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Short communication

Determination of the cyclic depsipeptide FK228 in human and mouse plasma by liquid chromatography with mass-spectrometric detection

Xiaohong Chen [a], Erin R. Gardner [b], William D. Figg [a,*]

[a] *Clinical Pharmacology Program, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA*
[b] *Clinical Pharmacology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA*

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Abstract

An analytical method was developed and validated for the quantitative determination of the cyclic depsipeptide FK228 (romidepsin, formerly FR901228; NSC 630176), a histone deacetylase inhibitor, in human and mouse plasma. Calibration curves were linear in the concentration range of 2–1000 ng/mL. Sample pretreatment involved a liquid–liquid extraction of 0.1 mL aliquots of plasma with ethyl acetate. FK228 and the internal standard, harmine, were separated on a Zorbax SB C18 column (75 mm × 2.1 mm, 3.5 μm), using a mobile phase composed of methanol and 0.2% formic acid. The column eluent was monitored by mass spectrometry with electrospray ionization. Accuracy and precision of four concentrations of quality control samples ranged from 101.5 to 106.4% and 0.7 to 3.5% in human plasma and 93.6 to 100.6% and 0.6 to 6.5%, in mouse plasma, respectively. This method represents a significant improvement over our previously published analytical assay for this agent, decreasing the sample volume requirements, increasing the accuracy and precision (through addition of a suitable internal standard), expanding the analytical range and validating in additional biological matrices. The developed method was applied to study the pharmacokinetics of FK228 in over 1000 clinical and preclinical samples.

Keywords: Depsipeptide; Human plasma; Mouse plasma; LC/MS

1. Introduction

FK228 (romidepsin, formerly FR 901228, NSC 630176; (*E*)-(1*S*, 4*S*, 10*S*, 21*R*)-7-[(*Z*)-ethylidene]4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8,7,6]tricos-16-ene-3,6,9,19,22-pentanone;) (Fig. 1) is a naturally occurring bicyclic tetrapeptide that was first isolated from *Chromobacterium violaceum* by Fujisawa Company [1,2]. This agent is a novel histone deacetylase inhibitor that has demonstrated potent cytotoxic activity against human tumor cell lines and *in vivo* efficacy against both human tumor xenografts and murine tumors [3,4]. Multiple phase I and phase II clinical trials of FK228 have been initiated at the National Cancer Institute [5–7].

An analytical method was previously developed and validated in our laboratory for the quantitation of FK228 in human plasma, in support of multiple clinical trials [8]. However, the lack of internal standard became a major concern in daily practice, since the assay was being used long-term to analyze large numbers of samples. Furthermore, the original assay was not suitable for analysis of preclinical mouse samples. These animal experiments require a reliable analytical method that only uses 100 μL plasma or less, as compared to the 500 μL that was required in the previous method. In addition, the original assay was not suitable for the analysis of samples following higher doses of the drug. As exposure increased, due to the rapid dose escalation design of the study, the original upper limit of quantitation (ULOQ), 100 ng/mL, was no longer sufficient. Attempts at extending the range failed due to signal fluctuation without the application of an internal standard. Due to these major limitations, we report here an improved analytical method for determination of FK228 concentration in human and mouse plasma based on liquid chromatography coupled with single-quadrupole mass-spectrometric detection.

* Corresponding author at: Clinical Pharmacology Research Core, National Cancer Institute, 9000 Rockville Pike, Building 10, Room 5A01, Bethesda, MD 20892, USA. Tel.: +1 301 4023622; fax: +1 301 4028606.
E-mail address: wdfigg@helix.nih.gov (W.D. Figg).

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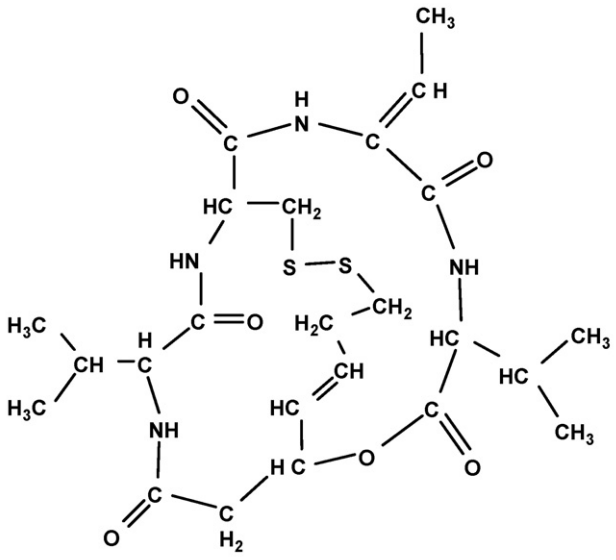
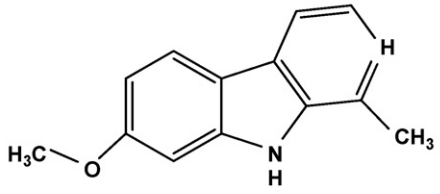


A. Structure of FK228



B. Structure of harmine, internal standard

Fig. 1. Structure of FK228 (A), and internal standard, harmine (B).

