Degradation and by-products identification of benzothiazoles and benzotriazoles during chlorination by LC-HR-MS/MS

Maria-Christina K. Nika[1], Anna A. Bletsou[1], Evagelos E. Gikas[2] and Nikolaos S. Thomaidis[1]

[1]Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis Zografou, 157 71 Athens, Greece.
[2] Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Athens, Panepistimiopolis Zografou, 157 71 Athens, Greece.
e-mail: *ntho@chem.uoa.gr*

Abstract

Nowadays, chlorination is the most prevalent disinfection method applied for water treatment in Europe. Chlorine can be supplied as sodium hypochlorite (NaOCl) which reacts in water to produce the disinfectants hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), otherwise known as free chlorine. Although the primary purpose of chlorination is the elimination of micropollutants via oxidation, several investigations have shown that chlorine reacts with micropollutants leading in the production of undesired by-products. 1,3-benzothiazoles (BTHs) and 1,2,3-benzotriazoles (BTRs) are classified as high production volume emerging environmental pollutants due to their broad industrial and domestic application, and even though recently several analytical methods have been applied for their determination , there is still a lack of research for their by-products' identification.

Initially, the degradation of three BTHs (BTH, 2-OH-BTH and 2-amino-BTH) and four BTRs (1-H-BTRi, TTRi, XTRi and 1-OH-BTRi) during chlorination was investigated by UHPLC-MS/MS (QqQ). Although chlorination appeared to be an insufficient degradation process for BTH and 1-H-BTRi, all their examined substituted derivatives seem to be significantly degraded when the molar ratio of sodium hypochlorite and the target analytes was between 5000:1 – 1000:1. Then, LC high resolution MS/MS (q-TOFMS) was used to investigate the formation of by-products in the chlorinated samples. Two suspect by-products of 2-amino-BTH and one of XTRi were tentatively identified based on their probable structure, mass accuracy, retention time and fragmentation and isotopic pattern. An interesting observation was the formation of 1-H-BTRi as a degradation product of 1-OH-BTRi during chlorination. Moreover, post-acquisition non-target treatment of the MS data revealed several unknown by-products of the tested analytes.

Experimental

Chlorination experiments:
- Amber vials
- Buffer CH_3COONH_4 1 mM (pH=7.00±0.2)
- Stock solutions of the analytes 1000 μg/g
- Commercial chlorine (4.8 g NaOCl in 100 g)
- Na_2SO_3 ⇒ quenching the residual chlorine and the oxidation reaction

Degradation:

Investigation of the molar ratio:
- Mix of all analytes (30 ng/g, 100 ng/g, 200 ng/g & 1 μg/g)
- Cl_2 (molar ratio: 30000:1, 10000:1, 5000:1 and 1000:1, respectively)

Kinetic experiment ($t_{1/2}$, k_{obs}):
Molar ratio which provokes at least **50% decrease**
1-H-BTRi &TTRi ⇒ 30000:1, XTRi, BTH & 2-OH-BTH
⇒ 10000:1, 1-OH-BTRi ⇒ 5000:1, 2-amino-BTH ⇒ 1000:1

By-products investigation:
TTRi, XTRi & 2-OH-BTH ⇒ 1000:1, 1-OH-BTRi ⇒ 2000:1, 2-amino-BTH ⇒ 1000:1 & 100:1

Column: Atlantis T3 (Waters)
ESI: positive
Mobile phase: f.a. 0.01% & ACN (gradient elution)
MS mode: SRM

Column: Acclaim C18 (Dionex-Thermo Scientific)
ESI: positive & negative
Mobile phases: amm. form./ MeOH (amm. ac. 10 mM) & H_2O/ MeOH (amm. form.10 mM)
MS mode: bbCID (full scan MS & MS/MS)

Results and discussions

Investigation of the molar ratio:

BTHs:
- *2-amino-BTH*: decrease over 90%, within the first 5 min, regardless of the molar ratio
- *2-OH-BTH*: decrease over 50% within the first 10 min
- *BTH*: not significantly reacting

BTRs:
- As molar ratio decreases, degradation rate of all analytes decreases
- *1-H-BTRi*: decrease over 50%, when molar ratio is 30000:1

