

UV METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FLAVOXATE HYDROCHLORIDE AND OFLOXACIN USING MIXED HYDROTROPIC AGENT**Md Tausif Iqbal*, Anjana Bhardwaj****Millennium College of Pharmacy, Nathu Barkheda Rd, Near Neelbad, Bhopal (M.P)***Corresponding Author's E mail: tausifiqbal21@gmail.com

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ABSTRACT

A simple, accurate, and eco-friendly UV spectrophotometric method was developed and validated for the simultaneous estimation of Flavoxate Hydrochloride (FHC) and Ofloxacin (OFC) in combined tablet dosage form using a mixed hydrotropic agent system. Poor aqueous solubility of both drugs was successfully overcome by employing a hydrotropic mixture of 2 M sodium citrate and 8 M urea (1:1), which provided significant solubility enhancement without interfering with the absorbance maxima of the drugs. FHC and OFC showed maximum absorbance at 291.0 nm and 250.0 nm, respectively. The method exhibited linearity in the concentration range of 5–25 µg/mL for both drugs with correlation coefficients (r^2) of 0.999. The proposed method was validated in accordance with ICH guidelines, demonstrating good accuracy with recovery values close to 100%, and excellent precision as indicated by low %RSD values for repeatability, intermediate precision, analyst-to-analyst variation, and reproducibility. The assay of marketed tablet formulation showed satisfactory results with percentage label claims within acceptable limits. The developed UV spectrophotometric method is rapid, economical, precise, and environmentally benign, making it suitable for routine quality control analysis of Flavoxate Hydrochloride and Ofloxacin in bulk and pharmaceutical dosage forms.

Keywords: Flavoxate Hydrochloride, Ofloxacin, UV spectrophotometry, Mixed hydrotropy, Method development, Validation, ICH guidelines.

INTRODUCTION

Flavoxate hydrochloride and ofloxacin are frequently co-formulated and co-prescribed drugs used in the management of urinary tract infections and associated bladder spasms. Flavoxate hydrochloride is a synthetic antimuscarinic agent that relieves smooth muscle spasm of the urinary tract, thereby reducing dysuria, urgency, and frequency of micturition. Ofloxacin is a broad-spectrum fluoroquinolone antibiotic effective against both Gram-positive and Gram-negative uropathogens, making the combination therapeutically significant in urinary disorders¹. For routine quality control and assay of

such combination products, UV-visible spectrophotometry is widely employed due to its simplicity, rapid analysis, cost-effectiveness, and suitability for routine laboratory use ².

However, the major limitation in UV spectrophotometric analysis of poorly water-soluble drugs like flavoxate hydrochloride is their low aqueous solubility, which often necessitates the use of organic solvents. These solvents may be toxic, expensive, and environmentally unfriendly ³.

To overcome this limitation, the concept of hydrotropy has gained considerable attention in pharmaceutical analysis. Hydrotropy involves the use of high concentrations of certain hydrotropic agents such as sodium benzoate, sodium citrate, sodium salicylate, and urea to enhance the aqueous solubility of poorly soluble drugs. Mixed hydrotropic systems, which utilize a combination of hydrotropic agents, offer synergistic solubilization effects at lower individual concentrations, thereby minimizing potential interference in spectrophotometric measurements ⁴⁻⁵.

The application of mixed hydrotropic agents in UV method development eliminates the need for organic solvents while maintaining accuracy and precision. This approach aligns with green analytical chemistry principles and has been successfully applied for the estimation of various poorly soluble drugs in bulk and pharmaceutical dosage forms ⁶.

Therefore, the present work aims to develop and validate a simple, accurate, precise, and eco-friendly UV spectrophotometric method for the simultaneous estimation of flavoxate hydrochloride and ofloxacin using a mixed hydrotropic agent system, in accordance with ICH validation guidelines, for routine quality control analysis.

