Determination of Pk_a Values of Antidiabetic Drugs from Mobility Data and Pharmaceutical Analysis by Capillary Electrophoresis

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Summary: In this study, the dissociation constants of six antidiabetic drugs (rosiglitazon maleate, pioglitazone, glimepride, glibenclamide, gliclazide and glipizide) have been calculated by capillary electrophoresis (CE) technique in water from the mobilities of compounds at several pH values. The dissociation constant values of antidiabetics had been checked with the ones formerly decided withinside the literature and additionally with the statistics expected through the SPARC on-line calculator and ACDLAB.
Also, easy, precise, green, accurate and completely validated CE technique for the analysis of glibenclamide in pharmaceutical medicine has been fully developed. The CE technique allowed quantitation over the ranges of 1.00-12.00 μg mL^{-1}. The detection and quantitation limits were determined as 0.036 μg mL^{-1} and 0.083 μg mL^{-1} respectively. Rosiglitazone was used as an internal standard and short analysis time (< 3 min.) was observed. The developed capillary electrophoretic technique could be used for ordinary analysis of the glibenclamide and this method can also be used for pharmacokinetic studies.

Keywords: CE Analysis, Green Chemistry, Rosiglitazone, Pioglitazone, Pharmaceutical Medicine.

Introduction

Most people with type 2 diabetes need more than one anti-hyperglycemic drug to achieve optimal blood sugar control. There are many drugs available to treat type 2 diabetes. Thiazolidinediones are in the form of oral antihyperglycemic medicines that increase glycemic check and peripheral insulin resistance. However, they have some features such as improve β-cell function, liver, skeletal muscle and adipose tissue to insulin effects for sensitizing to insulin effects [1]. The sulfonylurea group drugs also interact with the receptors in the pancreatic β-cells so that they can stimulate insulin release. Such treatment also increases the degree of reduced insulin. Sulphonylureas reduces the insulin clearance in the body and thus reduces the use of insulin [2].

Rosiglitazon maleate and pioglitazone belong to the group thiazolidinedione, are utilized in therapy of type 2 diabetes (non-insulin-dependent diabetes mellitus, NIDDM, also recognized as adult-onset diabetes) [3]. Glimepride, glibenclamide and gliclazide are the second generation of sulfonylurea that is used for lower blood sugar grades in NIDDM patients. These drug substances shown their effect by increasing of insulin secretion from β-cells of pancreas after binding to ATP reactive potassium channel [4]. Besides, sulphonylureas suppress glucose production in liver. Rosiglitazone is improved to glycemic mechanism in Type 2 diabetes mellitus in combinations with sulphonylurea and metformin [5].

Literature survey revealed that several analytical techniques assay for the antidiabetic drugs either alone or in combination with diverse drugs, in medicines, biological fluids or other samples including liquid chromatography (LC) [6-9] electrochemical determination [10], liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) [11], spectrophotometry using chemometry [12]. Only a few articles reported capillary electrophoresis (CE) methods including micellar electrokinetic chromatography (MEKC) for investigation of antidiabetic drugs [13-15]. In the literature, there have been one article regarding for the concurrent analysis of glibenclamide and impurities as IA and IB in dosage forms [16].

Capillary electrophoresis technique has been used very commonly for analysis of several drugs. When working with ionizable drugs, its electrophoretic behavior is based on the relationship between the electrophoretic mobility of each substance, the drug dissociation constant and the buffer pH [17-20]. This permits the determination of pK_a values from the relationship between pH and electrophoretic mobility [17, 21]. Determination of pK_a values with CE method has many advantages

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minimal sample consumption, separation of impurities and/or degradants from main drug, sensitive ultraviolet detection. CE method is a more selective analysis technique than potentiometry and UV. Unlike potentiometric pK_a determination, capillary electrophoresis can be used for small amounts of compounds with low solubility. This is particularly important when the analysis of pharmaceutical, biological and environmental chemicals because the standards are really costly and because of little quantities separated from biological and ecological species. Additionally, with capillary method, aqueous pK_a values of drugs can be determined precisely.

Nowadays, green chemistry is becoming important. Due to the increased awareness of environmental safety, efforts are needed in the green chemistry approach, such as waste generation, reduction of energy and natural resource use. Like carbon tetrachloride, chloroform, halogenated solvents are highly potent carcinogens. Some solvents also known to be neurotoxic. It is very important to choose less toxic solvents for separation and purification steps. Solvents have great harm to the environment due to their use in high amounts as well as their harm to human health [22]. Recommended alternatives to volatile organic solvents are water volatile supercritical carbon dioxide and ionic liquids.

