





CEDRA DCN 11-657-T1

Mylan Pharmatceuticals Inc. PRIL-0367

## Bioanalytical Report

**Quality Assurance Audits and Inspections**

Phase	Date of Audit or Inspection	Date Reported to CEDRA Project Director	Date Reported to CEDRA Management
Sample Aliquotting	October 28, 2003	October 28, 2003	October 29, 2003
Folders, Solution Preparation, Tables, Log Sheets, Training and Equipment Records	November 14, 2003	November 14, 2003	November 14, 2003
Report	December 4, 2003	December 4, 2003	December 5, 2003

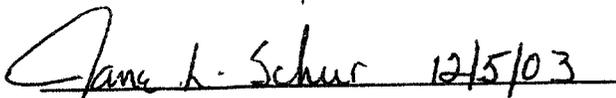
This study was audited and inspected by CEDRA Corporation Quality Assurance Unit and the findings reported on the dates listed above.

*Michel Malone for Robert Orozco 12/5/03*  
Robert Orozco  
Quality Assurance Unit  
CEDRA Corporation

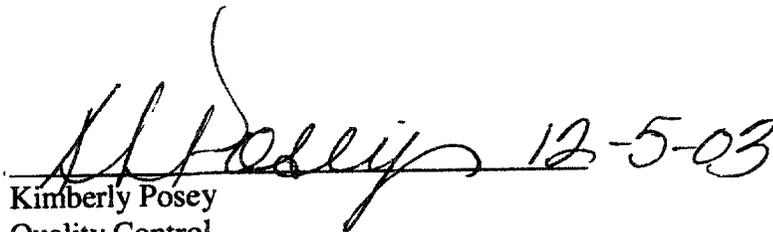


### GLP Compliance Statement

These analyses were conducted and reported in compliance with applicable US Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR 58, December 22, 1978, and all subsequent amendments, per CEDRA Corporation SOPs.

 12/5/03

Jane Schur  
Project Director  
CEDRA Corporation

 12-5-03

Kimberly Posey  
Quality Control  
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## **Bioanalytical Methodology**

### Method Summary

Heparinized human plasma samples for Mylan Pharmaceuticals Inc. Protocol PRIL-0367 were analyzed for omeprazole according to CEDRA procedure ATM-601, Revision 1, effective March 8, 2002 and ATM-601, Revision 2, effective November 6, 2003. The assay validation was finalized in April 2002 and reported under CEDRA DCN 11-350-V2 (see Method Validation Report section). The method used in this study was validated for a range of 1.00 to 500 ng/mL based on the analysis of 0.100 mL of human plasma. Plasma containing omeprazole and the internal standard, lansoprazole, was extracted with an organic solvent mixture. Following centrifugation, the upper organic layer was removed and evaporated before being reconstituted in mobile phase. An aliquot of the extract was injected onto a SCIEX API 3000 LC-MS-MS equipped with an HPLC column. Peak areas of the  $m/z$  344 $\rightarrow$ 194 product ion were measured against the  $m/z$  368 $\rightarrow$ 164 product ion of the internal standard. Quantitation was performed using weighted ( $1/x^2$ ) linear least squares regression analysis generated from calibration standards prepared immediately prior to each run.

### Preparation of Solutions

Stock solutions were prepared using the following reference standard compounds. Omeprazole was purchased from USP (lot H, 100% purity, CEDRA logbook 770-70). Lansoprazole was purchased from Sigma Chemical Company (lot 41K1743, CEDRA logbook 770-78). See Reference Standard Certificate of Analysis section for additional information. The heparinized human plasma used during analysis was purchased from Golden West Biologicals, Inc.

Calibration spiking solutions of omeprazole were prepared in methanol/sodium carbonate solution and stored at approximately 4 °C. Calibration standards were prepared to yield 1.00, 2.00, 5.00, 10.0, 50.0, 200, 450, and 500 ng/mL by fortifying 0.100 mL of human plasma with 10.0 µL of the appropriate spiking solution immediately prior to each analysis.

During preparation of quality control samples, human plasma was fortified with the QC intermediate solution at the appropriate concentrations. High, medium, and low QC samples were prepared at 375, 175, and 3.00 ng/mL of omeprazole. The QC samples were stored at approximately -70 °C in 0.100 mL aliquots. A very high dilution QC pool was prepared at 2500 ng/mL.

The internal standard working solution was prepared to yield a concentration of approximately 1.00 µg/mL of lansoprazole. Samples were fortified with 10.0 µL of this solution.

### Standard Curves

The peak area ratios (y) of omeprazole to the internal standard and the concentrations of the calibration standards (x) were fitted by a weighted ( $1/x^2$ ) linear least squares regression analysis to the equation  $y = a + bx$ , where “a” is the y-intercept and “b” is the slope of the calibration curve. The mean values of the slope, intercept, and  $R^2$  were 0.010410, 0.000994, and 0.9988, respectively.

Calibration standards used for each of the sample sets were entered as unknowns into the derived equation of the least squares regression line to obtain “back calculated” values. The CV ranged from 1.8% to 3.5% and the bias ranged from -1.0% to 1.0%. The LLOQ for this study was 1.00 ng/mL. The peak area ratios of the “unknown” samples were converted to concentrations of omeprazole using these computer-generated parameters. Detailed results from these analyses are shown in Table B I.

### Quality Control Samples

At least two samples from each QC pool (high, medium, and low) were processed along with each study sample run. The dilution QCs were included in the sample runs in which samples requiring a similar dilution scheme were analyzed. The CV for the four QC pools ranged from 6.9% to 12.5%. The bias ranged from -4.0% to -0.6%. Detailed results of this analysis are shown in Table B2.

### Sample Analysis

The Study Sponsor signed the protocol on October 15, 2003. On October 28, 2003, a total of two thousand three hundred twenty-five (2325) human plasma samples were received at CEDRA Corporation. Seventy-five empty tubes were also received in the shipment. On October 30, 2003, a total of two thousand three hundred twenty-one (2321) back-up human plasma samples were received at CEDRA Corporation. Seventy-nine empty tubes were also received in the shipment. The samples were received frozen and in good condition. Samples were logged in and stored at approximately -70 °C in a box clearly labeled with CEDRA's masterfile number 11-657-T1 and logbooks 949-46 and 962-11. Per client request, samples were to be stored at -70 °C, instead of -20 °C as indicated in the test method. However, due to limited freezer space, the back-up samples were stored at -20 °C. Samples were stored for 22 days at -70 °C starting on October 18, 2003 (date of first blood collection) until November 6, 2003 (end of sample analysis). Frozen stability has been established for 8 weeks.

Seventeen acceptable analytical runs were carried out between October 28, 2003 and November 6, 2003. Sample repeats were included in these analyses. Sample runs were valid only if at least two-thirds of the qualifying QC samples were within  $\pm 15\%$  of their theoretical values with at least 50% of the QCs at each level meeting this criteria.

The human plasma sample analysis results are presented in the Pharmacokinetic Report section. See Concentration vs Time Data Tables Section. Sample values below the LLOQ of



this assay are noted on the tables. Sample repeat analysis results are presented in Table B3. The analytical runs are shown in Table B4.

Additional Information

Subjects 06, 17, and 46 were not assayed upon the request of the sponsor.

Bioanalysis Archiving

The sample data, report, and electronic media will be archived off-site for a minimum of fifteen years according to applicable CEDRA SOPs. Study samples will be archived according to the applicable CEDRA SOP.

Contributors

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