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Simultaneous determination of glipizide and rosiglitazone unbound drug concentrations in plasma by equilibrium dialysis and liquid chromatography–tandem mass spectrometry

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Abstract

Glipizide and rosiglitazone are widely used to treat Type 2 diabetes. In order to investigate drug–drug protein binding interaction between glipizide and rosiglitazone, a method was developed and validated for simultaneously determining the free (unbound) fraction of glipizide and rosiglitazone in plasma employing equilibrium dialysis for the separation of free drug and liquid chromatography–tandem mass spectrometry (LC–MS/MS) for quantitation. Post-dialysis human plasma or buffer samples of 0.2 ml were extracted using a liquid–liquid extraction procedure and analyzed by a high performance liquid chromatography electrospray tandem mass spectrometer system. The compounds were eluted isocratically on a Zorbax SB-Phenyl column, ionized using an atmospheric pressure electrospray ionization source and analyzed in positive ion mode with multiple reaction monitoring. The ion transitions monitored were *m*/*z* 446 → 321 for glipizide, *m*/*z* 358 → 135 for rosiglitazone, and *m*/*z* 271 → 155 for tolbutamide (internal standard, IS). The chromatographic run time was 5 min per injection, with retention times of 2.3, 3.4 and 2.3 min for glipizide, rosiglitazone and IS, respectively. The calibration curves of glipizide and rosiglitazone were over the range of 1–2000 ng/ml ($r^2 > 0.9969$) in the combined matrix of human plasma and isotonic sodium phosphate buffer (1:1, v/v). The inter-assay precision and accuracy of the quality control samples were <10.9% of coefficient of variability and >93.5% and 94.5% of nominal concentration for glipizide and rosiglitazone, respectively. The lower limit of quantitation of both glipizide and rosiglitazone was 1.0 ng/ml. Both glipizide and rosiglitazone bound to plasma protein extensively (>99% bound). Glipizide and rosiglitazone free fraction averaged 0.678 ± 0.071 and 0.389 ± 0.061%, respectively, at plasma concentration of 1000 ng/ml. This developed method proves reproducible and sensitive and its application to clinical samples is also reported.

Keywords: Equilibrium dialysis; Glipizide; Rosiglitazone

1. Introduction

Glipizide and rosiglitazone are widely used to treat Type 2 (noninsulin-dependent) diabetes. Glipizide lowers blood glucose concentration by stimulating the pancreas to secrete insulin and in helping the body use insulin efficiently. Rosiglitazone is in a class of drugs called thiazolidinediones which work by increasing insulin sensitivity in target tissues, as well as decreasing hepatic gluconeogenesis [1]. The accurate determination of unbound (free) fraction of drug in plasma is essential in the therapeutic monitoring of drugs because only the unbound drug is available for distribution, elimination and pharmacodynamic interaction with receptors [2,3]. Especially for highly protein bound drugs (such as glipizide and rosiglitazone), fluctuations in the free fraction can impact the interpretation of total drug measurements. Unbound drug determinations may also be important for clinical studies where protein concentration may vary or drug displacement may occur. Methodologies for determination of unbound drug concentrations in plasma include equilibrium dialysis [4], ultrafiltration [5], ultracentrifugation [6], calorimetry [7], and capillary electrophoriesis

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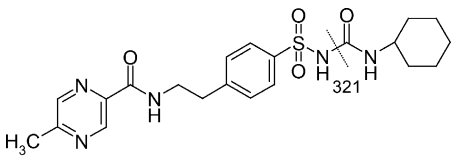
(CE) [8]. Extensive reviews on these methodologies including advantages and disadvantages of each methods can be found in the literature [9–11]. The protein binding has been reported to be 92–98% for glipizide and 99.8% for rosiglitazone, respectively [1]. However, to date, no published reports have dealt with the quantitation of the free fraction of glipizide and rosiglitazone in plasma. In order to evaluate a potential drug–drug protein binding interaction between glipizide and rosiglitazone, a sensitive and selective method was needed for simultaneously determining the free fraction of glipizide and rosiglitazone in plasma. Therefore, this paper describes a method employing equilibrium dialysis for separation of unbound drug from plasma and LC–MS/MS for the simultaneous quantitation of unbound and total glipizide and rosiglitazone in human plasma.

