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Development and Validation of RP-HPLC Method for Determination of Glibenclamide in Pharmaceutical Dosage Forms

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Abstract: A rapid, linear, precise, selective and accurate reverse phase HPLC method was developed and validated for simultaneous estimation of glibenclamide in tablet dosage form. The method employed for analysis using methanol as a solvent. The wavelength UV 300 nm was selected for estimation and linearity was observed in the concentration range of 160 - 240 µg/ml for glibenclamide respectively. The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The method can be employed for estimation of pharmaceutical dosage formulations with no interference from any other excipients and diluents. The resolution between the closest peaks glibenclamide was more than 1.5 gives a linear response (r2>0.999). The method can be used for quality control assay of the bulk and finished dosage form as single component. Moreover, the method was estimated as per ICH guidelines and the results were found to be within the acceptable range. Hence, the proposed method can be used for the routine quality control of the drugs and can also be applied to stability of glibenclamide in pharmacokinetic studies.
Keywords: Glibenclamide, Reverse phases HPLC, Validation.

Introduction

Glibenclamide is the most extensively used sulphonylurea in many parts of the world for the management of non-insulin-dependent diabetes mellitus (NIDDM)[1]. A search of the registry of drugs approved for marketing in Malaysia, kept at the drug evaluation and safety division of the national pharmaceutical control bureau, revealed a total of 32 glibenclamide preparations registered as at July 1999. Glibenclamide (1[[p-[2-(5-chloro-o-anisamido ethyl phenyl] sulfonyl]-3- cyclohexylurea) is a sulfonylurea derivative is a potent, second generation oral antidiabetic agent widely used for treatment of hyperglycemia in patients with type-II non-insulin dependent diabetes mellitus[1]. It acts mainly by stimulating endogenous insulin release from beta cells of pancreas[2]. HPLC methods coupled with UV detection, fluorescence detection[1,3], mass spectrometry [4,5,6] and capillary electrophoresis[7]. The method is

sophisticated and time consuming of simple RP-HPLC. Nowadays, RP-HPLC is using routine analytical technique due to its advantages[8,9,10]. Glibenclamide is documented to possess low aqueous solubility (Nawaz, 2001). Large inter and intra-individual responses following administration of glibenclamide preparations have also been reported[11]. Now a day's need for a simple, rapid, cost effective and reproducible method for assay of glibenclamide in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise RP- HPLC method for the analysis of glibenclamide in its tablet formulation. The objective of the current work is, therefore, to develop a simple RP-HPLC method for analysis of glibenclamide hydrochloride in tablet formulations.

Materials and methods

Materials

Glibenclamide working standard was a generous gift from paras chemicals, India. Silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used as a stationary phase. All chemicals and reagents were used analytical grade and were purchased from Sisco Chemicals, India. Semi euglucon 2.5 MG containing 10 mg Glibenclamide were purchased from Paras chemicals Pvt Ltd.

Instrumentation and chromatographic conditions

HPLC

The HPLC system consisted of Shimadzu – Prominence separation module equipped with pumps LC-10AT, Rheodyne valve injector with 20 µl fixed loop, UV-Visible detector SPD-20 with manual mode of injection. Single pan balance Shimadzu Libror AEG-220. {The data acquisition was made by Spincotech software 1.7 versions (Spinco Biotech Ltd., Chennai, India). The analysis was carried out at 248 nm with a Gemini C18 reversed phase column (Phenomenex, Torrance, USA) 150 × 4.6 mm i.d., 5µm dimensions at ambient temperature. The mobile phase consisted of 0.05% Triethylamine (pH-3.5, adjusted with ortho phosphoric acid), acetonitrile and methanol in the ratio of 55:15:30 v/v and was set at a flow rate of 1 ml min-1.

