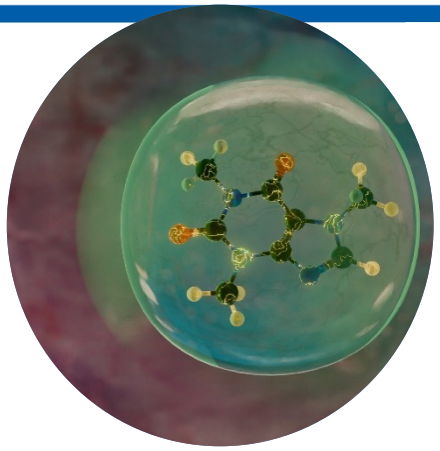

TN-1384

Separation of Irinotecan Hydrochloride and its Chiral Impurity per USP Monograph under Allowable Adjustments

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Introduction

Irinotecan hydrochloride is a chemotherapeutic agent classified as a DNA topoisomerase I inhibitor. Irinotecan is frequently administered alongside other drugs for the treatment of metastatic colon and rectal cancer, and occasionally for small cell lung cancer. The USP monograph specifies the use of an L40 column (4.6-mm × 25-cm; 10-µm), which is a Cellulose tris (3,5 dimethylphenylcarbamate) polymer column. In this technical note we demonstrate the utilization of Lux™- Cellulose-1, (5 µm 150 x 4.6 mm) as an alternative column for the separation of Irinotecan Hydrochloride and its chiral impurity per the allowable adjustments outlined in USP General Chapter <621>. The system Suitability per USP monograph is resolution NLT 2.5 between Irinotecan related compound D and Irinotecan, a percent relative standard deviation (%RSD) of NMT 5.0%, for standard solution and the Irinotecan related compound D peak should be visible for the sensitivity solution. All the system suitability requirements have been met by this column.

LC Conditions

Column: Lux™ Cellulose-1, 5 µm (Part No: 00F-4459-E0)

Dimensions: 150 x 4.6 mm

Mobile Phase-A: Hexane: Dehydrated Alcohol: Diethylamine in ratio of 250:250:1 ml, v/v/v.

Diluent: Dehydrated Alcohol: Diethylamine in ratio of 250:1

Isocratic Elution:

Run Time (min) %	Mobile phase-A
25	100

Flow Rate: 1.5 mL/min

Injection Volume: 12 µL

Temperature: 25^{0}C

LC System: Waters® Arc HPLC with PDA

Detection: UV @ 370 nm

Standard and System suitability solutions

Standard stock solution: 10 mg/ml of USP Irinotecan Related compound-D RS in Diluent.

System suitability solution: 0.1mg/mL Each Irinotecan Hydrochloride and Irinotecan Related compound-D in Diluent

Identification Solution: 1mg/mL of USP-Irinotecan Hydrochloride RS in Diluent.

Standard solution: 1.5 µg/mL of USP Irinotecan Related compound-D in Diluent, from the Standard stock solution.

Sensitivity solution: 0.5 µg/mL of USP Irinotecan Related compound-D reference standard in Diluent, from the Standard stock solution.

Sample solution: 1mg/mL Irinotecan Hydrochloride in Diluent.

All solutions were prepared as indicated in the USP monograph for the Irinotecan Hydrochloride

USP Irinotecan HCl (Catalog No.1347609), USP Irinotecan Related compound-D (Catalog No.1347653) were purchased from USP.

Standard and System suitability solutions

System suitability solution and standard solution were run on the Lux Cellulose-1, 5 µm 150 x 4.6 mm column.(Figure-1 on Page no:2)

Figures 1, 3 and 5 show system suitability solution chromatograms and Peak tables 1,3,5 on page no:2 show system suitability criteria Retention time, RRT, Resolution and S/N ratio values from sensitivity solution.

Figures 4 & 5, show standard solution chromatogram and Figure-5 table shows relative standard deviation (% RSD) of the six replicate injection of standard solution.

Allowable column Adjustments: L/dp Ratio- 25 % to 50%

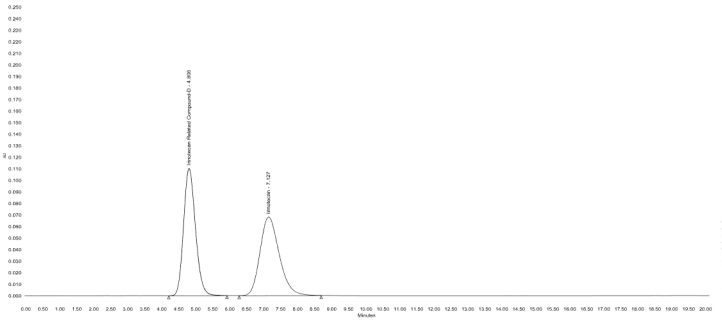
Column	Length (mm)	ID (mm)	dp (µm)	L/dp	Allowable Range (-25% to +50%)
Original	250	4.6	10	25	18.75 to 37.5
Alternative	150	4.6	5	30	Allowed

Conclusions

This USP monograph outlines the utilization of an L-40 column, measuring 10µm - 250 x 4.6mm. The 10µm particle columns are older generation columns with lower efficiencies compared to the 5µm. Utilizing the USP Allowable Adjustments Calculator, we have used Lux- Cellulose-1 column (5 µm, 150 x 4.6 mm) to evaluate its applicability. Method adjustments have also been considered in accordance with the Allowable Adjustments due to the change in the LC column.

The performance of this column was assessed based on the Relative Retention Time (RRT) of relative compound-D, resolution, signal-to-noise ratio, and the relative standard deviation (RSD) of the standard solution, all of which fell within acceptable limits. This indicates that the Lux-Cellulose-1 column (5 µm, 150 x 4.6 mm) can be used as an alternative to the 10 µm 250 x 4.6 mm column referenced in the monograph.

