Determination of Clarithromycin in Human Plasma by LC-EI Tandem Mass Spectrometry: Application to Bioequivalence Study

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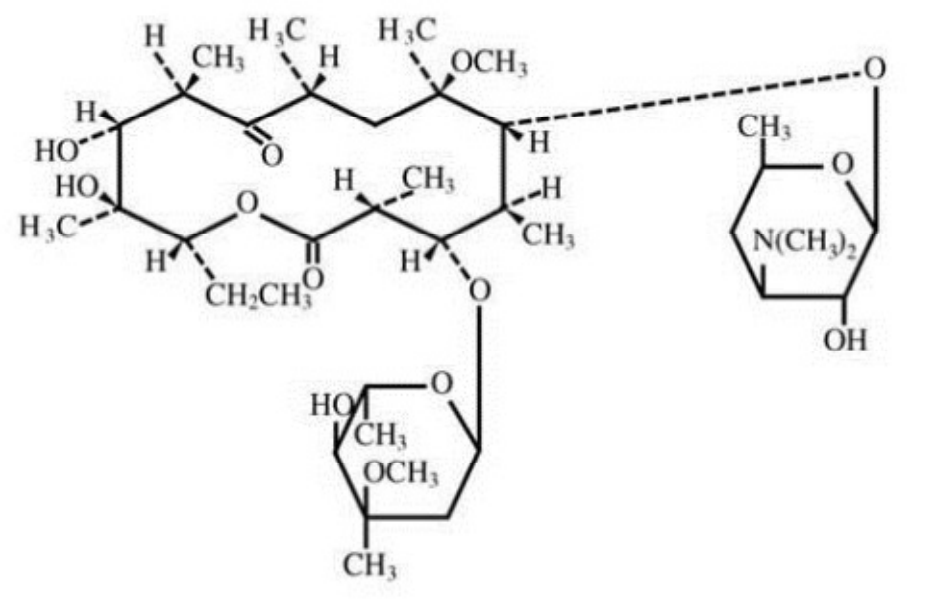
Clarithromycin - Background



- Chemical Name: 6-O-Methylerythromycin
- Molecular Formula: $C_{38}H_{69}NO_{13}$ (FW: 747.95)
- Solubility: Highly – Acetone ; Slightly –Methanol, Ethanol

  Practically insoluble in water.

Pharmacokinetic Study

- Bioavailability ; About 50%
- Peak plasma concentration: 2.41-2.85 µg/ml after single 500 mg dose.
- Time Max: 2-3 hrs.
- Half life: 3.5 - 4.5 hrs.

Brand & Generic Names

- Active ingredient : Clarithromycin
- Brand Names: APO-Clarithromycin, Biaxin, Chemmart, Clarac, Clarithexal, Clarithromycin AN , Clarithromycin-PS, Prevpac, Klacid
- Generic Names: Claritt, Clarimac and Clarex (Saudi Arabia)

References:

1. HPLC-Electrochemical: Biomedical Chromatography,15:8, (2001) 507.
2. HPLC- UV: J. of Chromatography B, 817:2 (2005) 193.
3. HPLC - Pre-column Derivatization Fluorescence Detector J. Chromatography 850:1-2 (2007) 359.
3. HPLC-UV: Pre-column Derivatization UV-Detector, Talanta, 71 (2007) 385.
4. LCMS-MS: J. of Pharmaceutical and Biomedical Analysis 43:4 (2007) 1460.
5. UPLCMS/MS: Chromatographia, 68 (2008) 617.

The objective of the study:

- To develop and validate a rapid, sensitive, and reproducible method to determine clarithromycin levels in small volumes of human plasma by LC-MS/MS.
- Assess the stability of clarithromycin under various conditions.
- Application of method in Bioavailable and Bioequivalence study.

Methodology:

Material/Reagents & Equipment

- Clarithromycin, erythromycin – Certified Purity ≥ 99%, from USP, Rockville, MD, USA.
- Acetonitrile, methanol (HPLC Grade),Triethylamine, Phosphoric acid (AR-Grade) (All from Fisher Scientific, NJ, USA).
- HPLC grade water prepared by reverse osmosis and further purified by passing through a Synergy Water Purification System (Millipore, Bedford, MA, USA)
- Drug free human plasma from Blood Bank King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.
- Instrument: MS/MS Micromass, Triple quadruple, HPLC, Alliance 2695, (Waters Associates Inc. Milford , MA, USA)

Standards and Quality Control Preparations

-Stock & Working Solutions:
-Clarithromycin & Erythromycin (0.1 mg/ml, methanol)

- Working Solutions:
  Clarithromycin : 10 µg/ml in drug free human plasma
  Erythromycin : µg/ml in methanol

- Calibration Curve (10 concentrations)
  Range: 5 ng/ml – 4.0 µg/ml plasma

- Quality Control Samples
  1: LLQ (5 ng/ml),
  2: 3xLLQ (1.5 ng/ml)
  3: 0.5 HLQ (2.0 µg/ml)
  4: 0.9 HLQ (3.6 µg/ml)

