ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 4, 1 SECTIO DDD 2011
Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University in Lublin, Poland

ANNA BERECKA, ANNA GUMIENICZEK*, ŁUKASZ KOMSTA,
TADEUSZ INGLOT, PATRYCJA MROCZKA

# *Elaboration and validation of a new capillary electrophoresis (CE) method in non-aqueous milieu for the analysis of hypoglycemic drug repaglinide in pharmaceuticals*

Opracowanie i walidacja nowej metody elektroforezy kapilarnej w środowisku niewodnym do oznaczania leku hipoglikemizującego repaglinidu w preparacie farmaceutycznym

## INTRODUCTION

Repaglinide is a short acting insulin secretagogue that acts probably by the sulfonylurea receptor SUR. It is rapidly absorbed and eliminated from the body and was developed in attempts to overcome the adverse effects associated with existing anti-diabetic compounds like sulfonylureas. These include hypoglycemia, secondary failure and cardiovascular side effects [3].

Capillary electrophoresis (CE) is more and more frequently applied as a highly effective analytical procedure. It has many advantages such as lower sampling volume than HPLC and equally high separation efficiency, allowing separation much complex mixtures, e.g. the drugs in combined dosage forms or the drugs and their impurities present in bulk or pharmaceutical formulations. To the best of our knowledge, there is no any previous report about the CE determination of repaglinide. On the other hand, some HPLC methods have been reported for determination of repaglinide in bulk or pharmaceuticals [1,4] and in plasma or serum [5,6,7]. Also, a micellar electrokinetic chromatography was applied for the same purpose [2]. Taking into account a small number of elaborated analytical methods for repaglinide, the aim of the present study was to elaborate a new CE method as alternative tool and to validate it with respect to robustness, linearity, accuracy, precision and recovery. Also, selectivity of our method in respect to known impurities of repaglinide (Fig. 1) described by European Pharmacopoeia [8] was proved. As concerns determination of repaglinide in the presence of its different impurities only two HPLC methods have been proposed so far [4,8].

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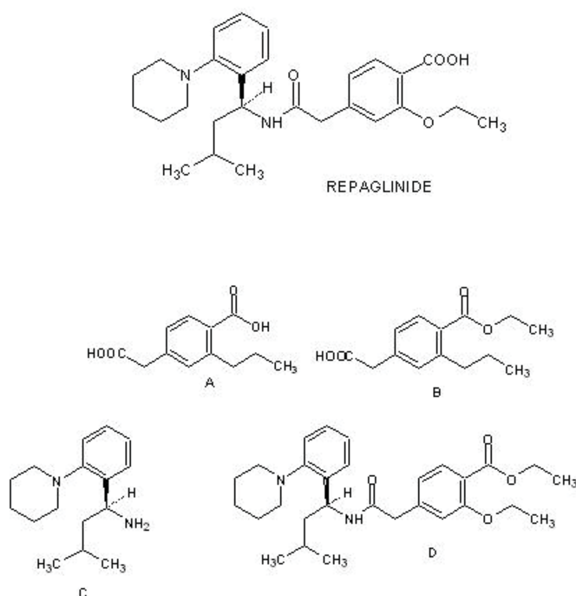


Figure 1. Chemical structures of repaglinide and its impurities A-D [8]

## MATERIAL AND METHODS

Chemicals and apparatus. Repaglinide pure substance and respective NovoNorm® tablets from NovoNordisk (Denmark) were used. Repaglinide for system suitability CSP [8] containing impurities A-D was purchased from LGC Standards (Poland). Gliclazide pure substance from Jelfa S.A. (Poland) was used as internal standard. All other chemicals were of analytical grade and were purchased from E. Merck (Germany). Deionized water was obtained using a deionizer SolPure 7 from Poll Lab (Poland).

Capillary electrophoresis was carried out using a PrinCE CE system (PrinCE Technologies, Netherland) equipped with a UV Lambda 1010 detector. Data acquisition and signal processing were performed using Dax Data Acquisition and Analysis software. A fused silica capillary tubing (Polymicro Technologies, USA) of 75 μm i.d. and 76 cm effective length (total length 90 cm) were used. Sample loading was achieved by hydrodynamic injection (10 mbar, 6 s). The running buffer was 0.01 mol/l solution of ammonium acetate in the mixture methanol-acetonitrile (3:7, v/v). Prior to use, the buffer was filtered through a 0.45 μm nylon membrane filter. Before start up, the capillary was preconditioned with 1 mol/l NaOH, 0.1 mol/l NaOH and deionized water, each for 10 min regular sequence, and finally with a running buffer for 5 min. Between runs, the capillary was rinsed with deionized water and finally with a running buffer, all for 5 min. The capillary was left filled with water between analyses. Our procedure was carried out at 30°C and a potential of 30 kV. The autosampler was also kept at 30ºC. The UV detection at 240 nm was applied.

