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Determination of Methylisothiazolinone and Methylchloroisothiazolinone in personal care products by HPLC-DAD

Pham Ngoc Thuy Vy^{1,2,3}, Tran Viet Hung³, Phan Nguyen Truong Thang³,
Trung Dang-Bao^{1,2}, Tran Thi Kieu Anh^{1,2,3}

¹Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam

²Vietnam National University Ho Chi Minh City, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam

³Institute of Drug Quality Control – Ho Chi Minh City (IDQC-HCMC), Vietnam

Corresponding author's e-mail: ttkanh@hcmut.edu.vn

Abstract. Preservatives are often utilized to prevent growth of bacteria and extend shelf-life of personal care products (PCPs). This causes an increase in the number of cases of allergic contact dermatitis to preservatives. This study focused on the determination of two isothiazolinones (MI and MCI) in PCPs by HPLC-DAD. Different pretreatment methods were examined for different sample matrices. Recoveries were over 80% with %RSD < 6% in three studied sample matrices (wet tissue, shampoo and cream) at three different spiked levels. The method was applied to determine MI and MCI in 84 PCPs (shampoo, shower gel, cream, *etc.*) purchased in Ho Chi Minh City, Vietnam from March to July in 2021. MI and MCI were detected in some PCPs.

1. Introduction

Along with the socio-economic development, human beauty needs increase dramatically which lead to the development of the worldwide cosmetic market. Personal care products (PCPs) are gradually expanded due to the diversification of the consumer. The quality of PCPs is an issue that should be placed on top of the list because it has been exposed to the body. Except for the main ingredients in cosmetic products, there are also many other harmful substances as colorants or preservatives to prevent mold contamination. Methylisothiazolinone (MI) and methylchloroisothiazolinone (MCI) are heterocyclic organic compounds, which have powerful activities against a broad spectrum of fungi and bacteria at very low concentration and at a broad pH range (2–6) [1]. The use of products with preservatives on the skin causes contact dermatitis for consumers. Hence, the European Union restricts the maximum level of these preservatives in cosmetics to 0.0015% for 3:1 MCI/MI mixture [2–4] and the use of MI on leave-on products is banned. That may help to monitor illegal cosmetics, ensure the health and safety of consumer usage.

The permissible concentration of MI and MCI is low and the formulation of cosmetics or PCPs generally includes complex mixtures of different compounds. Analysis of MI and MCI in cosmetics requires different analysis techniques and sample preparations. In terms of analysis techniques, many methods are used to analyze isothiazolinones namely GC-MS [5], HPLC-MS [6, 7], HPLC-DAD [8].



Although GS-MS delivers good separation efficiency and identification capabilities, derivatization of different classes of biocides caused limits of its applications. Obviously, HPLC-MS method has identification capabilities for the determination of biocides and does not require derivatization, however; the instrument is expensive and requires internal standards with high cost. Both MI and MCI absorb UV lights with the maximum wavelength at 274nm [8]. Thus, the common analytical methodology to identify and quantify these preservatives in cosmetics is HPLC method with UV or diode-array detector (DAD) [8]. In terms of sample preparation, due to the complex structural complexities of cosmetic ingredients, a mixture of solvent as methanol: NaH₂PO₄ [9], methanol:H₂O [10], chloroform and then methanol [6] were used for extractions of MI and MCI in different types of cosmetics such as shampoos containing plant extracts [9], detergents [10] or eyeshadows and liquid lipsticks [6].

MI and MCI were previously detected at high concentrations in many cosmetics [6, 9, 10]; however, there is the lack of information on detections of MI and MCI in cosmetics or PCPs in Vietnam. The aim of this work is to develop a quick, easy and effective sample pretreatment method for simultaneous determination of MI and MCI in different kinds of PCPs by HPLC-DAD and to survey such MI and MCI in PCPs in Vietnam.

2. Materials and methods

2.1. Chemicals and reagents

MI (95%), MCI (95%) (Figure 1) and potassium dihydrogen phosphate were purchased from Sigma-Aldrich (Switzerland). Methanol, acetonitrile, formic acid and orthophosphoric acid of HPLC-grade were purchased from Merck Vietnam (Ho Chi Minh City, Vietnam). The 0.02 M phosphate buffer pH = 3.0 was made by dissolving 2.72 g of potassium dihydrogen phosphate in 900 mL of water, changing pH = 3.0 ± 0.1 orthophosphoric acid if required and diluting with water to make 1000 mL.