HPTLC

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm × 10 cm), Camag win CATS software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on precoated silica gel 60 F254 TLC plates (10 cm × 10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

Preparation of standard stock and sample solutions

Stock solution of Glibenclamide RS was prepared by dissolving appropriate amounts of compounds in methanol to get a final concentration of 1000 µg ml-1. The linearity range was tested in the range of 80 and 100 µg ml-1 using methanol. For the analysis of pharmaceutical formulations, ten tablets of each drug were weighed and powdered individually. The mixture of formulations was prepared by weighing amount equivalent to labeled claim from the powdered formulations. To this, a suitable amount of methanol was added. The mixture was subjected to sonication for 30 min for a complete extraction of the drugs, and then filtered and diluted with mobile phase at a suitable concentration range and injected into HPLC system for the analysis.

Preparation of standard solutions

A stock solution of Glibenclamide was prepared by dissolving 100 mg in 100 ml methanol (1000 µg/ml). Further standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 80 µg/ml.

Sample preparation

Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to one tablet was dissolved in 50 ml methanol. To ensure complete extraction of the drug it was sonicated for 45 min. This solution was filtered through a Whatman no. 0.2 micron paper.

Validation procedure

The objective of the method validation is determined by the method is suitable for its intended purpose of ICH guidelines[12]. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 80 and 100 µg ml-1 prepared in triplicates to test linearity. The peak area of glibenclamide was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared glibenclamide test solution in the same equipment at a concentration value of 100% (100µg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same

concentration additionally on two consecutive days to determine intermediate precision. Peak area of the glibenclamide was determined and precision was reported as %RSD. Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of glibenclamide at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of glibenclamide recovered in the samples. Sample solution short term stability was tested at ambient temperature (20 ±100C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

Figure 1: Applicability of the RP-HPLC method for the analysis of the Glibenclamide linearity 100% levels

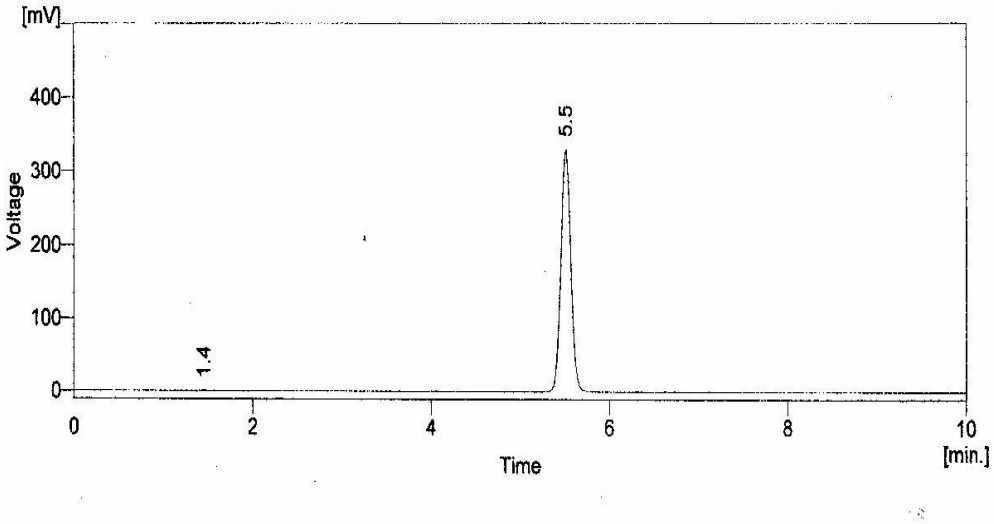

Table 1 : Applicability of the RP-HPLC method for the analysis of the pharmaceutical formulations

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.450	6.324	0.296
2.	5.500	2130.12	99.704
	Total	2136.4	100.00

Figure 2: Applicability of the RP-HPLC method for the analysis of the Glibenclamide linearity 120% levels

