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Der Pharmacia Lettre, 2016, 8 (13):214-222
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Preformulation study of furosemide

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ABSTRACT

The development of this orally and rapidly acting highly efficacious diuretic was a breakthrough. Its maximal natriuretic effect is much greater than that of other classes. The diuretic response goes on increasing with increasing dose: up to 10 L of urine may be produced in a day. It is active even in patients with relatively severe renal failure. The onset of action is prompt (i.v. 2-5 min., i.m. 10-20 min., oral 20-40 min.) and duration short (3-6 hours) The major site of action is the thick Asc LH (site II) where furosemide inhibits Na+- K+-2Cl cotransport. A minor component of action on PT has also been indicated. It is secreted in PT by organic anion transport and reaches Asc LH where it acts from luminal side of the membrane. It abolishes the corticomedullary osmotic gradient and blocks positive as well as negative free water clearance. K^+ excretion is increased mainly due to high Na^+ load reaching DT. However, at equinatriuretic doses, K+ loss is less than that with thiazides. Identification test was done by estimation of drug, infra-red spectroscopy, FTIR, UV-Spectroscopy, Melting point determination etc.

Keywords: Furosemide, IR-Spectroscopy, FTIR, UV-Spectroscopy, Melting point determination.

INTRODUCTION

Loop diuretics are diuretics that act at the ascending loop of Henle in the kidney. They are primarily used in medicine to treat hypertension and edema often due to congestive heart failure or renal insufficiency. While thiazide diuretics are more effective in patients with normal kidney function, loop diuretics are more effective in patients with impaired kidney function.[1] Loop diuretics act on the Na^+-K^+-$2Cl^-$ symporter (cotransporter) in the thick ascending limb of the loop of Henle to inhibit sodium, chloride and potassium reabsorption. This is achieved by competing for the Cl^- binding site. Because magnesium and calcium reabsorption in the thick ascending limb is dependent on the positive lumen voltage gradient set up by potassium recycling through renal outer medullary potassium channel, loop diuretics also inhibit their reabsorption. By disrupting the reabsorption of these ions, loop diuretics prevent the generation of a hypertonic renal medulla.[2] Without such a concentrated medulla, water has less of an osmotic driving force to leave the collecting duct system, ultimately resulting in increased urine production. Loop diuretics cause a decrease in the renal blood flow by this mechanism. This diuresis leaves less water to be reabsorbed into the blood, resulting in a decrease in blood volume. A secondary effect of loop diuretics is to increase the production of prostaglandins, which results in vasodilation and increased blood supply to the kidney.[3,4] NSAIDs block the COX pathway that synthesizes prostaglandins, so NSAIDs can reduce the efficacy of loop diuretics.[5] The collective effects of decreased blood volume and vasodilation decrease blood pressure and ameliorate edema.

Loop diuretics are principally used in the following indications:

- edema associated with heart failure, liver cirrhosis, kidney impairment, nephrotic syndrome
- hypertension adjunct in cerebral/pulmonary edema where rapid diuresis is required (IV injection)

They are also sometimes used in the management of severe hypercalcemia in combination with adequate rehydration.[6]

On the other hand, in critically ill patients with acute renal failure, loop diuretics do not appear to reduce mortality, reduce length of intensive care unit or hospital stay, or hasten any recovery of renal function.[7] A systematic review by the Cochrane Hypertension group assessing the anti-hypertensive effects of loop diuretics found only a modest reduction in blood pressure compared to placebo; the review highlights the need for more randomized control trials to be made available in order to construct a furnished assessment.[8]

MATERIALS AND METHODS

Materials and Equipments Used

Furosemide (Sanofi Aventis Pharma Mumbai), Gelucire 43/01 (Gattefosse(St Priest,Cedex, France). Acetone (Sd fine-chemicals). Potassium chloride (Sd fine-chemicals). Hydrochloric acid, Potassium dihydrogen phosphate, Sodium hydroxide pellets, Ethanol (Sd fine-chemicals), Dissolution rate test apparatus(Electrolab Pvt. Ltd. Mumbai), pH /mill voltmeter(Century instrument Pvt. Ltd.), UV-VIS spectrophotometer(Shimadzu Corp. Japan), Standard test sieves(HICON, Grover Enterprises, Delhi), Digital oven(Science tech Pvt. Ltd. India), Digital Electronic Balance (Shinko Denshi corp.Japan), Digital M. P. apparatus(Jindal Scientific instruments, Ambala), Single Pan Electronic Balance(Contech instrument pvt. Ltd.Mumbai), Magnetic Stirrer with Hot Plate(B.D. Scientific Industries, Delhi).

