UNIVERSITY OF NOTRE DAME
College of Science
DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

PHARMACEUTICAL ANALYSIS REPORT

5 July 2021

DISCLAIMER: The results herein are gathered for primary research purposes at the University of Notre Dame. This report and any attachments are intended for internal evaluation between the University of Notre Dame and the Liberia Medicines & Health Products Regulatory Agency. The results are not intended to discredit or replace the certificate of analysis provided by the manufacturer of the finished pharmaceutical product. If the results need to be used in an official capacity, further testing should be conducted at a laboratory with the appropriate jurisdiction.

Specific questions should be directed to:

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Report Prepared by:

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Kathleen Hayes	University of Notre Dame	DPAL Coordinator Analyst

I. SUMMARY

According to the United States Pharmacopeia (USP), finished pharmaceutical dosage forms of doxycycline must contain 90.0%-120.0% of the stated amount of active pharmaceutical ingredient (API). The University of Notre Dame has analyzed 13 samples of doxycycline representing 5 manufacturers and 5 batch numbers. Each doxycycline dosage form analyzed met the requirement for API content.

Active Ingredient	Number of Samples Analyzed	Number of Failing Samples	Comment on Medicine Quality	Average API Content (%)
Doxycycline	13	0	All passed	97.3%

II. RESULTS

A. HPLC SCREENING

Thirteen doxycycline samples collected in 2020 by covert shoppers in Liberia were selected for HPLC assay. One tablet from each package was assayed. If the sample failed assay, it was retested. All thirteen samples of doxycycline passed assay. We analyzed doxycycline made by five manufacturers, representing 5 batch numbers. All the samples had been stored in our 4˚C cold room.

Sample	P1 API Content (%)	P1 Retest API Content (%)	Brand	Manufacturer	Lot Number	Expiration Date
21L-0005	95.3	NA	DoxyNAN	Saintsun Pharma Co. Ltd, China	180944	9/1/2021
21L-0009	103.4	NA	Philco Doxy 100	Philco Pharma, Germany	1705044	9/1/2021
21L-0004	102.2	NA	DoxyLab	Laborate Pharmaceutical, India, Ltd	DAOC-003	10/1/2022
21L-0007	95.9	NA	DoxyLab	Laborate Pharmaceutical, India, Ltd	DAOC-003	10/1/2022
21L-0010	101.6	NA	DoxyLab	Laborate Pharmaceutical, India, Ltd	DAOC-003	10/1/2022
21L-0012	96.1	NA	DoxyLab	Laborate Pharmaceutical, India, Ltd	DAOC-003	10/1/2022
21L-0001	99.7	NA	DoxyNext	Osmed Formulation Pvt,Ltd, India	K40901	10/1/2022
21L-0003	121.6	105.3	DoxyNext	Osmed Formulation Pvt,Ltd, India	K40901	10/1/2022
21L-0008	91.5	NA	DoxyNext	Osmed Formulation Pvt,Ltd, India	K40901	10/1/2022
21L-0011	92.5	NA	DoxyNext	Osmed Formulation Pvt,Ltd, India	K40901	10/1/2022
21L-0013	96.2	NA	DoxyNext	Osmed Formulation Pvt,Ltd, India	K40901	10/1/2022
21L-0002	95.4	NA	Doxycycline	Soham Healthcare Inc, India	SC-027	4/1/2022
21L-0006	90.3	NA	Doxycycline	Sohan Healthcare Inc, India	SC-027	4/1/2022

III. METHODOLOGY

The procedures for quantifying the active ingredients in doxycycline finished pharmaceutical pills was based on the monograph published in the United States Pharmacopeia (USP). At times, minor modifications were made to the assay methodology. However, the accuracy, precision, and linearity of the methodology was verified according to USP <1226>. Sampling for the Distributed Pharmaceutical Analysis Lab is performed on a per pill basis, not as an average of 10 pills, since samples are generally composed of one dosage unit of the pharmaceutical product.

A. HPLC SYSTEM SUITABILITY

External calibration standards were created from analytical grade reagents. Five injections of the external standard were required to show a peak area within 2% relative standard deviation (RSD), and the range of retention times had to be within 0.3 minutes. After every five samples, the standard was injected as a quality check. It must have assayed within 2% RSD of the 5 initial injections and been within the 0.3 minute time range. If a quality check failed, data after the last passed quality check was discarded. Additional system suitability requirements information is available upon request.

B. HPLC INSTRUMENT CONDITIONS

Instrument: Waters 2695 High Performance Liquid Chromatograph
Column: XTerra C8, 5 μm, 4.6 mm x150 mm
Temperature: RT
Detector: Waters 2487, Analytical Wavelength: 360 nm

C. HPLC SAMPLE PREPARATION

Sample Concentration: 0.5 mg/mL
Sample Solvent: 18 MOhm water
Sample Injection Volume: 20 μL

D. HPLC STANDARD CONCENTRATION

Standard Concentration: 0.5 mg/mL
Standard Solvent: 18 MOhm water
Standard: Doxycycline hyclate, Sigma Aldrich, Lot # 069M4014V

E. HPLC MOBILE PHASE

Isocratic

30% Acetonitrile

70% 0.1% Trifluoroacetic acid in 18MOhm water (pH = 1.8)

APPENDIX

The appendix includes all the raw HPLC data and spreadsheet calculations for the samples and associated calibration checks.

APPENDIX

Data Collection Date: 30 June 2021

Data Collection Location: University of Notre Dame

Notebook	Date
KH004P067	6/30/2021

STD #	Doxycycline Peak Area
1	13548
2	13697
3	13644
4	13640
5	13638
AVG	13633
SD	54
%RSD	0.39
Pass?	pass

STD Conc (mg/mL)	Manufacturer	Lot #	Expiry date**	Mass Dox (g)	Volume (mL)	Used (mL)	Final Vol (mL)	Purity*	Conc (mg/mL)
						If no dilution, enter same value			
Doxycycline	Sigma Aldrich	069M4014V	current lot	0.01266	6.0	0.75	3.0	0.956	0.5042

*Express as pure doxycycline

**If using USP std, an expiry date is not assigned until they move to the next batch of standard, at which time the previous batch gets a one year expiry date. Enter "current lot."

