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DEVELOMENT AND VALIDATION OF UV-VIS SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF BUMETANIDE IN BULK AND ORAL TABLET DOSAGE FORM

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Abstract: A simple, accurate, precise, cost-effective, and linear UV-Vis spectrophotometric method has been developed for the simultaneous estimation of bumetanide in oral tablet dosage form. The method utilizes a mobile phase consisting of methanol and distilled water in a ratio of 10:90%v/v with a pH of 5.0. The linearity range for bumetanide was determined to be 5µg/ml to 25µg/ml, with correlation coefficients (r2) of 0.9995 for absorption maxima and 0.9998 for the area under the curve (AUC). The accuracy of the absorption maxima method was found to be 99.78%, while the AUC method yielded an accuracy of 99.89%. Low values of the limit of detection (LOD) and limit of quantification (LOQ) were obtained, measuring 2.62µg/ml and 7.94µg/ml, respectively, for absorption maxima, and 0.101µg/ml and 3.084µg/ml, respectively, for the AUC method, confirming the method's sensitivity. The method was successfully validated following the guidelines set by the International Council for Harmonisation (ICH). Parameters such as accuracy, precision, linearity, LOD, LOQ, and robustness were thoroughly investigated, and the obtained values were within the specified limits, indicating the method's reliability for the estimation of bumetanide in oral tablet dosage form.

Index Terms - Bumetanide (BUM), UV-Vis Spectrophotometry, ICH guideline, Absorbance maxima, Area under curve, Validation parameter.

INTRODCTION

Diuretics, also known as water pills, are medications commonly used to increase urine production and promote the excretion of excess fluid and electrolytes from the body. They play a crucial role in the management of various conditions, including hypertension, edema, and certain kidney disorders. Diuretics work by acting on different parts of the kidney to enhance the elimination of sodium, chloride, and water, leading to reduced fluid volume and decreased blood pressure. There are different types of diuretics, such as thiazides, loop diuretics, and potassium-sparing diuretics, each with their own mechanisms of action and specific indications. While diuretics can be highly effective in treating fluid-related conditions, it is essential to closely monitor electrolyte levels and kidney function to prevent potential imbalances or adverse effects. Proper dosage, regular monitoring, and close medical supervision are crucial in maximizing the benefits and minimizing the risks associated with diuretic therapy.

Bumetanide is a medication that belongs to the class of loop diuretics. It is primarily used to treat conditions such as edema (fluid retention) associated with congestive heart failure, liver disease, and kidney disorders.

Bumetanide belongs to a group of medicines called loop diuretics or "water pills." Bumetanide is given to help treat fluid retention (edema) and swelling that is caused by congestive heart failure, liver disease, kidney disease, or other medical conditions. It works by acting on the kidneys to increase the flow of urine.

Generic name:-Bumetanide

IUPAC Name: - 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid

Molecular formula: - C17H20N2O5S

Bumetanide, sold under the brand name Bumex among others, is a medication used to treat swelling and high blood pressure. This includes swelling as a result of heart failure, liver failure or kidney problem. It may work for swelling when other medications have not. For high blood pressure it is not a preferred treatment. It is taken by mouth, or by injection into a vein or muscle. Effects generally begin within an hour and lasts for about six hours. Bumetanide is a loop diuretics and works by decreasing the reabsorption of sodium by the kidneys

Bumetanide was patented in 1968 and came into medical use in 1972. It is on the World Health Organizations list of Essential Medicines. It is available as a generic medication . In 2020, it was the 270th most commonly prescribed medication in the United States, with more than 1 million prescriptions.[7] Mechanism of action: - Bumetanide interferes with renal cAMP and/or inhibits the sodium-potassium ATPase pump. Bumetanide appears to block the active reabsorption of chloride and possibly sodium in the ascending loop of Henle, altering electrolyte transfer in the proximal tubule. This results in excretion of sodium, chloride, and water ions hence, known as diuresis.

Side effect:-

1] Tinnitus 2] Anaphylaxis 3] low magnesium-dizziness, irregular heartbeats, feeling jittery, muscle cramps.

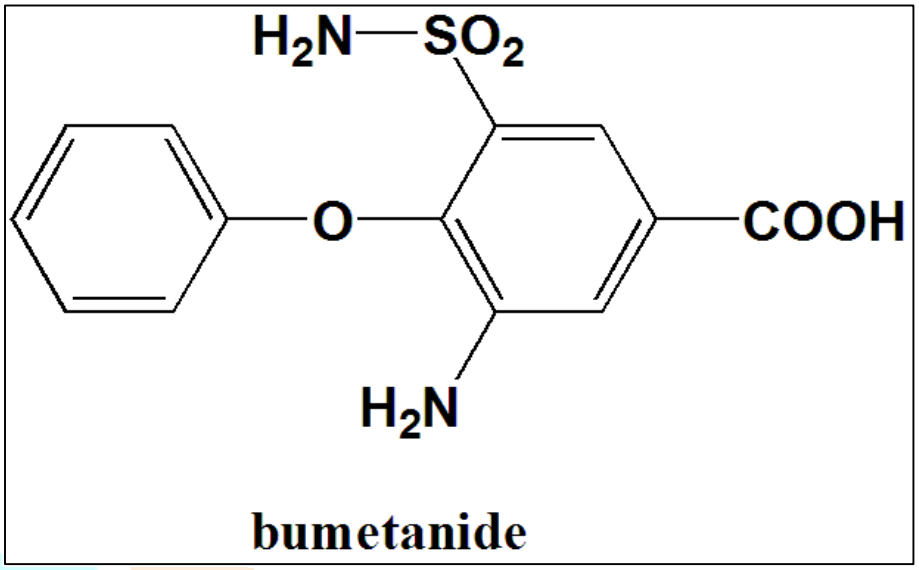

Figure no.1; Chemical Structure of bumetanide (3-butylamino-4-phenoxy-5-sulfamoyl-benzoic acid)

