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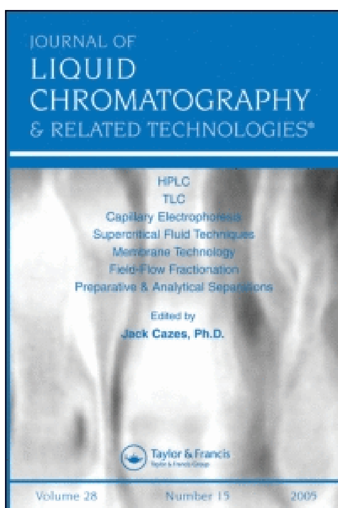

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SIMULTANEOUS ESTIMATION OF GLIBENCLAMIDE, GLICLAZIDE, AND METFORMIN HYDROCHLORIDE FROM BULK AND COMMERCIAL PRODUCTS USING A VALIDATED ULTRA FAST LIQUID CHROMATOGRAPHY TECHNIQUE

F. S. Bandarkar[a]; I. S. Khattab[a]
[a] Department of Pharmaceutics, Faculty of Pharmacy, Kuwait University, Safat, Kuwait

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SIMULTANEOUS ESTIMATION OF GLIBENCLAMIDE, GLICLAZIDE, AND METFORMIN HYDROCHLORIDE FROM BULK AND COMMERCIAL PRODUCTS USING A VALIDATED ULTRA FAST LIQUID CHROMATOGRAPHY TECHNIQUE

F. S. Bandarkar and I. S. Khattab

Department of Pharmaceutics, Faculty of Pharmacy, Kuwait University, Safat, Kuwait

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□ *A rapid, precise, sensitive, economical, and validated analytical method is reported for simultaneous separation and quantification of three anti-diabetic drugs,* viz., *glibenclamide (GLB), gliclazide (GLC), and metformin hydrochloride (MHC) using ultra fast liquid chromatography (UFLC). The separation of the three drugs was achieved using a XR-ODS C18 column (30°C) with a mobile phase comprised of acetonitrile-water-trifluoroacetic acid-triethylamine (54:46:0.1:0.1v/v) in isocratic elution mode at a flow rate of 0.38 mL/min and detected at 230 nm. System suitability tests essential for the assurance of quality performance of the method were performed. The method was validated for accuracy, precision, reproducibility, robustness, detection (LOD), and quantification (LOQ) limits according to FDA and ICH guidelines. MHC (Rt = 0.98 min), GLC (Rt = 4.10 min), and GLB (Rt = 6.40 min) separated with good resolution in a single chromatographic run of 7.5 min. Linear relationship ($r^2 > 0.999$) was observed between the peak area and concentration for all the three compounds within the range of 5–50 µg/mL. Accuracy ranged from 98 to 103% and the coefficient of variation for precision was found to be less than 3%; in all cases. LOD and LOQ values were 10 ng/mL and 20 ng/mL, respectively, for GLC and GLB; whereas 25 ng/mL and 35 ng/mL, respectively, for MHC. The method was found to be robust with minor changes in injection volume and column temperature. Validation results indicated that the method shows satisfactory linearity, precision, accuracy, and ruggedness. The extremely low flow rate, short run time, and simple mobile phase composition makes the method cost effective, rapid, non-tedious, and can also be successfully employed for simultaneous analysis of the three anti-diabetic drugs from commercial products.*

Keywords glibenclamide, gliclazide, metformin hydrochloride, simultaneous analysis, UFLC, validation

Address correspondence to F. S. Bandarkar, Department of Pharmaceutics, Faculty of Pharmacy, Kuwait University, P.O. Box 2492, Safat, Kuwait. E-mail: farzana@hsc.edu.kw or farzana_dawre@yahoo.com

