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DEVELOP A DIRECT CONTINUOUS FLOW INJECTION METHOD USING TURBIDITY DETECTION FOR THE QUANTIFICATION OF RISPERIDONE IN PHARMACEUTICAL AND BIOLOGICAL SAMPLES

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Abstract. This paper reports on the development and validation of a novel continuous flow injection analysis methodology employing turbidity detection (FIA-Turbidity) for the direct quantification of risperidone in biological and pharmaceutical specimens. The developed analytical method capitalizes on a unique chemical interaction where risperidone and cadmium chloride generate a distinctive white precipitate complex when combined in an acidic environment. This precipitate can be quantitatively assessed through turbidimetric measurement. A custom-built FIA equipped with a solar cell detection module was employed to monitor product turbidity values. This ad hoc FIA system leveraging a solar cell sensor was purposefully devised and applied over the course of the study to enable the quantitative measurement of turbidity changes. Risperidone standards were used to construct a linear calibration curve at concentrations ranging from 0.08 to 2.5 mmol/L, showing an excellent correlation (r^2) of 0.9944 indicating the high linearity and sensitivity of the proposed method. Various analytical parameters including, acetic acid concentration, cadmium chloride concentration, volume of injected sample, length of the reaction coil and flow rate were systematically analyzed and optimized to achieve optimal analytical performance. The rigorously determined limits of detection and quantification for the proposed FIA-Turbidity method were found to be 0.0137 and 0.0452 mmol/L, respectively. The developed flow-injection turbidimetric method was successfully used for the quantitative determination of risperidone concentration in pharmaceutical and biological samples, showing that it can rapidly and sensitively analyze this drug in different sample matrices It also provides method another environmentally friendly.

Keywords: *Risperidone, biological samples, pharmaceuticals, Flow Injection-Turbidity.*

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1. Introduction

Risperidone is an atypical antipsychotic belonging to the chemical class of benzisoxazole derivatives (Figure 1). It was first introduced in the early 1990s for the treatment of schizophrenia and other psychotic disorders (Cohen, 1994). The atypical antipsychotic drug risperidone is frequently used and has a distinct mode of action that sets it apart from other antipsychotics. Unlike other medications that solely target dopamine (D_2) receptors in the central nervous system, risperidone acts by antagonistic serotonin 5-HT_2A and dopamine D_2 receptors. Following oral administration, risperidone is rapidly and completely absorbed from the gastrointestinal tract. It then undergoes

extensive first-pass metabolism, primarily via hydroxylation and N-dealkylation reactions in the liver (Möller, 2005). The process of hydroxylating risperidone is facilitated by the cytochrome P450 isoenzyme CYP_2D_6, a protein that exhibits genetic variability. Consequently, individuals can be classified as either extensive metabolizers (EM) or poor metabolizers (PM) of risperidone. Poor metabolizers, comprising approximately 5-10% of the Caucasian population and around 1% of the Oriental population, fall into the latter category (Ono *et al*., 2002; Zhou, 2009). Risperidone is also approved for the treatment of severe depression or mixed episodes associated with bipolar I disorder (Wirshing *et al*., 1999). Given the narrow therapeutic window of risperidone, close monitoring of drug levels is important. By regularly checking risperidone blood concentrations, doctors can individualize treatment to best suit each person's needs, empowering patients to concentrate on their recovery (Malone *et al*., 2002; Alzand *et al*., 2024).

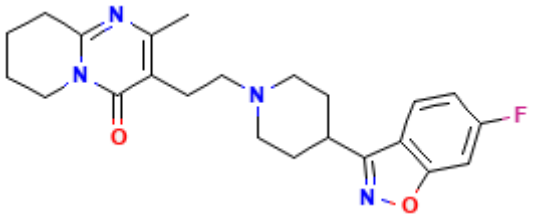


Figure 1. Shows the chemical structure of Risperidone

As the use of antipsychotic drugs such as risperidone has increased, the need for more rigorous screening methods has increased. This is because formulation stability, accurate distribution and dosing of risperidone are important for clinically monitored pharmacotherapy. The quantitation of the nonprescription antipsychotic risperidone in pharmaceutical formulations and biological matrices has been a focus of detailed research. Among the various analytical techniques that have been employed for this purpose, high-performance liquid chromatography (HPLC) has undoubtedly emerged as the workhorse methodology due to its inherent sensitivity and selectivity (Samala *et al*., 2013; Alam *et al*., 2024; Maljurić *et al*., 2020). More recently, ultra-high performance liquid chromatography (UHPLC) has also been applied to risperidone analysis (Konecki *et al*., 2023). Other studies have utilized HPLC coupled with mass spectrometry (HPLC/MS) (Zheng *et al*., 2021), Solid-phase extraction (Feyzi *et al*., 2023; Jeber *et al*., 2019), liquid-liquid extraction followed by LC separation (Gu *et al*., 2023), as well as densitometric (El-Sherif *et al*., 2005) and spectrophotometric (Jayanna *et al*., 2014; Dedania *et al*., 2011; Ashour & Kattan, 2013; Hammood *et al*., 2024) methods. Recent studied have showed that high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) enables more sensitive quantification of risperidone and its active metabolite 9-hydroxyrisperidone in serum compared to conventional analytical techniques (Feyzi *et al*., 2023; Gu *et al*., 2023; Aravagiri & Marder, 2000; Nozawa *et al*., 2024). However, HPLC techniques require sophisticated instrumentation and lengthy sample preparation steps, hindering their suitability for routine analysis in quality control laboratories or point-of-care settings. Flow injection analysis (FIA) has emerged as a viable alternative analytical technique that offers several distinct advantages (Turkey & Jeber, 2021a; Nesměrák *et al*., 2022). The benefits of FIA systems, which are inherently simple and automated, are numerous and enhance the advantages of traditional chromatographic techniques. FIA offers several benefits such as higher sample throughput, lower reagent use, less waste produced and reduced operating expenses. This

