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Hydrotropic Solubilization Green method for Quantitative determination of Azathioprine in Pharmaceutical products

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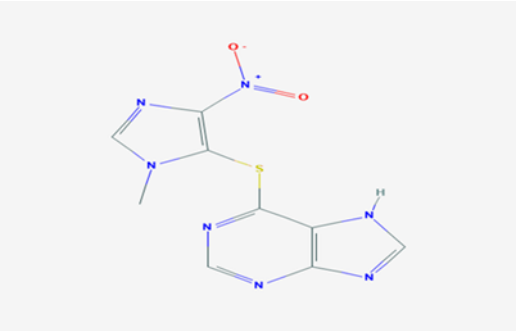
Abstract
Improving the aqueous solubility of drugs that are weakly water soluble can be done through variety of techniques. Hydrotropy is a solubility enhancement technique that uses hydrotropes such as sodium benzoate, sodium salicylate, sodium citrate, urea, ibuprofen sodium, and niacinamide to improve the solubility of poorly water-soluble drugs. Hydrotropic solubilization was employed in this study to solubilize weakly water-soluble drug like Azathioprine utilizing hydrotropic agents like sodium benzoate (2M) and sodium salicylate (2M).Azathioprine shows maximum absorbance at 279.45nm, when sodium benzoate is used as a hydrotropic agent. In the 5-25 µg/ml concentration range, this substance follows the beers law.Azathioprine shows maximum absorbance at 265.30nm, when sodium salicylate is used as a hydrotropic agent. In the concentration ranging from 5 to 25 µg/ml, this substance follows the beers law. The findings of studies have been statistically confirmed, as well as by re-examination validation studies. According to the ICH guidelines, validation parameters such as linearity, range, and assay were investigated.

Key Words: Solubility, Hydrotropic, First order derivative, Green method, Sodium Benzoate, Sodium Salicylate, Azathioprine

Introduction:

Azathioprine is poorly water-soluble drug and an immunosuppressant used to treat rheumatoid arthritis, Crohn's disease, and ulcerative colitis, as well as to avoid renal transplant rejection (Mallick and Malik, 2022).



Azathioprine

The therapeutic effectiveness of a medicine is determined by its bioavailability and, eventually, its solubility (Ali and Omer, 2022; Bhalani et.al. 2022). Solubility is a crucial characteristic for achieving the appropriate drug concentration in the systemic circulation and demonstrating pharmacological response (Nidhi et.al.; Agnihotri and Chauhan, 2020).Hydrotropic is a solubilization process in which a significant amount of a second solute increases the previous solute's water solubility (Maangar et.al. 2020; Kurkute et. al, 2022). Many poorly water-soluble drugs have been observed to benefit from concentrated aqueous hydrotropic solutions of sodium benzoate, sodium salicylate, urea, nicotinamide, sodium citrate, and sodium acetate (Sampathkumar et.al. 2013; Kumar and Singh, 2016; Hatwar et.al. 2022). In this current study, an effort is made to improve the aqueous solubility of the selected drug Azathioprine utilizing hydrotropic agents (Maheshwari et.al. 2007)like sodium benzoate (2M) and sodium salicylate (2M), as there is no reported method is available so far to enhance water solubility and establish linearity of selected drug molecules by hydrotropic solubilization technique.

Materials and Methods:

The drug solubility was tested by using different types of solubilisation agents and solubility was checked by using various molar concentrations of solubilising agents. The solubility details obtained are listed in Table 1.

Sl. No.	Drugs	Solubilizing Agents	Trails	Concentration	Result
01	Azathioprine	Sodium citrate	Trail-1	1M	No solubility & precipitation
			Trail-2	2M	No solubility & precipitation
		Sodium acetate	Trail-3	1M	No solubility & precipitation
			Trail-4	2M	No solubility & precipitation
		Urea	Trail-5	1M	No solubility & precipitation
			Trail-6	2M	No solubility & precipitation
		Sodium benzoate	Trail-7	1M	No solubility & precipitation
			Trail-8	2M	Completely soluble
			Trail-9	1M	No solubility &

		Sodium salicylate			precipitation
			Trail-10	2M	Completely soluble

Table 1: Identification of solubilizing agent

Procedure:

A. The first trial begins with a solubility test of the drug in a 1 M solution of the solubilizing agent in distilled water, performed in the order of preference. If complete solubility is achieved, no additional solubility tests are necessary.

