



APPENDICES

APPENDIX A

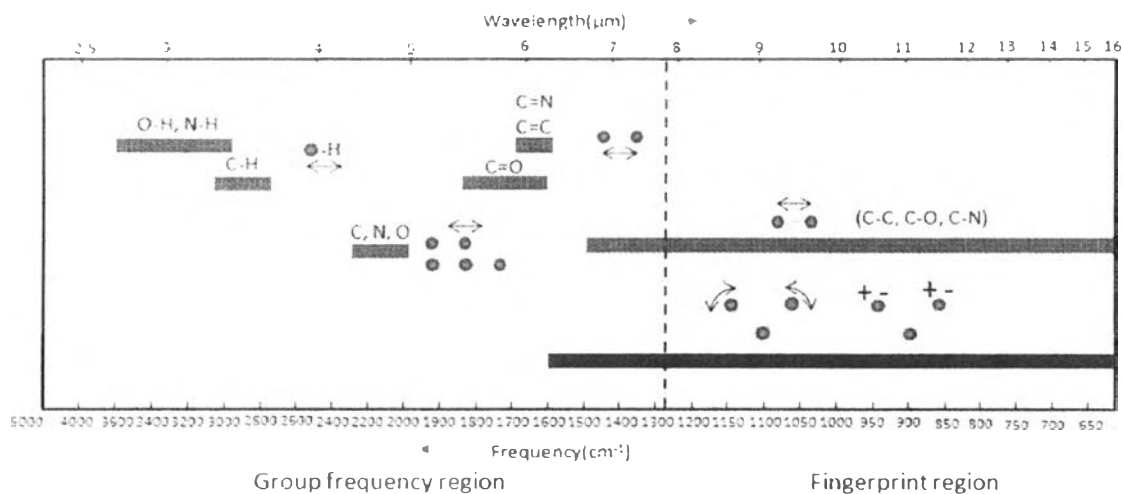


Figure 41 Group frequency and fingerprint regions of the mid-infrared spectrum.

([http://chemwiki.ucdavis.edu/@api/deki/files/9606/group_frequency1_\(1\).gif](http://chemwiki.ucdavis.edu/@api/deki/files/9606/group_frequency1_(1).gif) [Cited 2013 December 23])

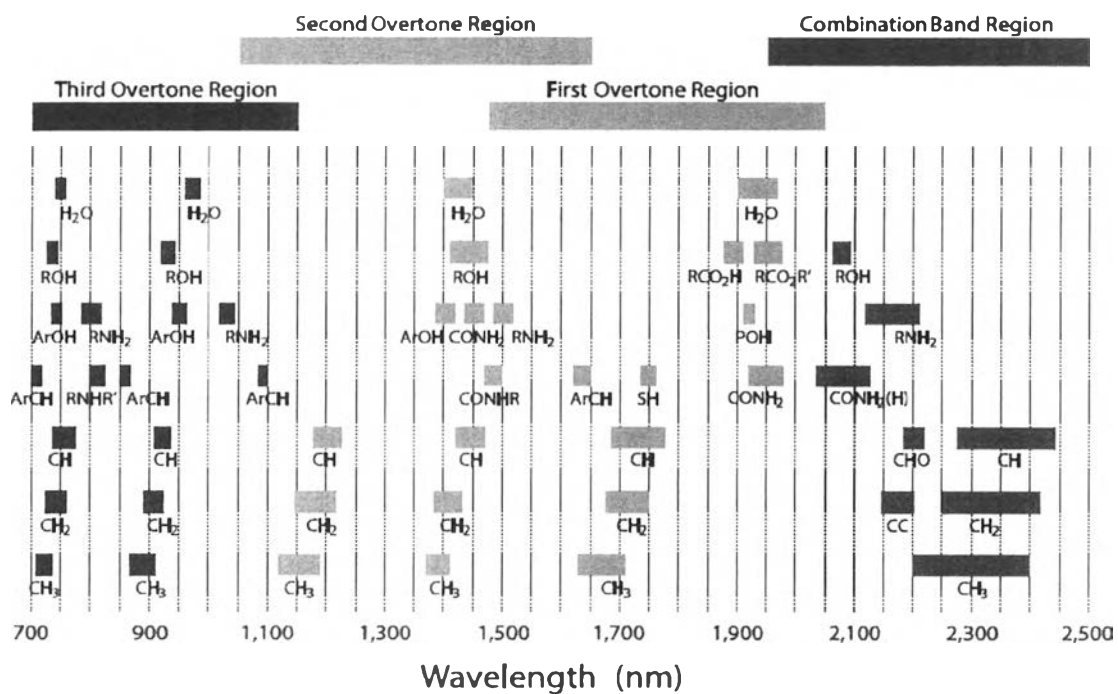


Figure 42 Group frequency and fingerprint regions of the near-infrared spectrum.

