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VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF FUROSEMIDE AND AMILORIDE HYDROCHLORIDE IN THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

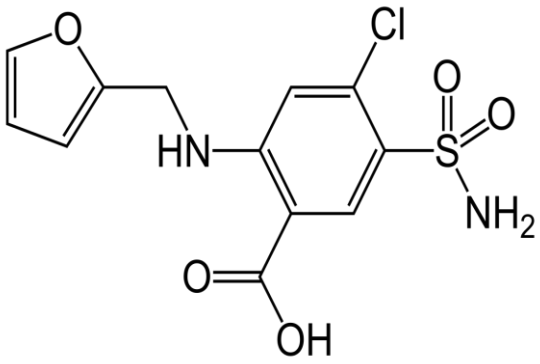
An accurate, precise, sensitive, spectrophotometric method was developed and validated for simultaneous estimation of Furosemide and Amiloride Hydrochloride in tablet form. The analysis was carried out in JASCO V 560 double beam UV Spectrophotometer. In simultaneous equation method, Furosemide and Amiloride Hydrochloride were quantified using their absorptivity values of at selected wavelengths, 274 nm and 362 nm respectively. Methanol was used as solvent for both drugs. The developed method was validated according to ICH guidelines for evaluation of accuracy, precision, sensitivity etc. The linearity range was found to be 1-11µg/ml for both drugs with correlation coefficient of 0.999 for both drugs. Recovery studies were conducted at three different concentration levels And average percentage in tablet dosage form was determined and found to be 99.58% for Furosemide and 99.61% for Amiloride Hydrochloride. So the developed method permits rapid determination of Furosemide and Amiloride Hydrochloride.

KEYWORDS: Furosemide, Amiloride Hydrochloride, Simultaneous Equation.

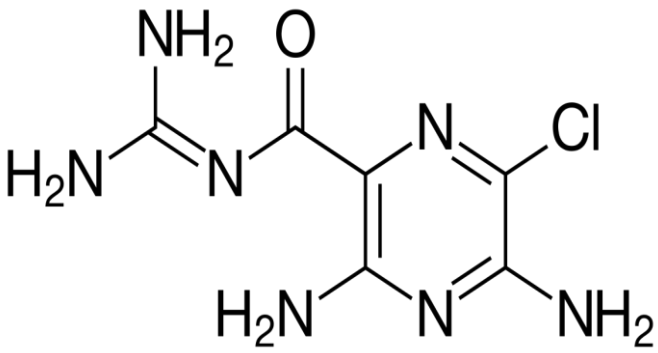
INTRODUCTION

Furosemide is high ceiling loop diuretics. The major site of action is the thick ascending limb of loop of Henle(TAL), Furosemide inhibits Na+-K+-2Cl- cotransport. . This is achieved by competitive inhibition at the chloride binding site on the co-transporter, thus preventing the

transport of sodium from the lumen of the loop of Henle into the basolateral institium. Consequently, the lumen becomes more hypertonic, which in turn diminishes the osmotic gradient for water reabsorption throughout the nephron. Because the thick ascending limb is responsible for 25% of sodium reabsorption in the nephron, Furosemide is very potent diuretic.



Amiloride is potassium sparing diuretics. Amiloride block the luminal Na^+ channels and directly inhibit K^+ excretion, while the net excess loss of Na^+ is minor, because this is only a small fraction of the total amount of Na^+ excreted in urine.[1]



Analytical method development and validation plays important roles in discovery, development and manufacture of pharmaceuticals.[2,3] As per the literature review, there are no analytical methods reported for the simultaneous estimation of Furosemide and Amiloride in combined Pharmaceutical dosage form, various publication are available regarding the UV spectrophotometric estimation, HPTLC and RP- HPLC determination of Amiloride and Furosemide either alone or in combination with other drugs in pharmaceutical dosage forms.

Objective was to develop and validate UV spectrophotometric method for the estimation of Amiloride Hydrochloride and Furosemide in the combined tablet dosage form by UV simultaneous equation method.

