### LC-MS/MS Method for Determination of Total Active Gentamicin in Brown Norway Rat Lung Tissue

#### **Authors:**

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#### Introduction:

- Genatmincin has four active components and belongs to a larger family of aminoglycoside antibiotics which are highly polar and water soluble
- Each of these active components needs to be measured and summed to provide an assessment of overall activity
- When delivered to the lung, the measurement of lung concentrations provides assurance that drug accumulation is limited but sufficient to overcome pulmonary infections
- Arobust liquid chromatographic-tandem mass spectrometric method has been established to quantify simultaneously all four active components of gentamincin in rat lung tissue. The method was validated over the linear range of 150 to 37,500 ng/g of total gentamicin
- The limit of detection was adequate to cover anticipated doses in toxicology studies; however, the assay can be extended by increasing sample size and using more sensitive instrumentation

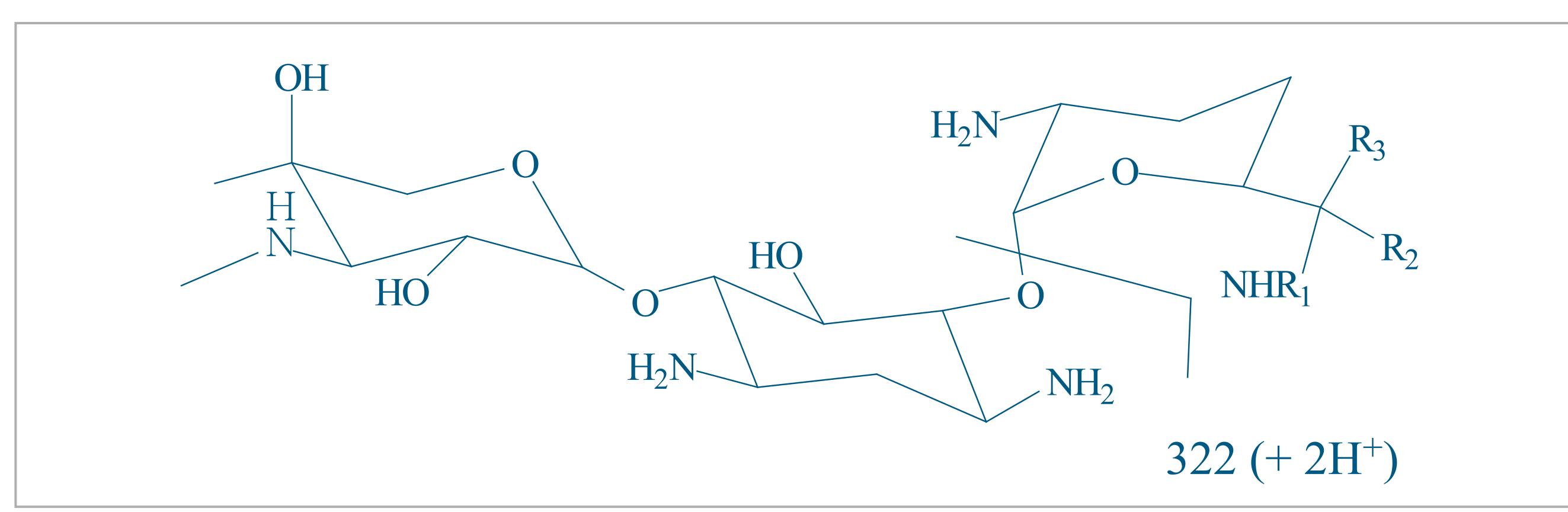


Fig. 1 Structure of gentamicin

#### Method and Experimental:

- Internal Standard: Gentamincin-d4 and Tobramycin
- Sample preparation: Lung tissue was homogenized with PBS buffer at the ratio of 1:4 (weight: volume)
- Extraction Principle: Homogenate was extracted by protein precipitation with trichloroacetic acid (TCA) followed with solid phase extraction
- (weak cation exchange)
- Liquid Chromatography:
- Autosampler: Perkin-Elmer Series 200
  Analytical Column: Discovery C18, 2x50 mm,
- Mobile Phase:
- A: 0.1% heptafluorobutyric acid in water
- B: 0.1% heptafluorobutyrci acid in acetonitrile
- MS/MS
- Sciex API 4000 with ESI (+) source
- MS/MS Transitions: Genatmicin: C1: 478→322
- C1a: 450→322 C2/C2a: 450→322
- The summation of all transitions was used for the quantitation of total gentamincin (Fig.1).

