Original Article

STABILITY INDICATING SPECTROPHOTOMETRIC AND TLC-DENSITOMETRIC METHODS FOR THE DETERMINATION OF ARIPIPRAZOLE IN BULK AND DOSAGE FORM

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ABSTRACT

Objective: Four different methods were used for stability indicating determination of aripiprazole in presence of its oxidation product in pure and its dosage form.

Methods: The described methods are namely, second derivative spectrophotometry, first-derivative of ratio spectra; bivariate and thin layer chromatography.

Results: The derivative spectrophotometric method was based on measuring the peak amplitudes of second derivative spectra of aripiprazole at 217.2 and 229 nm where zero crossing points obtained with its oxidation product, over a concentration range of 1.0 – 6.0µg.ml^{-1} for aripiprazole. The derivative ratio spectra method was based on measuring the peak amplitudes for aripiprazole at 209.8, 222, 246.8 and 283.2 nm using 5.0µg.ml^{-1} oxidation product as a divisor, over a concentration range of 1.0-6.0µg.ml^{-1} for aripiprazole. Bivariate method is used for determination of aripiprazole in presence of oxidation product over a concentration range of 1.0 – 6.0µg.ml^{-1} for aripiprazole. The method was based on measuring the absorbance at the selected wavelengths. A TLC separation with densitometric detection of aripiprazole was achieved using ethyl acetate: methanol [11:4, v/v] as developing solvent. The method allowed determination of aripiprazole in concentration ranges of 1.0-4.0 µg.spot[-1].

Conclusion: The proposed methods are accurate, precise and successfully applied for the determination of the studied drug in presence of its oxidation product in pure form and in pharmaceutical formulations containing them, so that these methods can be used as stability indicating methods for the determination of aripiprazole in quality control laboratories.

Keywords: *Aripiprazole, Bivariate, Ratio spectra, Second derivative, TLC.*

INTRODUCTION

Aripiprazole (ARP) chemically 7-[4-[4-(2, 3-dichlorophenyl)-1-piperazinyl]-3,4-dihydro-2(1H)- quinolinone (chemical structure shown in Fig. 1) is a recent atypical antipsychotic drug that is effective for the treatment of patients with schizophrenia or schizoaffective disorder [1]. It is belonging to the chemical class of benzisoxazole derivatives. It has potent partial agonist activity at dopamine (D2) receptors [2]. It is most commonly prescribed new drug worldwide for the treatment of schizophrenic illness [3]. Aripiprazole was determined by spectrophotometric [4, 5] gas chromatography-mass spectrometry [6], LC–MS/MS [7, 8], capillary electrophoretic [9] methods in biological fluids. Few HPLC techniques are reported [10-17] for the determination of aripiprazole in pharmaceutical dosage form, and most of them used different buffers as a mobile phase which is reducing the life span of the analytical column and preparation of buffer with the maintenance of proper pH is cumbersome process. Also an official HPLC method was described for the determination of ARP in pure form [18].
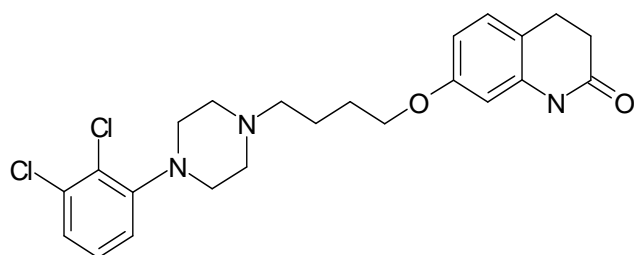
Literature survey revealed different stability indicating methods performed on ARP, but up to our knowledge, no definite data on the structure of degradation products were reported. The aim of this work was to develop a comparative study of recent, simple, sensitive, validated stability indicating methods that are of lower cost than the official HPLC method.

MATERIALS AND METHODS

Instruments

A dual-beam UV-visible spectrophotometer [Shimadzu, Japan] model UV-1601 PC, with 1cm quartz cells, connected to an IBM compatible computer was used. Bundled, UV-PC personal spectroscopy software version 2.21 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2nm with wavelength-scanning speed of 2800 nm min^{-1}.

TLC plates [20 cm x 10 cm, 0.25 mm] coated with silica gel 60 F254 [Merck, Germany] were used.

