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DEVELOPMENT AND VALIDATION OF RP-HPLC-UV/Vis METHOD FOR DETERMINATION OF PHENOLIC COMPOUNDS IN SEVERAL PERSONAL CARE PRODUCTS

(Pembangunan dan Validasi Kaedah Fasa Berbalik-HPLC-UV/Vis untuk Penentuan Sebatian Fenolik dalam beberapa Produk Penjagaan Diri)

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Abstract

A HPLC method with ultraviolet-visible spectrophotometry detection has been optimized and validated for the simultaneous determination of phenolic compounds, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as antioxidants, and octyl methylcinnamate (OMC) as UVB-filter in several personal care products. The dynamic range was between 1 to 250 mg/L with relative standard deviation less than 0.25%, (n=4). Limit of detection for BHA, BHT and OMC were 0.196, 0.170 and 0.478 mg/L, respectively. While limit of quantification for BHA, BHT and OMC were 0.593, 0.515 and 1.448 mg/L, respectively. The recovery for BHA, BHT and OMC ranged from 92.1-105.9%, 83.2-108.9% and 87.3-103.7%, respectively. The concentration ranges of BHA, BHT and OMC in 12 commercial personal care samples were 0.13-4.85, 0.16-2.30 and 0.12-65.5 mg/g, respectively. The concentrations of phenolic compounds in these personal care samples were below than maximum allowable concentration in personal care formulation i.e 0.0004 – 10 mg/g, 0.002 – 5 mg/g and up to 100 mg/g for BHA, BHT and OMC, respectively.

Keywords: Phenolic compounds, personal care products, RP-HPLC-UV/Vis, optimization and validation method.

Abstrak

Kaedah kromatografi cecair prestasi tinggi (KCPT) dengan pengesan spektrometri ultralembayung-boleh nampak telah dioptimumkan dan divalidasi untuk penentuan serentak sebatian fenolik seperti hidroksianisol terbutil (BHA) dan hidroksitoluena terbutil (BHT) sebagai antioksida, dan oktil metilsinamat (OMC) sebagai penapis-UVB dalam beberapa produk penjagaan diri. Julat dinamik adalah antara 1 ke 250 mg/L dengan sisihan piawai relatif kurang dari 0.25% (n=4). Had pengesanan untuk BHA, BHT dan OMC adalah 0.196, 0.170 dan 0.478 mg/L, masing-masingnya. Manakala had penentuan kuantitatif untuk BHA, BHT dan OMC adalah 0.593, 0.515 and 1.448 mg/L, masing-masingnya. Perolehan semula untuk BHA, BHT dan OMC adalah dalam julat dari 92.1-105.9%, 83.2-108.9% dan 87.3-103.7%, masing-masingya. Kepekatan BHA, BHT dan OMC dalam 12 sampel penjagaan diri komersial adalah dalam julat 0.13-4.85, 0.16-2.30 dan 0.12-65.5 mg/g, masing-masingnya. Kepekatan sebatian fenolik dalam sampel penjagaan diri adalah di bawah kepekatan maksimum dibenarkan dalam formulasi produk penjagaan diri iaitu 0.0004 – 10 mg/g, 0.002 – 5 mg/g dan hingga 100 mg/g untuk BHA, BHT dan OMC, masing-masingnya.

Kata kunci: Sebatian fenolik, produk penjagaan diri, RP-HPLC-UV/Vis, pengoptimaan dan validasi kaedah.

Introduction

Phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and octyl methylcinnamate (OMC) (see Figure 1) are active constituents in personal care products [**1,2**].

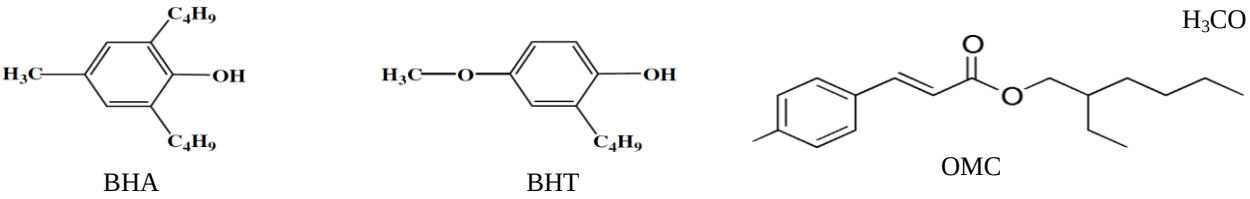

Figure 1: Structures of common phenolic compounds in personal care products.

BHA and BHT are added individually or in combination to prevent oxidative rancidity in personal care products [**3**], while OMC is used to absorbs UV-light between 280-320 mm to protect the skin from sunburn [**2**]. The amount of BHA and BHT in personal care formulation depends on the amount of sensitive compounds, such as α-hydroxy acids, ceramides, lipids, vitamins, oils and others, that are susceptible to oxidation by atmospheric oxygen to form unstable peroxide radicals [**4,5**]. BHA and BHT prevents the oxidation by inhibiting reactions promoted by oxygen and thus the formation of ketones and aldehydes that can give a products unpleasant smell and rancidity can be avoided [**5**]. Antioxidants prevent cosmetic formulations from peroxide radical because of their ability to neutralize those radicals through the transfer of hydrogen to this radical and stabilizing the antioxidant by resonance [**6,7**]. On the other hand, the amount of OMC added depend on type of product and part of body it applied [**2,8,9,10,11**]. Reversed phase HPLC with UV/vis detector (RP-HPLC-UV/Vis) is an important technique for phenolic compounds analysis because it have strong chromophores that absorb light in the wavelength region from 200 to 800 nm [**12**]. Application of RP-HPLC-UV/Vis in analysis of phenolic antioxidents such as BHA, BHT and OMC have been reported by several researchers [**2, 5, 13**]. The objective of this study is to determine the optimum analytical condition and validate the method for a simulataneous qualitative and quantitative analysis of phenolic compounds as well as to develop an analytical method evaluation and quality control of phenolic compounds by RP-HPLC-UV/Vis in personal care products.

