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Constructing a limit model and developing an HPLC-MS/MS analytical method for measuring isothiazolinone migration from children's sports protectors into sweat

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Infants and children have certain differences in physiological characteristics and behavioral patterns compared with adults, resulting in significantly higher exposure frequency and amounts to harmful chemical substances than those of adults when using similar products. To ensure the chemical safety of children when using sports protectors, we established a sweat migration limit model for isothiazolinones. Through this model, we determined the migration limits for MI, CMI and BIT to be 0.15 mg L⁻¹, 0.014 mg L⁻¹ and 0.15 mg L⁻¹, respectively. Furthermore, a corresponding isothiazolinone sweat migration detection method was established. The experiment focused on the influences of artificial sweat composition, pH value, and oscillation frequency and time on migration, ensuring that the pre-treatment migration process could restore the contact scene between the skin and the lining of the children's sports protectors and ensure sufficient migration efficiency. The experimental results showed that the three isothiazolinones exhibited linearity within a concentration range of 0.010–0.500 mg L⁻¹, with correlation coefficients (*R*²) exceeding 0.9990; the quantification limits of the method were 0.7–3.0 µg, which meets the requirements of the migration limits of the three isothiazolinones. The recoveries of the method are within the range of 87.2–114.8% with RSDs below 10%, and the RSDs of the intra-day precision and inter-day precision were less than 8%. The accuracy and precision of the method were between 87.2% and 114.8%, and the RSDs of intra-day and inter-day precision were below 8%. The accuracy and precision of the method meet the daily testing requirements. Different from the traditional isothiazolinone detection method, the method established in this study detects the migration amount of isothiazolinones rather than their total content in the samples, which is believed to be more scientific and efficient in protecting the skin health of infants and children.

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1. Introduction

Isothiazolinones are a class of organic compounds characterized by five-membered heterocycles. Due to their competence to inhibit the growth of microorganisms, they are widely used in cosmetics and personal care products to extend their shelf life. Additionally, they are commonly added into textile materials, plastics, leather and other materials, endowing these materials with effective antimicrobial and anticorrosive properties.^{1–4} Isothiazolinones perform significant disinfecting effects on

fungi and bacteria. They can penetrate bacterial cell membranes or fungal cell walls through diffusion, and the electron-deficient sulfur in their N–S bond can react with the nucleophilic groups in the cell, thereby reducing enzyme activity and ultimately leading to cell death in bacterial and fungal organisms.⁵ However, some studies indicate that isothiazolinones possess strong sensitizing potential for the skin and are among the primary contributors to allergic contact dermatitis (ACD) in humans.^{6–8} Commonly used isothiazolinones for antimicrobial and antiseptic applications include methylisothiazolinone (MI), methylchloroisothiazolinone (CMI), and 1,2-benzisothiazolin-3-one (BIT). Table 1 presents their chemical structures along with relevant information.⁹

The chemical safety of child products has consistently been a primary concern as consumer products designed for special demographics. In recent years, the improvements in living standards have led to heightened expectations regarding the


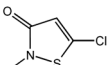
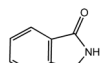
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Table 1 Characterisation of structures and chemical properties of isothiazolinones that are commonly used for antibacterial applications

Compounds	CAS no.	Molecular formula	Structural formula	Relative molecular mass (g mol ⁻¹)	Log <i>K</i> _{ow} ⁹
2-Methyl-4-isothiazoline-3-one	2682-20-4	C ₄ H ₅ NOS		115.2	-0.83
5-Chloro-2-methyl-4-isothiazolin-3-one	26172-55-4	C ₄ H ₄ ClNOS		149.6	0.401
1,2-Benzisothiazolin-3-one	2634-33-5	C ₇ H ₅ NOS		151.2	0.76

categories, functions, quality and safety of child products. This is particularly true for chemical safety, prompting greater scrutiny of harmful chemical substances present in these items. In reality, textile materials, plastics, leather, *etc.* are the main raw materials used in the manufacture of child products and pose a risk of exposure to isothiazolinones when children use these products (especially those that come into prolonged contact with their skin), which can potentially result in adverse effects to their skin health.

Based on the toxicity of isothiazolinone compounds, the U.S. EPA published a hazard assessment report in 2020 on isothiazolinone pesticides, detailing toxicological effects and skin sensitization risks for six isothiazolinone compounds. Furthermore, the U.S. Cosmetic Ingredient Review (CIR) issued a revised safety assessment of methylisothiazolinone (MI) in cosmetics in 2020, re-evaluating its safe usage levels.

In the field of toys and child products, only the European Union (EU) enforces the most comprehensive and strict regulations and has imposed a limit on isothiazolinone content. As early as 2005, EU introduced restrictions in the toy standard EN 71-9 regarding isothiazolinones,¹⁰ specifying that the concentrations of added MI, CMI and BIT should not exceed 10, 10 and 5 mg kg⁻¹, respectively. In the revised Toy Safety Directive 2009/48/EC in 2009,¹¹ the EU further tightened these restrictions, stipulating that the total amount of MI and CMI in water-based toy materials intended for children under 36 months should be below 0.25 and 0.75 mg kg⁻¹, respectively. However, the limits on the total amount cannot adequately reflect the pathway or actual exposure of harmful substances to children. In contrast, migration limits provide a more scientific and effective approach by restricting direct exposure of chemical substances to the human body. These limits are derived from dose-response data and human behavior pattern. Consequently, we researched the migration limits of isothiazolinones in sports protectors. The findings of this study were applied to inform the Chinese national standard GB/T 42801-2023 "General Technical Requirements for Daily Sports Protective Equipment for Children",¹² which was released in 2023. This study modelled the migration limits for MI, CMI and BIT to ensure the chemical safety of children using sports protectors. After setting the migration limit values for isothiazolinones, the corresponding

migration detection method was simultaneously developed to form a complete and effective means for detecting the migration of isothiazolinones in sweat from sports protector child products.

Currently, researchers have developed a variety of analytical techniques for the detection of isothiazolinones in different matrices, including high performance liquid chromatography (HPLC),^{13,14} gas chromatography mass spectrometry (GC-MS),¹⁵ high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS),^{16,17} *etc.* Among them, HPLC-MS/MS has emerged as one of the most important methods for detecting isothiazolinones due to its excellent structure determination capabilities and high sensitivity. Kawakami *et al.*¹⁸ utilized HPLC-MS/MS to analyze six isothiazolinones in deodorants, achieving detection limits of 0.012 to 0.032 mg L⁻¹. Zhong *et al.*¹⁹ established an HPLC-MS/MS method for the simultaneous quantitative analysis of six isothiazolinones in water-based adhesives used for food contact materials, with detection limits between 0.003 and 0.025 mg L⁻¹. Additionally, Alvarez-Rivera *et al.*²⁰ developed an HPLC-MS/MS approach to determine four isothiazolinones in cosmetics, reporting remarkably low detection limits of 0.0002–0.002 mg L⁻¹.

Although the available assays for isothiazolinone detection encompass a wide range of consumer products, they all focus on the total content rather than the detection of migration. Significantly different from the extraction pre-treatment process for total content detection, the detection of products in the migration limit category needs to determine the pre-treatment conditions according to the application scenario between the human body and the product. The objective is to ensure that pre-treatment accurately reflects the actual migration process of the chemical substance. Therefore, it is necessary to collect real human behavior data for support. For instance, in the detection method of migrating elements in the European standard EN 71-3,²¹ toys are immersed in simulated gastric fluid at a specific pH value during pre-treatment to replicate the actual migration process of gastric fluid in the human body. In this study, we developed a HPLC-ESI-MS/MS method for the rapid analysis and quantification of isothiazolinone sweat migration in common textile fabrics used in the lining of sports protectors based on the three migration limits calculated by the migration



limit model we established. The optimized migration-, chromatography-, and mass spectrometry conditions ensured that the migration pre-treatment of this method effectively simulates the actual migration process of the target substances in a relatively realistic way.

2. Modelling and calculation of migration limits

Taking into account the characteristics of children and sports protectors, this study constructed a limit value model for isothiazolinone migration from sports protectors to sweat in children based on the analysis of existing limit value models.^{22–24} The main steps of constructing a chemical safety limit value model are summarized as follows: (1) determine the dose–response data; (2) confirm the dimension of the chemical limit value based on the characteristics of the product in use; (3) introduce the product characteristics and parameters of the human behavioral patterns to establish a linkage between the dose–response data and the limit value based on the dimensional analysis method.

