GLYCOPYRROLATE (ROBINUL) FROM EQUINE URINE BY LC-MSMS USING: 500 mg CLEAN-UP® CCX2 EXTRACTION COLUMN

Part #:

CUCCX25Z – CLEAN-UP® CCX2 500 mg, 10 mL Tube

1. SAMPLE PREPARATION

Buffer 5 mL of urine to pH 7.0 by adding 3 mL of 100 mM phosphate buffer (pH 7.0).

Add (12.5 ng) of mepenzolate (internal standard).

Add 5 mL of H_2O to the sample.

Vortex or shake thoroughly.

Centrifuge for 5 min at 800 rpm.

2. CONDITION CLEAN-UP® EXTRACTION COLUMN

1 x 3 mL CH_3OH.

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM phosphate buffer (pH 7.0).

3. APPLY SAMPLE

Decant supernatant onto SPE column.

Load at 1 to 2 mL / min.

4. WASH COLUMN

5 mL of CH_3OH.

5 mL of D.I. H_2O.

Dry column (5 min > 10 inches Hg).

5. ELUTE GLYCOPYRROLATE

1 x 4 mL CH_3OH /0.5 M Ammonium Acetate buffer, pH 3.0 (95:5).

6. DRY ELUTE

Evaporate to dryness at 60 °C.

Reconstitute with 100 µL CH_3OH.

7. QUANTITATE

Inject 10 µL onto HPLC.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM

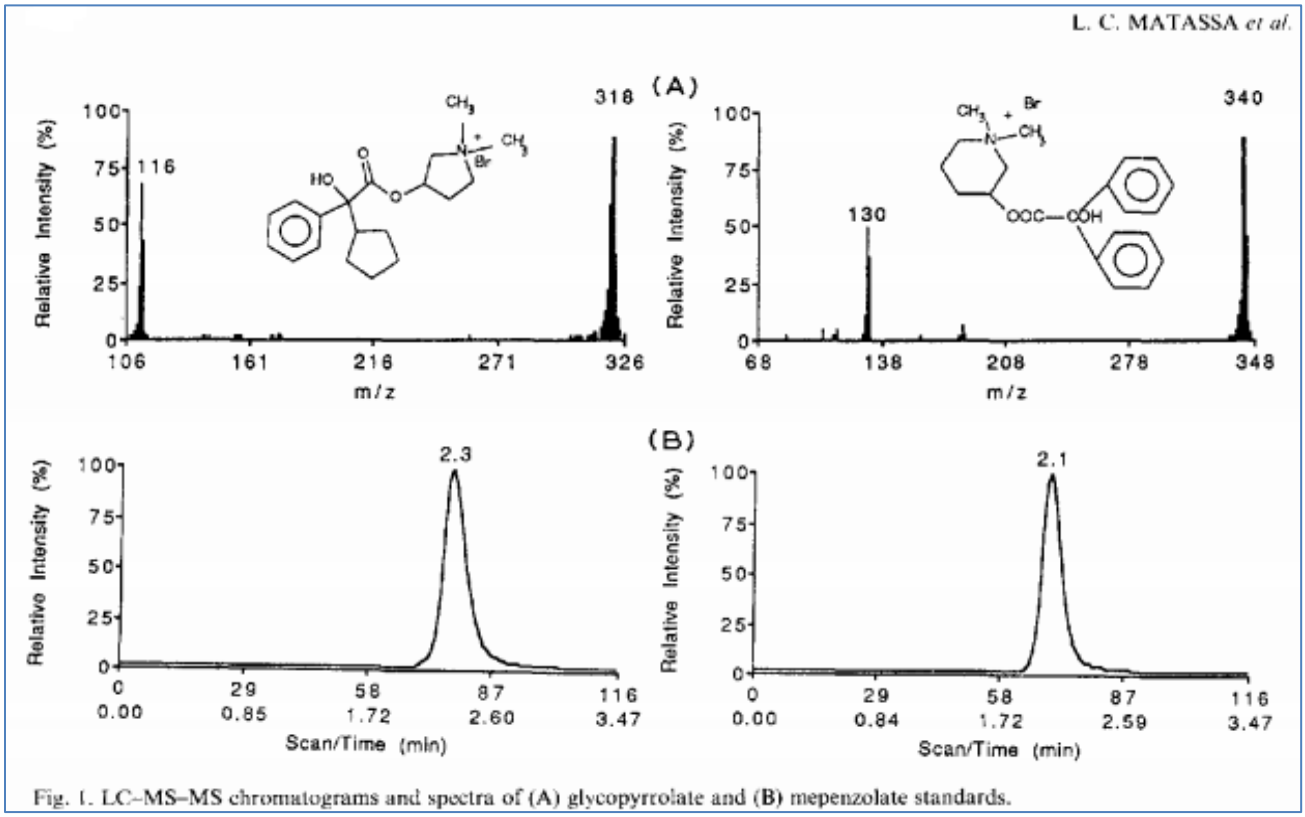

Fig. 1. LC–MS–MS chromatograms and spectra of (A) glycopyrrolate and (B) mepenzolate standards.

Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Glycopyrrolate	318	116	2.3
2. Mepenzolate (IS)	340	130	2.1

PARAMETERS

Mobile Phase A: Methanol

Mobile Phase B: 50mM Ammonium Acetate pH 3.0

Flow Rate: 0.8mL/minute

Polarity: Positive

Injection Volume: 10µl

LC Column: Hamilton PRP-1, 150 mm x 4.1 mm I.D. 5µm

Instrument: Sciex Model API III with Applied Biosystems 140A solvent delivery system

Isocratic Flow:

Time	%A	%B
0.00	80	20
10.0	STOP	

Reference: Matassa, L.C.et al. Solid-phase extraction techniques for the determination glycopyrrolate from equine urine by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry; Journal of Chromatography, 573 (1992) 43-48.