A Green Approach for the Analysis of Pimozide in Pharmaceutical Formulation, Stress Degradation, and Spiked Plasma Samples Using Spectrofluorimetry

Ceema Mathew[1], P. Sai Viswanath[1], Shashikala Metri[2], Prathibha Bharathi[1], D. Vijaya Durga[1]*
[1]Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Hyderabad-500090, India.
[2]Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Hyderabad,-500090, India.
nirujose2003@gmail.com

Abstract

A green analytical method was created and verified for the chosen antipsychotic medication, pimozide. The technique relies upon spectrofluorometric quantifying of pimozide in tablet and bulk dosage form using 0.1M sodium lauryl sulphate. At excitation wavelength, λ_{EX}= 282 nm and emission wavelength, λ_{EM}= 314 nm, the luminescence intensity of pimozide was determined. A linear relationship calibration curve is obtained within the 1-10 µg/mL concentration range. R^2= 0.9995 was the correlation coefficient found. A recovery study was conducted on spiked plasma samples. The work is extended to stress degradation samples for which the first derivative fluorescence spectra were employed. The analytical process was validated on par with ICH guidelines for the validation parameters. The results show that the procedure is appropriate for regularly inspecting quality in medicinal product formulations, bulk drugs and plasma samples.

Keywords: *Pimozide, Stress degradation, Sodium lauryl sulphate, Spectrofluorimetry, Validation*

1. Introduction

In comparison with absorption spectroscopy, fluorescence methods enjoy a superior role due to the inherent sensitivity of the technique and the limited number of molecules exhibiting fluorescence due to the structural rigidity requirement apart from the chromophore[1]. Furthermore, for analysing a molecule, two wavelengths are required, namely excitation and emission wavelengths, which can rarely be the same for two drugs and confer selectivity for the method[1]. Once it is found that the molecule is fluorescent, the next step is the proper solvent selection, as it can influence the emission intensity.

Pimozide (PMZ) (Chemical structure in Fig 1), a member of the diphenyl butyl piperidine group, is an orally active, potent typical antipsychotic drug that blocks dopaminergic receptors in the neurons of the CNS [2,3]. A vast survey on the published literature denotes UV [4-7], HPLC [8-10], HPTLC [11], RIA [12], and LC-MS [13-15] methods. The extensive literature survey revealed that no spectrofluorimetric method was used to analyse pimozide in its bulk and pharmaceutical formulation, stressed degradation samples and spiked plasma samples. Hence, the present search was undertaken to develop and validate a simple, sensitive spectrofluorimetric method for pimozide in its bulk and pharmaceutical dosage form, validating the procedure on par with ICH criteria, extension of the method for drug quantification from marketed tablets, stress degradation tests and drug estimation from spiked plasma samples. In this attempt, we have avoided the use of organic solvents at any step, and the entire process used 0.1M SLS and hence the method comes under green analysis.

*Ceema Mathew

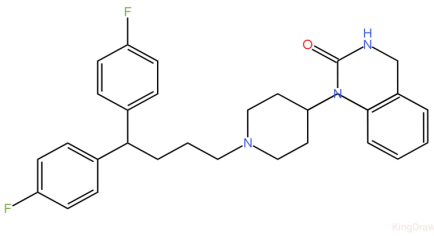

Figure 1. Chemical Structure of Pimozide

2. Materials and methods

2.1. Instrumentation

We used the Shimadzu 5301 Spectrofluorophotometer, facilitated the with RFPC software for recording the photo-luminescent process.

2.2.Drugs and chemicals

The complimentary sample of Pimozide (purity=99%w/w) supplied by Vasudha Pharmaceuticals Pvt Ltd, Hyderabad and Pimide tablets with a labelled strength of 4 mg manufactured by Dd Pharmaceuticals Ltd(India) was used for the work. Tween 80 (purity =98%v/v) and Tween 60 (purity = 98%v/v) were procured from Quality Deals India, Hyderabad. SLS (purity =99.5%) and urea AR grade(purity =99.5%w/w) were from Sisco Research Laboratories Pvt. Ltd. Methanol (purity =99.85%) was available from Qualigens, Mumbai. Double distilled water was prepared in-house.

