Appendix


4. **M. Rama Chandraiah** and Y. V. Rami Reddy, Spectrophotometric method for the determination of lomitapide in Bulk and dosage forms. *International Journal of Research in Chemistry and Environment (Communicated)*


3. Participated in International Seminar on “Current Trends In Environmental Toxicology and Experimental Therapeutics”, Vikrama Simhapuri University, Nellore, A.P., India, from 18th December to 19th December, 2012.

4. Participated in 7th National conference on “Thermodynamics of chemical, biological and environmental processes”, S.V. University, Tirupati, A.P., India, from 10th December to 12th December, 2012.

Sensitve Spectrophotometric Method for the Determination of Niguldipine in Pharmaceutical Formulations

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ABSTRACT
Simple, sensitive and rapid spectrophotometric methods were developed for the determination of Niguldipine in pharmaceutical formulations. Method-A was based on the extractive spectrophotometry and the developed color complex due to the formation ion association complex with Bromothymol blue (BTB) at pH 3.7. The formation of a yellow color solution exhibited maximum absorption at wavelength 380 nm. Method-B was based on the yellow color formation due to ion association complex between methyl orange (MO) and Niguldipine at pH 3.7 that exhibited maximum absorption at a wavelength of 430 nm. Both ion association complexes were extracted into chloroform and Beer’s law was obeyed over the concentration ranges 4-25 µg/ml-1 and 2-12 µg/ml-1 for methods A and B respectively. Both the methods have been successfully applied for the assay of the drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. The reliability and the performance of the proposed methods were established by point and interval hypothesis tests through recovery studies.

INTRODUCTION
Niguldipine, (8)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine di carboxylic acid, 3-(4,4-diphenyl-1-piperidinyl) propyl methyl ester. It is a calcium channel blocker and Aβ-adrenergic receptor antagonist(1). The chemical structure of Niguldipine is as shown in Figure 1.

![Figure 1. Structure of Niguldipine drug.](image)

Extractive spectrophotometric procedures are more popular in the assay of drugs due to for their high sensitivity. Therefore, ion-pair extractive spectrophotometry has been received considerable attention for quantitative determination of many pharmaceutical compounds. To the best of our knowledge, in the determination of Niguldipine, a few analytical methods like Stripping Voltametric(2) and High performance liquid Chromatography(3) studies have been reported in the literature and no reports were found in the usage of simple spectrophotometric methods. The methods described here are simple and sensitive and has been used for the routine quality control analysis of pharmaceutical formulations containing Niguldipine which involved in the formation of ion association complex with acidic dyes, bromothymol blue (BTB) which is extractable into chloroform and Beer’s law was obeyed over the concentration ranges 4-25 µg/ml-1 and 2-12 µg/ml-1 for methods A and B respectively. Both the methods have been successfully applied for the assay of the drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. The reliability and the performance of the proposed methods were established by point and interval hypothesis tests through recovery studies.

EXPERIMENTAL

Instrumentation
Spectral and absorbance measurements were made with Shimadzu UV/Visible double beam spectrophotometer (model 2450).

Reagents
All the reagents were used of analytical reagent grade. The solutions, bromothymol blue (BTB) (0.2%) for Method-A and methyl orange (MO) (0.1%) for Method-B were prepared daily.

Standard and sample solutions of Niguldipine
A standard stock solution containing 1 mg/mL was prepared by dissolving accurately, 100 mg of drug in 20 mL of methanol. After dissolved the Tablet completely, it was treated with 10 mL of 5.0 N HCl and 4 gm of zinc dust. The working standard solution containing 100 µg/mL was prepared for both A and B methods dilution with methanol.

Assay procedure

Method-A
Aliquots of standard and reduced Niguldipine (1mL = 100 µg/mL) solutions ranging from 0.1-0.5 mL of 100 µg were transferred into a series of 50 mL of separating funnels and then 2 mL of BTB (0.2%) was added to this solution. The total volume of the aqueous phase was made up to 10 mL with distilled water. Approximately, 10 mL of chloroform was added to each funnel, and the contents were shaken for 2 min. and then allowed to stand result separate the phases. The absorbance of chloroform layer was measured at 380 nm against the corresponding reagent blank. The amount of Niguldipine present in the sample solution was calculated from its calibration curve (Figure 2).

Method-B
The standard and reduced Niguldipine (1 mL = 100 µg/mL) solutions from 0.5-2.5 mL were taken into a series of 50 mL of separating funnels and then added 2 mL of MO (0.1%). The total volume of the aqueous phase was made up to 10 mL with distilled water. 10 mL of chloroform was added to each funnel, and the contents were shaken for 2 min. and then allowed to stand result separate the two phases. The absorbance of the chloroform layer was measured at 430 nm using corresponding reagent blank, and the calibration curve was drawn (Figure 4).

