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Research Article

Development of UV spectrophotometric methods and validation for estimation of furosemide in bulk and tablet dosage form by absorbance maxima and Area Under the Curve method

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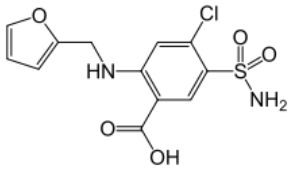
Keywords:
Furosemide (FUR),
Absorbance maxima method,
Area under curve method (AUC) and ICH guidelines.

Abstract

The present work was to develop two simple UV spectrophotometric methods for simultaneous estimation of furosemide (FUR) in bulk and tablet dosage form and validate as per ICH guidelines. Method A is absorption maxima method in which λmax was found to be 277 nm. Method B is area under the curve (AUC) in which area in the wavelength range of 258.40 nm – 293.80 nm was selected for analysis of furosemide. Linearity was observed in the concentration range 5-25µg/ml (r2 =0.999) for both the methods. The % assay for the marketed formulation for absorption maxima and area under the curve method was found to be 99.16%, and 99.20% respectively. The methods were validated with respect to linearity, precision and accuracy studies. Recovery studies for absorption maxima, and area under the curve was found to be 100.46%, and 100.86% respectively. The developed methods were validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines. Both the methods were found to be linear within the conc. Range of 5-25µg/ml for furosemide. The present methods were found to be simple, linear, precise, accurate and sensitive and can be used for routine quality control analysis for the estimation of furosemide in bulk and tablet dosage form.

1. Introduction

Furosemide (Fu) chemical name is 5-(aminosulfonyl)-4-chloro-2-[(2-furanyl methyl)amino)benzoic acid]. It has the following generic names: Frusemide, Fursemide, Aisemide, Beronald, Desdimin, Lasilix and others. The empirical formula is C12H11ClN2O5S corresponds to molecular weight of 330.77. Furosemide is white to slightly yellow, odourless, almost tasteless crystalline powder, slightly soluble in water, chloroform and ether soluble in acetone, methanol, dimethyl formamide [1] and in solutions of alkali hydroxides [2]. It melting point is 206°C, the pH of the aqueous solution is in the range 8.9 to 9.3. The UV spectrum of furosemide (0.01 mg/ml) in 0.1N NaOH was scanned from 190 to 400 nm using DMS 90 Varian spectrophotometer. It exhibited two maxima at 226 and 272 nm. Several methods have been reported for the determination of the components of this important drug (furosemide). Titrimetric methods [3-7] potentiometric methods [8,9] Ultraviolet methods, Colorimetric methods. Because of cost-effective and minimal maintenance, UV spectrophotometry is always preferred at small scale industries. Literature survey reveals that so far many UV spectrophotometric methods have been reported for the estimation of Furosemide in alone or in combination with other drugs [9]. But out of them only few methods included single estimation of furosemide. Therefore the main objective of the proposed methods were to develop simple, new and economic UV spectrophotometric methods for the estimation of furosemide in bulk and tablet dosage form and validate as per ICH guidelines.



Furosemide

2. Materials and Methods

2.1 Chemicals

Furosemide was supplied by Sanofi Aventis Andhari Mumbai, India. Tablet of furosemide 10mg (lasix) was procured from local pharmacy. Methanol S.D. Fine Chemicals, Mumbai, India) was used. All chemicals and reagents were of analytical reagent (AR) grade.

2.2 Instrumentation

A Shimadzu (Kyoto, Japan) model UV-1800 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.0 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical balance, Mettler Toledo (Model JL1503-C).

