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Greener liquid chromatography using a guard column with micellar mobile phase for separation of some pharmaceuticals and determination of parabens

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ABSTRACT

In this research, a greener chromatography employing a short column, Zorbax SB C18 cartridge (12.5 × 4.6 mm, 5 μm) commonly used as a guard column in a reverse phase high performance liquid chromatography (RP-HPLC), was utilized as the analytical column in conjunction with a more eco-friendly micellar mobile phase of sodium dodecyl sulfate (SDS) for separation tertiary mixtures of local anesthetics and antihistamines; and binary mixture of colds drugs; and quaternary mixture of some parabens with different separation conditions. The chromatographic behavior of these analytes was studied to demonstrate separation efficiency of this guard column in a micellar mobile phase. Moreover, this column and SDS mobile phase was exploited for determination of parabens in 64 samples of cosmetic product, both those that were produced locally in the community and those that were commercially manufactured. Linear calibration graphs of the parabens as detected at 254 nm were obtained in the range of 1–100 μmol L^{-1} with $R^2 > 0.9990$. Percentage recoveries were 92.4–109.2 with %RSD < 3, and the limit of detection and quantitation were 0.04–0.10 and 0.20–0.80 μmol L^{-1}, respectively. This analytical system is not only greener but also faster and employing simpler sample preparation than a conventional liquid chromatographic system.

1. Introduction

For two decades, research in Green Analytical Chemistry (GAC) has significantly grown. As presented in the review by de la Guardia et al. [1] in 2008, GAC has focused on replacement of toxic reagent, minimization of reagent consumption and waste generation. In 2010, Welch et al. [2], reviewed "Greening Analytical Chromatography" and described several main strategies, especially for liquid chromatography, which included a reduction of the amount of waste (concerning particle size of stationary phase and column diameter [3,4]), using more specialized equipment (such as ultra high performance liquid chromatography (UHPLC) [3–5], and microscale or nanoscale HPLC [6,7]). Alternative green liquid chromatography was demonstrated by Chen et al. [8] in 2010, using gradient stationary phase optimized selectivity liquid chromatography (SOSLC). Moreover, some researchers focused on applying greener mobile phase system such as an ethanol replacement of acetonitrile [9–12], subcritical water in high-temperature liquid chromatography [13], cyclodextrin modifier in hydro-organic mobile phase to reduce proportion of organic mobile phase and using a biodegradable micellar mobile phase substance [14–23].

Micellar liquid chromatography (MLC) is a reversed-phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant above its critical micellar concentration (CMC). Since its development in the 1990s it has been used to emphasize retention mechanisms or chromatographic behaviors [14–16] and applications in areas such as pharmaceuticals [16–18], cosmetics [19–21], environmental [22], and clinical [23] studies. Micelle is not only a greener mobile phase but a surfactant monomer also acts as a modifier attaching to the alkyl long chain boned stationary phases and creates a structure similar to an open micelle [14]. The surfactant monomer is also surrounded in a bulk aqueous phase, leading solutes to partition at three phase equilibriums which is different from the two phase equilibriums of classical RPLC. MLC has several advantages over other chromatographic methods because of the many possible

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adjustable conditions. These advantages include low toxicity, low volatility, the possibility of simultaneous separation of ionic and non-ionic compounds, direct injection of biological fluids, and high separation selectivity.

Despite a conventional long separation column, which is normally used in HPLC, provided good separation efficiency, it is very expensive and usually involves long separation time and very high pressure operation. Therefore, a short guard column is used to protect this analytical column. For uncomplicated separation, a short or ultra short column has been used in HPLC. For example, Le and Ma [24] used a 3-cm guard column to analyze arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA) within 3 min using a combination between fast HPLC and hydride generation atomic fluorescence spectrometry. Timmins [25] used short (3–5 cm length) or ultra short (4 mm length) HPLC columns for dissolution testing of antihypertensive drug formulations. Youngvises et al. [26] incorporated MLC with a short Zorbax SB C18 column, 12.5 mm in length and successfully separated and determined tolperisone and lidocaine in pharmaceutical formulations.

The aim of this work is to exploit a green analytical chemistry HPLC method by focusing on replacement of toxic reagent, minimization of reagent consumption, waste generation and analysis time by using a short guard column (Zorbax SB-C18 guard cartridge of 12.5 mm long, 4.6 mm i.d. and 5 μm particle size) in combination with micellar mobile phase (SDS) for determination of four parabens (methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP) in cosmetic samples. The proposed system was optimized by a simplex optimization procedure. Moreover, the chromatographic behaviors of these parabens and some drugs in the groups of antihistamines, local anesthetics and cold drugs were studied to present the efficiency of this guard column in micelle mobile phase.

2. Experimental

2.1. Reagents and chemicals

All reagents and chemicals used were of analytical-reagent grade. Methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP), *p*-aminobenzoic acid (PABA), procaine hydrochloride (PRO), lidocaine hydrochloride (LDC), prilocaine hydrochloride (PLC), bupivacaine hydrochloride (BVC), pheniramine maleate salt (PNM), chlorpheniramine maleate salt (CPM), diphenhydramine hydrochloride (DPM), pseudoephedrine hydrochloride (PSE), phenylpropanolamine hydrochloride (PPA), and tripolidine hydrochloride (TRI) were all purchased from Sigma (Steinheim, Germany). Sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium bromide (CTAB) were purchased from Fluka (Buchs, Switzerland). HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Water was deionized and purified on a DI water purification system (ELGASTAT Option3A, 13172G, Elga Ltd., Bucks, England) and used to prepare all solutions.

The cosmetic samples consisted of 10 manufactured products (sample No. 1–10) and 54 Thai community products (sample No. 11–64) in the forms of cream, lotions, shampoo, conditioner, and liquid soap. They were purchased from local markets and stores in Thailand. A placebo sample (without parabens) was prepared from a shampoo made at Rajamangala University of Technology, Thanyaburi, Pathum Thani province, Thailand.

2.2. Instrumentation and chromatographic conditions

Various high performance liquid chromatographs were used for different sets of sample, as described in the following sections. All systems were operated at room temperature using Zorbax SB-C18 column (12.5 × 4.6 mm i.d., 5 μm) and UV detection (Agilent Technologies, USA). SDS at a concentration higher than the critical micellar concentration with and without short chain alcohol modifier was used as a mobile phase. It is necessary to modify the stationary phase with a SDS mobile phase for about 30 min prior to use. All solutions were filtered through a 0.45 μm nylon membrane filter (Lubitech Technologies Ltd., China). All measurements were carried out with at least three replicates.