Analytical Conditions

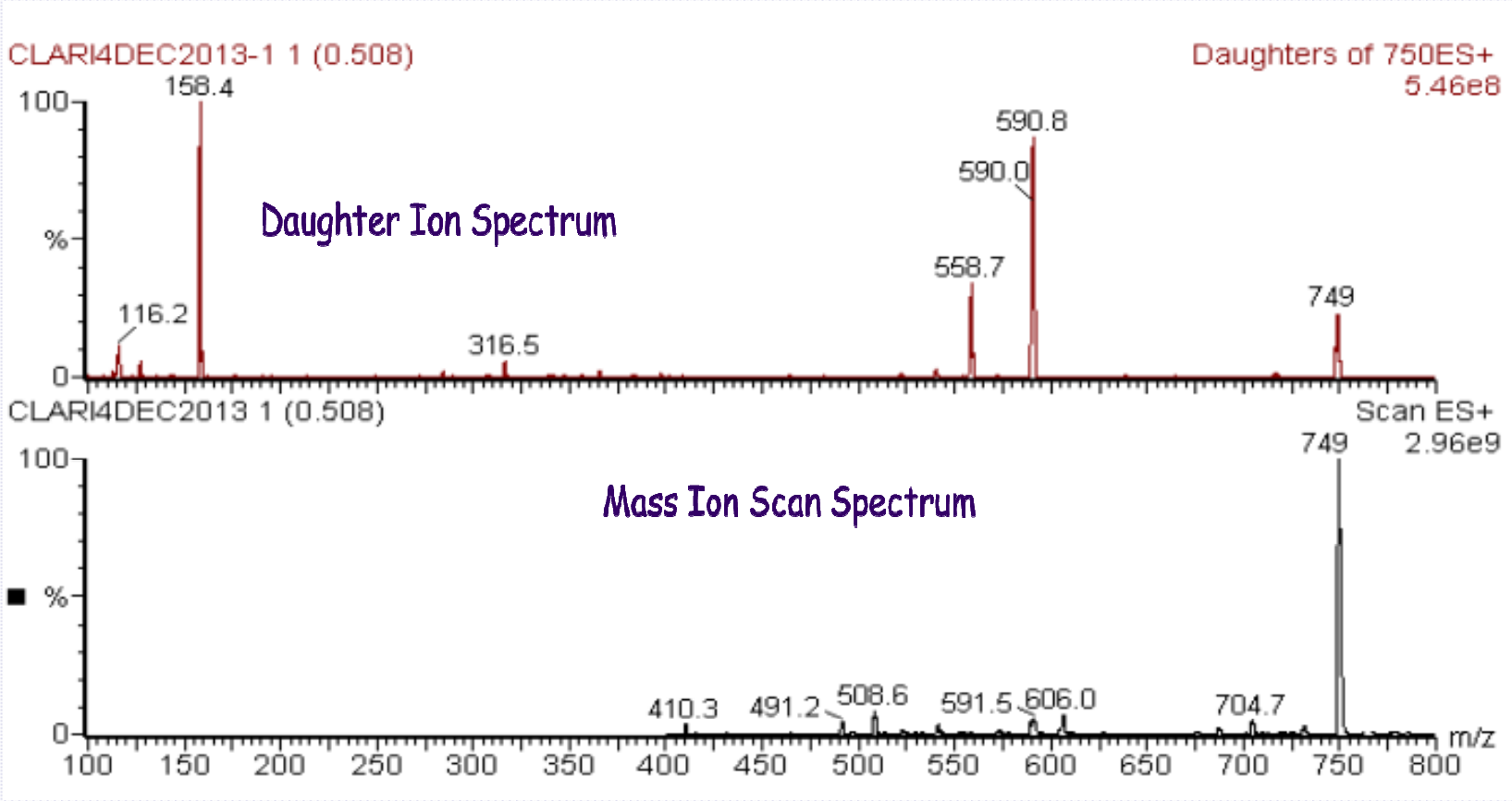
Liquid Chromatograph:

- Column: Atlantis dC18 (2.1 x 100 mm, 3 µm)
- Guard Column: Symmetry C18 (2.1 x 100 mm, 5 µm)
- Mobile Phase: Acetonitrile and 0.5% Triethylamine (PH=4, with phosphoric acid), (65: 35, V/V)
- Flow rate: 0.25 ml/min.

