Chapter 5

Conclusions

5.1 Development of MLC method for parabens analysis

The micellar liquid chromatographic with UV spectrometric method (MLC-UV) was successfully developed for determination of methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP) in various kinds of commercial cosmetics and Thai community cosmetics. Because of an expensive and long length analytical column and toxicity of organic mobile phase, Zorbax SB-C18 column with 12.5 mm length, 4.6 mm i.d. and 5 µm particle sizes (commonly used as guard column) was therefore applied as analytical column incorporating to the use of micellar mobile phase that is biodegradable substance.

Due to column Zorbax SB-C18 is short column, the possibility in application of guard column C18 replacement conventional analytical column in liquid chromatography was investigated. Some chromatographic behavior was studied to prove the performance of this column. It was found that the reciprocal value of capacity factors (1/k) of each paraben were linear to concentrations of surfactant as micelle foam in mobile phase ([M]) with the relation coefficient (R^2) > 0.99.

For the chromatographic separation, the suitable detection wavelength of all parabens and the suitable micellar mobile phase were first investigated. The types of surfactant, anionic surfactant (sodium dodecyl sulfate) and cationic surfactant (hexadecyltrimethylammonium bromide), were studied. The results showed that the suitable detection wavelength and the suitable micellar mobile phase were 254 nm and sodium dodecyl sulfate, respectively.

The optimal concentration of mobile phase and flow rate for simultaneous determination of four parabens obtaining from simplex optimization were then examined. It was found that the optimal conditions were 0.046 mol L^{-1} sodium dodecyl sulfate with a flow rate of 0.612 mL min^{-1} and detection at 254 nm. At these conditions, the chromatographic separation can be achieved within 7 minutes. The

advantage of the optimal conditions obtaining from simplex optimization was robustness of the method when the conditions were little variation.

For validation of the proposed method, working range, precision (repeatability and reproducibility), accuracy and detection limits of all parabens were examined. The calibration curves (working range) were constructed by plotting of peak areas versus the concentrations of each paraben, ranging from 1-100 μmol L^{-1}. All correlation coefficients (R^2) were higher than 0.9990. The precision in term of RSD in repeatability and reproducibility of the method were 0.51-3.76 and 1.24-2.65 %, respectively. The percentage recoveries of extraction step and detection limits of each paraben were in the range of 92.4-109.2 and 0.04-0.10 μmol L^{-1}, respectively. The proposed method was indicated that good linearity, satisfactory precision and sufficiently accurate method.

This method was applied for determination of parabens contents in cosmetic samples. Ten samples of commercial cosmetics and 54-samples of Thai community cosmetics were analyzed by using the developed method. A green extractant, sodium dodecyl sulfate was used to extract parabens in cosmetics, instead of organic solvent and/or solid phase extraction (SPE). It was found that the parabens contents in commercial cosmetics and Thai community cosmetics were within the EU requirement (0.40 %w/w for single paraben and 0.80 %w/w for mixed parabens in acid form).

It can be concluded that the developed MLC-UV method can be minimized analysis cost, chemical consumption and waste generation. This method was an effective and inexpensive method that was cheaper than the analysis of parabens using the most HPLC techniques (used conventional analytical column and organic solvent as mobile phase) about ten times.

5.2 Development of LC-MS/MS method for perchlorate analysis

The second part of this work involves the development of LC-MS/MS method for determination of perchlorate in environmental samples such as soil, drinking water, tap water and ground water. Under reversed phase liquid chromatographic conditions, ionic compounds are weakly retained. Therefore, ion-

interaction (ion-paring) reagent was added in chromatographic system for ionic separation on C18 column. This method was based on ‘ion interaction (ion pair) chromatography’.

First, the suitable cationic ion-pairing reagent and ion-pairing formation in liquid chromatographic system were investigated. It was found that the suitable cationic ion-pairing reagent and ion-pairing formation in LC system were hexamethonium bromide and on-column ion-pairing formation with directly injection of perchlorate, respectively. Perchlorate that formed with hexamethonium bromide obtained an adduct ($DClO_4^+$) at *m/z* 301 and 303. The isotopic ratio of $D^{\mathbf{35}}\mathbf{Cl}O_4^+$ (*m/z* 301) and $D^{\mathbf{37}}\mathbf{Cl}O_4^+$ (*m/z* 303) were 3:1 because of chlorine isotopic ratio (^{35}Cl and ^{37}Cl). For MS/MS detection with precursor ion of *m/z* 301, product ions of $DClO_4^+$ were *m/z* 128, 187 and 242 that were used as qualifying ions for confirmation the presence of perchlorate. Base peak which used as quantifying ion for quantitation of perchlorate content was *m/z* 242. Moreover, this developed confirmatory and quantitative LC-MS/MS method that used chlorine isotopic ratio and oxygen-labeled sodium perchlorate ($NaCl^{18}O_4$) as internal standard was performed for perchlorate analysis.

The liquid chromatographic conditions such as compositions of mobile phase and injection volume were then optimized. The results showed that injection volume was 100 µL loop and the optimal LC conditions were methanol and 10 µmol L^{-1} hexamethonium bromide (10 : 90) as mobile phase with a flow rate of 0.40 mL min^{-1}. The MS/MS conditions such as collision energy and scanning mode were investigated. It was found that the suitable scanning mode of ion-trap in MS/MS operation (Xcalibur software) was full scan mode and collision energy for dissociation step was 30.0 %.

Next, the analytical features such as linearity range, repeatability, reproducibility, recovery, limit of detection (LOD) and interferences study were studied for validation of the method. The linearity range of perchlorate was 4-1000 µg L^{-1} with correlation coefficient (R^2) at 0.9998. The precision in term of RSD in repeatability and reproducibility of the method were 9.80-15.54 and 15.57-19.77 %, respectively. The recoveries of drinking water and tap water were 86.59 and 80.10 %, respectively. The detection limit of the method was 2 µg L^{-1}. For interferences study,

chloride (Cl^-), nitrate (NO_3^-), nitrite (NO_2^-), carbonate (CO_3^{2-}), bromide (Br^-), iodate (IO_3^-) and iodide (I^-) at concentration of 50 mg L^{-1} were not interfered perchlorate analysis in environmental samples. The tolerant limits of phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) were 0.5 mg L^{-1}. However, in the case of sulphate, it required the sample clean-up prior to LC-MS/MS analysis. Solid phase extractions (OnGuard-Ba) were therefore used to remove the spectral interferences such as isotopic hydrogen sulphate (as same as mass of perchlorate at *m/z* 99). It was found that the isotopic hydrogen sulphate can be tolerated to approximately 100 mg L^{-1}.

The proposed method was applied for perchlorate analysis in environmental samples. Soils and ground water were collected from Chiang Mai. Drinking water was purchased from convenience store. Tap water was collected from Learning Center 5, Faculty of Science and Technology, Thammasat University. All samples were prepared by using OnGuard-Ba cartridges for interferences removal. The results showed that all samples were not found perchlorate.

It can be concluded that the developed LC-MS/MS method can be successfully analyzed perchlorate anion on reverse phase LC using dicationic ion-pairing reagent. This confirmatory and quantitative LC-MS/MS method was also illustrated that it was sufficiently sensitive and effective for measuring the trace perchlorate in environmental samples. All analytical performances were agreed with U.S. EPA requirements (EPA method 314.1 and 331.0).