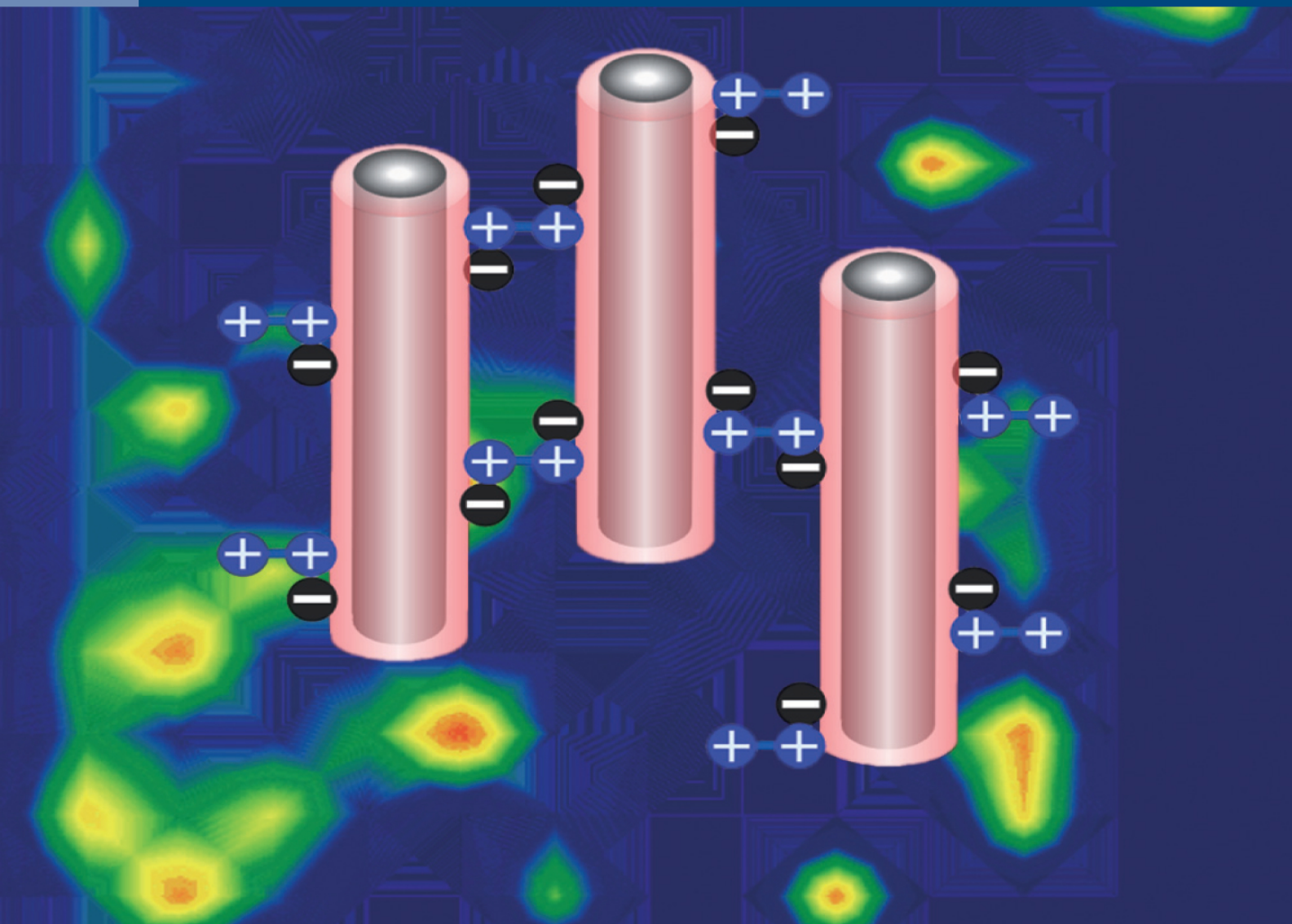


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Research Article

Determination of methylisothiazolinone and methylchloroisothiazolinone in cosmetic products by ultra high performance liquid chromatography with tandem mass spectrometry