Materials and Methods

Personal care samples

Four types of personal care products, namely sun cream, milk lotion, hair gel and hair oil were purchased from several local supermarkets in Kuching, Sarawak, Malaysia. Three commercial brands were collected for each type of personal care product.

Chemicals

The organic solvents (n-hexane, methanol, ethanol and acetonitrile) used for the analysis of phenolic compounds were of analytical grade (99.99% purity) from Merck (Darmstadt, Germany). Reverse-osmosis type quality water was used for analysis. BHA, BHT and OMC with purity 96%, 99.8% and 98%, respectively, were purchased from ACROS-ORGANICS (New Jersey, USA).

Preparation of standard solution

A 5000 mg/L stock solution of BHA, BHT and OMC in acetonitrile was prepared by weighing 1250 mg each of BHA, BHT and OMC in the flask and diluted with 100 mL acetonitrile. The mixture was shaken until a homogenous and clear solution was formed. Acetonitrile was then added to a final volume of 250 mL. The stock solution was covered with aluminum foil and stored in a freezer (4°C) and away from light for a maximum of one month. Prior to analysis, standard working solutions were prepared by diluting appropriate amounts of the stock solutions in acetonitrile.

Extraction procedure

Extraction of BHA, BHT and OMC from personal care samples was performed using reflux method according to the procedure described by Capitan-Vallvey et al. [**4,5**] with slight modification. Briefly, 0.1 to 1 g of personal care samples were accurately weighed in the 100 mL capacity round bottom flask. Prior to extraction, 25 mL n-hexane was added to the samples in order to remove lipids, fatty acids and volatile oils, and followed by addition of 15 mL acetonitrile. The sample was extracted under reflux for 30 minutes at 70°C with stirring. Extraction was performed

in triplicates. The mixture was transferred to separatory funnel and two layers, that are n-hexane and acetonitrile phases were formed. The n-hexane phase was repartitioned for two or three times using 10 mL of acetonitrile and shake vigorously. The n-hexane phase was removed and acetonitrile phase was collected. The acetonitrile phase (extract) was concentrated using a vacuum rotary evaporator at 45°C. The residue was redissolved with 10 mL of acetonitrile and filtered by membrane filter (Millipore, 0.5µm x 45 mm), then transferred into a 25 mL volumetric flask. It was diluted to 25 mL with acetonitrile.

High performance liquid chromatography (HPLC) analysis

The quantitative and qualitative analysis of phenolic compounds was performed on a Shimadzu HPLC system model LC-20AT equipped with four pumps and Shimadzu SPD-20 AV UV/Vis detector. Exactly 50 µL samples was injected and the chromatographic separation was performed on a RP-C_{18} Metacil (5µm) ODS column, 4.6 mm×250 mm. The analytical condition for HPLC analysis was according to Saad et al. [**14**] with a slight modification by using 280 nm as maximum wave length (λ_{max}), mobile phase system consisting of acetonitrile (phase A) and (water/acetic acid, 99:1, v/v) (phase B) and flow rate 0.8 ml/min. The resolution factor was calculated according to the equation used by Song & Wang [**15**].

Results and Discussion

Optimization of HPLC condition

Determination of the optimum wave length by spectrophotometer UV/Vis

The UV spectrum of BHA, BHT and OMC exhibited maximum absorption at 290, 275and 300 nm, respectively. The UV/Vis detector of RP-HPLC was fixed at 280 nm as maximum wave length (λ_{max}) for simultaneous determination.

Effect of the pH of mobile phase on resolution factor (R_s)

The pH is an important parameter to be optimized as it affects the ionization of phenolic compounds. Separation of BHA, BHT and OMC are sensitive to the pH because at low pH values BHA, BHT and OMC are ionized due to increase of protonation in mobile phase [**14,16,17,18**]. The analytical conditions for analysis of BHA, BHT and OMC were based on conditions recommended by Saad et al. [**14**], where a mixture of phase A (acetonitrile) with phase B (water:acetic acid) act as mobile phase with 280 nm as maximum wave length and 0.8 mL/min as flow rate of mobile phase. The pH was optimized by varying the percentage of acetic acid in order to adjust pH of the phase B of mobile phase at pH 3, 3.2, 3.5, 4 and 7. Decreasing pH value increases the separation and ionization of BHA, BHT and OMC, particularly between BHT and OMC. Figure 2 shows the effect of pH on the resolution factor (R_s) between BHT and OMC by varying the percentage of acetic acid in phase B of mobile phase from 0% to 2% (see Table 1).