2.1 Construction of the model

Based on the steps mentioned above, the initial focus is on determining the dose–response data. Dose–response data for sensitizing substances are known as the No Expected Sensitization Induction Level (NESIL), which represents the quantitative threshold exposure level that does not cause skin sensitization in humans.

Since NESIL is calculated based on animal and human experimental data, the Scientific Committee on Consumer Safety (SCCS) introduces uncertainty factors known as safety assessment factors (F_T) for correction.^{25,26} F_T is determined by four correction factors: individual differences between human races (F_{IV}), product matrix (F_{ME}), frequency or duration of exposure (F_{FD}), and application site of body (F_{SA}). Thus, the final F_T can be calculated by eqn (1):^{27,28}

$$F_T = F_{IV} \times F_{ME} \times F_{FD} \times F_{SA} \quad (1)$$

Based on the actual use scenarios of children's sports protectors and the F_T Table provided by SCCS, appropriate correction factor values were substituted into the calculation. Consequently, the total sensitization assessment factor F_T for isothiazolinones in children's protectors was determined to be 100.

Next, it is essential to clarify the dimension of the limit, which relates to the exposure route. When children place toys in their mouths, chemical substances primarily migrate into their bodies through saliva. For this reason, EN 71-9 (ref. 10) adopts mg L^{-1} as the dimension for such a type of migration. The transdermal sweat migration of child sports protectors is similar to the transoral saliva migration of toys. Therefore, it is reasonable that the migration limit dimension of isothiazolinones should be mg L^{-1} in this situation.

Subsequently, two parameters were introduced based on the dimensional analysis method: the contact area between the protector and the skin (A_{SC} in m^2) and the amount of sweat during exercise (L_S in L per day). These parameters facilitate formulating a linkage between the dose response data T_{NESIL} ($\mu\text{g per cm}^2$ per day) and the migration limit of isothiazolinones (V_{ML} in mg L^{-1}) as follows:

$$\frac{T_{\text{NESIL}} \times A_{\text{SC}}}{F_T} = 10L_S \times V_{\text{ML}} \quad (2)$$

Exercise sweat L_S can be calculated on the basis of the product of exercise sweat rate (R_S in $\text{L m}^{-2} \text{h}^{-1}$), skin area (A_{SC} in m^2) and exercise duration (T_S in h per day), represented by eqn (3):

$$L_S = R_S \times A_{\text{SC}} \times T_S \quad (3)$$

By associating eqn (2)–(4) can be obtained:

$$\frac{T_{\text{NESIL}} \times A_{\text{SC}}}{F_T} = 10R_S \times A_{\text{SC}} \times T_S \times V_{\text{ML}} \quad (4)$$

By simplifying eqn (4), the target eqn (5) can be achieved. Based on this equation, the migration limits of isothiazolinones from child sports protectors to sweat can be calculated:

$$V_{\text{ML}} = \frac{T_{\text{NESIL}}}{10R_S \times T_S \times F_T} \quad (5)$$

To use eqn (5) for calculating the isothiazolinones migration limits, the parameters of exercise duration (T_S) and sweat rate (R_S) need to be selected based on characteristics of child and product. According to China's Exercise Guidelines for Preschool Children (3–6 years old)²⁹ and Physical Activity Guidelines for Chinese Children and Youth,³⁰ the average exercise duration (T_S) for children was selected as 2 h per day in this study. Based on the statistical experimental data of children's local body sweating during intermittent exercise as reported by Arlegui *et al.*,³¹ this study clarified that the sweating rate interval for children wearing sports protectors is $200\text{--}600 \text{ mL m}^{-2} \text{h}^{-1}$. This range is deemed to adequately represent the actual conditions encountered in such scenarios.

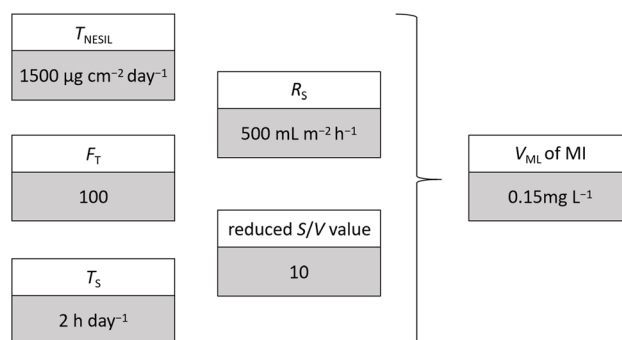


Fig. 1 Calculation of migration limit of MI.



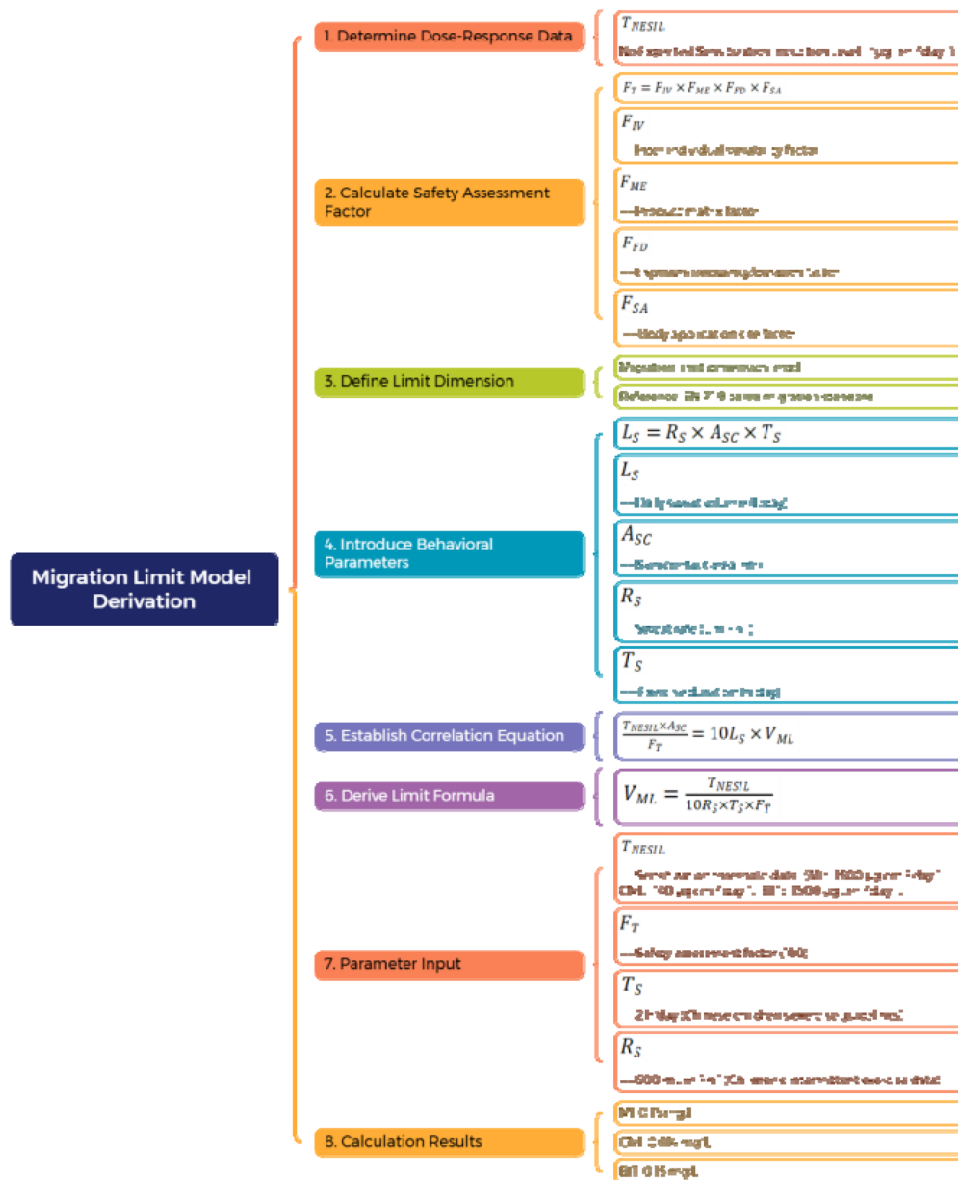


Fig. 2 Flowchart of migration limit model derivation.