Isothiazolinone biocides are broad-spectrum preservatives that are widely used in cosmetics, household, and industrial products. An increase in the number of cases of allergic contact dermatitis to isothiazolinone preservatives, namely, methylisothiazolinone and methylchloroisothiazolinone, have been recently noticed. The Food and Drug Administration relies on analytical methods to quantify levels of use of cosmetic ingredients and support enforcement action against products that are not in compliance with the law. In this study, an efficient ultra high performance liquid chromatography with tandem mass spectrometry method was developed and validated for the determination of methylisothiazolinone and methylchloroisothiazolinone in selected cosmetic products. The lower limit of quantitation was determined to be 0.1 $\mu\text{g/g}$ for both preservatives. A survey of 24 cosmetic products was conducted and found concentrations of methylisothiazolinone and methylchloroisothiazolinone ranging from not quantified, or below the lower limit of quantitation, to 89.64 $\mu\text{g/g}$ and not quantified to 10.31 $\mu\text{g/g}$, respectively.

Keywords: Cosmetics / Methylchloroisothiazolinone / Methylisothiazolinone / Preservatives
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1 Introduction

Isothiazolinone preservatives are widely used preservatives in cosmetics and personal care products [1, 2]. The most frequently found isothiazolinones in these products are methylisothiazolinone (MIT) and methylchloroisothiazolinone (MCI), which are also known as 2-methyl-3-isothiazolinone or 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-3-isothiazolinone or 5-chloro-2-methyl-4-isothiazolin-3-one, respectively. The structural formulas of these compounds are presented in Fig. 1. MIT is either used alone or in combination with MCI and a 3:1 MCI/MIT mixture is commercially sold under the name of Kathon, which is marketed to the cosmetics industry as Kathon CG (cosmetic grade) [3]. Since it is not permissible in the United States to use a trade name for the purpose of cosmetic ingredient labeling, these products list the active components of Kathon CG on their ingredient labels. According to the Mintel Database, the frequency of the use of MIT and Kathon CG has risen

over the last 10 years in a wide range of water-based cosmetics such as hair shampoos, conditioners, and skin care products. However, data on typical concentrations of use of MIT and MCI in cosmetic products sold in the United States is scarce.

Cosmetics sold in the United States are regulated by the Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act [<http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCA/default.htm>]. Under this act, cosmetic products and their ingredients (except for color additives) do not need FDA approval before the products are marketed. Cosmetics, however, are required to be safe for consumers under labeled or customary conditions of use. Companies and individuals who manufacture or distribute cosmetics have a legal responsibility for the safety of their products. FDA can take regulatory action against unsafe products on the market and typically such actions require data from analytical methods capable of detecting as well as quantifying compounds of interest. Hence, the goal of this study is to establish a method that enables efficient and reliable analysis of MIT and MCI from a number of cosmetic products.

A method to analyze these chemicals is important since both have been reported to have potential for induction of sensitization from dermal exposure [4–7]. Moreover, MIT was named Allergen of the Year for 2013 by the American Contact Dermatitis Society because of the increasing frequency of use of this preservative in cosmetic products and the increasing

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Abbreviations: ACN, acetonitrile; LLOQ, lower limit of quantification; MIT, methylisothiazolinone; MCI, methylchloroisothiazolinone; NQ, not quantified

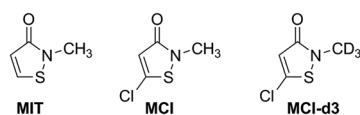


Figure 1. Structures of the isothiazolinone preservatives studied.

incidence of contact allergy reported to be associated with exposures to MIT [8, 9]. Such an analytical method, therefore, is crucial for gathering data on typical concentrations of use of MIT and MCI in cosmetics sold in the United States and to confirm that the target compounds have not been omitted from product ingredient labels.