S o l u t i o n s. The stock solutions at concentration of 6 mg/ml of repaglinide for system suitability CSP [8] and of 1 mg/ml of repaglinide and gliclazide standards were prepared separately in methanol. All these solutions were stored at 4°C and were found to be stable for at least 3 weeks.

S e l e c t i v i t y. The solution containing repaglinide for system suitability CSP [8] and gliclazide, both at concentrations of 0.6 mg/ml, was prepared in methanol and transferred to a CE vial.

L i n e a r i t y. The linearity was assessed using methanolic solutions in the concentration range of 0.12-0.32 mg/ml of repaglinide. Each solution contained 0.2 mg/ml of gliclazide (internal standard).

P r e c i s i o n o f t h e s y s t e m. The respective solutions at three different concentrations of repaglinide (0.14, 0.22 and 0.30 mg/ml), each containing 0.2 mg/ml of gliclazide were prepared in methanol and measured by CE method three times daily (intra-day precision). Intermediate precision was assessed by analyzing the similar concentrations on three separate days (one sample at each concentration once daily). The response factor was determined as the relationship between the ratio of peak areas (repaglinide versus internal standard) and respective concentration of the drug.

P r e c i s i o n a n d a c c u r a c y i n t a b l e t s. Twenty tablets were weighted and the average mass value was calculated. For each determination, an independent tablet powder was weighted.

The samples were obtained by weighting tablet powders equivalent to 10 mg of repaglinide. The samples were placed in 10 ml volumetric flasks containing 5 ml of methanol, sonicated for 10 min to dissolve the active ingredient, diluted to the mark and filtered by nylon membrane filters (0.45 μm). Then, 1.0 ml volumes were transferred to 5 ml flasks together with 1.0 ml of gliclazide solution at concentration of 1 mg/ml. Finally, the solutions were diluted to the mark with methanol and transferred to CE vials. The assay was repeated five times.

F o r t i f i e d s a m p l e s. The samples were obtained by weighting the tablet powders equivalent to 10 mg of repaglinide. They were placed in 10 ml volumetric flasks containing 5 ml of methanol, sonicated for 10 min to dissolve the active ingredient, diluted to the mark and filtered. Then, 0.6 ml volumes were transferred to 5 ml flasks together with 0.3, 0.6 ml and 0.9 ml of the standard solution of repaglinide at concentration of 1 mg/ml (50, 100 and 150 % addition) and 1.0 ml of gliclazide at concentration of 1 mg/ml. Finally, the solutions were diluted to the mark with methanol and transferred to CE vials. The completed procedure was repeated five times for each sample at each level of addition.

S t a t i s t i c a l c o m p a r i s o n. The results presented here and those obtained previously by our HPLC method [1] were statistically estimated by ANOVA. Then, a pair-wise comparison of precision by F-Snedecor test and of accuracy by t-Student and Wilcoxon tests was performed.

## DISCUSSION OF RESULTS

O p t i m i z a t i o n. In order to improve the migration times and peak shapes, different aqueous buffers like borate, phosphate and citrate at different pH were employed. For non-aqueous milieu, different concentrations of ammonium acetate and sodium acetate in the mixture of methanol-acetonitrile (3:7, v/v) were studied. Finally, 0.01 mol/l solution of ammonium acetate, showing the best resolution of repaglinide, its impurities A-D [8] and gliclazide as well as a stable baseline, was chosen. The separation voltage was set at 30 kV which affords a sufficient migration time and

acceptable current generation. The mean times of migration were ca. 9.5 and 8.7 min for repaglinide and gliclazide, respectively (Fig. 2).

