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The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma

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Abstract

This article describes the development of SPE and HPLC methods for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. Several extraction and HPLC methods have been described previously for the determination of each of these analytes in plasma separately. The simultaneous determination of these analytes is important for the routine monitoring of diabetic patients who take combination medications and for studying the pharmacokinetics of the combined dosage forms. In addition this developed method can serve as a standard method for the plasma determination of these analytes therefore saving time, effort and money. The recoveries of the developed methods were found to be between 76.3% and 101.9%. The limits of quantification were between 5 and 22.5 ng/ml. The intraday and interday precision (measured by coefficient of variation, CV%) was always less than 9%. The accuracy (measured by relative error %) was always less than 12%. Stability analysis showed that all analytes are stable for at least 3 months when stored at −70 °C.

Keywords: HPLC; Metformin; Glipizide; Gliclazide; Glimperide; Glibenclamide

1. Introduction

Currently the most commonly prescribed medications for Type 2 diabetes are metformin and the second generation sulfonylureas which include glipizide, gliclazide, glibenclamide and glimperide. For many patients with Type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control [1]. In such cases a combination of metformin and one of the sulfonylureas (SU) is used [2]. This combination can be achieved by taking each of the drugs separately or alternatively fixed formulations have been developed. Combinations of metformin and glipizide or gliclazide or glibenclamide are available commercially as single tablets. A combination tablet formulation is beneficial in terms of its convenience and patient compliance. The measurement of the plasma concentrations of antidiabetic medications is important for studying the pharmacokinetics of these drugs, for adherence and drug monitoring in diabetic patients and for diagnostic purposes in factitious hypoglycaemia.

The choice of treatment for diabetic patients is mainly dependent on the doctors' choice which should be dependent on the patients' clinical characteristics and the pharmacological properties of the various agents available, thus, for certain diabetic populations we can find patients who are prescribed glipizide, gliclazide, glimperide, glibenclamide, metformin or a combination of metformin and one of the sulfonylureas.

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Therefore, therapeutic monitoring requires the availability of a single method that can be used for all these possibilities in order to save time, cost ad effort.

Several procedures have been developed to be used as standard methods for the analysis of sulfonylureas [3–6]. However, none of these methods were suitable for routine analysis. Some of them used solvent extraction in sample preparation [4,5] which is a time consuming process and loss of sample may frequently occur during extraction due to emulsion formation in addition to the reported low recovery. Even though Paroni et al. [3] used solid phase extraction (SPE) utilising OASIS® HLB cartridges, they used five washing steps during the extraction process which is not practical and is time consuming, in addition they used a gradient elution HPLC method. The SPE method developed by Strausbauch et al. [6] was only validated for urine samples. A more important limitation for implementing the previously mentioned procedures for routine analysis of antidiabetic medications is that they were all developed for estimation of glipizide, gliclazide and glibenclamide but not for glimperide or metformin.

Until now glimperide was only analysed in biological matrices by an HPLC method using derivatisation and solvent extraction which is tedious and time consuming [7]. Glimperide is relatively new and it was reported that the commonly used methods for sulfonylurea determination have low sensitivity for glimperide determination [7]. Niemi et al. [8] used an HPLC–mass spectrometry (MS) method for studying the effect of rifampicin on the pharmacokinetics of glimperide, however, the method was not validated and the use of MS has the disadvantage of that it is not available in many laboratories.

There is no single published method for the simultaneous determination of both metformin and any of the sulfonylureas in biological fluids. A method for determination of metformin and glipizide or gliclazide [9] and a method for the estimation of metformin and glibenclamide from their combined dosage forms [10] have been described previously for use in studying pharmaceutical preparations but not for analysis in biological fluids. When studying the pharmacokinetics of a new formulation containing a combination of metformin and glibenclamide Martha et al. [1] used two separate methods one for measuring the concentration of metformin and the other for glibenclamide.

In summary, in order to save time and money routine therapeutic monitoring requires the availability of a single method that can be used for the simultaneous determination of antidiabetic medications in plasma. Although, many methods have been reported in the literature for the estimation of metformin, gliclazide, glibenclamide and glipizide individually there is no single method reported for the simultaneous estimation of metformin and sulfonylureas. The reported general procedures for sulfonylurea determination did not include glimperide or metformin. In a previous study [11] the first ion pair solid phase extraction technique was developed for the specific HPLC determination of metformin. In this study that method was optimized for the simultaneous determination of a combination of metformin and sulfonylureas.

2. Experimental

2.1. Reagents and materials

Metformin (M), phenformin (P), glibenclamide (glyburide) (Gb), tolbutamide (T), glipizide (Gp), potassium dihydrogenphosphate (KH_2PO_4), sodium dodecyl sulphate (SDS, sodium lauryl sulphate), decane sulfonic acid sodium salt and heptanesulfonic acid sodium salt were purchased from Sigma (Poole, England). Glimperide (Gm) was provided by Aventis Pharma (Frankfurt, Germany). Gliclazide (Gc) was extracted (according to a British Pharmacopoeial procedure, 1998) from Gliclazide 80 mg tablets which were purchased from Generics (Herts, UK). Acetonitrile, methanol, and tetrahydrofuran (THF) were of HPLC grade and purchased from Romil (Cambridge, UK). Membrane filters F-4500.45 μm were obtained from Gelman Laboratory (Portsmouth, UK). Solid phase extraction cartridges [Waters Oasis® HLB and MCX cartridges (1 ml, 30 mg)] were purchased from AGB, Belfast. Extraction was carried on a Waters extraction manifold. Blank blood was donated from Northern Ireland Blood Transfusion Centre.

2.2. Internal standards

For the simultaneous determination of M and Gb or Gm (Method 1) the internal standard was P and for the simultaneous determination of M and Gp or Gc (Method 2) the internal standard was T. Stock solutions of the internal standards (P and T) were prepared by dissolving 0.016 g in 100 ml of water (initially dissolved in a few drops of methanol) and then further diluted with water (1:40). When preparing samples for extraction 50 μl of this stock was added to 1 ml of plasma to produce an internal standard concentration of 200 ng/ml plasma.

2.3. Preparation of the mixed standards

For the determination of a combination of M and Gb or Gm (Method 1) the stock was prepared by dissolving 0.08 g of M and 0.04 g of Gb and Gm in 100 ml methanol. For the determination of a combination of M and Gp or Gc (Method 2) the stock was prepared by dissolving 0.04 g of Gp and 0.08 g of M and Gc in 100 ml methanol. From each of these two stock solutions 0.2 ml was taken and further diluted with water to 10 ml (this produced the first working standards with concentrations of 16 μg/ml for M and Gc and 8 μg/ml for G, Gb and Gp); these were further diluted with water to produce the remaining working standards. For Method 1; 50 μl of the aqueous solution of the internal standard (P) and 0.125 ml of the standards containing M, Gb and Gm were added to 1 ml plasma resulting in the following concentrations for M 50,