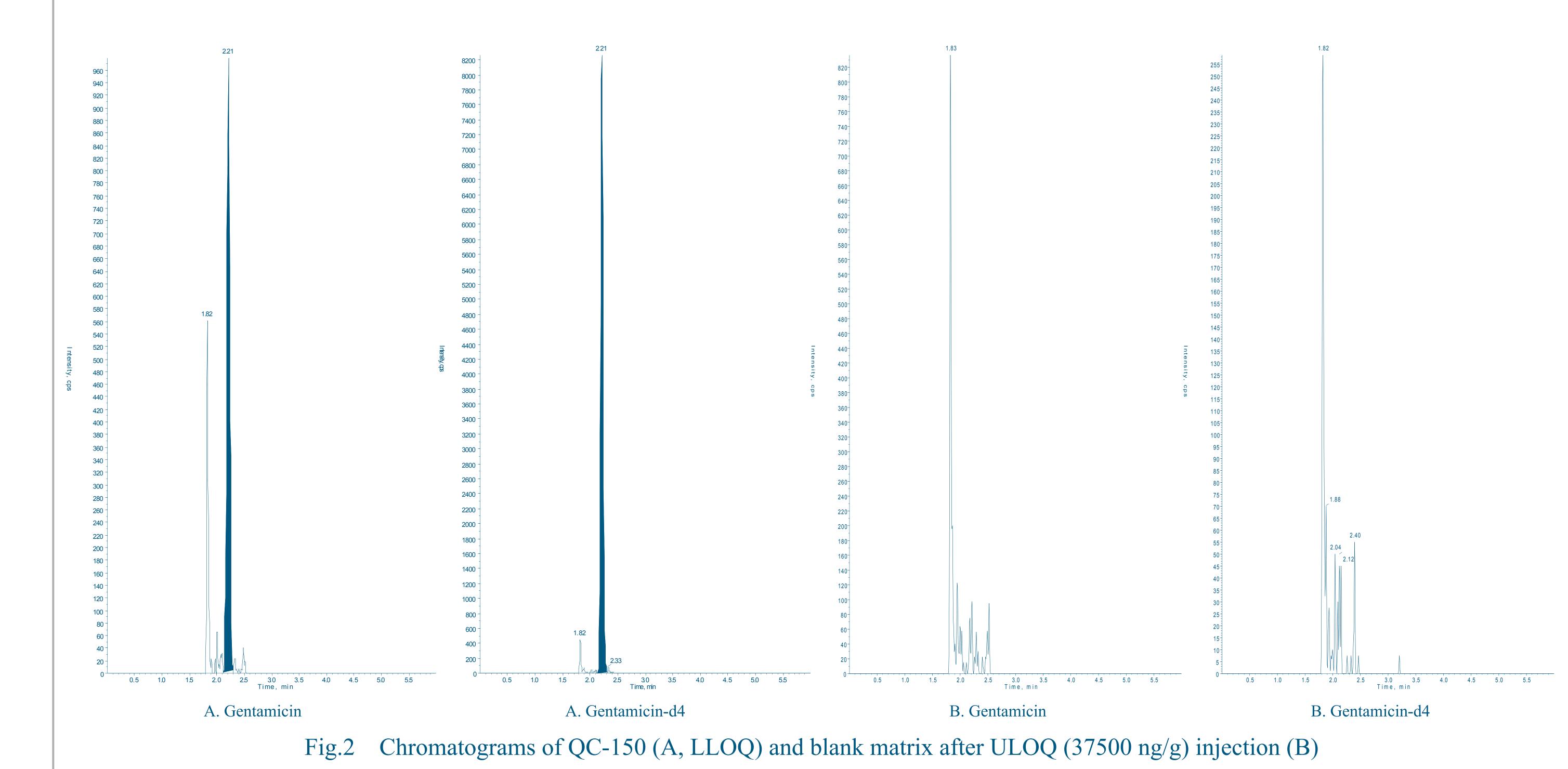
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#### **Extraction:**

- 50 μL of homogenate was precipitated with trichloroacetic acid

  Fig.3 Linear regress of the standard curve (150 ng/g to 37500 ng/g)
- Supernatant was load to an Oasis MCX SPE plate (Waters, 60mg).
- Gentamicin was eluted out with ammonium hydroxide methanol solution.
- The dried residue was reconstituted with mobile phase.

