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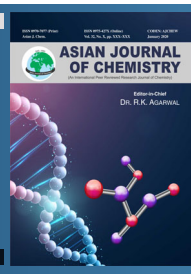

Development and Validation of Modern UHPLC-DAD Analytical Method for Simultaneous Determination of Repaglinide and Metformin in Pharmaceutical Dosage Forms

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For the simultaneous determination of repaglinide and metformin hydrochloride in bulk, an effective and simple UHPLC method was developed and validated and applied to marketed repaglinide and metformin products. The mobile phase used for chromatographic runs consisted of 30 mM phosphate buffer (pH 3.7) and acetonitrile (20:80, v/v) separation was implemented using isocratic mode on an Agilent Zorbax Eclipse Plus C18 (150 × 4.6 mm, 5 µm) column. Drug peaks were well separated and a 232 nm DAD detector observed them. The method was linear for repaglinide and metformin at the concentration range of 20-100 µg/mL, respectively. The method has been validated with respect to system suitability, specificity, accuracy, precision, robustness and ruggedness according to ICH guidelines. Repaglinide and metformin forced degradation studies were conducted for under acidic, base, neutral (peroxide), thermal and photo conditions.

Keywords: Repaglinide, Metformin, UHPLC, Degradation.

INTRODUCTION

Repaglinide (REPA) refers to the anti-diabetic family of meglitinides used to control type 2 diabetes [1]. Metformin hydrochloride (MET) is an anti-diabetic medication belonging to the class of biguanide [2]. Metformin hydrochloride shows its anti-diabetic action mainly by reducing the development of hepatic glucose. Meglitinides demonstrate their hypoglycemic influence by inhibiting ATP-sensitive potassium channels in the membrane of the pancreatic β-cells by inducing first-phase insulin secretion. This move is followed by a cascade of events which eventually stimulates the release of insulin from these cells decreasing the circulation of blood glucose [3]. Metformin hydrochloride is co-administered as a combined dosage form with the present drugs; subsequently, it has a different method of action to non-sulfonylurea insulin secretagogues (repaglinide, mitiglinide calcium and nateglinide), which gives a combination product more benefit than a single-component dosage form. Subsequently, the drugs cited by meglitinides are co-administered with metformin hydrochloride; the development of a technique for their simultaneous purpose with metformin hydrochloride was significant.

The metformin hydrochloride literature analysis was performed using HPLC-UV methods and alone with the column Nova-Pak silica [4], or simultaneously separation was accomplished using isocratic mode on an Alltima CN column [5]. HPLC-MS/MS approach for quantifying SGLT2 antagonists and metformin hydrochloride in plasma simultaneous and applying it to a pharmacokinetic test in fit volunteers [6]. However, the present work deals with multiple mixtures and is presenting a particular viewpoint for LC determination. As for the class of meglitinides, a few methods for their determination have been published. Metformin hydrochloride was calculated through means of spectrophotometry [7-9], HPLC [10-13] and HPTLC [14,15]. An LC method [16,17] for the simultaneous determination of repaglinide, HPTLC [18] and spectrophotometric [19,20] techniques are also reported. A variety of HPLC methods evaluating this were described for repaglinide combination with metformin hydrochloride [21-23] and even in multi-component mixtures [24,25]. The authors intend to establish appropriate HPLC methods for an immense range of medicinal products and at the similar time, appropriate for the study of counterfeits. Spectrophotometric

methods for calculating repaglinide have also been published and metformin hydrochloride binary mix [26] and HPTLC [27,28]. Most of the methods recorded for determination of repaglinide and metformin hydrochloride included plasma assay [29,30].

The present research has been effective in discussing and overcoming several problems in the assay of bulk drugs and dose forms. For instance, separation of repaglinide and metformin hydrochloride was accomplished in a short time, isocratic chromatographic sprint 6 min. Besides, under chromatographic conditions, aim and method development and validation of the method simple, precise, accurate and economic characteristics would be well illustrated further.

EXPERIMENTAL

Materials, reagents and pharmaceutical products: Repaglinide (certified to contain 99.44%) and metformin hydrochloride (certified to contain 99.10%) purchased from Clearsynth Labs Ltd. (Mumbai, India). EUREPA MF 2 (repaglinide 2 mg and metformin 500 mg tablets, Torrent Pharmaceuticals Ltd.) (India) attained from a local pharmacy. Analytical reagent grade orthophosphoric acid and potassium dihydrogen orthophosphate obtained from Finar Limited (Ahmedabad, India). HPLC grade acetonitrile and Water procured from S.D. Finechem Ltd. (India).

The UHPLC system used for the method development and validation consisted of Agilent, (CA, USA) equipped with a quaternary pump G4204A, Agilent DAD G4212A (Diode array detector), Agilent thermostat column compartment TCC G1316C and Agilent autosampler G4226A fitted with an Agilent thermostat G1330B, were used. Data acquisition, recording and chromatographic integration performed OpenLAB CDS Chem station (version A.01.05). All chromatographic evaluation and isolation conducted on the Agilent Zorbax Eclipse Plus C18 (150 × 4.6 mm, 5 µm) at 232 nm and the temperature held at 30 ºC. Phosphate buffer (4 g), pH 3.7 and acetonitrile at ratio (20:80, v/v), use as a mobile phase, a flow rate of 1 mL/min in isocratic mode and an injection volume of 5 µL for all.

Preparation of buffer solution: Buffer solution the concluding is collected of 4.0 g KH_2PO_4 in 1000 mL HPLC water, pH adjusted to 3.7 by orthophosphoric acid. Buffer solution filtered through (0.45 µ nylon membrane filter) and degassed for 20 min in a sonicator.