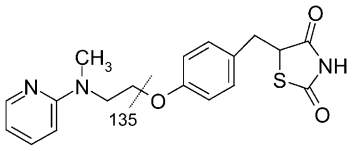
2. Experimental

2.1. Chemicals and reagents

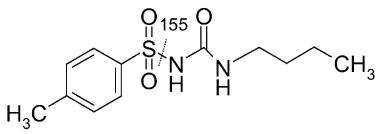
Glipizide (>98% purity) and tolbutamide as the internal standard (IS, 98% purity) were purchased from Aldrich (Milwaukee, WI, USA). Rosiglitazone (>99% purity) was purified at Avantix Laboratories, Inc. (New Castle, DE, USA). The chemical structures of glipizide, rosiglitazone and tolbutamide are shown in Fig. 1. Type I water was produced from an ultra high quality polishing system unit (UHQ-PS) (High Wycombe, Bucks, England). *N*-Butyl chloride (BC), methyl *t*-butyl ether (MTBE), acetonitrile, sodium hydroxide and hydrochloric acid were from Fisher Scientific (St. Louis, MO, USA). Ammonium acetate, sodium phosphate heptahydrate, potassium dihydrogen phosphate and sodium chloride were from Aldrich (Milwaukee, WI, USA), and triflouroacetic acid (TFA) was from Burdick & Jackson (Muskegon, MI, USA). Blank sodium heparinized human plasma was obtained from Bioreclamation, Inc. (Hicksville, NY, USA) and was stored frozen at −20 °C. Pooled human plasma from six different lots were used in this study. All mobile phase solvents were HPLC grade and all reagents were analytical reagent grade.



Glipizide



Rosiglitazone



Tolbutamide (IS)

Fig. 1. Chemical structures of glipizide, rosiglitazone, and the internal standard (IS, tolbutamide).

2.2. Equilibrium dialysis

The procedure for preparing plasma samples for free and total drug concentrations by equilibrium dialysis and LC–MS/MS is summarized in flow diagram (Fig. 2). Equilibrium dialysis was performed in cells made from Teflon with a compartment volume of 1.0 ml using a multi-equilibrium dialyser with variable speed drive unit (Amika Corp., Columbia, MD, USA). Dialysis membranes with very high permeability and a molecular weight cut-off of 10,000 Da were used (Harvard Apparatus, Holliston, MA, USA). Plasma samples were titrated to pH 7.4 ± 0.05 using either 1.0N hydrochloric acid or 0.1N sodium hydroxide. A 0.8 ml aliquot of plasma samples was dialyzed against 0.8 ml isotonic sodium phosphate buffer solution at pH 7.4 in a temperature controlled water bath at a nominal temperature of 37 ± 2 °C and rotated at a minimum of 22 rpm for 2.5 h. After dialysis was complete, the resulting plasma and buffer dialysates were promptly recovered from the Teflon cells and analyzed by LC–MS/MS after sample preparation.

2.3. Calibration standards and quality control samples

Standards and quality control samples (QCs) were made from two separate stock solutions (1 mg/ml in methanol for both glipizide and rosiglitazone). Two-in-one working calibration standards at concentrations of 1, 5, 20, 50, 200, 1000 and 2000 ng/ml were prepared daily in the combined matrix of blank human plasma and pH 7.4 isotonic sodium phosphate buffer (1:1, v/v). Six levels of two-in-one QC samples, 1, 2, 20, 200, 1000, and 2000 ng/ml, were prepared daily in the combined matrix for the determination of intra-assay and inter-assay accuracy and precision. Working standards and QCs were prepared by spiking the corresponding standard or QC acetonitrile solutions into the sample tubes, evaporating acetonitrile under nitrogen and dissolving the drugs with 1:1 (v/v) blank human plasma/isotonic sodium phosphate buffer (pH 7.4).

2.4. LC–MS/MS method

LC–MS/MS analyses were performed using a Hewlett Packard 1100 system (Wilmington, DE, USA) coupled to

