ISSN: 1674-0815

cjhmonline.com

DoI-10.564220/1674-0815

Chinese Journal of Health Management

Chinese Medical Association

Estimation of Aripiprazole and Paliperidone Hydrochloride Using Spectrophotometric and Chromatographic Techniques

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Article Information
Received: 12-11-2025
Revised: 08-12-2025
Accepted: 17-01-2026
Published: 15-03-2026

Keywords
Aripiprazole (ARI); Paliperidone hydrochloride (HCl) (PALI); High-performance liquid chromatography (HPLC) and UV spectrophotometric methods (UV).

ABSTRACT:

High-performance liquid chromatography (HPLC) and UV spectrophotometric methods were successfully developed and validated for the quantitative estimation of Aripiprazole and Paliperidone hydrochloride. The RP-HPLC method was optimized using an isocratic elution technique on a reversed-phase Kromstar C_{18} (250 × 4.6 mm, 5 µm) Column. The retention times were found to be 2.5 minutes for Aripiprazole and 5.0 minutes for Paliperidone hydrochloride, indicating efficient separation within a short run time. For the UV spectrophotometric method, measurements were carried out at 221 nm for Aripiprazole (Zero crossing point of Paliperidone hydrochloride) and 239 nm for Paliperidone hydrochloride (Zero crossing point of Aripiprazole). Both analytical techniques demonstrated excellent linearity over the selected concentration ranges, with correlation coefficients (R^2) greater than 0.999. The developed methods exhibited satisfactory precision and accuracy, with percentage relative standard deviation (%RSD) values below 2%, confirming good reproducibility and recovery. Furthermore, the results obtained by the HPLC method showed strong correlation with those from the UV spectrophotometric method, suggesting that UV spectroscopy can serve as a cost-effective, reliable, and less time-consuming alternative to chromatographic analysis. Overall, the proposed methods are sensitive, precise, and accurate, and were successfully applied for the assay determination and in-vitro dissolution studies of a marketed pharmaceutical formulation. The elution was carried out at a constant flow rate of 1 mL/min. Furthermore, the two advanced methods validated by the valuable ICH Q2 (R2) guideline, affirming their effectiveness as a successful analytical tool for the concurrent analysis of both in bulk and the synthetic mixture fabricated in the laboratory.

INTRODUCTION:

Aripiprazole, chemically *7-{4-[4-(2,3-dichlorophenyl) piperazin-1-yl] butoxy}-3,4-dihydroquinolin-2(1H)-one*, is an atypical (second-generation) antipsychotic used in the treatment of schizophrenia and bipolar disorder. It exhibits a unique pharmacological profile with differential engagement at dopamine D2 receptors. Aripiprazole acts as a partial agonist at dopamine D2 and serotonin 5-HT1A receptors and as an antagonist at serotonin 5-HT2A receptors. It shows predominant antagonistic activity at postsynaptic D2 receptors and partial agonistic activity at presynaptic D2, D3, and other serotonin receptor subtypes[1].

Paliperidone hydrochloride, chemically *(RS)-3-[2-[4-(6-fluoro-1,2-benzoxazol-3-yl) piperidin-1-yl] ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a] pyrimidin-4-one*, is an atypical (second-generation) antipsychotic agent used in the treatment of schizophrenia and related psychotic disorders. It is the major active metabolite of risperidone. Although its exact mechanism of action is not fully elucidated, paliperidone is believed to exert its therapeutic effects through antagonism of central dopamine D_2 and serotonin 5-HT_2A receptors. Fixed-dose combinations of paliperidone and risperidone have been reported to be beneficial in the management of antipsychotic-induced hyperprolactinemia[2-3].

Combination of Aripiprazole and Paliperidone hydrochloride was studied under clinical trial phase[4]. It was proved that therapy improved when the second atypical antipsychotic, Paliperidone hydrochloride, was added to Aripiprazole. Paliperidone hydrochloride with Aripiprazole has been largely isolated for treatment of antipsychotic-associated hyper-prolactinemia[5-8].

