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Development and Validation of RP-HPLC Method for the Quantification of Potential Genotoxic Impurities in Anti-psychotic drug Aripiprazole

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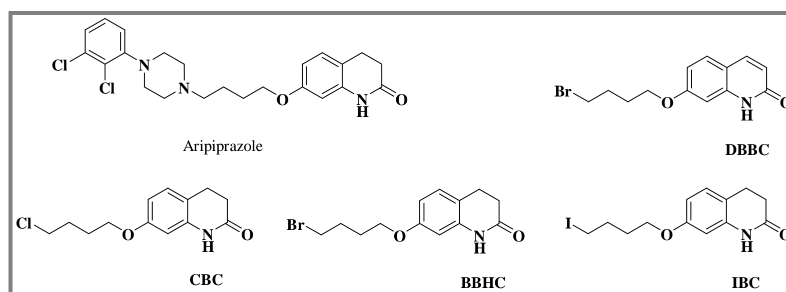
ABSTRACT

A simple, sensitive and validated RP-HPLC method is developed for the quantification of potential genotoxic impurities of Aripiprazole (APZ) drug substance, namely 7-(4-bromobutoxy)quinolin-2-(1H)-one (dehydrobromobutoxycarbostyril) (DBBC), 7-(4-chlorobutoxy)quinolin-2-(1H)-one (chloro butoxycarbostyril) (CBC),7-(4-bromobutoxy)-3,4-dihydro-2(1H)-quinolinone [7-(4-bromo butoxy)-3,4-dihydrocarbostyril](BBHC)and 7-(4-iodobutoxy)-3,4-Dihydro-2(1H)-quinolin-2-one (iodobutoxy carbostyril) (IBC).The analysis is performed on Alliance-Waters 2695 Separations Moldule® on C18, (150mm x 4.6mm, 5µm) (Make: Sunfire), maintained at temperature 45°C and UV detection at 215nm. The separation is accomplished using mobile phase, prepared by mixing a buffer (diluted 3 mL of orthophosphoric acid in 1000 mL of water) and acetonitrile in the ratio of 60:40%v/v. Flow rate is kept as 1.0 mLmin^{-1} and injection volume is 20µL. The proposed method is validated as per ICH guidelines in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy and specificity. The achieved limit of detection (LOD) values are 1.06, 1.22, 1.35 and 2.00; limit of quantification (LOQ) values are 3.20, 3.69, 4.08 and 6.07 µg g^{-1} and the average of accuracy values are 96.6, 93.5, 96.1 and 94.9 % for DBBC, CBC, BBHC and IBC respectively.

Graphical Abstract



Keywords: Aripiprazole, Anti-psychotic drug, Genotoxic impurities, Quantification, RP-HPLC method.

INTRODUCTION

Aripiprazole is chemically known as7-[4-[4-(2,3-Dichlrophenyl)-1-piperazinyl]butoxy]-3,4-dihydro carbostyril or 7-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butoxy]-3,4-dihydro-1*H*-quinolin-2-one. Aripiprazole is an atypical antipsychotic drug used to treat schizophrenia and schizoaffective disorders [1, 2]. Aripiprazole is metabolized by the cytochrome P450, isoenzymes 3A4 and 2D6.As a consequence of high inter individual variability in the expression of these enzymes; the aripiprazole concentration varies among healthy persons after administration of the drug [3]. The empirical formula of Aripiprazole is $C_{23}H_{27}C_{12}N_3O_2$ and molecular weight is 448.38. The recommended maximum dose is 30mgday^{-1}. The chemical structure of Aripiprazole is shown in figure 1.

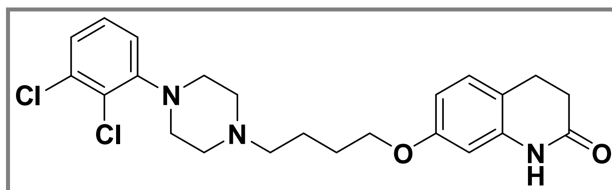


Figure 1. Chemical structure of Aripiprazoledrug substance (APZ).

During the synthesis of Aripiprazole drug substance, some of raw materials and intermediates may arise as impurities in the API. The chemical reactivity of these components frequently be converted into biological reactivity and can often be transformed as carcinogens or mutagens. It has been recognized that, the fate of the several genotoxic agents not entitled their retention within the final API due to elevated chemical relativity; especially if the formation is separated from the final API by various synthetic steps. Some of these known impurities are potential mutagens or carcinogens, but can be difficult or impossible to eliminate totally from synthetic sequence [4].