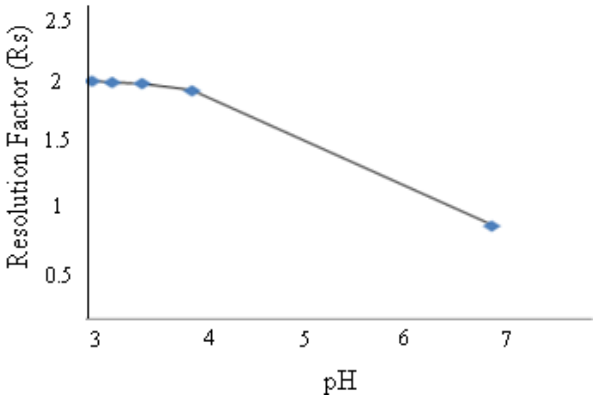

Figure 2: Variation of resolution factor between BHT and OMC at different pH values of phase B of mobile phase.

Table 1: Effect of acetic acid percentage in phase (B) on pH, resolution factors and total analysis time.

Acetic acid concentration (%, v/v)	0	0.5	1	1.5	2
pH value	7.0	4.0	3.5	3.2	3.0
Resolution factors (R_s)	0.79	1.92	1.98	1.99	2.00
Total time to elutes the analytes (min)	8.5	6.0	5.5	5.3	5.3

It was observed that the resolution factor (R_s) particularly for separation between BHT and OMC depended on the pH values of phase B of mobile phase. Mixture of water:acetic acid (99:1; v/v) of phase B as buffer solution at pH 3.5 was chosen after a compromise between resolution factors (R_s: 1.98 > 1.5) and total elution time to completely separated BHA, BHT and OMC were 5.5 minutes. BHA, BHT and OMC were eluted earlier at pH 3.5 compared to those at pH 4 and 7 (see Figures 3). The resolution factor was also better at pH 3.5 (R_s: 1.98 > 1.5) compared to pH 4 (R_s: 1.92 > 1.5) and pH 7 (R_s: 0.79 < 1.5).

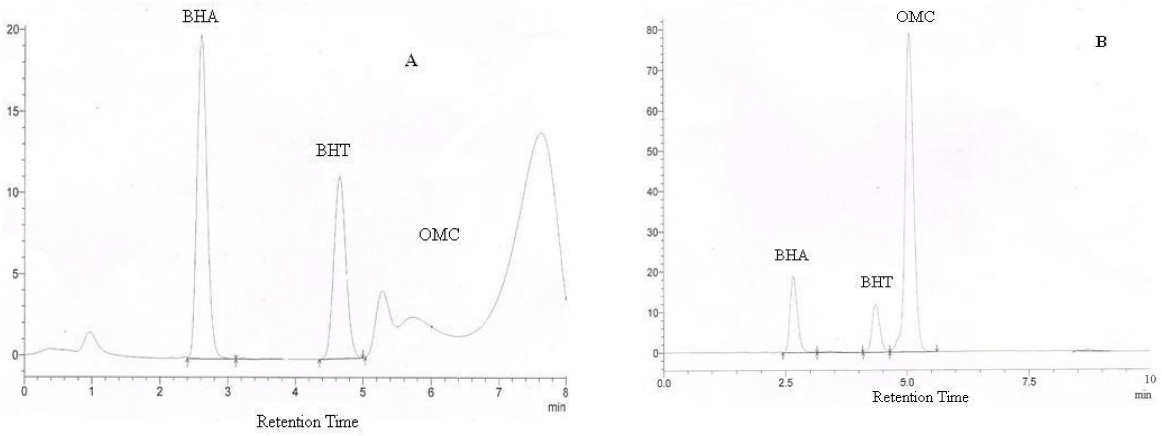

Figure 3: Chromatogram of BHA, BHT and OMC analyzed using RP-HPLC-UV/Vis at λ_{max}= 280 nm, (A: pH 7, Rs: 0.79 < 1.5 and B: pH 3.5, R_s: 1.98 > 1.5).

Effect the flow rate of mobile phase on retention time

Flow rate of mobile phase has important effect on retention time, peak area and little effect on separation for BHA, BHT and OMC. Table 2 shows gradient scaling of flow rates from 0.1 mL/min to 1.25 ml/min using RP-HPLC-UV/Vis.

Effect of mobile phase composition on retention time

The optimum composition of mobile phase for RPHPLC-UV/Vis analysis was determined by comparing the influence of different binary mixtures on retention times of BHA, BHT and OMC. Several mobile phase systems for RP-HPLC-UV/Vis analysis with detection at maximum wave length (λ_{max}) of 280 nm and flow rate of mobile phase at 0.8 mL/min were tested. These mobile phase systems were acetonitrile with water:acetic acid (99:1; v/v) (system A)[**14,19**], acetonitrile with methanol (system B) [**16,20**], ethanol with mixture of water:acetic acid (99:1; v/v)

(system C) [**4,11**] and acetonitrile with ethanol (system D) [**21**]. Figure 4 shows that better separation of BHA, BHT and OMC was achieved by using acetonitrile with mixture of water:acetic acid (99:1; v/v) as mobile phase.

Table 2: The retention times of BHA, BHT and OMC at different flow rate of mobile phase.