B. If the chemicals under test are insoluble in either the dilution or treatment mediums, go to trial 2 by adding a significant amount of medium (about 2 M) to try to dissolve the drugs. Further steps are not required if the chemicals under test are dissolved in a medium at a concentration of 2M. Stop attempting to dissolve the chemical if it does not dissolve in one medium or the other. There are no additional solubility tests needed if the drugs are soluble in any of these solvents.

C. If the drug remains insoluble in any of the trial 2 media, move on to trial 3 and change the solubilizing agent. There is no more solubility tests required if the drug is soluble. Table 1 contains the specifics.

Validation:

Statistics and re-examination studies have both validated the conclusions of previous investigations. Validation parameters such as linearity, range, and assay were investigated using marketed formulation of azathioprine at 279.45nm, At N=4 when sodium benzoate is used as a hydrotropic agent. Azathioprine at 265.30nm, At N=3 when sodium salicylate is used as a hydrotropic agent and UV first derivative absorption maximum to establish the testing conditions. The current approach, however, should be revalidated in accordance with ICH or USFDA guidelines. (Sharma et.al. 2018).Figures 1 to 8 and Tables 2 and 3 features data.

Sl.no.	**Concentration**	**Absorbance**
01	5µg/ml	0.5
02	10µg/ml	0.6
03	15µg/ml	0.7
04	20µg/ml	0.8
05	25µg/ml	0.9

Table 2:Linearity Data table and range of Azathioprine N=4 using Sodium Benzoate as solubilizing agent

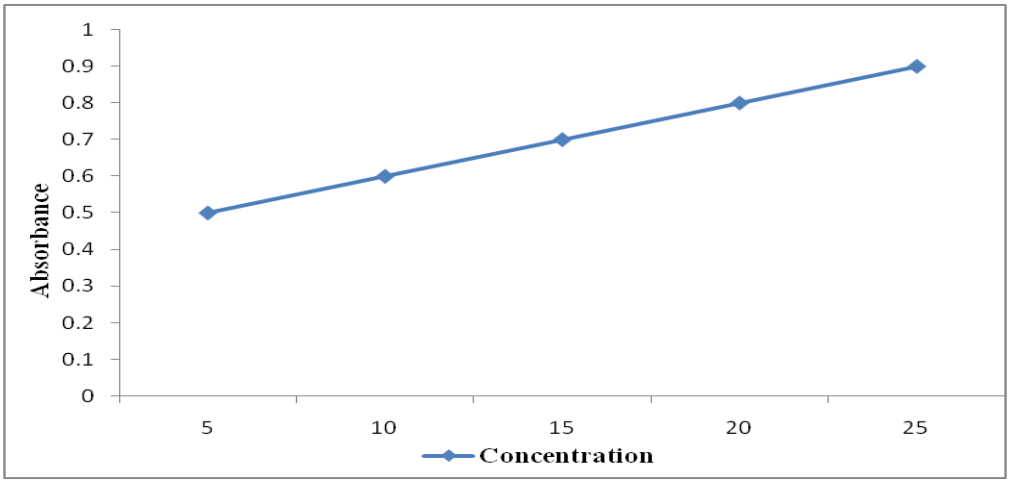


Figure 1:Linearity graph of experimental data for Azathioprine at N=4.

