Development of quantification methods for studying the emissions of isothiazolinones from building and consumer products into indoor environments

L Ducup de Saint Paul1,2,*, M Nicolas1 and E Quivet2
1 Centre Scientifique et Technique du Bâtiment (CSTB), Saint-Martin-d’Hères, France
2 Aix Marseille Univ, CNRS, LCE, Marseille, France
* lea.ducup@cstb.fr

Abstract. Isothiazolinones, a family of biocides, are used as preservatives in a wide range of consumer and building products, such as cleaning agents, paints, or cosmetics for their fungicide, bactericide, and algaeicide properties. Moreover, some isothiazolinones are classified as skin sensitizer (MIT, CMI, BIT and OIT) since they can cause eczema, oedema, or allergic contact dermatitis by dermal contact or inhalation. In this study, sensitive and robust analytical methods using GC-MS and UPLC-MS/MS as well as sampling and extraction protocols were developed to identify the emissions of seven isothiazolinones from building and consumer products. Two sampling supports (C18 Cartridge and PUF) were tested; Elution by acetonitrile for C18 Cartridge and ASE extraction using acetone and dichloromethane for PUF are chosen as extraction procedures. The first application of these developed methods on paints showed promising results.

1. Introduction

As per the definition in the European legislation, biocides are products which deter, destroy, render harmless, and control the effect on any harmful microorganism by chemical or biological means [1]. In Europe, products containing biocides are regulated by the Biocidal Products Regulation and classified into four main groups: disinfectants, preservatives, pest control, and other biocidal products (antifouling or taxidermist products) [1] [2].

Isothiazolinones have been incorporated as preservatives for their fungicide, algaeicide, and bactericide properties in a wide range of consumer (detergents, shampoos, paints, and varnishes) and industrial (polymers solutions, cooling fluids, and oils) products [3].

Historically, two isothiazolinone-type biocides were used as preservatives: BIT (1,2-benzisothiazol-3(2H)-one) used since the 1970s in industrial fluids and a mixture of CMI/MIT (5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-2H-isothiazole-3-one) (3/1) used since the 1980s in cosmetics [3] [4] [5]. MIT alone was introduced as preservative in cosmetics since the 2000s. Because of this, the number of allergic contact dermatitis has increased in an alarming way since 2009. Moreover, these three isothiazolinones in addition to BIT are classified as skin sensitizers. They can cause oedema, eczema, or allergic contact dermatitis by dermal contact or inhalation [2].

Isothiazolinones have been studied in many matrices such as cosmetics, paints, non-formalin adhesives, or waters. Gas and liquid chromatography are used for the separation of isothiazolinones and mass or tandem mass spectrometry with UV is used for their detection [4].

As isothiazolinones are volatile compounds and can be found in indoor environments, emissions of MIT, CMI, BIT, and OIT from building and cleaning products have been studied [4] [6] [7] [8].

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd
2. Objectives

Even if analytical Gas Chromatography – Mass Spectrometry (GC-MS) and Ultra Performance Liquid Chromatography - tandem Mass Spectrometry (UPLC-MS/MS) methods are already present in the literature for characterizing isothiazolinones, they were developed for studying only five of these compounds. Moreover, the study of the emission of isothiazolinones into indoor air is still rare. Most of the research done on these compounds is on their presence in water or content in consumer products and not in indoor air.

Therefore, the aim of this study is to develop sensitive and robust analytical GC-MS and UPLC-MS/MS methods to identify and quantify seven chosen isothiazolinones. In addition, sampling and measurement protocols will be developed to characterize their emission from building and consumer materials into indoor air.

3. Materials and methods

3.1. Selection of isothiazolinones

To the best of our knowledge, the quantification of isothiazolinones was done for a maximum of five isothiazolinones, i.e., MIT, BIT, CMI, OIT, and DCOIT [9]. In this study, due to their allergic properties (MIT, CMI, BIT, OIT, and BBIT) [10], occurrence in indoor environments (DCOIT) [11], and recent authorization from the European Commission for their usage in consumer products [12], seven isothiazolinones (Table 1) have been selected.

Table 1. The different used isothiazolinones to develop the UPLC-MS/MS and GC-MS methods.

| Isothiazolinones                  | Abbreviation | Molecular Formula | CAS No.   |
|----------------------------------|--------------|-------------------|-----------|
| 2-methyl-2H-isothiazole-3-one    | MIT          | C,H_NOS           | 2682-20-4 |
| 5-chloro-2-methyl-4-isothiazolin-3-one | CMI       | C,H_CLNOS         | 26172-55-4 |
| 1,2-benzoisothiazol-3(2H)-one   | BIT          | C,H_NOS           | 2634-33-5 |
| 2-methyl-1,2-benzoisothiazol-3(2H)-one | MBIT        | C,H_NOS           | 2527-66-4 |
| 2-butyl-1,2-benzoisothiazol-3(2H)-one | BBIT       | C,H_NOS           | 4299-07-4 |
| 2-octyl-2H-isothiazole-3-one    | OIT          | C,H_NOS           | 26530-20-1 |
| 4,5-dichloro-2-octylisothiazol-3(2H)-one | DCOIT   | C,H_CLNOS         | 64359-81-5 |

