RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPIC METHOD FOR ESTIMATION OF PROBENECID IN TABLET DOSAGE FORM.

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Manuscript Info	*Abstract*
	The objective of this research was to develop and validate an alternative accurate method for quantitative determination of probenecid in tablet dosage form. Probenecid is a highly lipid soluble 4-[(di-propyl amino) sulfonyl] benzoic acid having consistent uricosuric action for treatment of hyperuricaemia and gout. Probenecid was estimated using dilute sodium hydroxide at 243nm and the percentage recovery was found to be 100.2 ± 0.34. The method was tested and validated for various parameters according to the ICH guidelines. In proposed method relative standard deviation value less than 2% for the routine analysis of drug in tablet form. The limit of detection and limit of quantitation were 0.5µg/ml and 1.5µg/ml respectively. Results shows that the developed method is simple, reproducible and successfully for the estimation of probenecid in tablet dosage form without the interference of common excipients.

Introduction:-

Gout is a disease in which defective metabolism of uric acid causes arthritis, especially in the smaller bones of the feet, deposition of chalk-stones, and episodes of acute pain. It is caused by hyperuricemia and the deposition of monosodium urate monohydrate crystals in the joints or other connective tissues. Hyperuricemia can be caused by high purine intake, increased alcohol consumption, and obesity. Acute gouty attacks should be managed with non-steroidal anti-inflammatory drugs followed by appropriate uricosuric therapy (Mccarty, 1994; Groff et al., 1990).

Probenecid is a uricosuric and renal tubular blocking agent (Tripathi, 2009). Its generic name is 4-[(dipropylamino) sulfonyl] benzoic acid shown in fig. 1. It is a white or nearly white, fine, crystalline powder. Probenecid is soluble in dilute alkali, in alcohol, in chloroform, and in acetone; it is practically insoluble in water and in dilute acids (Kou, 2006; Guarino and Schanker, 1968). It inhibits the tubular reabsorption of urate, thus increasing the urinary excretion of uric acid and decreasing serum urate levels. Effective uricosuria reduces the miscible urate pool, retards urate deposition, and promotes resorption of urate deposits (Kou, 2006; Rang et al., 2008). Probenecid has also been reported to inhibit the renal transport of many other compounds including aminohippuric acid (PAH), aminosalicylic acid (PAS), indomethacin, sodium iodomethamate and related iodinated organic acids, 17-ketosteroids, pantothenic acid, phenolsulfonphthalein (PSP), sulfonamides, and sulfonylureas (Dyton et al., 1963).

Probenecid is effectively absorbed after 2- 4 hrs of oral administration with peak plasma concentration occurring at 1-5 hrs. Probenecid is 83-95% bound to plasma proteins and metablosim from boby mainly by side chain oxidation and glucuronide conjugation (Guarino and Schanker, 1968; IP, 2010; Kurian and Kurien, 2011). Several analytical techniques like derivative spectroscopy (Kurian and Kurien, 2011; Chaudhari et al., 2008; Jain, 1997), reverse phase high performance liquid chromatography (Shinde et al., 1994), high performance liquid chromatography (Vollmer et al., 1977), fluoremetric (Cunningham et al., 1978), ion-monitary (Forll et al., 1978) have been reported for

quantitative analysis of probenecid but some of these methods are complexes, costlier and time consuming. To overcome all these difficulties spectrophotometric analysis can be used as rapid, promising and reliable method for quantitative analysis of probenecid.

So, the aim of this study is to develop a new simple, rapid, reliable and precise UV spectrophotometric method for analysis of probenecid from the bulk drug and their formulation.

Experimental:-
Materials:-
Pure drug probenecid was obtained as a gift sample from Jackson pharmaceutical private limited, Amritsar. All the reagents used in this study were of analytical grade and the reagent solutions were prepared using double distilled water. A Shimadzu Corporation UV-1800 spectrophotometer & electronic balance was used.

Methods:-
Determination of maximum absorption & preparation of calibration graph of probenecid:-
The stock solution was prepared by dissolving 20 mg of pure sample of probenecid drug in 40 ml of 0.1N sodium hydroxide and then volume made up to 100 ml with phosphate buffer pH 6.8 to obtain 0.2mg/ml solution. The effect of dilution on absorption maxima was studied by diluting the above solution to 20μg/ml and scanned both the stock and diluted samples from 200-400 nm. Aliquots of different concentration of probenecid solution were made up with phosphate buffer pH 6.8.