Literature survey reveals stability indicating UV spectrophotometric [9-10], RP-HPLC [11-13], HPLC[14-15], HPLC in human plasma[16], GC-MS[17], UPLC-MS[18], HPLC-UV[19] method for the estimation of Aripiprazole alone and in combinations. Several analytical methods have been reported for the quantitative determination of Paliperidone hydrochloride such as UV spectrophotometry[20], RP-HPLC[21] and HPTLC[22], and LC-MS/MS[23].

From extensive literature survey, there is no any analytical method available in this combination. Given the widespread use of Aripiprazole and Paliperidone hydrochloride in the management of antipsychotic-induced hyperprolactinemia, the development of validated analytical methods for their simultaneous estimation is essential to ensure quality, safety, and efficacy of this combination intended for large patient populations. The objectives of the present work were to develop and validate a linear, accurate, precise, and sensitive first-order derivative UV method and RP-HPLC method for the simultaneous estimation of Aripiprazole and Paliperidone hydrochloride in synthetic mixture, providing a practical and robust solution for routine laboratory applications. These methods were validated according to ICH Q2 (R2) guideline[24] within all Validation Parameters.

2. MATERIALS AND ANALYTICAL CONDITIONS:

2.1 Reagents and chemicals:

Aripiprazole (Cadila Pharmaceuticals Ltd., Dholka), Paliperidone hydrochloride (Zydus Pharmaceuticals Ltd., Ahmedabad) were received as gift sample. Acetonitrile (HPLC grade) (Finar, Ahmedabad) Methanol (HPLC grade) (Finar, Ahmedabad) Water (HPLC grade) (Astron Chemical India) Ortho Phosphoric Acid 75 % (AR Grade) (Astron Chemical India). All other reagents employed were of high purity analytical grade. All weighing was done on a calibrated analytical balance. Calibrated glasswares were used throughout the work.

2.2 Instruments & Software:

The spectrophotometric measurements were performed using a UV-Visible spectrophotometer (Shimadzu-1900, UV Probe 2.7 version software) with a spectral bandwidth of 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells over the range of 200-400 nm. For chromatographic information acquisition and analysis, High-Performance Liquid Chromatography system Systronic RP-HPLC (LC-20-AD) (SPD-20 A) with UV Detector was utilized together. The pH of the buffer solution was observed utilizing the Chemi Line pH meter. The Scale-Tec analytical balance was utilized to weigh the samples. The HPLC mobile phase was subjected to sonication using an Sonicator- Digital Pro$^+$, PS-10A, (Broleo).

2.3 Analytical conditions:

In accordance with ICH Q2 (R2)[24] requirements, the analytical conditions for a simultaneous technique for the measurement of Aripiprazole and Paliperidone hydrochloride in UV and RP-HPLC were optimized and validated. For UV Spectroscopy Methanol was used as a Solvent. Detection wavelength (λmax) of ARI and PALI were 221 nm and 239 nm, respectively. The first-order derivative UV spectra were derived from the zero-order spectra using methanol as the solvent. Quantitative analysis was performed at the zero-crossing point (ZCP) of Aripiprazole at 239 nm for the estimation of Paliperidone hydrochloride, and at the ZCP of Paliperidone hydrochloride at 221 nm for the estimation of Aripiprazole. For RP-HPLC, Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) Column, ACN: Phosphate Buffer (pH 3): Methanol (65:25:10 % v/v/v) used as mobile phase at 1 ml/min flow rate. 242 nm was selected for HPLC method using U.V Detector. 20 µL injection volume was injected by using Hamilton syringe.

2.4 Preparation of Solutions:

2.4.1 Preparation of Stock Solution:

Accurately weighed 10 mg of Aripiprazole and 10 mg of Paliperidone hydrochloride were individually transferred into separate 100 mL volumetric flasks and dissolved in methanol. The solutions were sonicated to ensure complete dissolution, and the volume was made up to the mark with methanol to obtain standard stock solutions having a concentration of Aripiprazole 100 µg/mL of and 100 µg/mL of Paliperidone hydrochloride, respectively.