MIT and MCI have been previously analyzed using GC–MS, LC–MS, and HPLC–UV. The preservatives were studied in various matrices such as environmental matrices [10–12], household cleaning products [13, 14], pharmaceuticals [13, 15], juice [15], food packaging materials [16, 17], as well as cosmetics [13–15, 18, 19]. The wide variety of matrices requires different sample preparation and extraction techniques. Methanol solutions have proven to be fast and effective in extracting preservatives from a wide variety of matrices [13, 15, 17, 19]. Specifically for cosmetics, a mixture of water and methanol has proven to be the optimal extraction solvent [15]. The UHPLC–MS/MS method used in this study optimizes the extraction and chromatography parameters of Lin *et al.* [17] to accomplish an efficient determination of only MIT and MCI in cosmetic products. To our knowledge, it is the fastest and most sensitive method for identifying and quantifying MIT and MCI in cosmetic products.

2 Materials and methods

2.1 Chemicals

LC–MS-grade water, methanol (MeOH), and acetonitrile (ACN) were purchased from Fisher Scientific (Pittsburgh, PA). MIT was purchased from Fluka (Buchs, Switzerland), and MCI and MCI-d₃ were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). All chemicals were sold as $\geq 96\%$ pure. Two different Kathon CG lots were obtained from Dow Chemical (Midland, MI). 24 cosmetic products manufactured in the United States and labeled as containing MIT or MIT and MCI were purchased from the internet.

2.2 Instrumentation

The LC was carried out using a Nexera UHPLC (Shimadzu, Columbia, MD). The sample manager was set to 10°C during operation. The separations were carried out using a 1.7 μm HSS T3 UPLC[®] column (50 mm $\times$ 2.1 mm id, Waters, Milford, MA) coupled to a 0.5 μm KrudKatcher ultra HPLC in-line filter (0.004 in id, Phenomenex, Torrance,

Table 1. Scheduled SRM parameters for quantitation and confirmation transitions for isothiazolinones studied

Compound	RT (min)	Transition	DP (V)	CE (V)	CXP (V)
MIT-1	0.84	116→101	64	33	14
MIT-2	0.84	116→99	64	23	12
MCI-1	2.81	150→115	76	25	14
MCI-2	2.81	150→119	76	27	12
MCI-d ₃ -1	2.81	153→135	60	37	16
MCI-d ₃ -2	2.81	153→118	71	29	12

RT, retention time; CE, collision energy; DP, declustering potential; and CXP, cell exit potential. The first product ion (1) for each analyte was used for quantitation, and the second product ion (2) was used for confirmation.

CA). Elution was completed using a 5 min gradient program running at a flow rate of 0.5 mL/min with a 2 μL injection volume. Mobile phase A was composed of 0.1% formic acid in H₂O and mobile phase B was composed of 0.1% formic acid in MeOH. The gradient parameters were: Initial–0.75 min, 7% B; 0.75–4 min, 7% B to 90% B; 4–4.5 min, 90% B; 4.5–5 min, 90% B to 7% B; 3 min equilibration time. The LC eluent was introduced to the ion source only between 0.4 to 1.1 min and 2.3 to 3.2 min during the run using a Valco[®] valve switch to prevent contamination.

The LC was interfaced to an AB Sciex QTrap 6500 (AB Sciex, Foster City, CA) equipped with an IonDrive[™] Turbo V ion source (electrospray). UHPLC–MS/MS operation and data acquisition were controlled by the AB Sciex Analyst software version 1.6.2, and quantitation was completed using the AB Sciex MultiQuant software version 2.1. The ion spray voltage was set to 2500 V. The turbo heater was maintained at 500°C. Nitrogen was used as the curtain gas and collision gas. The curtain gas, ion source gas 1, and ion source gas 2 were set to pressures of 25, 40, and 50 psi, respectively. The entrance potential was set to 10 V for all transitions. The collision gas was set to “High.” The mass spectrometer was operated in scheduled multiple reaction monitoring (scheduled MRM[™]) mode. Other parameters (declustering potential, collision energy, and cell exit potential) were optimized for each transition (Table 1).