Pharmacodynamics: - Bumetanide is a loop diuretic of the sulfamyl category to treat heart failure. It is often used in patients in whom high doses of furosemide are ineffective. There is however no reason not to use bumetanide as a first choice drug. The main difference between the two substances is in bioavailability. Bumetanide has more predictable pharmacokinetic properties as well as clinical effect. In patients with normal renal function, bumetanide is 40 times more effective than furosemide

1] Protein binding: - 97%

2] Metabolism:- 45% is secreted unchanged. Urinary and biliary metabolites are formed by oxidation of the N-butyl side chain.

3] Route of elimination:- Oral administration of carbon-14 labeled Bumex to human volunteers revealed that 81% of the administered radioactivity was excreted in the urine, 45% of it as unchanged drug. Biliary excretion of Bumex amounted to only 2% of the administered dose.

4] Half life:- 60-90min[8]

1.1 Pharmaceutical analysis:

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as

pharmaceuticals are animals, plants, micro organisms, minerals and various synthetic products. The sample to be analysed is called as analyse and on the basis of size of sample, they can be classified as macro(0.1 g or more), semi micro (0.01 g to 0.1 g), micro(0.001 g to 0.01 g), sub micro (0.0001 g to 0.001 g), ultra micro (below 10-4 g), trace analysis(100 to 10000 ppm). Among all, the semi micro analysis is widely used.

TYPES:-

There are main two types of chemical analysis.

1.1.1 Qualitative (identification):-

Qualitative analysis is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not. Various qualitative tests are detection of evolved gas, formation of precipitates, limit tests, color change reactions, melting point and boiling point test etc.

Various types of Qualitative analysis:

A] Chemical methods:-

a) Titrimetric or volumetric method:-

It involves reaction of substance to be determined with an appropriate reagent as a standard solution, and volume of solution required to complete the reaction is determined. Volumetric methods require simple and less apparatus and they are susceptible of high accuracy. Various types of titrimetric methods are;
i)Acid-base titrations (neutralization reactions)

ii)Complexometric titrations

iii)Precipitation titration

iv)Oxidation reduction titrations

v) Non aqueous titrations

b) Gravimetric methods:-

In gravimetric analysis, a substance to be determined is converted into an insoluble precipitate in the purest form, which is then collected and weighed. It is the time consuming process. In electrogravimetry, electrolysis of the sample is carried out on the electrodes is weighed after drying. Thermogravimetry (TG) records the change in weight, differential thermal analysis (DTA) records the difference in temperature between test substance and an inert reference material, differential scanning Colorimetry (DSC) records the energy needed to establish a zero temperature difference between a test substance and reference material.

c) Gasometric analysis:-

Gasometry involves measurement of the volume of gas evolved or absorbed in a chemical reaction. Some of the gases which are analysed by Gasometry are CO2 , N2O,cyclopropane, amyl nitrate, ethylene, N2, helium etc.

B] Electrical methods:-

Electrical methods of analysis involve the measurement of electric current, voltage or resistance in relation to the concentration of some species in the solution.

Electrical methods of analysis include:

(a)Potentiometry
(b)Conductometry
(c)Polarography
(d)Voltametry
(e)Amperometry

Potentiometry measures electrical potential of an electrode in equilibrium with an ion to be determined. Conductometry measures electrical conductivity of an electrode with a reference electrode while Polarography, Voltametry and Amperometry measures electrical current at a micro-electrode.

C] Instrumental methods of analysis:-

Instrumental method involves measurement of some physical properties of the compound or a substance. These methods are employed for determination of minor or trace concentration of element in the sample. Instrumental methods are preferred due to their selectivity, high speed, accuracy and simplicity of analysis. Any change in the properties of the system are detected by measurement of absorbance, specific rotation, refractive index, migration difference, charge to mass ratio etc. Spectroscopic methods of analysis depend upon measurement of the amount of radiant energy of a particular wavelength emitted by the sample. Methods which include absorption of radiation are ultra violet, visible, infra red, atomic absorption, nuclear magnetic resonance spectroscopy etc. Emission methods involve heating or electrical treatment of the sample so that the atoms are raised to the excited state to emit the energy and the intensity of this energy is measured. Emission methods include emission spectroscopy, flame photometry, Fluorimetry etc. Chromatographic techniques and electrophoresis methods are separation methods for the mixure of compounds, but also applied for identification of compounds of mixtures. Various chromatographic techniques are GC, HPLC, TLC, HPTLC, PC etc.

Mass spectrometry involves vaporization of material using a high vacuum and the vapour is bombarded by a high energy electron beam. Vapour molecules undergo fragmentation to produce ions of varying size. These ions are differentiated by accelerating them in electrical field and then deflecting them in a magnetic field. Each kind of ion gives a peak in the mass spectrum.

D] Biological and microbiological methods:-

Biological methods are used when potency of a drug or its derivative cannot be properly determined by any physical or chemical methods. They are called bio-assays. Microbiological methods are used to observe potency of antibiotic or anti- microbial agents. In antimicrobial assay, inhibition of growth of bacteria of the sample is compared with that of the standard antibiotic. These methods include cup plate method and

turbidimetric analysis.

1.1.2 Quantitative (estimation):- Quantitative analytical techniques are mainly used to quantify any compound or substance in the sample. These techniques are based in (a) the quantitative performance of suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained, (b) the characteristic movement of a substance through a defined medium under controlled conditions, (c) electrical measurement, (d) measurement of some spectroscopic properties of the compound.