2.3 Solvent optimisation and wavelengths selection

We investigated PMZ for its fluorescence property in various solvents, including methanol, sodium lauryl phosphate (0.1M), urea (0.1M), phosphate buffer, and tween 80, and we found that it is soluble and fluorescent in all the solvents, with the magnitude of the fluorescence being maximum in the sample generated with 0.1M SLS (prepared by dissolving 2.888 gm of sodium lauryl sulphate in 100 mL of water).

2.4 Validation of analytical method

The methods listed below were employed to verify the approach for linearity, accuracy, Precision, LOD, and LOQ on par with ICH specifications [16].

2.4.1 Linearity: The PMZ reference material was measured and then placed in a 10 mL measuring container, amalgamated with 0.1M SLS, sonicated, and the volume was made up to the mark; 1 mL of the solution was further diluted to 10 mL using 0.1M SLS to produce an appropriate concentration of PMZ (100 µg/mL). The undiluted standard PMZ solution (100 µg/mL) was reconstituted using 0.1M SLS to yield an effective test solution with PMZ levels ranging from 1 to 10 µg/mL. A spectrofluorometer was then employed to analyze the solution. We noted the fluorescence level of the solution at a specific emitting wavelength. This procedure was used on three separate sets of freshly prepared samples. A measuring curve was built by graphing the fluorescence intensity versus the medicine dosage. We estimated the values of the coefficients and regression equation.

2.4.2 Accuracy: The strategy's validity has been assessed by computing PMZ restoration utilizing the standard additive method. The pimozide pill powdered has been infused with 80%, 100%, and 120% of the standard pimozide solution. The value of PMZ can be estimated by analyzing the fluorescence spectra at λEX at 282 nm and λEM at 314 nm. The accuracy parameter was validated by calculating the medicine recovered at every specified level. The process is done for triplicate preparation to estimate the extent of deviation from each set.

2.4.3 Precision: By measuring the equivalent output multiple times during the same day for three distinct PMZ dosages, the suggested spectrofluorimetric approach was tested for intra-day Precision. (2, 6, and 10 µg/mL). Comparably, the inter-day Precision of the proposed spectrofluorimetric approach was assessed using comparable PMZ levels, and the related reply was measured on three occasions on three distinct days of the week. The intra and interday precision results were expressed as a percentage of the standard deviation (% RSD).

2.4.4 Limit of Detection (LOD) and Limit of Quantification (LOQ): Quantification (LOQ) and detection (LOD) limits were calculated using the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) on par with the guidelines of the International Conference on Harmonisation. LOD = 3.3 x σ/S LOQ = 10 x σ/S where , σ= Response's standard deviation S= slope of the calibration study

2.5 Assay of PIMIDE tablet

Twenty standard formulations (PIMIDE tablets) carrying 4 mg of PMZ were measured precisely. The weight was averaged and processed into an ultra-fine powder. In a 5 mL volumetric flask, a measured amount of powder that was comparable to 5 milligrams of pimozide was subjected to ultrasonic dissolution with 0.1M SLS over 15 minutes. The bottle was shaken, and the volume was done to the required level with 0.1M SLS. The mixture of ingredients was filtered using Whatmann filter paper (NO. 41). One mL of the filtrate was shifted in a 10 mL volumetric flask, and the volume was 10 mL with the solvent (100 µg/mL). Further, a 5 µg/mL solution was prepared similarly with the same solvent. The result of this procedure is now used to calculate pimozide. The necessary amount of pimozide was calculated by quantifying the degree of fluorescence at the proper wavelengths (excitation at 282 nm and emission at 314 nm). The fluorometric reactions were entered in a mathematical equation reflecting the calibration graph of PMZ, and the drug concentration was computed. The assay parameters were determined using the drug concentration, dilution factor, tablet average weight, and tablet powder weight.

2.6 Degradation Studies

Stress degradation studies were done on PMZ using different stress factors such as acid,alkali, H2O2 and water and the process is given hereunder.

For acidic degradation, one mL PMZ conventional stock solution was shifted into a series of 10 mL volumetric flasks to obtain a concentration of 10 µg/mL. To this, freshly prepared 0.1M HCl was added to 1 mL of standard PMZ solution to prepare acidic degradation products. Then, they were kept in a water bath for up to 50mts, scanning at every ten mts interval to study the degradation process after the volume was done with 0.1M SLS.

