Analysis of isothiazolinone biocides in cosmetic products and detergents by HPLC

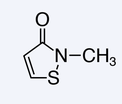
H. R. Wollseifen, T. Kretschmer, H. Riering,
MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany
Contact hwollseifen@mn-net.com

INTRODUCTION

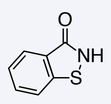
Despite their sensitizing effect isothiazolinone biocides are used in several cosmetics and cleaning products as antimicrobial agents. The most important isothiazolinone biocides are methylisothiazolinone (MIT), chloromethylisothiazolinone (CMIT), benzisothiazolinone (BIT), octylisothiazolinone (OIT), dichlorooctylisothiazolinone (DCOIT), and butylbenzisothiazolinone (BBIT). The use of these heterocyclic compounds is restricted by law [1, 2, 3]. If manufacturers use these preservatives, these substances must be declared on the packaging.

Due to very different polarities of these substances sample preparation and chromatographic separation are often not suitable for all target compounds. In the first part of this work we present a methodology for sample preparation of isothiazolinone analysis which includes a solid phase extraction (SPE) method for cosmetics and cleaning products. The second part of this work points out the chromatographic conditions on core-shell columns with pentafluorophenylpropyl (PFP) modification with UV and MS detection and illustrates limits of detection (LOD) and quantification (LOQ).

Methylisothiazolinone (MIT)

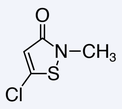


Benzisothiazolinone (BIT)

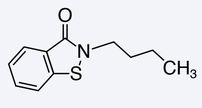


Octylisothiazolinone (OIT)

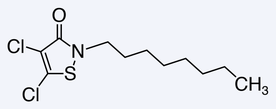
Chloromethylisothiazolinone (CMIT)



Butylbenzisothiazolinone (BBIT)



Dichlorooctylisothiazolinone (DCOIT)



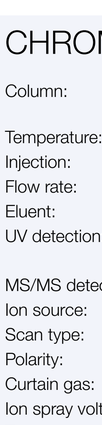
SAMPLE PREPARATION

Pre-extraction procedure

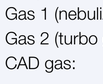
Dilute 5 g of sample in at least 50 mL water
Stir 10 min at room temperature
Add 0.5 mL formic acid
Fill up to 100 mL with water in a volumetric flask
Centrifuge 10 min at 6000 rpm at ambient temperature
Take the supernatant for the SPE

Solid phase extraction method for hair gel and dish soap

developed on CHROMABOND® HR-X polypropylene columns (3 mL, 200 mg, 85 µm)



Conditioning
3 mL methanol
3 mL water + 0.1 % formic acid



Sample application
1.0–5.0 mL sample solution with hydrostatic flow

Washing
3 mL water + 0.1 % formic acid, drying with air using a syringe

Elution
1 mL methanol
4 mL acetonitrile

Solvent changing
0.5 mL water as keeper
reduce volume to 0.6 mL under N_2 flow
fill up to 1.0 mL with water + 0.1 % formic acid

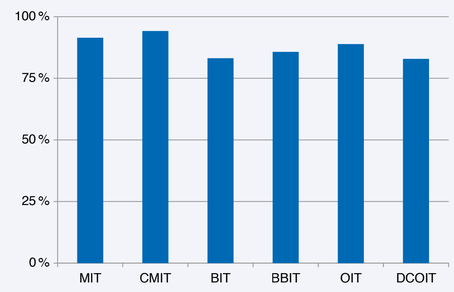
CHROMATOGRAPHIC METHOD

Column:	NUCLEOSHELL® PFP pentafluorophenylpropyl modification, multi-endcapping, particle size 2.7 µm, 100 x 2 mm ID
Temperature:	30 °C
Injection:	10 µL
Flow rate:	0.27 mL/min
Eluent:	A) water + 0.1 % formic acid; B) methanol; 5–95 % B in 3.6 min (1 min), 95–5 % B in 0.4 min (5 min)
UV detection:	260 nm, 275 nm, 315 nm (1 µg/mL for each compound)
MS/MS detection:	API 3200 AB SCIEX
Ion source:	Turbo Spray (ESI)
Scan type:	MRM
Polarity:	positive
Curtain gas:	10.00 psig
Ion spray voltage:	3000 V
Temperature:	550 °C
Gas 1 (nebulizer):	40 psig
Gas 2 (turbo gas):	40 psig
CAD gas:	8 psig

Analyte	Quantitative transition (m/z)	Qualitative transition (m/z)
MIT	115.5/98.1	115.5/69.3
CMIT	151.3/87.0	151.3/134.0
BIT	151.4/107.4	151.4/132.0
BBIT	207.3/149.5	207.3/131.8
OIT	213.4/100.5	213.4/56.0
DCOIT	281.2/168.0	281.2/42.3

RESULTS

SPE recoveries determined for standard solutions

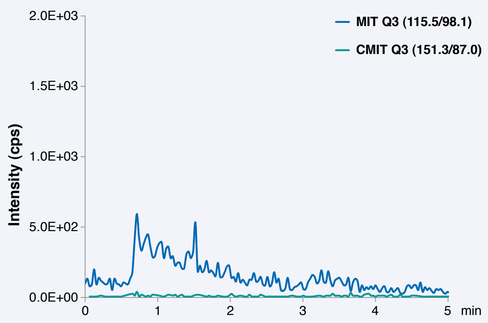


The recovery of all analytes is about 80 % or more. However, significant losses can occur at the exchange of the solvent if no keeper is used. In particular, the losses are greatest for the volatile analytes, MIT and CMIT.

