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Dedicated to the memory of
Professor Candin Liteanu on his 100th anniversary

QUANTITATIVE EVALUATION OF ARIPIPRAZOLE AND ITS FIVE RELATED CHEMICAL IMPURITIES FROM PHARMACEUTICALS USING A HPLC-DAD METHOD

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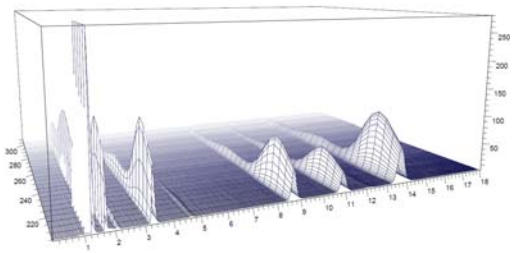
A reversed phase HPLC-DAD method was developed and validated for simultaneous determination of aripiprazole and five of its chemical related impurities in tablet dosage forms. The chromatographic separation of the studied compounds was achieved on a Zorbax SB-C18 column (150 mm x 4.6 mm, 5 µm particle size) and a mobile phase composed of methanol : water : orthophosphoric acid. The elution was isocratic with a flow rate of 1.5 mL/min, column temperature was set to 40ºC and the injection volume was 20 µL. Aripiprazole was detected at the wavelength of 254 nm, while its impurities were detected at 224 nm for a better sensitivity. The proposed method was fully validated through linearity, limit of detection, limit of quantitation, accuracy and precision. Forced degradation studies were also conducted in acid, alkaline, oxidative, photolytic and hydro-thermal conditions. The proposed method has been applied on commercial drug products for the determination of aripiprazole and its impurities.



INTRODUCTION

Aripiprazole, 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy}-3,4-dihydro-2(1*H*)-quinolinone, is a novel atypical antipsychotic drug used for the treatment of schizophrenia and schizoaffective disorders.[1-3] It presents a high affinity for dopamine D_2 and D_3 receptors,[4] serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2B} receptors.[5] The drug is metabolized by the cytochrome P450 isoenzymes CYP3A4 and CYP2D6 with dehydro-aripiprazole being the main metabolite.[6] Aripiprazole is a selective monoaminergic antagonist with high affinity for the serotonin Type 2 ($5HT_2$), dopamine Type 2 (D_2) and H_1 histaminergic receptors. It appears to mediate its antipsychotic effects primarily by partial agonism at the D_2 receptor. In addition to partial agonist activity at the D2 receptor, aripiprazole is also a partial agonist at the 5-HT_{1A} receptor and, like the

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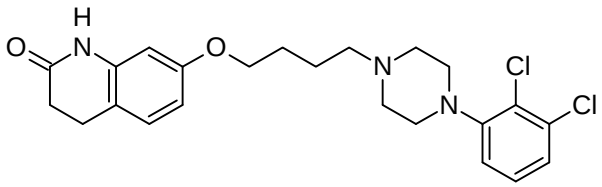
other atypical antipsychotics, it displays an antagonist profile at the 5-HT_{2A} receptor.[7] Aripiprazole has moderate affinity for histamine and alpha adrenergic receptors, and no appreciable affinity for cholinergic muscarinic receptors. It also blocks apomorphine-induced stereotypy and locomotors activity and does not produce stereotypy or increased locomotion when administered alone.[4] Aripiprazole is available as conventional tablets with normal dosage units between 5 mg and 30 mg per tablet. It is also available as oral disintegrating tablets, oral solution and intra muscular injection.

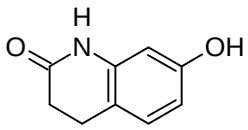
Determination of aripiprazole and its major metabolite in biological samples was carried out by LC-MS/MS,[8-10] LC-ESI-MS,[11-12], SPE–UPLC–MS/MS,[13] SPE-UPLC-DAD,[14] UPLC–ESI-MS/MS,[15] LC-DAD,[16-17] LC-UV,[18-20] GC-MS[21] and capillary-electrophoresis.[16,22]

Also there have been reported several methods for quantitation of aripiprazole and some of its impurities in pharmaceutical preparations including UV-Vis spectrophotometry,[23-24] LC-DAD,[25-26] and adsorptive stripping voltammetry.[27] Identification of degradation products of aripiprazole in tablets was achieved by means of a LC-MS method.[28] The chemical structure of aripiprazole and its related impurities studied in this paper are presented in Fig. 1 together with their chemical denomination.