2.2.1. Chromatograph for separation of some antihistamines, local anesthetics and cold drugs

For antihistamines (CPM, PNM and DPM) and local anesthetics (BVC, LDC and PLC), a liquid chromatograph, LC-20AT (Shimadzu, Japan) was used for the analysis featuring a quaternary gradient pump, UV detector and 20-μL loop injector.

For the study of cough and cold drugs (PSE, PPM, CPM and TRI), a Hewlett Packard 1100 (Agilent Technologies, USA) with a variable wavelength detector, an isocratic pump, a 20 μL loop injector and a HP 35600 integrator was used.

2.2.2. Chromatograph for parabens analysis

HPLC analyses were carried out with a Hewlett Packard 1090A (Agilent Technologies, USA) featuring a solvent delivery pump system (79835A), a diode array detector (79880A) and a 20-μL loop injector. Data acquisition and processing were controlled by the HP ChemStation software (ChemStation version A.08.03) from Hewlett Packard.

2.3. Stock standard solution preparation

The stock standard solutions of antihistamines, local anesthetics, cough and cold drugs were prepared at a concentration of 1000 mg L^{-1} by weighing 100 mg and dissolving each analyte in 20 mL methanol and diluting to 100 mL with 0.1 mol L^{-1} SDS. The stock standard solutions of methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP) were prepared at a concentration of 1×10^{-3} mol L^{-1} by weighing 0.0152, 0.0166, 0.0180 and 0.0194 g, respectively, and dissolving each of them in 10 mL methanol and diluting to 100 mL with DI water. The stock solutions can remain stable for one month if stored in a refrigerator at 4 °C. The working standard solutions were prepared daily by further dilution of the stock solutions with a mobile phase.

2.4. Sample preparation

Two milliliters of methanol were added to accurately weighed samples of approximately 0.5 g each and vortexed for 2 min (Vortex, KMC-1300V, Vision Scientific Co., Ltd., Korea). Then samples were sonicated (Ultrasonic bath, CT-430G1, Wah Luen Electronic Tools Co., Ltd., China) for 5 min and centrifuged (Centrifuge, 1040, Labquip International Co., Ltd., England) at 3000 rpm for 10 min. A 500 μL aliquot of the supernatant was transferred to a 5.0 mL volumetric flask. The sample was diluted to volume with a mobile phase (SDS). Further dilution might be needed in order to obtain the analyte concentrations in the range of quantification (1–100 μmol L^{-1}). The solution was filtered through a 0.45 μm nylon syringe filter (Lubitech Technologies Ltd., China) for the analysis.

2.5. The stock solution of SDS

The stock SDS 0.5 mol L^{-1} solution was prepared by weighing 144.2 g SDS, adding 700 mL deionized water and stirring using a magnetic stirrer for 15 min until it was dissolved. The solution

was further diluted to 1000 mL with deionized water. The mobile phases were prepared at a desired concentration by diluting the proper amount of this stock solution. Finally, the mobile phase was vacuum-filtered through 0.45 μm nylon membrane of 45 mm diameter, and then degassed in an ultrasonicator for 15 min.

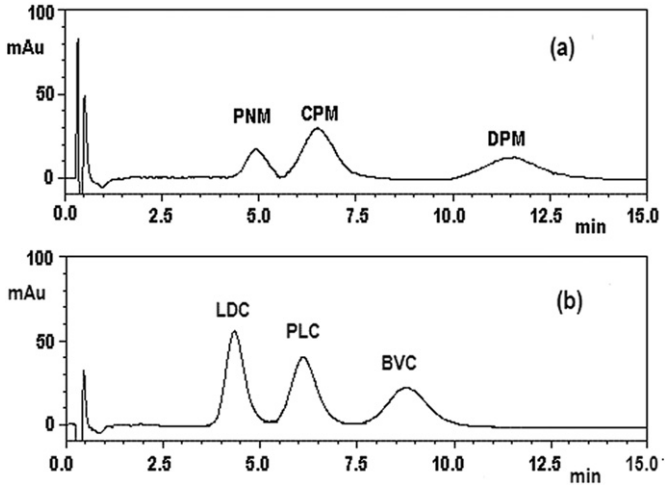

Fig. 1. Chromatograms of some pharmaceuticals obtained from a LC-20AT liquid chromatograph (Shimadzu) using Zorbax SB C18, 12.5 × 4.6 mm, id. 5 μm) as analytical column in 0.05 mol L^{-1} SDS:10% isopropanol mobile phase and 210 nm detection; (a) a mixture of some antihistamines; PNM, CPM and DPM 10, 25 and 10 mg L^{-1}, respectively and (b) a mixture of some local anesthetics, LDC, PLC and BVC (20 mg L^{-1}).

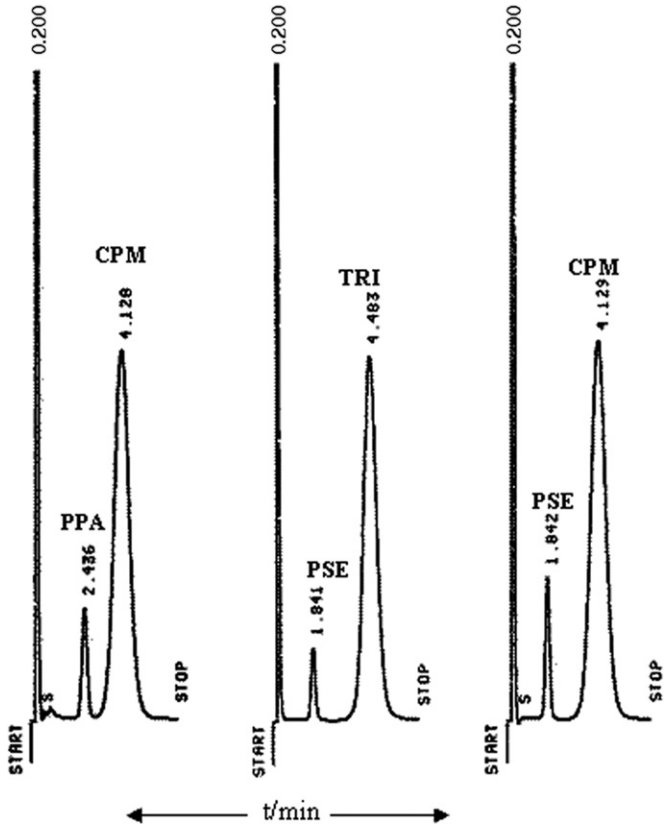

Fig. 2. Chromatograms of some binary mixtures of some formulation of cold drugs, PPA:CPM (20:40 mg L^{-1}), PSE:TRI (20:40 mg L^{-1}) and PSE:CPM (20:40 mg L^{-1}), using a Hewlett Packard 1100 with variable wavelength detector, isocratic pump, 20 μL loop injector and HP 35600 integrator. The mobile phase consisted of 0.10 mol L^{-1} SDS in phosphate buffer pH 3.0 mixed to 10% v/v isopropanol with 0.8 mL min^{-1}.