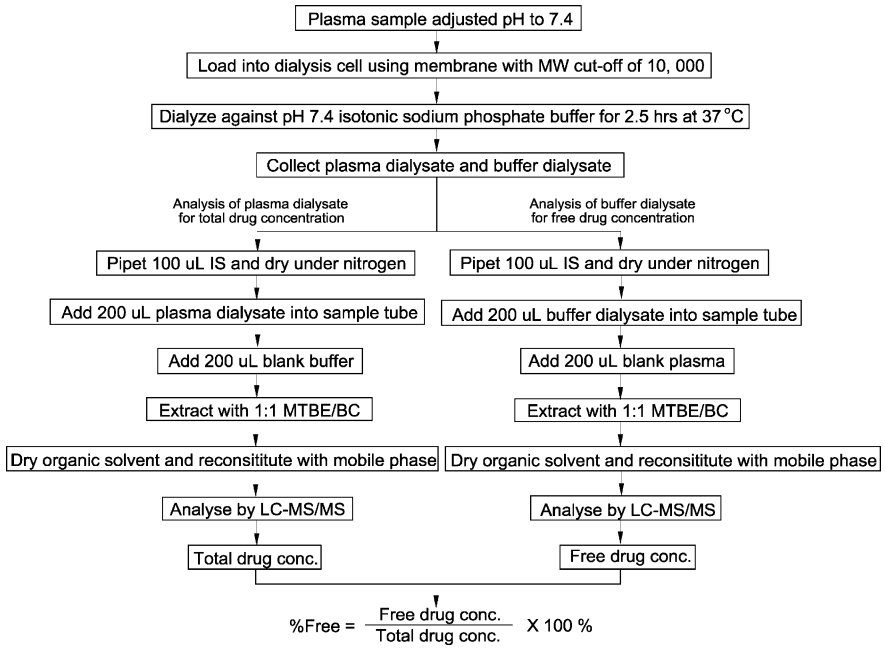


Fig. 2. A flow diagram for analyzing plasma samples for free and total drug concentrations by equilibrium dialysis and LC–MS/MS.

a Micromass Quattro LC triple-quadrupole mass spectrometer (Manchester, UK). The mass spectrometer was operated using an electrospray atmospheric pressure ionization source in positive ion mode (ESI^+) with multiple reaction monitoring (MRM). The analytical column was an Aglient Zorbax SB-Phenyl, 150 × 2.1 mm 5 μm (Wilmington, DE, USA). The mobile phase consisted of acetonitrile:water (50:50) with 10 mM ammonium acetate and 0.02% TFA at an isocratic flow rate of 0.3 ml/min. The sample injection volume was 10 μl and run time was 5 min. System carry-over was determined by injecting the highest calibration standard followed by a blank. No carry-over was observed.

Sensitivity of MRM was optimized by infusing a mixture of 50 ng/ml glipizide and rosiglitazone in the mobile phase. The capillary voltage was maintained at 3 kV. The cone and the extractor voltages were set to 20 and 3 V, respectively. The desolvation and ion source temperatures were 400 and 120 °C, respectively. Ions were activated at a collision energy of 20 eV and at an indicated argon pressure of 1.5×10^{-3} Torr. To assay all analytes, both quadrupoles were maintained at unit resolution and the transitions (precursor to daughter) monitored were m/z 446 → 321 for glipizide, m/z 358 → 135 for rosiglitazone, and m/z 271 → 155 for IS. The fragmentation patterns are shown in Fig. 1. The dwell time for each transition was 200 ms and the inter-channel delay was 20 ms.

MRM data were acquired and the chromatograms were integrated using MassLynx™ NT, version 3.2 software. A weighted ($1/x^2$) quadratic regression was used to generate calibration curves from standards and calculate the concentrations of samples.

2.5. Sample preparation

For calibration standards and QCs, 200 μl of standard or QC acetonitrile solutions and 100 μl of IS (500 ng/ml in acetonitrile) were added into the sample tubes, evaporated to dryness under nitrogen followed by adding 400 μl of 1:1 (v/v) blank human plasma/isotonic sodium phosphate buffer (pH 7.4) to the tubes. For post-dialysis plasma samples, 100 μl of IS (500 ng/ml in acetonitrile) was added into the sample tubes, evaporated to dryness under nitrogen followed by adding 200 μl of plasma sample and 200 μl of blank isotonic sodium phosphate buffer. For post-dailysis buffer sample, 100 μl of IS (500 ng/ml in acetonitrile) was added into the sample tubes, evaporated to dryness under nitrogen followed by adding 200 μl of buffer sample and 200 μl of blank plasma. All samples, standards and QCs were made slightly acidic by addition of 1 ml of 0.1 M aqueous ammonium acetate (pH 6), and were extracted into 4 ml of 1:1 methyl *t*-butyl ether/*n*-butyl chloride. The extraction tubes were shaken at high speed for 20 min followed by centrifugation at 4000 rpm for 20 min. The organic phase was transferred to clean glass tubes and evaporated to dryness in a 45 °C water bath under a nitrogen stream. The samples were dissolved in 100 μl of mobile phase and vortexed for 1 min. After transfer into glass inserts of autosampler vials and centrifugation for 5 min at 4000 rpm, an aliquot of 10 μl of each sample was injected onto the LC–MS/MS system.

