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Separation and quantitative determination of cinacalcet metabolites in urine sample using RP-HPLC after derivation with a fluorescent labeling reagent

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ABSTRACT

In this investigation, a novel strategy for separation and quantitative determination of four metabolites of cinacalcet (M2a-Glu, M2b-Glu, M7-Gly, and M8-Gly) in human urine is suggested. The analytical assay is based on a pre-column derivation procedure of cinacalcet metabolites with 1-pyrenyldiazomethane (PDAM) as a fluorescent labeling reagent, and subsequently separation and quantitative determination with reverse-phase high-performance liquid chromatography (RP-HPLC) coupled with a fluorescence detector. Metabolites were separated on a Microsorb-MV 100-5 C18 chromatography column (250 × 4.6 mm, 5 μm) using acetate buffer (pH 3.5):methanol (30:70 v/v) as mobile phase at a flow rate of 1.0 mL min^{-1}. The method was fully validated in terms of linearity ($r^2 > 0.996$; 1–10 ng mL^{-1}), precision (both intra-day and inter-day; RSD < 6.2%), accuracy (92–110%), specificity, robustness (0.15% < RSD < 4.1%), limits of detection (5×10^{-4} to 3×10^{-3} ng mL^{-1}) and quantification (2×10^{-3} to 1×10^{-2} ng mL^{-1}). According to the results, the proposed method can be useful in the routine analysis for the determination of cinacalcet metabolites in urine samples.

1. Introduction

In the patients with chronic kidney disease (CKD) the parathyroid hyperplasia and calcium/phosphorus disorders are occurred due to the increased level of serumal parathyroid hormone (PTH). This hyperplasia commonly also referred to secondary hyperparathyroidism (SHPT) [1]. SHPT develops early and worsens as kidney function disorders [2]. Traditional treatment, including dietary phosphorus restriction, phosphorus-binding agents, calcium supplementation, and 1,25-dihydroxy vitamin-D sterols has proven inadequate in successfully controlling of SHPT [3]. Cinacalcet hydrochloride is a calcimimetic agent that affected on the calcium receptor on the parathyroid cells and block secretion of PTH [4]. It is well established that cinacalcet can be used for treatment of SHPT and hypercalcaemia in patients with CKD receiving dialysis and parathyroid carcinoma, respectively [5]. Cinacalcet is mainly metabolized by multiple hepatic enzymes, primarily cytochrome P450 (CYP) 3A4, 2D6 and 1A2 [6]. *In vivo* studies have revealed that the cinacalcet is a strong inhibitor of cytochrome P450 isoenzyme CYP2D6 [7–9]. Cinacalcet is eliminated mainly through two major metabolism routes as follows: *N*-dealkylation, which lead to carboxylic acid derivatives and oxidation of the naphthalene ring system to form dihydrodiols [10]. In addition, the oxidative metabolites are conjugated before elimination. The circulating metabolites of cinacalcet are inactive, and <1% of the parent drug is excreted in the urine. The metabolism pathway is shown in Scheme 1.

Cinacalcet is not detected in urine samples of humans (mainly due to its low concentration; <1%), while its metabolites can be detected [10]. In addition, the concentrations of metabolites are trace amount in urine, so it is necessary to establish the method that characterized by high separation efficiency and low LOD and LOQ values. In this context, chemical derivation of the analytes is often used to enhance detection efficiency and sensitivity through signal improvement [11]. One of the most applied methods for signal increasing is labeling of analytes with a fluorescent agents. Such agents can be constructing or enhance the fluorescence intensity. The most common reagent for chemical derivation of the analytes possesses carboxyl functional group is diazomethane. Diazoalkanes easily reacted with carboxylic acid group without the addition of any catalyst, and may be useful for direct derivatization of both organic compounds and biomolecules [12]. The fluorescent diazomethyl derivative 9-anthryldiazomethane (ADAM, A1400) has

