APPLICATION

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Emily is an avid baker. She loves throwing themed parties to entertain friends.

Chiral Separation of the Drug Product Levetiracetam Using the United States Pharmacopeia Method on the Latest L51 USP Column - Lux® Amylose-1

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In this technical note, we report the enantiomeric separation of Levetiracetam using the approved United States Pharmacopeia method. We perform the chiral analysis on the latest L51 USP column, the polysaccharide-based chiral stationary phase Lux Amylose-1 column, and compare analytical result as well as performance to CHIRALPAK® AD-H® column.

Introduction

The United States Pharmacopeia (USP) establishes methods and provides reference standards for medicines, food ingredients, dietary supplement products, and active pharmaceutical ingredients (API). These USP methods and standards are used by regulatory agencies and pharmaceutical manufacturers to ensure that APIs or drug products are of the appropriate identity, as well as strength, quality, purity, and consistency. In the case of chiral drugs, such as Levetiracetam (**Figure 1**.) a chiral HPLC method is generally used to assess chiral purity.

Levetiracetam (trade name Keppra) is an anticonvulsant medication mainly used to treat seizure disorder (epilepsy), as well as psychiatric and neurologic conditions in humans and animals. As of November 2008, the drug is available as a generic brand in the United States and the United Kingdom.

Levetiracetam is the biologically active S-enantiomer of the racemic compound Etiracetam. A USP method using a L51 USP column is published to perform enantiomeric separation of a racemic mixture and quantify the R-enantiomer in the drug product Levetiracetam.

In this technote, we report the results for the limit of Levetiracetam (R-enantiomer) following the published USP method using the newest L51 USP column, Lux Amylose-1, and compare its performance to the well known CHIRALPAK AD-H (L51) column.

Materials and Methods

All analyses were performed using a Shimadzu SCL-10AVP LC system (Shimadzu Corporation, Canby, OR, USA) equipped with binary pump, in-line degasser, multi-wavelength UV detector and autosampler. The Lux Amylose-1 column used for analysis was obtained from Phenomenex (Torrance, CA, USA) and the CHIRALPAK AD-H column used was obtained from DAICEL® Corporation (Fort Lee, NJ, USA). USP Reference Standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were purchased from Honeywell (Morristown, NJ, USA).

USP sample and definition

System suitability solution: 0.1 mg/mL of USP Levetiracetam (RS racemic mixture) in mobile phase

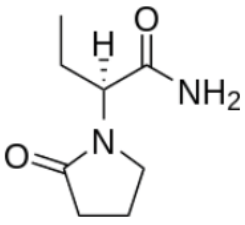
Sample solution: 10 mg/mL of USP Levetiracetam (S-enantiomer) in mobile phase

Each sample solution was subjected to the experimental conditions described below, first using Lux Amylose-1, followed by CHIRALPAK AD-H.

HPLC Conditions

Column:	Lux 5 µm Amylose-1 CHIRALPAK 5 µm AD-H
Dimensions:	250 x 4.6 mm
Mobile Phase:	Hexane/Ethanol (80:20)
Flow Rate:	1 mL/min
Temperature:	25 °C
Detection:	UV @ 215 nm
Sample:	1. Levetiracetam R-enantiomer 2. Levetiracetam S-enantiomer (biologically active compound)

Figure 1.
Chemical Structure of Levetiracetam



Results and Discussion

The USP HPLC analytical method for the limit of Levetiracetam (R-enantiomer) was evaluated on both the Lux® Amylose-1 and CHIRALPAK® AD-H® columns to compare product performance. The USP standards were dissolved in the mobile phase and injected on a Shimadzu HPLC system as described in the material and methods section.

The USP method gives guidelines for the relative retention time (RRT) of the Levetiracetam enantiomers in the system suitability section. According to the monograph, the R-enantiomer and S-enantiomer RRT should be 0.55 and 1.0 respectively for the system suitability solution.