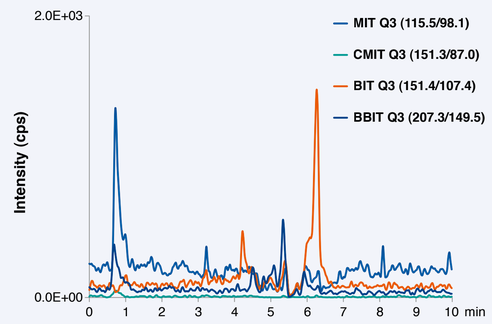
Mass spectrometry and UV detection results

Mass spectrometry detection in SPE eluates

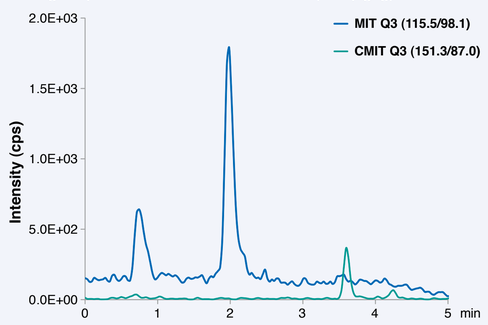
Hair gel without isothiazolinones



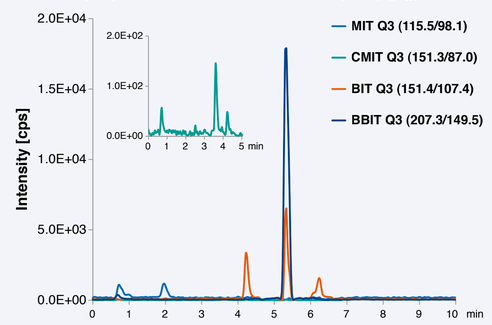
Dish soap without isothiazolinones



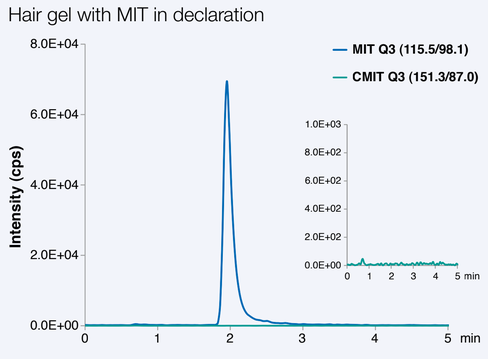
Hair gel spiked with isothiazolinones (0.1 µg/g)



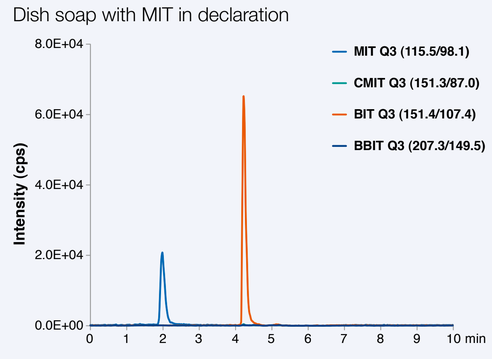
Dish soap spiked with isothiazolinones (0.1 µg/g)



Hair gel with MIT in declaration



Dish soap with MIT in declaration



CONCLUSION

The results of this work show that the solid phase extraction of isothiazolinones with CHROMABOND® HR-X is very well suited for hair gel and dish soap. The chromatographic method shows good retention for the six analytes on the NUCLEOSHELL® PFP phase, especially for the most polar MIT. The chromatographic separation takes only 10 min with reequilibration. The presented method allows detection of 0.6 µg MIT/g, 0.7 µg CMIT/g, 0.3 µg BIT/g, 0.1 µg BBIT/g, 0.2 µg OIT/g and 0.1 µg DCOIT/g and a quantification of 2.1 µg MIT/g, 2.6 µg CMIT/g, 1.2 µg BIT/g, 0.4 µg BBIT/g, 0.6 µg OIT/g and 0.2 µg DCOIT/g in hair gel. Therefore, it is possible to check the values required by cosmetics Regulation for MIT and CMIT in the amount of 3.75 µg MIT/g and 11.25 µg CMIT/g.

With the developed methodology the detection of 0.6 µg MIT/g, 1.9 µg CMIT/g, 0.1 µg BIT/g, 0.3 µg BBIT/g, 0.2 µg OIT/g and 0.5 µg DCOIT/g and the quantification of 2.0 µg MIT/g, 7.2 µg CMIT/g, 0.4 µg BIT/g, 1.0 µg BBIT/g, 0.7 µg OIT/g and 1.8 µg DCOIT/g are possible for detergents and cleaning agents. Therefore the method is suitable for verifying the labeling required by law.

REFERENCES

[1] REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products

[2] REGULATION (EC) No 648/2004 OF THE EUROPEAN PARLIAMENT 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