BBHC is one of the raw materials used in the preparation of Aripiprazole drug substance. The other compounds namely DBBC, CBC and IBC are the possible genotoxic impurities. All of these compounds are structurally alert and potential genotoxic impurities. Current regulatory guidelines for genotoxic impurities indicate to develop the analytical methods to meet the required daily intake limit of 1.5 mg day^{-1} of any individual impurity [5-7]. The limit of each impurity is considered as 50 µg g^{-1} with respect to Aripiprazole maximum daily dose, i.e 30 mg day^{-1}. The chemical structures of the genotoxic impurities are shown in figure 2.

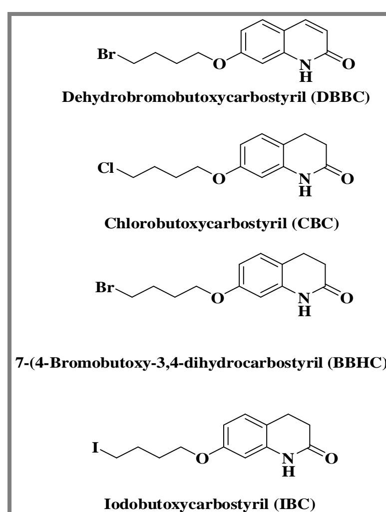


Figure 2. Chemical structures of genotoxic impurities of Aripiprazole

Numerous HPLC methods are reported in literature for the quantification of Aripiprazole drug substance, drug product and its related substances. Nandini R. Pai *et al.,* [8] described a validated HPLC method for quantitative determination of Aripiprazole and its impurities. In another report, Nandini R. Pai *et al.,* [9] described liquid chromatographic method for Aripiperazole. Narayana M B V *et al.,* [10] described a RP-HPLC method for Aripiprazole and its related substances. Despite, several methods described already in the literature, there is constraint for chromatographic method on HPLC for the determination of potential genotoxic impurities observed during the synthesis of Aripiprazole drug substance. HPLC techniques are advantageous for the determination of genotoxic impurities in pharmaceutical industry [11, 12]. In this perspective, we developed asimple RP-HPLC method for the determination of potential genotoxic impurities in Aripiprazole drug substance. In addition, this method is validated to meet the requirements of ICH validation guidelines [13]. To the best of our knowledge, this method is novel analytical technique to determine the potential genotoxic impurities in Aripiprazole drug substance.

MATERIALS AND METHODS

Chemicals, reagents, standards and samples: The investigated samples of Aripiprazole drug substance, analyzed impurities (for specificity experiment) were received as a gift from APL Research Centre-II Laboratories (A division of Aurobindo Pharma Ltd., Hyderabad). Orthophosphoric acid and Acetonitrile of GR grade were purchased from Merck research laboratories, India. Pure milli-Q water was obtained from Millipore purification system (Millipore®, Milford, MA, USA).

Instrumentation, buffers and chromatographic conditions: A Waters Alliance 2695 separation module equipped with 2487dual λ absorbance detector with Empower 3 data handling system [Waters Corporation, MILFORD, MA 01757, USA] was used. The analysis was performed on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with octadecylsilane groups chemically bonded to porous silica particles of 5µm diameter [Sunfire® C18, 5µm (150mm x 4.6mm) (Make: Sunfire)] maintained at a temperature of 45°C. Buffer was prepared by mixing 3 mL of orthophosphoric acid in 1000 mL of water. Further, mobile phase was prepared by mixing a buffer and acetonitrile in the ratio of 60:40% v/v. Diluent was prepared by a degassed mixture of acetonitrile, water and orthophosphoric acid in the ratio of 900:100:1 v/v. Flow rate was kept as 1.0 mL min^{-1}, injection volume was 20 µL, chromatographic data acquisition time was 30 min and UV detection was at 215 nm. Retention time of BBHC was at about 12 min. The pump was in isocratic mode (Figure 3).

Preparation of solutions: BBHC reference standard solution (0.25 µg mL^{-1}) was prepared by transferring 25 mg BBHC in 70 mL of diluent into a cleaned and dried 100 mL volumetric flask, sonicated to dissolve and made up to the mark with diluent. This solution (5 mL) was diluted to 50 mL with diluents and further 5 mL of this solution was diluted to 50 mL with diluent.

