CHAPTER III

EXPERIMENTAL

3.1 Chemicals

1. Methylene chloride (MDC),Commercial grade : White Group.
2. Pivaloyl chloride (PVCl),Commercial grade : Autochemical.
3. Acetone (DMK),Commercial grade : Shell, Thailand.
4. Triethylamine (TEA),Commercial grade : BASF.
5. N,N – Dimethylacetamide (DMAC), Commercial grade : BASF.
6. 2- Ethyl hexanoic acid (2- EHA), Commercial grade : BASF.
7. Sodium hydroxide (NaOH),Commercial grade : Chemical Enterprise, Thailand.
8. Hydrochloric acid (HCl),Commercial grade : Chemical Enterprise, Thailand.
9. 2,6-Lutidine (2,6-LTD),Commercial grade : Sumitomo Corporation, Japan.
10. 6-Aminopenicillanic acid (6-APA), Commercial grade : Synpac Phamaceuticals.Ltd, UK.
11. P-OH Phenylglycine Methyl Potassium Dane Salt (HPG.DS.), Ar grade : Nichimen Corporation, Japan.
12. Anhydrous potassium carbonate (K_2CO_3), Ar grade : Merck.
13. Molecular sieve zeolite type 4°A : Autochemical.
14. Calciumhydride (CaH_2), Ar grade : Fluka.
15. Ammonium sulfate (II) hexahydrate ($(NH_4)_2Fe(SO_4)_2.6\ H_2O$), Ar grade : Merck.

16. Amido sulfonic acid (H_2NSO_3H), Ar grade : Merck.
17. Silver sulphate (Ag_2SO_4), Ar grade : Merck.
18. Potassium dichromate ($K_2Cr_2O_7$), Ar grade : Carlo Erba.
19. Sulfuric acid (H_2SO_4), Ar grade : Merck.
20. Mercury sulfate ($HgSO_4$), Ar grade : Merck.
21. 1,10 – Phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$), Ar grade : Merck.
22. Ferrous sulfate ($FeSO_4.H_2O$), Ar grade : Merck.

3.2 Glasswares

1. 2-Necked round – bottom reactor, 500 cm^3 capacity.
2. Vigreux column, length 360 mm
3. Dropping funnel, 100 ml capacity.
4. Round – bottom flask, 250 cm^3 capacity.
5. 4- Necked round – bottom reactor, 1,000 cm^3 capacity.
6. 3- Necked round- bottom reactor, 1,000 cm^3capacity

3.3 Equipments

1. Karl Fischer (KF) : Titrino Model 701 KF, Swiss.
2. UV – VIS Spectrometer : Perkin Elmer Model BIO 20, U.S.A.
3. pH meter : Metrohm Model 691, Swiss.
4. Gas Chromatography (GC) : Packard instrument Model 437A, Netherland.
5. Digital Thermometer (-196^{O}C/ 1090^{O}C) : Fluke Model 52 K/J. USA.
6. Digital Thermocouple (-196^{O}C/ 1090^{O}C) : Fluke Model Digicou DT-240P, USA.

7. Vacuum Drying Oven	: W.C. Heraeus Gmblt . Model VRT 506, Germany.
8. Heating Mantle (600 W)	: Universal Model Series3, USA.
9. Microsyringe (10 µl)	: Hamilton-Benaduz Model Microliter® # 701, Schweiz.
10. Heavy stirrer	: Sicas Model Number 6, Italy.
11. Vacuum pump	: Vacuuband Model RD4, Germany.
12. 64S Multi-Dosimat	: Metrohm, Swiss.

3.4 Gas Chromatography

1. Column name	10% carbonwax 20M, 2% KOH on chromosorb W-HP.
2. Column length	2 m.
3. Carrier gas	Helium gas, flow rate 1 ml / 2 sec.
4. Back pressure	5.0 psi.
5.Column temperature	Initial temperature 60^{O} C. Final temperature 120^{O} C.
6. Injector temperature	180 ^{O}C.
7. Detector temperature	180 ^{o}C.
8. Detector type	TCD.
9. Sample size	1 µl.

