Clinical Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Short Communication

Improved determination of uracil and dihydrouracil in plasma after a loading oral dose of uracil using high-performance liquid chromatography with photodiode array detection and porous graphitic carbon stationary phase

Roberta Zilles Hahn [a], Andrés Fernando Andrade Galarza [b], Anelise Schneider [a], Marina Venzon Antunes [a], Gilberto Schwartsmann [b], Rafael Linden [a,*]

[a] Toxicological Analysis Laboratory, Instituto de Ciências da Saúde, Universidade Feevale, Novo Hamburgo, RS, Brazil
[b] Oncology Department, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 24 February 2015
Received in revised form 20 April 2015
Accepted 24 April 2015
Available online xxxx

Keywords:
Fluoropyrimidine drugs
Chemotherapy
Dihydropyrimidine dehydrogenase
HPLC-PDA
Porous graphitic carbon

ABSTRACT

Objectives: The aim of this study was to develop and validate a high-performance liquid chromatographic method for the measurement of plasma concentrations of uracil and dihydrouracil after administration of an oral loading dose of uracil in the context of evaluation of DPD enzyme activity.

Design and methods: Analytes were extracted from 500 μL plasma sampler with a mixture of ethyl acetate isopropanol (85:15, *v*/*v*) after protein precipitation with solid ammonium sulfate. The extract was inject in the porous graphitic carbon stationary phase, eluted with water and acetonitrile in gradient mode, allowing complete separation of uracil, dihydrouracil and the internal standard (5-fluorouracil). Chromatograms were monitored at 210 and 260 nm.

Results: Total chromatographic run time, including reequilibration, was 30 min. The assay was linear in the concentration range of 0.2 to 20 μg mL^{-1}. Accuracy was 98.4–105.3%, intra-assay precision was 5.1–12.1% and between-assay precision was of 5.3–10.1%. Analytes were stable in plasma at room temperature up to 6 h and for three freeze and thaw cycles. Processed samples are stable up to 12 h.

Conclusions: The developed method was fully validated and has significantly reduced running time when compared to previous assay using porous graphitic stationary phase, allowing complete resolution of uracil, dihydrouracil and internal standard. This assay might be suitable to investigate the eventual correlation between concentrations of uracil and dihydrouracil in plasma after an oral loading dose and DPD enzyme activity, with potential contribution to therapeutic drug monitoring.

© 2015 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

Introduction

Despite its use in many chemotherapy treatments, fluoropyrimidine drugs are associated to a high rate of severe adverse effects (31–34%) and, eventually, to toxic deaths (0.3%) [1]. One of the main reasons for fluoropyrimidine toxicity is a deficiency on the metabolic clearance mediated by dihydropyrimidine dehydrogenase (DPD) enzyme [2]. Approaches to identify patients with reduced DPD activity are based on the measurement of the concentrations of the endogenous compound uracil (U) and its DPD metabolic product, 5,6-dihydrouracil (UH2), either in peripheral blood mononuclear cells [3], blood plasma [4] or saliva samples [5]. Another alternative was proposed by van Stareven et al. [6] and is based on the measurement of plasma concentrations of U and UH2 after the administration of an oral loading dose of U, theoretically better reflecting DPD enzyme dynamics when high doses are administered to the patients.

Several methods based on high-performance liquid chromatography (HPLC) for measurement of U and UH2 in biological samples were described, usually based on reversed-phase (RP) separations [7,8]. However, considering the high hydrophilicity of U and UH2, RP separations usually require 100% aqueous mobile phases and long chromatographic runs to allow the separations of these analytes. An alternative separation strategy was described by Rémaud et al. [9], with the use of porous graphitic carbon (PGC) as stationary phase, with increased resolution of U and UH2. Despite its better separation capabilities, the method of Rémaud et al. [9] had a very long running (52 min) and equilibration (25 min) time, not amenable to clinical routine.

* Corresponding author at: Toxicological Analysis Laboratory, Instituto de Ciências da Saúde, Universidade Feevale, Rodovia ERS 239, n. 2755, CEP 93352-000 Novo Hamburgo, RS, Brazil.
E-mail address: rafael.linden@feevale.br (R. Linden).

http://dx.doi.org/10.1016/j.clinbiochem.2015.04.019
0009-9120/© 2015 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

Please cite this article as: Hahn RZ, et al, Improved determination of uracil and dihydrouracil in plasma after a loading oral dose of uracil using high-performance liquid chromatography with..., Clin Biochem (2015), http://dx.doi.org/10.1016/j.clinbiochem.2015.04.019

ARTICLE IN PRESS

Considering the potential clinical applications of a method for accurately classifying DPD activity, in this study we present an optimized and easily applicable liquid chromatographic method for determination of U and UH2 in human plasma after administration of an oral loading dose of U, based on PGC separation.

Material and methods

U, UH2, 5-fluoruracil (5-FU, internal standard) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile, ammonium sulfate, ethyl acetate and 2-propanol were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained through an Elga Purelab Ultra® apparatus from Elga Labwater (High Wycombe, UK). Considering the presence of endogeneous U and UH2, the matrix for preparation of calibration and QC samples was a 6% (*w*/*v*) aqueous solution of BSA.

U, UH2 and 5-FU stock and working solutions were prepared in ultrapurified water. Calibrators were prepared by adding the appropriate amount of U and UH2 solutions to 6% BSA solution (1:20, *v*/*v*) to obtain concentrations of 0.2, 0.5, 1, 2.5, 5, 10 and 20 μg mL^{-1}. Internal standard working solution (IS) was 5-FU (40 μg mL^{-1}). Quality control (QC) samples were prepared to obtain U and UH2 concentrations of 0.2 (quality control at the limit of quantification, QCLOQ), 0.25 (quality control low, QCL), 7.5 (quality control medium, QCM), and 15 μg mL^{-1} (quality control high, QCH).