MATERIALS AND METHOD

Chemicals and Reagents

- Furosemide R S from Yarrow chem
- Amiloride Hydrochloride R S from Sigma aldrich
- Methanol HPLC Grade from Merck India
- Amifru 40 Tablets.

Instruments

- JASCO V 560 double beam Spectrophotometer
- Shimadzu analytical Balance
- Sonicator

Preparation of standard solutions

Accurately weighed 10 mg of Furosemide RS and Amiloride Hydrochloride was quantitatively transferred into 100mL standard flask. It was then dissolved and the solution was made up to the mark using methanol to obtain a concentration of 100 µg/mL of Furosemide and Amiloride (solution A).From the solution A 1 mL was pipette out to a 10 mL standard flask and made up the volume with methanol. The solution had a concentration of 10µg/ml of Furosemide and Amiloride (solution B).

Preparation of sample solutions

Twenty tablets of AMIFRU 40 were weighed; average weight of the tablet was calculated and finely powdered. A quantity of powder equivalent to 100 mg of Furosemide (containing 12.5 mg of Amiloride Hydrochloride) was weighed accurately and transferred to a glass stoppered flask. The powder was extracted initially with 15 ml methanol by sonication for 10 minutes and filtered through Whatmann No. 1 filter paper to a 100 ml standard flask. The residue was further extracted twice with 10 ml of the methanol and transferred to the same standard flask through the sa,e filter paper. The volume was finally made up to the mark using methanol. The resulting solution had a concentration of 1000 µg/ ml of the 125 µg/ml of Amiloride hydrochloride.

From the above solution, accurately pipette out 2 ml and transferred to 100 ml standard flask. Then the volume was made up to the mark using methanol to obtain a concentration of 1.25 µg/ml of Amiloride Hydrochloride and 10 µg/ml of Furosemide.

Simultaneous equation method

Two wavelengths selected for this method are 274nm and 362 nm that are absorption maxima of Furosemide and Amiloride Hydrochloride respectively in methanol. The stock solutions of both drugs were further diluted separately with methanol to get series of standard solution of 1 to 11µg/ ml of Furosemide and Amiloride hydrochloride. The absorbance were measured at selected wavelengths and absorptivity's(A1%,1cm) for both the drugs at both wavelengths were determined as mean of six independed determinations. Concentrations in the sample were obtained by using following equations.

$$C_x = A_2a_{y1}\text{-}A_1a_{y2}/a_{x2}a_{y1}\text{-}a_{x1}a_{y2}$$

$$C_y = A1a_{x2}\text{-}A_2a_{x1}/a_{x2}a_{y1}\text{-}a_{x1}a_{y2}$$

Were A1 and A2 are absorbance of sample at 274 nm and 362 nm respectively ax1 and ax2 are absorptivity's of Furosemide at 274 nm and 362 nm respectively, ay1 and ay2 are absorptivity of Amiloride Hydrochloride at 274 nm and 362 nm respectively in diluted sample.(Table 1)

RESULTS

After stabilizing the instrument initially for 30 minutes, blank correction was done using methanol. Then 10µg/mL solution of both Furosemide and Amiloride Hydrochloride were scanned separately in UV region ranging from 200nm t0 400nm. The absorption spectra were observed with maximum absorption at 274nm and 362nm for Furosemide and Amiloride Hydrochloride respectively. The spectra obtained are given fig 1-2.

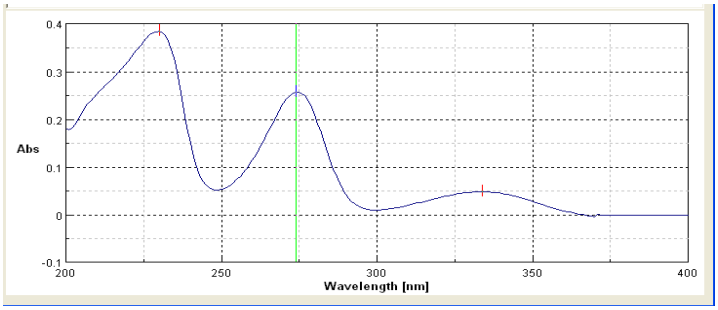


Fig. 1: UV absorption spectrum of Frusemide RS in methanol with absorption aximum at 274 nm.