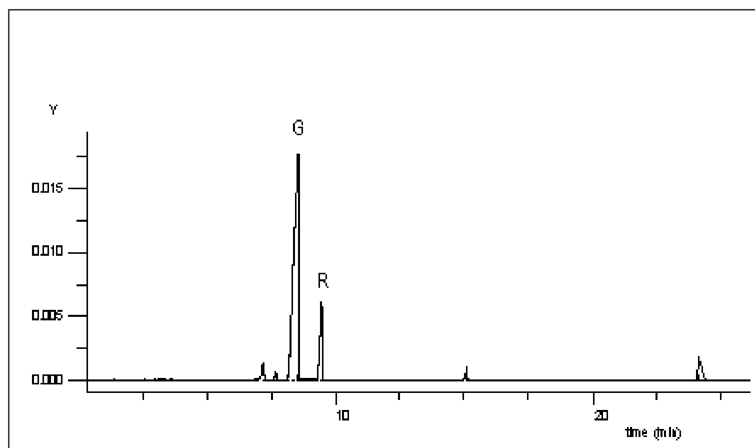


Figure 2. Electropherogram recorded for repaglinide (R) for system suitability CSP containing impurities (A-D) in the presence of the internal standard gliclazide (G)

R o b u s t n e s s. For the robustness study, the influence of deliberate small changes in the wavelength of detection (240±3 nm), in the separation voltage (30±2 kV) and in the temperature applied (30±2ºC) was tested. Then, the results obtained for all examined parameters were statistically estimated by ANOVA. Despite small changes in the migration times or peak areas, stability towards these changes was proved (Tab. 1).

Table 1. Results from the robustness testing.

| | Mean peak area ratio* (n=3) | SD | RSD (%) |
|---|---|---|---|
| Wavelength (237, 240, 243 nm ) | 0.9549 | 0.0030 | 0.31 |
| Temperature (28, 30, 32°C) | 0.9549 | 0.0025 | 0.26 |
| Voltage (28, 30, 32 kV) | 0.9524 | 0.0034 | 0.36 |
| | F = 4.20 p = 0.0796 | | |

*Repaglinide vs internal standard

S e l e c t i v i t y. The selectivity of the method was established by analyzing a mixture containing repaglinide and its impurities A-D [8] (Fig. 1) as well as the internal standard (gliclazide), under optimal method conditions. Because of lacking of pure standards of impurities A-D, they could not be identified on our electropherograms. However, resolution between all compounds observed was rather satisfactory and none of the additional peaks appeared at the same time than those of repaglinide and gliclazide (Fig. 2). The identity of the peaks for repaglinide and gliclazide was confirmed by simple comparison with the electropherograms obtained for respective standard solutions.

L i n e a r i t y. The six-point calibration curves were found to be linear as least squares regression gave good mean correlation coefficient r which was 0.9963. The residual data were distributed randomly around the zero line which indicates the correctness of the calibration plot (data not shown). Linearity

was tested by means of Mandel fitting test with quadratic equation as the alternative fitting and also by the Lack of Fit test. The linearity was proved so the linear equation was chosen as optimal (Tab. 2).

Table 2. Linear regression data of the calibration plot (n=5).

| Parameter | Mean value | SD | Confidence interval |
|---|---|---|---|
| a | 4.430714 | 0.232314 | 4.281855 – 4.579573 |
| b | 0.054863 | 0.038396 | 0.020571 – 0.089154 |
| r | 0.9963 | F = 3717.288 p = 2.5548E-31 | |
| Mandel test | | F = 2.3331 p = 0.1383 | |
| Lack of Fit test | | F = 2.620667 p = 0.06006 | |

Limit of detection (LOD) and limit of quantification (LOQ). A signal-to-noise ratio of approximately 3 was considered to be acceptable for estimating the detection limit. A signal-to-noise ratio of approximately 10 was considered to be acceptable for estimating the lowest concentration that could be quantified with acceptable precision and accuracy. The LOD and LOQ values obtained by the described CE method were 0.03 and 0.08 mg/ml of repaglinide, respectively.

Precision of the system. The RSD values for the intra-day and intermediate precision are shown in Table 3.

Table 3. Precision of the system in the standard solutions.

| | Intra-day precision (n=3) | | Intermediate precision | | |
|---|---|---|---|---|---|
| | | | (n=3) | | (n=9) |
| Amount examined (mg/ml) | Response factor* mean ± SD | RSD (%) | Response factor* mean ± SD | RSD (%) | Total RSD (%) |
| 0.14 | 4.4769 ± 0.15 | 3.32 | 4.3924 ± 0.04 | 0.85 | |
| 0.22 | 4.6333 ± 0.12 | 2.65 | 4.5763 ± 0.10 | 2.25 | 2.36 |
| 0.30 | 4.6452 ± 0.18 | 3.96 | 4.5438 ± 0.08 | 1.73 | |

*Relationship between the peak areas (repaglinide vs internal standard) and concentration of the drug.