For Alkaline Degradation, aliquots volume of 1 mL PMZ ordinary stock solution was transferred into a series of 10 milliLitre volumetric flasks to obtain a concentration of 10 µg/mL. To this, freshly prepared 0.1M NaOH, which is prepared for 10 mL, was added along to 1 mL of standard PMZ solution to prepare alkaline degradation products respectively kept in a water bath up to 40mts, scanning at every ten mts interval to study the degradation process after final volume was made up with 0.1M SLS.

For oxidative degradation, aliquots volume of 1 mL PMZ standard stock solution was transferred into a series of 10 mL volumetric flasks to obtain a concentration of 10 µg/mL. To this, 5 % of hydrogen peroxide solution is added to produce oxidative degradative products kept aside for a water bath of up to 15 mts, scanning at every five mts interval to study the degradation process after the final volume was made up with 0.1M SLS.

For hydrolytic degradation studies, a series of 10 mL volumetric flasks were loaded with aliquots, and a volume of 1 mL PMZ standard stock solution was transferred into a series of 10 milliLitre volumetric flasks to obtain a 10 µg/mL concentration. To these, 4 millilitres of water are added and kept aside for 15 minutes. The water is scanned every five minutes to check the degradation process after the final volume comprises 0.1M SLS.

2.7 Study on Biological Fluid

We transferred the drug-free human plasma of about 0.5 mL to a series of centrifuge tubes, and working solutions were spiked at various concentrations ranging from 1.0 to 10 µg/mL. 2 mL of acetonitrile was added as a protein precipitant. Each tube comprises 5 mL of the solvent used in the procedure and is centrifuged at 3000 rpm for approximately 30 minutes. Supernatants of each solution were scanned for fluorescence.

3. Spectrofluorimetric results for Pimozide

3.1 Optimized solvent and measurement wavelengths

After investigating pimozide solubility and fluorescence qualities, when compared to other solvents, 0.1M SLS had the maximum fluorescence intensity. As a result, stock solutions and further dilutions were carried out using 0.1M SLS. Though methanol also showed good fluorescence, we opted to use 0.1M SLS because the fluorescence intensity of pimozide in 0.1M SLS is higher, and we wanted to limit the use of organic solvents. Solvent selection details are depicted in Fig 2. With 0.1M SLS, the excitation wavelength of pimozide was found to be 282 nm. The emission spectrum of PMZ was acquired using the excitation wavelength after scanning in a wavelength range of 250-400 nm, and the emission wavelength was found to be 314 nm and given in Fig 3.

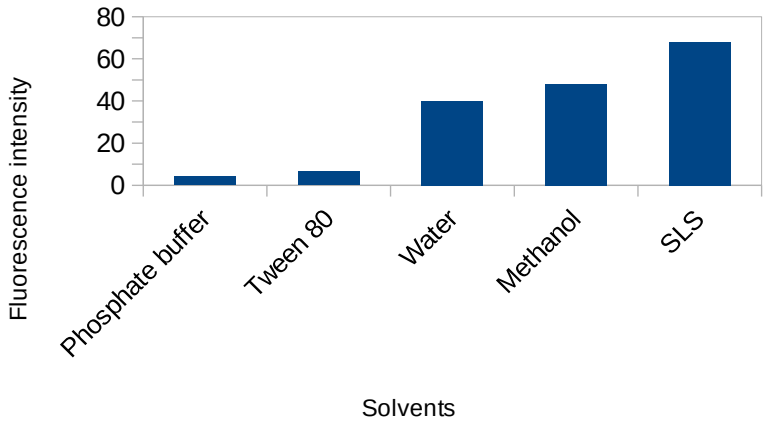

Figure 2. Solvent Selection for PMZ

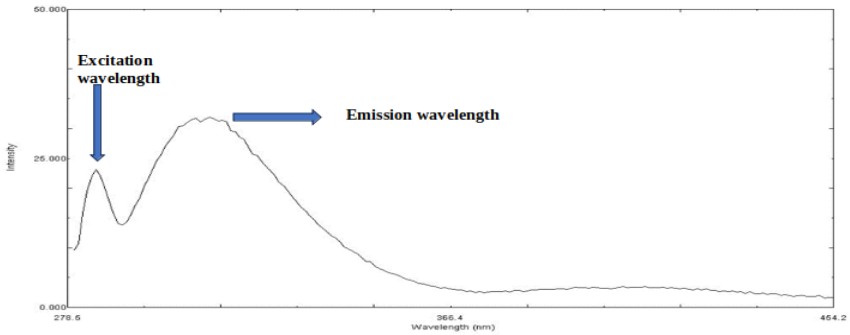

Figure 3. The Emission Spectra of PMZ

3.2 Analytical Method Validation

The validation findings for each parameter are discussed further below.

