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UV spectrophotometric method development and validation for estimation of furosemide in the bulk and tablets dosage form

V. Padmabushana Chary◆, G. Sushmitha*, B. Mani*, MD. Humera* and M. Chinnaeswaraiah**

Department of Pharmaceutical Analysis, Anurag Pharmacy College, Kodad-508206, Telangana, India

**Department of Pharmacy, Anurag Pharmacy College, Kodad-508206, Telangana, India*

***Department of Pharmacognosy, Anurag Pharmacy College, Kodad-508206, Telangana, India*

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Abstract

For the determination of furosemide in the bulk and its tablet dosage form, a simple, rapid, precise, and accurate UV spectrophotometric method was developed and validated. The maximum absorbance (λmax) of furosemide with this method can be observed at 229 nm. In accordance with ICH (International Conference on Harmonisation) standards, the method was validated. With a correlation coefficient (R^2) of 0.999, the drug followed linearity in the concentration range of 1-6 µg/ml. The accuracy of the proposed method was assessed by applying the standard addition technique where the mean % recovery was found in the range between 99.24% to 99.83%. The LOD and LOQ were found to be 0.14 µg/ml and 0.43 µg/ml, respectively. Since the proposed method is accurate, sensitive and affordable, it can be used for routine analysis of furosemide in bulk and tablet dosage form in quality control testing laboratories.

1. Introduction

Furosemide belongs to a group of medicines called loop diuretics also known as water pills and it is used to reduce the swelling (edema) caused by too much water in the body in people who have heart failure, liver or kidney disease (Spinosa Bosch *et al.,* 2008**).** The chemical name of furosemide is 4-chloro-2-[(furan-2-methyl)amino]-5-sulfamoyl benzoic acid (Supriya *et al.,* 2018) The main mechanism of action of furosemide is blocking of sodium-chloride co-transport system in the kidney tubules and the loop of henle, which normally reabsorb water and electrolytes from the urine (Shailesh and Mitesh, 2017).

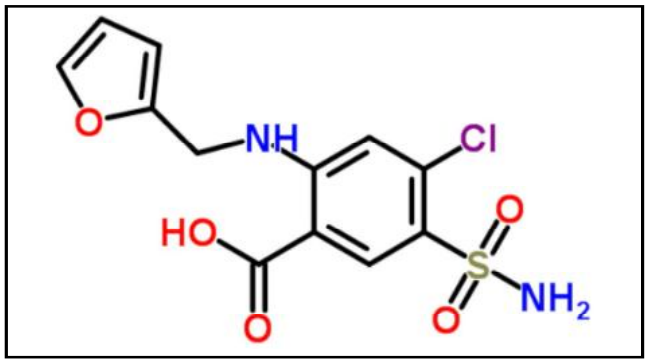

Figure 1: Furosemide chemical structure.

Corresponding author: Mr. V. Padmbhushana Chary

Assistant Professor, Anurag Pharmacy College, Kodad-508246, Suryapeta, Telangana, India

E-mail: padmabhushanchary@gmail.com

Tel.: +91-9949332171

Literature review revealed that there were several UV spectrophotometric methods reported for the estimation of furosemide in bulk and tablet dosage form (Gahandule and Banerjee, 2016.; Rahman Ahmed, 2020.; Supriya, Patel and Dhobale, 2018). In addition that separate UV spectrophotometry method was reported for the forced degradation study for furosemide without validation (Rohankumar *et al.,* 2019). There is one UV spectrophotometric (Patel and Sagar, 2012) and two HPLC methods (Rohankumar *et al.,* 2018.; Vijay Ram *et al.,* 2012) reported for the estimation of furosemide with spironolactone combination.

For optimization of the method, several trials were performed with different solvents such as chloroform, 0.1 N hydrochloric acid, methanol, acetone and 0.1 N sodium hydroxide and the drug was found to be insoluble in chloroform, hydrochloric acid, acetone and soluble in methanol (after sonicated for 10 min), 0.1 N sodium hydroxide solution. Finally, 0.1 N sodium hydroxide was found to be most suitable among the all solvents and absorption maxima with that solution was found at 229 nm.

2. Materials and Methods

2.1 Instruments

A UV-Visible spectrophotometer (Labindia) and UV-matched quartz cells (1 cm) were used for the measuring of λmax of the resultant solution of furosemide in the bulk and its pharmaceutical dosage form. An electronic weighing balance and a sonicator were used in this study.