positions FIA as a viable analytical methodology for quantifying pharmaceutical compounds like risperidone. Despite requiring less specialized equipment than chromatographic techniques, FIA can still deliver adequate selectivity and sensitivity when partnered with the right detector. The combination of FIA with turbidity show that it can potentially be an effective substitute for dosing in complex matrices (Jeber *et al*., 2019; Turkey & Jeber, 2021b, 2022b). The use of FIA and the combination of turbidity detection as an analytical tool has been on the increase recently because it can measure a wide range of chemicals. Some of the active pharmaceutical ingredients that have been reported in the literature employing the FIA-turbidity methodology include the determination of Mebeverine Hydrochloride (Turkey & Jeber, 2022a), Acetylcysteine (Suarez *et al*., 2007), Warfarin (Jeber & Turkey, 2021), Cyproheptadine hydrochloride (Jeber, 2020) and Ceftazidime (Hassan, 2023). These studies confirm the versatility and the potential of the analytical FIA-turbidity approaches in achieving accurate results in real sample analysis at low costs and with low environmental impact. Besides, non-separation FIA technology has a plethora of promises for the success of multi-pharmaceutical analyses. There is ample reason to believe that integrated FIA-turbidity can serve as a valuable analytical platform, considering that the integration of process simplifies the method, lessens the number of reagents needed and manages complex sample compositions. Its scope of application in pharmaceutical analysis appears poised for expansion, as demonstrated by a simplified workflow, conservation of reagents and management of intricate samples. The main aim of this paper was to establish and validate a FIA-Turbidity method for the expeditious, cost-effective and straightforward analysis of risperidone in different samples. This alternative analytical methodology has the promise to broaden capabilities for precise risperidone monitoring and better serve those receiving this critical antipsychotic drug. With its advantages of speed, low reagent uses and cost, simplified operation and capability to analyze complex matrices. The developed FIA-Turbidity method aims to determine the concentration of risperidone in different samples.

2. Experiment

2.1. Chemicals and reagents

All chemicals used in this study were of analytical reagent grade. Dilutions were performed using ultrapure water. A 10 mmol/L standard stock solution of risperidone (SDI, Samara, Iraq; M. Wt 410.49 g/mol) was prepared by precisely weighing 0.4105 g of the solid compound and dissolving it in 100 mL volumetric flask. A separate 0.3 mmol/L cadmium chloride working standard (BDH; M. Wt 183.32 g/mol) was obtained by dissolving 0.014 g $CdCl_2$ in 250 mL flask. Additionally, a 10 mmol/L acetic acid solution (BDH; MWt 60 g/mol) was generated through diluting 1 mL of 1 M acetic acid to a final volume of 100 mL. Great care was taken to accurately weigh reagents and fully dissolve solids in preparing these primary calibration standards from which secondary standards, blanks and samples were subsequently diluted, ensuring traceability and high quality of concentration values utilized in subsequent optimization and validation steps.

2.2. Apparatus

The two-channel manifold employed in the flow injection analysis system allowed for reproducible switching between sample and carrier streams, effectively transporting

analyte zones to the detector. A branched design incorporating the peristaltic pump, sample injection valve and reaction coil enabled precise control over timing and fluidic handling. Optimization of manifold dimensions and operating parameters was critical for ensuring stable baseline, sharp sample plugs and quantitative responses that could accurately assess risperidone either in standard or samples. A peristaltic pump from Ismatec (Switzerland, open valve mode) with variable speed control was utilized to precisely transport the carrier solution via plastic tubing with an inner diameter of 0.5 mm. The flow cell for optical detection was illuminated by an array of six white light emitting diode (LED) sources with a 2 mm path length. A CFI analyzer equipped with dual Ayah 6SX1-ST-2D solar cells served as the detector, transmitting signals across the 60 mm length of the flow cell. An injection valve was employed to introduce precise injection volumes of analytical standards and samples. Additionally, a Teflon reaction coil with an inner diameter of 0.5 mm was used to allow sufficient time for chemiluminescence reactions to occur prior to detection. A two-channel manifold was configured for the flow injection analysis (FIA) system. Recorded signals were plotted versus time using a voltage recorder (Figure 2), allowing visual assessment of risperidone peaks associated with risperidone concentration. These optimized parameters enabled sensitive and reproducible quantification of risperidone.