Validation of analytical method:-
Accuracy:-
The accuracy of an analytical procedure expresses the closeness of reference value and the observed value. Accuracy was investigated by using different concentration of probenecid in 3 replicates of each concentration by two different methods (ICH 2005; USP 2009). In first method, accuracy was reported as percent recovery by the assay of known amount of the drug with excipients. Accuracy was also determined by change the concentration of probenecid in final formulation. In first method, accurately weighed 20 tablets blend with different commonly used excipients. The powder equivalent to 100 mg of probenecid was transferred to 100 ml volumetric flask and dissolved with 40 ml 0.1N sodium hydroxide. This mixture was vigorously shaken by hand. The volume was made up with phosphate buffer pH 6.8 and filtered through Whatmann filter paper No. 41. Transferred 10ml of the filtrate into a 100 ml volumetric flask and made up the volume to mark with phosphate buffer pH 6.8. The respective absorbance of diluted samples was determined at 244 nm against phosphate buffer pH 6.8 as blank.

Precision:-
The precision of an analytical procedure expresses the degree of scatterness between a series of measurements obtained from multiple sampling of the same homogeneous sample and duplicate prepared samples under the prescribed conditions (ICH 2005; USP 2009).

❖ **Repeatability:-**
Repeatability expresses the precision under the same operating conditions. Repeatability was investigated by using 5 different concentrations of probenecid (5, 10, 15 20 and 25 μg/ml) in 3 replicates each.

❖ **Intermediate Precision:-**
Intermediate precision expresses within different days and different analyst. Probenecid was analysed in three independent replicates on the same day in morning and evening (intra-day accuracy and precision) and on three consecutive days (inter-day accuracy and precision). The relative standard deviations of intra-day and inter-day values were calculated. The Relative standard deviation of analysis of probenecid by different analysts was also calculated.

Linearity and Range:-
The linearity of an analytical procedure is its ability (within a given range) to test results which are directly proportional to the concentration (amount) of analyte in the sample. Aliquots of different concentration for probenecid were prepared upto the highest concentration, till linearity was observed and absorbance was recorded at

244 nm. The range of an analytical procedure was the interval between the upper and lower concentration of probenecid (ICH 2005; USP 2009).

Robustness:-
The evaluation of robustness should be considered during the development phase of formulation and depends upon the type of procedure taken up in the study. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. This was done by changing the concentration of sodium hydroxide (replaces the 0.1N sodium hydroxide by 0.01N sodium hydroxide) (ICH 2005; USP 2009).

Limit of Detection and Limit of Quantitation:-
The limit of detection (LOD) and Limit of quantitation (LOQ) were calculated according to ICH guidelines (ICH 2005; USP 2009). The LOD and LOQ were separately determined based on standard deviation of response of the calibration curve

$$LOD= 3.3σ/s$$
$$LOQ= 10σ/s$$

Where σ represents the standard deviation of Y-intercept & s is the slope of calibration curve.

Result and discussion:-
Determination of wavelength & Calibration graph of Probenecid:-
The U.V scan the standard solution of probenecid was done for the range 200 – 400 nm and absorption maximum was determined at 244 nm as shown in fig. 2 and fig. 3. The effect of dilution on absorption maxima was studied by diluting the above solution to 20μg/ml and observed parameter show no change in absorbance maxima on diluting the solution from 200μg/ml to 20μg/ml solution, which confirmed at 244 nm. Probenecid solution was found to follow Beer's law in concentration range of 0-28μg/ml. The correlation coefficient was found to be 0.999 and shown in fig. 4.

Result of the validation of analytical method of Probenecid:-
Accuracy:-
The mean percentage recovery values (100.2 ± 0.34) were close to the taken theoretical concentration and their %R.S.D. (0.33) values were within the acceptable range as per ICH guidelines. This indicates that the method has high accuracy. The validity and reliability of the proposed method was further accessed via recovery studies by the standard addition method. These results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods. The evaluated data is summarized in table 1and 2.

Precision:-
Precision was investigated by studying the repeatability and intermediate precision. In repeatability ranged from 5 to 25 μg/ml and results indicated the excellent precision under the same operating conditions. Intermediate precision express with in laboratory variation in inter- day, intra-day and different analysts. The % R.S.D. values for proposed analytical method were within the acceptable range as per ICH guidelines. The evaluated data is summarized in table 3, 4 and 5.

Linearity and Range:-
Probenecid aliquots were scanned for absorbance at wavelength 244 nm. The linearity was observed in concentration range of 0-40μg/ml with R^2 value 0.999 shown in fig. 5.