Flow rate (mL/min)	Retention time of BHA (minutes)	Retention time of BHT (minutes)	Retention time of OMC (minutes)
0.10	21.18	34.93	40.69
0.15	13.98	22.81	26.48
0.20	10.53	16.89	19.49
0.25	8.59	14.49	16.99
0.30	7.02	11.22	12.94
0.35	5.90	9.09	10.44
0.40	5.34	8.86	9.93
0.45	4.97	8.08	8.92
0.50	4.3	6.74	7.74
0.55	3.82	6.05	6.95
0.60	3.49	5.51	6.33
0.65	3.21	5.03	5.79
0.70	3.03	5.03	5.85
0.75	2.82	4.60	5.33
0.80	2.65	4.35	5.05
0.85	2.35	3.79	4.37
0.90	2.33	3.72	4.29
0.95	2.22	3.63	4.19
1.00	2.09	3.29	3.79
1.05	1.97	3.06	3.62
1.10	1.92	3.05	3.58
1.15	1.87	3.01	3.56
1.20	1.81	2.94	3.48
1.25	1.72	2.85	3.29

Validation method

The validation studies for BHA, BHT and OMC using RP-HPLC-UV/Vis were performed under the following optimized condition : maximum wave length at 280 nm , flow rate of mobile phase at 0.8 mL/min and mobile phase system consists of a mixture phase A (acetonitrile) with phase B (water:acetic acid; 99:1; v/v) with ratio (90A:10B; v/v). The analysis under this optimized condition was completed in approximately 8 minutes.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Eight standards solution of BHA, BHT and OMC in acetonitrile with concentrations of 1, 10, 25, 50, 75, 100, 125 and 250 mg/L were prepared. The calibration curves were obtained by plotting the peak area of chromatograms for BHA, BHT and OMC against the concentration (see Figure 5) in four replicates (n=4). Correlation coefficients (R^2) were 0.999 for all standards. Table 3 shows the results of analytical method validation obtained from the calibration curves of BHA, BHT and OMC analyzed on RP-HPLC-UV/Vis.

LOD for BHA and BHT by RP-HPLC-UV/Vis at 0.196 and 0.170 mg/L, respectively. These ranges were lower compared to those reported by Capitan-Vallvey et al. [**5**], Saad et al. [**14**] Campos & Figueiredo-Toledo [**22**] and Perrin & Meyer [**23**]. While, LOD for OMC by RP-HPLC-UV/Vis obtained in this study was 0.478 mg/L and this was lower compared to those reported by Chawla & Mrig [**2**], Salvador & Chisvert [**11**], Orsi et al. [**16**] and

Mazonakis et al. [**24**]. Thus, the LOD for BHA, BHT and OMC obtained in this study have been improved than those reported by previous studies due to the application of efficient mobile phase and detection system.