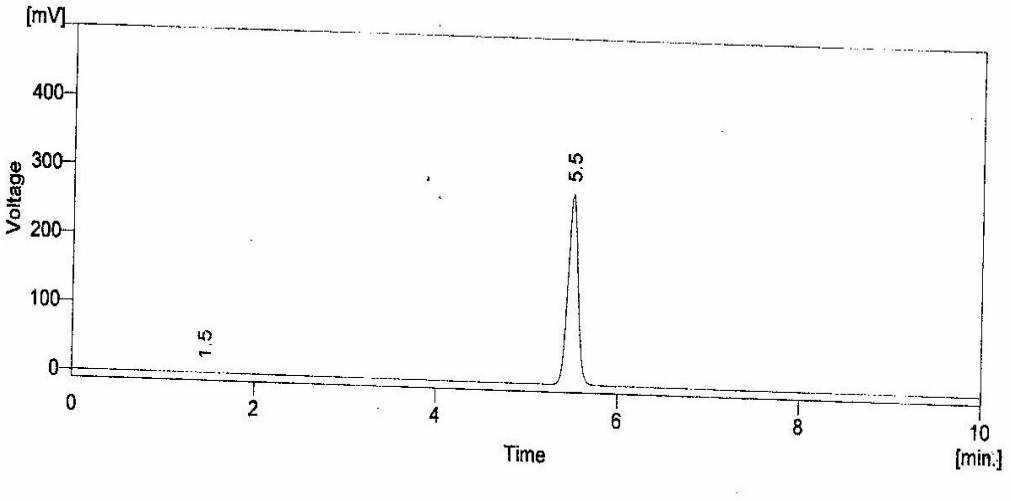

Table 2: Applicability of the RP-HPLC method for the analysis of the glibenclamide linearity formation

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.447	6.324	0.115
2.	5.510	2546.614	99.885
	Total	2549.553	100.00

Figure 3: Applicability of the RP-HPLC method for the analysis of the glibenclamide linearity formation Glibenclamide standard specificity

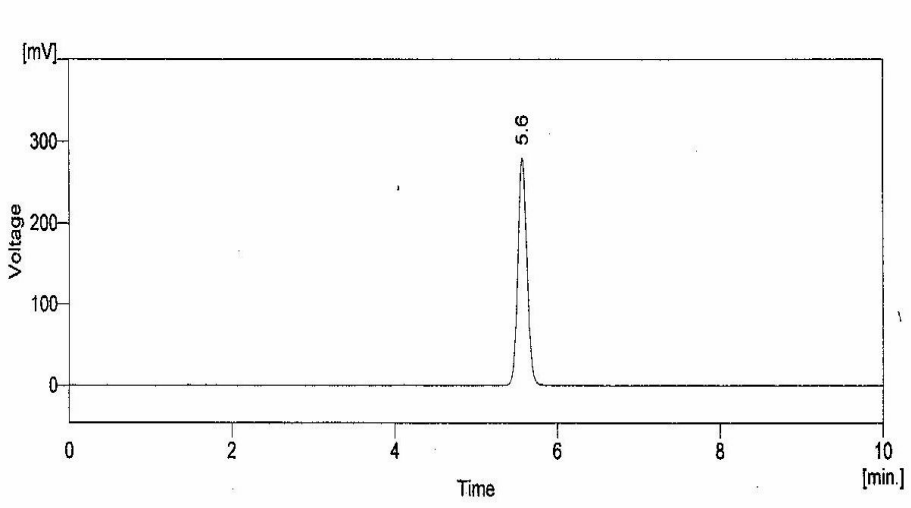

Table 3 : Applicability of the RP-HPLC method for the analysis of the glibenclamide standard specificity

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	5.573	2157.254	100.00
	Total	2154.254	100.00

Figure 4: Applicability of the RP-HPLC method for the analysis of the Glibenclamide accuracy (80%)