2.3 Preparation of standard solutions

A stock solution containing 5 $\mu\text{g/mL}$ of MIT and MCI in 50:50 H₂O/ACN v/v was prepared in a volumetric flask and stored at -20°C . A stock internal standard solution containing 250 ng/mL of MCI-d₃ in 50:50 H₂O/ACN v/v was prepared separately in a volumetric flask and stored at -20°C . Ten calibration solutions were prepared using the stock solution. The concentration ranges for the standard solutions were 0.1 to 500 ng/mL for MIT and 0.1 to 1000 ng/mL for MCI. A constant concentration of 25 ng/mL of the internal standard was added to each standard solution and was used for quantitation by plotting the ratio of analyte signal-to-internal standard

signal against the concentration of the analyte. Analyte confirmation was determined by the primary transition-to-secondary transition ratio. Any value within 15% of the theoretical ratio (0.734) was confirmed as a true-positive result.

2.4 Sample preparation

The cosmetic products surveyed included shampoos, conditioners, skin care lotions, gels, and moisturizers. In an attempt to keep sample preparation quick and effective over the wide range of matrices a simple extraction was used. Approximately 100–150 mg (~500 mg for Product 5) of the sample was weighed into a 15 mL conical centrifuge tube. To the tube were added 10 mL of 20% H₂O in MeOH and 100 μ L of a 25 μ g/mL solution of MCI-d₃ in 50:50 H₂O/MeOH. The mixture was shaken for 10 min on a Large Capacity Mixer (Glas-Col, Terre Haute, IN) and then centrifuged (Eppendorf 5804R Centrifuge, Hamburg, Germany) at 8000 rpm, or 8228 rcf (relative centrifugal force), for 5 min. A 100 μ L aliquot of the extract was mixed with 900 μ L of 50/50 H₂O/MeOH and then filtered through a 0.2 μ m PTFE Acrodisc CR 13 filter (Pall Life Sciences, Port Washington, NY) directly into an autosampler vial for LC–MS/MS injection. Each sample was prepared in triplicate.

3 Results and discussion

3.1 LC–MS/MS optimization

All parameters for MIT, MCI, and MCI-d₃ were optimized by infusing three separate 1 μ g/mL solutions of each analyte at a flow rate of 10 μ L/min. Positive ESI was used as the ionization source for all three compounds. Two transitions for each compound were chosen and were suitable for both quantitation (primary transition) and identification/confirmation (secondary transition). The precursor ions were determined to be the [M+H] ions while the product ions were chosen based on signal intensity.

In an attempt to optimize chromatography, four columns (Waters Acquity BEH C₁₈, Waters Acquity BEH Amide, Agilent Poroshell 120 PFP, and Phenomenex Kinetex HILIC) in addition to the column used by Lin et al. (Waters Acquity HSS T3) were tested with a goal of increasing the retention times of the analytes. Poor analyte retention was noticed with both the Phenomenex Kinetex HILIC and Acquity BEH Amide columns. Retention was sufficient with the Agilent Poroshell 120 PFP column, but peak widths were large. The Acquity BEH C₁₈ column gave similar results to the Acquity HSS T3 but with less retention. Several mobile phases (H₂O, H₂O w/0.1% FA, MeOH, MeOH w/ 0.1% FA, ACN w/0.1% FA) were also tested in conjunction with the columns. In terms of signal intensity, MeOH provided an advantage over ACN, and MeOH w/0.1% FA provided an advantage over MeOH. Ultimately, the Acquity HSS T3 column in combination with H₂O w/0.1% FA and MeOH w/0.1% FA as the mobile phases

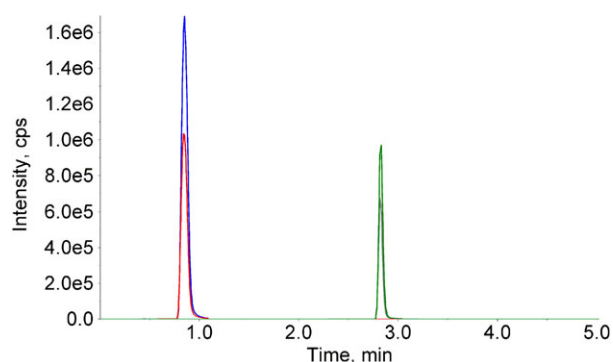


Figure 2. Chromatogram of the primary and secondary transitions of both MIT and MCI. Note: Identification of each analyte can be determined using the retention times in Table 1.