2.3. Sample collections

Serum samples were obtained from 20 adult health individuals at Al-Yarmouk Teaching Hospital in Baghdad, Iraq. The study was approved by the Ethics Committee of Mustansiriyah University College of Science. Blood samples were collected by qualified nurses and allowed to clot at room temperature before centrifugation. The separated serum was stored at -20°C until analysis. All samples were coded to maintain patient confidentiality. Tablets containing risperidone (2.0, 3.0 and 4.0 mg) were purchased from local pharmacies in Baghdad. Twenty randomly selected tablets from each of three different brands (60 tablets) were analyzed to evaluate inter-brand variability. Proper storage, handling and transportation of all biological samples were carried out according to guidelines set by Mustansiriyah University to protect sample integrity and patient safety/privacy. This ensured ethical procurement of real-world clinical specimens and pharmaceuticals to fully validate the proposed analytical method.

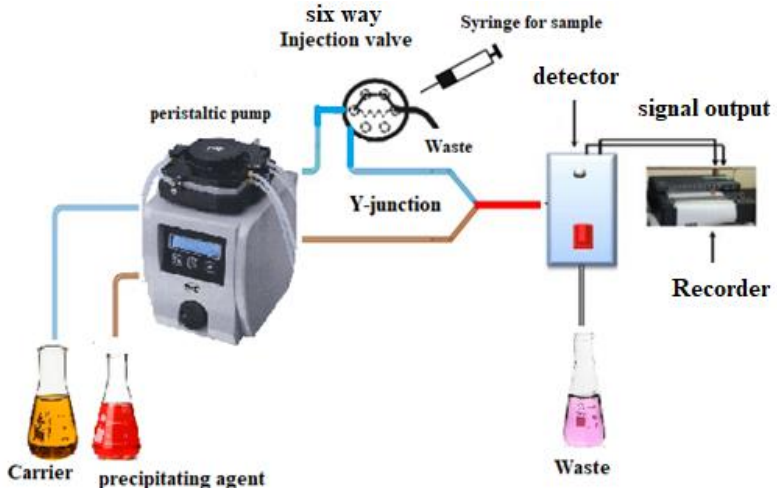


Figure 2. The construction of manifold system used for determination of risperidone

2.4. General procedure

A two-channel manifold system was connected to a solar cell detector for the analysis of risperidone via precipitation with cadmium chloride. Cadmium chloride functions as a precipitating agent, forming a white precipitate upon combination with risperidone. The first line utilized distilled water as the carrier stream and was joined to an injection valve to introduce 157 µL aliquots of the risperidone sample solution. This reagent line merged with the second line transporting the cadmium chloride precipitant at a confluence point. Downstream, the combined streams allowed sufficient mixing and reaction prior to reaching the flow cell detector (Figure 2). Visualization of the resulting turbidity peak allowed quantification of risperidone concentration in test samples via this optimized online precipitation-photometric detection methodology. All the optimized flow injection conditions were inserted in Table 1.

Table 1. Optimal Experimental Conditions for the FI-Turbidity Method

FI parameter	Studied Range	Chosen value
Flow rate (mL min $^{-1}$)	0.35-3.0	2.7
Sample volume (µL)	50-250	157
[$CdCl_2$], mmol L^{-1}	0.05-5.0	0.3
[CH_3COOH], mmol L^{-1}	3-20	10

3. Results and Discussions

The formation of this insoluble ion-pair complex during the reaction between risperidone and the chloride ions in the acidic medium is the basis for the turbidimetric detection in the FIA method described in the original abstract. Therefore, the mechanism of reaction can be proposed as follows: in the acidic environment, the nitrogen atom in the pyridine ring of risperidone becomes protonated, forming a positively charged species (Alhomrani *et al*., 2022). Meanwhile, the chloride ions from the cadmium chloride dissociate and are available in the solution. The positively charged risperidone species can then undergo an ion-pair formation reaction with the chloride ions, creating a neutral ion-pair complex. This complex is insoluble in the reaction medium, leading to the formation of a white precipitate that can be detected turbidimetrically. The proposed mechanism of the ion-pair complex formation can be illustrated in Figure 3. The figure provides a visual representation of the suggested process by which risperidone and cadmium chloride combine under acidic conditions to generate the precipitate used for turbidimetric detection and quantification of risperidone concentrations. In a summary, the mechanism of insoluble precipitate formation in reactions with chloride ions involves the nitrogen atom within the pyridine ring, leading to the formation of a unique precipitate. This unique characteristic ensures that the proposed method remains selective even in the presence of impurities and metabolites.

