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Advanced Separation Technologies Inc.

770-9P

Sensitivity and Selectivity - A Case Study of
LC/MS Enantioselective Resolution of Bupivacaine
Using Vancomycin as a Chiral Stationary Phase

J.T. Lee¹ Maria Esther Rodriguez Rosas² and Thomas E. Beesley¹

1 Advanced Separation Technologies Inc, Whippany, NJ, USA.

**2 Gerontology Research Center, National Institute on Aging, National Institutes of Health,
Baltimore, MD, USA.**

Website: www.astecusa.com; Email: info@astecusa.com

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Abstract

Racemic bupivacaine currently is the most widely used long-acting local anesthetic. However, numerous studies have shown that in most cases the levobupivacaine is less toxic. This study is to evaluate the sensitivity and selectivity issue of enantiomeric separation of this substance in biological matrix by various mobile phase designs on LC/MS platforms.

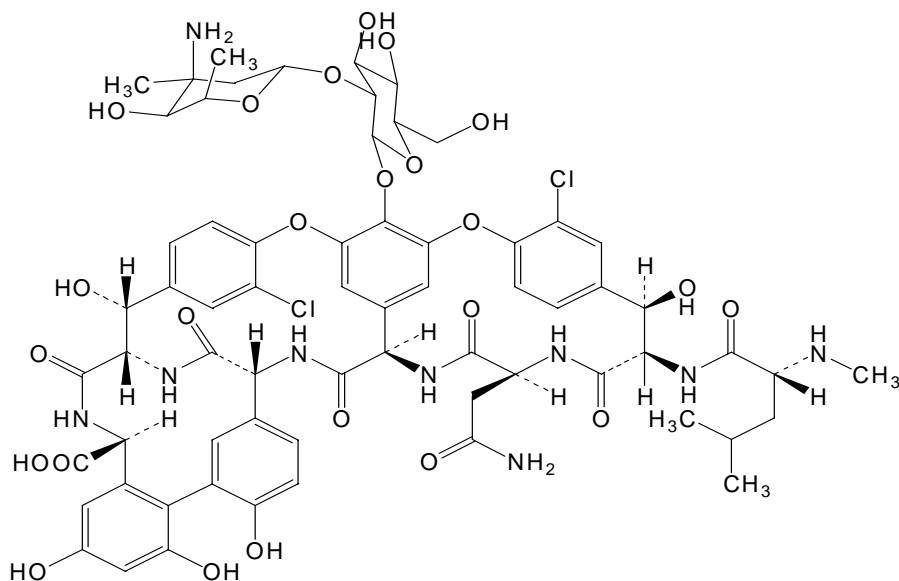
Enantioselective resolution of bupivacaine can be easily achieved by CHIROBIOTIC V and CHIROBIOTIC V2 columns in either polar ionic mode or reversed phase mode that is very MS-friendly. The polar ionic mode is composed of pure MeOH with small addition of acid and base or volatile salts while reversed phase mode contains a mixture of organic solvents and pH-adjusted buffer with volatile salts. The composition of mobile phase with the use of ESI mode dictates the outcomes of sensitivity and selectivity. Furthermore, the column dimensions will have major impacts on the resolution and the overall throughput.

Excellent linear relationships based on the peak heights were obtained in the range of 0.15ng-50ng/mL for each enantiomer standard with correlation coefficients greater than 0.999.

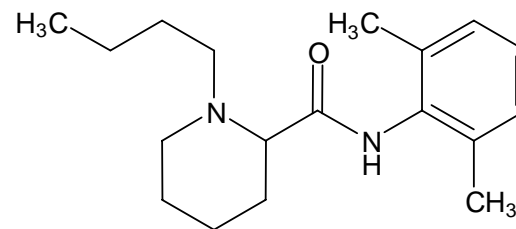
This presentation will cover several key variables including mobile phase compositions, flow rate, and finally the column dimensions that will affect the overall performance. Since Macrocylic glycopeptides chiral stationary phases have demonstrated to be highly robust and versatile in chiral assay on LC/MS platforms, the findings will be beneficial to pharmaceutical and biotech industries in the area of drug absorption, metabolism and pharmacokinetic studies.

Structure of Chiral Stationary Phase and Target Molecule

Vancomycin



Bupivacaine



Apparatus

The analytical system consisted of a Series 1100 Liquid Chromatography/Mass Selective Detector, LC/MSD (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser (G1379 A), a quaternary pump (1311 A), a thermostated autosampler (G1329 A), and a thermostated column compartment (G1316 A). The mass selective detector (MSD Quad SL, G1956 B) was equipped with atmospheric pressure ionization electrospray (API-ES, G2908 B) or atmospheric pressure chemical ionization (APCI, G1947 A) and an on-line nitrogen generation system (Whatman, Haverhill, MA, USA). The chromatographic system was interfaced to a 2.8 GHz HP Compaq computer (Hewlett-Packard, Palo Alto, CA, USA) running ChemStation software (Rev A.10.01[1635], 1990-2003, Hewlett-Packard) under Microsoft Windows XP.

