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DETERMINATION OF GLIBENCLAMIDE, METFORMIN HYDROCHLORIDE AND ROSIGLITAZONE MALEATE BY REVERSED PHASE LIQUID CHROMATOGRAPHIC TECHNIQUE IN TABLET DOSAGE FORM

Abstract

A simple, precise and accurate high performance liquid chromatography (HPLC) method was developed for the simultaneous estimation of metformin hydrochloride, rosiglitazone maleate, glibenclamide present in multicomponent dosage forms. Chromatography was performed on a 25 cm×4.6 mm i.d., 5- μ m particle, C₁₈ column with 78:22 (v/v) methanol:20 mM potassium dihydrogen phosphate buffer as mobile phase at a flow rate of 1.0 ml/min and UV detection at 238 nm for metformin hydrochloride, rosiglitazone maleate and glibenclamide. The total elution time was shorter than 9 min. This method was found to be precise and reproducible. The proposed method was successfully applied for the analysis of metformin hydrochloride, rosiglitazone maleate, glibenclamide as a bulk drug and in pharmaceutical formulation without any interference from the excipients.

Keywords: reverse phase high performance liquid chromatography, rosiglitazone maleate, metformin hydrochloride, glibenclamide.

Currently, the most commonly prescribed medications for the treatment of non-insulin dependent type 2 diabetes mellitus are drugs such as biguanides. For example, metformin hydrochloride, 1,1-dimethylbiguanide hydrochloride (Figure 1a), is an anti-hyperglycemic agent [1].

It improves glucose tolerance in patients with type 2 diabetes and reduces both basal and post-prandial plasma glucose [2]. Sulfonylurea glibenclamide (Gly), 1-[4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfonyl]-3-cyclohexylurea (Figure 1c), is a second generation hypoglycemic agent [3] that appears to lower blood glucose by stimulating the release of insulin from the pancreas [4-5]. Thiazolidinedione (TZD) derivatives such as rosiglitazone maleate (Rosi), chemically (\pm)-5-[4-[2-(*N*-methyl-*N*(2-pyridyl)amino)ethoxy]benzyl]-2,4-dionethiozolidine (Fi-

gure 1b) [6], are potent new oral antihyperglycemic agents that reduce insulin resistance in patients with type 2 diabetes by binding to peroxisome proliferator-activated receptors gamma (PPAR- γ) [7-9]. For many patients with type 2 diabetes, monotherapy with an oral anti-diabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control [10]. The use of combination of biguanides, sulfonylureas and TZDs is commonly observed in clinical practice. This combination can be achieved by taking each of the drugs separately or alternatively fixed formulations have been developed. Combinations of Met, Rosi and Gly are available commercially as single tablets. Although many methods have been reported in literature for the estimation of Met [11-28], Rosi [29-37] and Gly [38-43] individually, only a few methods are available for the simultaneous estimation of Met and Rosi [44-46], Met and Gly [47-50] and Rosi with Gly [51]. However, no analytical method has been published for the simultaneous analysis of three drugs combinations whether in pure forms or in the pharmaceutical preparation, which became the aim of this work. The method described is rapid, economical, precise, and

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accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [52].

