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Development and validation of a UPLC-MS/MS method for the quantification of isothiazolinones in the composition and emissions from consumer products

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ABSTRACT

Isothiazolinones, a family of biocides, are used as preservatives for their fungicidal, bactericidal, and algacidal properties. These compounds can be found in a wide range of consumer and building products, such as paints, varnishes, shampoos, and liquid detergents. A robust analytical UPLC-MS/MS method to identify and quantify seven isothiazolinones (MIT, CMI, BIT, MBIT, BBIT, OIT, and DCOIT) in consumer products and their emissions was developed and validated according to ISO/IEC 17025. The limits of detection (LODs) ranged from 0.14 $\mu\text{g L}^{-1}$ (BIT) to 0.54 $\mu\text{g L}^{-1}$ (CMI). The method was applied for the quantification of the seven isothiazolinones in four types of consumer products (i.e., cosmetics, air fresheners, cleaning products, and building products) and the indoor emissions from a paint. Matrix effects were observed for the shampoo (63-74%), the shower gel (67-84%), and the ceramic glass cleaner (53-57%). All isothiazolinones indicated by the manufacturer (i.e., MIT, CMI, BIT, OIT, and DCOIT) were detected in the products and successfully quantified by the UPLC-MS/MS method.

CLINICAL TRIAL REGISTRATION

Not applicable

KEYWORDS: Biocides; Isothiazolinones; UPLC-MS/MS; Consumption products

1. INTRODUCTION

Biocides are substances intended to destroy, deter, render harmless, or exert a controlling effect on any harmful organism by chemical or biological means. In Europe, products containing biocides are regulated by the Biocidal Products Regulation (BPR). Biocides are classified into four main groups and subdivided into twenty-two product types (PTs): disinfectants (PTs 1 to 5), preservatives (PTs 6 to 13), pest control agents (PTs 14 to 20), and other biocidal products, such as antifouling and taxidermist products (PTs 21 and 22) [1, 2].

Isothiazolinone biocides are used for their algacidal, fungicidal, and bactericidal properties as preservatives in many construction or consumer products, such as paints, varnishes, detergents, or shower gels [3-5]. Seven isothiazolinones are currently used in Europe: 2-methyl-2H-isothiazole-3-one (MIT), 5-chloro-2-methyl-4-isothiazolin-3-one (CMI), 1,2-benzisothiazol-3(2H)-one (BIT), 2-methyl-1,2-benzothiazol-3(2H)-one (MBIT), (2-butyl-1,2-benzisothiazol-3(2H)-one (BBIT), 2-octyl-2H-isothiazole-3-one (OIT), and 4,5-dichloro-2-octylisothiazol-3(2H)-one (DCOIT). Today, these seven isothiazolinones are allowed in ten PTs (Electronical Supplementary Material 1) [6]. However, a more relatively restrictive regulation for cosmetic products only approved MIT and the mixture of MIT/CMI in rinse-off products such as shampoos or shower gels [4, 7-10].

Because of the use of MIT and the mixture of MIT/CMI in cosmetics, the number of allergic contact dermatitis cases increased during the 1990s and 2010s [7, 10]. According to the Classification, Labelling, and Packaging Regulation (CLP), most isothiazolinones are classified as skin sensitizers [16-19]. These compounds may also cause edema, allergic contact dermatitis, or eczema by dermal contact or inhalation [4, 7, 16, 17]. Although DCOIT was not classified as a skin sensitizer, it may cause endocrine disruption in *Oryzias melastigma* by chronic exposure [18, 19].

Isothiazolinones have been already analyzed in construction and consumer products, especially in cosmetics, and in environmental matrices such as water, sludge, and indoor air (Electronical Supplementary Material 2). According to some studies, 3 to 6 isothiazolinones have been identified and quantified using high-performance liquid chromatography (HPLC) [20] or ultra(high)-performance liquid chromatography (UHPLC) [21] coupled to mass spectrometry (MS) [20-21]; or, to a lesser extent, gas chromatography (GC) coupled to MS [22], LC with UV and amperometric detection [23], and GC with flame thermionic detection (FTD), flame photometric detection (FPD), or electron capture detection (ECD) [23-25].

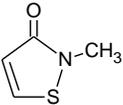
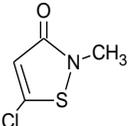
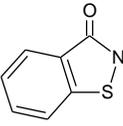
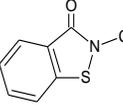
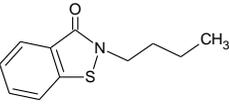
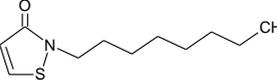
In this context, the main objective of this study was to optimize a more versatile analytic method using UPLC-tandem MS (MS/MS). It should make it possible to identify and quantify simultaneously, in the same chromatographic run, the seven isothiazolinones currently used in Europe (i.e., MIT, CMI, BIT, MBIT, BBIT, OIT, and DCOIT). In addition, the new UPLC-MS/MS method developed must be able to determine both the isothiazolinone levels in the product and its emissions. It was applied to the analysis of 10 consumer products, i.e., cosmetics, air fresheners, cleaning products, and building products, and to the characterization of paint emissions using a microchamber.