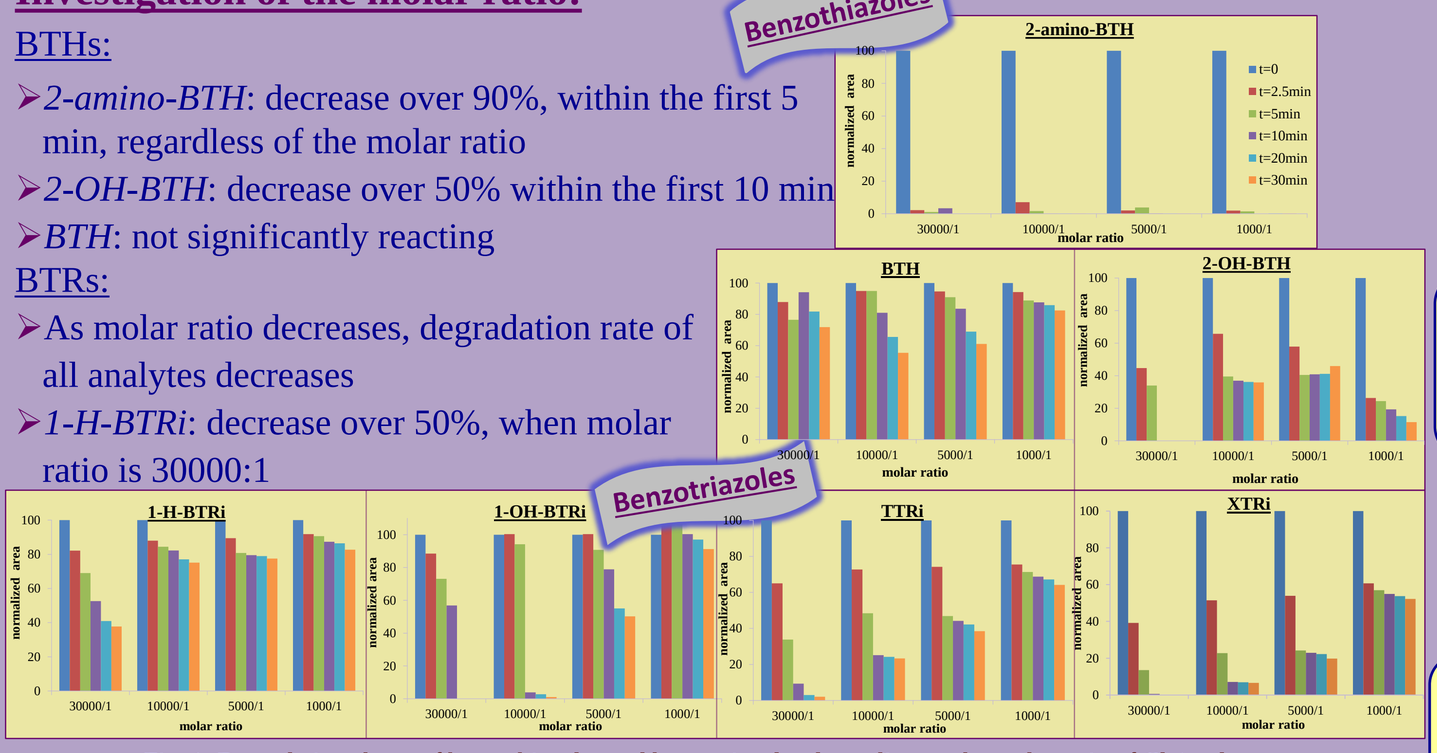

Fig. 1. Degradation charts of benzothiazoles and benzotriazoles depending on the molar ratio of Cl_2/analytes.

Kinetic experiment ($t_{1/2}$, k_{obs}):

Pseudo-first-order plots were revealed

Determination of $t_{1/2}$ and k_{obs} ***(the slope of the linear time-course plot of Ln([analyte]/ [analyte]$_0$)***

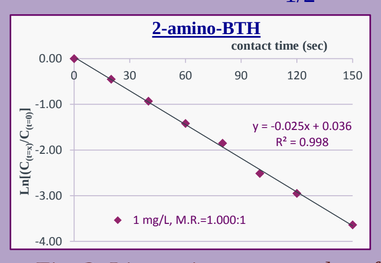

Fig. 2. Linear time-course plot of Ln([2-amino-BTH]$_t$/ [2-amino-BTH]$_0$).

According to their degradation rate:
- Very fast degraded ⇒ *2-amino-BTH*
- Fast degraded ⇒ *1-H-BTRi, TTRi, XTRi & 2-OH-BTH*
- Slowly degraded ⇒ *1-OH-BTRi & BTH*

Table 1. Determination of $t_{1/2}$ and k_{obs}.

