Chapter IV

Results and Discussion

Dicyclomine is a basic compound due to its tertiary amino group. Because of its basic property, dicyclomine, therefore, forms complex with acid dye, bromcresol green. In this study dicyclomine hydrochloride was determined by two methods. Method 1, dicyclomine free base was reacted with bromcresol green in chloroform to produce a yellow - colored complex which was determined spectrophotometrically. Method 2 involved the extraction of an acid - dye complex, dicyclomine hydrochloride and bromcresol green, in an optimum buffer pH, with chloroform. The extracted dye complex was determined spectrophotometrically.

Determination of Maximum Absorption Wavelength

A characteristic yellow - colored chloroform soluble complex was developed in both methods when dicyclomine was reacted with bromcresol green. The quantity of dicyclomine used in both methods was determined by measuring the intensity of color formed at the wavelength of maximum absorption. The absorption spectra of the dicyclomine - bromcresol green complex were obtained by scanning the developed color in the visible range from 350 - 550 nm. The maximum absorption of the complex formed in both methods showed the same absorption peak at 415 nm (Figure 1).

Determination of the Appropriate Normality of Sodium Hydroxide Solution Used in Liberating Dicyclomine Free Base from Dicyclomine Hydrochloride and the Completion of Extraction

The appropriate concentration of alkali and the completion of extraction in method 1 was determined by varying the normality of sodium hydroxide. Only the fifth and sixth chloroform extracts were then determined respectively. The experimental data was shown in Table 1. It was found that the fifth chloroform extract showed absorbance values of 0.037 when using 1 N sodium hydroxide and less than 0.005 for 2 - 6 N sodium hydroxide. Whereas the absorbance values of the sixth chloroform extract was o.o11 for 1 N sodium hydroxide and zero absorbance for 2 - 6 N sodium hydroxide. The results obtained indicated that the optimum normality of sodium hydroxide used was 2 N. The extraction was complete with five extractions.

Determination of the Effect of pH

Development of optimum conditions used in method 2, acid dye technique, involved proper selection of buffer pH. The pH of the aqueous phase was critical to the success of this method. The satisfactory determination of this technique required a minimum blank reading and a maximum absorbance reading for the drug - dye complex in chloroform. In order to achieve this requirement, the effect of pH of the buffer solution on the quantitative extraction of the drug - dye complex with chloroform has been examined. Complex formation

and extraction was studied at various pH values from 1 - 9. As shown in Table 2 and Figure 2, the acid dye, bromcresol green formed chloroform soluble complex with dicyclomine in the pH range 1 - 6; but the maximum absorbance value of the dye complex in chloroform occurred in the pH range 1 - 4. The results indicated that dicyclomine formed complex with bromcresol green in acid condition. Although the pH range 3 - 4 also gave the maximum absorbance value, the pH range 1 - 2 was selected to be the optimum pH for complex formation. Since at pH range 3 - 4 the tendency of aqueous chloroform mixtures to form emulsion occurred. For the proposed method, pH 2 was selected as the optimum pH for quantitative determination of dicyclomine hydrochloride and used throughout this study.

Determination of Stability of Dicyclomine - Bromcresol Green Complex on Time and Temperature

The stability of dicyclomine - bromcresol green complex was studied by determining the effect of time and temperature on the absorbance of dye complex. The absorbance of the complex solution in chloroform was measured at selected intervals of time within six hours at room temperature. The experimental data was shown in Table 3 and Figure 3. Method 1 showed a mean absorbance of 0.390 with coefficient of variation 0.53 %. Method 2 showed a mean absorbance of 0.373 with coefficient of variation 0.44 %. The absorbance of dicyclomine - bromcresol green complex obtained from both methods decreased slightly after two hours and then remained nearly constant in the peroid of six hours. From the presented data it was concluded that

the absorbance showed insignificant difference for a period of six hours in both methods.

The effect of temperature on the stability of dicyclomine - - bromcresol green complex was also investigated. The complex formed was treated at the temperature of 40, 50, 60 and 70° C compared to that treated at room temperature (25°C). The results were shown in Table 4 and Figure 4. The absorbances of complex at room temperature were 0.413 and 0.378 whereas that treated at 70°C were 0.404 and 0.374 in method 1 and 2, respectively. The results obtained from both methods showed that the absorbance of the complex decreased slightly after increasing the temperature. The slightly decreased absorbance at high temperature might be due to the dissociation of the complex. Therefore, it could be concluded that both methods were temperature - insensitive, since there was no significant change in absorbance after increasing temperature.

Since the maximum absorbance reading of the yellow - colored complex was produced immediately after mixing dicyclomine and bromcresol green, the time needed for maximum color intensity and heating process for the completion of reaction were not necessary in both methods. The complex was stable as long as six hours and was unaffected by time and temperature, therefore, the experiment could be done in any time intervals at room temperature.

Determination of Maximum Dye Concentration

The optimum dye concentration was investigated by using various volumes of bromcresol green solution (3×10^{-4} M) with a fixed volume of dicyclomine solution (2×10^{-4} M). The experimental data obtained from both methods were shown in Table 5. The typical curves showing the effect of bromcresol green concentration on absorbance were shown in Figure 5. The results showed that the absorbance increased up to a concentration Of 3 ml x (3×10^{-4} M) and then remained nearly constant to a concentration of 8 ml x (3×10^{-4} M) in both methods. Therefore, a required minimum dye concentration for both methods was 3 ml x (3×10^{-4} M); and in order to ensure an excess of dye used and to achieve a maximum absorbance, the concentration of 8 ml x (3×10^{-4} M) was then used in both methods for quantitative determination. Hence, the required ratio of dye to drug was 4 : 1.