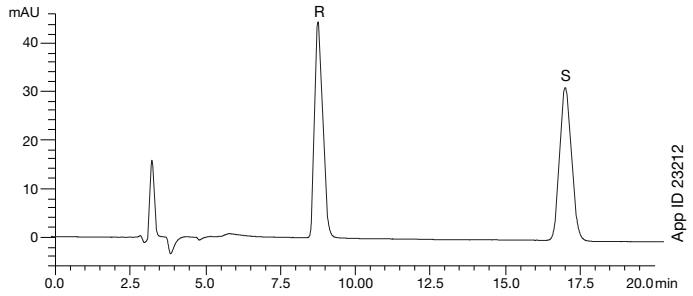
The resolution (Rs) criteria for the system suitability solution between the two enantiomers of Levetiracetam must be no less than (NLT) 4.0 and the amount of R-enantiomer shall be no more than (NMT) 0.8 % for the drug to meet the chiral purity acceptance criteria.

USP reference standards were first injected on the Lux Amylose-1 column following the USP monograph method. The retention times and resolution of the system suitability solution using the Lux Amylose-1 column are shown in **Figure 2a**. The retention times for the R- and S-enantiomers were 8.72 minutes (RRT 0.52) and 16.78 (RRT 1.0) minutes, respectively. The resolution obtained with the Lux Amylose-1 column was 13.9, well within the USP method criteria of no less than 4.0. Retention times and resolution for system suitability solution on CHIRALPAK AD-H column are shown in **Figure 2b**. The retention times for the R- and S-enantiomers were 8.66 minutes (RRT 0.52) and 16.61 (RRT 1.0) minutes, respectively. The results using the CHIRALPAK AD-H column are similar to Lux Amylose-1 for the RRT results but resolution was slightly lower with 13.4 vs 13.9 for the Lux column. The higher resolution is related to the better column efficiency of the Lux Amylose-1.

Figures 3a displays the chromatogram of Levetiracetam sample solution run on Lux Amylose-1 column. The calculated percentage of R-enantiomer is 0.29 %, well below the 0.8 % required by the USP method. **Figure 3b** shows the chromatogram of Levetiracetam sample solution run on CHIRALPAK AD-H column. The calculated percentage of R-enantiomer is 0.58 %, also adhering to the criteria of NMT 0.8 %. **Table 1** displays the corresponding results on both the Lux Amylose-1 and the CHIRALPAK AD-H columns for percentage of R-enantiomer in the sample solution.

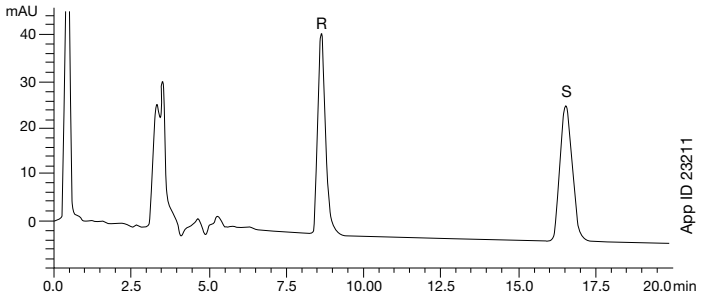
Figure 2.
Levetiracetam system suitability on Lux Amylose-1 and CHIRALPAK AD-H

Figure 2a.
System suitability solution on Lux Amylose-1



Peak	Ret Time	Area	Plates/m	Rs	Area%
1	8.72	765671	37686	-	48.42
2	16.78	815578	61626	13.90	51.58

Figure 2b.
System suitability solution on CHIRALPAK AD-H

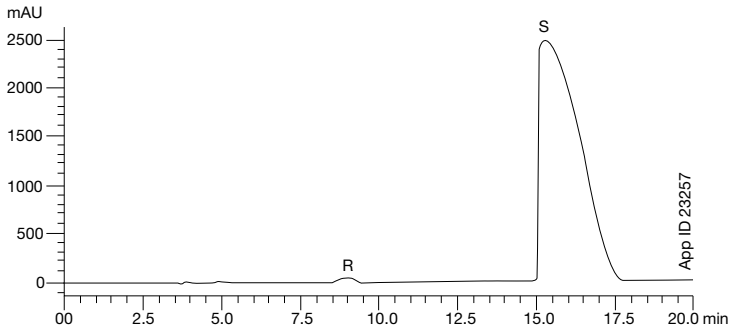


Peak	Ret Time	Area	Plates/m	Rs	Area%
1	8.66	780313	34961	-	50.15
2	16.61	775561	58599	13.39	49.85

