Identification of halogenated disinfection byproducts of nonylphenol in chlorinated wastewater effluent using novel high resolution GC/Q-TOF

Christiane Hoppe-Jones, Shawn Beitel, Sofia Nieto, Nathan Eno, Shane Snyder

Overview

- Background
- Experimental design
- Linear 4-nonylphenol – model compound
- Technical mixture
- Bioassay analysis
  - P53
  - ER

Disinfection By-Product Formation

organic DBP precursor ←---- natural organic matter (NOM)
algal organic matter (AOM)
wastewater effluent organic matter (EfOM)

+

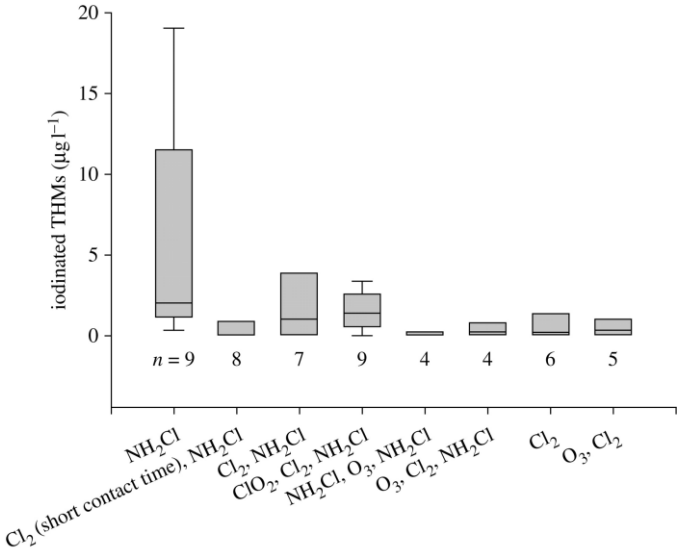
inorganic DBP precursor ←---- bromide (Br^-)
iodide (I^-)
nitrite (NO_2^-)

+

disinfectant ←---- chlorine (Cl_2)
chloramines (NH_2Cl)
chlorine dioxide (ClO_2)
ozone (O_3)
ultraviolet (UV)

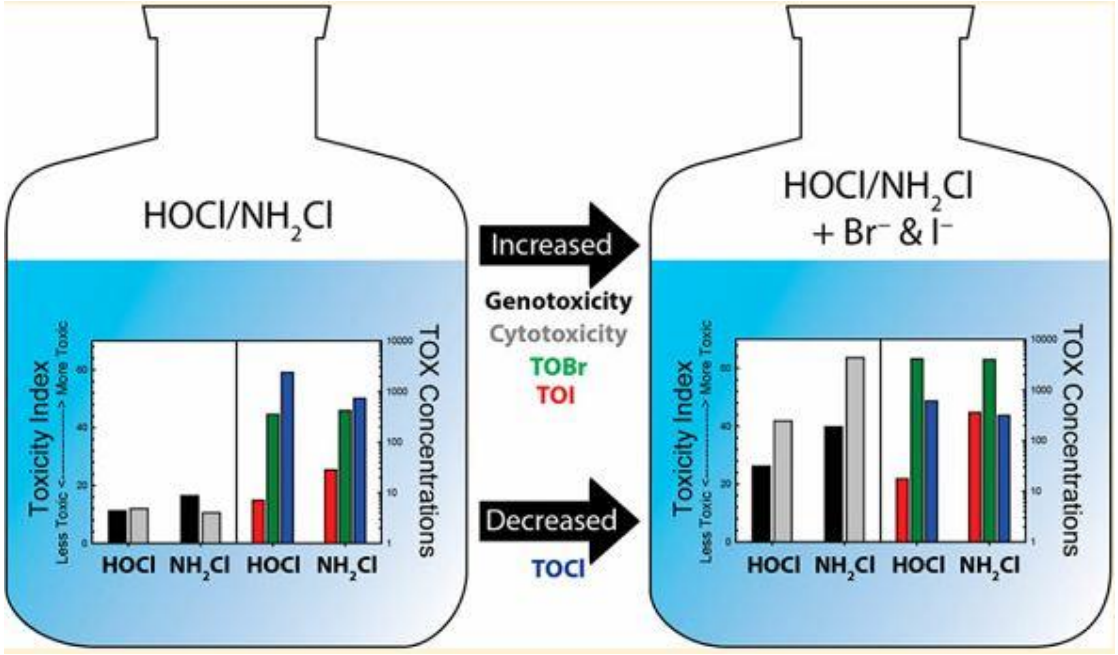
↓

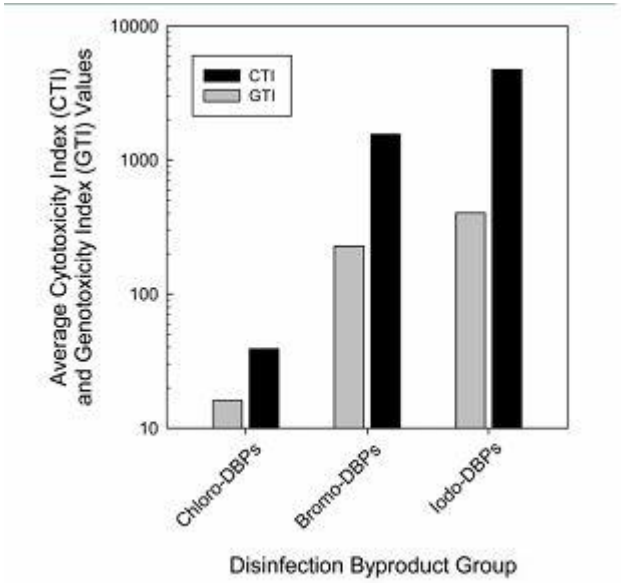
regulated and emerging DBPs ←---- trihalomethanes (THMs): CHX_3 (X = Cl, Br, I)
haloacetic acids (HAAs): $CH_iX_{3-i}COOH$ (i = 0–2)
haloacetaldehydes: $CH_iX_{3-i}CHO$
halonitromethanes (HNMs): $CH_iX_{3-i}NO_2$
nitrosamines: $R–\underset{\displaystyle R}{\underset{|}{N}}–N=O$



"The formation and control of emerging disinfection by-products of health concern" – Krasner, S.W. 2009, Phil. Trans. R. Soc. A 367: 4077-4095.

Toxicity of brominated and iodinated DBPs



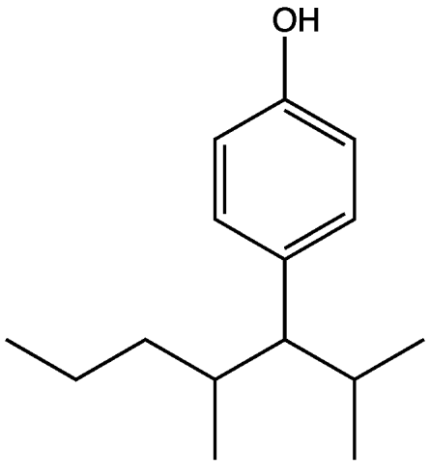


Cytotoxicity/Genotoxicity:
I-DBPs > Br-DBPs > Cl-DBPs

"Toxic Impact of Bromide and Iodide on Drinking Water Disinfected with Chlorine or Chloramines" – Yang, Y. et al. 2014, ES&T 48:12362-12369.

Why take a closer look and Nonylphenol?

- Used in antioxidants, lubricating oils, detergents, emulsifiers
- Used in the production of nonylphenol ethoxylates (detergents)
- Technical mixture of many isomers
- Known to have estrogenic activity
- On the EPA Drinking Water CCL4 Draft List



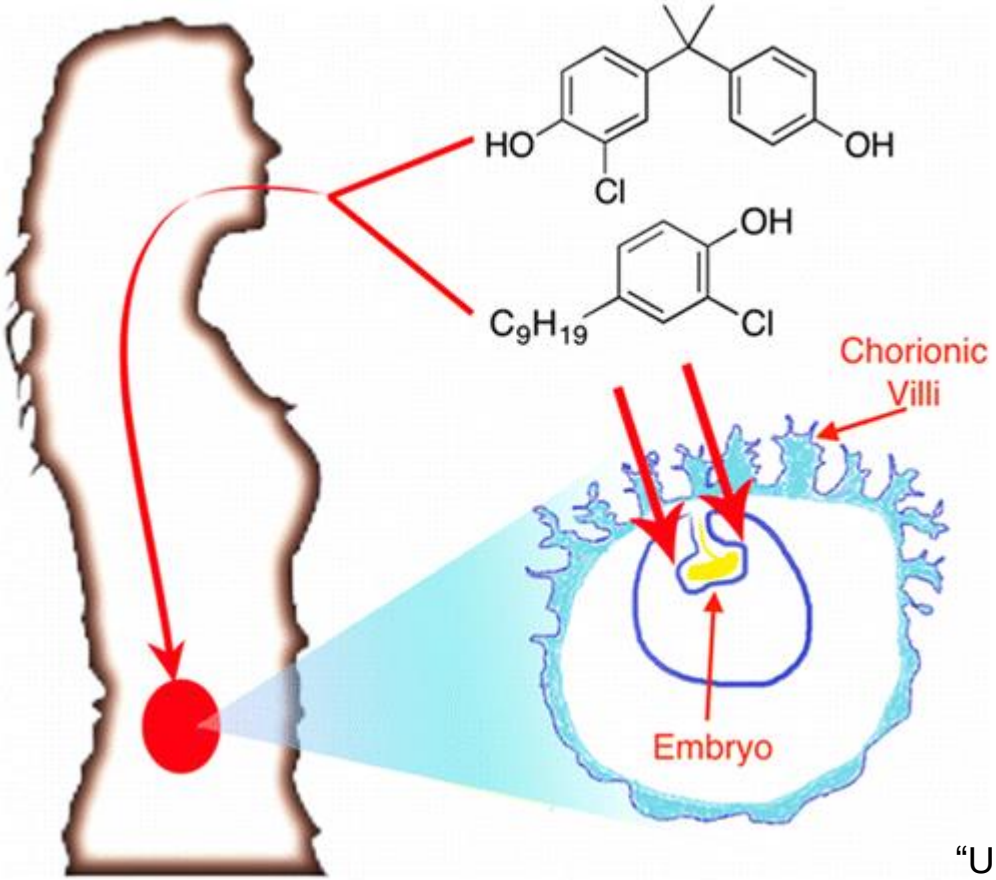
1999 *Environ. Sci. Tech. 33(16) 2814-2829*