Sample		Dox. Peak Area	Pill Total Mass (g)	Sample Mass (g)	Volume (mL)	Dox Dosage (mg)	mg Dox	%Error Dox	Dox Pass?	Comment	Total Mass (g)	MT Cap Mass (g)
21L-0001	26	13728	0.30943	0.01260	8.0	100	100	-0.3	pass			
21L-0002	27	15592	0.25899	0.01253	8.0	100	95	-4.6	pass			
21L-0003	28	16749	0.32218	0.01313	8.0	100	122	21.6	fail			
21L-0004	29	17643	0.29337	0.01499	8.0	100	102	2.2	pass			
21L-0005	30	17650	0.23041	0.01263	8.0	100	95	-4.7	pass			

		Dox. Peak Area
Cal. Check_1	25	13475
Error (%)		1.16
Pass?		pass

Sample		Dox. Peak Area	Pill Total Mass (g)	Sample Mass (g)	Volume (mL)	Dox Dosage (mg)	mg Dox	%Error Dox	Dox Pass?	Comment	Total Mass (g)	MT Cap Mass (g)
21L-0006	31	18658	0.27428	0.01676	8.0	100	90	-9.7	pass			
21L-0007	32	13995	0.29289	0.01265	8.0	100	96	-4.1	pass			
21L-0008	33	14461	0.28915	0.01352	8.0	100	92	-8.5	pass		0.35143	0.06228
21L-0009	34	15178	0.19571	0.01275	12.0	100	103	3.4	pass			
21L-0010	35	14603	0.29294	0.01246	8.0	100	102	1.6	pass			

		Dox. Peak Area
Cal. Check_2		13478
Error (%)		1.14
Pass?		pass

Sample		Dox. Peak Area	Pill Total Mass (g)	Sample Mass (g)	Volume (mL)	Dox Dosage (mg)	mg Dox	%Error Dox	Dox Pass?	Comment	Total Mass (g)	MT Cap Mass (g)
21L-0011	36	13449.000	0.31104	0.01338	8.0	100	93	-7.5	pass			
21L-0012	37	15939.000	0.29471	0.01446	8.0	100	96	-3.9	pass			
21L-0013	38	15460.000	0.30657	0.01457	8.0	100	96	-3.8	pass			
21L-0003	39	15107.000	0.32218	0.01368	8.0	100	105	5.3	pass	retest- pass		
NA		NA	NA	NA	NA	NA	NA	NA	NA			

	Dox. Peak Area
Cal. Check_3	13410
Error (%)	1.64
Pass?	pass

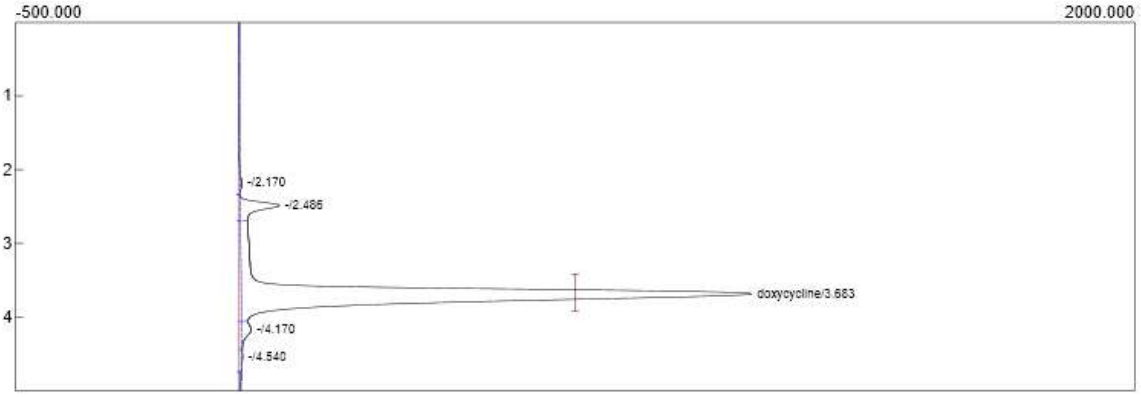
#USP requirements for tablets and capsules containing doxycycline: 90.0-120.0% of labeled amount on package

All yellow cells must have an entry, such as NA, to make the LC data explicitly clear.

This sheet only works for single point calibration

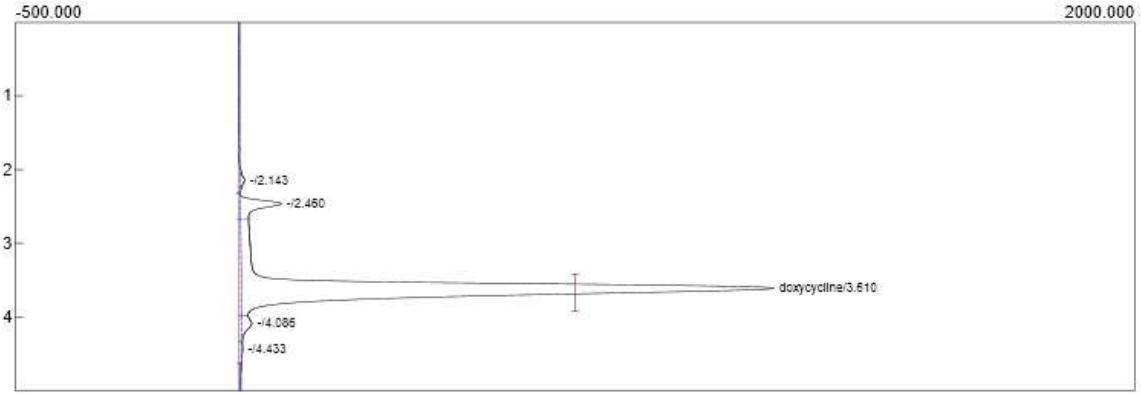
Password to unprotect sheet: password

Lab name: Notre Dame
Method: Syringe Injection
Data file: doxy std 01.CHR ()



