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Simultaneous determiantion of aripiprazole and escitalopram oxalate by HPLC

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Abstract: Simple, accurate, precise, and sensitive reverse-phase high-performance liquid chromatographic (HPLC) method was developed for the simultaneous measurement of ARI and ESC in pharmaceutical products by using a C18 column with dimensions of 250 mm x 4.6 mm and a particle size of 5 µm. The mobile phase consisted of a mixture of acetonitrile, methanol, and water in the ratio of 80:05:15 v/v/v. Orthophosphoric acid was added to adjust the pH to 7.00 ± 0.1. The detection wavelength used for measuring the absorbance of the components was 246 nm, and the Mobile Phase flow rate was set at 1.0 mL/min. The retention times of ARI and ESC were 5.63 and 3.65 minutes, respectively. The ARI and ESC correlation coefficients (R^2-values) were observed at 0.9995 and 0.9997, respectively, demonstrating a robust linear relationship between the analyte concentration and the observed absorbance. The linearity range for ARI was 20 to 120 µg/mL, while ESC was 60 to 360 µg/mL. The recovery percentages for ARI and ESC ranged from 99.43% to 100.65% and 99.58% to 100.15%, respectively. Additionally, all parameter uncertainty levels were below 2. The method was validated following ICH guideline Q2 (R1). The analytical method validated will be successfully applied to the pharmaceutical dosage form.

Keywords: Aripiparzaol; Escitalopram; HPLC; method validation.

1. Introduction

The class of benzisoxazole derivatives includes the hallucinogenic substance Aripiprazole (ARI) for treating schizophrenia. The serotonin type 2 (5HT2), dopamine type 2 (D2), H1 histaminergic receptor, and the 1 and 2 adrenergic receptors are all highly affine for the selective monoaminergic antagonist ARI. On other receptors, ARI has a less potent antagonistic action. The therapeutic and unfavorable effects of ARI's hostile activity at histamine H1 receptors may account for some other receptors with similar receptor affinities to dopamine and 5HT2 and the somnolence experienced with this medicine. The antagonistic actions of ARI on adrenergic a1 receptors may account for the orthostatic hypotension observed with this treatment. ARI's antipsychotic activity is likely due to a combination of

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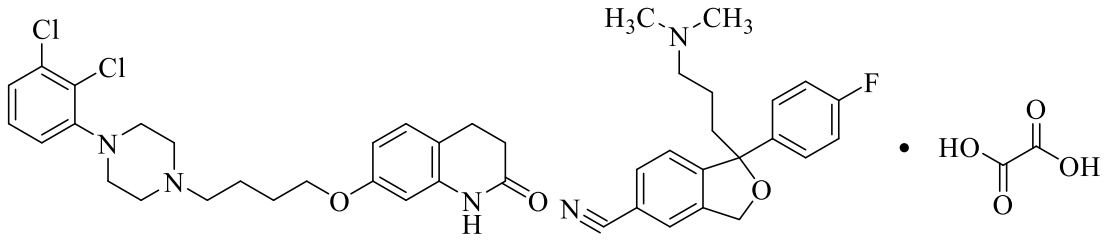
antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Antagonism at D2 receptors relieves positive symptoms, while antagonism at 5HT2A receptors relieves negative symptoms of schizophrenia [1-5].

Escitalopram Oxalate (ESC) is a drug belonging to the antidepressant class used as a selective serotonin reuptake inhibitor (SSRIs), which are chemically diverse and have 300–3000 times greater selectivity for the serotonin transporter than the norepinephrine transporter. The SSRIs prevent serotonin from being reabsorbed, which raises the concentration of neurotransmitters in the synaptic cleft and, eventually, increases postsynaptic neuronal activity [5-8].