Camag TLC scanner 3 S/N 130319 with WINCATS software and Camag Linomat 5 auto sampler [Muttenz, Switzerland] with Camag micro syringe [100µL] were used.

Materials

Samples

Reference ARP standard was kindly supplied by Al Andalus Pharmaceutical Company, Cairo, Egypt. The purity of ARP was found to be 99.30 ± 0.54 (n=6), according to its official method [18].

Pharmaceutical dosage form [Aripiprex® 10 and 30 mg tablets] batch No. 60365 and 60366 claimed to contain 10 and 30 mg aripiprazole, respectively, expressed as base per tablet, was kindly supplied by Al Andalus Pharmaceutical Company Cairo, Egypt.

Fig. 1: It Shows chemical structure of Aripiprazole

Reagents

All chemicals and reagents were of pure analytical grade.

-Methanol, ethyl acetate, acetonitrile and H_2O_2 (30%, v/v) were obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

Standard solutions

Stock standard solutions of ARP [0.1mg.mL^{-1}] in acetonitrile were prepared for both the spectroscopic and TLC-densitometric methods. All solutions were freshly prepared on the day of analysis.

Laboratory-prepared mixtures

Different aliquots of ARP stock solution [0.1mg.mL^{-1}] were transferred into a series of 10-ml volumetric flasks. Different aliquots from oxidation product (OXD) stock solution [0.1mg.mL^{-1}] were added to prepare different mixtures of ARP and OXD.

Procedures

Spectroscopic Methods

Second derivative spectrophotometric method

Aliquots from standard stock solution [0.1mg.mL^{-1}] of ARP were transferred into a series of 10-mL volumetric flasks. The volume was completed with acetonitrile to prepare solutions in concentration ranges of 1.0-6.0μg.mL^{-1} ARP. The zero order spectrum of each solution was recorded and then the ^2D spectra were computed using $\Delta \lambda = 4$ and scaling factor 100. The ^2D values of ARP were measured at 217.2 and 229 nm (where zero crossing obtained with OXD spectrum). Calibration graphs were constructed relating the peak amplitudes of (^2D) to the corresponding concentrations of ARP. The regression equations were then computed at the specified wavelengths and used for determination of unknown samples of ARP.

Derivative ratio spectrophotometric method

Aliquots from standard stock solution of ARP were transferred into a series of 10-mL volumetric flasks. The volume was completed with acetonitrile to prepare solutions in concentration ranges of 1.0-6.0μg.mL^{-1} ARP. The spectra of the prepared solutions were scanned from 200 nm to 400 nm and stored in the computer. The stored spectra of ARP were divided (amplitude at each wavelength) by the spectrum of 5.0μg.mL^{-1} of OXD. The first derivative of the ratio spectra (^1DD) with $\Delta \lambda = 4$ nm and a scaling factor = 10 was obtained. The amplitudes of the first derivative peaks of ARP were measured at 209.8, 222, 246.8 and 283.2 nm. Calibration graphs were constructed relating the peak amplitudes of (^1DD) to the corresponding concentrations of ARP. The regression equations were then computed at specified wavelengths and used for determination of unknown samples of ARP.

Bivariate method

Several dilutions of ARP and OXD were made from their stock solutions and were used for the bivariate calibration. Spectra of the obtained solutions were recorded and stored into the computer. The regression equations were computed at 210 nm and 216.2 nm. The concentrations of ARP and OXD were calculated using the parameters of the linear regression functions evaluated individually for each component at the same wavelength and substituting in the following equations:

$$C_{ARP} = m_{A2} (A_{AB1} - eA_{B1}) + m_{A1} (eA_{B2} - A_{AB2}) / m_{A2} m_{B1} - m_{A1} m_{B2}$$

$$C_{OXD} = A_{AB1} - eA_{B1} - m_{B1} C_{ARP} / m_{A1}$$

Where, A_{AB1} and A_{AB2} are the absorbance of ARP and OXD at 210 nm and 216.2 nm, respectively, e_{AB1} and e_{AB2} the sum of the intercepts of the linear calibration at the two wavelengths ($e_{AB1} = e_{A1} + e_{B1}$), m_A and m_b are the slopes of the linear regressions and C is the concentrations [μg.mL^{-1}]. The accuracy of the results was checked by applying the proposed bivariate method for determination of different samples of pure ARP. The concentrations were obtained from the corresponding regression equations from which percentage recoveries were calculated.