PREFORMULATION STUDY

Solubility study

White to slightly yellow, odorless crystalline powder. Practically insoluble in water, freely soluble in acetone, dimethylpharmamide, methanol and solutions of alkali hydroxides. Sparingly soluble in alcohol, slightly soluble in ether, very slightly soluble in chloroform.

Melting point determination [9]

Melting point apparatus, calibrated using L –ascorbic acid AR and sodium bicarbonate AR, was used for melting point determination of Furosemide by capillary fusion method. The melting point obtained was recorded and compared with literature value.

FTIR spectroscopy [10]

Fourier transform infrared (FTIR) spectra of furosemide HPMC K4M, Gelucire 43/01 and a physical mixture of these ingredient were recorded using KBr mixing method on FTIR instrument available at sophisticated analytical instrument facility (FTIR-Perkin Elmer- Spectrum Version 10.03.06).

UV spectrophotometric study [11]

Furosemide (10mg) was accurately weighed and transferred to a 50 ml volumetric flask. It was dissolved and diluted to 50 ml with pH 5.8 phosphate buffer (USP 27 / NF 22 2004) to obtain a final concentration of 100 µg / ml. Dilutions were made to obtain a concentration of 10 µg / ml and scanned for λ_{max} in a range of 200 – 400 nm in the spectrum basic mode for three consecutive days. Student t – test performed to check the significance in difference in absorbance values at 95 % confidence interval.

Selection of media

The pH values were selected based on the variable pH values in fasted and fed state gastric conditions for preparation of calibration curves. The pH selected was labeled as pH 5.8 phosphate buffer.

Scanning for λ_{max}

Furosemide (10mg) was accurately weighed and transferred to a 50 ml volumetric flask. It was dissolved and diluted to 50 ml with pH 5.8 phosphate buffer (USP 27 / NF 22 2004) and was diluted to get a final concentration of 10 µg / ml. the resultant solutions were scanned for λ_{max} in 200 – 400 nm in the spectrum basic mode.

Preparation of calibration curve

Aliquots of the stock solution of Furosemide (100 µg / ml) were pipetted out into a series of 10 ml volumetric flask and diluted with pH 5.8 phosphate buffer to get final concentration in the range of 2 – 10 µg / ml . The absorbances of the resultant solutions were measured at 271nm for pH 5.8 phosphate buffer. Freshly prepared solutions were made for the calibration curves on three consecutive days.

Validation of calibration curves

Assay validation of calibration curves were carried out as per the USP guidelines for the assay in category 1 and as per ICH Q2A guidelines. In validation procedure, calibration curves prepared in pH 5.8 phosphate buffer was run in triplicate for three days to determine between and within variations (Bolton 1997).

RESULTS AND DISCUSSION

DRUG IDENTIFICATION TESTS

Melting point determination

On calibration of the melting point apparatus with L - ascorbic acid AR (observed melting point 150 ^{o}C, reported melting point 141 -145 ^{o}C) and sodium bicarbonate AR (observed melting point 275 ^{o}C, reported melting point 270, a correction factor of -5 ^{o}C was documented. The correcting melting point of the drug was found to be 209^{o}C, which corresponds to the literature value of 206 - 210 ^{o}C (B.P 2003), and proves the identity and purity of drug.

FTIR

The IR spectrum was found concordant with the IR spectrum of furosemide reported in official monograph (B.P 2005) .

