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Validation and development of RP-HPLC method for quantification of glibenclamide in rat plasma and its application to pharmacokinetic studies in wistar rats

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ABSTRACT

Herein, we report a novel, simple, specific, accurate and cost-friendly validated reverse phase-high performance liquid chromatographic (RP-HPLC) method for the quantification of second generation sulphonylurea based antidiabetic drug, glibenclamide (GLB) in rat plasma and its application to calculate pharmacokinetic parameters in wistar rats. The internal standard used was flufenamic acid. The chromatographic separation was conducted on C_{18} column (250 mm × 4.6 mm x 5 μm, Agilent-Zorbax, SB) using isocratic elution with mobile phase containing Acetonitrile: Water (1:1; v/v) pH adjusted to 4.0 with 0.03 % glacial acetic acid and detected by photo-diode array as detector. Calibration curves made in the rat plasma were linear in the range of 50–1200 ng/ml with $r^2 = 0.998$. The LLOQ was 40 ng/ml. This method was effectively applied for pharmacokinetic studies of Glibenclamide following administration through oral route as solid dispersion formulation to Wistar rats. Several methods are available in the literature which can be employed for the quantification of Glibenclamide but such methods are tedious, provide lower

Abbreviations: M/s: Messrs, **IS**: Internal standard; RP-HPLC, Reversed Phase-High performance liquid chromatography; ICH, International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use; LOD, Limit of detection; LOQ, Limit of quantification.

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sensitivity, less simultaneous resolution and are time-consuming. Therefore the present methods suits best for the quantification of Glibenclamide from Wistar rats.

1. Introduction

Diabetes Mellitus is the most common metabolic disorder, and among the two types and type II is more prevalent in the population above 40 years of age. This is actually the un-controlled levels of blood sugar or glucose, termed as hyperglycemia [1,2]. The hyperglycemia over years leads to various other severe complications, if otherwise the blood glucose levels are not kept under control [3,4]. So, among different drugs used to control the erratic levels of blood sugar, Glibenclamide, also known as glyburide is potent second generation sulphonylurea drug with better therapeutic and pharmacological effect better than other antidiabetic drugs [5]. Glibenclamide is available as immediate release Daonil tablet (5, 20 mg) and as micronized tablet (Glynase, Prestab). So, the quantification of glibenclamide for different reasons like pharmacokinetic analysis, bioavailability, drug toxicity, etc is required of high demand. Various analytical methods were tried for the quantification studies but high performance liquid chromatography in its reversed phase mode appears the helpful approach of choice affording identification, separation and quantification of Glibenclamide in rat plasma. No doubt, few researchers has developed methods in rat plasma, but most of them are using buffers or else most complex chemicals for buffering purpose which make the analysis complex [6] but herein we report simple acetonitrile and water with 0.3 % glacial acetic for pH maintenance. The present method is simple, cheap and non-interfering chemicals. Also, the drug is very sensitive to pH changes, so it is better for this drug to carry bioanalysis in simple mobile phase instead of using complex buffers. Till date no one has reported cost-effective method of quantification of glibenclamide by RP-HPLC-DAD method. In continuation of our previous studies on application of chromatography for fingerprinting and chromatographic method development, herein a validated HPLC analytical method for the quantification of Glibenclamide in rat plasma and its application to pharmacokinetic studies in Wistar rats is reported.

2. Materials and methods

2.1. Chemicals and reagents

Glibenclamide (purity >99 %) and Flufenamic acid (purity >99 %) were purchased from M/s. Sigma Aldrich India Banglore. All the solvents and chemicals including glacial acetic, ethyl acetate, acetonitrile used were of HPLC grade and purchased from M/s. Merck, Himedia, respectively. Membrane filters (0.45 μm) were purchased from M/s. Nalgene. Deionized water for chromatographic purposes was produced in laboratory by Millipore (Direct Q-3UV).