![Figure 2. Absorption spectrum of Niguldipine with BTB/CHCl₃ system.](image)
Result and Discussion

Anionic dyes like BTB, MO form ion-association complexes with the positively charged drugs. The drug and dye stoichiometric ratio was calculated by the continuous variation and mole-ratio method and found to be 1:1 both with BTB and MO. The drug dye complex with two positively charged ions behaves as a single unit held together by an electrostatic force of attraction. The possible mechanism for the formation of ion-association complex between drug, and the reagents, BTB and MO were depicted in Figure 6 and Figure 7 respectively.

Optimization of variables

Optimum conditions are necessary for rapid and quantitative formation of colored ion-pair complexes with maximum stability and sensitivity which was established in preliminary experiments. Chloroform was preferred as better solvent for these methods for its quantitative extraction selectively. Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 380 nm for BTB and 430 nm for MO. The optical characteristics such as molar absorptivity, Beer’s law range and Sandell’s sensitivity are presented in Table 1. The regression analysis using the method of least squares was made for the slope (a), intercept (b) and correlation coefficient (r) obtained from different concentrations and the results are summarized in Table 1. The relative standard deviations and percent range of error (0.05 and 0.01) confidence limits were calculated for the eight measurements each. The experimental data revealed that method A is more sensitive than method B.

Table 1. Optical Characteristics, precision and accuracy of the proposed methods for Niguldipine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method-A</th>
<th>Method-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax (nm)</td>
<td>380</td>
<td>430</td>
</tr>
<tr>
<td>Beer’s law Limits (µg/mL)</td>
<td>4-25</td>
<td>2-12</td>
</tr>
<tr>
<td>Molar absorptivity (1 mole⁻¹ cm⁻¹)</td>
<td>6 x 10⁴</td>
<td>6.87 x 10⁴</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/cm²/0.001 absorbance unit)</td>
<td>0.0126</td>
<td>0.01443</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0134</td>
<td>0.0446</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.018</td>
<td>0.0028</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9832</td>
<td>0.04472</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.7285</td>
<td>0.1694</td>
</tr>
<tr>
<td>% of Relative standard deviation</td>
<td>0.92</td>
<td>0.79</td>
</tr>
<tr>
<td>0.05 level</td>
<td>±0.462</td>
<td>±0.741</td>
</tr>
<tr>
<td>0.01 level</td>
<td>±1.14</td>
<td>±0.954</td>
</tr>
</tbody>
</table>

Assay procedures

Aliquots of standard Niguldipine ranging from 0.1-0.5 mL were transferred into a series of 50 mL separating funnels. 2 mL of BTB (0.2%) and MO (0.2%) were added to the aliquots solution and the total volume of the aqueous phase was made up to 10 mL with distilled water. Chloroform (10 mL) was added in three initial amounts to each funnel and the contents were shaken for two minutes. The contents were allowed to stand until two phases are separate clearly and the absorbance of the chloroform layer was measured at 380 nm and 430 nm against the reagent blank. Satisfactory results were obtained for drug analysis in pharmaceutical formulations and the results were reproducible with low R.S.D. values. The average percent recoveries obtained were quantitative, indicating good accuracy of these
methods. The results of analysis of the commercial tablets and the recovery studies of drug suggested that there is no interfere for many excipients (such as Strarch, Lactose, Titanium dioxide, and Magnesium stearate) which are presented in Table 2.

Table 2. Assay and recovery of Niguldipine in dosage forms.

<table>
<thead>
<tr>
<th>SNO.</th>
<th>Pharmaceutical Formulation</th>
<th>Labelled amount(mg)</th>
<th>proposed method</th>
<th>Found by reference method ±S.D</th>
<th>% recovery by proposed methods ±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tablet-1</td>
<td>10</td>
<td>10.04 ± 0.015</td>
<td>0.067</td>
<td>2.169</td>
</tr>
<tr>
<td>2</td>
<td>Tablet-2</td>
<td>10</td>
<td>9.96 ± 0.012</td>
<td>0.075</td>
<td>2.540</td>
</tr>
<tr>
<td>3</td>
<td>Tablet-3</td>
<td>10</td>
<td>9.93 ± 0.095</td>
<td>0.057</td>
<td>2.169</td>
</tr>
<tr>
<td>4</td>
<td>Tablet-4</td>
<td>10</td>
<td>9.91 ± 0.009</td>
<td>0.045</td>
<td>2.474</td>
</tr>
<tr>
<td>5</td>
<td>Tablet-5</td>
<td>10</td>
<td>10.04 ± 0.082</td>
<td>0.262</td>
<td>2.175</td>
</tr>
<tr>
<td>6</td>
<td>Tablet-6</td>
<td>10</td>
<td>10.01 ± 0.008</td>
<td>0.391</td>
<td>2.638</td>
</tr>
</tbody>
</table>

Conclusions
The proposed methods are simple and sensitive and reproducible for the determination of Niguldipine in any pharmaceutical preparations and did not suffer any interference due to common excipients of tablets like talk starch and magnesium stearate, lactose etc.

