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Solid phase extraction—Non-aqueous capillary electrophoresis for determination of metformin, phenformin and glyburide in human plasma

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Abstract

Solid phase extraction (SPE) was coupled at line to capillary electrophoresis (CE) for the determination of three basic and neutral diabetic drugs (metformin, phenformin and glyburide) in human plasma. The SPE procedure employed a C_{18} cartridge to remove most of the water and proteins from the plasma sample. Analyte detectability was increased due to trace enrichment during the SPE process. Elution of metformin, phenformin and glyburide was achieved with methanol + 3% acetic acid. CE analysis was performed using a non-aqueous buffer, acetonitrile + 5 mM ammonium acetate + 5% acetic acid, which afforded rapid separation of metformin from phenformin within 3 min. Glyburide, with a migration time longer than 6 min, did not cause any interference. The present SPE–CE method, with an electrokinetic injection time of 6 s and UV detection at 240 nm, was useful for monitoring down to 1 μg/mL of metformin and phenformin in human plasma. When the electrokinetic injection time was increased to 36 s, the detection limits were improved to 12 ng/mL for metformin and 6 ng/mL for phenformin.

Keywords: Metformin; Phenformin; Glyburide; Human plasma; Solid phase extraction; Capillary electrophoresis; Non-aqueous buffer

1. Introduction

Metformin (or 1,1-dimethylbiguanide) is prescribed as an oral antihyperglycemic agent in the management of non-insulin-dependent diabetes mellitus (NIDDM) [1]. Metformin HCl (*N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride) has been the only clinically available drug that can significantly improve insulin sensitivity [2]. This drug improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. It decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization [3]. Glucovance combines metformin hydrochloride and glyburide, two antihyperglycemic agents with complementary mechanisms of action, to improve glycemic control in patients with type 2 diabetes. A new option in oral hyperglycemic therapy for type 2 diabetes mellitus was studied using a combination of metformin and rosiglitazone [4]. Metformin has clinically been used, in combination with a high protein–low carbohydrate diet, to restore normal menstrual cycles in teenage females with polycystic ovary syndrome (PCOS) [5]. Metformin is included in HIV clinical trials to assess its efficacy in the treatment of the metabolic disturbances (fat redistribution, insulin resistance and hyperinsulinemia) associated with HIV lipodystrophy syndrome [6].

When the metformin concentration in plasma becomes too high, metformin accumulation can induce serious metabolic complications such as lactic acidosis [6]. If lactic acidosis is suspected or diagnosed, monitoring of metformin plasma concentration would have much clinical value [7]. For this reason, the determination of plasma metformin should always be done during treatment. Indeed, when lactic acidosis occurs in metformin-treated patients (particularly due to renal failure), early determination of the metformin plasma concentration appears to be the best criterion for assessing this acute condition. Determination of metformin has previously been reported in human plasma, urine, breast milk and pharmaceutical preparations by high-performance liquid chromatography (HPLC) with spec-

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trophotometric detection [8–12], capillary electrophoresis (CE) using field-amplified sample stacking technique [13], spectrofluorimetry [14] and near infra-red reflectance spectroscopy [15]. The main advantages of CE over HPLC are the much higher separation efficiency, lower solvent (or buffer) consumption and shorter analysis time. One challenge with measuring very low metformin concentrations in human plasma (in pharmacokinetic studies) is due to the high polarity of this compound. It is difficult to extract the drug directly from biological fluids by solvent extraction. A method based on the extraction of metformin with Bromothymol Blue (as an ion pair) into chloroform had previously been described, using phenformin as internal standard [13].

In the present work, a new solid phase extraction (SPE) method was developed for the pre-treatment of human plasma samples on a C_{18} cartridge. Methanol + 3% acetic acid was used to concentrate the metformin in a small final volume. For the rapid separation of metformin from phenformin and other drugs by CE, the low conductivity sample can be electrokinetically injected onto the capillary head (providing a 100-fold concentration of metformin). This sensitivity enhancement was shown to be an effective and robust approach for the determination of ng/mL metformin levels in human plasma by UV detection at 240 nm.