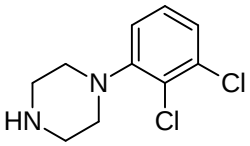
In the present study, a rapid, economical, precise and accurate stability indicating method has been developed for separation and quantitative estimation of aripiprazole and its chemical related impurities in tablet formulations. The maximum accepted level of each impurity in the pharmaceutical formulation is 0.15% relatively to the aripiprazole content.



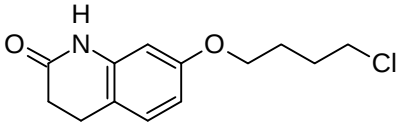
7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy}-3,4-dihydro-2(1*H*)-quinolinone
Aripiprazole



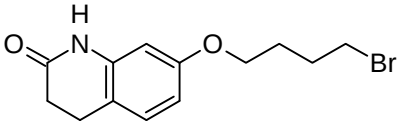
7-hydroxy-3,4-dihydro-2(1*H*)-quinolinone
Impurity A



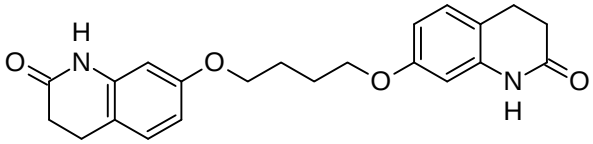
1-(2,3-dichlorophenyl) piperazine
Impurity B



7-(4-chlorobutoxy)-3,4-dihydro-2(1*H*)-quinolinone
Impurity C



7-(4-bromobutoxy)-3,4-dihydro-2(1*H*)-quinolinone
Impurity D



7,7'-[butane-1,4-diylbis(oxy)]bis(3,4-dihydro-2(1*H*)-quinolinone)
Impurity E

Fig. 1 – Chemical structures of the studied compounds.

EXPERIMENTAL

Material and Reagents

Reference standards of Aripiprazole and related chemical impurities (Fig. 1) were obtained from Cambrex Profarmaco Milano (Milan, Italy). Lactose monohydrate, modified corn starch, microcrystalline cellulose, hydroxypropyl cellulose and magnesium stearate were obtained as gift from Polipharma Industries SRL (Sibiu, Romania). HPLC gradient grade methanol and acetonitrile were procured from VWR (Vienna, Austria), while orthophosphoric acid (85%) was obtained from Sigma-Aldrich (Munich, Germany). Analytical grade sodium hydroxide, hydrochloric acid (35%) and hydrogen peroxide were purchased from Merck (Darmstadt, Germany).

Ultra-pure water with specific resistivity of 18.2 MΩ/cm was obtained using a Milli-Q Direct 8 System (Millipore, Bedford, MA, USA). All the solvents and samples were filtered through 0.45 µm nylon filter membranes (Phenomenex, Torrance, CA, USA) prior to their injection into the HPLC system.

Equipment

The HPLC system consisted of an Agilent 1200 series (Waldbronn, Germany) equipped with an on-line vacuum degasser, a quaternary pump, a vial autosampler, a thermostated column compartment and a diode array detector was employed for the chromatographic analysis. ChemStation software (revision B.04.02) was used for data acquisition and peak integration. The pH measurements were performed using inoLab 740 pH meter from WTW (Weilheim, Germany) and for ultrasonication of the sample solutions Sonorex Digitec DT 1028H (Bandelin, Berlin, Germany) was used.

Chromatographic conditions

The separation of the studied compounds was carried out on a Zorbax SB-C18 column 150 mm x 4.6 mm, 5 µm particle size (Agilent, Santa Clara, CA, USA). The mobile phase was a mixture of MeOH : water: H_3PO_4, 55:45:0.4 (v/v/v) with final pH of 2.0, adjusted with H_3PO_4. The elution was isocratic with flow rate of 1.5 mL/min, column temperature was set to 40°C and the injection volume was 20 µL. The chromatograms were recorded simultaneously at 224 nm and 254 nm, and whenever necessary, UV absorption spectra were recorded within the range of 200-400 nm. The low concentration limits of impurities that are accepted in aripiprazole drug formulations led to the choice of 224 nm as the detection wavelength for chemical related impurities, in order to attain the required level of sensitivity. For aripiprazole assay, the working concentration of 0.01 mg/mL is enough to ensure an acceptable detection at 254 nm.