Mass Spectrometric System:

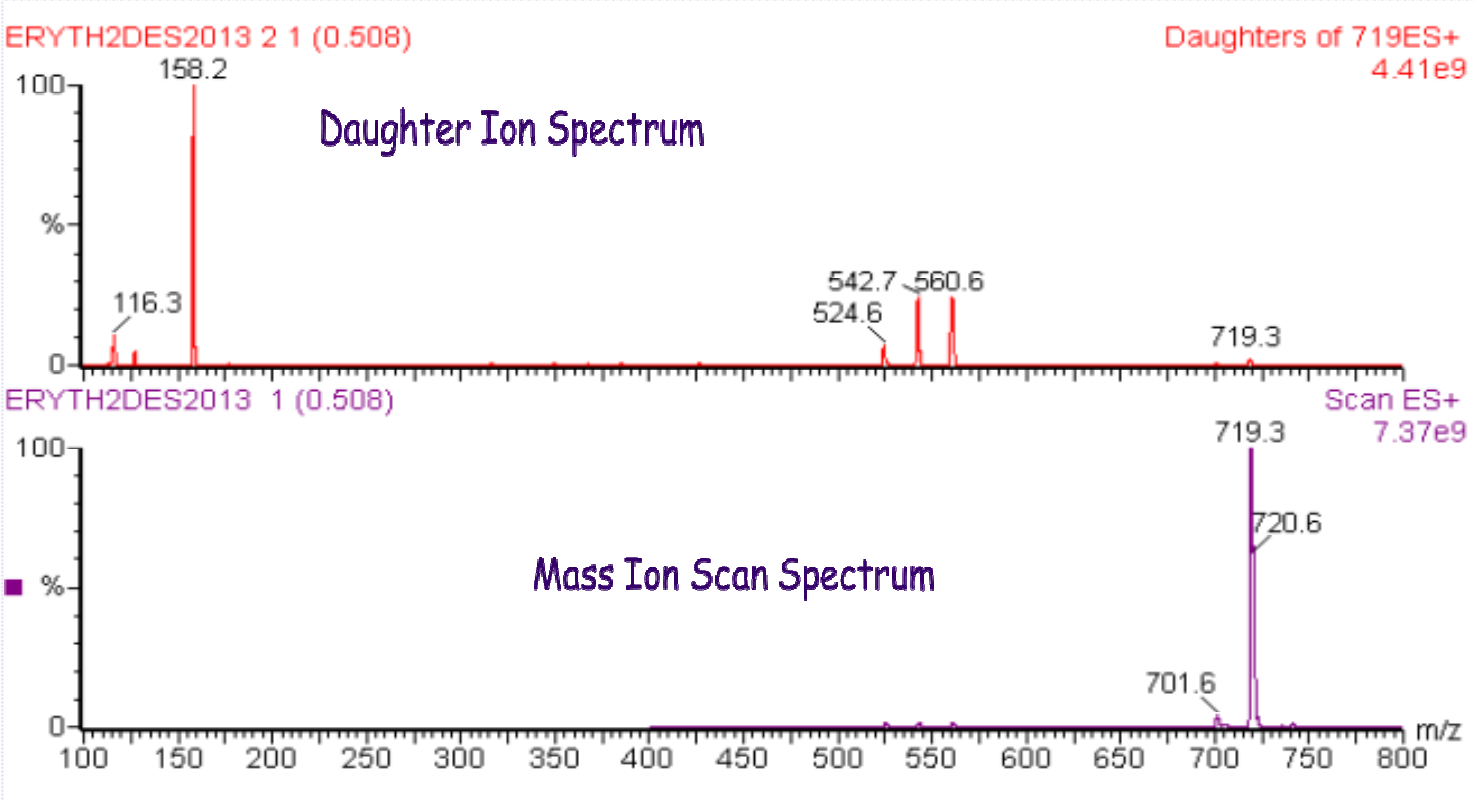
Spray: Electrospray ionization (positive)
Voltages: Capillary 4.0 kV, Cone 30 V
Temperature: Source 125°C, Desolvation 350°C
Cone Gas Flow: 600 L/hr.
Collision Energy ; 25 eV