3.2.1. Linearity: Within the concentration range of 1-10 µg/milliliter. The PMZ, the relationship within concentration (µg/mL) and the degree of fluorescence was linear. The correlation coefficient (R2) for PMZ was determined by linear regression analysis. The fluorescence intensity for PMZ at

the emission wavelength of 314 nm rose as the concentration increased. The PMZ linearity data is presented below in Table 1. The fluorescence spectra denoting the excitation and emission wavelengths are illustrated in Fig 4 as an overlay emission spectrum of PMZ, and the linearity graph is given in Fig 5.

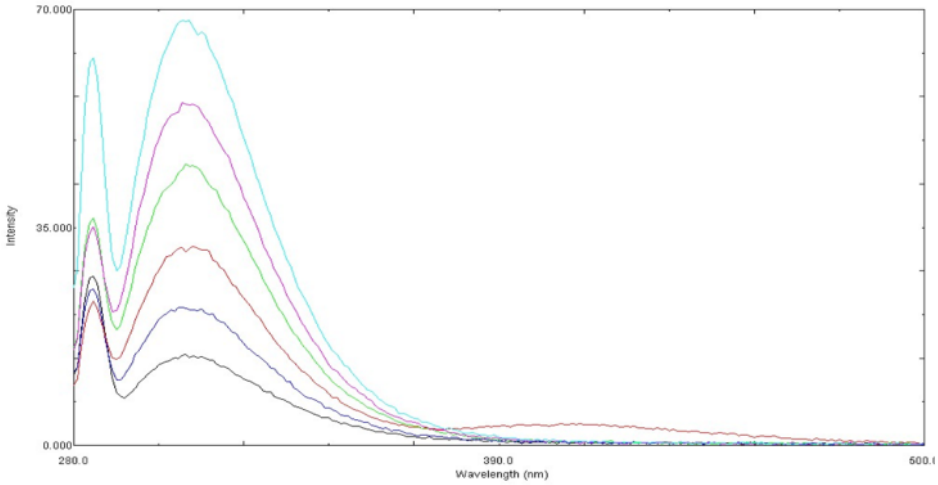

Figure 4. The Overlay Emission Spectra of PMZ

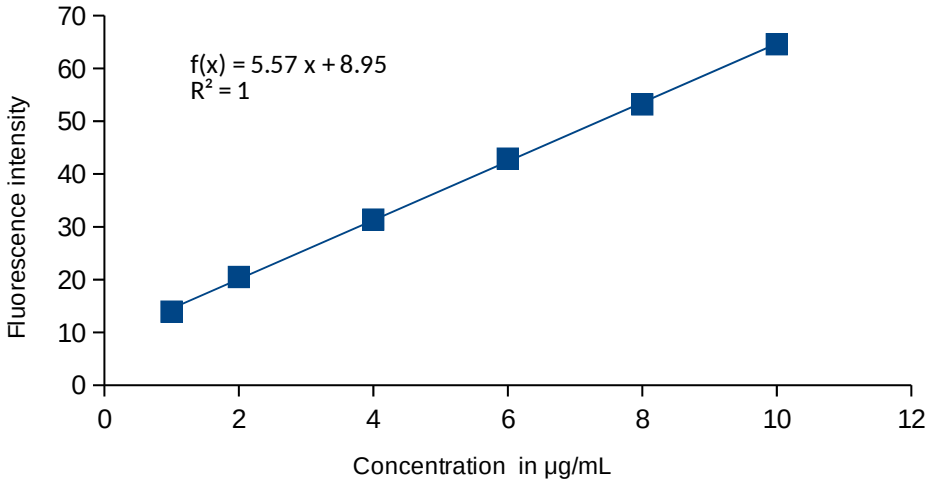

Figure 5. Linearity Graph of PMZ

Table 1: Linearity Data of Pimozide

S.NO	Con. (µg/mL)	Fluorescence Intensity AM±S.D (n=3)
1	1.0	13.925±0.012
2	2.0	20.499±0.028
3	4.0	31.369±0.051
4	6.0	42.902±0.054
5	8.0	52.55±0.066
6	10.0	65.974±0.014

3.2.2. Accuracy (Recovery Studies): The precision was ascertained using the traditional addition technique. In triplicate, three different levels of standards (80%, 100%, and 120%) were spiked to commercial pills. Table 2 determines the average percentage of recoveries and % RSD values. The per cent recovery of PMZ was determined to be good, ranging from 98.8 to 102.8%.