2.3 Method

2.3.1 UV-Spectroscopy Methods

A) Absorbance Maxima Method [11]

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflection spectroscopy in the ultra visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.[11]

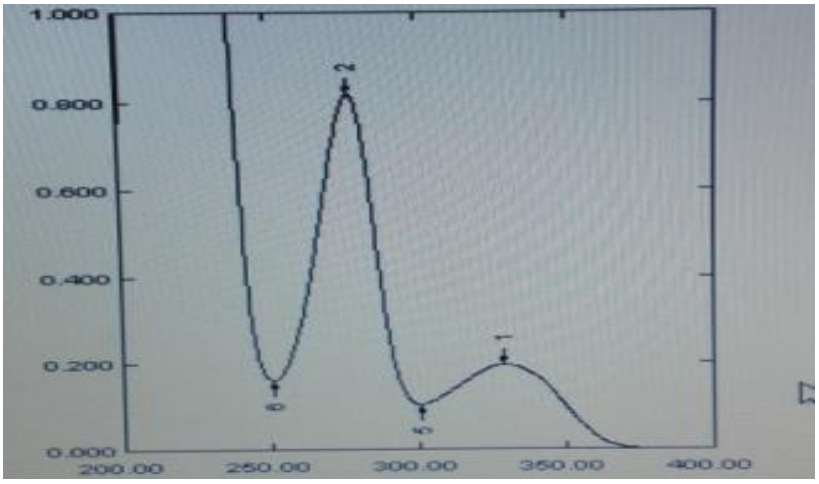


Figure 1: Absorbance maxima of the drug

B) Area under curve method

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis [14]. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration versus AUC [15].

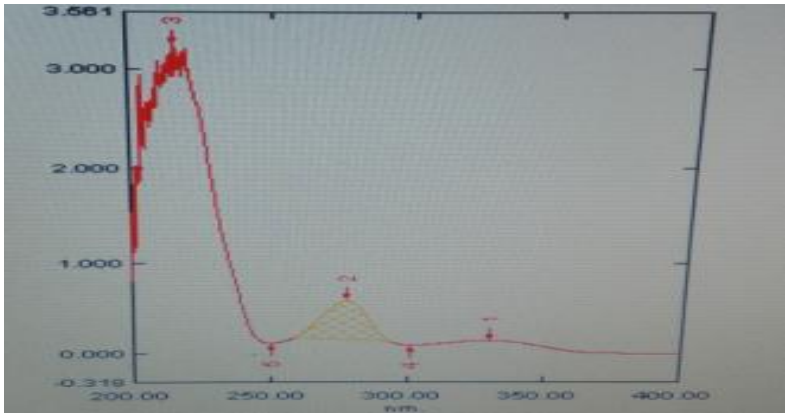


Figure 2: Area under curve shown in yellow colour of the drug

2.4 Experimental Work

a) To check the solubility of Furosemide

10 mg of furosemide was weighed and solubility of this sample was checked in double distilled water, methanol, ethanol, 1N NaOH, acetonitrile 0.1N HCL. The drug was found to be soluble in methanol was selected.[16]

b) To identify the λmax of furosemide

10 mg of the pure drug was accurately weighed and dissolved in 10ml methanol and the volume was made up to 10 ml with methanol to give a standard stock solution of 1000 µg/ml. Further 1000ppm withdrawn 2.5ml of Aliquote diluted to 25 ml of volumetric flask and prepare 100 ppm and Suitable dilutions were made with distilled water to get standard solutions of concentration: 5,10,15,20,25 µg/ml.

