Analytical Chemistry Insights

ORIGINAL RESEARCH

Simultaneous Determination of Sitagliptin and Metformin in Pharmaceutical Preparations by Capillary Zone Electrophoresis and its Application to Human Plasma Analysis

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Abstract: A novel, quick, reliable and simple capillary zone electrophoresis CZE method was developed and validated for the simultaneous determination of sitagliptin (SG) and metformin (MF) in pharmaceutical preparations. Separation was carried out in fused silica capillary (50.0 cm total length and 43.0 cm effective length, 49 µm i.d.) by applying a potential of 15 KV (positive polarity) and a running buffer containing 60 mM phosphate buffer at pH 4.0 with UV detection at 203 nm. The samples were injected hydrodynamically for 3 s at 0.5 psi and the temperature of the capillary cartridge was kept at 25 °C. Phenformin was used as internal standard (IS).
The method was suitably validated with respect to specificity, linearity, limit of detection and quantitation, accuracy, precision, and robustness. The method showed good linearity in the ranges of 10–100 µg/mL and 50–500 µg/mL with limits of detection of 0.49, 2.11 µg/mL and limits of quantification of 1.48, 6.39 µg/mL for SG and MF, respectively. The proposed method was successfully applied for the analysis of the studied drugs in their synthetic mixtures and co-formulated tablets without interfering peaks due to the excipients present in the pharmaceutical tablets. The method was further extended to the in-vitro determination of the two drugs in spiked human plasma. The estimated amounts of SG/MF were almost identical with the certified values, and their percentage relative standard deviation values (% R.S.D.) were found to be ≤1.50% (n = 3). The results were compared to a reference method reported in the literature and no significant difference was found statistically.

Keywords: sitagliptin, metformin, simultaneous determination, capillary zone electrophoresis, validation, pharmaceutical preparations, human plasma

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Introduction

As the number of individuals affected by diabetes is continuing to increase worldwide, the need for effective management assumes ever greater urgency. Newer classes of medications, particularly those which work via the incretin pathway, achieve glucose lowering and minimizing risks associated with more traditional therapies. Ideally, combination therapies should be well tolerated, convenient to take, have few contraindications, have a low risk of hypoglycemia and weight gain, and be reasonably effective over both the short and long term such as the combination of metformin (MF) and the dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin (SG).[1]

Sitagliptin (SG) 2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine; 7-[(3R)-3-Amino-1-oxo-4-(2,4,5-trifluoro phenyl) butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate (1:1) monohydrate (Fig. 1A) is a novel selective inhibitor of DPP-4.[2] SG has recently been approved in USA and Europe for treatment of type 2 diabetes mellitus (T2DM). Like other DPP-4 inhibitors, SG is responsible for the rapid degradation of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic peptide (GIP), which is released from the gut in response to food intake and reduces hemoglobin A_{1c} (HbA_{1c}), fasting and postprandial glucose by glucose-dependent stimulation of insulin secretion and inhibition of glucagon secretion.[3]

Metformin (MF) N,N-dimethyldiguanide hydrochloride (Fig. 1B), is an inexpensive biguanide oral antihyperglycemic agent,[2] it exerts its glucose-lowering

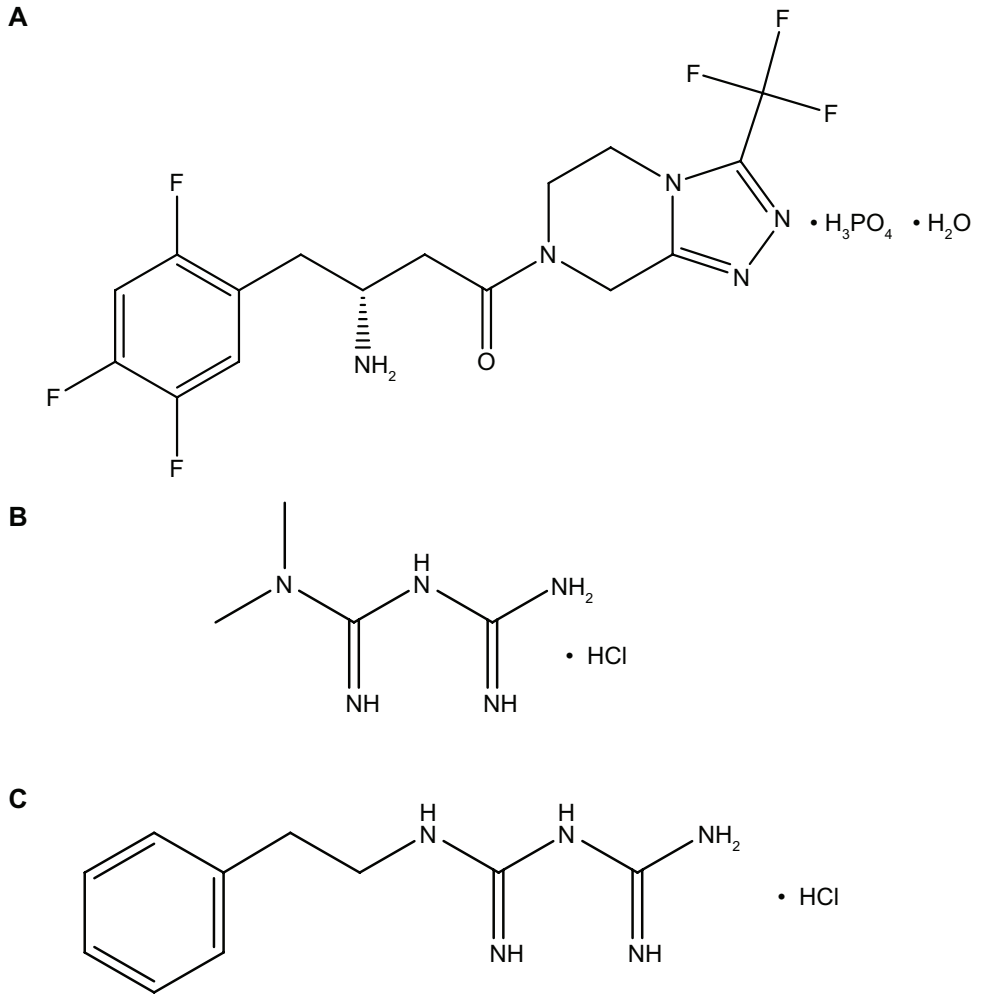

Figure 1. The structural formulae for the studied drugs. (**A**) sitagliptin phosphate monohydrate (SG), (**B**) metformin hydrochloride (MF) and (**C**) phenformin hydrochloride (IS).

effects primarily through increased hepatic insulin sensitivity and the resultant suppression of hepatic glucose output. MF may also modestly enhance glucose uptake in peripheral tissues and increase glucose metabolism in the splanchnic bed.[4]

SG and MF exert a complementary glucose-lowering effect and represent a well-tolerated option for patients requiring therapy for T2DM.[1]

Because SG is a new product and recently introduced in the market, it is not yet official in any of the pharmacopoeias but MF is official in British Pharmacopeia (BP)[5] and United State Pharmacopeia (USP).[6]

Literature survey reveals several methods for the determination of SG in pharmaceutical preparation or biological fluids including spectrophotometry[7,8] liquid chromatography-tendem mass spectroscopy (LC-MS/MS)[9–11] and only one capillary electrophoresis method.[12]

Several methods have also been described for the determination of MF either alone or in combination with various drugs, such as spectrophotometry,[13,14] LC,[15–17] LC/MS–MS[18–20] and capillary electrophoresis.[21–23]

Regarding SG and MF simultaneous analysis, two spectroscopic methods have been reported for the simultaneous determination of both drugs in pharmaceutical preparations[24,25] and only three chromatographic methods have been reported for their determination in pharmaceutical preparations.[26–28]

To the best of our knowledge, no capillary electrophoresis method was reported yet for the simultaneous determination of the studied drugs neither in pharmaceutical preparations nor in biological fluids.