Preparation of standard stock solutions: Accurately weighed 50 mg of repaglinide and metformin hydrochloride were separately transferred into 100 mL volumetric flasks and dissolved in 70 mL of the mobile phase mixture sonicated for 20 min. The final volume made up of the mark with the mobile phase mixture.

Preparation of working solution: Accurately calculated 1 mL aliquots from standard stock solutions were moved to volumetric flasks of 10 mL and finally completed to volume, utilizing REPA and MET as standard working solutions (50 mg/mL).

EUREPA MF 2 tablets, preparation: Twenty tablets EUREPA MF 2, claimed to contain 2 mg REPA and 500 mg MET were weighed precisely and then powder using a mortar pestle and fine particle size. The exact weight of this powder equivalent to the one tablet content was considered, transferred to a volumetric flask of 20 mL, added 10 mL of the mobile phase, sonicated for about 40 min and then completed with the same mobile phase to the volume. This solution (10 mL) was transferred to 20 mL volumetric flasks made up to the mobile phase mark and an additional 0.4 mL aliquot from Flask was transferred to 100 mL volumetric flasks. The mobile phase was added to the mark and filtered (Hydrophilic PVDF 0.22 µm) to produce a final concentration of 50 µg/mL repaglinide and 50 µg/mL metformin hydrochloride, respectively.

Method development and optimization: Method development and optimization certain mobile phases and columns initially checked to have all eluents on the same chromatogram due to the significant difference in chemical and physical properties of repaglinide and metformin hydrochloride. Based on the specificity, selectivity and correct chromatographic parameters of the formed peaks calculated in terms of peak symmetry, peak sharpness, resolution and tailing factor between the two peaks, column suitability and the used as a mobile phase in the improved method. We used the solvent as a mobile phase for all samples to confirm the minimum noise and eliminate any inappropriate solvent peaks.

Columns applied in our initial trials: Efforts were made by using four kinds of UHPLC columns Zorbax Eclipse Plus C18 (50 × 4.6 mm, 1.8 µm), Inertsil ODS-2 (150 × 4.6 mm, 5 µm), Zorbax Eclipse Plus C18 (150 × 4.6 mm, 5 µm), Phenomenex Synergi C18 (150 mm × 4.6 mm × 4 µm) for the improved method.

Types of buffers and different mobile phase evaluated using: Various concentrations of phosphate buffer with (20, 30, 40, 50 and 60 mM) then used to increase the polarity of the mobile phase resulting in a narrowed peak.

Several mixture solutions with various pH levels (2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0) were used to analyze the retention time and resolution of repaglinide and metformin hydrochloride under which the other chromatographic parameters were kept unchanged. A 30 mM and 3.7 pH phosphate buffer for the optimized method.

Selection of UV wavelength: Repaglinide has a λ_{max} at 241 nm and metformin hydrochloride has λ_{max} at 234 nm. The acceptable response obtained when both drugs were detected at 241 nm either independently or in combination.

Method validation: The standardized procedure for the simultaneous assessment of repaglinide and metformin hydrochloride was tested in accordance with the International Conference on Harmonization (ICH) Guidelines Q2 (R1) [32, 32] for the evaluation of limit of detection (LOD), limit of quantitation (LOQ), system suitability, precision, accuracy, specificity, robustness and ruggedness.

System suitability: Six replicates injections also determined the system's suitability repaglinide and metformin hydrochloride (50 µg/mL). The method developed was found suitable for use as tailing factor, repeatability, number of theoretical plates repaglinide and metformin hydrochloride resolution was within limits.

Precision, repeatability (intra-day precision) and intermediate precision (inter-day precision): The precision of the system and the method were tested by injecting six separate combinations of repaglinide and metformin hydrochloride samples (50 µg/mL) on the same day beneath the same operating conditions. Intermediate or inter-day precision was analyzed by evaluating the effects on three different days of six independent determinations.

Linearity and range: The standard repaglinide and metformin hydrochloride stock solution are diluted within the concentration range of (20-100 µg/mL). Triplicates of this concentration range were prepared and plotted on a calibration curve for repaglinide and metformin hydrochloride. This concentration range had been developed and plotted on a calibration curve for repaglinide and metformin hydrochloride. To ensure the linearity of the analytical method, the slope, intercept and correlation coefficient of the calibration curves (peak area *versus* concentration) was defined.

Accuracy study and recovery: The accuracy of the suggested method was verified by the placebo spiking process, which was achieved separately by spiking placebo with repaglinide and metformin hydrochloride at three different levels, 80%, 100% and 120%. Triplicate assessments of these three levels to determine the mean and % RSD were reported.

Method sensitivity, LOD and LOQ: LOD and LOQ for repaglinide and metformin hydrochloride were determined based on the linear regression equation:

$$LOD = 3.3 \times \frac{\text{Standard deviation (of response)}}{\text{Slope of calibration curve}}$$

$$LOQ = 10 \times \frac{\text{Standard deviation (of response)}}{\text{Slope of calibration curve}}$$

Robustness and ruggedness: Intentional minute changes were made in the chromatographic conditions such as wavelength, flow rate, temperature and pH of the buffer. Such differences were also tested for resolution between peaks of repaglinide and metformin hydrochloride, retention time, number of theoretical plates, asymmetric factor and % RSD.

Forced degradation study

Acid degradation: Forced degradation of repaglinide and metformin hydrochloride (50 µg/mL) by acid hydrolysis using 1 M HCl maintained for 2 h at 60 ºC. The sample was applied to the target after the stress was neutralized with NaOH and diluted with the mobile phase and filtered (Hydrophilic PVDF 0.22 µm) before the study.

