

新規な疎水性 / 親水性ハイブリッド ODS固定相を用いた 血漿試料直接注入による 血中ランソプラゾール濃度測定法

- 立木秀尚¹, 矢澤到²
東和薬品株式会社¹, インタクト株式会社²



TOWA PHARMACEUTICAL CO., LTD.

概要

前処理に関するアンケート



Novel Hydrophobic/Hydrophilic Hybrid ODS Column



Direct Injection LC-MS/MS Analysis

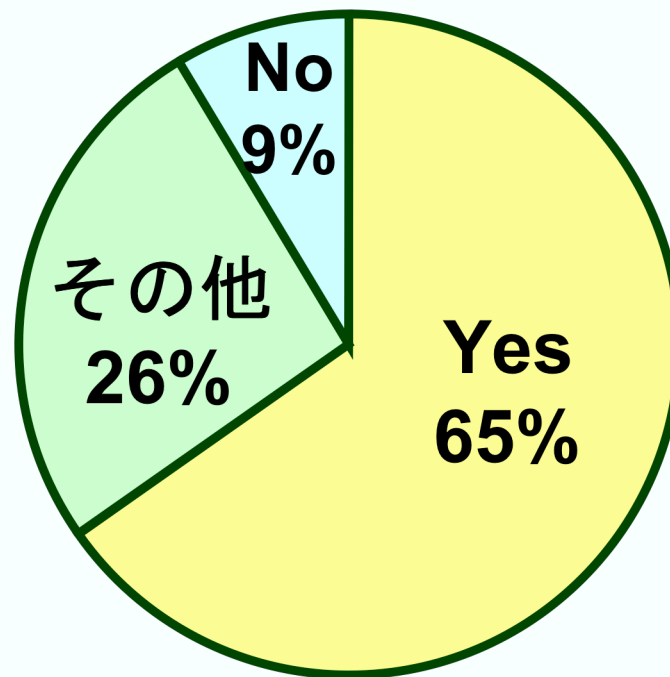


Clinical Trial



前処理に関するアンケート

Q1. 前処理はやりたくないですか？



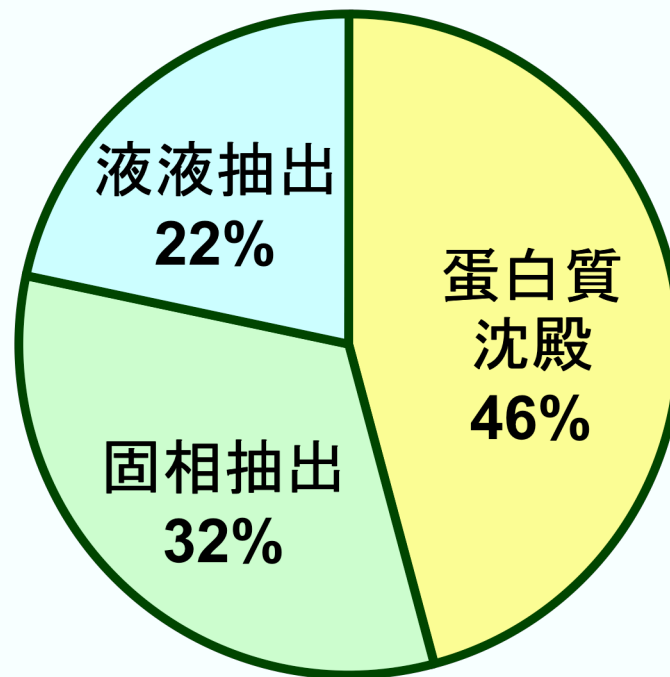
有効回答数 23

MS/MSユーザーズML 調査



前処理に関するアンケート

Q2. よく行なう前処理法は何ですか？



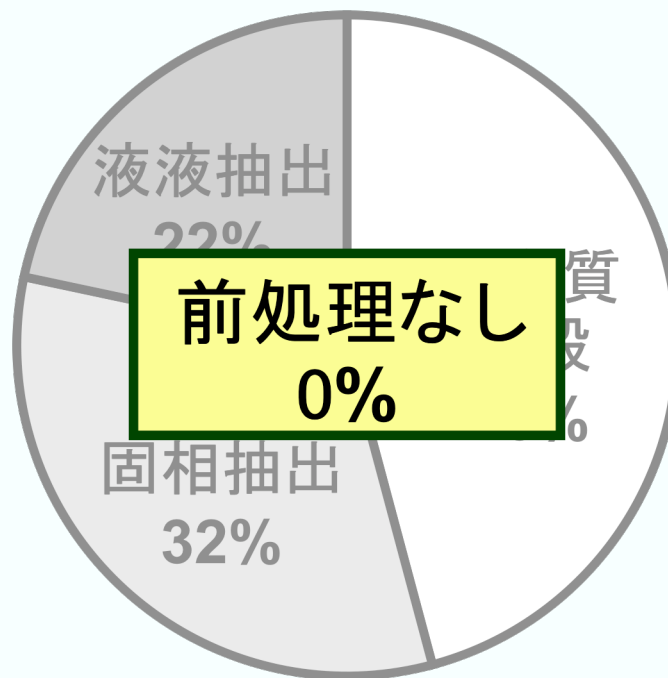
有効回答数 23

MS/MSユーザーズML 調査



前処理に関するアンケート

Q2. よく行なう前処理法は何ですか？



有効回答数 23

MS/MSユーザーズML 調査



生体試料直接注入の問題点

① 前処理カラムやカラムスイッチングが必要

② 汎用分析に適用しにくい

生体試料分析専用カラム

有機溶媒濃度に制限

リン酸緩衝液を常用（LC-MS分析が困難）

ODSが存在しない



生体試料直接注入の問題点 解決

① 前処理用カラムやカラムスイッチングは不要