MATERIAL AND METHODS

Material

Flavoxate Hydrochloride and Ofloxacin reference standards were obtained as gift samples from a reputed pharmaceutical manufacturer. Marketed tablet formulation containing Flavoxate Hydrochloride and Ofloxacin was procured from a local pharmacy. Analytical grade sodium citrate, urea, and other reagents used for the preparation of the mixed hydrotropic system were purchased from standard suppliers. Double distilled/RO water was used throughout the study. All chemicals and reagents employed were of analytical reagent (AR) grade and used without further purification. UV spectrophotometric analysis was carried out using a calibrated double-beam UV–Visible spectrophotometer with matched quartz cells.

Methods

Selection of solvent system

Flavoxate hydrochloride (FHC) and ofloxacin (OFC) were individually scanned in various hydrotropic agents in spectrum mode over the UV range of 200–400 nm to select a suitable solubilizing system. Among the different hydrotropic solutions evaluated, a mixed hydrotropic system consisting of **2 M sodium citrate and 8 M urea (1:1)** was found to be the most appropriate. Both drugs showed good solubility and stability in this hydrotropic mixture and exhibited well-defined and reproducible UV spectra with clear λ_{max} values. Importantly, the selected hydrotropic system did not show any spectral interference at the absorption maxima of either drug, ensuring accurate spectrophotometric estimation, as also reported by **Aher et al. (2015)**⁷. Additionally, the mixed hydrotropic agent provided a significant enhancement in aqueous solubility, with more than **7–8fold increase for FHC** and **6–7fold increase for OFC**, thereby facilitating the development of a simple, reliable, and eco-friendly UV spectrophotometric method without the use of organic solvents.

Establishment of stability profile

Stability of both drugs was observed by dissolving FHC and OFC in 2M Sodium citrate: 8M Urea (1:1) solution used as solvent. Solution of FHC and OFC was prepared in the conc. of 10 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution⁸.

Linearity range and calibration graph

Preparation of Standard and Working Solutions for FHC and OFC

Preparation of Standard Stock Solution (Stock-A):

Standard stock solutions were prepared by dissolving 10 mg of each drug separately in 8 mL of a mixed hydrotropic solution containing 2M Sodium citrate: 8M Urea (1:1) in a 1:1 ratio. The solutions were sonicated for 10 minutes to ensure complete solubilization of the drugs, and the volume was then adjusted to 10 mL with the same mixed hydrotropic solution to obtain a concentration of 1000 $\mu\text{g/mL}$ (Stock-A) for both FHC and OFC.

Preparation of Sub-Stock Solution (Stock-B):

From Stock-A, 1 mL aliquots of FHC and OFC were withdrawn using a pipette and transferred into separate 10 mL volumetric flasks. Each was diluted up to 10mL with distilled water, resulting in a concentration of 100 µg/mL (Stock-B) for both drugs.

Preparation of Working Standard Solutions:

For FHC: Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL were withdrawn from Stock-B and transferred into 10 mL volumetric flasks. Each flask was then diluted up to 10 mL with RO water to obtain working solutions of 5, 10, 15, 20, and 25µg/mL, respectively.

For OFC: Similarly, 0.5, 1.0, 1.5, 2.0 and 2.5mL aliquots were withdrawn from Stock-B, transferred into 10 mL volumetric flasks, and diluted up to 10mL with RO water to achieve concentrations of 5, 10, 15, 20, and 25 µg/mL, respectively ⁹.

Selection of Wavelength for Linearity:

Solutions of 10µg/mL of FHC and OFC were prepared separately and scanned in spectrum mode over the range of 200–400 nm. The maximum absorbance was observed at 276 nm for FHC and 224 nm for OFC. Both drugs demonstrated linearity in the concentration range of 5–25 µg/mL at their respective λ_{max} values. Calibration curves were plotted with absorbance versus concentration to confirm linearity¹⁰. The selected wavelengths for further linearity and analytical studies were 291 nm for FHC and 250 nm for OFC.

Selection of wavelength for linearity

Solutions of 10µg/ml of FHC and 10µg/ml OFC were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of FHC and OFC was observed at 291.0 nm and 250.0 nm, respectively.

FHC and OFC showed linearity in the concentration range of 5-25µg/ml and 5-25µg/ml at their respective maxima ¹¹. Calibration curve was plotted, absorbance versus concentration.