2.4.2 Preparation standard solution:

Accurately 1.0 ml of the mixture solution of Aripiprazole (100 µg/ml) and Paliperidone hydrochloride (30 µg/ml) was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with methanol. Final concentration of Aripiprazole was 10 µg/ml and Paliperidone hydrochloride was 3 µg/ml.

2.4.3 Preparation of standard working solution:

The concentration ranges of 5-25 µg/mL of Aripiprazole and 1.5-7.5 µg/mL of Paliperidone hydrochloride formed, from each stock solution, Aripiprazole (0.5, 1.0, 1.5, 2.0, and 2.5 ml) and Paliperidone hydrochloride (0.15, 0.30, 0.45, 0.60, and 0.75 ml) were pipetted out in ten different 10 ml volumetric flasks and made up to mark with Methanol to obtained 5, 10, 15, 20 and 25 µg/ml Aripiprazole of and 1.5, 3.0, 4.5, 6.0 and 7.5 µg/ml for, Paliperidone hydrochloride respectively. Under the optimized spectrophotometric conditions, the samples were analyzed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, the optimized chromatographic conditions, 20 µL of each standard working solution were injected into RP-HPLC system by Hamilton syringe and analyzed.

3. METHODOLOGY:

3.1. UV-spectrophotometric method:

A first-order derivative spectrophotometric technique was employed for the simultaneous quantification of Aripiprazole and Paliperidone hydrochloride in a synthetic mixture. Separate working standard solutions of each drug were scanned within the 200-400 nm wavelength range to generate their derivative spectra, enabling the determination of appropriate zero-crossing wavelengths for accurate quantitative analysis. Aripiprazole and Paliperidone hydrochloride standard stock solutions were prepared in Methanol at concentrations of 100 µg/mL and 100 µg/mL, respectively. Appropriate volume, 1.0 mL of Aripiprazole and 0.3 mL Paliperidone hydrochloride from standard stock solution were transferred to two separate 10 mL volumetric flasks and the volume was adjusted to mark with methanol to get concentration 10 and 3.0 µg/mL, respectively. The solutions were scanned separately in the UV-region i.e., 400-200 nm. The zero-order UV absorption spectra of Aripiprazole and Paliperidone hydrochloride in Methanol shown in Figure 1. The zero-order spectrum was processed to obtain first-derivative spectrum. The two first derivative spectra were overlaid which showed that Aripiprazole showed zero crossing at 239 nm, while Paliperidone hydrochloride showed zero crossing at 221 nm which showed in Figure 2. The amplitude values were measured at 221 nm ($\lambda 1$) (ZCP of PALI) for ARI and 239 ($\lambda 2$) (ZCP of ARI) for PALI showed in (Figure 2). To determine the wavelength for measurement, Aripiprazole (10 µg/ml) and Paliperidone hydrochloride (3 µg/ml) solutions were scanned between 200-400 nm. The zero order and first order overlay UV spectra of Aripiprazole and Paliperidone hydrochloride showed in Figure 1 and 2, respectively.

3.2. Reverse Phase High Performance Liquid Chromatography Method:

For RP-HPLC, the analysis was carried out using an isocratic elution technique using a mobile phase comprised of different mobile phases such as ACN: Phosphate Buffer (pH 3): Methanol (65:25:10 % v/v/v) at a flow rate of 1 mL/min found better separation of both the drug peaks. Prior to usage, the solvents were filtered through a 0.45 µm filter and sonicated for 30 min. The stationary phase was a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm), and the eluent was observed by a U.V Detector from 200 to 400 nm, alongside chromatograms extracted at 242 nm. The calibration curves were prepared by measuring the peak areas of ARI and PALI and plotted their values against the pertinent concentrations. In accordance, the equations for linear regression were calculated.

3.3 Method Validation:

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Q2(R2)[24]: Validation of Analytical Procedures established standards for the validation of the analytical procedures utilized in this investigation.

3.3.1 Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

3.3.2. Linearity and Range (n=6):

Linearity was studied by preparing standard solution at 5 different concentrations. The linearity range for Aripiprazole and Paliperidone hydrochloride were found to be 5-25 µg/ml and 1.5-7.5 µg/ml respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

3.3.3. Precision:

Precision expresses the closeness of agreement among individual test results obtained from repeated measurements of a homogeneous sample under specified conditions. It is assessed in terms of repeatability, intra-day precision, and inter-day precision.