INTRODUCTION

Currently, the most commonly prescribed medications for Type 2 diabetes are metformin and the second generation sulphonylureas, which include glipizide, gliclazide, glibenclamide, and glimperide.[1] For many patients with Type 2 diabetes, mono therapy with an oral anti-diabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control. In such cases, a combination of metformin and one of the sulphonylureas is generally used.[2,3] This combination can be achieved by taking each of the drugs separately or alternative fixed formulations have been developed. A combination tablet formulation is beneficial in terms of its convenience and patient compliance.[4] Various combinations of metformin with sulfonylurea are available commercially as single tablets. The objective of the present work was to achieve true high speed and high separation of a combination of three anti-diabetic drugs (*viz.*, Metformin Hydrochloride, Glibenclamide, and Gliclazide) without sacrificing either the basic performance or all-purpose applicability expected in HPLC analysis. Metformin hydrochloride, 1, 1-dimethyl biguanide hydrochloride, is an anti- hyperglycemic agent. It improves glucose tolerance in patients with type-2 diabetes and reduces both basal and postprandial plasma glucose. Gliclazide, 1-(3-aza bi-cyclo-[3,3,0]-oct-3-yl)-3-(*p*-tolyl sulfonyl) urea and Glibenclamide, 1-{4-[2-(5-chloro-2 methoxy benzamido) ethyl] benzene sulfonyl}-3-cyclohexyl urea, both belong to the category of second generation sulphonyl urea oral hypoglycemic agents, used in the treatment of non-insulin dependent diabetes mellitus (NIDDM). They act by stimulating insulin secretion from pancreatic beta cells by acting on the so called sulphonyl urea receptors.[5,6] The structures of the three drugs are shown in Figure 1.

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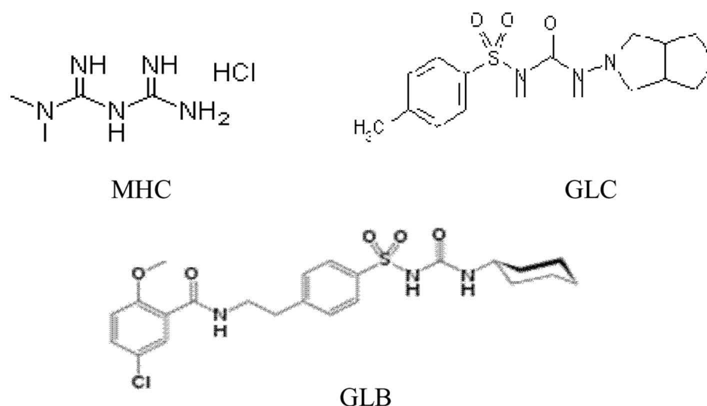

FIGURE 1 Chemical structures of MHC, GLC, and GLB.

Although, many methods have been reported in literature for the estimation of metformin, gliclazide, glibenclamide, and other sulphonylureas individually, only a few methods are available for the simultaneous estimation of these drugs.[7–9] Development and validation of analytical method for simultaneous estimation of glibenclamide and metformin HCl in bulk and tablets using UV-visible spectroscopy is reported by Patil et al.[10] However, it cannot be applied for more than two drugs in combination; also, the risk of excipient interference always exists in UV analysis.[11] HPLC methods for simultaneous determination of two drugs, metformin and glimperide or gliclazide from their combined dosage forms have been described previously for use in studying pharmaceutical preparations.[12,13] Simultaneous RP-HPLC method of analysis of a three-component tablet formulation containing Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glibenclamide, reported by Chaturvedi, employs phosphate buffer at a comparatively high flow rate i.e., 1.5 mL/min.[14] There is no single RP-HPLC method reported for simultaneous estimation of metformin, gliclazide, and glibenclamide which either do not involve acidic buffers or work at flow rates less than 1 or 1.5 mL/min in gradient elution mode leading to time consuming and expensive routine analysis.[15] Other methods reported, *viz*., capillary electrophoresis, ion-pair liquid chromatography, and micelle chromatography, may not be commonly available in all laboratories.[16–18] Various liquid chromatography-tandem mass spectrometry methods are reported for simultaneous plasma analysis of anti-diabetic drugs in combination; however, they are too sophisticated, involve tedious plasma extraction or derivatization procedures[19–24] and some include two mobile phase compositions.[25] Thus, practically, there is limited scope for these methods to be employed routinely for multi-component quality control formulation analysis.

The need for cost effective and speedier HPLC analysis at both the research and the commercial level is obviously increasing; however, basic performance, general-use functionality, ease of operation and robustness must be maintained with this enhanced analysis speed. If these needs are not fully met, the ultimate value of the system and the quality of the data obtained will be reduced. The two approaches most commonly applied for reducing the analysis time are either to shorten the column length or increase the mobile phase flow rate. When these approaches are attempted with a typical column and analytical condition, separation deteriorates (poor resolution) and large volumes of solvents are lost. The purpose of speeding up analysis is to improve throughput, but not by merely shortening the time required for a single analysis. The real goal is to reduce the total analysis cycle time, which includes shortening the intervals between analyses. In summary, in order to save time and money, a non-tedious

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routine analysis method that can be used for the simultaneous determination of combination medications is always of prime importance. The analytical method discussed in this article utilizes a simple mobile phase composition at an extremely low flow rate for the simultaneous determination of Metformin Hydrochloride (MHC), Gliclazide (GLC), and Glibenclamide (GLB) in bulk and from commercial formulations. The method was validated in accordance with FDA and ICH guidelines[26,27] and confirms that the analytical procedure employed is suitable and reliable for its intended use.

