CHAPTER 2

EXPERIMENTAL

2.1 Instruments, Apparatus and Chemicals

2.1.1 Instruments and Apparatus

1. Peristaltic pump : Eyela MP-3; Tokyo Rikakikai, Japan.
2. Six port injection valve : Anachem, Germany.
3. Colorimeter : Spectronic 21, Bausch&Lomb, USA.
4. UV-visible Spectrophotometer : Cary 1 E; Varian, Australia.
5. Quartz cell (1 cm) : Varian, Australia.
6. Flow through cell (1 cm) : Hellma, Germany.
7. Recorder : Hitachi, Japan.
8. Autopipet : Finnpipette, Finland.
8. Centrifuge : Dynac, USA.
9. Centrifuge tube
10. Pipette tip
12. Analytical Balance : HM-200; AND, Japan.
13. Analytical Balance : PM 400; Mettler, Switzerland.
14. pH-Meter : Metrohm 632, Switzerland.
15. High performance liquid chromatographic system : Perkin Elmer, USA., consisting of
    a. Autosample, series 200
    b. IC pump, series 200
    c. Vacuum degasser, series 200
    d. UV/vis detector, 785 A
    e. Chromatography workstation.
16. LC-Column, μ Bondapack CN (5 μm, 25 cm X 4.6 mm ID), Supelcosil, USA.
17. Guard column, μ Bondapack CN, Supelguard, USA.

18. Filter apparatus : Supelco, USA.
19. Ultrasonicator : Sonicor, USA.

2.1.2 Chemicals.

Chemicals used were analytical reagent grade except that specified and were used without further purification. The chemicals are listed as follows.

1. Sodium tetraborate : $Na_2B_4O_7$; E.Merck, Germany.
2. Bromocresol green : $C_{21}H_{14}Br_4O_5S$; E.Merck, Germany.
3. Sodium hydroxide : NaOH; Eka Nobel, Sweden.
4. Hydrochloric acid : HCl; BDH, England.
5. Potassium acid phthalate : $KHC_8H_4O_4$; Mallinckrodt, USA.
6. Chloroform : $CHCl_3$; E.Merck, Germany.
7. Acetonitrile : Cl_3CN (HPLC grade); J.T. Baker, USA.
8. Glacial acetic acid : CH_3COOH; E.Merck, Germany.
9. Triethylamine : $(C_2H_5)_3N$; BDH, England.
10. Sodium citrate : $C_6H_5Na_3O_7.2H_2O$; E.Merck, Germany.
11. Citric acid : $C_6H_8O_7.H_2O$; AJAX Chemicals, Australia.
12. Ammonium chloride : NH_4Cl; Riedel de Haen, Germany.
13. Lactose : $C_{12}H_{22}O_{11}$; Difco Laboratories, USA.
14. Sucrose : $C_{12}H_{22}O_{11}$, commercial grade; Mitphon, Thailand.
15. Camphor : $C_{10}H_{16}O$, commercial grade; Natural Camphor Industry, Thailand.
16. Diphenhydramine hydrochloride reference standard (99.4 % purity) : $C_{17}H_{21}NO.HCl$; Department of Medical Science, Thailand.
17. Dextromethorphen hydrobromide reference standard (100.1 % purity) : $C_{18}H_{25}NO.HBr$; Department of Medical Science, Thailand.
18. Bromhexine hydrochloride reference standard (99.6 % purity) : $C_{14}H_{20}Br_2N_2.HCl$; Department of Medical Science, Thailand.
19. Glyceryl Guaiacolate reference standard (98.3 % purity) : $C_{10}H_{14}O_4$; Department of Medical Science, Thailand.

2.2 Solutions

Deionized water was used for all solutions. Except the solution for HPLC, Milli Q water was used.

1. Diphenhydramine hydrochloride standard solutions

Stock diphenhydramine hydrochloride solution was prepared by dissolving an accurately weighed quantity of diphenhydramine hydrochloride reference standard in deionized water to obtain a solution having a known concentration of about 1000 µg/ml.

Working standards were freshly prepared by diluting the stock standard solution.