* Comparative separations may not be representative of all applications

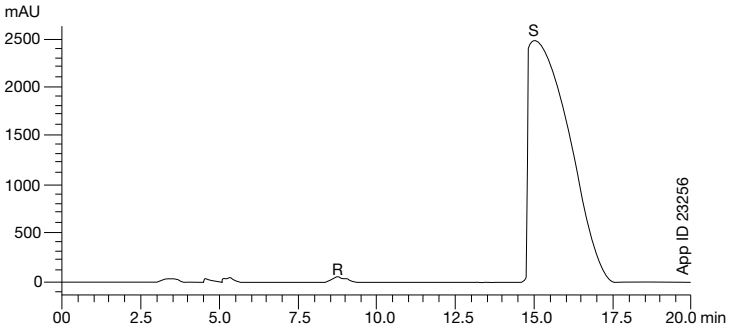
Figure 3.
Levetiracetam sample solution on Lux® Amylose-1 and CHIRALPAK® AD-H®

Figure 3a.
Sample solution on Lux Amylose-1



Peak	Ret Time	Area	Plates/m	Rs	Area%
1	8.67	448388	35398	-	0.23
2	15.44	194951304	6363	5.47	99.77

Figure 3b.
Sample solution on CHIRALPAK AD-H



Peak	Ret Time	Area	Plates/m	Rs	Area%
1	8.66	453801	35469	-	0.21
2	14.93	215663523	4568	4.6	99.80

* Comparative separations may not be representative of all applications

Table 1.
Percentage of the R-enantiomer in Levetiracetam sample solution on Lux Amylose-1 and CHIRALPAK AD-H.

USP L51 Column	ru	rs	cu	cs	%R
Lux Amylose-1	448388	765671	0.05	10	0.29
CHIRALPAK AD-H	453801	780313	0.05	10	0.58

The percentage of unwanted R-enantiomer in Levetiracetam sample solution is calculated using the given formula:

%R-enantiomer = [(ru/rs) x (cs/cu)] x 100

ru is the peak response of the R-enantiomer in the sample solution, rs is the peak response of the R-enantiomer in the standard solution, cs is the concentration of the standard solution, and cu is the concentration of the sample solution

Conclusion

The results shown in this technote demonstrate that the newest L51 USP column, Lux Amylose-1, produces equivalent results when compared to the CHIRALPAK AD-H column for the enantiomeric separation of Levetiracetam following the USP monograph for enantiomeric purity.

The resolution of the system suitability solution on both columns adhere to the criteria stated in the approved USP method for the Levetiracetam enantiomers. The resolution between the two enantiomers was calculated to be 13.9 on the Lux Amylose-1 column which is a better result than the CHIRALPAK AD-H column with a calculated resolution of 13.4. The difference is mainly explained by the better efficiency seen with the Lux Amylose-1 column.

Finally, the percentage of the R-enantiomer calculated from prepared standard and sample solutions was 0.29 % on the Lux Amylose-1 column and 0.58 % on the CHIRALPAK AD-H column, well below the USP criteria of NMT 0.8 %.

APPLICATION

Lux® Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
Amylose-1	00B-4732-E0	00D-4732-E0	00F-4732-E0	00G-4732-E0	AJ0-9336
				for ID:	3.2–8.0 mm

5 µm Semi-Prep Columns (mm)		SecurityGuard Cartridges (mm)
Phases	250 x 10.0	10 x 10.0‡
Amylose-1	00G-4732-N0	AJ0-9344
	for ID:	9–16 mm

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard Cartridges (mm)	
Phases	150 x 21.2	250 x 21.2	250 x 30	250 x 50	15 x 21.2**	15 x 30.0♦
Amylose-1	00F-4732-P0-AX	00G-4732-P0-AX	00G-4732-U0-AX	00G-4732-V0-AX	AJ0-9338	AJ0-9339
				for ID:	18–29 mm	30–49 mm

* SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282
‡ SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-9281
**HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8223
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8617
♦ HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8277
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8618

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