MACHEREY-NAGEL
Chromatography application note

MN application note 10/2018

Determination of isothiazolinones from dish-washing agents and cleaning agents

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Abstract

This application note describes the determination of isothiazolinones from dishwashing agents and cleaning agents using liquid extraction for sample pretreatment. The extracts are finally analyzed by HPLC-MS/MS.

Introduction

Nowadays, people are suffering more and more from allergies due to additives like scents, biocides and so on [1]. Therefore, the European food law sets maximum residue limits of 0.01 % for isothiazolinones in cleaning agents [2, 3]. For a combined use of Methylisothiazolinone (MIT) / Chloromethylisothiazolinone (CMIT) (3/1) the permitted amounts are less than 0.0015 %. Further isothiazolinones are Benzisothiazolinone (BIT), Octylisothiazolinone (OIT) and Butylbenzisothiazolinone (BBIT).

In addition, biocides have to be indicated on the packaging of detergents for consumer information.

Biocides are often used in a combination to achieve the maximum effect against microorganism. A mixture of Methylisothiazolinone and Butylisothiazolinone is often found in cleaning agents, the use of additional isothiazolinones is uncommon.

In this application note a HPLC-MS/MS method for the determination of isothiazolinones (MIT, CMIT, BIT, BBIT, OIT) was developed on NUCLEOSHELL® Bluebird RP 18 column and detergents by retrial are investigated.

Compounds of interest

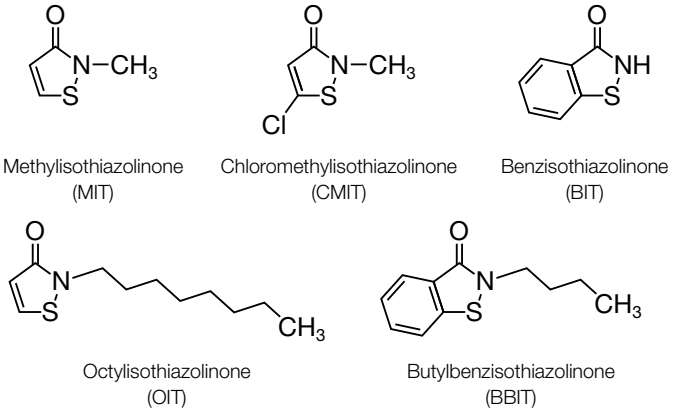

Figure 1: Compounds of interest.

Sample pretreatment

- Homogenize sample by stirring
- Weigh 2.5 g sample into a volumetric flask
- Fill up to 100 mL with water
- Shake mixture carefully for 5 min
- Filter sample solution through CHROMAFIL® PET-20/13 (REF 729222)

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions

Column:
EC 50/4.6 NUCLEOSHELL® Bluebird RP 18, 2.7 µm (REF 763432.46)

Eluent A:
0.1 % formic acid in water

Eluent B:
0.1 % formic acid in acetonitrile

Gradient:
from 0 % to 5 % B in 0.5 min, up to 95 % B in 2.0 min, hold 95 % B for 0.5 min, in 0.1 min down to 0 % B, hold 0 % B for 2.9 min

Flow rate:
1.3 mL/min

Temperature:
30 °C

Injection volume:
5 µL

MS conditions:

API 5500, ion source ESI, positive ionization mode, scan type SRM, detection window 90 s, curtain gas 35 psig, ion spray voltage 3000 V, temperature 500 °C, nebulizer gas 60 psig, turbo gas 45 psig, CAD medium

SRM transitions

Analyt	Retention time [min]	$[M–H]^-$	Q_1 (Quantifier)	Q_2 (Qualifier)
MIT	1.44	116.06	58.00	53.10
CMIT	1.80	149.98	87.00	135.00
BIT	1.89	152.00	109.00	134.10
BBIT	2.52	208.14	152.00	109.00
OIT	2.65	214.16	102.00	43.10

Table 1: SRM transitions for isothiazolinones.