3. Results and discussion

3.1. The preliminary test

In our previous work, Zorbax SB C18 (12.5 × 4.6 mm, 5 μm) and micellar mobile phase could be used successfully for determination of tolperisone and lidocaine hydrochloride. Here, additional applications have been shown in more complicated mixtures (tertiary mixtures) such as in the separation of antihistamines (chlorpheniramine maleate (CPM), dipheniramine (DPM) and pheniramine maleate (PNM)), and local hydrochloride anesthetics (lidocaine (LDC), bupivacaine (BVC) and prilocaine (PLC)). The chromatograms are shown in Fig. 1. Three antihistamines (PNR, CPM and DPM) were separated

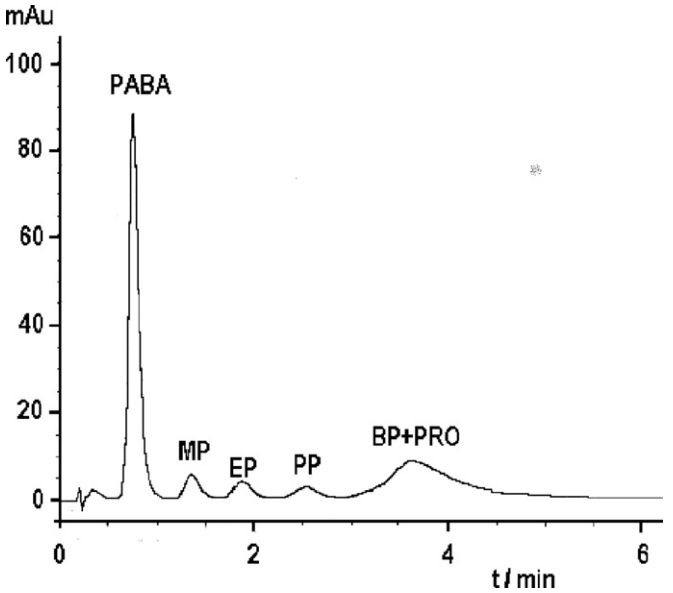

Fig. 3. Chromatograms of mixtures of PABA, MP, EP, PP and BP and PRO (each concentration 10 mg L^{-1}) using a Hewlett Packard 1090A featuring a solvent delivery pump system, a diode array detector, 20-μL loop injector. The ODS Hypersil (20 × 4.0 mm, 5 μm) was used with 0.1 mol L^{-1} SDS mixed to 0.5% v/v *n*-pentanol and flow rate 0.75 mL min^{-1}. The detection wavelength was 290 nm.

Table 1
The retention behaviors of some parabens, local anesthetics, antihistamines and cough and cold drugs.

Solute	$1/k = C_0 + C_1[M]$[a]			%RSD of 10-replicate injections	
	C_0	C_1	R^2	T_R	Peak area
MP	0.0632	1.1391	0.9983	1.6	1.5
EP	0.0303	0.9679	0.9979	1.8	1.9
PP	0.0148	0.7591	0.9978	2.0	1.8
BP	0.0101	0.5906	0.9980	2.2	2.7
LDC	−0.0035	0.8609	0.9997	1.7	1.7
PLC	−0.0015	0.5575	0.9999	1.6	2.4
BVC	−0.0033	0.5151	0.9903	1.1	2.0
PNM	0.0077	1.0048	0.9971	1.9	1.1
CPM	0.0012	0.7640	0.9988	1.8	2.5
DPM	0.0003	0.4794	0.9997	1.8	2.2
PRO	0.0822	0.9864	0.9510	1.7	1.5
PABA	0.5584	0.4203	0.9241	1.2	2.5
PSE	0.0221	1.1731	0.9980	1.1	2.1
PPA	0.0280	0.7663	0.9900	1.2	1.9
TRI	0.0011	0.5334	0.9974	1.6	2.4

[a] *k* is capacity factor, C_0 and C_1 are formal constants. [M] is concentration of micelles in the mobile phase and corresponds to the difference between total concentration of SDS and its critical micelle concentration (CMC).

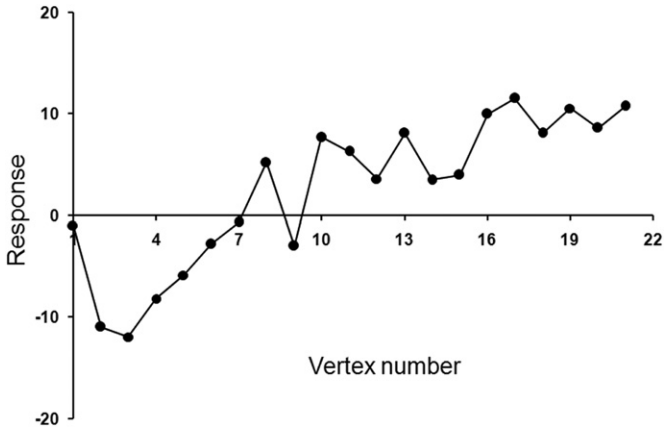

Fig. 4. Response versus vertex number for the variable-size simplex self-test results.

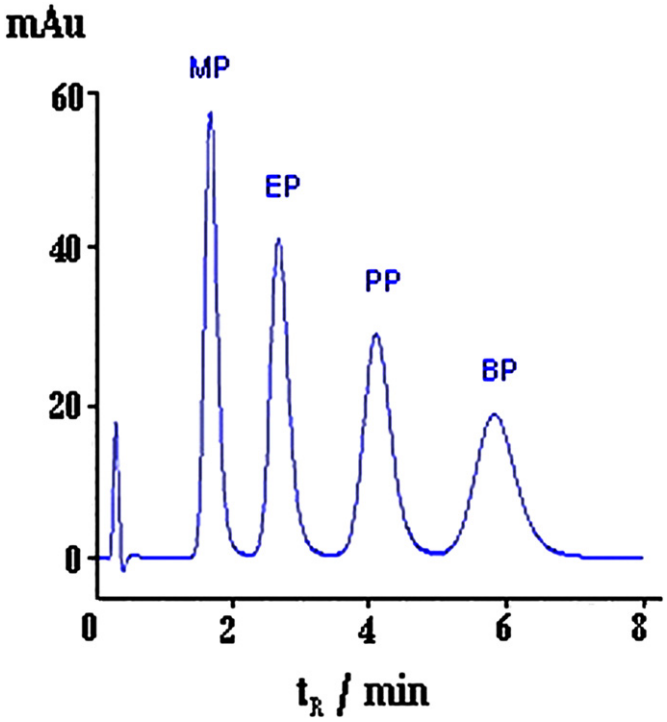

Fig. 5. Representative chromatogram of the standard parabens at concentration of 40 μmol L^{-1} obtained from the optimum conditions; mobile phase: SDS 45.6 mmol L^{-1}; flow rate: 0.612 mL min^{-1}; UV detection: 254 nm.