3.5 Experimental Procedures

3.5.1 Distillation of Mother Liquor and Triethylamine

1,500-2,000 ml Mother liquor and few boiling chips were added in round – bottom flask equipped with fractioning column and the distillation head (See Figure G-1 in Appendix G). The boiling point was recorded (boiling points of methylene chloride and of acetone are 39.5°C and 56.2°C, respectively) periodically for each interval, composition of drops from distillate was analyzed by GC.and KF (Karl Fischer Method of Water Determination). The percentage recovery was calculated.

Triethylamine was distillated similar to mother liquor but different of boiling point range depending on the temperature at the top of the column (boiling point of triethylamine is 89°C).

3.5.2 Separation of Triethylamine

Triethylamine was separated from mother liquor by adding 50% w/v NaOH in mother liquor in a small separatory funnel. The layers were separated by the aqueous layer by draining using bottom stopcock, and the composition of separated triethylamine was analyzed by GC and KF. Triethylamine was in upper layer as shown in Figure G-2 in Appendix G. The volume was recorded, and the percentage of triethylamine recovery was calculated [16].

The parameters of triethylamine separation are the reaction time, the reaction temperature and pH values.

3.5.3 Analysis of Impurity Triethylamine

The recovered triethylamine (from Section 3.6) was prepared and the 2,6-lutidine was added. The composition of recovered triethylamine was analyzed by GC. The impurities of triethylamine were measured from the peak areas and the retention times of chromatographic peak.

3.5.4 Demoisturization of Triethylamine by Adsorption and Absorption

Small amounts of water can be removed from the recovered triethylamine by allowing triethylamine to stand in direct contact with drying agent for a suitable period of time and followed by filtration . Some common drying agents are BaO, K_2CO_3, CaH_2 , molecular sieve type 4°A.

Five grams of each absorbent was added to 20 ml of recovered triethylamine in separatory funnel for 24 hours at ambient temperature and the solution was filtered. But solid NaOH and 50%w/v NaOH solution could be separated by draining the aqueous layer using the bottom stopcock. The filtrate and purified triethylamine were collected and the composition was analyzed by GC and KF. The percentage triethylamine recovery was calculated.

3.5.5 COD Analysis

The smaller sample portion of Amoxicillin trihydrate mother liquor was diluted to 20 ml and added in a 250 ml refluxing flask. 0.4 g of $HgSO_4$. Several glass beads were added, and mixed, while 30 ml sulfuric acid reagent was added very slowly to dissolve $HgSO_4$. 10 ml 0.0417 M $K_2Cr_2O_7$ solution was added and mixed. The flask was attached to condenser and cooled water was turned on.

The end of condenser was covered with a small beaker to prevent foreign material from entering refluxed mixture and the solution was refluxed for 2 hours. The condenser was cooled and washed down water. The refluxed condenser was disconnected and the mixture was diluted to about twice its volume with distilled water, cooled to room temperature and, was titrated by excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15ml (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator was not critical, the same volume was used for all titrations. At the end point of the titration, the first sharp color changed from blue-green to reddish brown. The blue-green might reappear. In the same manner, a blank contained the reagents and a

volume of distilled water equal to that of sample was refluxed and titrated. The COD value was calculated [17].

3.5.6 Preparation of Amoxicillin Trihydrate

a). Preparation of Mixed Anhydride

D(-) parahydroxyphenylglycine dane salt potassium methyl was added to anhydrous methylene chloride and the suspension was to be cooled to 10 °C. The dimethylacetamide was added in mixture and then cooled to–30°C. 2,6-lutidine was added in mixture and, followed by pivaloyl chloride at such a rate to maintain the temperature around –30°C. The reaction mixture was stirred further for 1 hour at –25 °C to 30 °C, then cooled to -60 °C.

b). Preparation of 6-APA Solution

A suspension of 6-APA in methylene chloride and water was cooled and treated with triethylamine at such a rate to keep the temperature at 12°C to15°C. The 6-APA solution with a pale yellow color would be formed.

c). Preparation of Amoxicillin Trihydrate

The 6-APA solution was added over 30 minutes to mixed anhydride and had been kept at temperature below –65°C. The reaction mixture was stirred further for 3 hours. The mixture was allowed to warm to –33°C, and water was added. The temperature rised to 0°C to 8°C. Concentrated hydrochloric acid was added and the mixture was stirred for 8 minutes at 5 °C to 8 °C. The layers were separated and acetone was added. The pH of the aqueous phase was adjusted to 5.0 with sodium hydroxide and the temperature was kept at 2°C to 5°C. The solution was stirred for 1 hour. Amoxicillin trihydrate was filtrated, washed with cold water and acetone, and dried in vacuum drying oven [3,4].

