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Validation of a UPLC–MS/MS method for the simultaneous determination of E6005, a phosphodiesterase 4 inhibitor, and its metabolite in human plasma

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ABSTRACT

E6005, a novel phosphodiesterase 4 inhibitor, is currently under clinical development for the treatment of atopic dermatitis. As ER-392710 (M11), a hydrolyzed metabolite, is a main metabolite, a simultaneous assay method for quantification of E6005 and M11 in human plasma has been developed and validated using ultra-performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS). E6005, M11, and each deuterium-labeled compound used as internal standard were extracted from 100 μL human plasma by solid phase extraction then chromatographed on an Acquity UPLC BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm) under gradient elution. The analytes were detected by selected reaction monitoring in the positive ion mode with the mass transition of *m/z* 473.1/163.0 and *m/z* 459.1/149.0 for E6005 and M11, respectively. E6005 and M11 were quantifiable ranging from 1 to 200 ng/mL with no carryover. Accuracy and precision in intra- and inter-batch reproducibility assays were within the acceptance criteria recommended by the regulatory bioanalytical guidelines. Various stability assessments including possible conversion of E6005 to M11 were thoroughly performed to demonstrate the stability of E6005 and M11 in human blood and plasma. The method was successfully applied to support clinical trials.

1. Introduction

Atopic dermatitis is one of major inflammatory skin diseases and corticosteroids have been often used for the treatment. As immediate antipruritic effects are not expected by the use of corticosteroids, it is highly expected to have alternative agents with prompt antipruritic effects. Among families of phosphodiesterases (PDEs), PDE4 expressed on various inflammatory cells is considered to play an important role in the inflammatory disorders [1] and PDE4 inhibitors showed anti-inflammatory effects in patients [2]. Although a number of PDE4 inhibitors have been developed for inflammatory diseases [3], adverse effects including nausea and emesis in clinical trials hindered further development [4]. High systemic exposure of PDE4 inhibitors may lead to side effects, thus dermal application would be an attractive option owing to expected lower systemic exposure of drugs. E6005 is a novel PDE4 inhibitor and currently under clinical development for the treatment of atopic dermatitis. E6005 potently inhibited human PDE4 with an IC_{50} of 2.8 nM as well as various cytokines [5]. E6005 also demonstrated pharmacological activities in mice with oxazolone- and mite-induced dermatitis [5]. In addition, a randomized vehicle-controlled clinical trial demonstrated that E6005 showed significant efficacy at 0.2% ointment [6] in humans. The antipruritic mechanism has not been fully addressed, but inhibition on the transient receptor potential vanilloid 1 (TRPV1)-madiated C-fibre depolarization [7] and proteinase-activated receptor 2-mediated leukotriene B4 production [8] have been suggested. These findings in both non-clinical and clinical studies support the development of topical E6005 for the treatment of atopic dermatitis.

Abbreviations: HQ, Chigh QC; IS, internal standard; LLOQ, lower limit of quantification; LQC, low QC; MQC, middle QC; PDE, phosphodiesterase; QC, quality control; RE, relative error; RSD, relative standard deviations; ULOQ, upper limit of quantification; UPLC–MS/MS, ultra-performance liquid chromatography with tandem mass spectrometry.

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It is important to monitor E6005 levels in systemic circulation (e.g. plasma) since its systemic exposure is considered as an indicator of possible adverse event findings. In vitro metabolism studies demonstrated that E6005 was metabolized to various metabolites including M11 (ER-392710) as a major metabolite (unpublished data), suggesting that M11 would be one of main metabolites of E6005 in clinical studies as well. We therefore have developed an assay method for the simultaneous determination of E6005 and M11 in human plasma to support clinical trials. Stability assessment of E6005 is crucial since E6005 was easily converted to M11 in whole blood and plasma in rodents (unpublished data). In the present study, possible conversion of E6005 to M11 has been fully evaluated in human whole blood and plasma in order to establish an accurate method for the simultaneous determination of E6005 and M11.

This paper presents a simple, selective, and reproducible validated bioanalytical method for E6005 and M11 by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS). The developed method was successfully applied for the determination of E6005 and M11 in plasma to support clinical trials.

2. Materials and methods

2.1. Materials

E6005 and M11 (ER-392710) were synthesized at Eisai Co., Ltd. (Ibaraki, Japan). ER-497652 and ER-497653 used as the internal standards (IS's) for E6005 and M11, respectively, were synthesized at Sekisui Medical Co., Ltd. (Ibaraki, Japan). The chemical name of E6005 was methyl 4-[({3-[6,7-dimethoxy-2-(methylamino) quinazolin-4-yl]phenyl}amino) carbonyl]benzoate. The purity of E6005 and M11 was 99.7% and 99.3%. The isotopic ratio of corresponding IS was less than 0.1%, demonstrating minimal contamination of the analytes. Their chemical structures are shown in Fig. 1. Drug-free blank human plasma with heparin sodium as an anticoagulant was purchased from Biopredic International (Saint Grégoire, France) and drug-free blank human whole blood was obtained from volunteers in Eisai Co., Ltd. with written consent. High-performance liquid chromatography (HPLC) grade acetonitrile, methanol, distilled water, and ammonium formate as well as special grade formic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of analytical grade.