Best HPLC Condition

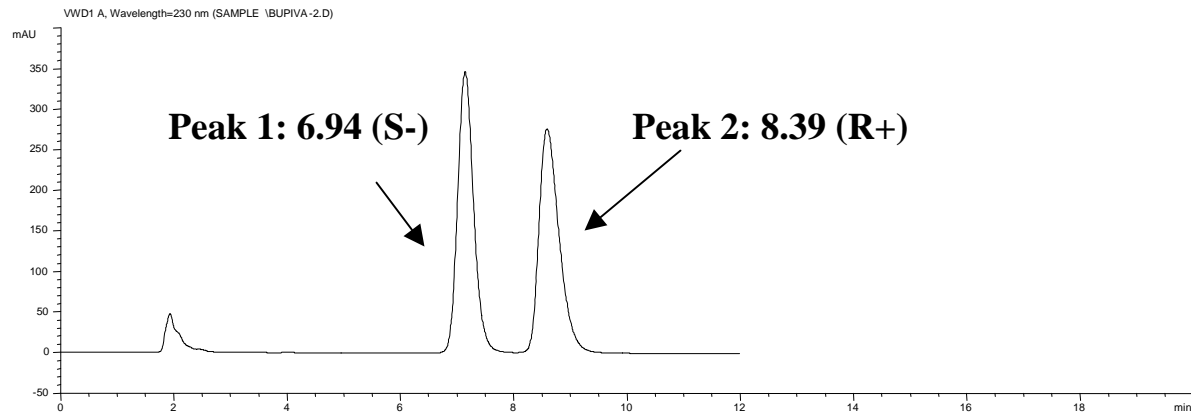
STD Bupivacaine 1mg/mL; Inj. Vol. : 2 μ L

Column: **CHIROBIOTC V2**, 150x2.1mm 5 μ

Mobile Phase: 90/10, MeOH/10mMNH₄OAc, pH 4.1

Flow Rate: 0.2 mL/min

UV: 230 nm



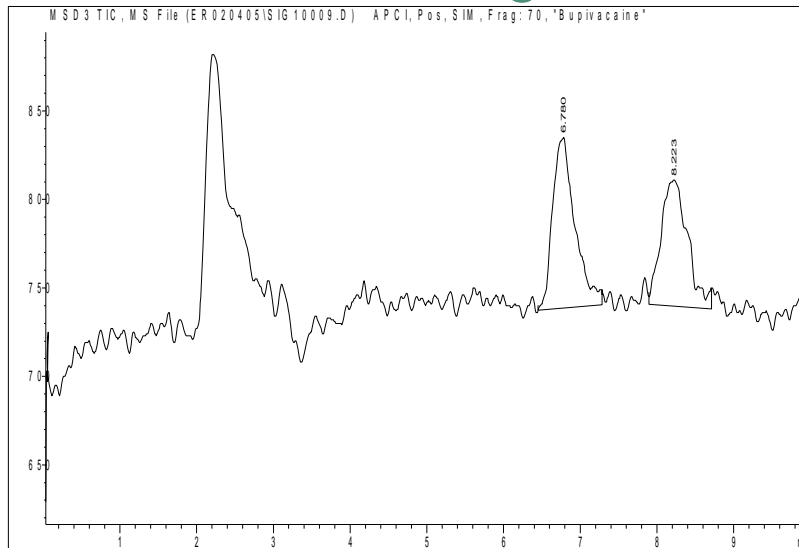
Sensitivity Comparison

APCI vs. ESI

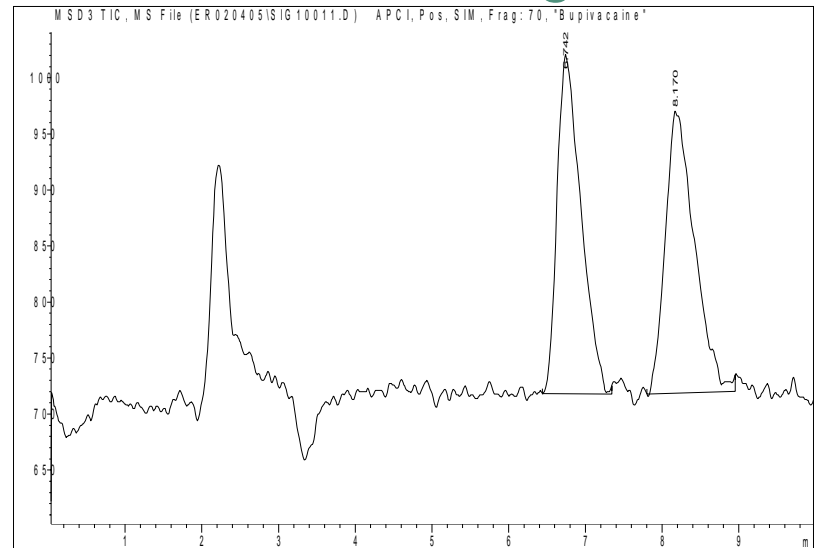
APCI mode

Corona Current: 3.5 V; Vaporizer Temperature: 350°C Fragmentor: 70;
Capillary Voltage: 1750 V; Drying Gas Temperature: 350°C
Drying Gas Flow: 7 L/min.; Nebulizer Pressure: 20 psig

LOD 0.06ng/mL



LLOQ 0.25ng/mL



- Column: **CHIROBIOTIC V2**, 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min