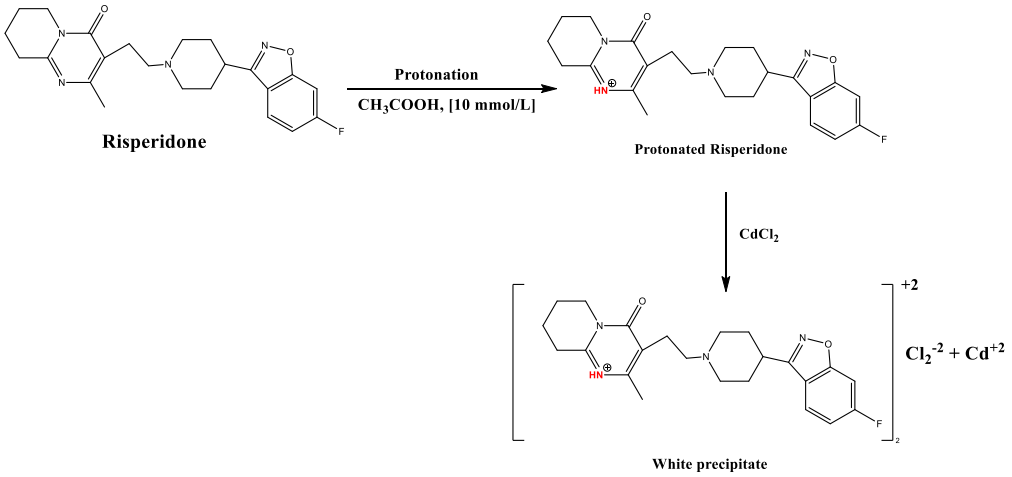


Figure 3. The proposed mechanism of the reaction between the risperidone and $CdCl_2$ in an acidic medium

Optimization of chemical and physical parameters of proposed method

The effect of precipitation reagent concertation

The study investigated various analytical parameters involved in the precipitation reaction, including the selection and concentrations of the precipitating reagent. $CdCl_2$ concentration was evaluated as the potential precipitating reagent. A series of standard solutions ranging from 0.05 - 5.0 mmol·L^{-1} $CdCl_2$ were injected under a constant flow rate of 1.4 mL min^{-1} (For both lines) to determine the optimal concentration. Results indicated 0.3 mmol·L^{-1} $CdCl_2$ produced the strongest risperidone response, likely due to formation of an appropriate particulate precipitate concentration. At this level, increased light scattering and reflection is thought to occur, reducing measured light intensity. Concentrations exceeding 0.3 mmol·L^{-1} $CdCl_2$ yielded diminished signals, as seen in Figure 4, possibly due to excessively large or dense precipitate particles inhibiting photodetection. Therefore, 0.3 mmol·L^{-1} $CdCl_2$ was selected as the optimal concentration for quantitative precipitation of risperidone.

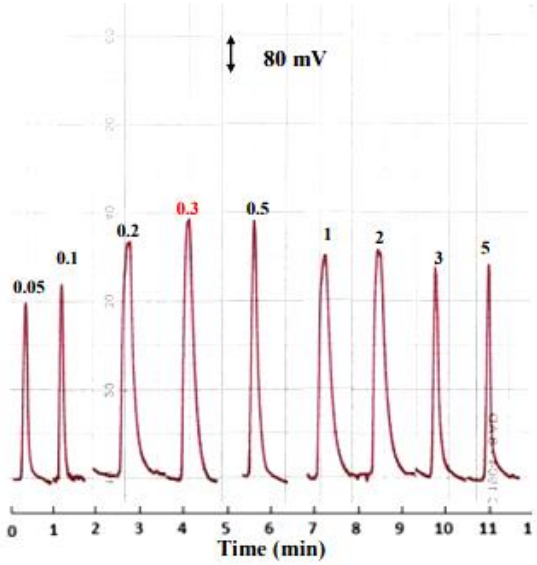


Figure 4. Impact of Varied Concentrations of Cadmium Chloride on Response Profile under the following experimental conditions: Flow rate of 1.4 mL·min^{-1}, sample volume of 100 µL and distilled water as carrier stream

3.1. The influence of acid type and concentration

To select the appropriate carrier and media for precipitation of risperidone with $CdCl_2$, preliminary experiments were conducted evaluating 10 mmol·L^{-1} solutions of sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4) and acetic acid (CH_3COOH) prepared in ultrapure water. Based on initial findings, acetic acid provided the strongest signals and was selected for further medium optimization studies. Acetic acid concentration was then investigated within the range of 3, 10, 15 and 20 mmol·L^{-1}. Results indicated 10 mmol·L^{-1} acetic acid produced the optimal response, as summarized in Table 2 and Figure 5. At higher concentrations, it is postulated that excessively large or dispersed precipitate particles reduced peak intensities. Therefore, 10 mmol·L^{-1} acetic acid was carried forward as the carrier and reaction medium for subsequent quantification of risperidone under optimized precipitation conditions using cadmium chloride.