Precision and accuracy in tablets. All results were homogenic and t test showed no significant differences between them and the declared amount of repaglinide (Tab. 4). All contents lied in a respective confidence interval, so the assay was proved to be sufficiently accurate.

Table 4. Accuracy and precision for repaglinide assay in powdered tablets (n=5).

| Amount expected (mg) | Amount found (mg) | Mean amount (mg) | Confidence interval (mg) | SD (mg) | RSD (%) | Recovery mean (%) |
|---|---|---|---|---|---|---|
| 0.5000 | 0.4985 - 0.5110 | 0.5046 | 0.4978 - 0.5115 | 0.007 | 1.36 | 100.92 |
| Q-Dixon test | Q = 0.632 < Qk = 0.642 | | | | | |
| t-Student test | t = 1412 < tαf = 2.571 | | | | | |

F o r t i f i e d  s a m p l e s. Accuracy of the method was proved by fortifying the respective amounts of powdered tablets with three concentrations of repaglinide at 50, 100 and 150% level of addition. The proposed method afforded mean recovery of 100.86% with the RSD value of 0.81% (Tab. 5).

Table 5. Accuracy data for repaglinide assay in the fortified samples (n=5).

| Addition (%) | Amount determined mean ± SD (mg/ml) | Recovery (%) | RSD (%) |
|---|---|---|---|
| 50 | 0.1818 ± 0.0033 | 100.81-101.23 | 0.19 |
| 100 | 0.2423 ± 0.0007 | 101.03-101.70 | 0.26 |
| 150 | 0.3046 ± 0.0005 | 101.25-101.66 | 0.17 |

S t a t i s t i c a l  c o m p a r i s o n. No significant differences, regarding accuracy and precision, were observed for the results presented here and those obtained previously by our previously published HPLC method [1] with gliclazide as internal standard and UV detection at 240 nm (Tab. 6).

Table 6. Statistical comparison of the results obtained in the fortified samples by CE and HPLC [1] methods.

| ANOVA | | Kruskal-Wallis test | | F-Snedecor test | | t-Student test | | Wilcoxon test | |
|---|---|---|---|---|---|---|---|---|---|
| F | p | H | p | F | p | t | p | W | p |
| 2.3135 | 0.1395 | 3.4076 | 0.1395 | 2.4417 | 0.1063 | -1.521 | 0.1414 | 68 | 0.0680 |

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SUMMARY

Capillary electrophoresis (CE) method in non-aqueous medium was developed and validated for determination of repaglinide in pharmaceutical formulation. CE was performed using a 75 µm x 90 cm fused silica capillary (76 cm effective length) and UV detection at 240 nm. 0.01 mol/l solution of ammonium acetate in the mixture of methanol-acetonitrile (3:7, v/v), 30 kV voltage, 30°C temperature and hydrodynamic injection (10 mbar, 6 s) were chosen as CE parameters. The solutions were prepared in methanol. The proposed CE method demonstrated sufficient stability and reproducibility with mean RSD values of less than 2.5%. The robustness, selectivity, linearity, accuracy and precision were sufficient for quantitative determination of repaglinide for different pharmaceutical purposes.

*Keywords:* capillary electrophoresis; validation; repaglinide; pharmaceuticals

STRESZCZENIE

Opracowano i zwalidowano metodę elektroforezy kapilarnej w środowisku niewodnym do oznaczania repaglinidu w preparatach farmaceutycznych. W metodzie użyto niemodyfikowaną powlekaną kapilarę kwarcową o długości 90 cm (76 cm długości do detektora), o średnicy wewnętrznej 75 µm oraz detekcję UV przy 240 nm. Zastosowano 0,01 mol/l roztwór octanu amonu w mieszaninie metanol-acetonitryl (3:7, v/v), napięcie 30 kV, temperaturę 30°C i nastrzyk hydrodynamiczny (6 s, 10 mbar). Roztwory przygotowano w metanolu. Proponowana metoda CE zapewnia wystarczającą stabilność i powtarzalność ze średnią wartością RSD poniżej 2,5%. Badanie odporności metody na zmiany, selektywności, liniowości, dokładności i precyzji wskazuje, że może być ona wykorzystywana do rutynowej kontroli jakości odpowiednich preparatów farmaceutycznych.

*Słowa kluczowe:* elektroforeza kapilarna; walidacja; repaglinid; preparaty farmaceutyczne