Chromatographic Methods

TLC-Densitometric method

Aliquots equivalent to 1.0-4.0μg.spot^{-1} of ARP standard solution [0.1mg.mL^{-1}] were applied in the form of bands on TLC plates. The band length was 4 mm and dosage speed was 150nL S^{-1}, the bands were applied 12.8 mm apart from each other and 15 mm from the bottom edge of the plate. Linear ascending development was performed in a chromatographic tank previously saturated with ethyl acetate: methanol [11:4, v/v] for 30 minutes at room temperature. The developed plates were air-dried and scanned at 255 nm using deuterium lamp, absorbance mode at 3 mm x 0.45 mm slit dimension and scanning speed of 20 mm S^{-1}. Calibration curves relating the optical density of each spot to the corresponding concentration of ARP were constructed. The regression equation was then computed for the studied drug and used for determination of unknown samples.

Assay of laboratory-prepared mixtures

A degraded sample of ARP was prepared as follows: 10.00 mg amount of intact ARP was refluxed for 2 hours with 5 ml 30% (v/v) H_2O_2. The resulting solution was tested for complete degradation by the thin layer chromatography technique using ethyl acetate: methanol (11: 4, by volume) as a mobile phase and detecting the spots at 255 nm. The solution was cooled, excess oxygen was removed and the solution was transferred into 100 ml volumetric flask. The volume was completed to the mark by acetonitrile. Aliquots of standard drug solution [0.1mg.mL^{-1}] were mixed with its degraded sample [0.1mg.mL^{-1}] in different ratios. The mixtures were analyzed using the suggested methods, and proceed as mentioned under each method, the concentrations were then calculated from the corresponding regression equations.

Assay of pharmaceutical formulation (Aripiprex® tablets)

Ten tablets were weighed from the dosage form and the average weight was calculated, tablets were crushed to furnish a homogenous powder and certain amount of powdered tablets were dissolved by the aid of an ultrasonic bath for 2 hours and filtered. The solutions were diluted to the same concentration of the appropriate stock solutions and proceed as described under each method.

RESULTS AND DISCUSSION

Preparation and Isolation of ARP Oxidation product

Being containing piperidine group in its structure, ARP is liable to oxidation. It was found that on refluxing ARP with 30% H_2O_2 for 2 hours, a complete oxidation was occurred.
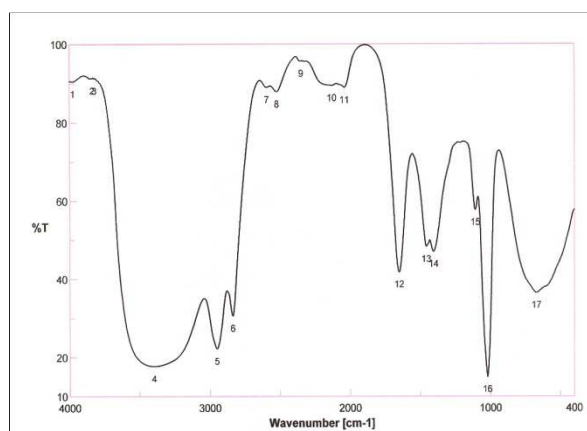

Fig. 2: It Shows IR spectrum of ARP oxidation product.

The resulting solution was tested for complete oxidation by the thin layer chromatography technique using ethyl acetate: methanol (11:

4, by volume) as a mobile phase and detecting the spots at 255 nm. The solution was cooled, applied in a band form on preparative TLC plate and developed in the mobile phase. After that the band corresponding to oxidation product was scratched, dissolved in acetonitrile, evaporated and purified. The isolated OXD solid was used to prepare the stock solution and further analyzed by infra-red and mass spectral analysis.