The use of capillary electrophoresis (CE) methods for pharmaceutical analysis has become increasingly popular in recent years. The wide range of pharmaceutical applications for which its use has proved successful, includes assay of drugs, determination of drug-related impurities, physicochemical measurements of drug molecules, chiral separation, and the analysis of vitamins and pharmaceutical excipients. The advantages of using CE for pharmaceutical analysis include its speed and low cost of analysis, reductions in solvent consumption and disposal, and the possibility of rapid method development. CE also offers the possibility that a single set of separation conditions can be applied for a wide range of analyses, giving efficiency savings.[29,30] Recently, CE techniques have been applied to the simultaneous determination of co-formulated drugs in dosage form.[31,32]

This study aimed to develop and validate a rapid, accurate, inexpensive and selective method by capillary electrophoresis applied to the routine quality control analysis of SG and MF simultaneously in their co-formulated tablets and in human plasma. SG and MF are co-formulated in a medicinally recommended ratio of 1:10 and 1:20 (calculated as sitagliptin base and metformin HCL). Analysis of such mixture with strong spectral overlapping is challenging. The proposed CZE method allowed the separation and quantitation of the two drugs with satisfied accuracy and precision. Because this method consumes no organic solvent, it can be considered a green method.

Experimental

Apparatus

The assay was developed and validated using a Beckman P/ACE 5510 system (Fullerton, CA, USA) equipped with an autosampler, a photodiode array (PDA) detector, a temperature controlling system(4 °C–40 °C) and power supply able to deliver up to 30 KV. Beckman P/ACE™ station software (version 1.2) was used for the instrument control, data acquisition and analysis.

Electrophoretic analyses were performed in a fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 50.0-cm-long (43.0-cm effective length) and 49.0 µm i.d. in normal mode, applying a voltage of 15 KV. The wavelength for analysis was 203 nm, hydrodynamic injection of samples for 3 s under a pressure of 0.5 psi and the temperature of the capillary cartridge was 25 °C.

The background running buffer (BRB) was filtered using 0.2 µm Anatop 25 Whatman inorganic membrane filter (Maidstone, England) and degassed using Branson Ultrasonic 5510 degasser (Danbury, CT, USA). A SympHonly (SB20) pH-meter (Thermo Orion Beverly, MA, USA) was used for pH measurements. Deionized water was prepared using a Barnstead NANO pure DIamond Analytical (USA) ultrapure water system.

Materials and reagents

All the chemicals used were of Analytical Reagent grade, and the solvents were of HPLC grade.

- Sitagliptin Phosphate Monohydrate (SG), Metformin Hydrochloride (MF) and Phenformin Hydrochloride (IS) were purchased from AvaChem Scientific (San Antonio, TX, USA), Selleck Chemicals, LLC (Houston, TX, USA) and Sigma-Aldrich (Saint Louis, MO, USA) with labeled purity of 98%, 99% and ≥97%, respectively. They were used as received without further purification.
- Janumet® 50–500 mg tablets; batch # mc20v005016803 and Janumet® 50–1000 mg tablets; batch # mc20v005016825 (Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., NJ, USA) were purchased from commercial sources in the local market. Each labeled to contain 64.25 mg sitagliptin phosphate monohydrate (equivalent to 50 mg sitagliptin as a free base) and 500 mg metformin hydrochloride and 64.25 mg sitagliptin phosphate monohydrate (equivalent to 50 mg sitagliptin as a free base) and 1000 mg metformin hydrochloride, respectively.
- Sodium hydroxide, acetic acid and sodium dihydrogen phosphate (JT Baker, NJ, USA)
- Orthophosphoric acid 85%, sodium acetate and boric acid (Fisher Scientific, NJ, USA).
- Acetonitrile, methanol and ethanol (Sigma-Aldrich, MO, USA).
- Human plasma was purchased from Sigma-Aldrich (Saint Louis, MO, USA) as 0.45 µm filtered and lyophilized powder then it had been reconstituted with deionized water and kept frozen until used after gentle thawing.

Standard and sample solutions

Standard solutions

Stock solutions 1.0 mg/mL of SG, MF and IS were prepared in deionized water. Working solutions were prepared by further dilution of the stock solutions with BRB to give a final desired concentration. The solutions were stable for at least three months when kept in the refrigerator at 2 °C.

Background running buffer (BRB)

Optimized BRB solution was 60 mM sodium dihydrogen phosphate buffer adjusted to pH 4.0 with orthophosphoric acid 85% and filtered through a 0.2 µm membrane filter.

Electrophoretic procedure

Before the first use, the capillary was conditioned by flushing with 1.0 M NaOH for 60 min, then with water for 30 min and BRB for 30 min at 0.5 psi pressure. At the beginning of each working day, the capillary was rinsed with 0.1 M NaOH for 10 min, water for 5 min and then with BRB for 5 min at 20 psi pressure. Before each injection, the capillary was preconditioned with 0.1 M NaOH (3 min), water (3 min) and BRB (3 min) at 20 psi pressure to maintain proper reproducibility of run-to-run injections. Injection was carried out under hydrodynamic pressure at 0.5 psi for 3 s. A diode-array UV detector was set at 203 nm with a bandwidth of 10 nm. The capillary temperature was kept constant at 25 °C and a voltage of +15 kV was applied.

Procedures

Construction of calibration graphs

Aliquots of the suitable drug stock or working standard solutions were transferred into a series of 4-mL vials so that the final concentrations were in the range of 10–100 µg/mL for SG and 50–500 µg/mL for MF. A constant 40 µL IS was added and the volumes were diluted to 1 mL with the BRB. The peak area ratio (peak area of the studied drug/peak area of IS) was plotted versus the final concentration of each drug in µg/mL to get the calibration graph. Alternatively, the corresponding regression equations were derived.

Analysis of SG/MF synthetic mixtures

Aliquots of SG and MF standard solutions keeping the pharmaceutical ratio of 1:10 and 1:20 were transferred into a series of 4-mL vials, diluted with BRB after addition of 40 µL IS and mixed well. The above procedure described under "*Construction of calibration graphs*" was then applied. The percentage recoveries were calculated by referring to the calibration graphs, or using the corresponding regression equations.

Analysis of the studied drugs in their co-formulated tablets

Ten tablets of each type were weighed after their coats were removed by carefully rubbing with a clean tissue wetted with methanol and the average weight was calculated. Tablets were crushed to a fine powder.

An accurately weighed amount of the finely powdered tablets, equivalent to 1.0 mg SG and 7.78 mg MF in case of Janumet® 50–500 and equivalent to 1.0 mg SG and 15.56 mg MF in case of Janumet® 50–1000, was transferred to 10-mL volumetric flasks and 6 mL of deionized water was added. The flasks were sonicated for 30 min and vortex mixing for 15 min by Fisher vortex (Fisher Scientific), and then diluted to the mark with deionized water. The solution was filtered through Whatman filter paper and then filtered again using 0.2 µm Whatman inorganic membrane filter. For analysis, an appropriate aliquot from the prepared sample solutions, spiked with 40 µL IS stock solutin (1.0 mg/mL), was diluted to 1 mL using BRB. The above procedure described under "*Construction of calibration graphs*" was then applied. The percentage recoveries were calculated by referring to the calibration graphs, or using the corresponding regression equations.

Procedure for spiked human plasma

Aliquots of human plasma (200 µL) spiked with increasing concentrations of the drugs were transferred into series of 4-mL vials. After dilution to 2 mL with BRB, extraction treatment was carried out on a C18 SPE cartridge (Extra-Sep™, Fisher Scientific). The cartridges were eluted with 2 mL methanol: BRB (50% v/v) and the eluates were collected in a new 4-mL vials. A blank experiment was carried out simultaneously. The above procedure described under "*Construction of calibration graphs*" was then applied. The percentage recoveries were calculated by referring to the calibration graphs, or using the corresponding regression equations.

All solutions were filtered through a 0.2 µm membrane filter and degassed for 5 min before injection to the CE system.

Results and Discussion

The proposed method permitted the separation of the two drugs in a reasonable time less than 8 min. Figure 2 shows a typical electropherogram for a synthetic mixture of the two drugs under the described elecctrophoretic conditions. The retention times for MF and SG were 4.85 and 7.30 min, respectively.