The composition of the complex was studied by the mole ratio method[29,30] which was determined by plotting the mole ratio of dye to drug against the absorbance (Figure 6). Two straight lines of different slope were obtained which the intersection showed a mole ratio corresponding to the combining ratio in the complex. The intersection occurred at the mole ratio of 1 : 1 for all methods suggested that the complex consisted of one mole of drug and one mole of dye. This is as anticipated since there is only one basic center in the dicyclomine molecule. Chemically, the color of product was similar to that formed by reaction between lidocaine or diphenhydramine

with bromcresol green[21,22]. These were in agreement with published data[31 - 33] concerning the reaction of sulfonphthalein dyes with compounds containing nitrogen group. The true nature of these colored products were not known, however, the structure might be postulated in the way similar to those amines and quaternary compound. Since the nature of reaction appeared to be a simple acid base reaction between the nitrogen of the tertiary amino group of dicyclomine and the sulfonic acid group of bromcresol green.

Determination of Adherence to Beer's Law

Calibration curves were constructed to check whether a linear relationship between concentration and absorption exists. The precision of the study was shown by the results of five experiments . The calibration curve obtained from each method obeyed Beer's Law, as shown in Table 6 and Figure 7.

Slopes of the calibration curves obtained from these two methods were different. The slope of the calibration curve from method 1 was 0.0600 which was slightly steeper than that from method 2 (with slope 0.0566), i.e. the sensitivity of the quantitative determination increased with the steep of the curve.

Method 1 showed linear absorbance - concentration relationship when the concentrations of dicyclomine hydrochloride were between 1.38 - 16.51 mcg/ml with 0.75 - 6.67 % of coefficient of varation. Whereas, method 2 showed linear absorbance - concentration relationship

when the concentrations of dicyclomine hydrochloride were between 1.37 - 16.46 mcg/ml with 0.69 - 11.76 % of coefficient of variation.

From the data obtained, method 1 not only showed linear concentration slightly higher than method 2, but also better reproducibility. Therefore, method 1 gave better sensitivity and reproducibility than method 2.

Comparison between Method 1 and Method 2

After studying the experimental parameters for the complete reaction and maximum absorption between dicyclomine and bromcresol green in both methods, the most suitable method was selected for quantitative determination of dicyclomine in pharmaceutical preparations. The conditions studied were summarized and shown in Table.7. The colored complex obtained from both methods was stable to time and temperature, and the composition of the complex was 1 : 1. The linear absorbance - concentration range in method 1 was 1.38 - 16.51mcg/ml and in method 2 was 1.37 - 16.46 mcg/ml, but method 1 gave better reproducibility as seen from the slope and % CV. Moreover, reagent used in method 1 is easy to prepare and stable for a long peroid of time. From the above discussion, method 1 was therefore selected as the method of choice for quantitative determination of dicyclomine hydrochloride.

Determination of the Percent Labelled Amount of Dicyclomine HCl in Dicyclomine HCl Tablet Using Method 1 and USP Method

The content of dicyclomine hydrochloride in dicyclomine hydrochloride tablet was determined by method 1 and official USP method. The results obtained were compared in Table 8. The mean percentage value for ten determinations was 98.66 with 0.55 % of coefficient of variation for method 1 and 97.51 with 0.29 % of coefficient of variation for the USP method. The data presented showed a good precision and a close relationship between the two methods. It was indicated that method 1 gave reproducibility results compare well with those obtained from the USP method.

The results obtained were within USP limit (93.0 - 107.0 %) thus this preparation was continue using for the purpose of testing the accuracy of the method.

Determination of the Percent Recovery of Dicyclomine HCl in Dicyclomine HCl Tablet Using Method 1 and USP Method

The accuracy of the proposed method was checked by determining the percentage recovery. Since other in-active excipients in the preparations might interfere with the absorbance of the sample, therefore, these interferences could be detected by adding standard dicyclomine hydrochloride of various amounts in to dicyclomine hydrochloride tablet, which the exact amount of dicyclomine hydrochloride was known and determined by using the proposed procedure. The

mean percent recoveries were calculated from five replicated determinations (Table 9). Method 1 gave the percent recoveries of 100.60, 101.53 and 100.86 with 0.96, 0.38 and 0.57 % of coefficient of variation for the weight of dicyclomine hydrochloride added : 2, 3 and 4 mg respectively. In USP method, the percent recoveries were 100.80, 100.63 and 100.86 with 1.24, 0.79 and 0.69 % of coefficient of variation for the weight of dicyclomine hydrochloride added as the same in method 1. The results showed that both method 1 and USP method produced good recoveries with high reproducibility. Therefore, the presence of other excipients produced no effect on the development and intensity of color and stability of the complex.

Comparative Analysis of Preparations Containing Dicyclomine HCl

To test the validity of the method, five commercially available formulations with different dosage forms were analyzed by method 1 compared with USP method. All results obtained were the mean value of five replicated determinations of each sample expressed in percentage of the amount labelled (Table 10). In tablet, the results were in close value with 0.49 - 0.71 % of coefficient of variation for the proposed method and 0.19 - 0.36 % for the USP method. In capsule, the coefficient of variation was 0.53 % for the proposed method and 0.63 % for the USP method. In syrup, the coefficient of variation were 1.04 - 1.52 % for the proposed method and 0.67 - 0.90 % for the USP method. The results obtained indicated that the suggested method

could be used for the estimation of dicyclomine hydrochloride in pharmaceutical preparations compared well with USP method.