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Research Article

Method Development And Validation Of Buparvaquone And Furosemide In Bulk Drug And Injection Dosage Form (Vet) By Simultaneous Estimation And Absorption Ratio Method

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ABSTRACT

A new, simple, precise, accurate, reproducible, and efficient Vierordt's method or simultaneous equation and Absorption Ratio Method was developed and validated for simultaneous estimation of Buparvaquone and Furosemide in bulk drug and Injection dosage form (vet). The Simultaneous estimation was performed on Lab India- UV 3000 & ELICO Sl210, Methanol used as solvent. Simultaneous Equation method was carried out at 251nm (λ max of Buparvaquone) and 273 nm (λ max of Furosemide). Absorption Ratio method was carried out at 251nm (λ max of Buparvaquone) 266 nm (Isobestic point). Linearity was observed in range of 2-10 µg/ml for Buparvaquone and Furosemide respectively. The correlation coefficient value was found to be 0.999 & 0.9994 for both drugs. Method was statistically validated as per ICH guidelines and can be successively applied for analysis of injection formulation. The proposed method was also found to be accurate, precise and robust. The method could be applied to routine quality control of pharmaceutical formulations containing Buparvaquone and Furosemide The percentage recovery at various concentration levels varied from 99.18 to 100.02% for Buparvaquone and 98.47 to 100.09% for Furosemide confirming that the projected method is accurate. It could be concluded from the results obtained in the present investigation that this method for simultaneous estimation Buparvaquone and Furosemide in bulk drug and Injection dosage form (vet) is simple, accurate, precise, and economical. The proposed method can be applied successfully for the simultaneous estimation of Buparvaquone and Furosemide in bulk drug and Injection dosage form (vet)The results demonstrated that the developed method warrants further development for use in the pharmaceutical industry, such as regular quality control analysis..

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INTRODUCTION

Buparvaquone (BUP) chemically 3-[(4-tert-butylcyclohexyl) methyl]-4-hydroxynaphthalene-1,2-dione(FIG-1) and is a hydroxynaphthoquinone antiprotozoal drug related to parvaquone and atovaquone8. It is a promising compound for the therapy and prophylaxis of all forms of theileriosis. Buparvaquone has been shown to have anti- leishmanial activity in vitro. It can be used to treat bovine East Coast fever protozoa in vitro, along with the only other substance known – Peganum harmala. It is the only really effective commercial therapeutic product against bovine theileriosis, where it has been used since the late 1980s.Buparvaquone resistance appears to be associated with parasite mutations in the Qo quinone-binding site of mitochondrial cytochrome b. Its mode of action is thus likely to be similar to that of the antimalarial drug atovaquone, a similar 2-hydroxy-1,4-naphthoquinone that binds to the Qo site of cyto chrome b thus inhibiting Coenzyme Q – cytochrome c reductase.

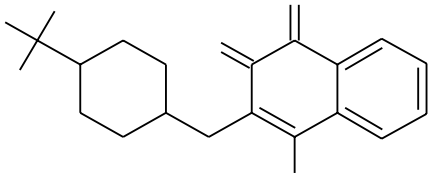

Fig-1

Furosemide (FUR)chemically 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid (FIG-2) and is a loop diuretic medication used to treat fluid build-up due to heart failure, liver scarring, or kidney disease8. It may also be used for the treatment of high blood pressure. It can be taken by injection into a vein or by mouth. When taken by mouth, it typically begins working within an hour, while intravenously; it typically begins working within five minutes.

Common side effects include feeling lightheaded with standing, ringing in the ears, and sensitivity to light. Potentially serious side effects include electrolyte abnormalities, low blood pressure, and hearing loss. Blood tests are recommended regularly for those on treatment. Furosemide is a type of loop diuretic that works by decreasing the reabsorption of sodium by the kidneys. Common side effects of furosemide injection include hypokalemia (low potassium level), hypotension (low blood pressure), and dizziness.

