Life Cycle Management of Analytical RP-HPLC Method Development for Assay of Abilify Disc melts in Immediate Release Dosage Form

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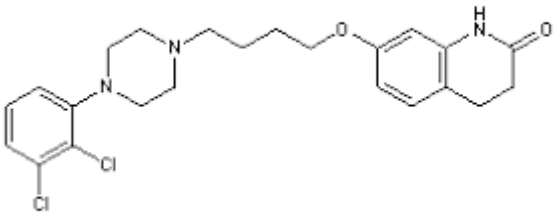
Abstract: In this research work of life cycle management of analytical RP-HPLC method development for assay of Ability Disc melt in immediate release dosage form, the RP-HPLC assay method was developed and validated for Ability Disc melt. Stress study is also carried out. The chromatographic conditions will be as, mobile phase is 0.05M Phosphate buffer pH2.0, Methanol, Acetonitrile in a ratio of 5:3:2, column used is Hypersil ODS C-18, 250 mm x 4.6 mm, 5.0 µm, Flow Rate1.5 ml/min, Injection volume 20 µl, Detection wavelength is 215 nm and run time is 25 min. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Ability Disc melt in immediate release dosage form.

Key words: Abilify Discmelt, Life Cycle Management, RP-HPLC Method Development, Validation, Immediate Release Dosage Form

Introduction:

Abilify Disc melt (Aripiprazole)) is a typical antipsychotic agent, which is used in a treatment of schizophrenia, bipolar I disorder and acute treatment of manic and mixed episodes [1-3]. It is also used in Tourette's disorder in pediatrics patients (16-18 years) in the dose range 5-20 mg/day patient weight less than 50 kg. It has chemical name 7-(-4-(4-2-3- dichlorophenyl)-1-piperazinyl)butoxy)-3,4-dihydrocarbostyr il (Figure 1). It is effective in the treatment of both negative and positive symptoms of schizophrenia disorder. This agent belongs to the class of benzioxazole with dose 10-15 mg/day. It has partial agonist effect towards 5- HT1A receptor, dopamine D2 receptor and antagonistic effect on 5- HT2 receptor. Its sides effects including weight gain, QTc prolongation and hyperprolactinemia [4]. On the basis of literature survey few analytical methods reported for the detection of Aripiprazole in pharmaceutical dosage forms and biological fluids include high performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), capillary electrophoresis and spectrophotometric methods have been describe for the determination of Aripiprazole in pharmaceutical preparations [5,7]. The purpose of the current study was to develop a validated HPLC method for determination of Aripiprazole in bulk and pharmaceutical dosage form, to achieve more accuracy, specificity and precision. The method validation was preformed according to ICH guidelines. The method designed for estimation of Aripiprazole is more superior than previously reported methods and water is used as major part of solvents and less use of hazardous organic solvents.



Structure of Abilify Discmelt (Aripiprazole)

Experimental:

- **Materials:**

1) Abilify Discmelt (Aripiprazole): Working standard and its claimed purity was 98.20%.
2) Abilify Discmelt (Aripiprazole): Tablet (label claim 10mg) and placebo, which was prepared and supplied by Instavision lab,.

Note: No any known Impurity reported.

- **Reagents and Chemicals:**

1) Acetonitrile: -HPLC grade, Rankem, India.
2) Methanol: - HPLC grade, Rankem, India.
3) Milli-Q water: - It was purified by Millipore Corporation's system.
4) Acetic acid: - Reagent Grade, Merck, India.
5) Hydrochloric acid: - Reagent Grade, Merck, India.
6) Sodium hydroxide: - Reagent Grade, Merck, India.
7) Hydrogen Peroxide (30%):- Reagent Grade, Merck, India.
8) Potassium di-hydrogen orthophosphate - AR Grade, Merck, India.