Sample solution: Aripiprazole drug substance sample solution of 5 mg mL^{-1} [5000 µg mL^{-1}] was prepared by using 50 mg of sample in 10 mL clean, dry volumetric flask, 7 mL of diluent was added to it and sonicated to dissolve. Volume was made up to the mark with diluent. Filtered the solution through 0.45µ (or finer) porosity membrane filter.

Suitability requirements: The column efficiency as determined from the BBHC peak was not less than 2500 USP plate count and USP tailing for the same peak was not more than 1.5. Relative standard deviation (RSD) for peak areas of BBHC obtained from six injections of the standard solution was not more than 5.0%.

Method development: The main aim of this method was to separate and quantify genotoxic impurities in the aripiprazole drug substance. In support of that, various chromatographic parameters were tested and optimized in order to achieve the optimum separation between the genotoxic impurities.

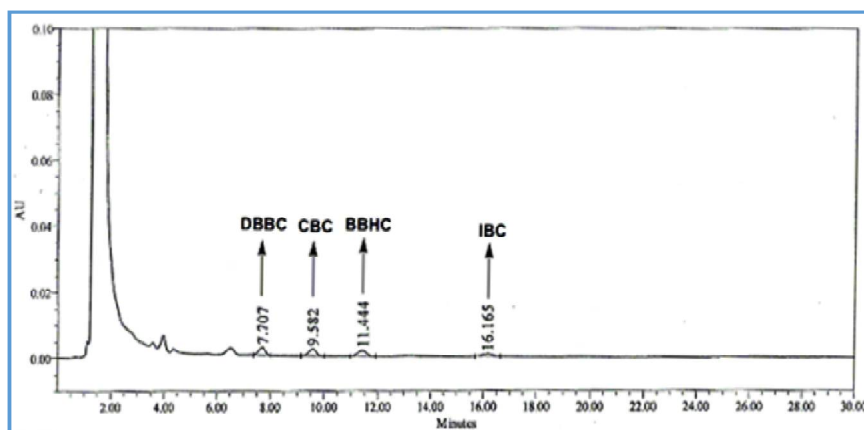


Figure 3. A representative chromatogram of potential genotoxic impurities.

Selection of Column: Reverse Phase compatible columns were selected to separate the genotoxic impurities on HPLC. Trials were carried out on YMC Pack Pro C18, 3µm (150mm x 4.6mm); Symmetry Sheild RP-18, 3.5µm (150mm x 4.6mm); X-bridgeC18, 5µm (150mm x 4.6mm); Sunfire® C8, 5µm (150mm x 4.6mm). Though, the tested columns resulted optimistic separation, Sunfire® C18, 5µm (150mm x 4.6mm) was found to be more suitable because of its endcaping technology. In addition, these columns were compatible with mass spectroscopy applications proving sharp peaks, high sensitivity, batch to batch reproducibility and symmetrical peak shapes with improved resolution. Finally, the desired separation was achieved with Sunfire® C18, 5µm (150mm x 4.6mm) reverse phase column.

Optimization of buffer and the pH of the buffer: After selection of the column, we focused on the selection, optimization of buffer solution effect of pH of buffer on retention and separation parameters were recorded over a pH range between 2.0 and 8.0. Several buffer solutions were tested for the separation of this method. Aqueous mono potassium phosphate (KH_2PO_4) of 0.05 molar solution at pH 6.0 (adjusted with KOH) afforded moderate separation and the peaks shapes were not impressive. Another trail experiment was performed using aqueous perchloric acid 1% solution but the retention and separation was not achieved. Subsequently, another trail experiment was performed using orthophosphoric acid 1% solution but the retention and separation was not achieved. Finally, the best separation was achieved using 3% aqueous orthophosphoric acid.

Optimization of the mobile phase composition: Subsequently, the optimization trails were carried out with Gradient programmes by using different aqueous buffers and with acetonitrile. In order to achieve shorter run time with good separation and peak shape, we opted for isocratic mode, prepared by mixing 3% aqueous orhophosphoric acid (buffer) and acetonitrile in the ratio of 60:40% v/v. Under these specified conditions, we achieved the best separation in shorter time.