#### Representative Chromatograms:



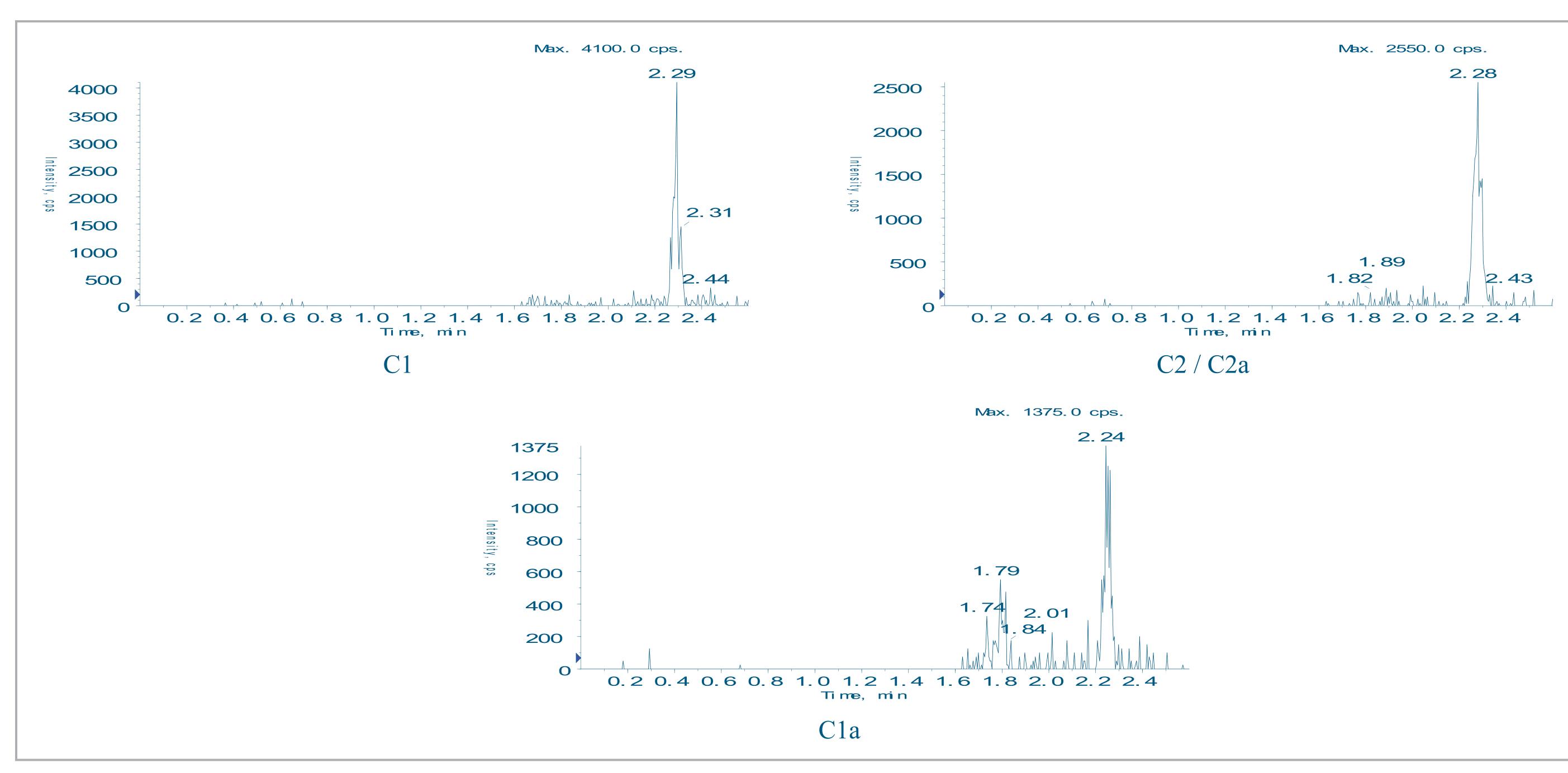


Fig.4 Chromatograms for gentamine (total) at 10 ng/g on the Sciex 5000 (Injection Volume: 15.0 μL; Extraction volume: 0.150 mL of homogenate)

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#### **Results:**

- Good sensitivity and was obtained for homogenate sample at 150 ng/g (Fig.2)
- Excellent linearity within the range of 150 ng/g to 37500 ng/g and the good accuracy and precision data were obtained in this study. (Table 1, Fig.3)
- Severe matrix effect was observed if only TCA precipitation or MCX SPE was used. The two-stage extraction minimized the matrix impact significantly. Additionally, the using of deuterated gentamicin leaded to much better results comparing with traditional tobramycin internal standard.
- The extraction recovery was about 80% over the calibration range. (Table 2)
- No significant carryover was observed in chromatograms. (Fig.2)
- The detection limit was extended to 10 ng/g when a Sciex API 5000 mass spectrometer was used. (Fig. 4).
- Similar results were obtained if APCI (+) ionization was used. Therefore, it could be used instead if additional interferences are detected.
- Lab automation had been used for many extraction steps which improved the production throughput.

Sample Name*	Conc. (ng/g)	Day 1		Day 2		Day 3	
		%CV	Accuracy	%CV	Accuracy	%CV	Accuracy
QC-150	150	8.94	111	8.45	105	6.80	99.7
QC-450	450	4.80	100	8.51	100	8.04	98.0
QC-4500	4500	5.41	100	6.44	105	8.08	98.8
QC-30000	30000	4.44	96.9	2.80	91.9	6.60	93.1

#### Table 2: Results from Stability and Extraction Recovery Tests

Sample Name	Concentration (ng/g)	%CV	Accuracy
QC-450 (8-h rt)	450	3.85	91.5
QC-450 (3-F/T cycles)	450	11.4	95.4
		Recovery (%)	
QC-450	450	78.3	
QC-4500	4500	82.0	
QC-30000	30000	86.8	

#### Conclusion:

- A robust and sensitive biaoanalytical method had been developed for total gentamicin measurement from rat lung tissue.
- The detection limit of quantitation can be modified easily by using different simple size and instrument.
- The similar methodology can be applied to the analysis of lung tissue containing other aminoglycoside antibiotics.

#### References

- 1. D. Heller; J. Peggins; C. Nochetto; M. Smith; O. Chiesa and K. Moulton; Journal of Chromatography B; 821(2005)22-30
- 2. J. Zimmer, S. Needham; J. McKinnel, B. Cass and D. Karr, Poster Presentation, ASMS 2008.