INTRODUCTION

Antidiabetics such as sulfonylurea and thiazolidinedione derivatives are commonly prescribed hypoglycemic drugs for the treatment of non-insulin-dependent type II diabetes mellitus. However, they can also be used as a stopper in race-horses by reducing the blood glucose level.

Pioglitazone hydrochloride, (RS)-5-[4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl]thiazolidine-2,4-dione hydrochloride (Fig. 1), is a member of the thiazolidinedione class, which exerts its glucose-lowering effect by binding to peroxisome proliferator-activated receptors gamma (PPARγ), thus increasing the receptor sensitivity to insulin.

Glimepiride, 3-ethyl-2,5-dihydro-4-methyl-N-[2-[4-[[trans-4-methylcyclohexyl]amino][carbamoyl]amino]-sulfamoyl]phenyl]ethyl]-2-oxo-1H-pyrrrole-1-carboxamide (Fig. 1), is a member of sulfonylurea drugs, which can increase the secretion of insulin by functioning islet β-cells. In the past few decades, several generations of sulfonylurea drugs have been developed for common use such as glimepiride. This generation of hypoglycemic drugs are much more potent and are therefore effective at much lower dosages.

Several analytical methods have been reported for the determination of pioglitazone HCl in bulk form, pharmaceuticals and biological fluids. Most of the reported methods are chromatographic methods and no official methods have been reported for the determination of pioglitazone HCl. The reported methods include: HPLC with UV detection, HPLC with tandem mass spectrometry, HPTLC, TLC, CE, MEKC, polarography, ion selective electrodes, UV spectrophotometry.

Glimepiride has been analyzed by HPLC with UV detection, HPLC with tandem mass spectrometry, HPTLC, MEKC, polarography, ion selective electrodes, UV spectrophotometry.

This paper describes a rapid and sensitive spectrofluorimetric method for the determination of the two antidiabetic drugs in the commercial pharmaceutical tablet preparations and biological fluids. Analytical quality criteria, including method sensitivity, precision and recovery, are discussed.
Fluorimetric measurements were performed using a spectrofluorimeter (Jasco model FP6200, Japan) equipped with Xenon discharge lamp and 1 cm quartz cell. The fluorescence intensity was measured at 512 nm using 298 nm for pioglitazone HCl excitation and at 522 nm using 286 nm for glimepiride excitation. The excitation and emission slit controls set at 10 nm.

Pure drug samples of pioglitazone HCl and glimepiride were kindly supplied by Chargen-Zert, Brand (Germany); dosage forms containing these drugs were obtained from commercial sources. Serum samples were supplied from (United Diagnostics Industry K.S.A.), urine samples were obtained from healthy volunteers.

The following reagents were used: methanol (BDH Ltd., UK); disodium tetraborate (BDH Ltd., UK) 0.02 M; dichloromethane (BDH Ltd., UK), diethyl ether (Merck, Germany); ethyl acetate (Merck, Germany); sodium sulfate (BDH Ltd., UK).

**General analytical procedure:** Stock solutions containing 1.0 mg/mL of pioglitazone HCl and glimepiride were prepared in methanol. Working solutions of pioglitazone HCl and glimepiride in the range of 0.005-1.3 µg/mL and 0.01-1.5 µg/mL, respectively transferred into 10 mL volumetric flasks and diluted to the mark with methanol. The fluorescence intensity was measured at 512 nm for pioglitazone HCl and at 522 nm for glimepiride. Calibration graphs were prepared by plotting the fluorescence intensity against the drug concentration.

**Analysis of tablets:** An accurately weighed amount of the powdered tablets equivalent to 10.0 mg of each drug was transferred into a 50 mL volumetric flask. Then methanol was added to each flask and completed to the mark. The contents of the flasks were sonicated for 20 min, filtered and analyzed as described above under general procedure.