2.6. Validation of the LC–MS/MS method

The method was validated for accuracy, precision, sensitivity, specificity, calibration function and reproducibility

according to the FDA guideline for bioanalytical method validation [12] over a concentration range of 1–2000 ng/ml using seven calibration standards in duplicate, each containing the two analytes of interest and five replicates of QC samples at each concentration level in three separate batch runs. Each batch run also contained additional samples such as stability samples for processing and storage.

Since the post-dialysis plasma and buffer samples were freshly generated and analyzed within 2 h after dialysis, only post-preparative stability and stock solution stability were determined while long-term stability at frozen condition was not determined. The extraction recoveries of glipizide and rosiglitazone were calculated by comparing the peak areas of extracted plasma standards to the peak areas of post-extraction plasma blanks spiked at corresponding concentrations. The matrix effect was evaluated by comparing the peak areas of post-extraction plasma blanks spiked with analytes and internal standard to those prepared in mobile phase at corresponding concentrations. The overall absolute recovery from human plasma was determined by comparing the peak areas of extracted plasma standards to those prepared in mobile phase. The method specificity was evaluated by screening six lots of blank sodium heparinized human plasma.

3. Results and discussion

3.1. LC–MS/MS method

The standards were prepared in the combined matrix of human plasma and isotonic sodium phosphate buffer (1:1, v/v) in order to quantify the free drug concentrations in buffer dialysate and the total drug concentrations in plasma dialysate using the same calibration curve. A Zorbax SB-Phenyl column with the mobile phase of acetonitrile and 10 mM ammonium acetate and 0.02% TFA (50:50, v/v) was used for separation, provided good separation of glipizide, rosiglitazone and IS, and maintained good peak shapes.

The electrospray ionization gave the optimum sensitivity for glipizide and rosiglitazone in positive ion mode. The Q1 mass spectrum of glipzide, rosiglitazone and IS showed protonated molecular ions $[M + H]^+$ at *m/z* 446, 358 and 271, respectively. The product ion scan spectrum of *m/z* 446 for glipizide, *m/z* 358 for roisglitazone and *m/z* 271 for IS showed high abundance fragment ions at *m/z* 321, 135 and 155, respectively (Fig. 3). The ion transitions of *m/z* 446 → 321 for glipizide, *m/z* 358 → 135 for rosiglitazone and *m/z* 271 → 155 for IS were chosen for multiple reaction monitoring.

3.2. Specificity, sensitivity and calibration curve range

Blank human plasma samples from six different subjects were extracted and analyzed for glipizide and rosiglitazone as a true blank (double blank), spiked with IS, or with glipizide and rosiglitazone as a single blank. There were no endogenous peaks that interfered with the quantitation of glipizide, rosiglitazone or IS. There was no interference from IS contributing to the glipizide or rosiglitazone *m/z* channels or from glipizide and rosiglitazone contributing to the IS *m/z* channel. The ratio of signal to noise from an extracted lower limit of quantitaion (LLOQ) sample (1 ng/ml) was at least 30 for glipizide and rosiglitazone. There was no significant lot-to-lot variation in matrix effect and no carry-over from highest calbration standard to blank sample observed. Calibration curves were over the concentration range of 1–2000 ng/ml using the quadratic regression with a weighting factor of the reciprocal of the concentration squared ($1/x^2$) for glipizide and rosiglitazone. The coefficients of determination (r^2) were 0.9969 or better for both glipizide and rosiglitazone. Representative chromatograms of blank combined matrix, blank combined matrix spiked with glipizide and rosiglitazone at LLOQ, an extracted post-dialysis buffer sample and an extracted post-dialysis plasma sample are shown in Figs. 4–7.