REFERENCE
Acceptance Letter

To,

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Dear Prof/Dr./Mr.
I have immense pleasure to inform you that your paper entitled “SIMULTANEOUS ESTIMATION AND VALIDATION OF GLISOXEPIDE IN PHARMACUETICAL FORMULATIONS BY RP-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY” have been accepted and the editorial board agrees to publish your paper in the forthcoming issue of Bulletin of Environment, Pharmacology and Life Sciences Volume 3 [1] 2013.

Thank You

Date: 11/11/2013
Place: AGRA

Dr. Manish Kumar
[BEPLS ]

Correspondence to: Dr. Manish Kumar, 27, B.N.Puram, Paschim Puri Road, Sikandra-Bodla, Agra-282007, U.P. INDIA. Email: manishzoology06@gmail.com. Mob: +91-9457202960, 9457053501.
SIMULTANEOUS ESTIMATION AND VALIDATION OF GLISOXEPIDE IN PHARMACEUTICAL FORMULATIONS BY RP-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

This paper describes a simple, precise and accurate HPLC method for the simultaneous estimation of Glisoxepide as the bulk drug and in tablet dosage forms. The separation was achieved using a reverse phase column (250 × 4.6 mm, 5µm, L7pack) at ambient temperature with an isocratic mixture of ammonium acetate and acetonitrile in the ratio of 30:70% V/V at a flow rate of 1mL/min and detection at 254 nm. The retention time for Glisoxepide was 1.247 min. The linearity for Glisoxepide was found to be 1 to 10 µg/ml and it obeys beer’s law with correlation coefficient of 0.9991. The percent recovery obtained for Glisoxepide was 99.6 ± 0.86 The method is accurate, precise and found to be suitable for the quantitative analysis of the drugs in combinational dosage forms.

Keywords: Glisoxepide, HPLC

INTRODUCTION

Glisoxepide is a well-known as a Glisoxepid, Glisepin, Pro-Diabon, Glisoxepida. It is an anti-diabetic drug from the group of sulfonylureas \(^{(1)}\) and it is one of the sulphonamide-derived oral anti-diabetic drug. It inhibits the uptake of bile acids into isolated rat hepatocytes. However, it inhibits taurocholate uptake only in the absence of sodium ions. Glisoxepide uptake could be further inhibited by blockers of the hepatocellular monocarboxylate transporter by the loop diuretic bumetanide, 4,4′-diisothiocyanato-2,2′-stilbenedisulfonate (DIDS) and Bisulphate\(^{(3-8)}\). These results are consistent with the transport of glisoxepide via the transport system for the unconjugated bile acid cholate. It has the structural formula and shown in Fig. 1. The developed method was simple, precise, specific and accurate. The statistical analysis prove that method is reproducible and selective for the analysis of Glisoxepide in bulk drug and tablet formulations.
The challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry. Different analytical techniques like preparative HPLC\textsuperscript{(9-14)}, liquid-liquid extraction\textsuperscript{(15-20)}, UV-Vis Spectrophotometry available for the assay and impurities of drugs, HPLC/UPLC\textsuperscript{(21-27)} is Glisoxepide, . The developed method was simple, precise, specific and accurate. The statistical analysis prove the method is reproducible and selective for the analysis of Glisoxepide in bulk drug and tablet formulations. This paper presents RP-HPLC method for simultaneous determination of Glisoxepide in bulk and its formulations.

![Structure of Glisoxepide](image)

**Fig 1. Structure of Glisoxepide**

**MATERIALS AND METHODS**

**Chemicals and reagents**

HPLC grade acetonitrile and ammonium acetate were used for the analysis. Water obtained from Milli-Q water system. Glisoxepide used in this study is procured from NEO Medichem private limited, Hyderabad. Formulation used for this study is 10 mg of Glisoxepide.

**Instrumentation**

Chromatographic separation was performed on a SHIMADZU chromatographic system equipped with LC-20AT pump. Variable wavelength programmable UV-visible detector SPD – 20A and Rheodyne (7725i) with 20µL fixed loop are used and data analysis is done by using SPINCHROM software.

**Chromatographic conditions**

Separation and analysis was carried out on C\textsubscript{8} (250 ×4.6 mm, 5µm, L7 pack) column. Mobile phase consisting of a mixture of acetonitrile and ammonium acetate in the ratio of 30:70 V/V was delivered at flow rate of 1 ml/min. with detection at 254 nm. The mobile phase was
filtered through a 0.45 µm membrane filter and sonicated for 15 min. Analysis was performed at ambient temperature.