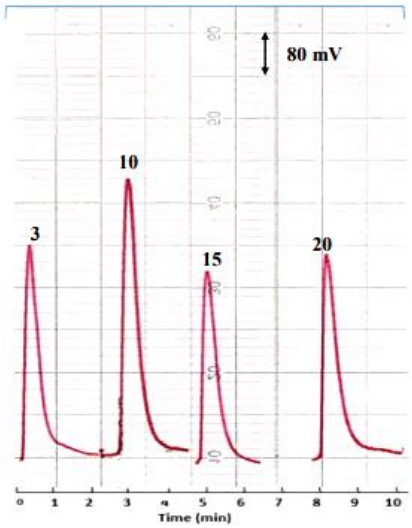


Figure 5. Summary of Response of acidic acid concentrations in the Risperidone-Cadmium Chloride Turbidity System under the following experimental conditions: Flow rate of 1.4 mL min^{-1}, sample volume of 100 µL and Cadmium Chloride concentration of 0.3 mmol L^{-1}

Table 2. The response profiles using variable concentrations of CH_3COOH in the Risperidone-Cadmium Chloride System

Concentration of acetic acid, mmol. L^{-1}	Detector Response, mV	RSD%
3	511	0.5212
10	660	0.3421
15	465	0.4504
20	490	0.3143

3.2. Effect of Flow Rates

The influence of flow rates on analytical response was investigated within the ranges of 0.4-4.0 mL/min for the carrier stream and 0.35-3.0 mL/min for the reagent line. All experiments were conducted under previously optimized conditions of 10 mmol/L acetic acid carrier solution, 2 mmol/L risperidone sample and 0.3 mmol/L cadmium chloride precipitant. Flow rates of 3.1 mL/min (carrier) and 2.7 mL/min (reagent) produced signals balancing sampling throughput with optimal sensitivity. At lower flow

rates, peak dispersion and baseline width (ΔtB) increased due to insufficient kinetic energy transporting the analyte zone. However, at the higher 3.1 and 2.7 mL/min flow rates, sharper peaks were observed as precipitation particles could clear the detection cell more rapidly. Therefore, 2.7 mL/min was selected for both lines to minimize dispersion effects while sustaining a suitable sample throughput, as illustrated in Figure 6.

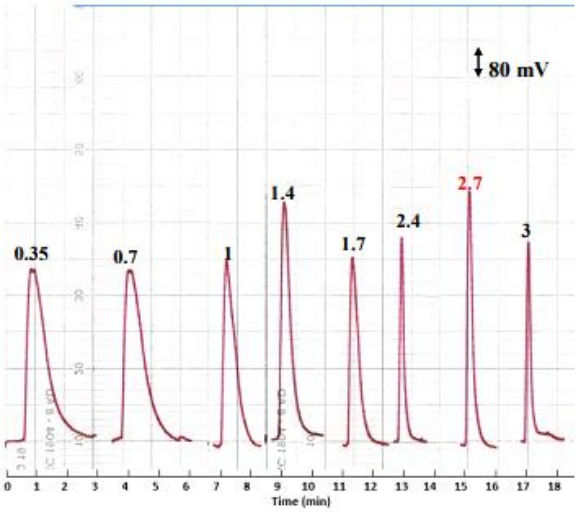


Figure 6. The response profiles of risperidone-cadmium chloride system used variable flow rates under the following experimental conditions: Acetic acid as carrier stream concertation of 10.0 mmol L^{-1}, sample volume of 100 µL and Cadmium Chloride concentration of 0.3 mmol L^{-1}

3.3. The effect of sample volume

To determine the optimal sample injection volume, studies were conducted utilizing sample loops of varying lengths constructed from 7.25 cm to 32.0 cm of 1 mm inner diameter Teflon tubing. Injection volumes tested spanned the range from 57 to 250 µL. Results indicated a sample volume of 157 µL generated the strongest analytical signal while maintaining appropriate peak width and dispersion under optimized flow and reagent conditions. Smaller injection volumes did not sufficiently precipitate risperidone for reliable quantification, while larger volumes exhibited peak broadening due to excessive dispersion. Therefore, 157 µL was selected as the optimized injection volume, balancing sensitivity, reproducibility and sampling throughput for the online precipitation-photometric methodology. Tables 3 summarizes the effect of using variable sample loops.

