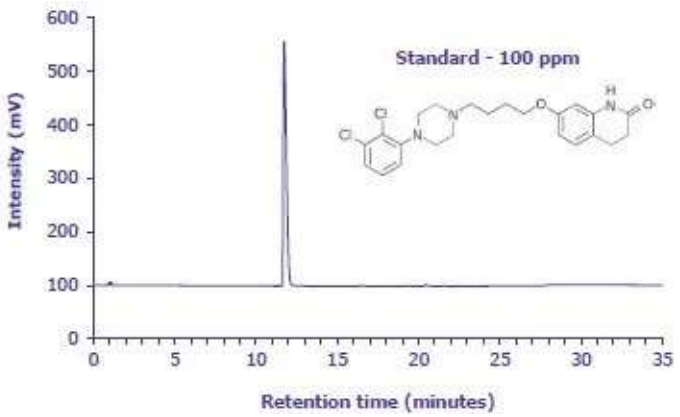


Chromatographic Data (System Suitability Solution)

Compound	Retention Time (min)	RRT	Tailing Factor	Resolution
Aripiprazole	12.0	1.0	1.4	—
Aripiprazole related compound F	12.5	1.05	1.3	2.8

Table 4. Chromatographic Data (System Suitability Solution)





Compound	Retention Time (min)	Tailing Factor	Theoretical plates
Aripiprazole	12.0	1.4	16,118

Table 5. Chromatographic Data (100ppm Standard Solution)

ASSAY AND RELATED SUBSTANCES VALIDATION DATA

A. Specificity

Compound	Retention time (min)	RRT	Tailing factor	Resolution
Aripiprazole	12.0	1.0	1.4	—
Aripiprazole related compound F	12.5	1.05	1.3	2.8

Table 6. Determined by injection of System suitability solution and determination of the retention time andrelative retention time for aripiprazole and aripiprazole related compound F.

B. Repeatability

Standard Solution	Aripiprazole (Area Units)	Aripiprazole Related Compound F (Area Units)
1	60,114	6,047,450
2	60,363	6,080,108
3	60,308	6,086,256
4	60,174	6,081,386
5	60,316	6,089,267
Mean	60,255	6,076,893
STDEV	105.6	16,868.6
RSD (%)	0.18	0.28

Table 7. Determined by injecting five samples with a Standard solution containing 100.0 ppm USP Aripiprazole and 1.0 ppm of USP Aripiprazole Related Compound F.

C. LINEARITY, LIMIT OF DETECTION (LOD), AND LIMIT OF QUANTITATION (LOQ)

Determined by injecting seven concentration levels from 0.1 to 1.5 ppm of USP Aripiprazole Related Compound F and nine concentration levels ranging from 1.0 to 150.0 ppm of aripiprazole.

LOD (ppb)	LOQ (ppb)
7.6	23

Table 8. Aripiprazole related compound F

LOD (ppm)	LOQ (ppm)
0.57	1.7

Table 9. Aripiprazole

(ppm)	Area Units
0.1	6,020
0.25	15,062
0.5	29,604
0.8	48,154
1.0	60,630
1.2	73,335
1.5	92,098

Table 10. Aripiprazole related compound F

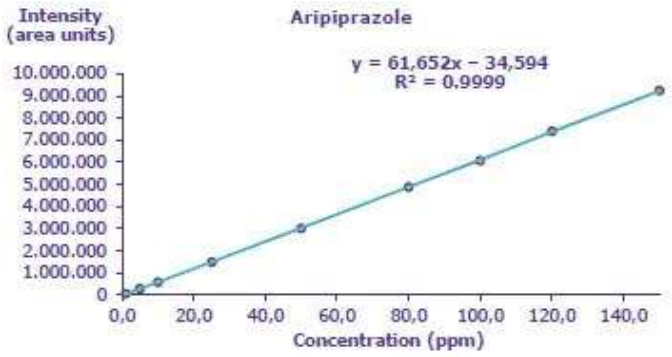
(ppm)	Area Units
1.0	63,167
5.0	292,127

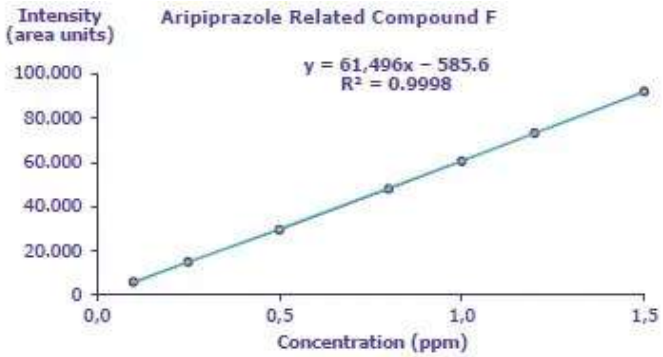
10.0	586,674
25.0	1,587,549
50.0	3,013,178
80.0	4,878,836
100.0	6,076,893
120.0	7,401,807
150.0	9,241,987

Table 11. Aripiprazole

D. LINEARITY, LOD, LOQ (CONT.)

Determined by injecting seven concentration levels from 0.1 to 1.5 ppm of USP Aripiprazole Related Compound F and nine concentration levels ranging from 1.0 to 150.0 ppm of aripiprazole.





D. LOQ Accuracy

Injection	Area units	SD Area units	RSD (%)
N=10	151,949	±424.9	0.28

Table 12. Determined by injecting 10 Standard solutions at LOQ level of USP Aripiprazole RS.

Recommended Products

- Acetic acid (glacial) 100% anhydrous for analysis—EMSURE® ACS, ISO, Reag. Ph. Eur. (Product No. **1.00063**)
- Acetonitrile (gradient grade for liquid chromatography)—LiChrosolv® Reag. Ph. Eur. (Product No. **1.00030**)
- Methanol (gradient grade for liquid chromatography)—LiChrosolv® Reag. Ph. Eur. (Product No. **1.06007**)
- Potassium bromide for IR spectroscopy—Uvasol® (Product No. **1.04907**)
- Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Product No. **1.50469**)
- Trifluoroacetic acid for spectroscopy—Uvasol® (Product No. **1.08262**)
- Water for chromatography (LC-MS grade)—LiChrosolv® (Product No. **1.15333**) or fresh water from the Milli-Q® system
- Aripiprazole United States Pharmacopeia (USP) Reference Standard (Product No. **1042634** USP)
- Aripiprazole Related Compound F United States Pharmacopeia (USP) Reference Standard (Product No. **1042689** USP)

Materials

Product No.	Description	SDS	Pricing
1.00063	**Acetic acid (glacial) 100%** anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	↓	Expand
1042634	**Aripiprazole** United States Pharmacopeia (USP) Reference Standard	↓	Expand
1042689	**Aripiprazole Related Compound F** United States Pharmacopeia (USP) Reference Standard	↓	Expand
1.06007	**Methanol** gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	↓	Expand
1.04907	**Potassium bromide** for IR spectroscopy Uvasol®	↓	Expand
1.50469	**Purospher® STAR RP-18 endcapped (3µm) Hibar® RT 100-4.6** suitable for HPLC		Expand
1.08262	**Trifluoroacetic acid** for spectroscopy Uvasol®	↓	Expand

Product No.	Description	SDS	Pricing
1.15333	**Water** for chromatography (LC-MS Grade) LiChrosolv®		Expand