2.2 Calculation of migration limits

In migration testing, the volume of the migration solution significantly influences the migrated concentration of the target substance. To solve this problem, the food contact materials (FCMs) regulation of the European Union and the EU standard EN 71-10 (ref. 32) have introduced the concept of area-to-volume ratio (S/V). According to the FCMs regulation, an average individual comes into contact with 6 dm² of packaging materials when consuming 1 L of food per day, resulting in an S/V value of (6 dm²)/(1 L). In contrast, the EU standard EN 71-10 defines a simple contact scenario for children. Specifically, it states that a maximum area of 10 cm² can be in contact with saliva from children who secrete approximately 100 mL. Consequently, its S/V value is (10 cm²)/(100 mL). This S/V value must follow the above rules during the migration testing of child sports

protectors, and is determined as (10 cm²)/(1 mL) by selecting an appropriate sweat rate (R_S). However, using only 1 mL of migration solution does not allow full infiltration of a 10 cm² sample in practical migration testing. To solve this problem, 10 mL of migration solution is used to migrate the 10 cm² sample in the pretreatment to ensure complete infiltration of the sample. Therefore, the S/V value calculated based on the model needs to be scaled down by a factor of 10 to account for this tenfold reduction observed during the actual migration.

Fig. 1 illustrates the process and results of applying the model to calculate the migration limit of methylisothiazolinone (MI). The parameters including T_{NESIL} , F_T , T_S and R_S selected according to the child sports protectors were inputted into eqn (5) for calculation. The resulting sweat migration limit for MI was found to be 0.15 mg L⁻¹. Utilizing the corresponding values of T_{NESIL} , the migration limits for CMI and BIT were calculated as



0.014 mg L⁻¹ and 0.15 mg L⁻¹, respectively. The derivation of the migration limit model of isothiazolinones was shown in Fig. 2.

3. Experimental

After obtaining the migration limits of three isothiazolinones, it is necessary to develop the corresponding migration detection method instead of using the conventional total content measurement.

The pre-treatment should try to restore the migration process of chemical substances. Simultaneously, the detection method should be able to meet the limit requirement and establish a standardized and complete chemical safety process to protect the health of children.

3.1 Apparatus, reagents and materials

1200 high-performance liquid chromatograph (Agilent products, USA), equipped with 60 MPa binary pump; API3200 triple quadrupole mass spectrometer (AB Sciex products, USA), equipped with electrospray (ESI) and atmospheric pressure chemical ionization source (APCI); SW23-type thermostatic water bath shaker (Julabo products, Germany); Orion pH meter (membraPure GmbH, Germany); Milli-Q Direct ultrapure water purification system (Merck Millipore, Germany); BS124S analytical balance (Sartorius, Germany).

Three reference materials of isothiazolinones, 2-methyl-4-isothiazolin-3-one (MI, purity ≥99.0%), and 5-chloro-2-methyl-4-isothiazolin-3-one (CMI, purity ≥99.9%) were purchased from Shanghai Anpel Laboratory Technologies. 1,2-benzisothiazolin-3-one (BIT, purity ≥98.0%) was purchased from Dr Ehrenstorfer company, Germany. Urea, sodium chloride, DL-lactic acid, L-histidine monohydrochloride monohydrate, sodium dihydrogen phosphate dihydrate, all analytical reagent, were purchased from Guangzhou Chemical Reagent Factory. Methanol (LC) and aqueous ammonia were purchased from Fisher Scientific Company. Experimental water was obtained from the Milli-Q system and filtered using a 0.22 μm membrane.

Self-made positive testing samples: isothiazolinones were added to three common textile materials: cotton, linen and nylon. Sampling samples: a total of 70 batches of scrapped commercially available textile fabrics were provided by the Textile Laboratory of Guangzhou customs Technology Center.

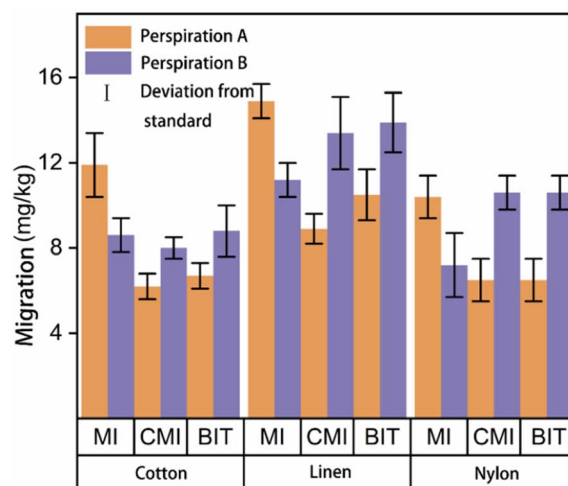


Fig. 3 Effect of different artificial perspirations on the migration of three isothiazolinones in different materials.

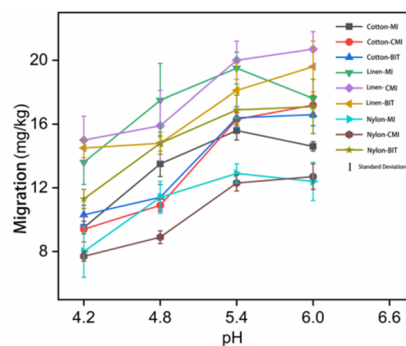


Fig. 4 Effect of sweat pH on the migration of three isothiazolinones.

3.2 Sample pretreatment

3.2.1 Preparation of positive textile samples. A certain amount of three types of negative textile materials (cotton, linen and nylon) were weighed out and carry out a series of treatments such as stirring, soaking, and air-drying in a mixture of solutions containing MI, CMI, BIT and methanol at varying concentrations ranging from 100–500 ppm. Ambient temperature was maintained between 25–30 °C and the samples were avoided from natural bright light. The samples were stirred with

Table 2 Basic information on two commonly used acidic artificial sweats

Classification	Perspiration composition	Typical application standards
Perspiration A	L-Histidine monohydrochloride monohydrate (C ₆ H ₉ O ₂ N ₃ · HCl · H ₂ O), 0.5 g L ⁻¹ Sodium chloride (NaCl), 5 g L ⁻¹ Sodium dihydrogen phosphate monohydrate dihydrate (NaH ₂ PO ₄ · 2H ₂ O), 2.2 g L ⁻¹	GB/T 3922-2013 GB/T 20385-2006 EN 16711-2:2015 ISO 105 E04:2013 ISO 17072-1:2019
Perspiration B	Sodium chloride (NaCl), 5 g L ⁻¹ Urea [CO(NH ₂) ₂], 1 g L ⁻¹ Lactic acid (mass fraction ≥ 88%), 1 g L ⁻¹	GB/T 19719-2005 GB/T 37647-2019 EN 1811:2023



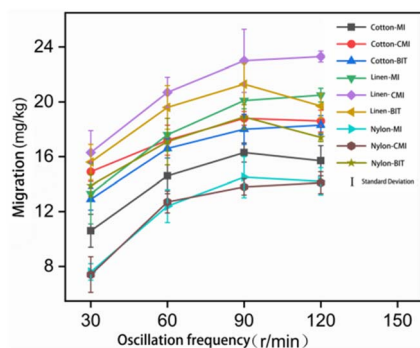


Fig. 5 Effect of water bath oscillation frequency on the migration of three isothiazolinones.

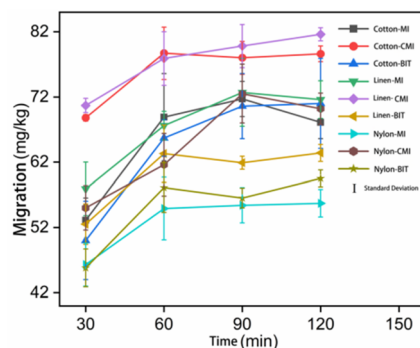


Fig. 6 Effect of water bath oscillation time on the migration of three isothiazolinones.

a glass rod for 1 min every 30 min for a total of six times to ensure thorough contact between the sample and the solution, and then left to stand overnight. After soaking, the samples were placed in a fume hood to dry for 2 h before being transferred to the natural environment for an additional 2 h of drying. Finally, the dried samples were sealed in numbered bags and stored at -18°C .

3.2.2 Preparation of artificial sweat. Two different types of artificial sweat were prepared. Artificial sweat A was formulated according to the International Standard ISO 17072-1:2019.²³ Specifically, 0.5 g of L-histidine monohydrochloride monohydrate, 5 g of sodium chloride, and 2.2 g sodium dihydrogen phosphate dihydrate were dissolved in deionised water and diluted to 900 mL. The solution was then transferred to a 1 L

volumetric flask with additional deionised water added to reach the mark.