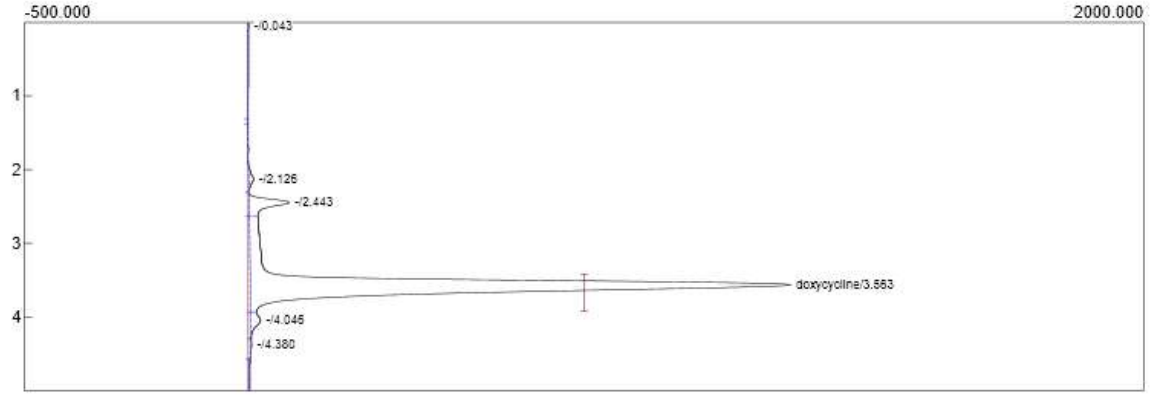
Component	Retention	Area	Height
-	2.170	51.8946	4.688
-	2.486	728.0415	87.571
doxycycline	3.683	13548.5027	1140.245
-	4.170	247.9528	21.163
-	4.540	12.9701	1.385
		14589.3617	

Lab name: Notre Dame
Method: Syringe Injection
Data file: doxy std 02.CHR ()



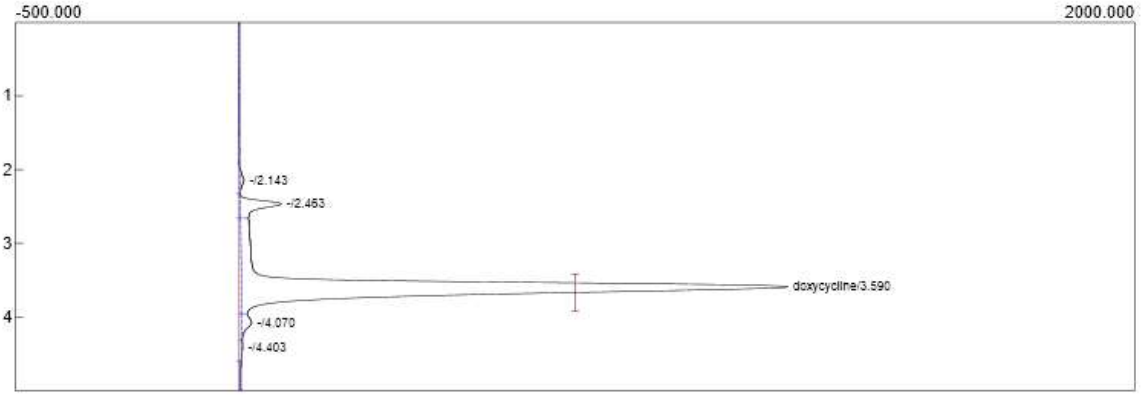
Component	Retention	Area	Height
-	2.143	104.2030	10.326
-	2.460	747.6656	91.309
doxycycline	3.610	13697.7097	1189.476
-	4.086	243.0242	21.706
-	4.433	12.3834	1.390
		14804.9859	

Lab name: Notre Dame
Method: Syringe Injection
Data file: doxy std 03.CHR ()



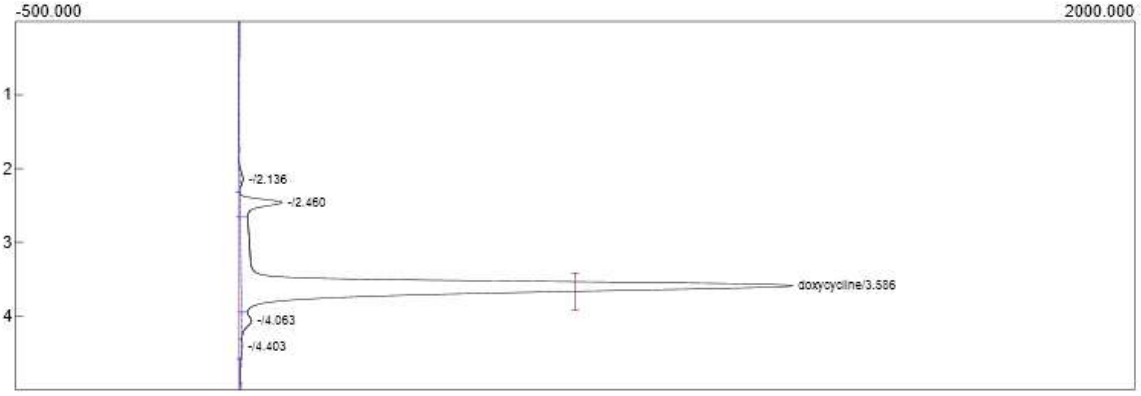
Component	Retention	Area	Height
-	0.043	10.7487	0.388
-	2.126	107.7888	10.875
-	2.443	723.2133	89.373
doxycycline	3.563	13644.8040	1207.149
-	4.046	235.8350	21.538
-	4.380	11.8878	1.383
		14734.2776	

Lab name: Notre Dame
Method: Syringe Injection
Data file: doxy std 04.CHR ()