Mass Spectra of Clarithromycin



Transition: 749 ⟶ 158.4

Mass Spectra of Erythromycin

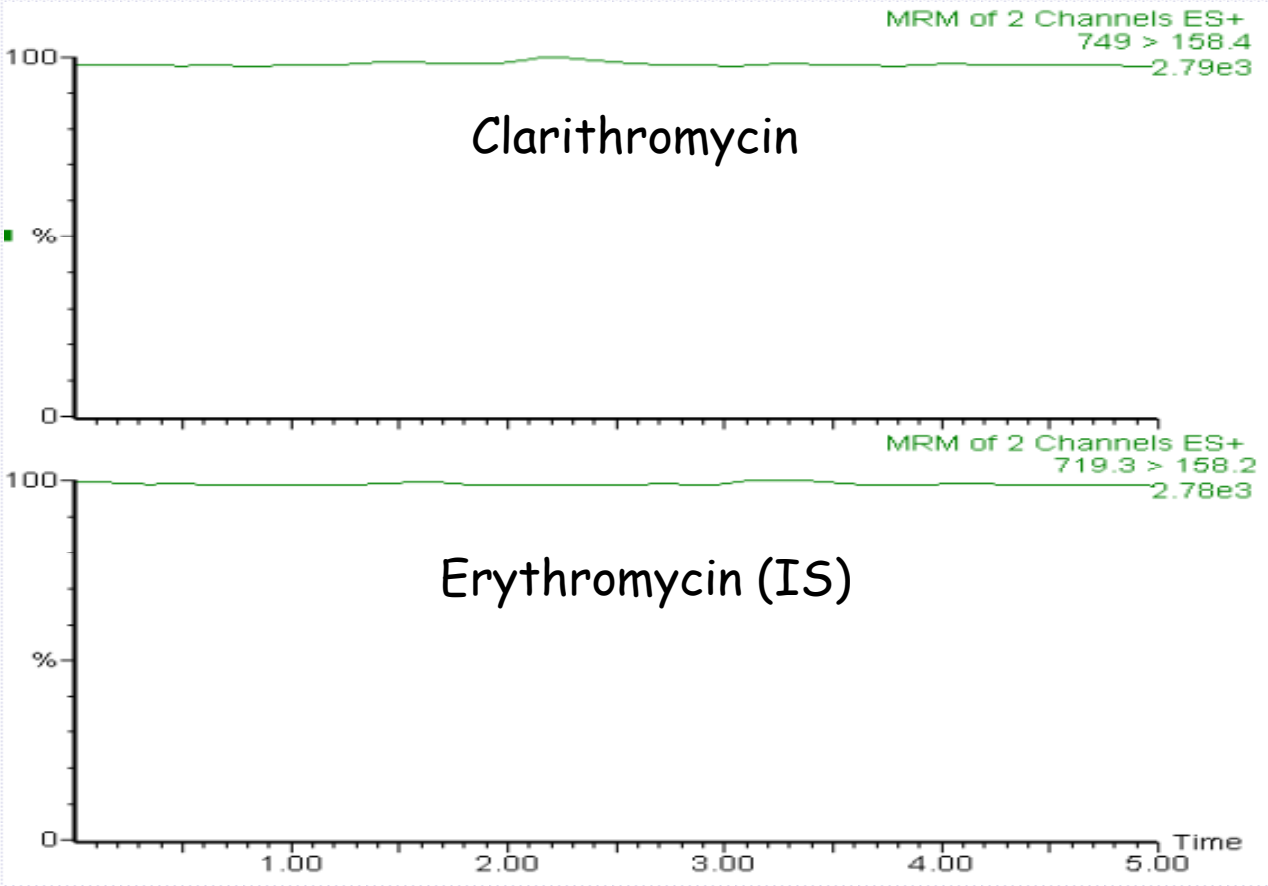


Transition: 719.3 ⟶ 158.2

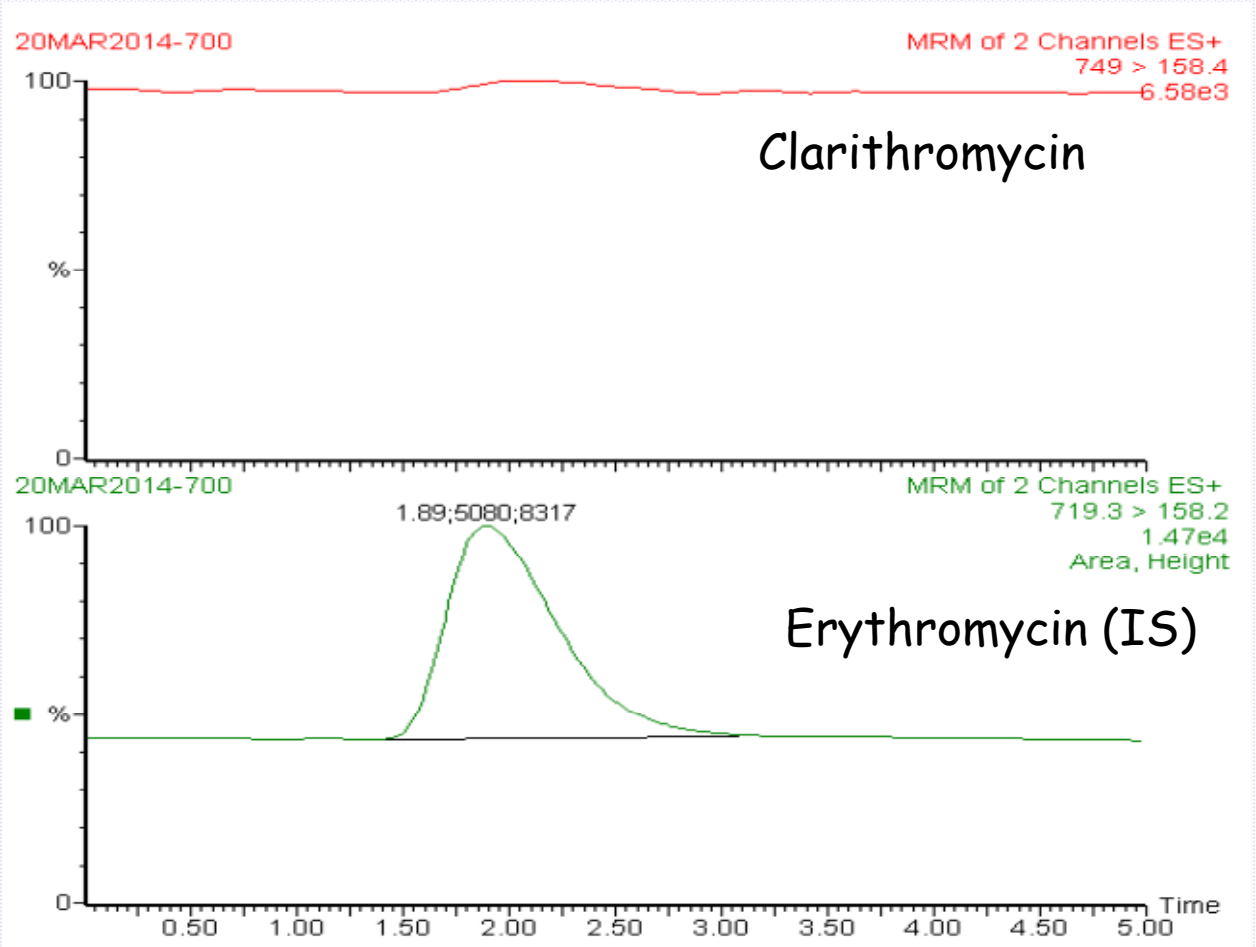
Plasma Sample Preparation

- Human plasma (0.2 ml) + Erythromycin, (Internal standard)
  (50 µl of 1.0 µg/ml, methanol)