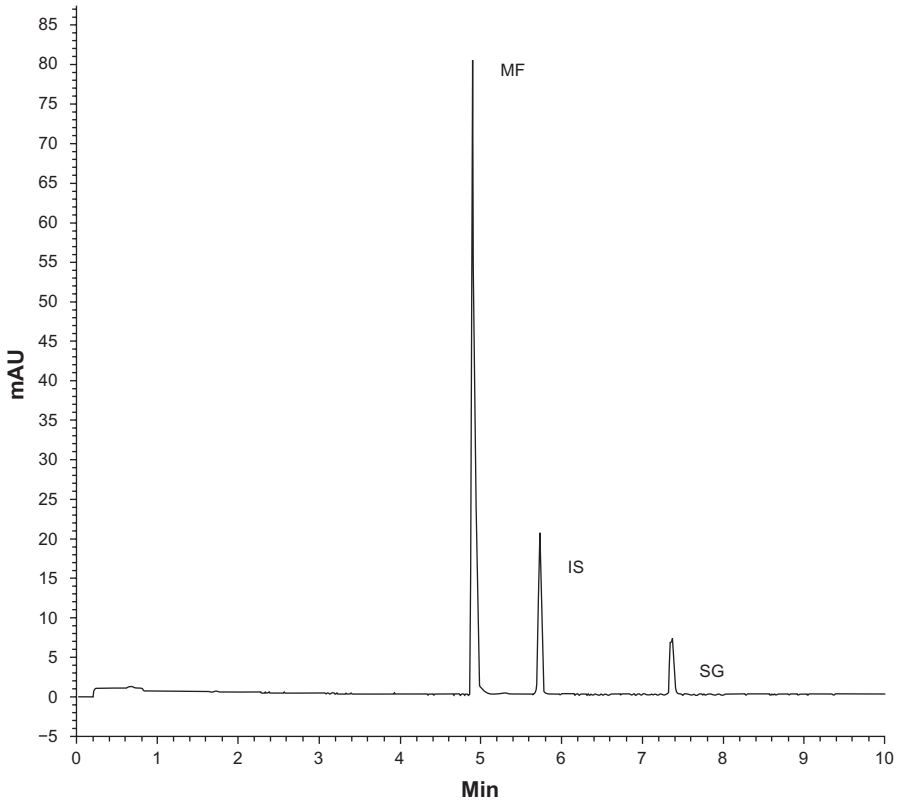

Figure 2. Typical electropherogram for a synthetic mixture; MF (311.3 µg/mL), IS (40 µg/mL) and SG (40 µg/mL) under the described elecctrophoretic conditions.

The proposed method offers good sensitivity as about 2.11 µg/mL of MF and 0.49 µg/mL of SG could be detected accurately. It also permitted the quantification of the drugs in their co-formulated tablets and in human plasma.

Optimization of CE conditions

In CZE the buffer type, pH and concentration is a key strategy for optimizing the separation of ionizable analytes as it determines the degree of the analyte ionization, its electrophoretic mobility and the magnitude of the electroosmotic flow (EOF). As SG has pK_a value of 7.7[27] and MF has pK_a value 11.5,[33] they are positively charged under acidic conditions and can be analyzed by CZE. Several running buffers such as phosphate, acetate and borate at different pHs and molarities were tested for CZE analysis and the best results were obtained in phosphate buffer taking into consideration of different parameters (migration time, resolution, peak shape, height, baseline noise and the electric current produced).

The effect of phosphate buffer pH

The effect of the buffer pH was investigated within the range of 3.0–6.0 at a 60 mM phosphate buffer concentration. The results demonstrated that SG was easily separated from MF in this pH range and both resolution and migration times decreased with increasing pH. At pH 6 a non-ionizable SG peak was appeared as its pK_a value is 7.7. Taking into consideration resolution, peak symmetry and migration time, pH 4.0 was selected as optimum pH (Fig. 3).

The effect of phosphate buffer concentration

Buffer concentration has also a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. The effect of phosphate concentration of running buffer was examined by varying the concentration from 10 to 100 mM. As shown in Figure 4, with an increasing in phosphate concentration, both resolution and migration times increased. A 60 mM concentration of phosphate buffer was chosen in order to reduce the analysis time while maintaining good resolution (≈14) and acceptable current (≈30 µA).

The effect of organic modifiers

The addition of organic modifiers to the running buffer was considered because they affect several parameters such as viscosity, dielectric constant and zeta potential. In order to investigate the effect of organic modifiers, methanol, ethanol and acetonitrile were added at various concentrations (5, 10 and 15% v/v) to the running buffer of 60 mM phosphate buffer pH 4.0. No appreciable improvements were observed, but migration times increased significantly with the addition of organic modifiers. Therefore, no organic modifiers were added to the running buffer in the proposed analysis method.

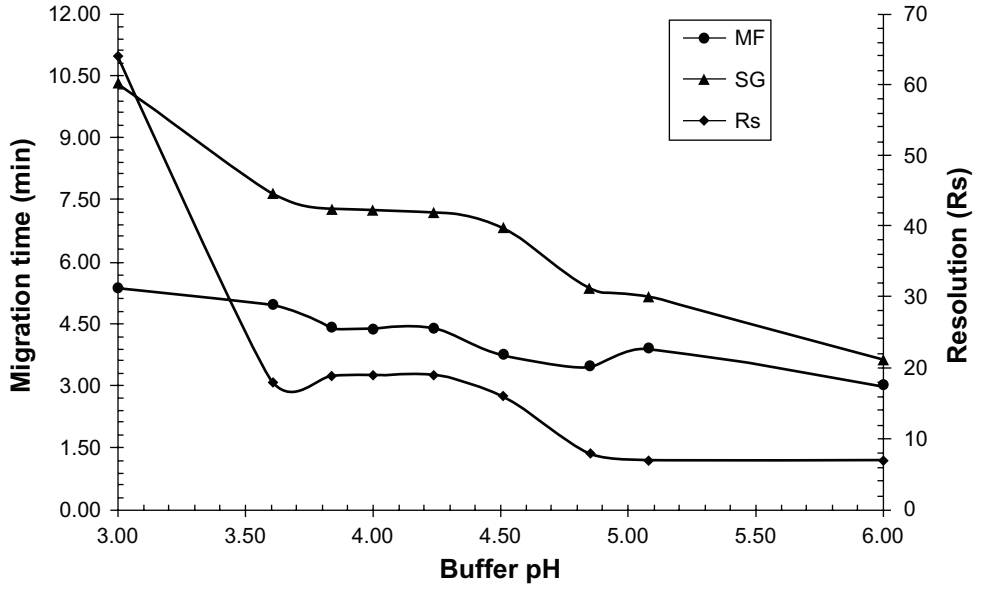

Figure 3. Effect of buffer pH on migration times and resolution.
Notes: Operating conditions: 60 mM phosphate buffer, injection (3 s), 15 kV, 25 °C, 203 nm (bandwidth 10 nm). (MF and SG: 0.1 g/mL).