The composition of amoxicillin trihydrate was analyzed by HPLC, KF and UV/VIS and the percentage yield was calculated.

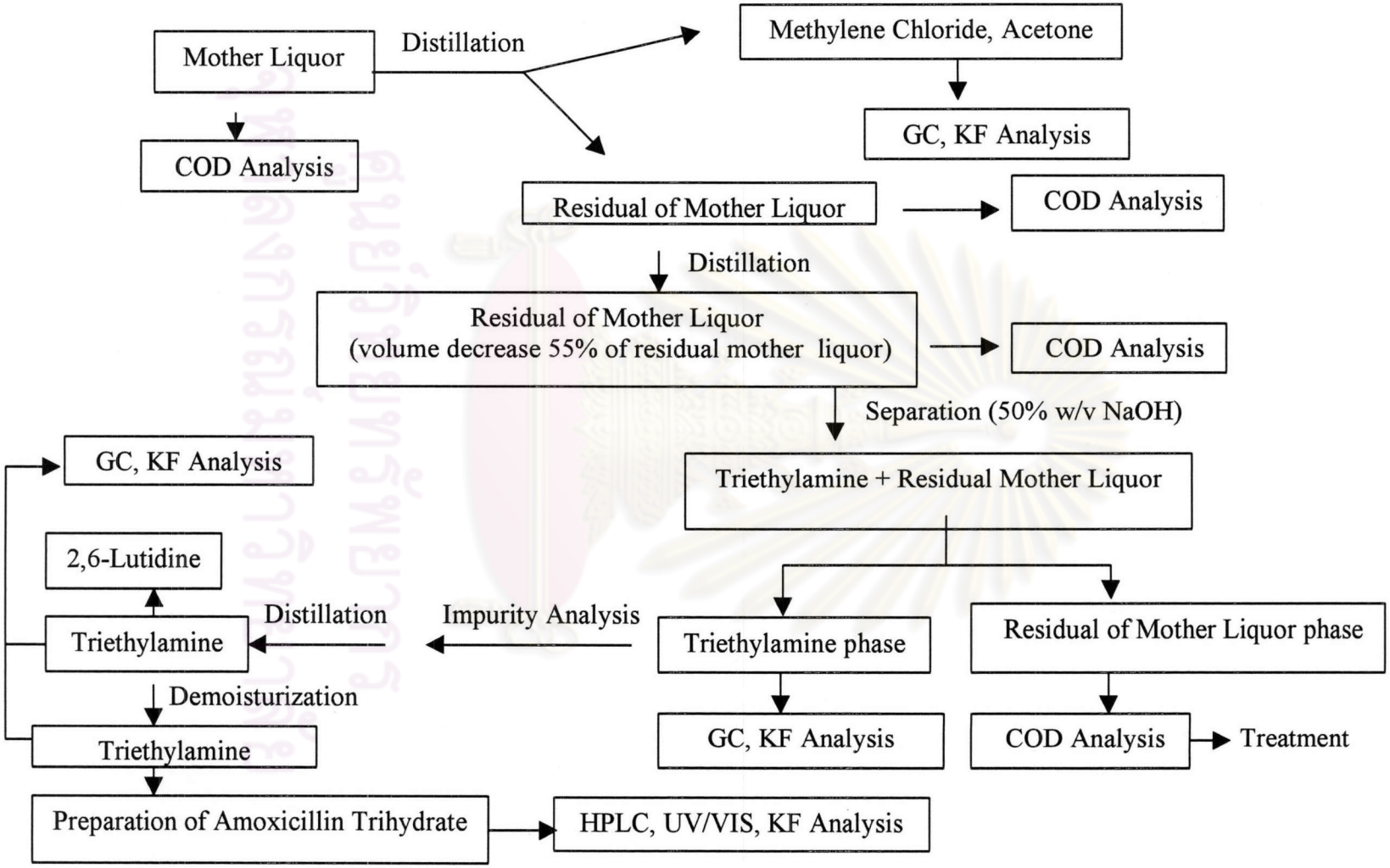

Figure 3.1 The overall schematic experimental of recovery triethylamine from amoxicillin trihydrate.