

APPLICATION REPORT

LC005

LC-MS and Astec Products

Since 1996, the LC-MS platform has gained increasing status as an analytical and developmental tool especially within the pharmaceutical industry. To date, of all the publications relating to chiral separations utilizing this technique, six papers have appeared applying Chiral Technologies products, one applying our CYCLOBOND and twelve citing our CHIROBIOTIC phases. Of particular interest is the combination of our column coupling technology with MS and tandem MS as the detector for both qualitative and quantitative analysis. Please refer to CHIROBIOTIC bibliography references 30 and 57.

One of the reasons that the CHIROBIOTIC phases have gained so much interest has to do with the success of the polar organic mode as a simple, effective mobile phase that results in an easy to validate method. CHIROBIOTIC phases avoid the use of inorganic buffers and rarely require the use of normal phase solvents like hexane. Instead, a typical mobile phase would be methanol with low concentrations (0.1– 0.001%, v/wt) of volatile salts like ammonium trifluoroacetate, ammonium formate or ammonium acetate, enhancing MS detection. Speed of analysis is another very favorable factor, especially when using the polar organic mode. Further, CHIROBIOTIC technology allows for the use of any solvent compatible with MS even halogenated solvents, which is not possible with competitive products.

We present below selected data, abstracted from the LC-MS publications in the CHIROBIOTIC bibliography, presented under appropriate summary headings.

Papers focusing on method development techniques:

Reference 28:

High-Throughput Chiral Liquid Chromatography/Tandem Mass Spectrometry. Bakhtiar, R., Tse, F.L.S, Rapid Commun. Mass Spectrom., 14, 1128 – 1135 (2000).

Analytes:	Fluoxetine, metoprolol, nicardipine, oxazepam, pindolol, propranolol, ritalinic acid, terbutaline
Columns:	CHIROBIOTIC V and T, 150 x 4.6mm
Mobile Phase:	Methanol/0.1% ammonium trifluoroacetate, v/wt

Reference 30:

Use of Atmospheric Pressure Ionization Mass Spectrometry in Enantioselective Liquid Chromatography. Bakhtiar, R., Ramos, L., Tse, F.L.S., Chirality 13, 63-74 (2001).

Analytes:	Threo-methylphenidate, methylphenidate, oxazepam, propranolol, pindolol, terbutaline
Columns:	CHIROBIOTIC V+R+T, 100 x 4.6mm
Mobile Phase:	Methanol/0.1% ammonium trifluoroacetate, v/wt
Detection:	2.61 pg/mL methylphenidate and ritalinic acid

Reference 115:

Chiral liquid chromatography-tandem mass spectrometric methods for stereoisomeric pharmaceutical determinations, Chen J., Korfmacher W.A., Hsieh Y., J. of Chrom. B., 820, 1-8 (2005).

Analytes:	Drug Discovery
Columns:	CHIROBIOTIC V & T
Mobile Phase:	Methanol/0.1% ammonium trifluoroacetate, v/wt.

Reference 117:

Evaluation of ethoxynonafluorobutane as a safe and environmentally friendly solvent for chiral normal-phase LC-atmospheric pressure chemical ionization/electrospray ionization-mass spectrometry, Ding J., Desai M., Armstrong D.W., J. of Chrom. A, 1076, 34-43 (2005).

Analytes:	15 drug racemates
Columns:	CHIROBIOTIC V & T
Mobile Phase:	Ethoxynonafluorobutane/EtOH vs heptane/EtOH

Note: reference 57 also an excellent paper for method development techniques.

Papers to review for nutraceuticals:

Reference 36:

Chiral Speciation and Determination of Selenomethionine Enantiomers in Selenized Yeast by HPLC-ICP-MS Using a Teicoplanin-based Chiral Stationary Phase. Mendez, S.P., Gonzalez, E.B., Medel, A.S., J. Anal. At. Spectrom. 15, 1109-1114 (2000).

