Quantitative Determination of Metformin by Capillary Electrophoresis with UV Detection

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Capillary electrophoresis with UV detection is a simple modern method which can be used for the quantitative determination of many drugs. This study aims to develop and validate a method for the quantitative determination of metformin by capillary electrophoresis with UV detection. Electrophoretic separation was performed at pH=4.0, voltage +15.0 kV, after sample injection for 3 seconds at a pressure of 0.5 psi. UV detection was done at 200 nm. The following validation parameters were determined: linearity (r = 0.9999), limit of detection (LD) = 13.933 μg/mL, limit of quantification (LQ) = 42.223 μg/mL, precision (RSD = 3.83%) and accuracy (average recovery 100.50%). The validation results will allow the use of the method for metformin assay in biological fluids.

Keywords: metformin, capillary electrophoresis, validation

In type 2 diabetes mellitus, defined as tissue resistance to insulin action combined with a relative deficiency of insulin secretion, a wide range of oral drugs are used. Metformin (N,N-dimethylimidodicarbonimidic diamides hydrochloride (figure 1) is an oral antidiabetic drug in the biguanide class recommended as the treatment of choice in type 2 diabetes. Its main advantage over sulfonylureas is that it does not cause weight gain or hypoglycemia [1].

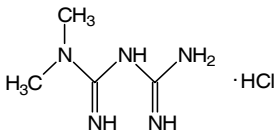

Fig. 1. Chemical structure of metformin hydrochloride

Metformin is currently one of the most widely used antidiabetic drugs [2]. The literature describes the recent the methods for quantitative determination including high performance thin layer chromatography [3], liquid chromatography combined with mass spectrometry (LC-MS) [4, 5], high performance liquid chromatography with UV detection (HPLC-UV) [6], voltametry [7], UV-VIS spectrophotometry [8, 9], capillary electrophoresis with UV detection [10-12].

Capillary electrophoresis is a new separation method that combines the electrophoretic principle with automated chromatography methods. It uses small amounts of sample, is quantitative, automated, and has a wide range of applications, human biological fluids included [13]. To the best of our knowledge, this method for metformin determination is not commonly found.

The purpose of this study was to develop and validate a simple, sensitive, economic method for metformin determination by capillary electrophoresis with UV detection, with applications in its quantitative analysis.

Experimental part

Materials and method

The study was performed by using a Beckman Coulter P/ACE System MDQ with UV detector, temperature control system (4 – 60°C) and a power supply capable of producing 30 kV. The software used was 32 Karat Software, Version 5.0, Build 1021.

Electrophoretic analysis was performed in a bare fused-silica capillary, total length 67 cm, 50 cm effective length, 50 μm ID, 375μm OD (Beckman Coulter Inc., USA) at a voltage of +15.0 kV. The wavelength at which the measurements were carried out was 200 nm, the sample was injected hydrodynamically at 0.5 psi for 3 s, and the system/column compartment temperature was 25°C.

Phosphate buffer was filtered using a 0.22μm membrane filter (Tehnokroma) to a KifLab vacuum pump model N86KT.18.

*p*H was determined by using a pH meter checker A873.1 (Hanna Instruments). The solutions were degassed with a SB-120DT Ultrasonic cleaner.

All reagents used were of analytical grade: metformin hydrochloride (Harman Finochem Ltd., India), sodium hydroxide (Lach-Ner, Czech Republic), sodium dihydrogen phosphate (UCB, Belgium), 85% orthophosphoric acid (Fisher Scientific, UK Kingdom), succinic acid (Austria), sodium acetate (Iasi Chemical Company), glacial acetic acid (Iasi Chemical Company).

Standard solution preparation

Standard solution of metformin hydrochloride was prepared in double distilled water at a concentration of 5000μg/mL.

Phosphate buffer preparation

The phosphate buffer solution was prepared from 60 mM sodium dihydrogenphosphate adjusted to *p*H=4.0 with 85% phosphoric acid. The solution was filtered through a 0.22μm membrane filter.

The prepared solutions were stored in the refrigerator (4°C).

Electrophoretic procedure

Operational parameters: the column was conditioned by washing for 5 min, at 20 psi, with 0.1 M NaOH, double-

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distilled water and phosphate buffer. Sample injection: 3 s, at 0.5 psi. Column and compartment temperature was kept constant at 25°C; the applied voltage was +15 kV.