Furosemide was patented in 1959 and approved for medical use in 1964.It is on the World Health Organization's List of Essential Medicines. In the United States, it is available as a generic medication. In 2019, it was the seventeenth most commonly prescribed medication in the United States, with more than 28 million prescriptions. It is on the World Anti-Doping Agency's banned drug list due to concerns that it may mask other drugs. It has also been used in race horses for the treatment and prevention of exercise-induced pulmonary hemorrhage.

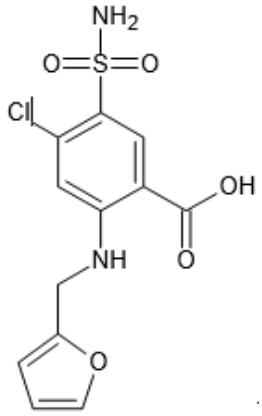

Fig-2

Literature survey publicized that certain UV spectroscopy 2 HPLC 1, 4, 12 methods were reported for the estimation of these drugs either individually for BPE in combined with other drugs. The other few methods such as UV spectroscopy 9 HPLC 5, 10, 11 methods were reported for the estimation of these drugs either individually for FSD in combined with other drugs. On the other hand simultaneous equation (SE) or Vierordt's method was not reported for this new combination. Simultaneous equation (SE) or

Vierordt's method is typically applied to estimate drug combinations that contain two drugs or more than two drugs in combined dosage form. Technical hitches involved in this method is very less when compared to other UV methods. Hence an attempt has been made to develop a simple and a reproducible SE method to ensure the safety and efficacy of this selected combination. The method was further validated as per ICH guidelines13 for the parameters like precision, accuracy, sensitivity, and linearity. The result of analysis was validated statistically and by recovery studies. This developed method was fully validated and applied successfully for the simultaneous estimation of BPE and FSD in pure and pharmaceutical dosage form.

MATERIAL AND METHOD

Materials

The selection of dosage form for the present study is BPE and FSD Injection. This drug was approved by CDSCO (Central Drug Standard Organization) at (27.06.2019)

Drug Sample

The raw material (Buparvaquone and Furosemide) was purchased from Akshay Trading Co. Mumbai.

Formulation

BUTASUN plus injection containing 50 mg of Buparvaquone and 50 mg of Furosemide was purchased from the Arka pharmaceuticals, Gujarat.

Reagents and Chemicals

All the chemicals used were of analytical grade procured from Fisher scientific, Maharashtra, India.

Selection of Solvent:

The solubility of drugs was determined in a variety of solvents as per Indian Pharmacopoeia Standards. Solubility was carried out in polar to non-polar solvents. The common solvent was found to be Methanol for the analysis of BPE and FSD for the proposed method.

Preparation of standard stock solution

Accurately weighed drug samples of both BPE and FSD (25mg each) were transferred into a suitable standard volumetric flask separately, dissolved in methanol and both the drugs were diluted to get final conc 10 µg/ml. These solutions were scanned in the UV region of 200 - 400 nm in 1cm cell against methanol as blank and the overlaid spectra was recorded. Wavelengths 251 nm (λ max of BPE) and 273 nm (λ max of FSD) were selected for the Simultaneous equation method (Figure: 3 and Figure: 4). For Absorption Ratio method, wavelengths 251 nm (λ max of BPE) and 266nm (isobestic point) were selected (Figure: 5).For both the methods 2- 10µg/ml concentration were taken for the preparation of calibration graph was given in Figure 7 and Figure 8.

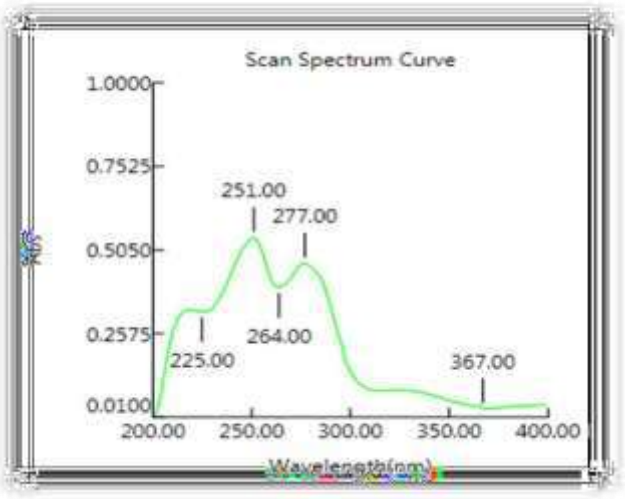

Figure 3: Spectrum of Buparvaquone (10µg/ml)