In this study, CE was chosen for the computation of pKa values of six antidiabetics in water. They include rosiglitazon maleate (RO), pioglitazone (PI), glimepride (GM), glibenclamide (GL), gliclazide (GC) and glipizide (GP). In our research, pK_a values of these studied antidiabetic drugs have not been determined by capillary technique so far. Also, a new, environmentally friendly, simple, rapid, and sensitive CE technique for the determination of GL in pharmaceutical medicine was developed. In the developed method, no organic solvents are used in the working buffer.

Experimental

Chemicals and Reagents

RO, PI, GM, GL, GC and GP were purchased from Sigma Corporation (St. Louis, Missouri, USA). Methanol was obtained from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Phosphoric acid, hydrochloric acid, formic acid, sodium tetra borate and sodium hydroxide was purchased from Sigma Corporation (St. Louis, Missouri, USA). The stock solution of the hypoglycemic agent was prepared by dissolving each hypoglycemic agent in MeOH (100 µg mL^{-1}).

Apparatus

All analyses were performed using Agilent Technologies' G7100A capillary electrophoresis instrument and a diode array detector. A fused silica capillary with 55 cm × 50 µm id (Agilent Tech. Ext. Light Path) with an effective length of 45 cm is used for analysis. pH/ion analyzer (Fisher Scientific AB 15) with Fisher Scientific combined pH electrode was utilized for measurements of pH.

Procedures

pK_a Analysis

Before the analysis, fused silica capillary was activated 5 min with methanol, 3 min with 1.0 M HCl, 3 min with H_2O, 20 min 1.0 M with NaOH and finally 3 min with water. Then buffer solution was used for 30 min for activation of separation capillary. Also, for daily use 30 minwith 1.0 M sodium hydroxide, 3 min with water and at the end 30 min with the running buffer were used for condition of capillary. Before using the buffer, filtered with 0.25 µm membrane filters (Millipore, Bedford, Massachusetts, USA) and degased it with ultrasound for 20 minutes. For pK_a analysis, buffer containing 25 mM phosphate was adjusted to pH 2.5-7.0 with 1.0 M sodium hydroxide. Pressure injection 10 psi for 10 s was used for injection of drug. A 26 kV voltage was chosen. Detection wavelength and temperature were 230 nm and 25 ^{0}C, respectively.

For each test pH, the working solution was introduced hydro dynamically into the capillary electrophoresis system three times for calculation of electrophoretic mobility values. The electrophoretic mobilities (m_e) for each drug were calculated using the neutral marker migration time (t_{eof}), each antidiabetics migration time (t), capillary length (LC), capillary length between the injection and the detector (LD), and used voltage (V) according to the following formula:

$$m_e = (LCLD / V) (1/t - 1/ t_{eof})$$

m_e/pH data which calculated from the inflection points of the m_e/pH curves, could be used for determination of pK_a values however the pH ranges studied here (0.5 units) do not permit an accurate prediction. NLREG 4.031 software was used for calculation of antidiabetic drugs pK_a values [23]. NLREG is a general nonlinear least squares regression program in which a set of first parameters is iteratively optimized until the minimum value of the objective function is reached. These parameters correspond to thermodynamic pKa and ionic mobility ($m_{e,\ A-}$). The objective function, U, is defined as the sum of squares of the difference between the calculated experimental and estimated

effective mobility ($m_{e,exptl}$ and $m_{e,pred,}$ respectively) in each running buffer,

$$U = \sum_{i=1}^{n} (m_{e,exptl,i} - m_{e,pred,i})^2$$

where n is the value of the m_e /pH data pair, defined as the average of three replicates. The estimated mobility values ($m_{e,pred}$) were determined using ionization constants, each electrophoretic mobilities, buffer solutions pH values and activity coefficients calculated from ionic strengths of buffers. The inflection points of the me/pH graphics can be used for initial estimates for pK_a and the initial datas for m_e,A were get from the experimental m_e /pH value (at intermediate and high pH data, respectively).

Analysis of Drug

For analysis of Glibenclamide (each tablet 5.00 mg Glibenclamide), ten tablets of GL were weighted and powdered. The stock of GL (1 mg mL^{-1}) was prepared in with methanol and was sonicated for 20 min. After filtration, solutions were injected to the CE system. Previously plotted calibration graph was used for calculation amount of glibenclamide in the tablets.