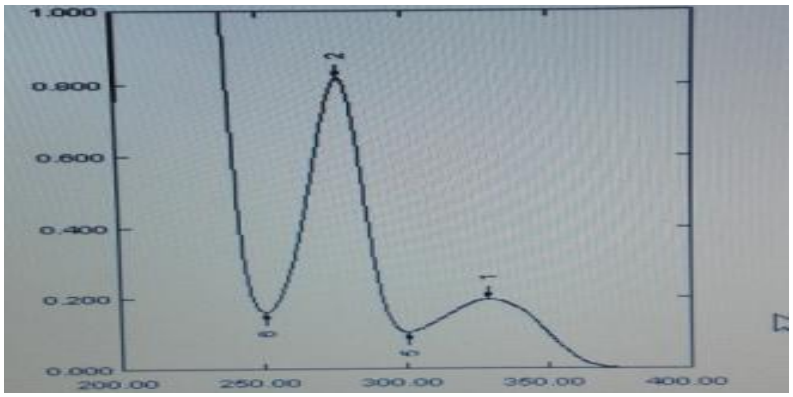


Figure 3: Standard solution of 15µg/ml

C) Sample preparation for analysis of Tablet formulation

Twenty tablets (furosemide) containing 10 mg of furosemide weighed, average weight calculated and triturated to fine powder and then weight equivalent 10 mg of furosemide transferred to 10 ml of volumetric flask containing proposed diluent, then sonicated for 15 minutes and filtered through Whatman filter paper no. 42 to form 1000µg/ml of furosemide stock solution of and final volume made upto mark with diluent. From this, 2.5 ml of aliquot transferred in 25 ml of volumetric flask containing diluent to form 100µg/ml of furosemide stock solution and further dilution of 5, 10, 15, 20, 25ppm and scanned in the range of 200-400 nm against methanol as blank at 277 nm and then drug content of solution was calculated by using standard calibration curve.[17]

2.5 Analytical Method Development

1. Accuracy [20]: It is closeness of the result obtained to the true value. It is often expressed as per cent age recovery by analyzing known added amounts of analyte. Also it can be determined by applying the procedure to quantitatively prepared samples.[18]

2. Precision: The precision of analytical procedure expresses closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. It may be considered at three levels: repeatability, intermediate precision and reproducibility. It is expressed as standard deviation or coefficient of variation[19].

3. Linearity: The linearity of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure is of precision, accuracy and linearity.[20]

3. Results and Discussion

Method A

A] Absorbance Maxima Method

Table 1: Calibration data of furosmide for absorbance maxima

Concentration (µg/ml)	Absorbance (Nm)
5	0.1922
10	0.5357
15	0.8327
20	1.133
25	1.4018

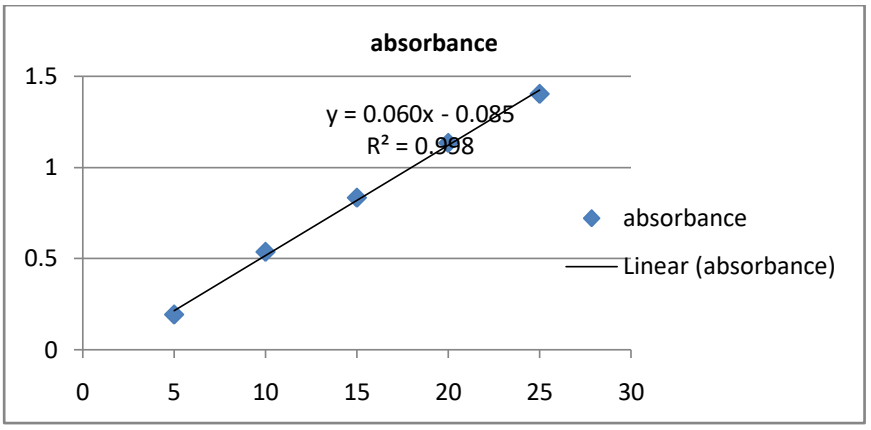


Figure 4: Calibration curve for furosemide

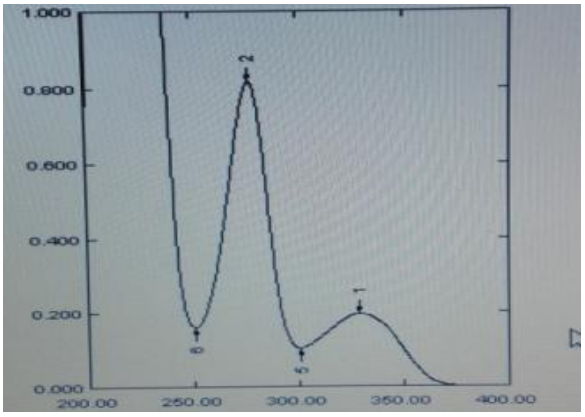


Figure 5: Spectrum of furosemide for absorbance maxima (15µg/ml)

