Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 846 (2007) 222–229
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of glibenclamide in human plasma by liquid chromatography and atmospheric pressure chemical ionization/MS-MS detection

Florin Albu [a], Cristina Georgiţă [a], Victor David [b], Andrei Medvedovici [a,b,*]

[a] *LaborMed Pharma S.A., Splaiul Independentei 319, Bucharest – 6, Romania*
[b] *University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Sos. Panduri 92, Bucharest – 5, Romania*

Received 19 July 2006; accepted 3 September 2006
Available online 29 September 2006

Abstract

A simple and fast method intended for large-scale bioequivalence studies for the determination of glibenclamide in plasma samples is presented. The chromatographic separation was achieved on a monolithic octadecyl chemically modified silicagel column and a mobile phase containing 42% aqueous 0.1% HCOOH solution (v/v) and 58% acetonitrile, at a flow rate of 1 mL/min, in isocratic conditions. Preparation of plasma samples was based on protein precipitation with acetonitrile. Gliquidone was used as internal standard. The target analytes were transferred into an ion trap mass analyzer via an atmospheric pressure chemical ionization interface. The precursor ions with mass 494 a.m.u. for glibenclamide and 528 a.m.u. for gliquidone were isolated, while in the second MS stage product ions 369 a.m.u. and 403 a.m.u., respectively, were monitored. The analytical process was characterized by a low limit of quantitation of 1.5 ng/mL. The mean recovery for glibenclamide was 98.1 ± 2.8% over a concentration interval ranging from 1 to 500 ng/mL. Intra-day and inter-day precision calculated over 2–400 ng/mL concentration interval ranged from 15.4% to 3.4%. Inter-sequence accuracy expressed as % bias from theoretical concentration values over the concentration interval of 10–400 ng/mL fall within −13.9% and +14.6%. The method was applied for evaluation of the bioequivalence between two formulations containing 3.5 mg glibenclamide per dose.

Keywords: Glibenclamide; Bioequivalence; Human plasma; HPLC; APCI; MS/MS; Method development; Method validation; Pharmacokinetic parameters

1. Introduction

Glibenclamide (known also as glyburide) is a sulfonylurea hypoglycaemic drug used for type 2 diabetes mellitus [1]. About 90% of the oral drug is absorbed from an empty stomach in 1.5–2 h. Food decreases absorption of glibenclamide. About 97% is bound to plasma albumin as a weak acid anion, and hence, it is susceptible to displacement by many weak acid drugs. Computation of the hydrophobicity parameter, octanol/water partition constant ($\log K_{o,w}$), by means of the fragment methodology [2] revealed a relatively high value, i.e. $\log K_{o,w} = 4.79$, that could explain the affinity to plasma matrix. Elimination occurs by hepatic metabolism resulting in a half-time of 1.5–5 h.

Literature data reports several methods for the determination of glibenclamide in biological samples. Most of them are based on the reversed-phase separation mechanism with C18 [3–13], or C8 stationary phases [14,15]. Normal-phase separation mechanism using silica stationary phase was also mentioned [16]. In almost all cases the detection was done by UV absorption spectrometry, excepting two approaches based on fluorescence [6,16], reaching 2–10 ng/mL as detection limits. The most common sample preparation procedure applied to plasma samples was liquid–liquid extraction, using different solvents, such as: dichloromethane, hexane, ethyl ether, benzene, toluene, ethyl acetate, or chloroform. Solid-phase extraction was also reported as convenient for plasma removal and analyte concentration from samples [6,10].

Recently, two new methods have been proposed for rapid determination of this drug from human plasma, which were based on electrospray mass spectrometry detection [17,18]. In the present study liquid–liquid with an atmospheric pressure chemical ionization (APCI) has been preferred to determine glibenclamide in plasma samples from healthy volunteers treated orally with this drug. Short separation times and high

* Corresponding author. Tel.: +40 723 330 423; fax: +40 21 221 36 92.
E-mail address: avmedved@yahoo.com (A. Medvedovici).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.09.005

sensitivity, without compromising the selectivity, are the main advantages of such a technique. In order to eliminate major sources of experimental errors, the sample preparation procedure used in this study is not based on the tedious liquid–liquid extraction [19]. Quantitation limit could be improved in the ng/ml range, even using moderate injection volumes (50 μL). The entire method has been applied to the bioequivalence study of two commercial formulations containing glibenclamide. Validation of the entire process is presented and discussed.

2. Experimental

2.1. Instrumentation

Experiments were performed with an Agilent 1100 series LC/MSD (Agilent Technology, Waldbronn, Germany) system consisting of the following modules: degasser (G1379A), quaternary pump (G1311A), thermostated autosampler (G1329A), column thermostat (G1316A), AP-ESI standard interface (G1948A), ion trap mass spectrometric detector SL series (G24450), and nitrogen generator (5183-2003). System control and data acquisition were made with the Agilent LC/MSD trap software version 4.2 incorporating the MSD Trap Control software version 5.1 from Brucker Daltronics. The system was operationally qualified before and after the bioequivalence study.