Selection of UV detector: The separation was studied in differ UV detector under different nano meters including 210, 215, 254 and 247. However, the best detection was achieved at 215 nm.

Optimization of column temperature: The development trails to optimize the column temperature were carried out at column temperature between 20-45°C. Considering the better separation and good peak shape, the column temperature was fixed at 45°C.

RESULTS AND DISCUSSION

Method Validation: After optimizing the suitable condition, we then focused on the method validation experiments as per the ICH Guideline [11], individually in terms of specificity or selectivity, LOD, LOQ, linearity, accuracy and precision.

Specificity: According to ICH Guidelines, specificity is the ability of the method to determine the individual analyte in presence of other related substances of drug substance. For specificity determination, the analytes (DBBC,CBC,BBHC and IBC), Aripiprazole related substances RS-1 and RS-2 solutions were prepared individually and injected into HPLC to confirm the retention times. Subsequently diluent, solutions of Aripiprazole drug substance, Aripiprazole drug substance spiked with DBBC, CBC, BBHC and IBC (spiked sample), Aripiprazole drug substance spiked with DBBC, CBC, BBHC, IBC and RS-1,RS-2(all spiked samples) were prepared and injected into HPLC to confirm any co-elution with analyte peaks from respective diluents. All of the related substances peaks and the peak homogeneity was verified for each analytes injected in Waters system with PDA detector using empower software and found to be pure (purity angle should be less than purity threshold). The specificity results are tabulated in table 1a and 1b.

Table 1a. Specificity Results

Sample Name	Name of the impurity	RT (spiked sample)	RRT (BBHC)
Analytes	DBBC	7.695	0.67
	CBC	9.583	0.84
	BBHC	11.418	1.00
	IBC	16.142	1.41
Other Impurities for Information	Dehydro APZ	1.431	0.13
	APZ 4,4′-dimer	1.367	0.12

Table 1b. Specificity Results

Name of the impurity	Control sample		Spiked sample	
	Purity angle	Purity threshold	Purity angle	Purity threshold
DBBC	1.374	1.563	1.216	1.465
CBC	1.521	1.917	1.398	1.657
BBHC	1.698	2.240	1.769	2.151
IBC	3.180	4.434	3.013	3.972

LOD and LOQ: To quantify the limit of detection (LOD) and limit of quantification (LOQ) for DBBC, CBC, BBHC and IBC impurities were determined based on response of analytes. The predicted concentrations of LOD and LOQ for these four impurities were verified for precision by analyzing the solutions containing DBBC, CBC, BBHC and IBC at about predicted concentrations and injected each solution six times into HPLC system as per method conditions. Results are tabulated in table 2.

Table 2. LOD/LOQ results

Name of the impurity	Conc(ppm)		%RSD (For Six injections)	
	LOD ($\mu g\ g^{-1}$)	LOQ ($\mu g\ g^{-1}$)	LOD	LOQ
DBBC	1.06	3.20	5.2	3.4
CBC	1.22	3.69	5.0	2.6
BBHC	1.35	4.08	9.0	4.4
IBC	2.00	6.07	9.8	5.1

Linearity: The linearity of the detector was determined by preparing a series of solutions using DBBC, CBC, BBHC and IBC at concentration levels from about LOQ to 150% level. The obtained data was subjected to statistical analysis by using a linear regression model figure 3-6. The statistical evaluations like slope, intercept, STEYX, Correlation coefficient and response factor values of linearity data is given in table 3.

Accuracy: Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries were determined by spiking DBBC, CBC, BBHC and IBC at four concentration levels from about LOQ to 150% levels (i.e LOQ, 50 $\mu g\ g^{-1}$, 100 $\mu g\ g^{-1}$ and 150$\mu g\ g^{-1}$)

Table 3. Linearity results

Name of the impurity	No. of points covered	Conc. Range (μg mL^{-1})	Slope	Intercept	STEYX	Correlation coefficient	Response factor
DBBC	10	0.016 - 0.375	194368	164	155	0.9999	0.76
CBC	10	0.018 – 0.376	171065	221	138	0.9999	0.86
BBHC	10	0.020 – 0.376	147401	211	196	0.9999	1.00
IBC	8	0.030- 0.323	102067	523	325	0.9996	1.44