Base degradation: Forced degradation of repaglinide and metformin hydrochloride (50 µg/mL) by base hydrolysis using 1 M NaOH maintained for 2 h at 60 ºC. The sample was applied to the target after the stress was neutralized with hydrochloric acid and diluted with the mobile phase and filtered (Hydrophilic PVDF 0.22 µm) before the study.

Hydrogen peroxide (neutral) degradation: Forced degradation of repaglinide and metformin hydrochloride (50 µg/mL) was observed under the impact of (3%) H_2O_2 maintained for 2 h at 60 ºC. The stressed sample was diluted with mobile phase and filtered (Hydrophilic PVDF 0.22 µm) before the study.

Thermolysis degradation: The effect of rising temperature on REPA and MET (50 µg/mL) was observed by heating the sample in refluxing apparatus at 60 °C for 48 h. The stressed sample was diluted with mobile phase and filtered (Hydrophilic PVDF 0.22 µm) before the study.

Photolytic degradation: The effect of UV light on the REPA and MET (50 µg/mL) stability was analyzed by 48 h illumination of the sample in UV light at 365 nm. The stressed sample was diluted with mobile phase and filtered (Hydrophilic PVDF 0.22 µm) before the study.

RESULTS AND DISCUSSION

Method development and optimization: Our goal in the present research was to establish a quick, inexpensive, selective and responsive system for the simultaneous determination of the antidiabetic class of meglitinides with repaglinide and metformin hydrochloride. Developing a sufficiently precise analytical method for discriminating against repaglinide and metformin hydrochloride has been highly challenging. Simple, HPLC with DAD detection was used to do this. Preliminary tests on C18 column at ambient temperature achieved separation between repaglinide and metformin hydrochloride Nevertheless, further optimization of chromatographic conditions was required to achieve separation of the two target products from the meglitinides along with demonstration of appropriate peak shape, selectivity, sensitivity and fulfillment of all other system suitability parameters. A study was performed to explain the impact of each parameter on component separation and sufficient elution to achieve the optimum conditions needed for the separation of target products; a buffer component of 30 mM potassium dihydrogen orthophosphate was measured at various pH values (2.5, 3.0, 3.5, 4.0 and 5.0); acetonitrile was observed at varies concentrations (20:80, 40:60, 60:40 and 80:20) and at different flow rates (0.8, 0.9, 1 and 1.2) were assessed.

Through referring to the selection step in the test, the optimum separation was obtained using a 30 °C and C18 column using a mobile phase consisting of 20% 30 mM potassium dihydrogen orthophosphate (pH adjusted by orthophosphoric acid to 3.7): 80% acetonitrile which flows at a rate of 1.0 mL/min. In a run time of 6 min, eluted peaks were observed at 232 nm. Deficiency of separation between repaglinide and metformin hydrochloride did not pose an incorrect outcome or undesirable matter as the two components fit the similar class of antidiabetics, which stops any option of uniting them in a single dosage type. Hence, their coordinated separation is not necessary. For repaglinide and metformin hydrochloride, respectively, retention periods (min) obtained under acceptable chromatographic conditions were 3.72 and 1.33 (Fig. 1). The requirements of the method, therefore, specified earlier under chromatographic conditions, are considered suitable to separate in the presence of the two oral antidiabetic drugs.

System suitability: The results obtained from six replication injections suggested that the parameters evaluated were within the appropriate range. Repaglinide and metformin hydrochloride were maintained consistently and well separated at

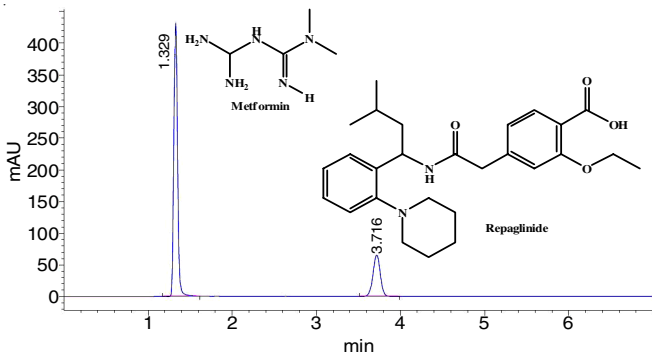

Fig. 1. UHPLC chromatogram of repaglinide and metformin hydrochloride. Chromatographic conditions: Zorbax Eclipse Plus C-18 (150 mm × 4.6 mm i.d., particle size 5 µm); mobile phase phosphate buffer (30 mM potassium dihydrogen phosphate, pH adjusted to 3.7 ± 0.02 with orthophosphoric acid) and acetonitrile (20:80 v/v); flow rate of 1.0 mL/min; and DAD detection at 232 nm

3.7 min and 1.3 min respectively, demonstrating excellent resolution of both peaks with an % RSD of the reported retention periods < 0.3 to suggest stable repeatability of replicate injections on the integrated UHPLC system used, the tailing factor for both repaglinide and metformin hydrochloride peaks never reached 1.0 in both peaks suggesting good peak symmetry (acceptance limit is < 2). The number of theoretical plates in all chromatographic runs was always > 2000 to ensure good column efficacy during the separation cycle established. Results are displayed in Table-1.