**Analysis of spiked urine and serum**

**A: For pioglitazone HCl:** An aliquot of urine or serum (1.0 mL) in a centrifuge tube was spiked with an aliquot of aqueous solution of pioglitazone HCl containing 10 µg and the tube was vortexed for 1 min, 0.2 mL of disodium tetraborate solution (0.02 M) was added and the tube again vortexed for 1 min. 5 mL of dichloromethane was added and the tube was shaken for 10 min. The tube was then centrifuged at 3000 rpm for 10 min at room temperature. The resulting organic layer was removed and the extraction was repeated two times with 5 mL of dichloromethane. The combined extracts were evaporated to dryness at room temperature and the residue was dissolved in 1 mL of methanol. The solution was transferred into a 10 mL volumetric flask and completed to volume with methanol, then analyzed as described above under the general procedure. A blank experiment was carried out adopting the above procedure.

**B: For glimepiride:** An aliquot of urine or serum (1.0 mL) in a centrifuge tube was spiked with an aliquot of aqueous solution of glimepiride containing 10 µg. 5 mL saturated sodium sulfate solution was added. Then, the solution was extracted with 5 mL of a mixture of diethyl ether-ethyl acetate (1:1, v/v). After phase separation by centrifugation, the organic phase was transferred to a beaker and evaporated to dryness. The aqueous phase was basified with 0.5 mL of aqueous sodium hydroxide (1 M) and extracted two times with 5 mL of the solvent mixture. The organic phase was transferred to the same beaker and evaporated to dryness. The combined residues were dissolved in 1 mL of methanol. The solution was transferred into a 10 mL volumetric flask and completed to volume with methanol, then analyzed as described above under the general procedure. A blank experiment was carried out adopting the above procedure.

**RESULTS AND DISCUSSION**

Pioglitazone HCl and glimepiride possess an aromatic ring that absorbs intensely and therefore have an intense band. So they were found to exhibit an intense native (Fig. 2).

**Application of the method**

**Analysis of pharmaceutical preparations:** The developed spectrofluorimetric method was applied to the tablet form.

**Different media such as water, methanol, ethanol, 0.1N NaOH, 0.1N H₂SO₄, acetone, acetonitrile, acetate buffer (pH 4.5), borate buffer (pH 7), borate buffer (pH 9.4) and dimethyl formamide were attempted. Maximum fluorescence intensity was obtained in methanol for both drugs, hence it was recommended throughout this work. Fig. 2 shows the excitation and emission spectra of both drugs in methanol.

The effect of methanol volume on the fluorescence intensity was also investigated using increasing volumes of methanol and completing to the mark with water. It was found that maximum fluorescence intensity was obtained when the flask is completely diluted with methanol.

The effect of different surfactants and sensitizers on the fluorescence intensity of pioglitazone HCl and glimepiride were studied by adding 1 mL of 0.1% of each surfactant solution to the methanolic 1 µg/mL drugs solutions. It is obvious from the results, that sodium dodecyl sulfate, cetyltrimethyl-ammonium bromide, cetylpyridinium chloride, Tween 20 and α-cyclodextrin caused an inhibitory effect on fluorescence intensity. In case of triton X 100 and γ-cyclodextrin the blank readings are over the range, but with gelatin the solution becomes turbid. Thus, no surfactant is used in this work.

**Application of the method**

**Analysis of pharmaceutical preparations:** The developed spectrofluorimetric method was applied to the tablet form.
formulations of the studied drugs. The recoveries based on the average of three replicate measurements are illustrated in Table-1. The results were compared with those obtained by the published spectrophotometric methods. Statistical analysis of the results obtained by the proposed and the published spectrophotometric method shows no significant difference between the two methods as regard to accuracy (t-test) and precision (F-test).

**Analysis of spiked biological fluids:** The high sensitivity attained by the proposed method allows the determination of the studied drugs in biological fluids. Pioglitazone HCl is rapidly absorbed after oral administration. Peak plasma concentrations are obtained within 2 h and bioavailability exceeds 80 %. Pioglitazone HCl is more than 99 % bound to plasma proteins. It is extensively metabolized by cytochrome P450 isoenzymes CYP3A4 and CYP2C9 to both active and inactive metabolites. It is excreted in urine and faeces and has a plasma half-life of up to 7 h. The active metabolites have a half-life of up to 24 h.