2.2 Materials

A pure standard drug of furosemide was procured from Yarrow Chemicals Private Limited, India. Tablets of furosemide 40 mg (Lasix)

was purchased from the local pharmacy. All the chemicals and reagents used for this study such as sodium hydroxide, acetone, chloroform and methanol were obtained from Merck. To prepare 0.1 N NaOH solution distilled water was used as a solvent.

2.3 Standard stock solution preparation

50 mg of accurately weighed furosemide was taken into 100 ml volumetric flask, which was then dissolved in 0.1N NaOH using a sonicator for up to 5 min. The final volume was then adjusted with 0.1N NaOH to get the desired final concentration of 500 µg/ml. To obtain a final concentration of 100 µg/ml, 10 ml of the above mentioned solution was put into a volumetric flask of 50 ml and then diluted with 0.1N NaOH.

2.4 Working standard solution preparation

Pipette out precisely 0.3 ml of the furosemide solution from the standard stock solution mentioned above into a 10 ml volumetric flask, then diluted it with 0.1 N NaOH solution to get the desired final concentration of 3 µg/ml.

2.5 Selection of suitable solvent for method development (Sai Krupa Raj *et al.*, 2022)

To optimize the method, 10 mg of accurately weighed furosemide was dissolved separately in various solvents such as chloroform, methanol, acetone, 0.1N hydrochloric acid (0.1 N HCl) and 0.1 N sodium hydroxide (0.1N NaOH). The standard drug was found to be soluble in 0.1N NaOH and it was selected as a solvent for method development.

2.6 Selection of wavelength for analysis of furosemide

To find out the wavelength maximum for furosemide, 10 µg/ml solution was prepared with the help of 0.1N NaOH solution. The resultant outcome was scanned in the UV region between 200 to 400 nm. The ultraviolet (UV) spectrum of furosemide revealed that its maximum absorption was found at 229 nm (Figure 2).

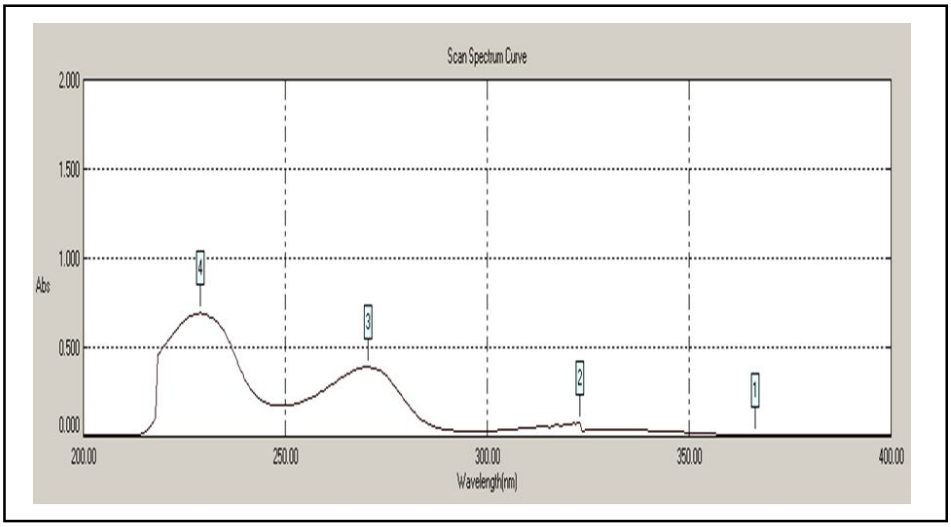

Figure 2: UV Spectra of furosemide.

3. Results

3.1 Validation of proposed UV spectrophotometric method

In accordance with the ICH specifications, the proposed method was verified for the following parameters (ICH, 2005).

Table 1: Results of linearity

Concentration (µg/ml)	Absorbance
1	0.118
2	0.226
3	0.327
4	0.439
5	0.534
6	0.656

3.1.1 Linearity

From the above standard stock solution series of furosemide (100 µg/ml), pipette out 0.1 to 0.6 ml of the solution into series of 10 ml of volumetric flasks separately and diluted up to the mark with 0.1N NaOH solution to obtain a concentration range of 1-6 µg/ml. These solutions were scanned at 229 nm with 0.1N NaOH as a blank solution. Table 1 represents the results of linearity. The calibration graph was plotted between concentrations versus absorbance to find out the correlation coefficient (Figure 3).