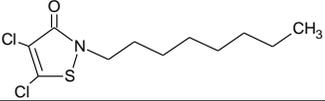
2. MATERIALS AND METHODS

2.1. Selection of isothiazolinones

Seven isothiazolinones were selected due to their allergen properties (MIT, CMI, BIT, OIT, and BBIT), their occurrence in indoor environments (DCOIT), and recent authorization by the European Commission for their usage in consumer products (MBIT). The selected isothiazolinones and their properties are presented in Table 1.

Table 1. Physicochemical properties of isothiazolinones.

| <i>Isothiazolinones</i> | <i>CAS No.</i> | <i>Molecular Formula</i> | <i>Molecular weight</i> (<i>g mol⁻¹</i>) | <i>Boiling point</i> (<i>°C</i>) | <i>Log Kow</i> |
|-------------------------|----------------|---|--|---------------------------------------|--------------------|
| MIT | 2682-20-4 |  | 115.2 | 130 ^a | -0.49 ^a |
| CMI | 26172-55-4 |  | 149.6 | 200 ^b | 0.75 ^c |
| BIT | 2634-33-5 |  | 151.2 | 328 ^d | 0.7 ^d |
| MBIT | 2527-66-4 |  | 165.2 | 267 ^e | 0.69 ^f |
| BBIT | 4299-07-4 |  | 207.3 | 332 ^g | 2.04 ^h |
| OIT | 26530-20-1 |  | 213.3 | 342 ⁱ | 2.61 ⁱ |

| | | | | | |
|-------|------------|---|-------|------------------|-------------------|
| DCOIT | 64359-81-5 |  | 282.2 | 322 ^j | 4.34 ^k |
|-------|------------|---|-------|------------------|-------------------|

^a [26], ^b [27], ^c [20], ^d [28], ^e [29], ^f [30], ^g [31], ^h [32], ⁱ [33], ^j [30], ^k [34]

2.2. Chemicals

Standards of isothiazolinones were purchased from Merck (MIT (purity \geq 98%), BIT (\geq 97%), OIT (\geq 98%)), Ark Pharma (BBIT (\geq 97%)), Carbosynth (MBIT (\geq 96%)), and Tokyo Chemical Industry (DCOIT (\geq 98%)). Sulfadimethoxine (\geq 99%) was purchased from Merck and used as the internal standard (IS). Acetonitrile (ACN, Optima®, LC/MS grade) and formic acid (FA, Optima®, LC/MS grade) were purchased from Fisher Scientific. Ultrahigh-quality (UHQ) water (resistivity of 18 M Ω cm at 25°C) was provided by a Direct-8 system from Millipore.

2.3. Selection of building and consumer products

To validate the developed UPLC-MS/MS method, it was applied for the identification and quantification of isothiazolinones in 10 different products corresponding to four classes of consumer products (i.e., cosmetics, air fresheners, cleaning products, and building products). The samples were selected as standard commercial products and were purchased from a local French market. According to the product composition declaration by the manufacturer, the products contained one to three isothiazolinones, including MIT, CMI, BIT, OIT, and DCOIT. Composition analysis was carried out immediately after opening the new product.

2.4. Consumer products analysis

According to extraction protocols dedicated to consumer products [35, 36], approximately 1 g of product was mixed with 5 mL of acetonitrile in a 15 mL centrifuge tube. The solution was placed in an ultrasonic bath for 15 min. After 15 min of centrifugation at 3500 rpm, 2 mL of the supernatant was directly filtered through a 0.22 μ m PTFE syringe filter. If necessary, depending on the product, the filtrate was diluted using an H₂O/ACN (90/10) mixture. The solution was then spiked with 20 μ L of the IS stock solution (10 μ g L⁻¹) and analyzed.

2.5. Quantitative estimation of matrix effect

To assess the matrix effect, as blank matrix is not available, the calibration-based method was used. The isothiazolinone standard solutions with known concentrations were prepared in solvent-only (i.e., a H₂O/ACN (90/10) mixture) and in their matrix-matched (i.e., a sample extract was prepared and spiked with the isothiazolinone at the same concentration levels). The matrix effect (ME, %) can be then calculated as a ratio of linear squared calibration slope in matrix-matched and in solvent-only following Equation 1:

$$ME = 100 \times \text{Slope}_{(\text{matrix-matched})} / \text{Slope}_{(\text{solvent-only})} \quad (\text{Equation 1})$$

ME value close to 100% (a value between 90 and 110% is deemed a negligible difference [37]) indicates no effect, less than 100% indicates ionization suppression, and over 100% indicates ionization enhancement due to coeluting sample compounds.