The infra-red spectral analysis of the isolated OXD, (Fig.2) in comparison with that of pure ARP, (Fig.3) showed the appearance of broad peak at 3397.9 cm^{-1} which is characteristic to (OH) group, while it was absent in the spectrum of pure drug. Also the spectrum showed the appearance of peaks at 1457 and 1405 cm^{-1} that are characteristic to (–NO) group, which were absent in the pure drug.
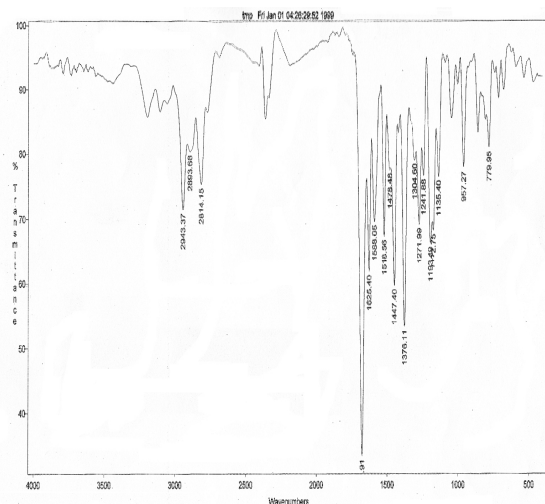

Fig. 3: It Shows IR spectrum of pure ARP.

The mass spectral analysis of the isolated OXD, (Fig.4) showed that the molecular ion peak was at 349 m/z, while that of pure drug was at 447 m/z, (Fig.5). Also, the isotopic ratio of two chlorine atoms that appeared in the fragmentation pattern of the mass spectrum of pure ARP (triplet form) didn't appear in the spectrum of OXD, so that this difference supported the suggested mechanism shown in figure 6.
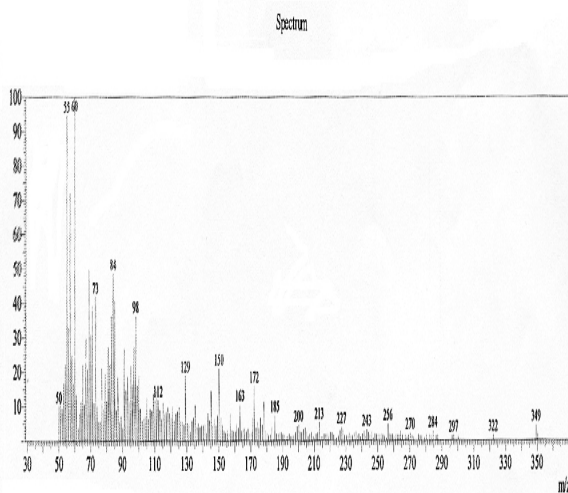

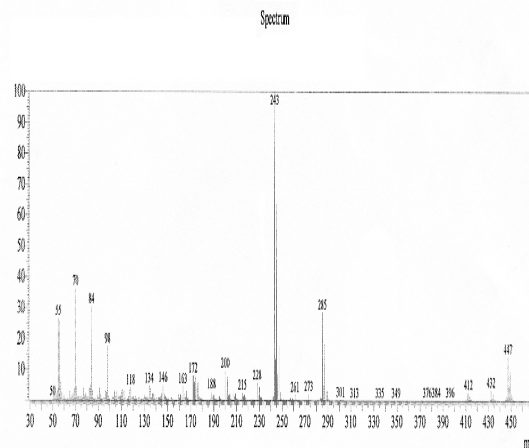**Fig. 4: It Shows Mass spectrum of ARP oxidation product.**
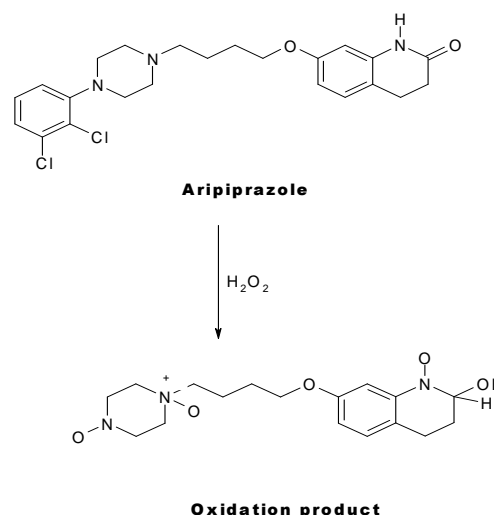

Fig. 5: It Shows Mass spectrum of pure ARP.