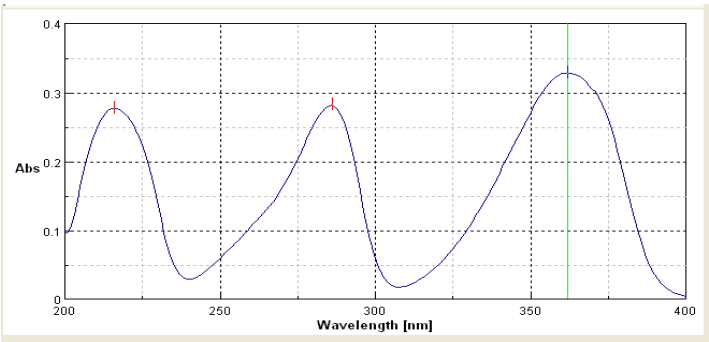


Fig 2: UV absorption spectrum of Amiloride RS in methanol with absorption maximum at 362 nm.

Validation of developed methods

Linearity

The linearity study was conducted to evaluate the linear relationship across the range of procedure. The linear response of Furosemide was determined by analyzing six different concentration of standard solution. The solutions were prepared by accurately pipetting out 0.1, 0.3, 0.5, 0.7, 0.9, 1.1 ml from stock solution A of Furosemide (100µg/ ml) into six different standard flasks and made up the volume using methanol. Absorbance of both druggiven in Table 1 The absorbance vs. concentration was plotted at 273 nm& 362 nm correlation coefficient and regression line equation was determined fig3-4.

Precision

Precision is a measure of degree of reproducibility or the repeatability of the analytical method under normal circumstances. Precision was considered at two levels- repeatability and intermediate precision.

- **Repeatability**

The repeatability of the method was studied by using six determinations of 100 % test concentration. The results are tabulated in table. The statistical validation is given in table 2.

- **Intermediate precision**

The intermediate precision was studied by using six Determination of the mixture of 1.25µg/ ml of Amiloride and 10 µg/ ml of Furosemide. The stock solution was prepared and analyzed at the same time on three consecutive days. The absorbance of the resulting solution was measured at 274 nm and 362 nm. The variations of the results on three days were analyzed and the stastical validation was done.

The results for 1 day, day 2 and day 3 are furnished in table respectively. The statistical validation data is furnished in table.3

Accuracy

Accuracy of the proposed method was determined by recovery study. The recover studies were performed by standard addition method at 15%, 20%,and 25% level and percentage recoveries were calculated. Table 4

Range

The range of the analytical procedure is determined from the linearity studies and depends on the intended application of the procedure. From the linearity studies, it is revealed that the range for the proposed analytical method is as follows.

Limit of detection and Limit of quantitation.

LOD and LOQ results are stated on table 5

Table 1: Absorption Data of Furosemide and Amiloride.

Sl. No	Concentration	Absorbance			
		FUROSEMIDE		AMILORIDE	
		274nm	362 nm	274 nm	362nm
1	1	0.0701	0.0127	0.0403	0.0674
2	3	0.2114	0.0378	0.1219	0.2028
3	5	0.3497	0.0616	0.2065	0.3347
4	7	0.4851	0.0871	0.2946	0.4745
5	9	0.6143	0.1126	0.3772	0.6143
6	11	0.7398	0.1375	0.4572	0.7395

