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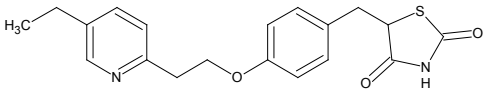
Comparison of Capillary Electrophoresis and UV Derivative Spectrophotometry for Determination of Pioglitazone, Glipizide and Repaglinide

Porównanie przydatności elektroforezy kapilarnej i spektrofotometrii pochodnych widma do oznaczania pioglitazonu, glipizydu i repaglinidu

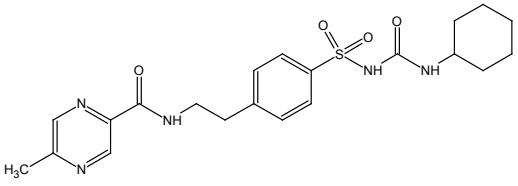
INTRODUCTION

Current treatment of type 2 diabetes can be subdivided into antihyperglycemic compounds such as thiazolidinediones and the hypoglycemic agents including sulfonylureas and benzoic acid derivatives. Thiazolidinediones, such as pioglitazone, are synthetic ligands for peroxisome proliferator-activated receptors (PPARs). They can increase glucose uptake and utilization in the peripheral organs and decrease gluconeogenesis in the liver thereby reducing insulin resistance. Sulfonylureas, such as glipizide, bind to an ATP-dependent K^+ (K_{ATP}) channel on the cell membrane of pancreatic β cells. This leads to increased fusion of insulin granulae with the cell membrane, and therefore increased secretion of insulin. Benzoic acid derivative repaglinide also binds to the K_{ATP} channel but a receptor for it is different from that of the sulfonylureas [2,3,5]. The chemical structures of the mentioned drugs are presented in Fig. 1.

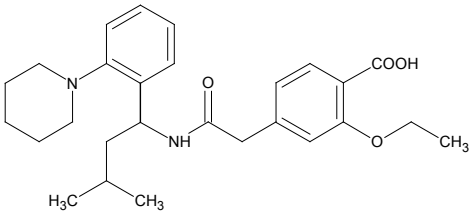
In recent years, capillary electrophoresis (CE) is more and more frequently applied as a highly effective analytical procedure. It has many advantages such as lower sampling volume and higher separation efficiency than HPLC, allowing to separate the complex mixtures. On the other hand, there is not any report about CE determination of pioglitazone, glipizide and repaglinide. Therefore, the aim of the present study was to elaborate and to fully validate new CE method for determination the mentioned drugs and to compare it with UV derivative spectrophotometric procedure. The spectrophotometry was proposed for its popularity, simplicity and less time and reagents consuming. As far as concerns UV spectrophotometry, some reports exist concerning pioglitazone [6], glipizide [1,4] and repaglinide [7,8]. However, previously described UV methods are not fully validated. In addition, the derivative spectrophotometric procedure has not been applied at all.



PIOGLITAZONE



GLIPIZIDE



REPAGLINIDE

Fig. 1. Chemical structures of the ezamined compounds

MATERIAL AND METHODS

Reagents and chemicals. All used chemicals were of analytical grade and were purchased from E. Merck (Germany). Purified water was obtained using a deionizer SolPure 7 from Poll. Lab. (Poland). The respective tablets were used: Actos® (30 mg of pioglitazone), Glipizide BP® (5 mg of glipizide) and NovoNorm® (0.5 mg of repaglinide).

Apparatus. CE 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. For UV spectrophotometry, a Perkin-Elmer (MA, USA) Lambda 15 UV double-beam spectrophotometer with quartz cells of 1 cm was used. This system provides the capability for data manipulation including smoothing and construction of derivative spectra from zero-order spectral data.

General procedures. CE was performed using a fused-silica capillary tubing (Polymicro Technologies, AZ, USA) of 75 µm i.d. and 65 cm effective length (total length 87 cm). Sample loading was achieved by hydrodynamic injection (10 mbar, 6 s). Before start-up, the capillary was preconditioned with 0.1 M 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 analysis and when was not in use. Analysis was carried out at 30°C and a potential of 30 kV. The autosampler was kept at room temperature (approximately 21ºC). The UV detection at 220, 270 and 310 nm for glipizide, repaglinide and pioglitazone was applied. The running buffer at pH 8.2 was prepared by mixing 94.5 ml Na_2HPO_4 solution (0.067 M) with 5.5 ml KH_2PO_4 solution (0.067 M). For pH control, a pH meter HI-9024 from Hanna Instruments (Germany) was applied. Prior to use, the buffer was filtered through a 0.45 μm nylon membrane filter.