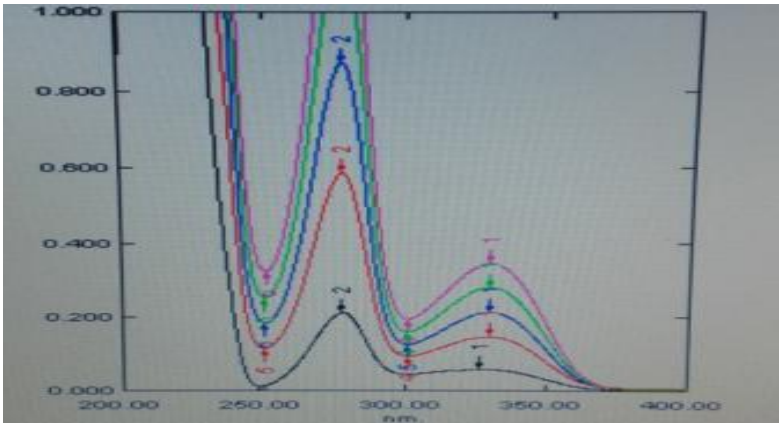


Figure 6: Overlay spectra of furosemide (5-25 µg/ml)

Table no.2: Parameters from the Calibration curve

Parameter	Observations
Calibration curve	Linear
Expression	Abs=A+B* conc or y=mx+c
Factor A	0.060
Factor B	0.080
Coefficient(r2)	0.998

Table 3: Analysis of pure drug

Sr. No	Amount Taken (µg/ml)	Absorbance nm	Amount of Drug Found (µg/ml)	% Amount Found
1	15	0.5357	7.511	112.67
2	15	0.5234	7.3067	109.6
3	15	0.4555	6.16	92.5
4	15	0.4675	6.375	95.625
5	15	0.4876	6.71	100.65
6	15	0.4354	5.84	87.6

Table 4: Statistical evaluation of pure drug

% Mean*	±S.D.*	%RSD*	±S.E.*
99.16	0.02	0.02	0.08

*Average of six determination

Step 3 Analysis of marketed formulation

Tablets were procured from local market (lasix by Sanofi India Limited) and average weight was determined. The powder equivalent to 10mg of furosemide was weighed accurately and dissolved in 10ml of methanol and filtered using whatmann filter paper # 42. The filtrate was appropriately diluted with methanol to give standard stock solution of 1000µg/ml. Further dilutions were made using distilled water 2.5 ml make 25 ml volumetric flask to give 100ppm and further dilution 5, 10, 15, 20, 25ppm. Absorbance was measured at 277 nm against standard solution.

Table 5: Calibration curve of marketed tablet-

Concentration	Absorption
5	0.0687
10	0.1324
15	0.1965
20	0.2543
25	0.3227

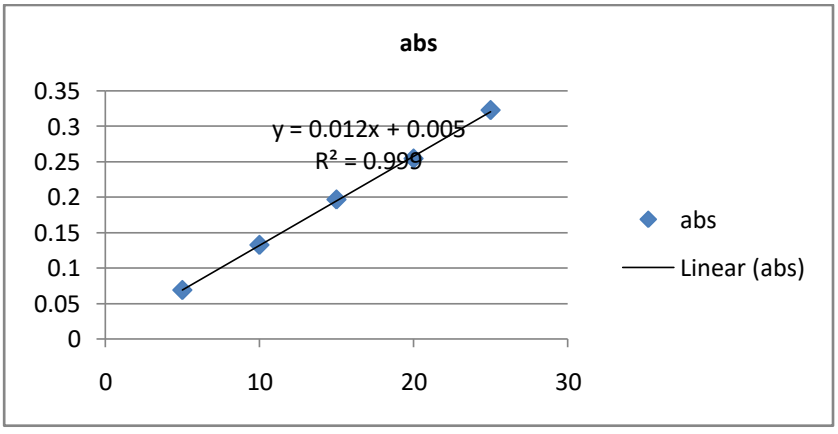


Figure 7: Calibration curve of marketed formulation (5-25ppm)