Sample preparation was based on liquid–liquid extraction. Briefly, 500 μL of either calibration, quality control or patient's samples (plasma) were added to glass tubes, followed by 75 μL of internal standard solution, followed by 30 s of vortex mixing. After, proteins were precipitated with 500 mg of ammonium sulfate, followed for 1 min of vortex mixing. The resulting mixture was added with 4 mL of a mixture of ethyl acetate and isopropanol (85:15, *v*/*v*) and mixed for 10 min in a rotatory mixer at 50 rpm. After 10 min centrifugation at 3000 g, the supernatant was transferred to an evaporation tube and dried at 56 °C under a gentle stream of air. The dried extract was reconstituted with 200 μL of ultrapurified water and centrifuged for 10 min at 10.000 *g*. An aliquot of 50 μL of supernatant was injected into the HPLC.

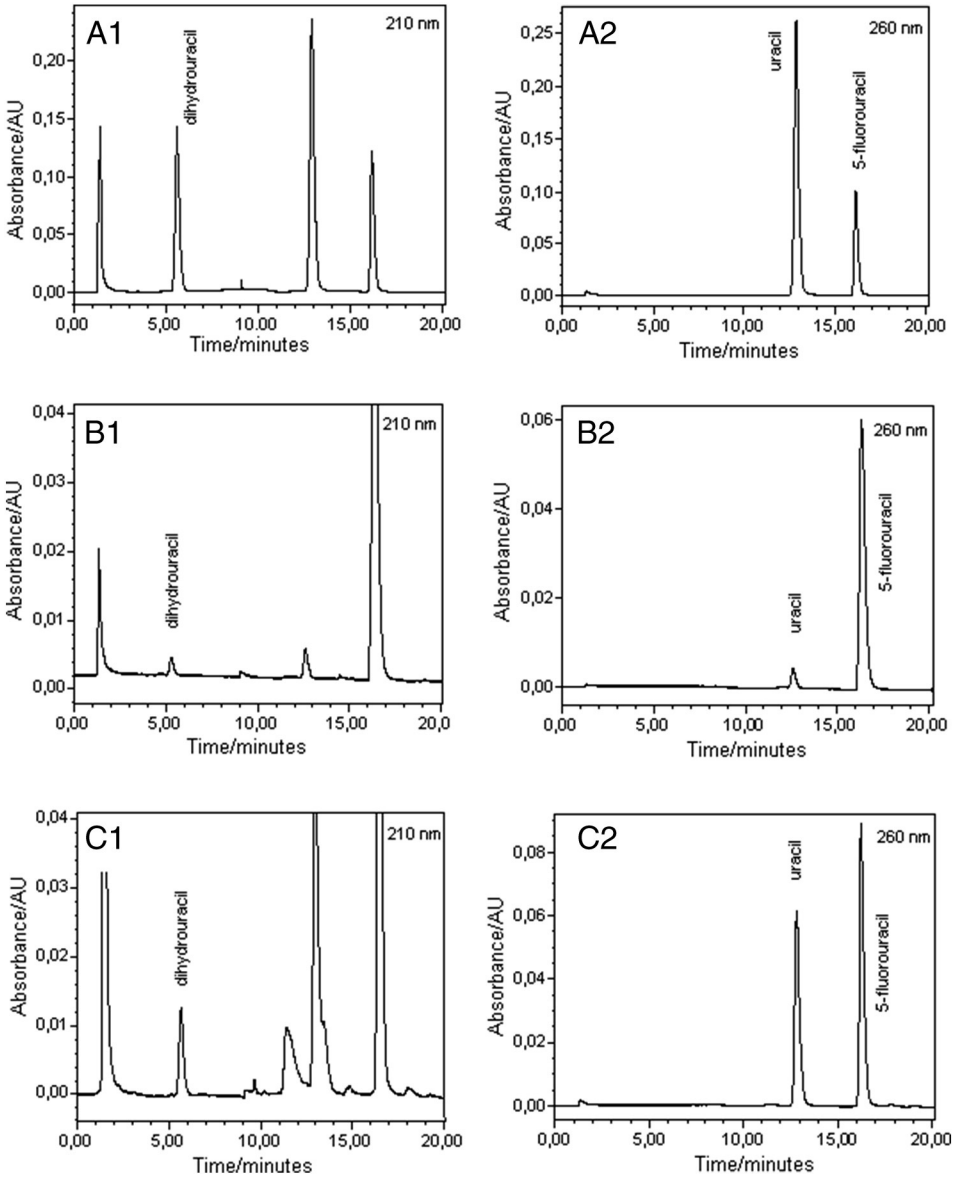

Fig. 1. Typical chromatograms of plasma extracts obtained at 210 and 260 nm. A: Quality control sample at low concentration (QCL) containing U and UH2 at 0.25 μg mL^{-1}; B: Quality control sample at high concentration (QCH) containing U and UH2 at 15.0 μg mL^{-1}; C: Patient sample 2 h after an oral loading dose of 1000 mg U containing U at 3.62 μg mL^{-1} and UH2 at 1.41 μg mL^{-1}.

Please cite this article as: Hahn RZ, et al, Improved determination of uracil and dihydrouracil in plasma after a loading oral dose of uracil using high-performance liquid chromatography with..., Clin Biochem (2015), http://dx.doi.org/10.1016/j.clinbiochem.2015.04.019

Download English Version:

https://daneshyari.com/en/article/8317213

Download Persian Version:

https://daneshyari.com/article/8317213

Daneshyari.com