Fig. 6: It Shows the suggested mechanism for ARP oxidation

Spectroscopic Methods

Second Derivative Spectrophotometric Method

Derivative spectrophotometry has been applied extensively to the simultaneous determination of substances with overlapping spectra which is frequently made on the basis of zero-crossing measurements. It can be applied for the determination of a drug in presence of another or a drug in presence of its degradation product by selecting a wavelength where contribution of one compound is zero or almost zero (ZCP), while the other to be determined has a reasonable value.

The zero – order absorption spectra of ARP and OXD are overlapped (Fig.7). First derivative absorption spectra of both ARP and OXD did not show any favorable zero crossing points, but on trying second derivative absorption spectra, they showed zero crossing points at 217.2 and 229 nm with good spectral characteristics and resolution (Fig.8). Linear calibration graph was obtained for ARP in concentration range of 1.0-6.0 µg.mL^{-1} by recording the peak amplitudes (^{2}D) at 217.2 and 229.0 nm and the regression equations were computed and found to be:

^{2}D $_{217.2}$ = 0.15 C + 0.30 r = 0.9996

^{2}D $_{229}$ = 0.08 C + 0.09 r = 0.9995

Where, ^{2}D is the peak amplitude of the second derivative curve for ARP, C the concentration of ARP (µg.mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be 99.30 ± 0.63 at 217.2 nm, 99.24 ± 0.64 at 229 nm. The linearity ranges and analytical data for the calibration graphs are listed in table 2. Results for analysis of laboratory-prepared mixtures with different proportions of ARP and its OXD are given in table 4.
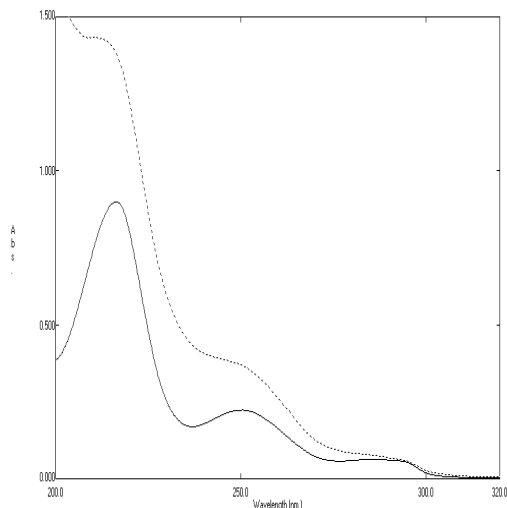

Fig. 7: It Shows absorption spectra of aripiprazole 5.0µg.ml^{-1} (—) and its oxidation product 5.0µg.ml^{-1} (.....) in acetonitrile.

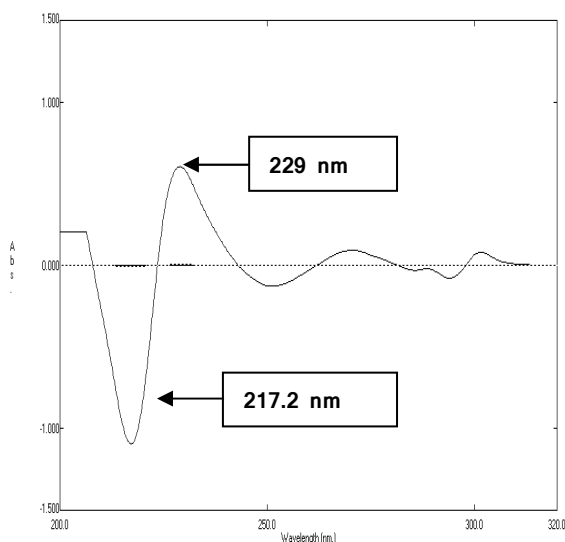**Fig. 8: It Shows Second derivative absorption spectra of aripiprazole 5.0µg.ml^{-1} (—) and its oxidation product 5.0µg.ml^{-1} (.....) in acetonitrile.**

Derivative Ratio Spectra Method

The derivative-ratio spectroscopy is a useful tool in quantification of drugs. It could be applied for the determination of ARP. The zero – order absorption spectra of ARP and OXD are overlapped (Fig.7). ARP can be assayed in presence of OXD by dividing the absorption spectra of different concentrations of ARP in the range of 1.0-6.0µg.ml^{-1} by the absorption spectrum of (5.0µg.ml^{-1}) OXD and then the first derivative of ratio spectra (^{1}DD) were recorded (Fig. 9).