Analytes:	Selenomethionine and selenethionine
Sample Prep:	Enzymatic hydrolysis/aqueous extraction
Column:	CHIROBIOTIC T, 250 x 4.6mm
Mobile Phase:	Methanol/water: 98/2
Flow Rate:	1.0 mL/min
Analysis Time:	Under 9 minutes
Detection:	0.8 µg/mL as selenium, Sensitivity 26x > crown ether

Reference 79:

Hybridation of Different Chiral Separation Techniques with ICP-MS Detection for the Separation and Determination of Selenomethionine enantiomers: Chiral Speciation of Selenized Yeast. S.P. Mendez, E.B. Gonzales, A. Sanz-Medel, Biomed. Chrom., 15, 181-188 (2001)

Analytes:	D and L selenomethionine
Sample Prep:	Enzymatic hydrolysis, filtration
Column:	CHIROBIOTIC T (10µm), 250x4.6mm
Mobile Phase:	2% Methanol in water v,v
Flow Rate:	1.0 mL/min.
Analysis Time:	7 min.
Detection:	0.8 µg/L (as Se)

Reference 103:

Analysis of Derivatized and Underivatized Theanine Enantiomers by High-Performance Liquid Chromatography/Atmospheric Pressure Ionization-Mass Spectrometry, M.J. Desai, D.W. Armstrong, Rapid Comm. Mass Spectrom., 18, 251-256 (2004)

Analyte:	Underivatized/derivatized Theanine
Sample Prep:	Standards and samples, without or with derivatization
Column:	CHIROBIOTIC T, 250x4.6mm
Mobile Phase:	
Flow Rate:	0.4 or 0.8 mL/min.
Analysis Time:	Underivatized, 10 min.; Derivatized, up to 40 min.
Detection:	10 ng/mL

Reference 107:

Comparative study of the instrumental couplings of high performance liquid chromatography with microwave-assisted digestion hydride generation atomic fluorescence spectrometry and inductively coupled plasma mass spectrometry for chiral speciation of selenomethionine in breast and formula milk, J.L.Gomez, V.Bernal-Daza, M.J.Villegas-Portero, Analytica Chima Acta, 520, 229-235, (2004)

Analyte:	D and L selenomethionine
Sample Prep:	precipitation, conc. SCX
Column:	CHIROBIOTIC T,(10u) 250 X 4.6mm
Mobile Phase:	Milli-Q water
Flow Rate:	1.0 mL/min
Analysis:	9 min
Detection limits:	3.1 (L), 3.5(D) ng/mL

Papers for clinical applications:

Reference 12:

Determination of the Enantiomers of Salbutamol and its 4-O- sulfate Metabolites in Biological Matrices by Chiral Liquid Chromatography/Tandem Mass Spectrometry, Joyce, K.B., Jones, A.E., Scott, R.J., Biddlecombe, R.A., Pleasance, S., Rapid Commun. Mass Spectrom. 12, 1899-1910 (1998).

Analytes:	Salbutamol and 4-O-sulfate metabolite
Sample Prep:	Robotic SPE, Processed 4000 human plasma samples
Column:	CHIROBIOTIC T, 250 x 4.6 mm
Mobile Phase:	Methanol/acetic acid/ammonium hydroxide; 100/0.5/0.1
Flow Rate:	2.0 mL/min
Analysis Time:	2.0 min (metabolite), 3.2, 3.7 min enantiomers
Detection:	LLQ 100 pg/mL and 5ng/mL, respectively

Reference 26:

Liquid Chromatographic/Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Enantiomeric Separation of d,l-threo-Methylphenidate Using a Macrocyclic Antibiotic as the Chiral Selector. Ramos, L., Bakhtair, R. Majumdar, T., Hayes, M., Tse, F., Rapid Commun. Mass Spectrom., 13, 2054-2062 (1999).

Analytes:	Methylphenidate (Ritalin®)
Sample Prep:	Liquid-liquid extraction
Column:	CHIROBIOTIC V, 150 x 4.6 mm Processed 2500 human plasma samples
Mobile Phase:	100 methanol/0.05% (wt) ammonium trifluoroacetate
Flow Rate:	1.0 mL/min
Analysis time:	6.1, 7.2 min
Detection:	LLQ 87 pg/mL

Reference 38:

Chiral Liquid Chromatography Tandem Mass Spectrometry in the Determination of the Configuration of 2-Hydroxyglutaric Acid in Urine, Rashed, M.S., Al Amoudi, M., Aboul-Enein, H.Y., Biomedical Chromatogr. 14, 317-320 (2000).

