Journal of Pharmaceutical and Biomedical Analysis 118 (2016) 96–100

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Simultaneous determination of 3-*O*-acetyloleanolic acid and oleanolic acid in rat plasma using liquid chromatography coupled to tandem mass spectrometry

Eunyoung Kim[a], Keumhan Noh[a], Sang Joon Lee[a], Beomsoo Shin[b], Joo Tae Hwang[c], Seung Woong Lee[c], Mun-Chul Rho[c,*], Wonku Kang[a,*]

[a] *Center for Metareceptome Research, College of Pharmacy, Chung-Ang University, Seoul 156-756, South Korea*
[b] *College of Pharmacy, Catholic University of Daegu, Kyoungbuk 712-702, South Korea*
[c] *Bio-Material Research Institute, Korea Research Institute of Bioscience and Biotechnology, Jeongeup 580-185, South Korea*

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form 13 October 2015
Accepted 19 October 2015
Available online 26 October 2015

Keywords:
3-*O*-Acetyloleanolic acid
Oleanolic acid
LC–MS/MS
Rat plasma

ABSTRACT

3-*O*-Acetyloleanolic acid (OAA) is a triterpenoid compound, and exerts an apoptosis in cancer cell lines, an inhibition of both atopic and allergic contact dermatitis in murine model, and a suppression of inflammatory bone loss in mice. OAA can be converted into oleanolic acid (OA) by hydrolysis *in vivo*, and OA exhibits several pharmacological effects as well. A liquid chromatographic method using tandem mass spectrometry (MS/MS) was developed for the simultaneous determination of OAA and OA in rat plasma. After liquid–liquid extraction with ethylacetate, both substances were chromatographed on a reversed phase column with a mobile phase of 0.1% formic acid aqueous solution and acetonitrile (1:9, v/v). The accuracy and precision of the assay were in accordance with FDA regulations for the validation of bioanalytical methods. This analytical method was successfully applied to monitor plasma concentrations of both substances over time following an intravenous administration of OAA in rats.

1. Introduction

3-*O*-Acetyloleanolic acid (10-acetoxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12, 12a,12b,13,14b-octadecahydro-2H-picene-4a-carboxylic acid; OAA; Fig. 1) is a triterpenoid compound isolated from the seeds of *Vigna sinensis* K. OAA exerts several pharmacological effects, such as inducing apoptosis in human colon carcinoma cells, which is mediated by upregulation of the death receptor DR5 [1], and inhibiting both atopic and allergic contact dermatitis in a murine model [2]. The substance also inhibits osteoclastogenesis via downregulation of PLCγ2-Ca^{2+}-NFAT signaling, and suppresses inflammatory bone loss in mice [3]. OAA at 10–50 mg/kg has been administered in mice to investigate these activities; however, its systemic exposure has not yet been measured.

It is expected that OAA can be converted into oleanolic acid (OA), resulting from hydrolysis of the ester at the 3-carbon position *in vivo*. OA has anti-inflammatory actions [4], anti-nociceptive activities [5], and anti-tumor effects [6]. Although plasma OA concentrations have been determined in mice [7], rats [8] and humans [9] by LC/MS/MS, there is no report on the determination of OAA in plasma or the time course of plasma OAA concentrations.

In this study, we developed a liquid chromatographic method using tandem mass spectrometry (MS/MS) for the simultaneous determination of OAA and OA in rat plasma. This analytical method was successfully applied to monitor plasma concentrations of both compounds over time following intravenous administration of OAA in rats.

2. Material and methods

2.1. Reagents and materials

OAA was synthesized and kindly donated by Korea Research Institute of Bioscience and Biotechnology (Jeongeup, Korea). OA and diclofenac (internal standard, IS) were purchased from Sigma (Seoul, Korea), and acetonitrile was obtained from Burdick & Jackson (Muskegon, MI, USA). All other chemicals and solvents were of the highest analytical grade available.