3.2. Chemicals

Standards of isothiazolinones were purchased from Merck: (MIT (purity ≥ 98%), BIT (purity ≥ 97%), OIT (purity ≥ 98%)), Ark Pharma (BBIT (purity ≥ 97%)), Carbosynth (MBIT (purity ≥ 96%)), and TCI (Tokyo Chemical Industry) (DCOIT (purity ≥ 98%)). From Sigma-Aldrich, 1-chlorodecane (purity ≥ 98%), 1-chlorotetradecane (purity ≥ 98%), and Bis(2-ethylhexyl) phthalate-3,4,5,6-d4 (DEHP-d4, purity ≥ 98%) were purchased as internal standards for GC separation while triphenylphosphate (purity ≥ 99%) was used for the UPLC separation.

3.3. Extraction protocol

According to the literature, two sampling supports were chosen: C18 Cartridge (Waters, Sep-Pak C18 plus short Cartridge, 360 mg sorbent) and polyurethane foam (PUF: 75 mm of length and 25 mm of diameter) [6] [7].

To test the extraction protocols (Table 2), 500 µL of a standard solution (a mixture of the seven isothiazolinones under study at 1 g/L) were spiked on each sampling support. The C18 Cartridge was eluted with 5 or 10 mL of acetonitrile, ethyl acetate, or methanol.

For the PUF, two protocols were tested:

- An Accelerated Solvent Extraction (ASE) using acetone and dichloromethane (50/50): the extraction lasts about 25 min at 100°C and 1100 psi.
- An ultrasonic extraction for 30 min with 45 mL of acetonitrile, hexane, or methanol.

The extracted solution was preconcentrated at 200 µL and injected in GC-MS and UPLC-MS/MS.
Table 2. The different tested solvents and protocols for the elution or extraction of isothiazolinones.

| Sampling support | Extraction        | Solvent          |
|------------------|-------------------|------------------|
| C18 cartridge    | Elution           | Acetonitrile     |
|                  |                   | Ethyl acetate    |
| PUF              | ASE               | Acetonitrile /   |
|                  |                   | Dichloromethane  |
| PUF              | Ultrasonic bath   | Acetonitrile     |
|                  |                   | Hexane /         |
|                  |                   | Dichloromethane  |

3.4. Development of the analytical method

3.4.1. Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS method was developed using gas chromatography (Autosystem XL, Perkin-Elmer) interfaced with a Quadrupole Mass spectrometer (TurboMass XL, Perkin-Elmer) equipped with electron ionization (70 eV). An RTX-5MS (5% diphenyl 95% dimethyl polysiloxane) GC column (60 m x 0.25 mm I.D., 0.25 µm film thickness) was used for the separation of the analytes.

3.4.2. Ultra Performance Liquid Chromatography tandem Mass Spectrometry (UPLC-MS/MS)

The UPLC-MS/MS method was developed using liquid chromatography (Acquity, Waters) interfaced with a Quadrupole-Time-of-Flight Mass spectrometer (Synapt G2 HDMS, Waters) equipped with an electrospray ion source (ESI). An HSS PFP (PentaFluoroPhenyl) column (100 mm x 2.1 mm I.D., 1.8 µm particle size) and a full loop injection volume (10 µL) were used. A gradient elution between Milli-Q water with 0.1 % formic acid and acetonitrile with also 0.1 % formic acid was used for the separation. The isothiazolinones were ionized in positive mode.

4. Results

4.1. GC-MS separation

After the optimization of the GC (retention time) and MS (selected ions) parameters, a triplicate of a mixture of seven standards of isothiazolinones was injected to validate the separation (Figure 1). According to the literature and based on the analysis of isothiazolinones in environmental waters, three internal standards, 1-chlorodecane, 1-chlorotetradecane, and DEHP-d₄, were added to the mixture. All the seven isothiazolinones were separated in less than 37 min (Figure 1). In the literature, GC separation of isothiazolinones ranged from 12 to 26 min for mixtures of MIT/CMI/BIT/OIT and MIT/CMI/BIT/OIT/DCOIT, respectively, but this was done using a 30 m length column [2] [13]. Preliminary results show limits of detection in the range between 5 and 15 mg/L depending on the compound.

Figure 1. GC-MS chromatogram of a mixture of the seven isothiazolinones under study in Acetonitrile: 1 = MIT (29 mg/L), 2 = CMI (27 mg/L), 3 = 1-chlorodecane (50 mg/L), 4 = BIT (26 mg/L), 5 = MBIT (31 mg/L), 6 = 1-chlorotetradecane (50 mg/L), 7 = OIT (44 mg/L), 8 = BBIT (36 mg/L), 9 = DCOIT (35 mg/L), 10 = DEHP-d₄ (50 mg/L)
4.2. UPLC-MS/MS separation

After the optimization of the UPLC (retention time) and MS (sampling cone, collision energy, and precursor and fragment ions) parameters, a triplicate of a mixture of seven standards of isothiazolinones was injected to validate the separation (Figure 2).