The UV spectra were recorded using 2 nm slit and 120 nm/min scanning speed. The derivatives were calculated automatically with the time constant 1 s and wavelength differences 3, 6 and 8 nm. The assay was performed at different analytical wavelengths using the peak-zero (P-0) and peak-to-peak (P-P) techniques. For pioglitazone, the procedure was carried out at 258 nm (D1) and 210 nm (D2) using P-0 technique. For glipizide, the analytical parameters were 237–214 nm with P-P (D1) and 292 nm with P-0 (D2). For repaglinide, the procedure was carried out at as first derivative assay at 251 nm using P-0 technique.

Preparation of solutions. For all compounds the stock solutions of 1 mg/ml were prepared in methanol. They were stored at 4°C and were found to be stable for at least 3 weeks. The working solutions were prepared in water-methanol (1:1) or methanol.

Precision. The respective solutions at three different concentrations were measured five times daily (intra day precision). Inter day precision was assessed by analyzing the same concentrations on three separate days (one sample at each concentration once daily).

Assay in the model mixtures.

CE method. For pioglitazone and glipizide, the preanalyzed samples were obtained by adding 5.0, 10.0 and 15.0 mg of pure compound to tablet powder equivalent to 10 mg of the drugs into 25 ml volumetric flasks containing 10 ml of methanol. For repaglinide, the samples were obtained by adding 2.0, 4.0 and 6.0 mg of pure compound to tablet powder equivalent to 2 mg of repaglinide. They were placed in 25 ml (50% addition) or 50 ml (100 and 150% addition) volumetric flasks containing methanol. All samples were sonicated for 10 min to dissolve the active ingredient. These extracts were diluted to volume with water–methanol (pioglitazone) or methanol (glipizide and repaglinide), filtered by nylon membrane filters (0.45 μm), once more diluted 1:10 (except for repaglinide) with respective solvent.

UV spectrophotometry. For pioglitazone and glipizide, the first step was the same as in CE procedure. For repaglinide, the samples were obtained by adding 2.0, 4.0 and 6.0 mg of pure drug to tablet powder equivalent to 0.6 mg of repaglinide and placed in 10 ml volumetric flasks containing methanol. Respective volumes such as 0.15 ml for pioglitazone and 0.1 ml for glipizide and repaglinide were transferred to 10 ml volumetric flasks and diluted to volume with methanol.

DISCUSSION OF RESULTS

Optimization of CE. In order to improve the migration times and peak shapes, borate, phosphate, citrate and glycine buffers were employed as a running buffer. In addition, the effect of concentration of the buffer was examined. Also, the pH effect was investigated within the range from

4.15 to 10.10. Generally, higher pH values increased electroosmotic flow which led to decreasing the analysis time. With phosphate buffer at concentration of 0.067 M and pH 8.2, the peak areas were found to be greater and the reproducibility of both migration time and area were the best. Furthermore with this electrolyte concentration the current generation was always far below 100 μA. Four voltages (15, 20, 25 and 30 kV) were studied and finally, the separation voltage was set at 30 kV which affords sufficient migration time. The effect of injection time was investigated, too. It was found that when the sampling time was prolonged, the peaks increased correspondingly. However, they were obviously broadening if the time was more than 6 s. As an example, the typical electropherogram for pioglitazone is shown in Fig. 2.