Table 3. The effect of using variable sample loop lengths on the response profile

Loop length, cm	Sample volume, µL	Detector Response, mV	RSD %
7.25	57	491	0.1605
12.72	100	573	0.1274
20.00	157	709	0.1104
25.46	200	668	0.3145
31.83	250	627	0.1318

3.4. Interferences study

To assess the method's reliability and precision, a comprehensive interference study was performed, examining the potential impact of various pharmaceuticals and serum

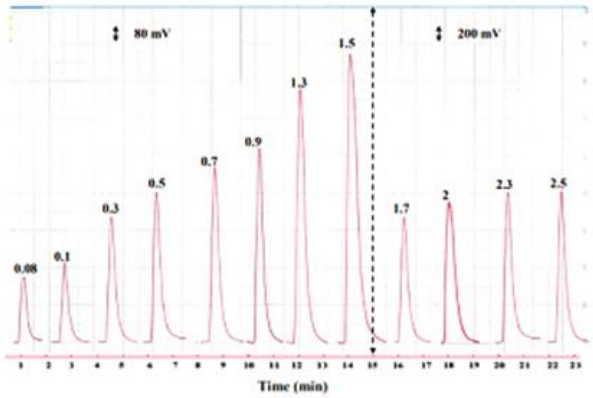
matrix components on the analytical technique's accuracy and reproducibility. Prior to examination, serum samples spiked with risperidone were mixed with varying quantities of potentially interfering chemicals, such as ascorbic acid, glucose, uric acid and bilirubin. Excellent recovery rates, regularly falling between 98 and 102%, were shown by the recently established FIA-Turbidity method for the quantification of risperidone, even in the presence of possible interfering compounds at physiological levels. The results of this study demonstrate that even when analyzing complex mixtures, the proposed method delivers accurate and consistent outcomes. Method validation included testing of samples containing common excipients expected in pharmaceutical formulations and no interference was observed, signifying the analyte (risperidone) could be precisely determined regardless of these compounds. Additionally, analogous results obtained with serum samples validate the approach's applicability to biological fluids. Overall, the evaluations reveal the proposed method maintains high precision and is impervious to potentially confounding materials routinely encountered in real-world dosage forms or bodily fluids.

3.5. Validation of the proposed method

The developed FIA-turbidity method for determination of risperidone was validated according to ICH guidelines (Borman & Elder, 2017) and ICH 10 FDA (Vazvaei-Smith *et al*., 2024). The method was validated for parameters such as linearity, range, interference effect, accuracy, precision, limit of detection and limit of quantification.

3.6. Linearity, LOD and LOQ of the proposed method

Linearity was determined by analyzing standard solutions over the concentration range of 0.08-2.5 mmol. L^{-1} and the method was found to be linear with correlation coefficient of 0.9899. Accuracy was determined by recovery studies via standard addition method to tablets and plasma samples. Recoveries from 98-102% indicated the method is accurate. Precision was determined by intra-day and inter-day RSD values which were <3% confirming the precision of the method as shown in Table 4. The method also had satisfactory sensitivity with LOD and LOQ of 0.013 and 0.045 mmol. L^{-1} respectively. The developed method was thus validated and found suitable for the intended quantification of risperidone in pharmaceutical formulations and biological samples. The plotted calibration curve and statistical information of linear equation of proposed method are illustrated in Figure 7 and Table 5 respectively.



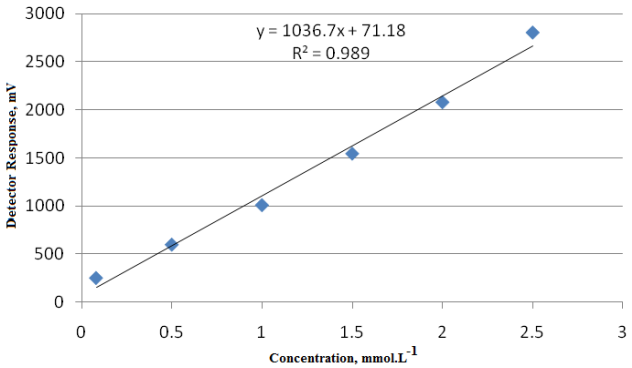


Figure 7. The response profile and plotted curve of calibration curve for the developed method

Table 4. The inter and Intra-day precisions of the developed method for the determination of risperidone

Method	Taken conc. mmol/L	Intra-day, (n=5)		Inter-day, *n=10*	
		Found, conc. mmol/L	RSD%	Found, conc. mmol/L	RSD%
FIA-turbidity	0.4	0.41	2.1	0.39	2.8
	0.8	0.79	1.3	0.78	2.4
	1.0	1.05	2.9	0.99	1.9
	1.5	1.52	2.5	1.48	1.1

Table 5. The statistical summary of linear regression equation of the proposed method

Statistical parameters	**Value**
Linear regression equation	Y= 1036.7141x+71.1822
Linearity (mmol/L)	0.08-2.5
LOQ (10*SD/b), (mmol/L)	0.0452
LOD (3 SD/b), (mmol/L)	0.0137
Slope, b	1036.7
Intercept, a	71.18
Coefficient of determination, R^2	0.9899
Correlation coefficient, r	0.9949

3.7. Repeatability of the proposed method

The repeatability of the newly developed FIA-turbidity method was assessed. Standard solutions of risperidone at 0.2 and 0.8 mmol/L were independently analyzed multiple times ($n = 6$) under optimized flow conditions. The resulting peak heights were recorded and the relative standard deviations (%RSD) were calculated to evaluate the repeatability of the measurements. As presented in Figure 8, good repeatability was achieved with %RSD values of 1.3% and 1.9% at 0.2 and 0.8 mmol/L risperidone, respectively. This suggests the method delivers consistent and reproducible measurements of analyte concentration over multiple assays of the same sample. The low %RSDs obtained verified the method's ability to accurately determine risperidone levels in quality control and authentic samples of interest in a precise manner. Overall, the data confirmed the suitability of the proposed FIA-turbidity method for the intended quantitative applications.