A recovery test was conducted to check the accuracy of the method and check the effectiveness of the additives. Therefore measured amount of the pure drugs, were put in pre-analyzed glibenclamide drug. Five replicate measurements were done for the recovery value and the obtained calibration graph was used for the calculations.

Result and Discussions

Determination of Dissociation Constants (pK_a)

For chromatographic and electrophoretic techniques, it is considerable to informed different physico-chemical properties. Among these, especially knowing the pK_a value is very important for absorption, evaluating bioavailability of drugs in the body.

Gliclazid, Glimepirid, Glipizid, and Glibenclamid have an acidic group (NH group of the sulfonylurea fraction), and the pKa value of the sulfonylurea fraction of the tested hypoglycemic drugs is usually in the range of 5.2 to 6.5. In addition, with these values, the compounds are considered to be characteristic of weak organic acids. Additionally, rosiglitazone and pioglitazone contain polar acidic head of 2,4-thiazolidinediones moiety. When the literatures were examined, it was seen that there is no data for determination of pK_a values of these antidiabetics by CE method. For pK_a determination, CE method has been used widely among the chromatographic techniques. The obtained pK_a values of these antidiabetic drugs, together with the values estimated by SPARC (SPARC) [24], ACDLAB (ACD Software) [25] and literature [26-29] are shown in Table-1. The ARChem physical chemistry calculator SPARC uses calculation algorithms based on basic chemical structure theory to estimate a wide range of reactivity parameters based only on molecular structure. Despite some shortcomings, SPARC is currently considered to be one of the best and most reliable publicly available chemical property prediction program. In addition, for almost any organic or inorganic structure, ACD / pK_a DB can accurately calculate the acid-base ionization constant (pKa value) at 25°C and the zero ionic strength. Except for very complex structures or poorly characterized substituents, the accuracy of calculation is usually better than ±0.2 pKa units, and its accuracy is usually within ±0.5 pKa units [25]. The CE method is based on measuring the relationship between the electrophoretic mobility of the analyte and the pH of the background electrolyte (BGE). By fitting the experimental points to an appropriate model depending on the properties of the complex and the number of ionizable groups, pKa can be easily calculated. However, in order to obtain reliable results, some general considerations should be considered before determining the pKa via CE technique. The most important parameters affecting mobility are pH, temperature and ionic strength [30, 31]. Other parameters that also affect mobility include BGE viscosity, buffer electrolysis due to applied voltage, and atmospheric CO_2 dissolution in the BGE solution [32, 33].

Narasimham and Barhate [26] use potentiometric and spectrophotometric titration at various temperatures (25 to 45°C) and ionic strength (0.15-0.5 M) in different mixtures of water and solvents (such as methanol, ethanol, acetonitrile and dioxane). Potentiometric titration and spectrophotometric titration were performed to determine the apparent ionization constant of the selected drug. Then the aqueous pKa values were obtained by Yasuda-Shedlovsky extrapolation. Remco [27] also used theoretical chemical methods to elucidate the molecular properties of sulfonylureas and certain glinides that lower blood sugar. On the other hand, no detailed information about determination conditions of pK_a values were given in literature [16, 28, 29]. Due to these differences, experimentally determined pK_a values by CE method could slightly be different from calculation programs and literature values given in Table-1.

In Fig. 1, graphs of effective mobilities against pH obtained for glimepride and gliclazide are given as examples.

