CHAPTER IV

CONCLUSIONS

4.1 Conclusions

In this research, three analytical methods, namely reverse flow injection analysis with simplex optimization, sequential injection analysis and micellar liquid chromatography were developed for determining of some pharmaceutical preparations. These methods are focus mainly on reduction of reagents and sample consumption, which should diminish the use and generation of hazardous substances in all the steps of the analytical procedure leading to be “Green Analytical Methods” These investigated works are as follows.

4.1.1Reverse Flow Injection Analysis for Determination of Chlortetracycline

A simple reverse flow injection analysis with spectrophotometric detector was developed for chlortetracycline (CTC) determination. The method is based on the reaction of CTC and yttrium (III) in tris – buffer pH 7.5 solution. A small volume of reagent solution (yttrium) was injected into a sample/standard (CTC) carrier steam. Then they were mixed in the reaction coil and followed by spectrophotometric detection at 392 nm. Because of the color forming of CTC – yttrium complex had limitation of the spectrophotometric detection. The use of surfactant as a micellar medium for increasing the signal detection was applied. The CTAB surfactant was used in this study. The chemicals and physicals condition optimizations were

observed by univariate method followed by the simplex method for confirming the results that were done by the univariate method. The suitable conditions were; wavelength 392 nm, Tris-buffer pH 7.5, yttrium concentration 10 ppm, CTAB concentration 5 x 10^{-3} mol l^{-1}, mixing coil length 50 cm, flow rate 2.5 ml min^{-1} and sample injection volume 200 μl. Under the optimum conditions, the calibration curve was constructed. It was linear over the range of 1.0 x 10^{-5} – 1.0 x 10^{-4} mol l^{-1}, with a correlation coefficient 0.9993 and a slope was 3208.52 absorbance unit l mol^{-1} cm^{-1}. The limit of detection (LOD) and the limit of quantification (LOQ) were 8.33 x 10^{-6} mol l^{-1} and 2.76 x 10^{-5} mol l^{-1} respectively. The recoveries for the standard addition method were found to be 99.20 – 101.60 % indicating that the proposed method was accurate. The relative standard deviation was 0.03 % for a series of 11 measurements of 5.0 x 10^{-5} mol l^{-1} CTC solution. This method has been successfully applied to the determination of CTC in commercial pharmaceutical preparations, comparing to the conventional spectrophotometric method. The results indicated that there is no significant difference between these two methods in mean verified by student's t – test value at a confident level of 95 %.

4.1.2 Sequential Injection Analysis for Determination of Zinc in Pharmaceutical Preparations

The SIA method was developed for spectrophotometric determination of zinc in pharmaceutical preparations. The method is based on the complexation between zinc and chromogenic reagent, PAN. Because the complex is insoluble in water, the extraction technique was necessary to apply for spectrophotometric method. . To avoid the extraction procedure, it was found that Zn – PAN complex can be

solubilized by a non – ionic surfactant. The pink colored Zn –PAN complex in micellar media is detected at 553 nm. The proposed method has been successfully applied to the determination of zinc in pharmaceutical preparations. To optimize the conditions, the SIA manifold in Fig2.2 was used the experimental parameters were optimized by the univariate method. The optimum conditions were wavelength at 553 nm, in the slightly basic medium at pH 9.5. The aspiration order of sample and reagents was mixed solution of buffer solution and masking agent, Triton X – 100, sample/standard solution then PAN. The flow rate of aspiration sample, reagent and flow rate of delivering the solution to detector was 25 µl s^{-1} and 40 µl s^{-1} respectively. The aspiration volume of buffer solution, Triton X – 100, sample/standard and PAN reagent were 30 µl, 30 µl, 50 µl and 30 µl respectively. The concentration of reagent, PAN, was 5×10^{-5} mol l^{-1} and 1.0 % v/v Triton X -100. Using the optimized parameters, the calibration curve was constructed. The linear range was studied over the range of 0.1 – 50 µg ml^{-1} with a regression equation: $Y = 10.714X + 0.0837$, with a correlation coefficient (R^2) of 0.9993, where Y and X represent SI signal as peak height in mV, and zinc concentrations in µg ml^{-1} respectively. The limit of detection and limit of quantification of the method were 0.02 µg ml^{-1} and 0.06 µg ml^{-1} respectively. The repeatability of the method was checked for 0.1and 0.5 µg ml^{-1} standard solutions, the % R.S.D. values were 2.27 and 1.25, respectively. The method was pursed by determining 10 standard solutions the inter day and intraday precisions were 2.39 and 1.29, respectively. The sample throughput was 40 h^{-1}. The developed SIA method has been satisfactorily applied to the determination of zinc in Centrum® tablets. Comparative determination of zinc in the same sample solutions by atomic absorption spectrophotometry (AAS) was also carried out. Results obtained by the