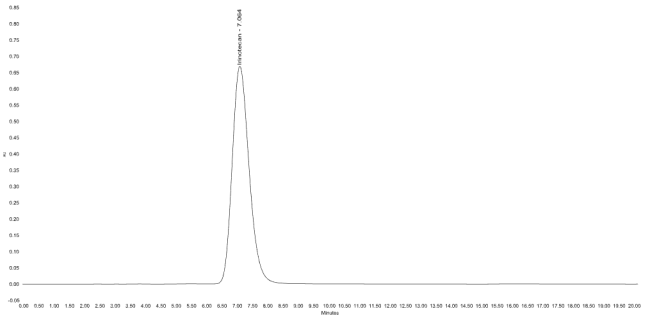
Figure 1. Irinotecan Hydrochloride System suitability solution



App ID 28536

Peak No.	Analyte	Retention Time	Area	% Area	RT Ratio	Resolution	USP Tailing Factor
1	Irinotecan Related Compound-D	4.805	1603142	50.03	0.67		1.2
2	Irinotecan	7.121	1595875	49.97	1.00	2.86	1.2

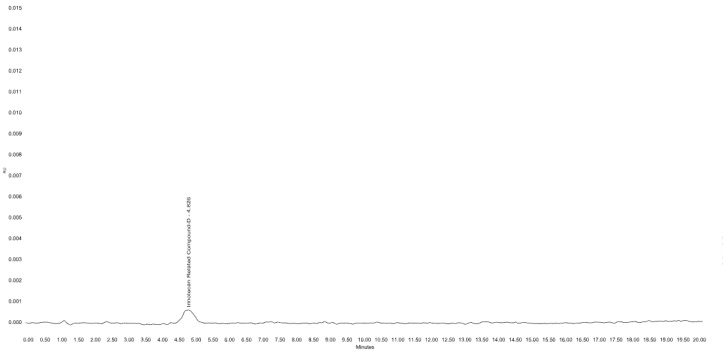
Figure 2. Irinotecan Hydrochloride USP- Identification solution



App ID 28537

Peak No.	Analyte	Retention Time	Area	% Area
1	Irinotecan	7.064	1537204	100.00

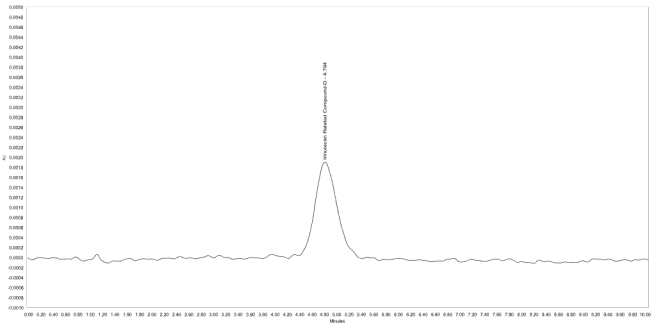
Figure 3. Irinotecan Hydrochloride USP- Sensitivity solution(0.5µg/ml)



App ID 28538

Peak No.	Analyte	Retention Time	Area	S/N Ratio
1	Irinotecan Related Compound-D	4.826	9941	10

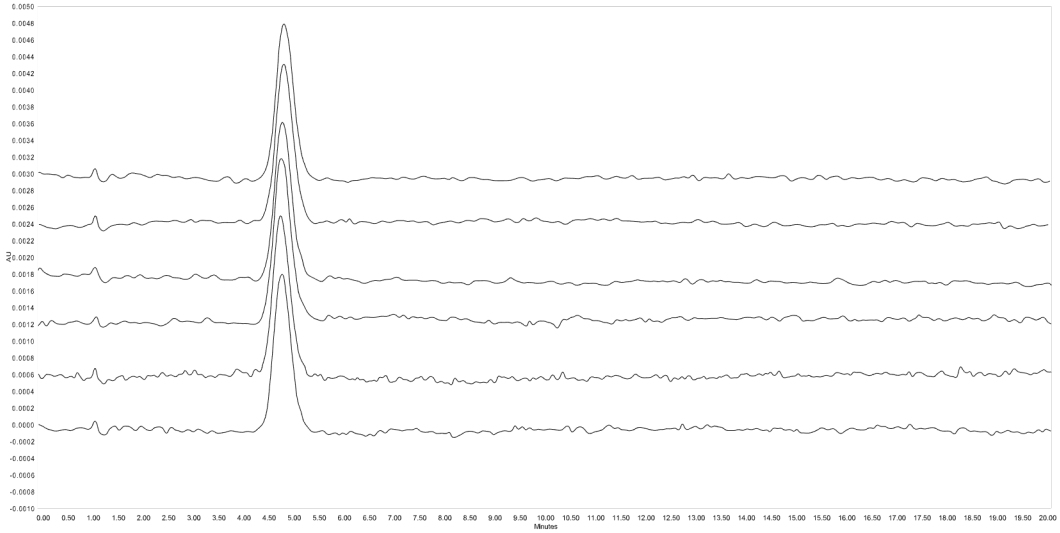
Figure 4. Irinotecan Hydrochloride USP- Standard solution (1.5µg/ml)



App ID 28539

Peak No.	Analyte	Retention Time	Area
1	Irinotecan Related Compound-D	4.817	26688

Figure 5. Irinotecan Hydrochloride USP- Standard solution (1.5µg/mL), replicate injections (N=6, overlayed) chromatogram.



App ID 28540

Injections (N=6)	Retention Time	Area
Mean	4.824	26608.66
SD	0.005	94.8
%RSD	0.1	0.35

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