2. Experimental

2.1. Materials

Metformin HCl, phenformin HCl and glyburide were obtained from Sigma (St. Louis, MO, USA). Stock solutions of 1 mg/mL metformin and 1 mg/mL phenformin were prepared in pure acetonitrile. They were stable when stored at 4 °C for 1 month. Standard solutions of 60 μg/mL each were prepared afresh by dilution with acetonitrile for daily use.

Acetonitrile (CH_3CN) of HPLC grade was purchased from Caledon (Georgetown, Ont.). Ammonium acetate (NH_4OAc) and water-free glacial acetic acid (HOAc) were obtained from Anachemia (Toronto, Ont.).

2.2. Capillary electrophoresis

CE separation was performed on a fused silica capillary (65 μm i.d., 360 μm o.d., 30 cm total length, 22.5 cm effective length) obtained from Polymicro Technologies (Phoenix, AZ, USA). The new capillary was flushed with 2 M NaOH followed by water. CE analyses were performed on a laboratory-built system that included a Spellman CZE1000R high voltage power supply. The non-aqueous buffer (pH 5.1) for CE was composed of 20 mM NH_4OAc and 5% HOAc in CH_3CN. When the buffer was run under an applied voltage of 15 kV, the capillary was equilibrated with the ambient room temperature of 22–25 °C. A Bischoff Lambda 1010 (Leonberg, Germany) UV detector was employed to monitor the elution of analytes at a wavelength of 240–280 nm. The detector output signal was acquired by both a Dionex 4270 integrator (Sunnyvale, CA, USA) and a personal computer running the Peak Simple Chromatography Data System (SRI model 203, Torrance, CA, USA). After each run, the capillary was conditioned by running the buffer at 15 kV for 1 min before the next analysis.

2.3. Plasma analysis

Human plasma samples were obtained from the Ottawa General Hospital (Ottawa, Ont., Canada). Each 0.5 mL plasma sample was spiked with metformin, phenformin (as an internal standard [16]) and glyburide (as a potential interference) from aqueous standard solutions. After dilution to 2 mL with deionized water, extraction treatment was carried out on a C_{18} SPE cartridge (T. Baker, 7020-03). The cartridge was dried by helium gas, before the extracted metformin, phenformin and glyburide were eluted with 2 mL of methanol + 3% acetic acid. The eluate was collected and electrokinetically injected to the capillary for CE analysis by electrokinetic injection (at 15 kV for 6–36 s). A standard calibration curve was constructed by performing CE–UV analysis of various metformin and phenformin standard solutions.

3. Results and discussion

3.1. Capillary electrophoresis

Fig. 1 shows the molecular structures of metformin ($pK_a = 13.1 \pm 0.5$) and phenformin ($pK_a = 12.7 \pm 0.5$) [17]. A CE method had previously been described for the determination of metformin in human plasma based on the extraction of the ion pair with Bromothymol Blue into chloroform. Phenformin was used as an internal standard and field-amplified sample stacking was employed [13]. When that method was adopted in our laboratory, the separation results turned out to be rather different. As shown in Fig. 2 using 50 mM phosphate buffer (pH 2.5) in water as the medium, separation of metformin from phenformin was attained (at 4.33 and 6.40 min, respectively). These migration times were deemed to be relatively long.

Song et al. had previously demonstrated a general method development process of starting at low pH for basic compounds [13]. Low pH (<3.0) would be ideal for basic compounds with cathodic CE. At a low pH, peak tailing interactions with the capillary would be minimized, providing the best peak shape. At mid pH (5.0–8.0), basic compounds might still have a positive charge but different selectivity would result. Changing the pH could be used to optimize selectivity in most CE method development.