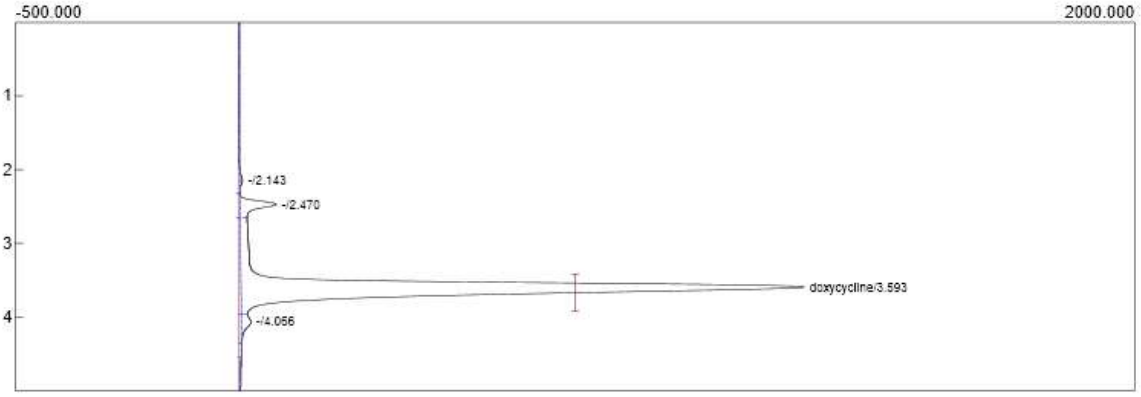
Component	Retention	Area	Height
-	2.143	95.8654	9.430
-	2.463	730.2420	91.338
doxycycline	3.590	13640.7316	1220.052
-	4.070	238.6380	21.556
-	4.403	12.4024	1.399
		14717.8794	

Lab name: Notre Dame
Method: Syringe Injection
Data file: doxy std 05.CHR ()



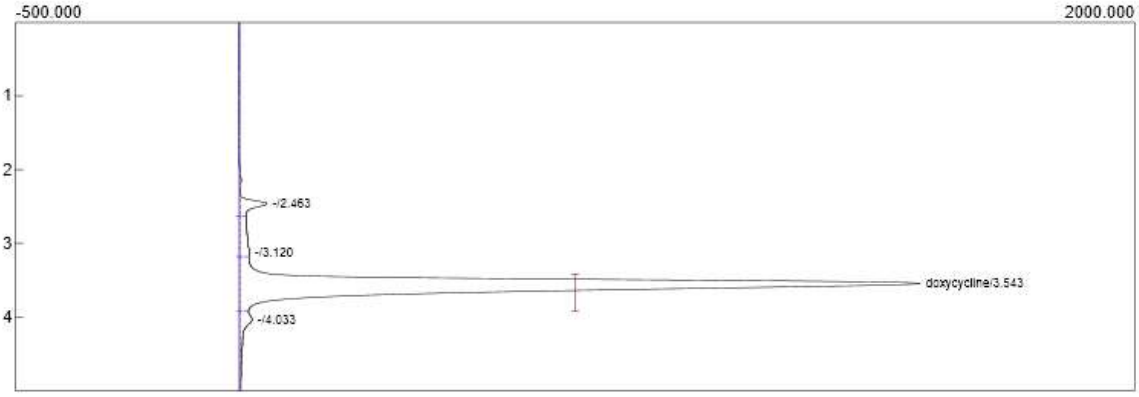
Component	Retention	Area	Height
-	2.136	85.6152	8.248
-	2.460	740.1230	93.381
doxycycline	3.586	13638.5108	1232.039
-	4.063	240.5496	21.954
-	4.403	12.3814	1.445
		14717.1800	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0001.CHR ()



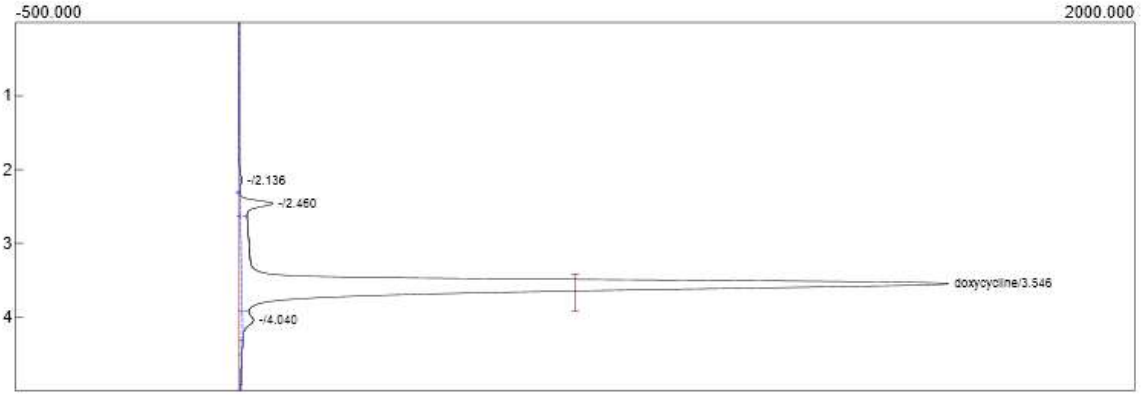
Component	Retention	Area	Height
-	2.143	58.6804	6.331
-	2.470	628.8977	80.956
doxycycline	3.593	13728.7828	1257.600
-	4.066	227.8032	20.338
		14644.1641	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0002.CHR ()



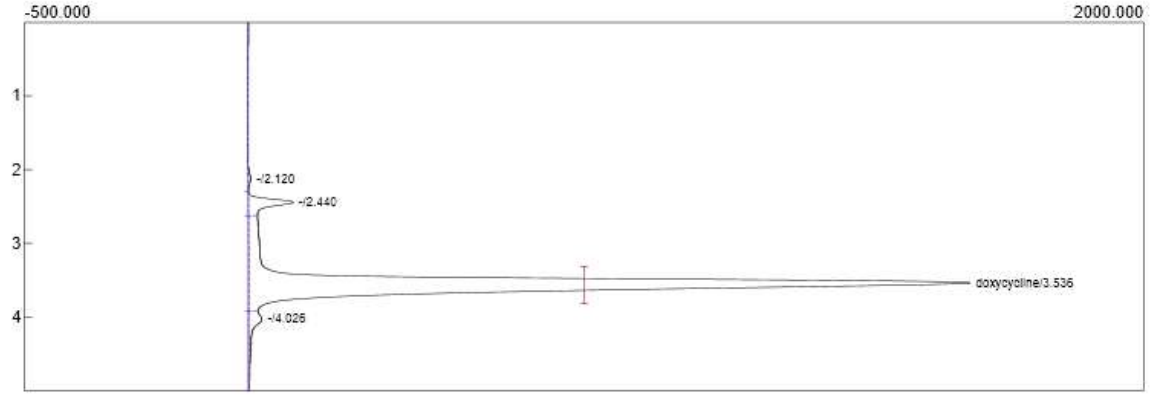
Component	Retention	Area	Height
-	2.463	457.9970	60.442
-	3.120	566.4417	20.573
doxycycline	3.543	15592.0054	1519.924
-	4.033	406.7240	26.021
		17023.1681	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0003.CHR ()



