Development and validation of zero and first-order derivative area under curve spectrophotometric methods for the determination of aripiprazole in bulk material and tablets

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Abstract
The proposed experiments explain simple, precise, specific and accurate UV spectrophotometry methods for the estimation of Aripiprazole in bulk and pharmaceutical formulation. Aripiprazole is a recent second generation atypical anti-psychotic drug used for the treatment of schizophrenia; four simple UV spectrophotometric methods were established for estimation of Aripiprazole, using double beam UV spectrophotometer (UV-2450, Shimadzu, Japan). Aripiprazole showed maximum absorbance (λ max) at 255 nm in methanolic HCl as a solvent. The calibration curve obeyed Beer-Lambert law in the concentration range of 5 -30 µg/mL. The % recovery was found to be in the range of 98 - 100 %. Precision value less than 2 in terms of % RSD indicates precise nature of developed methods. Validation of developed methods was carried out as per ICH guidelines. It was concluded that the results and statistical analysis amongst all four methods, AUC method is most simple, accurate, precise, and specific. All four methods can be used for routine analysis of Aripiprazole in bulk and pharmaceutical formulations.

Keywords: Aripiprazole, UV-Spectrophotometry, Derivatives Methods, Area Under Curve Method.

Introduction
Aripiprazole (ARP) is chemically 7-[4-[4-(2, 3-dichlorophenyl) -1-piprazinyl] butoxy] -3, 4-dihydro-(1*H*) –quinolinone (Fig. 1).[1] It is a recent second generation atypical anti-psychotic drug used for the treatment of schizophrenia and also used for the treatment of acute manic and mixed episodes associated with bipolar disorder. It has partial agonist activity at dopamine D_2 and D_3 receptors and serotonin 5-HT_1A receptors, and antagonist activity at 5-HT_2A receptors.[1,2]

Literature survey revealed that many analytical methods for the determination of ARP. HPLC[3-14] in pharmaceutical formulation and biological fluid has been reported. Liquid chromatography coupled with mass spectrometry,[15-23] UPLC-MS[24,25] and few spectrophotometric methods.[26-30] However, till date no UV spectrophotometry method has been developed for the determination of ARP in bulk and pharmaceutical formulation using derivative spectroscopic techniques, AUC, and amplitude technique.

At the same time, our objective was to establish zero order, first order derivative using AUC technique and Amplitude technique. The current work emphasizes simple, sensitive and effective UV spectrophotometry method for determination of ARP in bulk material and in pharmaceutical formulation. Further, the developed method was validated according to ICH guidelines.[32]

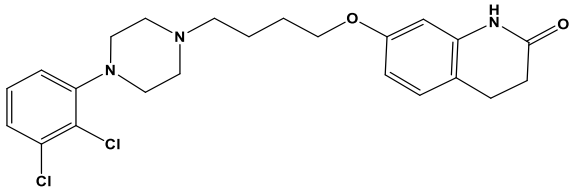

Fig. 1: Chemical Structure of Aripiprazole

Materials and Methods
Chemicals: Pure ARP was obtained as a gift sample from Lupin Pharmaceutical Ltd., Mumbai, India. The marketed formulation (ARZU) 10mg was purchased from local market. Methanol and Hydrochloric acid were used as solvent for this experiment.

Instrumentation: A double beam UV-VIS spectrophotometer (Model- UV-2450, Shimadzu, Japan) equipped with 10mm matched quartz cell and connected by computer operated in UV probe version 2.21 software was utilized in the current research work for all absorbance measurement. An electronic weighing balance (Model- AUX 120, Shimadzu, Japan) was used for weighing purpose.

Selection of Solvent: Solubility of ARP was checked in different solvents, it is partially soluble in methanoland freely soluble in 0.01M methanolic HCl after sonication for 18 min. Hence 0.01M methanolic HCl was chosen as a solvent throughout the method development and validation process and there was no degradation effect occurs in this solvent.

Preparation of standard stock solution and determination of lambda (λ max): The Standard stock solution (100 µg/mL) prepared by accurately weighed 10 mg of ARP, transferred in 100 mL volumetric flask

and volume was made up with methanolic HCl upto the mark.

From the standard stock solution 1mL of solution transferred to 10 mL volumetric flask. Further volume was made with same solvent upto the mark to obtained concentration (10 µg/mL). Resulting solution was scanned in the UV- region i.e. 400 – 200 nm. In zero order spectrums, ARP showed absorbance maximum at 255 nm (Fig. 2).