Robustness:-
The evaluation of robustness was performed by changing the concentration of sodium hydroxide (replacing 0.1N sodium hydroxide with 0.01N sodium hydroxide). This study found no significant variations by stated changes during the analysis of probenecid. The data evaluated is summarized in table 6.
Limit of Detection and Limit of Quantitation
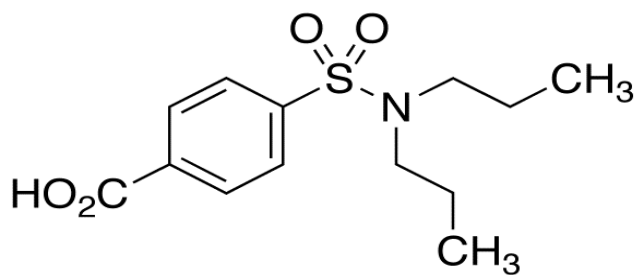The LOD & LOQ were 0.5 and 1.5μg/ml by using equations respectively.

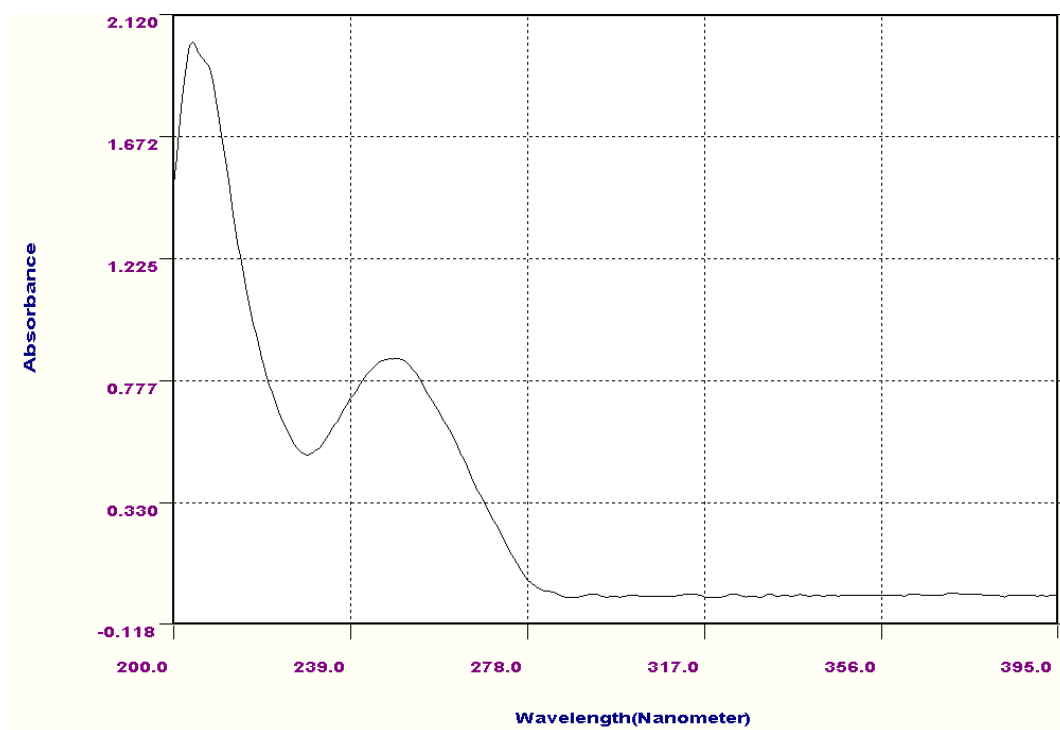**Fig.1: 4-[(dipropylamino) sulfonyl] benzoic acid (Probenecid)**
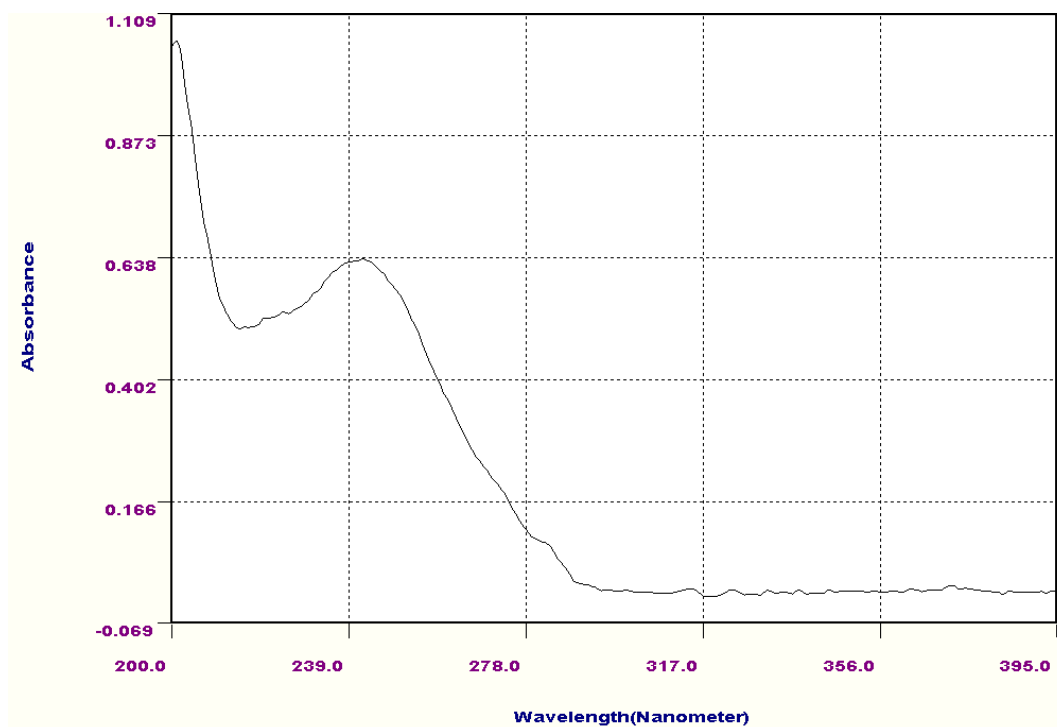

