Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Rosiglitazone and Metformin in Bulk and Tablet Dosage Form

Dhirender Singh Mittan\textsuperscript{a}, S.C. Dwivedi\textsuperscript{a}, Ashok Kushnoor\textsuperscript{b}

\textsuperscript{a}School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India.
\textsuperscript{b}Shri Gopichand College of Pharmacy, Baghapat, Uttar Pradesh, India.

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Abstract

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of rosiglitazone and metformin in bulk and tablet dosage form. Chromatographic analysis was performed on a C\textsubscript{18} column (250x 4.6 mm, 5\textmu m) with a mixture of Ammonium dihydrogen Phosphate buffer (pH 4.5): Acetonitrile in the ratio 65:35 as mobile phase, at a flow rate of 1.0 mL min\textsuperscript{-1}. UV detection was performed at 230 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of rosiglitazone and metformin were 7.19±0.044 and 5.57±0.038 min, respectively. Calibration plots were linear over the concentration ranges 12–32 \mu g mL\textsuperscript{-1} and 20–70 \mu g mL\textsuperscript{-1} for rosiglitazone and metformin, respectively. The Limit of detection was 1.100 and 0.712 \mu g mL\textsuperscript{-1} and the quantification limit was 3.66 \mu g mL\textsuperscript{-1} and 2.41 \mu g mL\textsuperscript{-1} for metformin and rosiglitazone, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 97.72\% to 100.46\%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of rosiglitazone and metformin in bulk and tablet dosage form.

Keywords:
Rosiglitazone, Metformin, RP-HPLC, ICH guidelines

1. Introduction

Rosiglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[[2-(5-ethylpyridin-2-yl) ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione. Rosiglitazone is an oral anti-diabetic agent and acts as an agonist at PPAR gamma receptors have acts primarily enhances tissue sensitivity to insulin. Metformin is an antihyperglycemic agent, which improves glucose tolerance in patients, chemically it is 3-(diaminomethylidene)-1,1-dimethylguanidine. Metformin is used for the treatment of with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. A literature survey reveals that various analytical methods like rosiglitazone by HPLC and MECK [1]. Simple HPLC method for the determination of rosiglitazone in human plasma [2], metformin in human plasma using ion-pair HPLC [8] Simultaneous HPLC estimation of metformin in combination
DEVELOPMENT AND VALIDATION OF A HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND GLIMEPIRIDE IN BULK AND TABLET DOSAGE FORM

Dhirender Singh1, S.C.Dwivedi1, Ashok Kushnoor2

1School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, (INDIA)
2Shri Gopichand College of Pharmacy, Baghpat, Uttar Pradesh, (INDIA)

E-mail of Corresponding author: chdsmittan@gmail.com

Abstract
A simple high-performance liquid chromatographic (HPTLC) method has been developed and validated for simultaneous determination of pioglitazone and glimepiride in bulk and tablet dosage form. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of Benzene: Ethyl acetate: Diethyl ether 6:3:1 v/v. The detection of spot was carried out at 254 nm. The calibration curve of pioglitazone was found to be linear between response was determined of 600 ng/ml to 3600 ng/ml with regression coefficient 0.9984 and calibration curve of glimepiride was found to be linear between 200-1200 ng/ml for glimepiride with regression coefficient of 0.9991. The limit of detection was 57.22 ng/ml and 16.67 ng/ml and the quantification limit was 190.73 ng/ml and 55.58 ng/ml for pioglitazone and glimepiride respectively. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 97.84 to 99.07 %. The proposed method is applicable to routine analysis of Pioglitazone in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

Keywords: Pioglitazone, Glimepiride, HPTLC, ICH guidelines

1. Introduction
Pioglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[(2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione. Pioglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamma receptors having effects primarily by reducing insulin resistance. Glimepiride is a second-generation sulfonylurea derivative...
Development and Validation of A HPTLC Method For Estimation of Pioglitazone in Bulk and Tablet Dosage Form

Dhirender Singh1,2, S.C. Dwivedi1, Ashok Kishnor2
School of Pharmacy, Satyam Gyan Vihar University, Jhunjhununj, (JNLU)
Shri Gopalchand College of Pharmacy, Bagpat, Uttar Pradesh, (HNDI)

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ABSTRACT

A simple, accurate, precise and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of pioglitazone in tablet dosage forms. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of Toluene: Ethyl acetate: Formic Acid 10:3:1 v/v. The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 100 to 9000 ng ml−1 with regression coefficient of 0.9992. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 98.34 to 99.40 %.