with a resolution factor of more than 1 in 0.05 mol L^{-1} SDS mixed to 10% v/v isopropanol with the flow rate of 0.6 mL min^{-1} as presented in Fig. 1(a). The retention time of PNR, CPM and DPM was 4.9, 6.5 and 11.6 min, respectively. Three local anesthetics (LDC, BVC and PLC) were separated in 10 min with the resolution factor of more than 1 as shown in Fig. 1(b), using the same mobile phase with the flow rate of 0.8 mL min^{-1}. The retention time of LDC, BVC and PLC were 4.3, 6.1 and 8.8 min, respectively. Moreover, some binary mixtures in formulation of the cold drugs such as PPA:CPM (20:40 mg L^{-1}), PSE:TRI (20:40 mg L^{-1}) and PSE:CPM (20:40 mg L^{-1}) were able to be separated in this short column using 0.10 mol L^{-1} SDS in phosphate buffer pH 3 mixed to 10% v/v isopropanol as a mobile phase with the flow rate of 0.8 mL min^{-1} and 210 nm detection. The resolution factors were more than 2 for each pair of the formulation of the cold drugs as shown in Fig. 2. The retention times of PSE, PPA, CPM and TRI in this condition were 1.8, 2.4, 4.1 and 4.5 min, respectively. However, this condition was unable to separate the mixture of TRI and CPM, neither nor the mixture of PSE and PPA, therefore, this system is still lacked of selectivity in separation of these cold drugs.

Another application of this short column is the separation of parabens; MP, EP, PP and BP; using 0.075 mol L^{-1} of SDS. The parabens were able to be separated not only in anionic surfactant mobile phase (sodium dodecyl sulfate, SDS) but also in cationic surfactant, hexadecyltrimethylammonium bromide (CTAB), with the resolution factor ≥ 1.5. The other guard column, ODS Hypersil (20 × 4.0 mm, 5 μm), was also applied to separate some parabens, PRO and PABA (which are formulated in some anti-aging products and PABA is hydrolysed product of PRO) using 0.1 mol L^{-1} SDS mixed with 0.5% v/v *n*-pentanol and the flow rate of 0.75 mL min^{-1} at the detection wavelength of 290 nm. The retention times of PABA, MP, EP, PP, BP and PRO were 0.7, 1.4, 1.9, 2.5, 3.3 and 3.6 min, respectively, and resolution factors for each two adjacent peaks were more than 1.5. The chromatogram was presented in Fig. 3. Unfortunately the peak of PRO and BP could not be separated in this condition.

3.2. Chromatographic behaviors

The retention behaviors of some parabens, local anesthetics, antihistamines and cough and cold drugs were studied in a micellar mobile phase at various concentrations of SDS, 0.025–0.150 mol L^{-1}. It was found that the reciprocal value of the capacity factor (k) of each solute is in a linear function of concentration of micelle in the mobile phase [M] as shown in Table 1. All equations corresponding to chromatographic behavior of other solutes in a micelle system with conventional analytical columns C8 and C18 were reviewed by Arunyanart and Cline Love [27] according to Eq. (1):

$$1/k = C_0 + C_1[\mathrm{M}] \quad (1)$$

Table 2
Some analytical features of the proposed method for determination of parabens (column: Zorbax SB-C18 (12.5 × 4.6 mm i.d., 5 μm); mobile phase: SDS 45.6 mmol L^{-1}; flow rate: 0.612 mL min^{-1}; UV detection: 254 nm).

Analytical features	MP	EP	PP	BP
Linearity				
Range (μmol L^{-1})	1–100	1–100	1–100	1–100
Slope ± S_m[a]	17.74 ± 0.13	18.30 ± 0.12	19.55 ± 0.13	18.63 ± 0.11
Intercept ± S_b[a]	4.75 ± 4.14	0.11 ± 3.98	−3.78 ± 4.06	0.01 ± 3.45
R^2	0.9996	0.9997	0.9997	0.9998
Repeatability (%RSD, n=10)				
Retention time of standard	3.8	2.1	1.1	0.5
Peak area of standard	3.0	1.2	1.0	1.3
Sample no. 6 (body lotion)[b]	2.2	ND	2.4	ND
Sample no. 50 (facial cream)[b]	1.4	ND	1.2	ND
Sample no. 60 (body lotion)[b]	1.8	1.9	2.6	2.4

Table 2 (*continued*)

Analytical features	MP	EP	PP	BP
Reproducibility (%RSD, $n=7$)				
Sample no. 6 (body lotion)[c]	3.2	ND	1.1	ND
Sample no. 50 (facial cream)[c]	1.2	ND	2.2	ND
Sample no. 60 (body lotion)[c]	3.0	2.7	3.2	2.9
LOD(3S/N) (μmol L^{-1})	0.040	0.050	0.075	0.100

[a] S_m and S_b: standard deviation of the slope and intercept, respectively.
[b] %RSD calculated from peak area.
[c] %RSD calculated from content of each paraben found in the samples.

Table 3
Recovery study of parabens by standard addition into a Placebo sample and some real samples.