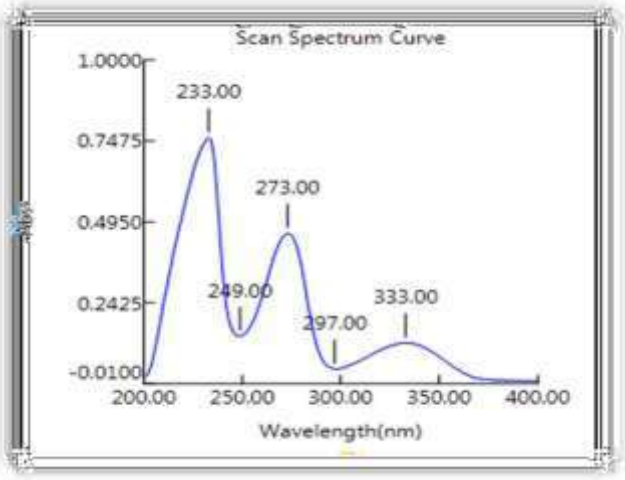

Figure 4: Spectrum of Furosemide (10µg/ml)

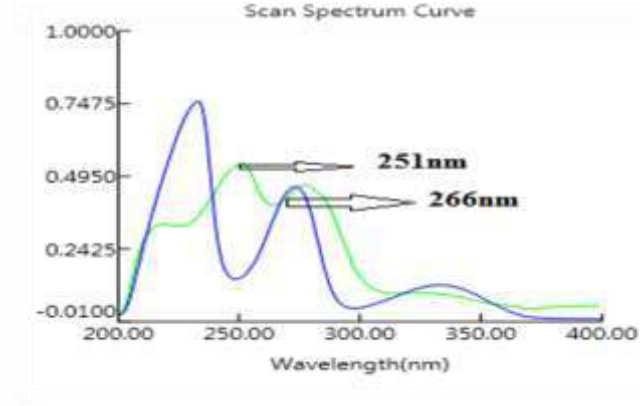

Figure 5: Overlay spectra of BUP and FUR

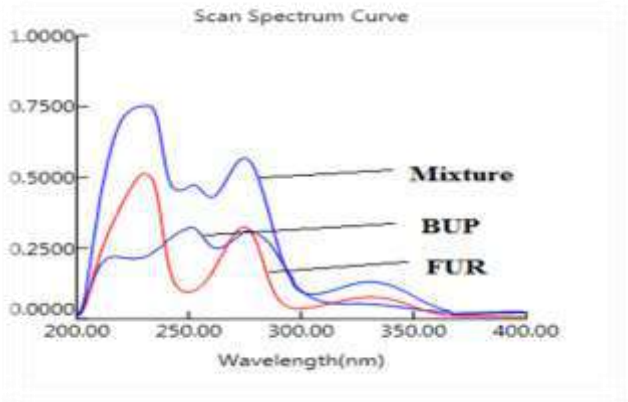

Figure 6: Overlay simultaneous spectra of BUP, FUR and MIX

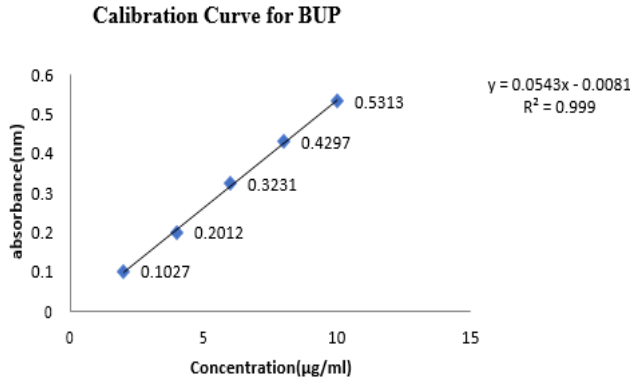

Figure 7: Calibration curve for BUP

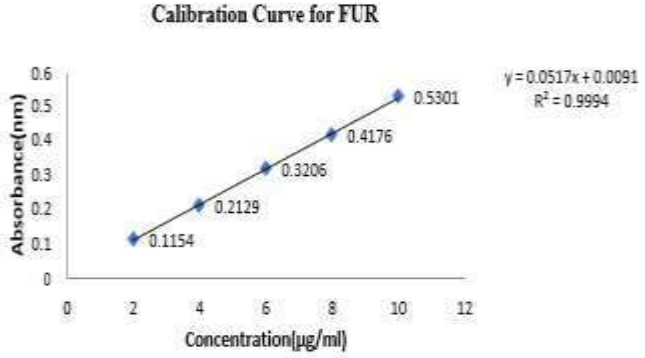

Figure 8: Calibration curve for FUR

Method development

UV spectrophotometric method for estimation Buparvaquone and Furosemide was carried by the Simultaneous and Absorption Ratio Method.

Analysis of Injection formulation

1ml of sample (BUTASUN Plus) containing 50mg of BUP and 50mg of FUR was accurately transferred into a 50 ml volumetric flask, dissolved in sufficient quantity of Methanol and the solution was sonicated for 15 minutes and diluted to the mark with Methanol and then it was suitably diluted to get final concentration of 6 µg/ml of BPE and 6 µg/ml of FSD for both the methods. The absorbance of sample solution was measured six times at all selected wavelengths for all the methods.

Recovery studies

The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drug standard solution was 6 µg/mL of BPE and 6 µg/mL of FSD for all the methods.

VALIDATION OF THE DEVELOPED METHODS

Linearity

Linearity was checked by diluting standard stock solution at five different concentrations. BPE was linear with the concentration range of 2-10 µg/ml and FSD showed linearity in the range of 2-10 µg/ml at 251nm and 273nm for Simultaneous method was shown in Fig:7. In Absorption Ratio method BPE and FSD was linear in the range of 2-10 µg/ml at 