Artificial sweat B was prepared in accordance with the EU standard EN 1811:2023.²⁴ In this case, 1 g of DL-lactic acid, 5 g of sodium chloride, and 1 g of urea were dissolved in deionised water and similarly diluted to a final volume of 900 mL before being transferred to a 1 L volumetric flask for further dilution with deionised water.

Both types of artificial sweat solutions should be used immediately after preparation, and it is essential to preheat them to $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ prior to use.

3.2.3 Standard solution preparation. MI, CMI and BIT standards were weighed out (0.0100 g each), dissolved in a small amount of methanol, transferred to a 100 mL brown volumetric flask and diluted with methanol to obtain a mixed stock solution C of three kinds of isothiazolinones with the mass concentration of 100 mg L^{-1} , which was stored at -18°C away from the light. Stock solution C was gradually diluted with artificial sweat A/B prepared in Section 3.2.2 to obtain the isothiazolinones mixed standard working solutions with concentrations of 0.010, 0.020, 0.050, 0.100, 0.200, 0.500, 1.000, 2.000 mg L^{-1} and stored in brown bottles away from the light at -4°C .

3.2.4 Migration in samples. The prepared positive sample was cut into pieces measuring $(10 \pm 1)\text{ cm}^2$ and placed into a 25 mL conical flask. Subsequently, 10 mL of preheated artificial sweat A/B was added at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, ensuring that the flask was tightly covered. The flask was placed in a constant temperature water bath shaker at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and oscillated in the dark to simulate migration for 90 min, with an oscillation frequency of 90 rpm. Immediately after oscillation, the migration solution was extracted using a 2 mL syringe and filtered through a $0.22\text{ }\mu\text{m}$ organic membrane before transferring the solution into a brown bottle, which is then ready for subsequent testing procedures.

3.3 HPLC-MS/MS analytical conditions

3.3.1 HPLC conditions. The analysis was conducted using an Agilent Poroshell 120EC-C18 column ($4.6\text{ mm} \times 50\text{ mm}$, $2.7\text{ }\mu\text{m}$). The mobile phase consisted of a methanol–water solution, and a gradient elution program was employed: the methanol concentration started at 10%, linearly increased to 60% in 0–2 min, reached 100% in 2–3 min, and was maintained for 6 min, resulting a total analytical time for 9 min. After each analysis, the system was equilibrated with a methanol–water solution (1 : 9, v/v) for 1 min. The flow rate of the mobile phase was 0.3

Table 3 Standardized oscillation time and frequencies established from sweat migration standards for consumer products

Standards	Oscillation time (min)	Oscillation frequency (r/min)	Related migration
GB/T 37647-2019	60	60	Migration of body fluids of elements in children's products and toys
GB/T 17593.1-2006	60	60	Sweat migration of heavy metals in textiles
GB/T 20385-2006	60	60	Sweat migration of organotin in textiles
GB/T 22930-2008	60 ± 5	100 ± 10	Sweat migration of heavy metals in leather and fur



Table 4 Optimized MRM parameters of three isothiazolinones

Target compounds	Parent ions (<i>m/z</i>)	Daughter ions (<i>m/z</i>)	Declustering potential (V)	Collision energy (V)	Collision cell exit potential (V)
MI	116.0	100.9 ^a	58.2	33.3	0.5
		58.1	58.2	40.4	0.5
		71.0	58.2	31.5	0.5
CMI	150.0	135.0 ^a	35.1	36.0	2.6
		87.1	35.1	58.9	2.2
		115.0	35.1	30.0	2.7
BIT	152.0	133.9 ^a	40.8	36.0	1.7
		108.9	40.8	33.4	3.4
		105.1	40.8	34.7	2.9

^a Parent ions with the highest response in MRM mode.

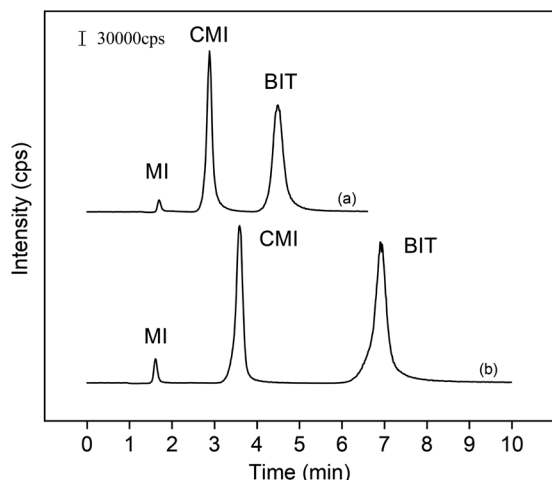


Fig. 7 Comparison of mobile phase chromatograms of methanol-water (a) and acetonitrile-water (b).

mL min⁻¹, with an injection volume of 15 μ L and the column temperature was 30 $^{\circ}$ C.

3.3.2 Mass spectrum conditions. In the positive ionization mode of electro-spray ionization (ESI), the ion source temperature was set at 600 $^{\circ}$ C. Both the ion source auxiliary curtain gas and collision gas were ultrapure nitrogen, with pressures of 0.138 MPa and 0.083 MPa, respectively. The multi-response monitoring (MRM) mode was employed for scanning, with a residence time of 100 ms for each MRM process. Stronger responding ion pairs were used for quantification, while weaker ones served for characterization purposes. The isothiazolinones in the samples were qualified according to the retention time of

the corresponding peaks and the abundance ratios of the peaks of the two MRM processes and quantified by the peak areas of the external standard method.

4. Results and discussion

4.1 Determination of migration conditions

4.1.1 Selection of artificial sweat. Sweat is essentially the filtrate of plasma, typically exhibiting weak acidity. Besides water, it contains major components such as sodium, chloride, potassium, urea, lactic acid, and ammonia.³³ When simulating sweat migration, it is essential that artificial sweat closely replicates the main components and pH values found in human sweat whenever possible. In various standards, two commonly used acidic artificial sweat are shown in Table 2, which are usually applied to relevant standards for the color fastness testing, migration detection of specific substances such as heavy metals and organotin compounds, and so on. In this study, we compared the migration effects of isothiazolinones in artificial sweat A and artificial sweat B using positive samples from different materials to determine which type of artificial sweat most effectively facilitates the migration of isothiazolinones.

The migration behaviors of MI, CMI and BIT in the three different material samples exhibit a consistent trend (Fig. 3). The migration effects of CMI and BIT in artificial sweat B are superior to those in artificial sweat A, while the opposite is true for MI. Considering the simultaneous migration efficiencies of MI, CMI, and BIT, artificial sweat B emerges as the more favorable choice. Furthermore, the chemical composition of artificial sweat B is more akin to the actual constituents of human sweat compared with artificial sweat A.

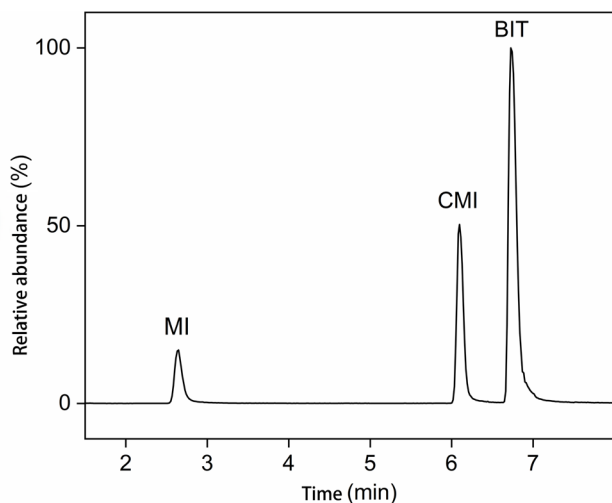
Table 5 Peak intensity reproducibility of three isothiazolinones in different mobile phases

Target compounds	Methanol-water		Acetonitrile-water	
	Intensity/cps	RSD/%	Intensity/cps	RSD/%
MI	16 276 \pm 591	3.6	30 691 \pm 3055	10.0
CMI	215 986 \pm 8070	3.7	220 307 \pm 13 319	6.0
BIT	143 423 \pm 9925	6.9	190 480 \pm 15 755	8.3



Table 6 Differences in half peak width $s(Y_{1/2})$, tailing factors (TF) and peak area of three isothiazolinones under gradient elution and isocratic elution conditions (methanol : water = 50 : 50)

Target substance	Gradient elution			Isocratic elution (methanol : water = 50 : 50)		
	$Y_{1/2}/\text{min}$	TF	Peak area	$Y_{1/2}/\text{min}$	TF	Peak area
MI	0.1023	1.15	19 646	0.1077	1.41	1341
CMI	0.0859	1.03	54 609	0.1183	1.59	16 213
BIT	0.1113	1.45	136 257	0.1530	1.56	49 128

**Fig. 8** Total ion chromatograms of three isothiazolinones (0.500 mg L^{-1}) with optimized chromatographic conditions.