The matrix effect was determined for the 10 consumer products (i.e., cosmetics, air fresheners, cleaning products, and building products) under study.

2.6. Paint emission analysis

Emission measurements were conducted in a 44 mL microchamber thermal extractor (μ -CTE, Markes International). The operating conditions were fixed at a temperature of 25°C and an air exchange rate of 68 h⁻¹, according to Clausen et al. [38]. Approximately 2 g of paint was introduced in the μ -CTE. The air sample was collected on a C18 Cartridge (Waters, Sep-Pak C18 Plus Short, 360 mg of sorbent) [39] at a dry air flow rate maintained at 50 mL min⁻¹ for 3 h, giving a total volume of filtered air of approximately 9 L. Our preliminary tests with a second sorption C18 cartridge attached downstream of the first showed no significant losses and consequently a 100% trapping efficiency by the first cartridge during air sampling. The sampled C18 cartridge was then immediately eluted with 5 mL of ACN. Afterward, the extracts were concentrated under a nitrogen flow using a concentration workstation (TurboVap II, Biotage) with a pressure of 2 bar and a water bath at 40°C until a 500 μ L extract was obtained. The concentration was adjusted with a H₂O/ACN (90/10) mixture. The solution was then spiked with 20 μ L of the IS stock solution (10 μ g L⁻¹) and analyzed.

2.7. Instrumentation

The UPLC-MS/MS method was developed using a liquid chromatograph (Acquity, Waters) interfaced with a quadrupole-time-of-flight mass spectrometer (Synapt G2 HDMS, Waters) equipped with an electrospray ionization

(ESI) source. The mass spectrometer was used in high-resolution mode, up to 18,000 full width at half maximum (FWHM) at 400 Th, and chromatograms were extracted with 0.02 Th mass accuracy. Separation was achieved with an HSS pentafluorophenyl (PFP) column (100 mm × 2.1 mm I.D., 1.8 μm particle size) and an Acquity UPLC HSS PFP precolumn (2.1 mm × 5 mm I.D., 1.8 μm particle size). The flow rate was maintained at 0.47 mL min⁻¹. Gradient elution with 0.1% FA in UHQ water (A) and 0.1% FA in ACN (B) was applied as follows: 10% B at 0 min and an increase to 100% B within 3 min, which was maintained for 2 min and returned to the initial conditions and held for 1 min. The injection volume was 10 μL. The column and the sampler were kept at 30°C and 5°C, respectively.

Analyses were carried out in positive ionization mode. A capillary voltage of 0.5 kV was used. The desolvation and source temperatures were 500 and 150°C, respectively. The cone gas flow rate and the desolvation gas flow rate were maintained at 10 L h⁻¹ and 1000 L h⁻¹, respectively. A dwell time of 0.20 scan s⁻¹ was chosen. The optimized values for the sampling cone and collision energy and obtained values of the precursor and product ions are presented in Table 2. Data acquisition and mass spectral data processing were conducted with MassLynx software (v.4.1, Waters).

Table 2. Precursor and product ions (intensity %) use for quantification and sampling cone and collision energies implemented for each isothiazolinone.

| <i>Compound</i> | <i>Precursor ions (m/z)</i> | <i>Product ions (m/z)</i> | <i>Sampling cone (V)</i> | <i>Collision energy (V)</i> |
|-----------------|-----------------------------|--|--------------------------|-----------------------------|
| MIT | 116.02 (100) | - | 30 | 7 |
| CMI | 149.98 (100) 151.98 (52) | 134.95 (17)/115.01 (7) 136.95 (20)/115.01 (7) | 40 | 13 |
| BIT | 152.02 (100) | 134.00 (10)/109.01 (11)/105.03 (5) | 30 | 8 |
| MBIT | 166.03 (100) | 151.01 (74)/109.01 (19)/137.01 (12)/123.03 (4) | 30 | 12 |
| BBIT | 208.08 (75) | 152.02 (100) | 20 | 7 |
| OIT | 214.13 (92) | 102.00 (100) | 30 | 7 |
| DCOIT | 282.05 (97) 284.05 (68) | 169.93 (100) 171.92 (69) | 20 | 6 |

Analyte quantification was based on the sum of the precursor and product ions. For MIT, there was no sufficiently intense product ion due to its low molecular weight. Therefore, the applied collision energy was only used to validate its presence in samples (i.e., confirmation ion m/z = 98.99).

2.8. Method validation

The method was validated, including the determination of linearity, limits of detection (LODs), limits of quantification (LOQs), retention time suitability, instrument repeatability, and interday repeatability according to the general requirements for the competence of testing and calibration laboratories ISO/IEC 17025 [40].