EXPERIMENTAL

Materials

Pure samples of MHC and GLB were received as gift samples from Kuwait Saudi Pharmaceuticals, Kuwait. GLC was purchased from Sigma Aldrich, Germany. Acetonitrile (HPLC – grade) was purchased from Merck, India. Millipore (Milford, USA) purification system was used for high purity water. All other chemicals and reagents employed were of analytical grade and were purchased from S.D. Fine Chemicals, India. Marketed formulations used for analysis were: Glucophage Tablets containing 850 mg of MHC (Batch No. 110540, Merck Sante s.a.s, France); Doanil Tablets containing 5 mg of GLB (Batch No. U014, Hoechst, France), and Diamicron Tablets containing 80 mg of GLC (Batch No. 1046506, Serdia, India for Servier, France).

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Analytical Method

High speed analysis of the three anti-diabetic dugs was performed using a validated Ultra Fast HPLC technique described in the following section.

Experimental Conditions

The chromatograph system comprised of a Shimadzu Prominence UFLC pump equipped with a PDA detector. The data was acquired and processed using Shimadzu LC Solutions software. Pre-filtered samples (10 μL) were injected into a Shim-pack XR-ODS column (3.0 mm I.D. × 100 mm L, 2.2 μm particle size) maintained at 30°C. The mobile phase system consisted of acetonitrile-water-trifluoroacetic acid-triethylamine (540:460:1:1v/v) and was run in isocratic mode at a flow rate was 0.38 mL/min through the column. The run time was 7.5 min. per injection and the elute was monitored at a wavelength of 230 nm.

Chromatographic Method Development and Optimization

Initial trial experiments were conducted, in order to select a suitable solvent system for accurate analysis and to achieve good resolution between the three drugs. Column chemistry, solvent type, solvent strength (volume fraction of organic solvents in the mobile phase), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The suitability of the mobile phase and the flow rate was decided on the basis of the sensitivity of the assay, resolution, time required for analysis, ease of preparation, and use of readily available cost-effective solvents. These included methanol–water (50:50v/v), acetonitrile–water, (50:50v/v), acetonitrile-water (60:40v/v), acetonitrile-water-glacial acetic acid (54:44:1v/v), acetonitrile-water-trifluoroacetic acid (55:40:5v/v), etc. A mobile phase system comprised of acetonitrile-water-trifluoroacetic acid-triethylamine (540:460:1:1%v/v) at a flow rate of 0.38 ml/min was found to be optimum. The mobile phase components were mixed together, filtered through nylon membrane filter of 0.45 microns diameter and degassed before use. The experimental work was performed in an air-conditioned room maintained at $22 \pm 1°C$.

Preparation of Stock Solution and Calibration Standards

MHC, GLC, and GLB (25 mg each) were weighed accurately and separately transferred to 25 mL volumetric flasks. GLC and GLB were dissolved in acetonitrile and diluted to produce stock solutions (I) containing 1 mg/ mL of the drug; whereas, MHC was first dissolved in 15 ml of distilled water and then diluted with acetonitrile to produce a 1 mg/ mL stock solution. All further dilutions were made with a solvent system comprising of 54:46 (v/v) acetonitrile: water. 10 mL of each of the stock solution I was diluted individually with the solvent in 50 mL volumetric flasks to produce stock solution II; containing 200 μg/mL of the drug. From stock solution II, combination working solutions were made after suitable dilution with the solvent, on each day of analysis.

Preparation of Resolution Mixture and System Suitability Standard

Individual working standard solutions (10 μg/mL) for all the three drugs was first prepared and chromatographed to identify the respective drug peaks. A combination solution containing all the three components at 10 μg/mL each was then prepared, using the individual stock solution II and was used as system suitability standard and resolution mixture.

