CHAPTER I

INTRODUCTION

Rationale for the Study

Currently, the cosmetic control in Thailand has been implemented by the Cosmetic Act B.E. 2535 (1992). Several cosmetic products including skin whitening products are classified as controlled cosmetic products under the cosmetic Act. According to Notification of the Ministry of Public Health published in Government Gazette volume 125 special section 157 d, dated 25 September 2008, the manufacturer agencies responsible for products in Thailand shall notify about the informations of cosmetic products to the Food and Drug Administration, Ministry of Public Health within 31 December 2010 [1]. In former time, an active ingredient such as hydroquinone used to be allowed to use in whitening products at the maximum limit of 2%. This substance is effective in reducing the creation of melanin, or pigment in the skin. However, it is a prohibited substance for the facial products since 1996 [2]. The hydroquinone is designated as a prohibited substance for the face according to the Notification by the Ministry of Public Health, in title assignation of prohibited substance for the face in the manufacture of cosmetics (No. 2), published in the Government Gazette volume 126 special section 46 d, dated 27 March 2009 [3]. Retinoic acid has been synthetically developed to treat acne, psoriasis and other skin diseases. However, it can cause skin irritation. Moreover, an excessive amount of retinoic acid can cause birth defects, therefore it is categorized as a prohibited substance for the face since 1989 [4]. The corticosteroids, namely betamethasone, betamethasone 17-valerate, dexamethasone, hydrocortisone acetate, prednisolone and triamcinolone acetonide, were prohibited substances for cosmetic products since 1976 [5]. Retinoic acid and all corticosteroids are still prohibited substances as shown in the Ministry of Public Health Notification, published in the Government Gazette volume 125 special section 80 d, dated 12 May 2008 [6].

Nowadays whitening products are very popular among Thais, especially teenagers. The problem of using prohibited substances in whitening cream still have

been found for a long time about twenty years, in particular those counterfeit products which out-of-control by regulator, e.g., marketed in temporary market or irregular imported products. According to the regulation, all kind of cosmetic products are controlled products all ingredients and notified number must be shown on their labeling, therefore the problems of those products containing prohibited substances, e.g., hydroquinone, retinoic acid and corticosteroids without labeling are increasingly occurred. From time to time, the screening test method could not detect trace amount of hydroquinone and retinoic acid in cosmetic products. Routinely, hydroquinone, retinoic acid and corticosteroids can be identified initially by Thin Layer Chromatography, and confirmed by HPLC method. However, these methods of detection are low in efficiency and very much time-consuming. Moreover, the confirmation method for corticosteroids has not yet been established. High Performance Liquid Chromatography with Mass Spectrometry (HPLC-MS) is rather new technology, which not only can separate compounds according to their chemical properties with high fidelity, but also can determine masses of such compounds which lead to identification of the molecules. A preliminary report indicates that the use of HPLC-MS may simplify the detection of corticosteroids. Determination of these banned substances using HPLC-MS is much more important in assuring the safety of cosmetic products than assaying by older techniques. The two main requirements, i.e. high selectivity and high sensitivity, achieved in the overwhelming majority of cases by the combined use of HPLC with MS. Developing new method using HPLC-MS, we will be able to simultaneously detect, confirm and determine the prohibited substances in cosmetic products, and will give consumers higher confidence.

Purpose of the Study

To establish new strategies to simultaneously detect, confirm and determine hydroquinone, retinoic acid, betamethasone, betamethasone 17-valerate, dexamethasone, hydrocortisone acetate, prednisolone and triamcinolone acetonide in whitening products using HPLC–MS in a single test.