Study of overlay spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of FHC and 10µg/ml of OFC were scanned in the spectrum mode over the range of 200-400 nm against water as blank and the overlain spectra of the two were recorded. FHC showed an absorbance peak at 291.0 nm,

whereas OFC at 250.0 nm. The overlain spectra also showed isoabsorptive points at 270.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method ¹².

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 291.0 nm and 250.0 nm that are λ_{max} of FHC and OFC respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1\text{cm}}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_{FHC} = \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \dots \dots \dots Eq. (1)$$

$$C_{OFC} = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \dots \dots \dots Eq. (2)$$

Where, A_1 and A_2 are absorbances of mixture at 291.0 nm and 250.0 nm respectively, a_{x1} and a_{x2} are absorptivities of FHC at λ_1 (291.0 i.e. λ_{max} of FHC) and λ_2 (250.0 i.e. λ_{max} of OFC) respectively and a_{y1} and a_{y2} are absorptivities of OFC at λ_1 and λ_2 respectively. C_{OFC} and C_{FHC} are concentrations of FHC and OFC respectively. The overlain spectra of both the drugs in 1:1 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio (A_2/A_1)/ a_{x2}/a_{x1} and a_{y2}/a_{y1}] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the FHC and OFC.

Validation of simultaneous equation method

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio ¹³.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of FHC and OFC to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels ¹⁴.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility¹⁵. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Analysis of tablet sample

Twenty marketed tablets of FHC and OFC were weighed and ground to a fine powder; amount equal to 10mg of FHC was taken in 10 ml volumetric flask. The OFC present in this amount of tablet powder was 10mg. Then 8 ml of 2M Sodium citrate: 8M Urea (1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times¹⁶⁻¹⁷.

Results and Discussion

The developed UV spectrophotometric method for the simultaneous estimation of **Flavoxate Hydrochloride (FHC)** and **Ofloxacin (OFC)** using a mixed hydrotropic system was systematically validated in accordance with **ICH Q2(R1) guidelines**, and the results demonstrate its reliability, accuracy, and suitability for routine quality control analysis.

The **overlain UV spectra (Figure 1)** clearly show well-defined and distinct absorption maxima for FHC at **291.0 nm** and OFC at **250.0 nm**, with no spectral interference between the two drugs. This confirms the appropriateness of the selected wavelengths for their simultaneous estimation and indicates the absence of interaction between the drugs in the hydrotropic solvent system.

Linearity studies (Table 1) revealed an excellent linear relationship between absorbance and concentration for both drugs over the range of **5–25 µg/mL**. The high correlation coefficients ($r^2 = 0.999$ for both FHC and OFC), along with low standard deviation and %RSD values, confirm the consistency and reproducibility of the analytical response. The regression equations further indicate good sensitivity of the method, as reflected by the steep slopes and minimal intercept values.

Accuracy of the method was confirmed through recovery studies at **80%, 100%, and 120%** levels (Table 2). The mean percentage recoveries for FHC ranged from **98.24% to 99.00%**, while for OFC they ranged from **98.88% to 98.96%**. The low SD and %RSD values (<1%) indicate minimal variability and affirm that common excipients present in the tablet formulation do not interfere with the estimation of either drug.

Precision studies, including repeatability, day-to-day variation, analyst-to-analyst variation, and reproducibility (Table 3), showed %RSD values well below the acceptable limit of **2%** for both drugs. These findings confirm that the method is precise under normal operating conditions and robust against minor variations in analytical parameters.

The applicability of the developed method was further validated by analysis of a commercial tablet formulation (Table 4). The assay results showed **99.33%** of label claim for FHC and **99.56%** for OFC, with low SD and %RSD values, demonstrating the method's suitability for routine quality control of combined pharmaceutical dosage forms.

The validation results establish that the proposed UV spectrophotometric method is **simple, accurate, precise, cost-effective, and environmentally friendly**, making it a reliable alternative to more complex analytical techniques for the simultaneous estimation of FHC and OFC in bulk and tablet dosage forms.