System Suitability Tests

The chromatographic system used for analyses must pass the system suitability limits before sample analysis can commence. The capacity factor,

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injection repeatability (n = 5), tailing factor, theoretical plate number, and resolution for the three drug peaks were the parameters tested on a combination solution containing 10 μg/mL each of all the three components; in order to assist the accuracy and precision of the developed HPLC system.

Method Validation

Linearity. A series of combination dilutions and standard curves were prepared over a concentration range of 5–50 μg/ml from stock solution II (200 μg/ml) of all the three drugs. The calibration curves were evaluated for intra-day and inter-day reproducibility (n = 6). The data of peak area versus drug concentration was treated by linear least square regression analysis, whereby the slope, intercept, and the correlation coefficient were determined.

Precision. Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions and expressed as relative standard deviation (RSD) or coefficient of variation (% CV). The standard error of the mean (SEM) was also determined as it gives an estimate of the standard deviation of the mean of all possible samples in a given sample size being analyzed. The three components of precision, i.e., repeatability, intermediate precision, and reproducibility, were determined as shown in the following sections.

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Repeatability. Injection Repeatability: Five injections of a combination solution containing 10 μg/mL of each drug were analyzed and (% CV) were calculated for injection repeatability. This parameter was under taken as an intricate part of the system suitability test.

Intra-day variation. Measurement of intra-day variation of the three component combination solution at three different concentrations (10, 20, and 30 μg/mL) was done by injecting the samples on the same day at intervals of two hours.

Analysis Repeatability. It is reflected by determining the relative standard deviation (RSD) of replicate samples of accuracy study, mentioned as follows.

Intermediate Precision (Inter-Day Variation). Measurement of inter-day variation of the three component combination solution at three different concentrations (10, 20, and 30 μg/mL) in triplicate, on three consecutive days determined the intermediate precision.

Reproducibility. The reproducibility of the method was checked by determining precision on the same instrument under the same experimental conditions, but a different analyst. For both intra-day and inter-day variation, solutions of the three component combination solution at three different concentrations (10, 20, and 30 μg/mL) were analyzed in triplicate.

Accuracy. Accuracy is the measure of how close the experimental value is to the true value and is expressed as % bias or mean relative error (%RE) of nominal compared with measured concentrations. Recovery studies by the standard addition method were performed in a view to justify the accuracy of the proposed method. Previously analyzed samples of a combination solution containing 10 μg/mL each of all the three components were spiked with 75, 100, and 125% excess of each drug standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. % Recovery, % Bias (% Relative Error), SEM, and RSD were calculated at every level.

Detection (LOD) and Quantification (LOQ) Limits. In order to estimate the limit of detection and limit of quantification, the blank sample was injected six times and the peak area of this blank was calculated (noise level was determined). The limit of detection was calculated to be three times the noise level and ten times the noise value gave the limit of quantification. The LOQ, as the lowest point on the calibration plot, should produce an identifiable and discrete peak response, as well as it should be reproducible with precision of 20% and accuracy of 80–120%.

Robustness. The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on analysis. Robustness was determined by altering the injection volume from 10 μL to 5 μL; and the column temperature from 30°C to 25°C.

Sample Solution Stability. The stability of the drugs in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for five days under laboratory bench conditions (25 ± 1°C) and under refrigeration (8 ± 1°C). From stock solution II of each drug (200 μg/ml), combination working solutions were made after suitable dilution with the solvent to get a final concentration of 10 μg/ml of each drug. The samples were analyzed immediately and after a period of one, three, and five days.

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Analysis of MLX from Marketed Tablets

The content of specific anti-diabetic drugs (used in the present study) was determined simultaneously in conventional tablets purchased from the local pharmacy (label claim: 850 mg of MHC per tablet; 80 mg of GLC per tablet; and 5 mg of GLB per tablet). Ten tablets each of MHC, GLC, and GLB were weighed. Their average weight was determined and they were ground, in individual batches, to fine powder using a porcelain mortar and pestle. An amount equivalent to the dose of each active ingredient was accurately weighed and transferred to three suitable volumetric flasks. The volume was adjusted with the solvent and the resultant solution was sonicated for 15 min. and filtered through a 0.45 μm nylon filter (Millipore, Milford, USA). From the resulting samples, suitable aliquots of the filtrate solution were transferred to a 50 mL volumetric flask and volume was made up to with the solvent to have a final concentration of 20 μg/ml of each drug. From this final solution, 10 μL was injected directly onto the HPLC column using the proposed method.