**Preparation of standard solution**

Accurately, weighed 10 mg of Glisoxepide transferred into a 100 mL volumetric flask then 5 mL methanol was added and shaken well to dissolve and sonicated for 5 min. Volume was made up to 100 mL with acetonitrile. The solution was further diluted with acetonitrile to achieve final concentration of 10 µg/mL of each drug and filtered through a 0.45 µm membrane filter before injection.

**Sample preparation and assay**

A pharmaceutical sample containing Glisoxepide 10 mg was weighed and transferred to 100 mL volumetric flask. The contents of the flask were dispersed in 30 mL of ammonium acetate and 70 mL of acetonitrile and shaken well to dissolve and sonicated for 30 min. and further dilution was made up to get 10 µg/mL concentration of each drug. The solution was filter through 0.45 µm membrane filter before injection. All determinations were conducted in triplicate. Both the standard and sample preparation was injected separately, and the peak area responses were recorded. The percentage of the formulations was calculated and given in Table-1.

**Table 1: Assay of formulation**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pharmaceutical Formulation</th>
<th>Labelled amount (mg)</th>
<th>proposed method</th>
<th>Found by reference method ±S.D</th>
<th>% recovery by proposed methods ±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amount found (mg)</td>
<td>t (Value)</td>
<td>F (Value)</td>
</tr>
<tr>
<td>1</td>
<td>Glisoxepide</td>
<td>5</td>
<td>4.98 ± 0.017</td>
<td>0.682</td>
<td>1.535</td>
</tr>
<tr>
<td></td>
<td>Glisoxepide</td>
<td>5</td>
<td>4.98 ±0.043</td>
<td>1.414</td>
<td>2.462</td>
</tr>
<tr>
<td></td>
<td>Glisoxepide</td>
<td>5</td>
<td>5.05 ± 0.028</td>
<td>1.426</td>
<td>1.124</td>
</tr>
</tbody>
</table>
RESULT AND DISCUSSION

The simultaneous estimation of Glisoxepide was carried out by RP-HPLC using ammonium acetate and acetonitrile as mobile phase in the ratio of 30:70 V/V and C₈ column as the stationary phase. The results of system suitability parameters such as tailing factor, asymmetry and number of theoretical plates are indicated satisfactory results and tabulated in Table-2. The retention time for Glisoxepide was found to be 1 mL/min. The resolution value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicate the complete separation of the drug. The linearity was studied the concentration range from 1-10 µg/mL. The regression coefficient value is 0.9994 respectively, the mean recovery for Glisoxepide was 99.0 to 100.0%, which is largely within the 90 to 110% range that is considered acceptable and reveals that the method is accurate.

The validation of the proposed method was verified by system precision and method precision. The system precision was evaluated by measuring the peak area response of Glisoxepide for five replicate samples of the standard solutions. The method precision was determined by quantifying the sample solutions as per the proposed method. The % RSD was found to be less than 2 proposed precision, accuracy. The specificity of the method was confirmed by injecting the placebo. Robustness of the method is determined by analyzing the samples in duplicate with varying the method conditions very small changes in flow rates, showed there were no marked changes in chromatographic behavior and content of the drug as evident from the low value of RSD indicating the method is robust. The method was also confirmed by ruggedness study analyzing the product day to day, analyst and instrument to instrument.

The data of ruggedness of Glisoxepide are found to be within the acceptance limit. Different validation parameters for the proposed HPLC method is summarized in Table-3 and chromatogram of Glisoxepide is shown in Fig- 2. The result obtained was in agreement with the labeled value of Glisoxepide formulations. The parameters are in the acceptable ranges.
**Fig 2. Chromatogram for GSP (M15)**

Peak table

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area%</th>
<th>Tailing factor (0%)</th>
<th>K'</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.247</td>
<td>11574</td>
<td>17.925</td>
<td>1.733</td>
<td>0.000</td>
<td>0.000</td>
</tr>
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</table>
Table-2. System suitability parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Glisoxepide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theoretical plates (N)</td>
<td>1216</td>
</tr>
<tr>
<td>2</td>
<td>Tailing factor</td>
<td>1.733</td>
</tr>
<tr>
<td>3</td>
<td>Assymetry</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>%RSD if peak retention time</td>
<td>0.136%</td>
</tr>
</tbody>
</table>

Table-3. Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glisoxepide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>1-10 µg/mL</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.9991</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>5 ng/mL</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.0 to 100.0</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>0.699%</td>
</tr>
<tr>
<td>Robustness (%RSD)</td>
<td>0.735%</td>
</tr>
<tr>
<td>Ruggedness (%RSD)</td>
<td>0.683</td>
</tr>
</tbody>
</table>

CONCLUSION

The proposed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of Glisoxepide and pharmaceutical dosage forms without any interference from the excipients.
REFERENCES


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