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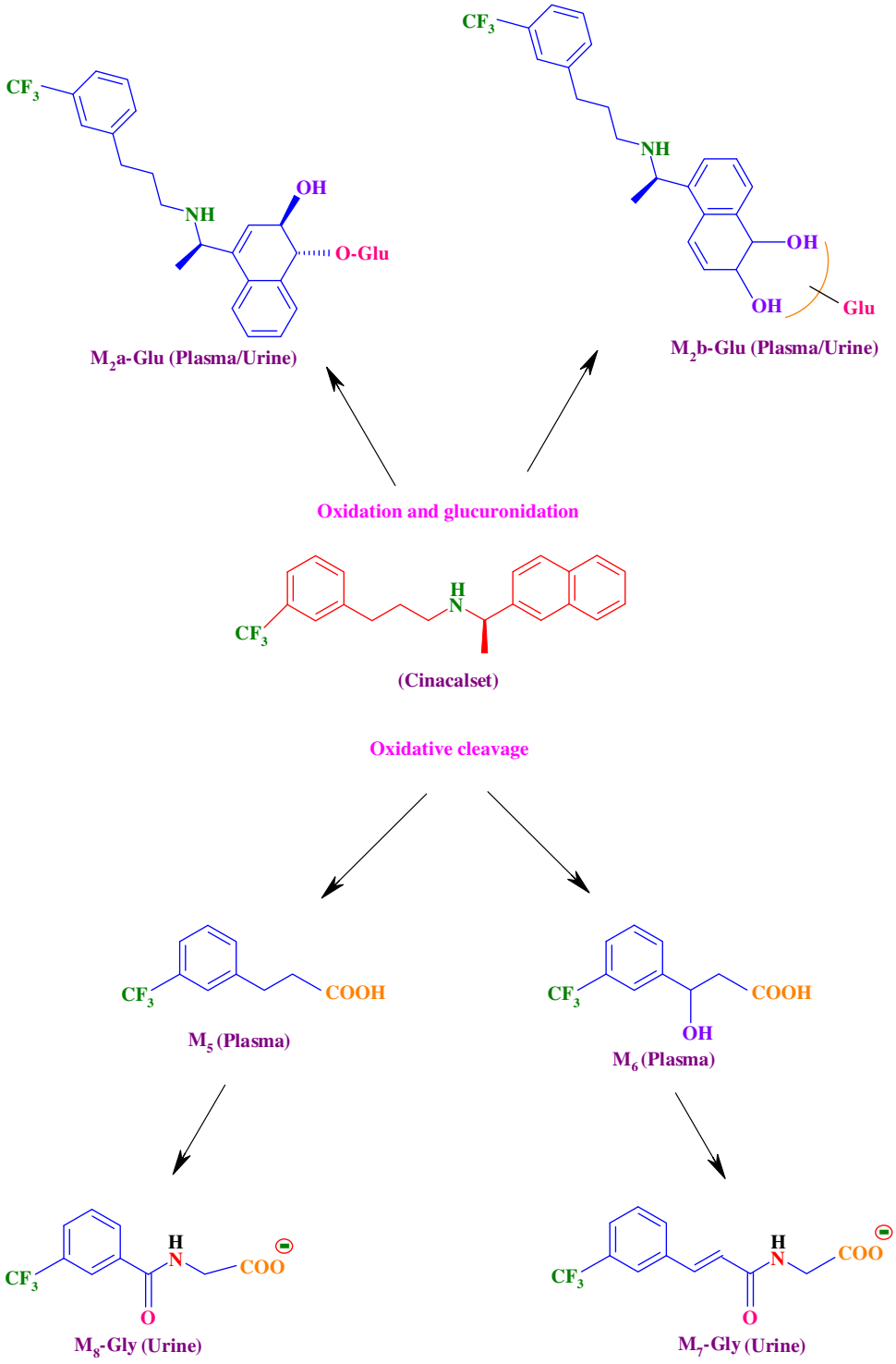

Scheme 1. Metabolic pathway of cinacalcet.

been commonly applied in derivatization of biomolecules. Unfortunately, ADAM is not very stable and may decompose during storage. In this respect, 1-pyrenyldiazomethane (PDAM, P1405) is recommended as a replacement for ADAM since it has higher chemical stability in comparison with ADAM [13–16]. Moreover, the detection limit for PDAM conjugates is reported to be about 20–30 femtomoles, which is five times better than reported for detection of ADAM conjugates [14]. Due to low concentrations of metabolites in human urine, a quantitative reaction between carboxylic acid group of the metabolites and PDAM is necessary. Several methods such as LC–MS/MS have been reported for determination of cinacalcet and its metabolites [17–20]. A low limit of quantitation (0.1 ng mL^{-1}) was obtained in all of the above mentioned methods, which was un-sufficient for pharmacokinetic studies of the cinacalcet and its urine metabolites.

The present study describes an efficient strategy for separation and quantitative determination of cinacalcet urinary metabolites (M2a-Glu, M2b-Glu, M7-Gly, and M8-Gly) in human urine samples. The proposed analytical assay is based on derivation of cinacalcet metabolites with a fluorescent labeling reagent