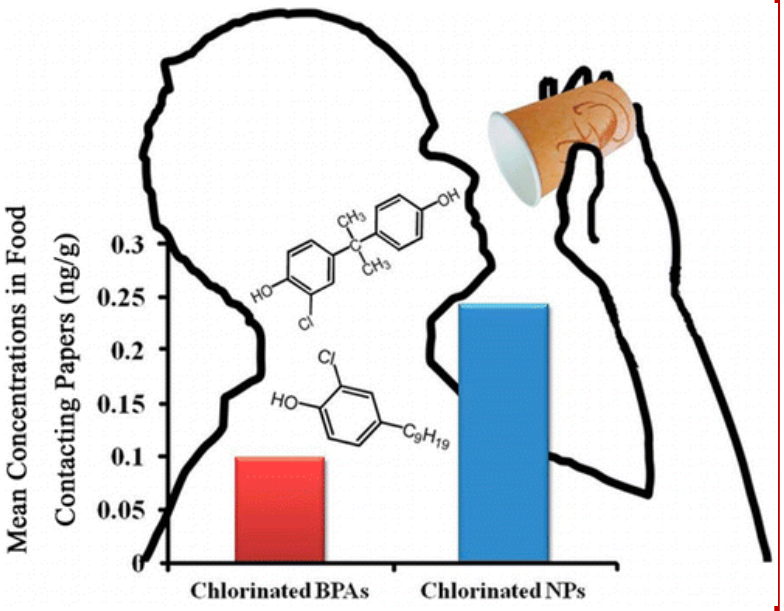
Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures

SHANE A. SNYDER,*,† TIMOTHY L. KEITH,† DAVID A. VERBRUGGE,† ERIN M. SNYDER,† TIMOTHY S. GROSS,‡ KURUNTHACHALAM KANNAN,† AND JOHN P. GIESY†

can operate through a number of both direct and indirect mechanisms of action, of particular concern are those compounds that mimic endogenous estrogens. The Safe Drinking Water Act Amendments of 1995 (Bill No. S.1316) and the Food Quality Protection Act of 1996 (Bill No. P.L. 104-170), which mandate comprehensive screening for estrogenic and anti-estrogenic chemicals, are examples of the increasing public concern regarding endocrine disruption. While it is known that many natural and synthetic chemicals are estrogenic, it is unclear whether the concentrations of estrogenic agents present in the environment are sufficient to cause adverse physiological effects. One aspect

Why take a closer look and Nonylphenol?



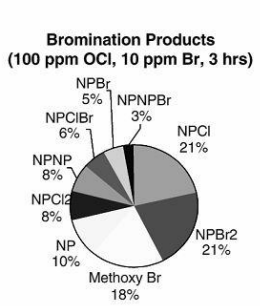


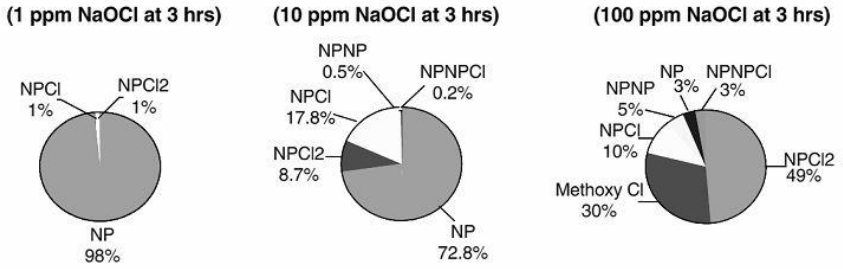
"Occurrence and Maternal Transfer of Chlorinated Bisphenol A and Nonylphenol in Pregnant Women and Their Matching Embryos" –
Chen, M. et al. 2016, ES&T 50(2):970-977.

"Ubiquitous Occurrence of Chlorinated Byproducts of Bisphenol A and Nonylphenol in Bleached Food Contacting Papers and Their Implications for Human Exposure" –
Zhou, Y. et al. 2015, ES&T 49(12):7218-7226.

Objectives

- Confirmation of halogenated by-products reported in the literature
- Formation of brominated and iodinated by-products
- Identify preferred pathway for three different oxidants:
  - Chlorine
  - Monochloramine
  - Chlorine dioxide
- Test Toxicity and Estrogenicity



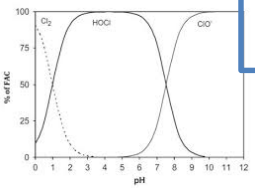


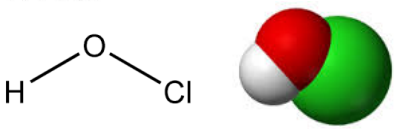
"Accurate-mass identification of chlorinated and brominated products of 4-nonylphenol, nonylphenol dimers, and other endocrine disrupters" – Thurman, M. 2006, J. Mass Spectrom. 41:1287-1297.

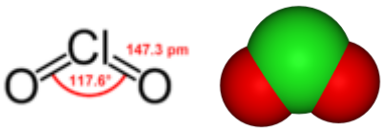
Nonylphenol Oxidation

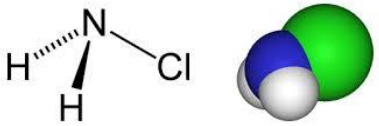
0.04M Phosphate buffer,
1ppm Nonylphenol
6.3uM Bromide, 6.3uM Iodide

4ppm Chlorine	4ppm Chlorine Dioxide	4ppm Monochloramine
10min	10min	10min
30min	30min	30min
1h	1h	1h
2h	2h	2h
5h	5h	5h









Sample Preparation and Analysis

Samples

- **LLE w/ Hexane**
  - GC-QTOF
  - GC-ICP-MS
- **SPE**
  - LC-QTOF
  - LC-QQQ
  - LC-ICP-MS
  - GC-QTOF
  - Bioassay
- **Direct injection**
  - IC-ICP-MS

Volatiles and semi-volatiles unknowns

Agilent 7200 GC-Q-TOF

Polar unknowns

Agilent 6540 LC-Q-TOF

Halogenated organics

Agilent 7900 ICP-MS

Oxahalides (BrO_3^-; IO_3^-) and Ions (Cl^-, Br^-, I^-)

Metrohm 850 IC+ Agilent 7900 ICP-MS

Halogenated volatiles and semi-volatiles

Agilent 7900 ICP-MS

Targeted analysis of identified DBPs

Agilent 6490 LC-MS/MS

Mutagenicity
Genotoxicity
p53 induction
Cytotoxicity
Oxidative stress
Estrogenic effects
Glucocorticoid effects …

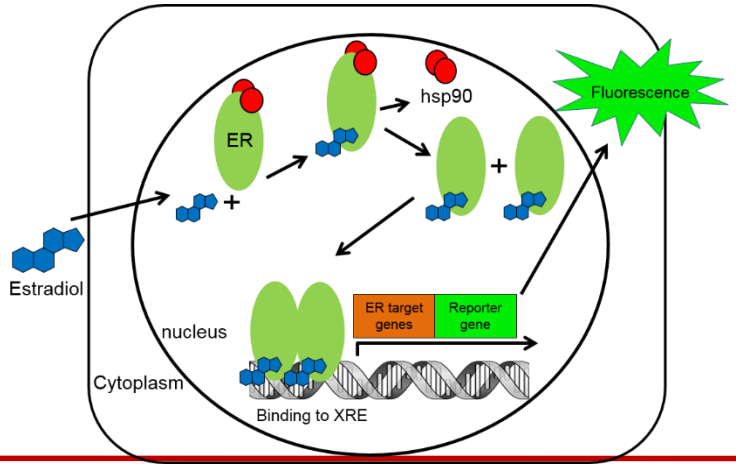
Bioassays

Sample Preparation and Analysis

- Quenching with Na Thiosulfate
- SPE extraction (HLB)
- Elution (MTBE, MeOH)
- Analysis on GC-qTOF
  - DB-5MS UI (30m x 0.25mm x 0.25um)
  - Scan m/z 50-1000

Agilent 7200 GC-Q-TOF

- Aliquot transferred to DMSO
- Analysis in bioasssays



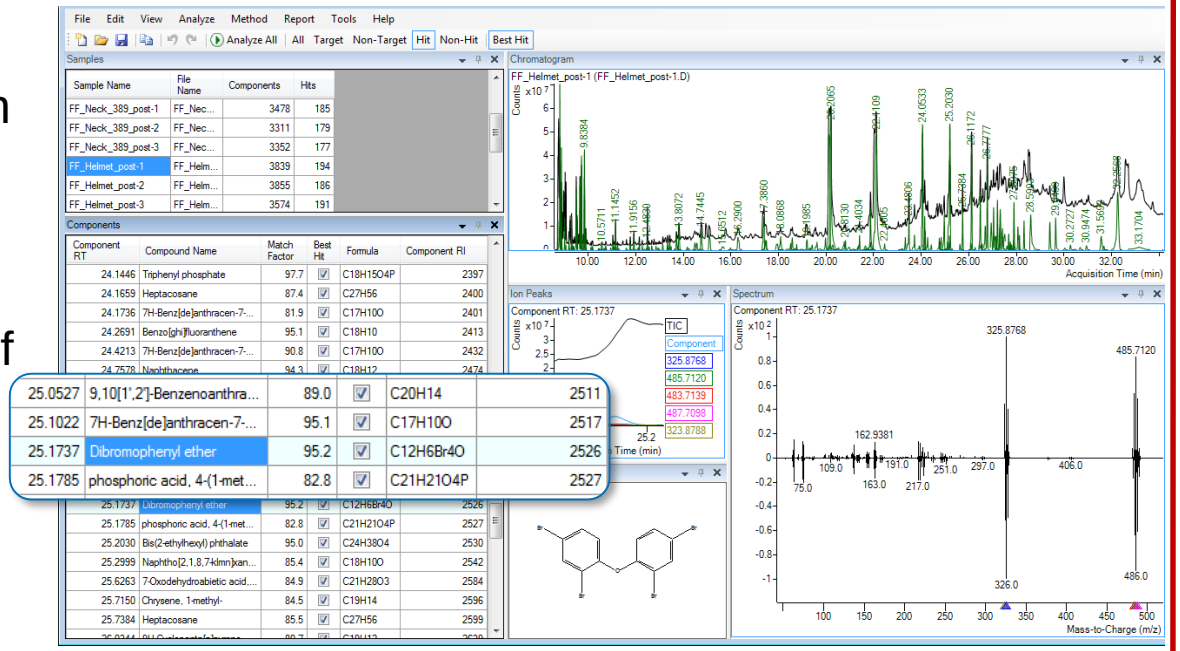
GC Analysis

Column	DB-5MS, 30 m, 0.25 mm, 0.25 µm
Injection volume	1 µL
Injection mode	10:1
Split/Splitless inlet temperature	280 °C
Oven temperature program	50 °C for 3 min 10 °C/min to 300 °C, 7 min hold
Carrier gas	Helium at 1.5 mL/min, constant flow
Transfer line temperature	300 °C
Ionization mode	Standard EI at 70 eV; low energy EI at 15 eV and 12 eV
Source temperature, 70eV/15 eV or less	240°C/200°C
Quadrupole temperature	150°C
Mass range	50 to 1200 m/z
Spectral acquisition rate	5 Hz