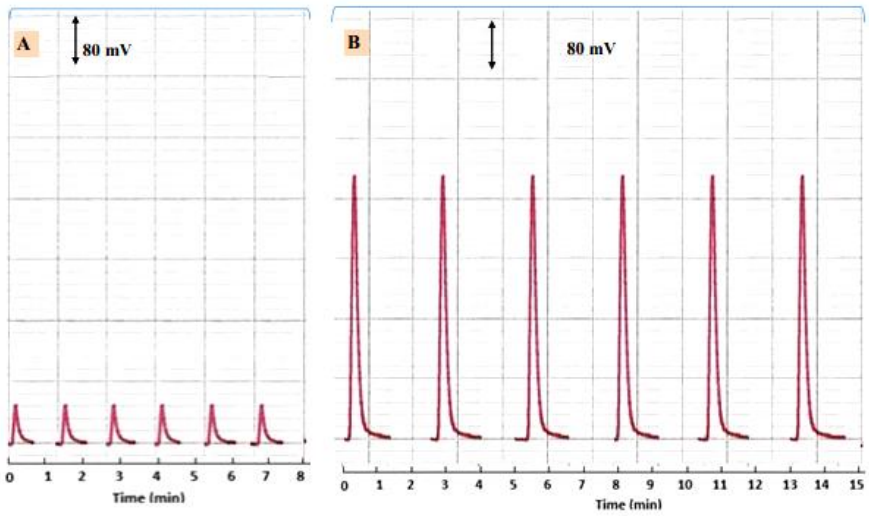


Figure 8. The response profiles of six successive measurements under optimized conditions

3.8. Selectivity of study

To further assess the selectivity of our proposed method, we conducted experiments to test the specificity against potential impurities and metabolites (Table 6). In pharmaceutical tablet formulation, various excipients are commonly used to improve the physical and chemical properties of medications. In this study, we investigated the impact of typical tablet excipients on the analytical determination of a compound risperidone at a concentration of 100 μg/mL. The chosen excipients were: Calcium dihydrogen orthophosphate, sodium alginate, lactose, starch, magnesium stearate, talc powder, calcium gluconate and sodium acetate. Each excipient was dissolved separately in the risperidone solution at proportions used for the formulation of pharmaceutical tablets. The purpose of this study was to assess the tolerance of the analytical method with regards to the extent these excipients interfered with the analysis of the risperidone. More specifically, the amounts of each excipient used were capped at levels that would guarantee a lack of interference with the analytical outcome. The criterion for acceptance was set at a threshold of ±1.5% recovery bias in the dosage concentration of the prepared solution of risperidone which nominal concentration was set at 100 μg/mL. The outcome of this study was presented in an aggregated form in the original publication in Table 6. This is very important in pharmaceutical analysis, measurement of the amount of active ingredient was found to be accurate and unaffected by the addition of the tablet's ingredients which includes the main component of the medicine, risperidone.

To quantify risperidone in biological specimens, the process was conducted as per the FDA M10 Bioanalytical method validation guidance. The procedure has been validated for specificity, accuracy, precision, LOD and LOQ. The in-depth evaluation of interference proved that our analytical method was robust towards a wide range of physiological and biochemical composition which showed a high tolerance to coexisting substances. Broad-spectrum clinical testing demonstrated remarkable selectivity and robustness with little analytical interference from important serum constitutions. The proteins estimated (albumin at 500 μg/mL) had no substantial interference on the method accuracy with only deviation of ±1.2% being obtained. Up to 300 μg/mL of the Lipid-related interferences were accounted for by the participants with a tolerance limit of ±1.5% from the readings. Metabolic markers bilirubin (conjugated), hemoglobin, uric

acid and creatinine were evaluated systematically and minimally disrupted the analytical signal. Even the more troublesome interferences such as ascorbic acid and cholesterol did not significantly compromise the method's precision with all interferences remaining within the tolerance limits of ±1.0-1.4%. The electrolytic components, including sodium chloride and calcium ions, further validated the method's exceptional selectivity, demonstrating consistent performance across varied serum environments. This comprehensive interference assessment conclusively demonstrates the proposed method's extraordinary resilience, exhibiting consistent accuracy and reliability across a diverse range of physiological conditions. The results unequivocally confirm the method's potential for robust clinical application, with all tested interferences failing to produce statistically significant deviations from the expected analytical outcomes.

