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Green and White Analytical Assessment of Spectrophotometric and Flow Injection Techniques for Quantifying Risperidone in Pharmaceuticals

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Risperidone is an important atypical antipsychotic used to treat schizophrenia, bipolar disorder, and autism-related irritability. It works by blocking the receptors for dopamine and serotonin. Accurate and eco-friendly methods for its determination in pharmaceuticals are essential for quality control. Two rapid and straightforward analytical approaches-spectrophotometric and flow injection analysis (FIA) were developed for the quantitative determination of Risperidone (RSP) in its pure form and in pharmaceutical formulations. Both methods rely on the formation of a purple ion-pair complex between RSP and Alizarin Red S (ARS), exhibiting a maximum absorbance at 534 nm. The calibration curves for Method A (spectrophotometric) and Method B (FIA) were linear within the concentration ranges of 10-125 $\mu g.mL^{-1}$ and 0.078-11.826 $\mu g.mL^{-1}$, respectively, adhering to Beer's law. The calculated limits of detection (LOD) and limits of quantification (LOQ) were 4.07 $\mu g.mL^{-1}$ and 12.33 $\mu g.mL^{-1}$ for Method A, and 0.037 $\mu g.mL^{-1}$ and 0.112 $\mu g.mL^{-1}$ for Method B. The methods demonstrated high precision with relative standard deviation (RSD) values below 1.25%. The environmental sustainability of the developed methods was assessed using several greenness evaluation tools, including the Analytical Eco-Scale (AES), ChlorTox, and Analytical GREEnness (AGREE) metrics, all of which indicated a strong green profile. Furthermore, White Analytical Chemistry (WAC) principles were evaluated using the RGB12 model, yielding whiteness scores exceeding 85, which confirms the method's balanced analytical performance. The validated procedures were effectively applied to the analysis of RSP in commercial pharmaceutical preparations.

Keywords: Risperidone (RSP), Alizarin Red S (ARS), UV-Vis, Green analytical chemistry, White analytical chemistry

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Risperidone (RSP) is a second-generation atypical antipsychotic medication, chemically known as 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl] ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido- [1,2-a]-pyrimidin-4-one, a member of the benzisoxazole derivative chemical family (Figure 1) [1, 2]. As an inhibitor of serotonin 5-HT2A and dopamine D2 receptors, risperidone is one of the most commonly prescribed antipsychotic drugs (stat pearls) worldwide [3]. RSP works well for treating bipolar disorders, neuroprotective, neuroinflammatory, antipsychotic, anti-manic, and acute and chronic schizophrenia [4]. Numerous analytical methods have been published for the determination of RSP in different matrices based on various techniques, among which are Spectrophotometric [5, 6], Spectrofluorometric [7], HPLC [8], UHPLC [9], Electrochemical [10], UPLC-MS/MS [11], DLLME-LC MS/MS [12], and flow injection analysis [13].

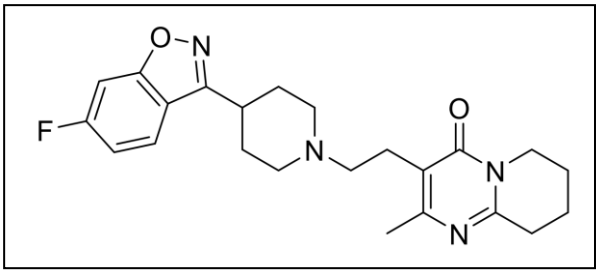

Figure 1. Chemical structure of risperidone (RSP).

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Given the widespread use of risperidone in clinical practice, there is a need to develop simple, rapid, and sensitive analytical methods for its determination in pharmaceutical preparations to ensure quality control and accurate dosage. Two approaches were used for the determination of RSP in its pure form and in pharmaceutical formulations. One of these is the conventional UV-Vis spectrophotometric method, which is widely utilized for quantitative analysis due to its high sensitivity and precision. This technique's success is also attributed to its ease of use, simplicity, and comparatively inexpensive instrument and operation costs [14]. Another approach, flow injection analysis (FIA), was also developed as a method for automated analysis and sample pretreatment. This technique is based on a continuous flow system where the sample, reagent, and carrier stream are continuously mixed and reacted, and then detected [15]. It has been widely used in drug estimation [16-19]. Its high sample throughput, simplicity of automation, accuracy, dependability, and reproducibility, as well as the fact that it uses comparatively fewer chemicals and sample material and is less prone to contamination, are the main factors contributing to its success [20, 21].