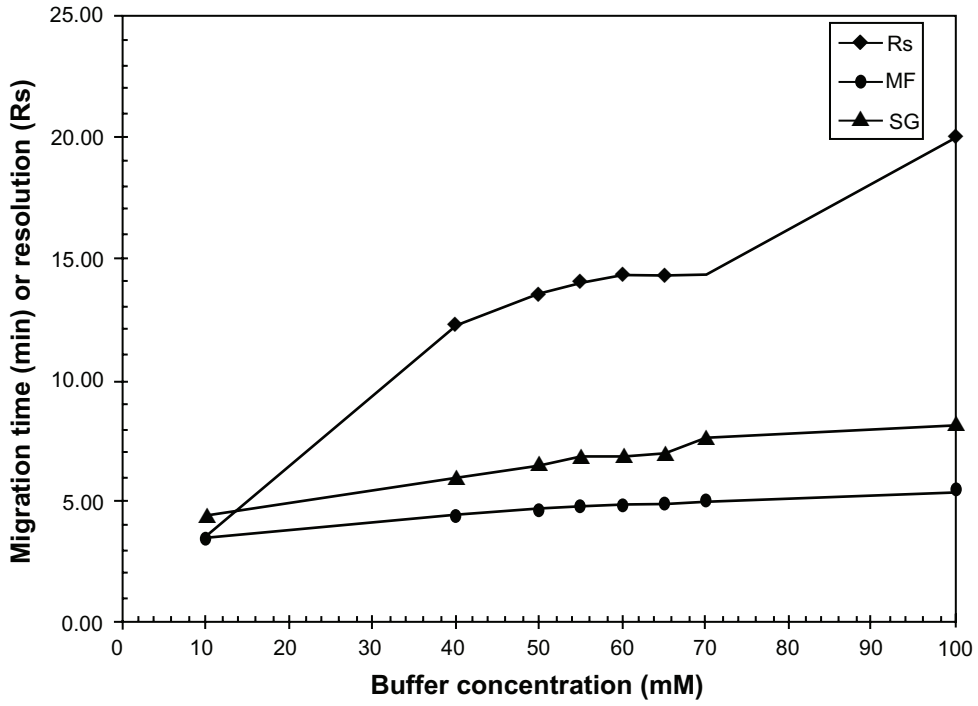

Figure 4. Effect of buffer conc. on migration times and resolution.
Notes: Operating conditions: pH 4.0 phosphate buffer, injection (3 s), 15 kV, 25 °C, 203 nm (bandwidth 10 nm). (MF and SG: 0.1 g/mL).

The effect of applied voltage

The applied voltage effect was investigated under the optimized conditions selected above from 5 to 30 kV. As expected, increasing the applied voltage increases the EOF, leading to shorter analysis time, better separation and higher efficiencies. However, lowering the applied voltage than 10 kV decreases the EOF so the peaks of MF and SG were not resolved and increasing applied voltage exhibits higher currents and produces Joule's heating. To limit this heating inside the capillary, the maximum applied voltage was chosen from an Ohm's plot (current versus voltage). The selected voltage was 15 kV (current (≈30 µA)).

The effect of injection time

Injection time affects the peak width and peak height. Sample solutions were hydrodynamically injected at 0.5 psi while the injection time was varied from 1.0 to 7.0 s. After 6.0 s, the peak widths of SG and MF were increased and the peak shapes were deformed, so 3.0 s was selected as the optimum injection time.

The effect of capillary cartridge temperature

Controlling the temperature of the capillary cartridge is important in capillary electrophoresis. The viscosity of the running buffer is dependent on capillary temperature, so changes in temperature cause changes in EOF, electrophoretic mobilities and injection volume. The influence of the temperature on analysis was investigated at 20, 25 and 30 °C. The selected temperature was 25 °C because it provided the best resolution and the generated current was not more than 30 µA.

Selection of internal standard (IS)

The use of an IS is recommended to compensate for injection errors, minor fluctuations effect of the migration time and improve the quantitative analysis. Then phenformin hydrochloride was chosen as it possesses pK_a value 11.8[33] and its molecular weight more than MF and less than SG so it will be positively charged under the optimized condition and as expected it eluted after MF and before SG.

Selection of the detection wavelength

In order to optimize sensitivity and detection limit of the method, a multiwavelength detection system (190–300 nm) was used in the CE system. For the simultaneous determination of SG and MF the maximum detection sensitivities (the best signal to-noise ratios) were obtained at 203 nm (bandwidth 10 nm). Figure 5 and Table 1 show the effect of detection wavelength on the proposed CZE method.

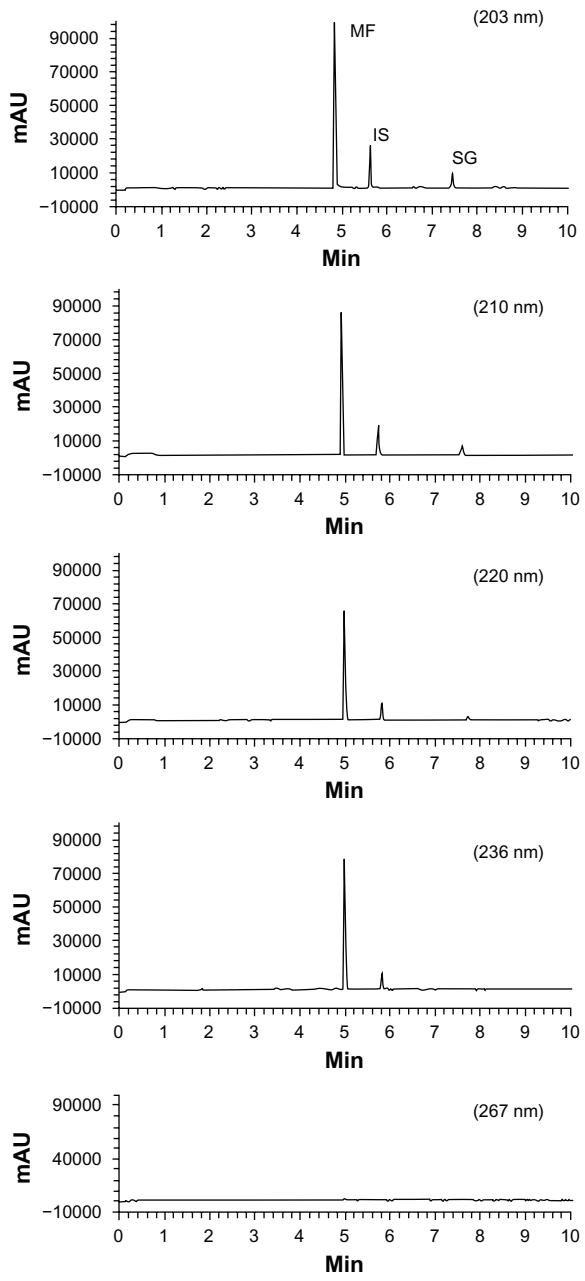

Figure 5. Selection of the detection wavelength.
Notes: Operating conditions: 60 mM phosphate buffer, pH 4.0, injection (3 s), 15 kV, 25 °C. (MF: 311.3 µg/mL, IS: 40 µg/mL, SG: 40 µg/mL).

Validation of the method

Validation of the proposed CZE method was performed with respect to stability, linearity and range, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision and robustness according to the ICH Guidelines.[34]

Linearity

A linear relationship was established by plotting the peak area ratio (the studied drug peak area/IS peak area) against the drug concentration as shown in Figure 6. The concentration ranges were found to be in linear in the range of 10–100 µg/mL and 50–500 µg/mL for SG and MF, respectively. Linear regression analysis of the data gave the following equations:

$$P = 0.0121\ C - 0.0597 \qquad (r = 0.9999) \text{ for SG}$$

$$P = 0.0149\ C - 0.0703 \qquad (r = 0.9999) \text{ for MF}$$

where P is the peak area ratio, C is the concentration of the drug in µg/mL and r is the correlation coefficient.

Statistical analysis of the data gave high value of the correlation coefficient (r) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small value of the percentage relative standard deviation and the percentage relative error (Table 2). These data proved the linearity of the calibration graph.

Limit of quantitation (LOQ) and limit of detection (LOD)

LOQ and LOD were calculated according to ICH Q2 (R1) recommendations using the following equation:[34]

$$LOQ = 10\ S_a/b \text{ and } LOD = 3.3\ S_a/b$$

where S_a = standard deviation of the intercept and b = slope of the calibration curve.

LOQ values were found to be 1.48, 6.39 µg/mL while LOD values were found to be 0.49, 2.11 µg/mL for SG and MF, respectively.

Accuracy and precision

To prove the accuracy and utility of the proposed method, the results of the assay of SG and MF were compared with those of the reference method.[24] Statistical analysis of the results using Student's *t*-test and variance ratio F-test[35] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 3).

The reference method depends on the analysis of SG—MF binary mixture by derivative spectrophotometry. Where, SG and MF were assayed by first derivative zero crossing technique.[24]

Table 1. Effect of detection wavelength on SG, MF and IS peak area: (311.3 μg/mL MF, 40 μg/mL IS and 40 μg/mL SG).

