

Chiral HPLC of Derivatized N-Protected Amino Acids Using Chirex™

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Introduction

The enantiomeric purity of a wide variety of amino acids can be easily determined using Chirex™ chiral HPLC columns. The ligand exchange phase 3126 is especially well suited for the direct resolution of most free amino acids, and was covered in Technical Note TN-1005. A number of derivatization reagents developed over the past two decades, however, have enabled analysts to improve not only the capabilities for resolving these compounds, but also to greatly extend the detection limits as well.

There are two approaches to the derivatization of amino acid enantiomers. The first uses chiral reagents to produce diastereomers that can then be separated using an ordinary achiral stationary phase such as a C18. The second approach employs derivatization with achiral reagents prior to separation on a suitable chiral stationary phase (CSP). Derivatization is usually performed by reaction at the amino function, and referred to as “N-protection”. A variety of applications using some popular fluorimetric and photometric reagents are shown in this application note. They offer the chiral chromatographer an excellent means to high-sensitivity detection of amino acid enantiomers.

Instrumentation & Equipment

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software was used for the data analysis. The HPLC columns used for the analysis are Chirex brand (Phenomenex, Torrance, CA, USA, see Ordering Information inside). Standards were purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), or Fluka (Ronkonkoma, NY), depending on availability.

Results & Discussion

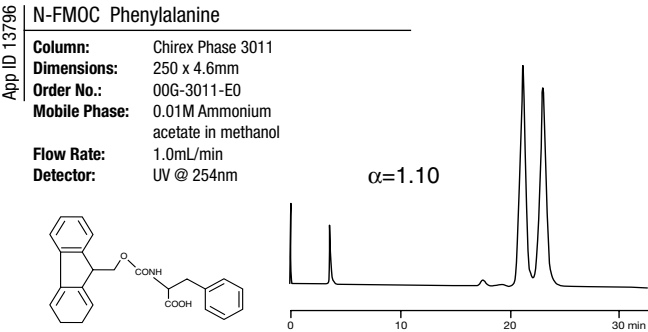
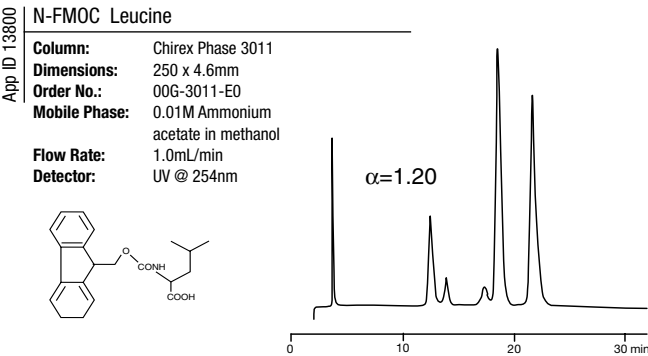
Of the various types of chiral stationary phases, or CSPs, the “Brush” or Pirkle Type is arguably the most useful and selective. The Phenomenex Chirex columns used for derivatized amino acid separations are of two types: The first consists of “Brush” type CSPs, consisting of an optically active amino acid derivative covalently bonded to γ -aminopropyl-silanized silica gel. An amide or urea linking of a π -electron group to the asymmetric center of the amino acid provides for π -electron interaction and an additional point of chiral recognition. Separations of derivatized amino acids detailed here are performed on the more versatile urea-type phases under simple reversed-phase conditions consisting of ammonium acetate in methanol.

The second column type examined, Chirex CSP 3126, is a ligand exchange type based on (D)-Penicillamine ionically bound to a hydrophobic support. This phase is particularly suited for the direct separation of amino acids and other ionic chiral compounds (as discussed previously in TN-1005). In fact, Chirex phase 3126 is considered one of the most effective columns for separating

underivatized amino acid racemates today. Separations of a few derivatized amino acid types are also performed under reversed-phase conditions, utilizing water-based solutions modified with a small percentage of methanol. Ionized samples form a transient diastereomeric complex by interacting with an added metal ion, such as copper(II), and the CSP.

Shown in the separations below, amino acid enantiomers are well resolved on four urea-type chiral phases Chirex 3010, 3011, 3012 and 3014 in the form of N-protected derivatives containing a free carboxylic acid group, such as tert-butoxycarbonyl (t-BOC), benzyloxycarbonyl (Z), 9-fluorenylmethoxycarbonyl (FMOC), benzoyl, phenylthiohydantoin(PTH), and 5-dimethylamino-1-naphthalenesulfanyl (dansyl) derivatives. Other amino acid enantiomers in the form of N-acetyl and N-formyl derivatives were also well resolved by chiral ligand-exchange on Chirex Phase 3126.

