

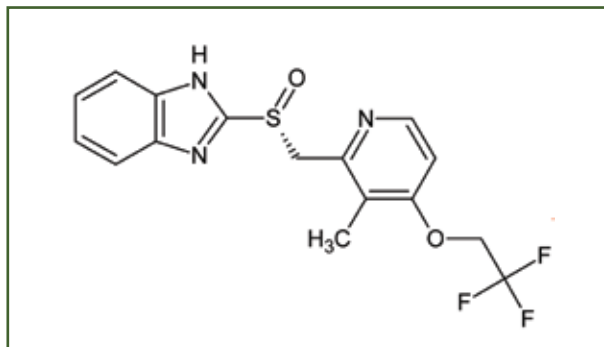
# When Stability, Solubility, and Chiral Selectivity Intertwine: A Quantitative Assay for Dexlansoprazole

## Authors

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## Introduction

- Dexlansoprazole is the R-enantiomer of lansoprazole and is used in the treatment of patients with erosive oesophagitis and non-erosive gastro-oesophageal reflux disease (GERD or GORD).



- Analytical challenges included chiral separation of the enantiomers, pH based instability, and low solubility.
- All challenges needed to be resolved while maintaining a rapid LC method.

## Method

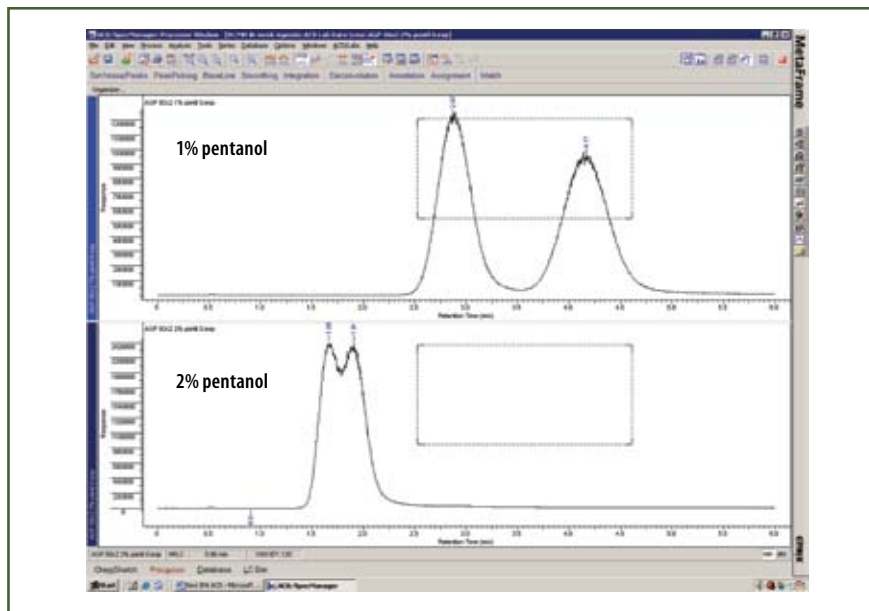
- 100  $\mu$ L of plasma was treated with ammonium acetate and prepared using a supported liquid extraction.
- The HPLC system consisted of Shimadzu LC-10AD HPLC pumps used in conjunction with a LEAP autosampler.
- Sciex API 5000 mass spectrometer was operated in the positive ion mode using ESI.
- Suitable chromatography was achieved on an  $\alpha$ 1-acid glycoprotein phase, 5  $\mu$ m HPLC column using a flow rate of 0.800 mL/min.
- The mobile phases consisted of pentanol and ammonium acetate.
- 5  $\mu$ L of the final extract was injected.

## Challenges

### 1. CHIRAL SEPARATION

- ACD SpecManager Software was used to assist in separating the enantiomers of lansoprazole (Figure 1A, 1B, 1C)
- Highly aqueous final conditions mandate aqueous reconstitution solvent.

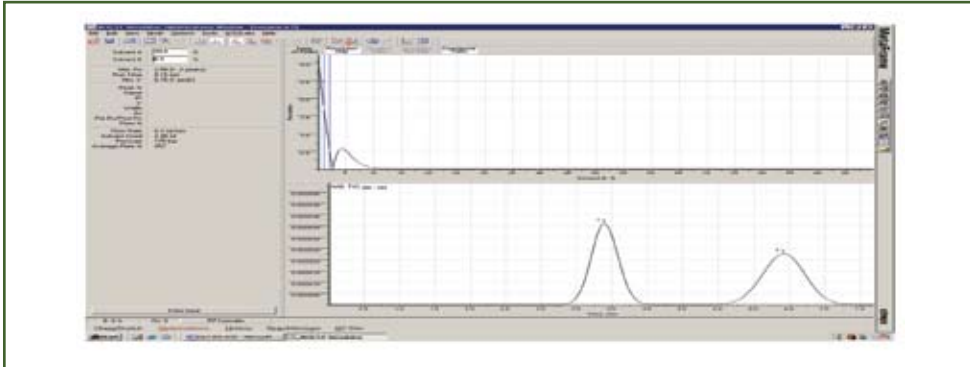
FIGURE 1A: Analyte wiff files imported to ACD SpecManager using pentanol as mobile phase B.