The developed UPLC-MS/MS method allowed a good separation of the seven isothiazolinones in less than three minutes (Figure 2). The UPLC-MS methods found in the literature, were developed only for mixtures of MIT/CMI and MIT/CMI/BIT [14] [15] with similar retention time windows.

Preliminary results show limits of detection in the order of few µg/L. These results indicate that the UPLC-MS/MS method is more sensitive than the GC-MS method.

4.3. Extraction protocol

Figure 3 shows the extraction efficiency of the C18 cartridge elution (a) and the PUF extraction using either ASE (b) or ultrasonic bath (c). Experimentations were repeated twice and the value of relative standard deviation (RSD) was lower than 20 % for all isothiazolinones.

The first results show that both supports can be used for extracting isothiazolinones, even if some solvents were not efficient in extracting CMI and BIT, e.g., the mixture acetonitrile/dichloromethane and hexane/dichloromethane.

For C18 Cartridge, the elution with 5 mL of acetonitrile seems like the best compromise.

For PUF, the extraction by ASE using a mixture of acetone and dichloromethane seems to be better even if it needs further optimization.
Figure 3. Results of the different extraction protocols by solvents and sampling supports: a) C18 Cartridges, b) PUF extracted by ultrasonic bath, and c) PUF extracted by ASE.

5. Atmospheric indoor application

Four glass plates were painted with 22.5 g of a paint containing two isothiazolinones, i.e., MIT and BIT. These plates were put in a chamber of 300 L with an air exchange rate at 1 vol/h. The sampling was carried out using a C18 Cartridge. The air flow rate was 460 mL/min for 24 h, giving a total volume of filtered air around 0.7 m³.

The C18 Cartridge was then eluted with 5 mL of acetonitrile. The eluate was preconcentrated to 200 µL and injected into the GC-MS.

The obtained results confirmed the emission of isothiazolinones, particularly MIT, from the studied paint (Figure 4). However, BIT was not detected in this first application. This can be explained based on two hypotheses: either BIT is not emitted from the chosen paint or the GC-MS limit of detection of BIT is too high.
Figure 4. GC-MS chromatogram of the emitted isothiazolinones from a paint: 1 = MIT, 2 = 1-chlorodecane, 3 = 1-chlorotetradecane, 4 = DEHP-d4

6. Conclusion
This project is still under progress. The first objective of this work in developing robust analytical GC-MS and UPLC-MS/MS methods was achieved. Seven isothiazolinones were well identified and separated. The UPLC-MS/MS method is more sensitive than the GC-MS method.

A first test to sample the emission of isothiazolinone by a paint was carried out with a C18 Cartridge as sampling support. This experimentation has demonstrated the ability of C18 Cartridge to retain MIT.

However, further experimentation should be conducted for the development of robust sampling and extraction protocols to characterize the emission of the seven chosen isothiazolinones from different building and consumer materials into indoor air. These to-be-developed analytical and measurement methods will allow us to predict the risk of human exposure to these compounds in indoor environments.

References
[1] Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market 16 February 1998 European Union Brussels
[2] Rafoth A, Gabriel S, Sacher F and Brauch H J 2007 J. Chrom. A 1164 74-81
[3] Garcia Hidalgo E 2017 Eth Zurich
[4] Lundov M D, Kolarik B, Bossi R, Gunnarsen L, Johansen J D 2014 Environ. Sci. Technol. 48 6989-94
[5] Pedersen N B 1976 Cont. Derma. 2 340-2
[6] Nagorka R, Gleue C, Scheller C, Moriske H J, Straff W 2015 Indoor Air 25 68-78
[7] Horn W, Jann O, Wilke O 2003 Atmos. Environ. 37 5477-83
[8] Bohn S, Niederer M, Brehm K, Bircher A J 2000 Cont. Derma. 42 196-201
[9] Wang C, Xie T, Xu R, Lin J, Li L 2017 World J. of Engi. and Tech. 5 1-18
[10] Aerts O, Goossens A, Lambert J, Lepoittevin J P 2017 Eur J Dermatol 27 115-22
[11] Friis U F, Menné T, Flyvholm M A, Bonde J P E, Lepoittevin J P, Le Coz C J, Johansen J D 2014 Cont. Derma. 71 65-74
[12] Execution reglementation 2017/2327 of European Commission for the authorization of 2-methyl-1,2-benzisothiazol-3(2H)-one as active substance in type product 6 14 December 2017 Offi J of the European Union
[13] Nakashima H, Matsunaga I, Miyano N, Kitagawa M 2000 Jour. of Health Sci. 46 447-54
[14] Wittenberg J B, Canas B J, Zhou W, Wang P G, Rua D, Krynitsky A J 2015 J. Sep. Sci. 38 2983-88
[15] Dang H, Liu D, Hou X, Wu Y, Wang B, Dong H, Xian Y 2017 Anal Methods 9 482-9