3. RESULTS AND DISCUSSIONS

3.1. Optimization of UPLC parameters

Given the available analytical techniques in the literature to study isothiazolinones (Electronical Supplementary Material 1), the optimal choice for their quantification in a complex matrix such as consumer products seems to be UPLC-MS/MS. MS/MS detection is a highly selective technique, and the effects of matrices are reduced while increasing the signal/noise ratio, unlike UV detection. Preliminary experiments have also shown greater sensitivity with the use of LC-MS/MS than with that of GC-MS, by up to three orders of magnitude.

The optimized separation in the UPLC-MS/MS method is shown in Fig. 1. A linear gradient curve profile using ACN and acidified water was optimal for isothiazolinone separation, especially that of OIT and BBIT. Under our conditions, the use of ACN as a mobile phase component prevented overpressure that is often generated by methanol. The seven isothiazolinones were well separated in less than 3 min.

In the literature, the most comprehensive studies on isothiazolinones cover mixtures of only 6 isothiazolinones, i.e., MIT, CMI, BIT, MBIT, OIT, and DCOIT [20] and MIT, CMI, BIT, BBIT, OIT, and DCOIT [46], with retention times for the last eluted isothiazolinone of 8.6 min (DCOIT) and 6 min (DCOIT), respectively.

3.2. Method validation

According to ISO/IEC 17025 [40] and the validation guideline ICH Q2(R1) [41], the chromatographic efficiency (plate number), resolution, LOD/LOQ, linearity (correlation coefficient, residuals, and relative standard deviation (RSD)), stability of intraday and interday retention times, instrumental repeatability and interday repeatability of the method were determined and the results are summarized in Table 3.

Table 3. Validation parameters for the quantification of isothiazolinones by UPLC-MS/MS.

| Compound | Plate number | Retention time (min) | Suitability of retention time | | LOD ($\mu\text{g L}^{-1}$) | LOQ ($\mu\text{g L}^{-1}$) | Linearity | | | Instrumental repeatability (RSD %) | Repeatability interday, critical value |
|----------|--------------|----------------------|-------------------------------|------------------|------------------------------|------------------------------|--------------------------------|-------|---------|------------------------------------|--|
| | | | Intraday (RSD %) | Interday (RSD %) | | | Range ($\mu\text{g L}^{-1}$) | R^2 | RSD (%) | | |

| | | | | | | | | | | | |
|--------------|------|------|-----|------|------|------|-----------------|-------|------|------|-------|
| MIT | 6297 | 1.18 | 2.8 | 0.49 | 0.29 | 0.89 | 0.91 – 18.23 | 0.998 | 11.4 | 6.8 | 0.526 |
| CMI | 5053 | 1.51 | 1.4 | 0.77 | 0.54 | 1.63 | 1.97 – 19.68 | 0.992 | 6.5 | 15.5 | 0.553 |
| BIT | 9087 | 1.62 | 0.7 | 0.36 | 0.14 | 0.42 | 0.26 – 18.51 | 0.999 | 18.4 | 4.5 | 0.566 |
| MBIT | 5614 | 1.90 | 0.0 | 0.00 | 0.50 | 1.50 | 0.39 – 28.16 | 0.998 | 8.9 | 4.5 | 0.568 |
| BBIT | 9773 | 2.31 | 0.9 | 0.25 | 0.43 | 1.29 | 0.25 – 17.64 | 0.996 | 7.8 | 5.1 | 0.553 |
| OIT | 7743 | 2.42 | 1.3 | 0.24 | 0.33 | 1.02 | 0.33 – 24.10 | 0.999 | 19.4 | 4.7 | 0.469 |
| DCOIT | 9559 | 2.69 | 1.7 | 0.21 | 0.42 | 1.26 | 1.84 – 16.58 | 0.996 | 3.6 | 2.7 | 0.560 |

Calibration curves were obtained for all the standard isothiazolinones at 6 different concentrations depending on the compound (Table 3). A linearity with correlation coefficients > 0.99 was achieved for all isothiazolinones, and the RSDs were lower than 20%. Relative residuals (i.e., ratio between the difference between experimental and calculated signal values ($y_i - \hat{y}_i$), and the calculated signal values (\hat{y}_i)) were all calculated less than 20%.

The intraday and interday repeatability of the retention times was calculated from the RSD of five injections of standard solutions of the highest level of calibration. The intraday and interday RSDs of retention times did not exceed 2.8% and 0.49% (MIT), respectively, which was above the ISO/IEC 17025 recommendations [40].

The quantitative interday repeatability was calculated from Cochran's Q test. Six-concentration level calibration curves were repeated on three different days, and the variance was calculated for each concentration level. Then, the sum of the variance was divided by the highest variance value and compared to the Cochran coefficient. The interday critical values were all lower than 0.722 corresponding to the Cochran coefficient associated with a risk value set of 1%.