2.2. Chromatographic method

A single monolithic Chromolith Performance RP-18e column (Merck, Germany), 100 mm length and 4.6 mm internal diameter fitted with a Chromolith Guard Cartridge RP-18e (10 mm × 4.6 mm) was used during the validation stage and entire bioequivalence study. Column was validated before and after study completion, by computing the lowest value corresponding to the height equivalent to the theoretical plate (HETP) in case of the fluoranthene peak (a variation from 1.47 to 1.53 μm was noticed during the whole process, meaning around 1100 injected samples). The column was thermostated at 40 °C.

Isocratic elution was applied, using a mobile phase containing 42% aqueous 0.1% (v/v) HCOOH solution and 58% acetonitrile, at a flow rate of 1 mL/min. Injection volume was 50 μL.

2.3. Interface parameters

The parameters controlling the APCI–MS interface were as following: drying gas (N_2) temperature: 300 °C; vaporizer temperature: 350 °C; drying gas flow: 5 L/min; pressure of the nebulizer gas: 60 psi; capillary voltage: 4500 V; high voltage end plate offset: −500 V; corona discharge: 5000 V.

MS signals were monitored as following: 0–2.4 min—divert valve oriented to purge; 2.4–3.8 min—divert valve oriented to APCI – analyte detection (product ion $m/z = 369$ a.m.u.); 3.8–5.5 min—divert valve oriented to APCI – internal standard detection (product ion $m/z = 403$ a.m.u.).

2.4. Materials

All solvents were HPLC grade from Merck (Darmstadt, Germany). Water for chromatography (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6 UV/UF instrument and used during experiments. Glibenclamide and I.S. (gliquidone) as standard reference substances were purchased from European Pharmacopoeia, Council of Europe, Strasbourg, France and from LGC Promochem, Wesel, Germany (glibenclamide, batch 1a, cat. no. EP G0325000, and internal standard, cat. no. BP 580).

2.5. Methodology and pharmacokinetic application

The developed method was applied to an open-label, randomized, two-period, two-sequence, crossover study, 24 healthy volunteers (male/female ratio = 19/5) with an mean age of 24 years received one dose of 3.5 mg glibenclamide of the tested product (T) and one of the reference product (R), in the sequence determined by randomization, with a 14 days wash-out period between consecutive administrations. The protocol of the study was formally accepted by the evaluation department of the Romanian National Drug Agency and received the approval of the Institutional Ethics Committee. Venous blood samples were collected pre-dose (0 h) and the following post-dose intervals of time: 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 24, 48 and 75 h. Medical examinations were performed in the screening and at the beginning of each study period (in-house day), in every single blood sampling days and at the end of each study period.

The pharmacokinetic parameters considered for evaluation of the bioequivalence between tested and reference products were: C_{max}—observed maximum plasma concentration of glibenclamide; T_{max}—sampling time of the maximum plasma concentration; t_{half}—terminal elimination half life time; AUC_{last}—area under plasma concentration/time plot until the last quantifiable value; AUC_{total}—area under plasma concentration/time plot extrapolated to infinity. Pharmacokinetic parameters were determined by means of the Kinetica™ software (version 4.4.1.) from Thermo Electron Corporation, U.S.A. The analysis of variance was performed on the pharmacokinetic parameters. Then, the 90% confidence intervals of the pharmacokinetic parameters characterizing the tested/reference products were determined.

3. Results and discussions

3.1. Choice of internal standard

An appropriate choice for the internal standard will attenuate errors induced during the sample preparation procedure, injection, and detection variability. In LC–MS/MS it is recommended to use an internal standard with a molecular weight very close to that of the analyte in order to narrow the scan range, to increase data acquisition and to obtain a higher sensibility [20]. Also the product ions must have similar molecular weights for the same reason. Two of the HPLC–MS methods already reported by the literature [17,19] proposed glipizide (molecular

weight = 445.55) as internal standard (I.S.) for the determination of glibenclamide (molecular weight = 493.14).

Gliquidone (1-cyclohexyl-3-*p*-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo2(1*H*)-isoquinolyl)ethyl] phenyl sulphonylurea (having the molecular weight 527.21) has been preferred as internal standard, due to its hydrophobic character closest to glibenclamide ($\log K_{o,w} = 4.65$) while glipizide is less apolar ($\log K_{o,w} = 3.34$). Similar behavior is expected during sample preparation stage as well as a chromatographic separation achieved faster. Glibenclamide and gliquidone are sulfonylurea derivatives having similar fragmentations pattern due to the breakage of S-N bound predominantly. Such process is enough probable, as far as the corresponding fragments can be produced directly in the APCI interface, with relatively low intensity. The breakdown of the precursor ions is completed in the CID stage, resulting in protonated sulfonamide fragments as product ions. MS and $(MS)^2$ spectra of both target analytes are given in Fig. 1.