2.2. Preparation of standard solutions

The stock solution of pure drug (Glibenclamide) was prepared by dissolving a weighed quantity (1 mg/ml) of pure drug in methanol and from this stock solution, eight working solutions of glibenclamide having different concentrations were prepared after required dilutions with methanol. These working solutions were kept in eight vials and capped tightly and stored at 4 °C. So, 25 μl from the eight different working solutions of glibenclamide were separately spiked into blank rat plasma to get eight calibration standards with concentration ranging from 50 to 1200 ng/ml for calibration curve.

2.3. Extraction and sample preparation

The plasma samples were prepared by addition of 100 μl of blank rat plasma to 100 μl of standard solution. The standard solution is primarily air dried by evaporating the entire solvent portion from it. Then, 200 μl of acetonitrile containing internal standard (Flufenamic Acid) having concentration of 10 μg/ml is added, which was added to denature the proteins in plasma. It is vortexed for 60 s and stored at 4 °C in a refrigerator. 500 μl of hexane-chloroform (70:30, v/v) was added and the tubes vortexed again for 60 s. The tubes were centrifuged at 4000 g for 10 min. The organic phase (1000 μl) separated is transferred to clean 5 ml tubes. The organic phase in each tube was dried under the nitrogen gas. The residue was dissolved in 100 μl of acetonitrile and transferred to 1.5 ml screw capped HPLC vials for chromatographic analysis [7–9].

2.4. Instrumentation and chromatographic conditions

The HPLC system (Agilent Infinity, 1200 series) equipped with C_{18} column (250 mm × 4.6 mm x 5 μm, Agilent-Zorbax, SB) and photodiode array detector was used for sample analysis. The mobile phase used was acetonitrile: water (1:1, v/v) pH adjusted to 4.0 with 0.03 % glacial acetic acid at the maintained flow rate of mobile phase at 1 ml/min. The mobile phase was degassed prior to use and always freshly prepared. The detection wavelength was set at 230 nm with an injection volume of 20 μL. By calculating the peak area ratio of (peak area Drug/peak area IS), the samples were quantified. All the analysis was carried out at room temperature at a wavelength of 230 nm. Flufenamic acid was used as internal standard. Under these chromatographic conditions, the mean retention time of glibenclamide and internal standard (Flufenamic acid) was found 9.38, and 15.56 min respectively (Fig. 1). The chromatograms of untreated blank plasma are shown in Fig. 2.

3. Method validation

The method was validated in rat plasma for linearity, selectivity, accuracy, precision, limit of detection, limit of quantification, and stability of analytes as per the guidelines drafted by European Medicines Agency (EMA) and in accordance with the International Conference on Harmonization [10,11].

3.1. Linearity

The linearity was calculated by plotting assay response versus concentration of the analyte. The assay response was obtained from spiked plasma samples, as assayed for ratio of peak area of analyte to peak area of IS. The calibration curve and correlation coefficient was determined by least sum of squares of regression analysis without weighting regression model. Plasma calibration samples or calibration standard samples were prepared in the mean concentration of 50–1200 ng/ml. Calibration curve was established by taking mean of each sampled before assayed in triplicate [12].

3.2. Sensitivity

The sensitivity or limit of detection (LOD) was calculated at a signal-to noise ratio of 3:1 [13].

3.3. Selectivity

The selectivity was found by taking blank rat plasma and checked for interferences due to endogenous compounds for glibenclamide and IS.

3.4. Lower limit of quantitation (LLOQ)

LLOQ was calculated using three replicates spiked plasma samples based on a signal-to-noise ratio of 10:1 [13,14].

3.5. Precision and accuracy

The precision and accuracy was determined by replicate analysis of 3 quality control samples (QC) comprising the analyte (glibenclamide) at low, medium and high mean concentration of 100 ng, 600 ng & 1200 ng/ml, respectively. Intra-day precision and accuracy was evaluated from three separate runs during a single day, while inter-day precision and accuracy was calculated during 5 different days [15].

3.6. Recovery studies

It is also known as extraction efficiency or extraction recovery. The recovery of glibenclamide was calculated by simply comparing peak area ratios of extracted analyte from QC samples to peak area ratios from reference standard solutions [13].