Intraday Precision (n=3): Solutions containing 5, 10, 15 µg/ml of Aripiprazole and 1.5, 3.0, 4.5 µg/ml of Paliperidone hydrochloride were analyzed three times on the same day and % R. S. D was calculated.

Interday Precision (n=3): Solutions containing 5, 10, 15 µg/ml of Aripiprazole and 1.5, 3.0, 4.5 µg/ml of Paliperidone hydrochloride were analyzed on three different successive days and % R. S. D was calculated.

Repeatability (n=6): Solutions containing 10 µg/ml of Aripiprazole and 3.0 µg/ml of Paliperidone hydrochloride were analyzed for six times and %R.S.D. was calculated. % R.S.D was not more than 2%.

3.3.4 Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.3.6. Accuracy (Recovery study) (n=3):

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50 %, 100 %, 150 % and the values were measured for Aripiprazole (10 µg/mL) and Paliperidone hydrochloride (3.0 µg/mL). This performance was done in triplicate. The accuracy of the method was determined by calculating recovery of Aripiprazole and Paliperidone hydrochloride by the standard addition method.

3.3.7. Assay as analysis of Synthetic Mixture:

The synthetic mixture of Aripiprazole and Paliperidone hydrochloride were prepared in the ratio of 10:3. Common excipients such as Micro crystalline cellulose (13 mg), Lactose (10 mg), Talc (6 mg), Magnesium stearate (4 mg), and Cross carmellose sodium (4 mg) were added in the motor pestle along with the drug Aripiprazole (10 mg) and Paliperidone hydrochloride (3 mg). Powder weight equivalent to 10 mg of Aripiprazole and 3 mg of Paliperidone hydrochloride was taken and transferred it in a 100 ml volumetric flask and make up to half mark with methanol. This solution is sonicated till the drug dissolves and was made up to the mark with methanol. This solution was filtered through whatmann filter paper. The mixture contains 100 µg/ml of Aripiprazole and 30 µg/ml of Paliperidone hydrochloride.

3.3.7.1 Preparation of sample solution:

Accurately 1 ml of the above mixture solution of Aripiprazole (100 µg/ml) and Paliperidone hydrochloride (30 µg/ml) was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Aripiprazole was 10 µg/ml and Paliperidone hydrochloride 3.0 µg/ml, then analysed using the previously described UV-spectrophotometric and chromatographic conditions. The concentrations of ARI and PALI were calculated using a regression equation.

3.3.8. Robustness:

The robustness of analytical methods becomes evaluated to decide their ability to face up to minor variations in approach situations. For the HPLC technique, samples have been subjected to evaluation below changed situations, which include adjustments inside the flow rate (± 0.1 mL/min), detection wavelength (± 2 nm), and natural content material (± 2 %) inside the mobile segment. The resulting results on machine suitability parameters have been intently monitored. In the times of Methods I and II, distinct analysts conducted sample analyses to evaluate the robustness of the strategies.

3.3.9 System Suitability Tests:

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

4. RESULTS & DISCUSSION:

4.1 UV SPECTROPHOTOMETRIC METHOD:

The first-order derivative UV spectrophotometric method offers superior selectivity, reduced spectral interference, and improved resolution compared with conventional zero-order UV methods, making it particularly advantageous for simultaneous and routine analysis of pharmaceutical formulations.

4.1.1 Selection of wavelength for Aripiprazole and Paliperidone hydrochloride:

The remarkable absorbance of Aripiprazole exhibited an absorption maximum at 221 nm, while Paliperidone hydrochloride showed an absorption maximum at 239 nm (Figure 1). To determine the wavelength for measurement, Aripiprazole (10 µg/ml) and Paliperidone hydrochloride (3 µg/ml) solutions were scanned between 200-400 nm. Absorbance maximum was obtained at their λmax 221 nm and 239 nm for measurement of Aripiprazole and Paliperidone hydrochloride, respectively. Overlain UV Spectra of Aripiprazole (10 µg/ml) and Paliperidone hydrochloride (3 µg/ml) in Methanol (Zero order) showed in Figure 1.