1.2 UV-Spectroscopy:-

UV spectroscopy or UV–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. Being relatively inexpensive and easily implemented, this methodology is widely used in diverse applied and fundamental applications. The only requirement is that the sample absorb in the UV-Vis region, i.e. be a chromophore. Absorption spectroscopy is complementary to fluorescence spectroscopy. Parameters of interest, besides the wavelength of measurement, are absorbance (A) or transmittance (%T) or reflectance (%R), and its change with time.

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. UV spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state. The basis of UV- Visible spectrophotometry is the absorption of the UV- visible radiation, which causes the electronic transition within the molecules by the radiant energy of definite and narrow wavelength of monochromatic radiation.

Light absorption in the UV-visible radiation causes the transition of an electron from a ground state and relaxation of energy takes place very rapidly. The important consequences of rapid relaxation of the excited states are not appreciably disturbed by absorption of light energy from any source. Therefore, the fraction of light absorbed from an incident beam is independent of the intensity of these beams. This fact was expressed quantitatively which was integrated to obtain Beer's and Lambert's law.[2]

1.2.1 Beer's and Lambert's law:-

$$\mathbf{A = \log I_o / I_t = abc}$$

Where,

A = Absorbance of the solution at a particular wavelength of the light beam.

I_o = Intensity of the incident light beam.

I_t = Intensity of beam after passing through solution.

a = Absorptivity of molecule at the wavelength of beam.

b = Path length of cell in cm.

c = Concentration of solution in g/lit.

1.2.1 Terms Used In Absorption Spectroscopy:

A] Transmittance: - The transmittance of a solution is the fraction of incident radiation transmitted by the solution.

$$T = I/I_o$$

Where,

I_o = intensity of incident radiation, I = intensity of transmitted radiation

B] Absorbance: - It is the negative logarithm of transmittance to the base ten.

$$A = - \log_{10} T = \log_{10} I_o/I$$

$$a = A / b\ c$$

Absorptivity (a)

A = Absorbance = Path length in cm, c = Concentration in g/l

C] Molar Absorptivity:-When concentration 'c' in equation A = a b c is expressed in mole/l and cell length in centimeter, absorptivity is called molar absorptivity.

$$A = b\ c$$

D] Beer – Lambert's Law: - It can be stated as the intensity of a beam of monochromatic light when passed through transparent medium decreases exponentially as the thickness and concentration of absorbing medium increases arithmetically.

$$\text{Log } I_o/I = A = abc$$

Beer's law said to be obeyed over a concentration range, if a plot of concentration against absorbance passes through origin and is a straight line.

E] Chromophore:- A chromophore is a covalently unsaturated group responsible for electronic absorption. *E.g.* C=C, C=O and NO_2.

F] Auxochrome:- An auxochrome represents a saturated group which when attached to a chromophore changes both the intensity as well as the wavelength of the absorption maximum.

E.g. OH, NH_2, Cl etc.[3]

1.2.3 Principle of UV -spectroscopy:-

- Basically, spectroscopy is related to the interaction of light with matter.
- As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules.
- When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state.
- Molecules containing π-electrons or nonbonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals.
- The more easily excited the electrons, the longer the wavelength of light they can absorb. There are four possible types of transitions (π–π*, n–π*, σ–σ*, and n–σ*), and they can be ordered as follows: σ–σ* > n–σ* > π–π* > n–π*

- The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum that aids in the identification of the compound.[4]

1.2.4 Instrumentation or part of UV-spectroscopy:-

A] Light Source:-

- Tungsten filament lamps and Hydrogen-Deuterium lamps are the most widely used and suitable light sources as they cover the whole UV region.
- Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