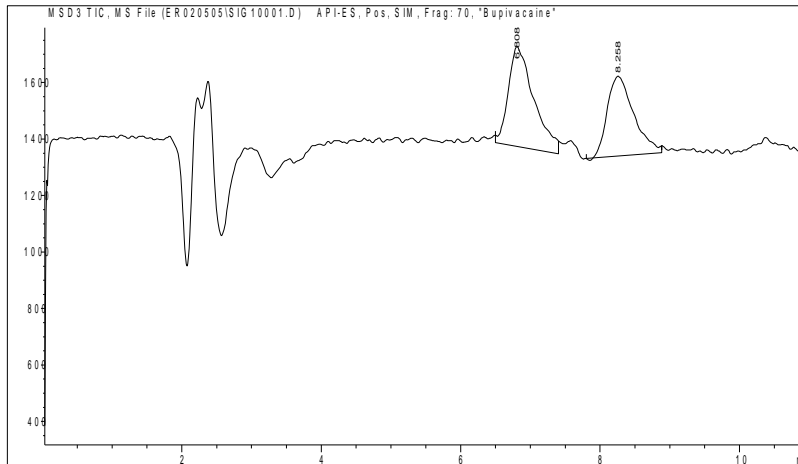
Sensitivity Comparison

APCI vs. ESI

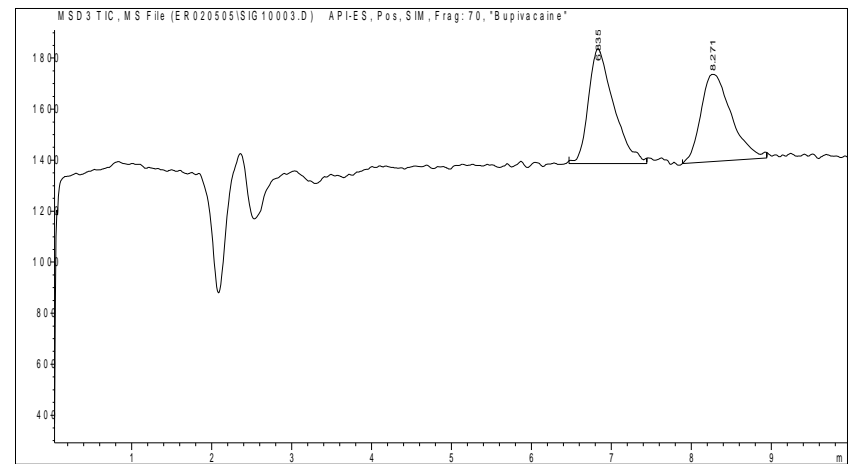
ESI mode

Fragmentor: 70; Capillary Voltage: 1750 V
Drying Gas Flow: 7 L/min; Nebulizer Pressure: 20 psig
Drying Gas Temperature: 350°C

LOD 0.015ng/mL



LLOQ 0.062ng/mL



- Column: **CHIROBIOTIC V2**, 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min

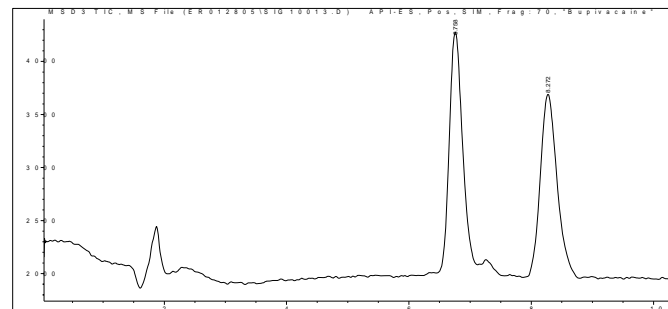
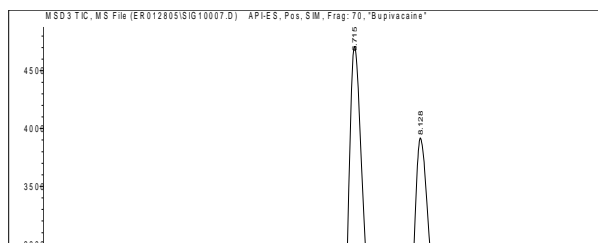
Sensitivity Comparison

Dimension Issue

- Column: CHIROBIOTIC V2, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Sample: 1ng/mL, 5 μ L inj

150x2.1 mm @ 0.2mL/min

150x4.6mm @ 1.0mL/min

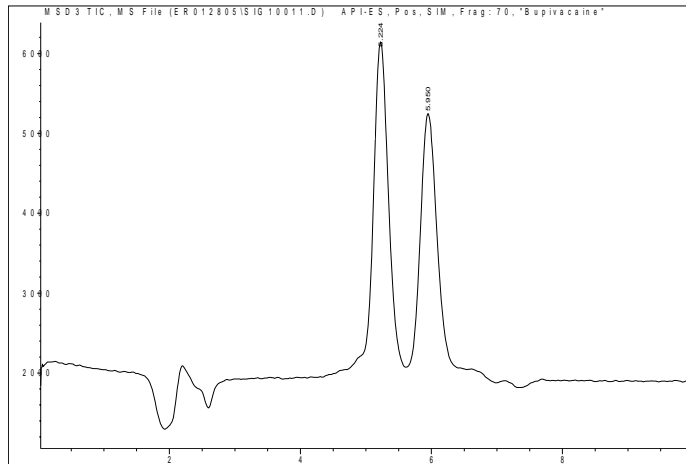


Sensitivity Comparison

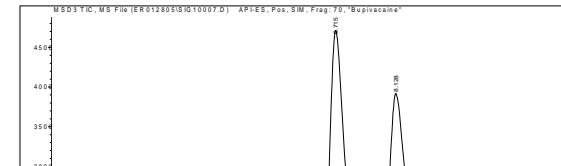
CHIROBIOTIC V2 vs.V

- Column: 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Sample: 1ng/mL, 5 μ L inj
- Flow Rate: 0.2 mL/min