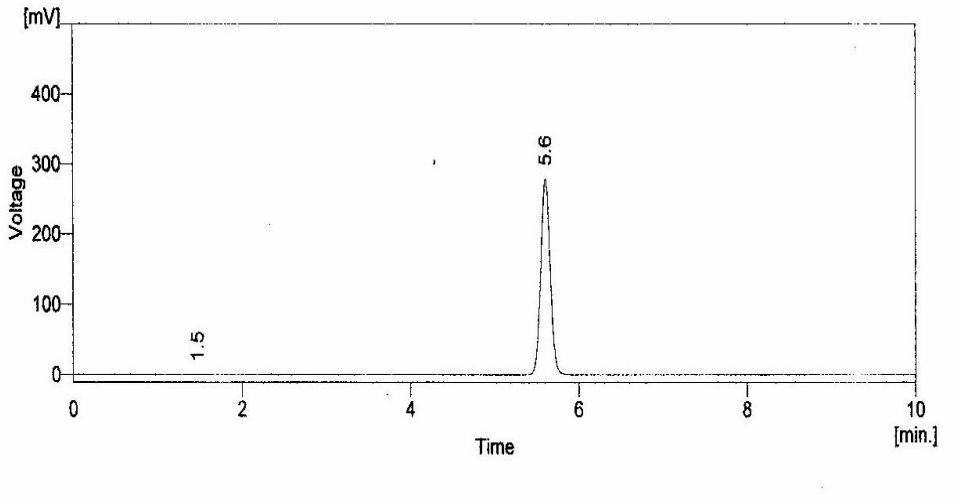

Table 4: Applicability of the RP-HPLC method for the analysis of the glibenclamide standard specificity

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.473	1.667	0.077
2.	5.607	2152.666	99.924
		2154.334	100.000

Figure 5: Applicability of the RP-HPLC method for the analysis of the Glibenclamide accuracy 80% levels

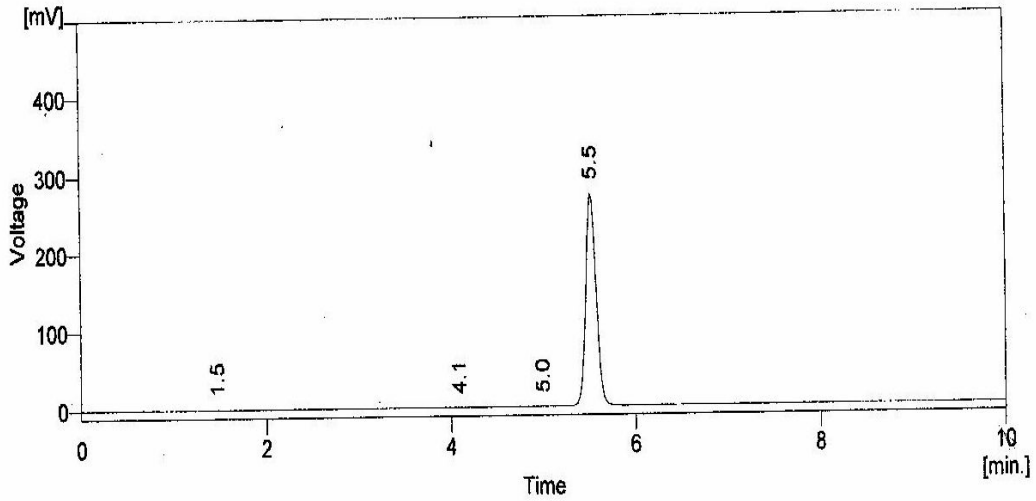

Table 5: Applicability of the RP-HPLC method for the analysis of the glibenclamide accuracy 80% levels

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.467	4.734	0.271
2.	4.087	3.081	0.176
3.	4.993	0.987	0.056
4.	5.520	1739.223	99.496
	Total	1748.025	100.000

Figure6: Applicability of the RP-HPLC method for the analysis of the glibenclamide accuracy 100% levels