Taking all these factors into consideration, artificial sweat B was selected as the artificial sweat for simulating migration in this study.

4.1.2 Determining the pH value of artificial perspiration.

Human sweat is colorless and slightly acidic, with a pH value generally ranging from 4.5 to 5.5. To achieve superior migration efficiency, it is essential to optimize the pH value of artificial sweat within the normal range of human sweat. Four pH values of 4.2, 4.8, 5.4, and 6.0 were selected to investigate the effect of pH on the migration of isothiazolinones in the positive samples made of different materials. In the experiment, dilute ammonia

water was used instead of sodium hydroxide solution to adjust the pH of artificial sweat. This choice circumvented potential reactions between sodium hydroxide and lactic acid in artificial sweat, while also addressing the absence of ammonia in the artificial sweat's composition.

There are relatively consistent migration patterns across three different material samples at different pH values (Fig. 4). The migration amount of MI increases with the increase of pH value until reaching a peak at 5.4, and tends to level off or even slightly decreases at 6.0. The migration amount of CMI and BIT increases with the increase of pH value and reaches the maximum at 6.0. Considering that MI, CMI, and BIT are typically involved simultaneously in migration processes, a pH value of 6.0 appears to be the most favorable choice based on these findings. Furthermore, some studies indicate that nitrogen-containing metabolites excreted during exercise can lead to a slight increase in sweat pH.^{34,35} Therefore, a pH value of 6.0 was selected for the artificial sweat.

4.1.3 Determining the oscillation frequency and time.

Repeated friction between the lining of a sports protector and a child's skin during exercise can lead to the migration of isothiazolinones into sweat, and the intensity of this friction is usually difficult to quantify through actual testing methods. In some consumer product standards related to skin contact, water bath oscillation is often used to simulate frictional interaction between the skin and the product. Additionally, the time for which a child wears a sports protector can affect the migration of isothiazolinones. Considering the usage characteristics of sports protectors for children, and referring to the oscillation frequency and time in the common sweat migration standards summarized in Table 3, oscillation frequencies of 30,

Table 7 Matrix effects of three isothiazolinones at varying concentrations ($n = 5$)

Concentration (mg L^{-1})		Material categories of fabrics					
		Cotton		Linen		Nylon	
	Target substance	Peak area ratio/%	RSD/%	Peak area ratio/%	RSD/%	Peak area ratio/%	RSD/%
0.050	MI	103.1 ± 5.2	5.0	95.0 ± 8.2	8.6	95.1 ± 7.2	7.6
	CMI	92.0 ± 4.9	5.3	105.2 ± 5.6	2.5	92.7 ± 4.8	5.2
	BIT	101.1 ± 5.0	5.3	95.0 ± 4.3	4.5	97.1 ± 6.3	6.5
0.500	MI	95.1 ± 1.9	2.0	99.9 ± 1.2	1.2	99.0 ± 1.5	1.5
	CMI	96.4 ± 3.5	3.7	109.8 ± 1.3	1.2	98.3 ± 1.5	1.5
	BIT	92.7 ± 8.7	9.3	102.0 ± 3.5	3.4	93.6 ± 2.7	2.7

Table 8 Linear equations, R^2 , method detection limits and quantification limits of the three isothiazolinones

Target substance	Concentration range/(mg L ⁻¹)	Correlation coefficient (R^2)	Linear equation	Detection limit/(μ g L ⁻¹)	Quantification limit/(μ g L ⁻¹)
MI	0.01–0.500	0.9992	$y = 1.1 \times 10^6 x + 1.33 \times 10^4$	0.9	3.0
CMI	0.01–0.500	0.9990	$y = 3.88 \times 10^6 x + 2.24 \times 10^4$	0.2	0.7
BIT	0.01–0.500	0.9996	$y = 7.05 \times 10^6 x + 6.4 \times 10^3$	0.2	0.7

Table 9 Spiked recoveries and relative standard deviations (RSDs) of three isothiazolinones in varying types of negative samples ($n = 5$)

Sample materials	Target substance	Level of addition (concentration of target in migration solution)					
		0.010 mg L ⁻¹		0.050 mg L ⁻¹		0.200 mg L ⁻¹	
		Recovery/%	RSD/%	Recovery/%	RSD/%	Recovery/%	RSD/%
Cotton	MI	99.0 \pm 6.3	6.3	102.6 \pm 9.0	8.8	91.2 \pm 3.0	3.3
	CMI	91.3 \pm 7.4	8.1	90.6 \pm 6.0	6.6	88.2 \pm 7.2	8.1
	BIT	87.9 \pm 6.7	7.6	99.3 \pm 8.2	8.3	114.2 \pm 7.7	6.8
Linen	MI	92.6 \pm 4.4	4.8	99.4 \pm 6.5	6.5	91.3 \pm 7.6	8.4
	CMI	98.7 \pm 9.3	9.4	97.1 \pm 6.0	6.2	113.1 \pm 6.0	5.3
	BIT	105.1 \pm 7.6	7.2	114.8 \pm 4.8	4.2	112.1 \pm 5.6	5.0
Nylon	MI	95.3 \pm 7.4	7.8	90.1 \pm 2.7	3.0	87.2 \pm 4.9	5.6
	CMI	91.1 \pm 5.2	5.7	109.0 \pm 8.4	7.7	95.3 \pm 2.5	2.6
	BIT	86.7 \pm 7.1	8.2	114.6 \pm 6.0	5.2	113.7 \pm 7.9	6.9

Table 10 Peak areas and relative standard deviations (RSDs) of intra-day and inter-day replicates of three isothiazolinones ($n = 5$)

Concentration (mg L ⁻¹)	Target substance	Intra-day		Inter-day	
		Peak area	RSD/%	Peak area	RSD/%
0.010	MI	407 \pm 13	3.1	440 \pm 24	5.5
	CMI	1104 \pm 26	2.3	1120 \pm 51	4.6
	BIT	2773 \pm 73	2.6	2600 \pm 109	4.2
0.050	MI	1950 \pm 107	5.5	2118 \pm 132	6.2
	CMI	5480 \pm 226	4.1	5541 \pm 121	2.2
	BIT	13 540 \pm 140	2.5	13 811 \pm 1052	7.6
0.200	MI	7305 \pm 217	3.0	7644 \pm 337	4.4
	CMI	20 118 \pm 982	4.9	21 179 \pm 1482	6.9
	BIT	53 548 \pm 1369	2.6	54 184 \pm 2408	4.4

60, 90, and 120 rpm, along with oscillation time of 30, 60, 90, and 120 min were selected as the inspection conditions.^{36–39}

Referring to the parameters of sweat migration standards for commonly used consumer products, a monofactor analysis method was used and a fixed oscillation time of 60 min was set to examine the effects on isothiazolinone migration at varying frequencies. The migration patterns for three different material samples across various oscillation frequencies exhibited relative consistency (Fig. 5). The migration amounts of the three isothiazolinones increased with the rise in oscillation frequency, reaching the highest point at 90 rpm, and then levelling off or slightly decreasing at 120 rpm. Consequently, an oscillation frequency of 90 rpm was selected for this study.

Subsequently, the effect of oscillation time on isothiazolinone migration was investigated at the determined oscillation frequency (Fig. 6). The migration amounts of the

three isothiazolinones in different material samples increased with prolonged oscillation time, reaching the maximum value at 60 min. After that, the migration amounts of the three isothiazolinones did not change significantly with the increase of oscillation time. To enhance efficiency and conserve time during experimentation, an oscillation time of 60 minutes was chosen for this study.