251nm and 266nm and was shown in Fig: 8. The calibration curves [n=5] were plotted between concentration and absorbance of drugs were measured. Optical parameters were calculated. The regression line relating standard concentrations of drug using regression analysis,

the calibration curves were linear in the studied range and equations of the regression analysis were obtained: and it was given in the table 1 for both the methods.

Table1: Optical Parameters for BUP and FUR for both Methods A and B

Parameter	BUPARVAQUONE	FUROSEMIDE
λ max (nm)		
Method A	251nm	273nm
Method B	251nm	266nm(ISOBESTIC POINT)
Linearity(µg/ml)		
Method A	2-10µg/ml	2-10 µg/ml
Method B	2-10 µg/ml	2-10 µg/ml
Correlation coefficient(r^2)		
Method A	0.999	0.9991
Method B	0.9992	0.9999
Regression equation (Y=mx+c)		
Method A	Y=0.0174x+0.002	Y=0.0332x+0.0143
Method B	Y=0.0537x-0.0039	Y=0.0424x+0.0004
LOD	0.0248	0.0155
LOQ	0.0752	0.0471

Table 2: Assay results for determination of BUP and FUR injection for method A andB

Mixture	Method	Label claim (mg)		Amount found (mg)		%Label claim± SDn=6	
		BUP	FUR	BUP	FUR	BUP	FUR
Injection	**Method A**	50	50	49.96	50.44	99.92±0.7808	100.98±0.0503
	Method B	50	50	49.42	50.41	99±0.1903	100.52±0.3173

Table 3: Intraday And Interday Analysis For Method A And Method B

Drug	Sample No.	Labelled amount (mg)	Percentage obtained		±SD		%RSD	
			Intraday(%)	Interday(%)	Intraday	Interday	Intraday	Interday
(Method A) BUP	1	50	100.01	100.14	0.0776	0.0866	0.0776	0.0864
FUR	2	50	100.84	100.70	0.0754	0.0971	0.0748	0.0964
(Method B) BUP	1	50	97.74	97.75	0.0754	0.0971	0.0748	0.0964
FUR	2	50	100.61	100.62	0.1594	0.1550	0.1585	0.1540