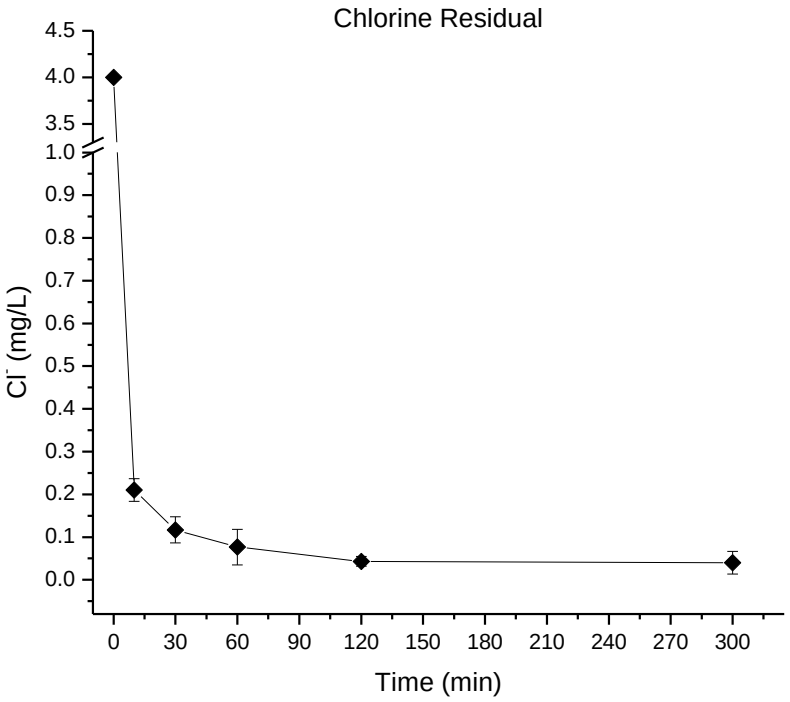
Agilent 7200 GC-Q-TOF

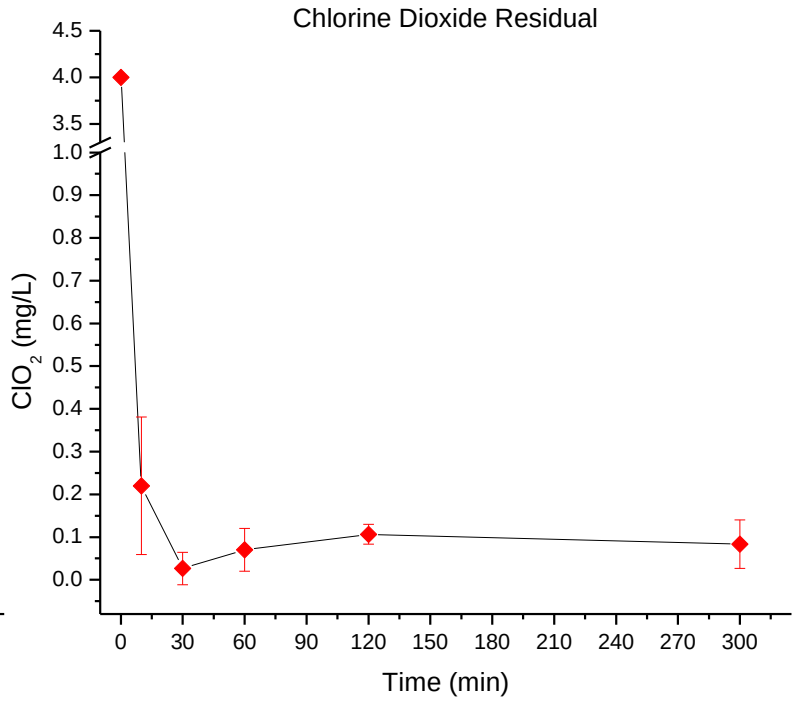
Data Analysis

- Data processed using SureMass in Unknowns Analysis B.08.00
- Compound identification using NIST14 EI library, confirmation by retention index (RI)
- Molecular ions of unknown brominated compounds were identified with the help of low electron energy
- Molecular ions were confirmed by evaluating the complete cluster for m/z, relative isotope abundance and isotope ratios using Molecular Formula Generator (MFG)



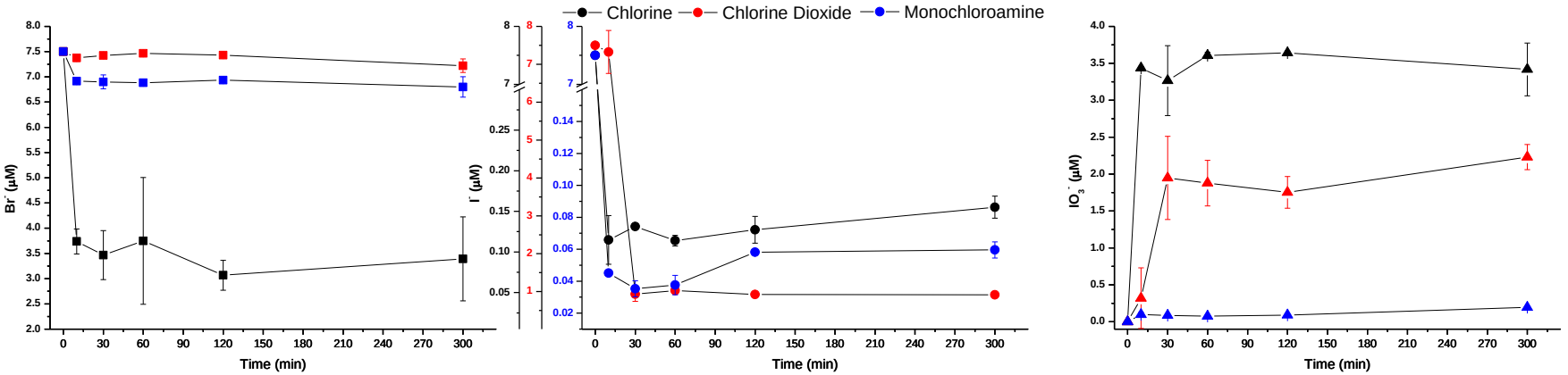
Chlorine/Chlorine Dioxide Residual



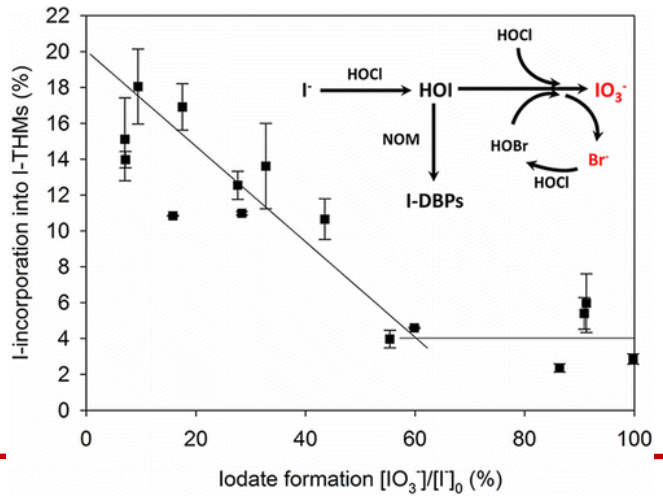


Bromide, Iodide and Iodate Results

Chlorine Chlorine Dioxide Monochloramine

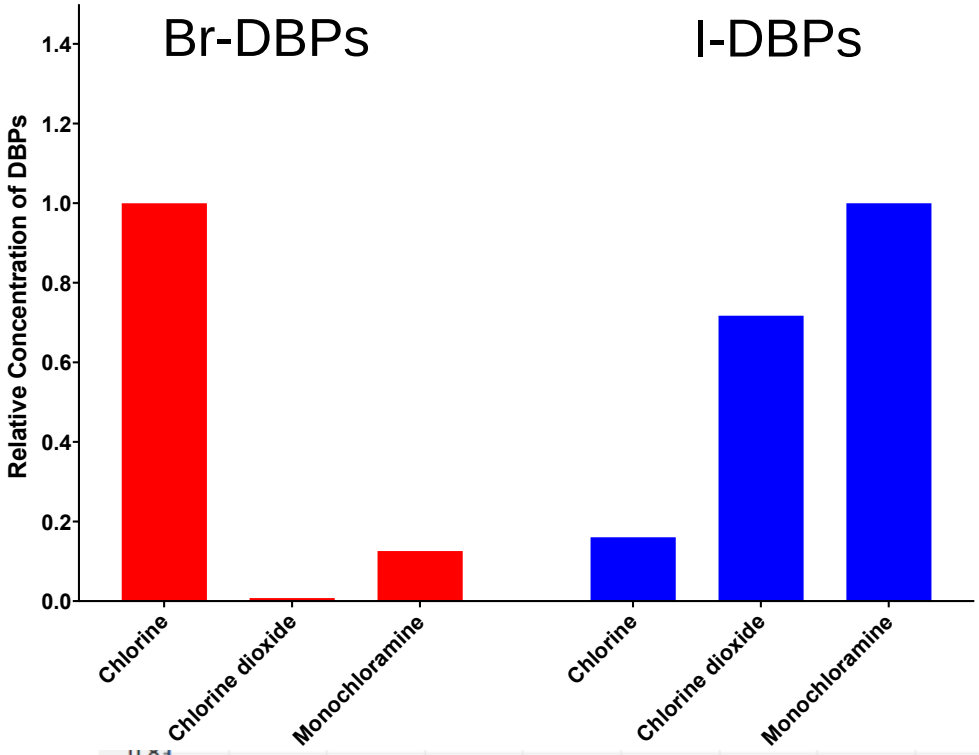


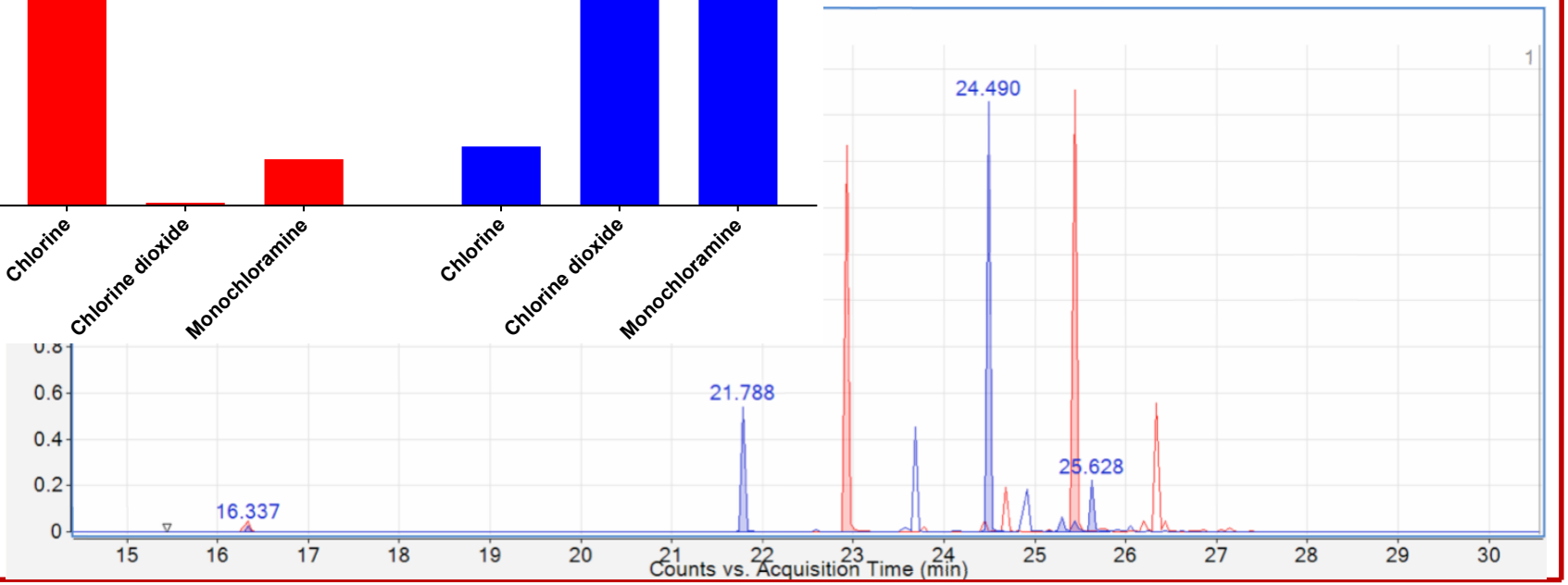
Br^- I^- IO_3^-



"Iodate and Iodo-Trihalomethane Formation during Chlorination of Iodide-Containing Waters: Role of Bromide" – Criquet, J. et al. 2012, *Environmental Science & Technology* 46, 7350-7357.