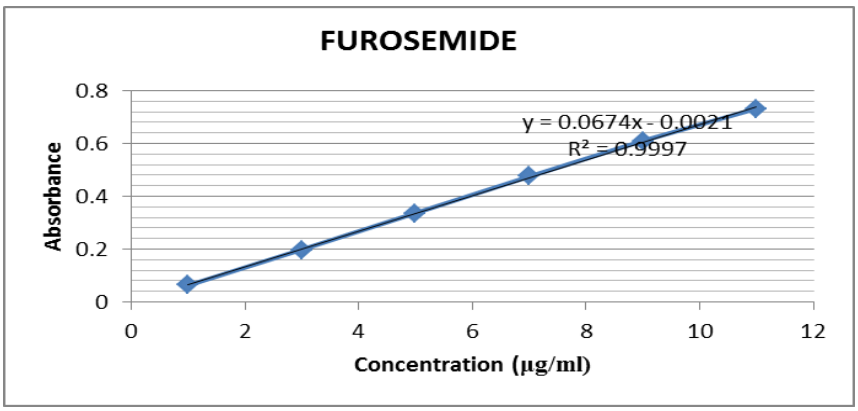


Fig. 3: Calibration plot of Furosemide.

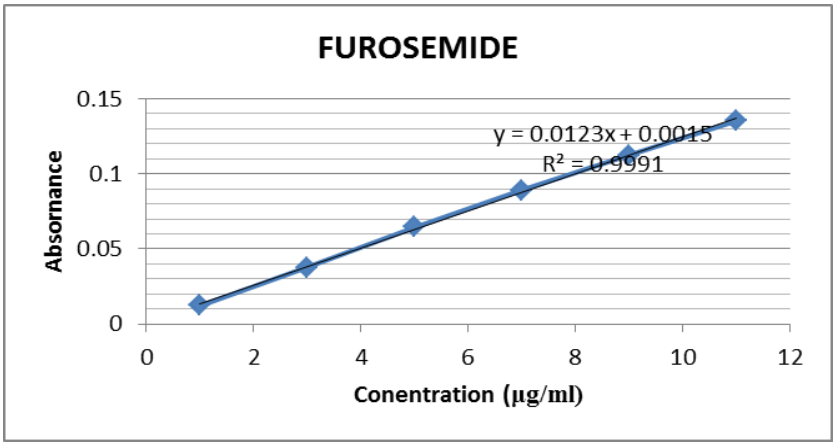


Fig. 4: Calibration plot of Furosemide 362 nm.

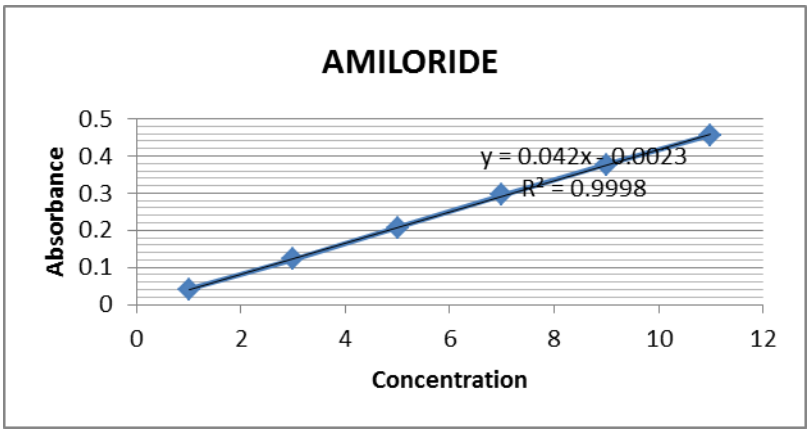


Fig 5: Calibration plot of Amiloride 362.