TABLE-1
SYSTEM SUITABILITY RESULTS FOR REPA AND MET

Parameters	REPA	MET
Peak area (A) mAs	433.30 ± 0.96	1565.50 ± 3.01
Relative standard deviation (RSD)	0.22%	0.19%
Retention time (tR)	3.72	1.33
Theoretical plates (N)	6949	3921
Symmetry factor (AS)	0.99	0.98
Resolution	10.61	–
Retention factor K'	6.46	1.66

Precision: The peak areas obtained after injecting six individual combined samples of repaglinide and metformin hydrochloride were repeatable and consistent for two consecutive days. The findings for both intra-day and inter-day determinations maintain the high precision and repeatability of the constructed system where all data was presented in % RSD and never surpassed 0.53 % (% RSD < 2 approval limit). Results for intra-day and inter-day precision displayed in Table-2.

TABLE-2
INTRA-DAY AND INTER-DAY PRECISION RESULTS

Analysis date	Intra-day		Inter-day	
	REPA	MET	REPA	MET
% Assay Mean	99.78	99.90	100.42	99.88
% RSD	0.53	0.29	0.37	0.52

Specificity: The analytical method was capable of detecting and assessing repaglinide and metformin hydrochloride in the presence of a typical tablet excipient matrix. The representative chromatogram of mobile phase, placebo and repaglinide and metformin hydrochloride, standard mixture, is displayed in Fig. 2. The specificity of the system was verified when the optimized conditions for detecting repaglinide and metformin hydrochloride (from manufacturer's excipients) were implemented in EREPA MF 2 tablets, respectively, representative repaglinide peak and metformin hydrochloride peak analysis of marketed tablets displayed in Table-3, while chromatograms in tablets are displayed in Fig. 3.

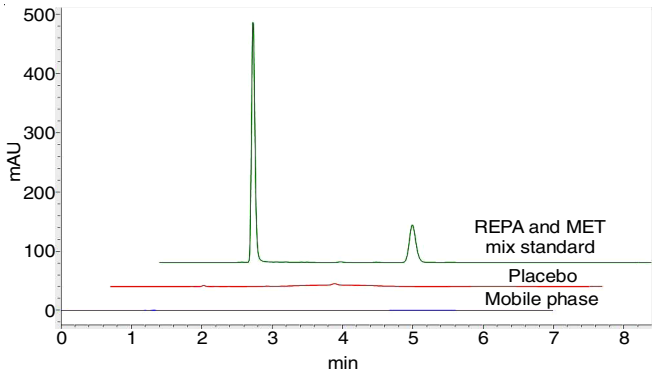

Fig. 2. Overlaid chromatograms of mobile phase, placebo and repaglinide and metformin hydrochloride standard mixture

Linearity: In the specified ranges, the analytical calibration curve constructed for both repaglinide and metformin hydrochloride was linear, indicated by the closeness of the correlation coefficient R^2 to 1 ($R^2 = 0.9999$). The linear regression equation for repaglinide is ($y = 8.174x - 3.810$, $R^2 = 0.999$) and the linear

TABLE-3
STUDY OF MARKETED TABLETS

Tablet (EREPA MF 2) Replicate number	Retention time		Area		Symmetric factor		Number of theoretical plates		Assay (%)	
	REPA	MET	REPA	MET	REPA	MET	REPA	MET	REPA	MET
1	3.718	1.326	434	1562	0.99	0.98	6924	3931	100.38	99.78
2	3.719	1.326	434	1566	0.99	0.98	6920	3921	99.58	99.69
3	3.719	1.327	434	1568	0.99	0.98	6981	3911	99.38	100.23
4	3.718	1.326	432	1565	0.99	0.96	6949	3931	100.07	99.54
5	3.719	1.326	433	1563	0.99	0.97	6973	3921	100.38	100.48
6	3.718	1.327	432	1570	0.99	0.98	6949	3911	100.81	99.63
Mean ± SD	3.719 ± 0.00	1.326 ± 0.00	433 ± 0.96	1565 ± 3.01	0.99 ± 0.00	0.97 ± 0.01	6949 ± 24.76	3921 ± 8.94	100.09 ± 0.54	99.89 ± 0.37
%RSD	0.01	0.04	0.22	0.19	0.00	0.86	0.36	0.23	0.54	0.37

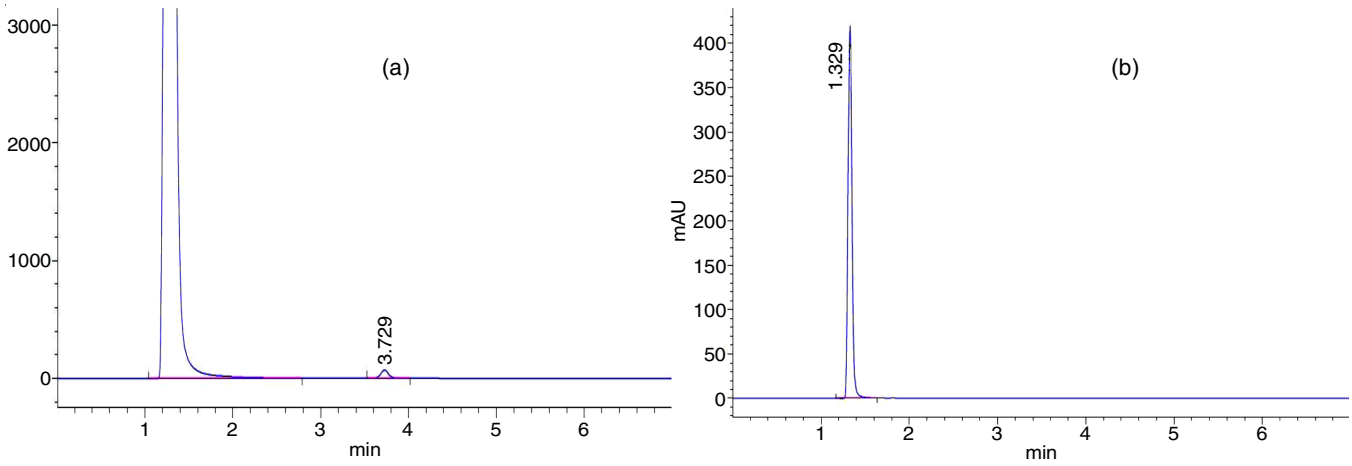

Fig. 3. UHPLC chromatogram of EREPA MF 2 capsule sample solution containing (a) 50 µg/mL repaglinide and (b) 50 µg/mL metformin hydrochloride