② 汎用分析でも使用可能なODSカラムで

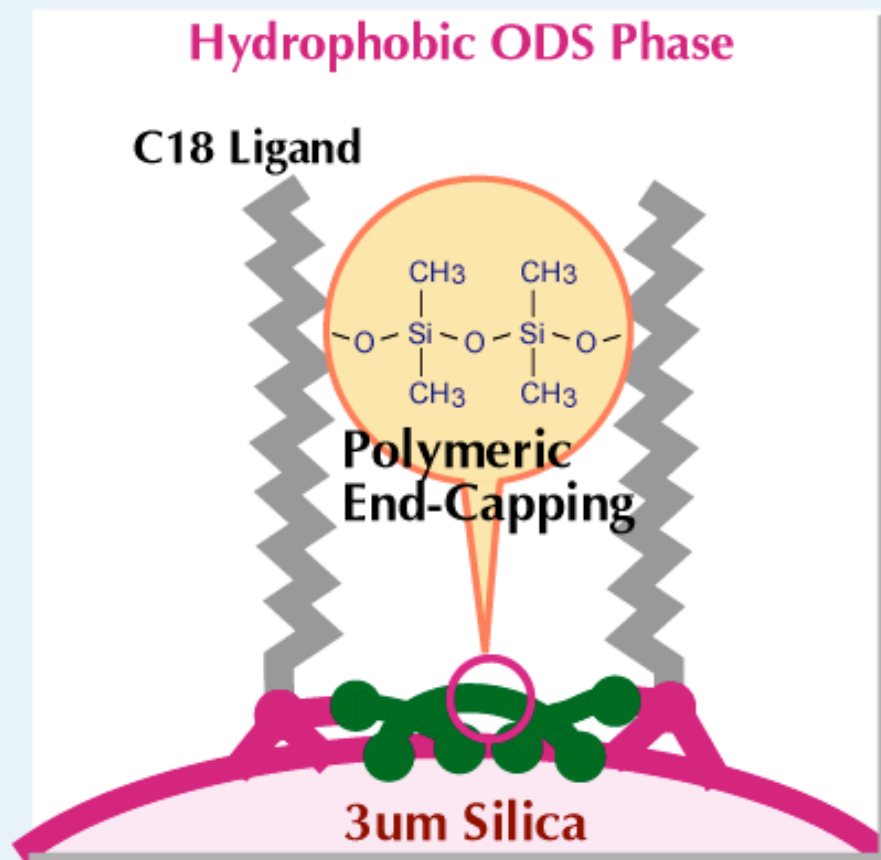
の条件を満たす分析法開発



新規ハイブリッド固定相の概念図

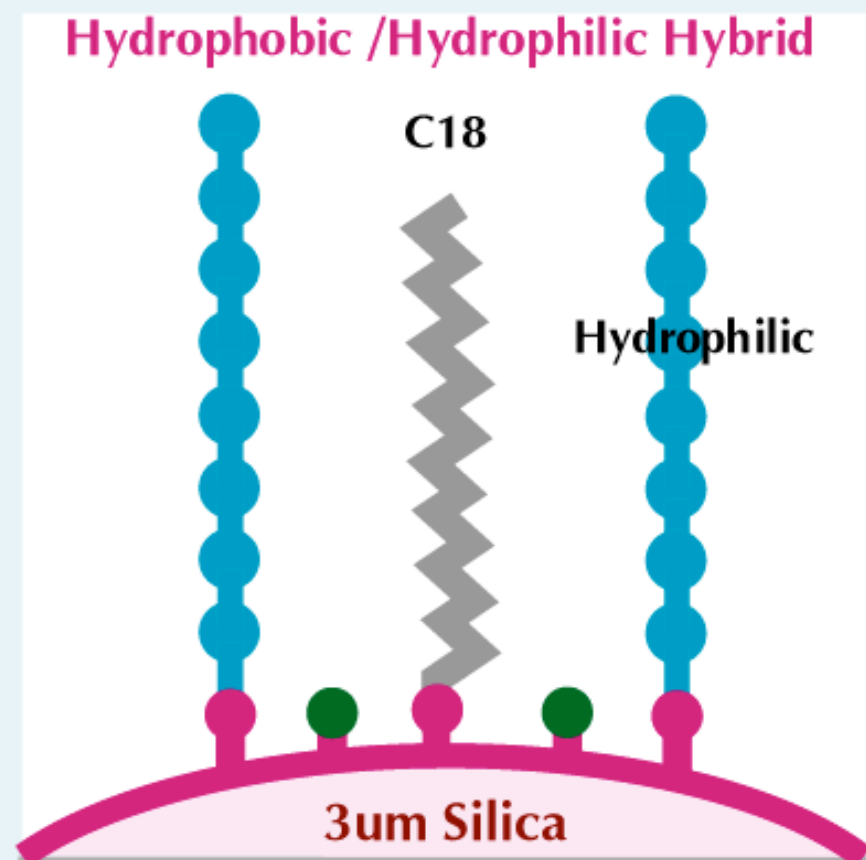
Cadenza CD-C18

Hydrophobic ODS Phase



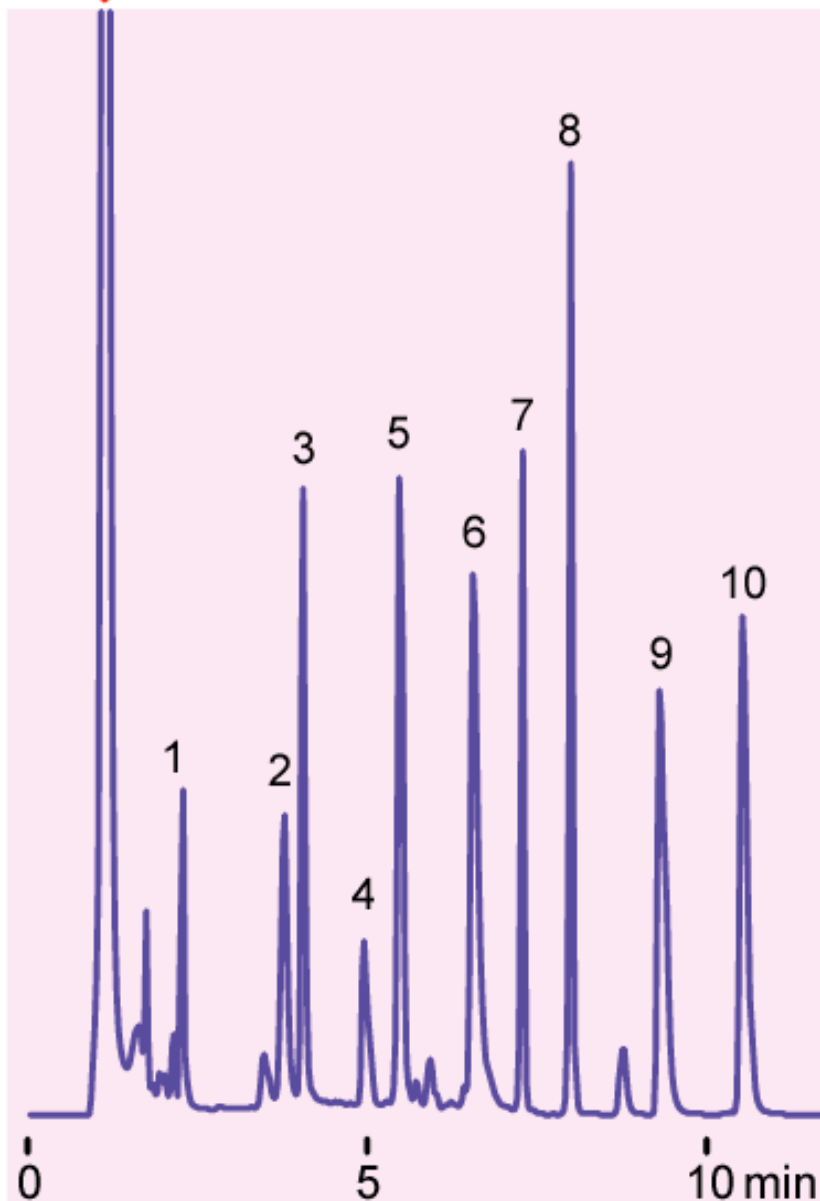
Cadenza **HS-C18**

Hydrophobic /Hydrophilic Hybrid



血清直接注入による逆相グラジエント

serum protein



- 1 acyclovir ($pK_a=2.5, 9.4$)
- 2 nicotinamide
- 3 theophylline
- 4 ibuprofen ($pK_a=4.3$)
- 5 sulfamethazine ($pK_a=7.4$)
- 6 N-acetylprocainamide ($pK_a=8.3$)
- 7 carbamazepine ($pK_a=7.0$)
- 8 progesterone
- 9 bacitracin (MW=1307)
- 10 nortriptyline ($pK_a=9.7$)

Cadenza HS-C18, 150 x 3 mm

A: 10mM ammonium acetate

B: acetonitrile

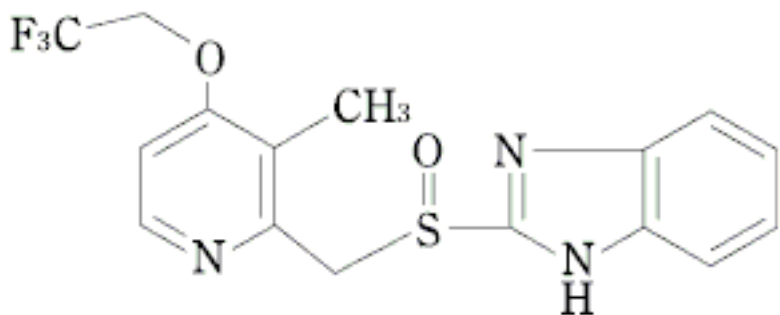
0-75%B (0-10min)