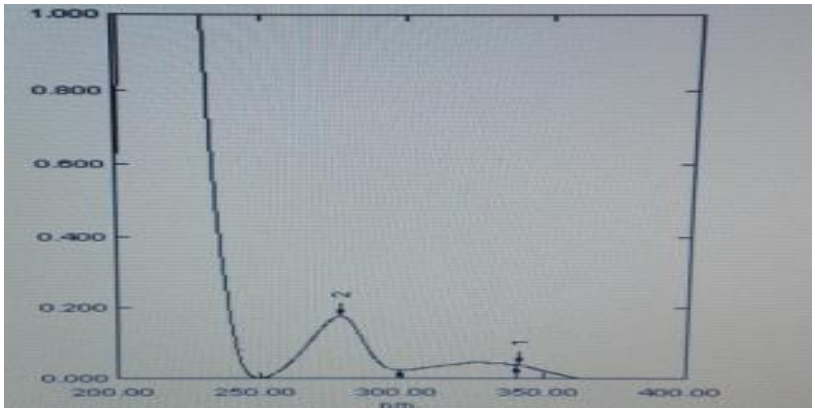


Figure 8: Spectrum of furosemide marketed formulation for absorbance maxima (15µg/ml)

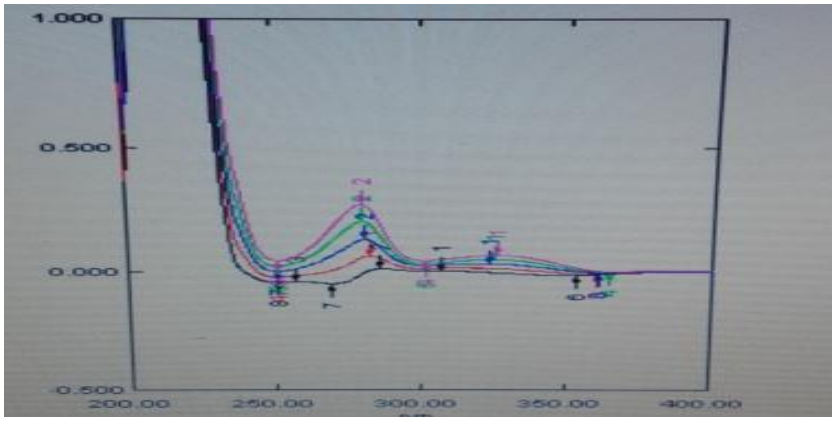


Figure 9: Overlay spectra of furosemide (5-25 µg/ml)

Table 6: Analysis of marketed formulation

Sr. No	Amount Taken (µg/ml)	Absorbance (Nm)	Amount of Drug Found (µg/ml)	% Amount Found
1	15	0.0996	7.8	117
2	15	0.0897	6.975	104.6
3	15	0.0845	6.5	98.12
4	15	0.0786	6.05	90.75
5	15	0.0786	6.05	90.75
6	15	0.0812	6.2	94

Table 7: Statistical evaluation of marketed formulation

% Mean*	±S.D.*	%RSD*	±S.E.*
99.20	1.54	1.554	0.54

*Average of six determination

Step 4-Validation

The developed method was validated as per ICH guidelines.

Linearity: The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of furosemide. Beer's law was obeyed in the concentration range 10-50µg/ml. The correlation coefficient was found to be 0.999309.

Precision: 100 mg of furosemide was weighed accurately and dissolved in 100ml of methanol. From the standard stock solution appropriate quantity of solution was taken to make further dilutions with methanol to give 10µg/ml. Absorbance was measured at 238nm against standard solution. This procedure was carried out 6 times.

Table 8: Analysis of inter-day precision

Sr. No	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	15	10.0700	100.70
2	15	9.9345	99.34
3	15	10.2088	102.08
4	15	10.04216	100.42
5	15	9.9317	99.31
6	15	9.9750	99.75

Table 9: Analysis of intra-day precision

Sr. No	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	15	10.02	100.70
2	15	9.890	98.34
3	15	10.00	100.08
4	15	9.97	99.42
5	15	10.020	100
6	15	10.0040	100

Table 10: Statistical evaluation of inter-day and intra-day precision studies

Parameter	% Mean*	± S.D. *	%RSD *	±S.E.*
Interday	100.26	1.0526	1.05	0.4297
Intraday	99.84	0.4978	0.50	0.2032

Accuracy:-

Accuracy studies were carried out by standard addition method. Pure furosemide was added at different levels i.e. 80%, 100% and 120% to drug sample present in tablet dosage form (100 mg furosemide in each tablet).