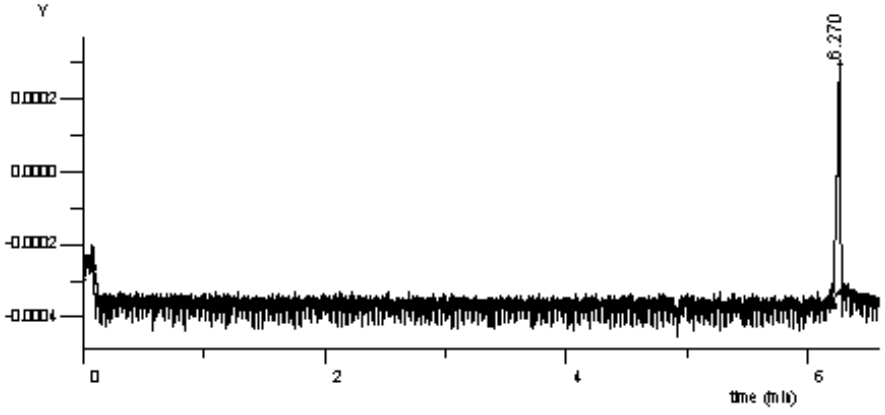


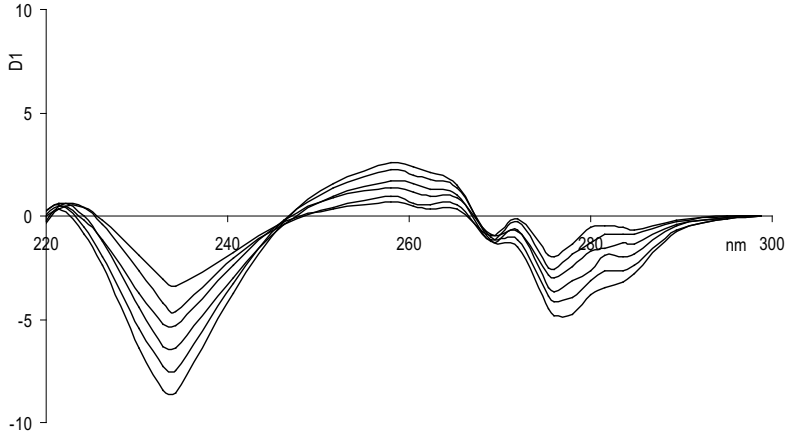
Fig. 2. Typical electropherogram of pioglitazone at concentration of 0.08 mg/ml

S p e c i f i c i t y . We compared the peak area or absorption of the analytes after 1, 3, 6, 12 and 24 h storage at room temperature (20 ± 2ºC) and monitored the presence of additional peaks in electropherograms and in spectra. For the both methods no observable peaks were noted.

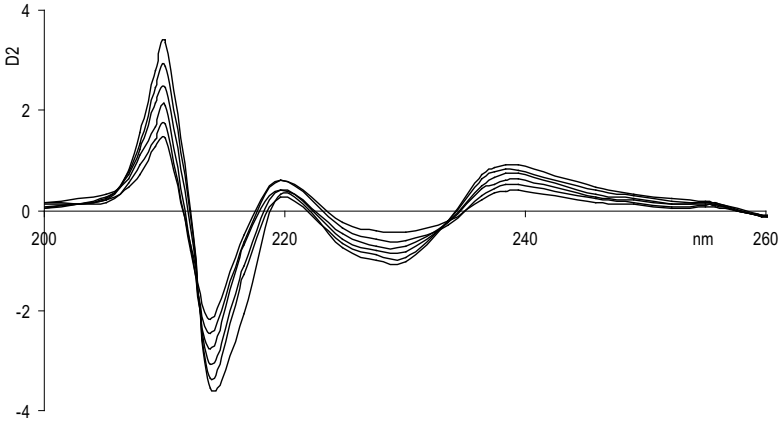
Linearity. The six-point calibration curves were found to be linear as least squares regression gave good correlation coefficients (Table 1). As an example, the D1 and D2 spectra recorded for different concentrations of pioglitazone are shown in Fig. 3.

P r e c i s i o n . The RSD values for the intra and inter day precision are shown in Tables 2 and 3.

R e c o v e r y i n t h e m o d e l m i x t u r e s . The results for recovery and accuracy studies are shown in Table 4.



(a)



(b)

Fig. 3. First D1 (a) and second D2 (b) derivative spectra of pioglitazone in the calibration range 0.006–0.016 mg/ml

Table 1. Linearity data for CE and UV D1 and D2 methods (n=6; the full names of the drugs are given in the text)