Green Analytical Chemistry (GAC) is a developing field that incorporates green chemistry ideas into analytical techniques with the aim of minimizing both environmental harm and human health hazards typically related to chemical analysis. The 12 principles of green chemistry, which offer a comprehensive framework for development and practical, environmentally friendly analytical methods, are the basis of GAC [22]. The widely accepted 12 principles of GAC aim to minimize the use of feedstocks, reagents, and energy, in-line sample collection, and solvent-free extraction techniques. Ultimately, GAC enhances the safety of both analysts and the environment [23]. Several tools and metrics have been developed to assess the greenness of analytical procedures including the National Environmental Methods Index (NEMI) [24], Analytical Method Volume Intensity (AMVI) [25], Green Analytical Procedure Index (GAPI) [26], Analytical Eco-Scale (AES) [27], Analytical GREEnness (AGREE) [28], Environmental Assessment Tool (EAT) [29], and ChlorTox [30], By employing safer solvents, renewable substances, and energy-efficient techniques, this strategy reduces the use of hazardous materials, energy consumption, and waste production, thereby minimizing the environmental effect of chemical processes [31]. However, these tools overlook the practicality of the method—an essential factor routinely encountered in analytical laboratories. This important aspect has been introduced into the White Analytical Chemistry (WAC) concept [32]. Nowak et al. [33] introduced WAC in 2021 as an expansion of the 12 GAC principles in order to overcome its limitations and provide a more comprehensive method for evaluating sustainability in analytical chemistry [34]. The Whiteness Assessment tool, which uses the RGB 12 model, combines the contributions of three different groups: red for analytical efficiency, green for green chemistry principles, and blue for practical aspects to assess the overall "whiteness" of an analytical procedure [35]. This study aimed to develop accurate, selective, precise, greenness, and whiteness procedures for the determination of RSP in pure form and its pharmaceutical preparations by forming a purple ion pair complex with ARS.

EXPERIMENTAL

Apparatus

UV-Vis Spectrophotometer (UV-8000T, Metash Instruments Co., Ltd., China) was used with 1.4 mL quartz cell for all spectrophotometric measurements, ultrasonic digital heating device (MANIKARN type), a digital pH meter (HANNA Bench model) to checking the pH of all prepared buffer solutions, Hotplate Stirrer (DAIHAN) to shake the samples, Analytical balance (Sartorius AG, Germany), Peristaltic pump (Standard Peristaltic Pump located in Lone, Zhangqiu District, Jinan City, Shandong, China) and Injection valve was a six-port medium pressure model (IDEX Corporation, United States).

Materials and Reagents

Risperidone pure sample was obtained from (Al-Kindi Company for Pharmaceutical Industries, Iraq), Alizarin Red S (BDH, England), Ethanol (BDH, England), Commercial Risperdone tablets (2 mg) Risnia-2 (Cipla, India), Respal 2 (JOSWE, Jordan), Ripharm (Pharma international company, Jordan) and Risnia-4 (4 mg) (Cipla, India), Boric acid (BDH, England), Sodium hydroxide (Fluka, Switzerland), Phosphoric acid (BDH, England), Acetic acid (BDH, England), Sodium acetate (Fluka, Switzerland), Nitric acid (BDH, England) and Hydrochloric acid (Fluka, Switzerland).

Stock Solutions

Standard Stock Solution

Risperidone (RSP) standard stock solution was prepared by accurately weighing 0.1 g of RSP (pure form), dissolving it in 20 mL of ethanol, and then diluting to 100 mL with distilled water in a volumetric flask to obtain a final concentration of 1000 $\mu g.mL^{-1}$. Similarly, a standard stock solution of ARS was prepared by dissolving 0.1 g of the reagent in 200 mL of distilled water to give a concentration of 500 $\mu g.mL^{-1}$. Working standard

solutions of both compounds were prepared by appropriate dilution with distilled water.

Commercial Stock Solution

Thirty pills were taken, each weighed individually, and then crushed and ground into a powder. A quantity equal to 0.05 g was taken and dissolved in 20 mL of ethanol, to which 80 mL of distilled water was added in a 100 mL volumetric flask. The flask was left to stir continuously for 30 min and was also placed in an ultrasonic device for 30 min to achieve complete dissolution of the drug. Using a Whatman filter paper No. 1, the solution was filtered.

Procedures

Method A

Different volumes (0.04-0.5 mL) of 1000 µg.mL^{-1} RSP were transferred into test tubes. Then, 1.04 mL of 500 µg.mL^{-1}of ARS was added. The mixtures were diluted to 4 mL with distilled water and then measured at λmax=534 nm in a UV-Vis spectrophotometer.

Method B

The flow injection analysis (FIA) system illustrated in Fig. 2 was designed for the determination of RSP in pharmaceutical formulations. The manifold consists of two channels: the first pumps distilled water, which serves as the carrier stream to transport the injected RSP 471 µL from the injection valve to the Y-junction. Simultaneously, the second channel delivers a 30 µg.mL^{-1} solution of ARS to the same Y-junction. At this point, the reaction occurs between RSP and ARS, forming a purple ion-pair complex. The resulting mixture is then directed toward the detector, which contains a 4 cm reaction cell and a green light source for measurement. Both channels pass through a peristaltic pump at a flow rate of 2.96 mL.min^{-1} to control the flow rate of the system.

Procedure for Commercial Tablets

The drug content in commercial tablets was determined using the prepared solution in paragraph (2.3.2), and the same procedures were applied above for the two methods.

RESULTS AND DISCUSSION

Absorption Spectra and Mechanism

The interaction between RSP and ARS leads to the formation of a purple ion-pair complex, which displays a maximum absorbance at 534 nm in Method A, as illustrated in Fig. 3. In contrast, Method B detects the complex through its peak intensity in the green region of the visible spectrum. In both techniques, the color intensity increases proportionally with RSP concentration, enabling accurate quantitative determination.