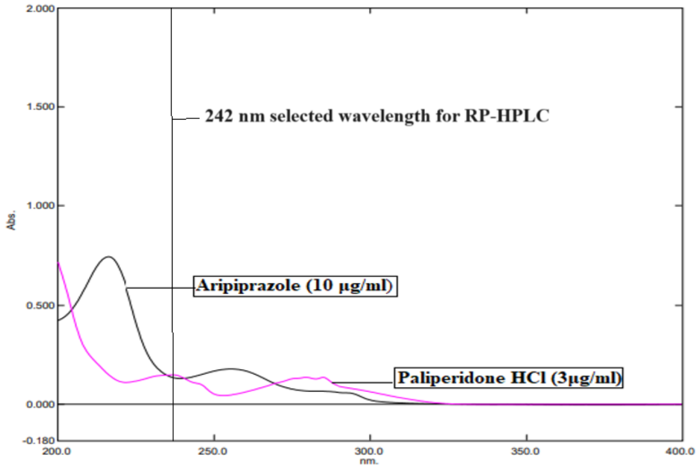

Figure 1: Overlain UV Spectra of Aripiprazole (10 µg/ml) and Paliperidone HCl (3 µg/ml) in Methanol

4.1.2 First order derivative UV Method Development:

The ARI and PALI overlapping absorption throughout the 200 - 400 nm range is shown by these spectra, which makes it more difficult to quantify the pharmaceuticals using traditional UV spectrophotometry without accounting for the overlap. The sum of the absorbances of the two compounds may be used to calculate the overall absorbance of a solution containing a combination of both at a certain wavelength. In situations where the levels of the two medicinal drugs overlap, the method entails figuring out the quantity of each drug using their zero-order spectra. The resulting absorbance spectra were derived to eliminate the interference of absorbing species. The first derivative corresponding to each absorption spectrum of each drug was recorded, using $\Delta\lambda = 2$ nm and scaling factor 4. The amplitude values were measured at 221 nm ($\lambda 1$) (ZCP of PALI) for ARI and 239 ($\lambda 2$) (ZCP of ARI) for PALI showed in (Figure 2). To determine the wavelength for measurement, Aripiprazole (10 µg/ml) and Paliperidone hydrochloride (3 µg/ml) solutions were scanned between 200-400 nm.

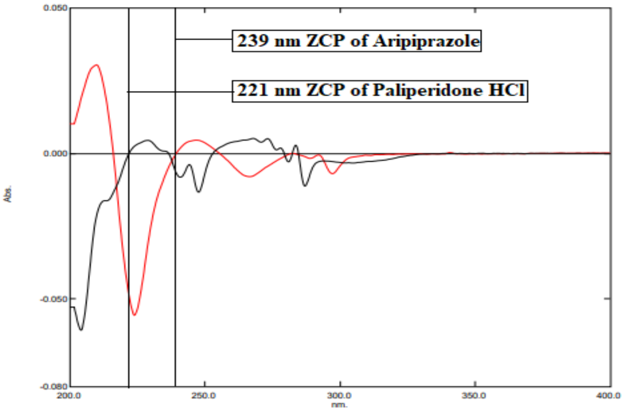

Figure 2: Overlain UV Spectra of Aripiprazole (10 µg/ml) and Paliperidone hydrochloride (3 µg/ml) in Methanol (First Order)

4.2 RP-HPLC METHOD:

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also performs better in terms of efficiency, stability and reproducibility. C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds. In addition to this UV detector is used which allows easy detection of the compounds in UV transparent organic solvents. Hence, C_{18} (250×4.6 mm) column of 5 µm particle packing was selected for separation of Aripiprazole and Paliperidone hydrochloride. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

4.2.1 Selection detection wavelength:

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 242 nm both drugs give good peak height and shape. So, 242 nm was selected for simultaneous estimation of Aripiprazole and Paliperidone hydrochloride in synthetic mixture. Overlain spectra of Aripiprazole (10 µg/ml) and Paliperidone hydrochloride (3 µg/ml) in Methanol has been shown in (figure 1).