Parameter		Piog	Glip	Repa
CE				
Calibration range (mg/ml)		0.04-0.14	0.04-0.14	0.12-0.20
Slope		2.289×10^{-4}	3.895×10^{-4}	2.111×10^{-3}
SE[a] for the slope		5.3×10^{-6}	1.19×10^{-5}	4.15×10^{-5}
Intercept		-3.55×10^{-6}	2.91×10^{-7}	-1.29×10^{-4}
SE[a] for the intercept		5.12×10^{-7}	1.29×10^{-6}	6.92×10^{-6}
Correlation coeficient (r)		0.9972	0.9975	0.9973
UV				
Calibration range (mg/ml)		0.006-0.016	0.004-0.014	0.002-0.012
Slope	D1	0.1902	1.1461	0.0405
	D2	0.1906	0.0307	-
SE[a] for the slope	D1	3.69×10^{-3}	0.0017	0.0027
	D2	0.0016	0.0002	-
Intercept	D1	-0.6026	-0.8144	-0.24362
	D2	0.2694	0.0071	-
SE[a] for the intercept	D1	0.0032	0.0118	0.0002
	D2	0.020	0.0039	-
Correlation coefficient (r)	D1	0.9989	0.9996	0.9994
	D2	0.9992	0.9994	-

[a]SE-standard error

Table 2. Precision of CE method (the response factor was determined as the relationship between the peak area and concentration of the respective drug; the full names of the drugs are given in the text)

		Intra day precision n=5		Inter day precision n=3		Inter day precision n=9
(mg/ml)		Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Total RSD (%)
Piog	0.05	0.168 ± 0.001	0.74	0.168 ± 0.002	0.99	
	0.09	0.169 ± 0.002	1.39	0.168 ± 0.003	1.58	1.39
	0.13	0.167 ± 0.003	1.68	0.166 ± 0.003	1.72	
Glip	0.05	0.402 ± 0.002	0.53	0.402 ± 0.002	0.55	
	0.09	0.401 ± 0.003	0.69	0.402 ± 0.003	0.73	1.39
	0.13	0.394 ± 0.004	1.10	0.394 ± 0.006	1.52	
Repa	0.13	1.45 ± 0.02	1.14	1.44 ± 0.02	1.35	
	0.17	1.42 ± 0.02	1.16	1.42 ± 0.02	1.07	1.22
	0.19	1.44 ± 0.02	1.26	1.43 ± 0.01	0.74	

Table 3. Precision of UV D1 and D2 methods (the full names of the drugs are given in the text)

		Intra day precision n=5				Inter day precision n=3				Inter day precision n=9	
(mg/ml)		Mean		RSD (%)		Mean		RSD (%)		Total RSD (%)	
		D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
Piog	0.007	99.94	100.17	0.14	0.19	99.89	100.17	0.17	0.19		
	0.011	99.90	100.13	0.24	0.28	99.87	100.13	0.21	0.30	0.19	0.23
	0.015	99.95	100.01	0.18	0.13	99.86	100.01	0.19	0.13		
Glip	0.005	99.64	99.61	0.30	0.21	99.73	99.60	0.26	0.19		
	0.009	100.14	100.33	0.07	0.14	100.14	100.31	0.07	0.10	0.28	0.24
	0.013	99.85	100.34	0.14	0.17	99.89	100.32	0.17	0.11		
Repa	0.003	99.74	-	0.17	-	99.65	-	0.09	-		
	0.007	100.08	-	0.17	-	100.20	-	0.04	-	0.25	-
	0.011	99.92	-	0.18	-	99.81	-	0.12	-		

Table 4. Validation of CE and UV D1 and D2 methods in the model mixtures (the full names of the drugs are given in the text)

Level of addition (%)		Recovery (%) n=5		RSD (%) n=5		Total recovery (%) n=15		Total RSD (%) n=15	
CE									
Piog	50	99.87		0.15					
	100	100.00		0.14		99.98		0.13	
	150	99.92		0.11					
Glip	50	99.95		0.22					
	100	99.93		0.28		99.97		0.20	
	150	100.04		0.13					
Repa	50	100.07		0.28					
	100	100.05		0.33		100.08		0.41	
	150	100.13		0.63					
UV		D1	D2	D1	D2	D1	D2	D1	D2
Piog	50	99.64	99.72	0.30	0.60				
	100	99.89	100.09	0.40	0.58	99.78	99.77	0.42	0.54
	150	99.82	99.50	0.57	0.32				
Glip	50	99.60	100.87	0.35	0.30				
	100	100.23	100.55	0.58	0.41	99.87	100.55	0.51	0.43
	150	99.77	100.22	0.43	0.37				
Repa	50	99.55	-	0.38	-				
	100	100.20	-	0.40	-	99.88	-	0.45	-
	150	99.89	-	0.36	-				

Statistical comparison. The multiple comparison of precision was done with Bartlett test and accuracy by ANOVA. We also decided to perform a pair-wise comparison of precision by F-Snedecor test and accuracy by t Student or Wilcoxon tests. For pioglitazone and repaglinide ANOVA test revealed no statistically significant differences between the results obtained. However, there were significant differences in precision examined by Bartlett test. For pioglitazone higher precision was achieved in D1 and D2 spectrophotometric methods than in CE. For repaglinide, CE method showed to be more precise than D1 procedure. For glipizide assay D1 method was shown to be more precise than D2 and CE methods. On the other hand, D2 spectrophotometric determination was more accurate than CE assay (Tab. 5).