3.7. Stability studies

Under different 5 conditions, the stability was determined using quality control standards. The stability was evaluated in stock solutions kept at 4 °c, then in rat plasma at room temperature (25 ± 1°c) and also, for 7 days at 4 °C. Freeze-thaw stability was evaluated by primarily freezing the reconstituted plasma samples, then stored at −20 °C and again thawed for three cycles at room temperature (25 ± 1 °C). After extraction, the stability of analyte reconstituted in methanol was determined for short-term stability at the bench-top and auto-sampler under room temperature for 24 h 3 replicates were analysed daily for concentration by HPLC quantitation [16,17].

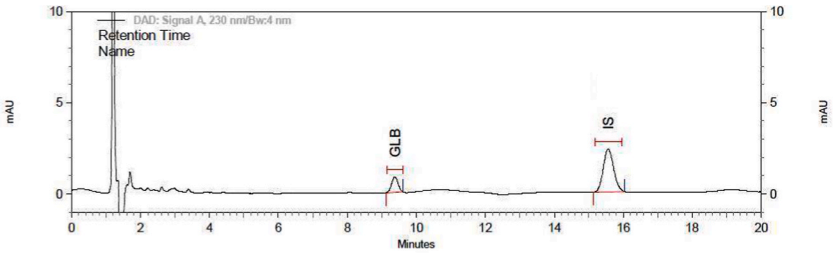

Fig. 1. Chromatogram of glibenclamide and internal standard (IS).

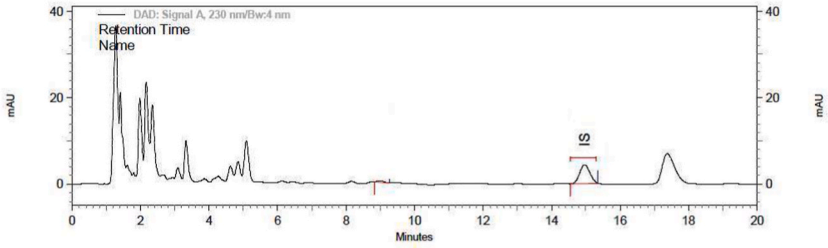

Fig. 2. Chromatogram of glibenclamide and internal standard (IS) in rat plasma.

3.8. IAEC approval for animal studies

The animal experiment was approved by Institutional Animal Ethics Committee (IAEC) at the University of Kashmir, Hazratbal, Srinagar, J&K, India under No: F(IAEC-Approval) KU/2017/08 Dated: 16-12-2017. The analytical study was performed at the Research Centre for Residue and Quality Analysis-Sheri Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar, J&K, India. Male wistar rats having body weights of approximately 250–300 g were used. The rats were maintained in a well ventilated animal house with alternating 12-h light/dark cycles at a room temperature of 22 ± 2 °C and a relative humidity of 50 ± 10 %. The rats were given commercial rat chow and water adlibitum. Twenty Four rats were randomly divided into four groups having six rats in each group and the animals were kept fasted for 12 h prior to start of experiment, but had free access to glucose-water solution throughout the experimental period to prevent any hypoglycemic condition arising due to Glibenclamide dosing.

3.9. Pharmacokinetic and stability studies

Each group of rats were given a single dose at 10 mg/kg body weight of plain Glibenclamide- (Group I), and Glibenclamide equivalent to 10 mg/kg body weight of marketed formulation- MF (Group II), physical mixture-PM_4(Group III), solid dispersion-SDE_4(Group IV) respectively, as suspension by oral gavage (i.e. before administration plain Glibenclamide was suspended in 0.5 % w/v solution of Carboxymethyl Cellulose and remaining formulations MF, PM_4 SDE_4 were dispersed in water). 100 μl of blood collected from the orbital venous plexus at the predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 8, 12, 24 h) were placed into pre-treated tubes with heparin. The samples were immediately centrifuged at 10,000 rpm for 15 min at 4 °C and the plasma obtained was refrigerated at −20 °C till further analysis. The maximum plasma concentration (C_{max}) and the maximum time to reach maximum plasma concentration (T_{max}) were determined directly from the curve of plasma concentration versus time. The trapezoidal rule was used for summation of the area under the plasma concentration versus time curve, from time 0 to t ($AUC_{0\text{-}t}$). Elimination rate constant (K_{el}) was calculated by the value of absolute slope of any three points lying on straight line during elimination phase or after C_{max}. Elimination half-life ($t_{1/2}$) is found simply using relationship, $t_{1/2} = 0.693/K_{el}$.