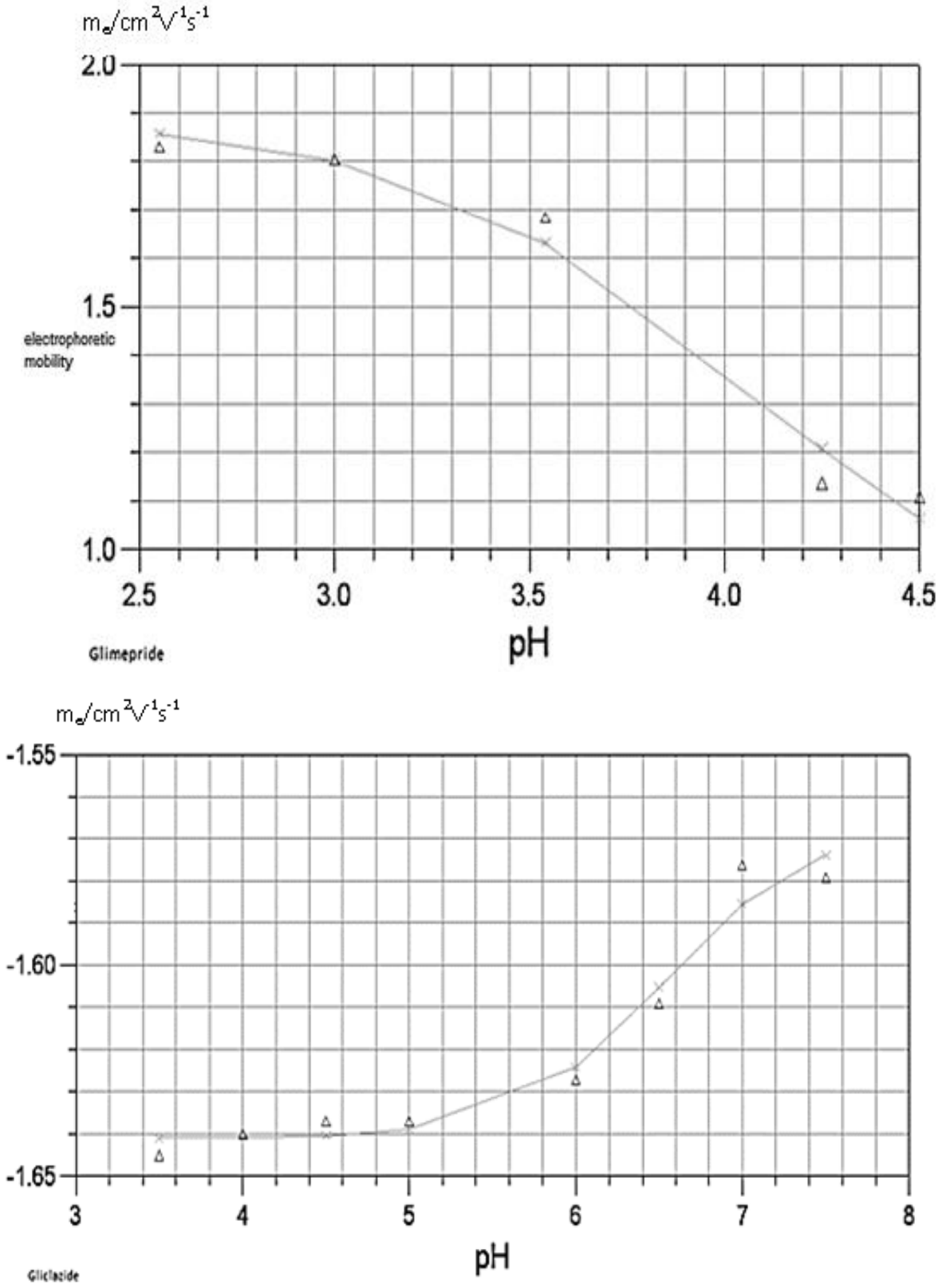

Fig. 1: Experimental electrophoretic mobilities of the glimepride and gliclazide as a function of pH of buffers.

Table-1: The pK_a values of studied compounds predicted by ACD Lab, SPARC and obtained by CE method.

	Lit. Value	ACD Lab	SPARC	CE
Rosiglitazone maleate	**6.1** [29] **6.8** [29]	**6.47 ± 0.1**	**5.32 6.38**	**6.50 ± 0.07**
Pioglitazone HCl	**5.26** [26] **5.59** [28]	**5.53 ± 0.2 6.35 ± 0.5**	**4.17 6.35**	**6.02 ± 0.25**
Glibenclamide	**5.3** [16]	**4.17 ± 0.3**	**5.57**	**5.25 ± 0.08**
Glimepride	**5.20** [27]	**4.99 ± 0.5**	**5.55**	**4.07 ± 0.25**
Gliclazide	**5.60** [27] **5.60** [26] **5.54** [28]	**5.03 ± 0.5**	**5.32**	**6.53 ± 0.16**
Glipizide	**5.25** [26] **5.16** [28] **5.20** [27]	**4.97 ± 0.5 13.04 ± 0.5**	**5.56 9.98**	**5.22 ± 0.11**

Determination of GL

For the determination of GL in pharmaceutical drugs, 20 mM $Na_2B_4O_7$ and 55 cm (effective length 45 cm) x 50 nm ID capillary column were selected as buffer. As device parameters, injection time was 10 seconds under 10 psi pressure, column temperature 25˚C, applied voltage +26 kV and DAD detector at 230 nm. Rosiglitazone was chosen as internal standard for analysis of GL. The calibration graph was plotted between 1-12 mg L^{-1}. Rosiglitazone concentration was kept constant at 6 mg L^{-1}. Electropherogram obtained for 8 mg L^{-1} standard GL and 6 mg L^{-1} RO are given in Fig. 2.