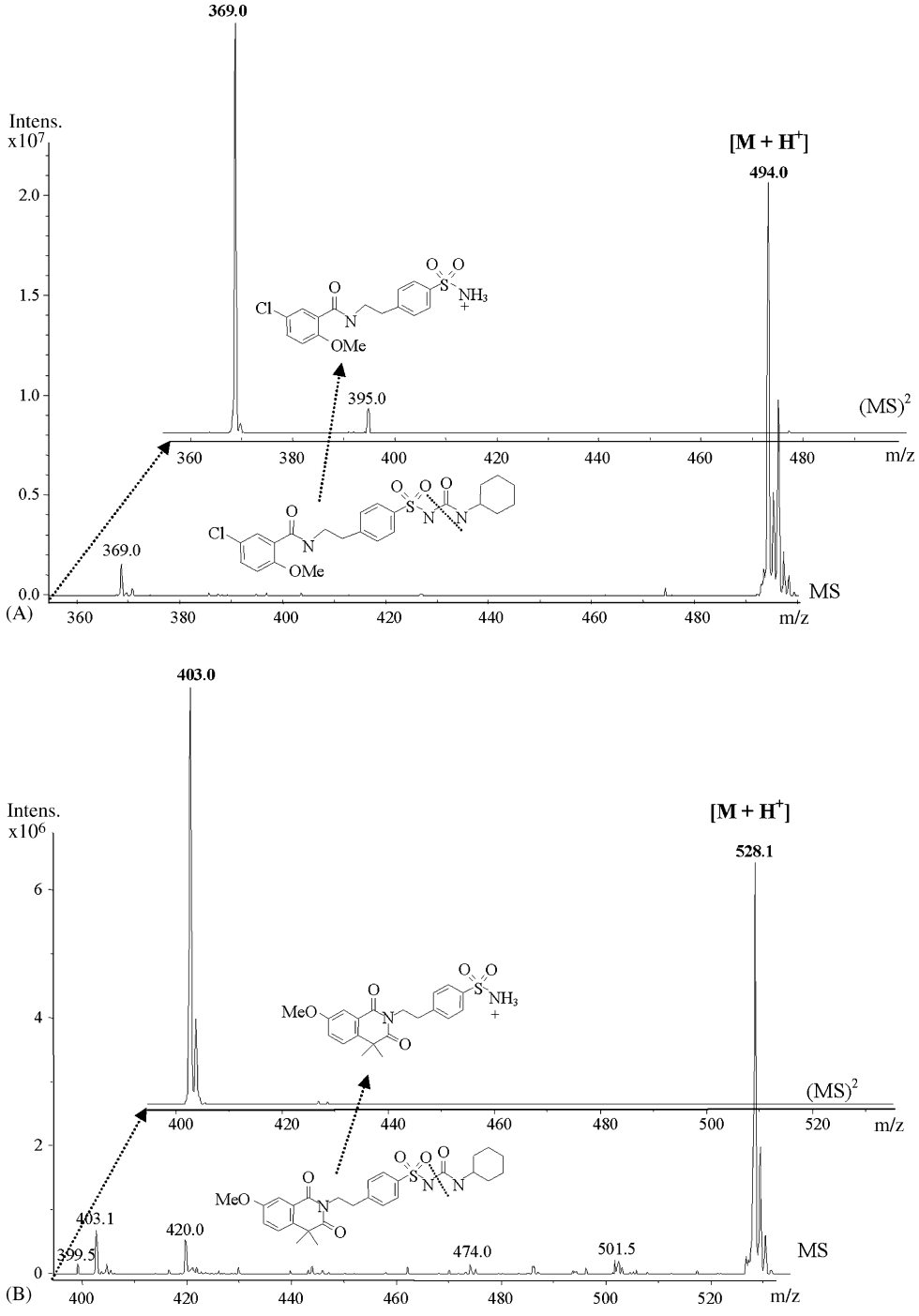

Fig. 1. Ionization pattern of glibenclamide (A) and internal standard (B) in the APCI interface.

Table 1
Recovery of glibenclamide from plasma samples at different concentration levels (the concentration of the internal standard is 500 ng/mL)

Analyte	Concentration (ng/mL)	Recovery (%)	RSD% ($n = 5$)
Glibenclamide	1	100.1	2.34
	50	97.5	0.90
	200	97.9	3.18
	500	96.9	2.01
	Average	98.1 ± 2.8	–
Internal standard	500.0	90.6	2.69

3.2. Sample preparation

The sample preparation method was finally set up to the following steps: −0.2 mL of a solution containing 0.1 μg of internal standard in acetonitrile is added to 0.2 mL of plasma sample and vortexed for 1 min at 2000 rpm; then, 0.2 mL of acetonitrile is added again, with a vortexing period of 3 min; sample was centrifuged 5 min at 800 × *g* (earth's gravitational field) (at 20 °C); to the resulting supernatant, 0.2 mL of water is added, followed by a vortex period of 3 min at 2000 rpm and injection of a 50 μL aliquot.

