Journal of Chromatography B, 869 (2008) 133–137

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Development and validation of an HPLC-UV method for iodixanol quantification in human plasma

Shripad D. Chitnis, Fatemeh Akhlaghi*

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI, USA

ARTICLE INFO

Article history:
Received 29 December 2007
Accepted 4 May 2008
Available online 10 May 2008

Keywords:
Assay
Concentration
Contrast media agent
GFR
HPLC-UV
Iodixanol
Iohexol
Kidney function

ABSTRACT

Iodixanol is a widely used iso-osmolar contrast medium agent. Similar to iohexol, it can also be a good exogenous marker for the measurement of glomerular filtration rate (GFR). This article describes the development and validation of an HPLC-UV method for quantification of iodixanol in human plasma. Internal standard, iohexol (20 μl, 1 mg/ml), and perchloric acid (30 μl, 20%, v/v) were added to plasma samples (300 μl), followed by neutralization with 10 μl potassium carbonate (5 M). Samples were centrifuged and 10 μl of the supernatant was injected onto a C_{18} EPS analytical column (3 μm particle size, 150 mm × 4.6 mm). The extraction method yielded >95% recovery for both iodixanol and iohexol. The mobile phase consisted of 0.1% (w/v) sodium formate buffer and acetonitrile. Iohexol and iodixanol peaks were eluted at ∼5 and 9 min, respectively using a fast gradient method. The assay lower limit of detection was 2.0 μg/ml and lower limit of quantification was 10 μg/ml. The calibration curves, assessed in six replicates, were linear over an iodixanol concentration range of 10–750 μg/ml. Intra- and inter-day accuracy was >95% and precision expressed as % coefficient of variation was <10%. This method is simple, accurate, precise and robust and can potentially be used for iodixanol quantification in large-scale clinical studies.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Chronic kidney disease is a worldwide epidemic and the number of patients suffering from this, and eventually reaching end stage renal disease (ESRD), is on the rise. An accurate method to measure kidney function, by the means of estimation of glomerular filtration rate (GFR), is therefore, required to diagnose chronic kidney diseases. GFR correlates well with the functional mass of the kidney [1]. It is measured as the renal clearance of a particular substance such as creatinine or inulin from plasma in unit time and typically expressed as ml/min [2].

In the clinical settings, calculated creatinine clearance (Clcr) values are widely used to estimate GFR [3]. Creatinine is produced endogenously during biosynthesis of aminoacids, is freely filtered through glomerulus and is excreted by the kidneys. Creatinine clearance can be calculated by collecting urine over a 24-h period or by measuring a single creatinine concentration in serum followed by calculation of Clcr using Cockcroft–Gault or comparable equations [3]. However, creatinine clearance often overestimates the GFR in different patient groups.

An ideal GFR marker is often considered as an agent that does not bind to plasma proteins, has no significant systemic metabolism and is freely excreted through glomerular filtration [4,5]. Traditionally, clearance of inulin, a simple sugar administered intravenously, is considered the gold standard for GFR measurement. The inulin clearance method has several disadvantages such as the unavailability of clinical grade dosage form for injection and the need for 24 h urine collection. Alternatively, the usefulness of contrast media agents for GFR estimation has been demonstrated by several investigators [6]. These agents include water-soluble iodinated pyridones [7], triiodinated benzoates (ionic monomers, e.g., iothalamate) [1,8] and nonionic monomers (e.g., iohexol) [6].

In the past decade, iodixanol (Visipaque®, Nycomed, Oslo, Norway) with a molecular weight of 1550.2 (iodine content 49.1%), has been introduced into the clinical practice (Fig. 1) [9]. Iodixanol is an iso-osmolar (low osmolality compared to other nonionic monomers and ionic compounds), nonionic dimeric and hydrophilic contrast medium agent; which produces less hemodynamic changes and in turn reduces the renal toxicity associated with the use of contrast media agents [9,10]. It is commonly used as a contrast agent during coronary angiography and contrast enhanced computer tomography (CECT) imaging of the head and body [11]. Iodixanol does not change GFR and is well tolerated in

* Corresponding author at: Clinical Pharmacokinetics Research Laboratory, Biomedical and Pharmaceutical Sciences, University of Rhode Island, 125 Fogarty Hall, 41 Lower College Road, Kingston, RI 02881, USA. Tel.: +1 401 874 9205; fax: +1 401 874 2181.
E-mail address: fatemeh@uri.edu (F. Akhlaghi).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.05.002