proposed SIA and AAS methods were compared favorably verified by the student t-test.

4.1.3 Determination of Active Ingredients in Cough – Cold Pharmaceutical Preparations by Micellar Liquid Chromatography

The micellar liquid chromatographic (MLC) technique was developed and validated for simultaneous determination of some active ingredients (acetaminophen, guaifenesin, phenylephrine, pseudoephedrine and phenylpropanolamine) in cough – cold pharmaceutical preparations. The method utilized a liquid chromatograph incorporated with column (150 x 4.6 mm i.d) packed with C8 (5 μm) using SDS with a small amount of modifier pentanol as mobile phase. The chromatographic conditions were optimized for determination of those drugs, simultaneously. The optimum conditions were consisted of mobile phase 0.150 mol l^{-1} SDS with 2.0 % v/v pentanol as modifier, phosphate buffer pH 7. The mobile phase flow rate for the drugs determination was 1.50 ml min^{-1} with the UV detection at 210 nm. The retention time of acetaminophen, guaifenesin, phenylephrine, pseudoephedrine and phenylpropanolamine were 1.256, 2.329, 3.991, 6.494 and 7.591 minutes respectively with the good separation. The calibration curve were linear over the range 1 – 25 μg ml^{-1} for acetaminophen, guaifenesin, phenylephrine and 10 – 50 μg ml^{-1} for others drugs with R^2 > 0.999. The LODs for acetaminophen, guaifenesin, phenylephrine, pseudoephedrine and phenylpropanolamine were 0.04, 0.3, 0.1, 0.6 and 0.7 μg ml^{-1},respectively. The LOQs for acetaminophen, guaifenesin, phenylephrine, pseudoephedrine and phenylpropanolamine were 0.15, 1.2, 1.0, 1.4 and 1.6 μg ml^{-1} respectively. The precision as intra-day and inter-day reproducibility was

characterized by the spread of the data from ten replicate determinations and RSD were less than 2.1 % for both of the studies. The reproducibilities were made by analyzing standard solutions along 1 month at three different drug concentrations (10, 15 and 20 μg ml^{-1}). The relative standard deviations (RSDs) were below 2.0 %. The accuracy of the method was performed by addition of known amounts of the studied drugs to a known concentration of the commercial pharmaceutical preparations (standard addition method). The recoveries of those drugs were found 99.07 – 101.80 %. The optimized procedure was applied to the analysis of several combinations of the studied cough – cold drug sample in USA. The results obtained from the proposed method were compared with those obtained from RPLC procedure using 55% methanol at pH 7 as mobile phase. It was found that there was no significant difference by t-test between the mean value obtained from both methods at 95 % confidential limit.

4.2 Suggestion for Further Works

For the MLC technique with SDS mobile phase should be developed to determine other protonated amine drugs both in formulations and biological fluids by direct injection.

An essential rule of care is that the micellar phase should never be allowed to remain motionless in a chromatographic system. Furthermore, a cleaning procedure has to be followed to keep the column in good condition. First, in order to prevent precipitation of the surfactant, the micellar mobile phase has to be replaced by 100% pure water and rinsed with it using from 10 to 20 column volumes. The pure water is

then replaced by 100% methanol and rinsed with at least 10 column volumes of this solvent. Finally, the power can be turned off with no risk of adsorbing surfactant inside the column.