- Add 4.0 ml- Tert. Butylmethylether
- Vertex 5 minutes then centrifuge at 6000 rpm for 10 minutes.
- Separate organic layer and evaporate solvent at 40 °C
- Reconstitute in mobile phase, inject volume 5.0 µL
- Run Time: 4 Minutes

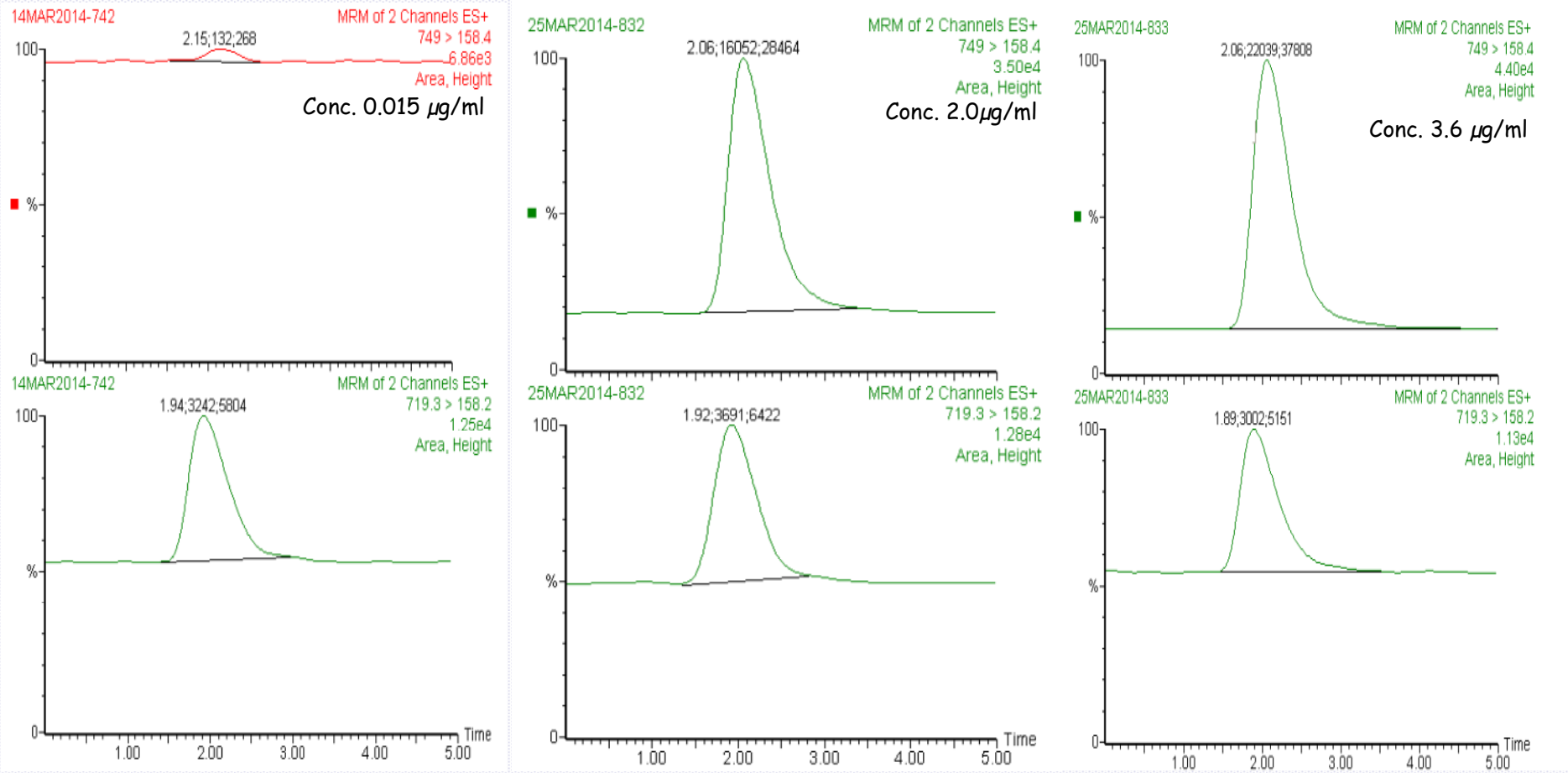
Representative MRM Chromatogram of blank human plasma



