Determination of CTS-1027 and its Metabolites CTS-1027-2 and CTS-1027-6 in Human Plasma by an LC/MS/MS Method

D.E. Lewiston¹, B.A. Babson¹, N.E. Henderson¹, P.C. Contreras², A. Spada²

¹MicroConstants, Inc., San Diego, CA 92121; ²Conatus Pharmaceuticals, Inc., San Diego, CA 92121

INTRODUCTION

CTS-1027, a novel matrix metalloproteinase inhibitor, is being investigated for the treatment of hepatitis C virus and other liver diseases. The purpose of this study was to develop and validate a method that provides a simple, rapid and specific route for the determination of CTS-1027 and its metabolites CTS-1027-2 and CTS-1027-6 in human plasma.

ANALYTICAL METHOD SUMMARY

INSTRUMENTATION

Agilent 1100 HPLC with a C18 HET-RP autosampler coupled to a Micromass Quattro Ultima tandem quadrupole mass spectrometer.

COLUMN

30 cm x 2.1 mm; 5 µm; analytical column (Waters, Milford, MA).

ANALYSIS

CTS-1027, CTS-1027-2, CTS-1027-6

IONIZATION MODE

Electrospray negative, multiple reaction monitoring (MRM).

MASS TRANSITIONS

CTS-1027: 432.20 > 267.05

CTS-1027-2: 432.25 > 267.06

CTS-1027-6: 432.25 > 270.06

HPLC MOBILE PHASE

10% methanol ammonium acetate in methanol/water/acetic acid-acetate acid solution (80/0.03/0.1, v/v/v), flow rate 0.3 mL/minute. An auxiliary pump was used to add 0.5% ammonium in methanol at a flow rate of 0.1 mL/minute post-column to enhance mass spectrometer response.

RETENTION TIMES

CTS-1027: 4.72 min.

CTS-1027-2: 4.59 min.

CTS-1027-6: 4.97 min.

CALIBRATION CURVE RANGES

0.150 to 150 ng/mL.

PLASMA VOLUME

0.2 mL.

ANTIDILUENT

Sodium Heparin.

SOIC EXTRATION METHOD


CALIBRATION EQUATION

Power.

ACCURACY AND PRECISION

INTERCLAY

CTS-1027 Theoretical Concentration (ng/mL)  |  CTS-1027-2 Theoretical Concentration (ng/mL)  |  CTS-1027-6 Theoretical Concentration (ng/mL)

Mean  |  Mean  |  Mean  |  Mean  |  Mean  |  Mean  

%CV  |  %CV  |  %CV  |  %CV  |  %CV  |  %CV

N=6  |  N=6  |  N=6  |  N=6  |  N=6  |  N=6

Accuracy and precision were determined by analyzing QC samples at three concentrations over the course of three separate analyses. Interday accuracy and precision were determined by analyzing six replicate QC samples at three concentrations analyzed within a single run.

CONCLUSIONS

A robust, specific, and simple assay for the analysis of CTS-1027, CTS-1027-2 and CTS-1027-6 in human plasma has been validated.

The method has been used to quantify human plasma in pharmacokinetic studies (See Poster #W4124 for details).