Aripiprazole Escitalopram oxalate

Figure 1. Chemical structures of the compounds of Aripiprazole (**1**) and Escitalopram (**2**)

ARI and ESC combination was approved on 24th June 2014 by FDA. This combination is patented by Otsuka Pharmaceutical Corporation, US Patent No: US8759350 B2 [9] at the same year of FDA approval. ARI is a novel atypical antipsychotic agent, and ESC is a selective serotonin reuptake inhibitor. Both drugs have frequently been used to treat major depression disorders in clinical traits [10]. Numerous methods have been reported for the quantitative assessment of ARI and ESC separately or when used with other medications. Most of the cited approaches involve high-performance liquid chromatography (HPLC) [11-28], employed to measure the quantities of ARI and ESC in concentrated samples, biological fluids, and pharmaceutical products. Nevertheless, it is worth noting that no HPLC procedure has been disclosed for the simultaneous determination of ARI and ESC within any mixture. Therefore, this study aimed to develop an HPLC method for the simultaneous analysis of AR1 and ESC in any matrix and validate it according to ICH Guide [11].

2. Experimental

2.1. Materials and Methods

The raw materials of ARI (99.5 %) and ESC (99.7 %) were procured as gift samples from Cadila Healthcare LTD, Ankleshwar. Methanol HPLC grade (Finar), HPLC grade water, Acetonitrile, Potassium dihydrogen Phosphate (Rankem), and phosphoric acid AR Grade (Astron) were used for development purposes.

2.2. Synthetic Mixture

A standard mixture solution of ARI: ESC (40:120 µg/mL) was made by adding 0.4 ml of ARI standard solution (1000 µg/mL) and 1.2 mL of ESC common solution (1000 µg/mL) to a classic 10 mL volumetric flask and diluting up to the mark with acetonitrile.

Table 1. Synthetic mixture composition from Patent [9]

Sr. No	Name of excipient	Quantity (mg)
1	ARI	5.0
2	Escitalopram	10.0
3	Starch	131.0
4	Magnesium Stearate	4.0
5	Lactose	100.0 qs
	Total	250

2.3. Instrumentation and Chromatographic Conditions

Chromatographic analysis was conducted utilizing a semi-automatic liquid chromatograph, Model-SPD 10 A-LC 10 AT (Shimadzu, Japan), pump-single pump systems, and UV-VIS Detector with Software-Win Chrome., an injector used in a microliter syringe (Hamilton Bonaduz AG, Switzerland). Weighing was accomplished using a semi-micro analytical balance (Germany's Sartorius CD2250). The pH measurements were performed using a tutor (313927, Eutech Instruments) and a magnetic stirrer (Remi) for mixing. Nylon membrane filters (0.22 µm, 47 mm D) were used for filtration after the solutions had been sonicated using an ultrasonic cleaner (D 120/1H, Trans-O-Sonic).

2.4. Preparation of Solutions

2.4.1. Preparation of Mobile Phase

The Mobile phase composition was composed of Acetonitrile:Methanol: Water (80:05:15 V/V/V), and pH adjusted to 7.0 with orthophosphoric acid was used. Isocratic elution mode was employed. Before use, the mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed.

2.4.2. Preparation Stock Solution

Transfer accurately weighs 10 mg of ARI and 10 mg of ESC into two separate 10 mL of a volumetric flask. Thus, for ARI and ESC, the concentrations are set at 1000 µg/mL for each substance. Following agitation for dissolution, the solution was shaken, and then acetonitrile was added to each volumetric flask to achieve the intended volume.

2.5. Method Validation

HPLC method validation was performed according to the International Council for Harmonization of technical guidelinesQ2 (R1).

2.5.1. Linearity and Range

The linearity of the method was evaluated by employing five different concentration levels ranging from 20-120 µg/mL for aripiprazole and 60-360 µg/mL for escitalopram oxalate (n=6). For every concentration, 20 µL injections were administered at a 1 mL/min flow rate, and the UV scan at 246 nm was applied to the resulting effluent. The process involved recording peak areas and creating calibration curves to establish a correlation between the integrated peak area and the respective concentration levels.