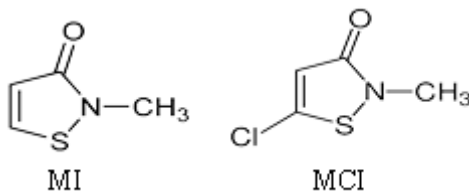


Figure 1. The chemical structures of MI and MCI.

Table 1. Physicochemical properties of MI and MCI.

Properties	Content	
Chemical name (INCI)	Methylchloroisothiazolinone	Methylisothiazolinone
Abbreviation	MCI	MI
IUPAC name	5-chloro-2-methylisothiazol-3(2H)-one	2-Methylisothiazol-3(2H)-one
CAS number	26172-55-4	2682-20-4
Chemical formula	C ₄ H ₄ ClNOS	C ₄ H ₅ NOS
Molecular weight	149.59	115.16
Synonyms	Chloromethylisothiazolinone Chloromethylthiazolone Methylchlorothiazolone	2-methyl-4-isothiazolin-2-one

INCI: International Nomenclature of Cosmetic Ingredients; IUPAC: International Union of Pure and Applied Chemistry; CAS: Chemical Abstracts Service.

2.2. Chromatographic conditions

The analysis was conducted on the Waters ACQ-rQSM instrument equipped with Waters 2998 photo diode array detector. The experiment was set at 274 nm for recording chromatograms. The chromatographic separation was carried out on the Gemini NX C18 Phenomenex column (25 cm × 4.6 mm, 5 μm) maintained at 25 °C. Mobile phase, solvent A, was 0.1% formic acid in distilled water, and solvent B was acetonitrile [3]. The flow rate was 1 mL/min and the injection volume was 25 μL. The gradient parameter of the mobile phase was as follows: initial - 5 min, 95% A; 5–10 min, 95% to 93% A; 10–20 min, 93% A; 20–23 min, 93% to 10% A; 23–25 min, 10% to 95% A; 25–30 min, 95% A.

2.3. Standard solutions

The stock solutions, MI (1838 mg/L) and MCI (1603 mg/L), were prepared separately in methanol and stored in refrigerator at 4 °C. Working standard solutions containing MI and MCI (from 0.3 to 20 ppm) were prepared in MeOH:H₂O (3:7, v/v). Before being used for chromatographic analysis, standard solutions were filtered through 0.45 μm nylon syringe filter membrane.

2.4. Sample preparation

A wide variety of PCPs were purchased from local supermarket and stored at room temperature. Based on the variations in PCPs formulation, sample treatments prior to chromatographic analysis were different. Clear liquid products were analyzed directly after diluting with distilled water or methanol if possible. Solid samples and liquid samples with high density or viscosity required ultrasonic extraction with different solvents before analysis [11]. In a previous study, sample matrices varied considerably medium water content, low water content and high water content [8]. Following that, shampoos, shower gel, cleanser, hand sanitizer (Group 1) are considered as high or medium water content whereas body cream and face cream are low ones (Group 2, Table 2).

Table 2. Classification of PCPs.

Classification	Sample names
Group 1	Shampoo, shower gel, cleanser, hand sanitizer
Group 2	Body cream, face cream
Group 3	Tissue

2.4.1. Group 1 (shampoo, shower gel, cleanser, hand sanitizer)

Approximately 1g of sample was extracted with 5 mL of solvent (mixture of methanol:NaH₂PO₄ (3:7, v/v)) [9] by vortexing in 1 min and ultrasonication for 30 min. After making up the volume to 10 mL with solvent, the extract was centrifuged for 10 min at 4000 rpm then filtered through 0.45 μm nylon syringe filter membrane before injecting to HPLC-DAD.

2.4.2. Group 2 (face cream, body cream)

Approximately 1 g of sample was firstly extracted with 1mL of chloroform [6] by vortexing for 1 min, 4 mL of methanol was added before the ultrasonication in 30 min. After making up the volume to 10 mL with methanol, the extract was centrifuged for 10 min at 4000 rpm then filtered through 0.45 μm nylon syringe filters membrane before injecting to system. In case the extract was cloudy, filtration using 0.22 μm nylon syringe filter membrane was required.