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Analysis of Excipients Influence on Developed Assay Method

The possibility of excipients interference was studied by addition of a known excess of pure drug to the stock assay solutions and determining % recovery by the developed method. Ten tablets each of MHC, GLC, and GLB were weighed. Their average weight was determined and they were ground, in individual batches, to fine powder using a porcelain mortar and pestle. An amount equivalent to the dose of each active ingredient was accurately weighed and transferred to three suitable volumetric flasks. A 100% excess of each pure drug was accurately weighed and added to the respective flasks. The volume was adjusted with the solvent and the resultant solution was sonicated for 30 min. and filtered through a 0.45 μm nylon filter (Millipore, Milford, USA). From the resulting samples, suitable aliquots of the filtrate solution were transferred to a 100 mL volumetric flask and volume was made up to with the solvent to have a final concentration of 40 μg/ml of each drug. A 10 μL sample was injected and analyzed using the proposed method.

Calculations and Statistical Analysis

Standard regression curve analysis was computed using EXCEL® software (Microsoft Corporation, USA) without forcing through zero. Means, standard deviation, % RE, and SEM were also calculated using the same software.

RESULTS AND DISCUSSION

Chromatographic Method Development and Optimization

Preliminary experiments were carried out to optimize the parameters affecting simultaneous estimation of the three drugs using the pre-selected reverse phase column [Shim-pack XR-ODS (3.0 mm I.D. × 100 mm L, 2.2 μm particle size)] and detection of eluted peaks was done by UV using a PDA detector. The solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized to avoid interference from solvent and formulation excipients. Other criteria, for example, time required for analysis, flow rate of mobile phase, symmetry of the eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. The UV spectra of the analytes were determined independently and in combination. It was observed that at wave length 230 nm all the three anti-diabetic drugs could be detected simultaneously with no mobile phase interference, good separation, sensitivity, and consistent baseline. The feasibility of various combinations of solvents such as acetonitrile and methanol with altered flow-rates (in the range 0.2–0.6 mL/min), was investigated for complete chromatographic resolution of the three anti-diabetic drugs with best sensitivity, efficiency, and peak shape. The acidic modifier, trifluoroacetic acid, in the mobile phase and an increase in the acetonitrile content could improve peak shape, whereas an increase in the water content not only broadened the peak but also resulted in extremely rapid desorption and elution of MHC. Finally, the use of acetonitrile: water: trifluoroacetic acid: tri-ethylamine in the ratio 540:460:1:1%v/v and at a flow rate of 0.38 mL/min was found to be optimum and provided adequate peak separation, with less tailing, and resulted in the best resolution among all the other combinations tested. All experiments were performed at 30°C temperature. Each chromatographic run was completed within 7.5 min. Under the optimum chromatographic conditions, the retention times obtained for MHC, GLC, and GLB were 0.978 (±0.013), 4.100 (±0.021), and 6.100 (±0.011) min, respectively (Figure 2a). Resolution between MHC and GLC and between GLC and GLB was 16.71 and 8.6, respectively.

System Suitability Tests

The results of the system suitability tests, recorded in Table 1, assure the feasibility and adequacy of the proposed method for simultaneous

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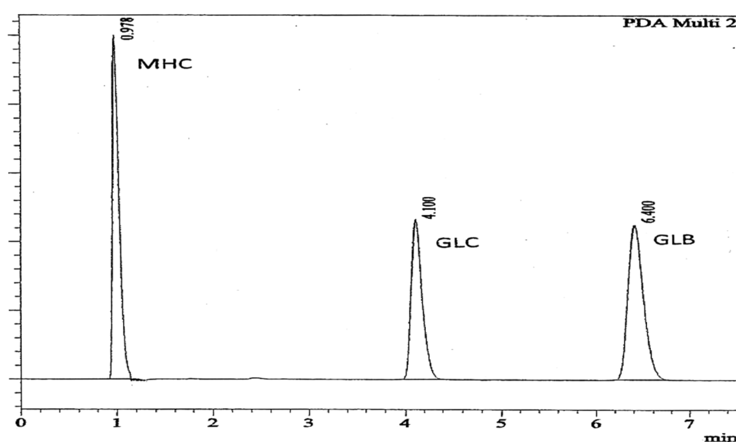
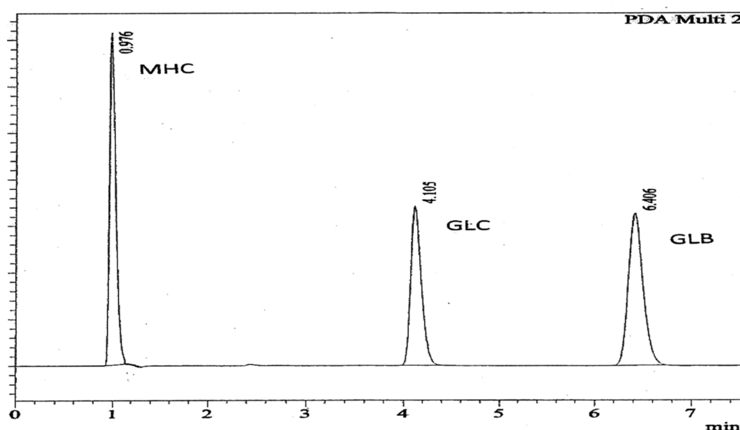