CHIROBIOTIC V



CHIROBIOTIC V2



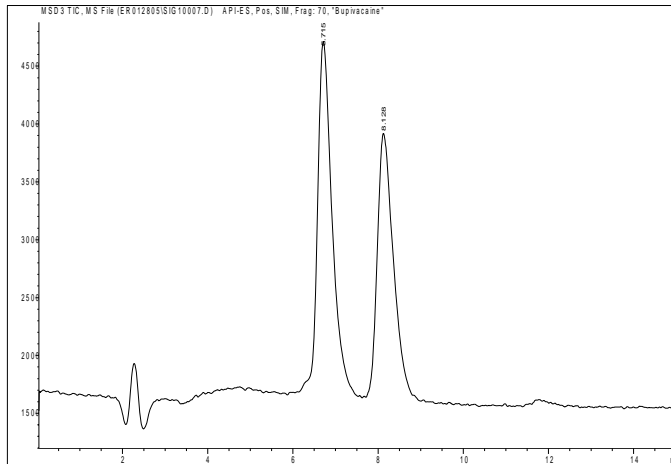
Selectivity Comparison

CHIROBIOTIC V2 vs. V

- Column: 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min

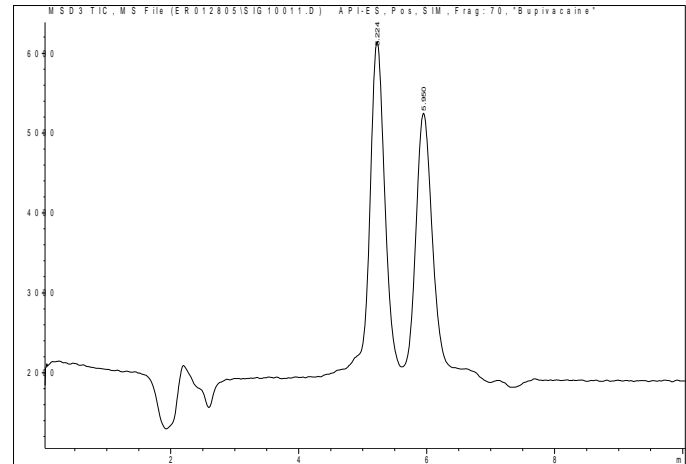
CHIROBIOTIC V2

$$\alpha = 1.30$$



CHIROBIOTIC V

$$\alpha = 1.21$$

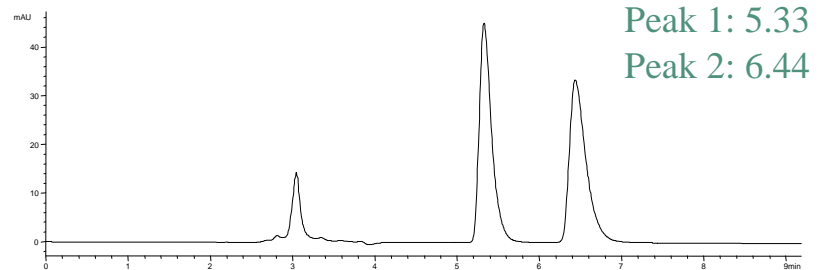


Polar Ionic Mode –Salt Effects

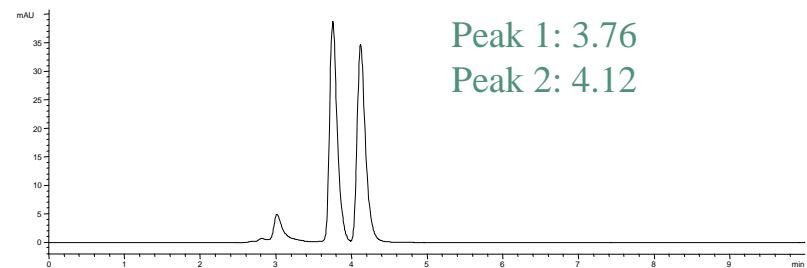
CHIROBIOTIC V2

- **Sample:** Bupivacaine
- **Column:** 250x4.6 mm

- **Mobile Phase:** 100/0.1w%, MeOH/NH₄TFA
- **Flow Rate:** 1 mL/min
- $\alpha = 1.44$



- **Mobile Phase:** 100/0.1w%, MeOH/NH₄Formate
- **Flow Rate:** 1 mL/min
- $\alpha = 1.37$