2.4.3. Group 3 (tissue)

Approximately 1 g of paper tissue was prepared by cutting it into small pieces (approximately 1 cm²). Then the sample was extracted with 5 mL of a mixture of methanol:H₂O (3:7, v/v) [3] by shaking in 1 min. Further extraction was performed by the ultrasonication for 30 min. After making up the volume

to 10 mL with solvent, the extract filtered through 0.45 μm nylon syringe filter membrane before injecting to system.

2.5. Method validation

The object of this validation is to show that an analytical technique is suitable for intended purpose. The analytical method for MI and MCI was validated for specificity, LOD, LOQ, linearity, precision and accuracy.

Different types of samples were used to optimize the conditions of sample preparation and chromatographic separation for development method. Blank matrix samples without containing target compounds represented as shampoo, tissue and cream were Olive, Let's green and Innisfree respectively. Recovery studies were performed with blank samples spiked with MI and MCI at three different concentration levels. Sample pretreatments were carried out as specific identification of each group.

The LOD and LOQ were performed using blank samples spiked with low concentration of MI and MCI. Sample pretreatments were carried out as specific identification of each group. The LOD was defined with a signal-to-noise ratio (S/N) of 3, and the LOQ was defined with an S/N of 10.

Matrix-matched calibration (MMC) was used to compensate for the matrix effects [12]. The matrix effects were defined as the influence of one or more co-extracted components from the sample on the measurement of MI and MCI concentrations. The presence of these effects is demonstrated by comparing the response produced from the MI and MCI in a pure solvent solution (methanol) with the blank samples (shampoo, tissue and cream), which were first extracted and then spiked with MI and MCI at the same concentration levels.

Matrix effects (ME%) were calculated using the equation:

$$\text{ME}\% = \frac{M_{\text{matrix}} - M_{\text{solvent}}}{M_{\text{solvent}}} \times 100\%$$

Where:

ME: the matrix effect

M_{matrix} : Slope of calibration curve in matrix

M_{solvent} : Slope of calibration curve in the pure solvent

2.6. Real samples

MI and MCI in 84 PCPs including rinse-off products (25 shampoos, 14 shower gels, 15 cleansers, 3 hand sanitizers) and leave-on products (4 wet tissues and 23 creams) were studied. Many of these products were labeled as containing isothiazolinones (MI and MCI). These PCPs were purchased from supermarkets and well-known wholesale markets (Binh Tay and Kim Bien) in Ho Chi Minh City, Vietnam from March to July in 2021.

3. Results and Discussion

3.1. HPLC-DAD determination

As shown in Figure 2, MI and MCI were separated under the HPLC-DAD condition. The retention times of the standard MI and MCI in a standard solution (methanol) were 5.663 and 16.702 minutes respectively (Figure 2a). There were no interfere peaks detected in the matrix-blank shampoo (Figure 2b and 2c) or the real shampoo sample (Figure 2d) at the retention times of MI and MCI.