Analytes:	D,L-2-hydroxyglutaric acid
Sample Prep:	Urine dilution (100 µL) with mobile phase (900 µL), filtration
Column:	CHIROBIOTIC R, 250 x 4.6 mm
Mobile Phase:	MeOH/TEAA, pH-7.0: 1/9
Flow Rate:	0.5 mL/min
Analysis Time:	Under 6 minutes
Detection Limits:	Not specified

Reference 40:

Simultaneous Analysis of Underivatized Amino Acids by Liquid Chromatography-Ionspray Tandem Mass Spectrometry Using a Teicoplanin Chiral Stationary Phase, Petritis, K., Valleix, A., Eflakir, C., Dreux, M., J. Chrom. A, 913, 331-340 (2001).

Analytes:	Underivatized 15 protein amino acids
Sample Prep:	Standards
Column:	CHIROBIOTIC T, 250 x 4.6 mm
Mobile Phase:	ACN/H ₂ O: 75/25
Flow Rate:	0.8 mL/min
Analysis Time:	Under 25 minutes
Detection limits:	0.25 to 5.0 µg/L depending on the amino acid

Reference 44:

Quantification of Methylphenidate (Ritalin®) in Rabbit Fetal Tissue Using Chiral Liquid Chromatography/Tandem Mass Spectrometry Assay, Bakhtier, R., Ramos, L., Tse, F.L.S., Letter to the Editor, Rapid Commun. Mass Spectrom., 16, 81-83 (2002).

Analytes:	Methylphenidate (Ritalin®) in rabbit fetal tissue
Sample Prep:	Cyclohexane extraction/evaporation
Column:	CHIROBIOTIC V, 150 x 4.6 mm
Mobile Phase:	MeOH/0.05% ammonium trifluoroacetate salt, v/wt
Flow Rate:	1.0 mL/min
Analysis Time:	7 minutes
Detection Limits:	LLQ 0.219 ng/gm tissue

Reference 46:

Enantiomeric Separation and Quantitation of Fluoxetine (Prozac®) in Human Plasma by Liquid Chromatography/Tandem Mass Spectrometry Using Liquid-Liquid Extraction in 96-well Plate Format, Shen, Z., Wang, S., Bakhtier, R., Rapid Commun. Mass Spectrom., 16, 332-338 (2002).

Analytes:	Fluoxetine (Prozac®) in human plasma
Sample Prep:	Ethyl acetate extract/evaporation
Column:	CHIROBIOTIC V, 250 x 4.6 mm
Mobile phase:	MeOH/0.075 % ammonium trifluoroacetate, v/wt
Flow Rate:	1.2 mL/min
Analysis Time:	8.4 minutes
Detection Limits:	2 ng/mL

Reference 53:

Determination of L-Pipecolic Acid in Plasma Using Chiral Liquid Chromatography-Electrospray Tandem Mass Spectrometry, Rashed, M.S., Al-Ahaidib, L.Y., Aboul-Enein, H.Y., Al-Amoudi, M., Jacob, M., Clinical Chemistry 47:12, 2124-2130 (2001).

Analytes:	Pipecolic acid in plasma, urine and cerebrospinal fluid
Sample Prep:	Acidified acetonitrile/evaporation/MeOH/water
Column:	CHIROBIOTIC T, 250 x 2.0 mm
Mobile Phase:	MeOH/H ₂ O: 60/40, v/v
Flow Rate:	200 µL/min
Analysis Time:	Under 12 minutes
Detection Limits:	0.5 to 80 µmol/L

Reference 54:

Chiral Liquid chromatography Tandem Mass Spectrometry in the Determination of the Configuration of Glyceric Acid in Urine of Patients with D-Glyceric and L-Glyceric Acidurias. Rashed, M.S., Aboul-Enein, H.Y., Al-Amoudi, M., Jakob, M., Al-Ahaideb, L.Y., Abbad, A., Shabib, S., Al-Jishi, E., Biomed. Chromatogr. 16, 191-198 (2002).

