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Analysis of isothiazolinones in environmental waters by gas chromatography–mass spectrometry

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Abstract

This paper describes an analytical method for the determination of five biocides of isothiazolinone type (2-methyl-3-isothiazolinone (MI), 5-chloro-2-methyl-3-isothiazolinone (CMI), 1,2-benzisothiazolinone (BIT), 2-octyl-3-isothiazolinone (OI), 4,5-dichloro-2-octyl-3-isothiazolinone (DCOI)) in environmental waters. The method is based on pre-concentration of the analytes by solid-phase extraction onto a mixture of a polymeric material and RP-C18 material and subsequent determination by gas chromatography–mass spectrometry (GC–MS). One of the target compounds (BIT) is derivatised with diazomethane after pre-concentration to improve its chromatographic performance. The method was optimised with respect to pre-concentration conditions (liquid–liquid extraction versus solid-phase extraction, solid-phase material, elution solvent and volume) and extensively validated. Applying the method to surface waters, groundwaters, and drinking waters, limits of detection between 0.01 and 0.1 μg/l could be achieved and the repeatability was below 10% for all compounds except for MI. Additional investigations showed that the stability of the isothiazolinones in environmental waters is limited and sample storage at 4 °C is mandatory to preserve the target biocides. First investigations of influents and effluents of a wastewater treatment plant showed that conventional wastewater treatment exhibits a high efficiency for removal of the isothiazolinones. In river waters, the target isothiazolinones could not be detected.

Keywords: Biocide; Isothiazolinone; Water analysis; Solid-phase extraction; Gas chromatography; Mass spectrometry; Derivatisation; Diazomethane

1. Introduction

The EU Biocidal Products Directive 98/8/EC defines biocides as active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means [1]. In the Directive four biocidal product types are distinguished: Disinfectants and general biocidal products, preservatives, pesticides, and other biocidal products. One important class of preservatives belongs to the chemical group of isothiazolinones, i.e. derivatives of 3(*2H*)-isothiazolinone. The most relevant representatives of this class of biocides are shown in Table 1.

Isothiazolinones are mainly used as effective preservatives for the control of microorganisms as fungi and bacteria in aqueous-based industrial products such as cleaning agents, paint formulations, resin emulsions, adhesives and in cosmetics, toiletries and household products such as shampoos, other hair and skin care products, fabric softeners or polishes [2,3]. Furthermore, some of them are widely used in pulp and paper industries, in cooling water circulations, or as wood preservatives [2,4,5]. Methylisothiazolinones like 2-methyl-3-isothiazolinone (MI) or 5-chloro-2-methyl-3-isothiazolinone (CMI) are commonly used as slimicides in pulp and paper making industries [2]. The cosmetic industry employs a 3:1 CMI/MI mixture as a broad-spectrum preservative whereas octylisothiazolinones like 2-octyl-3-isothiazolinone (OI) and 4,5-dichloro-2-octyl-3-isothiazolinone (DCOI) are mainly used for material and surface protection as wood preservatives, mildewcides and in anti-fouling paints [6].

Nowadays, especially MI and CMI are regarded as skin irritants and allergens and their use e.g. as preservatives in cosmetic products has been restricted to maximum authorised concentrations of 0.0015% for a 3:1 CMI/MI mixture or 0.01% for MI [7]. For the same reason, the chlorinated isothiazolinone CMI

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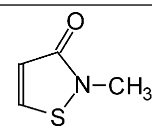
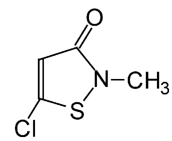
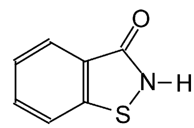
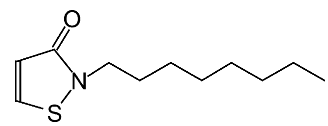
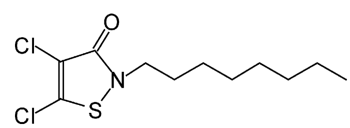
Table 1
Chemical structure, CAS number and molecular weight (MW) of the isothiazolinones under investigation

Compound	Chemical structure	CAS No.	MW (g/mol)
2-Methyl-3-isothiazolinone (MI)		2682-20-4	115.1
5-Chloro-2-methyl-3-isothiazolinone (CMI)		26172-55-4	149.6
1,2-Benzisothiazolinone (BIT)		2634-33-5	151.2
2-Octyl-3-isothiazolinone (OI)		26530-20-1	213.3
4,5-Dichloro-2-octyl-3-isothiazolinone (DCOI)		64359-81-5	282.2

CAS, chemical abstract service.

being a stronger sensitizer is increasingly substituted by 1,2-benzisothiazolinone (BIT) in cleaning agents, paints, adhesive, etc. [8].

Looking at their life-cycle and their patterns of use, several pathways are possible through which isothiazolinones might be released in the aquatic environment: A discharge by industrial wastewaters during production or processing must be taken into account as well as a release during usage e.g. in personal care products, in cleaning agents or in cooling waters. Additionally, leaching from materials containing isothiazolinones as protective coatings or paints cannot be excluded.

Due to their inherent properties as biocides, isothiazolinones must be regarded as possibly posing an ecotoxicological risk when discharged into the environment. Madsen et al. report LC50 values for a CMI/MI mixture of 0.003 mg/l for a green algae species, 0.16 mg/l for Daphnia magna, and 0.19 mg/l for rainbow trouts [9]. For BIT, the respective values are 0.15 mg/l (green algae), 1.35 mg/l (Daphnia magna), and 1.6 mg/l (rainbow trout), indicating that BIT is less harmful to water organisms than the CMI/MI mixture [9].

Despite their high production volumes, their wide-spread use and their negative eco-toxicological properties, up to now only few data on the occurrence and fate of isothiazolinones in the aquatic environment are available. This can mainly be attributed to the fact that analytical methods for the trace-level determination of these compounds in aqueous matrices are lacking.

Matissek describes amongst others a method for quantitative determination of methylisothiazolinones in cosmetic products by reversed-phase HPLC after purification and separation of interfering matrix components by flash chromatography [10]. For qualitative identification of methylisothiazolinone formulations, liquid–liquid extraction with ethyl acetate proved to be feasible to transfer methylisothiazolinones in an organic phase for subsequent gas chromatographic determination [10]. Nakashima et al. report on investigations about relevant specifications of MI, CMI, BIT and OI in non-formalin adhesives by either liquid–liquid or solid-phase extraction and subsequent detection by GC–MS [11].

Analytical methods for identification and determination of isothiazolinones in aqueous matrices, however, are hardly available. Thus, in the present paper the development of an analytical method for the trace-level determination of isothiazolinones in environmental waters (wastewater, surface water, groundwater, and tap water) is described. The method is optimised with respect to pre-concentration conditions and extensively validated. For testing the applicability of the method, wastewater and surface water samples are analysed and the stability of the compounds under investigation in different water matrices is investigated.

2. Experimental

2.1. Chemicals

2-Methyl-3-isothiazolinone (MI) with a purity of 100% was purchased from Sigma–Aldrich Chemie (Taufkirchen, Germany). 5-Chloro-2-methyl-3-isothiazolinone (CMI) was obtained as a 1.2% aqueous solution which also contained 0.3% MI from Fluka (Sigma–Aldrich Chemie, Taufkirchen, Germany). 2-Octyl-3-isothiazolinone (OI, 99.9% purity) was