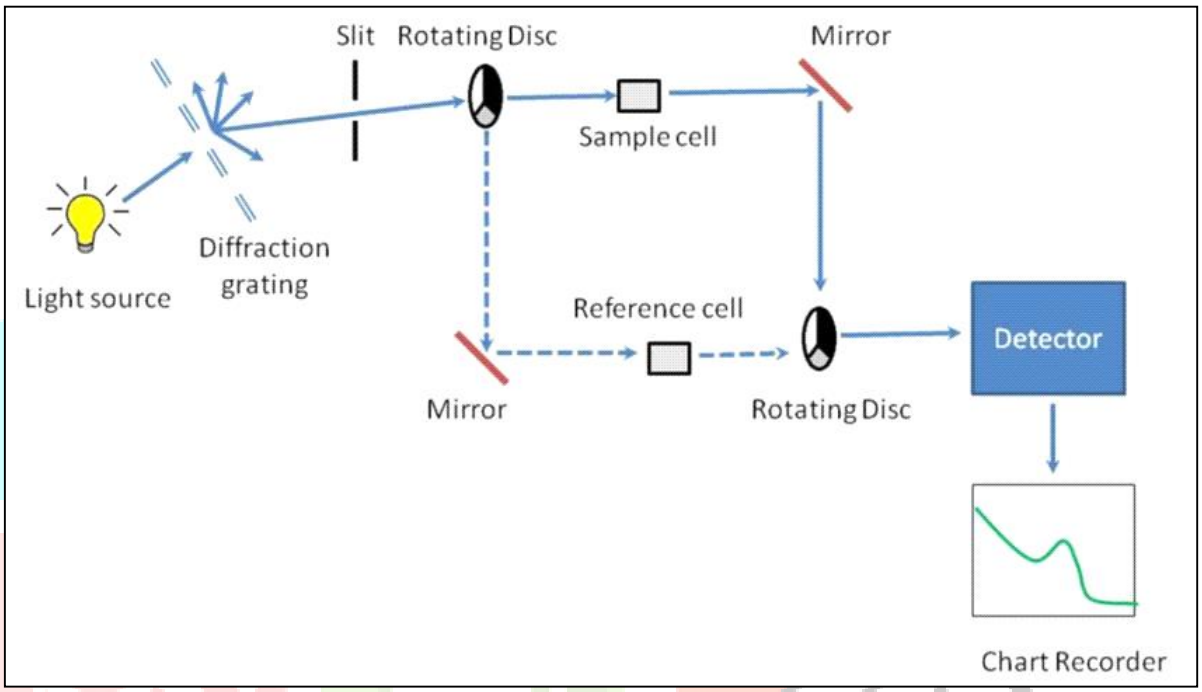

Figure no. 2 Block diagram of UV Spectrophotometer

B] Monochromator:-

- Monochromator generally are composed of prisms and slits.
- Most of the spectrophotometers are double beam spectrophotometers.
- The radiation emitted from the primary source is dispersed with the help of rotating prisms.
- The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelengths to pass through the slits for recording purposes.
- The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

C] Sample and reference cells:-

- One of the two divided beams is passed through the sample solution and the second beam is passé through the reference solution.
- Both sample and reference solution is contained in the cells.
- These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

D] Detector:-

- Generally, two photocells serve the purpose of the detector in UV spectroscopy.
- One of the photocells receives the beam from the sample cell and the second detector receives the beam from the reference.
- The intensity of the radiation from the reference cell is stronger than the beam of the sample cell. This results in the generation of pulsating or alternating currents in the photocells.

E] Amplifier:-

- The alternating current generated in the photocells is transferred to the amplifier.
- The amplifier is coupled to a small servometer.
- Generally, the current generated in the photocells is of very low intensity, the main purpose of the amplifier is to amplify the signals many times so we can get clear and recordable signals.

F] Recording devices:-

- Most of the time amplifier is coupled to a pen recorder which is connected to the computer.
- The computer stores all the data generated and produces the spectrum of the desired compound

1.2.5 Applications of UV Spectroscopy:-

A] Detection of Impurities:-

- It is one of the best methods for the determination of impurities in organic molecules.
- Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.
- By also measuring the absorbance at a specific wavelength, the impurities can be detected.

B] Structure elucidation of organic compounds:-

- It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of heteroatoms.
- UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation.
- UV absorption spectroscopy can characterize those types of compounds that absorb UV radiation thus used in the qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.
- This technique is used to detect the presence or absence of a functional group in the compound. The absence of a band at a particular wavelength is regarded as evidence for the absence of particular group.

- Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.
- Many drugs are either in the form of raw material or in the form of the formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at a specific wavelength.
- Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.
- UV spectrophotometer may be used as a detector for HPLC.

2. MATERIAL AND METHOD:-

Chemicals:-

Pure sample of bumetanide was received as sample from Reliable's lab. Methanol (K.R. chemicals), Distilled water (K.R Chemicals) India Research lab fine chemicals industries. Mumbai 400 002, Batch no-1001120219

Instrumentation:-

- A UV-PROFESSIONAl (analysis software), UV-VIS Spectrophotometer, Model no: - LT-2201 with fix bandwidth and quartz cell was used for spectral and absorbance measurements.
- Sonicator:-ultrasonic electronic instrument
- Weighing balance:- WENSAR

Glassware's: - volumetric flask, test tube, beaker, micropipette, measuring cylinder and are used in this study.

3. RESULT AND DISCUSSION:-

Validation of UV spectrophotometry method was done with respect to following parameter.

3.1 Linearity:-

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity was determined by plotting concentration verses corresponding absorbance.

Table No 1: Table show linearity data of bumetanide

Sr. no	Concentration µg/ml	Absorbance at 242nm	AUC at 242nm
1	5	0.2131	4.948
2	10	0.4112	10.056
3	15	0.6134	15.269
4	20	0.8129	20.333
5	25	0.9895	24.958

