HPLC for Ampicillin and Cefuroxime

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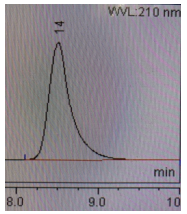
Abstract

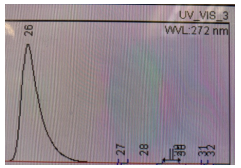
Appropriate levels of an active pharmaceutical ingredient (API) in medicines must be present in a medical product for the patient to receive therapeutic value. High performance liquid chromatography (HPLC) was used to develop a methodology to test for the API in Ampicillin and Cefuroxime drugs. Following the regulations of the Distributed Pharmaceutical Analysis Lab (DPAL) at the University of Notre Dame, the sustainability requirements of linearity, precision, accuracy/range, spike/degraded spike, limit of detection /quantitation, tailing factor, and number of theoretical plates were experimentally determined to meet the excepted standards.[1]

Instrumentation

On a Dionex HPLC machine (right picture) reverse phase chromatography was conducted with a c18 column (stationary phase).The mobile phase for Ampicillin was 20mM NaH_2PO_4 and Methanol (75:25) and for Cefuroxime 20mM NaH_2PO_4 and Methanol (80:20). UV detection was at 210 nm for Ampicillin and 280 nm for Cefuroxime.



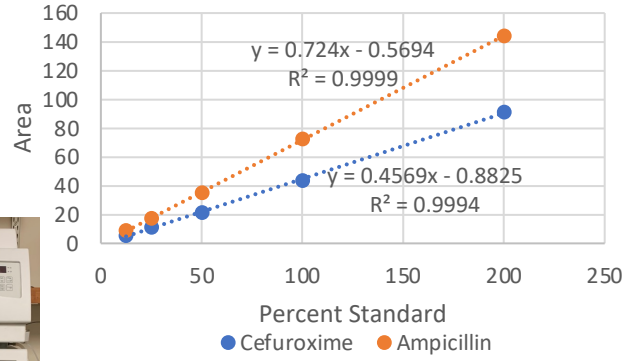
Ampicillin Chromatogram (right)



Cefuroxime chromatogram (left)

Linear Regression

6 calibration standards were plotted with their peak area generating a line of best fit. The R^2 value needed to be above 0.98. The y-intercept error x2 needed to be larger than the y-intercept. The y-intercept errors are 0.680 for Ampicillin and 3.602 for Cefuroxime.



Precision

6 injections of a 100% standard concentration were used to find the relative standard deviation (RSD). The RSD for ampicillin was 1.2% and 1.2% for cefuroxime, needing to be ≤ 2%.

Accuracy and Range

The average of 35,100, and 150% runs were used to find the measured experimental concentration and their percent error ≤ 2%. The relative standard deviation of the 200% external standard were 0.690% (Ampicillin) and 0.775% (Cefuroxime) both ≤2%.

Ampicillin	Theor. Conc.	Exp. Conc.	Percent Error
35%	0.0419	0.0421	0.480%
100%	0.102	0.0102	0%
150%	0.156	0.154	-1.28%
Cefuroxime	**Theor. Conc.**	**Exp. Conc.**	**Percent Error**
35%	0.0197	0.0202	2.54%
100%	0.0595	0.0596	0.168%
150%	0.118	0.118	0%

LOD and LOQ

Limit of Detection is 0.00207 mg/ml (Ampicillin) and 0.000468 mg/ml (Cefuroxime). Limit of Quantitation is 0.00691 mg/ml (Ampicillin) and 0.00156 mg/ml (Cefuroxime.)

LOD = 3*(Standard Deviation of x)

LOQ = 10*(Standard Deviation of x)

Spikes

From an Ampicillin tablet, a stock solution and a spike solution (30% more concentrated) were prepared, run, and subtracted from each other to get the amount of pure standard added 0.031 mg/ml with a percent recovery of 107%. An ampicillin tablet was heated at 60 degrees for one hour to degrade it. A stock solution and a degraded spike (30% more concentrated) were prepared, run, and subtracted from each other to get the amount of pure standard added 0.027 mg/ml with a percent recovery of 103%.

Calculations

A column efficiency measurement, the number of theoretical plates (N) are 6,620 for Ampicillin and 3368 for Cefuroxime. Tailing factor (T) is 1.5 for Ampicillin and for 1.6 for Cefuroxime.

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

$$T = \frac{a+b}{2a}$$

Conclusion and Acknowledgements

The ampicillin methodology is finished and ready to be submitted to DPAL for verification. For Cefuroxime, the spikes need to be completed. We thank the Asher Fund and FDC for supporting this summer research.

Lieberman, M. (2020). HPLC Methodology Manual. Distributed Pharmaceuticals Analysis Laboratory (DPAL). [1]