- **Instruments, Apparatus and equipment:**

1) High Performance Liquid chromatography system (HPLC): Agilent Liquid Chromatography with PDA detector
2) Chromatographic software:– E Z Chrome Elite
3) A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - UV- 2450, Shimadzu, Japan.
4) Analytical Balance: - AD 265S, Mettler Toledo, Sweetzerland.
5) pH Meter: - Labindia, India.
6) Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
7) Hot air oven: - Labline, India.
8) Photo stability chamber: - SVI equipment's, Germany

- **Chromatographic system:**

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1322A), binary pump (G1312A), auto injector (G1367C), column oven (G1310B), DAD detector (G1315C) and E Z Crome Elite (software).The published methods of analysis for determination and separation of Abilify Discmelt (Aripiprazole) in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Abilify Discmelt (Aripiprazole) .

- **Selection of Buffer in Mobile Phase: -**

0.05M Phosphate buffer pH2.0 with orthophosphoric acid was used to optimize the peak shape retention time and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (50:30:20) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

- **Selection of Mobile Phase: -**

Different ratios of Acetonitrile and Methanol was used to optimize the retention time of late eluting impurities and Methanol to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (Buffer: Methanol: Acetonitrile) [50:30:20 v/v] after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

- **Selection of Column:-**

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of polar and non-polar degradation products and impurities, a C_{18} column was preferred over other columns Hypersil ODS C-18, 250 mm x 4.6 mm, 5 µm column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

- **Selection of Diluents / Solvent for extraction:-**

Different solvents were tried including single solvent and combination of solvents like ACN: Water, Methanol: Water, in different concentrations, But Abilify Discmelt (Aripiprazole) tablet gets dissolved in Methanol. Hence first stock was prepared in methanol and followed by second dilution done in diluents as [Methanol: Acetonitrile: Buffer 30:20:50] same as that of mobile phase to reduce the peak shape related problems.

The results of all validation parameters are given in following tables and all lie well within the limit of acceptance criteria,
Various Method screening Trials has been taken using following different compositions.

- **Table for trials:**

Sr. No.	Trails Taken	Observation	Remarks
1	Buffer : Methanol (50:50 v/v), Flow rate 1.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	No Peak observed	Not Satisfactory
2	Buffer : Acetonitrile (50:50 v/v), Flow rate 1.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Peak observed later.	Not Satisfactory
3.	Buffer : Acetonitrile : Methanol (50:25:25 v/v), Flow rate 2.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Broaden peak observed	Not Satisfactory
4	Buffer : Acetonitrile : Methanol (50:25:25 v/v), Flow rate 1.5 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Tailing observed	Not Satisfactory
5	Buffer : Acetonitrile : Methanol (50:20:30 v/v), Flow rate 1.5 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Good peak shape observed	Satisfactory

Reason for validation: Non-Pharmacopeia method.

Design of experiment (DOE):
A smart DOE was performed with respect to components of mobile phase (like concentration of buffering agent/ buffer strength, pH of buffer, ratio of organic modifiers) and chromatographic parameter (like Flow rate and column temperature) as mentioned below.

1. KH_2PO_4 conc. 0.05M +/- 0.01
2. pH of buffer pH 2.0 +/- 0.2
3. Buffer ratio 500 mL +/- 50mL
4. Methanol 300 mL +/- 30mL
5. Acetonitrile 200 mL +/- 20mL
6. Flow rate 1.5 +/-0.2mL
7. Column temp 40+/- 5 °C

Method Validation:

- **Standard preparation:**

Weigh and transfer about 30 mg of Abilify Discmelt (Aripiprazole) reference standard to a 200 mL volumetric flask. Add about 140 mL of Methanol, sonicate to dissolve, make up the volume with solvent Methanol. Further dilute 10 mL of this solution and make up the volume 25 mL with mobile phase. (60 ppm)

- **Sample preparation**

Weigh accurately tablet powder equivalent to 15mg transfer into 100 mL volumetric flask add about 75 mL of methanol , sonicate at for about 45 min with intermittent shaking, keep to achieve room temperature make up to volume with methanol. Centrifuge the solution at 3500rpm for 20 minutes and further dilute 10mL of the supernatant to 25mL with mobile phase. (60 ppm)

- **Mobile phase Preparation**

Mixture of (Buffer:- 0.05M Phosphate buffer pH2.0) 500 mL buffer, 300 mL methanol, 200 mL Acetonitrile and filter through 0.45µ membrane filter and degas.

- **Blank Solution:**

Use mobile phase as blank.