Table 2: Accuracy Data of Pimozide

Spiking level	sampleConc. (µg/mL)	Conc. Of std	Total con. (µg/mL)	Amount recovered* (µg/mL)AM±SD	Recovery (%)	RSD** (%)
80%	4.0	3.2	7.2	7.12 ± 0.025	98.8	0.351
100%	4.0	4.0	8.0	8.06 ± 0.043	100.7	0.533
120%	4.0	4.8	8.8	9.05 ± 0.055	102.88	0.607

* triplicate ** Acceptance criteria: % RSD should not be more than 2

3.2.3. Precision: The method's repeatability (Intra-day precision) was determined using an Intra-day (n=3) analysis of three separate PMZ standard solutions at various strengths of 2, 6, and 10 micrograms/millilitre. For PMZ, the % RSD of repeatability was less than 2. The inter-day (n=3) analysis of 3 PMZ typical solutions at various concentrations of 2, 6, and 10 µg/mL indicated intermediate precision. For PMZ, the % RSD for Inter-day and Intra-day analysis was less than 2. This statistical data indicated high precision. The precision data are shown in the table 3 below.

Table 3: Precision Data of Pimozide

Conc. (µg/mL)	Intra-day		Inter-day	
	Conc. found (µg/mL) AM±SD (n=3)	% RSD*	Conc. found (µg/mL) AM±SD (n=3)	% RSD*
2.0	1.75±0.01	0.262	1.8±0.01	0.261
6.0	5.8±0.01	0.124	5.7±0.01	0.119
10	9.8±0.07	0.722	9.7±0.08	0.837

*Acceptance criteria: % RSD should not be more than 2

3.2.4.Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were found to be 7.28 ng/mL and 21.85 ng/mL for PMZ by the optimized spectrofluorimetric method.

3.3 Assay

The method's accuracy was determined by assaying commercially supplied tablets (Pimide) containing 4 mg of PMZ. The PMZ results are shown in Table 4.4 after being juxtaposed to the matching designated amounts. The quantity of PMZ detected was 4.006 mg, with an assay value of 100.15%. These amounts fall within the parameters. The formulation's %RSD was found to be 2, indicating that the proposed approach was accurate.

Table 4: Assay Results of PIMIDE

Label claim (mg)	Amount found AM±SD(mg)	Assay (%)	% RSD
4	4.006±0.0100	100.15%	0.249

3.5.Results of Degradation Studies

The acid degradation investigations were carried out using 0.1M HCl produced for 10 mL heated in a water bath for 10, 20, 30, 40 and 50 minutes at a temperature of 60°C. The First derivative spectrum of unstressed PMZ and the derivative spectrum of degraded samples at the above time intervals are depicted in Fig 6 and & 7, respectively.

The alkaline degradation tests were carried out using 0.1M NaOH produced for 10 mL heated at 60°C in a water bath for various time intervels for different periods (10, 20, 30, and 40 minutes). The degradation spectrum is depicted in Fig 8. The product degraded entirely after 40 minutes.

Oxidative degradation examinations were conducted using 5 mL of hydrogen peroxide (10%) mixed with 1 mL of standard stock solution set aside for varied time periods of 0, 5, 10, and 15 minutes. The degradation spectrum is depicted in Fig 9. It degraded after 15 minutes.

The Hydrolytic degradation studies have been done with the help of 4 mL water and added to 1 mL of Standard Stock solution kept for heating for different time intervals, 0,5,10 and 15 minutes, respectively. The degradation spectrum is shown in Fig 10. Complete degradation achieved after 15 minutes.