At the beginning of each working day all solutions were degassed at 20°C for 5 min.

Calibration curve construction

Suitable volumes of the stock solution were diluted with phosphate buffer so that the final concentrations were in the range 25-1000 μg/mL. The obtained peak area values were plotted to obtain a calibration curve and regression equation.

Method validation

The selectivity of the method was assessed by comparing the electropherograms obtained from the analysis of the working solution, metformin hydrochloride, and of a phosphate buffer control solution, respectively.

Based on the slope of the calibration curve the linearity, limit of detection and limit of quantification were established.

The precision of the method precision was determined by verifying the repeatability and intermediate precision. Three injections at 3 different concentrations were administered. To calculate the concentration of each solution the calibration curve equation was used.

The accuracy of the method was evaluated by "recovery" experiments. Three successive injections of metformin solution at three different concentrations were performed. Calibration curve equation was used to calculate the concentration of samples.

Data were statistically analyzed using Microsoft Excel (version 2007 Pro).

Results and discussions

The electrophoretic results depend on the electrophoretic mobility of the analyte, which is determined by the electrical charge and the size of its molecule [14]. Using the experimental conditions described above, the migration time for metformin was of 10.514 min (fig. 2).

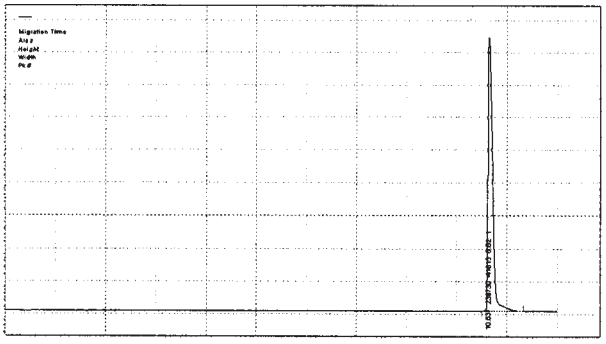

Fig. 2. Electropherogram of metformin 600 μg/mL, under the described electrophoretic conditions

Migration time was constant (RSD = 1.85%) (RSD = relative standard deviation) as shown in figure 3, where we plotted the overlapping electropherograms of metformin at various concentrations.

Acidic substances may be analyzed in their anionic form by capillary electrophoresis at high *p*H, while basic substances can be analyzed in cationic form at low *p*H [14]. Thus, given the basic character of metformin, a buffer solution with a *p*H value within the acidic range is used. To optimize this method, we studied the effect of *p*H value and buffer type used on migration time and peak area of metformin hydrochloride.

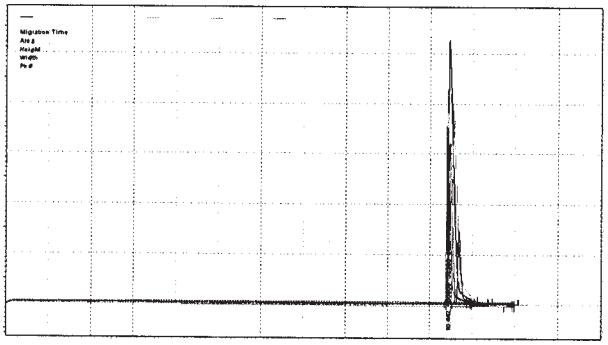

Fig. 3. Electropherograms of metformin (200, 400, 600, 800, 1000 μg/mL) under the described electrophoretic conditions

To assess the influence of the *p*H value of the buffer solution different phosphate buffer solutions were used. These solutions had the same molarity, 60 mM, and *p*H values of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0. Measurements were carried out with the same metformin solution, diluted with the buffer solution at the same concentration, according to the procedure outlined in "Electrophoretic procedure". The results are shown in figure 4. The *p*H value of the buffer solution should provide as many electrically charged molecules as illustrated by the higher value of the peak area. Also, a *p*H value closer to neutral has a protective effect on electrophoresis column and other components of the equipment. *p*H=4.0 was chosen as the optimum working *p*H. Similar results were obtained in other studies on metformin determination by capillary electrophoresis [12].

