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Ultraviolet-visible spectrophotometric method for estimation of the glibenclamide in presence of liposomal/proliposomal turbidity

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A simple and sensitive ultraviolet spectrophotometric method for quantitative estimation of glibenclamide in presence of lipid turbidity has been developed, to avoid false estimation due to diffraction by turbidity. UV detection has been performed at 232 nm, 227 nm and 237 nm and the calibration curve is plotted between resultant of absorbance of [232 nm – (227 nm + 237 nm)/2] and concentration of analyte. The calibration curve is found to be linear over the concentration range tested (2-20 µg/mL) with Limit of Detection of 0.27 µg/mL and Limits of Quantification 0.82 µg/mL. Percent relative standard deviations and percent relative mean error, representing precision and accuracy respectively, for clear as well as turbid solutions are found to be within acceptable limits i.e. always less than 0.69 and 0.41 respectively for clear solution and 0.65 and 0.47 respectively for turbid solution. The recovery studies are also found to be within acceptable limits (100.10 to 101.21%) for both types of solutions. Conclusively, our method is successfully applied for the determination of glibenclamide in clear as well as turbid solution and it is found that the drug analyte in both types of solutions can be detected from the same calibration curve accurately and precisely and glibenclamide entrapped in the liposomes or in proliposomal matrix is not detected.

Keywords: Glibenclamide, Liposomes, Proliposomes, Turbidity, UV spectrophotometry

The samples of dissolution of liposomes/proliposomes contain lipid turbidity. As the literature review revealed that the drug released is measured after separation of the lipid turbidity in cooling ultracentrifuges at high speeds[1,2]. During ultracentrifuge, at such a high speed, the liposomes might rupture and the drug is released to the dissolution medium all at once and can affect the actual concentration of the solution. Another method of dissolution of liposomes is using dialysis bags. The liposomal formulation is filled in the dialysis tubing and both the ends of it are tied with the help of tying clips. The tied tubing is then dipped in the dissolution medium. The drug thus released does not take the liposomal turbidity with it in the dissolution medium and can be detected easily without any interference due to turbidity[1,3]. This method increases the evaluation cost by adding the cost of dialysis tubing.

Derivative spectrophotometric methods, difference spectrophotometry and bichromatic methods can also be applied for elimination of background absorption and/or scattering but all these methods are more or less complex. Also derivative spectrophotometry results in more complex spectrum.

Various methods from different researchers are available for assay of GLIB. HPLC methods coupled with UV detection[4-6], fluorescence detection[7] or mass spectrometry[8] are the most commonly used methods for the determination of glibenclamide. These HPLC methods can be used for the purpose but are complex, expensive and time consuming extraction procedures. Also presence of turbidity might interact with the extraction process.

Thin layer chromatography (TLC) has been employed for the detection of glibenclamide[9]. Volta metric method was also used[10]. These methods too are time consuming, expensive and involve some of the reagents those may be harmful to human and to the environment.

The Literature survey also revealed that spectrophotometric methods have been employed for the determination of glibenclamide based on derivatization technique or coupling with another reagent[11-17].

None of the above mentioned methods can be used for estimation of dissolution samples of the liposomes or proliposomes of the drug without separation of turbidity i.e. separation of turbidity is required for estimation by these methods because turbidity results in false estimation of the drug due to diffraction or dissolution studies would be carried out using dialysis bags so that turbidity do not reach the dissolution medium. A high speed cooling ultracentrifuge is required for separation that might result in bursting of liposomes during separation leading to release of the entrapped drug all at once in the solution to give

increased concentrations and later one increases the cost of study by adding the cost of dialysis tubing.

A simple, easy to calculate, UV spectrophotometric method for estimation of glibenclamide in presence of liposomal turbidity is described here. The purpose of the research work is to develop and validate a simple and sensitive analytical method to avoid false estimation of glibenclamide due to diffraction by lipid turbidity. The method is successfully applied to quantitatively estimate the drug in presence of lipid turbidity during dissolution as well as for determining drug content of the liposomes/proliposomal matrices. By this method the drug entrapped inside the liposomes/proliposomal matrices is not detected.