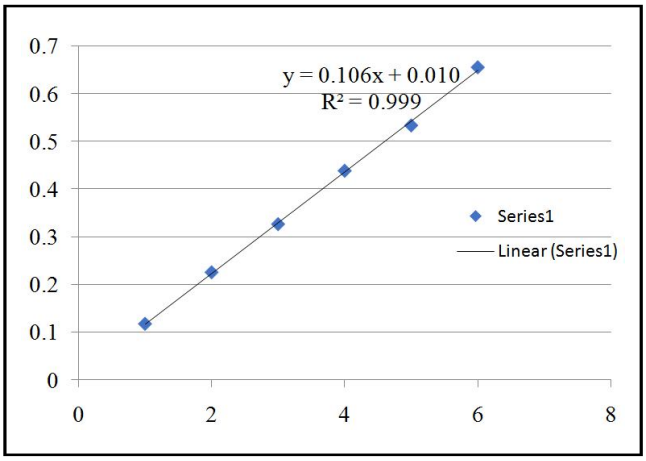

Figure 3: Calibration curve for furosemide.

3.1.2 Accuracy

Standard addition method used for determination of accuracy. It was carried out in triplicate at various concentration levels, including 50%, 100%, and 150% of targeted concentrations of drugs. Accuracy is determined by calculating per cent recovery and it was found to be in the range between 98-102% (Table 2).

$$\text{Amount found (50 \%)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \frac{50}{100} \times \frac{10}{50} \times \frac{0.2}{10} \times \frac{\text{Potency}}{100} \times 1000$$

$$\text{Amount found (100 \%)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \frac{50}{100} \times \frac{10}{50} \times \frac{0.4}{10} \times \frac{\text{Potency}}{100} \times 1000$$

$$\text{Amount found (150 \%)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \frac{50}{100} \times \frac{10}{50} \times \frac{0.6}{10} \times \frac{\text{Potency}}{100} \times 1000$$

$$\text{\% Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

Table 2: Results for accuracy

% Accuracy	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	% Mean recovery
50 %	2	1.964	98.2	
50 %	2	1.982	99.1	**99.24%**
50 %	2	2.008	100.4	
100 %	4	3.954	98.8	
100 %	4	3.972	99.3	**99.27%**
100 %	4	3.990	99.7	
150 %	6	5.981	99.6	
150 %	6	5.990	99.8	**99.83%**
150 %	6	6.009	100.1	

3.1.3 Precision

It was done by executing the intra-day and the inter-day precisions (Shailesh and Mitesh, 2017). It was carried out by analyzing six replicates of the same drug concentrations of furosemide and % RSD values were calculated. The per cent RSD values for both inter-day and intra-day values were found to be less than <2 (Table 3 and Table 4 are representing inter-day and intra-day precision, respectively).

Table 3: Inter-day precision results

Concentration (µg/ml)	Inter-day precision		
	1st - Day	2nd - day	3rd - Day
3	0.353	0.333	0.349
3	0.354	0.325	0.352
3	0.355	0.324	0.350
3	0.355	0.324	0.354
3	0.358	0.328	0.348
3	0.361	0.331	0.348
Mean	0.356	0.2807	0.3501
SD	0.002708	0.0035	0.002192
%RSD	0.758	1.068	0.599

Table 4: Intra-day precision results

Concentration (µg/ml)	Intra-day precision	
	Morning	Evening
3	0.351	0.352
3	0.353	0.354
3	0.354	0.357
3	0.355	0.356
3	0.361	0.360
3	0.364	0.365
Mean	0.356	0.357
SD	0.005046	0.004298
%RSD	1.404	1.20

3.1.4 Ruggedness

The ruggedness of the proposed method was evaluated by two different analysts under the same optimized conditions and then calculated % RSD value. The % RSD values were found to be <2 (Table 5).

Table 5: Results of ruggedness

Analyst-1		Analyst-2	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
3	0.316	3	0.333
3	0.329	3	0.329
3	0.320	3	0.320
3	0.320	3	0.334
3	0.325	3	0.324
3	0.329	3	0.322
Mean	0.323	Mean	0.327
SD	0.004879	SD	0.005354
%RSD	1.5097	%RSD	1.6373

3.1.5 Robustness

No significant changes were detected upon the deliberate variations in method parameter in terms of wavelength (maximum ± 1 nm) and the results were reported as % RSD. The % relative standard deviation (% RSD) values were found to be <2 (Table 6 representing the results for robustness).