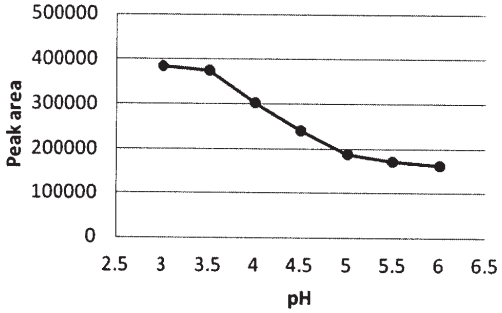

Fig. 4. Influence of buffer *p*H on peak area

To determine the effect of buffer type, phosphate, succinate and acetate buffer solutions were comparatively analyzed. The buffer solutions were prepared at the same concentration (60mM) and *p*H value (*p*H=4.0). Measurements were carried out in the same metformin solution, diluted with the buffer solution at the same concentration, according to the procedure outlined in "Electrophoretic procedure". According to the obtained peak area values (fig. 5), the best is the phosphate buffer solution. Phosphate buffer solution is also accessible, stable, with low risk of interactions with biological samples. Similar results were obtained in other studies on metformin determination by capillary electrophoresis [12, 15].

The literature describes methods for metformin determination by capillary electrophoresis with the use of various *p*H values and buffers. Thus, methods using *p*H=3.0 for phosphate buffer [15], *p*H=5.1 in non-aqueous media [16] and *p*H=6.7 for citrate buffer [10] have been described. Comparing these data with the experimental conditions in our study, the choice of a phosphate buffer *p*H=4.0 was justified by the aqueous medium, economically accessible, less polluting, a *p*H value that ensures effective separation

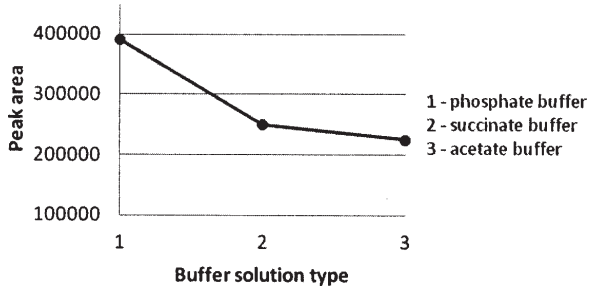

Fig. 5. Influence of buffer type (*p*H=4.0) on peak area

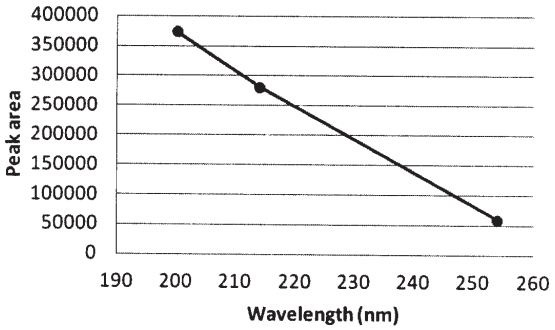

Fig. 6. Influence of wavelength on peak area

Parameter	Value
Linearity range (µg/mL)	25-1000
Limit of detection (LD) (µg/mL)	13.933
Limit of quantification (LQ) (µg/mL)	42.223
Regression equation	y = 403.02 · x + 1003.6 where: y = peak area x = concentration (µg/mL)
Intercept (a)	1003.6
Slope (b)	403.0237
Correlation coefficient (r)	0.9999
Standard error (SE)	1701.711

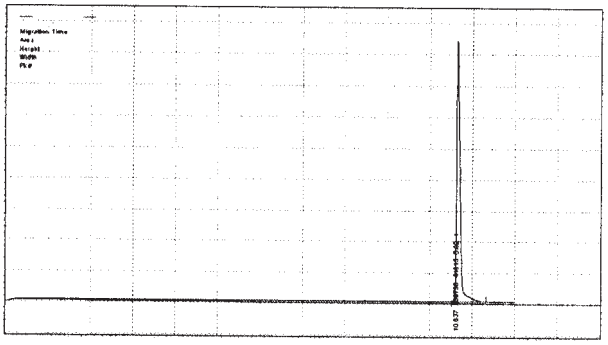

Fig. 7. Electropherograms of metformin 600 µg/mL and phosphate buffer, under the described electrophoretic conditions

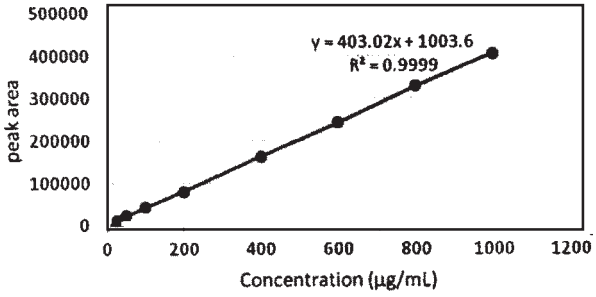

Fig. 8. Calibration curve

Table 1
VALIDATION PARAMETERS

of the analyte but without exposing the column and other system components to an overly acidic environment.