- **Optimized HPLC Parameters:**

Instrument : Agilant Liquid Chromatography with PDA detector
Column : Hypersil ODS C-18, 250 mm x 4.6 mm, 5.0 µm
Flow Rate : 1.5 mL/min
Injection volume : 20 µL
Column temperature : 40°C
Sample cooler Temperature : Ambient

Detection : 215 nm
Run time : 25 minutes

System Suitability Test:

Sr. No.	Parameters	Abilify Discmelt (Aripiprazole)
1.	Peak area	*1183271*
2.	No. of theoretical plates	*8529*
3.	Retention time (min)	8.212
4.	Asymmetry/USP Tailing	1.02
5.	% RSD	*0.35*

Linearity:

Linearity Level	Standard concentration	Concentration of Abilify Discmelt (Aripiprazole) (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	30.08	592833	0.9999
Level – 2	80%	48.12	946507	
Level – 3	100%	60.15	1186807	
Level – 4	120%	72.18	1425522	
Level – 5	150%	90.23	1765958	

Precision:

Sample Preparation	% Assay of Abilify Discmelt (Aripiprazole)
Test solution -1	99.57
Test solution -2	99.49
Test solution -3	99.40
Test solution -4	99.28
Test solution -5	99.59
Test solution -6	99.39
Mean	**99.45**
Standard Deviation	**0.12**
Relative Standard Deviation (%)	**0.12**

Intermediate precision:

Analysis performed during method precision study

Analyst: Analyst-I HPLC ID No.: ASR34

Make :Hypersil ODS,C18, 4.6mmx250mm, 5 µm
Column serial number. : 12058H

Sr. No.	% Assay of Abilify Discmelt (Aripiprazole)
Test solution-1	99.57
Test solution-2	99.49
Test solution-3	99.40
Test solution-4	99.28
Test solution-5	99.59
Test solution-6	99.39

Analysis performed during intermediate precision study
HPLC ID No.: ASR34
Make :Hypersil ODS,C18, 4.6mmx250mm, 5 µm
Column serial number : 05482J

Test solution-1	99.64
Test solution-2	99.53
Test solution-3	100.02
Test solution-4	99.32
Test solution-5	99.62
Test solution-6	99.82
Mean of twelve samples	**99.56**
Standard Deviation	**0.21**
Relative Standard Deviation (%)	**0.21**

Robustness:

- **Change the flow rate of Mobile Phase:**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in flow rate 1.30 mL/ min.	1	99.55
	2	99.30
Mean		**99.45**
Standard deviation		**0.12**
Relative standard deviation (%)		**0.12**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)
Method precision	1	99.57
	2	99.49
	3	99.40

	4	99.28
	5	99.59
	6	99.39
Change in flow rate 1.70 mL/ min.	1	99.11
	2	98.95
Mean		**99.35**
Standard deviation		**0.22**
Relative standard deviation (%)		**0.22**

- **Change in the Mobile Phase composition ±10%:**

Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Mobile Phase composition +10% {higher organic- Buffer: MeOH: ACN) (500:330:220)}	1	100.14
	2	100.69
Mean		99.69
Standard deviation		0.48
Relative standard deviation (%)		0.48
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Mobile Phase composition -10%. {Buffer:MeOH:ACN) (500:270:180)}	1	99.98
	2	101.01
Mean		99.71
Standard deviation		0.56
Relative standard deviation (%)		0.57

- **Change in the Temperature of the Column ±5°C:**

Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**

Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Temperature of the Column +5°C	1	99.66
	2	99.87
Mean		99.53
Standard deviation		0.18
Relative standard deviation (%)		0.19
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Temperature of the Column -5°C	1	100.14
	2	99.68
Mean		99.57
Standard deviation		0.26
Relative standard deviation (%)		0.27