Glimepiride is completely absorbed from the gastrointestinal tract. Peak plasma concentrations occur in 2 to 3 h and it is highly protein bound. It is extensively metabolized to two main metabolites, a hydroxy derivative and a carboxy derivative. The half-life of up to 9 h. Approximately 60 % of a dose is eliminated in the urine and 40 % in the faeces.

As a consequence, the proposed method appears to be convenient for the therapeutic drug monitoring in urine and serum. In addition, the spectrofluorimetric, requiring smaller volumes of samples, may be valuable for routine drug screening and diagnosis.

**Validation of the analytical procedure**

**Linearity:** The proposed method was tested for linearity. The regression plot showed a linear dependence of the fluorescence intensity on the studied drugs concentrations over the calibration range (0.005-1.3 µg/mL) for pioglitazone HCl and (0.01-1.5 µg/mL) for glimepiride. The LOD and LOQ as well as the slope and intercept were also clarified. Validation of the method was evaluated by statistical analysis of the regression line regarding the standard deviation of intercept δ0 and the standard deviation of slope δβ in Table-3.

**Precision and accuracy of the method:** The intra-day precision was evaluated through five replicate analysis of a sample containing 0.5 µg/mL of the studied drugs. The mean % recovery was 99.3 ± 0.43 for pioglitazone HCl and 99.6 ± 0.48 for glimepiride. The inter-day precision was determined by triplicate analysis of a sample containing 0.5 µg/mL of the studied drugs on three consecutive days. The mean % recovery was 99.4 ± 0.55 for pioglitazone HCl and 99.4 ± 0.36 for glimepiride. The reproducibility was investigated using 0.5 µg/mL for both drugs (n=15) and the RSD % < 2 which illustrates that the results were highly reproducible.
TABLE 2

PERFORMANCE DATA FOR THE SPECTROFLUORIMETRIC DETERMINATION OF THE STUDIED DRUGS IN URINE AND SERUM

<table>
<thead>
<tr>
<th>Compound</th>
<th>Serum Linear calibration range (µg/mL)</th>
<th>Serum Regression equation ( I = a + b C )</th>
<th>Serum Correlation coefficient ( r )</th>
<th>Urine Linear calibration range (µg/mL)</th>
<th>Urine Regression equation ( I = a + b C )</th>
<th>Urine Correlation coefficient ( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pioglitazone HCl</td>
<td>0.02-1.0</td>
<td>( I = 37.55 + 558.89 C )</td>
<td>0.99983</td>
<td>0.01-1.0</td>
<td>( I = 42.23 + 573.41 C )</td>
<td>0.99985</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>0.05-1.3</td>
<td>( I = 15.15 + 491.09 C )</td>
<td>0.99972</td>
<td>0.05-1.5</td>
<td>( I = 16.19 + 511.48 C )</td>
<td>0.99981</td>
</tr>
</tbody>
</table>

*Fluorescence intensity; °9 data points.

The accuracy of the proposed method was evaluated by analyzing standard solutions of the studied drugs. The % Found of the studied drugs compared with those obtained by the spectrophotometric methods[26,41] were given in Table-1. Statistical analysis[42] of the results, obtained by the proposed and the published methods,[26,41] using the student’s t-test and variance ratio F-test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively.

**Conclusion**

This study demonstrated the application of a spectrofluorimetric method for the determination of two anti-diabetic drugs which found to be simple, accurate, precise, reproducible and gives an acceptable recovery of the analytes. The proposed method can be directly and easily applied to the analysis of the pharmaceutical tablet formulations of the studied drugs. The method allows the determination of the two hypoglycemic drugs in both serum and urine after a very simple liquid-liquid extraction step. The proposed procedure showed clear advantages such as short period of real time of drug analysis and low limit of detection.

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