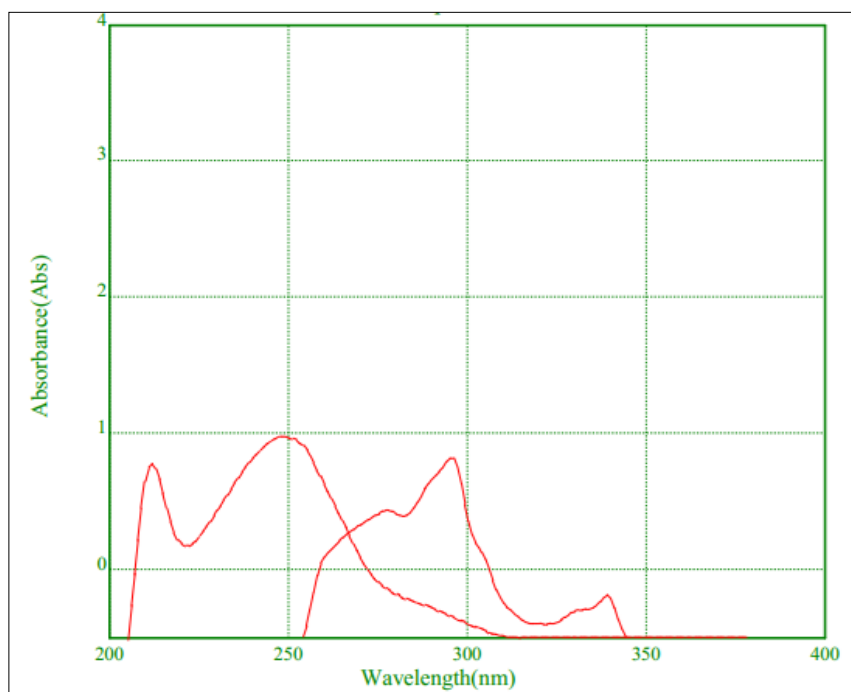


Figure 1: Overlain Spectra of both drugs

Table 1: Linearity statistics of linearity of FHC and OFC

Parameter	FHC	OFC
Linearity range (µg/ml)	5–25	5–25
λ_{max}	291.0 nm	250.0 nm
Regression equation	$y = 0.0505x + 0.003$	$y = 0.0407x + 0.009$
Correlation coefficient (r^2)	0.999	0.999
Slope	0.0505	0.0407
Intercept	0.003	0.009
SD range	0.001–0.002	0.001
%RSD range	0.056–0.511	0.082–0.531

Table 2: Summary of recovery studies of FHC and OFC

Drug	Recovery Level	Mean % Recovery	SD	% RSD
FHC	80%	98.89	0.471	0.476
	100%	99.00	0.398	0.402
	120%	98.24	0.708	0.720
OFC	80%	98.96	0.717	0.724
	100%	98.88	0.092	0.094
	120%	98.90	0.563	0.569

Table 3: Summary of precision studies for FHC and OFC

Precision Parameter	FHC Mean (%)	FHC SD	FHC %RSD	OFC Mean (%)	OFC SD	OFC %RSD
Repeatability	98.27	0.117	0.119	98.90	0.098	0.099
Day-to-Day Variation (Intermediate Precision)	98.51	0.150	0.153	98.54	0.101	0.102
Analyst-to-Analyst Variation	98.30	0.134	0.137	99.02	0.061	0.061
Reproducibility	98.35	0.112	0.114	98.22	0.103	0.104

Table 4: Analysis of tablet formulation of FHC and OFC

Drugs	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
FHC	200	198.65	99.33	0.116	0.125
OFC	200	199.12	99.56	0.265	0.274

CONCLUSION

A simple, rapid, and reliable UV spectrophotometric method was successfully developed and validated for the simultaneous estimation of **Flavoxate Hydrochloride (FHC)** and **Ofloxacin (OFC)** using a mixed hydrotropic agent system. The use of **2 M sodium citrate and 8 M urea (1:1)** effectively enhanced the solubility of both drugs without causing any spectral interference, thereby eliminating the need for organic solvents.

The developed method showed excellent **linearity** over the concentration range of **5–25 µg/mL** for both drugs, with high correlation coefficients ($r^2 = 0.999$). The method demonstrated satisfactory **accuracy**, with percentage recoveries close to 100%, and high **precision**, as evidenced by low %RSD values in repeatability, intermediate precision, analyst-to-analyst variation, and reproducibility studies. The assay results of the marketed tablet formulation confirmed the **applicability and reliability** of the method for routine quality control analysis.