N-FMOC Derivatives of Amino Acids



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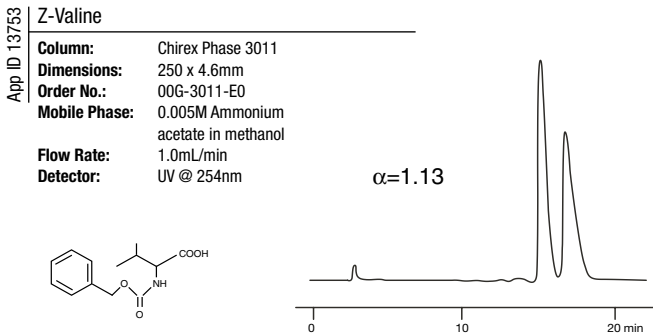
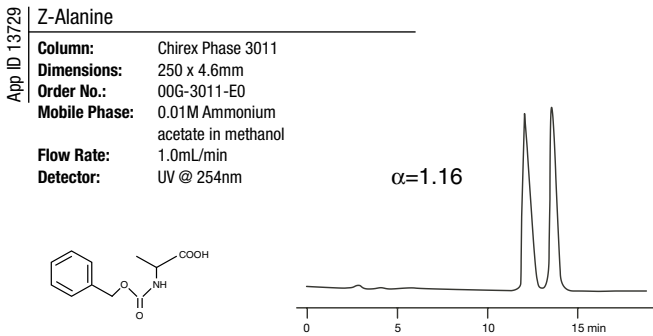


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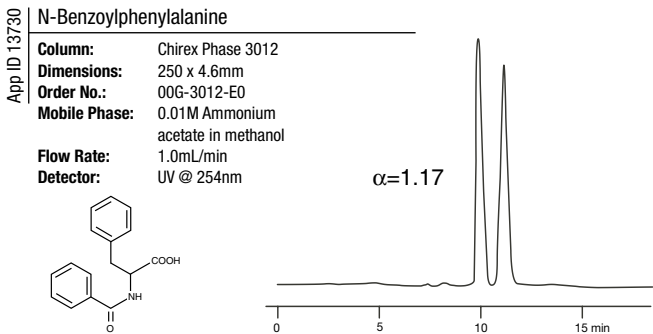
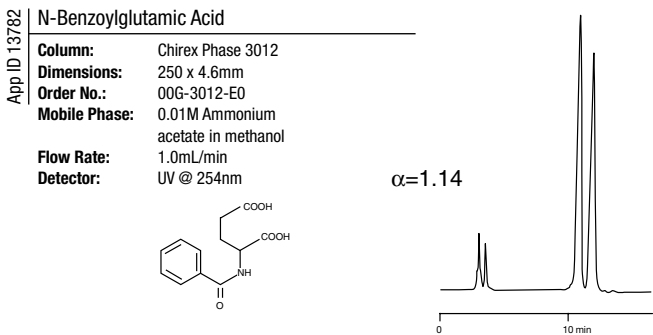
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Z-Derivatives of Amino Acids



N-Benzoyl Derivatives of Amino Acids



N-Dansyl Derivatives of Amino Acids

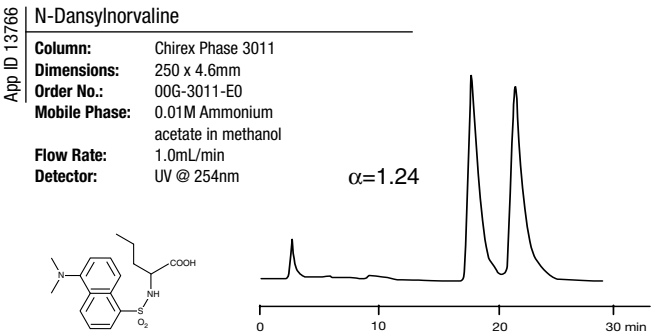
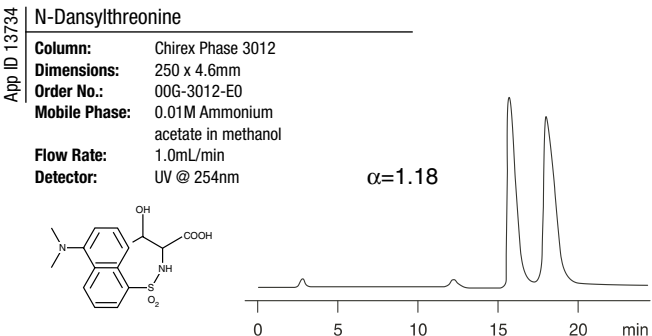


Table 1. Chiral HPLC of derivatized N-protected amino acids using Chirex CSPs.