(<http://www.spectroscopy-solutions.org/images//Articles/NIR/Jamrogiewicz/fig1.jpg> [Cited 2014 January 9])

APPENDIX B

HPLC method validation

- Wavelength selection

The ultraviolet spectra of azithromycin dihydrate showed the maximum of wavelength absorption at 210 nm. Therefore, 210 nm wavelength was selected to achieve the highest sensitivity for the study as followed by figure 39.

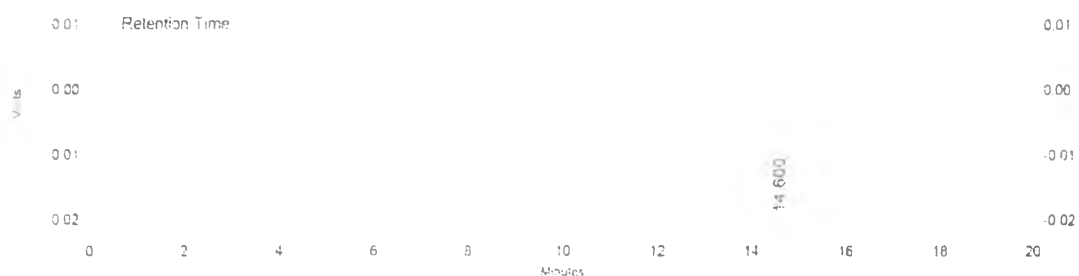


Figure 43 HPLC chromatogram of azithromycin dihydrate (model drug) at wavelength 210 nm.

- Selection of mobile phase

Combinations of acetonitrile and phosphate buffer were tested and the optimum condition, at acetonitrile-phosphate buffer (60:40, v/v), was reached. The obtained chromatogram showed a rapid separation with retention time of azithromycin dihydrate at about 15 minutes.

- Selection of HPLC stationary phase

The results were obtained by using octadecylsilane (ODS, 18). A good peak width was obtained on a C18 column with 250 mm length and 5 μ m particles size.

Linearity

The plot of peak area responses against concentration of azithromycin dihydrate is shown in Figure 40. The linear plot is over the concentration range 50-500 µg/mL yielding a regression equation $Y = 99877x - 62964$ with a coefficient of correlation of 0.9994 and with confidence intervals at $p = 0.05$.

Area under the curve (AUC)

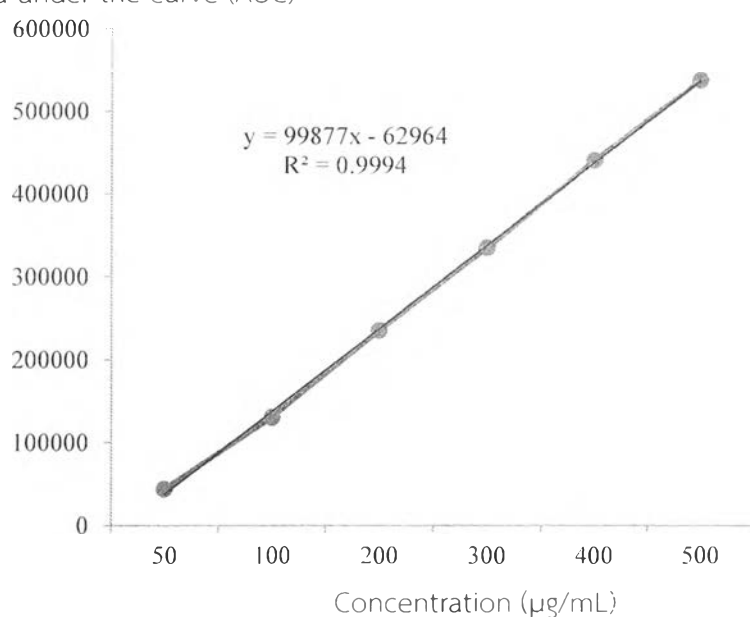


Figure 44 Linearity plot for azithromycin dihydrate drug substance.

Precision

The results obtained for repeatability studies and for intermediate precision are presented in Table 8. The precision of this method has a relative standard deviation (RSD) below 1.91% for repeatability and 1.51% for intermediate precision, which comply with the acceptance criteria proposed (RSD: not more than 2.0%)

Table 8 Intra and inter-day variations for the HPLC method for determination of azithromycin dihydrate.