Further, the same method was found to be applicable to quantitatively estimate the pure drug also. In the proposed method, there is no need to filter the samples to remove any undissolved drug before estimating because the undissolved drug in the samples does not absorb light instead it diffracts.

In this method, the turbidity added from outside is in the form of blank processed lipid components of liposomes/proliposomes without drug in the appropriate ratio such that they form liposomes when come in contact with the aqueous medium. Now, as the lipid molecules self assemble when come in contact with the aqueous medium to make the lamellar bodies, the prepared turbid samples can be the proper model for real liposomal and proliposomal formulations.

Experimental Section

Materials and Methods

Alcohol, double distilled water, sodium hydroxide and potassiumdihydrogenorthophosphate were procured from Loba Chemie Pvt. Ltd., Mumbai, India.

Glibenclamide (GLIB) was kindly gifted by Sun Pharmaceutical Industries Limited, Silvassa, India.

Stock Solution A

A 1000 µg/mL stock solution was prepared by dissolving 50 mg GLIB in ethanol in a 50.0 mL volumetric flask and making the volume up to the mark with the same solvent.

Stock Solution B

A 40 µg/mL working stock solution was prepared by diluting 10 mL of the stock solution A up to mark in a 250 mL volumetric flask with Phosphate buffer *p*H 7.5±0.1.

Stock Solution C

A 40 µg/mL working turbid stock solution was prepared by adding 16 mg of the blank, processed proliposomal components without drug to the previously prepared working stock solution B (40 µg/mL) in a 100 mL volumetric flask. The solution was sonicated for 15 min and made the volume up to mark with the same stock so as to ensure a drug: lipid ratio of 20: 80 (maximum in all working standards).

Instrument

A Shimadzu Pharmspec UV 1800 ultraviolet-visible spectrophotometer was used.

Methodology

Various aliquots of working stock solutions B and C were transferred to 10 mL volumetric flasks so as to prepare various alternate clear and turbid working standard dilutions of 2, 3, 6, 9, 12, 15 and 20 µg/mL and volume was made up to mark with phosphate buffer *p*H 7.4. The calibration curve was prepared from these dilutions against clear blank by taking the absorbances of the prepared standard dilutions at three wavelengths i.e. wavelength of maximum absorption λ_{max} (232 nm) and at two wavelengths equidistant on either side of λ_{max} i.e. 227 nm and 237 nm, taking the average of the later two, subtracting this average from the absorption at λ_{max} and plotting the result against concentration.

Results and Discussion

The UV spectra of the prepared clear as well as turbid solutions of GLIB were run between 400 nm and 200 nm. Both exhibited a prominent peak at 232 nm (Fig. 1) and it was selected for further studies as λ_{max}. Two other wavelengths of measurements used were 227 nm and 237 nm (equidistant on either side of λ_{max}) and the standard curve was prepared by plotting corrected absorbance $[A_{232} - (A_{227} + A_{237})/2]$