Instrumental repeatability was also calculated from the RSD of the five injections of the highest level of calibration and ranged between 2.7% (DCOIT) and 15.5% (CMI).

According to the validation guideline ICH Q2(R1) [41], the calculation of LOD and LOQ is as follows: $LOD = 3.3 \sigma / S$ and $LOQ = 10 \sigma / S$ where S is the slope of the calibration curve and σ is the standard deviation of the response. As LOD is obtained by calculation, when LOD is outside the linearity range (i.e., MIT, CMI, BIT, and DCOIT), this estimate is subsequently validated by the independent analysis ($n = 3$) of a solution prepared at the detection limit concentration. As a result, the LODs ranged between $0.14 \mu\text{g L}^{-1}$ (BIT) and $0.54 \mu\text{g L}^{-1}$ (CMI). LODs with the same order of magnitude ($0.2 \mu\text{g L}^{-1}$) were obtained using ultra-high-performance liquid chromatography/orbitrap high-resolution mass spectrometry for MIT, CMI, BIT, OIT, and DCOIT [42]. Lowest LODs

were calculated against those available in the literature for BBIT (70 pg vs. 4.3 pg for this optimized method) [43] and 2.5 $\mu\text{g L}^{-1}$ for MBIT [20].

3.3. Consumer product analysis

Isothiazolinones were analyzed in 10 consumer products. The comparison of the calibration slopes in matrix-matched and in solvent-only did not show a matrix effect (i.e., $90\% \leq \text{ME} \leq 109\%$) for 7 out of 10 products. For the shampoo and the shower gel, ME was estimated to be 63-84% for all isothiazolinones. For the ceramic glass cleaner, only OIT and BBIT were concerned by matrix effect, i.e., 57% and 53%, respectively. These matrix effects (Electronical Supplementary Material 4) lead to signal suppression. It is challenging to know the product component causing the matrix effect and then to remove the matrix effect. Only a matrix minimization (i.e., dilution) could be useful but with isothiazolinone in a sufficiently high concentration in consumer products to be quantified.

All the isothiazolinones indicated by the manufacturers in the 10 tested products were identified and quantified. On the other hand, OIT was quantified in paint, while this compound was not specified by the manufacturer at the time of the analysis. The concentrations of isothiazolinones found for each product are presented in Table 4. A chromatogram of each product category is presented as an example in Fig. 2.

Table 4. Results of analyses for building and consumer products.

| <i>Product</i> | | <i>Concentration of isothiazolinones (ppm)*</i> | | | | | | |
|------------------|-----------------------|---|------------|-------------|------------------|--------------|-------------|-------------|
| | | <i>MIT</i> | <i>CMI</i> | <i>BIT</i> | <i>OIT</i> | <i>DCOIT</i> | <i>MBIT</i> | <i>BBIT</i> |
| Cosmetic | Shampoo | 10.9 ± 0.9 | 44.7 ± 9.0 | - | - | - | - | - |
| | Shower gel | 8.6 ± 1.6 | 23.0 ± 5.5 | - | - | - | - | - |
| | Shower milk | 35.4 ± 4.0 | - | - | - | - | - | - |
| | Body cream | 41.3 ± 0.8 | - | - | - | - | - | - |
| Air freshener | Gel | - | - | 2.6 ± 0.1 | - | - | - | - |
| | Wick | 77.3 ± 0.8 | - | 57.4 ± 7.0 | - | - | - | - |
| Cleaning product | Floor cleaner | - | - | 1.8 ± 0.5 | - | - | - | - |
| | Ceramic glass cleaner | 8.1 ± 0.4 | - | 6.3 ± 0.2 | 0.9 ± 0.2 | - | - | - |
| Building product | Paint | 81.8 ± 7.3 | - | 119.3 ± 4.7 | 0.3 ± 0.1 | - | - | - |
| | Seal | - | - | - | - | 43.6 ± 13.5 | - | - |

*The uncertainties were based on replication of experiments (N = 2).

- : < LOD

Bolded values indicate that the isothiazolinone was not specified by the manufacturer at the time of the analysis

Cosmetics usually contain MIT and CMI [3, 44, 45, 46, 47] and sometimes contained BIT and OIT [3, 44] before they are banned by the European cosmetics regulation (Electronical Supplementary Material 1). In accordance with the product composition provided by the manufacturer, analysis of the 4 tested cosmetic products confirmed that they only contained MIT and CMI. The measured concentrations agreed with the literature for shampoos (MIT: from 0.38 ppm [3] to 163 ppm [44]; CMI: from 1.12 ppm [3] to 10.3 ppm [46]), shower gels (MIT: from 2.05 ppm [3] to 128 ppm [44]; CMI: from 3.35 ppm [3] to 8.59 ppm [45]), and shower milks (MIT: from 1.12 ppm [3] to 26.1 ppm [3]). The body cream under study showed a lower MIT concentration than the few measurements from aftershave and face creams (MIT: from 129.6 ppm [47] to 171 ppm [44]).