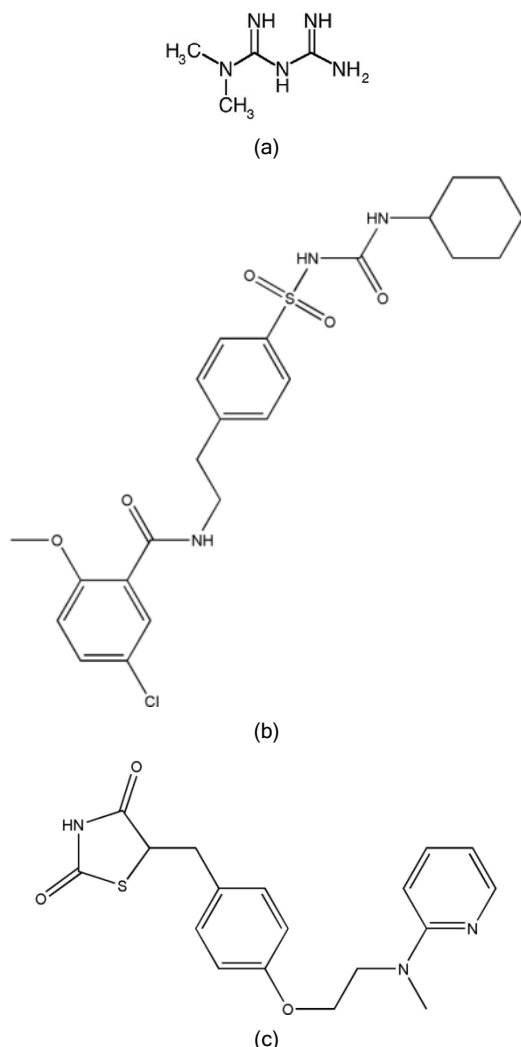


Figure 1. Chemical structures of a) metformin hydrochloride, b) glibenclamide and c) rosiglitazone.

EXPERIMENTAL

Materials and methods

Pharmaceutical grade working standards metformin HCl [Met] (batch no. 1997418), rosiglitazone maleate [Rosi] (batch no. 758001) and glibenclamide [Gly] (batch no. 2022198) were obtained from Ranbaxy Laboratories, Dewas, India.

The commercial tablet (brand name: Diabetrol 3D, Piramal Health Care, batch No. 20605016), label claim: 500 mg of Met, 2 mg Rosi and 5 mg of Gly per tablet) was purchased in March 2010 from a local pharmacy in Pune.

All chemicals and reagents were of HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The LC system consisted of a pump (Jasco PU-1580 intelligent LC pump) with auto injecting facility (AS-1555 sampler) programmed at 20 μ l capacity per injection. The detector consisted of a UV-Vis (Jasco UV 1575) model operated at a wavelength of 238 nm. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was HiQ Sil C18HS 250 mm \times 4.6 mm, 5.0 μ m (Kya Technologies Corporation). Different mobile phases were tested in order to find the best conditions for separation of Met, Rosi and Gly. The mobile phase contained 78:22 (v/v) methanol:20 mM potassium dihydrogen phosphate buffer and the flow rate was maintained at 1.0 ml/min UV detection was carried out at 238 nm (Figure 2). The mobile phase and samples was filtered using a 0.45 μ m membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

Standard solutions and calibration graphs for chromatographic measurement

Met, Rosi and Gly were weighed accurately and separately transferred to 10 ml volumetric flasks. All the drugs were dissolved in HPLC-grade methanol to prepare 1000 μ g/ml standard stock solutions. Calibration standards at five levels were prepared by appropriately weighed and mixed standard solutions in the concentration range of 50-250 μ g/ml for Met and 0.4-2.0 μ g/ml for Rosi and 0.6-3.0 μ g/ml for Gly. Samples were made in triplicate for each concentration and peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation

For the analysis of tablets, 20 tablets were weighed and finely ground in a mortar. The portion equivalent to 500 mg of Met, 2 mg of Rosi and 2.5 mg of Gly, was transferred in a 25 ml volumetric flask separately, 20 ml of methanol was then added, and sonication was done for 45 min with swirling. After sonication, the volume was made up to the mark with the diluent, and mixed well. The solution was filtered through Whatman filter paper (#41) then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times. The possibility of excipients interference with the analysis was examined.

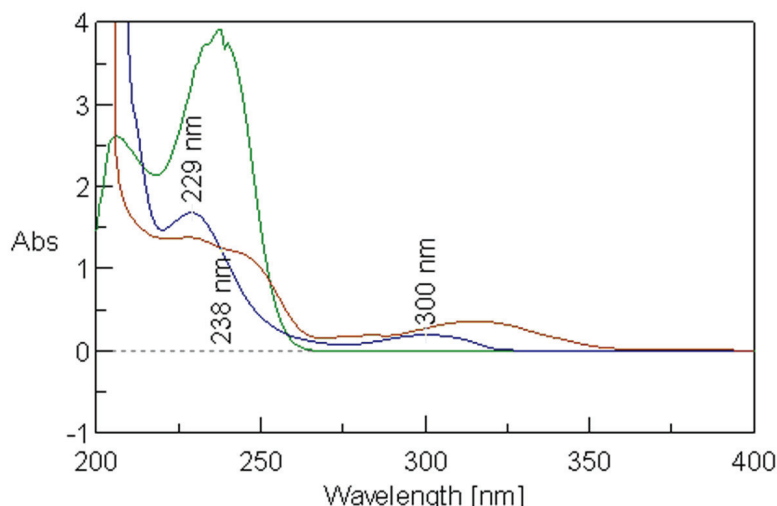


Figure 2. Overlaid UV spectra of metformin HCl rosiglitazone maleate and glibenclamide measured from 200 to 400 nm.