Concentration ($\mu\text{g/mL}$)	Intra-day precision (% RSD)	Inter-day precision (% RSD)
50	1.28	1.51
100	1.91	0.79
200	1.11	1.93
300	1.04	0.41
400	0.93	1.42
500	0.41	1.38

Accuracy

The results were expressed as percentage recoveries obtained for different azithromycin dihydrate concentration. Table 9 shows that the percent recoveries ranging from 91.70 to 104.04% with RSD ranging from 1.06 to 1.91% which comply with the acceptance criteria proposed (% Recovery range: 80-120%).

Table 9 Accuracy/recovery of the proposed method.

Concentration ($\mu\text{g/mL}$)	% Recovery	% RSD
80	95.30	1.08
120	99.31	1.52
160	98.01	1.45
250	91.70	1.91
350	104.04	1.06
450	93.35	1.63



Detection and quantitation limits (sensitivity)

Results showed that the detection and quantitation limits for azithromycin dihydrate using this method are 6 µg/mL and 19 µg/mL, respectively.

Table 10 The proportion amount percentage of Azithromycin dihydrate in mixture of AZD and EPO in PMS[®] MG15T high speed mixer.

% proportion amount of Azithromycin dihydrate in mixture of AZD and EPO					
Cycle	Calibration set (n=300)	Validation set (n=300)	Cycle	Calibration set (n=300)	Validation set (n=300)
1	95.54	93.20	11	72.49	74.57
2	89.37	91.55	12	73.38	73.65
3	87.08	89.57	13	71.29	72.25
4	86.61	87.31	14	71.78	71.80
5	83.51	85.27	15	71.36	72.00
6	84.66	82.74	16	72.38	71.97
7	79.54	77.78	17	71.83	72.28
8	78.76	77.76	18	70.28	71.25
9	78.43	76.13	19	69.32	71.39
10	76.63	76.14	20	70.56	70.23



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APPENDIX C

The Figure 45 shows that standard calibration curve was determined within range between 10-100 $\mu\text{g/mL}$ by UV-spectroscopy absorption at 213 nm.

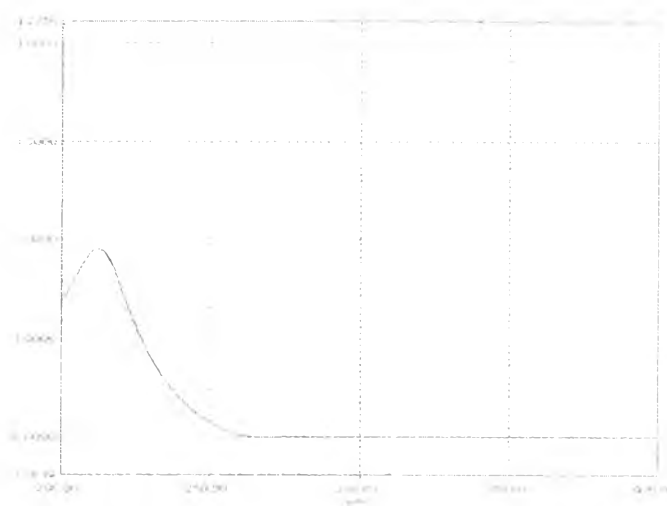


Figure 45 UV peak curve of azithromycin dihydrate.

The equation from standard calibration curve for azithromycin dihydrate in Figure 46 is $y = 0.001x + 0.0057$, $R^2 = 0.9992$.

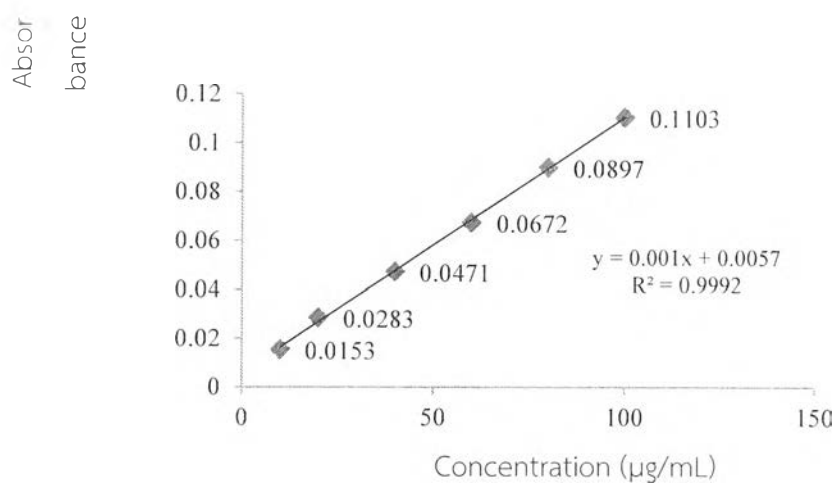


Figure 46 Standard calibration curve for azithromycin dihydrate concentration and UV-absorbance at 213 nm.



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VITA

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