According to European [12, 70], US [13], and Asian [14, 15] regulations, the mixture of MIT and CMI (ratio 1:3) as a preservative in rinse-off cosmetic products must have a maximum concentration of 15 ppm (Electronical Supplementary Material 1). In this respect, the concentrations of the MIT/CMI mixture measured for the shampoo and the shower gel were higher than the allowable concentrations in rinse-off cosmetics. In the other cosmetics, MIT is restricted to a maximum level of 100 ppm, and this concentration was not exceeded in the tested shower milk and body cream.

In the literature, the isothiazolinones found in air fresheners [25, 48], cleaning products [39, 44], and paints [10, 39] are MIT, CMI, BIT, and OIT. Their presence and concentrations in these consumer products depend on their role and the surface or air to be treated. To our knowledge, the identification of isothiazolinones in a sealant has never been reported in the literature. All other tested products complied with the relevant regulations (Electronical Supplementary Material 1). The presence of OIT (0.3 ± 0.1 ppm) in the paint was not indicated on the packaging at the time of the analysis. However, there was no requirement for this labeling because the manufacturer already indicated the presence of the two main isothiazolinones (i.e., MIT and BIT) in their paint. Since the analysis was carried out, now the manufacturer indicated the presence of OIT (Electronical Supplementary Material 4).

Measurements of isothiazolinones in several consumer products have shown the importance of having a sensitive and repeatable UPLC-MS/MS method for the detection of a broad spectrum of isothiazolinone compounds. The developed method allowed for highlighting the products containing isothiazolinones at levels exceeding the limits imposed by the market control.

3.4. Paint emission analysis

The tested paint was a low-cost product with the French labeling of building and decoration products [49] for volatile organic compound (VOC) emissions class “A+”, i.e., the emission measurements of 10 VOCs (i.e., formaldehyde, acetaldehyde, toluene, tetrachloroethylene, xylene, 1,2,4-trimethylbenzene, 1,4-dichlorobenzene, ethylbenzene, 2-butoxyethanol, and styrene) and the total VOC concentration were classified as low. Among the three isothiazolinones quantified in the paint, only MIT was quantified in the respective emissions, while the less volatile compounds OIT and BIT were not detected (Fig. 2).

These results from the paint emissions may be dependent on the form of application. Usually, in real conditions, the paint is extended to a given surface, increasing the contact with air and thus, potentially increasing the emissions. However, in our experimental conditions, approximately 2 g of paint was placed in a 44 mL microchamber 4 cm in diameter (i.e., 12.6 cm² of air-product contact). These application conditions could be less favorable for the emission of the paint although the use of a microchamber is a standard method for determining the emission of semi-volatile organic compounds by building products or furnishings [50].

More, Nagorka et al. [39] pointed out the difficulties in detecting BIT in air emissions from building products (i.e., paint and adhesive) using a C18 cartridge. These authors attributed the difference between the BIT concentrations measured in the product and the air emission to its low vapor pressure (3.7×10^{-4} Pa) and the complexity of the matrix product. The same assumption can be made for OIT, which was not detected in emissions in the microchamber. In addition to the low vapor pressure of OIT (4.9×10^{-3} Pa), its concentration in the paint under study was very low (0.3 ± 0.1 ppm).

BIT and MIT had close concentrations in paint composition, i.e., 119.3 ± 4.7 ppm and 81.8 ± 7.3 ppm, respectively. However, MIT was quantified in air emissions of the paint under study ($C_{MIT} = 31.4 \pm 1.4 \mu\text{g m}^{-3}$), probably because of its relatively high vapor pressure (8.3×10^{-2} Pa). From the concentrations of MIT in the paint emissions, it is possible to calculate its area-specific emission rate (SER, $\mu\text{g m}^{-2} \text{h}^{-1}$) using the following Equation 2:

$$SER_{MIT} = C_{MIT} \cdot k_{AER}/L \quad (\text{Equation 2})$$

According to the experimental under use, C_{MIT} is the concentration of MIT measured in the microchamber ($31.4 \mu\text{g m}^{-3}$), k_{AER} is the air exchange rate of the microchamber induced by the sampling flow rate (68 h^{-1}), and L is the paint surface per volume unit of the microchamber ($28.5 \text{ m}^2 \text{ m}^{-3}$).