2. Experimental

2.1. Chemicals

FK228 was supplied by Pharmaceutical Management Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, NCI (Bethesda, MD, USA). Internal standard, harmine, was purchased from Sigma–Aldrich (St. Louis, MO, USA). Formic acid (98%) was obtained from Fluka (through Sigma–Aldrich, St. Louis, MO, USA). Ethyl acetate (Fisher Scientific, Fairlawn, NJ, USA) and methanol (J.T. Baker, Phillipsburg, NJ, USA) are of HPLC grade. Deionized water was generated with a Hydro-Reverse Osmosis system (Durham, NC, USA) connected to a Milli-Q UV Plus purifying system (Billerica, MA, USA). Drug-free heparinized human plasma was obtained from the National Institutes of Health Clinical Center Blood Bank (Bethesda, MD, USA).

2.2. Preparation of stock solutions and standards

Master stock solutions of FK228 were prepared by dissolving the drug in absolute ethanol at a concentration of 1 mg/mL and stored in glass tubes at −20 °C. From the master stock solution, a working solution containing 40 μg/mL of drug in methanol was prepared each week and stored at −20 °C between uses. Serial dilutions were prepared from this working solution for the preparation of calibration and quality control (QC) samples. A master stock of the internal standard, harmine, was prepared at a concentration of 1 mg/mL in absolute ethanol. From the master stock, a working solution of the internal standard was prepared by dilution to 40 ng/mL in ethyl acetate, the extraction solvent. Both the master and working internal standard solutions were stored at −20 °C.

With each analytical run, calibration standards in drug-free human heparinized plasma were freshly prepared in duplicate at FK228 concentrations of 2, 5, 10, 25, 100, 500 and 1000 ng/mL, such that the total amount of methanol added was identical in each sample (4%). QC samples were prepared in batch at concentrations of 6, 75, 800 and 2000 ng/mL, by adding plasma to the required amount of working solution in a volumetric flask. After vortexing to ensure complete mixing, these QC samples were subdivided into 0.6 mL aliquots (100 μL for 2000 ng/mL QC samples) in cryovials and stored at −20 °C.

2.3. Sample preparation

Samples were prepared by spiking 240 μL of blank human plasma in a 2 mL cryovial with 10 μL of the appropriate FK228 working solution for a total volume of 250 μL. After vortexing for 15 s, 100 μL was transferred to each of two 5 mL disposable glass centrifuge tubes (Kimble, Vineland, NJ, USA) per concentration. Patient samples were allowed to thaw at room temperature, vortex-mixed for 20 s to ensure uniformity, and a volume of 100 μL of each sample was transferred into a glass tube. QC samples were also thawed at room temperature, vortex-mixed and then aliquotted out into each glass tube. To each tube, 600 μL of ethyl acetate containing 40 ng/mL internal standard was added, followed by an additional 1 mL of ethyl acetate. Tubes were then capped and vortex-mixed for 5 min, followed by centrifugation for 10 min at 1303 × *g*. The clear supernatant was transferred to a clean glass drying tube and evaporated to dryness under desiccated air in a water bath at 40 °C in a Zymark TurboVap LV (Hopkinton, MA, USA). The residue was reconstituted in 50 μL of a mixture of methanol/0.2% formic acid (55:45, v/v), and vortex-mixed for 15 s. Finally, each solution was transferred to a glass vial for injection. A volume of 15 μL of this solution was then injected into the chromatographic system.

2.4. Equipment

Experiments were conducted on an Agilent 1100 system (Agilent Technology, Palo Alto, CA, USA) which included a G1312 binary pump, a G1329 refrigerated autosampler, a mobile phase vacuum degassing unit, and a temperature-controlled column compartment, coupled with a single-quadrupole mass-spectrometric (MS) detector (Agilent 1100 MSD) equipped with an electrospray ionization source. The autosampler was maintained at 4 °C and the column was at 40 °C. An Agilent ZORBAX SB-C18 column (75 mm × 2.1 mm I.D.) packed with 3.5-μm packing material was employed. Samples were eluted using a step-wise gradient at a flow rate of 300 μL/min, comprised of 0.2% formic acid (A) and methanol (B). After 1 min at 60% A and 40% B, the methanol was increased to 80%