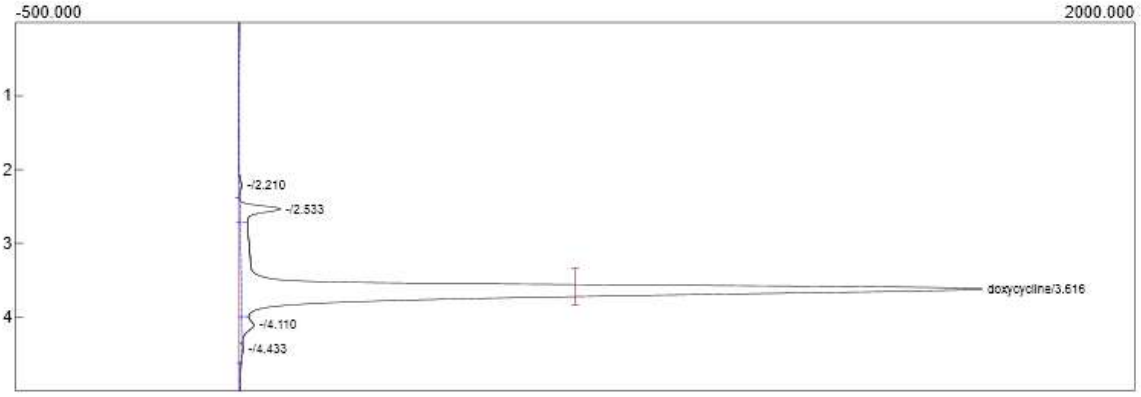
Component	Retention	Area	Height
-	2.136	46.6462	5.034
-	2.460	541.5352	73.073
doxycycline	3.546	16749.9076	1580.234
-	4.040	273.1652	24.939
		17611.2542	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0004.CHR ()



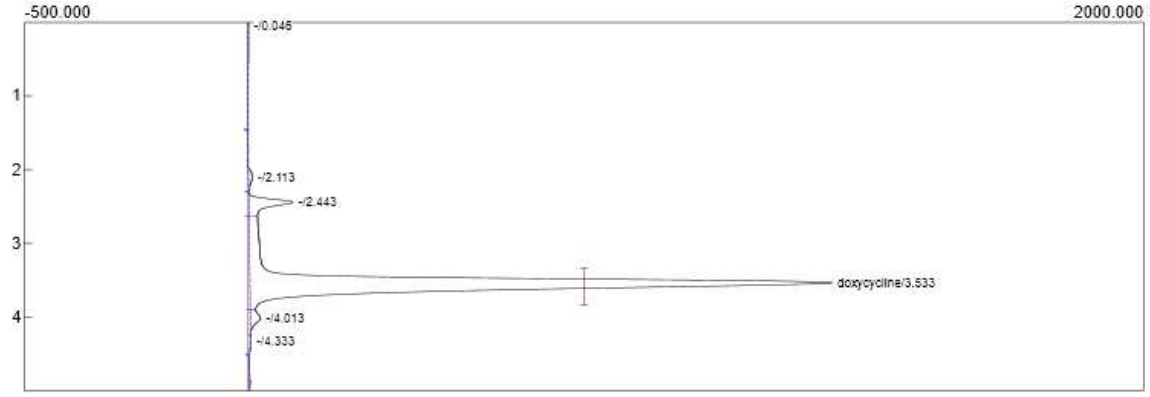
Component	Retention	Area	Height
-	2.120	55.5614	6.304
-	2.440	753.0838	99.643
doxycycline	3.536	17643.6806	1611.687
-	4.026	431.9265	28.570
		18884.2523	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0005.CHR ()



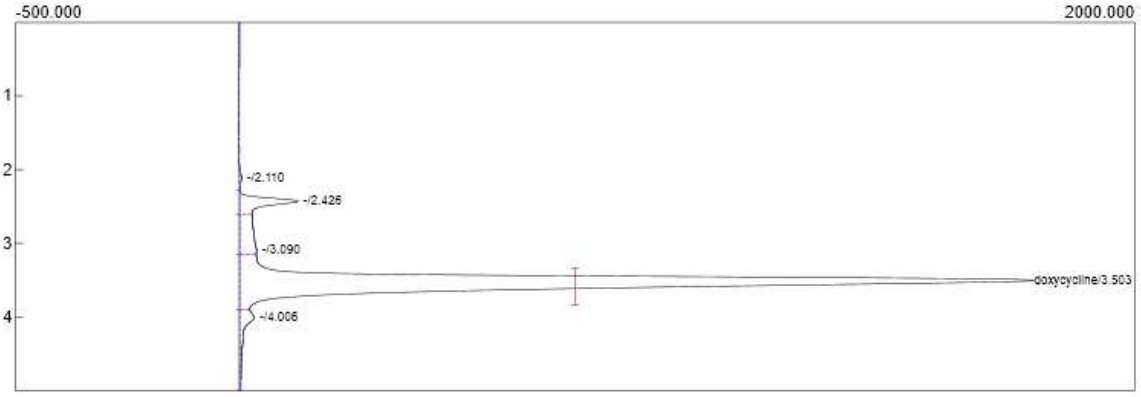
Component	Retention	Area	Height
-	2.210	54.1806	5.753
-	2.533	674.8254	90.528
doxycycline	3.616	17650.6196	1654.709
-	4.110	276.2506	26.019
-	4.433	15.1221	1.773
		18670.9983	

Lab name: Notre Dame
Method: Syringe Injection
Data file: cal check 01.CHR ()



