CHAPTER 3

MATERIALS AND METHODS

3.1 Apparatus

- Digital caliper
- Analytical balance (AND, HM-200, Japan)
- Hardness tester (Monsanto, Germany)
- Disintegration apparatus (Hanson Research QC21, USA)
- Dissolution apparatus (Pharmatest type PTWS3)
- Gas chromatography (Chrompack CP 9002, Netherlands) with a flame ionization detector, using a Chrompack CP 9050 autosampler, with a fused silica capillary column (30m, 0.32 mm i.d., film thickness 0.25 µm, Chrompack, Netherlands). Data processing software (Maestro Chromatography Data System, Chrompack) was used for retention time, compensation of gas chromatogram, and peak integration
- High Performance Liquid Chromatography (Perkin Elmer series 200 LC, USA) equipped with a UV detector (model 785A, Perkin Elmer, USA) linked to a data system (Turbochrom, Perkin Elmer, USA) for data acquisition and storage. C18 HPLC column (4.6 X 250 mm, 5µm, Hypersil, England) was used for dye analysis. C18 HPLC column (4.6 x250 mm, 5µm, Altech, USA) was used in dissolution study.

3.2 Materials

3.2.1 Standard substances

- Amphetamine sulphate, methamphetamine hydrochloride, ephedrine sulphate, caffeine phosphate, paracetamol and phenacetin. All standard compounds were supplied from the Drug and Narcotic Institute, Department of Medical Sciences, Ministry of Public Health, Thailand.
- Tartrazine (APS Ajax Finechem, Australia)
- Ponceau 4R (Fluka, Switzerland)
- Sunset yellow FCF (Wako-pure Chemical, Japan)

3.2.2 Chemical substances

- Diphenylamine (Sigma Chemical, USA)

- Methanol (Merck, Germany)
- Polyamide-6-powder (Research grade, Serva, Germany)
- Glacial acetic acid (Merck, Germany)
- Acetone (Merck, Germany)
- Ammonia solution (Merck, Germany)
- Acetronitrile (Merck, Germany)
- Diethylamine (BDH Chemicals, Ltd., England)
- Perchloric acid (Merck, Germany)
- Ammonium acetate (Merck, Germany)

3.3 Methods

The physico-chemical analyses required were conducted using a combination of techniques. To assess colours, pill colours were matched by eye against an appropriate colour chart (Figure 2). Dimensions were measured by a digital caliper. The dimension comprised three categories; diameter, thickness at the rim of the pills and thickness at the middle of the pills. An analytical balance was used to weigh the samples. The average hardness of samples was measured by a hardness tester. Gas chromatography was used to determine the qualitative and quantitative composition of the drugs and their adulterants (32). Disintegration tests were performed using a USP disintegration apparatus. Dissolution profiles also were also investigated using a dissolution apparatus. Methamphetamine content in relation to dissolution study and also food dyes in methamphetamine pills were analyzed and determined by a HPLC.

3.3.1 Sample preparation

3.3.1.1 Physico-chemical study of methamphetamine pills A total of 685 and 298 multi-pill samples seized by the police authorities in the northern region of Thailand during October 2002-March 2003 and October 2003- May 2004 were received and were included in the physical and chemical characterization study. Data from the study would help draw a more detailed picture of distribution patterns of methamphetamine pills and aid in development of drug profile data helpful to enhancing understanding of clandestine methamphetamine laboratories spread throughout Thailand. For physical characterization study, ten pills from each seized package of methamphetamine pills, which appeared to have the same visual

Code สียาบ้า

color	code	color	code	color	code	color	code	color	code
	089		099		060		059		035
	085		100		070		220		037
	139		090		075		239		043
	120		350		051		229		405
	130		080		063		019		055
	131		270		065		032		025
	110		040		069		033		

Figure 2 The colour index chart applied from CARAN d' ACHE, Switzerland

characteristics, were taken at random and checked for average dimension and hardness. Twenty pills were also taken randomly and weighed to determine the average weight. For chemical characterization study, 10 pills from each seized package of methamphetamine pills, which appeared to have the same visual characteristics, were selected. After the pills were ground into powder, 50 mg of powdered sample were dissolved, and volume was then adjusted to 10 ml with 0.5 mg/ml diphenylamine in methanol, following which 1 µl was injected into GC for analysis. Diphenylamine was used as an internal standard.