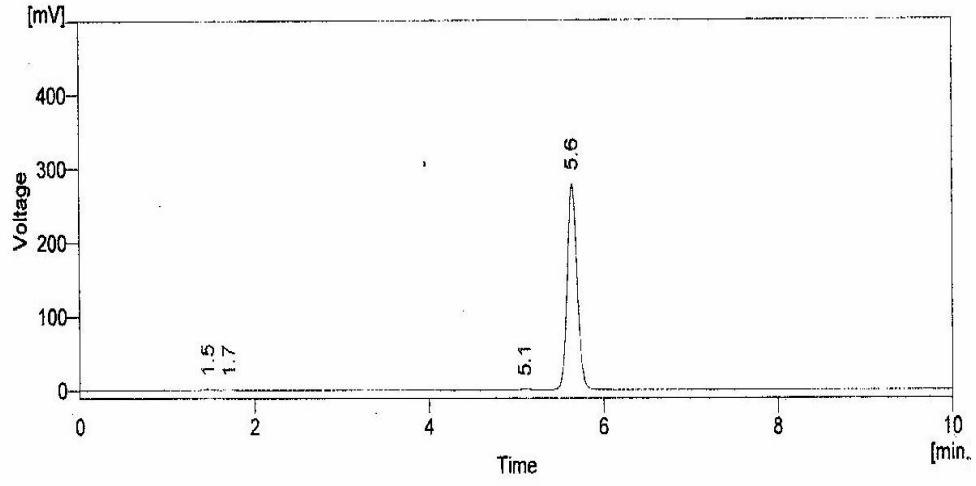

Table 6: Applicability of the RP-HPLC method for the analysis of the glibenclamide accuracy 100% levels

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.460	10.788	0.4970
2.	1.700	6.305	0.290
3.	5.090	3.549	0.163
4.	5.640	2150.178	99.049
	Total	2170.819	100.000

Figure 7: Applicability of the RP-HPLC method for the analysis of the glibenclamide accuracy 120% levels

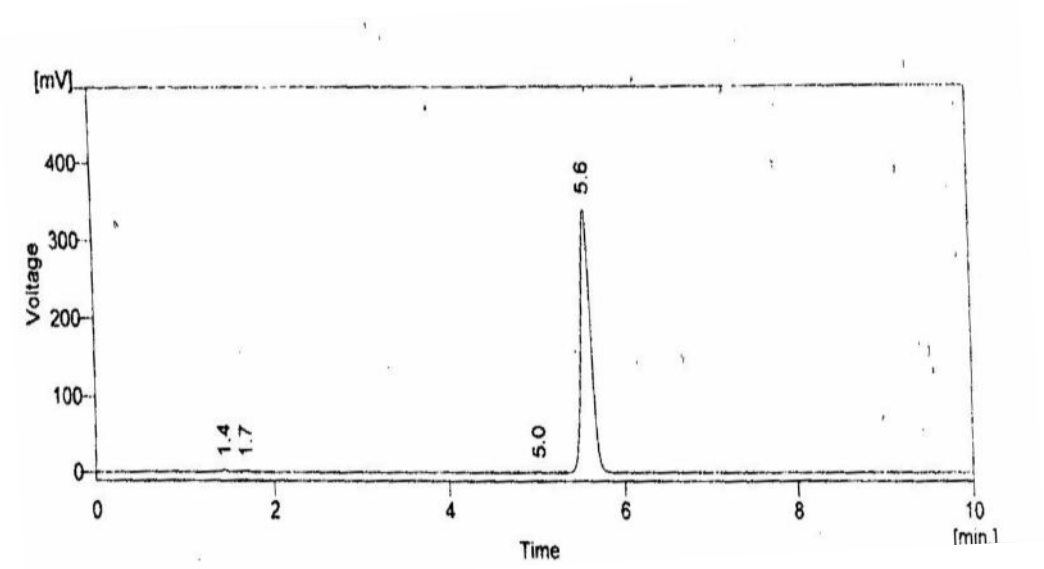

Table7: Applicability of the RP-HPLC method for the analysis of the glibenclamide accuracy formation

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.433	12.778	0.500
2.	1.677	6.550	0.256
3.	5.023	6.349	0.249
4.	5.583	3158.686	98.995
	Total	**2553.925**	**100.000**

Figure 8: Applicability of the RP-HPLC method for the analysis of the glibenclamide