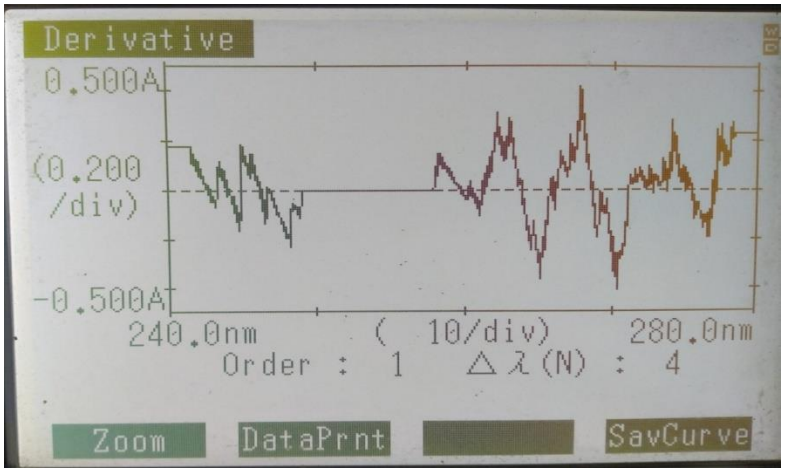


Figure 2: Drug concentration 5 microgram per ml

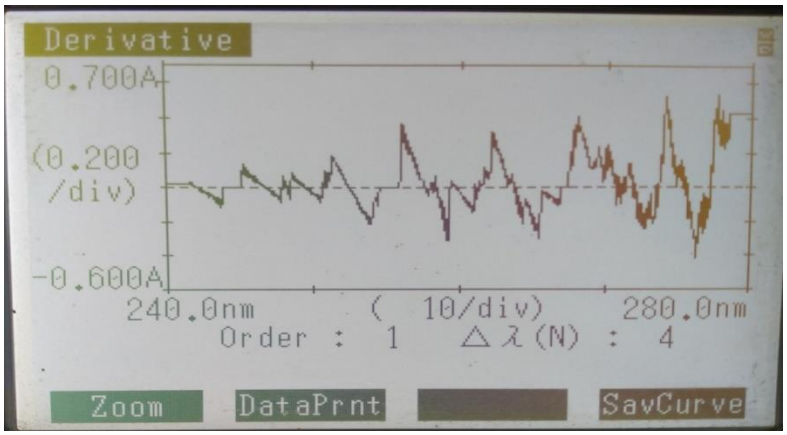


Figure 3: Drug concentration 15 microgram per ml

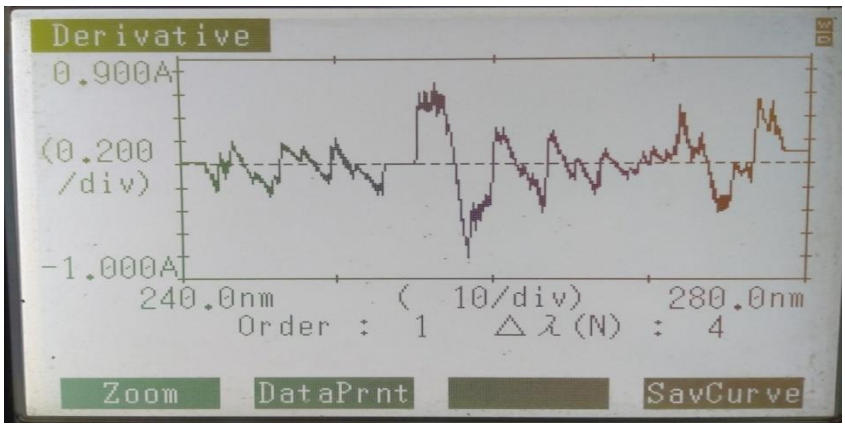


Figure 4: Drug concentration 25 microgram per ml

Sl. no.	Concentration	Absorbance
01	5µg/ml	1.1
02	10µg/ml	0.9
03	15µg/ml	0.7
04	20µg/ml	0.5
05	25µg/ml	0.3

Table 3:Linearity Data table and range of Azathioprine N=3 using Sodium Salicylate as solubilising agent