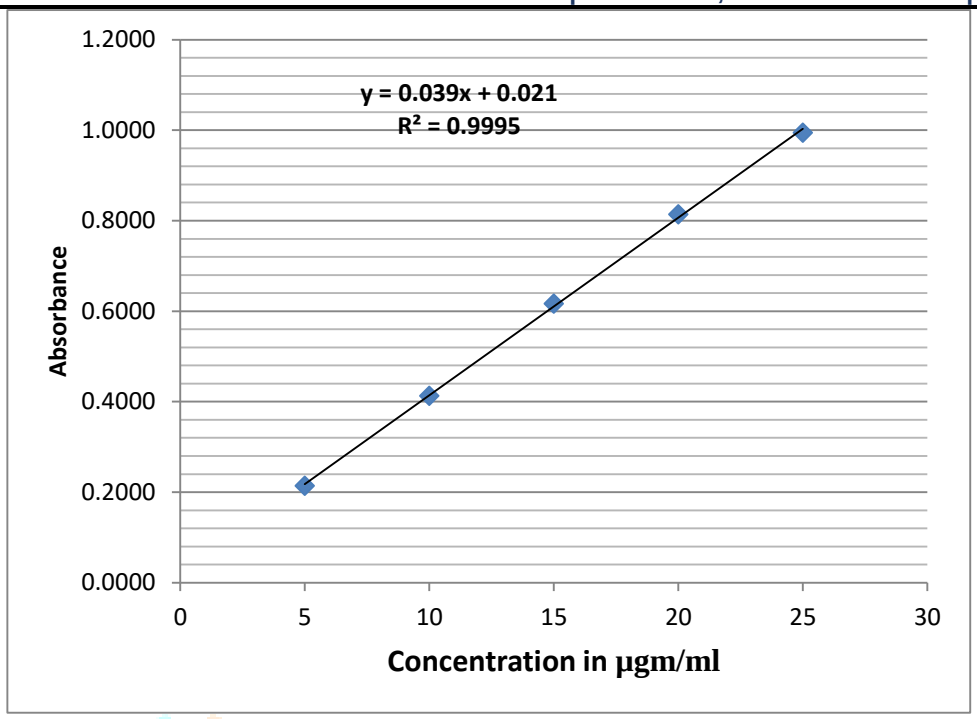

Figure No. 2; Calibration curve of Bumetinide

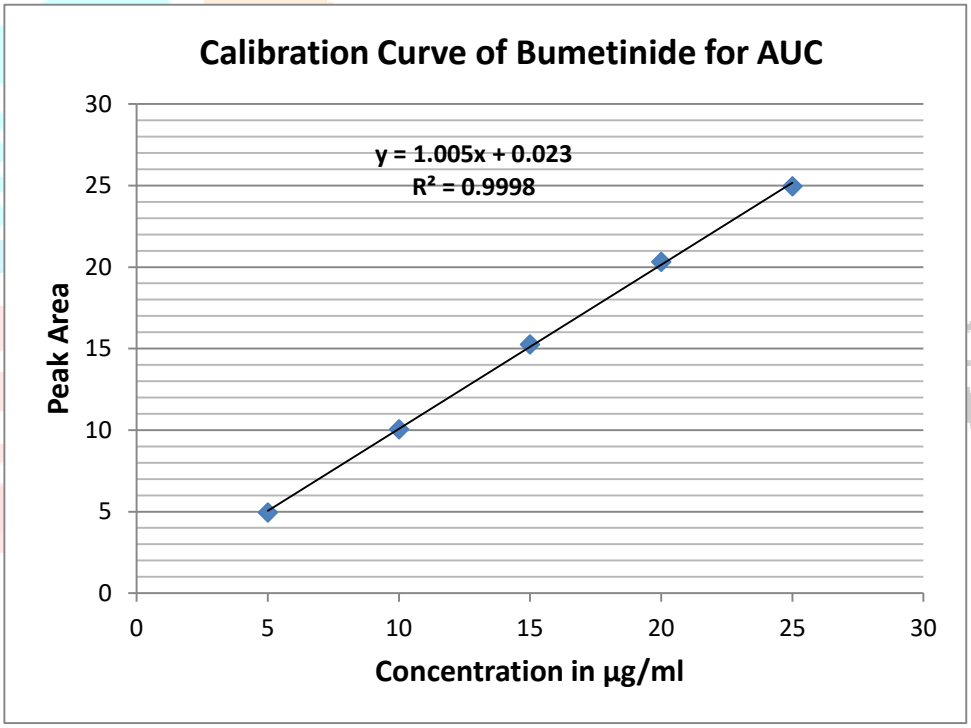

Figure No. 3; Calibration curve of Bumetinide for AUC method

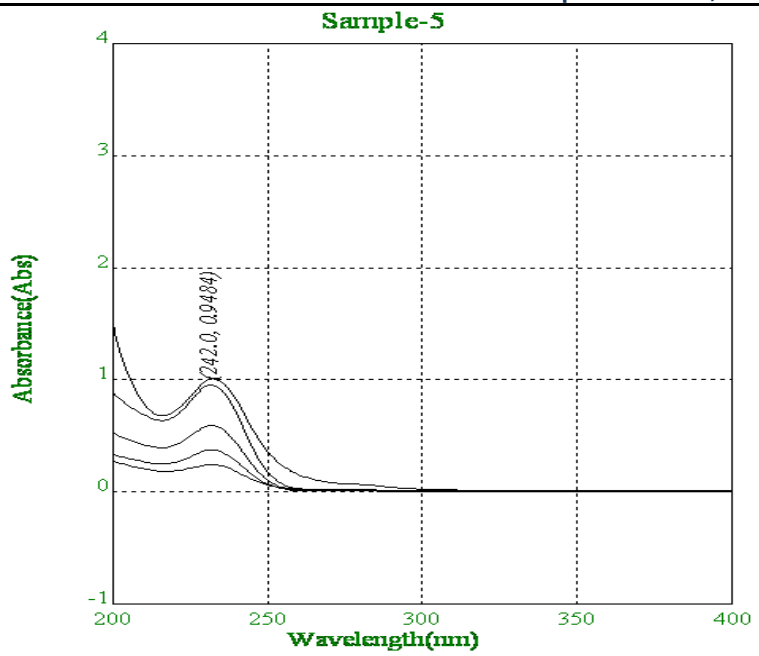

Figure No. 4; Overlay spectra of bumetanide for absorption maxima

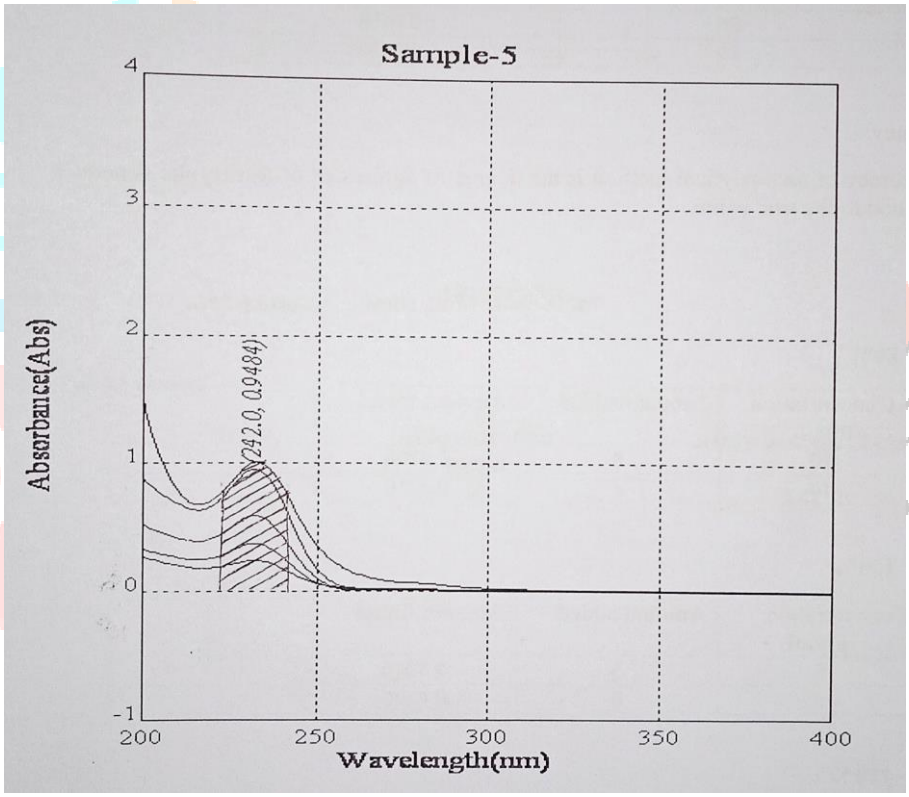

Figure No. 5; Overlay spectra of bumetanide for AUC

3.2 Precision:-

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings.

Table No 2: Table show the data of Intra-day and Inter-day precision

Sr. No.	Concentration	Intraday		Interday	
		Abs	AUC	Abs	AUC
1	10	0.4121	9.88	0.4124	9.55
2	15	0.6115	15.11	0.6114	15.14
3	20	0.8158	19.74	0.8148	19.84
4	**SD**	0.0017	0.0147	0.0019	0.14
5	**%RSD**	0.89	0.14	0.84	0.12

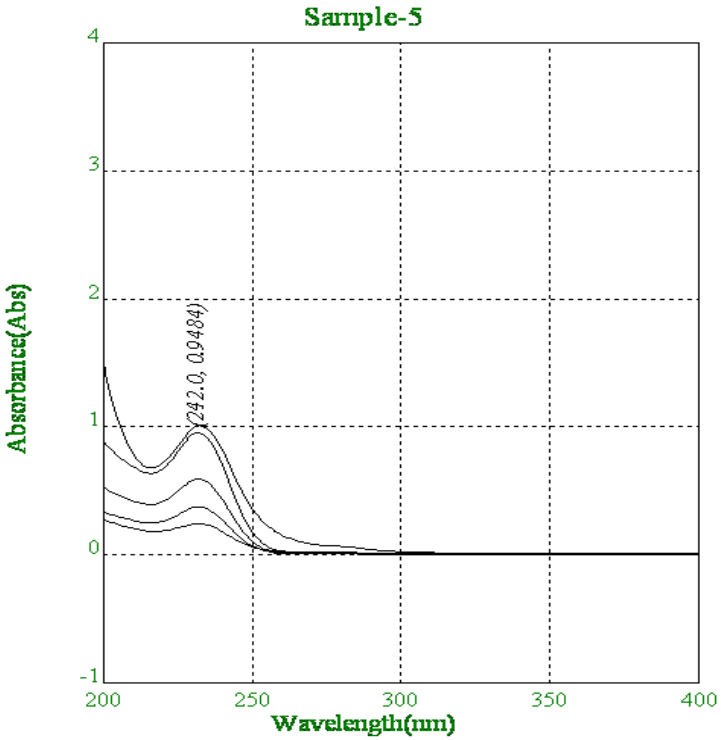

Figure No. 6; Overlay spectra of bumetanide for precision

3.3 Repeatability:-

Repeatability or test–retest reliability is the closeness of the agreement between the results of successive measurements of the same measure, when carried out under the same conditions of measurement.