Recovery studies:-

Table 11: Results of recovery studies

Level of recovery (%)	Amount taken (µg/ml)	Amount of std. added (µg/ml)	Absorbance (nm)	Total amount recovered (µg/ml)	% Recovery
80	10	8	0.3648	18.16	103.88
80	10	8	0.3641	18.13	103.46
80	10	8	0.3638	18.12	103.29
100	10	10	0.3914	19.46	101.81
100	10	10	0.3802	18.23	102.01
100	10	10	0.3820	19.45	101.69
120	10	12	0.4076	20.23	103.95
120	10	12	0.4062	20.09	103.27
120	10	12	0.4025	20.08	101.49

Table 12: Statistical evaluation of recovery studies

Level recovery	%mean	±S.D.*	%RSD*	±S.E.*
80	103.54	0.67	0.65	0.38
100	101.83	0.1616	0.16	0.093
120	102.90	1.1494	1.14	0.6636

*Average of three reading

METHOD B

B) AREA UNDER CURVE:-

Step 1-Preparation of Standard Stock Solution

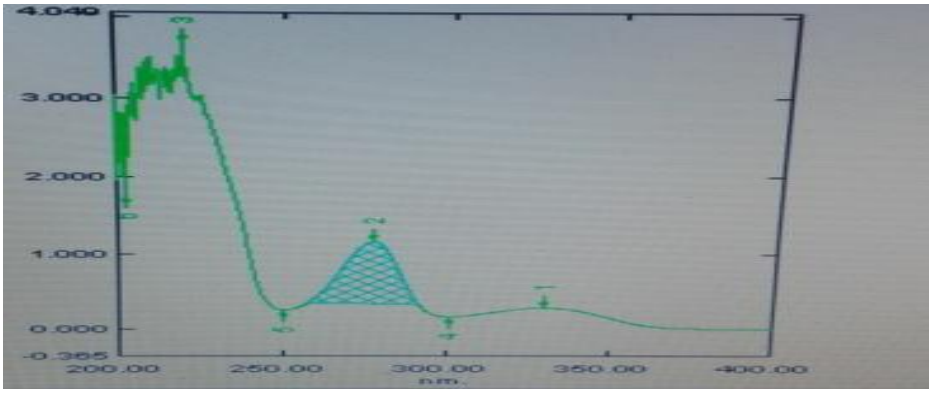


Figure 10: spectra of furosemide for AUC (5-25 µg/ml)

Table 13: Calibration data of furosemide for area under curve

Concentration	Area
5	3.123
10	6.342
15	9.76
20	12.87
25	15.89

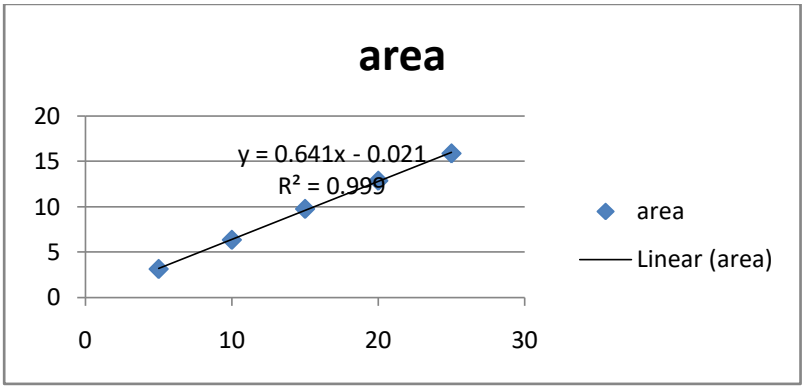


Figure 11: Calibration curve for furosemide for AUC

Table 14: Parameters from the Calibration curve

Parameter	Observations
Calibration curve	Linear
Expression	Abs=A+B* conc or y=mx+c
Factor A	0.641
Factor B	0.021
Coefficient(r2)	0.999

Table 15: analysis of pure drug:

Sr. No	Amount Taken (µg/ml)	AUC	Amount of Drug Found (µg/ml)	% Amount Found
1	15	3.1412	10.11	101
2	15	3.1101	10.00	100
3	15	3.901	9.980	99.80
4	15	3.123	10.10	101
5	15	3.897	9.87	98.87
6	15	3.1567	10.12	101

Table 16: Statistical evaluation of pure drug

% Mean*	±S.D.*	%RSD*	±S.E.*
100.46	0.01	0.018	0.006

*average of six determinations

Step 3-Analysis of marketed formulation

Table 17: Analysis of marketed formulation

Sr. No	Amount Taken (µg/ml)	AUC	Amount of Drug Found (µg/ml)	% Amount Found
1	15	3.1876	10.24	104
2	15	3.15	10.14	101
3	15	3.1234	10.02	100
4	15	3.09	9.99	99.9
5	15	3.897	9.987	99.87
6	15	3.124	10.021	100

Table 18: Statistical evaluation of marketed formulation

% Mean*	±S.D.*	%RSD*	±S.E.*
100.86	0.01	0.01	0.006

*average of six determinations

Step 4-Validation

The developed method was validated as per ICH guidelines.

Linearity: The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of furosemide. The Beer's law was obeyed in the concentration range 5-25µg/ml. The correlation coefficient was found to be 0.999309.

Precision: 100 mg of furosemide was weighed accurately and dissolved in 100ml of methanol to give concentration of 1000µg/ml. From the standard stock solution appropriate quantity of solution was taken further dilutions were made with methanol to give 10µg/ml. AUC was measured in the range 258.40-293.80nm. This procedure was carried out 6 times.

Table 19: Results of intra-day precision

Sr. No	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	**15**	10.0700	100.70
2	**15**	9.9345	99.34
3	**15**	10.2088	102.08
4	**15**	10.04216	100.42
5	**15**	9.9317	99.31
6	**15**	9.9750	99.75

Table 20: Results of inter-day precision

Sr. no	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	**15**	10.02	100.70
2	**15**	9.890	98.34
3	**15**	10.00	100.08
4	**15**	9.97	99.42
5	**15**	10.020	100
6	**15**	10.0040	100

Table 21: Statistical evaluation of inter-day and intra-day precision studies

Parameter	% Mean*	± S.D. *	%RSD *	±S.E.*
Interday	100.26	1.0526	1.05	0.4297
Intraday	99.84	0.4978	0.50	0.2032

*Average of six determinations

Accuracy

Accuracy studies were carried out by standard addition method. Standard sample of furosemide was added at different levels i.e. 80%, 100% and 120% to drug sample present in tablet dosage form.

Recovery studies

To tablet stock solution in 3 different volumetric flask, aliquots of 8ml, 10ml and12ml of the standard stock solution were added, volume was made upto 10ml with water to give concentration of 18 µg/ml (80%),20(100%) and 22(120%). Absorbance was determined at 277nm. Procedure was repeated 3 times for 80%, 100% and 120% for recovery studies.

Table 22: Results of recovery studies

Level of recovery (%)	Amount taken(µg/ml)	Amount of std. Added (µg/ml)	Area under curve	Total amount recovered (µg/ml)	% Recovery
80	10	8	5.523	17.34	100.88
80	10	8	5.545	17.35	100.46
80	10	8	5.589	17.38	98.76
100	10	10	6.2358	17.	100.56
100	10	10	6.234	18.23	100.90
100	10	10	6.298	19.45	100.90
120	10	12	6.734	20.23	98
120	10	12	6.785	20.09	100
120	10	12	6.7098	20.08	99.45

Table 23: Statistical evaluation of recovery studies

Level recovery	%mean	±S.D.*	%RSD*	±S.E.*
80	100.54	1.00	0.011	0.578
100	100.83	0.05	0.56	0.0345
120	99	0.75	6.33	0.43

*average of six determinations

4. Conclusions

Simple UV spectrophotometric methods have been developed and validated for the determination of furosemide in bulk and tablet dosage form. The results of the validation parameters show that the UV spectrophotometric methods were found to be accurate, precise and sensitive. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of furosemide in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

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