(a)



(b)

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FIGURE 2 UFLC chromatograms of 10 µL injection of a mixture of the three anti-diabetic drugs (20 µg/mL each) in (a) standard solution and (b) from commercial dosage forms.

TABLE 1 System Suitability Parameters

Parameter	Compound		
	MHC	GLC	GLB
Retention time (R_t)	0.979	4.098	6.396
Tailing factor (T_f)	1.1	1.25	1.08
†Injection repeatability (RSD)	0.713	0.628	0.827
‡Resolution (R_s)	–	16.71	8.6
Capacity factor (K′)	1.33	4.54	7.63
Theoretical plates (N)	1273.8	2371.6	3840.0

†RSD of peak areas of five consecutive injections at a concentration of 10 µg/mL of each drug.
‡Resolution between (MHC & GLC) & (GLC & GLB).

estimation of the three dugs in routine pharmaceutical application. The system suitability tests performed verified the resolution, column efficiency, and repeatability of the chromatographic system and ensured that the equipment, electronics, and analytical operations for the samples analyzed could be constituted as an integral system that can be evaluated as a whole. The capacity factor (k') was between 1–10, indicating good resolution with respect to the void volume. The RSD of peak areas of five consecutive injections was found to be less than 2%, thus showing good injection repeatability, and excellent chromatographic and environmental conditions. The tailing factor (T_f) for the three drug peaks was found to be close to 1, reflecting good peak symmetry. The resolution (R_s) between the peaks was found to be greater than 2, indicating good separation of the drug from each other. The values for theoretical plate number (N) demonstrated good column efficiency.

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Method Validation

Linearity

The range of reliable quantification was set at 5–50 μg/mL for each drug injected as a combination solution for simultaneous estimation in one run time. The standard curves had reliable reproducibility ($n = 6$) for all the three analytes across the calibration range. Table 2 shows the mean slope, intercept, and correlation coefficient values for the same. The polynomial regression data for the calibration plots showed good linear relationship with coefficient of correlation, $r^2 > 0.999$ in all cases over a wide range. The low values of the standard deviation, the standard error of slope, and the intercept of the ordinate showed the calibration plot did not deviate from linearity. The linear regression equations for the three drugs were:

$$\text{MHC}: \; y = 42555x - 16556 \; (n = 6, r^2 = 0.9995)$$

$$\text{GLC}: \; y = 32242x + 8269.9 \; (n = 6, r^2 = 0.9997)$$

TABLE 2 Linearity Parameters for Calibration Curve of the Three Drugs ($n = 6$)

Compound	Concentration range (μg/mL)	Slope		Intercept		r^2
		Mean ± SD	CV (%)	Mean ± SD	CV (%)	
MHC	5–50	42555 ± 1063.9	2.50	16556 ± 529.7	3.19	≥0.999
GLC	5–50	32242 ± 973.6	3.01	8269.9 ± 241.5	2.91	≥0.999
GLB	5–50	41420 ± 794.2	1.92	16937 ± 483.1	2.85	≥0.999

$$\text{GLB}: \ y = 41420x + 16937 \ (n = 6, r^2 = 0.9997)$$

where y is the peak area response (mAU) and x is the concentration (μg/ml).

Precision

Precision was measured in accordance with ICH recommendations.

Injection Repeatability. Five consecutive injections of a combination solution containing 10 μg/mL of each drug by the proposed method performed under the system suitability test showed excellent injection repeatability with RSD of less than 2% for MHC, GLC, and GLB.

