

Optimization and Validation of RP-HPLC-UV/Vis Method for Determination Phenolic Compounds in Several Personal Care Products

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Abstract

A sensitive and selective reversed phase 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 methylcinnamat (OMC) as UVB-filter in several personal care products. The optimum analytical conditions (280 nm as maximum wave length, 0.8 mL/min as flow rate, pH 3.5, mixture of phase A (acetonitrile) with phase B (water:acetic acid; 99:1; v/v) as mobile phase) were obtained by variation of flow rate, the pH value of phase B, different binary mixtures. The dynamic range was between 1 to 250 mg L⁻¹ with relative standard deviation better than 0.25%, (n=4). Limit of detection for BHA, BHT and OMC were 0.196, 0.170 and 0.478 mg L⁻¹, respectively. While limit of quantification for BHA, BHT and OMC were 0.593, 0.515 and 1.448 mg L⁻¹, respectively. Recovery study was performed by spiking standard of phenolic compounds at four different concentration levels using external standard addition method. The recovery for BHA, BHT and OMC were 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⁻¹, respectively. The concentrations of phenolic compounds in these personal care samples were below than maximum allowable concentration in personal care formulation i.e up to 1% (w/w) for BHA, up to 0.5% (w/w) for BHT and up to 10% (w/w) for OMC

Keywords:

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

1. Introduction

Phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) act as antioxidants and octyl methylcinnamat (OMC) as UVB-filter are active compounds in personal care products (see Fig. 1) [1,2].

BHA and BHT are added singly or in combination to prevent oxidative rancidity in personal care products [3]. While octyl methylcinnamat (OMC) is used to absorb the dangerous UV-light between 280-320 nm to protect the skin from sunburn [2]. The concentration of BHA and BHT in personal care formulation depends on the amount of sensitive compounds (alpha hydroxy acids, ceramides, lipids, vitamins, oils and other), that are susceptible to oxidation by the oxygen in the atmosphere making it possible for the unstable peroxide radicals [4,5]. BHA and BHT are able to inhibit reactions promoted by oxygen, thus avoiding the oxidation and are intended to prevent the appearance of ketones and aldehydes that can give a product a disagreeable smell and rancidity [5]. To prevent

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cosmetic formulations from peroxide radicals must using antioxidant compounds which have the ability to neutralize those radicals through the transfer of hydrogen to this radical, stabilizing the antioxidant by resonance [6,7]. While, the concentration of OMC depend on type of product and part of body it applied (face, hand, lips and other parts of human body) [2,8,9,10,11].

Reversed phase HPLC with UV/Vis detector (RP-HPLC-UV/Vis) is an important analytical technique with strong chromophores that absorb light in the wavelength region from 200 nm to 800 nm [12]. Numerous publications and research papers focus on separation methods to detect phenolic antioxidants as BHA and BHT, and phenolic UVB-filte as OMC in personal care products using RP-HPLC-UV/Vis [2,5,13]. The objective of this study is to determine the optimum analysis condition and validate the method for a simultaneous detection, identification, and quantification of phenolic compounds as well as to develop an analytical method for evaluation and quality control of phenolic compounds by RP-HPLC-UV/Vis in personal care products.

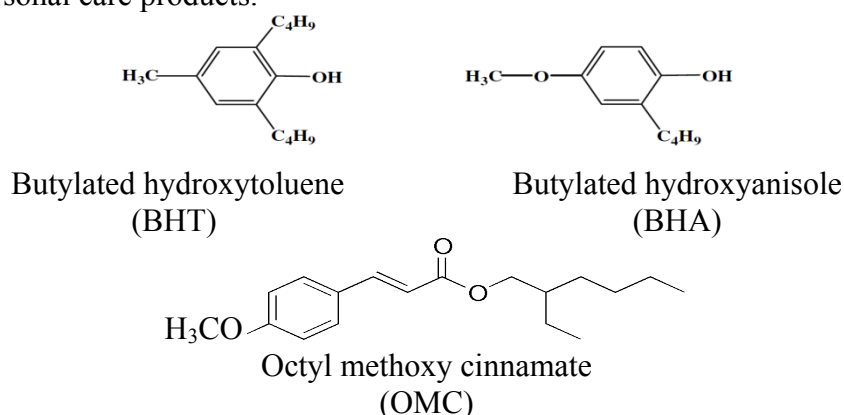


Fig.1. Structures of common phenolic compounds in personal care products.