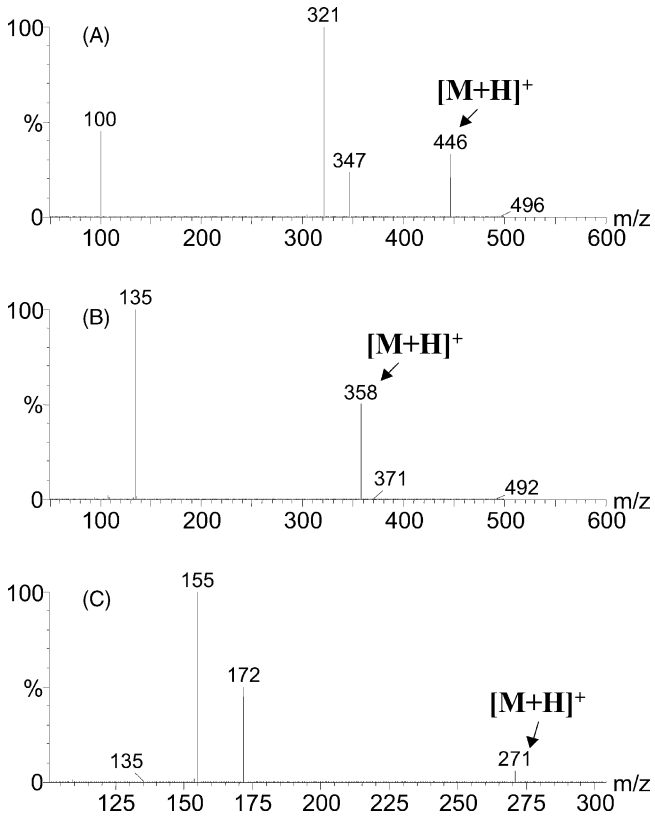


Fig. 3. Daughter scan spectrum of *m/z* 446 for glipizide (A), *m/z* 358 for rosiglitazone (B), and *m/z* 271 for IS.

3.3. Precision and accuracy

Table 1 shows the validation data on accuracy and precision of each standard concentration. The inter-assay coefficient of variation (CV) for the back-calculated calibration standards ranged from 3.4 to 12.6% for glipizide and 3.3

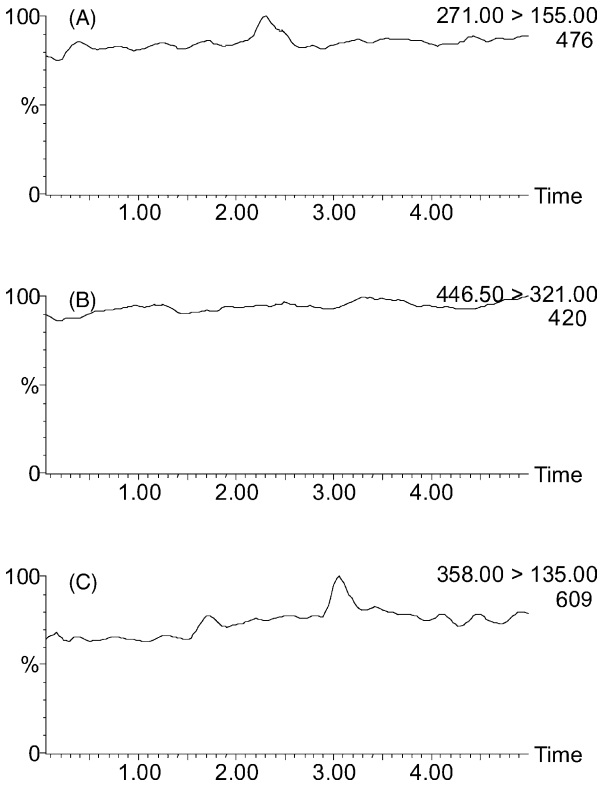


Fig. 4. Chromatograms of an extracted combined blank matrix (1:1 human plasma/buffer). (A) IS channel: *m*/*z* 271 → 155; (B) glipizide channel: *m*/*z* 446 → 321; (C) rosiglitazone channel: *m*/*z* 358 → 135.

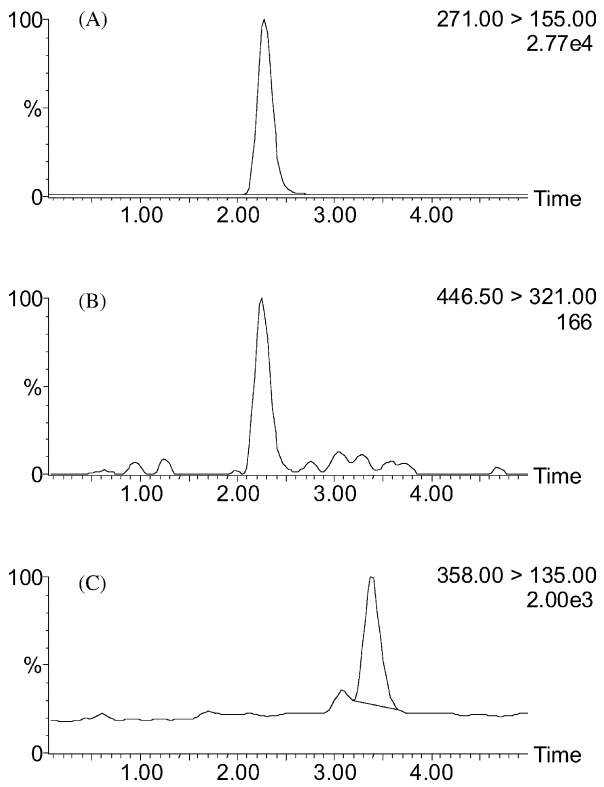


Fig. 5. Chromatograms of an extracted combined blank matrix (1:1 human plasma/buffer) spiked with glipizide and rosiglitazone at LLOQ (1 ng/ml). (A) IS channel: *m*/*z* 271 → 155; (B) glipizide channel: *m*/*z* 446 → 321; (C) rosiglitazone channel: 358 → 135.