Our first successful CE method employed 20 mM NH_4OAc and 5% HOAc in acetonitrile as the running buffer (pH 5.1), which was modified from the non-aqueous separation medium of 20 mM NH_4OAc and 1 M HOAc in acetonitrile–alcohol (50:49, v/v) reported by Siren et al. [18]. The CE analysis results are illustrated in Fig. 3, showing good resolution, high separation efficiency and short migration times for metformin (2.48 min) and phenformin (3.47 min). When the CE analysis was repeated by employing 20 mM NH_4OAc and 5% HOAc in water as the running buffer (pH 3.0), the results exhibited peak tailing, low separation efficiency and increased migration times as shown

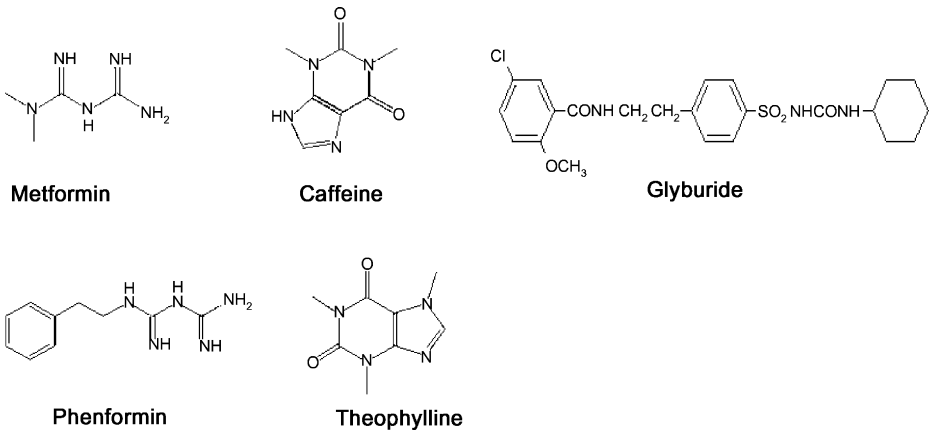

Fig. 1. Molecular structures of metformin (pK_a = 12.4), phenformin (pK_a = 11.3), caffeine (pK_a = 14.2), theophylline (pK_a = 8.7) and glyburide (pK_a = 5.3).

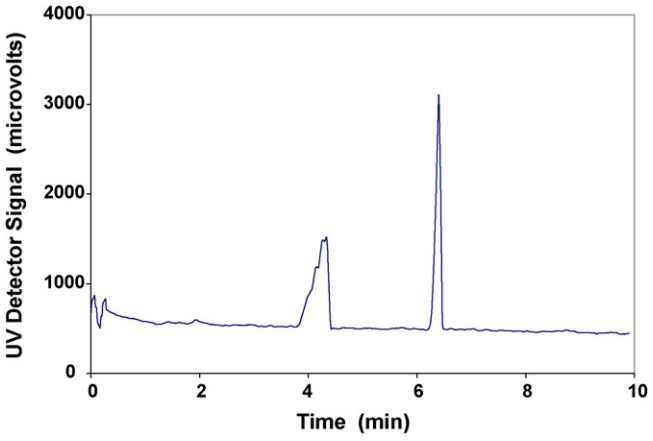

Fig. 2. Separation of metformin (at 4.33 min) from phenformin (at 6.40 min), 60 μg/mL each in aqueous sample. Phosphate buffer, 50 mM (pH 2.5) in water as the running buffer; CE under 15 kV; room temperature = 22 °C; electrokinetic injection at 15 kV for 6 s; UV detection at 240 nm.