Wavelength (nm)	Peak(s) area			% decrease in peak area (compared to 203 nm)		
	MF	IS	SG	MF	IS	SG
203	263498	61208	25475	0.00	0.00	0.00
210	208144	40353	18657	21.00	34.07	26.76
220	174725	26576	7199	33.69	56.58	71.74
236	182427	23085	--------*	30.76	62.28	100.00
267	3426	---------*	---------*	98.69	100.00	100.00

Note: *Not detected.

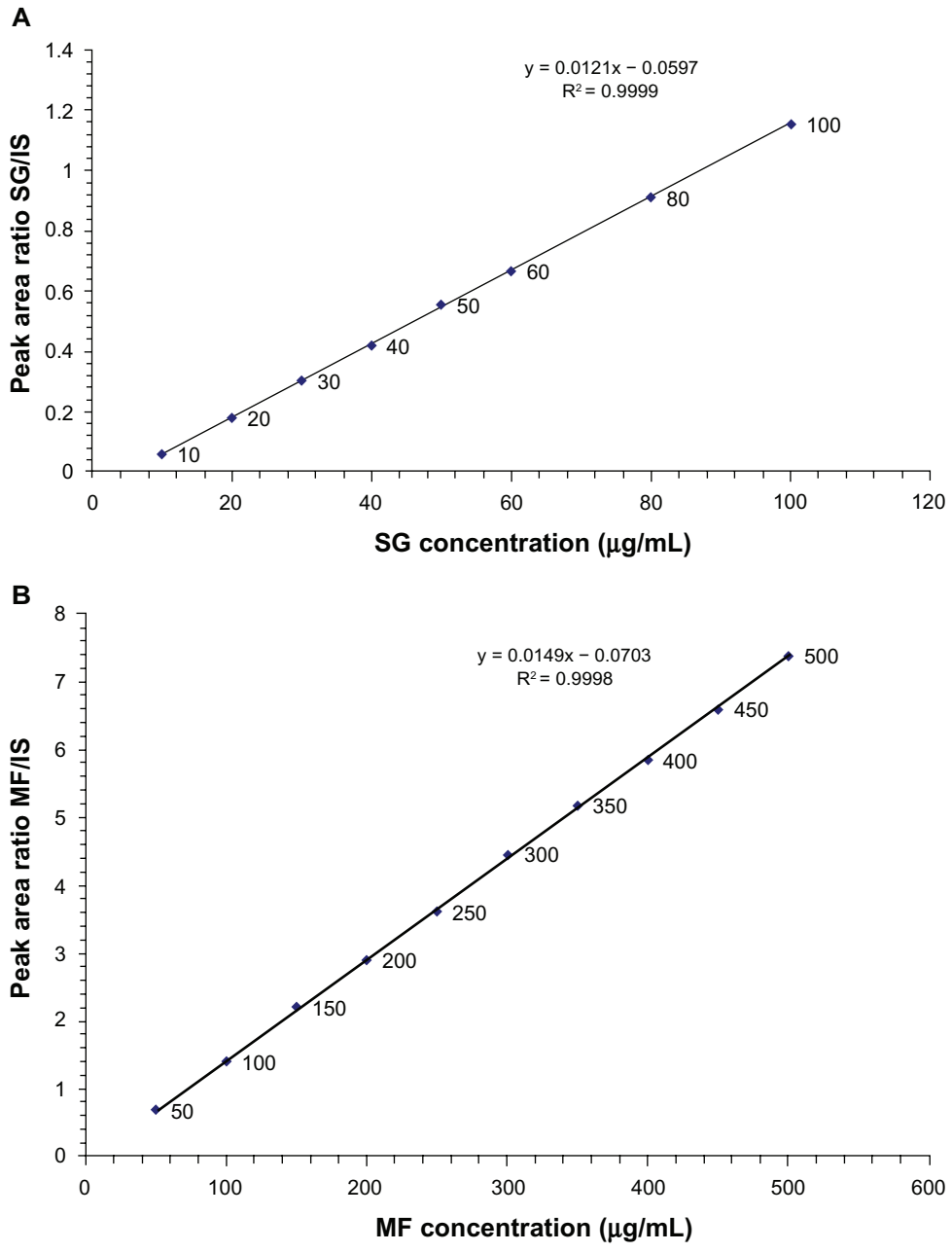

Figure 6. Calibration curve for the determination of (**A**) SG, (**B**) MF by the proposed CZE method under the described elecctrophoretic conditions.

Table 2. Performance data for the determination of the studied drugs by the proposed CZE method.

Parameter	SG	MF
Linearity range (μg/mL)	10–100	50–500
Intercept (*a*)	–0.0597	–0.0703
Slope (*b*)	0.0121	0.0149
Correlation coefficient (*r*)	0.9999	0.9999
SD of residuals ($S_{y/x}$)	0.0042	0.0287
SD of intercept (S_a)	0.0018	0.0095
SD of slope (S_b)	0.0001	0.0001
Percentage relative standard deviation, % RSD	0.769	0.960
Percentage relative error, % error	0.273	0.303
Limit of detection, LOD (μg/mL)	0.49	2.11
Limit of quantitation, LOQ (μg/mL)	1.48	6.39

Repeatability (intraday) and intermediate precision (interday) were assessed using three concentrations and three replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed method (Table 4).

Stability

Stability of the standard solutions of SG and MF, stored at 2 °C, were evaluated at various time points over 3 months. The concentrations of freshly prepared solutions and those aged for 3 months were calculated by the method developed and the difference between them was found to be less than 0.50%. These solutions can therefore be used during this interval of time without the results being affected.

Robustness of the method

The robustness of the proposed method was evaluated by the constancy of the peak area ratio with the deliberated changes in the experimental parameters; these parameters include (phosphate buffer pH 4.0 ± 0.2, Phosphate buffer concentration 60 ± 5 mM injection time 3 ± 1 s, applied voltage 15 ± 2 kV and capillary cartridge temperature 25 ± 2 °C). Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. These minor changes didn't greatly

Table 3. Assay results for the determination of the studied drugs in pure form by the CZE and reference methods.

Compound	Proposed method			Reference method[24]
	Amount taken (μg/mL)	Amount found (μg/mL)	% found	% found
SG	10.0	10.08	100.80	101.82
	20.0	19.93	99.66	100.89
	30.0	30.07	100.24	99.78
	40.0	39.69	99.23	99.15
	50.0	50.91	101.81	101.55
	60.0	60.05	100.09	
	80.0	80.12	100.15	
	100.0	100.20	100.20	
Mean ± SD			100.27 ± 0.77	100.64 ± 1.16
t			0.69 (1.79)	
F			2.21 (4.12)	
MF	50.0	49.71	99.43	100.00
	100.0	98.42	98.42	100.25
	150.0	151.89	101.26	100.33
	200.0	198.99	99.50	99.13
	250.0	246.55	98.62	100.40
	300.0	303.45	101.15	
	350.0	351.28	100.37	
	400.0	397.13	99.28	
	450.0	447.25	99.39	
	500.0	500.08	100.02	
Mean ± SD			99.74 ± 0.96	100.02 ± 0.52
t			0.59 (1.77)	
F			3.38 (5.99)	

Notes: Each result is the average of three separate determinations. The values between parentheses are the tabulated t and F values at $P = 0.05$.[35]

Table 4. Accuracy and precision data for the determination of SG and MF by the proposed CZE method.