0.4 mL/min (11MPa)

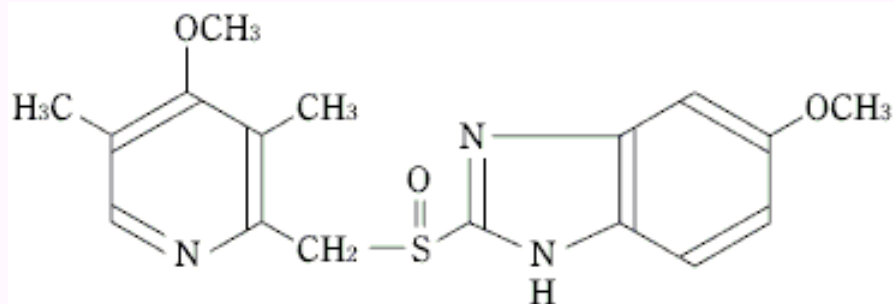
37 deg.C, 260 nm,

bovine serum 16uL (0.1-4.0 μ g)

Compounds



Lansoprazole
(Analyte)



Omeprazole
(Internal Standard)



Sample Preparation

*Direct Injection vs.
Protein Precipitation*

Direct Injection

- ↓ Plasma
- ↓ Centrifugation
- ↓ Addition of I.S.
9:1 H₂O (I.S.) to Plasma
- ↓ Injection

Protein Precipitation

- ↓ Plasma
- ↓ Centrifugation
- ↓ Protein Precipitation
4:1 ACN (IS) to Plasma
- ↓ Filtration
- ↓ Injection



Analytical Conditions

Direct Injection vs. Protein Precipitation

Direct Injection

Column

Cadenza HS-C18
20×4.6 mm, 3 μm (Imtakt)
with Guard Column

Mobile Phase

A, 10 mM ammonium formate
(pH3.0)

B, Acetonitrile

100 % A (0→0.5 min.)