2. 0.2 M Hydrochloric acid

17 ml of hydrochloric acid (37%) was diluted with deionized water to 1000 ml.

3. 0.2 M Sodium hydroxide

8.4 g of sodium hydroxide was dissolved in deionized water to produce 1000 ml.

4. 0.2 M Potassium biphthalate

40.85 g of potassium biphathalate [$KHC_6H_4(COO)_2$] was dissolved in deionized water, and diluted with water to 1000 ml.

5. Acid Phthalate Buffer, pH 2

50 ml of 0.2 M potassium biphthalate was placed in a 200 ml. volumetric flask, 49.5 ml of 0.2 M hydrochloric acid was added and then diluted to volume with water.

6. Acid Phthalate Buffer, pH 3

50 ml of 0.2 M potassium biphthalate was placed in a 200 ml volumetric flask, 22.3 ml of 0.2 M hydrochloric acid was added and then diluted to volume with water.

7. Acid Phthalate Buffer, pH 4

50 ml of 0.2 M potassium biphthalate was placed in a 200 ml volumetric flask, and 0.1 ml. of 0.2 M hydrochloric acid was added and adjusted with water to volume.

8. Acid Phthalate Buffer, pH 5

50 ml of 0.2 M potassium biphthalate was placed in a 200 ml. volumetric flask, and 22.6 ml of 0.2 M sodium hydroxide was added and adjusted to volume with water.

9. 0.01 M Borax

3.81 g of sodium tetraborate was dissolved in deionized water and diluted with water to 1000 ml.

10. 0.1 M Sodium Hydroxide

4.2 g. of sodium hydroxide was dissolved in water to produce 1000 ml.

11. Bromocresol Green Solution, 1 x 10^{-4} M

69.8 mg accurately weighed bromocresol green powder was dissolved in 2 ml 0.1 M sodium hydroxide and diluted to 1000 ml with acid phthalate buffer.

12. Mobile phase (for HPLC)

The mobile phase was prepared by mixing acetonitrile, water and triethylamine in proportion of 50:50:0.5, respectively. Then the mixed solution was adjusted with glacial acetic acid to a pH of 6.5. The mobile phase was filtered through 0.45 μm filter (millipore) and degassed for 10 min using a ultrasonic bath. The mobile phase was freshly prepared.

13. Diphenhydramine hydrochloride standard solution (for HPLC)

An accurately weighed quantity of diphenhydramine hydrochloride reference standard was dissolved in milli Q water to obtain a solution having a known concentration of about 0.5 mg per ml and filtered through 0.45 μm filter (millipore).

14. Sample preparation (for HPLC)

About 25 mg of Diphenhydramine hydrochloride accurately was weighed and transferred to a 50 ml volumetric flask, dissolved in the milli Q water, diluted with water to the volume and filtered through 0.45 μm filter (millipore).

2.3 Procedure

2.3.1 Determination of diphenhydramine hydrochloride by spectrophotometric flow injection analysis

Diphenhydramine hydrochloride in the sample was determined by forming the ion pair with bromocresol green which was extracted into chloroform layer and then the excess bromocresol green in aqueous layer was injected into the stream of sodium tetraborate solution (pH 9). The blue color of bromocresol green was monitored at 615 nm.

Dye concentration, extraction time and fia parameters (carrier solution, flow rate, sample volume, mixing coil length) were optimized. The interferences, the accuracy and precision of the developed method were evaluated.

2.3.2 Determination of diphenhydramine hydrochloride by HPLC [35]

The standard preparation and the sample preparation were injected the same volume separately into the nitrile (CN) column using acetonitrile-water-triethylamine solution as mobile phase with a flow rate about 1 ml per minute and detected at 254 nm. The chromatograms were recorded and the peak areas of the major peaks were measured. The quantity of diphenhydramine hydrochloride in the sample taken can be calculated by the formula:

$$50C(r_u/r_S)$$

which C is the concentration, in mg per ml, of diphenhydramine hydrochloride reference standard in the standard solution, and r_u and r_s are the peak areas obtained from the sample and the standard, respectively.