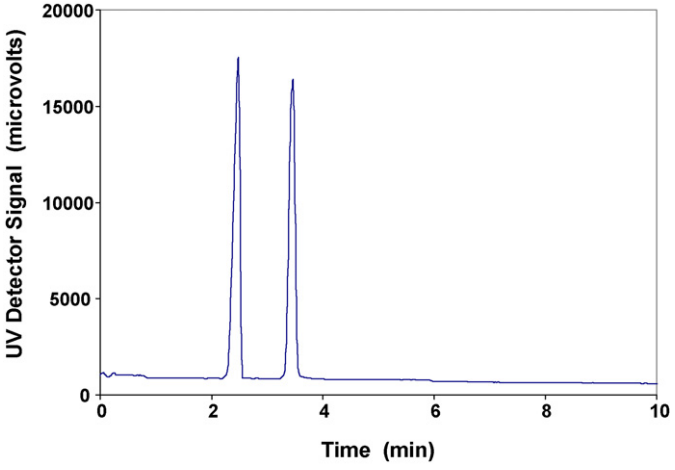

Fig. 3. Separation of metformin (at 2.48 min) from phenformin (at 3.47 min), 60 μg/mL each in aqueous sample. NH_4OAc (20 mM) + 5% HOAc in acetonitrile as the running buffer (pH 5.1); CE under 15 kV; room temperature = 22 °C; electrokinetic injection at 15 kV for 6 s; UV detection at 240 nm.

in Fig. 4. This comparison demonstrates that acetonitrile, a dipolar–aprotic solvent, was really contributing to the good separation of metformin and phenformin shown in Fig. 3. Note that the substitution of acetonitrile for water in the running buffer also caused an apparent change from pH 3.0 to 5.1.

In our second successful CE method using 5 mM NH_4OAc and 5% HOAc in acetonitrile as the running buffer (pH 4.5), the order of migration was metformin (2.98 min) followed by phenformin (3.23 min) for an applied voltage of 8 kV. The separation efficiency was good with resolution down to the baseline. These two analytes migrated well ahead of theophylline and caffeine (added as model compounds from the drug and food categories to test the selectivity of this CE method for metformin and phenformin determination), which eluted as one overlapping peak (7.62 min). Adequate baseline resolution was also observed when the CE–UV analysis was carried out at a higher applied voltage of 10 kV. All the three migration times decreased (2.28 min for metformin, 2.46 min for phenformin and 5.68 min for theophylline/caffeine). When the highest possible applied voltage of 15 kV was used without arcing, metformin (1.40 min) was just resolved from phenformin (1.49 min) at the baseline. This allowed a very rapid analysis time even in the presence of

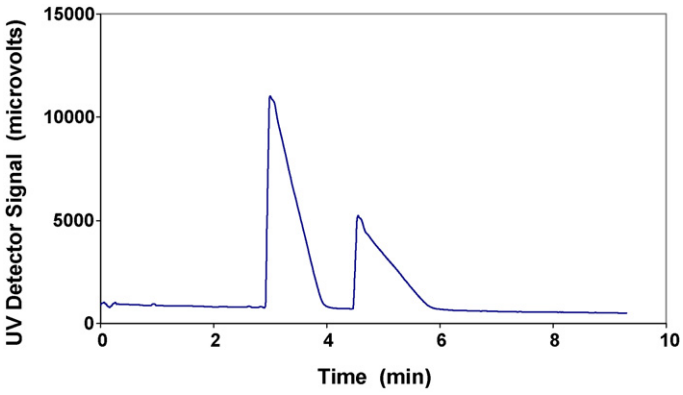

Fig. 4. Separation of metformin (at 3.00 min) from phenformin (at 4.55 min), 60 μg/mL each in aqueous sample. NH_4OAc (20 mM) + 5% HOAc in water as the running buffer (pH 3.0); CE under 15 kV; room temperature = 22 °C; electrokinetic injection at 15 kV for 6 s; UV detection at 240 nm.

theophylline and caffeine (3.25 min). Apparently, the high electrophoretic velocity of protonated metformin resulted in a very short migration time.