2. Experimental

2.1. Personal care samples

12 personal care samples were collected from local supermarket in Kuching city. Four types of personal care products were collected, i.e. sunscreen cream, milk lotion, hair gel and hair oil. The personal care samples were manufactured in Malaysia, Thailand, Indonesia and Philippines.

2.2. Chemicals

All chemical reagents used for analysis phenolic compounds by RP-HPLC-UV/Vis were analytical Grade (99.99%) of Merck (Darmstadt, Germany). The reagents include n-hexane, methanol, ethanol and acetonitrile. Reverse-osmosis type quality water was used during analysis. Standards of butylated hydroxyanisole BHA (96%), butylated hydroxytoluene BHT (99.8%), and OMC octyl methoxy cinnamate (98%) were purchased from Acros-Organics (New Jersey, USA).

2.3. Preparation of standard solution

An individual of 5000 mg L⁻¹ stock solution of BHA, BHT and OMC in acetonitrile was prepared by weighing equivalent accurately 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 formed and added with acetonitrile until 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.

2.4. Extraction procedure

Extraction of BHA, BHT and OMC from cosmetic samples was performed according to method described by Capitan-Vallvey et al. [4] and Capitan-Vallvey et al. [5] with slight modification. Briefly, 0.1 to 1 g 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 25 mL acetonitrile. The sample was then extracted by refluxing for 30 minutes at 70°C and stirring. Extraction was performed in triplicates. The crude extract was transferred to separatory funnel and two layers were formed, i.e n-hexane and acetonitrile phases. The n-hexane phase was repartitioned for two or three times using 10 mL of acetonitrile and shaken vigorously. The n-hexane phase was removed and acetonitrile phase was collected. The extract (acetonitrile phase) was concentrated using a vacuum rotary evaporator at 45°C. The residue was redissolved with 10 mL of acetonitrile and filtered by membrane filters (Millipore, 0.5µm x 45 mm), then transferred into a 25 mL volumetric. It was diluted to 25 mL with acetonitrile.

2.5. HPLC analysis

The quantitative and qualitative analysis of phenolic compounds was performed on Shimadzu HPLC system model LC-20AT equipped with four pumps and Shimadzu SPD-20 AV UV/Vis detector. 50 µL samples was injected and the chromatographic separation was performed on a RP-C₁₈ Metacil (5 µm) ODS column, 4.6 mm×250 mm. The HPLC analysis condition based on the report of Saad et al., (2007) with slight modification using 280 nm as maximum wave length (λ_{max}), acetonitrile (phase A) and (water/acetic acid, 99:1, v/v) (phase B) as mobile phase and 0.8 mL min⁻¹ as flow rate.

3. Results and discussion

3.1. Optimization of HPLC condition

3.1.1. Determination the optimum wave length by spectrophotometer UV/Vis

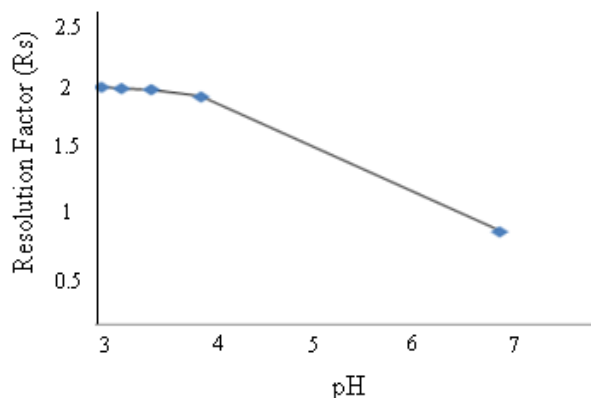
The UV spectrum of BHA, BHT and OMC exhibited maximum absorption at 290, 275 and 300 nm, respectively. For the RP-HPLC analysis, the UV/Vis detector was fixed at 280 nm as maximum wavelength (λ_{max}) for simultaneous determination.

