Develop Methods to Analyze Pharmaceutical Compounds Using HPLC

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Abstract

Since 2013, there have been 1500 reports of counterfeit drugs to WHO, especially from African, American, and European regions [1]. Tackling the problem of poor drug quality, the Distributed Pharmaceutical Analysis Lab (DPAL) provides a quality analysis to quantify active pharmaceutical ingredient contents with a goal to trigger a report to the MRA or WHO[2]. With the support of DPAL, we would like to perform a series of chromatography experiments using the HPLC in order to identify and quantify the active pharmaceutical ingredients (API) of Amoxicillin and Ciprofloxacin.

Introduction

Drug performance is dependent on the concentration of API in the vessel. One of the most accurate way to measure the concentration of API is High-performance liquid chromatography (HPLC). In our case, we are using reverse-phase HPLC, which consist of injecting the sample to an aqueous mobile phase and run through a non-polar solid stationary phase in a column. A full methodology include Linearity, Precision, Accuracy and Range, Spike Recovery, Degraded Spike Recovery, Limit of Detection and Quantitation, Theoretical Plates and Tailing Factors. In our research, we were testing Amoxicillin and Ciprofloxacin. Amoxicillin is a penicillin-type antibiotic, Ciprofloxacin is a quinolone antibiotics. Both are mainly used to treat bacterial injections.

Figure 1. Chromatogram obtained from the instrument

Materials and Method

Throughout our experiment, we performed serial dilution as needed to create necessary standards. For Amoxicillin, the mobile phase was pH5 20mM NaH_2PO_4 buffer and methanol in 95:5 ratio.The method consist of flow rate 1ml/minute, injection volume 10 uL, wavelength 228 nm, and 4 minutes run time. For Ciprofloxacin, the mobile phase was pH 3.0 0.29mM H_3PO_4 buffer and acetonitrile in 10:90 ratio. The method consist of flow rate 1ml/minute, injection volume 10 uL, wavelength 278 nm, and 6 minutes run time. In both case, the stationary phase was a Nova-Pak 60Å, 4 µm, 3.9 mm X 150 mm C18 column.

Figure 2. The Agilent HPLC instrument

Results

Linearity

We performed serial dilution of the 0.204mg/mL solution for Amoxicillin to get 5 more solution at lower concentrations. We obtained the peak areas for 6 standards in each cases and used that to make a linear regression.

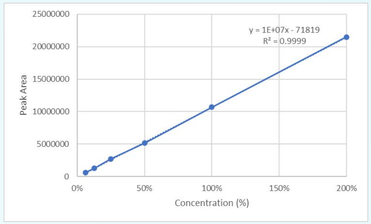

Figure 3. Linear regression and R2 values for Amoxicillin

Precision

We performed 6 consecutive injections of the 100% concentration standard. The peak areas were obtained to calculate the mean, standard deviation, and relative standard deviation.

	Amoxicillin (0.102mg/mL)	Ciprofloxacin (0.103mg/mL)
Mean	5295418	29031192
Standard deviation	105878	55255
Relative standard deviation	2.0%	0.19%

Table 1. Precision test data for Amoxicillin and Ciprofloxacin

Accuracy and Range

We prepared deficient, normal, and overdose standards and measured them against the 200% calibration standard. All standards need to be within +/- 2% of it true concentration and the relative standard deviation of the calibration standard needs to be within 2% to pass the test.

Concentration	Amoxicillin		Ciprofloxacin	
	Area	% Error	Area	% Error
35%	3760738	0.0357%	16606173	0.13%
100%	10799273	0.550%	48262709	1.85%
150%	15988249	0.756%	72426088	1.90%
RSD for cal. std.	0.325%		0.455%	

Table 2. Accuracy and Range test data for Amoxicillin and Ciprofloxacin

Spike Recovery

We prepared a blank and a spiked sample with the same stock solution which consisted of crushed expired tablets mix in water. the spiked sample has an extra 30% of the API. Peak areas were collected to calculate the percentage recovery, which has to fall with 90-110% to pass the test.

$$\%\ \text{spike recovery} = \frac{C_{\text{spiked sample}} - C_{\text{unspiked sample}}}{C_{\text{added}}} \times 100$$

Figure 4. Spike recovery formula

C tablet added (mg/mL)	Measured C different (mg/mL)	%Recovery
0.03006	0.0283	106%

Table 3. Spike recovery data for Amoxicillin

Degraded Spike Recovery

This test has similar preparation and using the same formula to calculate the percentage recovery as the Spike Recovery test. However, the stock solution was made using crushed expired tablets that had been treated in 60 degree Celsius oven for an hour.

C tablet added (mg/mL)	Measured C different (mg/mL)	%Recoveryn
0.03	0.0300	101%

Tablet 4. Degraded Spike recovery data for Amoxicillin

Limit of Detection (LOD) and Limit of Quantitation (LOD)

For Amoxicillin, we diluted 10-fold of the 100% standard solution, 0.102mg/mL and performed 7 consecutive injections of the new sample made. Peak areas were collected to calculate the standard deviation. LOD and LOQ values are 0.000219 and 0.000740 respectively. For Ciprofloxacin, we diluted 0.0064375mg/mL 50-fold.. The LOD and LOQ values are 0.0000453 and 0.000151

$$\text{LOD} = 3*(\text{Standard Deviation of x})$$
$$\text{LOQ} = 10*(\text{Standard Deviation of x})$$

Figure 5. LOD and LOQ formulas

Theoretical Plates and Tailing Factor

Unfortunately. we don't have a way to exact the chromatograph data from the program yet. Therefore, we closely took pictures of the graph and used the website Web Plot Digitizer to create axises and closely insert point follow the graph to get the approximate data for calculation. The theoretical plates and tailing factors for Amoxicillin are approximately 3083 and 0.98 for the 100% standard. For the 100% standard sample of Ciprofloxacin, the theoretical plates and tailing factors are 5747 and 1.17 respectively.

$$N = 5.54 \times \left(\frac{tr}{w1/2}\right)^2$$
$$T = \frac{a+b}{2a}$$

Figure 6. Theoretical Plates and Tailing Factors formulas

Conclusion and Future Work

Our methodology for Amoxicillin passed all the testing protocols and now we are compiling the data into methodology charts and send them to DPAL for validation.

However, for Amoxicillin, we are still in the process of finding way to extract the raw chromatogram data to better calculate the theoretical plates and tailing factor. for Ciprofloxacin, we need to re-make the sample to complete the Linearity test.

In the future, we would like to obtain tablet samples in order to continue working on the Spike and Degraded Spike tests. to complete our methodology for Ciprofloxacin.

Reference

1. World Health Organization. (2017). 1 in 10 medical products in developing countries is substandard or falsified. World Health Organization.
2. Lieberman, M. (2020). HPLC Methodology Manual. Distributed Pharmaceuticals Analysis Laboratory (DPAL).