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Chromatograms

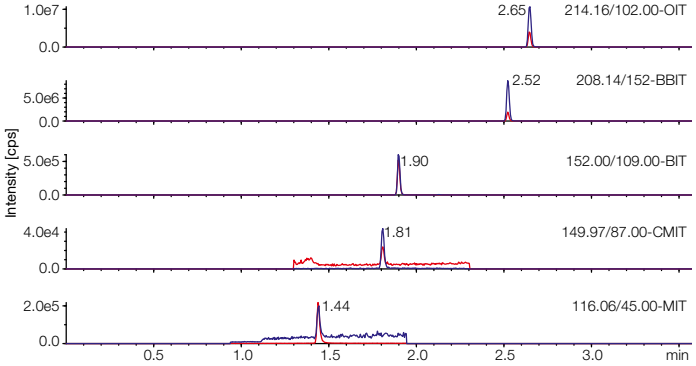

Figure 2: Chromatograms of standard mixture (β = 50 ng/mL for each analyte).

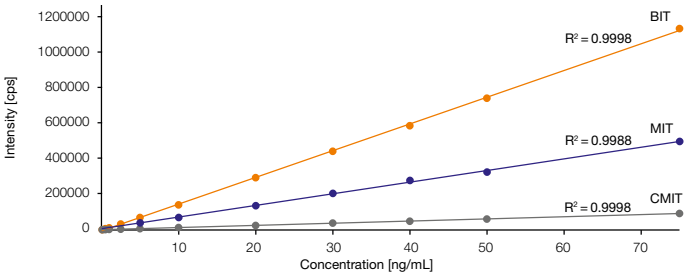

Figure 3: Calibration curves for MIT, CMIT, BIT (concentration range 0.5–75 ng/mL).

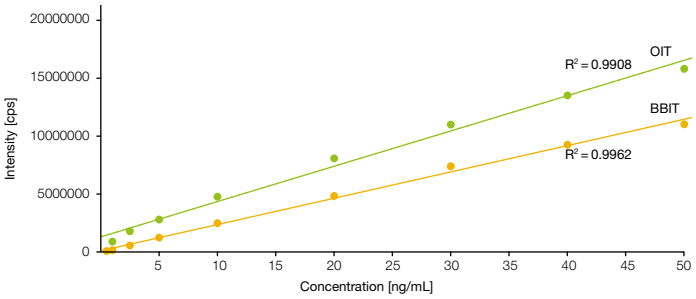

Figure 4: Calibration curves for OIT, BBIT (concentration range 0.5–50 ng/mL).

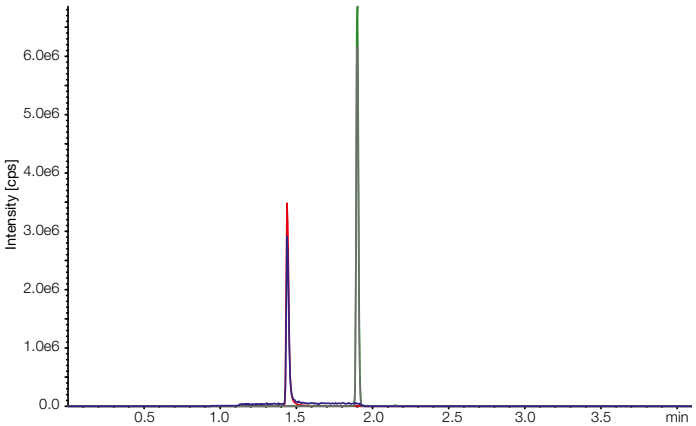

Figure 5: Chromatogram of dishwashing detergent sample A.

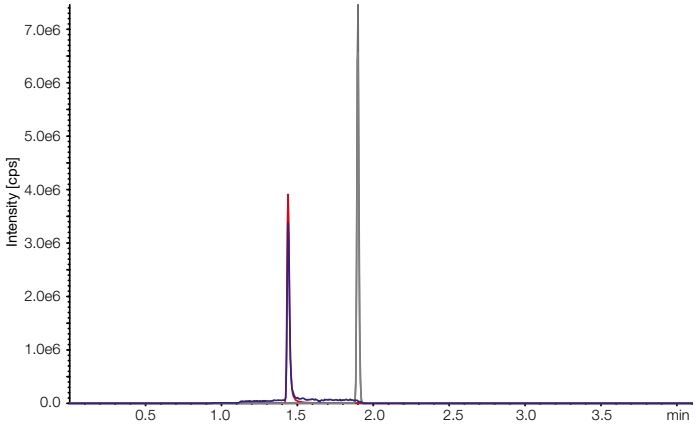

Figure 6: Chromatogram of dishwashing detergent sample B.

