

## Chapter II



## Experimental

- Apparatus - HPLC (Water Associates and Corporation).  
- NMR spectrometer model JFX-90Q (JEOL. Ltd).  
- pH meter (Beekman Instrument Inc.).
- Material - Codeine phosphate (From the department of Pharmacognosy, Faculty of Pharmaceutical Science, Chulalongkorn University).  
- Actifed Compound Linctus (Lot. No. ACL. 02067).., manufactured by The Wellcome Foundation Ltd. London, England.  
- Codeine phosphate injection from Pharmacy department of Central Chest Hospital.

All solvents used were AR. grade.

Phosphorus-31 nuclear magnetic resonance experiment

Phosphorus-31 nuclear magnetic resonance experiments were performed at 25<sup>o</sup>c with a JEOL FX 90Q spectrometer operating at 90 MHz (at The Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok).

NMR experimental conditions for analysis of codeine phosphate were : Sweep width 15015 Hz, pulse width

12  $\mu$ sec (45°), repetition time 5.7 second, Time spent for each sample 10 minutes.

The peak positions of Codeine phosphate were read out by the computer of the instrument.

Each sample was triplicated analysis and the mean of the intensity of peak was observed.

#### Measurement of Intensity of peak

The intensity of peaks were measured; first, to make the base line by drawing a straight line through the most of highest part of noise, then measured the peak intensity. Drew another line through the most of lowest part of noise and measured the peak intensity. The average of two peak intensities then were used as the intensity of the peak.

#### Effect of pH

##### 1) Preparation of various pH Codeine phosphate solution

Codeine phosphate solution were prepared in various pH, but in the same concentration (15 mg/ml). The solution was prepared by weighing 15 mg. of codeine phosphate and dissolved in a little volume of distilled water, then adjusted to a certain pH with Hydrochloric acid or Sodium Hydroxide, to make the final volume to be 10 ml. The pH of each solution was measured by Beckman Chem-Mate

pH meter. The nine solutions of Codeine phosphate were prepared by the method mentioned above for nine various pH.

## 2) Analysis using $^{31}\text{P}$ -NMR

Each prepared codeine phosphate solution about 2 ml. was transferred into the NMR tube and the tube was placed in the NMR probe. The  $^{31}\text{P}$  -NMR was operated using the condition mentioned above. The spectra were obtained and measurement of the Intensity were made. To show the relation of intensity and pH of solution, the intensity against the pH of solution were plotted.

### Effect of viscosity

#### 1) Method of measurement of viscosity

The measurement of viscosity of syrup using Ostwald viscometer was done as the following step:

- Set the Ostwald viscometer in the vertical position.
- Pipet 5 ml. of the syrup into Ostwald viscometer.
- Compare the time taken for passing two points of the syrup and water at the same temperature.
- Determine the ratio of density of each syrup and water with pycnometer :

compare the weight of syrup and water that has the same volume as pycnometer.

- Calculate the viscosity using the equation

$$\frac{\eta_1}{\eta_2} = \frac{d_1 t_1}{d_2 t_2}$$

where

$\eta_1, \eta_2$  = viscosity of water and syrup

$d_1, d_2$  = density of water and syrup

$t_1, t_2$  = time used of water and syrup

### 2) Preparation of Syrup B.P.

Sucrose 667 grams was weighed and dissolved in purified water, then heated. The syrup obtained was adjusted with boiling water to 1,000 grams. The viscosity of syrup B.P. was measured with Ostwald viscometer using the method of measurement of viscosity.

### 3) Preparation of various viscosity of codeine phosphate syrup

First, the viscosity of syrup B.P. was measured with Ostwald viscometer in order to know the range of viscosity of syrup that should be studied. Then the five various viscosity syrup; 24.66, 30.46, 41.26, 48.37 and 65.22 centipoise were prepared by adding distilled water into the syrup B.P. The viscosity of each syrup were

measured with Ostwald viscometer using the method which mentioned above. Then, 100 mg. of codeine phosphate was added into each prepared syrup. The final solution was shaken to obtain clear solution.