3.2. Plasma analysis

Metformin HCl tablets contain 500–850 mg of metformin HCl. In addition, each tablet contains the following inactive ingredients: povidone, magnesium stearate and hydroxypropyl methylcellulose (hypromellose) coating. There are a lot of ionic substances and proteins in human plasma. Ineffective stacking and unsatisfactory electropherograms would be obtained unless the plasma samples are deionized. For CE analysis of human plasma and serum, solid phase extraction was considered to be a suitable sample preparation technique. The commercially available C_{18} SPE chemistry was hence evaluated for binding metformin and phenformin in spiked plasma samples. In general, the pH of the sample may be two pH units below the pK_a of the analyte molecules [19]. This requirement was easily met in the current method as the acidic pH for the extraction was definitely much lower than the pK_a (12.4) of metformin and pK_a (11.3) of phenformin. In addition, drug binding to specific plasma transport proteins (albumin and lipoproteins) is an integral part of many types of intermolecular interactions in a cellular or organ environment. When a small molecule binds to a plasma protein, the interaction is typically the result of strong ionic and hydrophobic interactions. As blood contains several hundred proteins, there is a high probability that many small molecules will exhibit some level of binding [20]. The extraction method was optimized by including an elution step from the cartridge with methanol + 3% HOAc (pH 3.0). This elution solvent was adapted from our previously reported works on molecularly imprinted solid phase extraction for the screening of metformin and antihyperglycemic biguanides [21,22].

When metformin and phenformin were next eluted from the cartridge with methanol + 3% HOAc, a high recovery and precision was achieved for both compounds. Since metformin and phenformin became protonated in the acidic sample matrix to form cations, high stacking efficiencies were afforded by the modest matrix conductivity during electrokinetic injection. Fig. 5 correlates the CE–UV peak areas and conductivity in different sample matrices, which were chosen based on the criterion of different electrical conductivities. These results indicate that methanol + 3% HOAc was an optimal sample matrix (conductivity = 76 μS/cm). A more acidic matrix like methanol + 3% TFA (conductivity = 610 μS/cm) actually made it worse due to its increased conductivity, as illustrated in Fig. 6. Furthermore, deionized water (conductivity = 107 μS/cm) was second to methanol + 3% HOAc, while 20 mM ammonium acetate and 5% acetic acid in acetonitrile (conductivity = 1220 μS/cm) was the worst matrix with the lowest stacking efficiency.

A typical CE analysis of the methanol + 3% HOAc eluate from C_{18} SPE of human plasma is demonstrated in Fig. 7. The CE condition was changed from Figs. 3–7 for the sake of faster electro-osmotic flow and hence more rapid migration of metformin and phenformin. No endogenous peak was observed interfering with metformin (1.75 min) or phenformin (2.70 min).

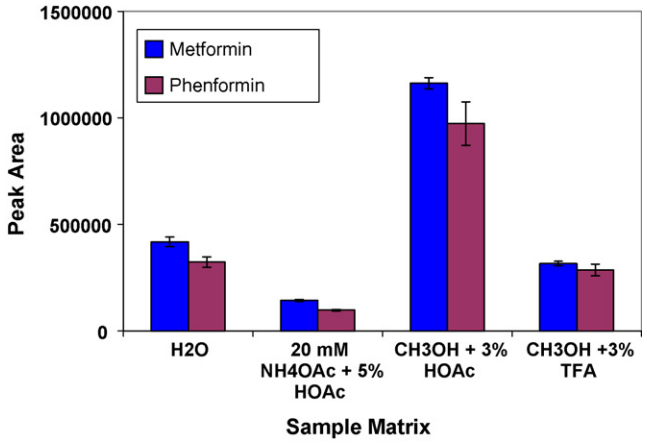

Fig. 5. Effect of sample matrix on the CE–UV peak areas for 60 μg/mL metformin and 60 μg/mL phenformin. Ammonium acetate (20 mM) and 5% acetic acid in acetonitrile as running buffer; CE under 15 kV at 13 μA; room temperature = 22 °C; electrokinetic injection at 15 kV for 6 s; UV detection at 240 nm.

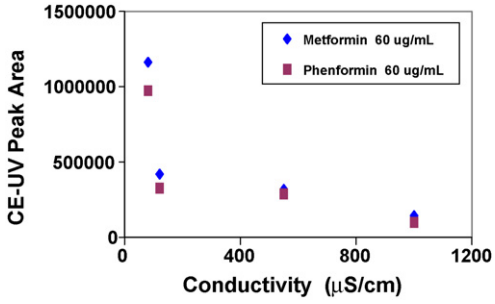

Fig. 6. Dependence of CE–UV peak areas for 60 μg/mL metformin (♦) and 60 μg/mL phenformin (■) on sample conductivity. Ammonium acetate (20 mM) and 5% acetic acid in acetonitrile as running buffer; CE under 15 kV at 13 μA; room temperature = 22 °C; electrokinetic injection at 15 kV for 6 s; UV detection at 240 nm.