Standard and sample preparation

Stock standard solution of aripiprazole with the concentration of 1.25 mg/mL was prepared by accurately weighing the corresponding amount and dissolving it with mobile phase in a 100 mL volumetric flask.

Equal amounts from each impurity were added to a 10 mL volumetric flask, dissolved and brought to mark with acetonitrile. Next a dilution of 1:100 was made with mobile phase in order to obtain a stock standard impurities solution with the concentration of 18.75 µg/mL of each impurity. The stock standard solutions were used for the study of the validation parameters.

For sample preparation, 20 tablets of aripiprazole were weighed and crushed to a fine powder using a grinding mortar. For aripiprazole assay, an amount equivalent to 10 mg of aripiprazole was transferred into a 100 mL volumetric flask and sonicated with 60 mL of mobile phase for 15 minutes with occasionally shaking. Finally, the volume of the sample solution was made up to 100 mL with mobile phase so that the expected concentration was 0.1 mg/mL of aripiprazole. Subsequently, the solution was filtered. For impurity profile, an amount equivalent to 100 mg of aripiprazole was transferred into a 100 mL volumetric flask and sonicated with 60 mL of mobile phase for 30 minutes with periodic shaking. The flask was brought to mark with mobile phase and the expected concentration was 1.0 mg/mL of aripiprazole. The impurity profile solution was then filtered.

Forced degradation studies

Acidic, alkaline and peroxide degradation studies for aripiprazole were conducted at ambient temperature using HCl, NaOH and H_2O_2. From the stock standard solution 8 mL were transferred to several 10 mL volumetric flasks and diluted with degradation medium so that the initial concentration of 1.0 mg/mL of aripiprazole was obtained, while the concentration of the degradation medium was 1 M HCl, 1 M NaOH and 3% H_2O_2, respectively. For thermal, humidity and photolytic studies, mobile phase was used as the dissolution medium and the conditions were applied as per the Q1A (R2) ICH guidelines.[29] Photolytic degradation was carried out according to Option 2 from Q1B of ICH guidelines[30] by exposing the aripiprazole sample to light illumination of 1.2 million lux hours and an integrated near ultraviolet energy of minimum 200 W·h /m^2. Thermal studies were conducted at 80ºC and 90% relative humidity.

RESULTS AND DISCUSSION

Specificity

The specificity of the method was investigated in order to demonstrate the discrimination between the studied compounds by spiking known amounts of impurities and aripiprazole with the placebo formulation. For a better visualization of the chromatographic peaks separation, the synthetic sample was dissolved and diluted so that the final concentration of all the analytes was 0.01 mg/mL. Also, placebo and solvent samples were injected in order to detect any peak that could interfere with the studied compounds. The excipients used for placebo formulation were: lactose monohydrate, modified corn starch, microcrystalline cellulose, hydroxypropyl cellulose and magnesium stearate. Fig. 2 illustrates a 2-D and a 3-D chromatogram of the synthetic sample recorded at 254 nm.