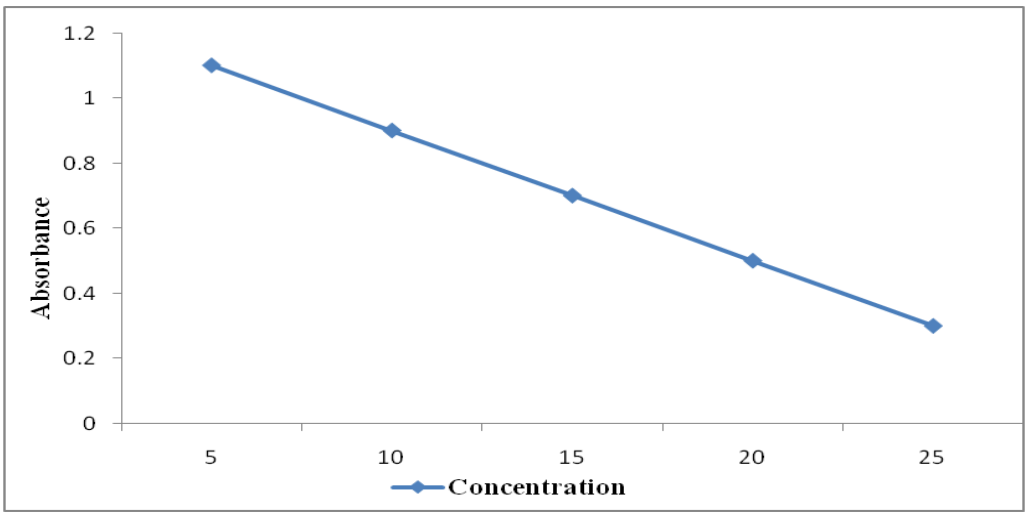


Figure 5:Linearity graph of experimental data for Azathioprine at N=3.