Table 6: Results for robustness

Concentration (µg/ml)	228 nm	229 nm	230 nm
3	0.342	0.342	0.339
3	0.330	0.331	0.329
3	0.346	0.333	0.331
3	0.333	0.337	0.335
3	0.336	0.339	0.337
3	0.342	0.344	0.341
Mean	0.338167	0.3376	0.3353
SD	0.00561	0.004607	0.00423
% RSD	1.658	1.364	1.261

3.1.6 Limit of detection and limit of quantification

Limits of detection (LOD) and quantification (LOQ) were established by taking into account the intercepts standard deviation and slope of the calibration curve for the analyte. It is done by using the following formulas and the results were found to be <1, so the proposed method was sensitive.

$$\text{LOD} = 3.3 \times \text{SD of y-intercept/S}$$
$$= 3.3 \times 0.0047/0.1078$$
$$= 0.14\ \mu g/ml$$
$$\text{LOQ} = 10 \times \text{SD of y-intercept/S}$$
$$= 10 \times 0.0047/0.1078$$
$$= 0.43\ \mu g/ml$$

where SD is the standard deviation and S is the slope of the calibration curve. The values of SD and slope are the mean of three determinations obtained from calibration curve.

3.1.7 Assay of marketed formulation

15 tablets weighed accurately (Lasix 40 mg), the average weight was determined. The tablets were then broken down into fine powder. Accurately weighing 50 mg of the equivalent powder, it was then put into a 100 ml volumetric flask and the final volume was made up with 0.1 N NaOH. 10 ml of this solution was pipette out into a 50 ml volumetric flask and then diluted with 0.1 N NaOH solution. To get the final concentration of 3 µg/ml, 0.3 ml of above solution was then further diluted to 10 ml. The resultant solution was determined by scanning in the UV region of 200-400 nm. Table 7 discloses assay results.

Table 7: Results for assay

Formulation	Trade name	Label claim	Amount found (mg/tablet) ± SD	% Mean assay ± SD
Furosemide	Lasix	40 mg/tablet	39.076 ± 0.0811	97.69% ± 0.1950

% Mean assay is the average of three determinations (n = 3).

$$\text{Assay} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times 100$$
$$= \frac{0.422}{0.432} \times 100$$
$$= 97.69\%$$

3.1.8 Solution stability

For this study, the prepared working standard solution was kept at room temperature for 24 h. Then after the resulting solution was examined in the UV region of 200 to 400 nm. Then resultant UV spectrum was compared with a freshly prepared working standard solution and found that there was no significant change. Figures 4 and 5 are representing the UV spectrum for solution stability.

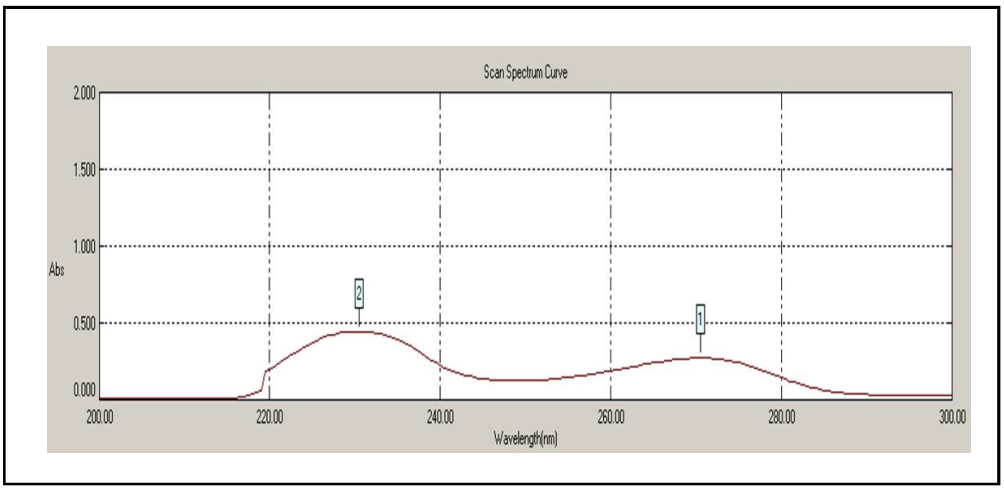

Figure 4: UV spectra of working standard solution at 0 h.