Addition of the organic solvent to plasma in two distinct stages (first aliquot contains internal standard) allows a better reproducibility in terms of recovery for the I.S. The sample preparation procedure was characterized by high recoveries for both glibenclamide and I.S., which are independent with respect to concentration, as it can be observed from the Table 1. An acceptable recovery (90.6%) has been obtained for the internal standard at the concentration value used through the study. Recoveries for the target compounds were calculated against spiked aqueous solutions prepared in the same way as the plasma samples.

As a conclusion, protein precipitation by means of the addition of an organic solvent readily keeps glibenclamide and I.S. dissolved. Dilution of the supernatant with water makes the composition of the sample closer to the composition of the mobile phase, to avoid solvent focusing effects on injection of higher volumes. An injection volume of 50 μL was preferred, but a 10–18 folds increase of this parameter is possible without affecting peak symmetry and retention. If necessary, the quantitation limit can be easily reduced to 100 pg/mL by increasing the injection volume.

3.3. Selectivity of the chromatographic method

The chromatographic method separates target compounds with an increased resolution ($R_S = 3.6$). Tandem mass spectrometric detection adds its own selectivity against the plasma endogenous components still remaining in samples after preparation. During the method validation the selectivity was been proved for six blank plasma samples. Additionally, in all predose collected plasma samples from volunteers participating to the study, no endogenous interference was observed. In Fig. 2 two overlaid chromatograms are given in order to prove the selectivity of the chromatographic method at very low level of concentration.

Residual peak areas in blank samples over the whole study ranged from 1.3% to 14.5% (mean value 6.6%) from the peak area of glibenclamide corresponding to LLOQ (2 ng/mL).

The method was also checked for selectivity against the major degradation impurity of the target drug, (named according to Eur. Ph. V Ed. as glibenclamide impurity A [21]. No interference was observed.

3.4. Robustness of the chromatographic method

Several parameters of the chromatographic process have been studied as part of the validation of the entire analytical process.

Retention of both analytes increases with the increase of the proportion of the aqueous constituent of the mobile phase (C_{aq})

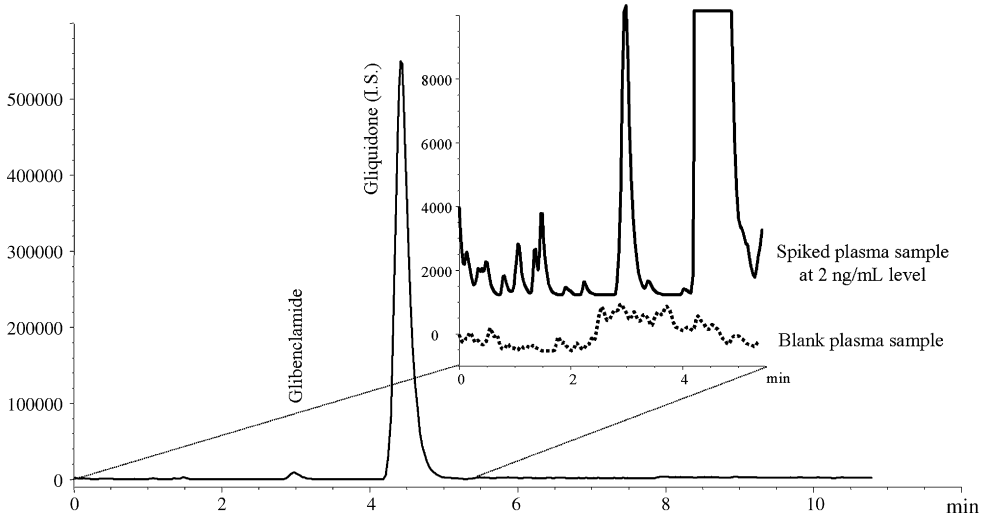

Fig. 2. Chromatogram of a plasma sample spiked with 2 ng/mL glibenclamide and 500 ng/mL internal standard, with a zoomed window for the analyte of interest, overlaid to the blank plasma (pointed line).