4. Results and discussions

4.1. Method validation by HPLC

The developed HPLC method was validated according to the ICH guidelines [18] using the various analytical parameters: linearity, precision, accuracy, specificity, robustness, detection, and quantification limits.

4.1.1. Linearity

Linearity of the developed method was evaluated by constructing calibration curves at six concentration levels over a concentration range of 50–1200 ng/ml. The calibration curve was developed by plotting peak area versus concentrations (n = 6) using linear regression analysis with the help of the HPLC EZchrome software. Good correlation coefficient 0.999 was observed. Other quantification parameters such as LOD and LOQ have also been calculated (Table 1). To estimate the LOD and LOQ, blank methanol was applied six times and standard deviation of the analytical response was determined. The limits of detection (LOD) (signal/noise >3) and the limits of quantification (LOQ) (signal/noise>10) were determined by analyzing dilutions of a solution containing all the compounds. The sensitivity or LOD was calculated to be 33 ng/ml while as lower limit of quantification was 40 ng/ml and the mean retention times of glibenclamide was 9.01 min. In order to reduce the matrix interference during the method validation, matrix match calibration experiment was performed over the concentration range of 50–1200 ng/ml. The spectra for all these compounds are

Table 1
Calibration curve, LOD and LOQ.

Analyte	Range (ng/ml)	Regression Equation	r	LOD (ng/ml)	LOQ (ng/ml)
1	50–1200	y = 0.0006x+0.0229 ± 0.003	0.998	33.0	40.0

provided as a supplementary data (Fig. S1 and Table S1 & S2).

4.1.2. Specificity

The specificity of the method was ascertained by analyzing standards and sample. The peak purity of glibenclamide was assessed by comparing the spectra at peak start, peak apex and peak end position. The peak purity values of the compounds was found to be more than 998 (ideal value, 1000), which shows that the peak was pure with no co-eluting or interfering substances. This proves that method is specific. Also no interfering peaks were found co-eluted with glibenclamide, and a good separation of peaks was seen by using the mobile phase, i.e. of glibenclamide and IS. The spectra for all these compounds are provided as a supplementary data (Fig. S2).

4.1.3. Precision and repeatability

The intra-day precision ranged from 0.32 to 5.17%, while inter-day precision ranged from 0.54 to 5.53% of the QC standards. The data is given in Table 2. The intra-day accuracy ranged from 0.463 to 5.23%, while inter-day accuracy ranged from 0.286 to 4.13% of the reference samples. The obtained results were found within the acceptable limits, as both intra-day and inter-day precisions and accuracies for the LOQ must be with ±20 % and higher concentration within ±15 %. The spectra for all these compounds are provided as a supplementary data (Fig. S3).

4.1.4. Accuracy

The recovery experiment was conducted to evaluate the accuracy of the method. Four different concentrations (43, 125, 663 and 1127 ng/ml) were used for carrying out the recoveries studies. The recoveries were determined by adding known concentrations of the each standard solution into the sample solution (extract) in triplicate. The average percentage recoveries were evaluated by calculating the ratio of detected amount versus added amount. Recovery (%) was calculated by the following this equation:

Recovery (%) = (found amount – original amount)/spiked amount × 100.

The accuracy was calculated from the test results as the percentage of recovery and the results have been summed up in Table 3. The extraction efficiency or recoveries was found 96.53 ± 4.57% (Fig. 3).

4.1.5. Robustness of the method

The robustness of developed method was calculated by introducing small changes in certain chromatographic conditions, i.e., amount of mobile phase, flow rate of mobile phase, temperature, relative humidity. The variations in HPLC analysis because of these changes were ≤1.51 %RSD was found less than 5 %, indicating thereby the robustness of the method.