Parameters	SG concentration (μg/mL)			MF concentration (μg/mL)		
	10.0	20.0	30.0	155.6	311.3	466.9
Intraday						
% found	101.02	99.39	100.23	99.02	99.80	100.33
	100.36	98.97	99.56	98.94	100.22	99.75
	99.86	100.26	99.91	99.98	99.35	101.2
($\bar{x}$)	100.41	99.54	99.90	99.31	99.79	100.43
±SD	0.58	0.66	0.33	0.57	0.44	0.73
% RSD	0.58	0.66	0.34	0.58	0.44	0.73
% error	0.34	0.38	0.19	0.34	0.25	0.42
Interday						
% found	98.36	101.06	98.19	99.54	98.61	101.60
	99.02	100.98	99.86	100.13	99.09	100.11
	100.67	99.35	100.29	98.70	100.06	98.90
($\bar{x}$)	99.35	100.46	99.45	99.46	99.25	100.20
±SD	1.19	0.97	1.11	0.72	0.74	1.35
% RSD	1.20	0.96	1.12	0.72	0.75	1.35
% error	0.69	0.56	0.64	0.42	0.43	0.78

Note: Each result is the average of three separate determinations.

affect the peak area ratio and migration times of the studied drugs (Table 5).

Specificity

The specificity of the method was investigated by observing any interference encountered from common tablet excipients and it was confirmed that the signals measured was caused only by the analytes. Each film-coated tablet of Janumet® contains the following inactive ingredients: microcrystalline cellulose, polyvinylpyrrolidone, sodium lauryl sulfate, and sodium stearyl fumarate. In addition, the film coating contains the following inactive ingredients: polyvinyl alcohol, polyethylene glycol, talc, titanium dioxide, red iron oxide, and black iron oxide. It was found that these compounds did not interfere with the results of the proposed method. As shown in Figures 7 and 8 the tablets and plasma electropherograms did not show any additional peaks when compared to SG/MF synthetic mixture electropherograms which confirm the specificity of the method.

Table 5. Robustness data (40 μg/mL SG, 311.3 μg/mL MF and 40 μg/mL IS).

Parameters	Migration times (min)		Peak area ratios	
	SG	MF	SG	MF
Standard	7.30	4.85	0.38	4.07
Buffer pH				
3.84	7.35	4.79	0.38	4.07
4.24	7.20	4.80	0.38	4.07
Buffer concocentration (mM)				
55	7.25	4.75	0.37	4.06
65	7.35	4.85	0.38	4.07
Injection time (s)				
2	7.25	4.78	0.36	3.97
4	7.27	4.83	0.38	4.07
Applied voltage (kV)				
13	7.55	5.15	0.38	4.15
17	7.05	4.65	0.40	4.05
Capillary temp. (°C)				
23	7.45	4.90	0.38	4.10
27	7.30	4.80	0.37	4.11

Note: Each result is the average of three separate determinations.

Applications

Analysis of SG/MF in synthetic mixtures and co-formulated tablets

The proposed CZE method was applied to the simultaneous determination of SG and MF in synthetic mixtures in the medicinally recommended ratio of 1:10 and 1:20. Furthermore, the proposed method was successfully applied to their determination in co-formulated tablets (Fig. 7). The results shown in Tables 6 and 7 are in good agreement with those obtained using the reference method.[24] Statistical analysis of the results

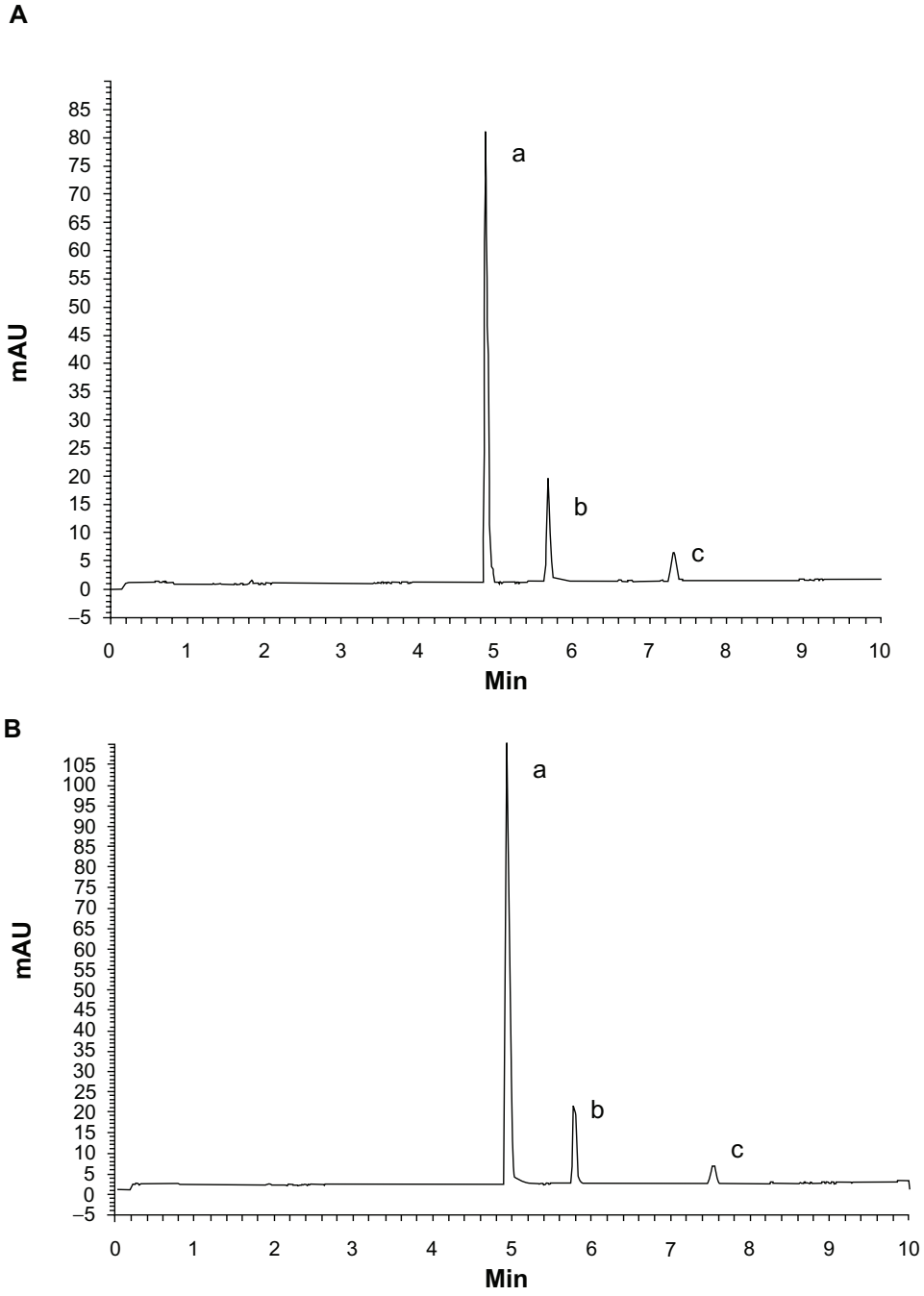

Figure 7. Application of the proposed method for the simultaneous determination of SG and MF in co-formulated tablets. (**A**) Janumet® 50–500 tab. (a) MF: 311.3 µg/mL (b) IS: 40 µg/mL (c) SG: 40 µg/mL. (**B**) Janumet® 50–1000 tab. (a) MF: 466.9 µg/mL (b) IS: 40 µg/mL (c) SG: 40 µg/mL.

obtained using Student's *t*-test and variance ratio F-test[35] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively.