Optimization of HPLC method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for Met, Rosi and Gly. The mixed standard stock solution (200 $\mu\text{g/ml}$ of Met, 0.8 $\mu\text{g/ml}$ of Rosi and 1.0 $\mu\text{g/ml}$ of Gly) injected in HPLC. Different ratios of methanol and potassium dihydrogen phosphate buffer at different pH and molarities were tested.

Method validation

The method was validated according to the ICH guidelines. The following validation characteristics were addressed: linearity, accuracy, precision, and specificity, limits of detection and quantitation and robustness.

Linearity and range

Calibration standards at five levels were prepared by appropriately weighed and mixed standard solutions. From the mixed standard stock solution (50–250 $\mu\text{g/ml}$ for Met and 0.4–2.0 $\mu\text{g/ml}$ for Rosi and 0.6–3.0 $\mu\text{g/ml}$ for Gly). Each concentration was injected six times into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

Method repeatability was obtained from *RSD* (relative standard deviation, %) by repeating the analysis six times for three concentrations in the same day for intra-assay precision. The repeatability of sample injection and measurement of peak area for active compound were expressed in terms of *RSD*. Intermediate precision was assessed by repeating the analysis on three different days. The repeatability and intermediate precision variation was carried out at

three different concentration levels (50, 150 and 250 $\mu\text{g/ml}$ for Met, 0.4, 1.2 and 2 $\mu\text{g/ml}$ for Rosi and 0.6, 1.8 and 3 $\mu\text{g/ml}$ for Gly).

Limit of detection and quantification

In order to estimate the limit of detection (*LOD*) and limit of quantification (*LOQ*), blank methanol was injected 6 times following the method as described in the instrumentation Section. The signal-to-noise (*S/N*) ratio was specified as 3:1 for *LOD* and 10:1 for *LOQ*. The *LOD* and *LOQ* were experimentally verified by diluting known concentrations of standard solutions of Met, Rosi and Gly until the average responses were approximately 3 or 10 times the standard deviation (*SD*) of the responses for 6 replicate determinations.

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ± 0.1 ml/min), mobile phase composition (methanol ± 2 ml). These chromatographic variations were evaluated for resolution between Met, Rosi and Gly.

Solution stability

To assess the solution stability, three different concentrations of (2, 4 and 6 $\mu\text{g/ml}$) were prepared from sample solutions and kept at room temperature for 8 days. These solutions were compared with freshly prepared standard solutions.

System suitability

The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between Met, Rosi and Gly peaks were defined.

Specificity

Extracts of commonly used placebos were injected to demonstrate the absence of interference with the elution of the Met, Rosi, and Gly. For determining selectivity of the method, a powder blend of typical tablet excipients containing lactose monohydrate, mannitol, maize starch, povidone K30, citric acid anhydrous granular, sodium citrate, natural lemon and lime flavor and magnesium stearate was prepared and analyzed. All chromatograms were examined to determine if the compounds of interest co-eluted with each other or with any additional excipients peaks.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample to which known amounts of Met, Rosi and Gly standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed, and the powder was extracted and analyzed by running chromatograms in optimized mobile phase. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%) and *RSD* were calculated.

Analysis of marketed formulation

The marketed formulation was assayed as described above. The peak areas were measured at 238 nm and concentrations in the samples were determined using multilevel calibration developed on the same LC system under the same conditions, and analyzed using linear regression as described earlier.

RESULTS AND DISCUSSION

Method development and optimization

The HPLC procedure was optimized with the aim of developing a suitable LC method for the analysis of Met, Rosi and Gly in fixed dose combined dosage form. Initially, methanol and water in different ratios were tried. However, a broad peak shape was obtained for Met, so water was replaced by potassium dihydrogen phosphate buffer (20 mM), and mixture of methanol and potassium dihydrogen phosphate buffer in different ratios were tried. It was found that methanol:potassium dihydrogen phosphate buffer (20 mM) at a ratio of 78:22, v/v, resulted in acceptable retention time (t_R 2.51 min for Met, 3.90 min for Rosi and 8.12 min for Gly), plates, and good resolution for Met, Rosi and Gly at a flow rate of 1.0 ml/min (Figure 3).