3.3.1.2 For development of disintegration and dissolution profiles A total of 38 methamphetamine multi-pill samples were included in the study. Specifically, the pills with wY logo (14 samples from 14 seizure cases), WY logo (15 samples from 15 seizure cases) and wy logo (9 samples from 9 seizure cases) were included in this phase of the study. To determine disintegration times, 6 pills from each sample were checked using water at 37°C as a medium. To investigate dissolution rates, 5 samples of each different logo marking were selected. Six pills from each sample group were studied. Dissolution characteristics of the methamphetamine pills were determined using a USP Dissolution apparatus 1. 750 ml of water was used as the dissolution medium. At predetermined time intervals (5, 10, 15, 30, 45, 60 min), 5.0 ml of the

release medium was collected through a filter assembly and replaced with the same volume of fresh water. The amount of methamphetamine was determined by HPLC.

3.3.1.3 For determining food dyes in methamphetamine pills A multi-pill methamphetamine samples in the three popular orange shades seized by the police authorities in the northern region of Thailand was received and were included in the study. Samples (500 mg) were dissolved in 10 ml of water. pH was adjusted to acid with glacial acetic acid, and polyamide powder 2 gm was added, followed by transfer into chromatographic column, washing with hot water (20 ml) 3 times, and acetone 15 ml 1 time. Fractions were collected after passing through the eluting solution (methanol: ammonia solution = 95: 5) and evaporation of the eluted fraction until it was dry. Then a small amount of water was added to dissolve the residue followed by transfer to a 10 ml volume flask, and injection into HPLC with a constant flow rate 1.0 ml/min. The injection volume was 20 µl.

3.3.2 Standard compound preparation

3.3.2.1 For the physico-chemical study of methamphetamine pills Ten mg of each standard compound was dissolved and adjusted in volume to 10 ml with 0.5 mg/ml diphenylamine in methanol, and then 1 µl was injected into GC for analysis.

3.3.2.2 For the study of dissolution profiles. Ten mg of methamphetamine hydrochloride was dissolved and adjusted in volume to 10 ml with water, and 20 µl was injected into HPLC for analysis.

3.3.2.3 For determining food dyes in methamphetamine pills Each 100 mg each of sunset yellow FCF, tartrazine and ponceau 4R standard were dissolved with water and adjusted to 10 ml volume as a stock standard solution. To make a working standard, pipetting of the stock standard solution 5 ml to bring the volume to a final concentration of 250 µg/ml with mobile phase was done.

3.3.3 The conditions of gas chromatography

Injection of samples was made at the split ratio 20:1. The injector temperature was 260◦ C and detector temperature was 280◦ C. The oven temperature was programmed as follows: initial temperature, 130◦C, initial hold, 2 min; temperature program rate, 15◦C /min; final temp, 280◦ C, final hold, 10 min. The carrier gas was nitrogen at a flow rate of 2 ml/min.

3.3.4 The conditions of high performance liquid chromatography

3.3.4.1 For dissolution study of methamphetamine pills The eluent consisted of acetronitrile: 1% aqueous ammonium acetate: 2.5% aqueous diethylamine 40:45:15 (pH 8-9), perchloric acid was added to achieve a pH level of 5.9. Flow rate was set at 1.5 ml/min. The eluent was monitored by measuring UV absorbance at 254 nm. The eluent was injected to HPLC at a volume of 20 µl (32, 33).

3.3.4.2 For study of dye analysis in methamphetamine pills The eluent consisted of tetrabutylammonium hydroxide 0.005 M (pH 4.5): acetronitrile = 60:40. The eluent was monitored by measuring UV absorbance at 254 nm.

3.3.5 Data analysis

A profile was developed of the distribution patterns of methamphetamine pills collected during October 2002 – February 2003 was developed. The prime purpose of this analytic work is to enable the samples to be described in fine detail, thus developing key data for in the subsequent delineation of geographical areas of methamphetamine distribution. This delineation was accomplished using a specialized computer program in conjunction with Geographic Information System software (GIS). Predictive discriminant analysis was done using SPSS software. The relevant data from physico-chemical investigation of methamphetamine pills studied during October 2003- May 2004 was evaluated and interpreted by using ANOVA to highlight the key differences amongst the groups of pills as sorted by logo.