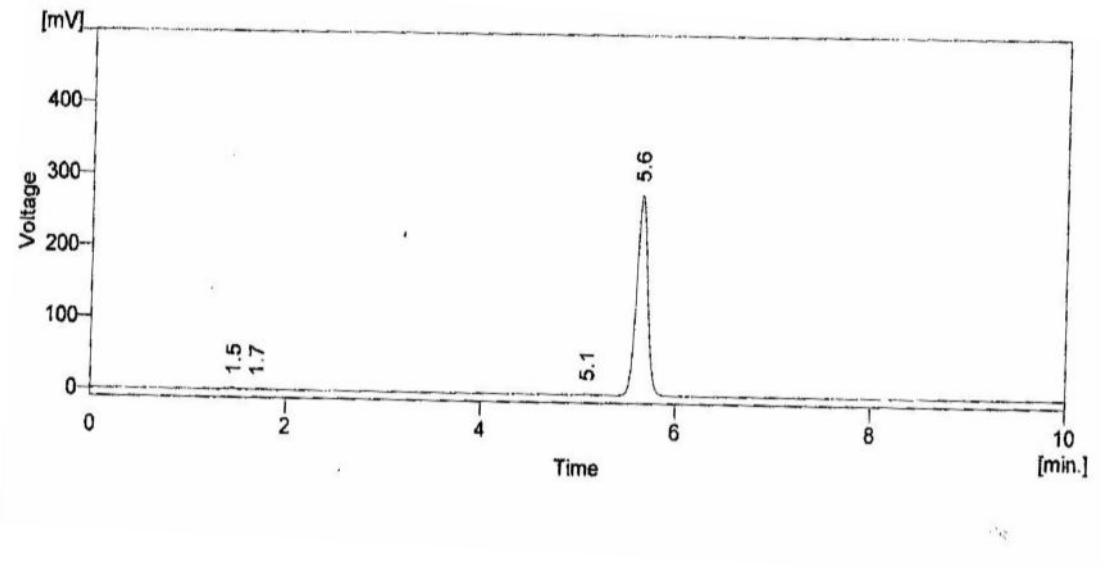

Table 8: Applicability of the RP-HPLC method for the analysis of the glibenclamide

	Reten. Time (min)	Area (mV.s)	Area (%)
1.	1.460	11.499	0.528
2.	1.700	6.305	0.290
3.	5.090	7.158	0.329
4.	5.640	2151.294	98.853
	Total	2176.255	100.000

Results and discussion

Optimization of Chromatographic Conditions the drugs were soluble in organic solvents like methanol and acetonitrile. During the development phase, the mobile phase containing methanol-water and methanol-buffer solutions, resulted in peaks with poor resolution and the acetonitrile-water resulted in asymmetric peaks with a greater tailing factor ($0 \geq 2$) and high run time. The successful use of both acetonitrile and methanol, along with 0.05% TEA in water, pH adjusted to 3.5 (based on the pKa values of the drugs 6-7) reduced tailing and resulted in good peak symmetry and resolution. The optimized mobile phase contained 0.05% TEA in water, acetonitrile, and methanol in the ratio of 55:15:30 at a flow rate of 1 ml min-1. The analytes were monitored at 248 nm and the retention times were found to be 5.5 and 5.5 min for GBM for 100 and 120% respectively (Figure 1a and 2).

Validation of the developed method

The proposed method was validated as per the guidelines in ICH for its linearity, accuracy, precision, specificity and selectivity, robustness and stability etc. Linearity. The linearity was tested for the concentration range of 80, 100, 120µg ml-1 and the calibration curve was constructed and evaluated by its correlation coefficient. The correlation coefficient (r2) for all the calibration curves was consistently greater than 0.99 ± 0.004. Accuracy and Precision. The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percent difference (% bias) between the measured mean concentrations and the corresponding nominal concentrations. The accuracy of the proposed method was tested by recovery experiments by adding known amounts of each anti-diabetic drug corresponding to 80, 100 and 120% of the label claim from the respective standard solution. The accuracy was then calculated as the percentage of each anti-diabetic drug recovered by the assay (Figure, Table 5, 6 and 7). The precision of the proposed method was assayed by replicate injections of anti-diabetic drugs mixture.