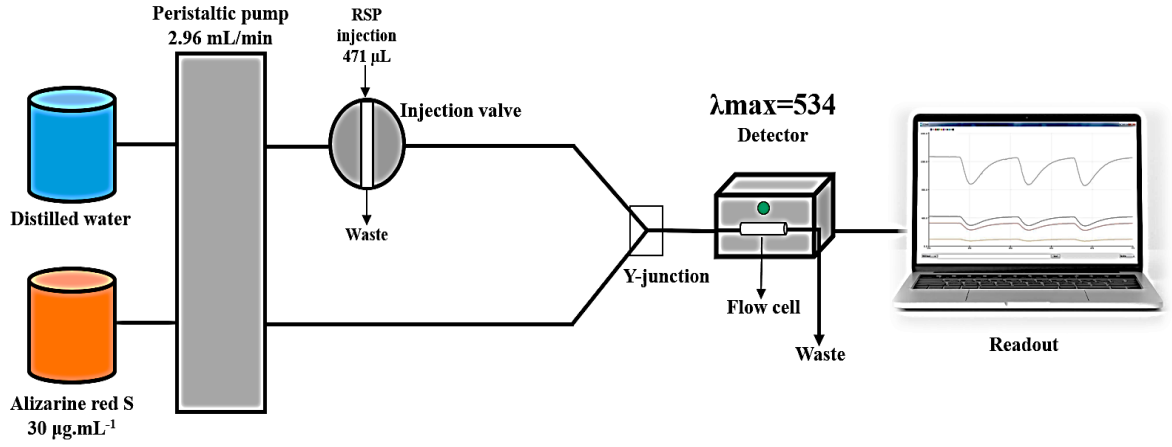

Figure 2. Schematic representation of the Flow Injection Analysis (FIA) manifold.

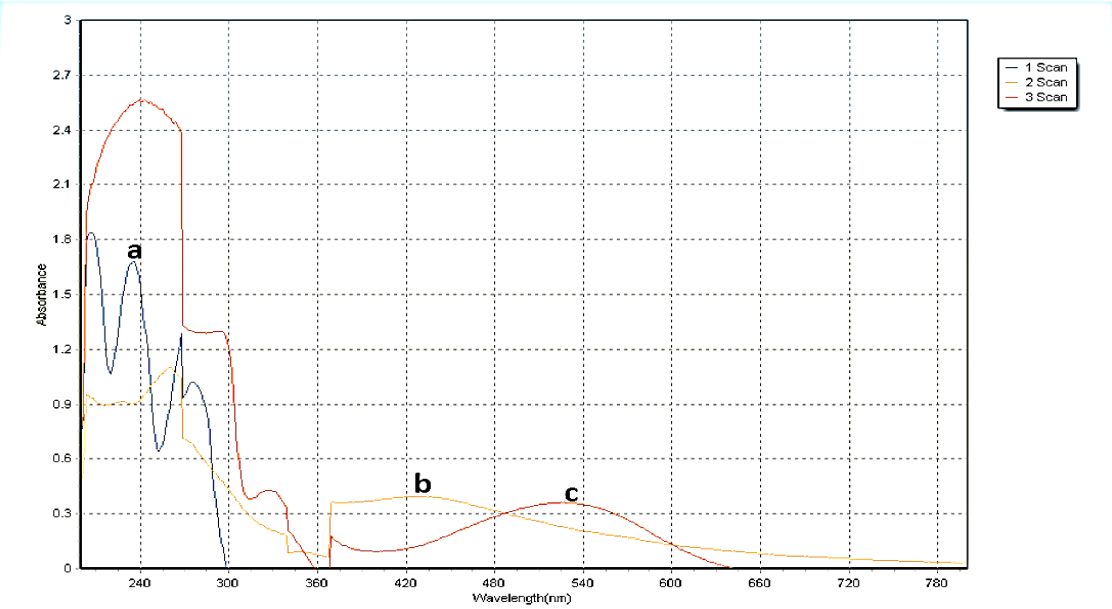

Figure 3. Absorption spectra of (a) Risperidone, (b) Alizarin Red S, and (c) their ion-pair complex, all recorded against distilled water as the blank.

The nitrogenous medication and anionic dyes, like ARS, combine to produce an ion-pair complex [36]. Because RSP is an alkaline medication, it forms a purple ion-pair complex with the anionic dye. Protonation of the benzisoxazole ring and in pyrimidin-4-one is extremely difficult because of the resonance effect. Therefore, the nitrogen in the piperidine ring is the only location that is vulnerable to protonation [5]. In aqueous solution, ARS, a sodium salt of 3,4-dihydroxy-9,10-dioxo-2-anthracene sulfonic acid, ionises and acquires a negative charge [37]. A 1:1 molar ratio between RSP and ARS was confirmed, as shown in Fig. 6. Accordingly, the proposed reaction mechanism is illustrated in Figure 4.