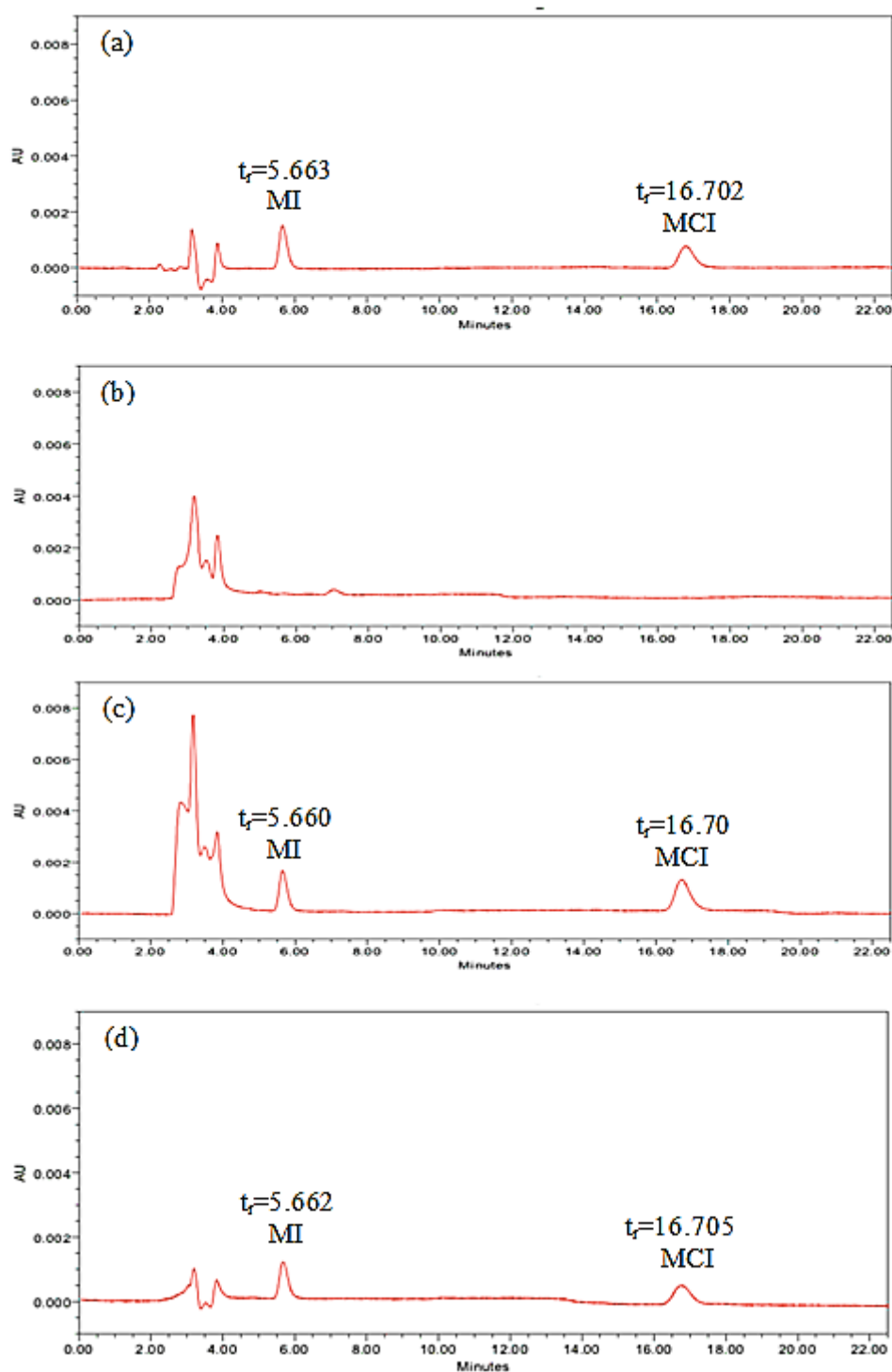


Figure 2. HPLC-DAD chromatograms of (a) a standard solution of MI (3.676 mg/L) and MCI (3.848 mg/L) in methanol, (b) a blank shampoo sample, (c) the blank shampoo spiked with MI (3.87 mg/kg) and MCI (6.76 mg/kg) and (d) a real shampoo sample with MI and MCI detected.

3.2. The LODs and LOQs of the method

The LODs of sample were in the 0.083–0.304 (mg/kg) range whereas the LOQs were in 0.276–1.015 (mg/kg) range (Table 3) in different sample matrices. The LODs and LOQs of the studied method

were comparable to LODs and LOQs of previous studies using HPLC-DAD technique [9] and HPLC-MS [3].

Table 3. The LODs and LOQs of the method for MI and MCI.

Compound	LOD (mg/kg)			LOQ (mg/kg)		
	Shampoo	Tissue	Cream	Shampoo	Tissue	Cream
MI	0.083	0.174	0.102	0.276	0.581	0.343
MCI	0.304	0.304	0.304	1.015	1.015	1.015

3.3. Linearity parameter and matrix effect

The linear was evaluated by means of calibration curves in the following six different concentrations (MI at 0, 0.3676, 1.838, 3.676, 9.190, 18.38 mg/L and MCI at 0, 0.3848, 1.924, 3.848, 9.620, 19.24 mg/L) in solvent (methanol) and matrix-matched of three samples (shampoo, tissue and cream) (Figure 3). The coefficient of determination (R^2) for the calibration curves was above 0.99 for the target compounds (Table 4).