0→100 % B (0.5→2.5 min.)

100 %B (2.5→3.5 min.)

Flow Rate 500 μL/min.

Temperature 40 deg. C

Injection Volume 10 μL

MS/MS ESI, Positive, MRM

Protein Precipitation

Column

ODS Column
20×4.6 mm, 5 μm
without Guard Column

Isocratic

A, 10 mM ammonium formate
(pH3.0)

B, Methanol

C, Acetonitrile

A/B/C = (27.5 : 35.0 : 37.5)

0 - 8.5 min.

Flow Rate 70 μL/min.

Temperature 40 deg. C

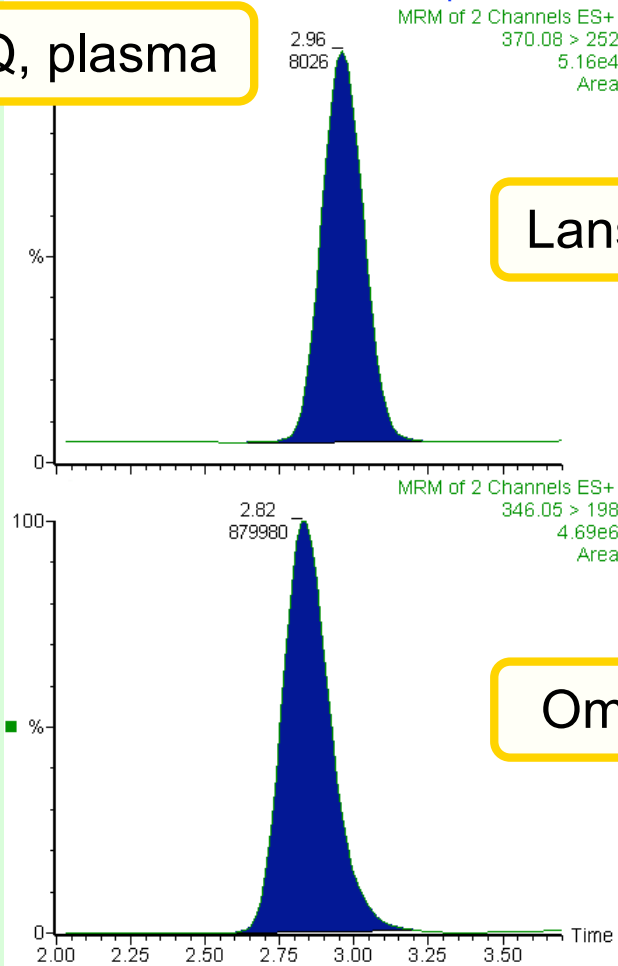
Injection Volume 1 μL

MS/MS ESI, Positive, MRM

Chromatogram

*Direct Injection vs.
Protein Precipitation*

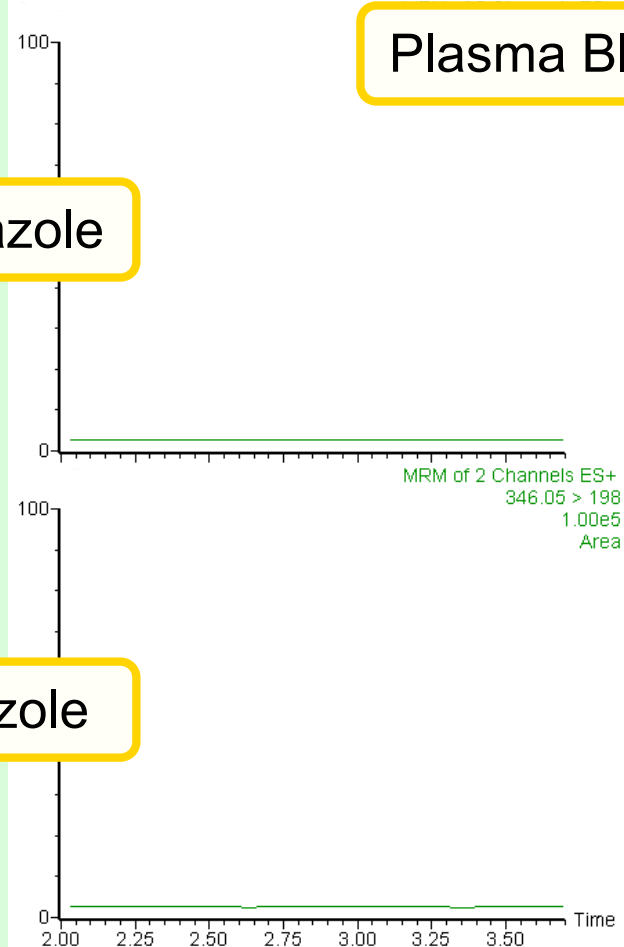
LOQ, plasma



Lansoprazole

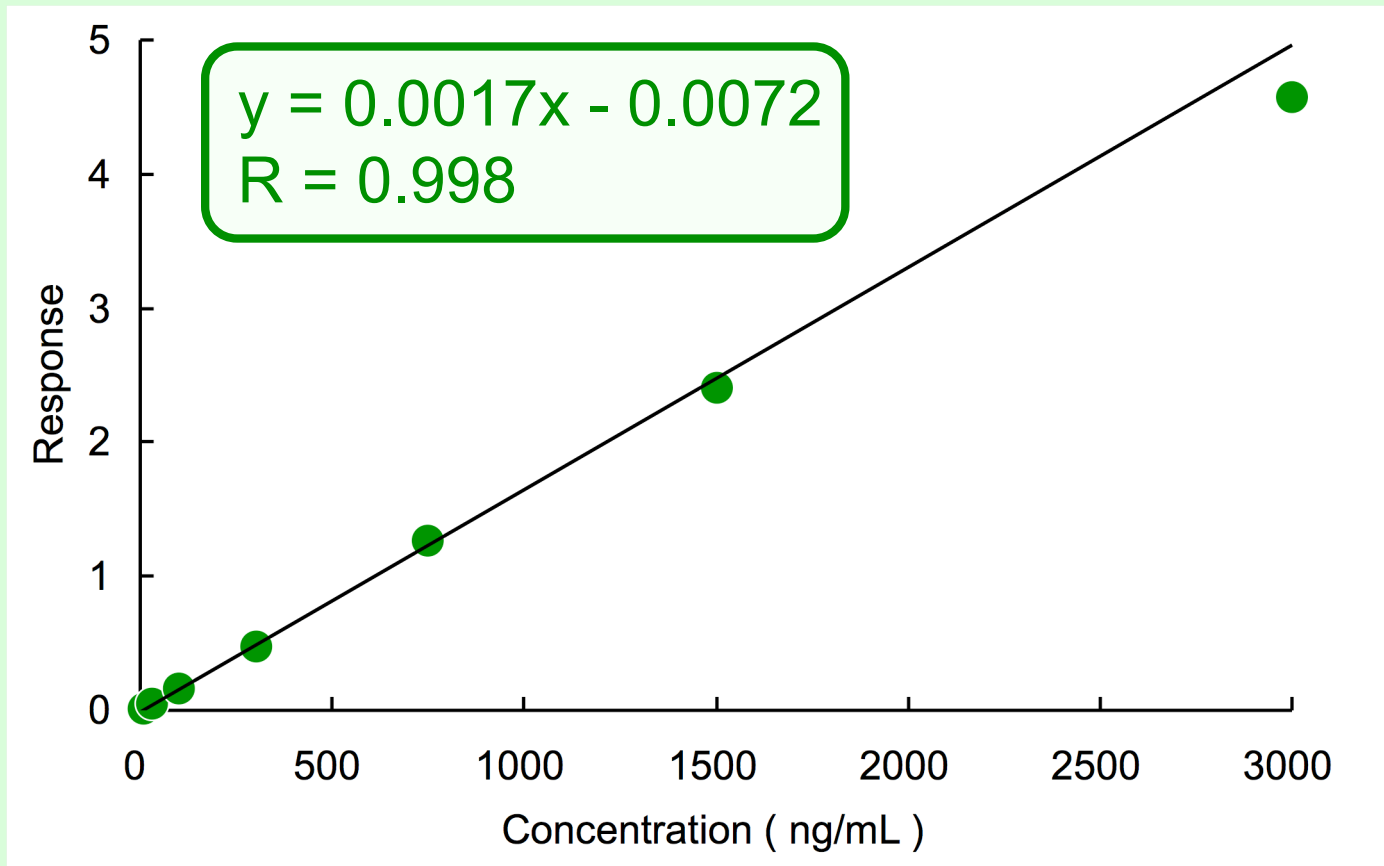
Omeprazole

Plasma Blank



Linearity