regression equation for metformin hydrochloride is ($y = 21.73x + 42.71$, $R^2 = 0.999$) are displayed in Tables 4 and 5. The calibration curves that were produced by plotting peak area against concentration displayed linear relation. Calibration curves with corresponding residual plots repaglinide and metformin hydrochloride are displayed in Fig. 4.

TABLE-4
LINEARITY RESULTS OF REPA AND MET

STD Concentration range (µg/mL)	Peak area		Found concentration	
	REPA	MET	REPA	MET
20	159	467	19.23	19.08
30	243	677	29.48	28.84
40	326	912	39.80	39.76
50	395	1155	48.29	51.02
60	490	1351	59.94	60.16
70	568	1571	69.65	70.39
80	655	1804	80.33	81.19
90	734	1989	90.14	89.78
100	809	2192	99.36	99.21

TABLE-5
LINEARITY PARAMETERS FOR THE REPA AND MET

Linearity parameter	REPA	MET
Range (µg/mL)	20-100	20-100
Slope	8.17	21.73
Intercept	3.81	42.71
Regression coefficient (r^2)	0.999	0.999
Standard error of Intercept	4.07	15.34
Standard deviation of intercept	12.20	46.02
Confidence limit of the slope	8.17 ± 0.78	21.73 ± 0.97
Confidence limit of the intercept	3.81 ± 3.83	42.71 ± 8.42

Recovery: The accuracy of the experimental, analytical method was tested by evaluating the added analytes in the placebo matrix in triplicates at three separate levels (80, 100 and 120%) and represented in terms of percentage recovery from the spiked form of repaglinide and metformin hydrochloride. The similarity of the observed analyte values to the theoretical concentrations reported at various rates demonstrated the trueness/accuracy of the proposed method where repaglinide and metformin hydrochloride > 99% recovered from the spiked excipients. Details for recoveries from repaglinide and metformin hydrochloride are displayed in Table-6.

TABLE-6
PERCENT RECOVERY RESULTS REPA AND MET

Drug	Simulated dosage nominal (%)	% Mean (n = 3)	RSD (%)	RE%
REPA	50	100.02 ± 0.70	0.7	0.02
MET	50	100.19 ± 0.64	0.64	0.19
REPA	100	99.96 ± 0.27	0.27	-0.04
MET	100	100.10 ± 0.22	0.22	0.1
REPA	150	99.65 ± 0.75	0.75	-0.35
MET	150	99.83 ± 0.40	0.4	-0.17

LOD and LOQ: The calculated LOD and LOQ were 1.64 mg/mL, 4.93 mg/mL for repaglinide and 2.33 mg/mL, 7.06 mg/mL for metformin hydrochloride, respectively are displayed in Table-7.

TABLE-7
VALUES OF LOD AND LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
REPA	1.64	4.93
MET	2.33	7.06

Robustness and ruggedness: No significant adjustments observed when adding minor variations to the chromatographic conditions ensuring the method is robust to small intentional modifications introduced in terms of the wavelength, flow rate, temperature, pH of the buffer used. Increasing the previous parameters was modified, thus holding the other chromatographic system parameters unchanged. Retention time, theoretical plates, symmetric factors have not changed significantly by adding the various conditions maintaining the robustness of the method described. The retention time, theoretical plates, symmetric factors under different conditions are summarized in Table-8.

Forced degradation study: Repaglinide and metformin hydrochloride were pressured under different conditions and UHPLC was subject to separation of the samples. Significant drug degradation peaks were observed under basic and neutral