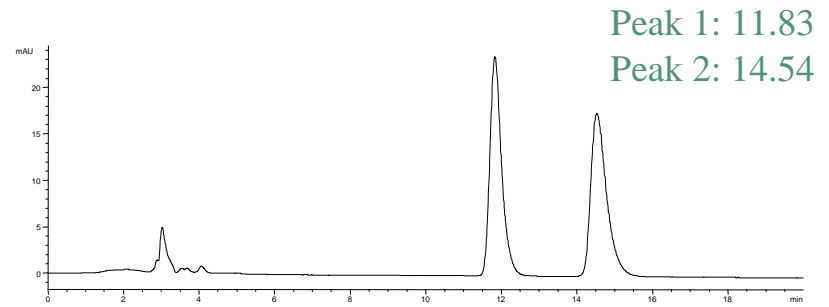


Reversed Phase Mode-1

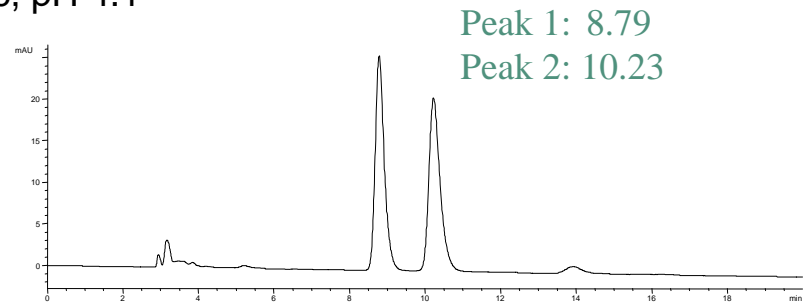
CHIROBIOTIC V2

- **Sample:** Bupivacaine
- **Column:** 250x4.6mm

- **Mobile Phase:** 90/10, MeOH/10mM NH₄OAc, pH 4.1
- **Flow Rate:** 1 mL/min
- $\alpha = 1.30$



- **Mobile Phase:** 80/20, MeOH/20mM NH₄OAc, pH 4.1
- **Flow Rate:** 1 mL/min
- $\alpha = 1.24$

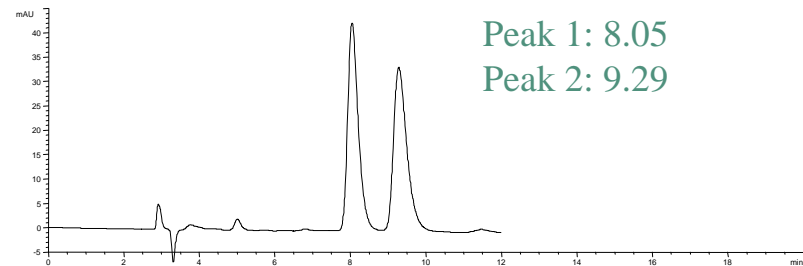


Reversed Phase Mode-2

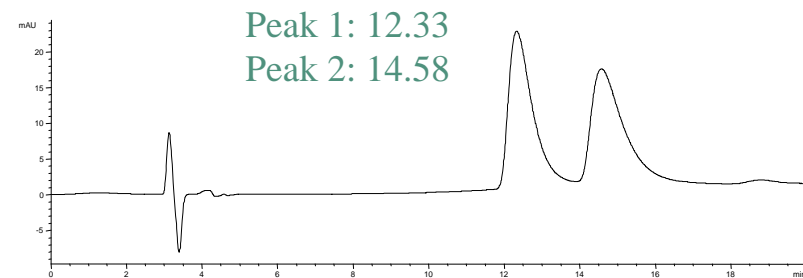
CHIROBIOTIC V2

- **Sample :** Bupivacaine
- **Column:** 250x4.6mm

- **Mobile Phase:** 50/50, MeOH/10mM NH₄OAc, pH 4.1
- **Flow Rate:** 1 mL/min
- $\alpha = 1.24$