- **Change in the pH of the Buffer ±0.1Unit:**

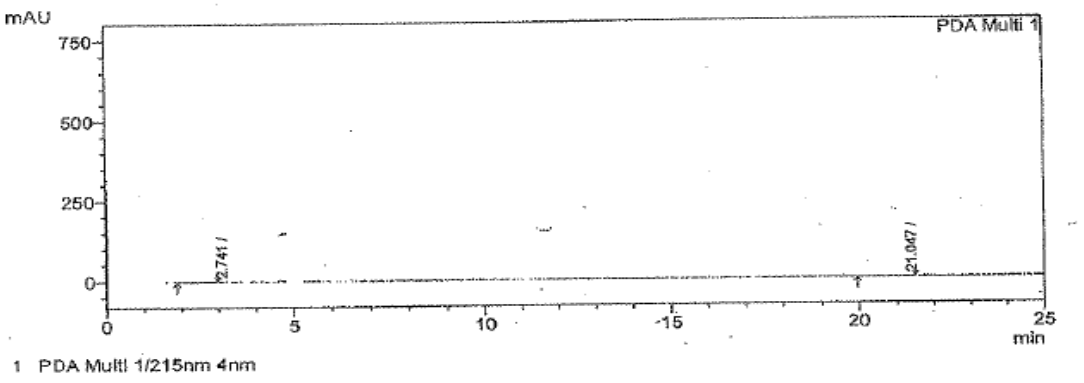
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in pH of the buffer by + 0.1 unit	1	101.01
	2	100.65
Mean		99.80
Standard deviation		0.65
Relative standard deviation (%)		0.65
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57

	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in pH of the buffer by - 0.1 unit	1	99.98
	2	100.15
Mean		99.61
Standard deviation		0.30
Relative standard deviation (%)		0.31

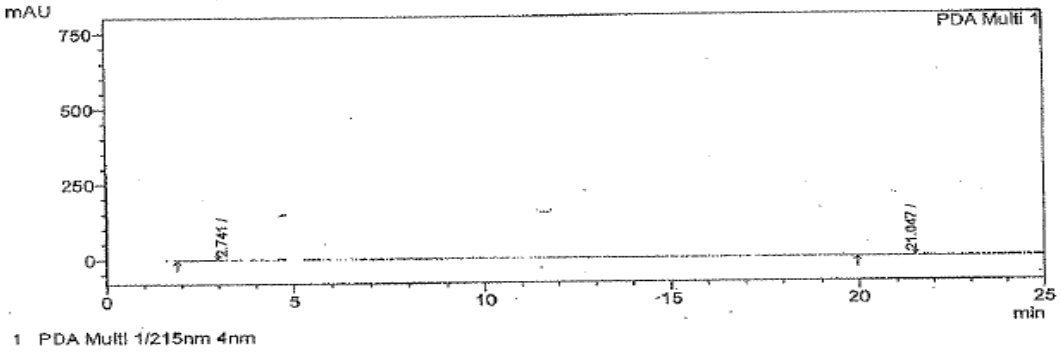
- **System suitability parameters:**

Parameter			**Theoretical Plates**	**Tailing Factor**	**%RSD**
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	**Specificity**				
	1.1	Specificity-Part-A	8952	1.07	0.13
	1.2	Specificity-Part-B	8213	1.05	0.15
2	**Linearity and Range**		8201	1.03	0.17
3	**Accuracy study (Recovery)**		8135	1.02	0.16
4	**Precision**				
	4.1	Method precision (Repeatability)	8546	1.04	0.14
	4.2	Intermediate Precision (Ruggedness)	8451	1.06	0.12
5	**Robustness**				
	5.1	Change flow rate by ± 10% (1.3 ml/minute and 1.8 ml/minute).	8212 8672	1.09 1.01	0.60 0.46
	5.2	Change the column temperature by ± 5°C (35°C and 45°C)	8014 7753	1.08 1.28	0.60 0.85
	5.3	Change the mobile phase Organic components by ± 10%	7845 8911	1.09 1.20	0.68 1.02
	5.4	Change the mobile phase Buffer pH by ± 0.1 Unit	8162 6785	1.11 1.29	0.57 1.36