2.5.2 Accuracy

The accuracy of the method was evaluated using the standard spiking technique, with recovery studies conducted at three distinct levels: 80%, 100%, and 120%. The recovery percentages for ARI and ESC were computed using their respective regression equations, and subsequently, the mean recoveries and standard deviation values for both substances has been calculated. Moreover, a standard addition technique has been performed to confirm the accuracy of the developed method.

2.5.3. Precision

The precision of the method was demonstrated through investigations of interday and intraday variations. For the intraday studies, three injections of standard solutions with varying concentrations were conducted, and the response factors of the drug peaks as well as the percentage relative standard deviation (RSD) were computed. In the case of between-day variation studies, three sets of injections involving standard solutions with different concentrations were administered over three consecutive days, with the response factor of drug peaks and the percentage RSD calculated accordingly.

2.5.4. Specificity

The develop method that was employed to evaluate the specificity of a mixture containing ARI and ESC that had been prepared in the laboratory. Furthermore, the specificity was confirmed by evaluating system suitability parameters, including capacity, resolution, and selectivity factors for the distinct chromatographic peak.

2.5.5 Sensitivity

LOQ and LOD are the sensitivity of the proposed method. By using a linear regression model, these parameters were calculated from a standard deviation of the intercept of calibration curves and the mean slope values. A series of concentrations of drug solution and its impurities were injected; LOD and LOQ were established by the slope method as mentioned below.

$$LOQ = 10 \times \text{Standard deviation of the response / Slope of mean}$$
$$LOD = 3.3 \times \text{Standard deviation of the response / Slope of mean}$$

2.5.6. Robustness and Ruggedness

Robustness was determined using a minor modification to the HPLC conditions. By Varying the flow rate, Mobile Phase Composition, pH changes in wavelength, and robustness of the method were evaluated, and the percentage RSD for retention time was calculated. Ruggedness: The significant Variations in HPLC conditions were implemented to assess the ruggedness. This method was determined by change in analyst and change in HPLC columns.

2.5.7. System Suitability Testing Parameters

An overall system suitability testing was done to determine if the operating system was performed properly. Parameters such as resolution, tailing factor and theoretical plates were determined. Various factors, including resolution, tailing aspect, and theoretical plates, were investigated and measured as part of this analysis.

2.5.8. Chromatographic Separation

Both standard and sample solutions were introduced into the column. The chromatogram was conducted for a suitable duration using a degassed mobile phase, a mixture of Acetonitrile: Methanol: Water (80:05:15 v/v/v), and a UV detector set at a wavelength of 246 nm. The chromatogram was terminated upon achieving complete separation. Parameters such as peak area, height, retention time, and resolution were captured using Win Chrome software.

2.6. Analysis of Synthetic Mixture

The levels of ARI and ESC within a synthetic mixture were assessed and compared them to established standards for ARI and ESC. The assessment outcomes for a synthetic variety containing ARI (40µg/mL) and ESC (120µg/mL) are displayed in Table 1. The percentage analysis demonstrates the absence of additive disruptions, indicating the proposed technique's effectiveness for assessing commercial formulations containing ARI and ESC. This chromatographic approach has been validated for the simultaneous measurement of ARI and ESC.

3. Result and Discussion

3.1. Method Development and Optimization

Various combinations of solvents with varying proportions and pH values were employed and are outlined in Table 3. The optimal polarity required for adequate movement, differentiation, and clear distinction between ARI and ESC peaks was achieved using a blend of acetonitrile, Methanol, and Water in a ratio of (80:05:15 v/v/v). This specific composition resulted in well-defined, separate, and symmetrical peaks upon elution, with no tailing observed. As the stationary phase is primarily non-polar, the more polar ESC component has eluted first, driven by its higher affinity for the opposite mobile phase. Conversely, the less polar ARI component was eluted later due to its stronger attraction to the non-polar stationary phase. The chromatograms of different mobile phase trials have been included as evidence of the experimental efforts undertaken during method development.