Table 2
Intra- and inter-day precision for spiked plasma samples

Spiked concentration (ng/mL)	Parameter	Intraday ($n=10$)		Interday ($n=6$)	
		Mean ± 2s	RSD %	Mean ± 2s	RSD %
2	Peak area (units × 10^6)	0.082 ± 0.026	16.3	0.093 ± 0.018	9.8
	Analyte/I.S. peak area ratio	0.015 ± 0.008	14.6	0.014 ± 0.003	9.8
	Experimental conc. (ng/mL)	1.99 ± 0.6	15.4	2.13 ± 0.4	10.3
50	Peak area (units × 10^6)	1.768 ± 0.314	6.1	2.068 ± 0.473	11.4
	Analyte/I.S. peak area ratio	0.287 ± 0.022	3.9	0.305 ± 0.058	9.4
	Experimental conc. (ng/mL)	46.9 ± 3.6	3.9	49.8 ± 9.4	9.4
200	Peak area (units × 10^6)	6.405 ± 0.801	6.3	8.389 ± 2.458	14.7
	Analyte/I.S. peak area ratio	1.156 ± 0.090	3.9	1.235 ± 0.196	7.9
	Experimental conc. (ng/mL)	188.8 ± 14.6	3.9	201.9 ± 32	7.9
400	Peak area (units × 10^6)	12.953 ± 1.434	5.5	14.557 ± 3.591	12.3
	Analyte/I.S. peak area ratio	2.270 ± 0.172	3.8	2.296 ± 0.155	3.4
	Experimental conc. (ng/mL)	371.1 ± 28.1	3.8	375.3 ± 15.4	3.4

following polynomial dependences ($t_R = a + b_1 C_{aq} + b_2 C_{aq}^2$): (i) for glibenclamide: $a = 8.448$; $b_1 = -0.388$; $b_2 = 0.00616$; $r^2 = 0.9980$; (ii) internal standard: $a = 22.566$; $b_1 = -1.152$; $b_2 = 0.01715$; $r^2 = 0.9991$, $C_{aq} \in [36\%, 48\%]$. By means of these relationships one can calculate that a maximum variation of ±0.5% in the composition of the mobile phase will induce variations of the retention time values for both compounds within their normal variation interval ($t_R \pm 2$ s, RSD = 2%). No influence on peak area values has been observed. Chromatographic resolution ranged between 2.7 and 7.5.

Relation of retention with column temperature (°C) was studied in the interval of 35–45 °C, leading to the following linear relationships ($t_R = a + bt$): (i) glibenclamide: $a = 3.769$; $b = -0.019$; $r^2 = 0.9806$; (ii) Internal standard: $a = 5.938$; $b = -0.038$; $r^2 = 0.9808$. Temperature variations within the range 40 ± 4 °C place retention time values for the both compounds within their normal interval of variation. In such conditions, chromatographic resolution ranged between 2.9 and 4.5.

Experiments performed with addition of 0.05%, 0.1% and 0.2% formic acid in the aqueous component of the mobile phase induce no significant variations in the retention behaviour of the target analytes and detector response.

Changes of the acidic additive in the aqueous component of the mobile phase do not significantly influence the retention behaviour of the target analytes. The use of acetic acid in the aqueous component of the mobile phase does not significantly affect the ionization yield of the target analytes. The use of trifluoroacetic acid as additive in the mobile phase increases the ionization yield for both compounds, practically doubling the peak areas. For column safety reasons, trifluoroacetic acid was not used as mobile phase additive (also considering that method sensitivity is not critical).

Chromolith columns from three different batches have been tested for the reproducibility of retention data. Moreover, a single one has been used during the entire process of validation and bioanalytical study (about 1100 injections of prepared plasma samples), without registering almost any loss in terms of efficiency, proving the performances of whole analytical process as well as the quality of the analytical column.

Precision of the MS detection was in accordance to the typical values obtained with the ion trap mass analyzer (within 12% as RSD%). For instance, injection volumes of 50 μL aqueous 5% acetonitrile solutions containing 200 ng/mL glibenclamide and 200 ng/mL internal standard were characterized by relative standard deviations of the peak areas of 7.5% for glibenclamide and 9.7% for the internal standard ($n = 6$). This was the reason why the sample preparation method development has been focused on minimizing error inducing sources.

Peak areas of glibenclamide are significantly increased (by a factor of 5) in plasma samples obtained with citrate as anticoagulant in comparison to plasma samples obtained with potassium edetate, heparin–lithium or heparin–ammonium anticuagulants.

3.5. Precision

Precision was checked on spiked plasma samples at four concentration levels of glibenclamide (2, 50, 200 and 400 ng/mL). Table 2 enlists experimental results obtained during the evaluation of precision, considering as parameters the absolute peak area of glibenclamide, the peak area ratio between glibenclamide and I.S., and the corresponding calculated concentration (applying the regression equation obtained under the linearity study). Results obtained at the first concentration value likely fall outside the accepted ±15% interval, confirming the LLOQ of the method somewhere at this concentration level.

During the study completion, for each analytical sequence, quality control (QC) samples were considered at three concentration levels (10, 100 and 400 ng/mL, respectively). Intra-sequence precision was evaluated for 16 QC sets, in terms of concentration (calculation was made by using the linear regression equation obtained for the calibration corresponding to the sequence). The following results were obtained: for 10 ng/mL, the mean calculated concentration was 9.45 ± 1.26 ng/mL, with an RSD% of 6.7%; for 100 ng/mL, the mean calculated concentration was 99.3 ± 13.5 ng/mL, with an RSD% of 6.8%; for 400 ng/mL, the mean calculated concentration was 398 ± 63.7 ng/mL, with an RSD% of 8%.