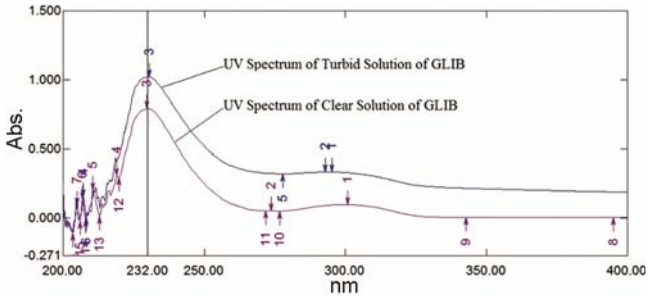

Fig. 1 — Overlay spectra of GLIB in clear and turbid solutions

Table 1 — Analytical parameters using the proposed method

No.	Parameter	Value
1	Analytical wavelengths (nm)	227, 232 & 237
2	Equation used for standard curve	$A_{232} - (A_{227} + A_{237})/2$
3	Linearity range (µg/mL)	02-20
4	Regression equation (A = aC+b)[a]	A=0.00519C + 0.00019
	Slope (a)	0.00519
	Intercept (b)	0.00019
5	SD of intercept (n = 6)	6.11×10^{-5}
6	LOD ($3.3(SD_{intercept}$/Slope)) (µg/mL)	0.04
7	LOQ ($10(SD_{intercept}$/Slope)) (µg/mL)	0.12
8	Correlation coefficient	0.99955

[a]A= Absorbance and C= Concentration

Table 2 — Test for residuals

Predicted conc. (µg/mL)	Observed conc. (µg/mL)	Residual amount (µg/mL)
2	1.782595	0.217405
3	3.025369	-0.02537
6	6.138728	-0.13873
9	9.09955	-0.09955
12	12.12139	-0.12139
15	14.84939	0.15061
20	19.94894	0.05106

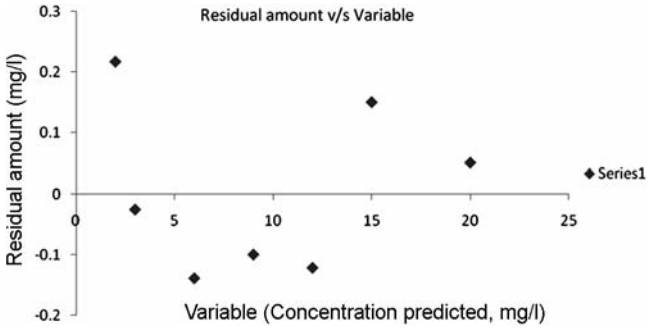

Fig. 2 — Residual amount v/s variable curve

on Y-axis against concentration on X-axis. The Beer's law was validated from 2-20 µg/mL. The dilutions were prepared alternate clear and turbid to prove that the effect of turbidity had been overcome. The linear regression equation was found to be A = 0.00519C + 0.00019, where A is the absorbance, and C is the concentration, with correlation coefficient of 0.99955. The limit of detection and limit of quantification determined with acceptable accuracy and precision were 0.04 and 0.12 µg/mL respectively. The low values of limit of detection (LOD) and limit of quantification (LOQ) showed negligible scatter of points with respect to line of regression. The method was validated for assessment of both clear as well as turbid solutions and within acceptable range validation parameters indicated that the developed method was specific, rapid, reliable, accurate, precise and reproducible.

Method validation

The validity of the method for linearity, specificity, accuracy, repeatability and precision according to recommendations were tested (ICH Guidelines Q2 (R1), 2005)[18]. The results are shown in Tables 1-4. The LOD were determined by establishing the minimum level at which the analyte can be reliably detected. The LOD and LOQ were calculated according to the following equation:

$$LOD = 3.3\frac{SD_{intercept}}{Slope} \quad \ldots (1)$$

$$LOQ = 10\frac{SD_{intercept}}{Slope} \quad \ldots (2)$$

where $SD_{intercept}$ is standard deviation of the intercept of regression line and Slope is the slope of calibration curve. The proposed method was evaluated for linearity (linear regression analysis as shown in Table 1 as well as test for residuals as shown in Table 2 and Fig. 2), accuracy as percent relative mean error (%RME) and the precision as percent relative standard deviation (%RSD) (Table 3). The recovery studies were also performed for the developed method (Table 4).

Specificity

The method was found to be specific as indicated by a less than 0.03% difference in corrected absorbance of 10 µg/ml clear and turbid solutions of GLIB.