Sample no.	Added (μmol L^{-1})	Found (μmol L^{-1}) $\pm$ SD ($n=3$)				% Recovery			
		MP	EP	PP	BP	MP	EP	PP	BP
Placebo	5.0	4.9 ± 0.2	5.1 ± 0.1	4.8 ± 0.1	5.0 ± 0.2	97.5	100.9	96.4	99.4
(shampoo)	10.0	9.6 ± 0.2	10.2 ± 0.3	9.7 ± 0.2	10.1 ± 0.1	95.9	101.7	96.8	100.8
	20.0	20.4 ± 0.1	20.6 ± 0.3	20.3 ± 0.2	20.1 ± 0.2	102.0	102.9	101.6	100.6
	40.0	38.4 ± 0.6	39.2 ± 0.4	38.1 ± 0.5	38.7 ± 0.8	96.1	97.9	95.3	96.8
	60.0	57.8 ± 0.9	57.7 ± 1.5	57.4 ± 0.9	59.4 ± 1.4	96.4	96.1	95.7	98.9
	80.0	$79.3 \pm 1,2$	79.3 ± 1.9	78.2 ± 1.0	80.6 ± 1.5	99.1	99.1	97.7	100.8
6	10.0	10.5 ± 0.2	9.8 ± 0.1	10.2 ± 0.2	9.3 ± 0.3	105.3	97.6	102.3	93.1
(Body lotion)	20.0	21.6 ± 0.3	18.8 ± 0.2	20.4 ± 0.1	18.6 ± 0.3	107.9	94.1	101.9	92.9
	30.0	32.8 ± 0.4	28.4 ± 0.3	29.9 ± 0.8	27.7 ± 0.6	109.2	94.7	99.5	92.4
	40.0	43.0 ± 0.8	38.6 ± 0.6	39.8 ± 1.3	37.0 ± 1.5	107.5	96.4	99.5	92.4
	50.0	54.5 ± 1.4	51.1 ± 1.8	51.2 ± 1.0	49.6 ± 2.6	109.0	102.1	102.3	99.2
17	10.0	10.4 ± 0.2	10.0 ± 0.3	9.9 ± 0.2	10.0 ± 0.5	103.5	100.0	99.1	99.6
(Shower gel)	20.0	19.6 ± 0.2	19.8 ± 0.7	19.6 ± 0.9	20.2 ± 0.6	98.1	98.7	98.2	101.0
	30.0	29.4 ± 1.0	29.6 ± 0.9	29.9 ± 0.6	29.8 ± 1.1	97.8	98.8	99.7	99.4
	40.0	38.8 ± 0.5	40.0 ± 0.6	40.3 ± 1.0	39.9 ± 1.2	97.1	100.1	100.8	99.7
	50.0	48.4 ± 0.8	50.8 ± 0.9	51.4 ± 1.2	51.5 ± 0.9	96.9	101.7	102.8	103.0
50	10.0	10.0 ± 0.5	10.2 ± 0.3	9.8 ± 0.2	10.0 ± 0.2	100.4	101.8	98.0	99.8
(Facial cream)	20.0	20.1 ± 0.4	19.5 ± 0.2	19.7 ± 0.8	19.6 ± 0.6	100.3	97.5	98.6	98.0
	30.0	31.1 ± 0.8	30.3 ± 1.2	31.5 ± 0.9	30.8 ± 1.1	103.8	101.0	104.9	102.7
	40.0	41.8 ± 1.0	40.8 ± 0.8	41.8 ± 1.0	41.5 ± 0.6	104.5	101.9	104.4	103.9
	50.0	51.8 ± 0.6	50.5 ± 0.5	51.5 ± 1.2	50.7 ± 1.0	103.6	101.0	103.0	101.3
60	10.0	9.9 ± 0.6	9.9 ± 0.1	10.2 ± 0.3	10.1 ± 0.2	98.5	98.8	102.4	100.5
(Body lotion)	20.0	19.5 ± 0.5	19.9 ± 0.4	20.2 ± 0.4	20.7 ± 0.5	97.6	99.6	100.9	103.5
	30.0	30.1 ± 0.3	30.5 ± 0.6	30.4 ± 1.0	31.0 ± 0.7	100.2	101.7	101.4	103.3
	40.0	40.0 ± 0.8	40.6 ± 1.1	41.2 ± 0.9	41.3 ± 1.0	99.9	101.4	103.0	103.2
	50.0	49.7 ± 0.7	50.6 ± 1.0	50.9 ± 1.3	51.8 ± 1.6	99.5	101.2	101.8	103.5

Table 4
The content of parabens in some cosmetic products (manufactured and community products) analyzed by the proposed method.

No.	Sample	Parabens content/mg $kg^{-1} \pm$ SD			
		MP	EP	PP	BP
1	Revitalift milky foam	475 ± 7	682 ± 18	386 ± 5	1220 ± 30
2	Gentle cleansing milk	2260 ± 60	ND	63 ± 1	ND
3	Revitalift aqua-milky toner	818 ± 24	141 ± 5	96 ± 1	255 ± 12
4	Skin lightening cream	605 ± 15	ND	793 ± 18	ND
5	Hand & nail lotion	718 ± 16	ND	592 ± 16	ND
6	Body cream	745 ± 14	ND	872 ± 19	ND
7	Milk yoghurt body scrub	1740 ± 20	ND	1780 ± 30	ND
8	Firming pre-holiday balm	2320 ± 20	ND	1030 ± 20	ND
9	Dry skin relief moisture lotion	1400 ± 20	ND	780 ± 12	ND
10	Whitening immediate sun protection	1370 ± 30	394 ± 9	435 ± 9	ND
16	Orange honey shower gel	166 ± 5	146 ± 4	107 ± 3	280 ± 6
18	Shower gel 1	ND	ND	ND	238 ± 3
19	Shower gel 2	ND	ND	ND	233 ± 1
29	Tamarind herbal cleansing cream	ND	ND	74 ± 2	ND
31	Hand liquid soap	89 ± 2	ND	ND	ND
32	Herbal shampoo	339 ± 6	ND	136 ± 3	ND
41	Kaffir lime & moss shampoo	114 ± 1	18 ± 1	114 ± 4	ND
46	Milky conditioner	214 ± 12	ND	41 ± 1	ND
49	Young rice milk sunscreen cream	952 ± 5	ND	1020 ± 20	ND

Table 4 (*continued*)

No.	Sample	Parabens content/mg kg^{-1} ± SD			
		MP	EP	PP	BP
50	Young rice milk night cream	1040 ± 10	ND	1160 ± 30	ND
51	Whitening cream	4310 ± 130	ND	ND	ND
53	Snow lotus cream	1300 ± 30	300 ± 4	174 ± 2	505 ± 17
54	Whitening night cream	915 ± 12	ND	818 ± 14	ND
59	Tamarind body scrub	2880 ± 20	ND	160 ± 2	ND
60	Herbal body lotion	175 ± 3	49 ± 1	29 ± 1	66 ± 1
61	Carrot body lotion	4200 ± 40	ND	300 ± 9	ND
64	Armpit whitening cream	3910 ± 50	ND	4750 ± 100	ND

SD: standard deviation (n=3) and ND: not detectable (The limits of detection of MP, EP, PP, and BP in the unit of mg kg^{-1} were 0.24, 0.33, 0.54 and 0.78, respectively.)