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

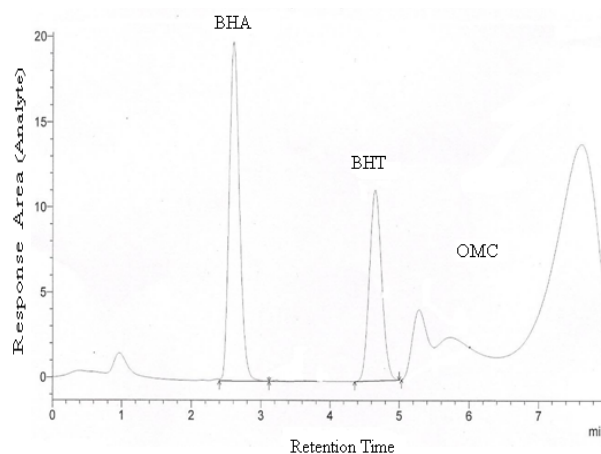
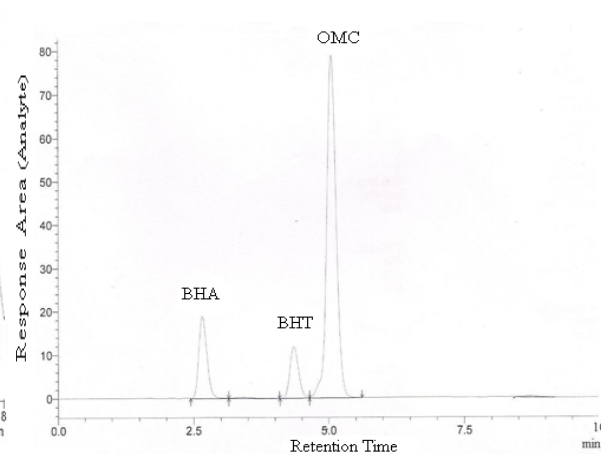
pH is an important parameter to be optimized as it affects the ionization of phenolic compounds. Separation of BHA, BHT and OMC are sensitive to the pH values because at low pH values phenolic antioxidants are ionized due to increase of protonation in mobile phase [14,15,16,17]. The analytical conditions were used for analysis BHA, BHT and OMC based on the recent report by Saad et al. [16], mixture phase A (acetonitrile) with phase B (water:acetic acid) as mobile phase, 280 nm as maximum wave length and 0.8 mL min⁻¹ 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, respectively. Decreasing pH value increases the separation and ionization of BHA, BHT and OMC, especially 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).

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

Acetic acid concentration (% v/v)	0	0.5	1	1.5	2
pH value	7	4	3.5	3.2	3
Resolution factors (R_s)	0.79	1.92	1.98	1.99	2
Total time of elute the analytes (minutes)	8.5	6.0	5.5	5.3	5.3

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

It was observed that the resolution factor (R_s) particularly for separation between BHT and OMC depend 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 time of elute of BHA, BHT and OMC (5.5 minutes). BHA, BHT and OMC at pH 3.5 elute earlier compared to at pH 4 and 7 (see Fig. 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).