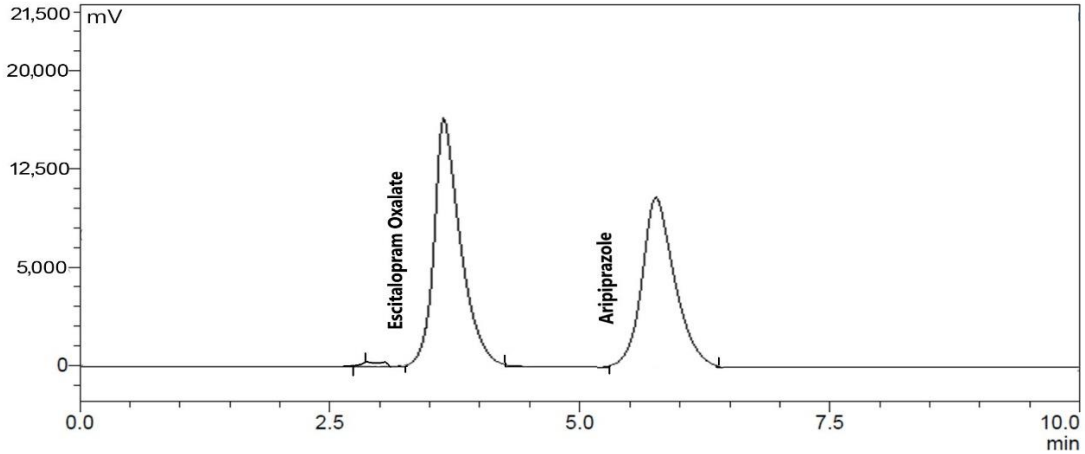


Figure 3. Chromatogram of ARI and ESC in ratio of 40:120 µg/mL

3.2 Method Validation

3.2.1 Linearity and Range

The RP-HPLC method demonstrated linear behavior over concentration ranges of 20-120 µg/mL for ARI and 60-360 µg/mL for ESC. The equations representing the regression relationships can be found in Table 2. Furthermore, Figure 4 shows an HPLC chromatogram 2D overlay, displaying the elution profiles of ARI (20-120 µg/mL) and ESC (60-360 µg/mL).