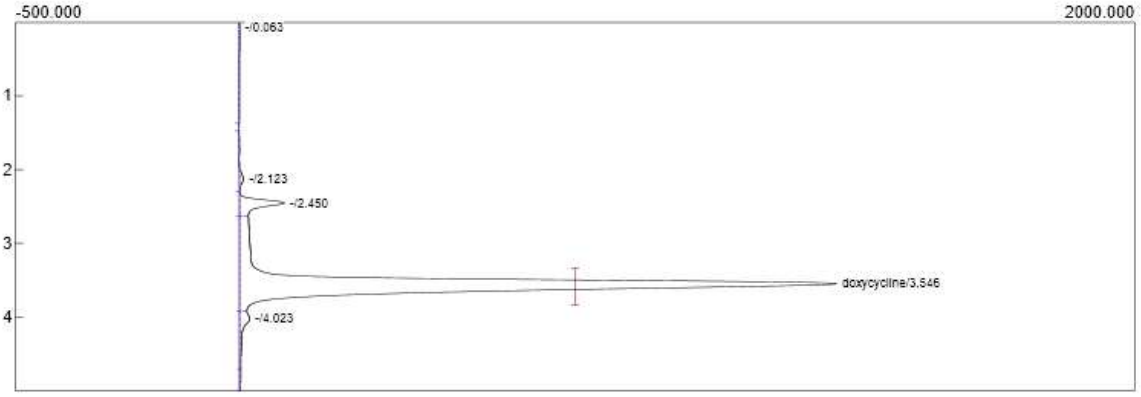
Component	Retention	Area	Height
-	0.046	12.3572	0.273
-	2.113	100.8460	8.955
-	2.443	754.8595	98.045
doxycycline	3.533	13475.3748	1300.585
-	4.013	232.9754	22.515
-	4.333	13.4082	1.626
		14589.8191	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0006.CHR ()



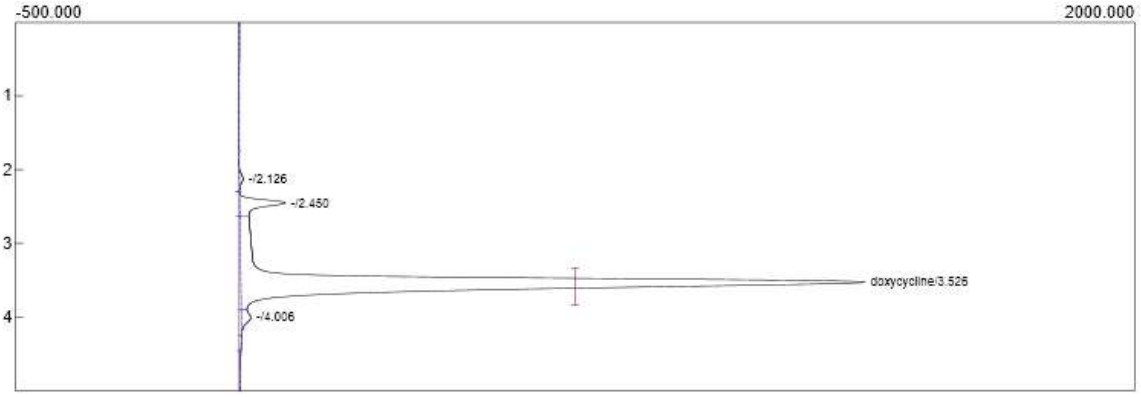
Component	Retention	Area	Height
-	2.110	44.7920	4.504
-	2.426	974.5054	129.898
-	3.090	1030.7965	36.677
doxycycline	3.503	18658.8510	1779.641
-	4.006	473.8195	30.757
		21182.7644	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0007.CHR ()



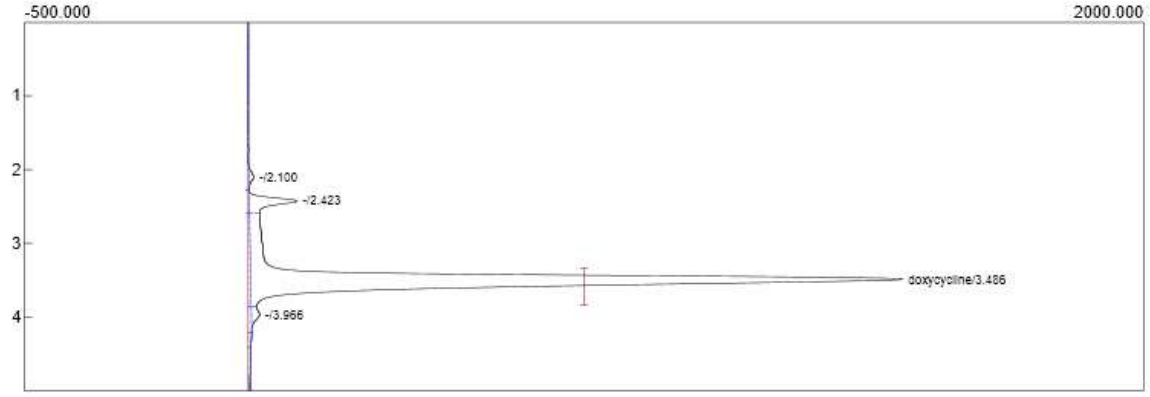
Component	Retention	Area	Height
-	0.063	22.0513	0.684
-	2.123	79.0724	9.130
-	2.450	738.1389	99.161
doxycycline	3.546	13995.5390	1332.890
-	4.023	285.4408	20.729
		15120.2424	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0008.CHR ()



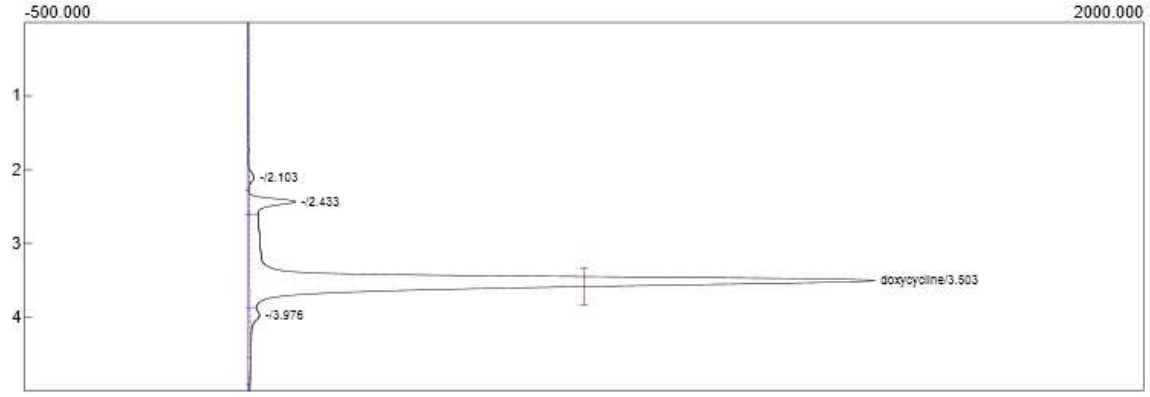
Component	Retention	Area	Height
-	2.126	74.5384	8.754
-	2.450	756.1858	101.906
doxycycline	3.526	14461.7374	1394.520
-	4.006	217.2857	20.798
		15509.7473	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0009.CHR ()