to 8.8% for rosiglitazone and the nominal concentrations ranged from 96.7 to 103.4% for glipizide and 97.0 to 102.0% for rosiglitazone, respectively. The precision and accuracy data for QCs are summarized in Table 2. Inter-assay CV values were less than 10.9% for glipizide and 7.6% for rosiglitazone and the nominal concentrations ranged from 93.5 to 103.7% for glipizide and 94.5 to 101.2% for rosiglitazone, respectively. Intra-assay CV values were less than 9.1% for glipizide and 8.9% for rosiglitazone and the nominal concentrations ranged from 83.9 to 107.8% for glipizide and 98.2 to 108.0% for rosiglitazone, respectively. These CV and nominal concentration values indicated reproducible LC–MS/MS conditions and that the assay is consistent and reliable.

Table 1
Precision and accuracy of calibration standards ($N = 6$)

Nominal (ng/ml)	Glipizide			Rosiglitazone		
	Mean	%CV	%Nominal	Mean	%CV	%Nominal
1	1.03	12.6	103.0	0.970	6.4	97.0
5	5.05	5.9	101.0	5.02	8.8	100.4
20	20.4	5.1	102.0	20.3	4.1	101.5
50	51.7	5.9	103.4	50.1	3.3	100.2
200	201	6.4	100.5	204	3.4	102.0
1000	967	3.6	96.7	986	5.4	98.6
2000	2036	3.4	101.8	2010	3.6	100.5

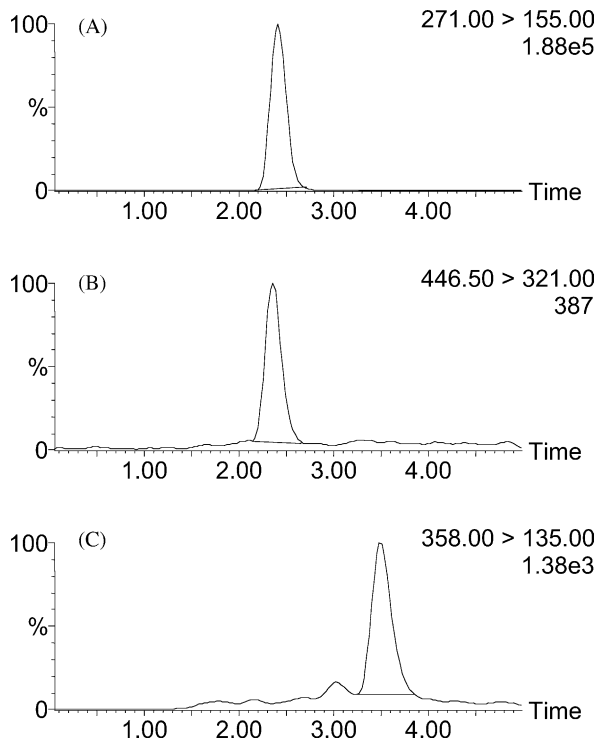


Fig. 6. Chromatograms of an extracted post-dialysis buffer sample (subject 1010, Day 21, 4 h). (A) IS channel: *m*/*z* 271 → 155; (B) glipizide channel: 446 → 321; (C) rosiglitazone channel: *m*/*z* 358 → 135.