3.6. Calibration and quantitation limit

A calibration study was first made for the I.S., to acknowledge that the planned concentration level spiked to plasma samples (500 ng/mL) fits within the linearity domain. The studied concentration interval ranged from 100 to 1000 ng/mL of I.S. spiked to plasma samples. The linear regression ($y = a + b \times C$; y—peak area of the I.S.; C—concentration of I.S. spiked to plasma samples in ng/mL; a—intercept = 3319 ± 143694; b—slope = 13064 ± 259) was characterized by a correlation coefficient of 0.99971.

Calibration was realized over a wide range of glibenclamide concentration in spiked plasma samples (2–400 ng/mL) and a fixed concentration of I.S. (500 ng/mL). Calibration function ($Y = A + BC$; Y—glibenclamide/I.S. peak area ratio; C—glibenclamide concentration in plasma samples, ng/mL) was characterized by the following parameters: $b = 6.12 \times 10^{-3} \pm 3.1 \times 10^{-5}$; $a = 6.2 \times 10^{-4} \pm 3.4 \times 10^{-3}$; $r = 0.99998$.

Over the linearity procedure carried out for glibenclamide (eight concentration levels, six replicates per concentration), the RSD% characterizing I.S. peaks was 8.3%.

Evaluation of the quantification limit (LOQ) and subsequent calculation of the low limit of quantitation (LLOQ) and the limit of detection (LOD) has been achieved in three different ways: (1) $\mathrm{LOQ} = [(10 s_A) - A]/B$, where B is the slope of the linear regression, A the intercept and s_A the standard deviation calculated for A; LLOQ = LOQ/2; LOD = LOQ/3.33 [22]; (2) $\mathrm{LOQ} = [2t(s_A + s_B C_{av})]/(B + 2t s_B)$, where s_B is the standard deviation calculated for B, C_{av} the mean concentration value from the set used for the linear regression and t is the Student coefficient considered for $n - 2$ ($n = 9$) degrees of freedom and a confidence level of 99% ($t = 3.365$) [23]; (3) interpolation in the plot representing the variation of the calculated RSD% for each set of replicate injections versus the concentration values corresponding to each set.

From the experimental dataset, the LOQ computed with algorithm 1 is 3.8 ng/mL. Calculation methods 2 and 3 produce 1.5 ng/mL as results. Considering also data from precision at a concentration level of 2 ng/mL it clearly results that an LOQ of 3 ng/mL should be considered as a realistic value.

During study completion, a calibration was performed for each volunteer. Bulk blank plasma samples were spiked at 2, 10, 50, 100, 200 and 400 ng/mL with glibenclamide and at 500 ng/mL with I.S. 24 aliquots at each concentration level from bulk spiked plasma samples were placed in separate vials and frozen at −40 °C. One set of calibration plasma samples was thaw at the same time as samples from one volunteer, prepared in the same manner and analyzed within the same chromatographic sequence. The normal variation interval of the intercepts resulting by computation of the linear regressions was $[-3.36 \pm 19.3] \times 10^{-3}$, while the same interval for slopes was $[6.8 \pm 2.5] \times 10^{-3}$.

It was observed that the slopes characterizing the linear regressions computed over the whole study registered a positive trend. This situation may be explained by the negative trend registered for peak areas of the I.S. over the study. The decrease of the detector response in time against I.S. may result from the gradually deposition of residuals in the APCI interface, resulting in a reduction of the ionization yield. The supposition is confirmed by the shape of the plot of I.S. peak area versus time, each interface cleaning process being followed by an increase of the detector response. Detector response for glibenclamide seems less sensitive to interface fouling. However, the trend was compensated by the calibration frequency (one calibration for samples belonging to each volunteer). The back interpolations of the experimental data in the linear regression equations lead to results falling within the allowed variation interval (±15%).

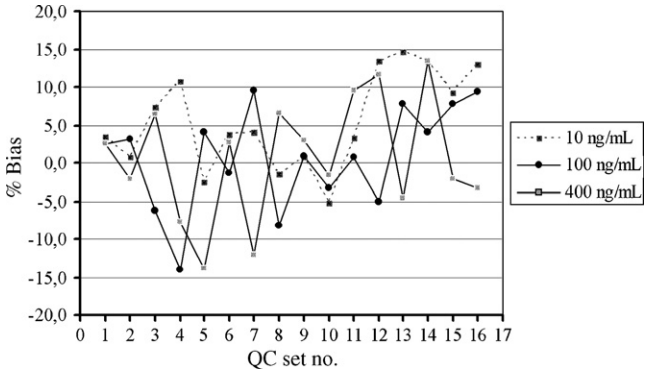

Fig. 3. % bias obtained for QC samples over the study, as an accuracy indicating tool.

3.7. Accuracy

The accuracy of the method may be evaluated from the QC samples analyzed over the study (16 sets). Intra-sequence accuracy, estimated as the bias (calculated as percentage) of the QC samples against the theoretical concentration values, acts as an accuracy indicator. Fig. 3 indicates the variation of the % bias, at the three concentrations chosen for the QC sets (low level—10 ng/mL; medium level—100 ng/mL and high level—400 ng/mL), over the whole study. All results are within the accepted interval.