Biological application

MF is slowly absorbed after oral administration. The reported peak plasma concentrations of 0.59 to 1.30 mg/L and 1.80 to 4.0 mg/L were attained in about 2 hours following oral dose of 50 mg and 150 mg, respectively.[33] SG is rapidly absorbed from gastrointestinal tract with absolute oral bioavailability of about 87%. The maximum plasma concentration of SG was reported 950 nM (0.497 mg/L) following a single oral dose of 100 mg.[36] The electropherograms for human plasma

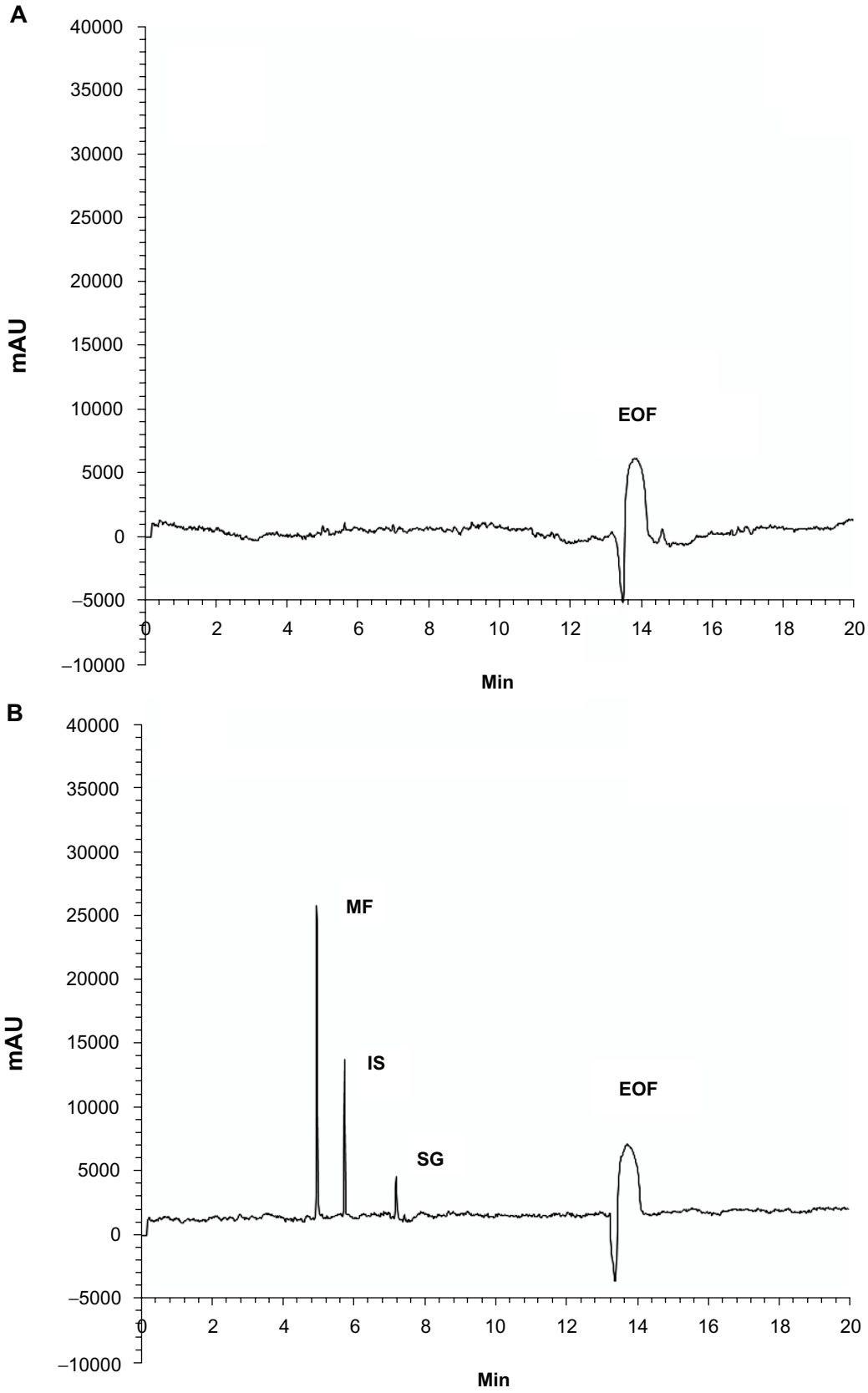

Figure 8. Application of the proposed method for the simultaneous determination of SG and MF in spiked human plasma performed in long time (20 min) to show that there is no additional peak observed for the plasma protein. (**A**) Blank plasma sample. (**B**) Plasma sample spiked with 50 μg/mL MF, 10 μg/mL IS and 10 μg/mL SG.

Table 6. Assay results for the determination of the studied drugs in their synthetic mixture and reference methods.

Prepared ratio	Amount taken (µg/mL)		% found[a]			
	SG	MF	Proposed CZE method		Reference method[24]	
			SG	MF	SG	MF
1:10	10.0	77.8	100.24	100.02	101.82	100.00
	20.0	155.7	98.76	100.01	100.89	100.25
	30.0	233.5	99.24	99.69	99.78	100.33
	40.0	311.3	100.37	100.10	99.15	99.13
	50.0	389.1	99.92	101.06	101.55	100.40
($\bar{x}$)			99.71	100.18	100.64	100.02
±SD			±0.69	±0.52	±1.16	±0.52
Student's *t*-test			1.56 (2.31)[b]	0.47 (2.31)		
F-test			2.78 (6.39)	1.01 (6.39)		
1:20	10.0	155.6	101.19	100.51	101.82	100.00
	20.0	311.3	101.50	99.69	100.89	100.25
	30.0	466.9	100.23	99.86	99.78	100.33
					99.15	99.13
					101.55	100.40
($\bar{x}$)			100.97	100.02	100.64	100.02
±SD			±0.66	±0.43	±1.16	±0.52
Student's *t*-test			0.45 (2.45)	0.01 (2.45)		
F-test			2.99 (19.25)	1.45 (19.25)		

Notes: [a]The average of three separate determinations; [b]the figures between parentheses are the tabulated values of *t* and F at $P = 0.05$.[35]

Table 7. Assay results for the determination of the studied drugs in their co-formulated tablets.

Preparation	Amount taken (µg/mL)		Amount found[a] (µg/mL)		% found		Reference method[24]	
	SG	MF	SG	MF	SG	MF	SG	MF
1-Janumet® 50–500 tablets	50.0	389.1	49.89	388.24	99.78	99.78	97.59	98.33
	40.0	311.3	39.69	309.46	99.22	99.41	99.13	99.87
	30.0	233.5	29.62	232.31	98.74	99.49	99.41	99.10
	20.0	155.7	19.93	155.75	99.65	100.03		
	10.0	77.8	9.85	77.89	98.53	100.11		
($\bar{x}$)					99.18	99.76	98.71	99.10
±SD					±0.55	±0.31	±0.98	±0.77
% RSD					0.55	0.31	0.99	0.77
% error					0.25	0.14	0.58	0.45
t					0.90 (2.45)[b]	1.76 (2.45)		
F					3.22 (6.94)	6.10 (6.94)		
2-Janumet® 50–1000 tablets	30.0	466.9	29.92	464.94	99.72	99.58	100.58	100.17
	20.0	311.3	20.28	308.78	101.42	99.19	99.56	100.13
	10.0	155.6	10.23	156.24	102.34	100.41	99.65	101.00
($\bar{x}$)					101.16	99.73	99.94	100.43
±SD					±1.32	±0.62	±0.57	±0.49
% RSD					1.31	0.63	0.57	0.49
% error					0.76	0.36	0.33	0.28
t					1.46 (2.78)	1.54 (2.78)		
F					1.37 (19.00)	1.60 (19.00)		

Notes: [a]The average of three separate determinations; [b]the figures between parentheses are the tabulated values of t and F at $P = 0.05$.[35]

Table 8. Assay results for the determination of the studied drugs in spiked human plasma using the proposed method.

Compound	Amount taken (µg/mL)	Amount found (µg/mL)	% found
SG	10.0	10.09	100.93
	20.0	19.71	98.53
	30.0	29.99	99.98
($\bar{x}$)			99.81
±SD			±1.21
% RSD			1.21
% error			0.70
MF	50.0	49.26	98.51
	60.0	58.92	98.2
	70.0	70.64	100.91
($\bar{x}$)			99.21
±SD			±1.48
% RSD			1.49
% error			0.86

of blank and that spiked with the studied drugs are shown in Figure 8. The blank plasma exhibited no peaks in the electropherogram. The results are abridged in Table 8.

Conclusion

A new, simple, accurate and sensitive CZE method was explored for the simultaneous determination of SG and MF in binary mixture. The developed method has distinct advantages regarding analysis time, sensitivity and cost compared with those of the previously reported methods. Limits of detection of 0.49, 2.11 µg/mL and limits of quantitation of 1.48, 6.39 µg/mL for SG and MF, respectively were obtained. It could be applied to the analysis of the drugs in their co-formulated tablets. It also offers the possibility to determine the studied drugs in in human plasma. It seems to be very promising for the therapeutic drug monitoring of patients undergoing treatment with the studied drugs.