Various mobile phases, such as Methanol: Water, Acetonitrile: Water, ACN: Buffer, Methanol: Buffer in different proportion was tried. The combination of ACN: Phosphate Buffer (pH 3): Methanol (65:25:10 % v/v/v) provided optimum polarity for proper migration, separation and resolution of Aripiprazole and Paliperidone hydrochloride. Under these conditions, the eluted peaks were well defined and resolved. The mobile phase ACN: Phosphate Buffer (pH 3): Methanol (65:25:10 % v/v/v) was selected because it was found to ideally resolve the peaks with retention time 2.5 min and 5 min for Aripiprazole and Paliperidone hydrochloride, respectively. Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for separation of Aripiprazole and Paliperidone hydrochloride with Flow rate of 1.0 ml/min.

4.3 VALIDATION PARAMETERS OF THE UV METHOD:

4.3.1 Linearity and range:

For ARI and PALI, the absorbances ranged from 5-25 µg/mL at 221 nm and 1.5-7.5 µg/mL at 239 nm showed in Figure 3 and 4, respectively.

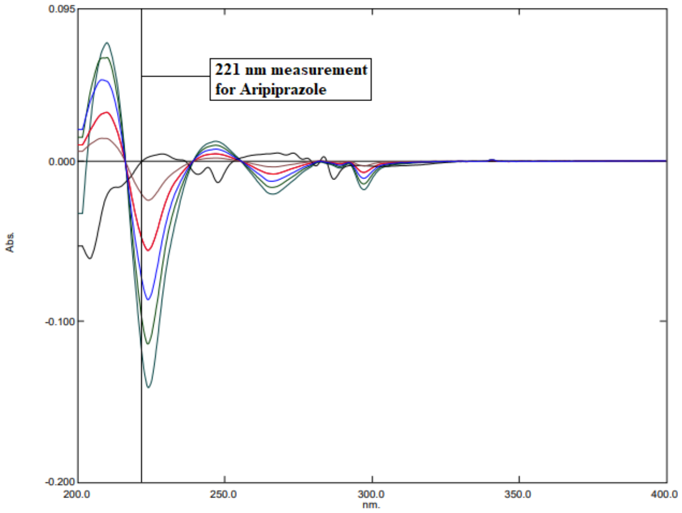

Figure 3: Overlain UV Spectra of Aripiprazole (5-25 µg/ml) at 221 nm

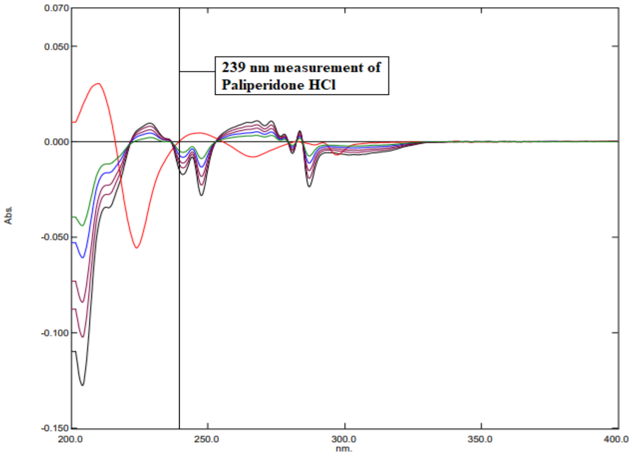

Figure 4: Overlain UV Spectra of Paliperidone hydrochloride (1.5-7.5 µg/ml) at 239 nm

The linearity range for Aripiprazole and Paliperidone hydrochloride were found to be 5-25 µg/ml and 1.5-7.5 µg/ml, respectively. A linear relationship was found and calibration curve was plotted for concentration vs. absorbance. For ARI, the calibration curve equation y = 0.0051x + 0.0069, while for PALI, it was y = 0.002x + 0.002. Results showed that the correlation coefficient (R^2) was between 0.9969 and 1. (Table 1)