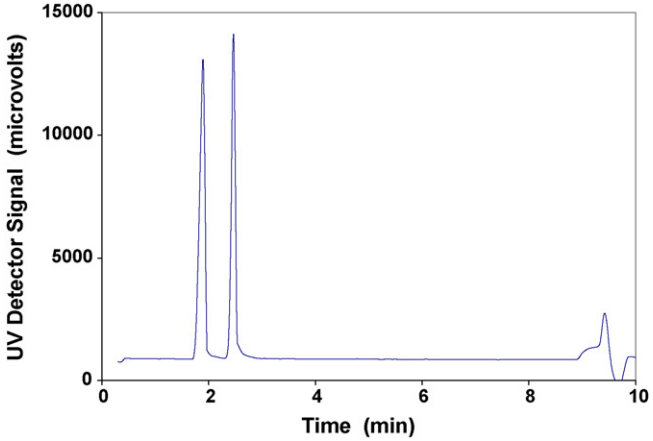

Fig. 7. Typical CE analysis of the methanol + 3% acetic acid eluate from C_{18} SPE of spiked human plasma. Rapid separation of metformin (at 1.75 min) and phenformin (at 2.70 min) from theophylline and caffeine (8.9–9.2 min), 60 μg/mL each in the plasma sample. NH_4OAc (5 mM) + 5% HOAc in acetonitrile as the running buffer (pH 4.5); CE under 15 kV; room temperature = 22 °C; electrokinetic injection at 15 kV for 6 s; UV detection at 240 nm.

These two analytes migrated well ahead of theophylline and caffeine, which eluted as overlapping peaks (8.9–9.2 min). These results verified that metformin is negligibly bound to plasma proteins. No metabolites or conjugates of metformin could be identified. At the usual clinical doses and dosing schedules of metformin, steady state plasma concentrations of metformin are typically ~1 μg/mL. Peak metformin plasma levels would not exceed 5 μg/mL, even at maximum therapeutic doses of 850–1500 mg during controlled clinical trials [7]. Note that the relative height of metformin against phenformin is significantly lower in Fig. 7 compared to that in Fig. 3 although the concentration of the two drugs in each experiment was the same (60 μg/mL). It suggests that metformin could not be extracted quantitatively from plasma in Fig. 7. Although the extraction method might not be optimized, the method of standard additions can always be adapted to yield accurate results for metformin determination. For blank plasma sample, the blank matrix electropherogram exhibited no metformin or phenformin peaks at these migration times. Only a couple of small system peaks due to baseline perturbation by the sample solvents were observed in the time window from 8.9 to 9.2 min.

3.3. Linear dynamic range and detection limit

When metformin hydrochloride capsules were administered in a previous study by Song et al. and blood samples were assayed at regular time intervals, the results showed that the concentration of metformin in plasma reached a maximum of $C_{max} = 1.9$ μg/mL at $t_{max} = 3$–4 h [13]. A recent report by Tache et al. showed the variation of metformin concentration in plasma samples (from 12 healthy human volunteers) as a function of time after a single 500 mg oral dose administration. The maximum metformin concentration was 1.2–1.3 μg/mL, as found at 2–3 h after dose administration [11]. In the present SPE–CE method development, standard calibration curves were constructed over a concentration range from 1 to 30 μg/mL metformin and phenformin in plasma. With an electrokinetic injection time of 6 s and UV detection at 240 nm, linearity was observed from 1 to 15 μg/mL ($R^2 = 0.9600$ and 0.9506 and slopes = 7106 and 12,300 arbitrary units per μg/mL, for metformin and phenformin, respectively). Significant scattering of data points appeared between 15 and 30 μg/mL. Hence, the method would be best used for monitoring metformin and phenformin from 15 down to 1 μg/mL in human plasma. When the electrokinetic injection time was increased from 6 to 36 s, the metformin and phenformin peak areas increased linearly ($R^2 = 0.9804$ and 0.9650, respectively). Further increase of injection time to 42 s began to cause splitting of the metformin peak. Using an optimal injection time of 36 s, progressive dilution of the 1 μg/mL plasma sample with methanol + 3% acetic acid for CE–UV analysis demonstrated improvement in detection limits down to 12 ng/mL for metformin and 6 ng/mL for phenformin. However, precision and deviation from linearity were compromised.