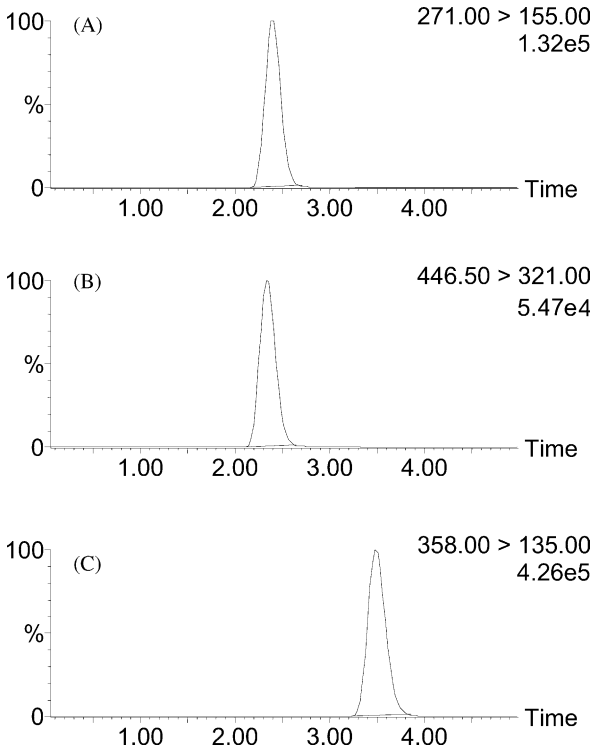


Fig. 7. Chromatograms of an extracted post-dialysis plasma sample (subject 1010, Day 21, 4 h). (A) IS channel: *m/z* 271 → 155; (B) glipizide channel: *m/z* 446 → 321; (C) rosiglitazone channel: *m/z* 358 → 135.

3.4. Recovery and matrix effect

The extraction recovery was determined by comparing the peak areas of extracted standards at 0.5 and 2000 ng/ml in triplicate to the peak areas of post-extraction combined matrix blanks spiked at corresponding concentrations. Extraction recovery from the combined matrix ranged from 85.2 to 86.7% for glipizide, 84.3 to 93.5% for rosiglitazone and 66.1 to 71.9% for IS, respectively. The overall absolute recovery ranged from 80.2 to 81.6% for glipizide, 73.4 to 77.6% for risoglitazone and 49.6 to 58.9% for IS, respectively, indicating the matrix effect was less than 17% for glipizide and rosiglitazone and 25% for IS, respectively.

3.5. Stability of the analytes

Glipizide and rosiglitazone were stable throughout the equilibrium dialysis (37 °C up to 6 h) and extraction procedure. Extracted analytes were allowed to stand at ambient temperature in mobile phase for at least 63 h prior to LC–MS/MS analysis, with no observed effect on quantitation. Stability of stock solutions was also investigated. When stock solutions of glipizide and rosiglitazone were stored at a nominal temperature of 4 °C for one month or at room temperature for 6 h, the analytes were stable.

3.6. Precision of free glipizide and rosiglitazone determination

The equilibrium dialysis time between plasma and buffer was investigated. The plasma spiked with a fixed amount of glipizide and rosiglitazone (1000 ng/ml) was dialyzed against isotonic sodium phosphate buffer (pH 7.4) for 1, 2, 4 and 6 h. Both glipizide and rosiglitazone reached equilibrium after 2 h. Therefore, a dialysis time of 2.5 h was used for all subsequent equilibrium dialysis. The adsorption of glipizide and rosiglitazone to the membrane and Teflon cell walls was also investigated. There was no drug loss due to adsorption. The precision of free glipizide or rosiglitazone determination using equilibrium dialysis and LC–MS/MS was evaluated by dialyzing plasma spiked with 1000 ng/ml of glipizide (or rosiglitazone) or in the presence of 1000 ng/ml of rosiglitazone (or glipizide) against pH 7.4 isotonic sodium phosphate buffer followed by LC–MS/MS analysis as described. The free fraction of glipizide and rosiglitazone and the precision of the determination were

Table 2
Precision and accuracy of quality control samples

Nominal (ng/ml)	Glipizide			Rosiglitazone		
	Mean	%CV	%Nominal	Mean	%CV	%Nominal
Intra-assay ($N = 5$)						
1	0.839	9.1	83.9	0.982	1.7	98.2
2	1.91	4.2	95.5	1.98	3.5	99.0
20	21.6	2.2	107.8	21.6	2.9	108.0
200	210	3.6	104.8	209	8.9	104.7
1000	937	3.3	93.7	1002	4.4	100.2
2000	1988	5.1	99.4	2033	5.7	101.7
Inter-assay ($N = 15$)						
1	0.984	9.6	98.4	1.00	5.6	100.0
2	2.02	10.9	101.2	1.89	6.7	94.5
20	20.7	4.5	103.7	20.0	7.6	100.2
200	207	3.9	103.7	202	6.4	101.2
1000	935	2.9	93.5	965	4.4	96.5
2000	1971	5.2	98.5	1975	6.2	98.7

Table 3
Precision of glipizide and rosiglitazone unbound drug (%Free) determination by equilibrium dialysis and LC–MS/MS

	Glipizide		Rosiglitazone	
	Alone	With rosiglitazone (1000 ng/ml)	Alone	With glipizide (1000 ng/ml)
Mean ($N = 5$)	0.678	0.721	0.389	0.408
S.D.	0.071	0.037	0.061	0.047
%CV	10.5	5.1	15.7	11.5