4.2 Optimization of mass spectrum conditions

4.2.1 Selection of ionization mode. Due to the high polarity of isothiazolinones, electrospray ionization (ESI) was selected as the ionization source for mass spectrometry in this study. The ionization effects of ESI positive and negative ion modes on the three types of isothiazolinones (MI, CMI, and BIT) were individually investigated, revealing that only the ESI positive ion mode yielded stable characteristic fragments of the parent ions.



Table 11 Statistics of the proficiency testing of migration detection of three isothiazolinones in variable types of samples

Sample materials	Target substance	M^a mg kg ⁻¹	S_r^b mg kg ⁻¹	CV _r ^c %	r^d mg kg ⁻¹	S_R^e mg kg ⁻¹	CV _R ^f %	R^g mg kg ⁻¹
Cotton	MI	43.6 ± 3.5	3.3	7.5	9.2	3.5	8.0	9.8
	CMI	16.6 ± 1.9	1.4	8.6	4.0	2.0	12.3	5.7
	BIT	7.5 ± 0.7	0.6	8.3	1.7	1.0	13.7	2.9
Linen	MI	27.8 ± 1.8	2.3	8.2	6.4	3.0	10.7	8.3
	CMI	15.8 ± 1.4	1.3	8.1	3.6	2.3	14.6	6.5
	BIT	11.0 ± 0.8	0.9	8.0	2.5	1.7	15.7	4.8
Nylon	MI	19.5 ± 2.1	1.3	6.9	3.8	2.7	13.8	7.5
	CMI	32.3 ± 2.7	2.6	8.0	7.3	3.7	11.4	10.3
	BIT	51.7 ± 5.8	3.9	7.6	11.0	4.6	8.9	12.8

^a Mean, M . ^b Repeatability standard deviation, S_r . ^c Repeatability coefficient of variation, CV_r. ^d Repeatability limit, $r, r = 2.8 \times S_r$. ^e Reproducibility Standard Deviation, S_R . ^f Reproducibility coefficient of variation, CV_R. ^g Reproducibility limit, $R, R = 2.8 \times S_R$

Table 12 Migration results of isothiazolinones in positive samples of sports protectors for children ($n = 5$)

Name of sample	Migration concentration/(mg L ⁻¹)		
	MI	CMI	BIT
Dark blue sample #1	0.014	—	—
Light blue sample #2	—	—	0.013
Black sample #3	0.010	0.012	0.009
Black sample #4	—	—	0.023

Consequently, the ESI positive ion mode was employed as the ionization source for subsequent analyses.

4.2.2 Optimization of MRM parameters. The multiple reaction monitoring (MRM) parameters represent a critical component in tandem mass spectrometry, as appropriate parameter settings can enhance the response of the target substances by dozens of times. The MRM parameters of the three isothiazolinones were optimized separately and the findings were summarized in Table 4.

4.3 Optimization of chromatographic conditions

4.3.1 Optimization of the mobile phase. Mobile phase system of methanol–water and acetonitrile–water was evaluated for the selection of mobile phase (Fig. 7). The target compounds can be completely and effectively separated using either methanol–water or acetonitrile–water. However, the precision of target compounds were better in the mobile phase system of methanol–water than acetonitrile–water (Table 5). Furthermore, Lin *et al.*¹⁶ shows that the responses of target compounds are stronger using methanol–water as the mobile phase rather than methanol–water with acids or buffer. Thus, methanol–water was chose as the mobile phase for this study.

4.3.2 Optimization of the elution program. Given that the solvent of the standard solution comprised methanol–artificial sweat mixture, a methanol–water solution was chosen as the initial mobile phase to ensure compatibility between the standard solution, mobile phase and sample matrix. The study compared the separation effects of isocratic elution and

gradient elution on the target compounds and employed the tailing factor (TF) to assess the chromatographic peak shape of the target substance. The findings show that the isocratic elution procedure (methanol:water = 50 : 50) could separate the target compound, although the quantification limit of MI ($S/N = 10$) did not meet the migration limit of 0.14 mg L⁻¹ as determined by the sweat migration model. Therefore, a gradient elution procedure was deemed necessary. At the initial stage of the gradient elution program, the initial proportion of methanol was set at 10%, which was linearly increased to 100% within 15 min, and a standard solution containing three isothiazolinones with a concentration of 0.500 mg L⁻¹ was analyzed. MI peaked near 6 min and CMI and BIT peaked near 12–13 min. Further adjustments to the gradient elution procedure are necessary to increase separation efficiency and reduce daily analysis time. In subsequent experiments, a steeper gradient elution program was employed to linearly increase the methanol proportion to 60% within 2 min, resulting in MI being eluted at 2 min. Then, the methanol proportion was linearly increased to 100% between 2–3 min and maintained until 9 min, ensuring that CMI and BIT were completely eluted. The column was equilibrated for 1 min to prepare for the next analysis. Table 6 summarizes the differences in half-peak width $s(Y_{1/2})$, tailing factors (TF) and peak areas of the three isothiazolinones (0.500 mg L⁻¹) under gradient elution and isocratic elution conditions (methanol:water = 50 : 50).

Compared with isocratic elution, the half-peak widths and tailing factors of MI, CMI and BIT are significantly reduced using gradient elution, and the chromatographic peak shape becomes more symmetrical. The peak area enhancement rates of MI, CMI and BIT were 14.65, 3.37 and 2.77 times higher, respectively. The peak area enhancement rate of MI was the highest, and the method quantification limit ($S/N = 10$, based on 10 times signal-to-noise ratio) satisfied the migration limit of 0.14 mg L⁻¹ for MI. In conclusion, employing gradient elution for target substances led to sharper and more symmetric peak shapes while greatly increasing peak areas, and the sensitivity of the method satisfied the migration limits of the three isothiazolones.

Finally, the optimized gradient elution program was used to analyze the mixed standard solution containing the three



isothiazolones (0.500 mg L^{-1} for each isothiazolone). The total ion chromatogram revealed an advanced peak time of MI at 2 min, and the peaks of CMI and BIT appeared at 6–7 min, resulting in a shortened overall analytical time of 7 min and significantly improved detection efficiency (Fig. 8).

4.4 Matrix effect

Based on the external standard method, it is necessary to assess the influence of the matrix effect of textile materials. The matrix effect can be calculated according to eqn (6).⁴⁰

$$\text{Matrix effect(\%)} = \frac{B}{A} \times 100\% \quad (6)$$

where, A and B are the response values of the target compound in the solvent and blank sample, respectively. Herein, the peak area of the mass spectrum is used to represent the matrix response value. The influence of the matrix effect can be neglected when the value of matrix effect is between 90–110%.

In accordance with the pre-treatment method described previously, negative textile samples made of cotton, linen and nylon were subjected to migration. The filtrate of these samples was recovered and used to prepare standard solutions of the three isothiazolinones at 0.050 and 0.500 mg L^{-1} . Subsequently, their matrix effects were compared with those of the standard solutions prepared with artificial sweat at the same concentration, and the summarized results are presented in Table 7. The response value ratios (peak area ratio) of the three isothiazolinones in different matrices were within 90–110%, with relative standard deviations (RSDs) all below 10%. Similar conclusions were also obtained by Zhang *et al.*,⁴¹ indicating that the matrix effect of textile materials is relatively weak and can be ignored. In summary, it is reasonable to use artificial sweat for the preparation of standard solutions to construct the standard curve and employ the external standard method for quantitative calculation due to the negligible impact on matrix effects.

4.5 Method validation

4.5.1 Standard curve, linear range and detection limit.

Mixed standard solutions of three isothiazolinones at mass concentrations of 0.010 , 0.020 , 0.050 , 0.100 , 0.200 , and 0.500 mg L^{-1} were prepared following the previously described method and subjected to HPLC-MS/MS on-line analysis, with five repetitions ($n = 5$) for each concentration point to assess their linearity within this concentration range. The linear equations were obtained by fitting the average of five measurements. The linear relationships of the three isothiazolinones were excellent, with correlation coefficients (R^2) exceeding 0.9990 for all compounds (Table 8). The lowest point of the standard curve is at 0.010 mg L^{-1} , which was utilized to calculate the method detection limit ($S/N = 3$) and method quantification limit ($S/N = 10$) using the signal-to-noise ratio method (Table 8). The limits of detection (LOD) and quantification (LOQ) were well below the migration limits.