AbuRuz et al. reported a determination method of metformin in plasma using ion pair solid phase extraction (IPSPE) technique and ion pair liquid chromatography [23]. In their method, the limit of detection and quantification was 3 and 5 ng/mL, respectively, which is more sensitive than the present CE method. In comparison with liquid chromatography (LC), however, CE offers the advantages of high efficiency and resolution, plus minimal sample and solvent requirements (the latter of which translates to low operating cost and environmental friendliness).

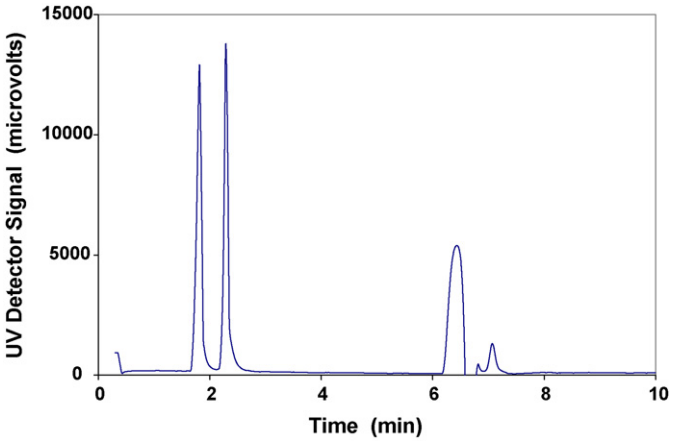

Fig. 8. CE analysis of the methanol + 3% acetic acid eluate from C_{18} SPE of spiked human plasma. Rapid separation of metformin (at 1.78 min) and phenformin (at 2.50 min) from glyburide (6.43 min), 60 μg/mL each in the plasma sample.

3.4. Internal standard method

Alternatively, the internal standard method was evaluated with an electrokinetic injection time of 6 s. By plotting the ratio of metformin and internal standard (phenformin) peak areas versus metformin concentration, a straight line was obtained in the concentration range of 0.2–3.5 μg/mL. The limit of detection was 0.1 μg/mL (S/N = 3). At the limit of quantification, an acceptable R.S.D. of 20% ($n = 5$) was obtained.

3.5. Glyburide

Glyburide, which is an oral blood-glucose-lowering drug of the sulfonylurea class, is usually combined with metformin hydrochloride. Under the brand name of Glucovance, these two antihyperglycemic agents (with complementary mechanisms of action) could improve glycemic control in patients with type 2 diabetes [24,25]. The SPE–CE–UV method was applied to analyze human plasma for metformin, phenformin and glyburide simultaneously. Using 3% acetic acid in methanol as the sample matrix, glyburide (at 6.43 min) was totally separated from metformin (at 1.78 min) and phenformin (at 2.50 min) as shown in Fig. 8. These results demonstrated how easy it was to separate the neutral glyburide molecules from the positively charged metformin and phenformin cations by CE.