4.2. Stability and pharmacokinetic studies

4.2.1. Stability

The concentrations for GLB at 125 and 1127 ng/ml samples deviated within ±15 % of the nominal concentrations in a series of stability tests, viz. In rat plasma for 6 h at room temperature, in rat plasma for 7 days at 4 °C, Freeze-thaw stability, Short term Bench-top for 7 h & auto-sampler under room temperature for 24 h (Table 4). The results were found to be within the assay variability limits during the entire process (Figs. S4-S8).

4.2.2. Pharmacokinetic studies

From the pharmacokinetic parameters (Tables 5 and 6), it was found that the $AUC_{0–24h}$of the solid dispersion formulation 'SDE_4'(5636 ng/ml) represents greater improvement than that of marketed formulation (3747 ng/ml) and$AUC_{0–24h}$ of solid dispersion formulation 'SDE_4' was almost 2-fold compared with marketed. It was also found that C_{max} of solid dispersion formulation was 610.0 ng/ml and thus the difference was highly significant ($P < 0.05$) compared with C_{max} of marketed formulation (287.5 ng/ml). The T_{max} of solid dispersion SDE_4 (1 h) and marketed formulation (3 h) was faster than that of marketed formulation. The MRT values for SDE_4 (8.69 h) was also significantly different ($P < 0.05$) from marketed tablet (9.22 h). The relative bioavailability of SDE_4 with respect to plain Glibenclamide was obtained as 150.41 and 79.1 %, respectively. The value of Relative Bioavailability was also significantly different ($P < 0.05$) from plain Glibenclamide. The hydrophilicity of Glibenclamide increases through solid dispersion technique. All these could explain that the $T_{1/2}$of solid dispersion formulation was shorter than that of marketed formulation. As the plasma concentration and $AUC_{0–24h}$increased with administered solid dispersion 'SDE_4' formulation. The considerable increase in the C_{max} with simultaneous reduction in T_{max} in SDE_4 could be attributed to increased solubility of Glibenclamide in SDE_4 due to both solubilization effect of Poloxamer-188 on Glibenclamide and conversion of crystalline Glibenclamide to amorphous form. The oral absorption of

Table 2
Intra- and inter-day precision of area of HPLC method.

Conc. level of QC Standard (ng/ml)	Intra-day precision		Inter-day precision	
	Mean response (±SD) (n = 3)	CV%	Mean response (±SD) (n = 5)	CV%
43	40.75 ± 2.11	5.17	41.22 ± 2.28	5.53
125	121.65 ± 2.54	2.08	120.62 ± 1.98	1.64
663	652.14 ± 2.11	0.32	647.29 ± 3.29	0.50
1127	1132.22 ± 4.21	3.18	1132.77 ± 6.28	0.55

Table 3
Recovery study.

Conc. level of QC Standard (ng/ml)	% Recovery (n = 3)
43	91.45 ± 1.33
125	94.56 ± 2.43
663	90.24 ± 3.29
1127	96.53 ± 4.57

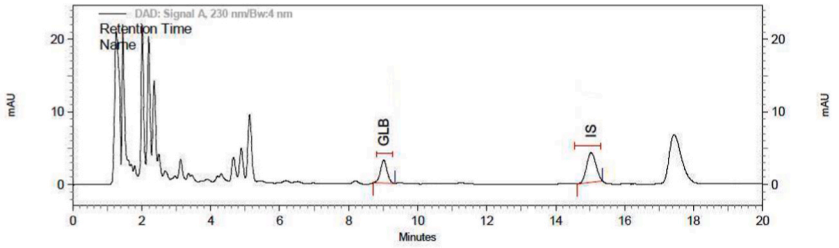

Fig. 3. Chromatogram of spiked glibenclamide and internal standard (IS) in rat plasma.

Table 4
Stability data GLB quality controls in rat plasma.