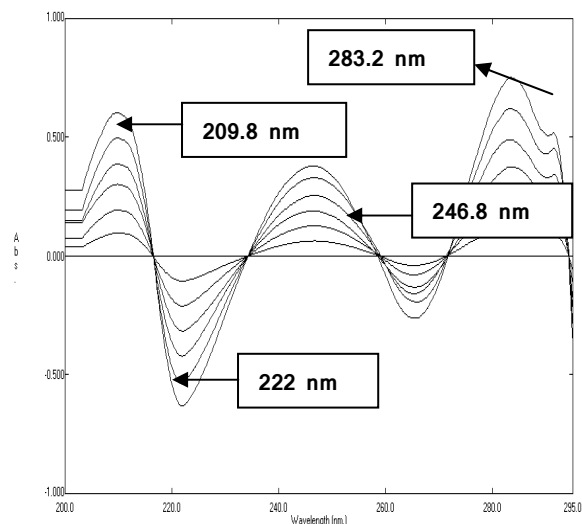**Fig. 9: It Shows first-order of ratio spectra of different concentrations of ARP (using5.0µgmL^{-1} OXD as divisor).**

It was found that upon dividing by 5.0µg.mL^{-1} of OXD, best results were obtained in terms of sensitivity, repeatability and signal to noise ratio. Linear calibration graph was obtained for ARP in concentration range of 1.0-6.0 µg.mL^{-1} by recording the peak amplitudes at 209.8, 222, 246.8 and 283.2 nm using 5.0µg.mL^{-1} of OXD as a devisor. The regression equations were computed and found to be:

^{1}DD = 0.1 C – 0.008 (r^2 = 0.9995), at 209.8 nm

^{1}DD = 0.11 C - 0.002 (r^2 = 0.9998), at 222 nm.

^{1}DD = 0.07 C - 0.051 (r^2 = 0.9995), at 246.8 nm

^{1}DD = 0.13 C - 0.003 (r^2 = 0.9997), at 283.2 nm

Where, ^{1}DD is the peak amplitude of the first derivative ratio curve for (ARP/OXD), C the concentration of ARP (µg.mL^{-1}) and r^2 is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be 99.39 ± 0.52 at 209.8 nm, 99.16 ± 0.95 at 222 nm, 99.41 ± 0.46 at 246.8 nm and 99.38 ± 0.71 at 283.2 nm. The linearity ranges and analytical data for the calibration graphs are listed in table 2. Results for analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 4.

Bivariate method

The bivariate calibration method may be competitive and in some cases even superior to commonly use derivative spectrophotometric methods as applied for the resolution of binary mixtures. The advantage of bivariate calibration method is its simplicity and the fact that derivatization procedures are not necessary. Unlike other chemometric techniques, there is no need for full spectrum information and no data processing is required. Calibration function was calculated (*r* > 0.9990), mi- and ei-values were taken for the bivariate algorithm. In order to apply the bivariate method to the resolution of binary mixture of ARP and its OXD, we first select the signals of the two components located at six wavelengths; 210.0, 216.2, 220.0, 225.0, 230.0, and 250.0 nm. The calibration curve equations and their respective linear regression coefficients are obtained with the aim of ensuring that there is a linear relationship between the absorbance values and the concentrations. All the calibration curves at the selected wavelengths showed satisfactory linear regression coefficients (*r* > 0.9990). The slope values of the linear regression were estimated for both components at the selected wavelengths and used for determination of the sensitivity matrices *K*, proposed by Kaiser's method [19]. The determinants of these matrices were calculated and the wavelength set was selected

for which the highest matrix determinant value was obtained as shown in table 1. For the bivariate method determination of ARP and its OXD, this was done using 210.0 nm and 216.2 nm. The linearity ranges are listed in table 2. Results of analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 4.