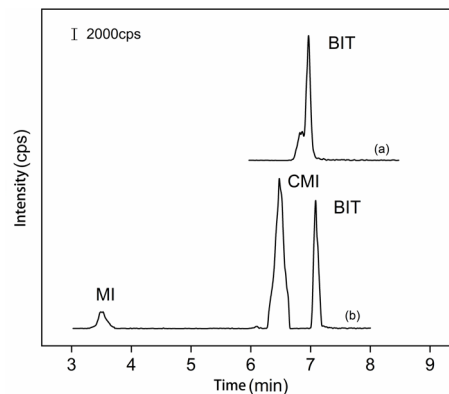


Fig. 9 Total ion chromatograms of isothiazolinones in black sample no. 4 (a) and black sample no. 3 (b)

4.5.2 Recovery and precision. The spiked recovery test was conducted on the negative textile samples, with low (0.010 mg L^{-1}), medium (0.050 mg L^{-1}), and high (0.200 mg L^{-1}) concentrations selected as the spiked levels for recovery validation. The experiment was repeated five times ($n = 5$) for each sample to validate the accuracy of the method. The spike recoveries and relative standard deviations (RSDs) are presented in Table 9. The recoveries for MI ranged from 87.2% to 102.6% with RSDs between 3.0% to 8.8% for CMI ranged from 88.2% to 113.1% with RSDs between 2.6% to 9.4%, and ranged from 86.7% to 114.8% for BIT with RSDs between 4.2% to 8.3%. The recoveries of the three isothiazolinones fell within the range of 86.7% to 114.8%, and the RSDs were all below 10%. These findings indicate excellent method accuracy.

Additionally, the experiments were repeated for intra-day and inter-day measurements at low (0.010 mg L^{-1}), medium (0.050 mg L^{-1}) and high (0.200 mg L^{-1}) concentrations, with each test conducted five times ($n = 5$) to verify the precision of the method. The obtained intra-day and inter-day peak areas of the target compounds and their corresponding RSDs are detailed in Table 10. The intra-day RSDs of the three isothiazolinones at varying concentrations were between 2.3% and 5.5%, while the inter-day RSDs ranged from 2.2% to 7.6%, confirming the method's high precision.

4.5.3 Inter-laboratory proficiency testing. Seven laboratories were organized to conduct proficiency validation on the developed sweat migration detection method. For each participating laboratory, the samples and standard solutions required for the experiment were uniformly configured with reference to Sections 3.2.1 and 3.2.4 above. In this case, the experiment was repeated three times ($n = 3$) for each sample, and the final experimental results are listed in Table 11. The repeatability coefficient of variation (CV_r) of the method ranged from 6.9% to 8.6%, while the reproducibility coefficient of variation (CV_R) ranged from 8.0% to 15.7%. These results are in accordance with the requirements, thereby confirming that the proficiency testing of the method meets the required standards.





Table 13 Summary results of method validation

Target substance	Concentration range/(mg L ⁻¹)	Correlation coefficient (<i>R</i> ²)	Linear equation	LOD/(μg L ⁻¹)	LOQ/(μg L ⁻¹)	Recovery/%	Precision/%	CV _i /%	CV _R /%
MI	0.01–0.500	0.9992	$y = 1.1 \times 10^6 x + 1.33 \times 10^4$	0.9	3.0	87.2–102.6	3.0–8.8	6.9–8.2	8.0–13.8
CMI	0.01–0.500	0.9990	$y = 3.88 \times 10^6 x + 2.24 \times 10^4$	0.2	0.7	88.2–113.1	2.6–9.4	8.0–8.6	11.4–14.6
BIT	0.01–0.500	0.9996	$y = 7.05 \times 10^5 x + 6.4 \times 10^3$	0.2	0.7	86.7–114.8	4.2–8.3	7.6–8.3	8.9–15.7

4.5.4 Testing of market samples. Seventy batches of various brands of child protectors available on the market were tested to examine the practical efficacy of the established detection method. Isothiazolinones were detected in four samples, while all other samples tested negative (Table 12). Among them, only MI was detected in dark blue sample no. 1, with a migration concentration of 0.014 mg L⁻¹. BIT was found in the light blue sample no. 2 and the black sample no. 4, with migration concentrations of 0.013 mg L⁻¹ and 0.023 mg L⁻¹, respectively. Additionally, all three isothiazolinones were detected in the black sample no. 3. The total ion chromatograms of sample no. 3 and 4 are presented in Fig. 9. The chromatographic peaks of the target substances in the figure displayed irregularities characterized by a certain degree of burr phenomenon, which may be attributed to the lower concentration of isothiazolinones detected in the actual samples. Although the migration amounts of all the samples did not exceed the calculated migration limits for the three isothiazolinones based on the established migration limit model, there remains a potential risk associated with their presence in the sports protector. When children use protectors, there is a potential for these substances to migrate into sweat and pose risks to skin health.

5. Conclusions

We first constructed a sweat migration model to calculate the sweat migration limits of three isothiazolinones, which were found to be 0.15 mg L⁻¹, 0.014 mg L⁻¹ and 0.15 mg L⁻¹. Furthermore, an HPLC-ESI-MS/MS method was established to determine the migration amount of isothiazolinones in sweat from sports protectors used by children.

The key aspect of the established detection method lies in pre-treatment. The final migration conditions were determined by combining the Chinese national standard conditions for sweat migration with the practical scenarios of children engaging in sports activities while wearing protectors.

The optimized pre-treatment conditions included utilizing artificial sweat containing lactic acid, urea, sodium chloride at a pH of 6.0 as the migration solution. Samples were subjected to oscillation in a water-bath at a frequency of 90 rpm and maintained at a constant temperature of 37 °C for 90 min to simulate the migration process effectively. At the same time, the detection response and the analysis efficiency of the target substances were significantly enhanced by optimizing the mass spectrometry parameters and chromatographic parameters. The experimental results indicated that the mass concentrations of the three isothiazolinones exhibited linearity within the range of 0.010–0.500 mg L⁻¹ with correlation coefficients (*R*²) exceeding 0.9990. The quantification limits of the method ranged from 0.7 to 3.0 μg L⁻¹, which were lower than 1/10 of their respective migration. The recoveries of the method fell within the range of 87.2–114.8%, with RSDs below 10% (*n* = 5), and both the intra-day and inter-day RSDs were less than 8% for repeated measurements (*n* = 5) across varying concentrations. The summary results of method validation are listed in Table 13.

The method demonstrated accuracy and precision, satisfying the standard's requirement for routine testing application. The

method established in this study differs from the traditional method for detecting the total amount of isothiazolinones since it focused on the migration amount. The detection method combined with the migration limit values of isothiazolinones calculated by our model constitute a standardized and comprehensive chemical safety process. This ensures that the skin of infants and children is protected from the allergic hazards of isothiazolinones when wearing sports protectors. The limits of three types of isothiazolinones and the sweat migration analytical method have been adopted by the Chinese national standard (GB/T 42801-2023) and are used for the detection of isothiazolinone-based antimicrobial agents in children's sports protectors.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Notes and references