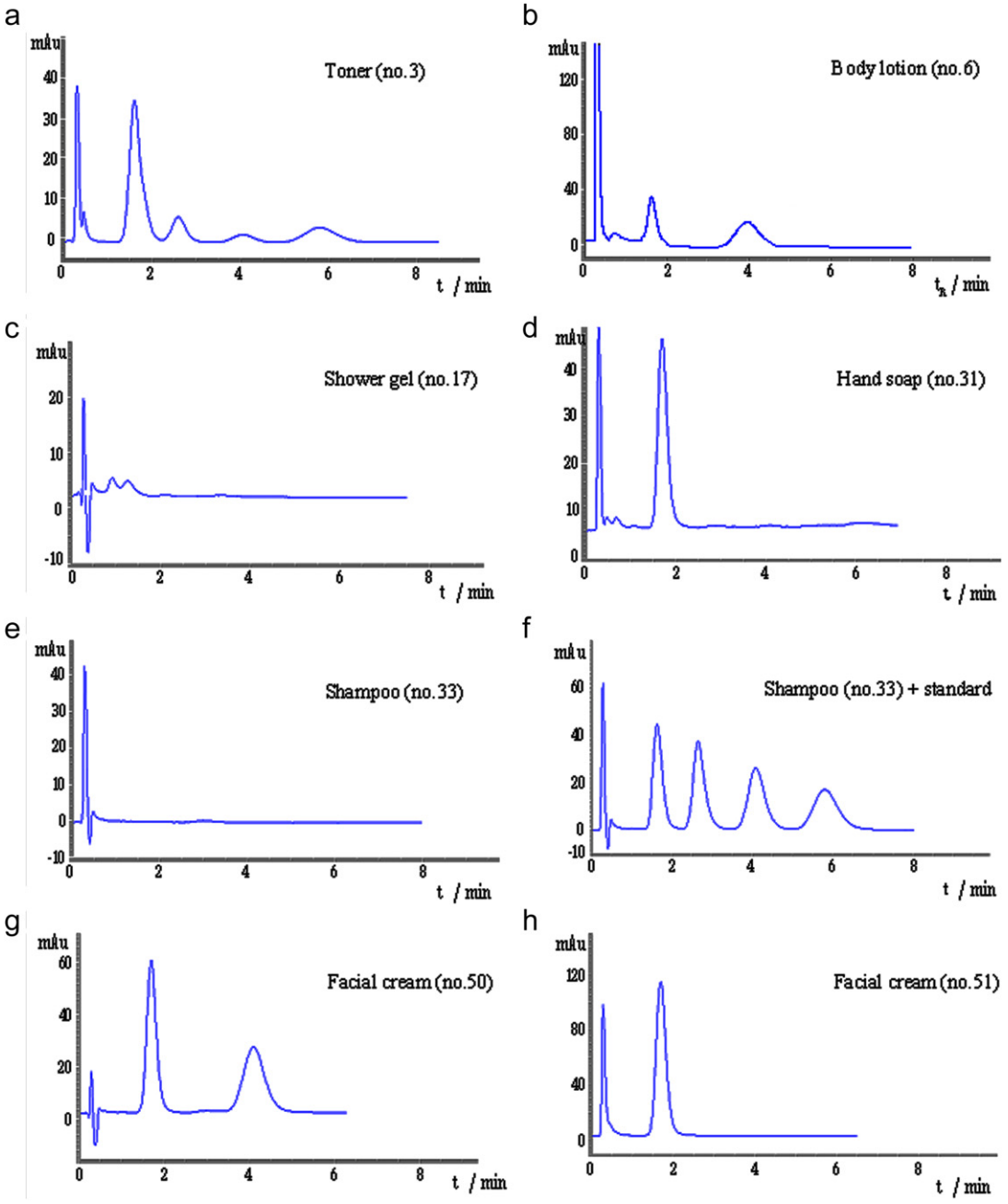

Fig. 6. Chromatograms of some samples (a) toner (sample No.3), (b) body lotion (sample No.6), (c) shower gel (sample No.17), (d) hand soap (sample No.31), (e) shampoo (sample No.33), (f) shampoo (sample No.33) with 40 mg L^{-1}standard parabens added, (g) facial cream (sample No.50) and (h) facial cream (sample No.51).

where k is capacity factor, C_0 and C_1 are formal constants. [M] is concentration of micelle in the mobile phase, and [M] is equal to the difference between total concentration of SDS and its critical micelle concentration (CMC).

For the study of chromatographic behaviors of some local anesthetics and antihistamines, 10% v/v isopropanol was mixed with SDS, whereas for PRO and PABA, 0.5% v/v *n*-pentanol was added in SDS mobile phase. However, in the study retention behaviors of parabens, there was no need to add short chain alcohol in the micellar mobile phase.

The results indicated that a short column of only 12.5 mm in conjunction with a micellar mobile phase was able to separate these analytes. Therefore, this short column and SDS mobile phase was chosen for parabens determination in cosmetic samples in the following experiment.

3.3. Determination of parabens

HPLC with a Zorbax SB-C18 column 12.5 mm in length was used with micellar (SDS) mobile phase for determination of MP, EP, PP and BP at the wavelength 254 nm. Before analysis of parabens, the concentration of SDS and the flow rate were optimized using the simplex optimization method.

3.3.1. Simplex optimization of the chromatographic condition

In this method, the aim was to search for optimal conditions using simplex optimization that provide maximum response that giving good separation in short analysis time. This was considered from the resolution of four chromatographic peaks of MP, EP, PP and BP as a function of the flow rate and the concentration of micellar mobile phase (SDS). The objective of our work was to elute the last peak (BP) within 7 min (which is the shortest analysis time of BP from the literature reviews by HPLC technique [18,19,28–35] and with the resolution factors not less than 1.5 for all the analytes. The chromatographic response function (*CRF*) given by Eq. (2), was selected as the criterion for the optimization process [36,37].

$$CRF = \sum_{i=1}^{n-1} R_i + n^a - b|T_A - T_L| + c(T_1 - T_0) \quad (2)$$

where R_i is the resolution factor between adjacent peaks pairs (four peaks give three values which are R_1, R_2 and R_3); n is the number of peaks detected (four peaks, $n=4$); T_A is a specified analysis time (7 min as mentioned above); T_1 and T_L are the retention time of the first and the last peaks from the experiment, respectively; T_0 is the specified minimum retention time (1.2 min for this work, set from the preliminary study). Values of a–c are constant which is assigned based on the importance of the parameter. The most important parameter in this case is the difference between T_A and T_L, for seeing the last peak in specified time, therefore value of "b" is 5, whereas "a" and "c" is 1.