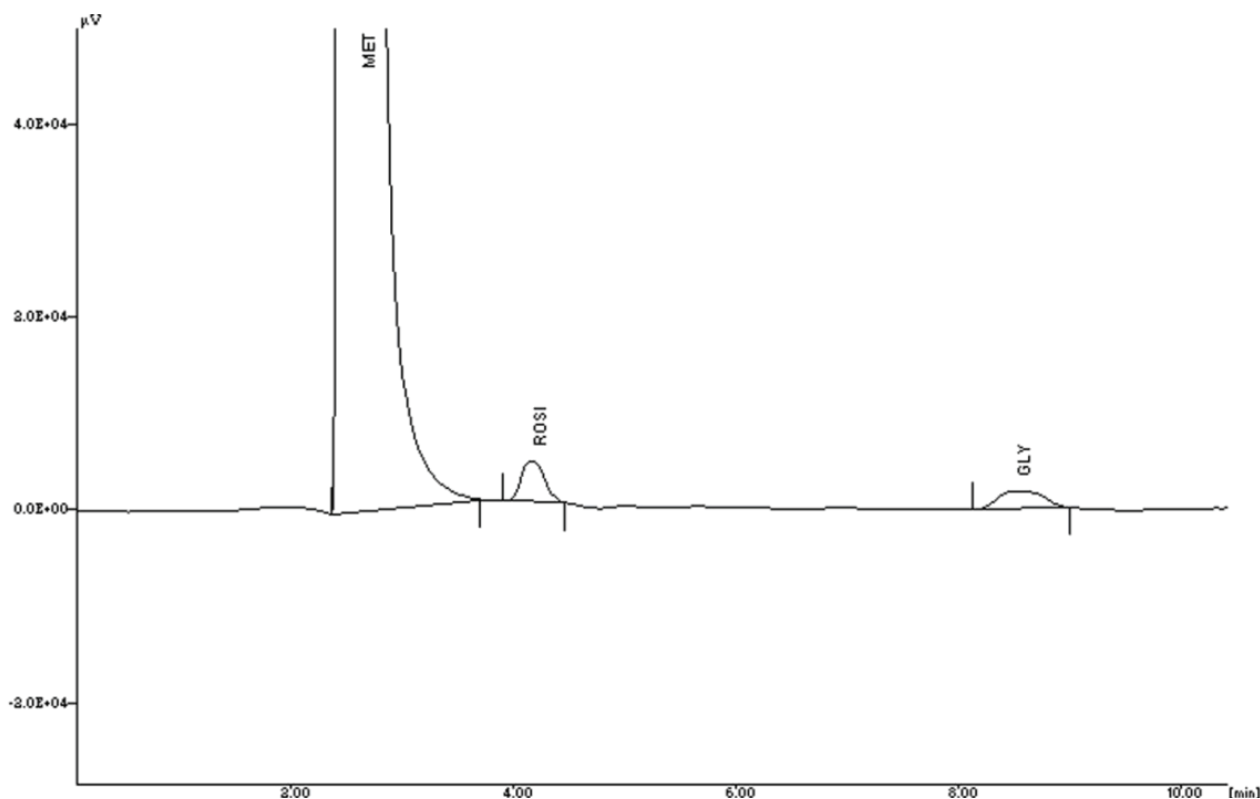


Figure 3. Chromatogram of metformin hydrochloride (200 $\mu\text{g/ml}$), t_R : 2.35 min; rosiglitazone maleate (0.8 $\mu\text{g/ml}$), t_R : 3.90 min; glibenclamide (1 $\mu\text{g/ml}$), t_R : 8.12 min; measured at 238 nm, mobile phase: methanol/potassium dihydrogen phosphate buffer (20 mM) (78/22, v/v).