Table No 3: Repeatability of Bumetanide

Sr. No.	Concentration	Absorbance	Amount found	AUC	Amount found	SD	%RSD
1	20	0.8153	20.41	0.7989	20.07	0.0015	0.29
2	20	0.8187	20.49	0.7991	20.08		
3	20	0.8795	22.01	0.7991	20.08		
4	20	0.8156	20.41	0.7992	20.09		
5	20	0.8179	20.47	0.7991	20.08		

3.4 Accuracy:-

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value.

Table No 4: Table show accuracy data of Bumetanide

Drug taken	Amt. added	ABS	Amount found	%Amount found	SD	%RSD
80%						
5	4	0.3708	8.97	99.66	0.004	0.040
5	4	0.3710	8.97	99.66	0.004	0.040
100%						

5	5	0.4118	10.020	100.2	0.004	0.036
5	5	0.4120	10.025	100.25	0.004	0.036
120%						
5	6	0.4478	10.943	99.48	0.002	0.017
5	6	0.4477	10.941	99.46	0.002	0.017

Table No 5: Table show accuracy data of Bumetanide

Drug taken	Amt. added	AUC	Amount found	%Amount found	SD	%RSD
80%						
5	4	8.97	3.98	99.78	0.007	0.091
5	4	8.97	3.97	99.66	0.007	0.091
100%						
5	5	10.02	5.020	100.2	0.009	0.072
5	5	10.035	5.035	100.35	0.009	0.072
120%						
5	6	10.95	5.963	99.68	0.003	0.031
5	6	10.94	5.941	99.46	0.003	0.031