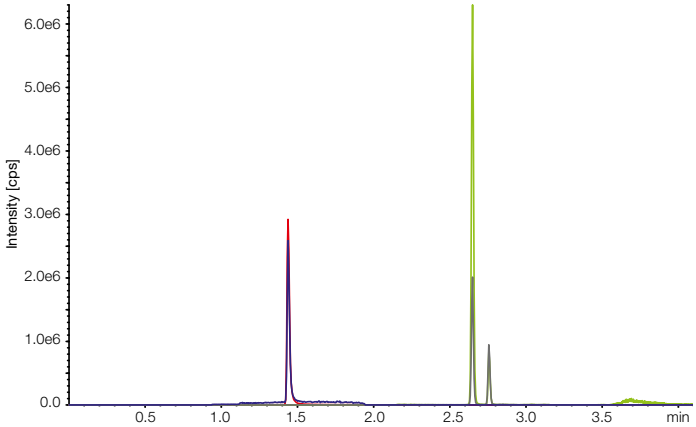

Figure 7: Chromatogram of dishwashing detergent sample C.

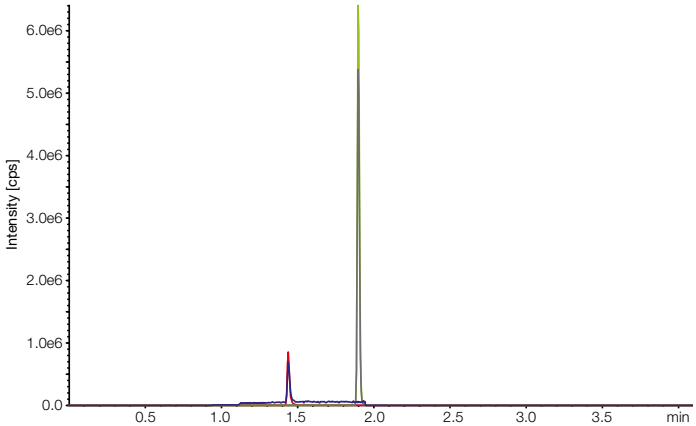

Figure 8: Chromatogram of cleaning agent sample.

Results

Sample	Amount of MIT [%]	Amount of BIT [%]	Amount of OIT [%]
dishwashing detergents sample A	0.0027	0.0014	-
dishwashing detergents sample B	0.0022	0.0005	-
dishwashing detergents sample C	0.0024	-	0.0001
cleaning agent sample	0.0006	0.0021	-

Table 2: Results for the amounts of MIT, BIT and OIT in dishwashing detergents and a cleaning agent.

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Conclusion

The results show that the determination of isothiazolinones from dishwashing agents and cleaning agents could be carried out successfully with all the tested products. Regarding the different types of detergents, the amounts of MIT, BIT and OIT were below the maximum residue limits by law. The results are corresponding to product declaration. Customers are informed of used additives as required by law.

The identification and quantification of isothiazolinones were carried out by ESI mass spectrometry on an EC 50/4.6 mm NUCLEOSHELL® Bluebird RP 18 column. Using this special octadecyl core-shell phase with hydrophilic endcapping leads to high interaction for the most polar analyte MIT and high retention as shown in figure 2. Further chromatograms of sample extracts are presented in figure 5–8.

In summary, the presented application describes a quick and convenient method for the determination of isothiazolinones from cleaning agents.

References

[1] BfR Expert Opinion No. 001/2007, 27 September 2006, Allergies caused by consumer products and foods.

[2] REGULATION (EC) No 648/2004 OF THE EUROPEAN AND OF THE COUNCIL of 31 March 2004 on detergents.

[3] REGULATION (EU) No 528/2012 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 May 2012 concerning the making available on the market and use of biocidal products.

Additional information

The following application regarding “Determination of isothiazolinones from dish washing agents and cleaning agents” and further applications can be found on our online application database at *www.mn-net.com/apps*

HPLC: MN Appl. No. 128730

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 763432.46	EC 50/4.6 NUCLEOSHELL® Bluebird RP 18, 2.7 µm
REF 729222	CHROMAFIL® PET-20/13
REF 702293	Screw neck vials N 9, 1.5 mL
REF 702107	N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red