## Challenges continued

FIGURE 1B: Lansoprazole computational results from ACD LC Simulator

0.5% Pentanol 2x50 mm



1.2 % Pentanol 2x50mm

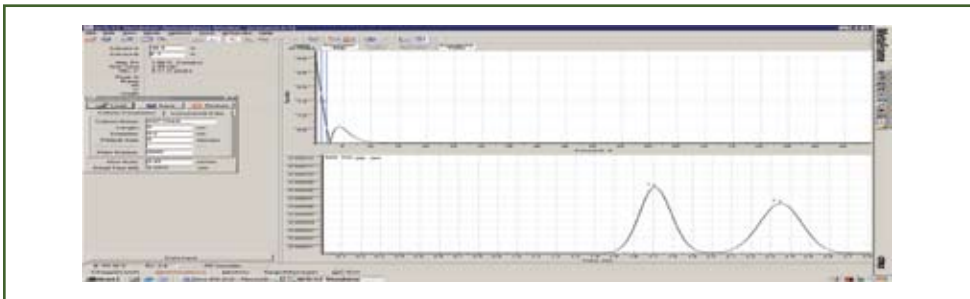
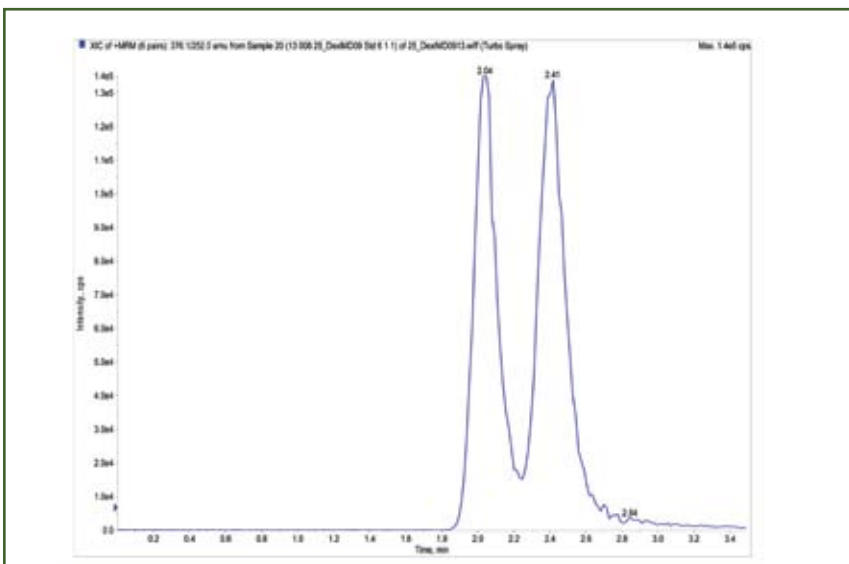


FIGURE 1C: Lansoprazole chiral separation using results from ACD LC simulator prediction



## Challenges continued

### 2. STABILITY

- Dexlansoprazole was found to be unstable in acidic conditions.
- Extraction screen showed very low recoveries at acidic pH (Figure 2A).
- First test extraction yielded very low recoveries.