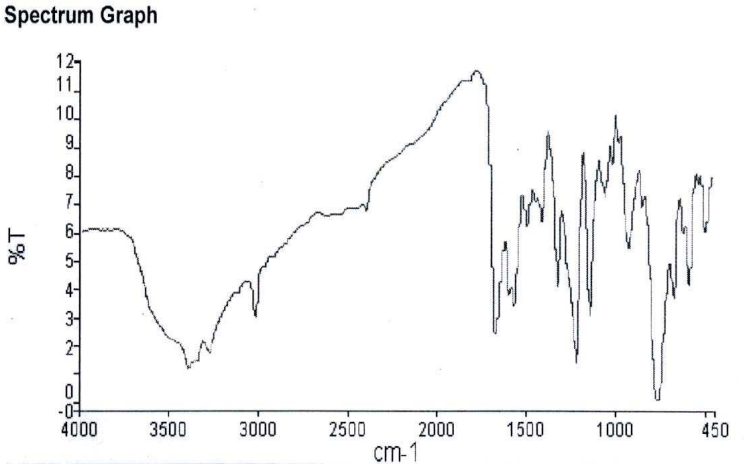

Figure:1 IR spectra of furosemide

UV spectrophotometric study

Spectrophotometric study was carried out in order to determine the λ_{max} of Furosemide in pH 5.8 phosphate buffer. 10 µg / ml solution of Furosemide in the test medium when scanned for absorption maxima in the range of 200 - 400 nm, exhibited the results tabulated in Table 1 on three consecutive days.

Table:1 Scanned λ_{max} and the absorbance values of same sample of Furosemide prepared in pH 5.8 phosphate buffer at three consecutive days.

Day	Strength	Scanned λ_{max}	Absorbance
1	10 µg / ml	271 nm	**1.300**
2	10 µg / ml	271 nm	**1.298**
3	**10 µg / ml**	**271 nm**	**1.305**

The scanned λ_{max} were found to be similar as that of reported λ_{max} (271 nm, reference) and the difference in absorbance value for three determinations was found to be insignificant at 95 % confidence interval.

CALIBRATION CURVE

Selection of media

Fasting state pH is usually steady and approximates 2 and food buffers, neutralizes gastric acid, thus increasing the pH up to about 6.5. Floating drug delivery systems are usually administered in fed state, as during the fed state onset of MMC is delayed resulting in slowdown of gastric emptying rate. Therefore, pH values in fed state conditions for preparation of calibration curves was selected.

Scanning for λ_{max}

The solutions of having a concentration of 10 µg / ml in pH 5.8 phosphate buffer was scanned in 200 -400 nm in spectrum basic mode and the results are tabulated in Table no. 3.2.

Table 2: Table for scanned λ_{max} of Furosemide in pH 5.8 phosphate buffer

S.No.	Solvents	Experimental λ_{max}(nm)
1	10 µg / ml solution of Furosemide in pH 5.8 phosphate buffer	271

Preparation of calibration curves

Calibration curves of Furosemide was prepared in pH 5.8 phosphate buffer on three consecutive days at λ_{max} 271 nm. The absorbance values (mean of three determinations) with their standard deviations at different concentrations in the range of 2 – 10 µg / ml are tabulated on Table 3.3 and represented in Figure 3.2 .Furosemide was found to obey Beer– Lambert' s law in the concentration range of 2 – 10 µg / ml with regression coefficient (r^2) values 0.9999 in pH 5.8 phosphate buffer. The regression equations were calculated as y = 0.1262 + 0.1173x.

Table 3: Calibration curves data of Furosemide using pH 5.8 phosphate buffer

S.No.	Concentration (mcg /ml)	Absorbance
		pH 5.8 phosphate buffer
1	2	0.361
2	4	0.596
3	6	0.830
4	8	1.065
5	10	1.300

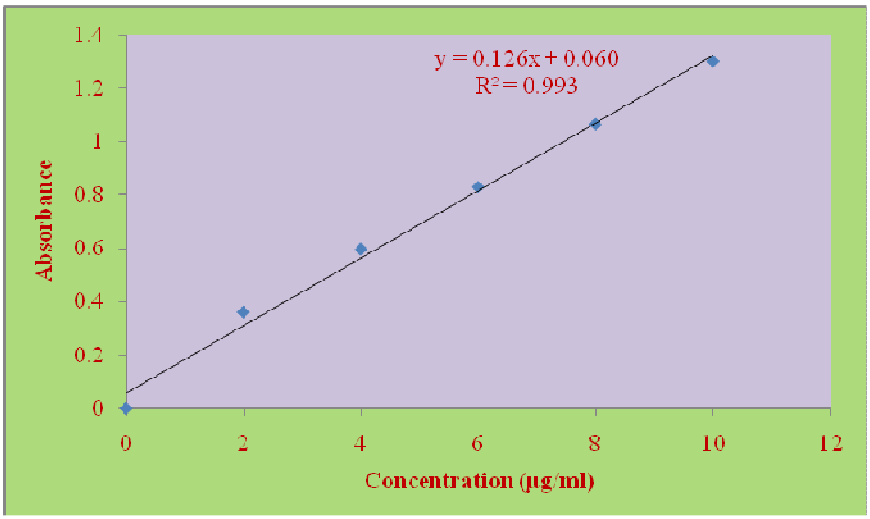

Figure2: Calibration curve of Furosemide in pH 5.8 phosphate buffer.