Table 3 — Precision and accuracy of proposed method (n = 6)

Solution	Conc. Taken (µg/mL)	Intra-day			Inter-day		
		Found ± S.E.[a, b] (µg/mL)	Precision (%RSD)	Accuracy (%RME)	Found ± S.E.[a,b] (µg/mL)	Precision (%RSD)	Accuracy (%RME)
Clear Solution	8	8.04 ± 0.02	0.69	0.46	8.04 ± 0.02	0.62	0.45
	10	10.05 ± 0.02	0.49	0.47	10.06 ± 0.02	0.45	0.59
	12	12.05 ± 0.03	0.52	0.42	12.05 ± 0.03	0.55	0.45
Turbid Solution	8	8.06 ± 0.02	0.61	0.70	8.05 ± 0.02	0.59	0.65
	10	10.04 ± 0.02	0.48	0.44	10.05 ± 0.02	0.51	0.50
	12	12.04 ± 0.02	0.42	0.37	12.08 ± 0.03	0.54	0.63

Conc., Concentration
[a]Average of six determinations
[b]Mean ± Standard error
%RSD, Percentage relative standard deviation
%RME, Percentage relative mean error

Table 4 — Recovery of the proposed method (standard addition method, n = 6)

Dosage forms	Concentration taken, Cs (µg/mL)	Concentration added, Ca (µg/mL)	Proposed method Recovery [a, b] (%) ± %RSD
Pure GLIB	5	-	100.49 ± 0.47
		3	100.51 ± 0.75
		5	100.01 ± 0.63
		7	101.16 ± 0.68
Proliposomes of GLIB	5	-	100.42 ± 0.40
		3	100.62 ± 0.75
		5	100.08 ± 0.63
		7	101.20 ± 0.68

[a] Average of six determinations
[b] % Recovery = [(Ct–Cs)/Ca]x100, where Ct = Total concentration, Cs = Concentration taken and Ca = Concentration added

Linearity

The curve was found to be linear as shown by linear regression equation and the regression coefficient of the formed standard curve as shown in Table 1. The linearity was also be proved by test for residuals. A random pattern of residuals supports the linearity[19] as shown in Table 2 and Fig. 3.

Precision

To test the precision of the proposed method a certain amount of GLIB was assayed by the proposed method six different times a day (Intraday precision) and six different days (Interday precision) in both clear and turbid solutions. The low values of %RSD indicated that the developed method is precise. Table 3 shows the obtained values for precision (%RSD < 0.69 and 0.61 for clear and turbid solutions respectively).

Accuracy

The accuracy was similarly determined by analysing different levels of drug concentrations from independent stock solutions (with and without turbidity) (n=6) as percent relative mean error six different times a day (Intraday accuracy) and six different days (Interday accuracy). The low values of %RME indicated that the developed method is accurate. Table 3 shows the obtained values for accuracy (%RME < 0.59 and < 0.70 for clear and turbid solutions respectively).

The accuracy was also studied by recovery studies. The percent recovery of the added known amounts of the drug to a known concentration of the sample was always found to be 100.01-100.51% for clear solution and 100.08-101.20% for turbid solution (Table 4).

Robustness

The method was found to be robust as indicated by consistency of the absorbance with the deliberate minor changes in the experiment, such as sonication time, *p*H of the buffer by ±0.1.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by proposed method were found to be respectively 0.04 and 0.12 µg/mL respectively.

Non-detection of analyte that was entrapped inside the formulation

The in-house formulation was added to a known sample and detected by the proposed method. It was found that the entrapped analyte was not detected by the proposed method.

Conclusion

The proposed method has been proved to be simple, precise, rapid and reliable. The method is validated by evaluation of the validation parameters as described in the ICH2QR guideline for specificity, linearity, LOD values, LOQ values, inter- and intra-day precision, accuracy and robustness which are obtained during the validation studies and were found to be within acceptable limits.

Moreover, the method is fast with respect to analysis time as compared to sophisticated chromatographic techniques. No expensive instrumentation and no expensive organic solvents are required.

The method can be successfully employed for GLIB quantification in all types of pharmaceutical formulations and liquid samples of GLIB.

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