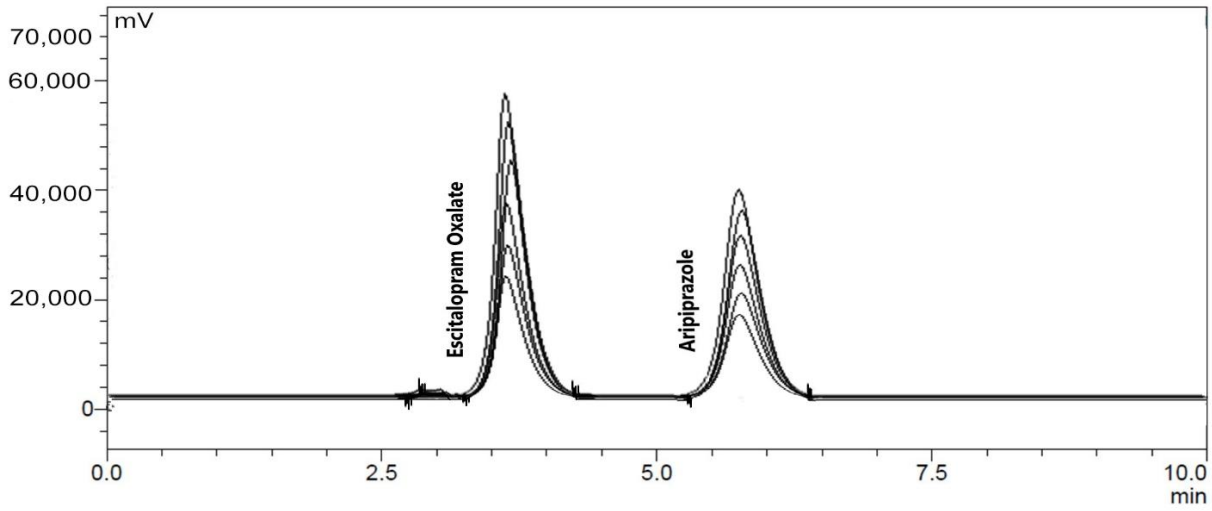


Figure 4. 2D Overlain Chromatogram for six concentration of ARI (20-120 µg/mL) and ESC (60-360 µg/mL)

Table 2. Calibration data for ARI and ESC *(*n*=5)

Sr. No	Concentration (µg/mL)		Peak Area* ± SD ARI	%RSD ARI	Peak Area* ± SDESC	%RSD ESC
	ARI	ESC				
1	20	60	162631±1452	0.893	130607±1096	0.839
2	40	120	266761±1408	0.528	249763±1534	0.614
3	60	180	373408±2027	0.543	356191±1932	0.542
4	80	240	467821±3397	0.726	458445±1640	0.357
5	100	300	562089±1888	0.335	562873±5201	0.924
6	120	360	656737±1532	0.233	671223±5135	0.765

Table 3. Chromatographic conditions of the applied method

Parameter	Condition
Mobile Phase	: Acetonitrile: Methanol: Water (80:05:15v/v/v)
pH	:7.0 by Orthophosphoric acid
Diluent	: Acetonitrile
Column	:C_{18} ,250 mm x 4.6 mm, 5µm
Wavelength	:246 nm
Injection Volume	:20µL
Flow rate	:1.0 mL/min.
Run time	:10 min.

3.2.2. Accuracy

The accuracy of the proposed methods was calculated from the corresponding regression equations. Favorable % yields were achieved and are displayed in Table S1. Additionally, it was evaluated using the standard addition method on a synthetic mixture. good recoveries were obtained, revealing the good accuracy of the proposed methods and proving that excipients did not interfere.

3.2.3. Precision

The proposed method provides acceptable intra- and Interday variation, indicating the good precision of the method and revealing that it is suitable for the quality control of the suggested components. The Relative Standard Deviation (% RSD) percentage remained below 2.0%, indicating the method's precision. Table S2 validates the consistency, and the developed techniques exhibited satisfactory intermediate accuracy.

3.2.4. Specificity

The specificity of the suggested approaches was clearly demonstrated through the HPLC chromatograms depicted in Figures S3 and S4 within the Supporting Information.

Table 4. The example parameters for system suitability test of the method

Parameters	**Observed Values**	
	ARI*	**ESC***
Retention Time(min)	8.11 ± 0.0456	4.23±0.0236
Peak Area	689554	866727
Theoretical plates	3706 ± 8.36	3646 ±9.23
Tailing Factor	1.432 ±0.065	1.561± 0.0356
Resolution	-	9.352 ± 0.0523

3.2.5. Sensitivity

The suggested lower limits of detection (LOD) and quantification (LOQ), which are displayed in Table S4, are proof of the increased sensitivity attained by the developed techniques. These results further show the methods meet the established criteria for detection limits.

3.2.6. Robustness

This assessment was conducted under various chromatographic conditions, with the evaluation focused on the Rt value of the band. The outcomes of the robustness investigations are presented below in Table 5.

3.3. Assay

The simultaneous estimation of ARI and ESC in a synthetic mixture was done using the suggested method. The assay result values are shown in Table 5. The % assay for ARI and ESC was 100.2 and 100.15 with a % RSD of 0.37 and 0.25, respectively.

Table 5. Analysis Data of Synthetic Mixture *(*n*=3).