#### 4) Analysis using $^{31}\text{P}$ NMR

Each prepared codeine phosphate syrup about 2 ml. was transferred into the NMR tube and the tube was placed in the NMR probe. The  $^{31}\text{P}$  -NMR was operated using the condition directed above. The spectra were obtained and measurement of the intensity were made. To show the relation of intensity and viscosity of solution, the intensity against the viscosity of solution were plotted.

#### Effect of concentration

Codeine phosphate was weighed and dissolved in distilled water and diluted with the same solvent to make five final concentration 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml and 25 mg/ml. Codeine phosphate solution were prepared in various concentration and analyzed by using  $^{31}\text{P}$  -NMR then the intensity of peaks were plotted against the concentration in order to set up the suitable concentration for making a standard curve.

#### Assay of Codeine phosphate injection using $^{31}\text{P}$ NMR

##### Preparation of standard solution

Codeine phosphate was weighed and dissolved

in distilled water and diluted with the same solvent to make five final concentrations, i.e. 5 mg/ml, 7.5 mg/ml, 10 mg/ml, 12.5 mg/ml and 15 mg/ml. Analyzed these solution by phosphorus-31 nuclear magnetic resonance. The intensity of the peaks were measured at least three times. The means of the intensity of each concentration of solution were converted to logarithm and used to plot against the concentration to prepare a standard curve.

#### Preparation of unknown sample

Transfer codeine phosphate injection about 5 ml. to a tight-container and were analyzed by phosphorus-31 nuclear magnetic resonance.

#### Procedure

Each codeine phosphate solution about 2 ml. was transferred into the NMR tube and the tube was placed in the NMR probe. The  $^{31}\text{P}$  -NMR was operated using the condition mentioned above. Each solution were measured at least three times, converted to logarithm and used to calculate the amount of codeine phosphate in the injection by the equation as follow:

$$y = -0.15644 + 0.055212x$$

where,  $y = \log$  intensity of peak

$x =$  concentration (mg/ml)

The equation was set up from the data of Standard curve by the linear regression method.

Assay of Codeine phosphate injection using U.S.P. method

Transfer an accurately measured volume of Codeine phosphate injection, equivalent to about 75 mg. of Codeine phosphate, to a small separator. About 15 ml. of water and 2 drops of phosphoric acid were added, then extracted with four 10- ml. portions of chloroform and collected the chloroform extracts in a separator. The water was added to wash the chloroform extract containing the sample in separator. Discarded the chloroform extracts. Rendered the solution alkaline with 6N. ammonium hydroxide, and completely extracted the alkaloid with successive 15 ml. portions of chloroform. Evaporated the combined chloroform solution on a steam bath nearly to dryness. The residue was dissolved in about a ml. of methanol, heating, if necessary, added methyl red T.S. and titrated with 0.02 N. sulfuric acid VS. to a faint pink color. Added about 40ml. of freshly boiled, cooled water, and completed the titration with 0.02N. sulfuric acid VS.

Each ml. of 0.02N. sulfuric acid is equivalent to 8.128 mg. of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$ .

## Assay of Codeine phosphate syrup using $^{31}\text{P}$ NMR

### Preparation of Syrup B.P.

Sucrose 66.7 grams were dissolved in purified water by heating until the clear syrup was obtained. The syrup was adjusted with boiling purified water to 100 grams.

### Preparation of Standard Codeine Phosphate syrup

An exact amount of codeine phosphate was dissolved in syrup B.P. to make the final concentration of 7.5, 10.0, 12.5 and 15.0 mg/ml respectively.

### Preparation of Standard curve

Each of standard Codeine phosphate syrup about 2 ml. was transferred to the NMR tube and placed in sample probe. The  $^{31}\text{P}$  - NMR was operated using the condition mentioned above. The intensity of each peak was measured and used log intensity to plot against the concentration to construct a standard curve. The equation for the calculation of codeine phosphate was set up in order to used in the analysis of Codeine phosphate syrup.