Representative MRM Chromatogram of plasma spiked with IS



Representative MRM Chromatograms of plasma spiked with Clarithromycin and IS



Method Validation

Parameters	Acceptable limits
Specificity	: Blank plasma (6) Commonly used drugs
Recovery	: Consistent
Linearity	: Analyte Conc./Response : (6-8, Zero and Blank)
Accuracy & Precision	: ±15% (3 levels) :±20% for LLQ
Stability	: Confirm

Specificity

- 6 different batches of human plasma screened
- Eight commonly used medications:
  Acetaminophen, Ibuprofen, Aspirin, Omeprazole, Nicotinic acid, Ascorbic acid, Ranitidine and Caffeine.

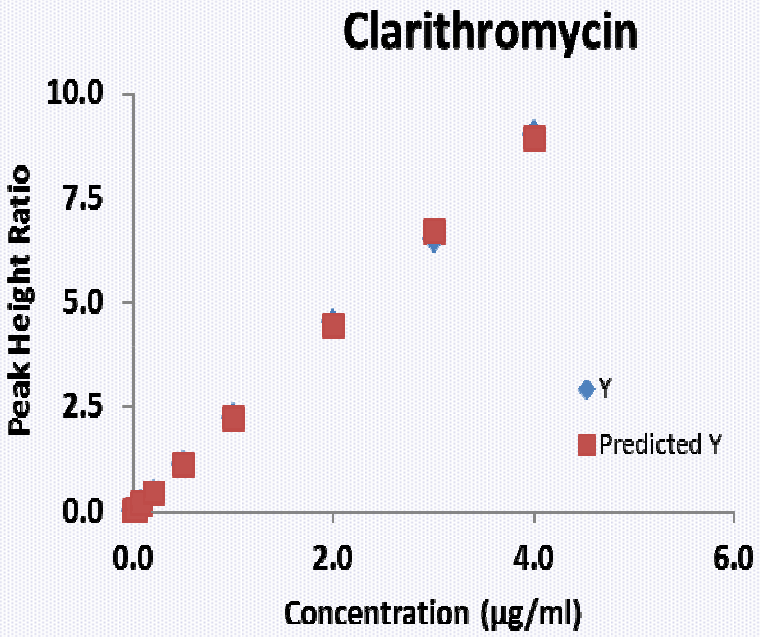
Recovery from human plasma

Clarithro-mycin (µg/ml)	Mobile Phase		Human Plasma		Recovery (%)	Mean (%)
	Mean Height	SD	Mean Height	SD		
0.005	122	4.36	120	12.16	98	94
0.015	322	4.58	325	8.02	101	
2.0	38439	1188	34299	1028	89	
3.6	67845	1601	58253	997	86	
IS (1.0)	17260	838	17024	453	99	99

Linearity

Nominal Conc. (µg/ml)	CLA-PH	IS-PH	Ratio	Measured Conc. (µg/ml)	Acc. (%)
0.005	766	43847	0.0175	0.006	116
0.010	991	39553	0.0251	0.009	92
0.020	2097	43542	0.0482	0.020	98
0.080	6907	38113	0.1812	0.079	99
0.200	25597	58360	0.4386	0.194	97
0.500	97180	85465	1.1371	0.507	101
1.000	149208	66571	2.2413	1.001	100
2.000	231682	51170	4.5277	2.024	101
3.000	326101	50037	6.5172	2.915	97
4.000	456860	50136	9.0439	4.046	101

Representative Standard Calibration Curve



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9997
R Square	0.9994
Adjusted R Square	0.9994
Standard Error	0.0789
Observations	10
Intercept	0.0020
X Variable 1	2.2337