4. Conclusion

The simultaneous determination of metformin, phenformin, glyburide and other drug compounds in human serum has

been demonstrated by capillary electrophoresis with solid phase extraction. The present SPE–CE method is unique in that rapid separation of metformin and phenformin from glyburide and other drug compounds is the merit of judicious optimization based on the basic, neutral and acidic functionalities of various SPE and CE steps. CE analysis was best performed using a non-aqueous buffer, acetonitrile + 5 mM ammonium acetate + 5% acetic acid. This buffer afforded rapid separation of metformin from phenformin (within 3 min) and glyburide (within 7 min), depending on the sample matrix composition. Potential applications of this newly developed SPE–CE methodology may fall into two pharmaceutical and biomedical analysis areas. First, the absolute bioavailability of a 500 mg metformin hydrochloride tablet given under fasting conditions is approximately 50–60%. Studies using single oral doses of metformin tablets, from 500 to 2550 mg, have indicated that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin, compared to the same tablet strength administered fasting. Second, intravenous single dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (i.e., no metabolites have been identified in humans) nor biliary excretion. The clinical relevance of these two related areas is important and further investigation would be greatly facilitated by the present SPE–CE methodology.

Acknowledgments

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References

[1] http://www.rxlist.com/cgi/generic/metformi.htm.
[2] A. Nourparvar, A. Bulotta, U. Di Mario, R. Perfetti, Trends Pharmacol. Sci. 25 (2004) 86.
[3] http://www.rxlist.com/cgi/generic2/glucovance_cp.htm.
[4] V. Fonseca, J. Rosenstock, R. Patwardhan, A. Salzman, JAMA 283 (2000) 1695.
[5] C.J. Glueck, P. Wang, R. Fontaine, T. Tracy, L. Sieve-Smith, J. Adolesc. Health 29 (2001) 160.
[6] http://www.aegis.org/pubs/aidswkly/2000/AW000903.html.
[7] C.K. Chu, Y.T. Chang, B.J. Lee, S.Y. Hu, W.H. Hu, D.Y. Yang, J. Chin. Med. Assoc. 66 (2003) 505.
[8] K.H. Yuen, K.K. Peh, J. Chromatogr. B 710 (1998) 243.
[9] O. Vesterqvist, F. Nabbie, B. Swanson, J. Chromatogr. B 716 (1998) 299.
[10] C.L. Cheng, C.H. Chou, J. Chromatogr. B 762 (2001) 51.
[11] F. Tache, V. David, A. Farca, A. Medvedovici, Microchem. J. 68 (2001) 13.
[12] M. Zhang, G.A. Moore, M. Lever, S.J. Gardiner, C.M.J. Kirkpatrick, E.J. Begg, J. Chromatogr. B 766 (2001) 175.
[13] J.Z. Song, H.F. Chen, S.J. Tian, Z.P. Sun, J. Chromatogr. B 708 (1998) 277.
[14] S.S.M. Hassan, W.H. Mahmoud, M.A.F. Elmosallamy, A.H.M. Othman, Anal. Chim. Acta 378 (1999) 299.
[15] I.H.I. Habib, M.S. Kamel, Talanta 60 (2003) 185.
[16] R. Huupponen, P. Ojala-Karlsson, J. Rouru, M. Koulu, J. Chromatogr. 583 (1992) 270.
[17] Calculated Using Advanced Chemistry Development (ACD) Software Solaris V4.67, http://www.cas.org/SCIFINDER/SCHOLAR.
[18] H. Siren, T. Hiissa, Y. Min, Analyst 125 (2000) 1561.
[19] C. Franke, H. Westerholm, R. Niessner, Water Res. 31 (1997) 2633.
[20] H. Yuan, J. Pawliszyn, Anal. Chem. 73 (2001) 4410.
[21] E.P.C. Lai, S.Y. Feng, Microchem. J. 75 (2003) 159.
[22] S.Y. Feng, E.P.C. Lai, E. Dabek-Zlotorzynska, S. Sadeghi, J. Chromatogr. A 1027 (2004) 155.
[23] S. AbuRuz, J. Millership, J. McElnay, J. Chromatogr. B 798 (2004) 203.
[24] http://www.rxlist.com/cgi/generic2/glucovance_cp.htm.
[25] X. Chen, Q. Gu, F. Qiu, D. Zhong, J. Chromatogr. B 802 (2004) 377.