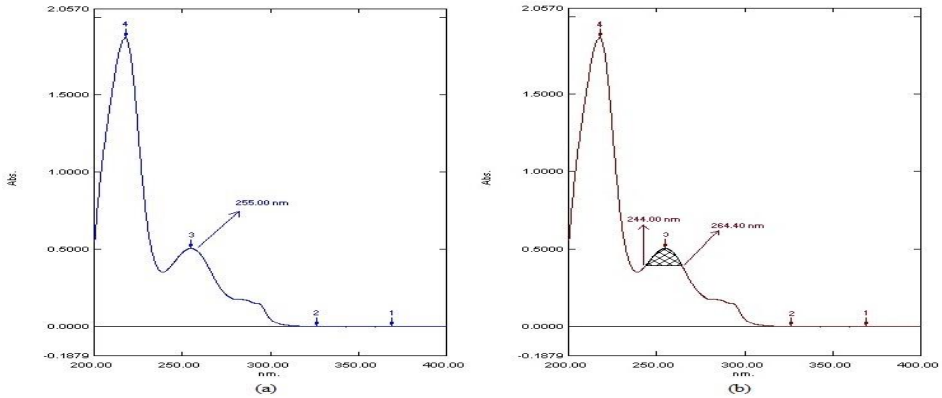

Fig 2: Zero Order Spectrum (a) and Area under Curve; (b) between selected wavelengths of Aripiprazole

Method A (Zero order derivative) and Method B (Zero order derivative-AUC): For method A and method B the standard stock solutions of ARP (100 µg/ml) were further diluted to prepared different sets of standard solution of ARP ranging from (5-30 µg/mL). For this aliquots of 0.5 – 3.0 mL of standard stock solution were separately pipette out and transferred to series of volumetric flask having capacity upto 10 mL then volume was made upto the mark with methanolic HCl solvent. For method A absorbance in zero order derivative spectrum was determined at 255 nm shown in (Fig. 2(a)).While for method B AUC in zero order spectrum was selected in between 244 nm and 264.40 nm shown in (Fig. 2(b)). The calibration curves of method A and method B were constructed by plotting concentration versus absorbance and AUC of zero order spectrum respectively, shown in (Fig. 3).

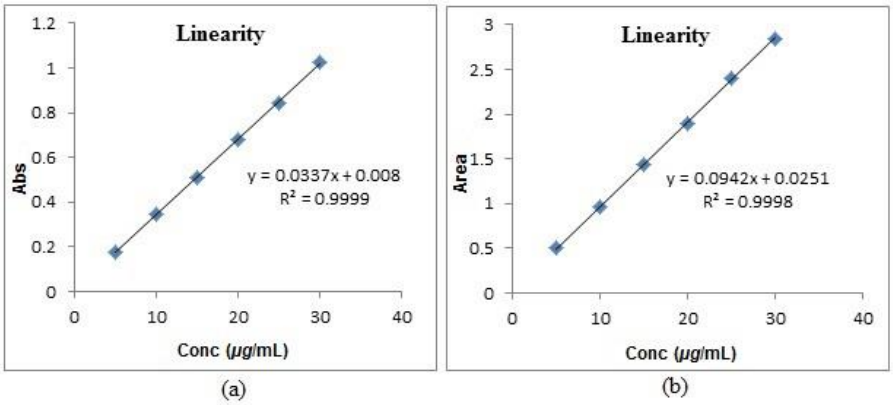

Fig. 3: Calibration Curve of Aripiprazole for Method A (a); and Method B (b)

Method C (First order derivative) and Method D (First order derivative-AUC): For method C and method D spectra of previous solution derivatized into first order spectra using UV probe 2.21 version software with delta 4 and scaling factor 10. In method C amplitude was determined at 266.40 nm shown in (Fig. 4(a)). While for method D AUC in first order spectrum was selected in between 259.80 nm and 273.40 nm shown in (Fig. 4(b)). The calibration curves of method C and D were constructed by plotting concentration versus amplitude and AUC of first order spectrum respectively, shown in (Fig. 5).

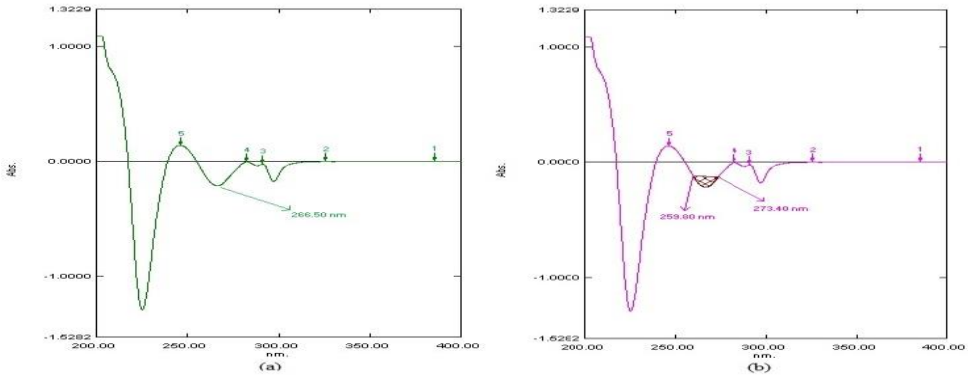

Fig. 4: First Order Derivative Spectrum (a) and Area Under Curve; (b) between selected wavelengths of Aripiprazole