Table 5. Statistical comparison between the methods (values appearing in parenthesis denote p values; the full names of the drugs are given in the text)

	Methods	F-Snedecor	t Student	Wilcoxon
Piog	CE–UV D1	7.59 (0.006)*	0.43 (0.68)	53.5 (0.82)
	CE–UV D2	2.13 (0.28)	0.41 (0.69)	54.5 (0.76)
	UV D1–UV D2	16.16 (0.0003)*	0.79 (0.45)	57.0 (0.62)
Glip	CE–UV D1	66.37 (7.50×10^{-7})*	1.61 (0.14)	60.0 (0.47)
	CE–UV D2	3.43 (0.08)	2.52 (0.02)*	75.0 (0.06)
	UV D1–UV D2	19.32 (0.0001)*	2.24 (0.05)	74.0 (0.08)
Repa	CE–UV D1	13.08 (0.001)*	1.22 (0.25)	61.0 (0.43)

*significant at $p < 0.05$

CONCLUSIONS

In spite of some statistical differences, the proposed CE and UV derivative spectrophotometry demonstrated sufficient specificity, linearity, accuracy and precision with very low RSD values. Generally, CE was more selective while the derivative spectrophotometry was more sensitive and precise. In addition, spectrophotometry required shorter analysis time.

The elaborated methods were successfully used for determination of the mentioned antidiabetics in respective formulations, only requiring a simple extraction before analysis. For the examined compounds, pioglitazone, glipizide and repaglinide, they are first CE methods which may be applied in quantitative analysis. Also, for these antidiabetics, the derivative spectrophotometry has not been described previously.

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SUMMARY

Two new methods, capillary electrophoresis (CE) and UV derivative spectrophotometry were developed and validated for determination of three oral antidiabetics, pioglitazone, glipizide and repaglinide in pharmaceutical formulations. CE was performed using a 75 μm x 87 cm fused silica capillary (65 cm effective length) and UV detection. The phosphate buffer at pH 8.2, 30 kV voltage, 30°C temperature and hydrodynamic injection (10 mbar, 6 s) were chosen as CE parameters. UV spectrophotometry was carried out using first (D1) and second (D2) derivative spectra obtained by the peak-zero (P-0) and peak-to-peak (P-P) techniques. Precision and accuracy of the both methods were compared. In spite of some statistical differences, the results showed that the both methods are suitable for quantitative determination of these five oral antidiabetics and can be applied for different pharmaceutical purposes.

Keywords: oral antidiabetics, determination/validation, capillary electrophoresis, UV derivative spectrophotometry

STRESZCZENIE

Opracowano dwie nowe metody oznaczania trzech doustnych leków przeciwcukrzycowych, pioglitazonu, glipizydu i repaglinidu, w preparatach farmaceutycznych, przy użyciu elektroforezy kapilarnej (CE) oraz spektrofotometrii pochodnych widma. W metodzie CE użyto niemodyfikowaną powlekaną kapilarę kwarcową o długości 87 cm (65 cm długości do detektora), o średnicy wewnętrznej 75 μm oraz detekcję UV. Zastosowano bufor fosforanowy o pH 8,2, napięcie 30 kV, temperaturę 30°C i nastrzyk hydrodynamiczny (6 s, 10 mBar). W metodzie spektrofotometrycznej zastosowano pierwszą (D1) i drugą (D2) pochodną widma, wykorzystując techniki „pik-zero" (P-0) i „pik do piku" (P-P). Opracowane metody porównano pod względem precyzji i dokładności. Otrzymane wyniki wskazują, że obydwie metody mogą być wykorzystane do rutynowej kontroli jakości odpowiednich preparatów farmaceutycznych.

Słowa kluczowe: doustne leki przeciwcukrzycowe, oznaczanie/walidacja, elektroforeza kapilarna, spektrofotometria pochodnych widma