The proposed method is applicable to routine analysis of pioglitazone in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

Key words: Pioglitazone, HPTLC, ICH guidelines

INTRODUCTION

Pioglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus. Chemically it is C21H24N7O3. Pioglitazone has been evaluated by HPLC, HPTLC and solid phase extraction method in human serum and Human Plasma. Pioglitazone has been analysed by HPLC and HPTLC by various authors. So, there is a need for a simple and rapid method for the estimation of pioglitazone in bulk and pharmaceutical formulations.

EXPERIMENTAL DETAILS

Materials

Pioglitazone (Assigned purity 99.95 %) was obtained from the vendors. Standard solution of pioglitazone was prepared by dissolving 10 mg of standard pioglitazone in 100 ml volumetric flask containing sufficient amount of methanol to dissolves the drug. Stock solution was stored at -20 °C until used. The standard solution was further diluted with methanol to obtain concentration of 100, 200, 300, 500, 750 and 1000 ng ml−1.

Equipment

The instrument used in the present study was Shimadzu SPD-10A UV-vis spectrophotometer, Hitachi 2000, Hitachi High-Tech Co., Ltd. (Japan) equipped with a 10 mm pathlength quartz cell. The HPLC system consisted of an Autosampler (Kumon, Japan), a Shimadzu LC-10AT pump and a Shimadzu SPD-10A UV-vis spectrophotometer. The data was processed using Shimadzu DC-2000 mass spectrometer.

RESULT AND DISCUSSION

The developed method was validated according to the ICH guidelines. The results are represented in Table 1.

Fig 1: Chemical structure of Pioglitazone
DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR ESTIMATION OF PIOGLITAZONE IN BULK AND TABLET DOSAGE FORM

Dhirender Singh1, S.C. Dwivedi2, Ashok Kushnur2
1School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, (INDIA)
2Shri Gopichand College of Pharmacy, Baghpat, Uttar Pradesh, (INDIA)

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ABSTRACT
Purpose: A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for determination of pioglitazone in bulk and tablet dosage form.
Method: Chromatographic analysis was performed on a C18 column (250x 4.6 mm, 5µm) with a mixture of Methanol: Phosphate buffer (pH 4.0) in the ratio 70:30 as mobile phase, at a flow rate of 1.0 mL min⁻¹. UV detection was performed at 260 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity.
Result: The retention times of pioglitazone was 6.66±0.0366 min respectively. Calibration plots were linear over the concentration ranges 30–80 µg mL⁻¹. The Limit of detection was 0.365 and the quantification limit was 1.218 µg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 100% to 102.52%.
Conclusion: Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of pioglitazone in bulk and tablet dosage form.

KEYWORDS: Pioglitazone, RP-HPLC, ICH guidelines

INTRODUCTION
Pioglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-{[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl}-1,3-thiazolidine-2,4-dione. Pioglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamma receptors have acts primarily by reducing insulin resistance. A literature survey reveals that various analytical methods like pioglitazone by HPLC and MECK, HPLC and solid phase extraction method in human serum, Human Plasma, pioglitazone have been analysed by HPLC. Simultaneous HPLC analysis of pioglitazone and glimepiride, Simultaneous HPLC
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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR ESTIMATION OF ROSIGLITAZONE IN BULK AND TABLET DOSAGE FORM

Dhirendra Singh\textsuperscript{1}, S.C.Dwivedi\textsuperscript{1}, Ashok Kushnoor\textsuperscript{2}

1. School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, (INDIA)
2. Shri Gopichand College of Pharmacy, Baghpat, Uttar Pradesh, (INDIA)