Conceptual framework

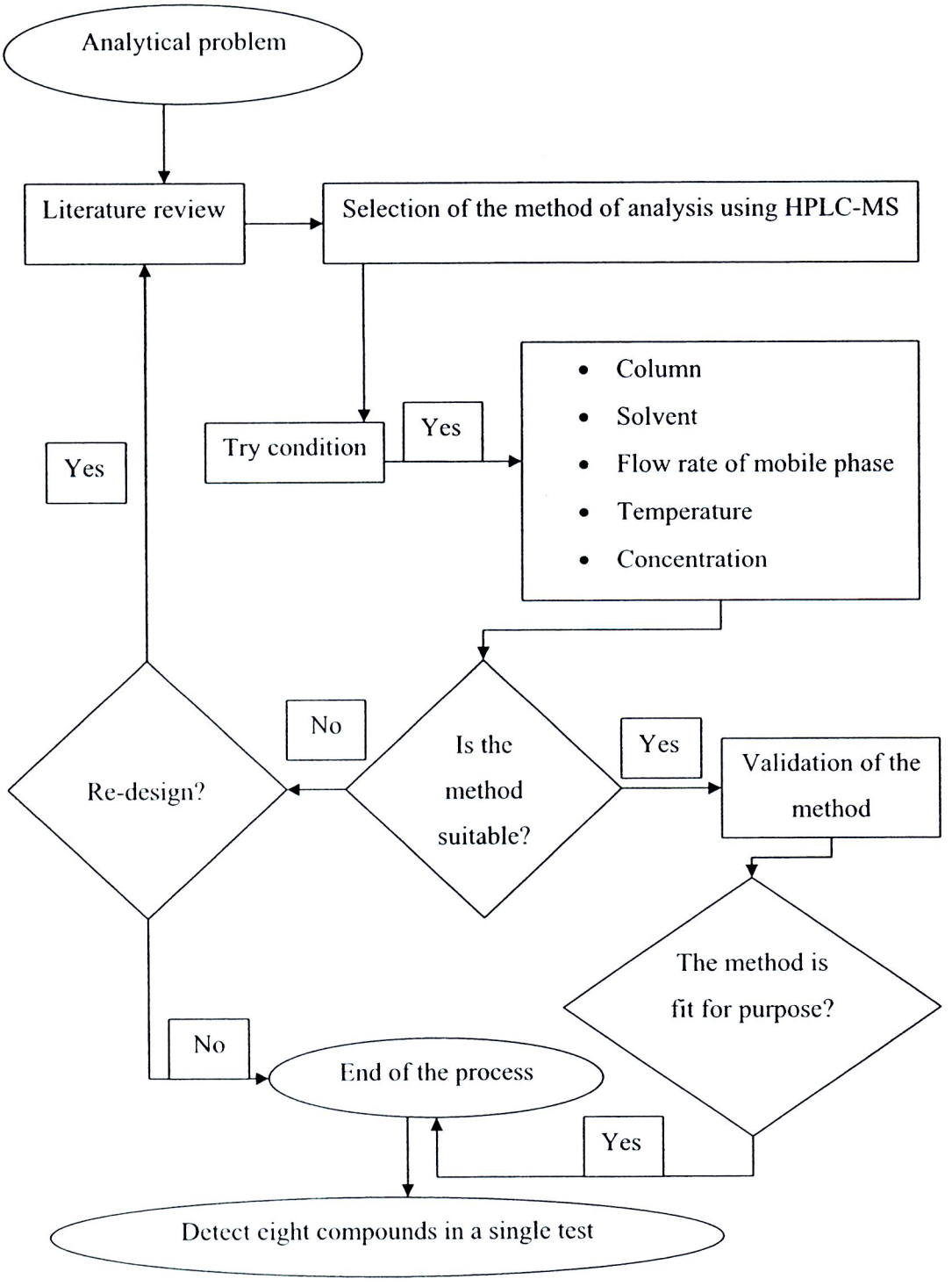

Figure 1 Conceptual framework of the study

Hypothesis

Determination of hydroquinone, retinoic acid and corticosteroids, e.g., betamethasone, betamethasone 17-valerate, dexamethasone, hydrocortisone acetate, prednisolone and triamcinolone acetonide, in whitening products using HPLC–MS will give high selectivity and high sensitivity and can be performed in a single test.

Scope of the Study

The analytical technique, HPLC-MS, is being developed to quantitative analysis of hydroquinone, retinoic acid and corticosteroids, e.g., betamethasone, betamethasone 17-valerate, dexamethasone, hydrocortisone acetate, prednisolone and triamcinolone acetonide in whitening products (cream) in a single test. The developed method should be validated covering validation parameters, e.g., specificity/selectivity, linearity and range, recovery, precision, limit of detection (LOD), limit of quantitation (LOQ) and measurement uncertainty (MU) in Table 1.

Table 1 Acceptance criteria and scope of the study

Validation parameter	Experiment	Evaluation	Acceptance criteria
Specificity/ Selectivity	Analysis of standard, matrix blank and spiked matrix blank	Checking for interfering signals	Absence of interfering signals
Linearity and range - system linearity	At least 6 concentration levels of standard and preferable triplicate or more per level	Evaluation of linear model	$r \geq 0.99$
- method linearity	Analysis of matrix blank which was spiked with analytes at 3 concentration levels (50%, 100%, 150% of sample); analysis of 5 replicates per level		
Recovery	Analysis of matrix blank which was spiked with analytes at 3 concentration levels (50%, 100%, 150% of sample); analysis of 5 replicates per level	Calculation of % recovery	% recovery 90-110

Table 1 (Cont.)

Validation parameter	Experiment	Evaluation	Acceptance criteria
Precision	- Repeatability Analysis of sample of 5 replicates	Calculation of precision data as % RSD of analyte in sample	%RSD $\leq$ 5,
	- Intermediate precision Analysis of sample of 5 replicates repeat in 5 days	Calculation of precision data as % RSD of analyte in sample and analysis of variance (ANOVA)	%RSD $\leq$ 5, $p > 0.05$
LOD	Analysis of matrix blank which was spiked with analytes at lowest concentrations of analyte that could be detected	Checking for evaluation of S/N	S/N $\geq$ 3
LOQ	Analysis of matrix blank which was spiked with analytes at lowest concentrations of analyte that could be quantified	Checking for evaluation of S/N, calculated accuracy and precision data	S/N $\geq$ 10, % recovery 90-110 and % RSD $\leq$ 5
MU	Evaluated measurement uncertainty of the developed method	Calculated expanded uncertainty at 95% confidence level Result $\pm$uncertainty	Combined standard uncertainty should not exceed the predicted Horwitz's RSD_r x 2

Expected benefits

1. HPLC-MS can be used to confirm the banned substances in whitening products for safety of consumers.

2. The new method could probably be further developed for ASEAN cosmetic standard method (ACM).

Expected outcomes

We expect the use of HPLC-MS to effectively separate, simultaneously detect, confirm and determine all eight analytes, e.g., hydroquinone, retinoic acid, betamethasone, betamethasone 17-valerate, dexamethasone, hydrocortisone acetate, prednisolone and triamcinolone acetonide contaminated in a single test.