2.2. Ultra-performance liquid chromatography with tandem mass spectrometry

An Acquity system consisting of two pumps, an on-line degasser, an auto-sampler, and a controller (Waters, MA, USA) was used as an ultra-performance liquid chromatography (UPLC). Gradient elution was achieved using the mobile phase consisting of (A) water–acetonitrile-1 mol/L ammonium formate (950:50:5, v/v/v) and (B) water–acetonitrile-1 mol/L ammonium formate (100:900:5, v/v/v) at a flow rate of 0.25 mL/min. The gradient program is as follows: a linear increase of mobile phase (B) from 5% to 95% for 2.0 min, then an isocratic elution of 95% (B) for 2.0 min to elute the analytes, followed by having the system equilibrated with 5% (B) for 1.5 min. The total run time per assay was 5.5 min. Analytes were chromatographed on Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters) maintained at 40 °C.

E6005, M11, and the IS's were analyzed on a triple quadrupole mass spectrometer Quattro Premier XE (Waters) in positive ion electrospray mode. Mass spectrometer conditions were optimized and following analytes-independent ionization conditions were used in the validation study: desolvation gas flow, 800 L/h; cone gas flow, 180 L/h; collision gas flow, 0.35 mL/min; desolvation temperature, 370 °C; source temperature, 125 °C; capillary voltage, 1300 V. The cone voltage and collision energy were set at 65 V and 55 eV, respectively, for all the analytes including the IS's. Detection of ions from the analytes was carried out in the multiple reaction monitoring by transition of precursor/product ions at m/z 473.1/163.0 and m/z 459.1/149.0 for E6005 and M11, respectively, and at m/z 477.2/167.0 and m/z 463.2/153.0 for the IS's of E6005 and M11, respectively.

2.3. Preparation of calibration and quality control samples

A stock solution of E6005 and M11 was separately prepared by dissolving in methanol to yield a final concentration of 100 μg/mL as free base. The standard working solutions were prepared by mixing the stock solutions of E6005 and M11 at a volume ratio of 1:1 then diluting with acetonitrile–methanol (1:1, v/v). Calibration samples were prepared by fortifying standard working solutions to blank human plasma to make final concentrations of 1, 2, 10, 20, 80, 100, 160, and 200 ng/mL for both E6005 and M11. Stock solutions of the two IS's were separately prepared in methanol at 10 μg/mL and mixed at 1:1 (v/v), then the mixture was diluted with acetonitrile–methanol (1:1, v/v) to make an IS working solution (200 ng/mL). The standard and IS working solutions were stored below −20 °C and used within 181 days in which stability was ensured.

Quality control (QC) samples including the lower limit of quantification (LLOQ), low QC (LQC), middle QC (MQC), high QC (HQC), and the upper limit of quantification (ULOQ), were prepared at concentrations of 1, 3, 30, 160, and 200 ng/mL. QC samples for the assessment of dilution integrity were prepared at 1000 ng/mL.

2.4. Sample extraction procedures

Aliquots (100 μL) of plasma samples were fortified with 30 μL of the IS working solution (200 ng/mL) followed by the addition of 850 μL of formic acid solution (1%, v/v). The mixture was then loaded onto solid phase extraction plate, Oasis HLB 96 well plate (5 mg, Waters), conditioned by methanol (1 mL) followed by water (1 mL). The extraction plate was centrifuged at 4 °C (700 × g, 5 min), then was washed twice by 1.2 mL of water followed by 0.6 mL of water–methanol (9:1, v/v). Analytes retained on the cartridge were eluted with 0.8 mL of water–acetonitrile-formic acid (600:400:1, v/v/v) then the cartridge was centrifuged at 4 °C (110 × g, 6 min) to obtain eluate completely. Aliquots (7 μL) of the eluate were injected to the UPLC–MS/MS system.

2.5. Method validation

2.5.1. Linearity and carryover

Calibration samples containing both E6005 and M11 (1–200 ng/mL) were extracted and assayed to find whether calibration curves showed linearity across 13 assay batches. The calibration curve was constructed by weighted (1/concentration^2) least squares linear regression. Linearity was evaluated for E6005 and M11 to ensure that relative error (RE) at each concentration was not greater than ±15% (±20% for the LLOQ). Trends in RE across eight concentrations were also evaluated by plotting nominal concentrations of the analytes and their corresponding RE.

Carryover was assessed by injecting blank samples just after ULOQ samples. Peak areas of any interferences in blank samples