Precision & Accuracy

	INTRA-DAY (n=10)				INTER-DAY (n=20)			
Nominal (µg/ml)	Measured (µg/ml)	SD	CV (%)	Bias (%)	Measured (µg/ml)	SD	CV (%)	Bias (%)
0.005	0.0053	0.0007	13.1	5.1	0.0047	0.0004	9.5	-5.9
0.150	0.0164	0.0018	10.7	9.0	0.0171	0.0016	9.6	12.2
2.000	1.8219	0.0647	3.6	-9.0	1.8699	0.0529	2.8	-7.0
3.600	3.3252	0.0953	2.9	-7.6	3.3878	0.0854	2.5	-6.3

Stability: Processed & unprocessed samples

Storage Condition	Nominal (µg/ml)	Measured (µg/ml	SD	Stability (%)
Base line/None	0.015 3.600	0.015 3.394	0.002 0.056	
Processed 24 h. RT	0.015 3.600	0.015 3.343	0.002 0.056	100 98
48 h. (-20º)	0.015 3.600	0.015 3.291	0.002 0.060	104 97
Unprocessed 24 h. RT	0.015 3.600	0.012 3.421	0.001 0.175	83 101
14 wks (-20º)	0.015 3.600	0.013 3.342	0.002 0.506	94 93
FT: Cycle-1	0.015 3.600	0.012 3.306	0.001 0.144	83 99
FT: Cycle-2	0.015 3.600	0.015 3.163	0.002 0.229	104 95
FT: Cycle-3	0.015 3.600	0.013 3.403	0.001 0.112	87 102

Ruggedness & Robustness

Ruggedness: Mobile Phase:

- Altering Strength of Triethyleamine
- Proportion of Acetonitrile

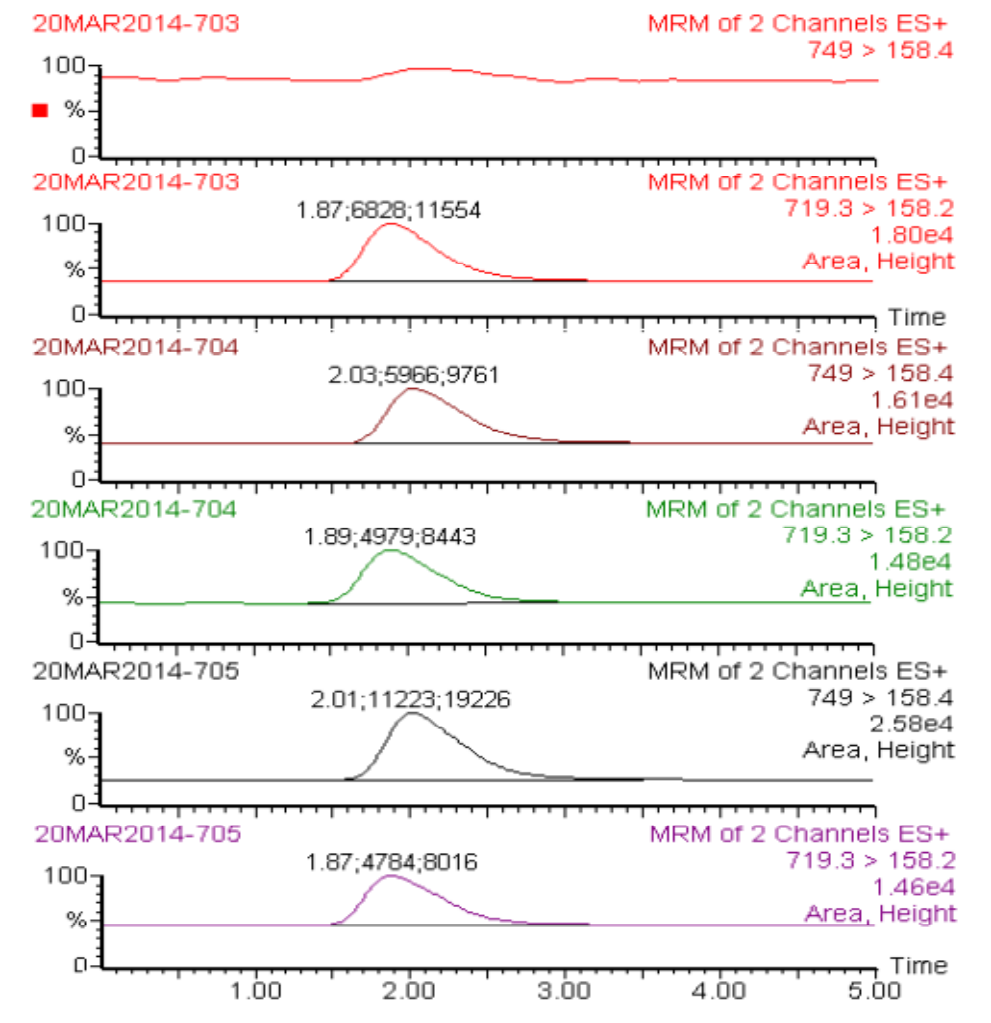
Robustness: Analyst

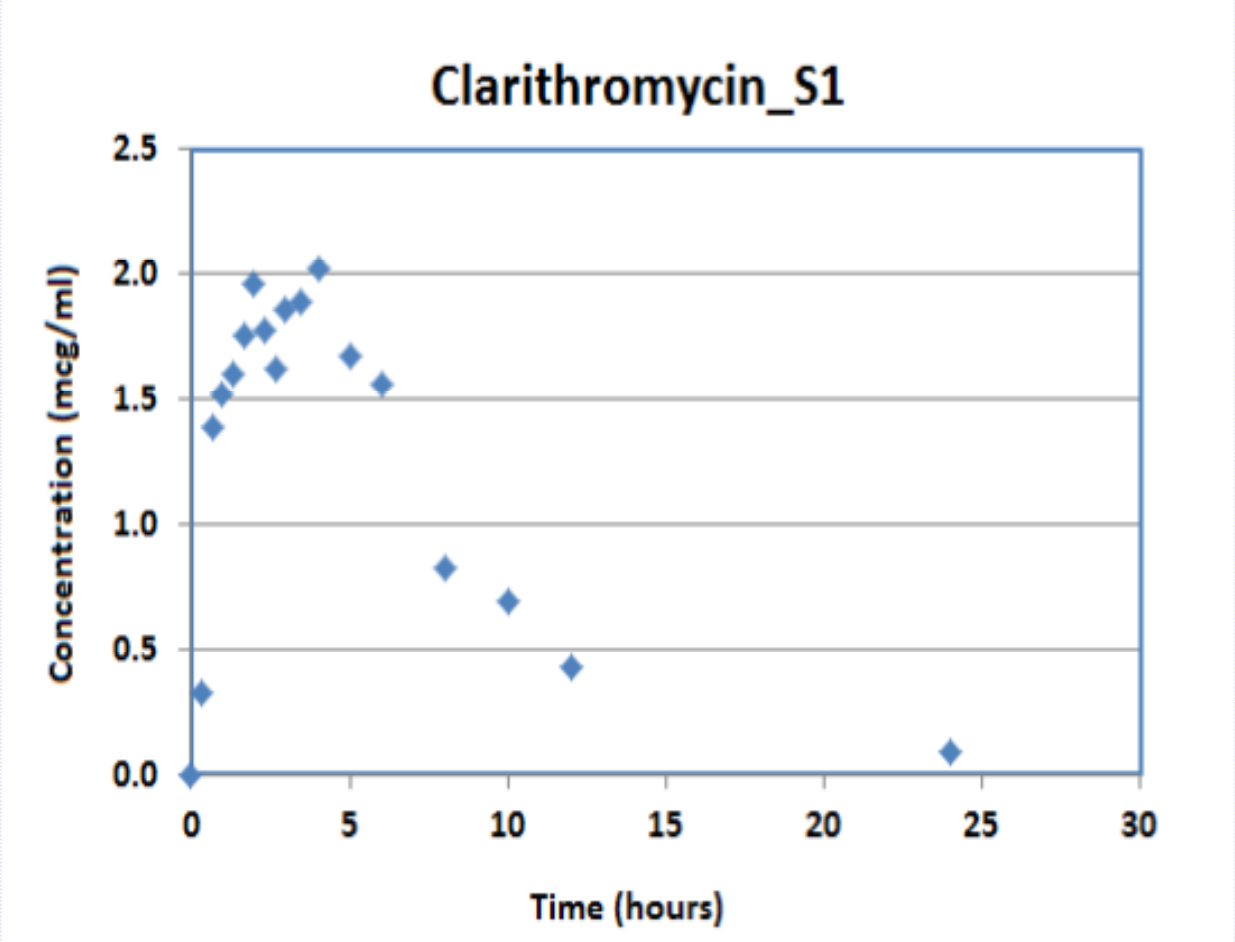
- Split Analysis

Method Application

- No. of Samples collected: 18 within 24 hrs.
- Processed: According to method
- Analyzed: LC-MS/MS

Typical MRM chromatograms of plasma sample obtained from healthy volunteer before and 1 & 2 hrs. after oral a single 500 mg Clarithromycin dose.




Clarithromycin_S1
Concentration (mcg/ml)
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
Time (hours)

Measured levels

- Samples collected from a health volunteer before and after ingestion of a single oral dose of 500 mg clarithromycin analyzed according method.

- Measured concentration:
  Range 0 - 2.03 µg/ml.

Conclusions

- A simple, precise, and accurate assay for the measurement of clarithromycin in human plasma was developed and fully validated.
- The assay was successfully applied to monitor stability of clarithromycin under various condition routinely encountered by the laboratory.
- The assay was applied to determine the level of clarithromycin in 0.2 ml plasma sample obtained from a healthy volunteer.

Acknowledgements

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Thanks for your Attention

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