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

3.1.3. Effect the flow rate of mobile phase on retention time

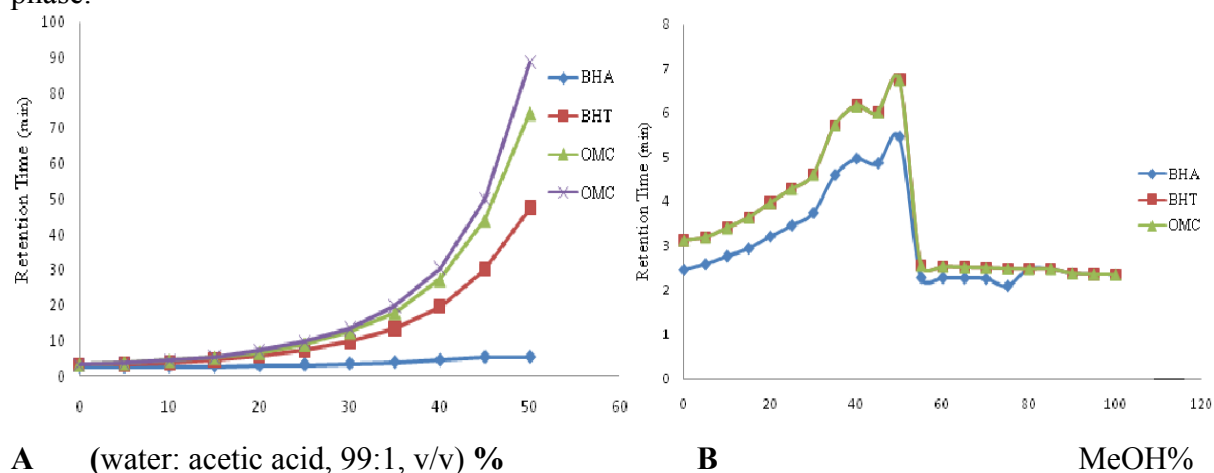
Flow rate of mobile phase has important effect on retention time, peak area and little effect on separation for BHA, BHT and OMC. Table 2 shows gradient scaling of flow rates from 0.1 mL min⁻¹ to 1.25 mL min⁻¹ using RP-HPLC-UV/Vis at 280 nm with mixture of phase A (acetonitrile) and phase B (water:acetic 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

3.1.4. Effect of mobile phase composition on retention time

Fig.4 shows that the optimum composition of mobile phase was determined by comparing the influence of different binary mixtures were used in previous studies on retention times of BHA, BHT and OMC using RP-HPLC-UV/Vis such as acetonitrile with mixture of water:acetic acid (99:1; v/v) (A) [16,18], acetonitrile with methanol (B) [14,19], ethanol with mixture of water:acetic acid (99:1; v/v) (C) [4,11] and acetonitrile with ethanol (D) [20] at 280 nm as maximum wave length (λ_{max}) and 0.8 mL min⁻¹ as flow rate of mobile phase.



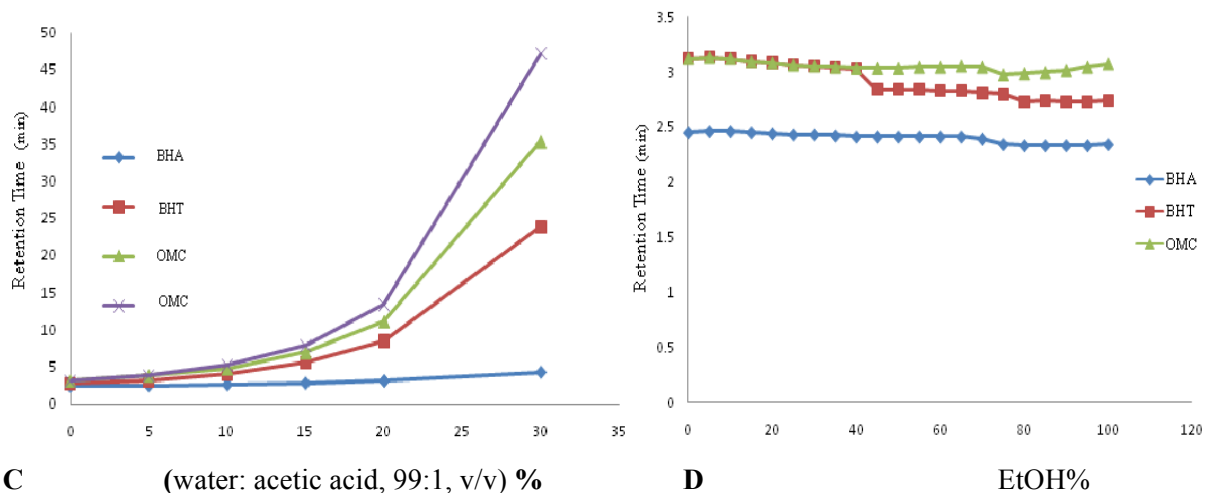


Fig. 4. Effect of mobile phase composition on retention time of BHA, BHT and OMC.