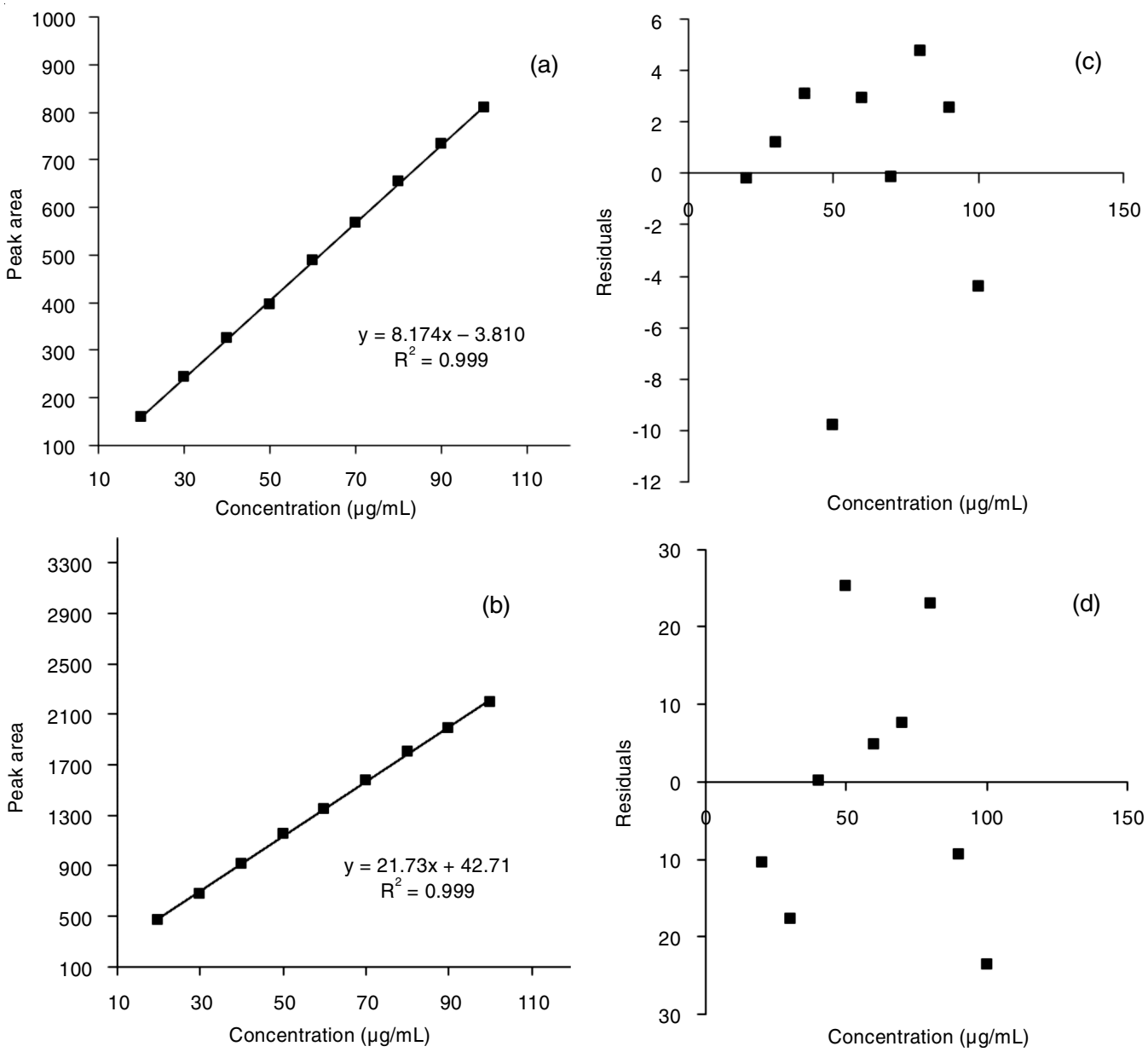

Fig. 4. Linearity plots for repaglinide (a) and metformin hydrochloride (b) with corresponding residual plots for the repaglinide (c) and metformin hydrochloride (d)

TABLE-8
ROBUSTNESS AND RUGGEDNESS RESULTS OF REPAGLINIDE (REPA) AND METFORMIN HYDROCHLORIDE (MET)

Parameter	Conditions	% RSD (n = 3)		Retention time		Number of theoretical plates		Symmetric factor	
		REPA	MET	REPA	MET	REPA	MET	REPA	MET
Change in λ_{max} 241 ± 2	239	0.15	0.3	3.61	1.34	7560	4985	0.99	0.97
	243	0.73	0.19	3.62	1.35	7257	4978	0.99	0.96
Change in flow rate 1 ± 2	0.8	0.58	0.25	4.51	1.68	8938	6783	0.99	0.98
	1.2	0.87	0.46	3	1.12	5887	3946	0.97	0.99
Change in temp. 30 ± 5	25	0.32	0.29	3.73	1.34	7187	5149	0.91	0.93
	35	0.2	0.19	3.52	1.34	7135	4893	0.99	0.97
Change in pH 3.7 ± 2	3.68	0.39	0.33	3.61	1.33	7828	5243	0.96	0.98
	3.72	0.33	0.23	3.84	1.35	7916	5276	0.94	0.98
Ruggedness									
Different analyst	Analyst 1	0.18	0.21	3.72	1.33	6942	3921	0.99	0.98
	Analyst 1	0.57	0.32	3.72	1.33	6957	3924	0.99	0.97

(H_2O_2) conditions. Lastly, the terms of acid, light and thermal stress did not encourage the formation of degradation products. The chromatograms of pure drugs and their stressed samples were seen in Fig. 5b-f. Table-9 reported peak retention time, repaglinide and metformin hydrochloride recovery percentage degradation under different stress conditions.

Conclusion

For the simultaneous separation and quantification of repaglinide and metformin hydrochloride in bulk, laboratory-prepared mixture and pharmaceutical preparations, the suggested UHPLC approach has the advantages of simplicity, precision, accuracy and convenience. The proposed UHPLC approach can,