Assay validation of calibration curves

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Assay validation must demonstrate that the analytical procedure is able to accurately and precisely predict the

concentrations of unknown samples (Bolton1997). The calibration curves have thus been validated for the assay of active constituent i.e. Furosemide, using the following discussed parameters.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision was studied to find out intra and inter day variation in the calibration curve of Furosemide prepared in pH 5.8 phosphate buffer.

For the intraday precision , calibration curves prepared in pH 5.8 phosphate buffer were run in triplicate in same day for 3 times and for the interday precision , calibration curves were prepared in pH 5.8 phosphate buffer were run for three days and % RSD were calculated for both the cases which should be less than 2 % (Siddiqui et al 2006 ; Philip and Pathak 2006).Table no. 3.4 shows the intraday precision studies and Table no. 3.5 shows the interday precision studies for calibration curves prepared in pH 5.8 phosphate buffer .

Table 4: Interday precision study for calibration curves prepared in pH 5.8 phosphate buffer

S.No	Concentration (mcg /ml)	pH 5.8 phosphate buffer		
		Absorbance	Mean	S.D
1	2	0.361 0.364 0.363	0.362	0.0015
2	4	0.596 0.599 0.594	0.596	0.0025
3	6	0.830 0.835 0.833	0.833	0.0025
4	8	1.065 1.069 1.066	1.067	0.0020
5	10	1.300 1.303 1.309	1.304	0.0045

Table 5: Interday precision study for calibration curves prepared in pH 5.8 phosphate buffer

S.No	Concentration (mcg /ml)	pH 5.8 phosphate buffer		
		Absorbance	Mean	S.D
1	2	0.361 0.358 0.365	0.361	0.0035
2	4	0.596 0.586 0.590	0.591	0.0050
3	6	0.830 0.838 0.836	0.835	0.0041
4	8	1.065 1.071 1.068	1.068	0.0030
5	10	1.300 1.305 1.310	1.305	0.0050

For precision of calibration curve prepared in pH 5.8 phosphate buffer, the range of S.D was 0.0015– 0.0045 for the intraday and 0.0030 – 0.0050 for the interday.

Linearity and range

Linearity of an analytical method is its ability to elicit test results that are directly, or by a well – defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. Table 3.8 shows the linearity and range data for calibration curves prepared in different buffers.