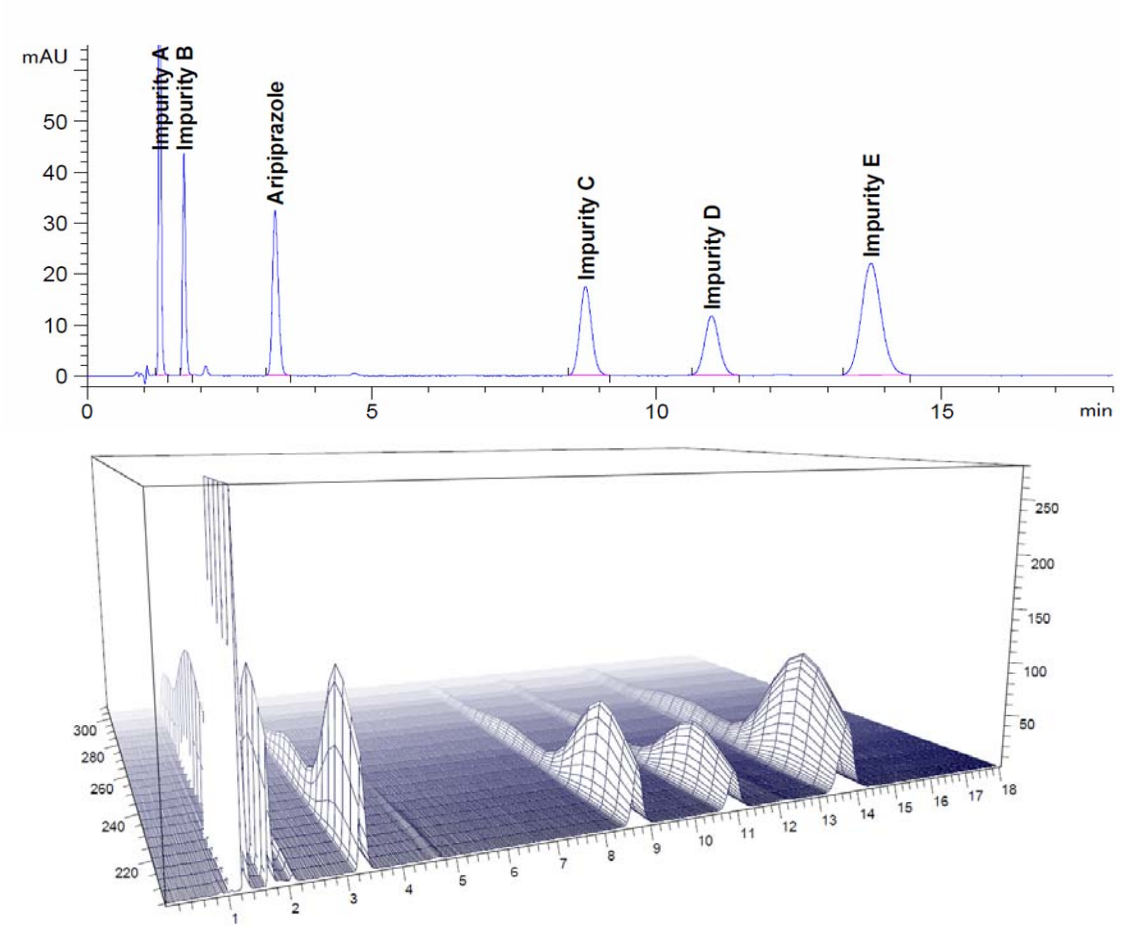


Fig. 2 – Example of a 2-D chromatogram of aripiprazole and its impurities recorded at 254 nm (above) and a 3-D chromatogram of the same mixture (below).

By comparing the chromatograms from placebo, solvent and spiked sample solutions there have not been found any peak to interfere with aripiprazole main peak or its chemical related impurities. The separation parameters obtained are presented in Table 1. In Fig. 3 it can be observed the separation of the studied compounds in the synthetic sample solution that contains all the impurities at their maximum acceptance level (0.15% of aripiprazole content, in this case 1.5 μg/mL). Also, in this synthetic sample solution, aripiprazole concentration is 1.0 mg/mL, corresponding to the sample preparation for impurity profiling, as described above in experimental part.

The proposed method was demonstrated to efficiently separate the active pharmaceutical ingredient from the excipients and chemical related substances.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Linearity of the analytical method was checked by preparing and injecting five standard solutions within the range of 50 – 150% of the nominal working concentration. For each concentration level three replicates have been prepared using the stock standard solution. After the injection and chromatographic run of each standard solution, the calibration curve of the peak area versus concentration was evaluated. The nominal concentrations for each of the studied compounds together with the regression parameters are given in Table 2. In all cases, the correlation coefficients obtained using least-squares linear regression model of the calibration curve were greater than 0.9995. Further evaluation of residual plots was carried out to check the calibration model's adequacy. The residuals did not exceed 2% of the peak area corresponding to the nominal working concentration (the middle of the range) for each of the studied compound. The method was proven to obtain chromatographic signals which are directly proportional to the concentration of the analytes over the entire range.