* Corresponding authors. Fax: +82 28167338.
E-mail addresses: rho-m@kribb.re.kr (M.-C. Rho), wkang@cau.ac.kr (W. Kang).

http://dx.doi.org/10.1016/j.jpba.2015.10.030
0731-7085/

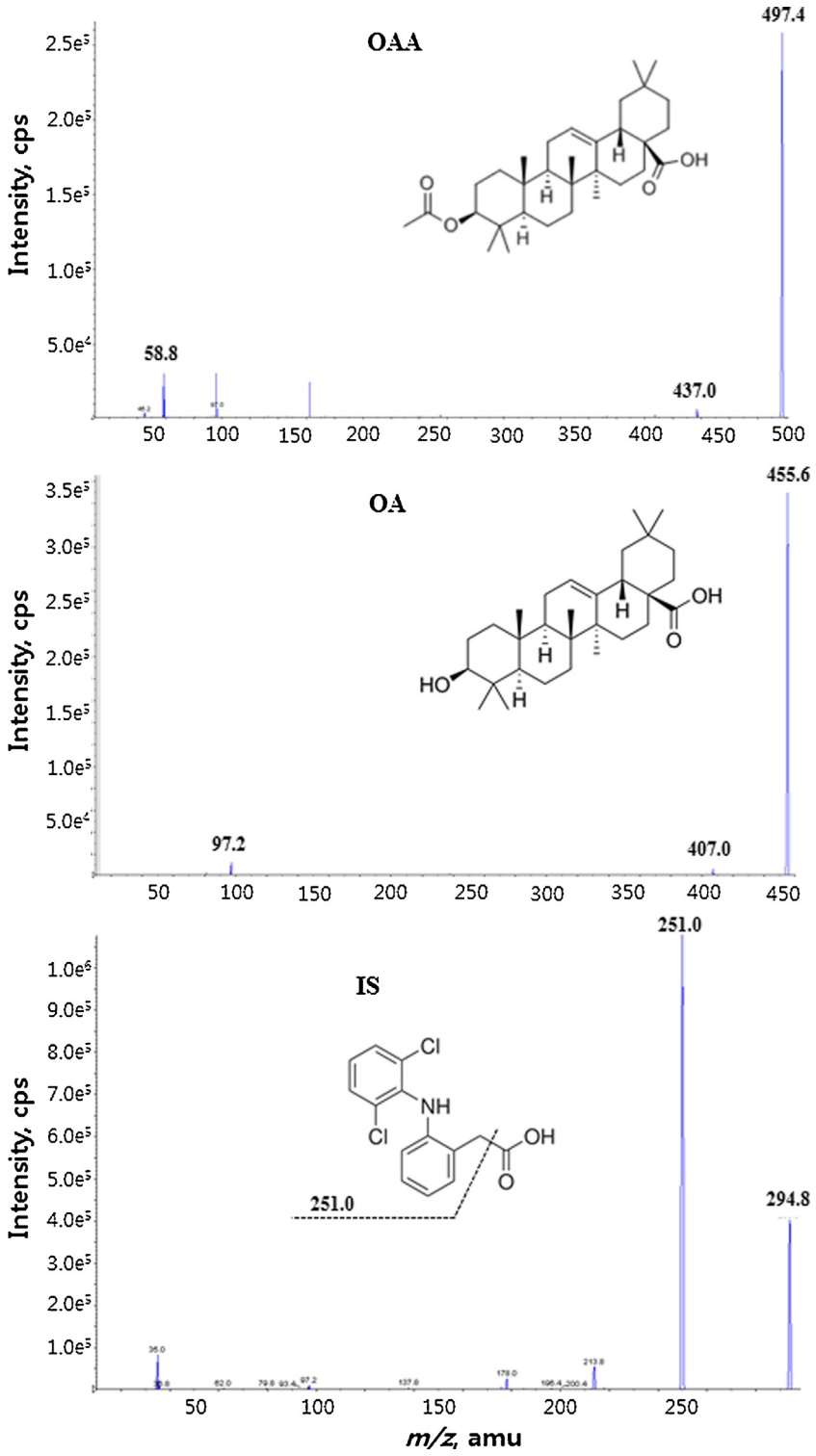

Fig. 1. Mass spectra of 3-*O*-acetyloleanolic acid (OAA), oleanolic acid (OA) and the IS (diclofenac).

2.2. Preparation of standards and quality controls

OAA, OA, and the internal standard (IS) were dissolved in methanol to a concentration of 1.0 mg/mL. OAA and OA solutions were then serially diluted with methanol, and 5 μL of a mixture of both diluted solutions was added to 90 μL drug-free plasma to obtain final concentrations of 0.01, 0.05, 0.1, 0.5, 2, and 10 μg/mL for both OAA and OA. Using linear regression, six calibration graphs were derived from the ratio between the area under the peak of each compound and the IS. Quality control samples were prepared with 90 μL blank rat plasma by adding 5 μL of serially diluted solutions of each substance to determine the lower limit of quantification (0.01 μg/mL), as well as low (0.03 μg/mL), intermediate (1 μg/mL), and high concentrations (8 μg/mL). These samples were used to evaluate the intra- and interday precision and accuracy of the assay.