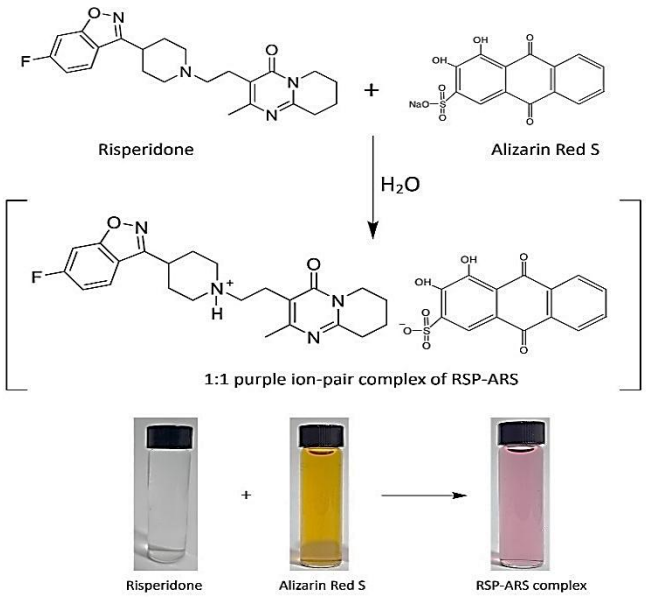

Figure 4. Proposed reaction mechanism illustrating the formation of the purple ion-pair complex between risperidone (RSP) and Alizarin Red S (ARS).

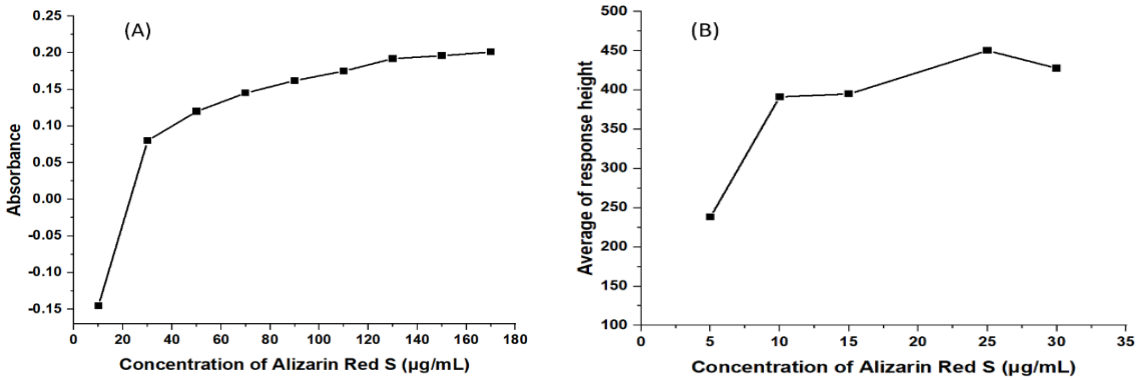

Figure 5. Influence of Alizarin Red S concentration on absorbance in (a) Method A, and (b) Method B.

Methods of Optimizations

Effect of the Alizarine Red S Concentration

In Method A (spectrophotometric analysis), the optimal concentration of ARS was determined by varying its concentration from 10 to 170 µg.mL^{-1}, while maintaining a fixed concentration of RSP at 25 µg.mL^{-1}. The results indicated that maximum absorbance was achieved at 130 µg.mL^{-1} of ARS, beyond which no significant increase in absorbance was observed, suggesting the completion of the complexation reaction. These findings are illustrated in Figure 5-a.

Similarly, in Method B (Flow Injection Analysis, FIA), the effect of ARS concentration was evaluated over the range of 5 to 40 µg.mL^{-1}, with RSP fixed at 100 µg.mL^{-1}. The highest absorbance was recorded at 25 µg.mL^{-1} of ARS, after which further increases in reagent concentration did not affect absorbance, confirming that the reaction had reached completion. These results are presented in Figure 5-b.

Effect of pH

The impact of pH on the formation of the colored complex was evaluated in both methods by preparing a series of Britton-Robinson (BR) buffer solutions spanning pH 2 to 12, as well as acetate buffer solutions within the pH range of 3 to 5. It was observed that acidic conditions promoted dissociation of the complex, reducing its stability, while basic conditions led to the reaction of ARS with hydroxide ions, adversely affecting the complex formation and its absorbance. Consequently, to achieve optimal and consistent results, the reaction was conducted without the addition of any buffer solution.

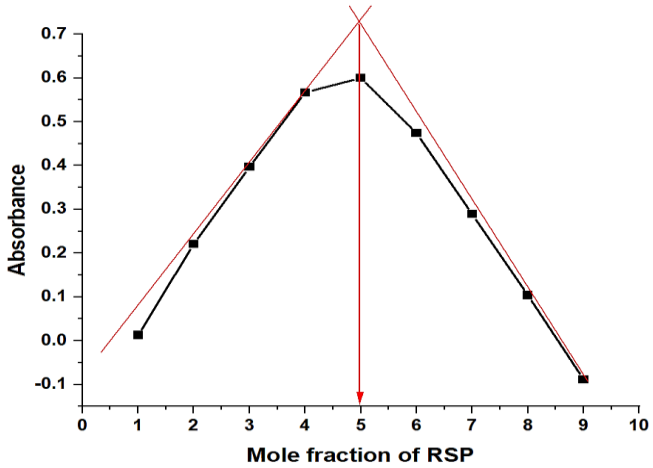

Figure 6. Job's continuous variation plot illustrating the 1:1 stoichiometric ratio of the formed RSP-ARS complex.