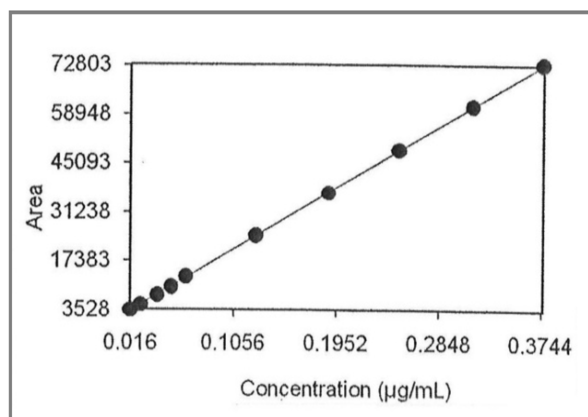


Figure 3. Linearity plot of DBBC.

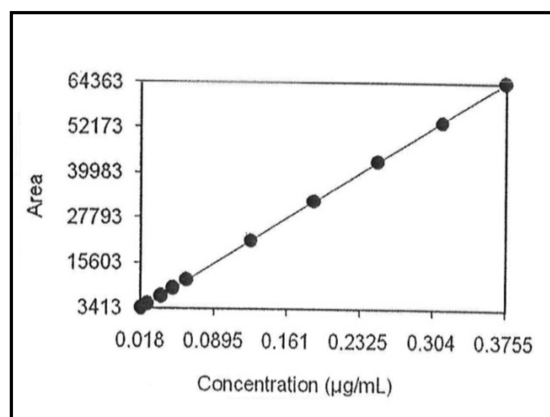


Figure 4. Linearity plot of CBC.

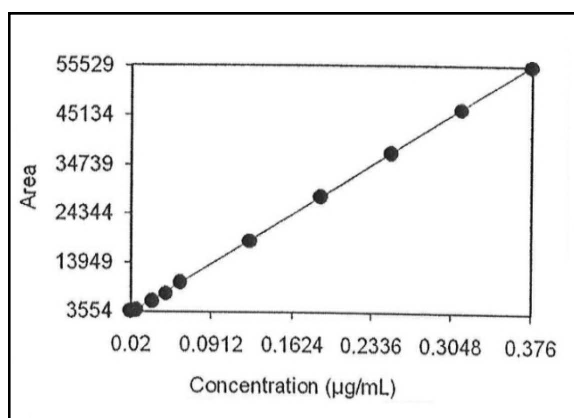


Figure 5. Linearity plot of BBHC.

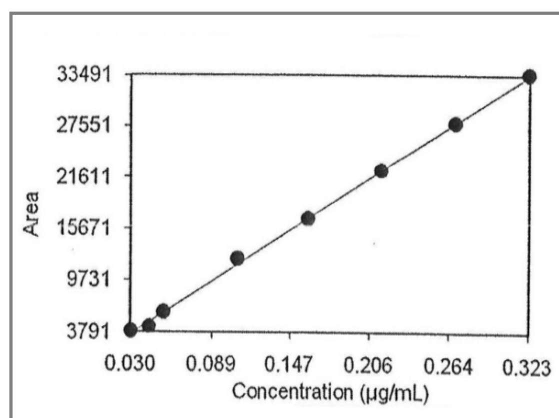


Figure 6. Linearity plot of IBC.

into Aripiprazole drug substance. These samples were prepared as per respective test procedure and analyzed in triplicate and the percentage recoveries were calculated. The percentage recovery values for analytes ranged from 95.7-97.6, 92.0-95.8, 94.9-96.9 and 93.8-96.4 and the average percentage recovery of four levels (twelve determinations) were 96.6, 93.5, 96.1 and 94.9 for DBBC, CBC, BBHC and IBC respectively. The fully validated accuracy results are shown in table 4.

Precision: System precision was established by preparing the standard solutions of individual analytes as per methodology and analysed by injecting six replicates. Repeatability was the intra-day variation (method precision) and the intermediate precision was the inter-day variation (ruggedness). Method precision was demonstrated by preparing six sample solutions individually using a single batch of Aripiprazole drug substance spiked with DBBC, CBC, BBHC and IBC at a known concentration level (about 50µg/g)and injected each solution and determined the content of analytes. Achieved results like %RSD and 95% confidence interval for six determinations are summarized in table 5.