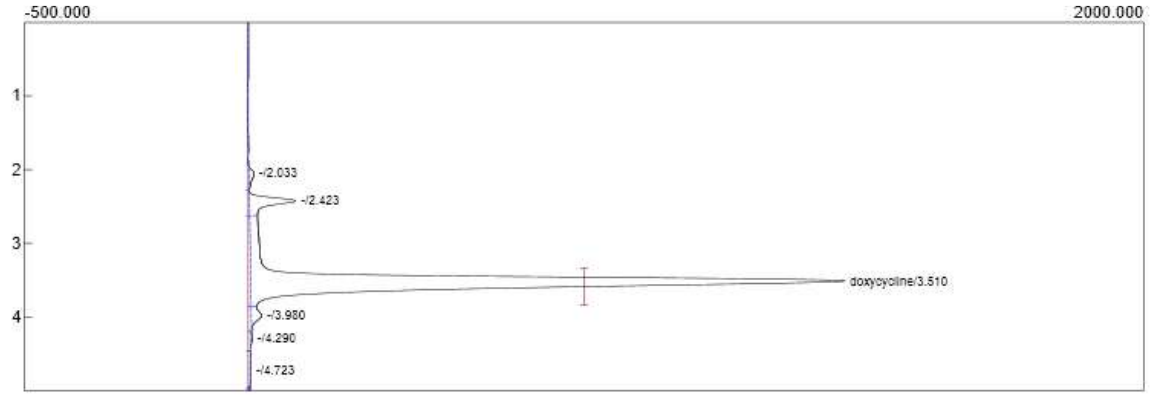
Component	Retention	Area	Height
-	2.100	94.4298	11.008
-	2.423	789.8638	106.344
doxycycline	3.486	15178.5525	1456.693
-	3.966	194.6700	18.590
		16257.5161	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0010.CHR ()



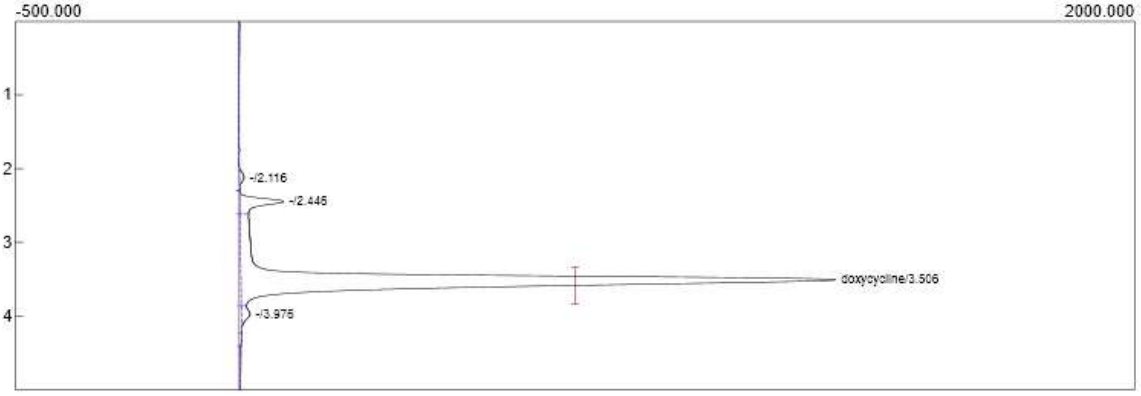
Component	Retention	Area	Height
-	2.103	100.7038	12.075
-	2.433	760.2372	103.891
doxycycline	3.503	14603.3302	1397.258
-	3.976	280.3792	21.223
		15744.6504	

Lab name: Notre Dame
Method: Syringe Injection
Data file: cal check 02.CHR ()



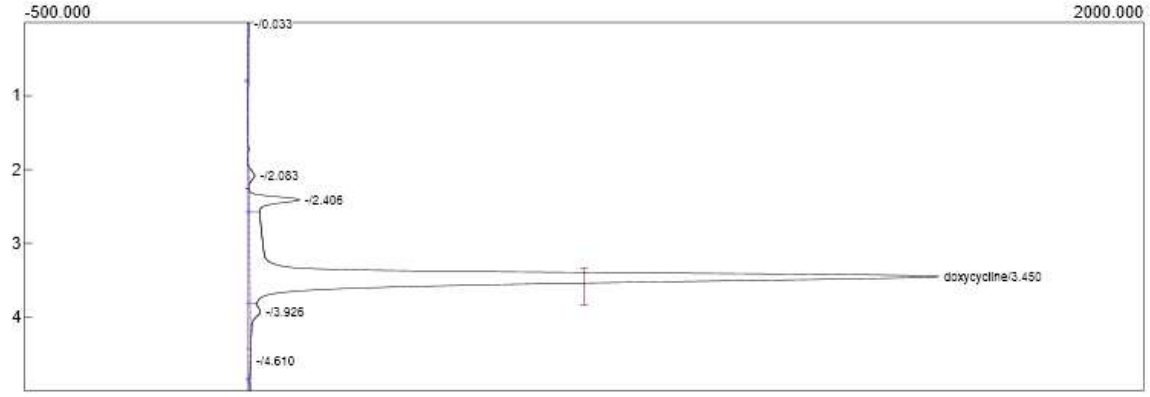
Component	Retention	Area	Height
-	2.033	112.6581	10.981
-	2.423	775.5932	102.956
doxycycline	3.510	13478.9226	1326.675
-	3.980	231.7793	22.700
-	4.290	13.1926	1.620
-	4.723	20.0433	1.527
		14632.1891	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0011.CHR ()