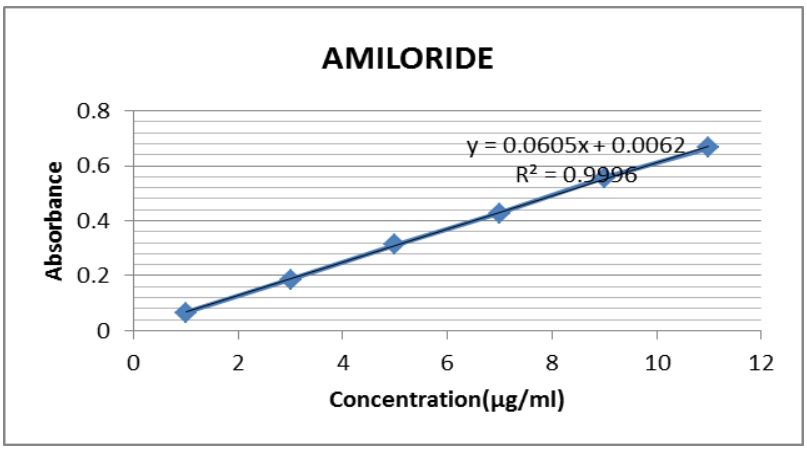


Fig 6: Calibration plot of Amiloride 274 nm.

Table 2: Results of repeatability study- Statistical Validation data.

Components	Mean of % label claim	Standard deviation (SD)	Relative standard deviation (%RSD)
Furosemide	99.45	0.32359	0.3221
Amiloride	99.45	0.22447	0.2257

Table 3: Statistical Validation- Intermediate precision study.

Components	Mean% Label claim (n=1)	Standard deviation (SD)	Relative standard deviation (%)
Furosemide	99.6038	0.1459	0.1456
Amiloride	99.3727	0.3960	0.3931

Table 4: Recovery study- statistical validation data.

Level of % recovery	Mean % recovery		Standard deviation		% RSD	
	FRU	AMI	FRU	AMI	FRU	AMI
15%	100.17	99.18	0.04079	0.1320	0.4072	0.1331
20%	98.54	99.68.	0.2138	0.0818	0.217	0.8210
25%	99.41	99.66	0.8504	0.0814	0.0855	0.0817

Table 5: LOD and LOQ Results

Method Parameters	Furosemide		Amiloride Hydrochloride	
	274 nm	362 nm	274 nm	362 nm
LOD (µg/ml)	0.0985	0.275	0.1571	0.33
LOQ(µg/ml)	0.2985	0.833	0.4761	1.0

DISCUSSION

This research work was done to develop simple, acute and economic UV method for the simultaneous estimation of Furosemide and Amiloride Hydrochloride in tablet dosage form. The two drugs ere soluble in methanol and also excellent UV detection in methanol, So methanol was chosen as the desirable solvent for simultaneous estimation of these drugs. Furosemide in methanol shows a sharp at 274 nm and Amiloride Hydrochloride shows sharp peak at 362 nm. Both the absorption maxima wavelength employed for further spectrophotometric measurements. In the quantitative assay of two components in the mixture by Simultaneous equation method, absorbance are measured at two wavelength ie, 274 nm and 362 nm. Calibration graph were plotted for each drugs. For both Furosemide and Amiloride linear relationship between concentration and absorbance was observed for 1-11µg/mL. AMIFRU 40. A marketed formulation 9containing Furosemide 40 g and Amiloride 5 mg was analyzed by proposed method and result were obtained was:

Amount of furosemide=39.852 mg.

Amount of Amiloride Hydrochloride=4.980mg.

Percentage label claim of Fursemide=99.6 % w/w.

Percentage label claim of Amiloride Hydrochlorie=99.6% w/w.

Validation of proposed method was performed accordance with ICH guildeline.

CONCLUSION

The proposed UV Spectrophometric method enable the simultaneous determination of Furosemide and Amiloride in their binary mixture with good accuracy and precision, either in laboratory prepared samples or combined dosage form. The mehod was successfully applied for the determination of Furosemide and Amiloride in Pharmaceutical dosage forms as no interference was observed due to excipients or other components present. Hence, this method can be conveniently used for routine quality control analysis of Furosemide and Amiloride in their pharmaceutical formulations.

ACKNOWELEGMENT

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