Table 1

Chromatographic parameters of the synthetic sample

Compound	Retention time (min)	Relative retention time*	Capacity factor (k)	Resolution	Symmetry factor	USP tailing factor
Impurity A	1.280	0.387	0.243	-	0.80	1.210
Impurity B	1.704	0.516	0.654	5.31	0.75	1.247
Aripiprazole	3.304	1.000	2.208	11.59	0.80	1.178
Impurity C	8.751	2.649	7.496	19.46	0.89	1.094
Impurity D	10.966	3.319	9.647	5.31	0.92	1.084
Impurity E	13.762	4.165	12.361	5.11	0.91	1.091

* with respect to aripiprazole

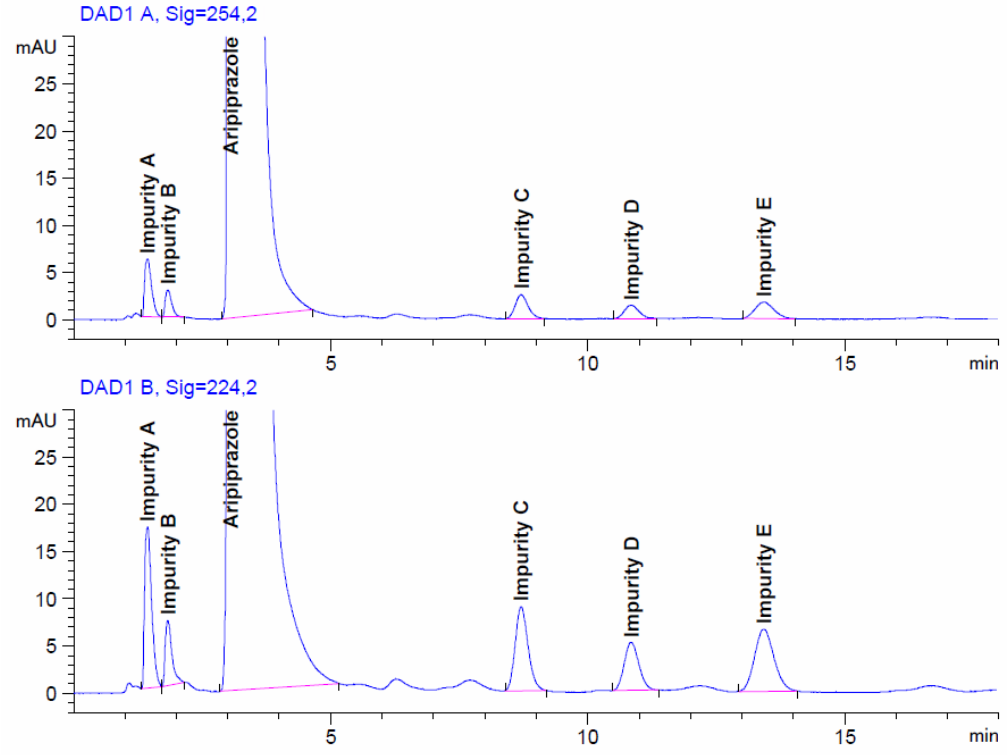


Fig. 3 – The chromatogram recorded at 224 and 254 nm of a synthetic sample solution containing all the impurities at the maximum acceptance level (1.5 µg/mL) and aripiprazole at 1.0 mg/mL.

Table 2

The results of the linearity experiments; x - concentration; y - peak area (mAU s)

Analyte	Nominal concentration (µg/mL)	Range (µg/mL)	Regression equation	Correlation coefficient (r)	Standard error of estimate (SEE)	F (Fischer test)
Aripiprazole	100	50 – 150	y = 22.881x – 6.615	0.9998	16.68	35304.5
Impurity A	1.50	0.75 – 2.25	y = 104.894x + 0.153	0.9998	1.33	26334.6
Impurity B	1.50	0.75 – 2.25	y = 35.963x + 5.286	0.9995	0.62	14234.7

Table 2 (continued)

Impurity C	1.50	0.75 – 2.25	y = 99.452x – 2.361	0.9997	1.40	21478.7
Impurity D	1.50	0.75 – 2.25	y = 68.812x – 3.789	0.9997	0.99	20360.9
Impurity E	1.50	0.75 – 2.25	y = 108.951x – 9.753	0.9997	1.65	18339.7