Intra-Day and Inter-Day Precision. Repeatability of sample injection was determined as intra-day variation measured at intervals of two hours; whereas inter-mediate precision was determined by measuring inter-day variation for three consecutive days. All sample combinations were injected in triplicate at three different concentrations, the results for which are listed in Table 3. The low RSD values ($\leq$3%) indicate the sensitivity and repeatability of the proposed method.

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Reproducibility. It was checked by measuring the precision of the proposed method with analysis being performed on three different concentrations (10, 20, and 30 μg/mL), by another analyst. There was no significant difference observed (RSD $\leq$ 3%) in the intra-day and inter-day precision, confirming reproducibility of the method.

Accuracy

The accuracy of the method was confirmed by studying the recovery at three different concentrations, 75%, 100%, and 125% of those expected by spiking a previously analyzed test solution with additional drug standard solutions, the analysis being done in replicate (n = 3). The %RSD and % relative error in all cases were within the acceptable limit ($\leq$3%). It is evident from the results of accuracy study, reported in Table 4, that the proposed method enables very accurate quantitative simultaneous estimation of MHC, GLC, and GLB.

Detection (LOD) and Quantification (LOQ) Limits

The limit of detection was found to be 10 ng/mL for GLC and GLB; whereas, 25 ng/mL for MHC. The drugs peaks could be detected without any baseline noise disturbances (>3 times) at these concentrations. The limit of quantification was 20 ng/mL for GLC and GLB; whereas,

TABLE 3 Precision of the Method

	Intra-day precision			Inter-day precision		
Conc. (μg/mL)	Mean area ± SD*	SEM†	RSD	Mean area ± SD*	SEM†	RSD
MHC						
10	413901 ± 2963	1710.7	0.72	418304 ± 6645	3836.6	1.59
20	832198 ± 9896	5713.6	1.19	834074 ± 9463	5463.6	1.13
30	1227863 ± 7368	4254.0	0.60	1217282 ± 14756	8519.6	1.21
	Intra-day precision			Inter-day precision		
Conc. (μg/mL)	Mean area ± SD*	SEM†	RSD	Mean area ± SD*	SEM†	RSD
GLC						
10	328051 ± 2132	1230.9	0.65	338890 ± 4384	2531.2	1.29
20	657944 ± 6861	3961.3	1.04	659773 ± 7974	4603.9	1.21
30	960238 ± 8893	5134.5	0.93	951681 ± 9566	5523.1	1.01
	Intra-day precision			Inter-day precision		
Conc. (μg/mL)	Mean area ± SD*	SEM†	RSD*	Mean area ± SD*	SEM†	RSD
GLB						
10	436146 ± 3843	2218.8	0.88	421821 ± 3299	1904.7	0.78
20	856881 ± 9859	5692.3	1.15	851426 ± 10148	5859.1	1.19
30	1254407 ± 11985	6919.7	0.96	1262155 ± 12528	7233.3	0.99

*SD = Standard deviation (n = 3).
*RSD% = SD/Mean × 100.
†SEM = Standard error of mean.

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35 ng/mL for MHC. The analytes' response at these concentration levels were >10 times the baseline noise. The precision and accuracy at these concentration levels for the three drugs were within the acceptable range (<3% of the CVs and <4% of the relative errors). This indicated the method can be used for simultaneous detection and quantification of these three drugs over a wide concentration range.

Robustness

There was no significant change observed in the retention time, peak shape, and resolution of the three drugs when the samples were analyzed by altering the injection volume from 10 μL to 5 μL; and the column temperature from 30°C to 25°C, thus assuring that the proposed methods capability and robustness are unaffected by small variations in the method parameters.

Sample Solution Stability

From stock solution II, stored under refrigeration (8 ± 1°C) and at laboratory temperature (25 ± 1°C) combination working solutions were made