3.2. Validation method

The validation study for BHA, BHT and OMC using RP-HPLC-UV/Vis was performed under the optimized conditions at 280 nm as maximum wave length, 0.8 mL/min as flow rate of mobile phase, mixture phase A (acetonitrile) with phase B (water:acetic acid; 99:1; v/v) as mobile phase with elution ratio (90A:10B; v/v) during the analysis time (8 minutes).

3.3. Linearity and limits of detection (LOD), quantification (LOQ)

Eight standards solution of BHA, BHT and OMC in acetonitrile concentrations of 1, 10, 25, 50, 75, 100, 125 and 250 mg L⁻¹ were prepared. The calibration curves obtained by plotting the peak area of chromatograms for BHA, BHT and OMC against the concentration are presented in Fig. 5, with four replicates (n=4). Correlation coefficients (R²) were 0.999 for all standards. Table 3 shows the validation of analytical method obtained from the calibration curves of BHA, BHT and OMC analysed on RP-HPLC-UV/Vis.

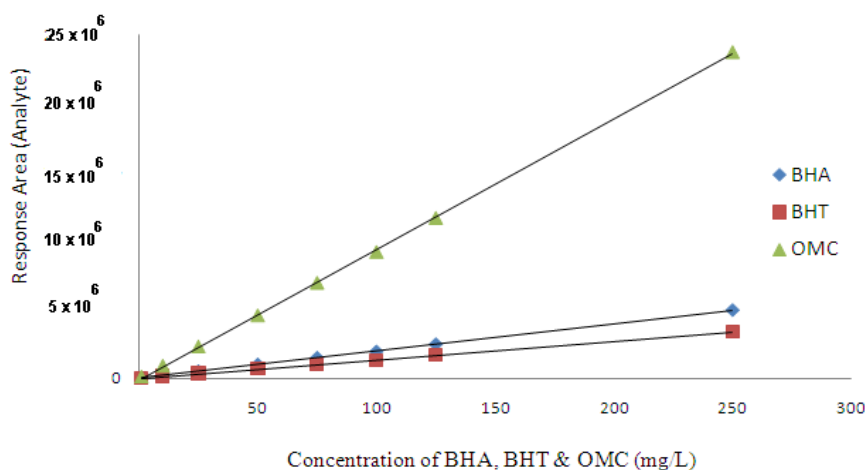


Fig. 5. Calibration curves for BHA, BHT and OMC analysed on RP-HPLC-UV/Vis at λ_{max} =280 nm, 0.8 mL min⁻¹ and (water: acetic acid, 99:1, v/v) as mobile phase.

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

Compound	Retention time (minutes)	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

LOD for BHA and BHT by RP-HPLC-UV/Vis in this study (0.196 and 0.170 mg L⁻¹, respectively) are low compare with previous publications for LOD of BHA and BHT reported by Capitan-Vallvey et al. [5] (1.8 and 2.1 mg L⁻¹, respectively), by Saad et al. [16] (0.5 and 0.5 mg L⁻¹, respectively), by Campos & Figueiredo-Toledo [21] (0.6 and 2.7 mg L⁻¹, respectively), by Perrin & Meyer [22] (2 and 2 mg L⁻¹, respectively). While, LOD for OMC by RP-HPLC-UV/Vis in this study (0.478 mg L⁻¹) is low compare with previous publications for LOD value of OMC have reported by Chawla & Mrig [2] (1.38 mg/L), Salvador & Chisvert [11] (0.9 mg L⁻¹), Orsi et al. [14] (0.8 mg/L) and Mazonakis et al. [23] (1.11 mg L⁻¹). Thus, the LOD for BHA, BHT and OMC in this study are better compared to previous studies.