Amino Acid Derivative	Chirex Phase	Alpha Factor	App ID No.
t-BOC-Derivatives (Butyloxycarbonyl)			
t-BOC-Leucine	3012	1.09	14064
t-BOC-Phenylalanine	3012	1.09	13784
t-BOC-Valine	3012	1.10	14063
N-FMOC Derivatives (9-Fluorenylmethyloxycarbonyl)			
N-FMOC-Leucine	3011	1.20	13800
N-FMOC-Phenylalanine	3011	1.10	13796
N-FMOC-Valine	3011	1.12	13798
Z-Derivatives (Benzyloxycarbonyl)			
Z-Alanine	3011	1.16	13729
Z-Asparagine	3010	1.12	13760
Z-Leucine	3011	1.17	13731
Z-Norvaline	3011	1.13	13755
Z-Phenylalanine	3012	1.08	13762
Z-Serine	3011	1.09	13758
Z-Valine	3011	1.13	13753
N-Acetyl Derivatives			
N-Acetylalanine	3126	1.17	14052
N-Acetylleucine	3126	1.39	14058
N-Acetylmethionine	3126	1.27	13728
N-Acetylvaline	3126	1.50	14055
N-Formyl Derivatives			
N-Formylvaline	3126	1.37	13722
N-Formylmethionine	3126	1.25	13721
N-Benzoyl Derivatives			
N-Benzoylglutamic acid	3012	1.14	13782
N-Benzoylleucine	3012	1.11	14460
N-Benzoylphenylalanine	3012	1.17	13730
N-Benzoylphenylglycine	3012	1.13	14461
N-Benzoylvaline	3012	1.19	13778
N-Dansyl Derivatives			
N-Dansylnorvaline	3011	1.24	13766
N-Dansylphenylalanine	3011	1.27	13771
N-Dansylthreonine	3012	1.18	13734
N-Dansyltryptophan	3010	1.15	13774
N-Dansylvaline	3011	1.28	13763
PTH Derivatives (Phenylthiohydantoin)			
PTH-Valine	3014	1.12	13921

If you would like more information on these chiral columns or any of the applications listed, please contact Phenomenex. Also, if you are new to chiral HPLC or are doing method development work call us today to reserve your **FREE** copy of our 70-page **Guidebook to Chiral HPLC Method Development**.



References

- Handbook of Derivatization Reactions for HPLC, by G. Lunn and L.C. Hellwig, Wiley, 1998.
- Toyo'oka, T. J. Biochemical and Biophysical Methods, 54 (1-3), (2002) 25-56.
- Oi, N., Kitahara, H., Aoki, F., Kisu, N. J. Chromatogr., 689:195, 1995.
- Oi, N., Kitahara, H., Aoki, F. J. Chromatogr., 694:129,

Ordering Information:

Chirex is available in a wide range of phases and column sizes, from analytical to preparative. The columns discussed in this paper are listed below. Contact us for all others.

5µ Analytical Columns

Chirex Brush Type Urea-Derivatized Phases, 250 x 4.6mm ID		
Phase	Description	Order No.
3010	(S)-VAL and DNB Covalent Urea	00G-3010-E0-TN
3011	(S)-LEU and DNB Covalent Urea	00G-3011-E0-TN
3012	(R)-PGLY and DNB Covalent Urea	00G-3012-E0-TN
3014	(S)-VAL and (R)-NEA Covalent Urea	00G-3014-E0-TN
3017	(S)-PRO and (S)-NEA Covalent Urea	00G-3017-E0-TN
3018	(S)-PRO and (R)-NEA Covalent Urea	00G-3018-E0-TN
3019	(S)-LEU and (S)-NEA Covalent Urea	00G-3019-E0-TN
3020	(S)-LEU and (R)-NEA Covalent Urea	00G-3020-E0-TN
3022	(S)-ICA and (R)-NEA Covalent Urea	00G-3022-E0-TN

Chirex Ligand-Exchange Type Phase, 150 x 4.6mm ID		
Phase	Description	Order No.
3126	(D)-Penicillamine	00F-3126-E0-TN

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