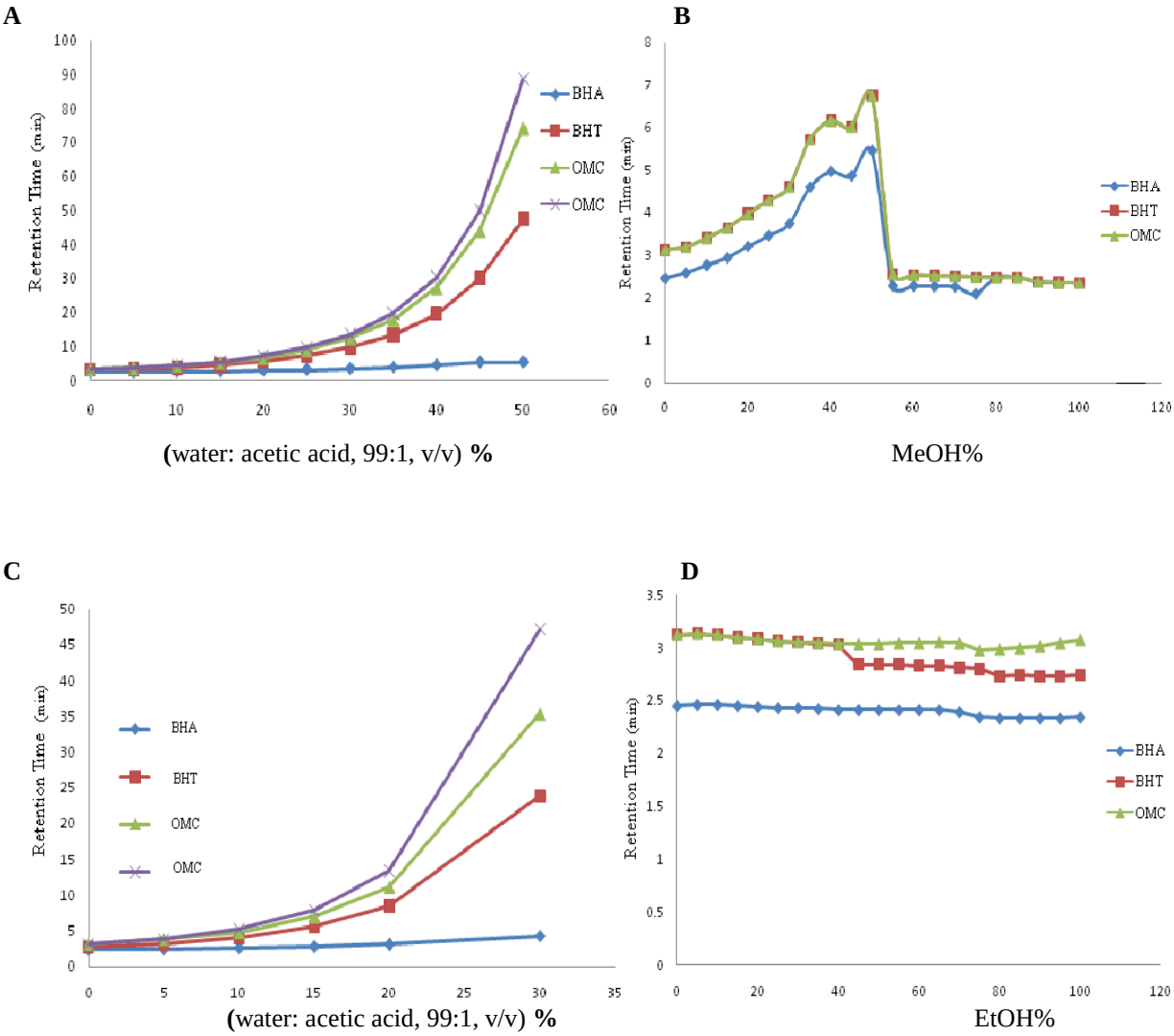

Figure 4: Effect of mobile phase composition on retention time of BHA, BHT and OMC.

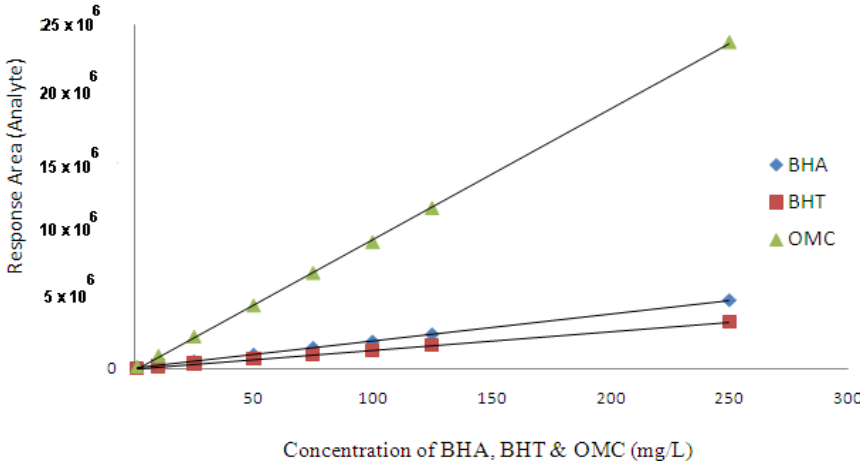

Figure 5: Calibration curves for BH, BHT and OMC analysed on RP-HPLC-UV/Vis at λ_{max}=280 nm, 0.8 ml/min and (water: acetic acid, 99:1, v/v) as mobile phase.

Table 3: Validation of analytical method for BHA, BHT and OMC by RP-HPLC-UV/Vis.

Compound	R.T (min)	Calibration Equation	R^2	RSD %	LOD (mg/L)	LOQ (mg/L)
BHA	2.60	y=19673x + 2579	0.999	0.18	0.196	0.593
BHT	4.35	y= 13410x – 5551	0.999	0.17	0.170	0.515
OMC	4.95	y= 95019x – 14004	0.999	0.25	0.478	1.448

Comparison with LOD reported in literatures:

Phenolic compounds	LOD (mg/L)							
	[**2**]	[**5**]	[**11**]	[**14**]	[**16**]	[**22**]	[**23**]	[**24**]
BHA	-	1.8	-	0.5	-	0.6	-	-
BHT	-	2.1	-	0.5	-	2.7	-	-
OMC	1.38	-	0.9	-	0.8	-	0.478	1.11

Recovery efficiency and Method Performance

The relative recoveries for phenolic compounds were determined by using the external standard addition method at four spiking levels of 1, 5, 10 and 25 mg/L by comparing with a standard chromatogram of similar concentration. Mean recoveries for each spiking level were determined three times with four replicates representing at each time (see Table 4).

Table 4: Results of recovery study for BHA, BHT and OMC by RP-HPLC-UV/Vis at λ_{max}= 280 nm.

Spiked (mg/L)	Relative Recovery (%, n=12)					
	BHA	RSD %	BHT	RSD %	OMC	RSD %
1	105.9	2.64	108.9	7.69	103.7	2.53
5	102.3	3.72	102.8	4.02	94.6	1.95
10	99.7	1.65	95.9	3.13	93.3	1.45
25	92.1	1.18	83.2	2.24	87.3	1.27

Recoveries of BHA and BHT were in the range of 92.1-105.9% and 83.2-108.9%, respectively. These recovery efficiencies are comparable to those reported by Saad et al. [**14**] which ranged between 96.7-101.2 and 73.9-94.6 %, respectively, by using the similar external standard addition method. The recovery of OMC in this study was in the range of 87.3-103.7% . This is comparable to those reported by Mazonakis et al. [**24**] which ranged between 87.6-101.3 %.

Real samples Analysis

Four types of personal care products such as sunscreen cream, milk lotion, hair gel and hair oil with three different samples for each type were analyzed for their BHA, BHT and OMC content. These samples were analyzed three times with four replicates for each time. The results for analysis of real samples using the optimized RP-HPLC-UV/Vis method are presented in Tables 5, 6, 7 and 8.