3.4. Recovery efficiency and method performance

The relative recoveries for phenolic compounds were determined by using the external standard additions methodology at four spiked levels 1, 5, 10 and 25 mg L⁻¹ by comparison with a standard chromatogram of similar concentration. Mean recoveries for every spiked level were determined at 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} =280nm.

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

The recovery ranges of BHA and BHT in this study (92.1-105.9, 83.2-108.9 %, respectively) are better than previous paper by Saad et al. [16] (96.7-101.2, 73.9-94.6 %, respectively) using the external standard addition methodology. While, the recovery range of OMC in this study (87.3-103.7 %) is similar with earlier study reported by Mazonakis et al. [23] (87.6-101.3 %).

3.5. Analysis real samples

Four types of personal care products such as sunscreen cream, milk lotion, hair gel and hair oil with three different samples for every type were analyzed for their BHA, BHT and OMC content as can be seen in Tables 5, 6, 7 and 8. Every real samples were analysed three times with four replicates for each time.

Table 5. Concentration of BHA, BHT and OMC in sunscreen 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 ⁻¹)	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	62.1±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

n.d: not detected or below detection limit.

Table 5 shows that concentration ranges of BHA and BHT in three different commercial products of sunscreen cream, namely Aiken, Nivea and Gervenne (1.82-4.85 and 1.01-1.33 mg g⁻¹, respectively) are higher than concentration range of BHA and BHT in other commercial sunscreen products reported by Yang et al. [3] (0.003-0.026 and 0.006 mg g⁻¹, respectively). While, The concentration of BHT in these sunscreen products (1.01-1.33 mg g⁻¹) is lower than concentration of BHT in other commercial products of sunscreen products reported by Capitan-Vallvey et al. [4] (2.263 mg g⁻¹). On other hand, the concentration range of OMC in these sunscreen products (16.23-65.50 mg g⁻¹) is low compare with previous studies for concentration range of OMC in other commercial sunscreen products reported by Chawla & Mrig [2] (56.12-91.02 mg g⁻¹), Wang & Chen [8] (18.3-80.1 mg g⁻¹), Chisvert et al. [9] (19.5-90.5 mg g⁻¹), Orsi et al. [14] (20-74 mg g⁻¹) and Chisvert et al. [24] (5.8-77.8 mg g⁻¹).

Table 6 shows that concentration ranges of BHA and BHT in three different commercial products of milk lotion, namely Nivea, New Trendy and Garnier (2.74-450 and 0.73-2.30 mg g⁻¹, respectively) are high compared with previous studies for concentration range of BHA and BHT in other commercial products of milk lotion reported by Yang et al. [3] (not detected and not detected), Capitan-Vallvey et al. [4] (0.127 and 0.610 mg g⁻¹), Capitan-Vallvey et al. [5] (not detected and 0.408 mg g⁻¹) and Tsai & Lee [25] (not detected and not detected). The concentration range of OMC in these milk lotion samples (8.99-17.00 mg g⁻¹) are low compared with previous studies for concentration range of OMC in other commercial products of milk lotion reported by Salvador & Chisvert [11] (30.2-74.1 mg g⁻¹) and Mazonakis et al. [23] (70-75 mg g⁻¹).

Table 7 shows concentration ranges of BHA and BHT and OMC in three different hair gel products, namely De Boy, Beyond and Elite (1.28-1.51 and 0.16-0.22 mg/g, respectively) are high compare with previous studies for concentration range of BHA and BHT in other commercial hair gel samples reported by Yang et al. [3] (not detected and not detected, respectively) and Garcia-Jimenez et al. [26] (not detected and not detected, respectively). While, the concentration range of OMC in these hair gel samples (0.12-0.84 mg/g) are higher than concentration of OMC in other commercial hair care products reported by Gao & Bedell, [27] (not detected).

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

Commercial Name	Country of Origin	Phenolic Compounds	Mean Concentration (mg g ⁻¹)				
			(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±0.3	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±0.3	15.13±0.3	17.0	1.86

n.d: not detected or below detection limit.