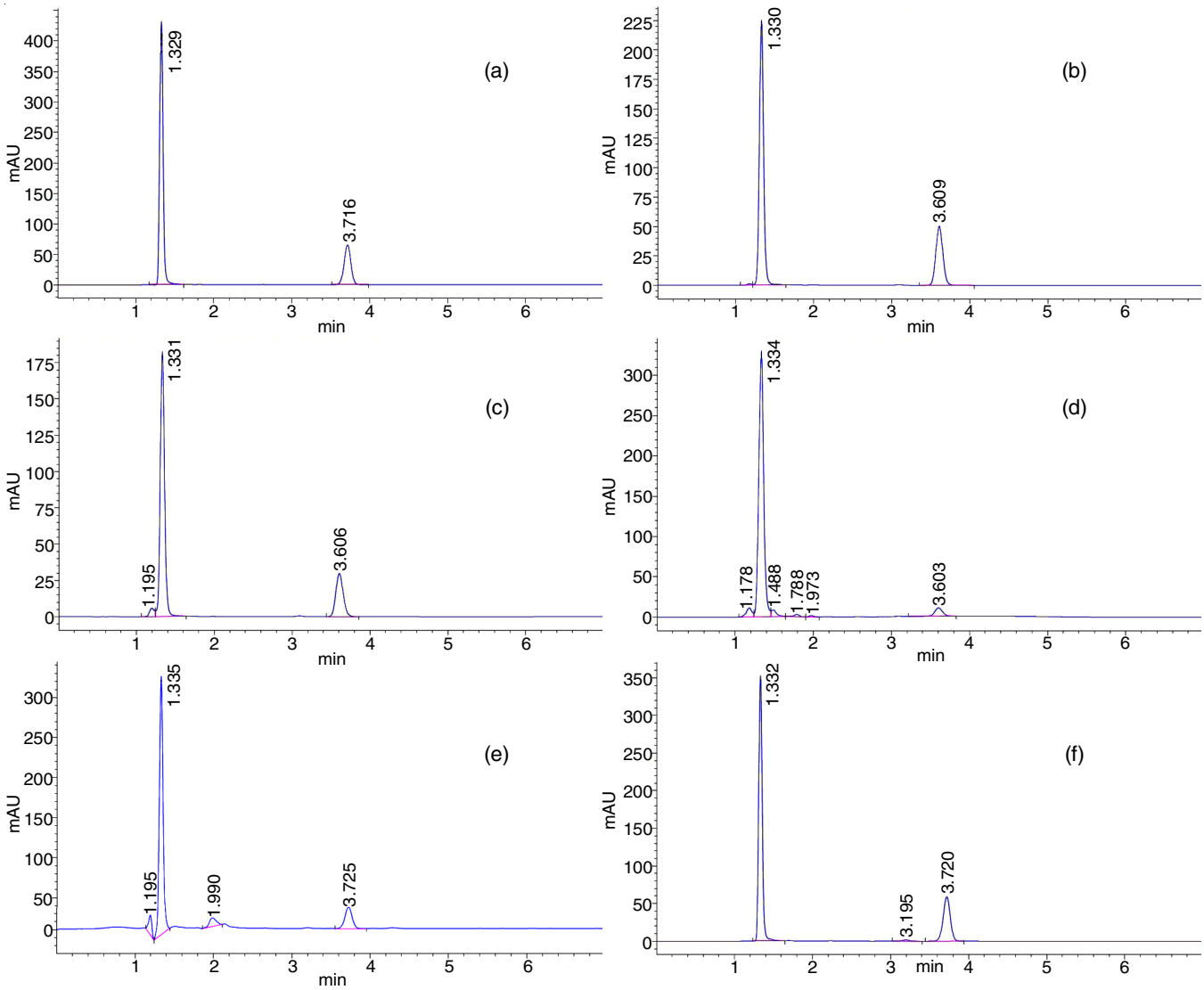

Fig. 5. (a) UHPLC chromatogram of a standard sample solution containing REPA and MET. UHPLC chromatogram of REPA and MET obtained from degradation studies, (b) Acid hydrolysis (1 M HCl at 60 °C for 1 h); (c) Base hydrolysis (1 M NaOH at 60 °C for 1 h), (d) Oxidative degradation (3% H_2O_2 at 80°C for 1 h), (e) Thermal degradation (60 °C for 48 h), (f) Photo degradation at 25°C for 48 h with UV radiation at 365 nm)

TABLE-9
DEGRADATION STUDY OF REPAGLINIDE AND METFORMIN HYDROCHLORIDE

Condition	Repaglinide				Metformin hydrochloride			
	Rt	Recovery ± SD	%RSD	% Drug degraded	Rt	Recovery ± SD	%RSD	% Drug degraded
Acid hydrolysis	3.62	84.89 ± 0.35	0.41	14.67	1.33	98.56 ± 0.50	0.51	1.15
Base hydrolysis	3.62	74.43 ± 0.58	0.77	24.78	1.33	75.79 ± 0.44	0.58	22.99
Oxidative degradation	3.61	70.13 ± 0.65	0.93	28.83	1.33	91.97 ± 0.63	0.69	6.95
Thermal degradation	3.72	91.83 ± 0.42	0.85	7.92	1.33	93.85 ± 0.61	0.65	5.47
Photo degradation	3.72	96.49 ± 0.35	0.36	3.17	1.33	98.63 ± 0.43	0.43	0.96