Stoichiometry

The stoichiometric ratio of the RSP and ARS complex was determined using the continuous variation (Job's) method. Solutions of RSP and ARS, each at a concentration of 3.6×10^{-4} M, were mixed in varying volumes while maintaining a constant total volume of 10 mL. The absorbance was measured for each mixture, and a plot of absorbance versus the mole fraction of RSP revealed a maximum at a mole fraction of 0.5, indicating a 1:1 molar ratio between RSP and ARS. This finding supports the proposed mechanism and structural model of the ion-pair complex, as illustrated in Figure 6.

Effect of Temperature and Time

In method A, the influence of temperature on the stability of the formed colored complex was examined within the range of 25–50°C. The absorbance remained consistent throughout this range, indicating that the complex is stable under these conditions; therefore, room temperature (25°C) was selected for the analysis. Additionally, the effect of time was evaluated, revealing that the complex maintained its stability for several hours without significant changes in absorbance.

Physical Parameters of the FIA Method

Effect of Sample Volume

In this study, different lengths of Teflon tubes were used, ranging from 10 to 70 cm with an internal diameter of 1 mm. Thus, the sample volume varied between 78.5-549 µL, while the rest of the parameters remained constant, namely, the ARS concentration was 25 $\mu g.mL^{-1}$ with RSP 100 $\mu g.mL^{-1}$, and the flow rate was 2.96 $mL.min^{-1}$. The measurements were carried out using a green light source in the detector, and the results showed an increase in the signal when the sample volume was increased up to 471 µL. After this volume, a slight increase in the signal occurred, which is almost negligible. This is evidence of the completion of the reaction at 471 µL of RSP. The result is shown in Table 1.

Table 1. The effect of sample volume on method B (FIA Method).

Volume length (cm)	Sample volume (µL)	Average of Detector response n=3	Standard Deviation [a]	RSD% [b]	Confidence interval [c]
10	78.5	450	6.88	1.35	450 ± 17.11
15	117	542	3.74	0.69	542 ± 9.30
20	157	553	5.36	0.97	553 ± 13.33
25	196	629	6.60	1.05	629 ± 16.41
40	314	761	4.41	0.85	761 ± 10.96
60	471	971	1.45	0.15	971 ± 3.60
70	549	1009	3.53	0.35	1009 ± 8.78

[a] $\mathbf{SD}= \sqrt{\frac{\sum(x-\bar{x})}{n-1}}$, [b] $\mathbf{RSD\%} = \frac{SD}{\bar{x}}$, [c] $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma n-1}{\sqrt{n}}$

Table 2. Effect of Flow Rate on Signal Intensity in Method B (FIA Method).

Flow rate (mL/min)	Average of Detector response n=3	Standard Deviation	RSD%	Confidence interval
1.5	794.66	1.51	0.19	794.66 ± 3.75
2.23	845.00	3.97	0.47	845.00 ± 9.87
2.96	971.66	1.45	0.15	971.66 ± 3.60
3.63	765.00	3.44	0.45	765.00 ± 8.55
4.36	769.00	1.23	0.16	769.00 ± 3.06

Effect of Flow Rate

The influence of the peristaltic pump flow rate for both the carrier stream and the ARS line was investigated within the range of 1.5 to 4.36 mL.min^{-1}, while all other parameters were kept constant. The ARS concentration was maintained at 25 µg.mL^{-1}, and 471 µL of 100 µg.mL^{-1} RSP was used. Results indicated that the signal intensity increased with rising flow rates, reaching a maximum at 2.96 mL.min^{-1}. Beyond this point, further increases in flow rate led to a decline in signal intensity. Consequently, 2.96 mL.min^{-1} was selected as the optimal flow rate for both lines. The findings are summarized in Table 2.

Effect of Reaction Coil

The effect of reaction coil length was studied by keeping the remaining parameters constant, with ARS concentration at 25 µg.mL^{-1}, 471 µL of 100 µg.mL^{-1} RSP and 2.96 mL.min^{-1} as flow rate by using variable coil lengths of 10, 20, 30, and 40 cm were tested, in addition to a manifold with no coil. The reaction coil was made of Teflon tubing with an inner diameter (I.D.) of 2 mm, corresponding to internal volumes of approximately 314, 629, 943, and 1,256 µL for 10, 20, 30, and 40 cm lengths, respectively. The results showed that using no reaction coil produced the strongest signal. This suggests that there is no need for further residence time because the complexation process between RSP and ARS occurs instantly. Consequently, the reaction coil was excluded from the final design to reduce dispersion and preserve peak sharpness. The result is shown in Figure 7.

Validation of Development Method

Under optimal experimental conditions, ARS was added to different volumes of RSP, forming a purple ion-pair complex that recorded the highest absorbance in the visible spectrum at 534 nm. The calibration graph was created by plotting absorbance against the initial concentration of RSP and found to be linear with a good correlation coefficient (r) shown in Figure 8. The International Conference on Harmonisation (ICH) guidelines for the validation of analytical procedures were followed in calculating the limits of detection and quantification of methods. Precision was also studied, and small RSD% values (<1.25) were found, indicating that the current method's precision is acceptable; all parameters are illustrated in Table 3. OriginPro 2020b software used calibration data to create the linear regression equation.