was selected because it produced optimal peak shape, sensitivity, and retention times. An increase in the flow rate from 0.3 to 0.5 mL/min combined with the addition of 0.1% FA to the unbuffered mobile phases increased signal intensity ~7.5-fold most likely due to smaller peak widths. A 3 min equilibration time was necessary to reequilibrate the column and prevent any peak shape aberrations. One complete sample run analysis, including equilibration time, required only 8 min. A chromatogram displaying the primary and secondary transitions for both analytes is shown in Fig. 2.

3.2 Method validation

Method validation was based on the “Guidelines for the Validation of Chemical Methods for the FDA Foods Program” [<http://www.fda.gov/ScienceResearch/FieldScience/ucm273423.htm>]. Nine- and ten-point calibration curves were created for MIT and MCI, respectively. The accuracies at each level for both analytes ranged from 90 to 110%. The lower limits of quantitation (LLOQs) were established to be the lowest points on the calibration curves for both MIT and MCI, where the accuracy of the calculated concentration was within 20% of the actual concentration and S/N ratio of the primary (quantitative) transition was calculated to be at least 10:1. The LLOQ for a representative 100 mg sample was determined to be 0.1 μ g/g for MIT (%RSD = 0.78) and for MCI (%RSD = 1.7), which was at least an order of magnitude lower in MIT and/or MCI concentration than any incurred sample. A weighting factor of $1/x$, where x is the analyte concentration, was applied, and all r values (correlation coefficient) were greater than 0.995. Both intraday (Table 2) and interday (Table 3) instrument precision were determined by sequentially injecting the calibration standards either within a single day (intraday) or over the course of eight days (interday). The autosampler was set at 10°C and the standards were stable over the course of eight days during the interday instrument precision testing. All stock and working solutions were stored at –20°C over the course of four months and were considered stable based on the comparable responses for freshly prepared

Table 2. Intraday instrument precision

Run	MIT			MCI		
	<i>r</i>	Slope	Intercept	<i>r</i>	Slope	Intercept
1	0.9998	0.0953	0.0018	0.9998	0.0312	0.0003
2	0.9999	0.0971	0.0027	0.9995	0.0315	−0.0002
3	0.9997	0.0952	0.0033	0.9997	0.0311	0.0005
4	0.9996	0.0950	0.0026	0.9991	0.0315	−0.0001
5	0.9998	0.0946	0.0030	0.9997	0.0313	−0.0001
6	0.9996	0.0947	0.0027	0.9996	0.0312	0.0006
Mean	0.9997	0.0953	0.0027	0.9996	0.0313	0.0002
%RSD	0.01	0.94		0.03	0.53	

Table 3. Interday instrument precision

Day	MIT			MCI		
	<i>r</i>	Slope	Intercept	<i>r</i>	Slope	Intercept
1	0.9998	0.0953	0.0018	0.9998	0.0312	0.0003
2	0.9992	0.0865	0.0026	0.9993	0.0314	−0.0003
3	0.9994	0.0832	0.0025	0.9997	0.0308	0.0004
4	0.9993	0.0842	0.0019	0.9989	0.0302	0.0004
5	0.9991	0.0789	0.0029	0.9999	0.0309	0.0001
8	0.9953	0.0761	0.0025	0.9996	0.0312	0.0002
Mean	0.9987	0.0840	0.0025	0.9995	0.0310	0.0002
%RSD	0.17	7.92		0.04	1.37	

Table 4. Recovery and repeatability studies at the “low, medium, and high” levels of the calibration curves for both MIT and MCI (*N* = 3)