For estimating the optimum wavelength for UV determination of metformin the same metformin solution was analyzed at 200 nm, 214 nm and 254 nm. Measurements were carried out according to the procedure outlined in "Electrophoretic procedure". The results are shown in figure 6. The highest peak area value was at 200 nm. Similar studies used metformin detection wavelength of 240 nm [16], 254 nm [15] or 203 nm [12].

Validation of the capillary electrophoresis method with UV detection

The method was validated according to ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) requirements [17] and other data in the literature [18, 19].

Comparing the electropherograms of metformin and phosphate buffer made under the same conditions, no additional peaks that cause interference in the analysis were found (fig. 7). It results that the method is selective for metformin.

The linearity of the method was demonstrated by peak area analysis related to concentration, as shown in figure 8. The limit of detection (LD) and limits of quantification (LQ) were calculated according to the formulas:

$$LD = 3.3 \times SE / b$$
$$LQ = 10 \times SE / b,$$

where: SE = standard error, b = calibration curve slope.

Validation parameters obtained from the calibration curve are presented in table 1. One advantage of this method was the study of wide linear range (25-1000 µg/mL), unlike other values mentioned in some studies in the literature (50-500 µg/mL) [12].

For determining the precision of the method the relative standard deviations less than 5% (3.83 and 3.39%) were obtained, according to table 2.

The accuracy of the method was characterized by a mean recovery of 100.50%, RSD = 2.47%.

When comparing this method with high performance chromatographic methods, it resulted that capillary electrophoresis has the advantage of lower cost due to the use of more economical aqueous solvents and easier to

Table 2
PRECISION

Metformin concentration (µg/mL)	Method precision		Intermediate precision	
	Peak area	Recovery (%)	Peak area	Recovery (%)
200	84072	103.05	247580	101.96
	86564	106.14	237672	97.87
	85769	105.16	243697	100.36
600	241348	99.39	79590	97.49
	247898	102.10	78128	95.68
	238497	98.21	78037	95.56
1000	396889	98.22	412969	102.21
	388784	96.21769	372199	92.10
	385363	95.36886	388763	96.21
Statistical data	Mean = 100.43% SD* = 3.85 RSD** = 3.83%		Mean = 97.72% SD = 3.31 RSD = 3.39%	

* SD – standard deviation

** RSD – relative standard deviation

Table 3
ACCURACY

Metformin concentration (µg/mL)	Peak area	Calculated concentration (µg/mL)	Recovery (%)
200	82446	202.07	101.03
	83928	205.75	102.87
	82702	202.71	101.35
600	251169	620.72	103.45
	246583	609.34	101.55
	247907	612.62	102.10
1000	389529	964.02	96.40
	395364	978.50	97.85
	395587	979.05	97.90
Statistical data	Mean = 100.50% Min = 96.40% Max = 103.45% SD = 2.49 RSD = 2.47%		

purchase equipment accessories (e.g. separation columns). In addition, the analysis by capillary electrophoresis had similar results with the methods for metformin determination by HPLC-UV.

Conclusions

The present study describes the development of a simple, modern and economic method for the quantitative determination of metformin by capillary electrophoresis with UV detection. The method was validated by determining the following parameters: linearity range 25-1000µg/mL, r = 0.9999, LD = 13.933µg/mL, LQ = 42.223µg/mL, precision (RSD = 3.83%) and accuracy (mean recovery 100.50%). All reagents are stable, inexpensive and available in analytical laboratories. The method is also "environmentally friendly" because it does not use organic solvents and all steps of the experiment were carried out in an aqueous medium. The validated method will be used for the determination of metformin in biological samples and pharmaceutical products. Thus, we aim at establishing the conditions for optimal processing and analysis of biological samples.

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