### Preparation of sample

A quantity of Codeine phosphate 100 mg. was dissolved in Syrup B.P. to make the final syrup 10 ml.



### Procedure

The sample about 2 ml. was transferred into the NMR tube and placed in the NMR probe. The  $^{31}\text{P}$  - NMR was operated using the condition mentioned above. The Intensity of each peak was measured and calculated the amount of codeine phosphate in the syrup by the equation as follow:

$$y = 0.26886 + 0.027987x$$

where,  $y =$  log intensity of peak  
 $x =$  concentration (mg/ml)

### Assay of Codeine phosphate syrup using High Performance Liquid Chromatography

#### Apparatus

A high performance liquid chromatograph equipped with a fixed wavelength (254 nm.) detector and recorder was used. (Water Associates and Cooperation).

#### Chromatographic condition

The chromatographic solvent was 0.05M.  $\text{KH}_2\text{PO}_4$  in water containing 13% (v/v) methanol. The temperature was ambient and the solvent flow rate was 2.0 ml/min.

Column was  $\mu$ bondapack  $\text{C}_{18}$  of Water Associated (30 cm. long and 3.9 mm. o.d., particle size 10  $\mu$  ).

### Preparation for standard solution

A standard solution containing 20 mg. of codeine phosphate and 12.5 mg. of chlorpheniramine maleate in 100 ml. of chromatographic solvent was prepared.

### Preparation for unknown sample

Dissolved 100 mg. of codeine phosphate in syrup 10 ml. A 2 ml. of codeine phosphate syrup and 12.5 mg. of chlorpheniramine maleate were transferred to 100 ml. volumetric flask and diluted to 100 ml. with chromatographic solvent to make a clear solution.

### Procedure

A unknown sample 60 l was injected into the loop. For purposes of comparison, an identical volume of the standard solution was injected after the assay was eluted. The peak area was integrated by computer. Calculate amount of codeine phosphate using the ratio of area under peak of standard and unknown. The amount of Codeine phosphate in unknown sample was calculated by the formula:

$$\text{Amount of Codeine PO}_4 \text{ in sample} = \frac{A_1 B}{A_2}$$

where,  $A_1$  = ratio of area under the peak (Unknown).

$A_2$  = ratio of area under the peak (Standard).

B = concentration of Codeine PO<sub>4</sub> in Standard solution.

Assay of Codeine phosphate in Actifed Compound Linctus using <sup>31</sup>P NMR

Preparation of standard curve

The preparation of standard curve was done as same as Assay of Codeine phosphate syrup.

Preparation of unknown sample

A 10 ml. of Actifed Compound Linctus was transferred into a 25 ml-volumetric flask and exactly amount 80 mg. of codeine phosphate powder was added into the mixture to make the concentration to be 10 mg/ml then mixed in order to make a clear syrup.

Procedure

Transferred the sample about 2 ml. into the NMR tube and placed in the NMR probe. The <sup>31</sup>P -NMR was operated using the condition mentioned above. The intensity was measured and calculated the amount of codeine phosphate in the linctus by the equation as follow:

$$y = 0.26886 + 0.027987x$$

where, y = log intensity of peak

x = concentration (mg/ml)



Assay of codeine phosphate in Actifed compound linctus by High Performance Liquid Chromatograph.

Preparation of standard solution

A standard solution containing 20 mg. of codeine phosphate and 12.5 mg. of chlorpheniramine maleate in 100 ml. of chromatographic solvent was prepared.

Preparation of unknown sample

A 10.0 ml. of the Actifed compound linctus and 12.5 mg. of chlorpheniramine maleate were transferred to 100 ml. volumetric flask and diluted to 100 ml. with the chromatographic solvent to make a clear solution.

Procedure

Injected 60  $\mu$ l of unknown sample into the loop. For purposes of comparison, an identical volume of the standard solution was injected after the assay was eluted. The peak area was integrated by computer. Calculated amount of codeine phosphate in linctus from ratio of area under the peak using the equation for the Assay of Codeine phosphate syrup by High Performance Liquid Chromatography.