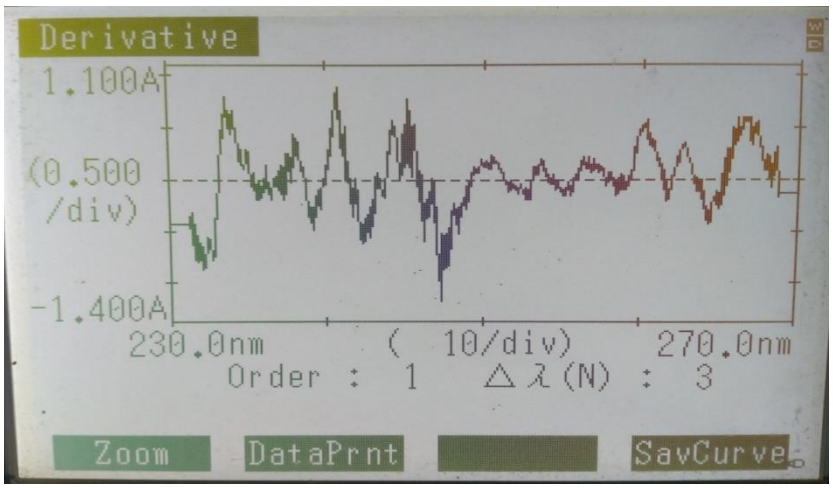


Figure 6:Drug concentration 5 microgram per ml

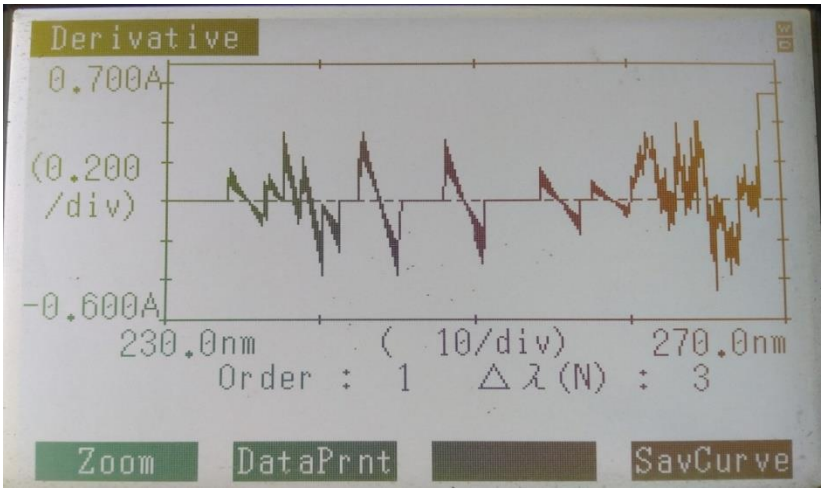


Figure 7: Drug concentration 15 microgram per ml

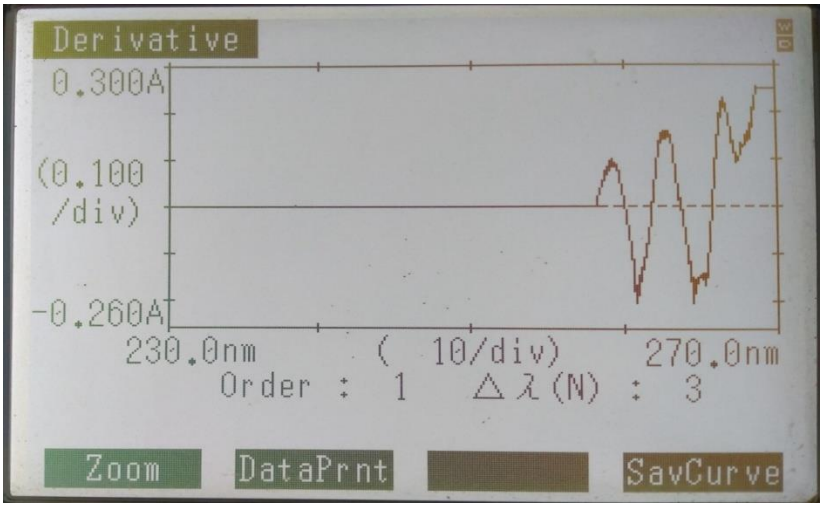


Figure 8: Drug concentration 25 microgram per ml

Assay:

1) Assay of Azathioprine is performed when sodium benzoate is used as a hydrotropic agent.

Sl No.		Concentration	Absorbance	% Purity
01	Standard	22 µg/ml	0.84	101.19%
02	Test	22 µg/ml	0.85	

Table 4: Assay Table of Azathioprine

2) Assay of Azathioprine and Dexamethasone performed when sodium salicylate is used as a hydrotropic agent**.**

Sl No.		Concentration	Absorbance	% Purity
01	Standard	23µg/ml	0.5	98%
02	Test	23µg/ml	0.49	

Table5: Assay Table of Azathioprine

Sl No	Solubilizing agents	Concentration	Scanning range	Absorption maxima	Absorbance	Graph obtained
01	Sodium Benzoate	5-25µg/ml	240-280nm	279.45nm	Increased absorbance	Linear
02	Sodium Salicylate	5-25µg/ml	230-270nm	265.30nm	Decreased absorbance	Linear

Table 6: Comparison Tables of Hydrotropic Agents

Results and Discussion:

The solubility of the weakly water-soluble drug Azathioprine was improved by introducing the second solutes, Sodium benzoate (2M) and Sodium salicylate (2M). Sodium benzoate and sodium salicylate were chosen since the drugs were insoluble in other solubilizing agents. Other solubilizing agents used include sodium citrate, sodium acetate, and urea. The drug Azathioprine is not solubilized in sodium citrate at concentrations of 1M and 2M, and a clear solution is not formed. Similar to sodium citrate, Sodium acetate has been tried at various molarities, but no clear solution has been formed, and the same is true for Urea. However, medicine is entirely soluble in Sodium benzoate (2M) and Sodium salicylate (2M), and the graph is linear. The rate of solubility increases as the graph becomes more linear.

According to ICH guidelines, the developed technique was validated for various parameters such as range, linearity, and assay, with satisfactory results. The objective of this project was to design a quantitative analytical approach for the quantitative estimation of selected bulk medicine made for cost-effective routine analysis, based on the above results and discussion. The above study is superior since the UV visible spectrophotometer employed is simple to operate and cost effective.
Two hydrotropic agents are employed to make drug completely soluble in water; therefore, a comparison of the two hydrotropic agents has been made.
Azathioprine is poorly water-soluble drug. As a result, hydrotropic compounds are utilized to enhance the water solubility of this drug. Sodium benzoate and sodium salicylate are used as hydrotropic agents.In comparison to sodium benzoate, this drugrequires less quantity of sodium salicylate to be completely water soluble.
In the presence of sodium benzoate as a hydrotropic agent, Azathioprine scanned in the range of 240-280nm in the concentration range of 5-25g/ml. Azathioprine is scanned in the same concentration range as in the presence of sodium benzoate when sodium salicylate was used as a hydrotropic agent. However, azathioprine scanned between 230 and 270 nm.
In the presence of sodium benzoate as a hydrotropic agent, Azathioprine exhibits increased absorbance. While Azathioprine exhibits decreased absorbance in the presence of sodium salicylate as a hydrotropic agent.

Conclusion:

The newly developed Hydrotropic Solubilization process has been successfully used to improve the solubility of drug that is weakly aqueous soluble Azathioprine is accurate and exact. To improve the solubility of the first solute, sodium benzoate and sodium salicylate are added as second solutes. Quantitative estimation is carried out by using UV Visible spectrophotometer. Validation parameters like Range, Linearity and Assay are carried out.
As the solubility of a drug increases, so does its bioavailability, resulting in an increase in the medicine's efficiency. For a broader area of research, Accuracy, precision, repeatability, and reproducibility are some of the validation parameters that can be used.

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