

CHAPTER III

EXPERIMENTAL



Apparatus

A Spectra Physics Model SP 8810 Chromatograph with a Chrom-A-Scope Model 1970 variable-wavelength absorbance detector (BarSpec System Inc., U.S.A.) controlled by HP Vectra VL2 computer and Rheodyne 7125 injector (Altech, U.S.A.) with 20 μ L loop for injection

A LiChrosorb RP-8 and RP-18 column 10 μ m. 4.6 mm X 25 cm. I.D.

A Millipore Alpha-Q water purification system (Millipore, France)

Eldex Heater Column CH-150 (Eldex Laboratories Inc., U.S.A.)

A Water Bath DT CB11 (Hetotherm, U.S.A.)

Micropipettes 10-100 and 100-1000 μ L (Socorex, SWISS)

Cellulose Nitrate Filter 0.45 μ m (Whatman Limited, Japan)

Cellulose Acetate Filter 0.45 μ m (Sartorius, Germany)

Chemicals

1. Phenolic Compounds

Phenol of analytical reagent grade was purchased from Merck Darmstadt , Germany.

Paracetamol , methylparaben , ethylparaben and propylparaben of analytical grade were donated by the Government Pharmaceutical Organization of Thailand.

All of the standard chemicals were of greater than 99.5 percent purity.

2. Organic Solvents

Methanol of analytical grade (AR Grade) was purchased from Merck Darmstadt , Germany.

3. Water

Water was purified on a Millipore Alpha-Q water purification system and filtered through a 0.45 μm cellulose nitrate filter.

Preparation of Standard Solutions

A 10 ml methanolic stock solution of phenols contained 13.6 mg phenol , 0.6 mg paracetamol , 0.8 mg methylparaben , 1.4 mg ethylparaben and 2.3 mg propylparaben was prepared. Subsequently , 20 μ L each of these methanolic solutions were mixed with various amounts of water (0 to 980 μ L) and diluted to 1000 μ L with methanol. These freshly prepared solutions were employed as sample solution.

Study of the Effect of Solvent Strength on Chromatographic Behavior of Analytes on Two RP-HPLC Columns

The solution to be used as the mobile phase was methanol-water (60:40) which was degassed in an ultrasonic bath for least 30 min prior to use. The column was equilibrated with at least 30 mL of mobile phase before injecting a sample solution and was thermostated precisely to ± 0.1 $^{\circ}$ C by circulating water from a water bath between 20 $^{\circ}$ C and 25 $^{\circ}$ C and Eldex column heater between 30 $^{\circ}$ C and 35 $^{\circ}$ C.

The procedure for the study of solvent strength on chromatographic behavior on two RP-HPLC columns , i.e., RP-8 and RP-18 can be described as follows :

All sample solutions were freshly prepared and injected onto the column under the chromatographic conditions as listed in Table 3.1 and 3.2

Table 3.1 Chromatographic conditions for the study of effect of solvent strength on chromatographic behavior of analytes on RP-8 column.

HPLC Parameter	HPLC Condition
Analytical Column	LiChrosorb RP-8 10 μ m 4.6 mm X 25 cm I.D.
Mobile Phase	Methanol/Water , 60 : 40 (v/v)
Flow Rate	1.0 mL/min
Column Temperature	Between 20 °C and 35 °C
Detector	UV 254 nm

Table 3.2 Chromatographic conditions for the study of effect of solvent strength on chromatographic behavior of analytes on RP-18 column.

HPLC Parameter	HPLC Condition
Analytical Column	LiChrosorb RP-18 10 μ m 4.6 mm X 25 cm I.D.
Mobile Phase	Methanol/Water , 60 : 40 (v/v)
Flow Rate	1.0 mL/min
Column Temperature	Between 20 °C and 35 °C
Detector	UV 254 nm