Matrix	Spiked conc. (ng/mL)	Calc. conc. MIT/MCI (ng/mL)	% Recovery MIT/MCI	% RSD MIT/MCI
Shampoo	1	1.11/0.969	111/97	4.5/2.6
Shampoo	75	82.0/72.2	109/96	2.2/0.33
Shampoo	150	152/139	101/93	2.8/4.0
Conditioner	1	1.09/1.04	109/104	3.2/2.9
Conditioner	75	78.3/73.4	104/98	1.8/2.7
Conditioner	150	148/143	99/95	1.3/0.64
Lotion	1	0.993/0.995	99/100	5.6/6.8
Lotion	75	74.8/72.6	100/97	6.6/5.6
Lotion	150	148/145	99/97	0.86/0.68

calibration standard solutions. Recovery studies were carried out for both MIT and MCI at three concentration levels (1, 75, and 150 ng/mL, or 1, 75, and 150 µg/g assuming a 100 mg sample) and in three different blank sample matrices (shampoo, conditioner, and lotion). A known amount of analyte was spiked into the sample and taken through the sample preparation process in triplicate. The recovery of the analyte from the matrix was calculated as a percentage, and repeatability was determined using %RSD. Recoveries ranged from 99–111% for MIT and 93–104% for MCI with repeatabilities of less than 7% (%RSD; Table 4).

Table 5. Calculated % concentration of Kathon CG samples (*N* = 3)

Kathon CG	Calc. conc. MIT/MCI (%)	% RSD MIT/MCI	% Difference from declared
Lot A	0.417/1.32	2.2/0.52	10/17
Lot B	0.407/1.28	1.0/0.59	6/13

3.4 Kathon CG analysis

Two different lots of Kathon CG were analyzed separately in triplicate and the % concentrations of MCI and MIT were calculated based on their respective calibration curves. Analysis of lot A determined the concentrations of MCI and MIT to be 1.32 and 0.417%, respectively, and 1.28 and 0.407%, respectively, in lot B (Table 5). In the certificate of analysis, the declared % concentrations of MCI and MIT in lot A were 1.13 and 0.379%, respectively, and in lot B were 1.13 and 0.382%, respectively. The ratio of the calculated % concentrations of MCI to MIT was compared to that of the declared % concentrations. The ratios of MIT to MCI were calculated to be 3.155 for lot A and 3.135 for lot B. The declared ratios of MCI to MIT were 2.985 for lot A and 2.950 for lot B. There was only a 6% difference between the calculated and declared ratios. Therefore, the calculated ratio of MCI to MIT may indicate whether Kathon CG was the sole source of the two preservatives in a cosmetic product.

3.5 Cosmetic product survey

A total of 24 cosmetic products were analyzed. These products were either shampoo/conditioners or skin care products, such as body lotions, moisturizers and body cleansers (Table 6). These cosmetics were characterized as either leave-on, leave-in, or rinse-off based on exposure from the intended use of each product. Rinse-off cosmetics are products that remain in contact with human skin only for a limited time and are then rinsed off. In contrast, leave-on products remain on the skin for at least 1 h and essentially a longer period of time than rinse-offs. Leave-ins are products such as conditioners that are usually not rinsed out until the next time a person shampoos but are not fully in contact with skin.

The ranges of MIT and MCI concentrations determined for the different cosmetic matrices were as follows (Table 6): rinse-off shampoo/conditioners: 1.26–68.71 µg/g MIT and NQ (not quantified)–4.05 µg/g MCI; leave-in shampoo/conditioners: 3.35–77.63 µg/g MIT and NQ–10.31 µg/g MCI; rinse-off skin care products: 43.97–79.20 µg/g MIT and NQ–0.26 µg/g MCI; and leave-on skin care products: NQ–89.64 µg/g MIT and NQ–2.76 µg/g MCI.