Table 4
Dose schedule of glipizide and rosiglitazone in a drug–drug protein binding interaction clinical study

Study day	Glipizide (15 mg)	Rosiglitazone (4 mg)
1	×	–
2–7	Washout	–
8 and 9	–	×
10	×	×
11–20	–	×
21	×	×

(×) Drug received; (–) No drug received.

summarized in Table 3. Both glipizide and rosiglitazone were bound extensively to plasma protein (>99% bound). Glipizide free fraction averaged 0.678±0.071% and 0.721± 0.037% with CV less than 10.5% at a plasma concentration of 1000 ng/ml and in the presence of 1000 ng/ml of rosiglitazone, respectively. Rosiglitazone free fraction averaged 0.389 ± 0.061% at a plasma concentration of 1000 ng/ml, and 0.408 ± 0.047% in presence of 1000 ng/ml of glipizide with CV less than 15.7%. The results were calculated without consideration of observed slight volume shifts during dialysis.

3.7. Applicability of method

The developed method proved reproducible and sensitive and was applied to human plasma samples from a study of drug–drug protein binding interaction between glipizide and rosiglitazone. In this study, a total of 12 male and 12 female subjects received multiple doses of rosiglitazone with and without glipizide according to the dose schedule listed in Table 4. The Student's *t*-test was used to assess differences under experimental conditions. A *P*-value of less than 0.05 was considered significant. When administered glipizide alone, the mean %Free value was 1.15 ± 0.20%. Upon concurrent adminstration of rosiglitazone, the glipizide %Free remained the same, 1.15 ± 0.18 and 1.14 ± 0.15% for Days 10 and 21, respectively. No statistically significant difference was detected. The drug–drug protein

Table 5
Protein binding (%Free) summary for glipizide and rosiglitazone drug–drug protein binding interaction study[a]

		N[b]	Mean	S.D.	%CV
Glipizide					
Glipizide alone (Day 1, 4 h)	Male	10	1.18	0.27	22.9
	Female	11	1.13	0.10	8.8
	Overall	21	1.15	0.20	17.4
Glipizide + multiple dose of rosiglitazone (2 days) (Day 10, 4 h)	Male	12	1.20	0.22	18.3
	Female	11	1.10	0.11	10.0
	Overall	23	1.15	0.18	15.7
Glipizide + multiple dose of rosiglitazone (14 days) (Day 21, 4 h)	Male	10	1.15	0.19	16.5
	Female	10	1.14	0.10	8.8
	Overall	20	1.14	0.15	13.2
Rosiglitazone					
Rosiglitazone alone (Day 8, 4 h)	Male	10	0.432	0.182	42.1
	Female	11	0.313[a]	0.042	13.4
	Overall	21	0.370	0.139	37.6
Glipizide + multiple dose of rosiglitazone (2 days) (Day 10, 4 h)	Male	10	0.386	0.100	25.9
	Female	11	0.297[a]	0.033	11.1
	Overall	21	0.339	0.084	24.8
Glipizide + multiple dose of Rosiglitazone (14 days) (Day 21, 4 h)	Male	9	0.436	0.144	33.0
	Female	10	0.328[a]	0.047	14.3
	Overall	19	0.379	0.116	30.6

[a] Statistically significant difference when compared to male subjects, $P < 0.05$.
[b] Outliers were excluded before statistical analysis.

binding interaction study data are summarized in Table 5. When rosiglitazone was given alone (Day 8, 4 h), the mean %Free value was $0.370 \pm 0.139\%$. Upon concurrent administrations of glipizide, the binding of rosiglitazone remained the same. On Days 10 and 21, the %Free values were 0.339 ± 0.084 and $0.379 \pm 0.116\%$, respectively. No statistically significant difference was detected. However, a small gender effect was detected for rosiglitazone. The plasma protein binding for rosiglitazone in female was higher than in male as shown by a decreasing %Free of approximately 16%.

4. Conclusions

A novel and reliable method for simultaneous determination of glipizide and rosiglitazone free fraction in human plasma using equilibrium dialysis for the separation of free (unbound) drug and LC–MS/MS for quantitation has been developed and validated. This method proved reproducible and sensitive and has been used successfully to support a clinical drug–drug protein binding interaction study between glipizide and rosiglitazone. No binding interaction was observed between glipizide and rosiglitazone in this clinical study. However, gender difference was observed for rosiglitazone, as shown by a higher binding in female than in male.

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