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A sensitive method for determination of COL-3, a chemically modified tetracycline, in human plasma using high-performance liquid chromatography and ultraviolet detection

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Abstract

COL-3, 6-deoxy-6-desmethyl-4-desdimethylamino-tetracycline, is a matrix metalloproteinase inhibitor currently in clinical development. A HPLC–UV method to quantitate COL-3 in human plasma was developed. COL-3 was extracted from plasma using solid-phase extraction cartridges. COL-3 is separated on a Waters Symmetry Shield RP8 (3.9 mm × 150 mm, 5 μm) column with EDTA (0.001 M) in sodium acetate (0.01 M, pH 3.5)–acetonitrile mobile phase using a gradient profile at a flow rate of 1 ml/min for 22 min. Carryover was eliminated by using an extended needle wash of methanol:acetonitrile:dichloromethane (1:1:1, v/v/v). Detection of COL-3 and the internal standard, chrysin, was observed at 350 nm. COL-3 and chrysin elute at 8.9 and 9.9 min, respectively. The lower limit of quantitation in human plasma of COL-3 was 75 ng/ml, linearity was observed from 75 to 10,000 ng/ml. A 30,000 ng/ml sample that was diluted 1:50 with plasma was accurately quantitated. This method is rapid, widely applicable, and suitable for quantifying COL-3 in patient samples enabling further clinical pharmacology characterization of COL-3.

Keywords: COL-3; Tetracycline; Matrix metalloproteinase inhibitor; HPLC; Pharmacokinetics

1. Introduction

COL-3, 6-deoxy-6-desmethyl-4-desdimethylaminotetracycline, is a non-antimicrobial, chemically modified tetracycline with documented anti-tumor effect [1]. COL-3 is being tested in clinical trials in patients with refractory advanced cancers, increasing the need to develop a rapid, accurate method for the detection of COL-3 [1–4].

Two published methods quantitated COL-3 in the concentration range of 30–10,000 ng/ml [5,6]. The first method utilized a HPLC/MS and two standard curves [5], while the second method utilized a HPLC/MS/MS and one standard curve [6]. The concentration range of both assays was sufficient to characterize the pharmacokinetics of COL-3 for up to 1 week and weekly pre-treatment trough levels after administration of doses of 36–98 mg/m^2 in a phase I clinical trial [7,8].

In another phase I clinical trial, COL-3 was being administered at escalating doses starting at 25 mg/m^2 on a continuous daily dosing schedule, only pre-treatment trough concentrations being obtained [9]. In this trial, several patients received a drug-metabolizing enzyme-inducing anticonvulsant

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agent, which could result in increased COL-3 metabolism and low plasma concentrations. The challenge was to develop an assay with suitable sensitivity allowing quantitation over a broad concentration range, and a method that did not use mass spectrometric detection since many laboratories do not have that capability. As a result, we have developed a high-performance liquid chromatography method using ultraviolet detection (HPLC/UV) that quantitates COL-3 in plasma in the range of 75–10,000 ng/ml.

2. Experimental

2.1. Materials

COL-3 (97.0% pure) was a gift from CollaGenex Pharmaceuticals, Inc. (Newtown, PA, USA). Chrysin (96.0% pure) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Heparinized human plasma was purchased from Pittsburgh Blood, Inc. (Pittsburgh, PA, USA). All other reagents were of the highest grade commercially available.

2.2. Preparation of stock solutions, calibration standards, and quality controls

COL-3 at a concentration of 1 mg/ml (corrected for purity) was dissolved in Tween 80/ethanol (1:1, v/v) in Sigmacote®-treated glassware. The solution was sonicated for 20 min and then allowed to sit at room temperature for at least 1 h prior to use. COL-3 was stable for at least 1 month when stored at −80 °C and protected from light. The stock solution was serially diluted in Tween 80/ethanol (1:1, v/v) on each day of analysis to prepare working standards of 250, 100, and 25 μg/ml. Microliter amounts of COL-3 stock solution were added into pooled human plasma to prepare a calibration curve and quality controls (QC). The standards were prepared at 75, 200, 350, 600, 900, 1250, 2500, 5000 and 10,000 ng/ml, and the QCs at 150, 2000, and 8000 ng/ml. An additional dilutional QC was prepared at 30,000 ng/ml. The dilutional QC was diluted 1:50 with blank pooled human plasma prior to sample preparation to ensure patient samples could be diluted. All standards and quality controls were prepared fresh daily. For long-term and freeze-thaw stability, quality controls were stored at −80 °C.

Chrysin was dissolved at a concentration of 250 μg/ml in absolute methanol. Chrysin was stable for 2 weeks when stored at −20 °C and protected from light [5].

2.3. Assay procedure

An amount of 5 μl of chrysin (250 ng/μl) was added to 500 μl plasma sample on ice. The sample was acidified using 20 μl of 50% phosphoric acid and vortex-mixed for 30 s. An amount of 500 μl of the acidified sample was transferred onto an activated OASIS HLB 1 cc (30 mg) extraction cartridge (Waters Corporation, Milford, MA, USA) that was conditioned and equilibrated by washing with 1 ml of methanol (100, v/v) and 1 ml of Milli-Q water. The cartridge was washed twice with 1 ml of methanol/water (5/95, v/v). Analytes were eluted with 1 ml of methanol (100, v/v). After evaporation, the samples were reconstituted with glacial acetic acid (1/99, v/v) in acetonitrile/0.01 M sodium acetate, pH 3.5 (30/70, v/v) and 20 μl of the resulting solution was injected into the HPLC system.

Table 1
Gradient profile

Time (min)	% Solvent A[a]	% Solvent B[b]
0	70	30
4	70	30
9	20	80
10.5	20	80
11	30	70
16	30	70
17.5	70	30
22	70	30

[a] EDTA (0.001 M) in sodium acetate (0.01 M, pH 3.5).
[b] Acetonitrile.

2.4. Chromatographic conditions

Chromatographic analysis was performed using a Waters Model 2690 separations system (Milford, MA, USA) equipped with a Waters Model 2487 UV detector. Separation of the analyte was achieved at ambient temperature using Waters Symmetry Shield C_8 (3.9 mm × 150 mm, 5 μm particle) column protected by a Waters C_{18} guard column (Milford, MA, USA). A gradient profile, as shown in Table 1, consisted of EDTA (0.001 M) in sodium acetate (pH 3.5, 0.01 M)-acetonitrile with a flow rate of 1 ml/min and a total run time of 22 min. An extended needle wash was utilized and consisted of a mixture of methanol–acetonitrile–dichloromethane (1:1:1, v/v/v) [10]. Column eluant was monitored at 350 nm. The instrument was controlled by ChromPerfect Spirit software version 4.4.23 (Justice Laboratory Software, Denville, NJ, USA).

2.5. Validation procedures

COL-3 working standards were added to human plasma to prepare calibration samples over the range of 75–10,000 ng/ml. Calibration curves were computed using the ratio of the peak area of COL-3 and internal standard by using a weighted (1/[nominal COL-3 concentration]) linear regression analysis. The assay lower limit of quantitation (LLOQ) was determined to be 75 ng/ml for COL-3. The LLOQ was determined by meeting the following two criteria: a signal to noise ratio of the peak areas larger than 10 and the values for precision and accuracy less than 20%.

Method validation runs were performed on 9 days. Each analytical run consisted of a calibration curve using single samples with duplicate samples at the lower limit of quantiation (LOQ) and upper limit of quantiation (ULQ) and QC