Table 4. Accuracy results

Accuracy parameter	Accuracy of DBBC(Average of 3 replicates)			
	LOQ level	**50 $\mu g\ g^{-1}$ level**	**100 $\mu g\ g^{-1}$ level**	**150 $\mu g\ g^{-1}$ level**
Added ($\mu g\ g^{-1}$)	3.164	24.84	49.71	74.47
Recovered ($\mu g\ g^{-1}$)	3.060	24.24	47.97	71.25
Recovery (%)	96.7	97.6	96.5	95.7
%RSD	0.7	2.6	0.9	0.3
Average Recovery (%)			96.6	
	Accuracy of CBC(Average of 3 replicates)			
	LOQ level	**50 $\mu g\ g^{-1}$ level**	**100 $\mu g\ g^{-1}$ level**	**150 $\mu g\ g^{-1}$ level**
Added ($\mu g\ g^{-1}$)	3.662	24.82	49.68	74.43
Recovered ($\mu g\ g^{-1}$)	3.394	22.84	46.39	71.29
Recovery (%)	92.7	92.0	93.4	95.8
RSD	1.1	0.3	0.2	1.5
Average Recovery (%)			93.5	
	Accuracy of BBHC (Average of 3 replicates)			
	LOQ level	**50 $\mu g\ g^{-1}$ level**	**100 $\mu g\ g^{-1}$ level**	**150 $\mu g\ g^{-1}$ level**
Added ($\mu g\ g^{-1}$)	4.069	24.95	49.93	74.8
Recovered ($\mu g\ g^{-1}$)	3.942	23.95	48.13	70.97
Recovery (%)	96.9	96.0	96.4	94.9
RSD	1.6	1.0	0.5	0.4
Average Recovery (%)			96.1	
	Accuracy of IBC (Average of 3 replicates)			
	LOQ level	**50 $\mu g\ g^{-1}$ level**	**100 $\mu g\ g^{-1}$ level**	**150 $\mu g\ g^{-1}$ level**
Added ($\mu g\ g^{-1}$)	6.013	24.87	49.78	74.57
Recovered ($\mu g\ g^{-1}$)	5.798	23.34	47.26	70.51
Recovery (%)	96.4	93.8	94.9	94.6
RSD	1.3	0.9	0.2	0.4
Average Recovery (%)			94.9	

Table 5. Precision results

Sample ID	Results (ppm)							
	DBBC		CBC		BBHC		IBC	
	MP[§] (Set-I)	IP[§§] (Set-I1)	MP (Set-I)	IP (Set-I1)	MP (Set-I)	IP (Set-I1)	MP (Set-I)	IP (Set-I1)
Sample 1	47.64	45.4	46.33	47.38	48.10	51.13	47.31	45.82
Sample 2	47.88	46.55	46.37	48.5	48.29	50.55	47.10	47.0
Sample 3	48.40	44.41	46.47	47.01	47.99	49.42	47.38	47.83
Sample 4	48.66	44.89	46.71	48.84	48.31	50.36	46.66	49.44
Sample 5	48.67	45.51	46.26	49.38	48.19	51.17	46.66	46.43
Sample 6	47.87	45.06	46.12	48.5	48.13	50.06	47.00	46.12
	Statistical Analysis							
Mean	48.19	45.3	46.38	48.27	48.13	50.45	47.00	47.3
SD	0.45	0.73	0.2	0.9	0.17	0.67	0.32	1.35
%RSD	0.9	1.6	0.4	1.9	0.4	1.3	0.7	2.9
95% confidence Interval (±)	0.47	0.77	0.21	0.94	0.18	0.7	0.34	1.42
	Overall Statistical Analysis							
Overall Mean	46.75		47.32		49.29		47.05	
Overall SD	1.67		1.17		1.3		0.93	
Overall RSD (%)	3.4		2.5		2.6		2.0	
Overall 95% confidence Interval (±)	1.02		0.74		0.83		0.59	

[§]MP: Method Precision; [§§]IP: Intermediate precision

APPLICATION

This developed and validated chromatographic technique is new and useful to quantify the genotoxic impurities of Aripiprazole drug substance at low level. This RP-HPLC method is

applicable in generic pharmaceutical industry for routine analysis.

CONCLUSION

A simple and efficient RP-HPLC method was described for the quantification of potential genotoxic impurities namely DBBC, CBC, BBHC and IBC of Aripiprazole drug substance. The results of various validation parameters proved that the method is specific, sensitive, precise and accurate and the method can be introduced into routine testing.

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