MIT was listed as an ingredient in all 24 products surveyed, but it was not detected in skin care product 12. MCI was listed as an ingredient in seven of the 24 products, that is, four out of ten shampoo/conditioners and three out of 13

Table 6. Quantitation results for cosmetic products containing MIT and MCI ($N = 3$)

Product	MIT (%RSD) ($\mu\text{g/g}$)	MCI (%RSD) ($\mu\text{g/g}$)	Calculated MCI:MIT ratio	Exposure type
Shampoo/conditioner 10	77.6 (0.59)	NQ	—	LI
Shampoo/conditioner 3	68.7 (5.3)	NQ	—	RO
Shampoo/conditioner 5	61.0 (12)	NQ	—	RO
Shampoo/conditioner 8	44.5 (3.1)	NQ	—	LI
Shampoo/conditioner 11	38.9 (6.1)	NQ	—	LI
Shampoo/conditioner 9	32.5 (0.30)	NQ	—	LI
Shampoo/conditioner 4	5.60 (0.20)	NQ	—	RO
Shampoo/conditioner 6	3.49 (6.1)	10.3 (6.6)	3.0	LI
Shampoo/conditioner 7	3.35 (2.8)	8.72 (1.8)	2.6	LI
Shampoo/conditioner 2	1.60 (2.4)	2.20 (2.4)	1.4	RO
Shampoo/conditioner 1	1.26 (8.1)	4.05 (9.0)	3.2	RO
Skin care product 4	89.6 (2.3)	NQ	—	LO
Skin care product 2	79.2 (3.1)	NQ	—	RO
Skin care product 5	78.5 (3.9)	NQ	—	LO
Skin care product 1	71.8 (0.74)	0.260 (0.84)	<0.010	RO
Skin care product 6	69.0 (0.26)	NQ	—	LO
Skin care product 7	67.7 (2.8)	NQ	—	LO
Skin care product 8	67.3 (1.2)	NQ	—	LO
Skin care product 9	65.1 (2.8)	NQ	—	LO
Skin care product 3	44.0 (2.2)	NQ	—	RO
Skin care product 10	28.8 (4.3)	NQ	—	LO
Skin care product 13	1.38 (1.3)	2.76 (2.9)	2.0	LO
Skin care product 11	0.393 (20)	0.305 (20)	0.79	LO
Skin care product 12	NQ	NQ	—	LO

NQ, not quantified/below LOQ; RO, rinse-off; LI, leave-in; LO, leave-on.

skin care products. In contrast to MIT, the analytical detection of MCI concurred at the levels of what was declared on the ingredient lists. For the products that contained MCI, with the exception of skin care product 1, the MIT concentration was relatively lower (maximum of $3.49 \mu\text{g/g}$) than in products containing only MIT. Overall, there is not a clear correlation between MIT and/or MCI concentrations and if the product is rinse off, leave in, or leave off.

Three of the seven products that contained both MIT and MCI had MCI/MIT ratios similar to that of Kathon CG ($\sim 3:1$) and probably Kathon CG was their source. The MCI/MIT ratios for the other four products were calculated to be 2:1 or lower (Table 6). It appears that Kathon CG may not be the only source of these two preservatives for cosmetic formulations. Alternatively, reactions of MCI and/or MIT with other cosmetic ingredients within a given product may explain the unexpected ratios of MCI/MIT observed [10].

4 Concluding remarks

A UHPLC–MS/MS method for the determination of MIT and MCI in cosmetic products was developed and validated using 24 cosmetic products. The concentrations of MIT ranged from 0.39 – $89.64 \mu\text{g/g}$ in the 23 of the 24 products that

contained MIT. The concentrations of MCI ranged from 0.26 – $10.31 \mu\text{g/g}$ in the seven of the 24 products that contained MCI.

The MCI/MIT ratios calculated for products surveyed in this study were not always the expected ratio of 3:1 when Kathon CG is the source of these preservatives. A previous survey of skin creams had a similar observation [20]. Furthermore, Rastogi et al. suggested that the reason for non 3:1 MCI/MIT ratios may be that MCI reacts with other cosmetic ingredients within a given product.

The analytical method described here may be applied to a wide variety of cosmetic products for the determination of MIT and MCI. For example, this method will be suitable to monitor the frequency of incorrect labeling of MIT and MCI on cosmetic product ingredient lists. Ingredient labels that are inaccurate or missing can pose a challenge to health care professionals and consumers when looking for a causative agent to explain skin reactions and trying to avoid products that contain these potential skin allergens.

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The authors have declared no conflict of interest.

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