therefore, be used to control the quality of the above drugs with sufficient selectivity and efficiency in a short time and with low solvent usage. Such elements have been identified to see the requirements of the chromatographers for pharmaceutical drug quality evaluation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.M. Amin and M.S. Arbid, *Appl. Physiol. Nutr. Metab.*, **42**, 181 (2017); https://doi.org/10.1139/apnm-2016-0429
2. E. Sanchez-Rangel and S.E. Inzucchi, *Diabetologia*, **60**, 1586 (2017); https://doi.org/10.1007/s00125-017-4336-x
3. R. Walker and C. Whittlesea, eds.: E.A. Hackett and S.N.J. Jackson, Diabetes mellitus, In: Clinical Pharmacy and Therapeutics Churchill Livingstone Elsevier, Amsterdam, vol. 369, Chap. 44, p. 685 (2013).
4. F. Al-Rimawi, *Talanta*, **79**, 1368 (2009); https://doi.org/10.1016/j.talanta.2009.06.004
5. A. Gedawy, H. Al-Salami and C.R. Dass, *J. Food Drug Anal.*, **27**, 315 (2019); https://doi.org/10.1016/j.jfda.2018.06.007
6. B.C.L. Dias, M.M. Fachi, M.L. de Campos, F.L.D. Degaut, R.G. Peccinini and R. Pontarolo, *Biomed. Chromatogr.*, **33**, 1 (2019); https://doi.org/10.1002/bmc.4663
7. R.H. Majithia, D.A. Khodadiya and V.B. Patel, *Heliyon*, **6**, e03855 (2020); https://doi.org/10.1016/j.heliyon.2020.e03855
8. S. Mowaka, M.S. Hendy and W.A. Zaghary, *J. Anal. Chem.*, **75**, 742 (2020); https://doi.org/10.1134/S1061934820060180
9. P. Cholke, M. Shirsath, Y. Temak, A. Kagde and R. Lagad, *Int. J. Res. Pharm. Pharm. Sci.*, **3**, 31 (2018).
10. M.M. Sebaiy, S.M. El-Adl, M.M. Baraka and A.A. Hassan, *Egypt. J. Chem.*, **62**, 429 (2019); https://doi.org/10.21608/EJCHEM.2018.4394.1388
11. V. Kumar D. and J.V.L.N.S. Rao, *Int. Res. J. Pharm. Med. Sci*., **1**, 16 (2018).
12. W.A. Dayyih, M. Hamad, E. Mallah, A.A. Dayyih, R. Awad, Z. Zakaria and T. Arafat, *Int. J. Pharm. Sci. Res.*, **9**, 2965 (2018); https://doi.org/10.13040/IJPSR.0975-8232.9(7).2965-72
13. M.A. Shende and B.R. Budde, *J. Drug Deliv. Ther.*, **9**, 30 (2019); https://doi.org/10.22270/jddt.v9i1.2256
14. S.D. Bhende, M.B. Varanasi and K. Abbulu, *J. Chromatogr. Sci.*, **58**, 418 (2020); https://doi.org/10.1093/chromsci/bmz124
15. N.S. Abdelhamid, M.T. Elsaady, N.W. Ali and W.G. Abuelazem, *Anal. Chem. Lett.*, **9**, 418 (2019); https://doi.org/10.1080/22297928.2019.1622450
16. D.G. Han, J. Kwak, S.W. Seo, J.M. Kim, J.W. Yoo, Y. Jung, Y.H. Lee, M.S. Kim, Y.S. Jung, H. Yun and I.S. Yoon, *Pharmaceutics*, **11**, (2019); https://doi.org/10.3390/pharmaceutics11080382
17. S.S. Joshi, R.R. Nahire, N.R. Shastri, K.V. Surendranath and J. Satish, *Acta Chromatogr.*, **24**, 419 (2012); https://doi.org/10.1556/AChrom.24.2012.3.6
18. K. Deshpande, P. Ranaware, A.R. Madgulkar and M.C. Damle, *Res. J. Pharm. Technol.*, **6**, 158 (2013).
19. M.M. Fouad and N.S. Rashed, *J. Global Trends Pharm. Sci.*, **5**, 1844 (2014).
20. P.B. Parejiya, P.K. Shelat, R.C. Patel, B.S. Barot and A.K. Shukla, *Eurasian J. Anal. Chem.*, **6**, 53 (2011).
21. S.R. Patel, P.V. Kabra, R.V. Kimbahune, R. Markad and L.V.G. Nargund, *J. Appl. Pharm. Sci.*, **2**, 227 (2012); https://doi.org/10.7324/JAPS.2012.2738
22. M.A. Mahrouse and N.T. Lamie, *Microchem. J.*, **147**, 691 (2019); https://doi.org/10.1016/j.microc.2019.03.038
23. L.K. Soni, T. Narsinghani and M. Jain, *J. Liq. Chromatogr. Rel. Technol.*, **35**, 385 (2012); https://doi.org/10.1080/10826076.2011.601492
24. T. Gulsun, Y. Akdag, N. Izat, M. Cetin, L. Oner and S. Sahin, *Pharm. Dev. Technol.*, **0**, (2020); https://doi.org/10.1080/10837450.2020.1772290
25. S.T. Hassib, E.A. Taha, E.F. Elkady and G.H. Barakat, *J. Chromatogr. Sci.*, **57**, 697 (2019); https://doi.org/10.1093/chromsci/bmz042
26. F. Farouk, B.A. Moussa and H.M.E.-S. Azzazy, *Spectroscopy*, **26**, 297 (2011); https://doi.org/10.1155/2011/975284
27. N. Shah, B. Suhagia, R. Shah and N. Patel, *Indian J. Pharm. Sci.*, **69**, 834 (2007); https://doi.org/10.4103/0250-474X.39447
28. R.S. Sakhare, S.S. Pekamwar and D.P. Mohkare, *Indian J. Pharm. Educ. Res.*, **51**, S8 (2017); https://doi.org/10.5530/ijper.51.2s.44
29. G. Derosa, A. Mugellini, L. Ciccarelli, G. Crescenzi and R. Fogari, *Diabetes Res. Clin. Pract.*, **60**, 161 (2003); https://doi.org/10.1016/S0168-8227(03)00057-3
30. D. Hoelscher, P.L. Chu and W. Lyness, *Clin. Drug Investig.*, **28**, 573 (2008); https://doi.org/10.2165/00044011-200828090-00004
31. International Conference on the Harmonization, ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology, Q2 (R1) (2005).
32. International Conference on the Harmonization, ICH Harmonized Tripartite Guidelines, Stability Testing of New Drug Substances and Products Q1A (R2) (2003).