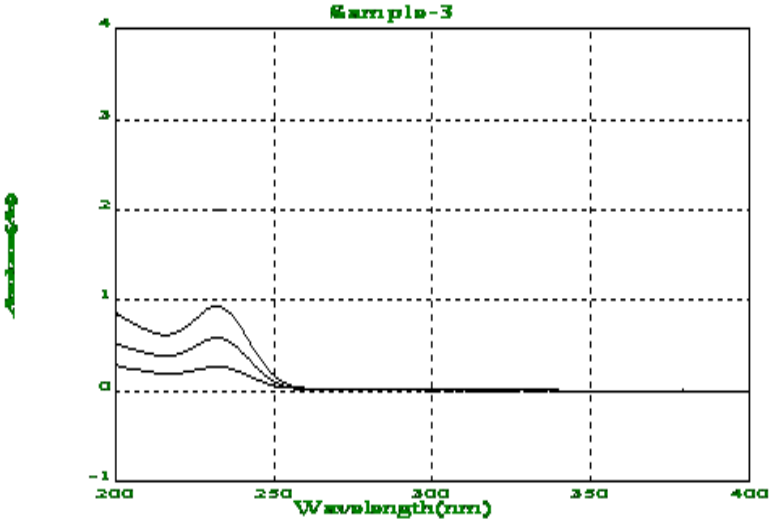

Figure No. 7; Overlay spectra show accuracy data of Bumetanide

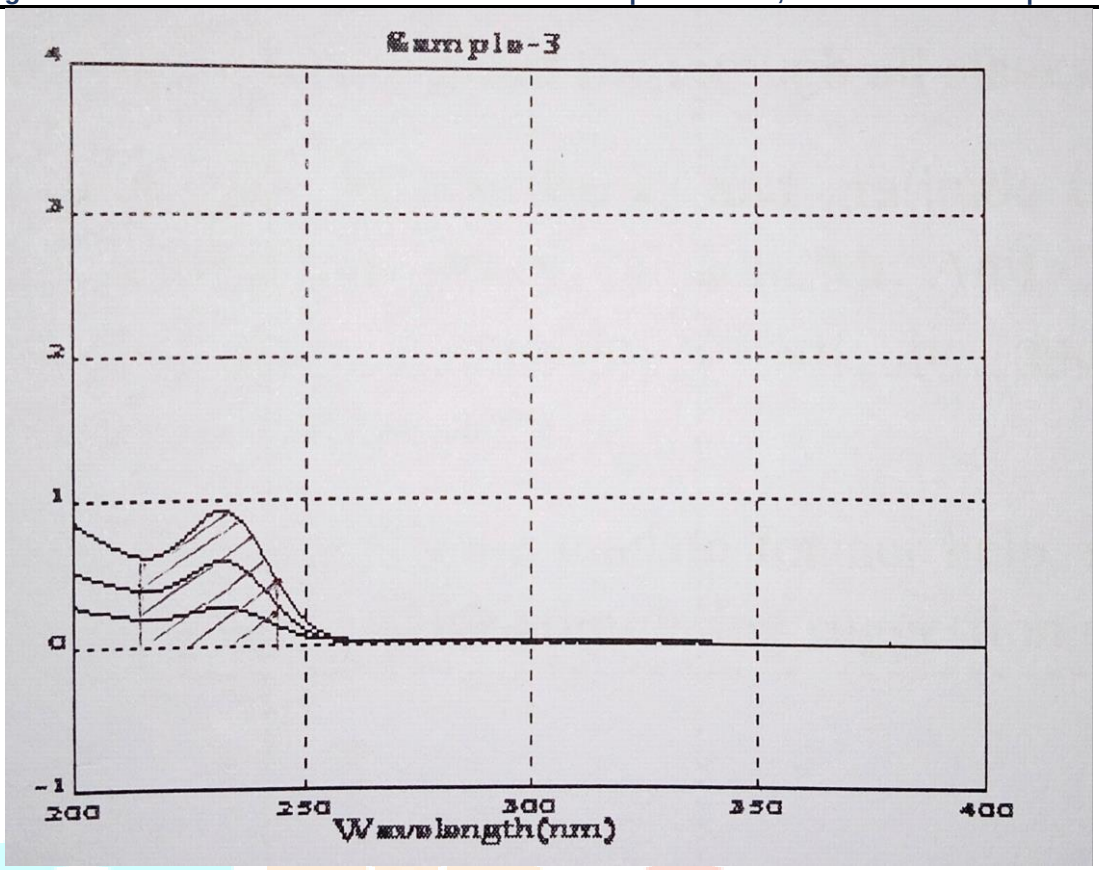

Figure No. 8; Overlay spectra show accuracy data of Bumetanide for AUC

3.5 Tablet assay:-

An assay is an investigation or analytic procedure for assessing or measuring the presence, amount or functional activity of drug

Table No 6:- Table show Tablet assay data of Bumetanide

Sr. No.	Conc.	Abs.	Amt found	AUC	Amt found	%Amt found	SD	%RSD
1	10	0.5804	10.01	10.024	10.024	100.13	0.00	0.10