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 ⁻¹)	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

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 ⁻¹)	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

Table 8 shows concentration ranges of BHA and BHT in three different commercial hair oil products, namely Elite, Gervenne and Johnsons (0.13-3.89 and 0.18-1.54 mg g⁻¹, respectively) is high compare with previous studies for concentration of BHA and BHT in other commercial products of hair oils reported by Capitan-Vallvey et al. [4] (0.031 and 0.100 mg g⁻¹, respectively) and Capitan-Vallvey et al. [5] (not detected and 0.659 mg g⁻¹, respectively). While, the concentration range of OMC in these hair oil samples (0.57-3.40 mg g⁻¹) are higher than concentration of OMC in other commercial products of hair oil reported by Fent et al. [28] (not detected).

4. Conclusion

The analytical method by RP-HPLC-UV/Vis in this study is modern for simultaneous determination of common phenolic compounds in personal care products. The optimum parameters that can be used as follow; binary mixture of phase A (acetonitrile) and phase B (water /acetic acid, 99:1, v/v) as mobile phase with elution ratio (90 A: 10 B, v/v) during the analysis time (8 minutes), pH 3.5 of phase B (using acetic acid for adjust it), 0.8 mL/min as flow rate and 280 nm as maximum wave length. The satisfactory results of optimization and validation methods are quick, accurate, sensitive, excellent recoveries, convenient and effective for phenolic compounds. The developed method was successfully applied to fingerprint analysis of personal care products as well as quantify the relevant phenolic compounds markers present in these 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 TF, Lee MR (2008) Determination of antioxidants and preservatives in cosmetics by SPME combined with GC-MS. *Chromatographia*, 67: 425-431.
2. Chawla HM, 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 TJ, Tsai FJ, Chen CY, Yang TCC, Lee MR (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 LF, Valencia MC, Nicolas EA (2002) Flow-through sensor for determination of butylated hydroxytoluene in cosmetics. *Analytical Letters*, 35(1): 65-81.
5. Capitan-Vallvey LF, Valencia MC, Nicolas EA (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 SP, Chen WJ (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 MC (2001a) 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 EA, Oliveira DAGD, Kedor-Hackmann ERM, Santoro MLRM (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 MIA, Ramanathan S, Mansor SM, Nair NK, Croft SL, 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 MR, Lin CY, Li ZG, Tsai TF (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. Orsi DD, 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.
15. 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.
16. Saad B, Sing YY, Nawi MA, Hashim N, Ali AM, Saleh MI, Ahmad K (2007) Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chemistry*, 105: 389–394.
17. 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.
18. Dondi D, Albin 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.
19. 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.
20. 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.

21. Campos GCMD, Toledo MCF (2000) Determination of BHA, BHT and TBHQ in fats and oils by high performance liquid chromatography. *Brazilian Journal of Food Technology*, 3: 65-71.
22. 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.
23. Mazonakis NE, Karathanassi PH, Panagiotopoulos DP, Hamosfakidi PG, Melissos DA (2002) Cleaning validation in the toiletries industry. *Journal of Analytical Chemistry for Anti-Counterfeiting Trade Agreement*, 467: 261-266.
24. Chisvert A, Pascual-Marti MC, Salvador A (2001b) Determination of UV-filters in sunscreens by HPLC. *Journal of Analytical Chemistry*, 369: 638–641.
25. Tsai TF, Lee MR (2008) Determination of antioxidants and preservatives in cosmetics by SPME combined with GC-MS. *Chromatographia*, 67: 425-431.
26. Garcia-Jimenez JF, Valencia MC, Capitan-Vallvey LF (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.
27. Gao T, Bedell A (2001) Ultraviolet damage on natural gray hair and its photoprotection. *Journal of Cosmetic Science*, 52: 103-118.
28. Fent K, Kunz PY, Zenker A, Rapp M (2009) A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor) 2-ethyl-hexyl-4-trimethoxy-cinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. *Marine Environmental Research*, 10: 1016-1018.