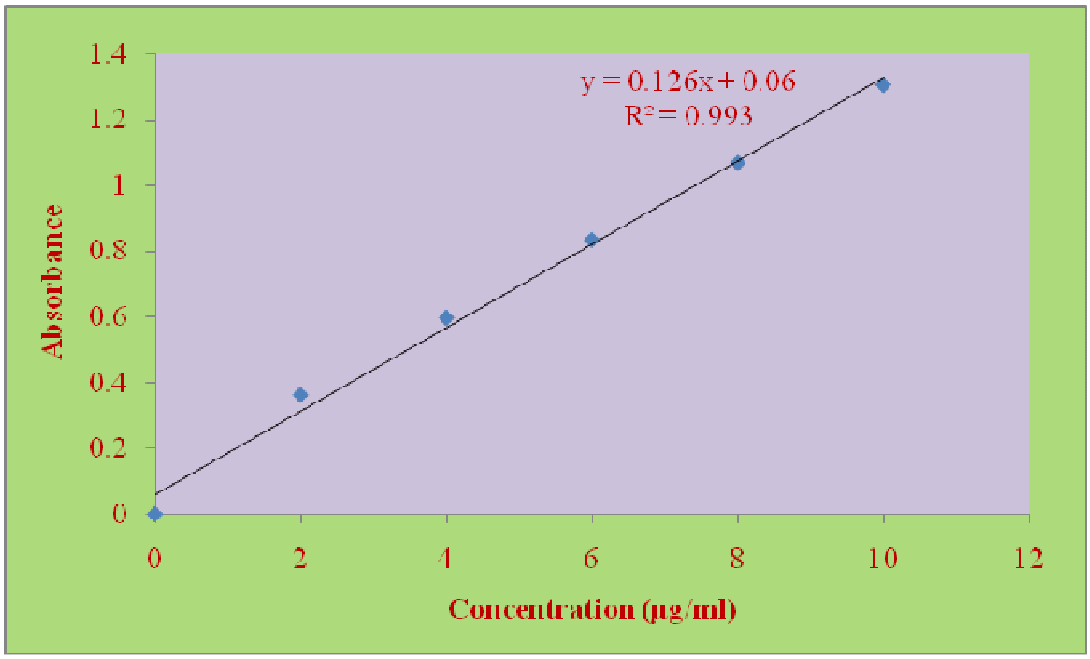

Figure 3: Intraday variation in calibration curve of Furosemide in pH 5.8 phosphate buffer.

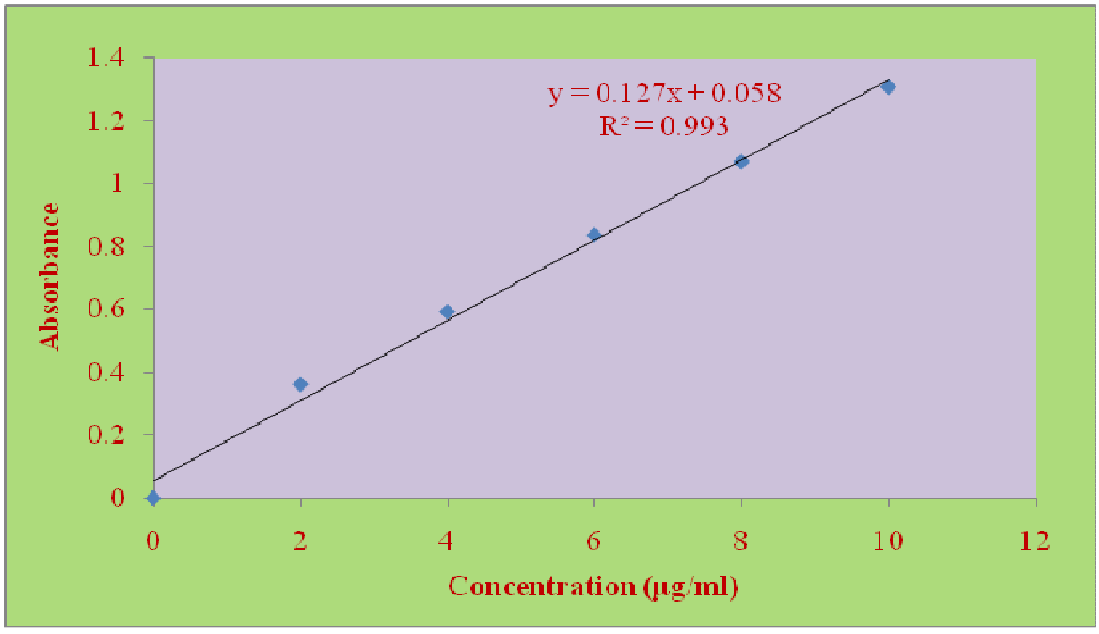

Figure 4: Interday variation in calibration curve of Furosemide in pH 5.8 phosphate buffer.

Limit of detection and limit of quantitation

Limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under a stated experimental conditions and the limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. These two parameters are required for assay validation as per ICH Q2A guidelines. Limit of detection and limit of quantitation of calibration curves were calculated (Siddiqui et al 2006; Cartensen and Rhodes 2000) which was based on the standard deviation of y – intercept of regression line (SD) and the slope (S) of the

calibration curves at levels approximating the LOD and LOQ, LOD = 3.3 (SD /S) and LOQ =10(SD /S). LOD and LOQ of calibration curve of Furosemide prepared in pH 5.8 phosphate buffer are shown in Table 6.

Table 6: Other validation parameters of calibration curve prepared in pH 5.8 phosphate buffer.