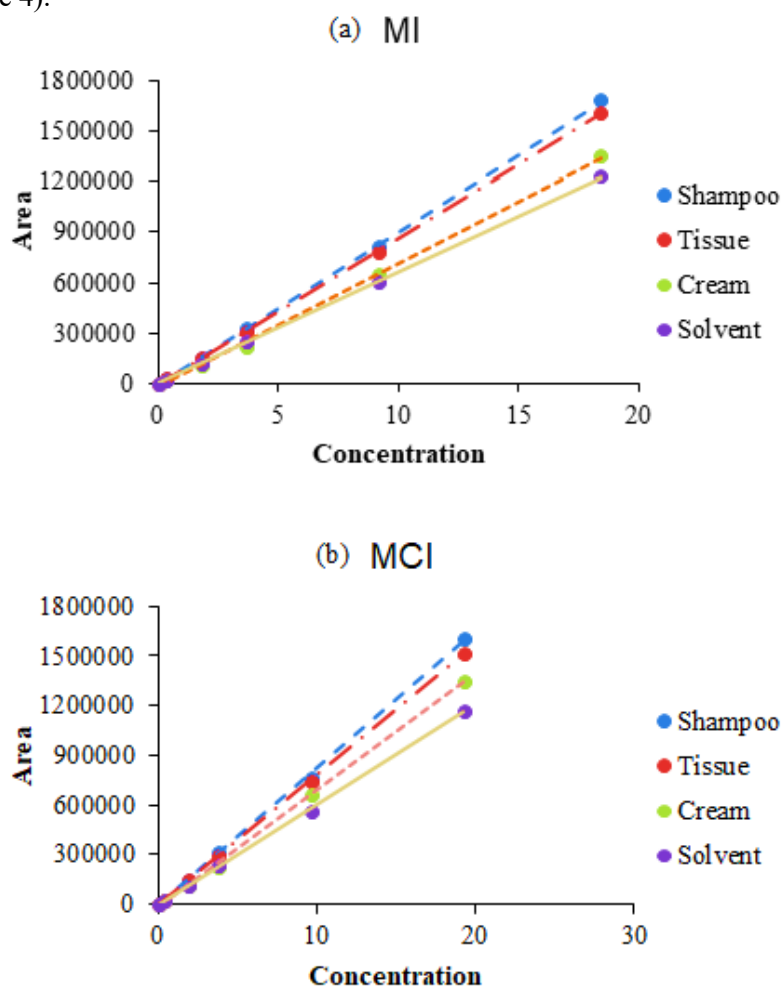


Figure 3. Comparison matrix effect of three different sample matrices (shampoo, tissue, cream) on standard curves of (a) MI and (b) MCI.

Table 4. Matric effect and linearity parameter in solvent and in sample extracts.

Compound	Solvent		Matric-matched shampoo			Matric-matched tissue			Matric-matched cream		
	Slope	R ²	Slope	R ²	ME%	Slope	R ²	ME%	Slope	R ²	ME%
MI	67048	0.999	91602	0.999	36.6	87450	0.999	30.5	72219	0.995	7.7
MCI	60317	0.999	83116	0.998	37.7	78768	0.999	30.5	70749	0.995	17.2

Depending on the value of this percentage, matric effects were classified into different categories. A percentage between -20% and 20% was considered as no matrix effect. A medium matrix effect occurred when the values were between 20% and 50% [12]. The ME for both MI and MCI in shampoo were 36.6% and 37.7% respectively, the ME for those in tissue were 30.5% (Table 4). These percentages considered as a medium signal enhancement and illustrated that shampoo and tissue were affected by the matric effect. Whereas, the ME for MI and MCI in cream were 7.7% and 17.2% indicated that no interfering peak appeared and did not significantly suppress or enhance the response of the instrument. Thus, to avoid these effects and ensure reliable results it is necessary to use a matric-matched calibration curve for shampoo and tissue samples. Calibration curve in the solvent can be used for accurate quantification in cream samples.

3.4. The recovery studies

Recovery studies in three different sample matrices (shampoo, tissue, cream) were performed at three concentration levels (MI at 1.93, 9.69, 19.3 mg/kg and MCI at 1.02, 6.77, 16.9 mg/kg). In this experiment, recoveries were achieved in triplicate and ranged from $83.2-103.2\%$ for both MI and MCI (Table 5) with the %RSD value of $0.2-5.14\%$. Previous studies had shown that recoveries at three distinct concentrations were within $86.5-101.8\%$ [9], $69-119\%$ [11] and %RSD values varied from $2.5-5.2\%$ [9]. In fact, the analysis process for compounds with low concentration in complex sample matrices was complicated and difficult, however; the result was acceptable when compared to prior research [9, 11]. Thus, the approach method is suitable for determination of MI and MCI in complex matrices and these results were with the accepted limit for recovery each concentration level according to AOAC [9].