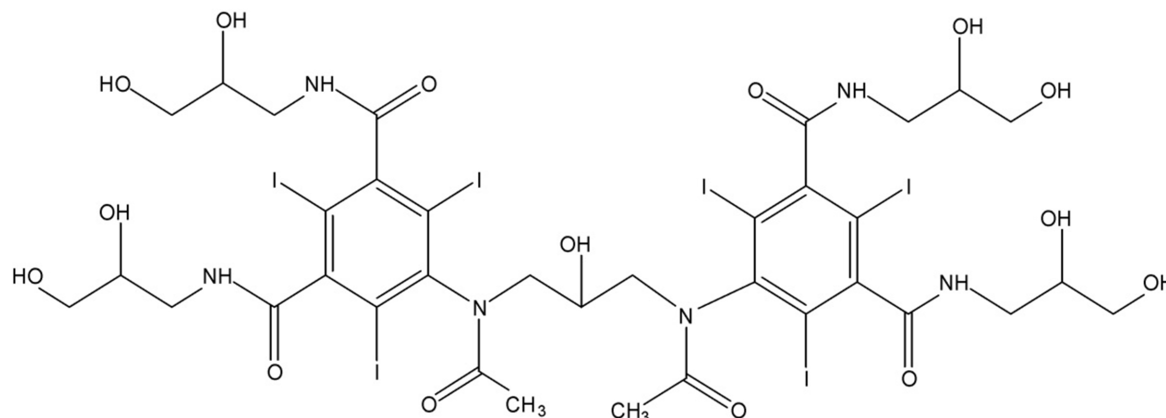

Fig. 1. Chemical structure of iodixanol. Iodixanol: 5,5′-[(2-hydroxy-1,3-propanediyl)bis(acetylimino)] bis [*N*,*N*′-bis(2,3-dihyd roxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide].

patients with normal renal function or in patients with severe renal failure [12,13].

Upon intravenous administration of iodixanol, peak plasma concentration is achieved rapidly and it distributes exclusively in extracellular fluids with a distribution half-life of ∼0.5 h. Iodixanol does not undergo systemic metabolism and >97% of the dose is excreted in urine by glomerular filtration [9,14]. Furthermore, the renal clearance of iodixanol shows a good agreement with the renal clearance of ^{125}I-iothalamate, which itself is a well-established GFR marker [15]. In conclusion, iodixanol may prove to be an ideal candidate for the measurement of GFR when compared to other iodinated contrast media agents.

Several methods have been published for quantification of iodixanol in plasma, urine and feces using HPLC-UV, X-ray fluorescence spectroscopy and neutron activation analysis [14,16–18]. The method described by Jacobsen [16] requires an automated online dialysis system that is not widely available. In addition, the other published methods lack detailed description of the chromatography or extraction procedures and present limited assay validation. In the present study, we have developed a simple, robust and rapid HPLC-UV assay, which utilizes a simple protein precipitation method and commercially available USP grade internal standard to quantify iodixanol in human plasma. The present assay, is a modified version of a method we have previously published to analyze the concentration of iohexol in human plasma [5]. Furthermore, the assay was validated according to guidelines provided by the Food and Drug Administration of the United States (USFDA) [19].

2. Experimental

2.1. Chemicals and reagents

Iodixanol (99% pure) and iohexol (99% pure, used as internal standard) were purchased from United States Pharmacopeia (USP, Rockville, MD). Perchloric acid (70%, v/v) was obtained from Acros Organics (Fair Lawn, NJ), HPLC grade acetonitrile and methanol from Pharmco Products (Brookfield, CT) and potassium carbonate from Fisher Scientific (Fair Lawn, NJ). Purified deionized water was prepared using Milli Q50 (Millipore, Bedford, MA) water purification system. For the preparation of in-house quality control and calibration samples, human plasma from normal donors was purchased from Rhode Island Blood Center (Providence, RI).

2.2. Assay conditions

Quantitative analysis of iodixanol in human plasma was performed using an HPLC-UV analytical system. The instrument consisted of a Hitachi D-7000 series (San Jose, CA) interfaced with an auto-sampler fitted with a 200 μl sample loop, a quaternary pump, a column oven and a variable wavelength UV detector set at 254 nm. The Hitachi System Manager (HSM) software was used to integrate the chromatograms. All quantifications were based on the peak area ratio of iodixanol to internal standard and all calculations were performed using Microsoft Excel (MS office 2000). The analytical column used was a C_{18} EPS analytical column (3 μm particle size, 150 mm × 4.6 mm) manufactured by Alltech Associates (Deerfield, IL). A 2 μm pore size pre-column filter (Supelco, Bellefonte, PA) was attached to the column and both the pre-column and analytical column were maintained at 30 °C.

Iodixanol exists in three isomeric forms however, the mobile phase composition and gradient were adjusted such that all three isomers were eluted as a single peak at ∼9.3 min. The optimum chromatographic conditions were achieved using a fast gradient system of sodium formate buffer and acetonitrile. Mobile phase was filtered and degassed by passing through a 0.45 μm nylon filter (Millipore, Bedford, MA) under vacuum followed by sonication for 15 min.

The mobile phase consisted of different percentage of Solution A (sodium formate buffer, 0.1% (w/v) pH 7.4) and acetonitrile. At the beginning of each run, Solution A was pumped for 1 min at a flow rate of 1 ml/min. At 1.1 min, the mobile phase composition was changed (over a period of 30 s) to 4% acetonitrile and 96% Solution A and the flow rate was increased to 1.2 ml/min. Between 6.6 and 11.0 min, the mobile phase composition was maintained at 30% acetonitrile and 70% Solution A at 1.2 ml/min flow rate. The column was then re-equilibrated from 11.1 to 15.0 min with 100% Solution A. To minimize carryover, the sample loop was washed between injections with 10% acetonitrile in deionized water. Before each batch of samples, the analytical column was equilibrated with Solution A for at least 1.5 h at a flow rate of 1 ml/min.

2.3. Protein precipitation method

Frozen calibrators, QC standards and working stock solution of internal standard were thawed at 37 °C using a thermostatic shaking water bath (Precision Scientific, Chicago, IL). Aliquots of calibrators and QC standards (300 μl) were added to 1.5 ml micro-

Download English Version:

https://daneshyari.com/en/article/1215320

Download Persian Version:

https://daneshyari.com/article/1215320

Daneshyari.com