Table 1: Linearity data of ARI and PALI for UV Spectrophotometry & HPLC Method

Parameters	**UV Spectrophotometry**		**HPLC**	
	ARI at 221 nm	**PALI at 239 nm**	**ARI at 242 nm**	**PALI at 242 nm**
Linearity Range	5-25 µg/mL	1.5-7.5µg/mL	5-25 µg/mL	1.5-7.5µg/mL
Regression Equation	y = 0.0051x + 0.0069	y = 0.002x + 0.002	y = 72.48x − 12.10	y = 172.85x + 118.30
Correlation Coefficient	0.9969	1	0.9992	0.9997
LOD	0.22	0.18	0.066	0.042
LOQ	0.67	0.55	0.201	0.127

4.3.2 Precision

In terms of precision, both Inter-day, Intraday and Repeatability measurements were conducted at three distinct concentrations 5, 10 & 15 µg/mL for ARI and 1.5, 3.0 & 4.5 µg/mL for PALI in triplicate over three consecutive days and on the same day. The absorbance of the same solutions was measured. For repeatability, 10 µg/mL for ARI and 3.0 µg/mL for PALI were measured. The resulting RSD values for Intraday, Inter-day precision, and Repeatability were showed in Table 2, respectively.

Table 2: Precision study of ARI & PALI for UV Spectrophotometry & HPLC

Sr. No.	Parameters	UV Spectrophotometry		HPLC	
		Aripiprazole	**Paliperidone hydrochloride**	**Aripiprazole**	**Paliperidone hydrochloride**
1	Intraday Precision (%RSD, n=3)	0.89-1.50	1.00-1.20	0.33-0.65	0.42-0.68
2	Interday Precision (% RSD, n=3)	0.92-1.54	1.09-1.28	0.37-1.35	0.48-0.71
3	Repeatability (% RSD, n=6)	0.95	1.11	0.83	0.56

4.3.3 Accuracy:

To decide the accuracy of the technique recuperation, change into accomplished by means of standard addition approach. To pre-analysed pattern acknowledged quantity of general ARI and PALI spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of ARI and PALI. Accuracy was carried out by the Recovery Studies (standard addition method). The results were stipulated in triplicate and the accuracy indicated by % recovery. For UV, The % Recovery was obtained in range of 99.50%-99.73% for Aripiprazole and 98.88%-99.86% for Paliperidone hydrochloride. For HPLC, 99.90%-99.96% for Aripiprazole and 99.77%-99.86% for Paliperidone hydrochloride were showed in Table 3.

Table 3: Recovery study for UV Spectrophotometry & HPLC Method

UV method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Total Std Amt (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Mean Recovery ± SD(n=3)**
Aripiprazole	50	10	5	15	14.93	99.55±1.01
	100	10	10	20	19.90	99.50±1.00
	150	10	15	25	24.93	99.73±0.61
Paliperidone hydrochloride	50	3	1.5	4.5	4.49	99.77±0.44
	100	3	3.0	6.0	6.00	98.88±1.66
	150	3	4.5	7.5	7.49	99.86±0.26
RP-HPLC Method						
Aripiprazole	50	10	5	15	14.99	99.94±0.0270
	100	10	10	20	19.98	99.90±0.0115
	150	10	15	25	24.99	99.96±0.0152
Paliperidone hydrochloride	50	3	1.5	4.5	4.49	99.77±0.0185
	100	3	3.0	6.0	5.99	99.83±0.0380
	150	3	4.5	7.5	7.49	99.86±0.0230

4.3.4 LOD and LOQ:

The minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 0.22 µg/mL for ARI at 221nm and 0.18 µg/mL for PALI at 239 nm, the quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured which was found to be 0.67µg/mL for ARI and 0.55 µg/mL for PALI both 242nm (Table 1). The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs.

4.3.5 Assay as Analysis of Synthetic mixture:

From assay, Final concentration of Aripiprazole was 10 µg/mL and Paliperidone hydrochloride 3 µg/mL were run into UV and The Percentage assay of Aripiprazole and Paliperidone hydrochloride were found to be 99.83% and 99.33%, respectively. Its results showed in Table 4.