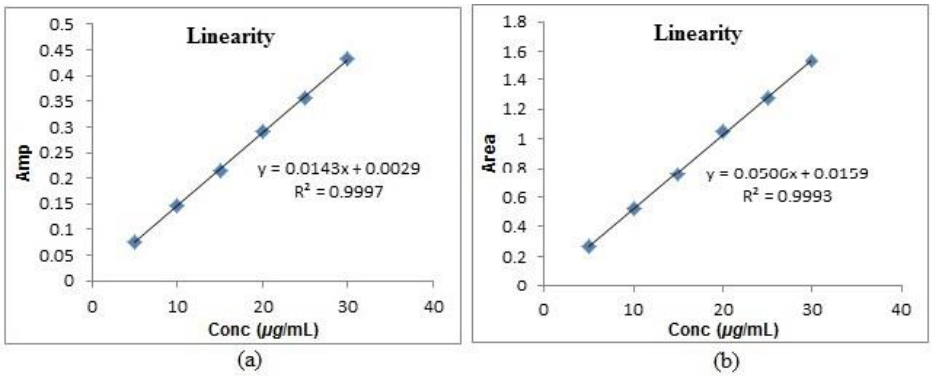

Fig. 5: Calibration Curve of Aripiprazole for Method C (a); and Method D (b)

Study of marketed formulation: For analysis of marketed formulation twenty tablets of ARP (ARZU having label claim 10 mg) were weighed and transferred to clean dry mortar then tablet grounded to fine powder by a pestle. Fine powder of tablet equivalent to 10 mg of ARP was transferred to 100 mL volumetric flask containing 70 mL methanolic HCl and sonicated for 10 min. After ultra-sonication volume was made upto the mark with methanolic HCl and filter through whatman filter paper (no. 41). From the filtrate sets of same concentration was separately transferred to 10 mL volumetric flask and volume was made upto the mark to get final concentration of 15 µg/mL. The absorbance recorded and concentrations in the sample were determined from linearity equation.

Validation of Method

The propose method was validated with regard to various parameter i.e. linearity, precision, accuracy, limit of detection, limit of quantification and ruggedness according to ICH guide line.[32]

Linearity: The linearity of the "methods A, B, C, and D" was evaluated by analysis of six standard solutions of ARP of concentrations 05, 10, 15, 20, 25, and 30 µg/mL. The calibration curve was obeyed in the concentration of range 5 - 30 µg/mL and the graph was plotted between concentrations versus absorbance, amplitude and AUC.

Accuracy: The accuracy of all methods was evaluated by measurement of recovery. To the pre-analyzed sample solutions (10 µg/mL in all method), known amounts of stock standard solutions were added at different levels, that is, 80%, 100%, and 120%. The solutions were reanalyzed through the developed methods. The experiments were repeated for three times at each level for method.

Precision: Precision of the methods was studied in the form of intra-day and inter-day variations. For "all methods," precision was found out by analyzing the 10, 15, and 20 µg/mL of ARP solutions separately for intra-day and inter-day variations.

Sensitivity: For sensitivity measurements, solution of ARP analyzed by developed methods and estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) which were calculated using formulae "LOQ = 10 × N/B" and "LOD = 3.3 × N/B," where "N" is average standard deviation of the amplitudes or peak areas of the ARP (n = 3), taken as a measure of noise, and "B" is the slope related to calibration curve.

Repeatability: In "all methods" repeatability was estimated by analyzing of 15 µg/mL solution of ARP for six times.

Ruggedness: For "all methods" ruggedness of developed methods was determined by analyzing of 15

µg/mL solution of ARP by two different analysts using similar operational and environmental conditions.

Result and Discussion

Method validation: ARP was validated with respect to the following parameters linearity, accuracy, precision, sensitivity, LOD, LOQ, and ruggedness. The results were found to be acceptable as per guidelines. The data of regression analysis shown in (Table 1).