Fig. 2: UV scan of the standard solution of Probenecid
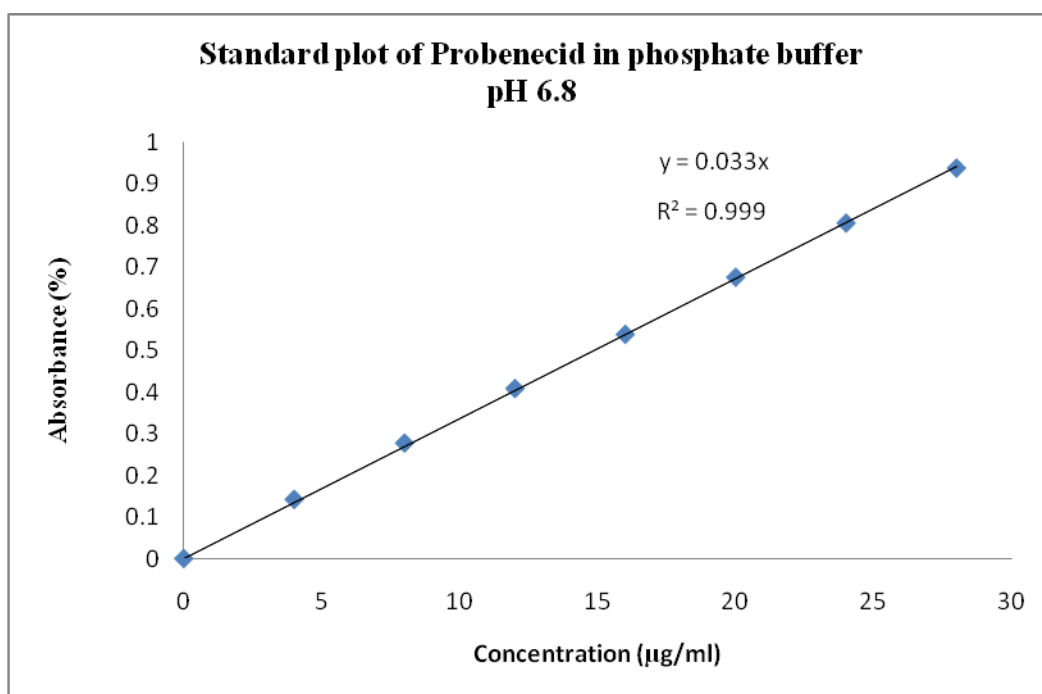
Fig. 3: UV scan of the diluted standard solution of Probenecid
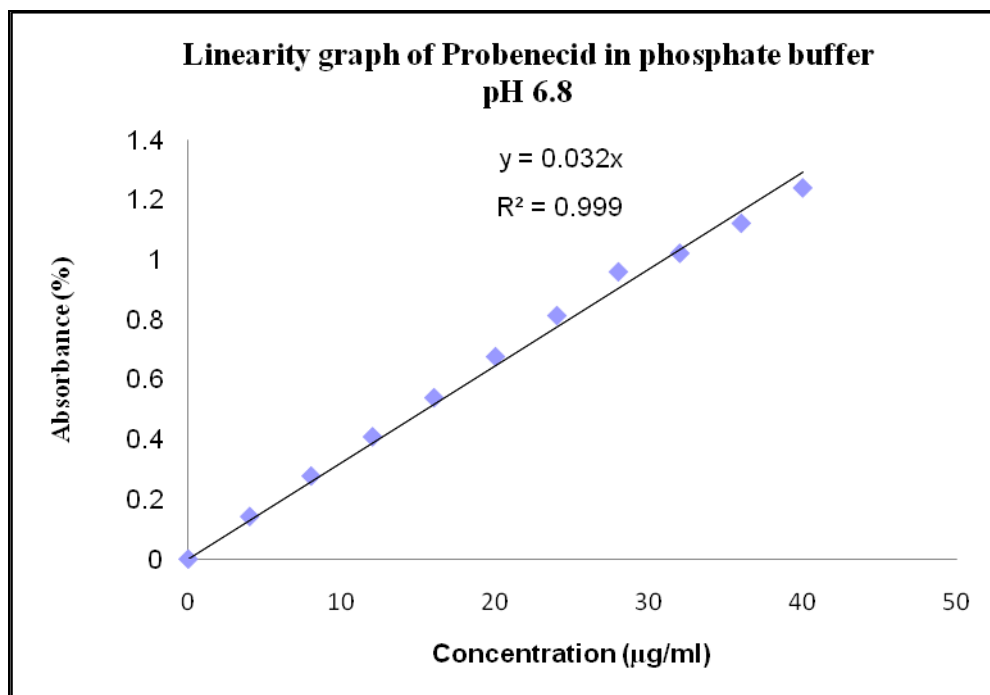