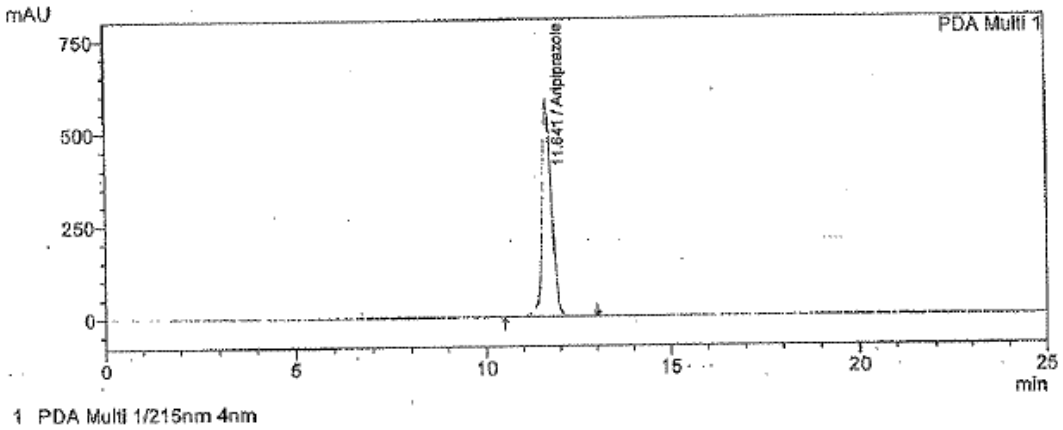
Chromatograms:



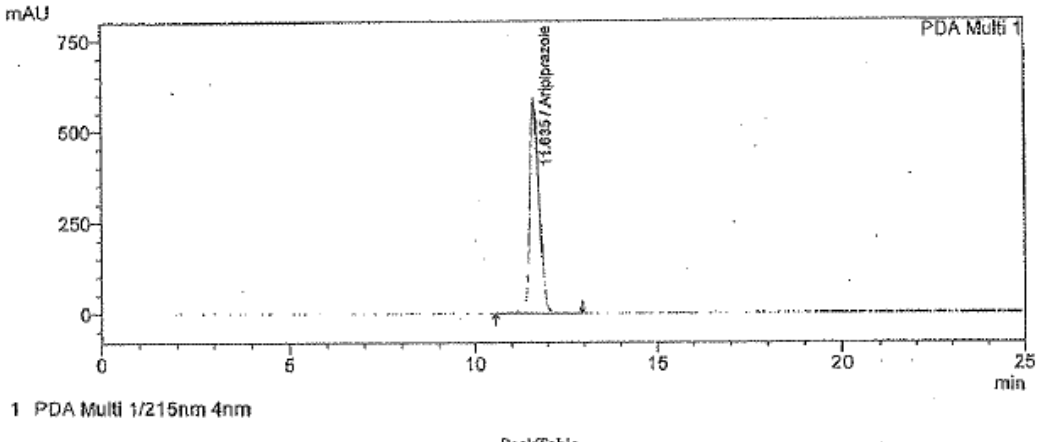
Blank



Placebo



Standard



Sample

Result and Discussion:

- **Specificity Part-A**

There is no interference of blank and placebo peaks with the main peak. All impurities are well separated from the main peak. The main peak purity and known impurities purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method can be termed as specific.

- **Specificity Part-B**
  - Degraded impurities in all sample preparation are well separated from the main peak.
  - Peak purity for the main peak in sample preparation is well within the limit of acceptance criteria.
  - Hence the method can be termed as specific

- **Linearity and Range**

The areas obtained are directly proportional to the concentration of analyze in the sample. Hence the method considered as linear in the range considered.

- **Accuracy**
  The recovery at each level and mean recovery meets the established acceptance criteria. Hence, the method can be termed as accurate in the considered range.

- **Precision**
  The results obtained lie well within the limit of acceptance criteria. Hence the method can be termed as precise and rugged.

- **Filter media interference**
  The results obtained lie well within the limit of acceptance criteria. Hence there is no interference from filter media.

- **Robustness**
  No significant changes observed in system suitability parameters. Hence, the method can be termed as robust.

- **System Suitability**

The mean values of system suitability parameters lay well within acceptance criteria, hence the method is suitable. Since the results are within the limit of acceptance criteria for all validation parameters, therefore, the method can be considered as validated and suitable for intended use.

Conclusion:

The proposed method for determination of Abilify Discmelt (Aripiprazole) is simple, specific, rapid, linear, accurate, precise, rugged, robust, sensitive as well as selective and suitable for routine analysis in laboratories.

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