Table 1: It Shows Application of Kaiser Method for the selection of the Wavelength set for aripiprazole (ARP) – its oxidation product (OXD) mixture

λ / λ	210 nm	216.2 nm	220 nm	225 nm	230 nm	250 nm
210 nm	0	140	136	28.3	44.6	46.8
216.2 nm		0	10.7	59.3	102.3	13.5
220 nm			0	59.7	95.8	9.5
225 nm				0	39.6	76
230 nm					0	29.9
250 nm						0

## Chromatographic Method

TLC-Densitometric method

TLC densitometry overcomes the problem of overlapping absorption spectra of mixture of drugs by separating these components on TLC plates and determining each ingredient by scanning the corresponding chromatogram. The TLC densitometric method has the advantage of simultaneously determining the active ingredients in multi-component dosage forms [20].

A TLC-densitometric method could be used for determination of ARP in presence of OXD without prior separation. Different solvent systems were tried for the separation and a satisfactory results were obtained by using a mobile phase composed of ethyl acetate: methanol [11:4, v/v] where R_f = 0.73 and 0.29 for ARP and OXD, respectively.

The separation allowed the determination of different concentrations of ARP in presence of OXD with no interference, (Fig.10). The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 255 nm over a range of 1.0-4.0µg.spot^{-1} for ARP, where a linear response was obtained, regression equation was found to be:

A= 4.58 C + 2.64 r = 0.9997 (for ARP).

Where A is the area integrated under the peak x 10^{-3} for ARP, C is the concentration in µg.spot^{-1}and r is the correlation coefficient.
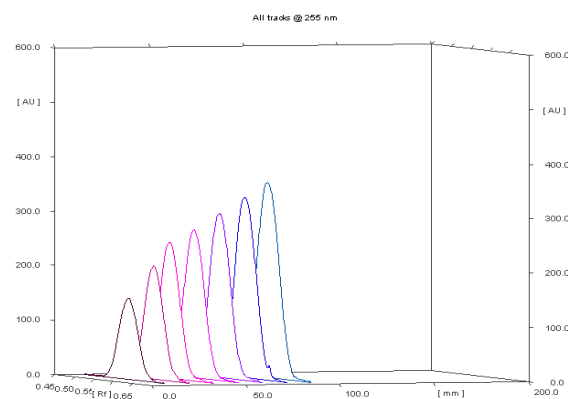
Fig. 10: It Shows a 3D linearity graph of ARP (R_f = 0.73) over a concentration range (1.0- 4.0µg. spot^{-1}).

The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recovery was found to be 98.98 ± 0.93 for ARP. To assess the specificity, accuracy and selectivity of the TLC method for assay of ARP without interference from its OXD, synthetic mixtures of both ARP and OXD at various concentrations within the linearity range were prepared and analyzed.

Table 2: It Shows assay parameters and validation of the proposed methods for determination of ARP

parameters	The proposed methods								
	Bivariate method		[1]DD method				[2]D method		TLC method
							217.2 nm	229 nm	
	210 nm	216.2 nm	209.8 nm	222 nm	246.8 nm	283.2 nm			
LOD (µg.ml^{-1})	0.15	0.06	0.06	0.07	0.09	0.05	0.08	0.10	0.17 (µg.spot^{-1})
LOQ (µg.ml^{-1})	0.47	0.17	0.19	0.21	0.26	0.16	0.25	0.29	0.53 (µg.spot^{-1})
Range (µg.ml^{-1})	1.0-6.0	1.0-6.0	1.0-6.0	1.0-6.0	1.0-6.0	1.0-6.0	1.0-6.0	1.0-6.0	1.0-4.0 (µg.spot^{-1})
Slope	0. 15	0.18	0.10	0.11	0.07	0.13	0.15	0.08	4.58
Intercept	0.004	0.009	-0.008	- 0.002	-0.051	- 0.003	0.30	0.09	2.64
Mean ± S.D.	99.31 ± 0.35	99.10 ± 0.90	99.39 ± 0.52	99.16 ± 0.95	99.41 ± 0.46	99.38 ± 0.71	99.30 ± 0.63	99.24 ± 0.64	98.98 ± 0.93
Correlation Coeff. (r)	0.9997	0.9993	0.9995	0.9998	0.9995	0.9997	0.9996	0.9995	0.9997
RSD% ᵃ*	0.22 -0.24	0.12-0.15	0.12-0.16	0.14-0.17	0.16-0.18	0.11-0.13	0.13-0.15	0.18-0.20	0.41 – 0.60
RSD% ᵇ*	0.38-0.42	0.27-0.32	0.37-0.40	0.42-0.44	0.47-0.50	0.32-0.34	0.33-0.35	0.47-0.49	0.48-0.65

*[a,b] **Intra-day and inter-day (n=3) relative standard deviations of samples of concentrations 3.0 and 4.0µg.ml^{-1} of ARP for spectroscopic methods and 2.0 µg.Spot^{-1} of ARP for TLC method.**

A statistical comparison of the results obtained by the proposed methods and the official one for ARP [18] is shown in table 3. The values of the calculated T and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed methods and the official one [18].