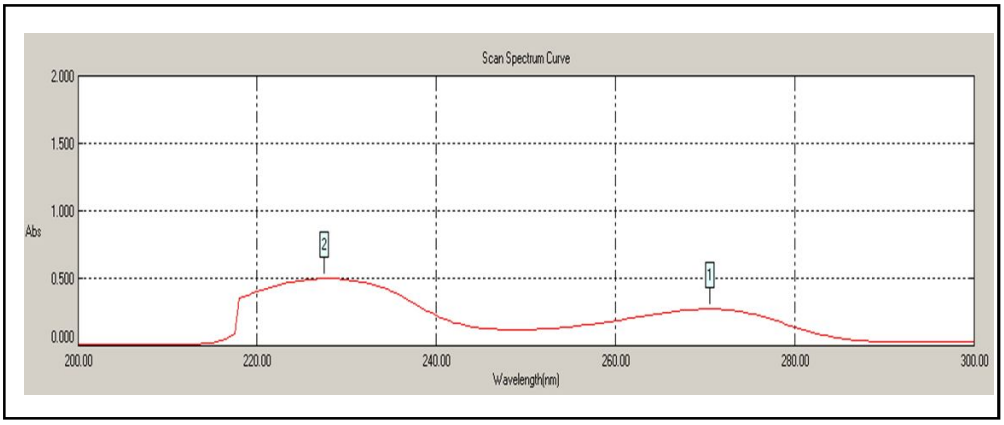

Figure 5: UV spectra of working standard solution after 24 h.

Table 8: Summary of validation parameters

Parameter	Results
Absorption maxima (nm)	219
Linearity range	1-6 µg/ml
Regression equation	y = 0.106x + 0.010
Slope	0.106
Intercept	0.010
Correlation coefficient (R^2)	0.999
Accuracy (mean % recovery)	99.24% to 99.83%
Intra-day precision (% RSD)	< 2
Inter-day precision (% RSD)	< 2
Robustness (% RSD)	< 2
Ruggedness (% RSD)	< 2
LOD	0.14 µg/ml
LOQ	0.43 µg/ml
% Mean assay ± SD	97.69 % ± 0.1950

4. Discussion

The present study is aiming to develop and validate a simple, sensitive, rapid and economical UV spectrophotometric method for the determination of furosemide in the bulk and its tablet dosage form. 0.1 N sodium hydroxide solution was selected as a solvent for method optimization and the absorption maximum of furosemide solution (10 µg/ml) was found at 229 nm. The developed method was validated according to ICH guidelines. To get a 1-6 µg/ml concentration range, the standard stock solution was appropriately diluted to series of 10 ml volumetric flasks separately for performing linearity. The correlation coefficient (R^2) from the calibration curve was found to be 0.999. The precision of the recommended method was evaluated at intra-day, inter-day precision with six replicates of working standard solution and the accuracy was evaluated at three concentration levels such as 50, 100 and 150 %. The % recovery results for accuracy in the range between 99.24% to 99.83% and % RSD values for precision were found to be < 2, indicating the developed method was accurate and precise, respectively. The current method of ruggedness was tested by two different analyst and the % RSD was discovered to be within the acceptable limit. Robustness of proposed UV method was established by modifying wavelength maxima of ± 1 nm and % RSD values were found to be in the range between 1.261 to 1.658. The results for LOD and LOQ obtained by the proposed method were found to be 0.14 µg/ml and

0.43 µg/ml, respectively, indicated that the followed method was sensitive. The assay parameter is also successfully finished with this method and it was found to be 97.69 %. Table 8 discloses the summary of validation parameters.

5. Conclusion

From the above results, it could be concluded that the method developed for the determination of furosemide in bulk and tablet dosage form was found to be simple, economical, accurate, precise and sensitive. The proposed method was validated in accordance with ICH guidelines. The results are reproducible for precision, robustness and ruggedness and their % RSD values were found to be < 2. Based on the limit of detection and limit of quantification results, the developed method has shown good sensitivity than the reported methods. The developed method can be used for routine analysis for the determination of furosemide in bulk and tablet dosage form. The application of the current proposed method can be further useful for forced degradation studies (ICH, 2003).

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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