3.8. Stability of analytes and samples

Stability studies for glibenclamide were made on spiked plasma samples having concentrations of 2, 50, 200, and 400 ng/mL. The stability of the I.S. stock solution in acetonitrile (10 μg/mL) was also checked over an 8 days period, at 48 h sampling interval. Before each analysis, the I.S. stock solution was spiked to a blank plasma sample at 500 ng/mL level; sample was processed according to the procedure and injected to the chromatographic column.

Freeze and thaw stability was studied for five consecutive cycles, from −40 °C to ambient (thaw process was unassisted).

Long-term stability was studied over 8 days, at 24 h sampling interval.

Short-term stability was made over 48 h interval. Frozen spiked plasma samples were thawed unassisted at room temperature and analyzed after 4, 12, 24 and 48 h, respectively.

Table 3
Results obtained during the stability study

Procedure	Concentration of glibenclamide								I.S.	
	2 ng/mL		50 ng/mL		200 ng/mL		400 ng/mL		Peak area	
	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%
Freeze and thaw ($n=5$)	2.16	2.9	57.3	7.0	225.8	7.9	432.0	4.3	–	–
Long term ($n=8$)	2.05	15.5	50.4	7.5	200.1	5.5	399.9	0.9	–	–
Short term ($n=5$)	2.13	5.6	54.2	9.5	226.1	6.6	440.7	2.4	–	–
Post preparative ($n=7$)	2.11	4.3	57.2	13.2	227.9	9.6	438.8	5.6	–	–
I.S. stock solution ($n=5$)	–	–	–	–	–	–	–	–	5.557×10^3	12.9

Table 4
Statistics of pharmacokinetic parameter

Drug	Statistic	C_{max} (ng/mL)	T_{max} (h)	T_{half} (h)	AUC_{last} (ng/mL h)	AUC_{tot} (ng/mL h)
Tested (T)	Mean	203.8	2.65	7.93	953.1	1043.4
	%RSD	53.4	34.1	40.2	52.6	55.7
Reference (R)	Mean	229.8	1.92	7.09	927.2	993.7
	%RSD	34.0	30.5	39.0	47.9	52.6
90% confidence interval for the ratio of the means T/R		75.9–91.6	–	–	95.1–108.1	97.2–111.0

Post-preparative stability was evaluated by analyzing processed spiked plasma samples at 1, 3, 6, 12, 18 and 24 h after preparation, on storage bench top, at room temperature.

Results obtained during stability evaluation study are given in Table 3.

3.9. Bioequivalence study

The main pharmacokinetic parameters obtained on study completion are given in Table 4.

The mean concentration profiles and the corresponding variation intervals obtained for the reference (R) and tested (tested) drugs are given in Fig. 4.

It can be observed that the 90% confidence interval for the pharmacokinetic parameter C_{max} falls within the second admissibility range, according to regulations in place (75–135%, while the normal criterion refers to 80–120% interval). Data presented in references [24–26] discuss the linear dependence appearing between the maximum plasmatic concentration of glibenclamide and the maximum decrease of sugar concentration in blood. According to the cited researches, the difference observed between maximum plasma concentrations for reference and tested products correspond to a maximum decrease of sugar concentration in blood of about 0.2 mmol/L. As a concentration of 202 ng/mL glibenclamide generates an effective decrease of the sugar concentration in blood of 3 mmol/L, it clearly results that the difference between tested and reference products represents about 6.7% from the maximum effect. According to source [27], glibenclamide is a substrate for the cytochrome P450 (CYP)2C9 enzyme, which is polimorphically expressed. This may represent a possible explanation of a marked inter-individual variation in the pharmacokinetics of the drug. One can conclude that the difference observed for C_{max} does not affect the therapeutic effects of the tested drug.

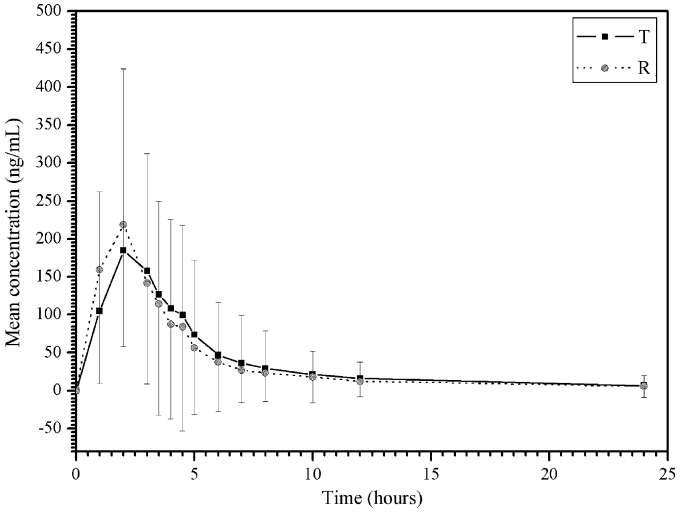

Fig. 4. Mean concentration profiles of glibenclamide obtained for the reference (R) and tested (T) drugs over the bioequivalence study.