Parameters	pH 5.8 phosphate buffer
Linearity Correlation coefficient	0.9999
y – intercept	0.1262
slope	0.1173
Range	2 -10 µg / ml
LOD	0.321 µg / ml
LOQ	1.300µg / ml

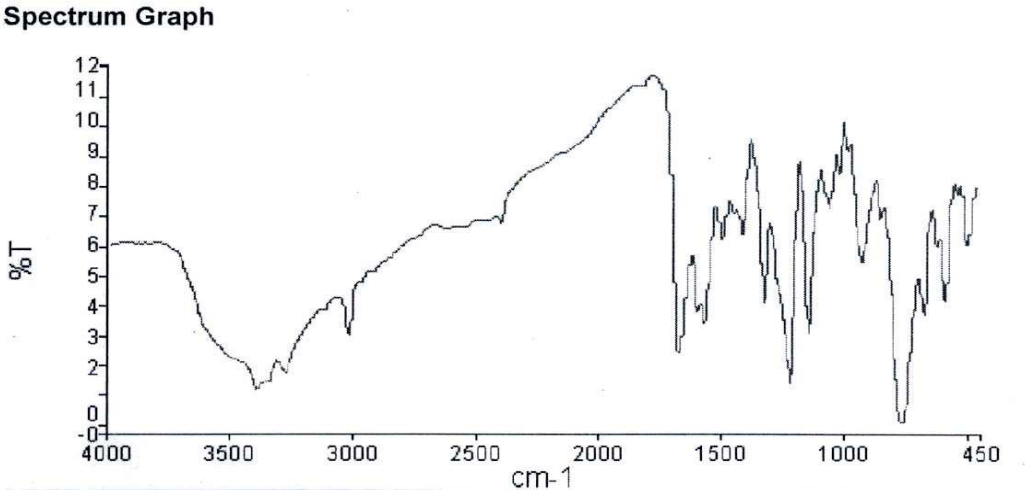

Figure 5: IR spectra of pure drug

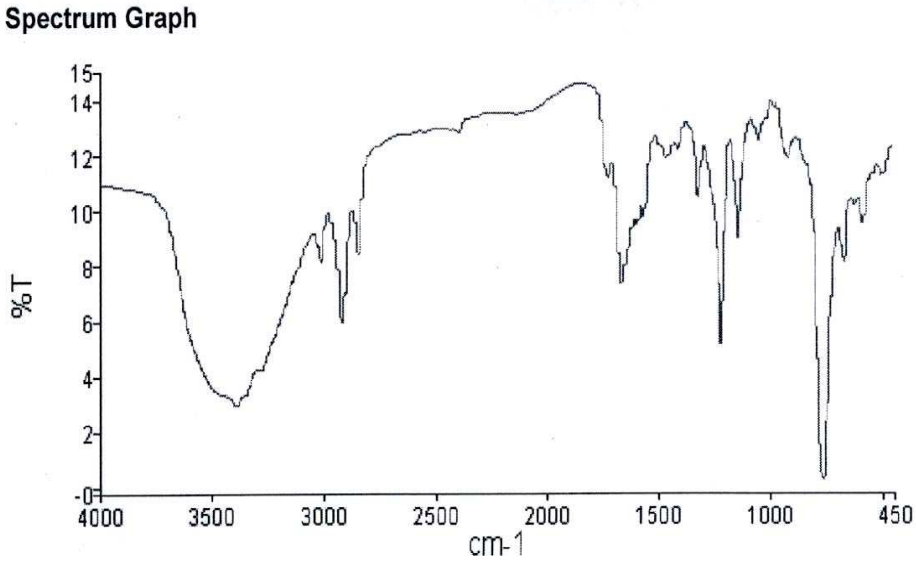

Figure 6: IR spectra of furosemide +gelucire 43/01+HPMC K4100

Compatibilaty studies

Compatibility studies were perform using IR spectrophotometer .the IR spectrum of pure drug and physical mixture of drug and polymer were studied the. Drug –excipient interaction play a vital role with the respect to release of drug

from the formulation amongst others. FTIR technique have been used here to study the physical and chemical interaction between and excipient used.it has been observed that there is no chemical interaction between furosemide and polymer used .it was observed that there were no changed in these main peak in IR spectra of mixture of drug and polymer, which show there were no physical interaction because of some bond formation between drug and polymer

The peak obtained in the spectra of each formulation correlate with the peak of drug spectrum. This indicate that the drug was compatible with the formulation component