By using these CE conditions, short analysis time (< 3 min.) and a satisfactory chromatographic peak resolution was achieved as given in Fig 3. For glibenclamide and rosiglitazone maleate symmetrical and sharp single peaks were achieved without using organic solvent.

For CE method, system suitability features such as migration time, capacity factor, tailing, resolution and selectivity values are presented in Table 2. The data obtained meet USP requirements.

Table-2: System Suitability Parameters for GL Determination by CE-DAD.

	GL	RO	Recommended value
Migration time (min)	**2.368**	**2.535**	-
Tailing factor (T)	**0.97**	**0.98**	**≤ 2**
Capacity factor	**0.328**	**0.422**	**≥ 1**
Resolution (R)	**1.286**	-	**> 2**
Selectivity factor (α)	**1.286**	-	**> 1.15**
RSD% (for migration time)	**0.145**	**0.183**	**≤ 1**

The relationship between drug concentration and the response was linear and the proposed CE method permitted quantitation over the 1.00-12.00 µg mL^{-1}. The calibration graph was get with the linear least squares regression. Regression equation was found y = 0.12 x – 0073 with the high coefficient of correlation (0.999). The LOD and LOQ values were determined as 0.0356 (µg mL^{-1}) and 0.083 (µg mL^{-1}) respectively for glibenclamide.

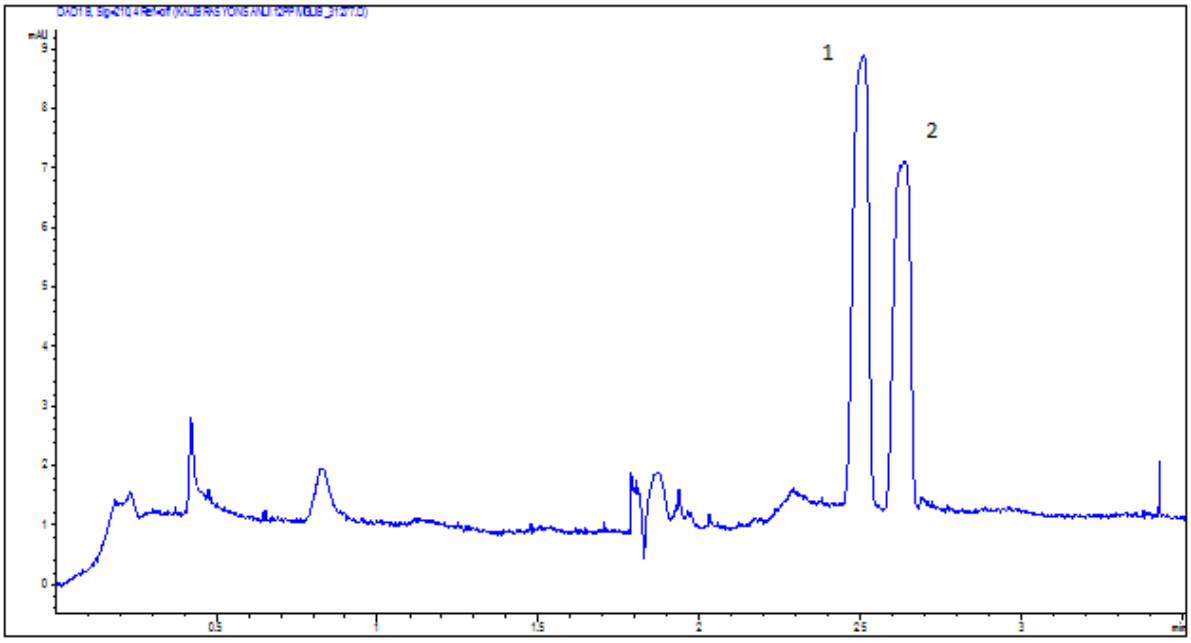

Fig. 2: Electropherogram obtained from a standard mixture of studied drugs under optimum conditions, containing 8 µg mL^{-1} GL (1) and 6 µg mL^{-1} RO (2).