4. Conclusions

The method developed in this study is simple, fast, and robust. It provides a high throughput, acceptable precision and accuracy, together with a low limit of quantitation of 1.5 ng/mL. Sensitivity can be further increased at least by a factor of 10 by increasing the injected volume. Data related to method development and validation is presented. The method can be successfully applied to bioequivalence studies for glibenclamide formulations, as it is illustrated for two products (reference and tested) containing 3.5 mg of active substance per tablet. Trends observed during study completion are discussed and their impact on the analytical results is emphasized. Pharmacokinetic parameters were determined and discussed.

References

[1] A.R. Gennaro (Ed.), Remington: The Science and Practice of Pharmacy, 2000, p. 1374.
[2] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83.
[3] M. Nieder, C. Dilger, H. Jaeger, J. High Resolut. Chromatogr. Chromatogr. Commun. 9 (1986) 102.
[4] H. Emilsson, S. Sjoberg, M. Svedner, I. Christenson, J. Chromatogr. Biomed. Appl. 56 (1986) 93.
[5] S. Othman, O. Shaheen, I. Jalal, A. Awidi, W. Al-Turk, J. Assoc. Off. Anal. Chem. 71 (1988) 942.
[6] R.N. Gupta, J. Liq. Chromatogr. 12 (1989) 1741.
[7] B.J. Starkey, G.P. Mould, J.D. Teale, J. Liq. Chromatogr. 12 (1989) 1889.
[8] C. Arcelloni, I. Fermo, A. Calderara, M. Pacchioni, A.E. Pontiroli, R. Paroni, J. Liq. Chromatogr. 13 (1990) 175.
[9] T. Rydberg, E. Wahlin-Boll, A. Melander, J. Chromatogr. Biomed. Appl. 102 (1991) 223.
[10] H. Noguchi, N. Tomita, S. Naruto, S. Nakano, J. Chromatogr. Biomed. Appl. 121 (1992) 266.
[11] K.I. Al-Khamis, Y.M. El-Sayed, K.A. Al-Rashood, M. Al-Yamani, Anal. Lett. 27 (1994) 1277.
[12] I. Niopas, A.C. Daftsios, J. Pharm. Biomed. Anal. 28 (2002) 653.
[13] M. Ionescu, T. Galaon, V. David, A. Medvedovici, Rev. Roum. Chim. 49 (2004) 351.
[14] L. Zecca, S. Trivulzio, A. Pinelli, R. Colombo, O. Tofanetti, J. Chromatogr. Biomed. Appl. 40 (1985) 203.
[15] M.E. Abdel-Hamid, M.S. Suleiman, Y.M. El-Sayed, N.M. Najib, M.M. Hasan, J. Clin. Pharm. Ther. 14 (1989) 181.
[16] J.G. Pearson, E.J. Antal, K.S. Albert, P.G. Welling, Anal. Lett. 18 (1985) 891.
[17] B.M. Chen, Y.Z. Liang, F.Q. Guo, L.F. Huang, F.L. Deng, X. Chen, Y.L. Wang, Anal. Chim. Acta 514 (2004) 185.
[18] I.I. Salem, J. Idrees, J.I. Al Tamimi, J. Chromatogr. B 799 (2004) 103.
[19] M.R.L. Moura, G. de Nucci, S. Rath, F.G.R. Reyes, Anal. Bional. Chem. 378 (2004) 499.
[20] A. Medvedovici, F. Albu, C. Georgita, V. David, Biomed. Chromatogr. 19 (2005) 549.
[21] European Pharmacopoeia 5.0, vol. 2, Council of Europe, Strasbourg, 2005, p. 1659.
[22] J.N. Miller, Analyst 116 (1991) 3.
[23] C. Liteanu, I. Rica, Statistical Theory and Methodology of Trace Analysis, Chapter 6—Detection Theory of Analytical Signals, Ellis Horwood, Chichester, 1980, p. 165.
[24] G. Neugebauer, G. Betzien, V. Hrstva, B. Kaufmann, E. von Mollendorf, U. Abshagen, Int. J. Clin. Pharmacol. Therap. 23 (1985) 453.
[25] H. Jaeger, C. Dilger, K.H. Molz, G. Pabst, Z.B. Salama, Therapiewoche 36 (1986) 654.
[26] K.H. Molz, R. Klimmek, C. Dilger, G. Pabst, W. Weber, M. Muller, H. Jaeger, Arzneim. Forsch./Drug Res. 39 (1989) 1280.
[27] M. Niemi, I. Cascorbi, Clini. Pharmacol. Therap. 72 (2002) 326.