Nominal Concentration (ng/ml)	Stability	Mean ± SD (n = 6) (ng/ml)	Accuracy (%)	Precision (% CV)
125	Rat plasma for 6 h at room temperature	121 ± 1.43	97	1.18
	Rat plasma for 7 days at 4 °C	118 ± 1.47	95	1.24
	Freeze-thaw stability	115 ± 1.63	92	1.41
	Short term Bench-top for 6 h	119 ± 1.21	95	1.01
	Auto-sampler under room temperature for 24 h	114 ± 1.86	91	1.63
1127	Rat plasma for 6 h at room temperature	1113 ± 1.78	98.75	0.16
	Rat plasma for 7 days at 4 °C	1109 ± 2.51	98.4	0.22
	Freeze-thaw stability	1111 ± 1.41	98.5	0.12
	Short term Bench-top for 7 h	1114 ± 2.13	98.41	0.19
	Auto-sampler under room temperature for 24 h	1113 ± 2.16	98.75	0.21

Table 5
Comparative data of in-vivo drug release of plain GLB, MF Daonil, PM_4 & SDE_4.

Time (hr)	Conc. (ng/ml)			
	Plain GLB ±SD (n = 6)	MF Daonil ±SD (n = 6)	PM_4 ±SD (n = 6)	SDE_4 ±SD (n = 6)
0.5	35.0 ± 0.81	95.0 ± 0.51	110.0 ± 0.98	179.5 ± 0.98
1.0	48.5 ± 0.75	152.5 ± 0.98	195.5 ± 0.51	610.0 ± 0.93
1.5	59.0 ± 0.51	206.5 ± 0.75	235.0	397.0 ± 0.99
2.0	76.0 ± 0.54	255.0 ± 0.54	363.5 ± 0.93	347.0 ± 1.97
3.0	133.5 ± 0.51	287.5 ± 0.41	290.0 ± 0.97	338.5 ± 0.99
4.0	238.5 ± 0.54	216.0 ± 0.54	195.5 ± 0.87	337.0 ± 0.83
8.0	201.5 ± 0.75	195.5 ± 0.75	179.0 ± 0.51	323.0 ± 0.87
12.0	114.0 ± 0.83	173.0 ± 0.92	134.0 ± 0.88	232.5 ± 0.87
24.0	38.5 ± 0.51	56.0 ± 0.91	80.5 ± 0.91	75.0 ± 0.99

Table 6
Pharmacokinetic parameters.

Pharmacokinetic parameters	Plain GLB Mean ± SD (n = 6)	MF Daonil Mean ± SD (n = 6)	PM_4 Mean ± SD (n = 6)	SDE_4 Mean ± SD (n = 6)
C_{max} (ng/ml)	238.5 ± 0.54	287.5 ± 0.41	363.5 ± 0.93	610.0 ± 0.93
T_{max} (h)	4.07 ± 0.017	3.06 ± 0.020	2.06 ± 0.013	1.07 ± 0.026
$AUC_{0\text{-}24}$ (ng*hml^{-1})	2807 ± 23.97	3747 ± 22.19	3592 ± 11.27	5636 ± 19.84
K_{el} (h^{-1})	0.075 ± 0.002	0.197 ± 0.005	0.437 ± 0.011	0.497 ± 0.005
$t_{1/2}$ (h)	9.24 ± 0.006	3.517 ± 0.004	1.585 ± 0.005	1.394 ± 0.003
MRT (h)	9.23 ± 0.009	9.22 ± 0.007	9.42 ± 0.007	8.69 ± 0.006
Relative Bioavailability (%)	79.91	___	95.86	150.41

Glibenclamide from solid dispersion 'SDE$_4$'formulation resulted in 2-fold enhancement in oral bioavailability in comparison with marketed formulation. The enhancement in oral bioavailability of Glibenclamide from SDE$_4$ was possible due to the use of the hydrophilic carrier (Poloxamer-188) in the formulation of SDE$_4$ prepared by solvent evaporation method using ethanol as the solvent in comparison with PM$_4$, plain Glibenclamide and marketed formulation. Poloxamer-188 is known to enhance the oral bioavailability of lipophilic drugs by enhancing their wettability, surface area, solubility and dissolution rate. Therefore, the significant enhancement in oral bioavailability of Glibenclamide from solid dispersion 'SDE$_4$'formulation was probably due to the presence of Glibenclamide in amorphous form and Poloxamer-188 also prevents recrystallization of amorphous Glibenclamide back to the initial crystalline state by inhibiting nucleation and crystal growth during in-vivo dissolution. Further, the poloxamer-188 has also a huge role to maintain the supersaturation during in-vivo dissolution. The pk parameters are different for all the four, viz., Plain Glibenclamide, Physical Mixture (PM$_4$), Marketed Formulation (Daonil Tablet) and Solid Dispersion (SDE$_4$). The reason behind the differences in PK parameters, is that *in-vivo* drug release v/s time is different in different formulations (Fig. 4). The more data are provided as a supplementary data (Table S4- S8).