*Direct Injection vs.
Protein Precipitation*

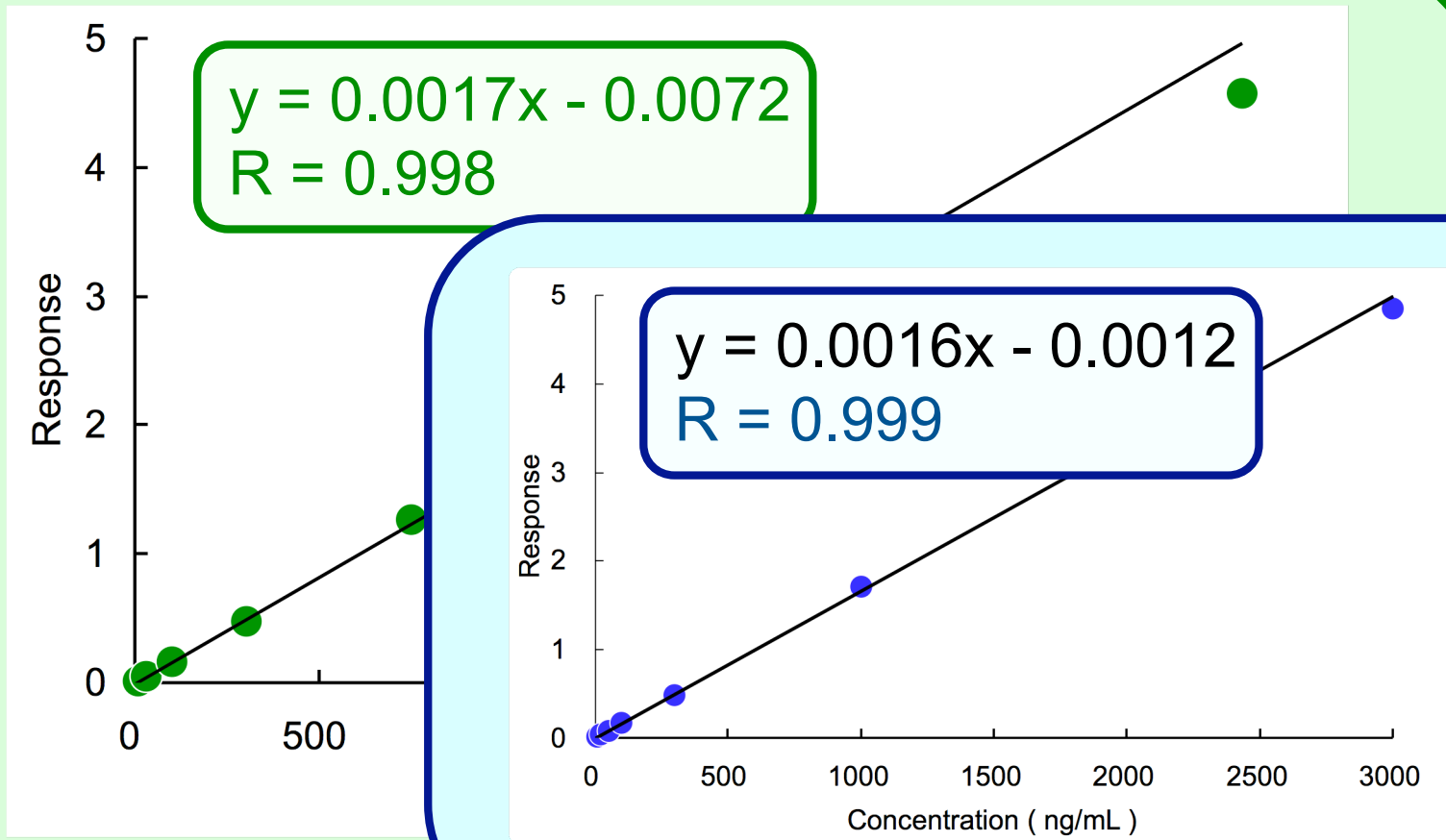


10 - 3000 ng/mL, Weight $1/x^2$, $n = 3$



Linearity

*Direct Injection vs.
Protein Precipitation*



10 - 3000 ng/mL, Weight $1/x^2$, $n = 3$



Accuracy and Precision

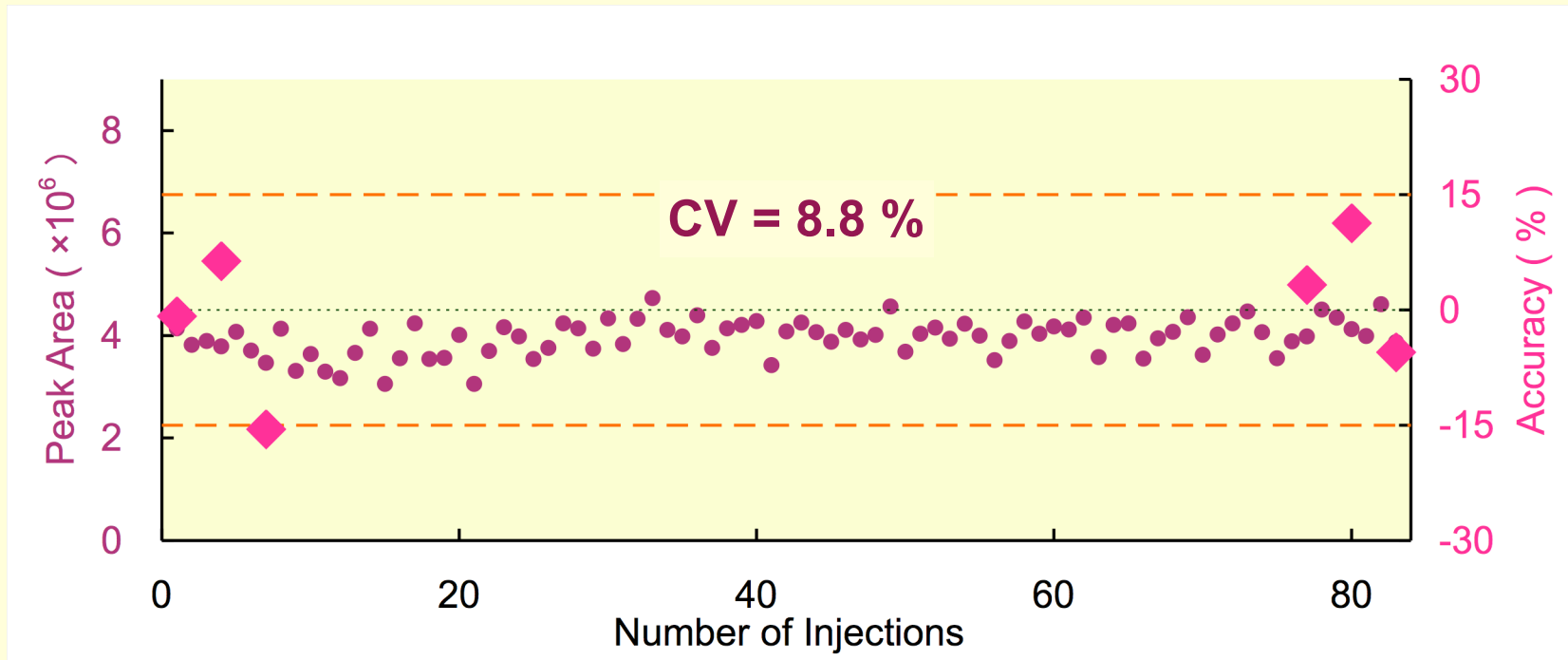
*Direct Injection vs.
Protein Precipitation*