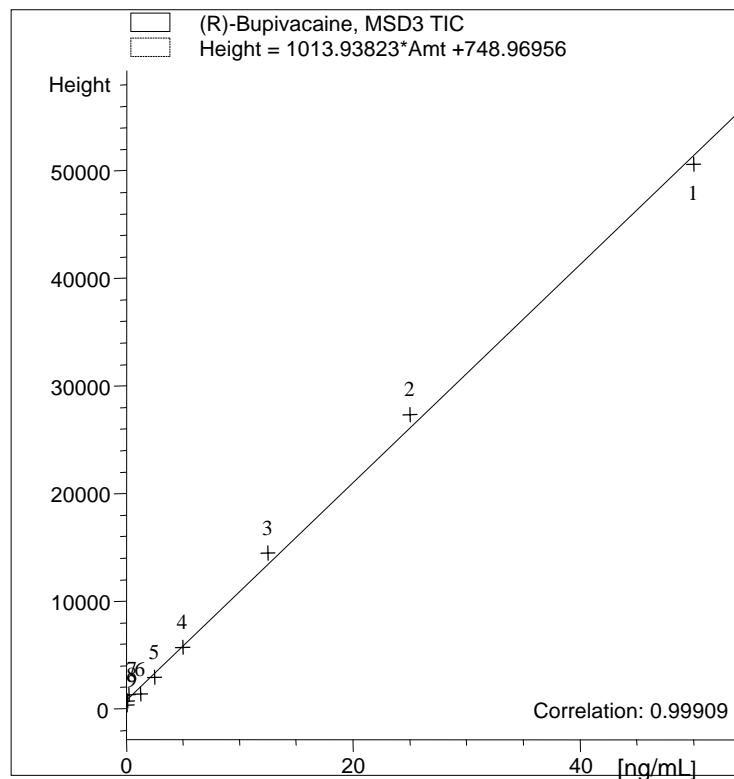
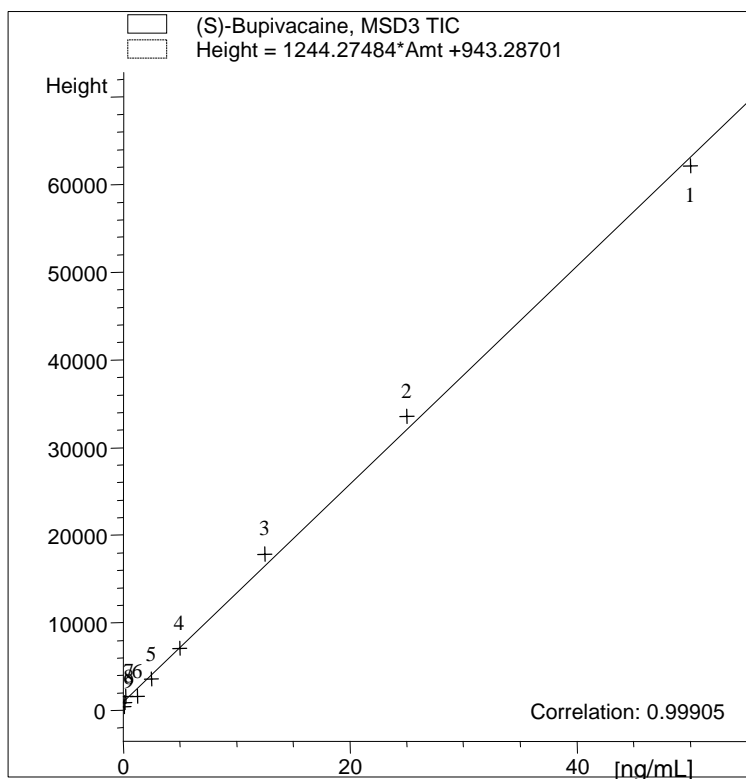


- **Mobile Phase:** 25/75, MeOH/10mM NH₄OAc, pH 4.1
- **Flow Rate:** 1 mL/min
- $\alpha = 1.24$



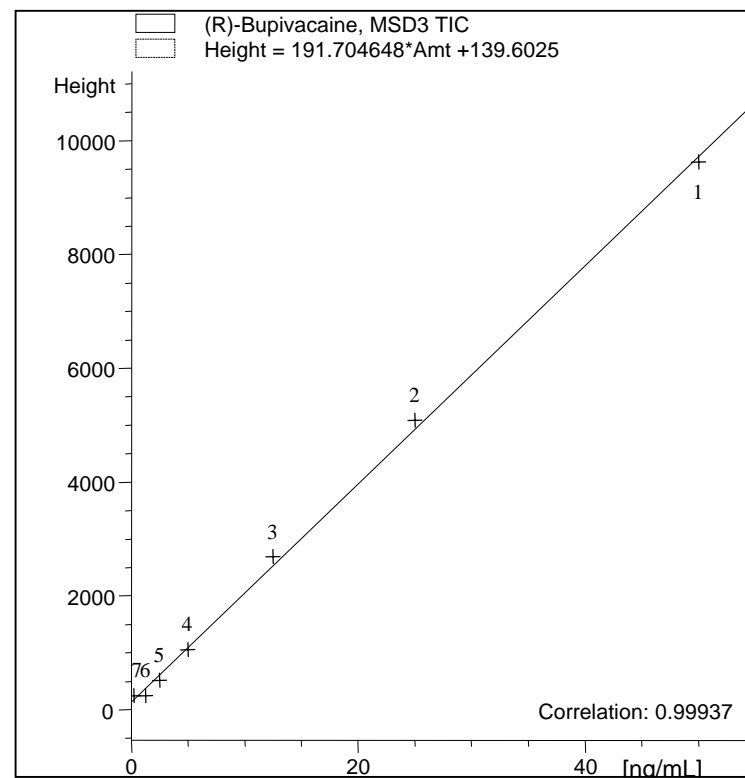
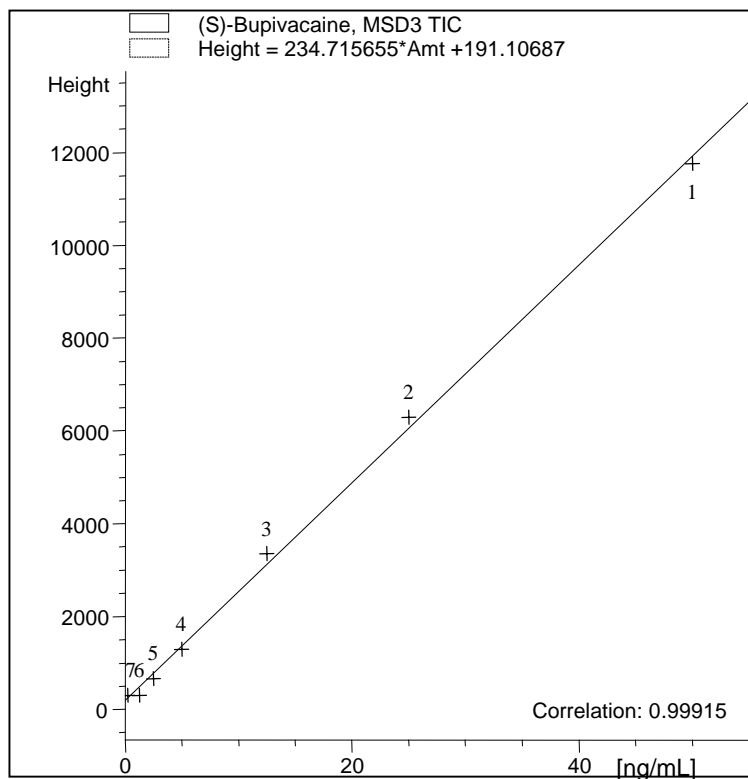
Calibration Curves for Std Bupivacaine Solutions-ESI