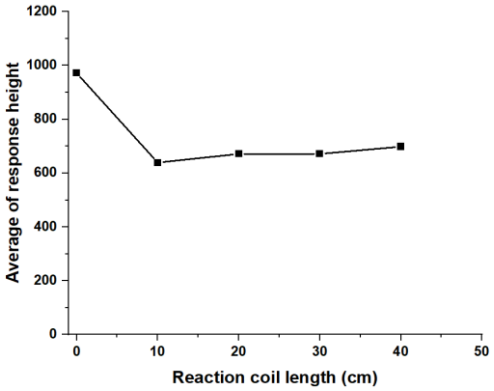

Figure 7. The effect of the reaction coil on method B.

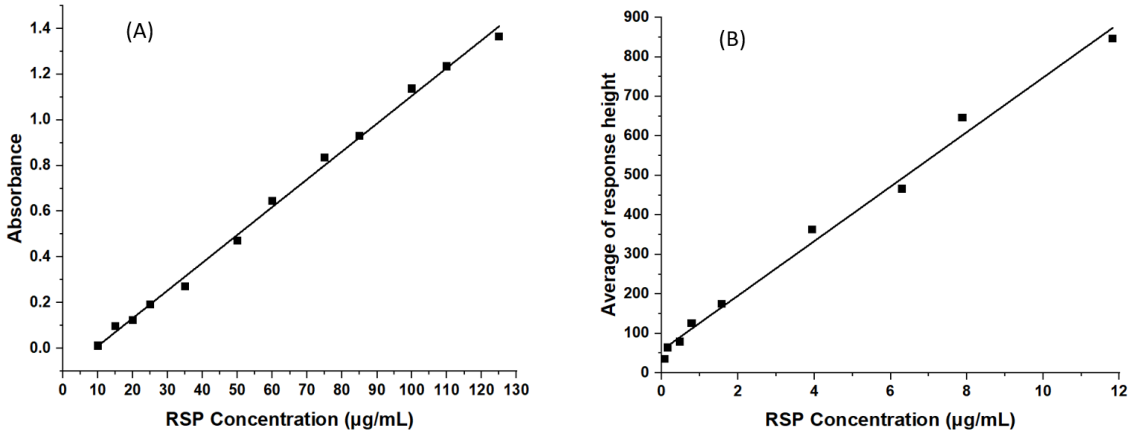

Figure 8. Calibration curves for the proposed analytical methods: (A) Method A, (B) Method B.

Table 3. Regression equations, optical parameters, and statistical data for the proposed methods.

Parameter	Method A	Method B
λmax, nm	534	Green light
Beer's law limits, μg.mL^{-1}	10-125	0.078-11.826
Molar Absorptivity, L.mol^{-1}.cm^{-1}	4.2×10^{3}	8.69×10^{6}
Sandell sensitivity, μg.cm^{-2}	0.0822	1.45×10^{-5}
Limit of detection, μg.mL^{-1}	4.15	0.037
Limit of quantification, μg.mL^{-1}	12.59	0.112
Intercept (a)	-0.11033	57.8121
Slope (b)	0.01216	68.9797
Standard deviation of intercept (S_a)	0.01531	13.0540
Standard deviation of slope (S_b)	2.17×10^{-4}	2.42545
Variance (S_a^2)	2.34×10^{-4}	170.407
Correlation coefficient (r)	0.9984	0.9957
Relative standard deviation RSD%*	< 1	< 1.25

*Mean value of four determinations.

Application to Formulations

The proposed methods were successfully applied to the determination of RSP in two commercial pharmaceutical formulations for each method. Method A was applied to Risnia-2 and Respal 2, while method B was applied to Risnia-4 and Rishparm. The recovery studies were conducted to evaluate the potential interference of excipients with the active drug. High recovery percentages, good precision, and little interference were shown in the obtained data, validating the method's dependability for regular quality control. All results are shown in Table 4.

A one-sample t-test was performed using IBM SPSS Statistics 22 software (IBM Corp., Armonk, NY, USA) to assess the statistical difference between the labelled claim and the values obtained by the developed methods. According to Table 5, at a 95% confidence interval and degree of freedom (DF)= 2, the calculated t-values for methods A and B were less than the tabulated t-value of 4.303 [38]. Moreover, the P-values that were obtained were higher than 0.05. indicating no significant difference between the labelled content and the values obtained by the developed methods. This confirms the accuracy and applicability of the proposed methods for the determination of risperidone in pharmaceutical formulations.

Table 4. Quantitative determination of risperidone formulations by two proposed methods and application of the standard addition method.

Tablet brand name	Add	Found	Rec%±SD*	RSD%	RE%
Method A					
Risnia-2	20	20.55	102.75 ± 0.002	0.50	2.75
Respal 2	20	19.10	95.50 ± 0.015	3.81	-4.50
Method B					
Risnia-4	1.57	1.56	99.36% ± 2.30	1.32	-0.64
	3.94	3.89	98.73% ± 1.15	0.32	-1.27
	6.30	6.47	102.70% ± 0.57	0.12	2.70
Rispharm	1.57	1.65	105.10% ± 1.52	0.82	5.10
	3.94	3.95	100.25% ± 3.78	1.03	0.25
	6.30	6.37	101.11% ± 1.73	0.36	1.11

*Mean value of three determinations. SD: standard deviation, RSD: Relative standard deviation, RE: relative error.