	Nominal Concentration (ng/mL)					
	10		300		3000	
Accuracy (Mean, %)	-1.9	5.5	4.3	-0.4	-6.4	0.7
Precision (CV, %)						
Intra-batch (n = 3)	2.7	4.0	2.4	3.1	2.5	2.8
Inter-batch (n = 6)	5.0	6.1	4.0	5.1	6.6	4.8



Quality Control

Clinical Trial



Lansoprazole 30 mg Single Dosa (One Healthy Adult male)

● - IS Peak Area

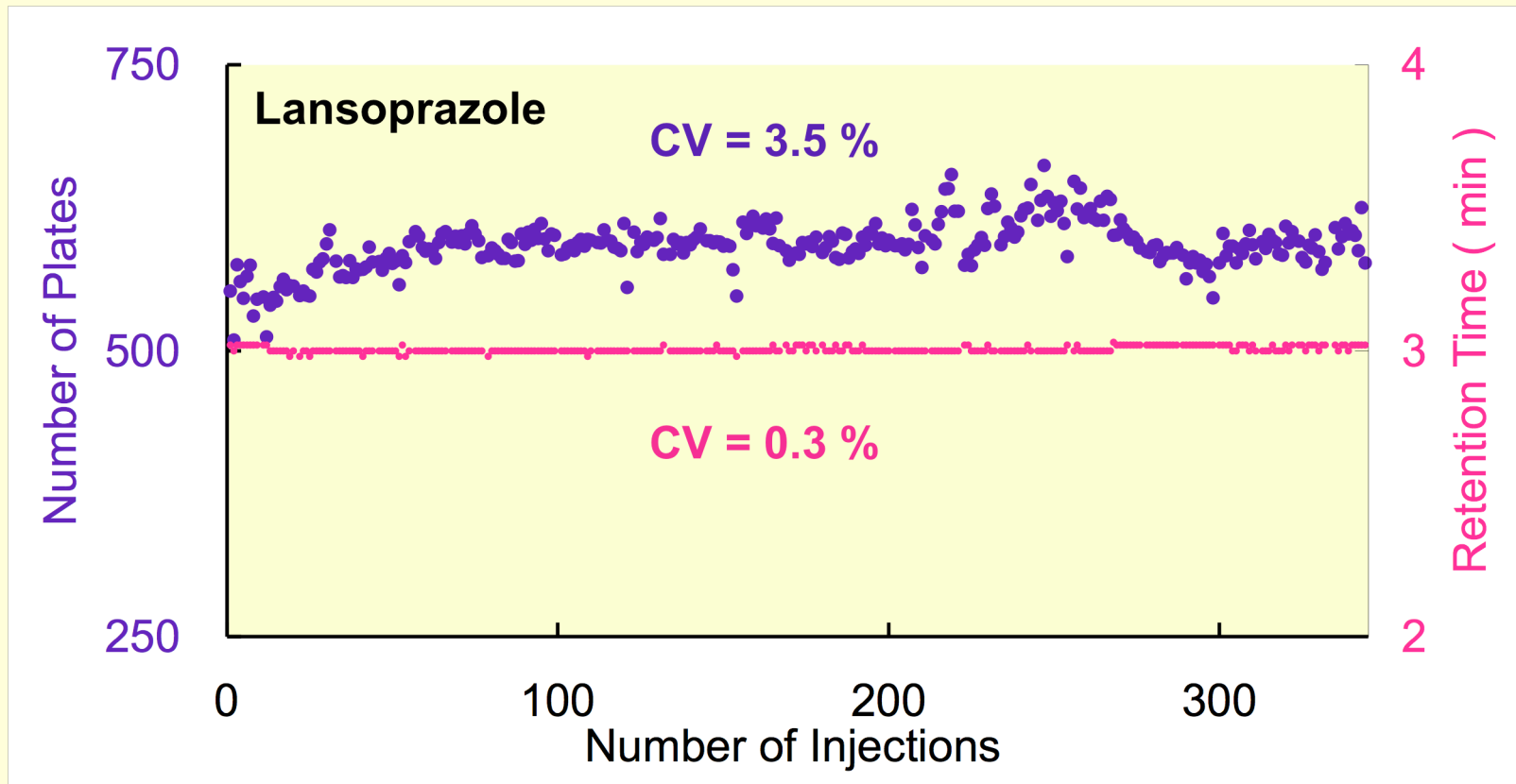
◆ - QC Samples (10, 300 and 3000 ng/mL); QC Range = ± 15 %