Table 4: Analysis of synthetic mixture for UV Spectrophotometry & HPLC Method

UV- Method				
Name of Drug	**Amount in synthetic mixture (µg/ml)**	**Mean Amount found (µg/ml)**	**% Assay ± SD (n=3)**	**%RSD**
Aripiprazole	10	9.98	99.83 ± 0.05	0.057
Paliperidone hydrochloride	3	2.98	99.33 ± 0.33	0.335
RP-HPLC Method				
Aripiprazole	10	10.01	100.1±0.038	0.0379
Paliperidone hydrochloride	3	2.99	99.66±0.056	0.056

4.4. VALIDATION PARAMETERS OF RP-HPLC METHOD:

4.4.1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. It was proved by comparing the chromatogram of mobile phase, test preparation solution to show that there was no interference of mobile phase and excipients peaks with peak of Aripiprazole and Paliperidone hydrochloride.

4.4.2. Linearity

The RP-HPLC chromatogram of (5-25 µg/ml) Aripiprazole and Paliperidone hydrochloride (1.5-7.5 µg/ml) at 242 nm. The Peak Area was found. Linearity data was showed in table 1. Calibration curves were plotted between concentrations and peak areas. The regression equation of calibration curve was generated $y = 72.48x - 12.10$ for ARI and $y = 172.85x + 118.30$ for PALI. The correlation coefficient (R^2) values were observed to be 0.9992 and 0.9997 (Table 1).

4.4.3. Precision

A concentration of 5, 10 and 15 µg/mL for Aripiprazole and 1.5, 3.0 and 4.5 µg/mL for Paliperidone hydrochloride. At same day three-time interval, the absorbance of the finished solution was measured in a 1.0 cm cell at a chosen wavelength. Likewise, on the first, second, and third days, the absorbance of the same solutions was measured. Every solution is made in triplicate and examined. The resulting RSD values for Inter-day and Intraday precision were show in table 2, respectively.

4.4.4. Accuracy

To decide the accuracy of the technique recuperation, change into accomplished by means of standard addition approach. To pre-analysed pattern acknowledged quantity of general Aripiprazole and Paliperidone hydrochloride spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of fashionable Aripiprazole and Paliperidone hydrochloride. The results were studied in triplicate and the accuracy changed into indicated by % recovery.

4.4.5. LOD and LOQ

LOD Values were found to be 0.066 and 0.042 µg/mL for Aripiprazole and for Paliperidone hydrochloride respectively. LOQ Values were found to be 0.201 and 0.127 µg/mL, respectively for Aripiprazole and for Paliperidone hydrochloride. These results showed in Table 1.

4.4.6. Assay

From assay, Final concentration of Aripiprazole was 10 µg/mL and Paliperidone hydrochloride 3 µg/mL were injected into HPLC System and The Percentage assay of Aripiprazole and for Paliperidone hydrochloride were found to be 100.1% and 99.66 %, respectively. Results showed in Table 1.

4.4.7. Robustness

Chromatographic analysis was used to analyse the effects of changes in analysts, and the results showed that there was no statistically significant difference in the% RSD. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches' robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, mobile phase composition, and detection wavelength. It was determined that the created approaches were essential as a result.

5. CONCLUSION:

The present work successfully demonstrates the development and validation of first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Aripiprazole and Paliperidone hydrochloride in a synthetic mixture. Both methods were validated as per ICH Q2 (R2) guidelines and exhibited excellent linearity, accuracy, precision, and sensitivity. The UV method provides a cost-effective and rapid alternative for routine analysis, while the RP-HPLC method offers enhanced specificity and robustness suitable for advanced quality control laboratories. All Developed and Validated Methods were found to be Accurate, Economical, Reproducible and Precise. There was no interference of any Excipients in the determination of Drugs from Synthetic Mixture. So, this Method can be applied for routine Quality Analysis.

ACKNOWLEDGEMENT:

The authors are grateful to Smt. N. M. Padalia Pharmacy College, Ahmedabad, for encouragement and for providing the necessary facilities to carry out this research work.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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