Sr. No	Formulation (Synthetic Mixture)		% Assay* ARI ± SD	%RSD	% Assay* ESC ± SD	%RSD
	ARI	ESC				
1	40	120	100.2 ± 0.377	0.370	100.15 ± 0.0253	0.250

3.4 Uncertainty Estimation

Measurement uncertainty is a crucial aspect in all analytical procedures, as it directly impacts the reliability and accuracy of the measured outcome and quantified result. Uncertainty stems from diverse sources such as sampling, solution preparation, experimental parameters, instrumentation, calibration, and other factors contingent upon the sample type. These various influences can collectively introduce uncertainties into the outcome, revealing the potential magnitude of errors. Thus, it becomes imperative to integrate uncertainty analysis alongside the reported findings. In the context of the present methods, the principal contributors to uncertainty were standard preparation, calibration curve slope, sample recovery, and repeatability. These factors were evaluated following guidelines from sources such as the EURACHEM guide, GUM documents, and previously documented methodologies [27-32]. The cumulative uncertainty was determined using the equation (1).

$$\textit{Combined uncertainty (U)} = \sqrt{(\text{ustd})^2 + (\text{ucal})^2 + (\text{urec})^2 + (\text{urep})^2} \quad (1)$$

3.4.1 Uncertainty in the Standard Sample (ustd)

According to the supplier's certificate, the purity of ARI is reported as 99.5%. The provided uncertainty is considered to follow a rectangular (uniform) distribution, resulting in a relative standard delay, u_{std}, that is equal to $\frac{100-99.7}{\sqrt{3}}$ = 0.173 %.

As indicated in the supplier's certificate, ESC's purity is reported 99.7%. The uncertainty provided is treated as a rectangular (uniform) distribution, resulting in an equal relative standard uncertainty, u_{std} $\frac{100-99.6}{\sqrt{3}}$ = 0.231%

3.4.2. Uncertainty in the Slope Of calibration Curve plot (u_{cal})

A calibration graph was generated using spreadsheet software to calculate the slope and standard error. The corresponding data is presented in Table 6. The uncertainty linked to the calibration curve was assessed using the provided equation.

$$u_{cal} = \frac{\text{standard error of slope x } 100}{\text{slope}}$$

Table 6. Data of slope of the calibration curve

	ARI	ESC
Slope	4929	1783
Standard Error	103.55	35.48
u_{cal}	2.101	1.990

3.4.3. Uncertainty in Recovery of Sample (u_{rec})

Sample recovery uncertainty was assessed based on the average recovery from the accuracy data. Comprehensive recovery outcomes are available in Table 9.

Table 7. Data of sample recovery

	ARI	**ESC**
Mean Recovery	99.60	100.6
u_{rec}	0.376	0.252

3.4.4. Uncertainty Associated with Repeatability (u_{rep})

Repeatability uncertainty was determined by examining the relative standard deviation of repeatability data for ARI and ESC. The relevant data points are furnished in Table 7.

3.4.5. Expanded Uncertainty

Using equation 1, the total uncertainty was computed, followed by determining the expanded uncertainty at a 95% confidence level. This was achieved by multiplying the combined uncertainty with the coverage factor (k=2). The corresponding values for combined and expanded uncertainty are detailed in Table 8.

Table 8. Data of combined and Expanded uncertainty

Analyte	u_{std}	u_{cal}	u_{rec}	u_{rep}	**Combined uncertainty**	**Expanded uncertainty**
ARI	0.173	2.101	0.376	0.642	2.235	4.471
ESC	0.283	1.990	0.252	0.736	2.156	4.312

4. Conclusion

The outcomes show that the suggested method displayed sensitivity, accuracy, precision, and repeatability when ARI and ESC Synthetic Mixture were evaluated simultaneously. The ICH guideline Q2 (R1) validation determined this methodology's suitability for routine estimation. According to the results, this method can be applied to systematic assessment. The HPLC method, which has the advantage of being more repeatable, is the initial method created for the rapid analysis of this binary mixture using a single wavelength.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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