Table 1: Optical Characteristics of Aripiprazole

Parameters	Method A	Method B	Method C	Method D
Beer-Lambert's range(µg/mL)	05-30	05-30	05-30	05-30
Lambda Max (nm)	255	244.00-264.40	266.50	259.80-273.40
Slope	0.0337	0.0942	0.0143	0.0506
Intercept	0.008	0.0251	0.0029	0.0156
Correlation coefficient	0.9999	0.9998	0.9997	0.9993

Linearity: From the linear regression data it was cleared that for "methods A, B, C, and D" calibration curves shown in (Fig. 3 & 5) linear relationship over the concentration range of 5-30 µg /mL for ARP shown in (Table 1).
Accuracy: For determination of accuracy of the developed methods, pure drug solution was added in pre-tested sample solution at three different concentration levels; 80 %, 100 %, 120 %. The results were depicted in (Table 2). The % Recovery values indicate that the accuracy of the methods was found to be satisfactory.

Table 2: Accuracy studies

Drug	Methods	Initial amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL, n = 3]	% Recovered	% RSD
Aripiprazole	A	10	8	17.92	98.97	0.62
		10	10	19.98	99.76	0.67
		10	12	21.96	99.64	0.30
	B	10	8	17.94	99.26	0.43
		10	10	19.89	98.92	0.18
		10	12	21.95	99.59	0.49
	C	10	8	17.98	99.74	0.92
		10	10	20.03	100.30	0.81
		10	12	21.88	99.03	0.66
	D	10	8	17.99	99.88	1.24
		10	10	20.04	100.40	0.17
		10	12	22.01	100.07	0.39

n- number of determinations

Precision

Intra-day: For intraday precision studies three replicates of three different concentration 10, 15, 20 µg/mL was analyzed at different time in same day. The % RSD and data demonstrate in (Table 3).

Table 3: Precision studies

Drug	Methods	Concentration [µg/mL]	Intra-day [n = 3]	% RSD	Inter-day [n = 3]	% RSD
Aripiprazole		10	9.92	0.88	10.01	0.44
	A	15	14.91	0.61	14.92	0.59
		20	20.05	0.34	20.03	0.10
		10	9.91	0.34	10.05	0.17
	B	15	14.93	0.62	14.95	0.20
		20	19.97	0.06	20.18	0.48

		10	9.89	0.43	10.01	0.43
	C	15	14.90	0.55	14.86	0.75
		20	20.19	0.19	20.20	0.21
		10	10.02	0.19	10.10	0.45
	D	15	14.75	0.14	14.80	0.12
		20	20.18	0.21	20.10	0.19

n- number of determinations

Inter-day: For inter-day precision three replicates of three different concentrations 10, 15, 20 µg/mL was analyzed in different day subsequently. The % RSD and data demonstrate in (Table 3).
Sensitivity: The LOD and LOQ for ARP in "method A" were found to be 0.26 µg and 0.77 µg, in "method B" 0.37 µg and 1.11 µg, "method C" 0.24 µg and 0.72 µg, while "method D" 0.29µg and 0.87 µg.
Repeatability: The results of repeatability in terms of % RSD for "methods A, B, C, and D," were less than 2 indicates precise nature of developed methods. Results are shown in (Table 4).

Table 4: Repeatability studies

Drug	Methods	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found [n =6]	Mean ± SD	% RSD
	A	15	15.03	100.92	100.92 ± 0.59	0.59
	B	15	15.28	101.90	101.90 ± 0.60	0.59
Aripiprazole	C	15	15.01	100.05	100.05 ± 0.86	0.86
	D	15	14.97	99.80	99.80 ± 1.19	1.19

n- number of determinations

Ruggedness: The results of ruggedness were in acceptable range that is % RSD values < 2 for all the developed methods as given in (Table 5). The results prove no statistical differences between different analyst using same operational and environmental condition. Hence it signifies that the developed methods are rugged in nature.

Table 5: Ruggedness studies

		Analyst I		Analyst II	
Drug	Methods	% Amount found ± SD [n = 3]	% RSD	% Amount found ± SD [n = 3]	% RSD
Aripiprazole	A	98.84 ± 0.66	0.66	101.50 ± 1.90	1.87
	B	99.99 ± 0.79	0.79	100.33 ± 0.97	0.97
	C	100.33 ± 0.16	1.06	98.55 ± 0.88	0.90
	D	99.13 ± 0.79	0.80	99.29 ± 0.69	0.69

n- number of determinations

Analysis of Tablet Formulation: From ARP tablet formulation amount of ARP estimated by using methods A, B, C and D were found to be 99.55%, 100.08%, 99.04%, and 99.27%, respectively. The % amount found from tablet formulation shows that there was no interference from excipients present in tablet formulation.

Conclusion

All methods were established for quantitative analysis of ARP in bulk and tablet formulation by using derivative spectroscopic technique and AUC technique of UV-spectrophotometry. The results and statistical analysis show that amongst all methods, AUC method is most simple, accurate, precise, and specific. Therefore, these all methods can be used for routine analysis of ARP in bulk and pharmaceutical formulation.

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Disclosure of Interest

The authors have none to declare

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