Validation

Linearity

Linearity was evaluated by analysis of working standard solutions of Met, Rosi and Gly of five different concentrations. The range of linearity was from

50-250 $\mu\text{g/ml}$ for Met, 0.4-2.0 $\mu\text{g/ml}$ for Rosi and 0.6-3.0 $\mu\text{g/ml}$ for Gly. The regression data obtained are represented by calibration curves (Figures 4-6). The results showed that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration of each drug

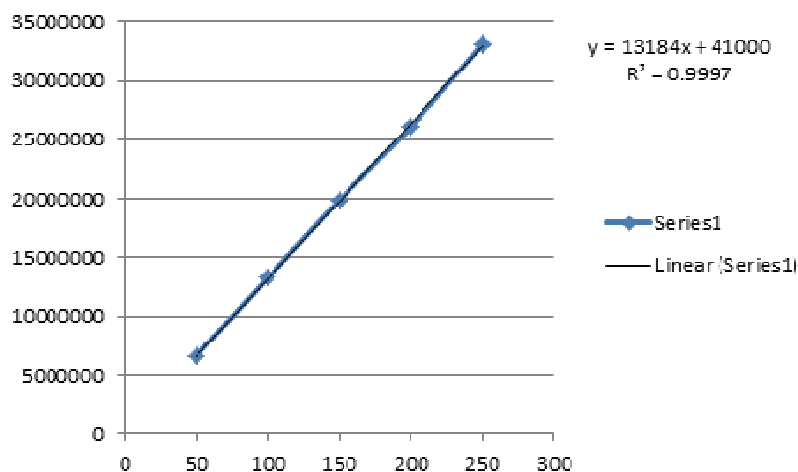


Figure 4. Calibration curve of Met.

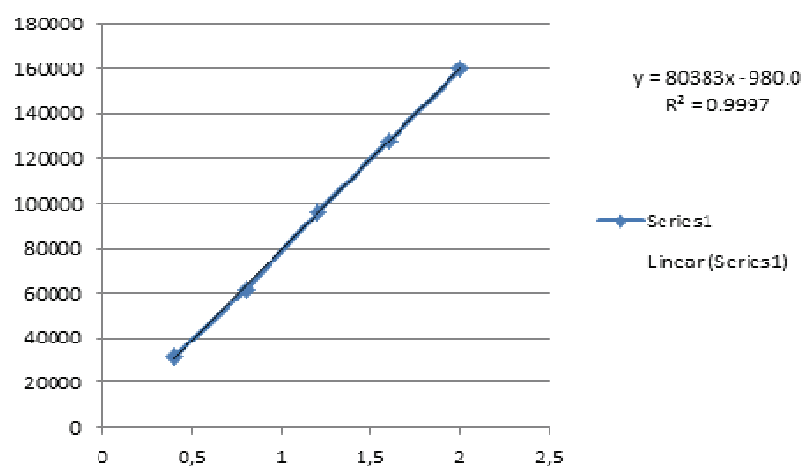


Figure 5. Calibration curve of Rosi.

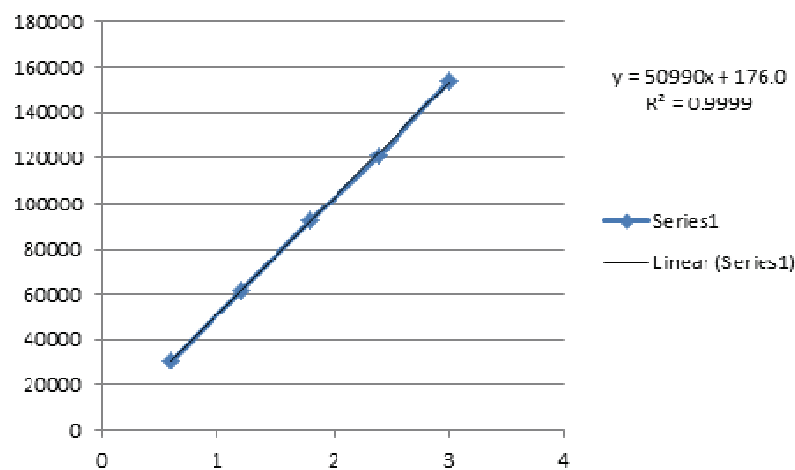


Figure 6. Calibration curve of Gly.

Precision

The results of the intra-day and inter-day precision experiments are given in Table 1. The developed method was found to be precise, with RSD values for intra-day and inter-day precision < 2%, as recommended by ICH guidelines. Separation of the drugs was found to be similar when analysis was performed on different chromatographic systems on different days, as shown in Table 1.