- 1 N. Hunziker, The 'isothiazolinone story', *Dermatology*, 1992, **184**(2), 85–86.
- 2 R. Valsecchi, P. Leghissa, S. Piazzolla, T. Cainelli and P. Seghizzi, Occupational Dermatitis From Isothiazolinones In The Nylon Production, *Dermatology*, 1993, **187**(2), 109–111.
- 3 S. A. Febriana, H. Soebono, P. J. Coenraads and M. L. A. Schuttelaar, Contact allergy in Indonesian patients with foot eczema attributed to shoes, *J. Eur. Acad. Dermatol. Venereol.*, 2015, **29**(8), 1582–1589.
- 4 K. Aalto-Korte, K. Alanko, M.-L. Henriks-Eckerman and R. Jolanki, Antimicrobial allergy from polyvinyl chloride gloves, *Arch. Dermatol.*, 2006, **142**(10), 1326–1330.
- 5 J. O. Morley, A. J. O. Kapur and M. H. Charlton, Structure-activity relationships in 3-isothiazolones, *Org. Biomol. Chem.*, 2005, **3**(20), 3713–3719.
- 6 A. Herman, O. Aerts, L. de Montjoye, I. Tromme, A. Goossens and M. Baeck, Isothiazolinone derivatives and allergic contact dermatitis: a review and update, *J. Eur. Acad. Dermatol. Venereol.*, 2019, **33**(2), 267–276.
- 7 M. Leiva-Salinas, L. Francés and J. F. Silvestre, Update on Allergic Contact Dermatitis Due to Methylchloroisothiazolinone/Methylisothiazolinone and Methylisothiazolinone, *Actas Dermo-Sifiliográficas*, 2014, **105**(9), 840–846.
- 8 J. Magdaleno-Tapia, C. Valenzuela-Onate, J. M. Ortiz-Salvador, M. Garcia-Legaz-Martinez, A. Martinez-Domenech, M. Alonso-Carpio, *et al.*, Contact allergy to isothiazolinones epidemic: Current situation, *Contact Dermat.*, 2020, **82**(2), 83–86.
- 9 National Library of Medicine, [Internet], 2022, [cited December 29 2022].
- 10 EN 71-9:2005+A1:2007:Safety of Toys – Part 9: Organic Chemical Compounds – Requirements. CEN/TC 52, 2007.
- 11 Directive 2009/48/EC of the European Parliament and of the Council of 18 June 2009 on the Safety of Toys, 2009.
- 12 GB/T 42801-2023, General Technical Requirements for Daily Sports Protective Equipment for Children, National Standards of the People's Republic of China, 2023.
- 13 M. Hefnawy, A. Al-Majed, M. Mohammed, A. Al-Ghusn, A. Al-Musallam, N. Al-Sowidan, *et al.*, Fast and Sensitive Liquid Chromatography Method for Simultaneous Determination of Methylisothiazolinone, Salicylic Acid and Parabens in Cosmetic Products, *Curr. Anal. Chem.*, 2017, **13**(5), 430–438.
- 14 L. Abad-Gil, S. Lucas-Sanchez, M. Jesus Gismera, M. Teresa Sevilla and J. R. Procopio, Determination of paraben-, isothiazolinone- and alcohol-type preservatives in personal care products by HPLC with dual (diode-array and fluorescence) detection, *Microchem. J.*, 2021, **160**, 105613.
- 15 A. Rafoth, S. Gabriel, F. Sacher and H.-J. Brauch, Analysis of isothiazolinones in environmental waters by gas chromatography-mass spectrometry, *J. Chromatogr. A*, 2007, **1164**(1–2), 74–81.
- 16 Q. B. Lin, T. J. Wang, H. Song and B. Li, Analysis of isothiazolinone biocides in paper for food packaging by ultra-high-performance liquid chromatography-tandem mass spectrometry, *Food Addit. Contam., Part A*, 2010, **27**(12), 1775–1781.
- 17 J. B. Wittenberg, B. J. Canas, W. Zhou, P. G. Wang, D. Rua and A. J. Krynsky, Determination of methylisothiazolinone and methylchloroisothiazolinone in cosmetic products by ultra high performance liquid chromatography with tandem mass spectrometry, *J. Sep. Sci.*, 2015, **38**(17), 2983–2988.
- 18 T. Kawakami, M. Tahara and Y. Ikarashi, Analysis of isothiazolinone preservatives in household deodorizers and air fresheners through solid-phase extraction and liquid chromatography-tandem mass spectrometry, *J. Liq. Chromatogr. Relat. Technol.*, 2021, **44**(11–12), 564–569.
- 19 H. Zhong, Z. Li, S. Chen, Y. Zeng, J. Zheng, Y. Zeng, *et al.*, Simultaneous Quantitative Analysis of Six Isothiazolinones in Water-Based Adhesive Used for Food Contact Materials by High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS), *Molecules*, 2019, **24**(21), 3894.
- 20 G. Alvarez-Rivera, T. Dagnac, M. Lores, C. Garcia-Jares, L. Sanchez-Prado, J. Pablo Lamas, *et al.*, Determination of isothiazolinone preservatives in cosmetics and household products by matrix solid-phase dispersion followed by



- high-performance liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A*, 2012, **1270**, 41–50.
- 21 EN 71-3: 2019+A1: 2021, *Safety of Toys Part 3: Migration of Certain Elements*, European Committee for Standardization, 2021.
 - 22 GB/T 41531-2022, *Determination of Phenol and Bisphenol A in Textiles*, National Standards of the People's Republic of China, 2022.
 - 23 ISO 17072-1:2019, *Leather-Chemical Determination of Metal Content-Part 1: Extractable Metals*, International Organization for Standardization, 2019.
 - 24 EN 1811:2023, *Reference Test Method for Release of Nickel from All Post Assemblies Which Are Inserted into Pierced Parts of the Human Body and Articles Intended to Come into Direct and Prolonged Contact with the Skin*, European Committee for Standardization, 2023.
 - 25 G. F. Gerberick, M. K. Robinson, S. P. Felter, I. R. White and D. A. Basketter, Understanding fragrance allergy using an exposure-based risk assessment approach, *Contact Dermat.*, 2001, **45**(6), 333–340.
 - 26 *Opinion on Skin Sensitisation Quantitative Risk Assessment for Fragrance Ingredients (QRA2)-Submission I SCCS/1589/17-Final Opinion*, Publications Office of the European Union, 2018.
 - 27 S. P. Felter, M. K. Robinson, D. A. Basketter and G. F. Gerberick, A review of the scientific basis for uncertainty factors for use in quantitative risk assessment for the induction of allergic contact dermatitis, *Contact Dermat.*, 2002, **47**(5), 257–266.
 - 28 A. M. Api, D. A. Basketter, P. A. Cadby, M.-F. Cano, G. Ellis, G. F. Gerberick, *et al.*, Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients, *Regul. Toxicol. Pharmacol.*, 2008, **52**(1), 3–23.
 - 29 G. Hong-yan, G. Jian-jun, L. Dong-mei, Q. Sha, W. Jian-xin, *et al.*, Physical activity guideline for Chinese preschoolers aged 3-6 years, *J. Child Health Care*, 2020, **28**(6), 714–720.
 - 30 Y. T. Zhang, S. X. Ma, C. Chen, S. J. Liu, C. F. Zhang, Z. B. Cao and F. Jiang, Physical activity guide for Chinese children and adolescents, *Chin. J. Evidence-Based Pediatr.*, 2017, **12**(6), 401–409.
 - 31 L. Arlegui, J. W. Smallcombe, D. Fournet, K. Tolfrey and G. Havenith, Body mapping of sweating patterns of pre-pubertal children during intermittent exercise in a warm environment, *Eur. J. Appl. Physiol.*, 2021, **121**(12), 3561–3576.
 - 32 EN 71-10: 2005, *Safety of Toys - Part 10: Organic Chemical Compounds – Sample Preparation and Extraction*, European Committee for Standardization, 2005.
 - 33 Whitehouse AGR, The dissolved constituents of human sweat, *Proc. Roy. Soc.*, 1935, **B117**(803), 139–154.
 - 34 S. Wang, G. Zhang, H. Meng and L. Li, Effect of Exercise-induced Sweating on facial sebum, stratum corneum hydration, and skin surface pH in normal population, *Skin Res. Technol.*, 2013, **19**(1), E312–E7.
 - 35 V. F. Curto, N. Angelov, S. Coyle, R. Byrne, S. Hughes, N. Moyna, *et al.*, *My Sweat My Health: Real Time Sweat Analysis Using Wearable Micro-fluidic Devices*, 2011, pp. 196–197.
 - 36 GB/T 37647-2019, *Toys and Children's Products General Principles for the Migration Test of Specific Elements*, China Standard Press, 2019.
 - 37 GB/T 17593.1-2006, *Determination of Heavy Metals in Textiles Part 1 Atomic Absorption Spectrophotometry*, China Standard Press, 2006.
 - 38 GB/T 20385-2006, *Textiles Determination of Organic Tin Compounds*, China Standard Press, 2006.
 - 39 GB/T 22930-2008, *Leather and fur Chemical tests Determination of heavy metal content*, China Standard Press, 2008.
 - 40 S. J. Lehotay, K. Mastovska, A. R. Lightfield and R. A. Gates, Multi-Analyst, Multi-Matrix Performance of the QuEChERS Approach for Pesticide Residues in Foods and Feeds Using HPLC/MS/MS Analysis with Different Calibration Techniques, *J. AOAC Int.*, 2019, **93**(2), 355–367.
 - 41 L. Zhang, X. Luo, Z. Niu, X. Ye, Z. Tang and P. Yao, Rapid screening and identification of multi-class substances of very high concern in textiles using liquid chromatography-hybrid linear ion trap orbitrap mass spectrometry, *J. Chromatogr. A*, 2015, **1386**, 22–30.