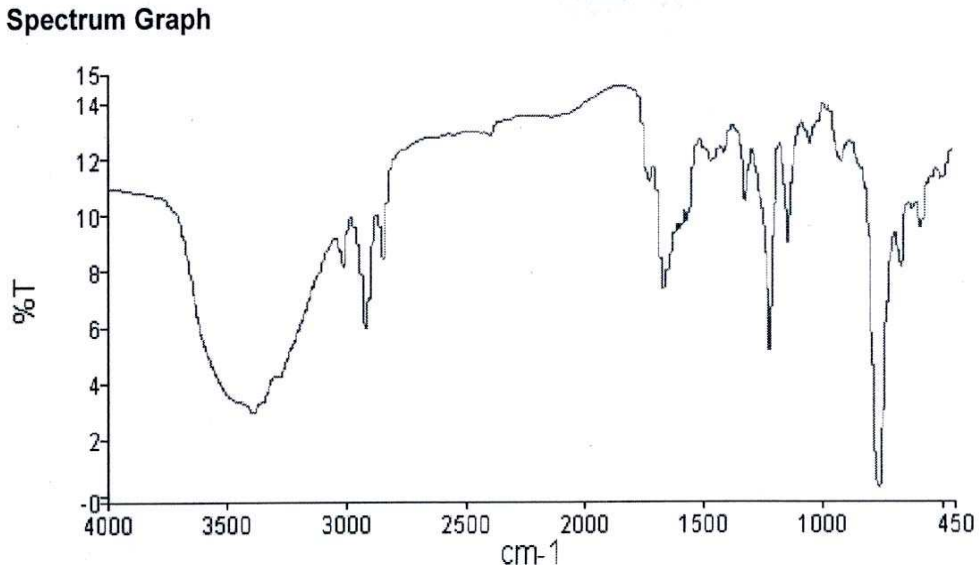

Figure 7: IR spectra of gelucire 43/01

CONCLUSION

Furosemide, is a loop diuretics that prevent that the body from absorbing too much salt, allowing the salt to instead be passed in urine. It is used in treatment of congestive heart failure and odema. Furosemide belongs to the biopharmaceutical classification system class IV i.e. furosemide has low permeability and low solubility. It oral bioavailability is 40-60%.The poor aqueous solubility and poor dissolution rate of the drug may have negative impact on it bioavailability. Estimation of furosemide was carried spectrophotometric ally by UV method at271nm.the pre -formulation study involving FTIR show that no interaction between drug and polymer. The stability study indicates that there is no degradation of drug in the formulation. Hence the furosemide was selected for the formulation .As it was important the overall bioavailability of furosemide. it absorption throughout the intestine was also focused. The sustain release floating granules of the furosemide is made by the melt granulation technique. Such formulation is achieve sustained released of drug in intestine, so that sustain absorption can be achieve. The drug released profile of the developed formulation in compression with the marketed formulation indicated a definite improvement in the drug release pattern throughout gastro intestine PH.

REFERENCES

[1] Wile, D (**2012**). *Annals of Clinical Biochemistry*. 49 (5): 419–31.
[2] Rose, BD (**1991**). *Kidney International*. 39 (2): 336–52.
[3] Liguori, A.; A. Casini; M. Di Loreto; I. Andreini; C. Napoli (**1999**). *European Journal of Clinical Pharmacology*. 55 (2): 117–124.
[4] Miyanoshita, A.; M. Terada; H. Endou (**1989**). *The Journal of Pharmacology and Experimental Therapeutics*. 251 (3): 1155–1159.

[5] Nies, A. S. (**1988**). "Renal effects of nonsteroidal anti-inflammatory drugs". Agents and Actions. Supplements. 24: 95–106.
[6] Rossi S, ed. (**2004**). Australian Medicines Handbook 2004 (5th ed.). Adelaide, S.A.: Australian Medicines Handbook Pty Ltd.
[7] Davis, A. (**2006**). *Emergency Medicine Journal*. 23 (7): 569–70.
[8] Musini, VM; Rezapour, P; Wright, JM; Bassett, K; Jauca, CD (**2015**). *Cochrane Database of Systematic Reviews* (5): CD003825. doi:10.1002/14651858.CD003825.pub4.
[9] Indian Pharmacopoeia, **2010**. Vol. I and II, Government of India, Ministry of Health and Family Welfare. Published by the Indian Pharmacopoeia Commission, Ghaziabad.
[10] Gupta R. and Pathak k., (**2008**) *Drug Dev. Ind. Pharm*., Vol. 1, pp.1-8.
[11] United State Pharmacopoeia27/ National Formulary 22 (**2004**), Asian Edn., United state Pharmacopeial Convention Inc., Rockville, pp.2622.