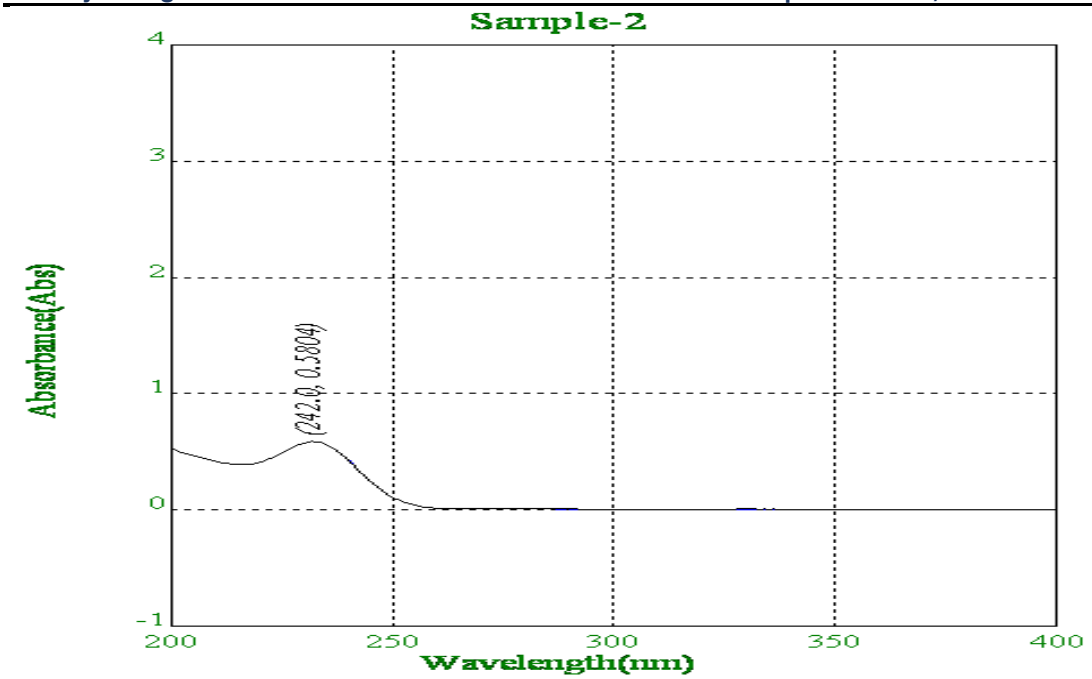

Figure No. 9; Overlay spectra show accuracy data of Bumetanide

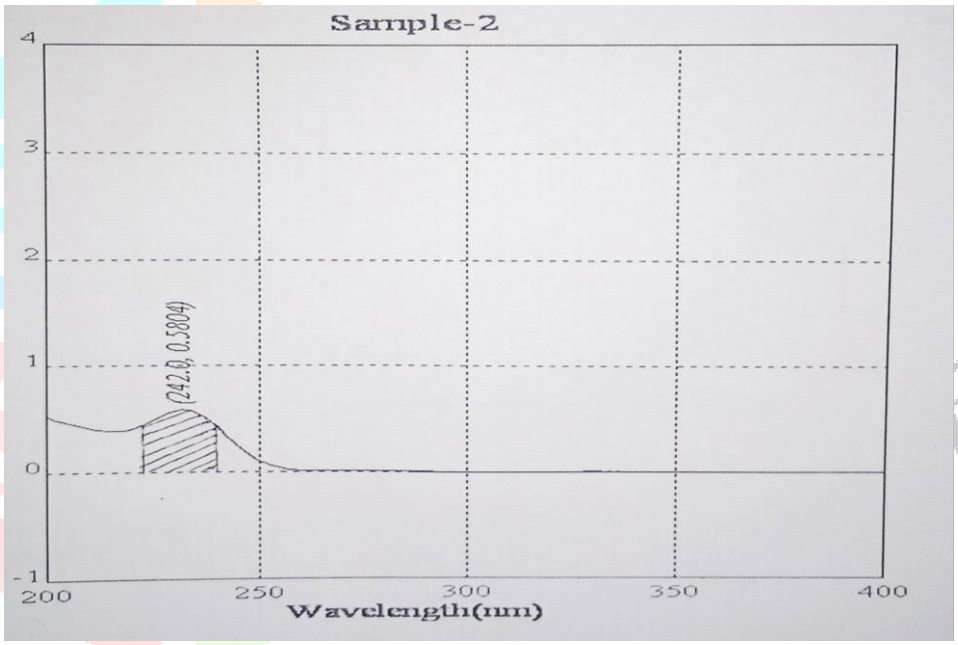

Figure No. 10; Overlay spectra show accuracy data of Bumetanide for AUC

3.6 Ruggedness:-

Table No 7; Ruggedness data of Bumetanide

Sr. No.	Concentration	Analyte	Amount found	%Amount found
1	20	0.8175	20.23	100.24

3.7 LOD and LOQ

The LOD and LOQ were found to be 2.623µg/ml and 7.948µg/ml for absorption maxima, 0.0101µg/ml and 3.084µg/ml for AUC respectively, these low values of LOD and LOQ confirmed that the method is sensitive.

Table No. 8; LOD and LOQ data of Bumetanide

Parameters	Absorption maxima	AUC
LOD (µg/ml)	2.623	0.0101
LOQ (µg/ml)	7.948	3.084

4. CONCLUSION

UV-PROFESSIONAL (analysis software) UV-Visible Spectrophotometer Model NO:-LT2201 was used to develop accurate and precise method employing Methanol: water (10:90 % v/v) as a mobile phase . The methods that have been developed for the estimation of BUM in bulk and dosage form are Absorption maxima and. The method involves absorbance measurement at 242 nm (λ_{max} of BUM for absorption maxima). The method followed Beer's law in the range of 5-25 µg/ml for both methods at selected wavelengths. Correlation coefficient was found in the range of 0.9995 and 0.9998 for Abs maxima and AUC respectively. The proposed methods were first applied to bulk powder and results indicated the BUM could be estimated accurately and precisely by these methods. The developed methods were then employed for the analysis of marketed formulation. The developed methods were validated as per ICH guidelines.

5. ACKNOWLEDGEMENT

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