5. Conclusion

A simple, specific, precise and accurate method for quantification of glibenclamide was developed and validated in rat plasma and was applied for pharmacokinetics to determination the concentration of glibenclamide in wistar rats dosed with different formulation of the glibenclamide. Although, several methods have been developed previously for this drug as reported, but this is the low-cost, fast and more accurate as the samples have been prepared by less costly chemicals and procedures, like protein precipitation technique also, use of nitrogen for drying has shown better results compared to earlier methods. The stability studies conducted proved that the samples could retain their potency for up to 30 days, if stored under the stated conditions. Based on our results, the developed method features good quantification parameters and can serve as effective quality control method for standardization of drugs and pharmaceutical products.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Khalid Bashir Mir: Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft. **Vidushi Abrol:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Conceptualization, Validation. **Taha U. Wani:** Methodology, Project administration, Validation. **Ishrat Jan:** Formal analysis, Resources, Software. **Nasseb Singh:** Conceptualization, Data curation, Investigation, Software, Validation, Visualization. **Nisar A. Khan:** Supervision. **Alamgir A. Dar:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Rania Mohammad Sabri Sultan:** Formal analysis, Funding acquisition, Methodology, Software. **Showkat A. Lone:** Funding acquisition, Methodology, Validation, Visualization. **Mohamed A.M. Iesa:** Conceptualization, Data curation, Project administration, Resources. **Sadeq K. Alhag:** Data curation, Formal analysis, Funding acquisition. **Laila A. Al-Shuraym:** Funding acquisition, Project administration, Software, Visualization. **Nawal Helm:** Visualization. **Ammar AL-Farga:** Conceptualization, Methodology, Resources, Software.

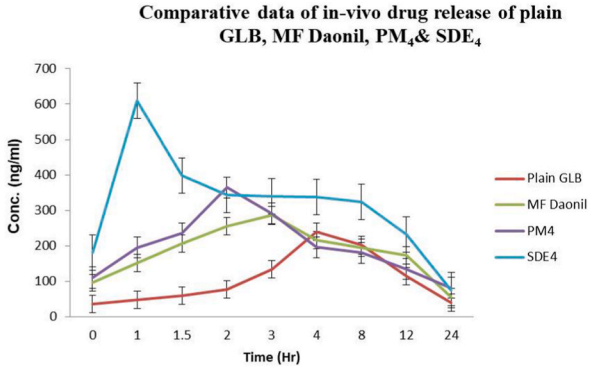

Fig. 4. Comparative data of *in-vivo* drug release of plain GLB, MF Daonil, PM$_4$& SDE$_4$.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20876.