Table 5 shows that concentration ranges of BHA and BHT in three different commercial products of sunscreen cream, namely Aiken, Nivea and Gervenne ranged between 1.82-4.85 and 1.01-1.33 mg/g, respectively. These range were higher than those reported by Yang et al. [**3**]. While, the concentration of BHT in these sunscreen products was lower than concentration of BHT in other sunscreen products as reported by Capitan-Vallvey et al. [**4**]. On other hand, the concentration range of OMC in these sunscreen products (16.23-65.50 mg/g) was lower compared to those reported by Chawla & Mrig [**2**], Wang & Chen [**8**], Chisvert et al. [**9, 25**] and Orsi et al. [**16**].

Table 5: Concentration of BHA, BHT and OMC in sunscreen samples determined by RP-HPLC-UV/Vis at λ_{max}= 280 nm.

Commercial Name	Country of Origin	Phenolic Compounds	Mean Concentration (mg/g) (1) (n=4)	(2) (n=4)	(3) (n=4)	Average (mg/g)	RSD %
Aiken	Malaysia	BHA	4.80±0.10	4.90±0.07	4.90±0.05	4.85	1.50
		BHT	1.30±0.06	1.40±0.07	1.28±0.03	1.33	3.88
		OMC	62.10±0.60	65.9±0.41	68.5±0.51	65.5	0.77
Nivea	Thailand	BHA	3.31±0.09	3.03±0.08	3.43±0.07	3.26	2.43
		BHT	1.16±0.06	1.03±0.04	0.85±0.04	1.01	4.47
		OMC	27.68±0.4	30.72±0.3	25.48±0.6	27.96	1.58
Gervenne	Malaysia	BHA	1.93±0.08	1.81±0.06	1.72±0.08	1.82	3.92
		BHT	n.d	n.d	n.d	n.d	n.d
		OMC	16.66±0.4	14.61±0.5	17.43±0.4	16.23	2.68

Comparison:

Phenolic compounds	Concentration (mg/g) reported in literatures [**2**]	[**3**]	[**4**]	[**8**]	[**9**]	[**16**]	[**25**]	This study
BHA	n.d	0.003-0.026	-	-		-		1.82-4.85
BHT	0.408	0.006	2.263	-		-	n.d	1.01-1.33
OMC	56.12-91.02	-	-	18.3-8`0.1	19.5-90.5	20-74	5.8-77.8	16.23-65.50

n.d: not detected or below detection limit.

Table 6 shows that concentration ranges of BHA and BHT in three different commercial products of milk lotion, namely Nivea, New Trendy and Garnier (2.74-4.50 and 0.73-2.30 mg/g, respectively) were higher compared to concentration of BHA and BHT in other milk lotion products reported by Yang et al. [**3**], Capitan-Vallvey et al. [**4,5**] and Tsai & Lee [**26**]. The concentration range of OMC in these milk lotion samples (8.99-17.00 mg/g) was lower

compared with concentration of OMC in milk lotion products reported by Salvador & Chisvert [**11**] and Mazonakis et al. [**24**].

Table 6: Concentration of BHA, BHT and OMC in Milk lotion samples using RP-HPLC-UV/Vis at λ_{max}= 280 nm.

Commercial Name	Country of Origin	Phenolic Compounds	Mean Concentration (mg/g)				
			(1) (n=4)	(2) (n=4)	(3) (n=4)	Average (mg/g)	RSD %
Nivea	Thailand	BHA	4.51±0.12	4.46±0.05	4.55±0.04	4.50	1.57
		BHT	1.96±0.09	2.58±0.07	2.37±0.06	2.30	3.21
		OMC	13.4±0.26	12.5±0.17	15.6±0.21	13.83	1.55
New Trendy	Malaysia	BHA	3.92±0.15	4.15±0.11	4.42±0.09	4.16	2.82
		BHT	n.d	n.d	n.d	n.d	n.d
		OMC	7.82±0.38	8.68±0.32	10.48±0.31	8.99	3.79
Garnier	Indonesia	BHA	2.96±0.09	2.47±0.10	2.79±0.09	2.74	3.32
		BHT	0.64±0.03	0.83±0.02	0.71±0.03	0.73	3.26
		OMC	20.41±0.38	16.64±0.30	15.13±0.30	17.0	1.86

Comparison:

Phenolic compounds	Concentration (mg/g) reported in literatures						
	[**3**]	[**4**]	[**5**]	[**11**]	[**23**]	[**25**]	This study
BHA	n.d	0.017	n.d	-	-	n.d	2.74-4.50
BHT	n.d	0.610	0.408	-	-	n.d	n.d – 2.30
OMC	-	-	-	30.2-74.1	70-75	-	8.99-17.00

n.d: not detected or below detection limit.

Table 7 shows concentration ranges of BHA and BHT in three different hair gel products, namely De Boy, Beyond and Elite were in the range between 1.28-1.51 and 0.16-0.22 mg/g, respectively. Yang et al. [**3**] and Garcia-Jimenez et al. [**27**] reported that BHA and BHT was not detected in several hair gel products analysed. While, the concentration of OMC in these hair gel samples was in the range of 0.12-0.84 mg/g. OMC was not detected in hair care products screened by Gao & Bedell [**28**].

Table 7: Concentration of BHA, BHT and OMC in hair gel samples determined by RP-HPLC-UV/Vis at λ_{max}= 280nm.