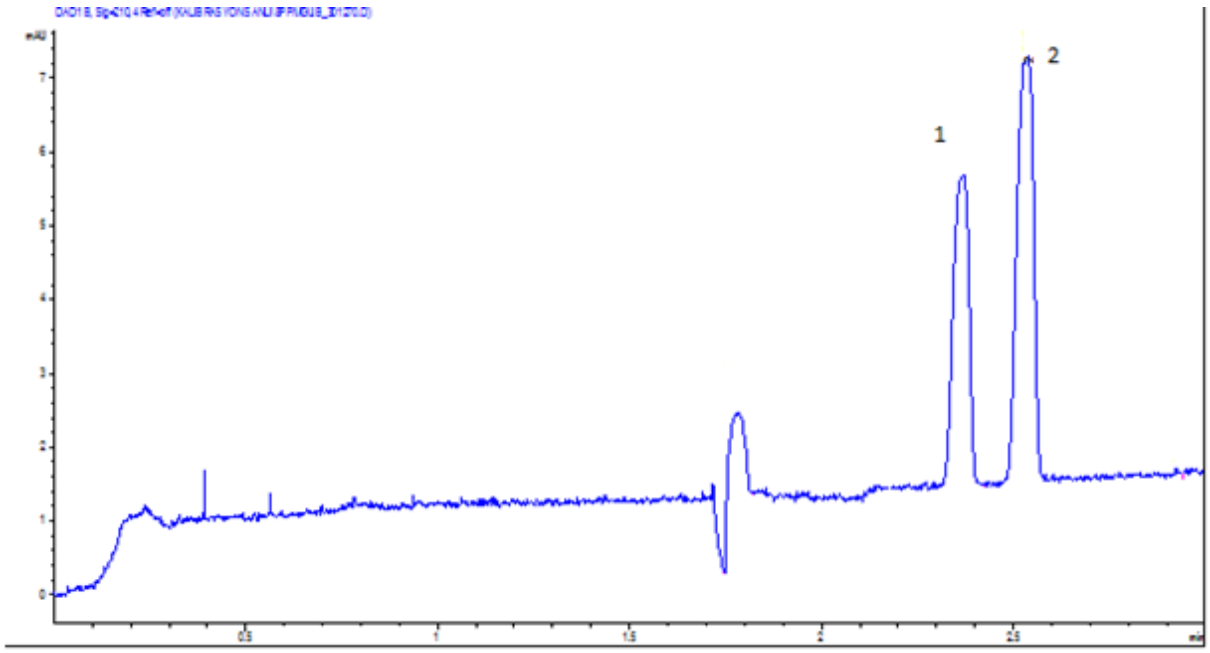

Fig. 3: Electropherogram obtained from tablet dosage forms, containing 6 µg mL $^{-1}$ GL (1) and 6 µg mL $^{-1}$ RO (2).

Intra-day and inter-day precision values were evaluated for two different concentrations (4 and 8 g µg mL $^{-1}$) of GL five times on the same day and different day to obtain the relative standard deviation (RSD %) needed to evaluate the precision. The RSD% values for the intra-day variation were found as 0.647 and 0.211. Also the RSD% values for inter-day were found 0.150 and 0.437. These results supported good precision of the method.

Table-3: Results of the assay and the recovery analysis of GL in pharmaceutical dosage forms.

	GL
Labeled claim (mg)	**5.000**
Amount found (mg)[a]	**5.025**
RSD (%)	**0.655**
Added (mg)	**5.000**
Found (mg)[a]	**5.010**
Recovery (%)	**100.20**
RSD% of recovery	**0.958**

[a] Each value of the mean five experiments.

Fig. 3 shows an electropherogram of the pharmaceutical dosage form GL and RO (IS). The studied compounds appeared as symmetrical single peaks, forming shapely, very well separated from the other one. In addition, no interfering peak was occurred in the analysis due to medicine excipients. The use of suggested CE technique was confirmed by means of replicate results of medicine formulations and the achieved data were analyzed statistically and given in Table 3. The suggested method could be appropriate for simultaneous quantitation and determination of GL in several biological samples. As a result of the high recovery data obtained from the analysis, it was seen that the CE method developed was not affected by the interference of the excipients situated in the pharmaceutical medicine.

Conclusion

The CE method developed is a fast, green, simple method with low LOD and LOQ values for the analysis of glibenclamide in drug formulations. Total analysis time of drug shorter than 3 min. In addition, this article is the first study for estimation of pK_a values of rosiglitazon maleate (RO), pioglitazone (PI), glimepride (GM), glibenclamide (GL), gliclazide (GC) and glipizide (GP) by CE method in water.

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Conflict of Interest

None of the authors declare any conflicts of interest.

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