Table 3

LOD and LOQ of the chromatographic method

Compound	LOD		LOQ		RSD % ** (n = 6)
	(ng/mL)	% *	(ng/mL)	% *	
Aripiprazole	250.0	-	500.0	-	5.12
Impurity A	37.5	0.00375	75.0	0.0075	4.21
Impurity B	75.0	0.0075	225.0	0.0225	5.16
Impurity C	37.5	0.00375	75.0	0.0075	8.36
Impurity D	75.0	0.0075	150.0	0.0150	5.29
Impurity E	75.0	0.0075	150.0	0.0150	6.60

* relative to the aripiprazole content (see sample preparation);
** determined for LOQ level.

LOD and LOQ were estimated as signal-to-noise ratio[31] of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentration. Precision study was also carried out at the LOQ level by calculating the RSD percentage of the peak area of six consecutive injections. The results obtained are presented in Table 3. It can be seen that lower LOD and LOQ were obtained for the related impurities, compared to aripiprazole. This is due to the fact that the detection wavelength of 224 nm was set for impurities. The aim of obtaining very low LOD and LOQ for aripiprazole is not necessary nor justified, the method being designed to quantify aripiprazole as the main active pharmaceutical ingredient in tablets at a reasonable working concentration of 0.1 mg/mL. The need for a lower LOD and LOQ for the impurities is clear, because the maximum accepted level is 0.15 % relative to the aripiprazole content. So the proposed method has to be able to quantitatively estimate low concentration of chemical related impurities.

Accuracy and precision

The accuracy of the analytical procedure was evaluated at three concentration levels over the entire range of the assay by spiking to placebo samples known amounts of standards. Each concentration level was prepared in three replicates. Accuracy was expressed as the percent recovery calculated as the ration between the experimental concentration and the theoretical concentration. The recovery values determined are shown in Table 4 together with the relative standard deviation (RSD %) of the replicates. The recovery for aripiprazole ranged between 98.43 – 100.59%, while for the impurities the recovery ranged between 92.75 – 108.79%.

Precision has been estimated by repeatability and its intermediate estimate. Six replicate injections were performed at 100% assay concentration for aripiprazole and at the maximum accepted concentration level for its impurities. Intermediate precision was checked by evaluating the repeatability of the entire analytical procedure in the same laboratory, on two different days by two different investigators, on two different chromatographic systems and on two different columns (different batch, same manufacturer). The results are shown in Table 5. The proposed method was found to have good accuracy and precision.

Table 4

Accuracy of the proposed method (n = 3)

Analyte	Concentration level	Theoretical concentration (μg/mL)	Assayed concentration* (μg/mL)	Recovery (%)	Mean recovery (%)	RSD (%)
Aripiprazole	50%	50.0	49.81 ± 0.48	99.61	99.54	1.38
	100%	100.0	100.59 ± 1.65	100.59		
	150%	150.0	147.64 ± 0.89	98.43		
Impurity A	50%	0.75	0.79 ± 0.01	104.68	104.77	0.59
	100%	1.50	1.56 ± 0.00	104.33		
	150%	2.25	2.37 ± 0.01	105.30		
Impurity B	50%	0.75	0.69 ± 0.03	92.75	98.96	6.11
	100%	1.50	1.49 ± 0.05	99.23		
	150%	2.25	2.36 ± 0.01	105.09		
Impurity C	50%	0.75	0.76 ± 0.00	101.96	101.85	0.65
	100%	1.50	1.52 ± 0.01	101.55		
	150%	2.25	2.30 ± 0.01	102.35		
Impurity D	50%	0.75	0.72 ± 0.01	95.92	98.97	2.56
	100%	1.50	1.49 ± 0.01	99.57		
	150%	2.25	2.28 ± 0.01	101.41		
Impurity E	50%	0.75	0.82 ± 0.00	108.79	104.92	2.80
	100%	1.50	1.55 ± 0.01	103.03		
	150%	2.25	2.32 ± 0.01	102.94		

* mean ± standard deviation.