Corresponding author*: chdsmittan@gmail.com
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ABSTRACT
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated of rosiglitazone in bulk and tablet dosage form. Chromatographic analysis was performed on a C\textsubscript{18} column (250x 4.6 mm, 5\textmu m) with a mixture of Ammonium dihydrogen phosphate buffer (pH 4.5) : Acetonitrile in the ratio 65:35 as mobile phase, at a flow rate of 1.0 mL min\textsuperscript{-1}. UV detection was performed at 230 nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The retention times of rosiglitazone was found to be 7.19±0.145 respectively. Linearity was observed in concentration ranges of 12–70 \mu g mL\textsuperscript{-1} The limit of detection was 0.725 and the quantification limit was 2.41 \mu g/ml. The accuracy of the proposed method was determined by recovery studies and found to be 98.26\% to 101.37\%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of rosiglitazone and in bulk and tablet dosage form.

KEY WORDS: Rosiglitazone, RP-HPLC, ICH guidelines

1. INTRODUCTION
Rosiglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus; chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione. Rosiglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamma receptors have acts primarily enhances tissue sensitivity to insulin. A literature survey reveals that various analytical methods like rosiglitazone by HPLC and MECK\textsuperscript{2}, Simple HPLC method for the determination of rosiglitazone in human plasma\textsuperscript{3,4}, Simultaneous HPLC estimation of metformin in combination with rosiglitazone\textsuperscript{5}, rosiglitazone with gliclazide in tablet\textsuperscript{6}, rosiglitazone and gemfibrozil in human plasma\textsuperscript{7}, Simultaneous LC-UV estimation of rosiglitazone and glimepiride in plasma\textsuperscript{8}, rosiglitazone and glimepiride in human plasma\textsuperscript{9}. Simultaneous LC-ESI-MS method for estimation of rosiglitazone and N-desmethyl rosiglitazone in human plasma\textsuperscript{10}.
But these methods are sophisticated, expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproducible HPLC method for the
DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND METFORMIN IN BULK AND TABLET DOSAGE FORM

Dhirender Singh1, S.C.Dwivedi1, Ashok Kushnoor2

1School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India
2Shri Gopichand College of Pharmacy, Baghpat, Uttar Pradesh, India

E-mail of Corresponding Author: chdsmittan@gmail.com

Abstract
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of pioglitazone and metformin in bulk and tablet dosage form. Chromatographic analysis was performed on a C18 column (250x 4.6 mm, 5µm) with a mixture of Methanol:Phosphate buffer in in the ratio 68:32 as mobile phase, at a flow rate of 1.0 mL min⁻¹. UV detection was performed at 260 nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The retention times of pioglitazone and metformin were 7.24±0.051 and 2.54±0.038 min respectively. Calibration plots were linear over the concentration ranges 10–35 µg mL⁻¹ and 15–40 µg mL⁻¹ for pioglitazone and metformin respectively. The Limit of detection was 0.382 and 0.131 µg/ml and the quantification limit was 1.27 µg/ml and 0.436 µg/ml for metformin and pioglitazone respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.65% to 98.90%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of pioglitazone and metformin in bulk and tablet dosage form.

Keywords: Pioglitazone, Metformin, RP-HPLC, ICH guidelines

1. Introduction
Pioglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4-dione. Pioglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamma receptors have acts primarily by reducing insulin resistance. Metformin is an antihyperglycemic agent, which improves glucose tolerance in patients, chemically it is 3-(diaminomethylidene)-1,1-dimethylguanidine. Metformin is used for the treatment of with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

A literature survey reveals that various analytical methods like pioglitazone by rapid determination of metformin in human plasma using ion-pair HPLC5 Simultaneous estimation of metformin, pioglitazone, and Glimepride by RP-HPLC2, Simultaneous spectrophotometric estimation of three component tablet formulation Containing pioglitazone, metformin and Glibencamide3,

Simultaneous Estimation of metformin in combination with rosiglitazone by RPHPLC6 & in human plasma by liquid chromatography/tandem mass spectrometry with electrospray ionization5, liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimepride in plasma7. But these methods are sophisticated, expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproducible simultaneous HPLC method for the estimation of pioglitazone and metformin in bulk and its formulation.

Fig. 1: Chemical structure of Pioglitazone and Metformin