Commercial Name	Country of Origin	Phenolic Compounds	Mean Concentration (mg/g)				
			(1) (n=4)	(2) (n=4)	(3) (n=4)	Average (mg/g)	RSD %
De Boy	Malaysia	BHA	1.23±0.05	1.27±0.04	1.33±0.04	1.28	3.14
		BHT	0.17±0.01	0.24±0.01	0.26±0.01	0.22	3.40
		OMC	0.11±0.01	0.15±0.01	0.12±0.01	0.13	4.52
Beyond	Malaysia	BHA	1.28±0.04	1.36±0.06	1.49±0.05	1.38	3.37
		BHT	0.13±0.01	0.19±0.01	0.16±0.01	0.16	4.05
		OMC	0.31±0.01	0.24±0.01	0.36±0.02	0.30	3.48
Elite	Malaysia	BHA	1.42±0.06	1.48±0.03	1.63±0.04	1.51	2.76
		BHT	0.17±0.01	0.11±0.01	0.23±0.01	0.17	4.48
		OMC	0.81±0.03	0.93±0.02	0.79±0.02	0.84	2.69

Comparison:

	Concentration (mg/g) reported in literatures			
Phenolic compound	[**3**]	[**26**]	[**27**]	This study
BHA	n.d	n.d	-	1.28-1.51 mg/g
BHT	n.d	n.d	-	0.16-0.22 mg/g
OMC	-	-	n.d	0.12-0.84 mg/g

n.d: not detected or below detection limit.

Table 8: Concentration of BHA, BHT and OMC in hair oil samples determined by RP-HPLC-UV/Vis at λ_{max}= 280nm.

Commercial Name	Country of Origin	Phenolic Compounds	Mean Concentration (mg/g) (1) (n=4)	(2) (n=4)	(3) (n=4)	Average (mg/mL)	RSD %
Elite	Malaysia	BHA	3.96±0.04	3.93±0.03	3.85±0.05	3.89	1.06
		BHT	0.89±0.02	0.87±0.02	0.84±0.01	0.87	2.11
		OMC	0.83±0.02	0.82±0.01	0.80±0.01	0.82	1.37
Gervenne	Malaysia	BHA	0.11±0.01	0.12±0.01	0.15±0.01	0.13	4.66
		BHT	1.44±0.05	1.61±0.05	1.57±0.06	1.54	3.25
		OMC	3.42±0.06	3.29±0.07	3.48±0.05	3.40	1.75
Johnsons	Philippines	BHA	0.34±0.01	0.29±0.01	0.26±0.01	0.30	3.40
		BHT	0.19±0.01	0.22±0.01	0.14±0.01	0.18	4.13
		OMC	0.51±0.02	0.63±0.01	0.56±0.01	0.57	2.19

Comparison:

	Concentration (mg/g) reported in literatures			
Phenolic compounds	[**4**]	[**5**]	[**28**]	This study
BHA	0.031	n.d	-	0.13-3.89
BHT	0.100	0.659	-	0.18-1.54
OMC	-	-	n.d	

Conclusion

A convenient and rapid RP-HPLC-UV/Vis has been developed for the estimation of common phenolic compounds in several types of personal care products. The optimum parameters that can be used are as follows; binary mixture of phase A (acetonitrile) and phase B (water /acetic acid, 99:1, v/v) as mobile phase with the elution ratio (90 A: 10 B, v/v) with the analysis time (8 minutes), pH 3.5 of phase B (using acetic acid for adjust it), 0.8 mL/min flow rate of 0.8 mL/min and maximum detector wave length at 280 nm. The method is fast, accurate, sensitive, provide excellent recoveries, convenient and effective for the simultaneous quantification of phenolic compounds for routine analysis in quality control of commercial cosmetic products. The developed method can be used to fingerprint the relevant phenolic compounds markers present in personal care products under optimum parameters. This method can be applied to analyze the phenolic compounds in commercial cosmetic and food products.