References

1. El-Shaheny RN, El-Enany NM and Belal FF. Analysis of ofloxacin and flavoxate HCl either individually or in combination via a green chromatographic approach with a pharmacokinetic study of ofloxacin in biological samples. *Analytical methods*. 2015;7(11):4629-39.
2. Carson C and Naber KG. Role of fluoroquinolones in the treatment of serious bacterial urinary tract infections. *Drugs*. 2004;64(12):1359-73.
3. Singhal A, Saini U, Chopra B, Dhingra AK, Jain A and Chaudhary J. UV-Visible spectroscopy: a review on its pharmaceutical and bio-allied sciences applications. *Current Pharmaceutical Analysis*. 2024;20(3):161-77.
4. Kulkarni NS, Ghule SB and Dhole SN. A review on hydrotropic solubilization for poorly water soluble drugs: analytical application and formulation development. *Research Journal of Pharmacy and Technology*. 2019;12(7):3157-62.
5. Joshi J, Nainwal N and Saharan VA. Review on hydrotropy: A potential approach for the solubility enhancement of poorly soluble drug. *Asian J. Pharm. Clin. Res.* 2019;12(10):19-26.
6. Kuchekar AB, Gawade A and Boldhane S. Hydrotropic solubilization: an emerging approach. *Journal of Drug Delivery and Therapeutics*. 2021;11(1-s):200-6.

7. Jain N, Jain R, Kulkarni S, Jain DK and Jain S. Ecofriendly spectrophotometric method development and their validation for quantitative estimation of Pramipexole Dihydrochloride using mixed hydrotropic agent. *J Chem Pharm Res.* 2011;3(1):548-52.
8. Aher BO, Jain NP, Jain UN, Paithankar AR, Bagul TP and Gaikwad SS. Novel Spectrophotometric Estimation of Acyclovir Using Hydrotropic Solubilizing Agent. *Innovational Journal of Chemistry.* 2015; 1(3): 22-32.
9. Lurdhu Mary K and Manohar babuan S. Ecofriendly Spectroscopic Method for Estimation of Etraverine by Using Hydrotropic Agents. *World journal of pharmacy and pharmaceutical sciences.* 2015; 4(3): 655-662.
10. Maheshwari RK, Prasad S, Pandey P and Wanare G. Novel Spectrophotometric Analysis of Piroxicam Tablets Using Ibuprofen Sodium as Hydrotropic Solubilizing Agents. *International Journal of Pharmaceutical Sciences and Drug Research.* 2010; 2(3): 210- 212.
11. Rane J, Thakre V, Bakal RL and Patil S. Novel Spectrophotometric Estimation of Glliclazide by Using Mixed Hydrotropic Solubilization Phenomenon. *Journal of drug discovery and therapeutics.* 2015; 3(27): 8-10.
12. Khan MA. Enhancement of Solubility of Poorly Water Soluble Drugs Diclofenac Sodium by Mixed Solvency Approach, *International Journal of Research and Development in Pharmacy and Life Sciences.* 2015; 4(6): 1835-1837.
13. Chauhan A, Mittu B and Chauhan P. Analytical method development and validation: a concise review. *J. Anal Bioanal Tech.* 2015; 6(1): 1-5.
14. Ravisankar P, Gowthami S and Rao GD. A review on analytical method development. *Indian J Res Pharm Biotech.* 2014; 2(3): 1183.
15. Srivastava RK and Kumar SS. An updated review: analytical method validation. *Eur J Pharm Med Res.* 2017; 4: 774-84.
16. Devi TP, Setti A, Srikanth S, Nallapeta S, Pawar SC, Rao JV, et al. Method development and validation of paracetamol drug by RP-HPLC. *J Med Allied Sci.* 2013; 3: 8.
17. Daksh S, Goyal A and Pandiya CK. Validation of analytical methods strategy and significance. *Int J Res Dev Pharm Life Sci.* 2015; 4: 1489-97.