Table 5. One-sample t-test for comparison between the measured concentrations and the label claim of the pharmaceutical formulations.

Methods	Tablet brand name	Label claim (mg)	Found (mg)	P-value	t-calculated	t-tabulated*
Method A	Risnia-2	2	1.938	0.429	-0.983	4.303
	Respal 2	2	2.030	0.667	0.500	
Method B	Risnia-4	4	4.010	0.851	0.214	
	Rispharm	2	2.043	0.286	1.441	

*Tabulated t value at DF= 2, and 95% confidence level.

Green Analytical Chemistry (GAC)

Green analytical chemistry (GAC) is known as the improvement of analytical procedures to make them safe, nontoxic, ecologically benign, and economical in their use of substances, energy, and waste production [39]. GAC aims to replace polluting methods with clean ones. It finds practical substitutes for the off-line treatment of wastes and residues [40]. Even chemists' small-scale operations, like laboratory research, can negatively impact the environment. This may happen through careless disposal of used reagents and chemical waste. Over a number of years, the chemical community has created various activities for these reasons [41]. Green analytical chemistry promotes using energy-efficient equipment, reducing waste production, and reducing hazardous chemicals and reagents [42]. Consequently, it has revolutionised chemical monitoring. This is achieved through solvent-free approaches, real-time environmental tracking, and strategies that minimize waste [43]. To assess the greenness of analytical procedures, several metrics have been developed, including NEMI [24], AMVI [25], GAPI [26], AES [27], AGREE [28], EAT [29], and ChlorTox [30]. These tools consider factors like hazardous substance use, energy consumption, and waste creation to assess and lessen the environmental impact of analytical processes [31].

The ideal green analysis, which has a value of 100, forms the foundation of the analytical Eco-Scale concept. If the analytical process deviates from ideal green analysis, penalty points are given for each category: number of reagents, hazards, energy, and waste. The score is calculated by subtracting total penalty points from 100. On the AES scale, a score below 50 means inadequate green analysis. A score over 50 but below 75 means appropriate analysis, while a score above 75 means excellent green analysis [27]. The main advantages of the AES tool are its simplicity, its ability to provide quantitative data on environmental effects, and its capacity to assess multiple aspects of ecological sustainability [44]. In our current methods, the AES score was 92 for method A and 90 for method B. This demonstrates that both are excellent green methods, according to Tables 6-A and 6-B. The pictograms and hazard information used for penalty points were obtained from the Safety Data Sheets (SDS) provided by Sigma-Aldrich for each substance.

Table 6-A. Analytical evaluation of our suggested methods for the eco-scale system greenness.

Reagents	Number of Pictograms	Penalty Points
ARS	0	0
Ethanol	2	4
Instruments		
UV-Vis Spectrophotometer	< 0.1 kWh per sample consumption	0
Hot plate		0
Occupational hazard	Analytical process hermetization	0
Waste	1–10 mL, Degradation	4
Total penalty points		8
AES score		**92**

Table 6-B. Analytical evaluation of our suggested methods for the eco-scale system greenness.

Reagents	Number of Pictograms	Penalty Points
ARS	0	0
Ethanol	2	4
Instruments		
Peristaltic pumps	< 0.1 kWh per sample consumption	0
Hot plate		0
Occupational hazard	Analytical process hermetization	0
Waste	>10 mL, Degradation	6
Total penalty points		10
AES score		**90**

Based on safety data sheets released by various manufacturers, ChlorTox Base is a database on the chemical hazards of common reagents used in chemical laboratories [45]. The first assumption concerns the reference substance, chloroform, which we selected for this reason. Estimating the substance-of-interest's (CH sub) overall chemical hazard and contrasting it with the standard's (CH CHCl3) overall chemical hazard forms the basis of the method. Second, it is necessary to precisely quantify and account for the mass of the material of interest needed for a single analysis or measurement (m sub) [30]. According to the following equation:

$$\text{ChlorTox} = \frac{\text{CH sub}}{\text{CH CHCl3}} \times \text{m}_{\text{sub}}$$

CHsub represents the chemical risk associated with the substance under investigation, while $CHCHCl_3$ refers to the chemical hazard of standard chloroform. msub indicates the mass of the substance required for a single analysis. The safety data were acquired from the Safety Data Sheet (SDS) provided by Sigma-Aldrich. Our current methods demonstrate low values in the ChlorTox scale of 0.0478 g for method A and 0.0239 g for method B, indicating that they are very low total chemical risk, green, and environmentally friendly methods. As shown in Table 7-A, B.

In 2020, the Analytical GREEnness Calculator (AGREE) was developed by Pena-Pereira et al [28]. The degree of greenness of a certain approach is indicated by AGREE using a colour-coded scale consisting of red, yellow, and green. Using software, a clock-like symbol representing the analytical method's ultimate score, ranging from 0 to 1, is produced. This method is thorough, adaptable, simple to understand, and provides outcomes that are both clear and informative [46]. The result was 0.72 for method A and 0.69 for method B, which indicates that the methods are acceptable in terms of greenness and environmentally friendly. As shown in Figure 9.

Table 7-A. Data for the ChlorTox scales of method A.