- Column: CHIROBIOTIC V2, 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min



Calibration Curves for Std Bupivacaine Solutions-APCI

- Column: CHIROBIOTIC V2, 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min



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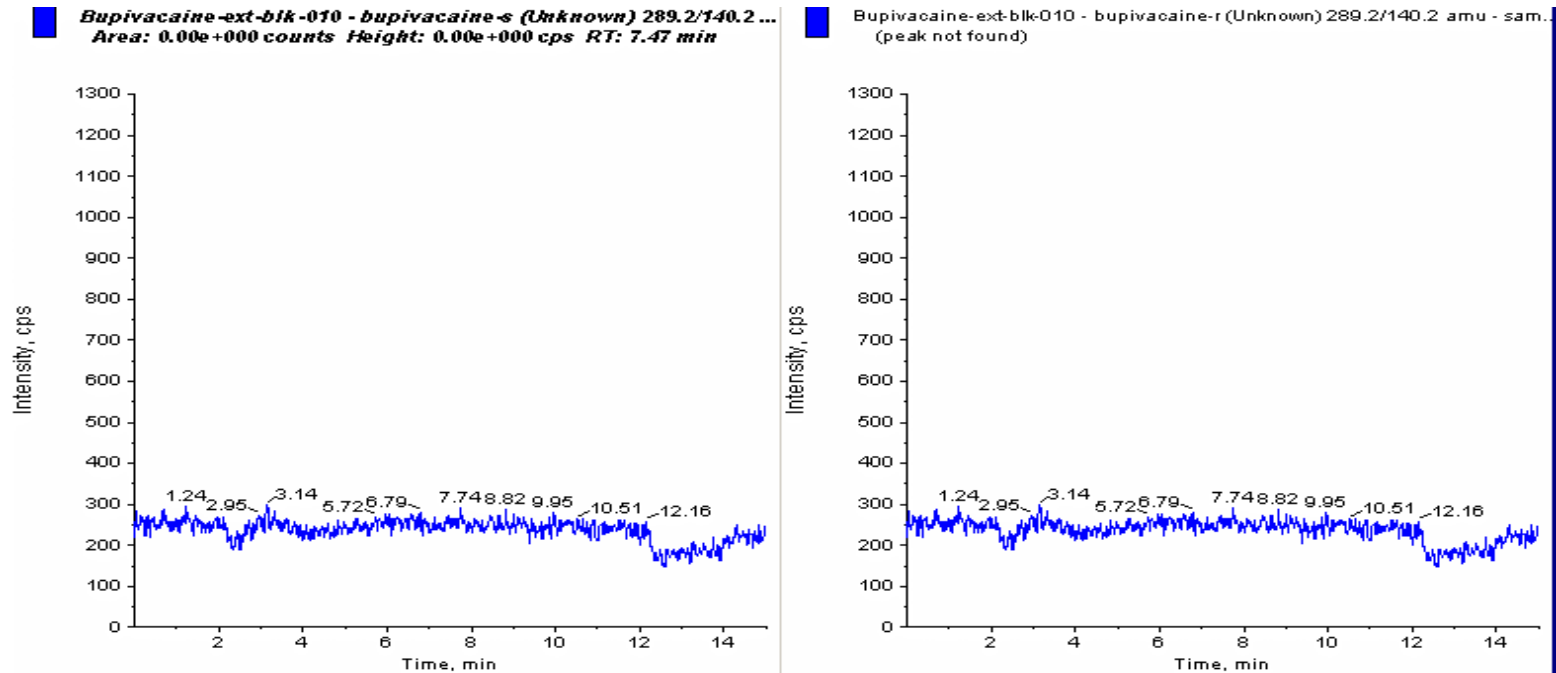
SPE Extraction Method

(Human Plasma Matrix)

- Transfer a 0.5 mL aliquot of sample into a 96-deep-well (2-mL) plate.
- Using a repeater pipette, add 500 μ L of 0.1 M acetic acid in water to each sample. Cover the plate and vortex briefly.
- Load the sample plate on the Tomtec and follow the SPE procedures below.
 - Precondition the SPEC. PLUS.96-Well SCX (Ansys, Cat. No. 598-04) with 0.3 mL of methanol.
 - Precondition with 0.3 mL of 0.1 M acetic acid in water
 - Load the sample on the SPE plate slowly
 - Wash the SPE plate with 0.3 mL of 0.1 M acetic acid in water: methanol (50:50) twice.
 - Elute the analyte with 0.2 mL of 2% ammonium hydroxide in methanol (2:98) twice (collect the eluate with a round deep well 1-mL 96-well plate).
- Evaporate the eluate at 40°C for 20-30 min to dryness.
- Reconstitute residue in 0.5 mL of 70:30 methanol/10 mM ammonium acetate, pH 4.1. Cover plate and vortex for ~50 seconds.
- Inject 10 μ L into LC/MS/MS system.