Table 5. The recovery of MI and MCI in different sample matrices.

Compounds	Conc. (mg/kg)	Recovery (%)	RSD (%)	
Shampoo	MI	1.93	99.9	4.2
		9.69	87.5	1.6
		19.3	91.9	2.4
	MCI	1.02	89.0	0.8
		6.77	89.7	1.3
		16.9	85.4	2.3
Tissue	MI	1.93	88.3	3.7
		9.69	89.1	2.5
		19.3	89.6	0.9

		1.02	88.1	0.2
	MCI	6.77	87.4	1.6
		16.9	88.3	1.8
		1.89	88.4	1.7
Cream	MI	9.45	83.2	3.7
		18.9	96.7	2.0
		4.71	103.2	3.0
	MCI	9.43	102.3	4.3
		23.6	92.4	5.14

3.5. Real samples

A total of 84 well-known and unknown brand samples including shampoo, shower cleansing, cleanser, hand sanitizer, face cream, body cream and wet tissue were collected from supermarkets, stores and wholesale markets in Ho Chi Minh City to evaluate the potential of this method and to determine concentrations of MI and MCI in real samples. In general, MI and MCI were detected in all kinds of studied PCPs except wet tissue (Table 6). The concentration of MI in samples ranged from 1.2–6.9 mg/kg and that of MCI ranged from 0.6–20.1 mg/kg.

Total of 1948 products were screened for the presence of MI and MCI in detergents and cosmetics in Switzerland between March and May 2015 [3]. Only MI was detected at high concentrations (> 100 ppm) in two face creams and one hand cream. In another study, 320 cosmetic samples (lipstick and nail polish) collected in Korea (in 2019) were screened for the presence of MI and MCI [6]. Both MI and MCI were detected in two kinds of samples with MI in the range of 11.45–34.63 mg/kg and MCI in the range of 17.19–35.24 mg/kg. In our study, the result showed that MI and MCI concentrations in renowned brand products (Unilever, P&G, Shishedo, L'oreal, etc.) and purchased from the supermarket were within acceptable level limit whereas some unknown brands acquired from wholesalers were above that. Typically, 2 of the total 14 shower cleansings, 1 of the total 3 hand sanitizers and 2 of the total 23 creams were found to have an exceeded the limit (larger than 15 ppm). Among 25 shampoos and 15 cleansers, none of them were in the excess of permissible level. Notably, some items did not have MI and MCI stated on the label, yet the method detected them whereas others did but they were not found in the test. Furthermore, missing ingredients on the labels of products may make it difficult for health care professionals and consumers to avoid items containing these potential skin allergens. Frequent monitoring MI and MCI in PCPs should be required to protect health customers.

Table 6. The number of MI and MCI detections in the real samples.

	Shampoo ^a	Shower cleansing ^a	Cleanser ^a	Hand sanitizer ^a	Face and body cream ^b	Wet tissue ^c	Total
The number of samples	25	14	15	3	23	4	84
The number of MI	2	n.d.	1	n.d.	1	n.d.	3

detection (mg/kg)	MCI	2	3	n.d.	n.d.	2	n.d.	7
	MI & MCI	7	4	n.d.	3	1	n.d.	15

a: Group 1, b: Group 2, c: Group 3, n.d.: not detected

4. Conclusions

Isothiazolinone can cause allergic contact dermatitis and are banned in cosmetic ingredients. The developed method has been validated and applied for detecting the presence of MI and MCI in a wide variety of PCPs. A total of 84 PCPs were screened to evaluate the practicality of the method and to determine concentrations of MI and MCI in the real samples. The presence of MI and MCI in among those products were found throughout the experiment, proven that the successful application of the method. The research demonstrates that the approach can be used to identify MI and MCI in three distinct types of cosmetic goods. The methodology might be used guarantee reliable and consistent of monitoring of substandard cosmetics, and therefore maybe beneficial in public health.

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