Fig. 4: Calibration graph of Probenecid

Fig. 5: Linearity graph of Probenecid

Table 1: Result for Accuracy of Probenecid

Probenecid taken (mg)	Excipients added	Probenecid recovered (%)*± S.D	%R.S.D
100	HPMC 50 cps: (100 mg), Microcrystalline cellulose:(100mg), Lactose: (50mg)	100.2±0.34	0.33

*Every reading is average of 3 samples

Table 2: Standard addition of Probenecid for accuracy

Probenecid taken (mg)	Probenecid recovered (%)*± S.D	%R.S.D
80	98.52±0.49	0.49
100	101.1±0.35	0.34
120	100.03±0.22	0.22

*Every reading is average of 3 samples

Table 3: Results for Repeatability Precision Probenecid

Amount of drug taken (µg/ml)	Average amount of Probenecid found* (µg) ± S.D	%R.S.D
5	5.03±0.018	0.36
10	10.04±0.031	0.31
15	15.06±0.034	0.22
20	19.98±0.080	0.40
25	24.95±0.029	0.11

*Every reading is average of 3 samples

Table 4: Results for Intermediate Precision of Probenecid for inter and intra day study

Amount of Probenecid (µg/ml)	Average amount of Probenecid found in intra day studies* (µg/ml)		Average amount of Probenecid found in inter days studies* (µg/ml)			Precision (intra day)		Precision (inter day)	
10	morning	Evening	1st day	2nd day	3rd day	S.D	% R.S.D	S.D	% R.S.D
	10.01	10.03	9.99	10.02	10.01	0.023	0.23	0.046	0.46

*Every reading is average of 3 samples

Table 5: Results for Intermediate Precision of Probenecid by different Analyst

Type of analysis	Probenecid taken (µg/ml)	Average amount of Probenecid found (µg/ml)*	S.D	%R.S.D
Analyst 1	10	10.01	0.056	0.56
Analyst 2	10	9.99	0.075	0.75

*Every reading is average of 3 samples

Table 6: Results for Robustness of Probenecid

Type of analysis		Probenecid taken (µg/ml)	Average amount of probenecid found (µg/ml)* ± S.D	%R.S.D
Concentration	Volume			
By 0.1N sodium hydroxide	5ml	10	10.02±0.019	0.19
	10ml	10	9.97±0.017	0.17
	15ml	10	10.01±0.034	0.034
By 0.01N sodium hydroxide	10ml	10	10.04±0.067	0.66

*Every reading is average of 3 samples

Conclusion:-

All parameters of validation were found according to ICH guidelines i.e % R.S.D value is less than 2. So, this shows the analytical method is simple, sensitive and rapid and it can be conveniently employed for the routine analysis and the quality control of probenecid in pharmaceutical dosage forms. Analysis of authentic samples containing probenecid showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in pharmaceutical preparations.

Acknowledgment:-

Reference

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