Table 6. Studying the impact of common interferences on the developed method using 100 μg ml^{-1} of drug

Spiked excipients	Tolerance limits μg ml^{-1}
Sodium acetate	490
Talc powder	550
Magnesium stearate	1150
Calcium gluconate	390
Sodium alginate	1250
Calcium dihydrogen orthophosphate	1400
Lactose	850
Starch	490
Protein (Albumin)	±1.2
Triglycerides	±1.5
Bilirubin (Conjugated)	±1.0
Hemoglobin	±1.3
Ascorbic Acid	±1.1
Uric Acid	±1.4
Creatinine	±1.2
Cholesterol	±1.3
Calcium Ions	±1.1
Sodium Chloride	±1.2

3.9. Real-World Application of the Proposed Method in Analysis of Pharmaceutical and Serum samples

To further assess the practical utility of the proposed analytical approach, experimental validation studies were conducted by applying the method to the quantification of risperidone in relevant sample matrices. Furthermore, the proposed method was tested on human serum samples spiked with known amounts of risperidone (0.5, 1.0 and 2.0 mg). These experiments aimed to evaluate the practical applicability of the newly developed FIA-Turbidity method for determining the concentration of risperidone concentrations in real-world sample matrices commonly encountered in in pharmaceutical or biological samples. To assess pharmaceutical tablet analysis, risperidone standards were injected in triplicate (*n=3*) at concentrations of 0.0, 0.9 and 2.0 mmol/L into the FIA-Turbidity system. The quantitative results from tablet samples showed the method was unaffected by typical excipients and were in excellent agreement with labeled values and with those results obtained from the official method (HPLC-UV). The suitability for biological matrices was also assessed. Risperidone recovery from serum was determined in triplicate at fortification levels of 0.5, 1.0 and 2.0 mmol/L.

Recoveries fell within 98-110%, validating the method's ability to accurately analyze risperidone from complex biological matrices. The developed FIA-turbidity method was further validated by applying the statistical analysis between the proposed method and reference method. The obtained results were statistically compared to those of a validated reference HPLC-UV method (Alves, 2020). For tablets and serum samples, risperidone concentrations determined by the proposed and reference methods were found to correlate very well with each other (R^2 = 0.9944). No significant differences were observed between the techniques according to t-test and F-test at 95% confidence level. These results demonstrate excellent agreement between the proposed FIA-turbidity method and current reference technique for accurate quantification of risperidone in complex clinical and pharmaceutical samples. Thus, the method is considered suitable for adoption as an alternative technique. These results, summarized in Table 7, confirm the developed method is suitable for quantitative application to real pharmaceutical formulations and biological specimens as required.

Table 7. Assay findings for determining risperidone in authentic commercial samples employing both official and proposed methods

Sample	Claimed Dosage, *mg*	Spiked *mg*	$t^{[a]}$	$F^{[a]}$	Found			
					Proposed method		Officially method	
					FI-Tur. *mg*	Rec%	HPLC *mg*	Rec%
Tablet	2.0	0.0	0.21	1.23	2.11	105.5	2.20	110.00
		1.0	0.32	2.12	1.98	99.00	1.95	97.50
		2.0	0.11	2.03	2.05	102.5	2.01	100.50
Tablet	3.0	0.0	0.09	0.98	3.05	101.66	3.15	105.00
		1.0	0.07	0.18	3.09	103.00	2.99	99.66
		2.0	0.10	0.17	2.98	99.33	3.02	100.66
Tablet	4.0	0.0	0.12	2.05	4.12	103.00	4.02	100.50
		1.0	0.33	2.01	4.07	101.75	4.11	102.75
		2.0	0.42	1.98	3.97	99.25	4.01	100.25
Serum	-	0.5	0.08	1.12	0.52	104.00	0.49	98.00
		1.0	0.09	1.09	1.02	102.00	1.10	110.00
		2.0	0.11	1.05	1.96	98.00	2.01	100.50

[a]= The tabulated values of F and t at 95 % confidence interval, p= 0.05 are 2.168 and 2.09 repetitively

4. Conclusion

In this study, it was successfully developed and validated a novel flow injection analysis method coupled with turbidity detection for the rapid quantification of risperidone. Through optimization of key experimental parameters such as precipitating agent, acid medium, flow rates and sample volume, we were able to achieve sensitive and reproducible detection of risperidone concentrations ranging from 0.08 to 2.5 mmol/L. The method demonstrated satisfactory accuracy, precision and sensitivity with limits of detection and quantification of 0.0137 and 0.0452 mmol/L respectively. Validation as per ICH guidelines showed the method to be linear, accurate and precise for intended applications. Interference studies confirmed no effects from common pharmaceutical excipients or endogenous serum components on analyte recovery. Most importantly, application of the method to real pharmaceutical and serum samples yielded results in good agreement with a reference HPLC-UV method, verifying its ability to directly analyze risperidone in complex matrices. In comparison to established chromatographic

techniques, the proposed FIA-turbidity methodology offers significant advantages of lower cost, greater simplicity and higher sample throughput. These attributes make it especially suited for routine therapeutic drug monitoring and quality control applications in clinical laboratories and pharmaceutical industries.

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