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References

1. Tsai, T.F., Lee, M.R. (2008). Determination of antioxidants and preservatives in cosmetics by SPME combined with GC–MS. *Chromatographia*, 67: 425-431.
2. Chawla, H.M., Mrig, S. (2009). Simultaneous quantitative estimation of oxybenzone and 2-ethylhexyl-4-methoxycinnamate in sunscreen formulations by second order derivative spectrophotometry. *Journal of Analytical Chemistry*, 64(6): 585–592.
3. Yang, T.J., Tsai, F.J., Chen, C.Y., Yang, T.C.C. & Lee, M.R. (2010). Determination of additives in cosmetics by supercritical fluid extraction on-line headspace solid-phase microextraction combined with gas chromatography–mass spectrometry. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 668:188-194.
4. Capitan-Vallvey, L. F., Valencia, M. C., Nicolas, E. A. (2002). Flow-through sensor for determination of butylated hydroxytoluene in cosmetics. *Analytical Letters*, 35(1): 65-81.
5. Capitan-Vallvey, L. F., Valencia, M. C., Nicolas, E. A. (2004). Solid-phase ultraviolet absorbance spectrophotometric multisensor for the simultaneous determination of butylated hydroxytoluene and co-existing antioxidants. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 503: 179-186.
6. Porat, Y., Abramowitz, A., Gazit, E. (2006). Inhibition of amyloid fibril formation by polyphenols: Structural similarity and aromatic interactions as a common inhibition mechanism. *Chemical Biology and Drug Design*, 67: 27–37.
7. Stockmann, H., Schwarz, *K.*, Huynh, T. (2000). The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. *Journal of Surfactants and Detergents*, 77(5): 535-542.
8. Wang, S.P., Chen, W.J. (2000). Determination of *p*-aminobenzoates and cinnamate in cosmetic matrix by supercritical fluid extraction and micellar electrokinetic capillary chromatography. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 416: 157-167.
9. Chisvert, A., Salvador, A., Pascual-Marti, M.C. (2001). Simultaneous determination of oxybenzone and 2-ethylhexyl 4-methoxycinnamate in sunscreen formulations by flow injection-isodifferential derivative ultraviolet spectrometry. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 428: 183-190.
10. Dutra, E.A., Oliveira, D.A.G.D., Kedor-Hackmann, E.R.M., Santoro, M.L.R.M. (2004). Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Brazilian Journal of Pharmaceutical Sciences*, 40(3): 381-385.
11. Salvador, A., Chisvert, A. (2005). An environmentally friendly (green) reversed-phase liquid chromatography method for UV filters determination in cosmetics. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 537: 15-24.
12. Venkatesh, G., Majid, M. I. A., Ramanathan, S., Mansor, S. M., Nair, N. K., Croft, S. L., Navaratnam, V. (2008). Optimization and validation of RP-HPLC-UV method with solid-phase extraction for determination of buparvaquone in human and rabbit plasma: application to pharmacokinetic study. *Biomedical Chromatography*, 22: 535–541.
13. Lee, M. R., Lin, C. Y., Li, Z. G., Tsai, T. F. (2006). Simultaneous analysis of antioxidants and preservatives in cosmetics by supercritical fluid extraction combined with liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1120: 244-251.
14. Saad, B., Sing, Y.Y., Nawi, M. A., Hashim, N., Ali, A. M., Saleh, M. I., Ahmad, K. (2007). Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chemistry*, 105: 389–394.
15. Song, D., Wang, J. 2003. Modified resolution factor for asymmetrical peaks in chromatographic separation. *Journal of Pharmaceutical and Biomedical Analysis*, 32: 1105-1112.
16. Orsi, D.D., Giannini, G., Gagliardi, L., Porra, R., Berri, S., Bolasco, A., Carpani, I., Tonelli, D. (2006). Simple extraction and HPLC determination of UV-A and UV-B filters in Sunscreen. *Chromatographia*, 64: 9-10.
17. Fang, F., Jing-Ming, L., Qiu-Hong, P., Wei-Dong, H. (2007). Determination of red wine flavonoids by HPLC and effect of aging. *Food Chemistry*, 101: 428–433.
18. Neungnapa, R., Jia, Z., Xuewu, D., Bao, Y., Jianrong, L., Yueming, J. (2008). Effects of various temperatures and pH values on the extraction yield of phenolics from litchi fruit pericarp tissue and the antioxidant activity of the extracted anthocyanins. *International Journal of Molecular Sciences*, 21:105-116.

19. Dondi, D., Albini, A., Serpone, N. (2006). Interactions between different solar UVB/UVA filters contained in commercial suncreams and consequent loss of UV protection. *The Royal Society of Chemistry and Owner Societies*, 5: 835-843.
20. Perrin, C., Meyer, L. (2003). Simultaneous determination of ascorbyl palmitate and nine phenolic antioxidants in vegetable oils and edible fats by HPLC. *Journal of the American Oils Chemist's Society,* 80 (2): 115 -118.
21. Tsuji, S., Nakano, M., Terada, H., Tamura, Y., Tonogal, Y. (2005). Determination and confirmation of five phenolic antioxidants in foods by LC/MS and GC/MS. *Japanese Society of Food Hygienically*, 46(3): 63-71.
22. Campos, G.C.M.D., Toledo, M.C.F. (2000). Determination of BHA, BHT and TBHQ in fats and oils by high performance liquid chromatography. *Brazilian Journal of Food Technology*, 3: 65-71.
23. Perrin, C., Meyer, L. (2002). Quantification of synthetic phenolic antioxidants in dry foods by reversed-phase HPLC with photodiode array detection. *Food Chemistry*, 77: 93–100.
24. Mazonakis, N.E., Karathanassi, P.H., Panagiotopoulos, D.P., Hamosfakidi, P.G., Melissos, D.A. (2002). Cleaning validation in the toiletries industry. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement,* 467: 261-266.
25. Chisvert, A., Pascual-Marti, M.C., Salvador, A. (2001b). Determination of UV-filters in sunscreens by HPLC. *Journal of Analytical Chemistry*, 369: 638–641.
26. Tsai, T. F., Lee, M. R. (2008). Determination of antioxidants and preservatives in cosmetics by SPME combined with GC-MS. *Chromatographia*, 67: 425-431.
27. Garcia-Jimenez, J.F., Valencia, M.C., Capitan-Vallvey, L.F. (2007). Simultaneous determination of antioxidants, preservatives and sweetener additives in food and cosmetics by flow injection analysis coupled to a monolithic column. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 594: 226-233.
28. Gao, T., Bedell, A. (2001). Ultraviolet damage on natural gray hair and its photoprotection. *Journal of Cosmetic Science*, 52: 103-118.
29. Fent, K., Kunz, P.Y., Zenker, A., Rapp, M. (2009). A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor) 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. *Marine Environmental Research*, 10: 1016-1018.