Table 1. Repeatability and intermediate precision; $n = 6$, average of 50, 150 and 250 $\mu\text{g/ml}$ for Met; 0.4, 1.2 and 2 $\mu\text{g/ml}$ for Rosi and 0.6, 1.8 and 3 $\mu\text{g/ml}$ for Gly

Compound	Intraday		Interday	
	Mean % of assay	RSDI %	Mean % of assay	RSDI %
Met	100.89	1.14	99.89	1.09
Rosi	100.71	1.03	99.54	0.37
Gly	100.03	0.51	100.53	0.29

LOD and LOQ

The LOD and LOQ values were found to be 0.02 and 0.06 $\mu\text{g/ml}$ for Met, 0.01 and 0.032 $\mu\text{g/ml}$ for Rosi and 0.003 and 0.01 $\mu\text{g/ml}$ for Gly (Table 2).

Table 2. Limit of detection and quantitation

Compound	LOD	LOQ
	RSDI %	
Met	0.009	0.72
Rosi	0.55	0.28
Gly	0.12	0.43

Specificity

Extracts of commonly used placebos were injected to demonstrate the absence of interference with the elution of the drugs. The results demonstrated that there was no interference from other materials in the tablet formulation, thereby confirming the specificity of the method (Fig. 7).

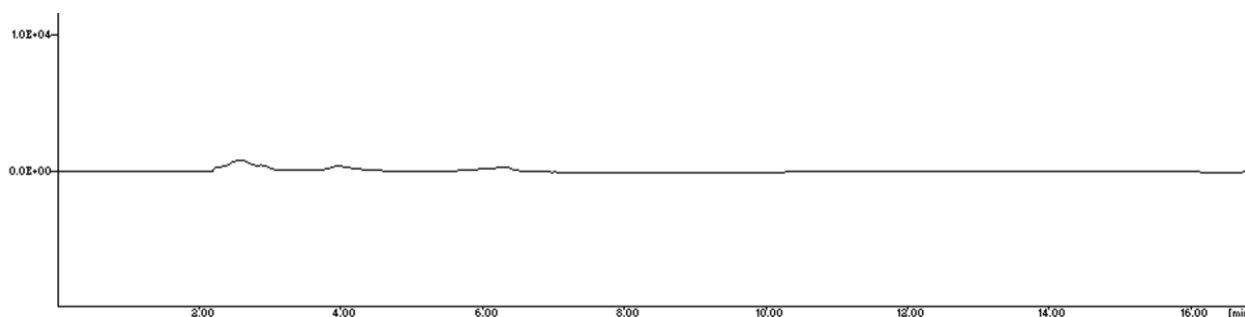


Figure 7. Representative chromatogram obtained for the commonly used excipients.

System suitability

System suitability parameters such as the number of theoretical plates, HETP and peak tailing were determined. The obtained results are shown in Table 3.

Robustness of the method

To ensure the insensitivity of the developed HPLC method to minor changes in the experimental conditions, it is important to demonstrate its robust-

ness. None of the alterations caused a significant change in resolution between Met, Rosi and Gly, peak area, RSD, %, tailing factor and theoretical plates (Table 4).

Table 3. Statistical analysis of parameters required for system suitability testing of the proposed HPLC method

Parameter	Met	Rosi	Gly
Theoretical plates	35547.65	3563	3213.85
Resolution	-	3.01	11.99
Peak asymmetry	1.31	1.35	1.25
RSDI %	0.32	0.09	0.04

Solution stability studies

Three different concentrations 2, 4 and 6 $\mu\text{g/ml}$ were prepared from sample solution and stored at room temperature for 8 days. They were then injected into the HPLC system and no additional peaks were found in the chromatogram, indicating the stability of Met, Rosi and Gly in the solution (Table 5).

Table 4. Robustness testing ($n = 6$)

Chromatographic factor	Level	Retention time, t_R / min			Resolution (R_s)			Asymmetry (A_s)		
	-	Met	Rosi	Gly	Met	Rosi	Gly	Met	Rosi	Gly
Flow rate, ml/min	0.9	2.30	3.78	7.98	0	6.01	2.39	1.13	1.46	1.38
	1.0	2.35	3.90	8.12	0	3.01	11.99	1.31	1.35	1.25
	1.1	2.39	3.70	4.00	0	6.49	2.48	1.01	1.30	1.25
Methanol content, %	76	2.40	3.99	8.50	0	3.70	12.06	1.70	1.66	1.40
	78	2.35	3.90	8.12	0	3.01	11.99	1.31	1.35	1.25
	80	2.30	3.10	4.08	0	6.09	2.01	1.30	1.34	1.24