The simplex optimization was started by introducing the lower and upper limit boundary condition for two variables; concentration of SDS (0.04–0.10 mol L^{-1}) and flow rate (0.5–1.0 mL min^{-1}). In the preliminary optimization study using univariate method for estimation the suitable concentration of SDS, if SDS concentration was out of the range 0.04–0.10 mol L^{-1}, the mixtures of parabens was not well separated (resolution factor was less than 1.5). The same results were obtained when flow rate was not in the indicated range. The relationship between chromatographic response and vertex number was plotted in Fig. 4.

The results showed that *CRF* of vertex number of 16–21 were slightly varied and could not be improved further (with standard deviation = 1.3). The vertex number of 17 offered the highest *CRF* which was the maximum response. Therefore, these conditions were selected. The optimum conditions for simultaneous determination of the four parabens were 45.6 mmol L^{-1} SDS with a flow rate of 0.612 mL min^{-1} and detection wavelength at 254 nm. The results showed that the separation could be completed within 7 min under these conditions, as shown in Fig. 5. The retention times of MP, EP, PP and BP were 1.5, 2.7, 4.0 and 6.0 min with resolution factors more than 2 for all adjacent peaks.

3.3.2. Study of some analytical features

3.3.2.1. Linearity. Linearity was studied by performing three replicate injections at nine standard concentrations 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 75.0 and 100.0 μmol L^{-1}. The calibration curves were obtained from peak area versus concentration. The results of the equations and the correlation coefficients (R^2) of MP, EP, PP and BP were reported in Table 2. The calibration curves showed good linearity over the concentration range 1–100 μmol L^{-1} and the correlation coefficients of the four parabens were higher than 0.9990.

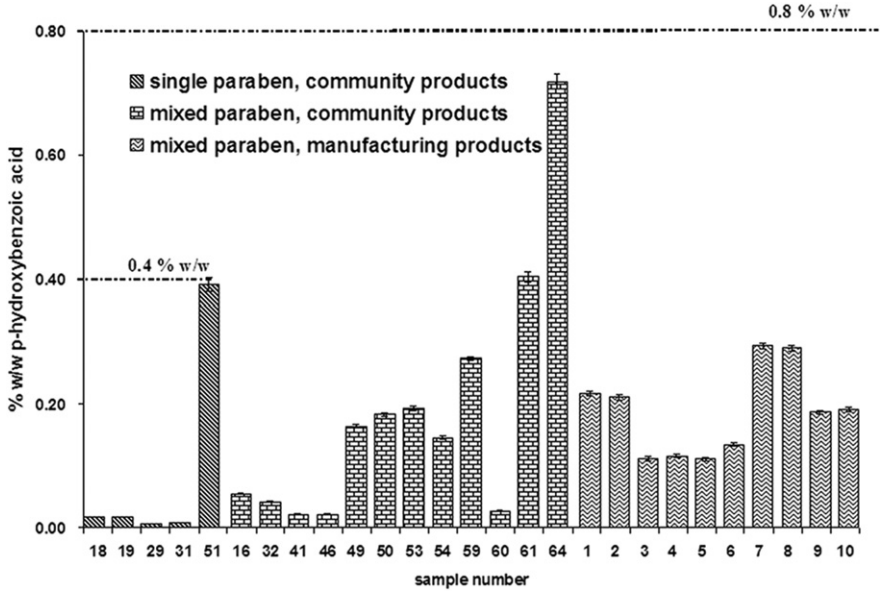

Fig. 7. Parabens contents equivalent to *p*-hydroxybenzoic acid (PHBA) in manufactured products and Thai community products which consisted of single paraben or mixed paraben.

Table 5
Comparative review of parabens determination in cosmetics by LC techniques.

Year	Column	Mobile phase	Flow rate (mL min^{-1})	Detection[a]	Analysis time[b] (min)	Solvent consumption (mL)	Analyte	Linearity (mg L^{-1})	LOD[c] (μg L^{-1})	Ref.
2000	RP Lichrospher C18 column (125 × 4.0 mm, 5 μm)	MeOH:1% CH_3COOH (gradient 35–60% MeOH)	1.0	UV (260 nm)	20	20	MP EP PP BP	1.0–40 1.0–40 1.0–40 1.0–40	20 30 30 50	Labat et al. [28]
2005	Zorbax Eclipse XDB-C8 (150 × 4.6 mm, 5 μm)	MeOH:H_2O (60:40)	1.0	CL	9	9	MP EP PP BP	0.004–7 0.005–9 0.006–10 0.006–10	1.9 2.7 3.9 5.3	Zhang et al. [29]
2005	Licrosorb ODS (250 × 4.6 mm, 5 μm)	2% Brij-35 (pH 3.0): propanol (80:20)	1.0	UV (254 nm)	25	25	MP EP PP BP	5–150 5–150 5–150 10–150	25 25 25 50	Memon et al. [19]
2006	RP C18 column (250 × 4.6 mm, 5 μm)	MeOH:H_2O (gradient 65–98.5% MeOH)	0.9	MS	12	11	MP EP PP BP	0.01–1.0 0.02–2.0 0.02–2.0 0.02–2.0	4.7 13.5 13.4 19.3	Lee et al. [30]
2008	RP Bondclone C18 column (300 × 3.9 mm, 10 μm)	MeOH:H_2O (gradient 57–98% MeOH)	1.4	UV (220 nm)	15	21	MP PP	30–55 8.0–18	2350[d] 570[d]	Gaona-Galdos et al.[31]
2008	RP Kromasil C18 column (150 × 4.6 mm, 5 μm)	0.04 M SDS and 0.1% (v/v) trichloroacetic acid:1-butanol (99:1)	1.0	UV (260 nm)	25	25	MP PP	5–94 2–42	290[d] 750[d]	Kulikov et al. [18]
2010	RP C18 column (250 × 4.6 mm, 5 μm)	CH_3CN:H_2O (50:50)	0.5	C-CAD	25	12	MP EP PP BP	5.3–400 4.6–400 3.0–400 2.0–400	2100 1500 700 500	Márquez-Sillero et al. [32]
2010	RP 18 LichroCART (125 × 4.0 mm, 5 μm)	MeOH:H_2O (70:30)	1.0	UV (250 nm)	7	7	MP EP PP BP	0.2–2.5 0.15–2.5 0.05–2.5 0.03–2.5	200[e] 150[e] 50[e] 30[e]	Melo and Queiroz [33]
2010	SpeedRod RP monolithic column (50 × 4.6 mm)	CH_3CN:H_2O (gradient 3–20% CH_3CN)	3.0	UV (254 nm)	15	45	MP EP PP *i*-BP *n*-BP	0.3–50 0.3–50 0.3–50 0.6–50 0.6–50	100 100 100 200 200	Zotou et al. [34]
2011	Waters XTerra™ C8 analytical column (250 × 4.6 mm, 5 μm)	0.025 mol L^{-1} Na_2HPO_4 (pH 7.0): CH_3CN (40:60)	1.0	Amperometric detection	8	8	MP EP PP	0.25–25[f] 0.25–25[f] 0.25–25[f]	400[g] 400[g] 400[g]	Martin et al. [35]
2012	Zorbax SB C18 (12.5 × 4.6 mm, 5 mm)	SDS 0.0456 mol L^{-1}	0.612	UV (254 nm)	7	4	MP EP PP BP	0.152–15.2 0.166–16.6 0.180–18.0 0.194–19.4	6.1 8.3 13.5 19.4	The proposed method