TABLE 4 Accuracy of the Method

Amount (%) of drug added	Theoretical content (μg/mL)	Conc. found (μg/mL) ± SD*	Recovery (%)	SEM	RE† (%)	RSD (%)
MHC						
0	20	20.14 ± 0.382	100.70	0.221	0.70	1.89
75	35	35.47 ± 0.437	101.34	0.252	1.34	1.23
100	40	40.83 ± 0.581	102.08	0.335	2.08	1.43
150	45	45.79 ± 0.764	101.76	0.441	1.76	1.67
GLC						
0	20	20.39 ± 0.434	101.95	0.251	1.95	2.12
75	35	35.63 ± 0.519	101.18	0.299	1.80	1.46
100	40	40.71 ± 0.385	101.78	0.222	1.78	0.95
150	45	46.02 ± 0.627	102.27	0.362	2.27	1.36
GLB						
0	20	19.94 ± 0.281	99.7	0.162	0.30	1.41
75	35	35.14 ± 0.313	100.40	0.181	0.40	0.89
100	40	40.34 ± 0.601	100.85	0.345	0.85	1.49
150	45	44.89 ± 0.542	99.76	0.313	0.24	1.21

*n = 3.
†RE (%) = % Relative error = (Mean assayed concentration − added concentration)/Added concentration × 100.

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after suitable dilution with the solvent, on each day of analysis. There was no significant change in analyte composition (10 μg/mL) until a period of 5 days. The mean RSD between peak areas, for the samples stored under both refrigeration and ambient conditions was found to be less than 3%, suggesting that the individual drug stock solution II (200 μg/mL), can be stored without any degradation for the time interval studied.

Specificity and Selectivity

The selectivity of the method is depicted by the three sharp well resolved peaks for MHC, GLC, and GLB obtained at their respective retention times, i.e., 0.978, 4.100, and 6.400 min, respectively. The specificity of the method was assessed by comparing chromatograms obtained from drug standards (Figure 2a & 2b) with that obtained from tablet solutions. The retention times of the drug standards and the drugs from sample solutions were identical, confirming the specificity of the method. The method was also selective because there was no interference observed from any of the excipients in the tablets formulations tested.

Analysis of MLX from Marketed Tablets

The validated HPLC method was applied for the simultaneous determination of MHC, GLC, and GLB in tablets. A final sample solution

TABLE 5 Assay Result of Commercial Conventional Tablets

Compound	Brand name	Label claim (mg)	Amount found (mg)	RSD (%)	Assay (%)
MHC	Glucophage	850	856.37 ± 1.98	0.23	100.75
GLC	Daonil	5	5.13 ± 0.11	2.14	102.60
GLB	Diamicron	80	81.49 ± 0.57	0.69	101.86

TABLE 6 Drug Recovery from Commercial Conventional Tablets to Study Influence of Excipients on the Developed Assay Method

Compound	Brand name	Conc. found* (μg/mL) ± SD**	Recovery (%)	SEM	RE (%)	RSD (%)
MHC	Glucophage	39.83 ± 0.865	99.57	0.508	0.425	2.17
GLC	Daonil	41.05 ± 0.715	102.62	0.413	2.625	1.74
GLB	Diamicron	40.76 ± 0.36	101.90	0.208	1.900	0.88

*Theoretical content of each drug = 40 μg/mL.
**n = 3.

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containing a combination of the three drugs (20 μg/mL each) was injected. A typical chromatogram identical to that of the drug standard solution was obtained without any excipients interference (Figure 2). The formulation assay results, expressed as a percentage of the label claim, are shown in Table 5. The results indicate that the amount of each drug in the tablets corresponds to the requirements of 95–105% of the label claim. The low RSD values (<3%) confirmed the suitability of this method for routine analysis of MLX in pharmaceutical dosage forms.

Effect of Excipients on the Proposed Assay Method

The influence of excipients on the developed method of analysis was studied by performing recovery studies on the marketed tablets by addition of 100% excess of pure drug to the individual assay stock solutions. The results depicted in Table 6 show good extraction capability and recovery (100 ± 3%) of all the three drugs under test devoid of any excipients interference, thus ensuring the efficiency of the proposed method.

CONCLUSION

Thus, to summarize, the proposed UFLC method of analysis was found to be precise and accurate, as depicted by the statistical data of analysis. The developed method is non-tedious, with a very simple mobile phase composition, extremely small flow rate (0.38 ml/min), and relatively short run

time, i.e., 7.5 min. utilizing an isocratic elution mode with single wavelength monitoring. The validated method allows analysis of approximately 100 injections in fourteen hours, consuming not more than 350 ml of the mobile phase. All these factors enable rapid quantification and simultaneous analysis of the three anti-diabetic drugs in bulk and from commercial formulations without any excipient interference. It can, therefore, be concluded that the reported method could find practical application as an economical and rapid quality-control tool for simultaneous analysis of the three drugs from their combined dosage forms in both research and industrial quality-control laboratories.

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