Table 5

Results for intermediate precision of the analytical process

Compound	Retention time (min, n = 6)		Assay (% of declared, n = 6)		Mean (%)	RSD (%)
	Investigator 1*	Investigator 2**	Investigator 1*	Investigator 2**		
Aripiprazole	3.300	3.297	100.20	102.35	101.27	1.15
Impurity A	1.291	1.282	98.31	101.54	99.93	1.76
Impurity B	1.710	1.709	98.71	102.18	100.44	2.16
Impurity C	8.713	8.697	103.18	101.98	102.58	1.05
Impurity D	10.961	10.940	98.02	96.09	97.06	1.29
Impurity E	13.760	13.720	97.50	96.02	96.76	1.14

* results obtained in day 1.
** results obtained in day 2.

Force degradation and solution stability

The scope of the degradation studies was to help identifying the likely degradation products of aripiprazole, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule. All stress degradation studies were performed at an initial concentration of 1.0 mg/mL for 48 hours. Samples were withdrawn at appropriate times and subjected to LC analysis to evaluate the ability of the proposed method to separate aripiprazole from its potential degradation products. Photodiode array detector was employed to check and to ensure the homogeneity and purity of Aripiprazole peak in all the stressed sample solutions.

Assessment of mass balance in the degraded samples was carried out and the results are presented in Table 6. It have been found that a major degradation product forms under oxidative conditions which seems to be the N-oxide impurity of aripiprazole.[32]

The chemical stability of the stock solutions containing the studied compounds in mobile phase has been investigated by storage for a period of 48 hours at room temperature (25°C). All the compounds were found to be stable in mobile phase for 48 hours at 25°C. There have not been detected any other peaks in the chromatograms during the stability studies.

Analysis of commercial drugs

The proposed method has been applied for quantitative evaluation of aripiprazole and chemical related impurities from three dosage units of ABILIFY®: 10, 15 and 30 10 mg/tablet. The obtained results (given in Tables 7 and 8) are in agreement with the labeled content for aripiprazole, with chemical impurities being below the acceptance limit.

Table 6

Results for forced degradation studies of aripiprazole

Stress condition	24 hours		48 hours		Remarks
	Recovery (%)	Mass balance (%)*	Recovery (%)	Mass balance (%)*	
Mobile phase	98.56	98.92	98.23	98.81	No degradation product detected
Acid hydrolysis	97.66	99.05	97.54	98.86	No degradation product detected
Base hydrolysis	99.10	99.80	98.40	99.56	No degradation product detected
Oxidation	20.16	96.16	18.15	96.45	One major degradation product formed
Thermal degradation	98.02	98.89	98.66	99.71	No degradation product detected
Photolytic degradation	97.15	97.93	97.24	98.06	No degradation product detected

* represent the sum between the recovery and all other chromatographic peaks.

Table 7

Aripiprazole assay on commercial pharmaceutical products

Active ingredient	Abilify® 10 mg/tablet		Abilify® 15 mg/tablet		Abilify® 30 mg/tablet	
	Found* (mg/tablet)	Recovery (%)	Found* (mg/tablet)	Recovery (%)	Found* (mg/tablet)	Recovery (%)
Aripiprazole	9.89 ± 0.05	98.90 ± 0.50	14.78 ± 0.24	98.53 ± 1.60	31.08 ± 0.19	103.60 ± 0.63

* mean ± standard deviation

Table 8

Impurity profile of commercial pharmaceutical products containing aripiprazole

	Acceptance level*	Abilify® 10 mg	Abilify® 15 mg	Abilify® 30 mg
Impurity A		0.03 %	< LOD	0.01 %
Impurity B		0.01 %	< LOD	0.01 %
Impurity C	< 0.15 %	< LOD	< LOD	< LOD
Impurity D		< LOD	< LOD	< LOD
Impurity E		< LOD	0.01 %	< LOD

* relative to aripiprazole content

CONCLUSIONS

A new HPLC method has been developed and validated for simultaneous quantitative evaluation of aripiprazole and five of its chemical related impurities from commercial drugs. The chromatographic conditions were adjusted in such a way that the chemical-related impurities or the excipients did not interfere with aripiprazole. The proposed method was validated and proven to have adequate selectivity, good linearity, accuracy, precision, low limits of detection and quantification. Furthermore, the assay was applied, with good results, for quality control of commercial drugs and could be successfully applied to bulk aripiprazole or in the analytical control of aripiprazole synthesis.

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