References

[1] A.D. Association, Gestational diabetes mellitus, Diabetes Care 27 (2004) S88, https://doi.org/10.2337/diacare.27.2007.S88.
[2] S. Bastaki, Diabetes mellitus and its treatment, Int. J. Diabetes Metabol. 13 (3) (2005) 111–134, https://doi.org/10.1159/000497580.
[3] V. Rani, G. Deep, R.K. Singh, K. Palle, U.C. Yadav, Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies, Life Sci. 148 (2016) 183–193, https://doi.org/10.1016/j.lfs.2016.02.002.
[4] A.H. Barnett, Diabetes and hypertension, Br. Med. Bull. 50 (2) (1994) 397–407, https://doi.org/10.1093/oxfordjournals.bmb.a072899.
[5] A.J. Krentz, R.E. Ferner, C.J. Bailey, Comparative tolerability profiles of oral antidiabetic agents, Drug Saf. 11 (4) (1994) 223–241, https://doi.org/10.2165/00002018-199411040-00002.
[6] A. Ahmad, R. Khan, K. Alkharfy, Development and validation of RP-HPLC method for simultaneous estimation of glibenclamide and thymoquinone in rat plasma and its application to pharmacokinetics, Acta Chromatogr. 27 (3) (2015) 435–448, https://doi.org/10.1556/achrom.27.2015.3.3.
[7] T.U. Wani, K.B. Mir, A. Raina, A.A. Dar, I. Jan, N.A. Khan, T.A. Wani, J.A. Sofi, G. Hassan, H.S. Almoallim, Simultaneous quantification of losartan potassium and its active metabolite, EXP3174 in rabbit plasma by validated HPLC-PDA, biomed, Chromatography 37 (8) (2023), e5645, https://doi.org/10.1002/bmc.5645.
[8] I. Jan, A.A. Dar, A.A. Wani, M. Mukhtar, J.A. Sofi, G. Hassan, Risk assessment, development and validation of a GC-ECD-based method for the quantification of cypermethrin from green pea, Biomed. Chromatogr. 36 (7) (2022), e5373, https://doi.org/10.1002/bmc.5373.
[9] M.H. Bhat, M. Fayaz, A. Kumar, A.A. Dar, A.K. Jain, Chromatographic method for determination of the amino acid content in Dioscorea bulbifera L. tubers by RP-HPLC, Pharmaceut. Sci. 25 (1) (2019) 65–69, https://doi.org/10.15171/PS.2019.10.
[10] I.C.H. Guideline, Validation of analytical procedures: text and methodology, Q2 (R1) 1 (20) (2005) 5.
[11] A.A. Dar, P.L. Sangwan, N. Singh, A. Kumar, Method validation and simultaneous quantification of five triterpenoids from Codonopsis ovata by high-performance thin-layer chromatography, J. Planar Chromatogr.-Mod. TLC 32 (3) (2019) 251–256, https://doi.org/10.1556/1006.2019.32.3.11.
[12] A.A. Dar, A. Raina, A. Kumar, Development, method validation and simultaneous quantification of eleven bioactive natural products from high-altitude medicinal plant by high-performance liquid chromatography, Biomed. Chromatogr. 36 (8) (2022), e5408, https://doi.org/10.1002/bmc.5408.
[13] A.A. Dar, P.L. Sangwan, I. Khan, N. Gupta, A. Qaudri, S.A. Tasduq, S. Kitchlu, A. Kumar, S. Koul, Simultaneous quantification of eight bioactive secondary metabolites from Codonopsis ovata by validated high performance thin layer chromatography and their antioxidant profile, J. Pharm. Biomed. Anal. 100 (2014) 300–308, https://doi.org/10.1016/j.jpba.2014.07.034.
[14] F. D.A, Guidance for Industry: Bioanalytical Method Validation, 2001.
[15] J.A. Sofi, A.A. Dar, I. Jan, G. Hassan, S.R. Dar, A.H. Mughal, N.A. Dar, Development and validation of GC-ECD method using QuEChERS for pesticide residue determination in cucumber, Biomed. Chromatogr. (2023), e5647, https://doi.org/10.1002/bmc.5647.
[16] N. Singh, A.A. Dar, A. Kumar, A simple and efficient approach for the synthesis of 1, 3-oxazolidines from β-amino alcohols using grinding technique, ChemistrySelect 3 (48) (2018) 13675–13681, https://doi.org/10.1002/slct.201802369.
[17] M.H. Bhat, M. Fayaz, A. Kumar, A.A. Dar, A.K. Jain, Development of an efficient micropropagation system for Dioscorea bulbifera L. and phytochemical profile of regenerated plants, J. Genet. Eng. Biotechnol. 20 (1) (2022) 107, https://doi.org/10.1186/s43141-022-00382-9.
[18] I.C.H. Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), International conference on harmonization, Geneva, Switzerland, 2005.