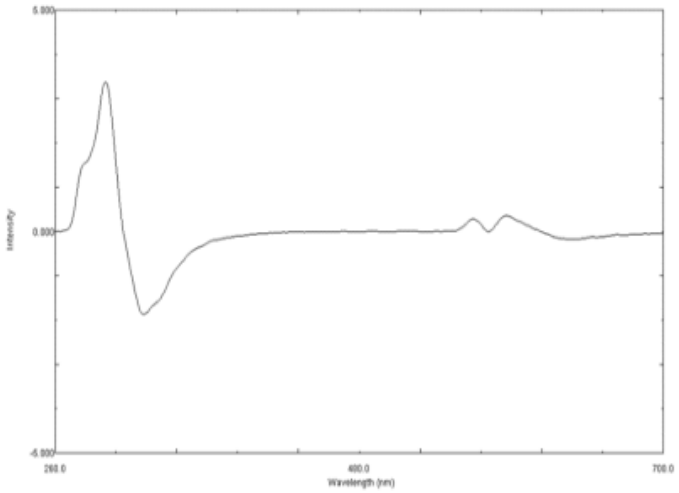

Figure 6. First Derivative Fluorescence Spectrum of Unstressed Pimozide

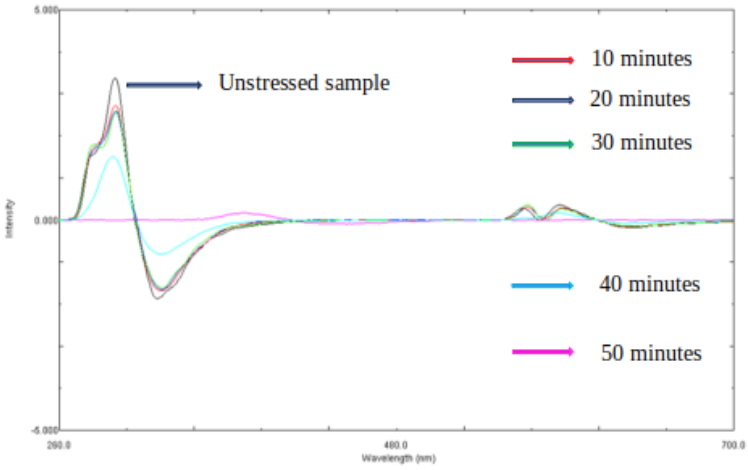

Figure 7. First Derivative Fluorescence Spectrum of Acidic Degradation

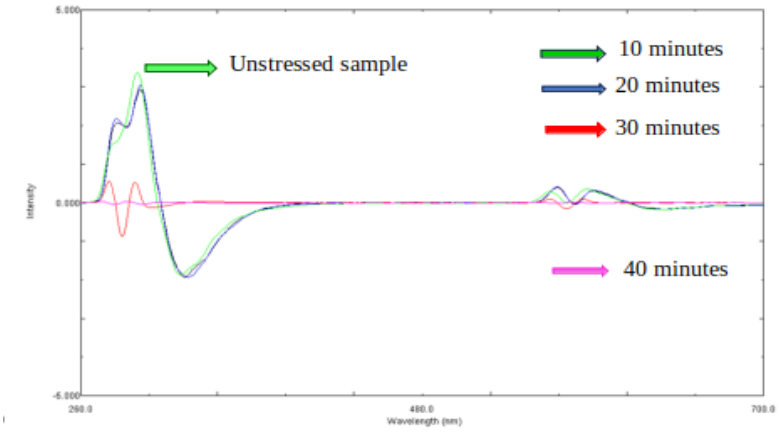

Figure 8. First Derivative Fluorescence Spectrum of Alkaline Degradation

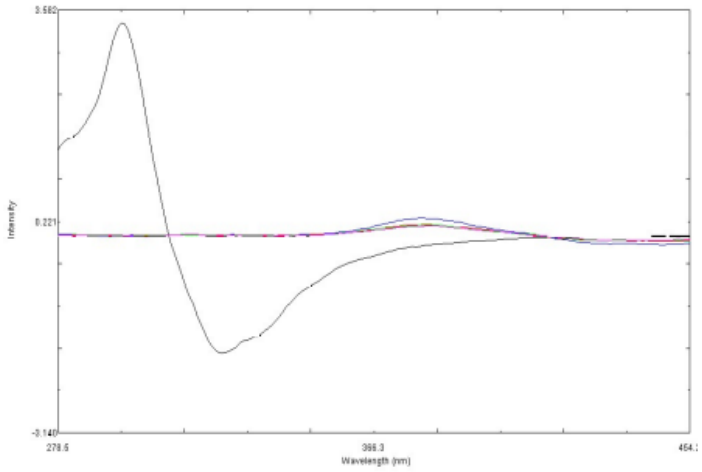

Figure 9. First Derivative Fluorescence Spectrum of Oxidative Degradation

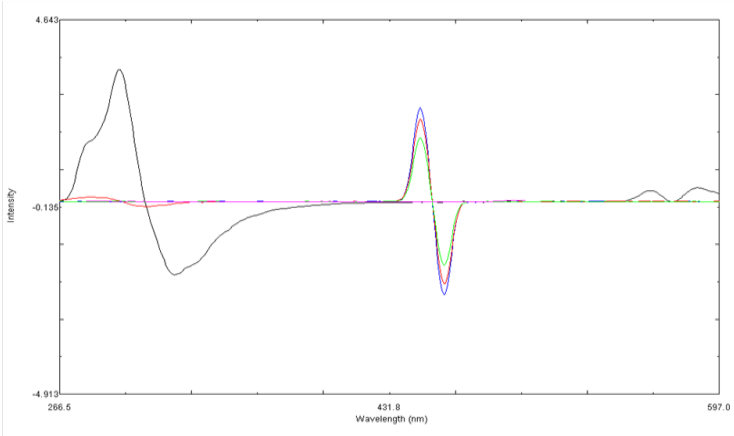

Figure 10. First Derivative Fluorescence Spectrum of Hydrolytic Degradation

3.6. Application To Biological Fluids

The very sensitive approach was employed for PMZ determination in spiked human plasma, and the concentration range of PMZ for human plasma investigations is 1-6 µg/ml, as shown in the fig.11. The percentage recovery obtained in a range of 95.3 to 98.5 is shown in table 5.

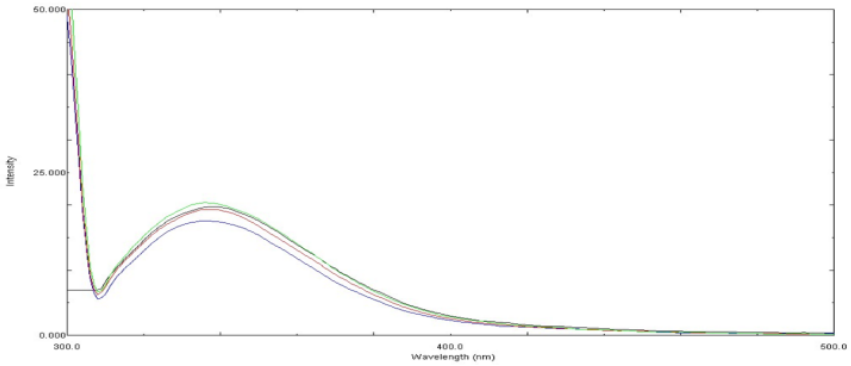

Figure 11. Overlay Emission Spectra Of PMZ in Spiked Human Plasma (1-6 Mg/Ml)

Table 5. % Recovery Data In Spiked Plasma Sample

Concentration (µg/mL)	% Recovery[a]	%RSD
1.0	95.3	0.87
2.0	97.8	0.75
4.0	98.2	1.24
6.0	98.5	1.34
8.0	96.8	1.22

[a] Average of three replicates.

4. Conclusion

Although numerous methods for determining and validating pimozide have been established individually, the current report is a precise, simple, and accurate approach using spectrofluorimetric. We arrived at the following conclusions after the study based on the results obtained. An appropriate spectrofluorimetric technique was created using 0.1M SLS in water. In the diluent impact research, PMZ fluoresced in 0.1M SLS and was thus chosen for further examination in the current approach. We preferred to use it as the solvent inorder to avoid the use of organic solvents, thereby developing an environmentally friendly method. The wavelength of pimozide emission was measured to be 314 nm, and the excitation wavelength was found to be 282 nm. Linearity was observed for PMZ in the 1-10 µg/mL range (R^2=0.9995).The validation results are on par with ICH criteria and accuracy and precision was achieved as the per cent RSD was less than 2. The testing value for the PIMIDE tablet is 100.15%. Pimozide detection and quantification limits were 7.28 ng/mL and 21.85 ng/mL respectively, thus proving a very sensitive method and hence it can be beneficial for the drug content experiments in biological samples. The percentage recovery for spiked human plasma is 97.45%. The degradation process is examined using the first derivative emission spectrum; the process is fast in oxidative conditions followed by hydrolytic, alkaline and acidic conditions.

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