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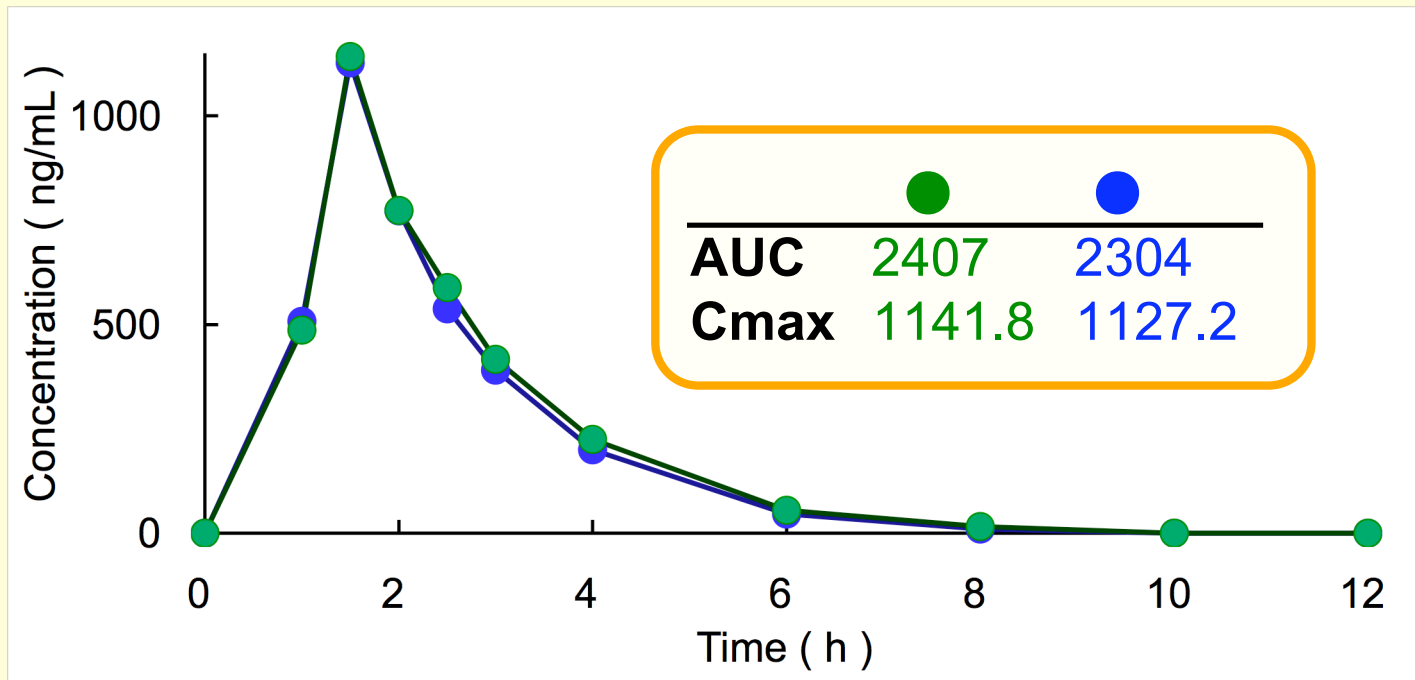
Longevity

Clinical Trial



Plasma Concentrations

Clinical Trial



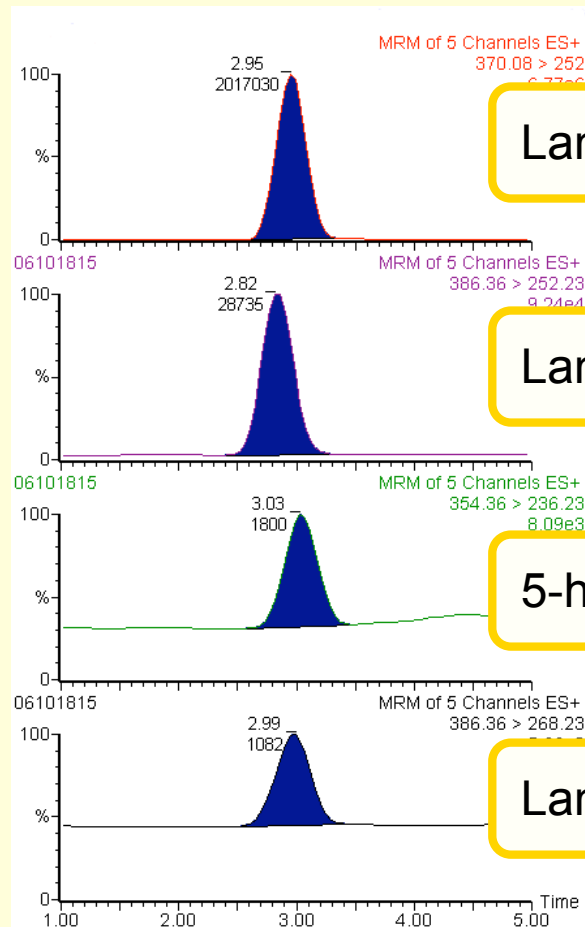
- - Direct Injection
- - Protein Precipitation

AUC - ng·h/mL
Cmax - ng/mL



Metabolites

Clinical Trial



Lansoprazole

Lansoprazole Sulfide

5-hydroxy Lansoprazole

Lansoprazole Sulfone



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まとめ

**Novel Hydrophobic/Hydrophilic Hybrid ODS Column
Cadenza HS-C18**

**Direct Injection LC-MS/MS Analysis of
Lansoprazole in human plasma**

**Method Validation
Clinical Trial
Direct Injection vs. Protein precipitation**



まとめ

**Novel Hydrophobic/Hydrophilic Hybrid ODS Column
Cadenza HS-C18**

**Direct Injection LC-MS/MS Analysis of
Lansoprazole in human plasma**

Satisfactory

Direct injection vs. Protein precipitation



まとめ

**Novel Hydrophobic/Hydrophilic Hybrid ODS Column
Cadenza HS-C18**

**Direct Injection LC-MS/MS Analysis of
Lansoprazole in human plasma**

Direct Injection vs. Methanol Precipitation

Excellent?



まとめ

**Novel Hydrophobic/Hydrophilic Hybrid ODS Column
Cadenza HS-C18**

**Direct Injection LC-MS/MS Analysis of
Lansoprazole in human plasma**

Direct Injection vs. Extraction

**Perfect?
Satisfactory?**