Blank in Human Plasma

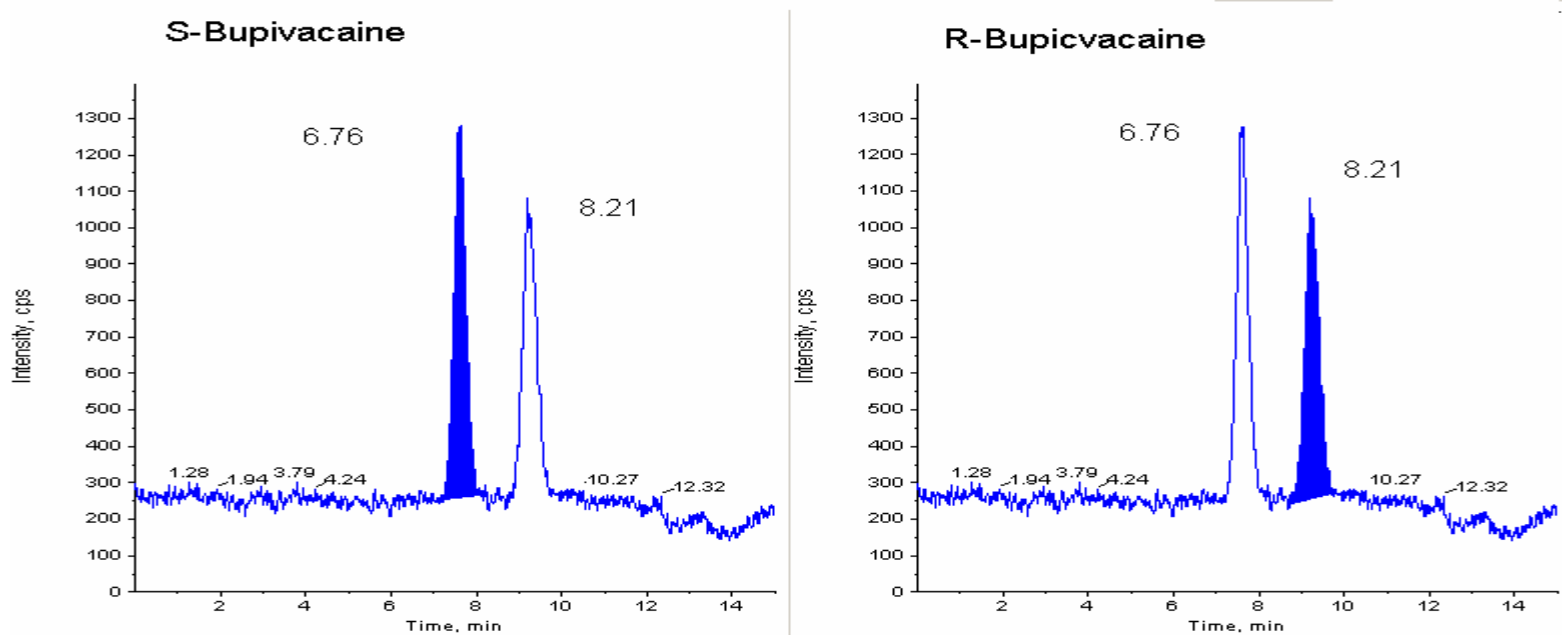
- Column: CHIROBIOTIC V2, 150x2.1 mm, 5 μ
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min



Bupivacaine by LC/MS-ESI

- Column: CHIROBIOTIC V2, 150x2.1 mm
- Mobile Phase: 90/10, MeOH/10mM NH₄OAc, pH 4.1
- Flow Rate: 0.2 mL/min

Bupivacaine in plasma



Summary

Sensitivity (Ratio):

LC/MS Mode	ESI:APCI	=	4.0:1.0
Column ID (mm)	2.1: 4.6	=	1.5:1.0
CHIROBIOTIC	V:V2	=	1.3:1.0

Selectivity (α):

CHIROBIOTIC	V2:V	=	1.30: 1.21
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Calibration:

Excellent Correlation Coefficients	>	0.999
Linear Range	=	0.15ng-50ng/mL

Conclusions

- CHIROBIOTIC V2 and V showed exceptional selectivity towards basic drugs, and they are complementary in terms of sensitivity and selectivity.
- Unique multi-modal characteristics from 100% organic to high aqueous can be utilized to obtain best separation needs.
- Unique mobile phase designs can be tailored on LC/MS platforms in biological matrix for both sensitivity and selectivity issues.
- Unique ionic interaction between CSP and analyte is key to successful chiral recognition.