Table 4: Ruggedness Study For Method A And Method B

S. No	Drug	Condition	Mean %	±SD	%RSD
1	**(Method A)** Buparvaquone	Analyst 1	100.04	0.2271	0.2270
2	Buparvaquone	Analyst 2	99.88	0.0556	0.0557
3	Furosemide	Analyst 1	100.59	0.1928	0.1917
4	Furosemide	Analyst 2	100.71	0.1258	0.1249

1	**(Method B)** Buparvaquone	Analyst 1	98.57	0.1289	0.1308
2		Analyst 2	98.51	0.1662	0.1687
3	Furosemide	Analyst 1	99.44	0.2098	0.2110
4		Analyst 2	99.73	0.1921	0.1926

Table 5: Results of the recovery study by proposed method A

Drug	Percentage	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	S. D	% R.S.D	S. E
BUP	80 100 120	6 6 6	4.8 6 7.2	10.7865 11.9695 13.1839	4.7865 5.9695 7.1839	99.67 100.34 99.60	0.1738	0.1746	0.1003
					Mean	99.53			
FUR	80 100 120	6 6 6	4.8 6 7.2	10.854 12.11 13.3145	4.854 6.11 7.3145	100.98 101.98 101.70	0.5158	0.5079	0.2978
					Mean	101.55			

Table 6: Results of the recovery study by proposed method B

Drug	Percentage %	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	S. D	% R. S. D
BUP	80 100 120	6 6 6	4.8 6 7.2	10.6173 11.8071 12.9756	4.6173 5.8071 6.9756	96.19 96.78 96.88	0.3728	0.3859
					Mean	96.61		
FUR	80 100 120	6 6 6	4.8 6 7.2	10.9624 12.1519 13.3779	4.9624 6.1519 7.3779	103.38 102.53 102.47	0.5089	0.4951
					Mean	102.43		

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation and it was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated and given in the table 2. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated and given in the table 3.

Accuracy

Percent Accuracy of an analysis was determined by systemic error involved. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Recovery studies were carried out for all the methods by spiking standard drug in the injection formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines. The result was given in the table 5 and table 6.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test

results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. The result was given in the table 4.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated, in accordance with ICH guidelines, LOD = 3.3σ/S and LOQ = 10 σ/S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The result was given in the table 1.

RESULTS AND DISCUSSION

The selected drugs Buparvaquone and Furosemide were estimated by using simultaneous estimation and Absorption Ratio method as per ICH guidelines. The method was validated for all validation parameters as per ICH guidelines. The linearity range for BUP and FUR was 2- 10 µg/ml with R2 value of 0.999 and 0.9994 respectively was given in Table: 1. the % RSD for intraday and inter day precision and inter day precision was <2% (Table: 1). The method has been validated in assay of injection dosage forms. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits. SD values suggest that the precision of the method was further confirmed. The ruggedness of the method was confirmed by performing the analysis with the different analysts and different instruments. The % average of synthetic mixture was found to be 99.92 for BUP and for FUR 100.98(Table.2) The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation. The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of BPE and were found to be 0.7808 and 0.0503 for FSD respectively (Table: 2). The low % RSD values suggest that the method has good precision. The recovery studies were performed and the report was given in the table 5 and table 6 for Method A and Method B.

CONCLUSION

The results indicate that the proposed UV spectrophotometric methods are simple, rapid, precise and accurate. The developed UV spectrophotometric methods were found suitable for determination of BUP and FUR as bulk drug and in marketed Injection dosage formulation without any interference from the excipients. The validated methods produce results within known uncertainties that are helpful to continuing drug development and provide emerging knowledge supporting the product. The time and effort that is devoted into developing scientifically sound and robust analytical methods should be aligned with the drug development stage. Statistical analysis proves that, these methods are repeatable and selective for the analysis of BUP and FUR. Thus the above study's findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms.

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