#### EXPERIMENTS:

1. Stability in plasma vs. protein precipitated plasma (Figure 2B)
2. Also demonstrates storage considerations.

FIGURE 2A: Sample prep screen for Dexlansoprazole

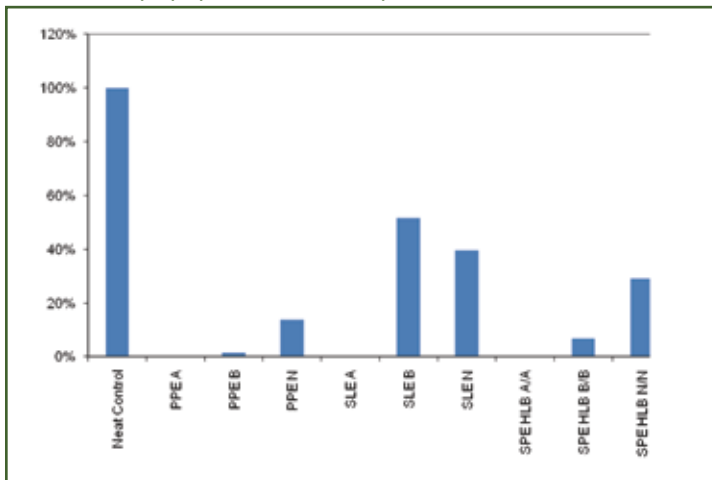
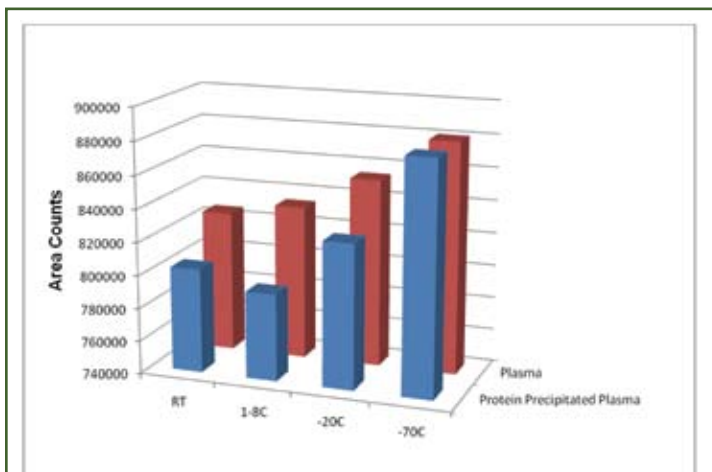


FIGURE 2B: Stability in plasma vs. protein precipitated plasma





## Challenges continued

### 3. SOLUBILITY

- Must avoid acidic pH range to ensure stability.
- Solubility tests confirm storage temperature conditions.

#### EXPERIMENTS:

1. Extract storage stability/solubility tested with varying percent of organic as well as varying temperatures (Figure 3A)
2. Reconstitution solvent dramatically affects sensitivity. (Figure 3B)

FIGURE 3A: Stability and solubility testing of dexlansoprazole in reconstitution solvent

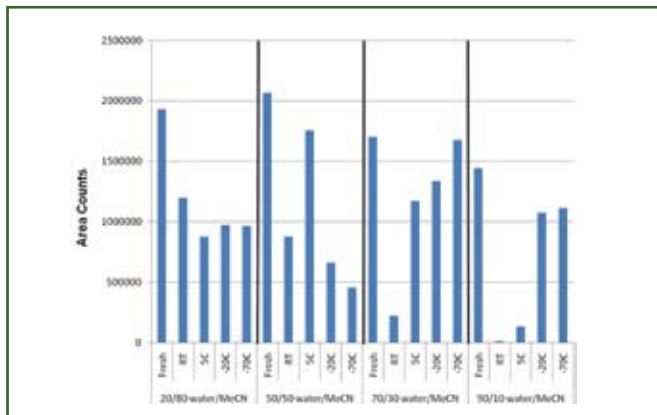
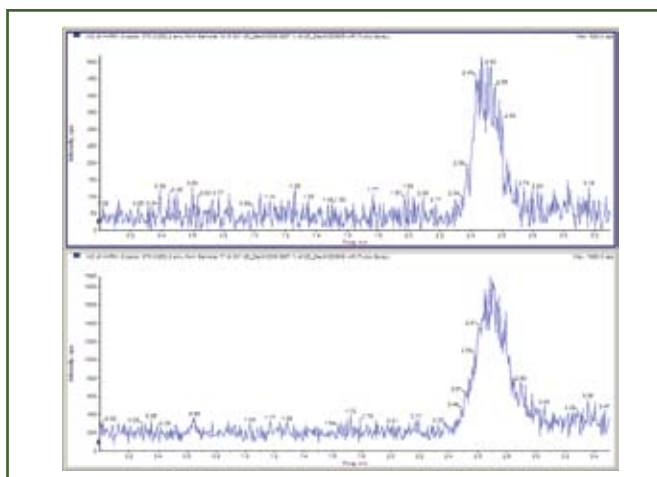