As a result, the SER_{MIT} of the tested paint was $74.3 \mu\text{g m}^{-2} \text{h}^{-1}$, which was in agreement with those available in the literature for other paints [39], i.e., from 97 to $231 \mu\text{g m}^{-2} \text{h}^{-1}$ after 3 d in a chamber test. Therefore, the

experimental conditions in the microchamber (flow, temperature, relative humidity, amount of product) and the choice of sampling cartridge will need to be optimized to better characterize emissions of all the isothiazolinones.

5. CONCLUSION

A UPLC-MS/MS method was developed to identify and quantify seven currently used isothiazolinones and was validated according to ISO/IEC 17025. The seven isothiazolinones were eluted in a 3 min chromatographic run. With the low LODs/LOQs obtained, the UPLC-MS/MS method was successfully used for the isothiazolinone quantification in four types of consumer products (i.e., cosmetics, air fresheners, cleaning products, and building products) and in the emissions from a paint.

Among the 10 tested products, the MIT and MCI concentrations in rinse-off cosmetic products were higher than regulation levels for cosmetics, thus showing the need for a rapid and sensitive analytical method for a market surveillance purpose.

Despite the analytical sensitivity, it was not possible to quantify emissions of all isothiazolinones present in the paint. The experimental conditions in the emission microchamber need to be optimized.

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DECLARATIONS

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Conflicts of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

Léa Ducup de Saint Paul performed the experiments, analysed the data and wrote the manuscript; Sylvain Ravier performed the experiments and wrote the manuscript; Henri Wortham, François Maupetit, Mélanie Nicolas and Etienne Quivet wrote and reviewed the manuscript.

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FIGURE CAPTIONS

Fig. 1 UPLC-MS/MS chromatogram of a mixture of the seven isothiazolinones under study in acetonitrile/water (10/90): 1 = MIT (1.14 min – 11.8 $\mu\text{g L}^{-1}$), 2 = CMI (1.48 min – 12.8 $\mu\text{g L}^{-1}$), 3 = BIT (1.61 min – 13.3 $\mu\text{g L}^{-1}$), 4 = MBIT (1.91 min – 20.3 $\mu\text{g L}^{-1}$), 5 = sulfadimethoxine (1.94 min – 0.2 $\mu\text{g L}^{-1}$), 6 = BBIT (2.34 min – 12.7 $\mu\text{g L}^{-1}$), 7 = OIT (2.47 min – 17.4 $\mu\text{g L}^{-1}$), and 8 = DCOIT (2.76 min – 12.0 $\mu\text{g L}^{-1}$)

Fig. 2 Chromatograms of A) 7 isothiazolinone standards and sulfadimethoxine and extracts of B) paint, C) shower gel, D) floor cleaner, E) air freshener, and F) indoor air emission of the paint. 1 = MIT, 2 = CMI, 3 = BIT, 4 = MBIT, 5 = sulfadimethoxine (IS), 6 = BBIT, 7 = OIT, and 8 = DCOIT