Diabetes mellitus is a heterogeneous group of disorders characterized by abnormalities in carbohydrate, protein, and lipid metabolism[13]. For many patients with Type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to maintain effective control[14]. Drugs belonging to classes such as sulfonyl ureas (*e.g.* glipizide, glibenclamide, glimepiride) and thiazolidinedione (TZD) derivatives (pioglitazone, rosiglitazone) (structures shown in Figure 1), are the commonly prescribed hypoglycemic drugs for the treatment of non-insulin dependent type II diabetes mellitus. Thiazolidinedione classes of drugs exert their glucose-lowering effect by binding to Peroxisome Proliferator.

Activated Receptors gamma (PPARγ), thus increasing the receptor sensitivity to insulin[15,16,17]. Sulfonylurea drugs act by increasing the secretion of insulin by the functioning β-cells of the pancreas. Such a combination can be attained by taking each of the drugs separately or alternatively by fixed formulations. A combination tablet formulation is beneficial in terms of its convenience and patient compliance. The human dose of the drugs was 2 mg for rosiglitazone and glimepiride, 5 mg for glipizide and glibenclamide, and 15- 30 mg for pioglitazone. These drugs were found to be more than 98-99% bound to plasma protein with a half life of about 2-7 h after single oral dose except glibenclamide which was extensively bound to serum proteins and had a half life of 10.

Development of the optimum mobile phase

Initially chloroform: methanol (8.0:2.0 v/v) in varying ratio was tried. Then toluene: ethyl Acetate: methanol was tried in different ratios in order to achieve good resolution. The mobile phase of toluene: ethyl acetate: methanol (8.0: 0.5: 1.0, v/v/v) gave good resolution with Rf value 0.45 for Glibenclamide and a sharp and symmetrical peak. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature (Figure 2.). The analytical wavelength, 229 nm, was chosen on the

basis of the absorption spectrum recorded in the range 200–800 nm.

Validation parameters

Linearity

Linearity for Glibenclamide was observed in the range of 40−300 mV/spot with a correlation coefficient of 0.296 and the linear regression equation was y = 6.296x + 28.36 (Figure 1 and 2). Glibenclamide was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.296 and 99.704% of accuracy RT (middle, end) = 5.500.

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 5.607. The results shown in Figure 3 & 4 revealed intra- and inter-day variation of GLY at three different concentration levels 80, 100, 120 ng/spot. The % RSD for within and day-to-day analysis was found to be 0 ≤ 2% (Figure 3).

Robustness

The standard deviation of peak area was calculated for each parameter and % R.S.D. was found to be less than 2%. The low values of % R.S.D as shown in Table 3 indicated robustness of the method.

Specificity

The peak purity of GLY (Glybenclamide) was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.077 and 99.92% of accuracy (middle, end) = 5.573. Good correlation (r=5.573) was also obtained between standard and sample spectra of Glibenclamide in Figure 4 and 5.

Recovery studies

The proposed method when used for extraction and subsequent estimation of GLY from pharmaceutical dosage form after spiking the pre-analyzed sample with 80, 100 and 120 % of label claim of Glibenclamide afforded recovery of 99.70-100 % as listed in Fig 6 and 7. The data of summary of validation parameters are listed in Figure 6 and 7.

Analysis of marketed formulations

A single spot at Rf 0.52 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The results, given in Figure 8, indicate that the amount of drug in the tablets is within the requirement of 98.853– 100 % of the label claim. A new RP-HPLC method has been developed for the identification and quantification of Glibenclamide in formulations. The method was found to be simple, sensitive, precise, accurate and specific for estimation and can be conveniently employed for the routine quality control analysis of Glibenclamide from tablets.

Conclusion

A RP-HPLC method for the detection and quantification of glibenclamide from dissolution studies had been successfully developed, with acceptable retention times of the drug and internal standard peaks, of less than 4 minutes per assay. The RP-HPLC method is able to detect glibenclamide concentrations as low as 0.05mg/ml with a Relative Standard Deviation ranging between 0.08% and 1.6%. Apart from the greater precision and sensitivity attained using this RP-HPLC method, the specificity offered is undoubtedly another advantage compared to the UV method of analysis.

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