[a] UV: Ultraviolet detection; CL: Chemiluminescence detection; MS: Mass spectrometry; C-CAD: Corona-charged aerosol detector.
[b] Analysis time for only parabens.
[c] The limit of detection (LOD) was an estimation of 3S/N.
[d] The limit of detection (LOD) was an estimation of 3σ.
[e] The limit of quantitation (LOQ) was an estimation of 10S/N that the variation coefficient was lower than 5.0%.
[f] The working range of parabens 0.25–25 mg L^{-1}, however, for linearity ranges were 0.0125–0.500% w/w by adding standard parabens in blank shampoo samples.
[g] Detectability = $ks_{y/x}$/m, where $k = 3$, $s_{y/x}$ is the standard deviation of the residuals and m is the slope of the analytical curve.

3.3.2.2. Precision. To determine the precision of the system, the mixture of standard parabens (10.0 μmol L^{-1} of each paraben) and sample solutions were injected ten times. The results are given in Table 2. In all instances, the RSD values of peak area response and retention time were less than 3%, indicating good repeatability of the assay system. To determine the method precision, seven aliquots of each sample (sample No. 6, 50 and 60) were extracted using the same procedure as described in Section 2.4 and then analyzed. The values of RSD were less than 4% which was a satisfactory reproducibility of the method as summarized in Table 2.

3.3.2.3. Limit of detection (LOD) and quantitation (LOQ). Here, the LODs were defined as the amount of compound that would still give a signal three times greater than the noise of the baseline, by injecting progressively lower concentration. The limits of detection were 0.040, 0.050, 0.075 and 0.100 μmol L^{-1} for MP, EP, PP and BP, respectively (Table 2). The limit of quantitation (LOQ) were 0.20, 0.30, 0.50 and 0.80 μmol L^{-1}, respectively (10S/N).

3.3.2.4. Percentage recoveries. The recovery studies were carried out by spiking six known amounts of MP, EP, PP and BP in placebo shampoo (ranging from 5 to 80 μmol L^{-1}). Three replicated samples were prepared at each concentration and results were averaged. As shown in Table 3, the percentage recoveries of placebo at each level were within 95.3 to 102.9. The results obtained for the accuracy study from 4 samples, by spiking five known amounts of parabens (ranging from 10 to 50 μmol L^{-1}), are presented in Table 4. For body lotion (sample No. 6), shower gel (No. 17), facial cream (No. 50) and body lotion (No. 60), the recoveries data of parabens ranged from 92.4 to 109.2, 96.9 to 103.5, 97.5 to 104.9 and 97.6 to 103.5%, respectively. Therefore, recovery values demonstrated that the method was sufficiently accurate within the desired range.

3.4. Analysis of samples

The proposed method was also applied for monitoring MP, EP, PP and BP present in cosmetics distributed in Thailand. Chromatograms of some samples are shown in Fig. 6. The amount of each paraben was determined by external calibration graph in the range 1–100 μmol L^{-1}. As summarized in Table 4, all manufacturing products (sample No. 1–10), and 17 out of 54 Thai community products (sample No. 11–64) were found to contain parabens.

According to the EU regulation the content of paraben in the form of *p*-hydroxybenzoic acid (PHBA) must not exceed 0.4% w/w for single paraben formulation and 0.8% w/w for mixed paraben formulation. The content of acidic form of parabens (PHBA) in manufactured products (sample No. 1–10) and Thai community products (sample No. 11–64) were calculated by conversion the amount of each paraben from mg kg^{-1} to % w/w PHBA equivalent. It was found that the amount of paraben in all samples were within the EU regulation as presented in Fig. 7.

3.5. Comparison of the proposed method with some published HPLC methods for determination of parabens

This proposed method with short column 12.5 mm length and SDS mobile phase was compared to the HPLC methods published in 2000–2011 [18,19,28–35] for the determination of parabens. The parameters that were compared include chromatographic conditions, some analytical features and waste generation or solvent consumption as shown in Table 5. The most advantageous point of this proposed method over the other HPLC methods is the lower solvent consumption. Only 4 mL of mobile phase (SDS, biodegradable substance) was used per analysis with short run time. Moreover, no organic solvent was added in the mobile phase. In addition, the cost of column is cheaper than the conventional C18 column (12.5–30.0 cm length). Monolith column is also a novel column for HPLC. However, in determination of parabens, approximately 45 mL of acetonitrile (gradient 3–20%) was required as mobile phase per analysis [34]. Although the detection limit of this proposed method was not as low as the method of Zhang et al. [29] which employed chemiluminescence as a detection unit, it is sufficient for detecting parabens in complicated samples. Moreover, in this work the sample preparation procedure using methanol and SDS micelle extraction which is simpler and faster than the procedure used in the above literatures could be employed due to the high separation selectivity of micelle.

4. Conclusions

This research proposed the use a commercially available guard column (Zorbax SB-C18) as an analytical column in micellar liquid chromatographic separation of some local anesthetics, antihistamines, cold drugs and homologous series of parabens. The system provided very good separation efficiency with short run time (7 min) and consumed low amounts of an environmental friendly mobile phase (SDS). Moreover, a simpler sample preparation procedure could be used for sample clean-up prior to the analysis. Under the optimum condition, the system was successfully demonstrated for determination of four parabens in 64 samples of cosmetic products, both those that were produced locally in the community and those that were commercially manufactured. The proposed method might be suitable to be used in routine analysis for industry that used conventional HPLC, instead of procurement of high cost of UPLC or fast HPLC. The proposed strategy is a greener analytical chromatography which might be useful for other applications as well.

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