Reagent/Solvent	CH_{sub}/CH_{CHCl3}	m_{sub} (g)	ChlorTox (g)	Total ChlorTox (g)
ARS	0	0.0043	0	0.0478
Ethanol	0.26	0.1841	0.0478	

Table 7-B. Data for the ChlorTox scales of method B.

Reagent/Solvent	CH_{sub}/CH_{CHCl3}	m_{sub} (g)	ChlorTox (g)	Total ChlorTox (g)
ARS	0	0.00083	0	0.0239
Ethanol	0.26	0.09205	0.0239	

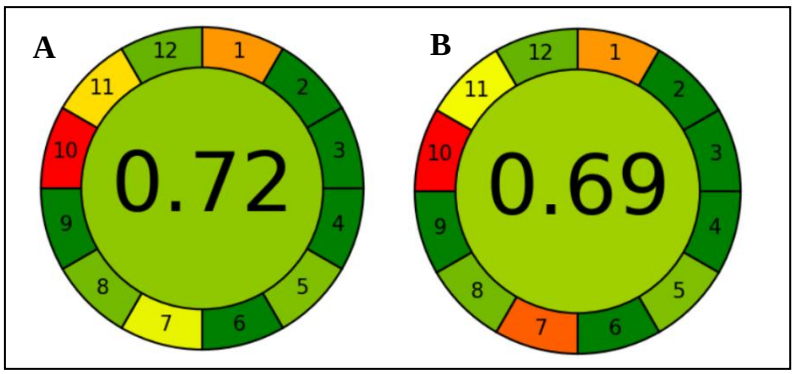

Figure 9. Analytical AGREE scale for (a) method A, (b) method B.

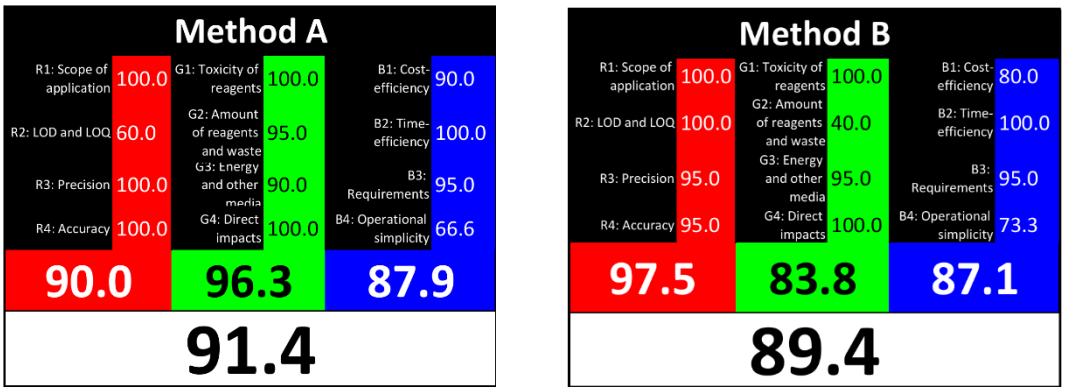

Figure 10. RGB12 whiteness evaluation of the two proposed methods by using the analytical RGB12 algorithm.

White Analytical Chemistry (WAC)

White analytical chemistry (WAC) is a concept of sustainable development analytical chemistry, which is an extension of green analytical chemistry. In 2021, Nowak et al. created and developed WAC [47], emphasising different factors that affect ecological aspects (green), the quality and performance of a method from an analytical perspective (red), and its practicality (blue) [48]. Three primary attributes-Red (R), Green (G), and Blue (B)—are defined by three categorised colours. Each category is further subdivided into four parameters that are pertinent to analytical performance, ecological impact, and commercial measures, respectively. This was accomplished using the RGB12 algorithm tool. Consequently, the net colour created by combining the three colours is white [49]. The Excel template spreadsheet that is readily available is utilised (see Supplementary data in Nowak et al.'s study) [33]. Each criterion is scored on a scale from 0 to 100, where a score of 100 reflects an excellent alignment of the method with its intended application, while a score of 0 represents the least favourable outcome. Moreover, under certain conditions, outstanding performance in specific aspects may be rewarded with scores exceeding 100 points. The proposed methods demonstrated excellent whiteness, scoring 91.4 for method A and 89.4 for method B, which confirms their sustainability, as shown in Figure 10.

CONCLUSIONS

Two sensitive, accurate, and environmentally sustainable analytical methods were successfully developed for the determination of risperidone in pharmaceutical formulations. The first method employed conventional UV-Vis spectrophotometry, while the second utilized flow injection analysis (FIA), which offers advantages such as rapid analysis, low reagent and sample consumption, high reproducibility, and suitability for automation. The environmental impact of both methods was systematically evaluated using multiple green chemistry assessment tools, including the Analytical Eco-Scale (AES), ChlorTox, AGREE, and the RGB12

algorithm for white analytical chemistry. The results confirmed excellent greenness and whiteness profiles, affirming the methods' compliance with modern sustainable analytical practices. Both techniques exhibited high sensitivity, precision, and robustness, making them suitable for routine quality control. Future investigations may explore the application of these methods to biological matrices or extend their use to structurally related pharmaceutical agents. Moreover, further development of the FIA method through automation or miniaturization could enhance its utility in high-throughput pharmaceutical analysis.

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