Analyte	k_{obs}	$t_{1/2}$
1-H-BTRi	0.063 min $^{-1}$	11.4 min
1-OH-BTRi	0.023 min $^{-1}$	31.1 min
TTRi	0.241 min $^{-1}$	3.22 min
XTRi	0.265 min $^{-1}$	2.74 min
BTH	0.020 min $^{-1}$	35.1 min
2-OH-BTH	0.098 min $^{-1}$	5.50 min
2-amino-BTH	0.024 sec $^{-1}$	30.4 sec

By-products investigation:

- ***2-amino-BTH***: 2-amino-5-chloro-1,3-benzothiazol & 2-amino-5,6-dichloro-1,3-benzothiazol were tentatively identified ***(mass accuracy/ t_R/ fragmentation & isotopic pattern)***
- ***XTRi***: chloro-5,6-dimethyl-benzotriazole was detected ***(MS/MS spectrum ⇒ low intensity ⇒ further structure elucidation would not be confident)***
- ***1-OH-BTRi:*** 1-H-BTRi was produced ***(confirmation with reference standard)***

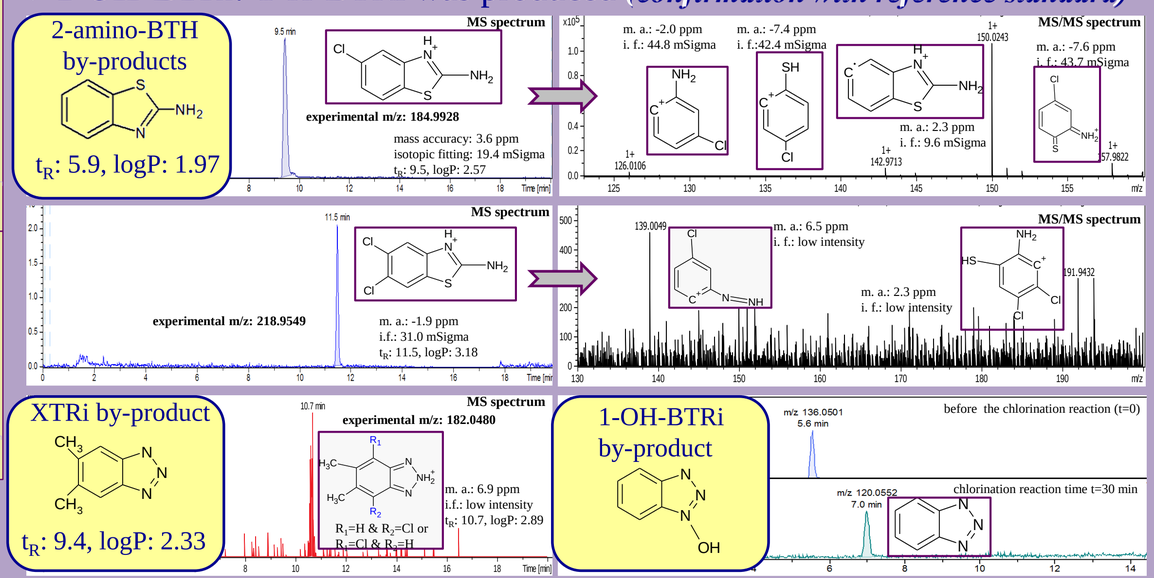

Fig. 3. MS and MS/MS spectrums of the identified by-products.

Background subtraction ***(chlorinated-zero time sample)*** ⇩ Revelation of "hidden" peaks ***(unknown by-products can now be identified)***

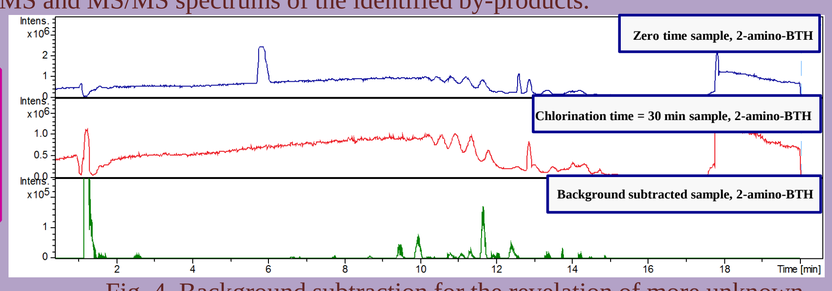

Fig. 4. Background subtraction for the revelation of more unknown by-products.

Conclusions

- The degradation rate of the benzotriazoles seems to increase proportionally to the molar ratio of Cl_2/ analyte. Benzothiazoles are either degraded within the first 10 min, or they do not significantly react.
- Chlorination kinetic parameters (k_{obs} and $t_{1/2}$) were determined for all the analytes, that were classified according to their degradation rate (very fast, fast and slowly degraded).
- One and two by-products were tentatively identified in the chlorinated samples of XTRi and 2-amino-BTH, respectively, while 1-H-BTRi seems to be produced by the chlorination of 1-OH-BTRi. Furthermore, numerous by-products' peaks were revealed in the chlorinated samples of 2-amino-BTH and XTRi.

Acknowledgments
This research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – ARISTEIA 624 (TREMEPOL project).

European Union
European Social Fund
OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co-financed by Greece and the European Union