Author Contributions

Conceived and designed the experiments: GP, MW, FB, NE, MS. Analysed the data: GP, MS. Wrote the first draft of the manuscript: MS. Contributed to the writing of the manuscript: GP. Agree with manuscript results and conclusions: GP, MW, FB, NE. Jointly developed the structure and arguments for the paper: GP, MS. Made critical revisions and approved final version: GP, MS. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

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References

1. Barnard K, Cox ME, Green JB. Clinical utility of fixed combinations of sitagliptin—metformin in treatment of type 2 diabetes. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. 2010;3:363–72.
2. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, Fourteenth Edition, O'Neil MJ, Heckelman PE, Koch CB, Roman KJ, editors, Merck & Co., Inc., Whitehouse Station, NJ, USA, 2012.
3. Gallwitz B. Sitagliptin: Profile of a novel DPP-4 inhibitor for the treatment of type 2 diabetes. *Drugs Today*. 2007;43(1):13–25.
4. Goodarzi MO, Bryer-Ash M. Metformin revisited: Re-evaluation of its properties and role in the pharmacopoeia of modern antidiabetic agents. *Diabetes Obes Metab*. 2005;7:654–65.
5. The British Pharmacopoeia 2009, The Stationery Office: London; Electronic version; pp. 3813–6.
6. The United States Pharmacopoeia 30, the National Formulary 25, US Pharmacopeial Convention: Rockville, MD, 2007; Electronic version.
7. Pritam J, Amar C, Bhargav D, Shani P, Santsaran P, Hiren S. Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in formulation. *Int J Drug Dev and Res*. 2011;3(4):194–9.
8. Pathade P, Imran M, Bairagi V, Ahire Y. Development and validation of stability indicating UV spectrophotometric method for the estimation of sitagliptin phosphate in bulk and tablet dosage form. *Journal of Pharmacy Research*. 2011;4(3):871–3.

9. Hess C, Musshoff F, Madea B. Simultaneous identification and validated quantification of 11 oral hypoglycaemic drugs in plasma by electrospray ionisation liquid chromatography—mass spectrometry. *Anal Bioanal Chem*. 2011;400(1):33–41.
10. Zeng W, Xu Y, Constanzer M, Woolf EJ. Determination of sitagliptin in human plasma using protein precipitation and tandem mass spectrometry. *J Chromatogr B*. 2010;878(21):1817–23.
11. Jiu X-F, Shang D, Chen Y, et al. A high performance liquid chromatography method for the quantitative determination assay of sitagliptin in rat plasma and its application in pharmacokinetics study. *Journal of Chinese Pharmaceutical Sciences*. 2011;20:63–9.
12. Lange ADC, Gasperin FT, Barth AB, Todeschini V, Volpato NM, Schapoval EES. Application of factorial design and study of photodegradation kinetics for the determination of sitagliptin by capillary electrophoresis. *Curr Anal Chem*. 2012;8(1):116–23.
13. Bodar JD, Kumar S, Yadav YC, et al. Development of the spectrophotometric method for the simultaneous estimation of piogliazone and metformin. *International Journal of Pharmaceutical Sciences*. 2011;2(3):236–43.
14. Doredla NR, Shanmugasundaram P, vaishnav H. Method development and validated of simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide in pure and tablet dosage form by spectrophotometric multi component method. *Int J Chem Tech Res*. 2011;3(4):2011–7.
15. AbuRuz S, Millership J, McElnay J. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. *J Chromatogr B*. 2005;817(2):277–86.
16. AbuRuz S, Millership J, McElnay J. Determination of metformin in plasma using a new ion pair solid phase extraction technique and ion pair liquid chromatography. *J Chromatogr B*. 2003;798(2):203–9.
17. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Rapid determination of metformin in human plasma using ion-pair HPLC. *J Pharm Biomed Anal*. 2003;31(1):197–200.
18. Koseki N, Kawashita H, Niina M, Nagae Y, Masuda N. Development and validation for high selective quantitative determination of metformin in human plasma by cation exchanging with normal-phase LC/MS/MS. *J Pharm Biomed Anal*. 2005;36(5):1063–72.
19. Wang Y, Tang Y, Gu J, Fawcett JP, Bai X. Rapid and sensitive liquid chromatography—tandem mass spectrometric method for the quantitation of metformin in human plasma. *J Chromatogr B*. 2004;808(2):215–9.
20. Chen X, Gu Q, Qiu F, Hong D. Rapid determination of metformin in human plasma by liquid chromatography–tandem mass spectrometry method. *J Chromatogr B*. 2004;802(2):377–81.
21. Hamdana II, Bani Jaber AK, Abushoffa AM. Development and validation of a stability indicating capillary electrophoresis method for the determination of metformin hydrochloride in tablets. *J Pharm Biomed Anal*. 2010;53:1254–7.
22. Wei S-Y, Yeh H-H, Liao F-F, Chen S-H. CE with direct sample injection for the determination of metformin in plasma for type 2 diabetic mellitus: An adequate alternative to HPLC. *J Sep Sci*. 2009;32:413–21.
23. Zhai H, Wu Y, Huang B, Huang Q, Yang Bi, Chen Z. Rapid determination of metformin hydrochloride in metformin hydrochloride tablets by capillary electrophoresis. *Huaxue Yanjiu Yu Yingyong*. 2008;20(7):923–926. [Through SciFinder® Scholar (Chemical Abstracts): April 23, 2012].
24. El-Bagary RI, Elkady EF, Ayoub BM. Spectroflourometric and spectrophotometric methods for the determination of sitagliptin in binary mixture with metformin and ternary mixture with metformin and sitagliptin alkaline degradation product. *Int J Biomed Sci*. 2011;7(1):62–9.
25. Khan G, Sahu D, Agrawal YP, Sabarwal N, Jain A, Gupta AK. Simultaneous estimation of metformin and sitagliptin in tablet dosage form. *Asian Journal of Biochemical and Pharmaceutical Research*. 2011;1(2):352–8.
26. Martín J, Buchberger W, Santos JL, Alonso E, Aparicio I. High-performance liquid chromatography quadrupole time-of-flight mass spectrometry method for the analysis of antidiabetic drugs in aqueous environmental samples. *J Chromatogr B*. 2012;895–6:94–101.
27. Chellu SN, Malleswararao, Mulukutla V, Suryanarayana, Mukkanti K. Simultaneous determination of sitagliptin phosphate monohydrate and metformin hydrochloride in tablets by a validated UPLC method. *Sci Pharm*. 2012;80:139–52.
28. El-Bagary RI, Elkady EF, Ayoub BM. Liquid chromatographic determination of sitagliptin either alone or in ternary mixture with metformin and sitagliptin degradation product. *Talanta*. 2011;85:673–80.
29. Hendrickx A, Debbymangelings, Heyden YV. Capillary methods for drug analysis. *J AOAC Int*. 2011;94(3):667–702.
30. Altria KD, editor. *Quantitative Analysis of Pharmaceuticals by Capillary Electrophoresis*. View eg, Wiesbaden; 1998:1–285.
31. Suntornsuk L. Recent advances of capillary electrophoresis in pharmaceutical analysis. *Anal Bioanal Chem*. 2010;398:29–52.
32. Thanh Ha PT, Hoogmartens J, Schepdael AV. Recent advances in pharmaceutical applications of chiral capillary electrophoresis. *J Pharm Biomed Anal*. 2006;41:1–11.
33. Moffat AC. Clarke's Analysis of Drugs and Poisons, The Pharmaceutical Press: London, 2006; Electronic version.
34. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Current Step 4 Version, Parent Guidelines on Methodology Dated Nov 6, 1996, Incorporated in Nov 2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed Apr 23, 2012.
35. Miller JN, Miller JC. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed, Pearson Education Limited, Harlow, England, 2005, p. 256.
36. http://www.globalrph.com/DPP-4-inhibitors.htm. Accessed May 1, 2012.