FIGURES

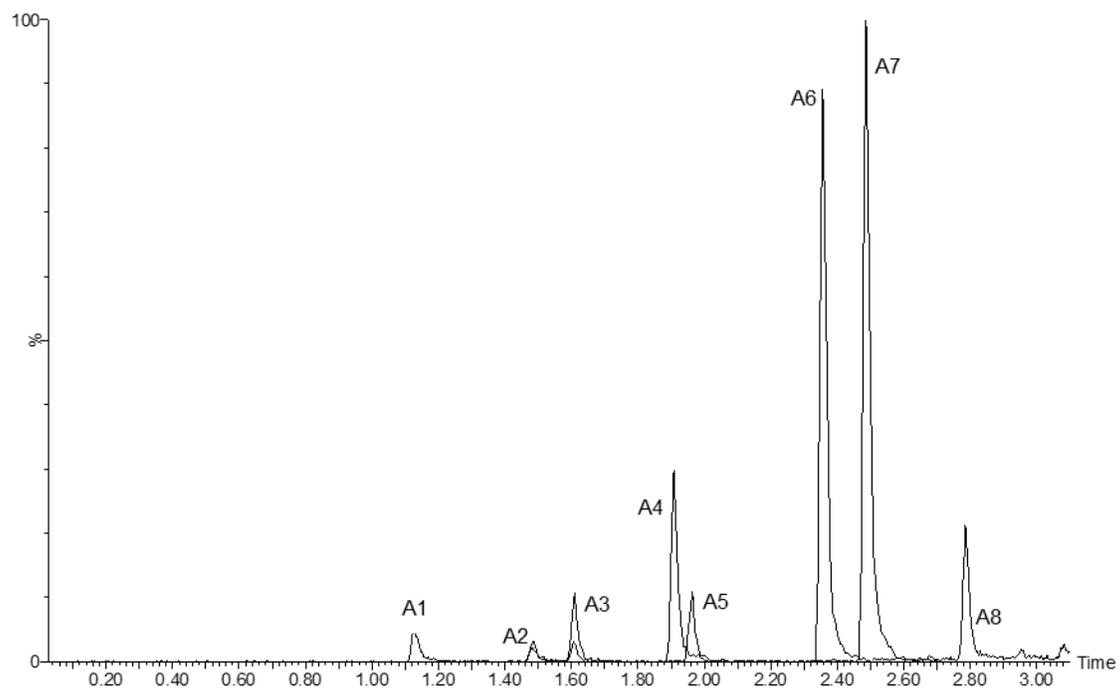
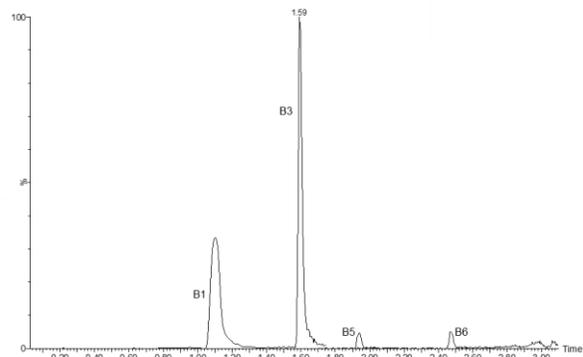
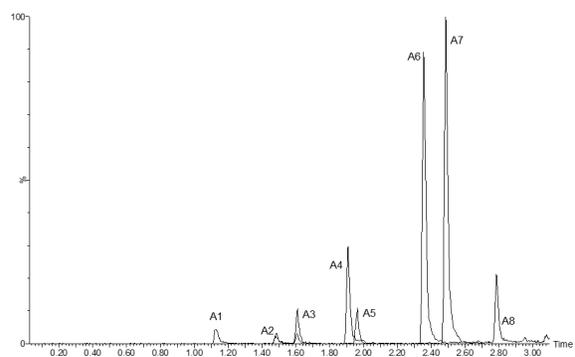
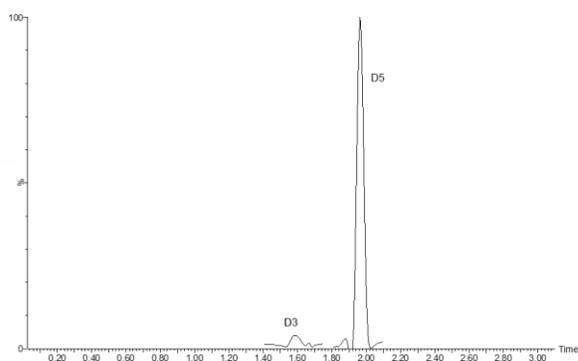
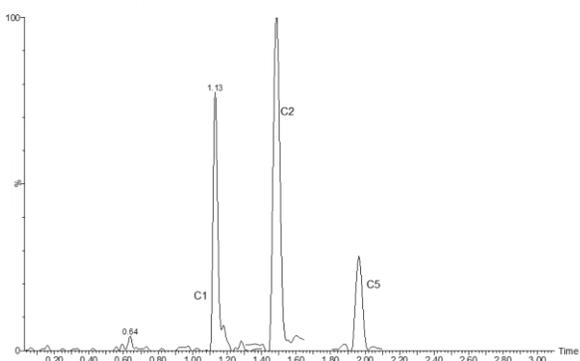


Fig. 1



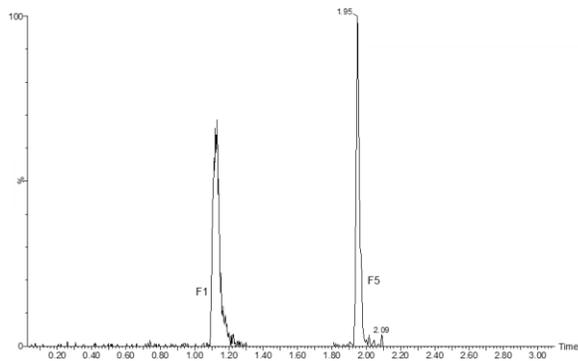
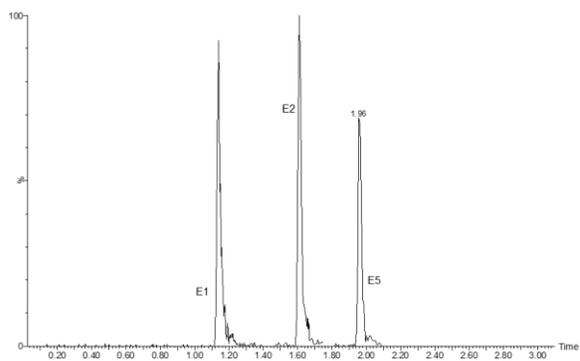
A

B



C

D



E

F

Fig. 2