FIGURE 3B: Representative LLOQs comparing reconstitution solvents



Challenges continued

TABLE 1: Inter-assay precision				
Nominal Conc.	LLOQ QC 2.00 ng/mL	Low QC 6.00 ng/mL	Medium QC 200 ng/mL	High QC 1600 ng/mL
Mean Observed Conc.	2.15	5.88	195	1560
%Bias	7.5	-2.0	-2.5	-2.5
Between Run Precision (%CV)	2.7	2.6	3.8	4.5
Within Run Precision (%CV)	7.1	5.1	2.5	2.9
Total Variation (%CV)	7.6	5.7	4.5	5.3
n	18	18	18	18
Number of Runs	3	3	3	3

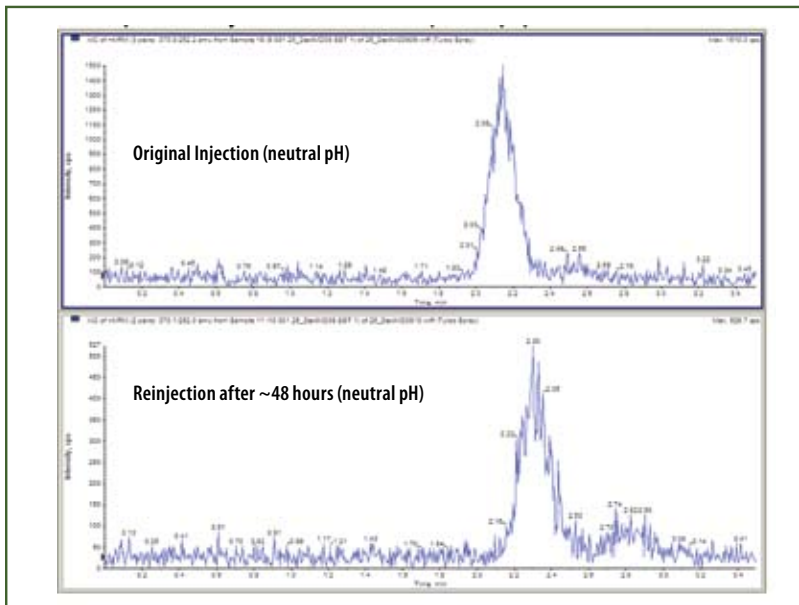
**4. STABILITY—POST EXTRACT**

- Upon reinjection stability testing, a large drop in sensitivity was observed (Figure 4A).

EXPERIMENTS:

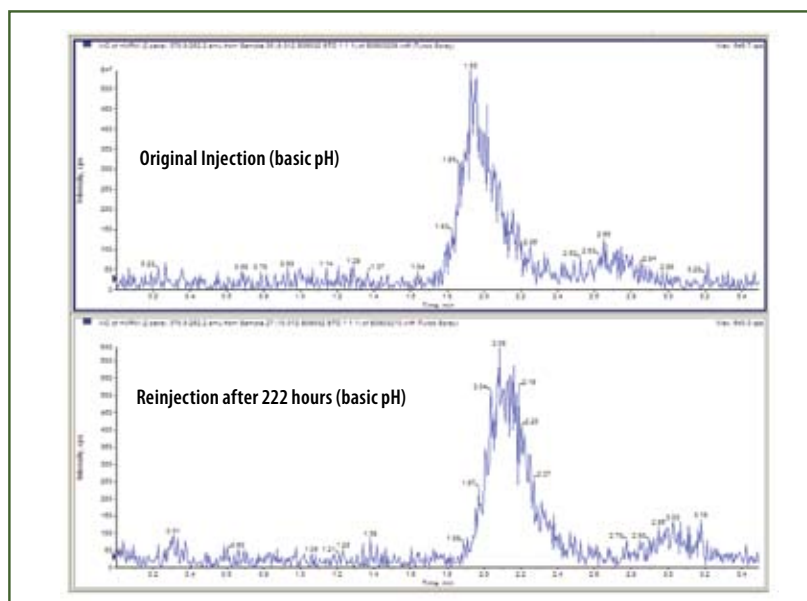
1. Raise pH to stabilize. (Figure 4B)

FIGURE 4A: Representative LLOQs comparing original injection and reinjection in neutral reconstitution solvent.



## Challenges continued

FIGURE 4B: Representative LLOQs comparing original injection and reinjection in basic reconstitution solvent.



## Conclusion

- A rugged and efficient method was developed for the analysis of Dexlansoprazole in human plasma from 2.00 – 2,000 ng/mL.
- A high level of inter-assay accuracy/precision was demonstrated for this assay (Table 1).
- Stability in matrix at room temperature (7 hrs) and -20C (104 days) was established.
- Selectivity using 6 lots of matrix was acceptable with no interfering peaks.
- Upon stabilization of the final extract, reinjection reproducibility was established for 222 hours.