Table 5. Stability of drugs in sample solutions; $n = 6$, average of (50, 150 and 250 $\mu\text{g/ml}$ for Met, 0.4, 1.2 and 2 $\mu\text{g/ml}$ for Rosi; 50, 150 and 250 $\mu\text{g/ml}$ for Met and 0.6, 1.8 and 3 $\mu\text{g/ml}$ for Gly)

Parameter	Met	Rosi	Gly
RSDI %	0.74	0.04	1.10

Recovery studies

Good recoveries of Met, Rosi and Gly were obtained at various added concentrations for the tablets (Table 6).

Analysis of a commercial formulation

Experimental results of the amount of Met, Rosi and Gly in tablets, expressed as a percentage of label claims were in good agreement with the label claims, thereby suggesting that there is no interference from any of the excipients that are normally present in tablets. Fixed dose combination tablets were analyzed using the proposed procedures (Table 7).

The summary of validation parameters is listed in Table 8.

Table 8. Summary of validation parameters

Parameter	Met	Rosi	Gly
Linearity range, $\mu\text{g/ml}$	50-100	0.4-2	0.6-3.0
Correlation coefficient	0.9997	0.9997	0.9999
Limit of detection, $\mu\text{g/ml}$	0.02	0.01	0.003
Limit of quantitation, $\mu\text{g/ml}$	0.06	0.03	0.01
Recovery ($n = 6$)	100.19	99.50	99.08
Precision (RSDI %)			
Intraday	1.14	1.03	0.51
Interday	1.09	0.37	0.29
Robustness	Robust	Robust	Robust

CONCLUSION

The new HPLC method described in this paper provides a simple, convenient and reproducible approach for the simultaneous identification and quantification that can be used to determine metformin hydrochloride, rosiglitazone maleate, glyburide in routine quality control.

Table 6. Recovery studies ($n = 6$)

Label claim	Amount of drug added, %	Total amount, mg	Amount recovered, mg	Recovery, %
Met 500 mg	80	900	899.28	99.92
	100	1000	1008.3	100.83
	120	1100	1098.02	99.82
Rosi 2 mg	80	3.6	3.601	100.05
	100	4	3.97	99.38
	120	4.4	4.35	99.08
Gly 2.5 mg	80	4.5	4.45	98.91
	100	5	4.96	99.32
	120	5.5	5.44	99.03

Table 7. Applicability of the HPLC method for the analysis of the pharmaceutical formulations

Sample	Label claim, mg	Drug content, %	RSDI %
Met	500	100.39	0.08
Rosi	2	99.38	0.28
Gly	2.5	99.88	0.14

Nomenclature

Met: Metformin hydrochloride

Rosi: Rosiglitazone maleate

Gly: Glibenclamide

ICH: International Conference on Harmonisation

HPLC: High Performance Liquid chromatography

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NAUČNI RAD

ODREĐIVANJE GLIBENKLAMIDA, METFORIN- -HIDROHLORIDA I ROSIGLITAZON-MALEATA U TABLETAMA REVERSNO-FAZONOM HROMATOGRAFIJOM POD VISOKIM PRITISKOM

Razvijena je jednostavna, precizna i tačna HPLC metoda za simultano određivanje metformin-hidrohlorida, rosiglitazone-maleata i glibenklamida u multikomponentnim lekovitim preparatima. Određivanje je izvedeno na C₁₈ koloni dimenzija 25 cm×4.6 mm i.d., i veličina čestica 5 μm. Kao mobilna faza korišćen je rastvarač metanol:kalijum-dihidrogenfosfat (20 mM) 78:22 (v/v) sa protokom 1,0 ml/min. Metformin-hidrohlomid, rosiglitazon-maleat i glibenklamid su detektovani na 238 nm. Ukupno vreme analize je kraće od 9 min. Metoda je precizna i reproduktivna i uspešno je primenjena za određivanje metformin hidrohlorida, osiglitazone osigli i glibenklamida u aktivnim supstancama i farmaceutskim formulacijama bez interferencije sa dodatim ekscipijensima.

Ključne reči: RP-HPLC, rosiglitazon-maleat, metformin-hidrohlomid, glibenklamid.