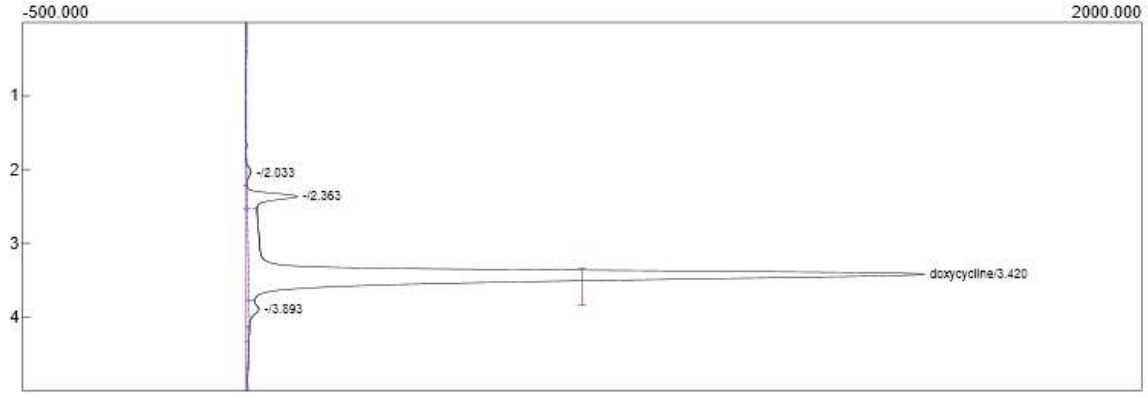
Component	Retention	Area	Height
-	2.116	91.8110	10.625
-	2.446	715.3044	97.801
doxycycline	3.506	13449.0116	1328.866
-	3.976	197.9728	19.244
		14454.0998	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0012.CHR ()



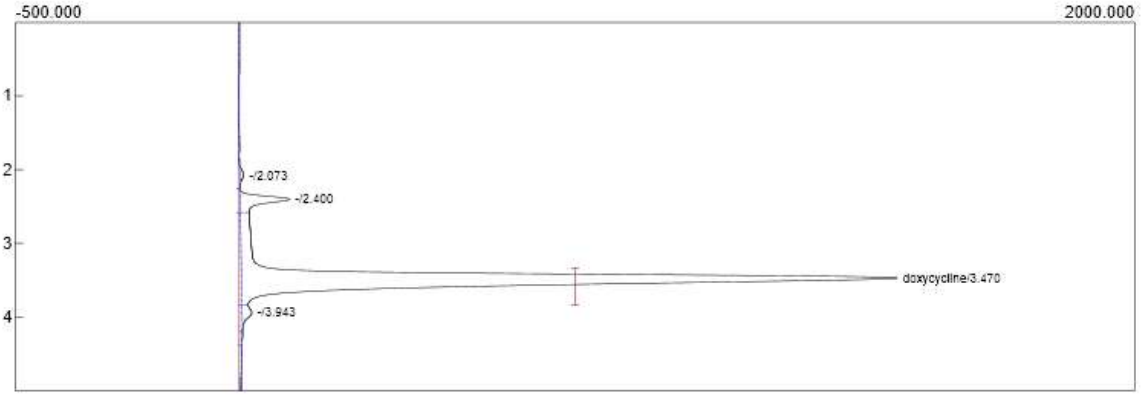
Component	Retention	Area	Height
-	0.033	10.5455	0.532
-	2.083	114.2900	13.637
-	2.406	819.5016	111.922
doxycycline	3.450	15939.7030	1538.945
-	3.926	286.8245	22.788
-	4.610	14.1558	1.141
		17185.0204	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0013.CHR ()



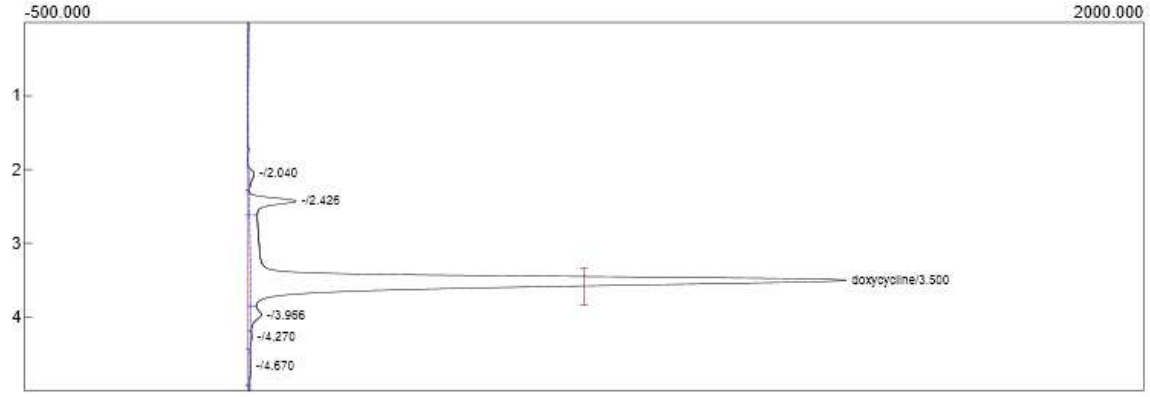
Component	Retention	Area	Height
-	2.033	82.2654	9.778
-	2.383	815.0547	111.720
doxycycline	3.420	15460.5845	1510.475
-	3.893	222.0966	21.491
		16580.0012	

Lab name: Notre Dame
Method: Syringe Injection
Data file: 21L-0003 try 2.CHR ()



Component	Retention	Area	Height
-	2.073	78.7109	9.015
-	2.400	810.4192	110.354
doxycycline	3.470	15107.2994	1465.199
-	3.943	226.6944	21.444
		16223.1239	

Lab name: Notre Dame
Method: Syringe Injection
Data file: cal check 03.CHR ()



Component	Retention	Area	Height
-	2.040	115.2795	11.885
-	2.426	774.4160	104.105
doxycycline	3.500	13410.4159	1330.954
-	3.966	228.6334	22.538
-	4.270	12.2351	1.532
-	4.670	19.8840	1.519
		14560.8639	