Analytes:	Glyceric acid in urine
Sample Prep:	1:1 dilution with mobile phase, filtration
Column:	CHIROBIOTIC R, 250 x 2.0 mm
Mobile Phase:	MeOH/0.1% TEAA, pH 4.1: 1/9
Flow rate:	0.3 mL/min
Analysis Time:	Under 5 minutes
Detection Limits:	Specifies ~125 ng creatinine

Reference 57:

Use of On-line-Dual-Column Extraction in Conjunction with Chiral Liquid Chromatography Tandem Mass Spectrometry for Determination of Terbutaline Enantiomers in Human Plasma, Yuan-Qing Xia, David Q. Liu, and Ray Bakhtiar, Department of Drug Metabolism, Merck Research Laboratories, Rahway, New Jersey. Chirality 14:742-749 (2002).

Analytes:	Terbutaline in human plasma
Sample Prep:	On-line SPE
Column:	CHIROBIOTIC T, 100 x 4.6 mm or R+V+T
Mobile Phase:	MeOH/0.05% ammonium trifluoroacetate salt, v/wt
Flow Rate:	1.2 mL/min
Analysis Time:	5.5 min
Detection Limits:	1 ng/mL

Note: Good comparison of single column to coupled column CHIROBIOTIC phases.

Reference 83:

Automated Online Dual-column Extraction Coupled with Teicoplanin Stationary Phase for Simultaneous Determination of (*R*) - and (*S*)-propranolol in Rat Plasma Using Liquid Chromatography – Tandem Mass Spectrometry, Xia, Y-Q, Bakhtiar, R., Franklin, R.B., J. of Chromatogr. B, 788, 317-329 (2003).

Development and validation an automated online sample extraction method for the quantification of (*R*)- and (*S*)-propranolol from rat plasma using CHIROBIOTIC T with MS/MS detection.

Analytes:	R,S-Propranolol in plasma
Sample Prep:	96 well, 2 online Oasis HBL (EC-1 & EC-2) extraction columns
Column:	CHIROBIOTIC T, 100 x 4.6 mm
Mobile Phase:	Methanol/0.05% ATFA
Flow Rate:	1.5 mL/min
Analysis Time:	10 min
Detection Limits:	2ng/mL

Reference 84:

Quantification of Methylphenidate in Rat, Rabbit and Dog Plasma Using a Chiral Liquid-Chromatography/Tandem Mass Spectrometry Method, Application to Toxicokinetic Studies, Bakhtiar, R., Ramos, L., Tse, F.L.S., Analytica Chimica Acta 469, 261-272 (2002).

Development and validation of an enantioselective reversed phase LC/tandem MS method for methylphenidate. Over 2500 injections on a single column.

Analytes:	L and D-Methylphenidate in plasma
Sample Prep:	Semi-automatic liquid-liquid extraction
Column:	CHIROBIOTIC V, 150 x 4.6 mm
Mobile Phase:	Methanol/0.05% ATFA
Flow Rate:	1.0 mL/min
Analysis Time:	6 min
Detection Limits:	1 ng/mL

Reference 89:

LC-MS Method for the Determination of Albuterol Enantiomers in Human Plasma Using Manual Solid-Phase Extraction and a Non-Deuterated Internal Standard, Jacobson, G.A., Chong, F.V., Davies, N.W., J. of Pharm. and Biomed. Analysis, 31 1237-1243 (2003).

Analytes:	R,S-Albuterol in plasma
Sample Prep:	SPE
Column:	CHIROBIOTIC T, 250 x 4.6 mm
Mobile Phase:	MeOH/HOAc/NH ₄ OH; 1000/5/1
Flow Rate:	1.3 mL/min
Analysis Time:	5 min
Detection Limits:	0.25 ng/mL

Papers for Amino Acids and Peptides:

Reference 102:

Analysis of Native Amino Acid and Peptide Enantiomers by High-Performance Liquid Chromatography/Atmospheric Pressure Chemical Ionization Mass Spectrometry, M.J. Desai, D.W. Armstrong, J. Mass Spectrom., 39, 177-187 (2004)

Analyte:	Underivatized Amino Acids
Sample Prep:	Standards
Column:	CHIROBIOTIC T/TAG, 250x4.6mm or 250x2.0mm
Mobile Phase:	Various
Flow Rate:	0.4 or 0.8 mL/min.
Analysis Time:	20 min. or less
Detection:	250pg – 10ng/mL

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