Table 3: It Shows statistical analysis of the results obtained by applying the proposed methods and the official one for the analysis of pure ARP.

Values	The proposed methods									Official method**
	Bivariate method		¹DD method				²D method		TLC method	
	210 nm	216.2 nm					217.2 nm	229nm		
			209.8	222	246.8	283.2				
Mean	99.31	99.10	99.39	99.16	99.41	99.38	99.30	99.24	98.98	99.53
S.D.	0.35	0.91	0.52	0.95	0.46	0.71	0.63	0.64	0.93	0.62
N	6	6	6	6	6	6	6	6	6	6
Variance	0.12	0.83	0.27	0.90	0.21	050	0.40	0.41	0.86	0.38
T[2.23]*	0.76	0.96	0.42	0.80	0.38	0.39	0.65	0.71	1.20	....
F[5.05]*	3.17	2.18	1.40	2.37	1.81	1.32	1.05	1.08	2.26	....

*The figures in parenthesis are the corresponding tabulated values at P=0.05. ** Official method is USP HPLC method for ARP [18].

Analysis of Laboratory-prepared mixtures

The validity of the proposed methods for the determination of ARP in presence of OXD was assessed by analysis of laboratory prepared mixtures containing different ratios of ARP and OXD and calculating the concentrations from the corresponding regression equations. The results are shown in table 4.

Analysis of Tablets

The validity of the proposed methods for the analysis of the pharmaceutical formulations and the effect of possible interferences from common excepients were studied by assaying Aripiprex® tablets (labeled to contain 10 and 30 mg of ARP per tablet), the results are present in table 5.

Table 4: It Shows determination of ARP in laboratory prepared mixtures with OXD by the proposed methods.

Drug	²D method		Bivariate method		¹DD method				TLC method
	217.2 nm	229 nm	210 nm	216.2 nm	209.8 nm	222nm	246.8 nm	283.2 nm	
ARP	99.26 ± 0.67	99.29 ± 0.93	99.58 ± 0.75	99.34 ± 0.64	99.23 ± 0.77	± 99.07 0.69	99.44 ± 1.04	99.29 ± 0.28	99.94 ± 0.78

Table 5: It Shows determination of ARP in dosage forms [Aripiprex®10 and 30 mg tablets] by the proposed methods.

Drug	²D method		Bivariate method		¹DD method				TLC method
	217.2 nm	229 nm	210 nm	216.2 nm	209.8 nm	222nm	246.8 nm	283.2 nm	
ARP in Aripiprex 10 mg B.N:60365	99.40 ± 0.27	99.46 ± 0.62	99.65 ± 0.31	99.23 ± 0.61	98.65 ± 0.32	99.34 ± 0.52	99.04 ± 0.33	98.37 ± 0.46	98.84 ± 0.52
ARP in Aripiprex 30 mg B.N:60366	99.17 ± 0.40	99.62 ± 0.23	99.27 ± 0.62	98.86 ± 0.55	98.37 ± 0.46	98.76 ± 0.33	98.52 ± 0.41	99.62 ± 0.23	98.47 ± 0.64

CONCLUSION

The proposed methods are accurate and precise and could be used as stability indicating methods for the determination of ARP in presence of its OXD and in their pharmaceutical formulation without prior separation. The most striking feature of the spectrometric methods is their simplicity and rapidity. For spectroscopic methods there was no need for time-consuming sample preparation steps such as tank saturation that is needed for the TLC procedure. The TLC-method has some advantages such as a short run time, large sample capacity and minimal volume use of solvent. With this method, one can gain the advantages of speed, low-cost, and environmental protection without sacrificing accuracy.

CONFLICT OF INTEREST

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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