GC-ICP-MS

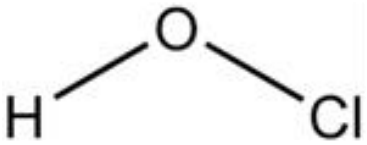

Br-DBPs
I-DBPs
Relative Concentration of DBPs
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Chlorine
Chlorine dioxide
Monochloramine
Chlorine
Chlorine dioxide
Monochloramine

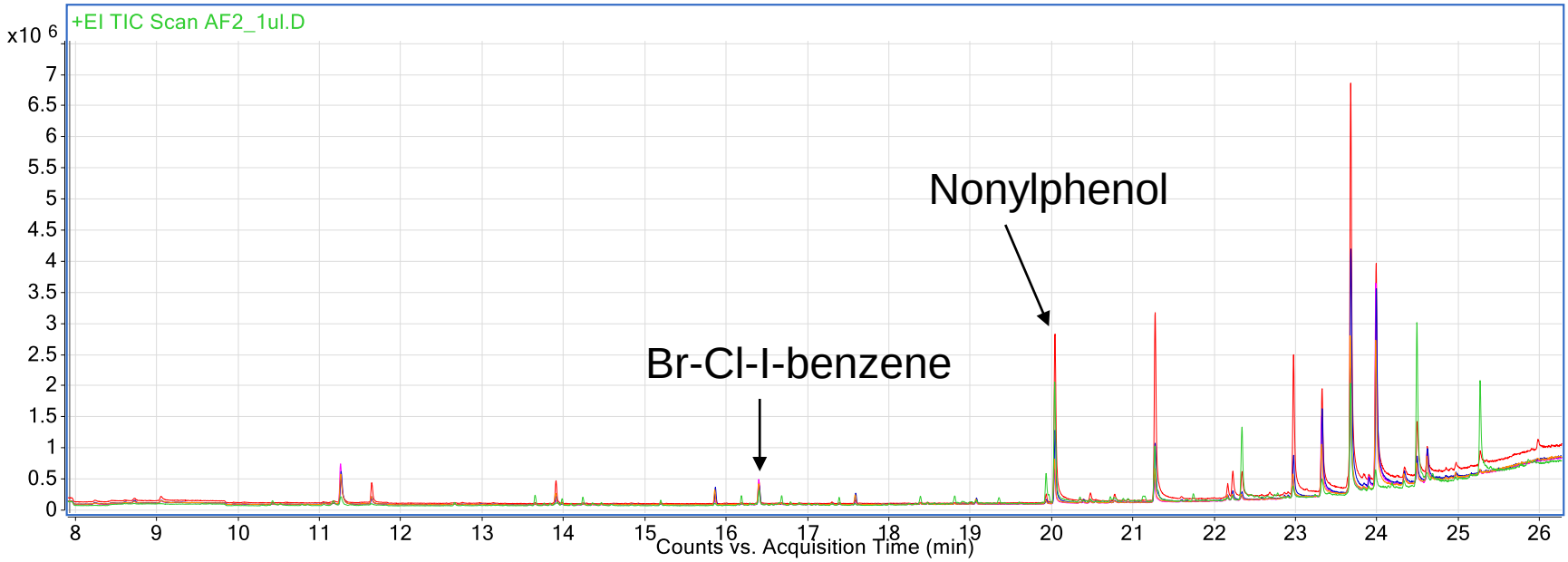

1
24.490
21.788
25.628
16.337
0.6
0.4
0.2
0
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Counts vs. Acquisition Time (min)

Oxidation with chlorine

DBP formation after oxidation with chlorine:

- 10min
- 30min
- 1h
- 2h
- 5h

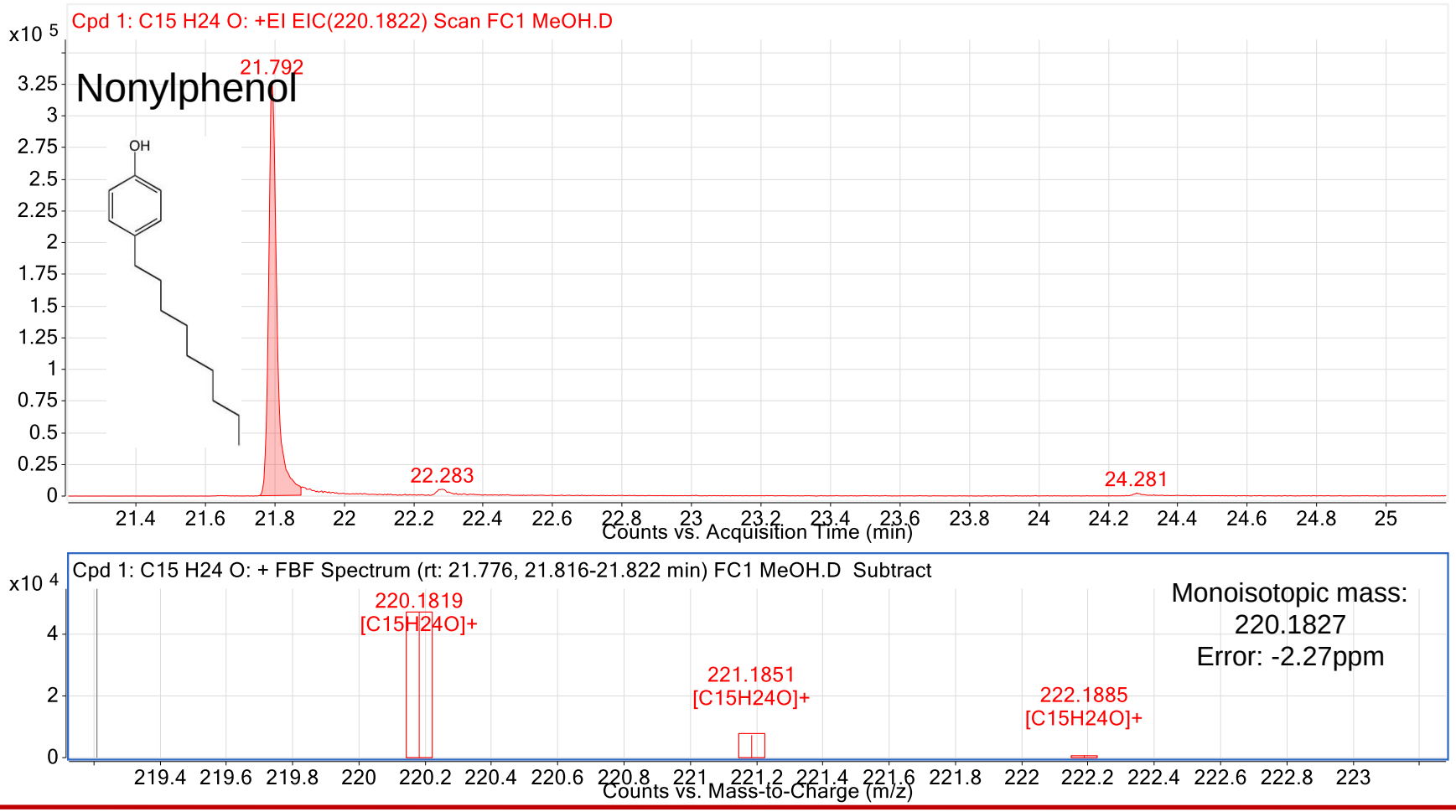




Oxidation with Chlorine

Monohalogenated DBPs:

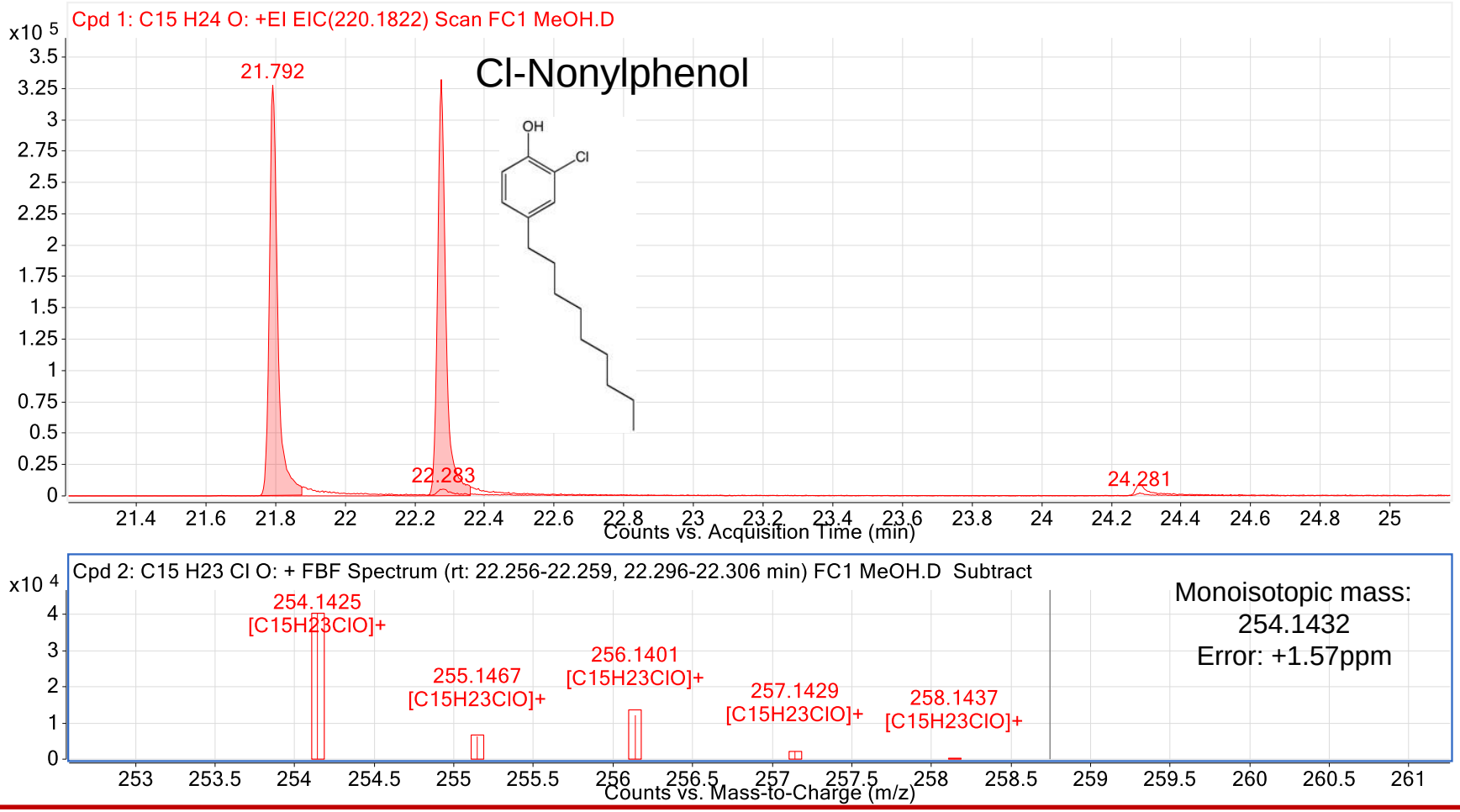
NP+Cl_2



Oxidation with Chlorine

Monohalogenated DBPs:

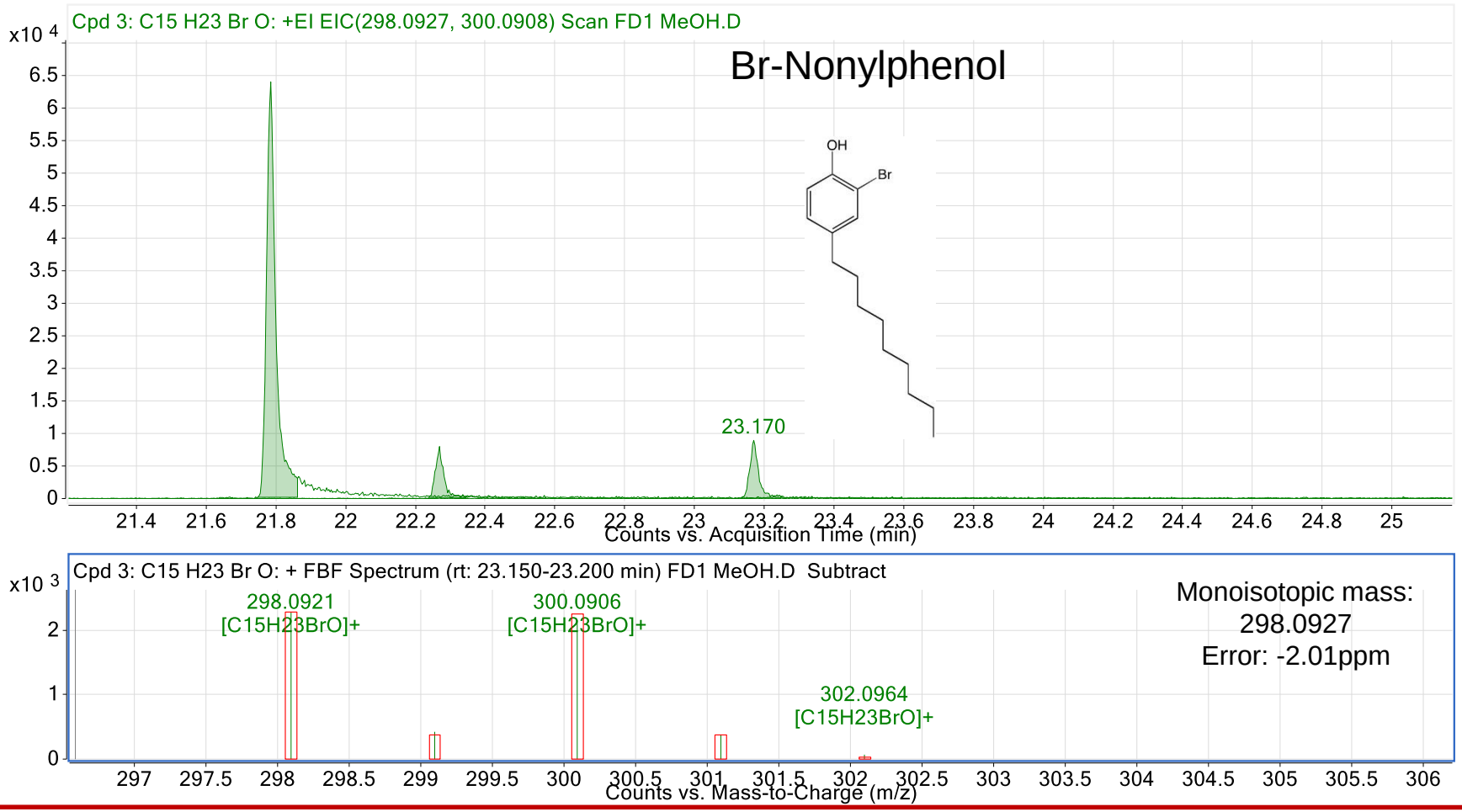
NP+Cl_2



Oxidation with Chlorine

Monohalogenated DBPs:

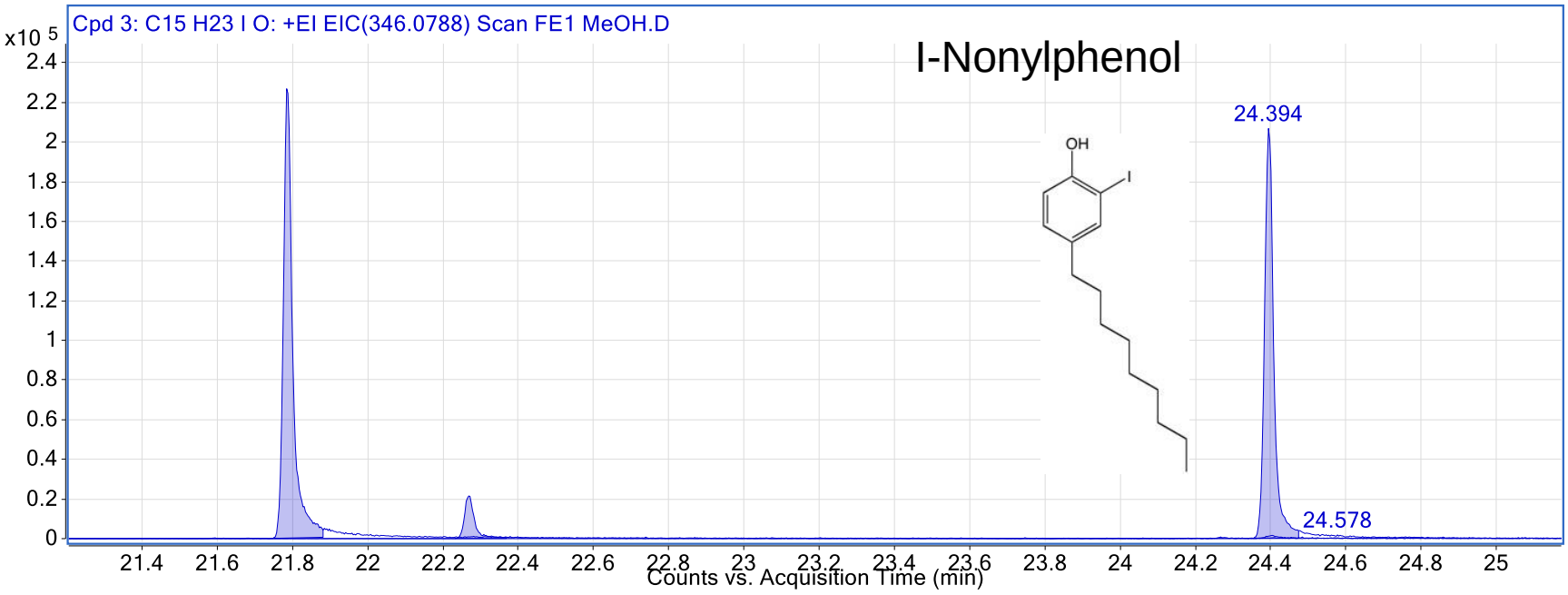
NP+Cl_2+Br^-



Oxidation with Chlorine

Monohalogenated DBPs:

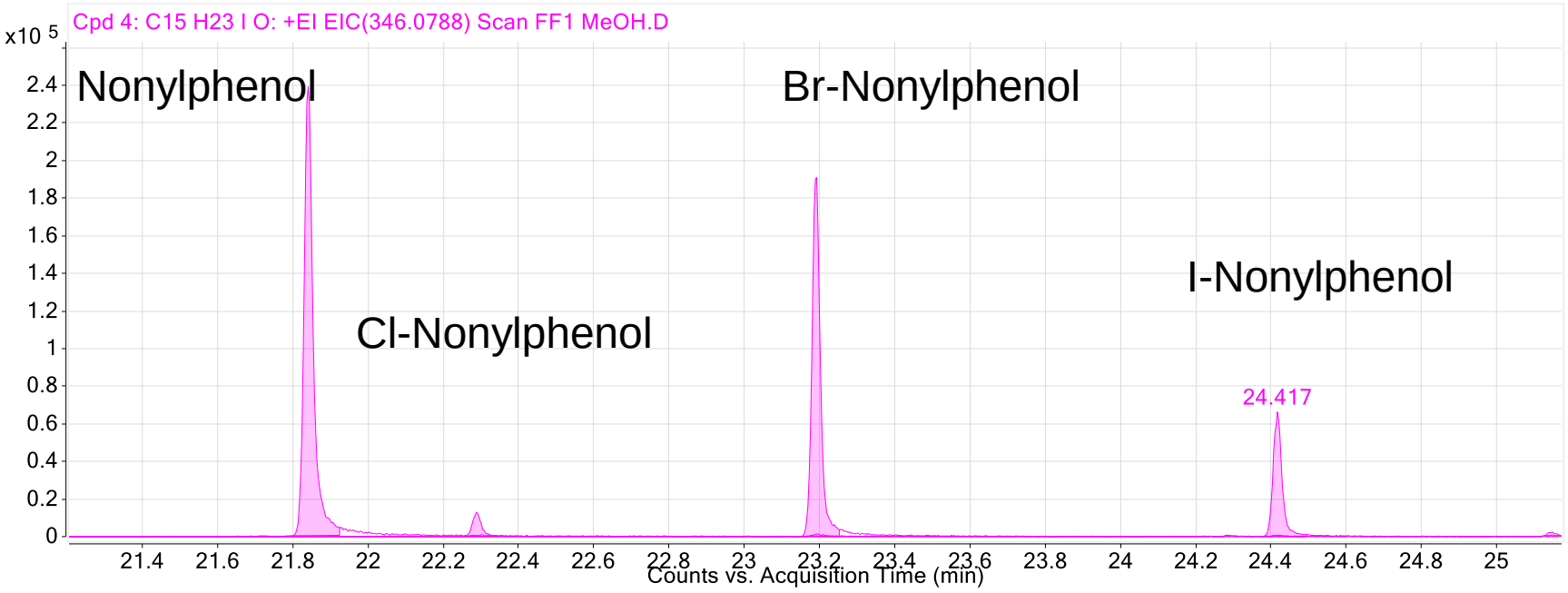
NP+Cl_2+I^-



Oxidation with Chlorine

Monohalogenated DBPs:

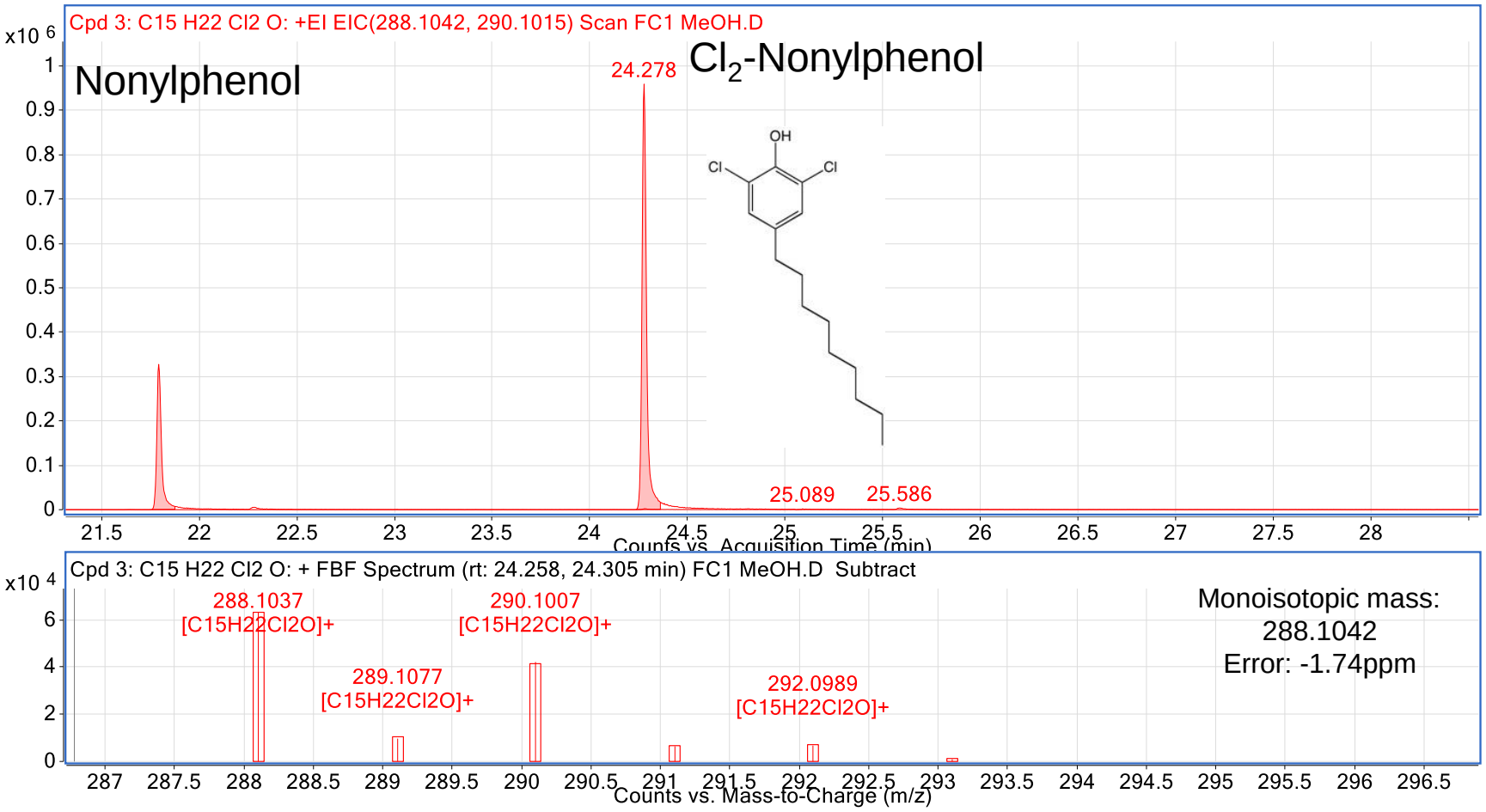
$NP+Cl_2+Br^-+I^-$



Oxidation with Chlorine

Dihalogenated DBPs:

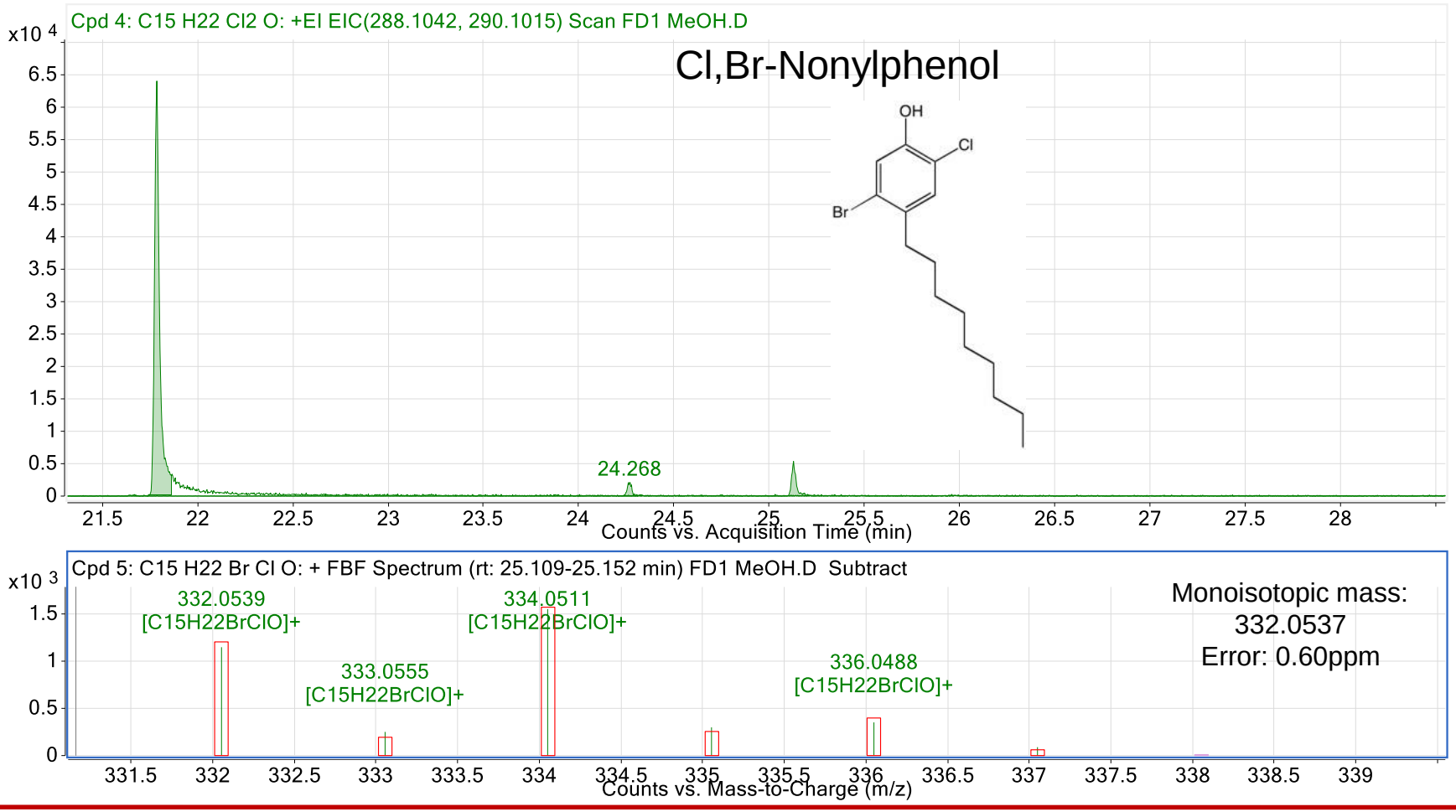
NP+Cl_2



Oxidation with Chlorine

Dihalogenated DBPs:

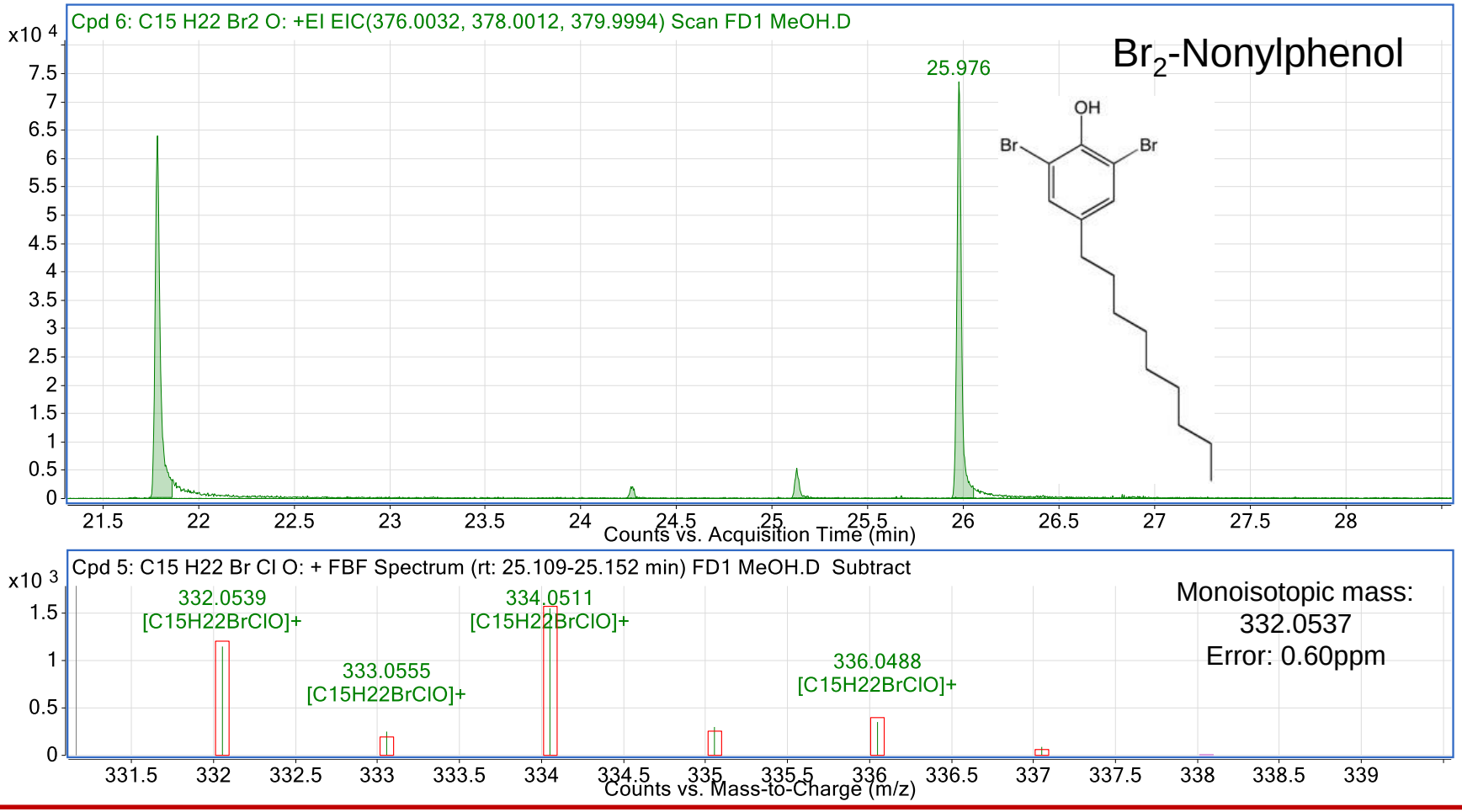
NP+Cl_2+Br^-



Oxidation with Chlorine

Dihalogenated DBPs:

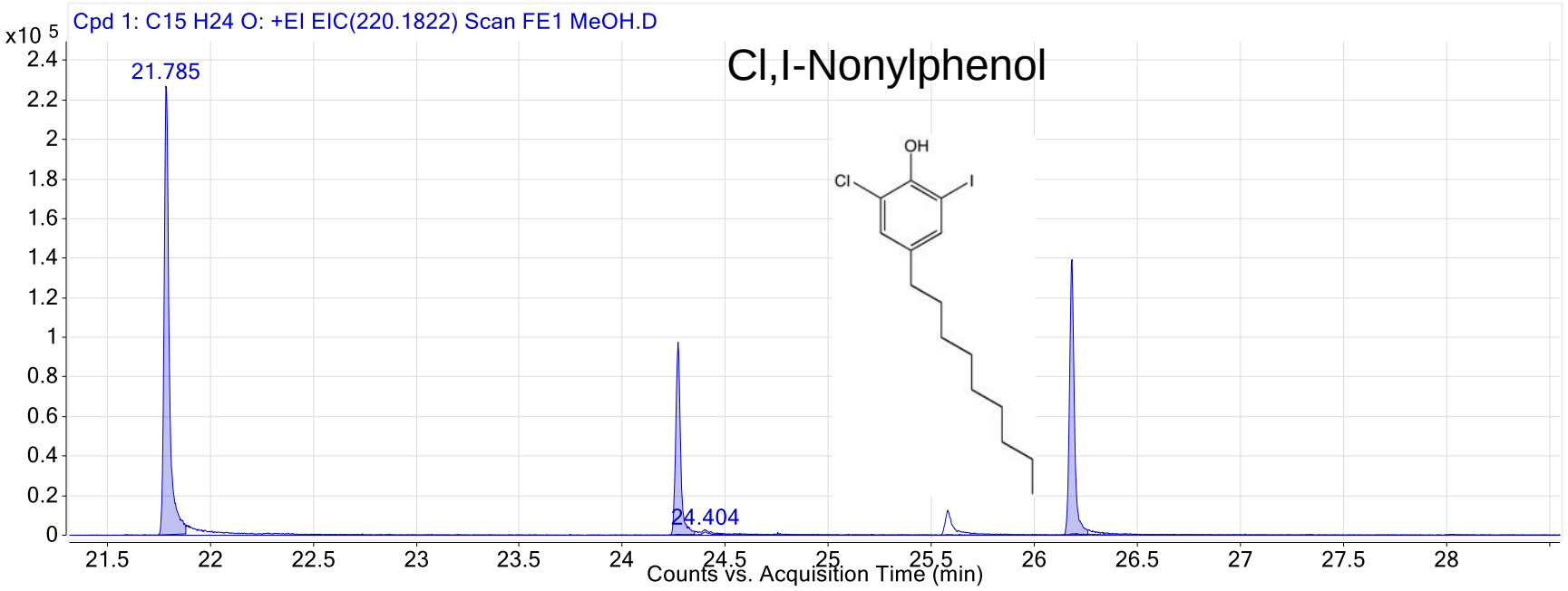
NP+Cl_2+Br^-



Oxidation with Chlorine

Dihalogenated DBPs:

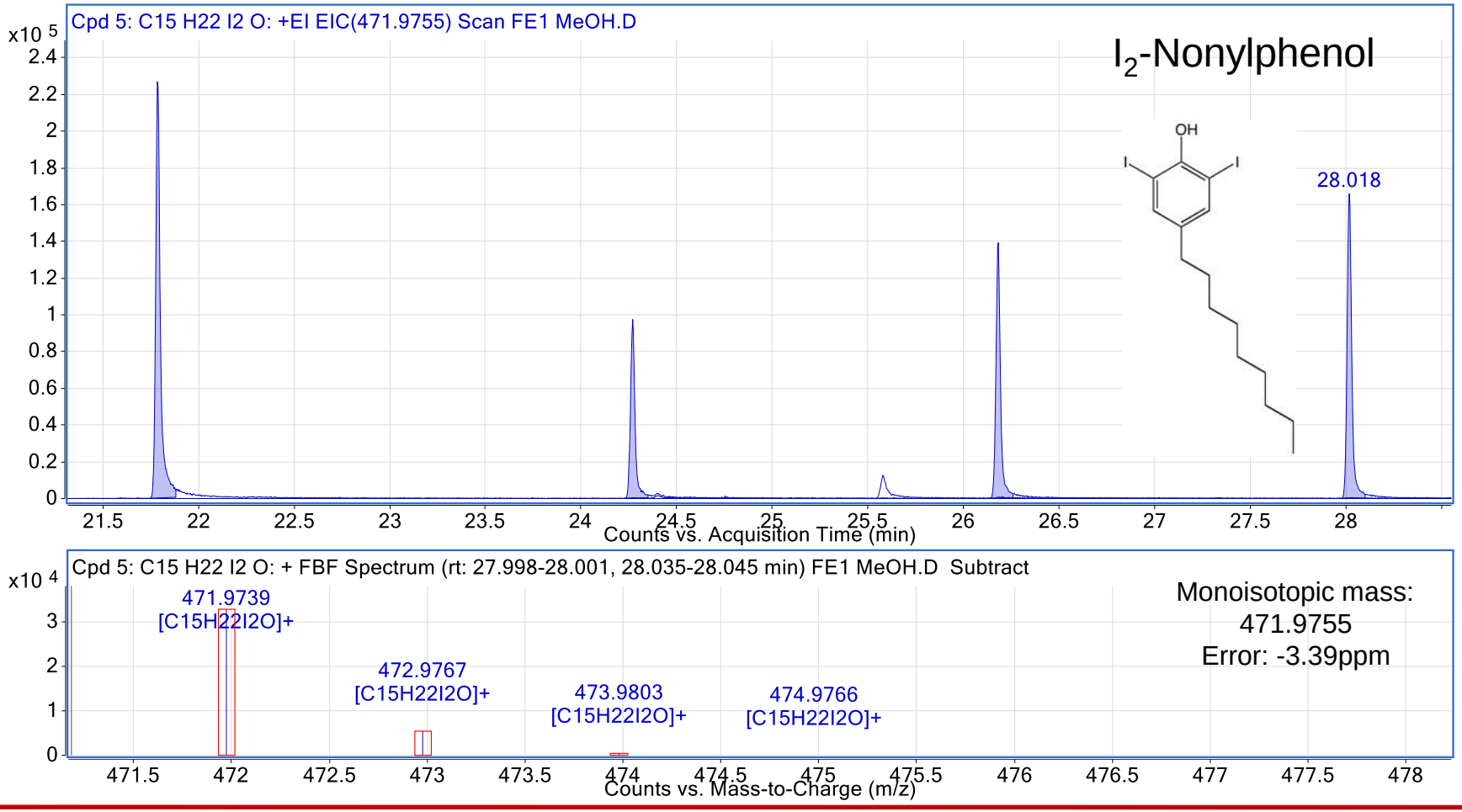
NP+Cl_2+I^-



Oxidation with Chlorine

Dihalogenated DBPs:

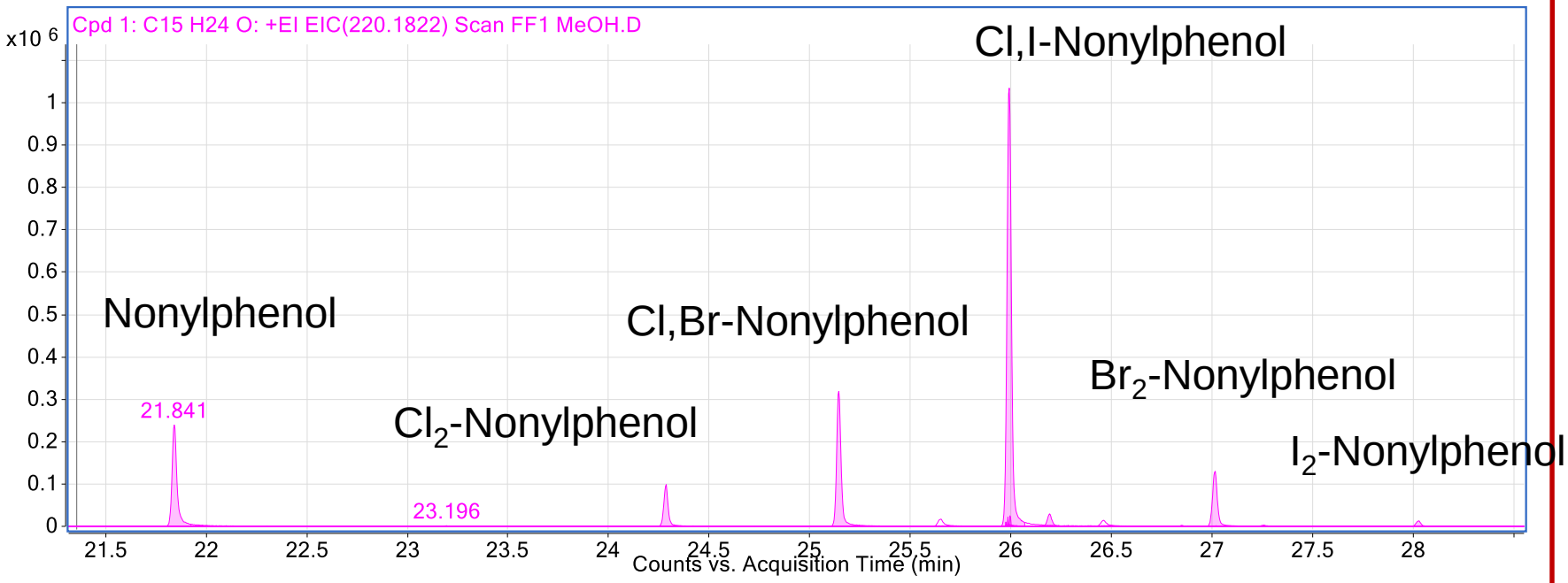
NP+Cl_2+I^-



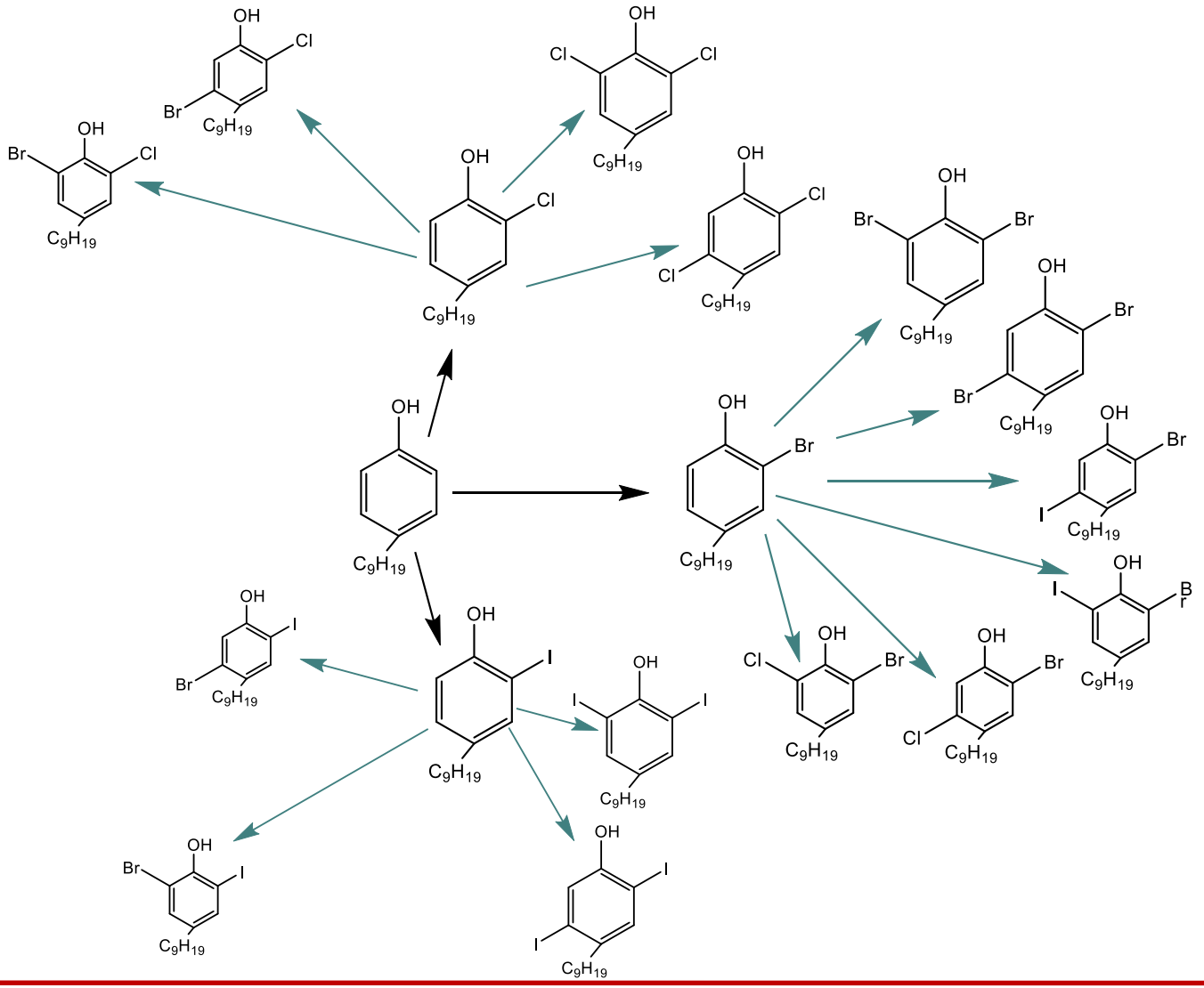
Oxidation with Chlorine

Dihalogenated DBPs:

NP+Cl_2+Br^-+I^-



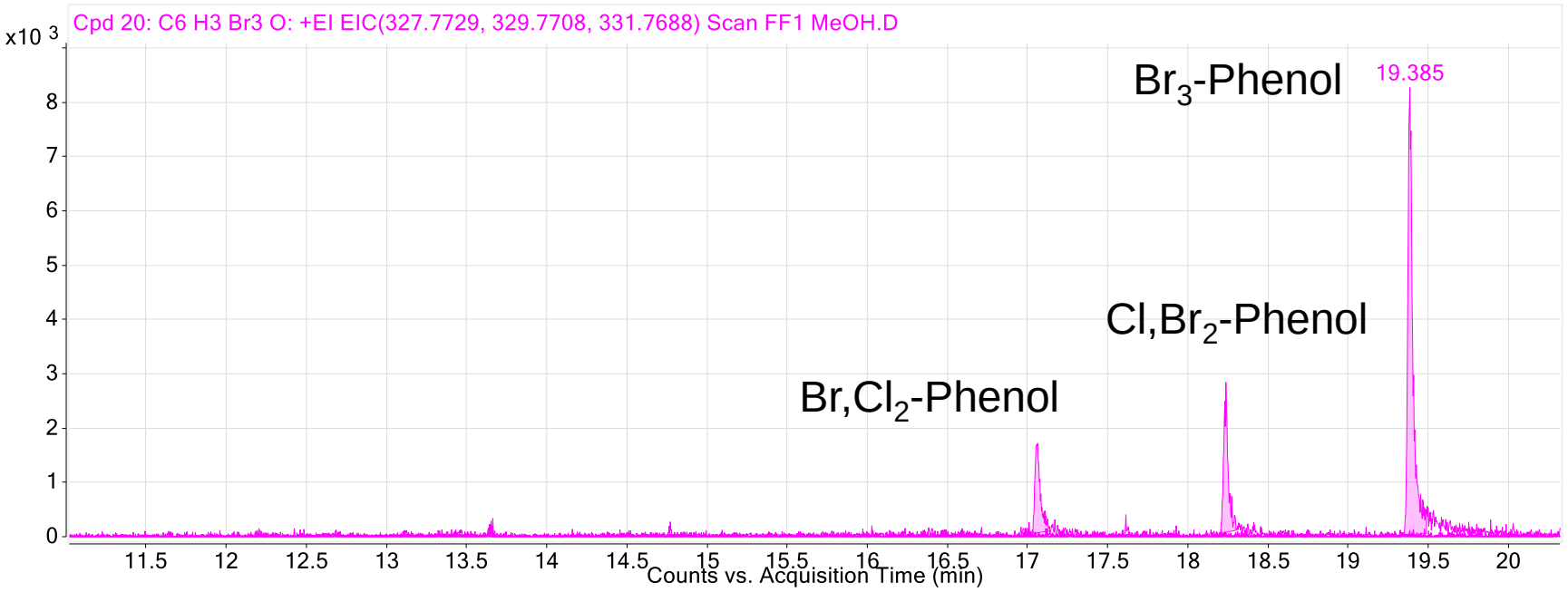
Identified DBPs after Chlorination



Oxidation with Chlorine

Halogenated Phenol DBPs:

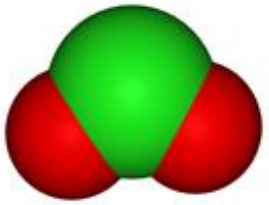
NP+Cl_2+Br^-+I^-

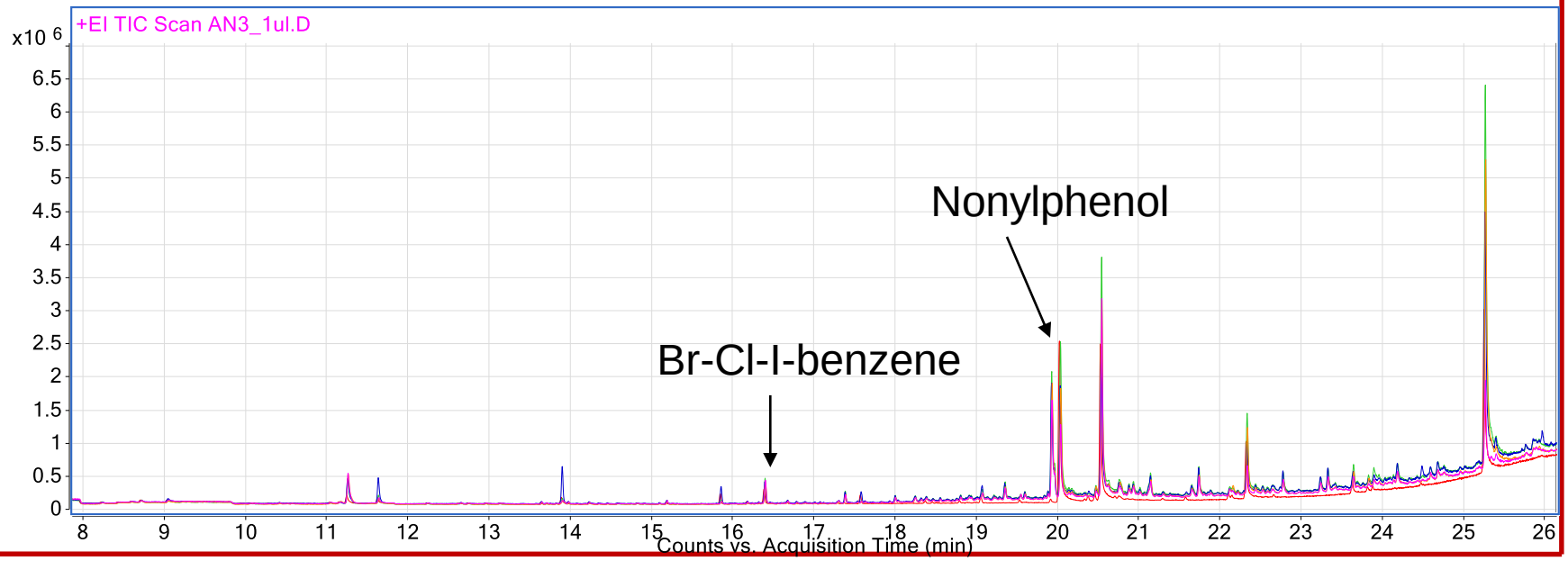


Oxidation with Chlorine Dioxide

DBP formation after oxidation with chlorine dioxide:

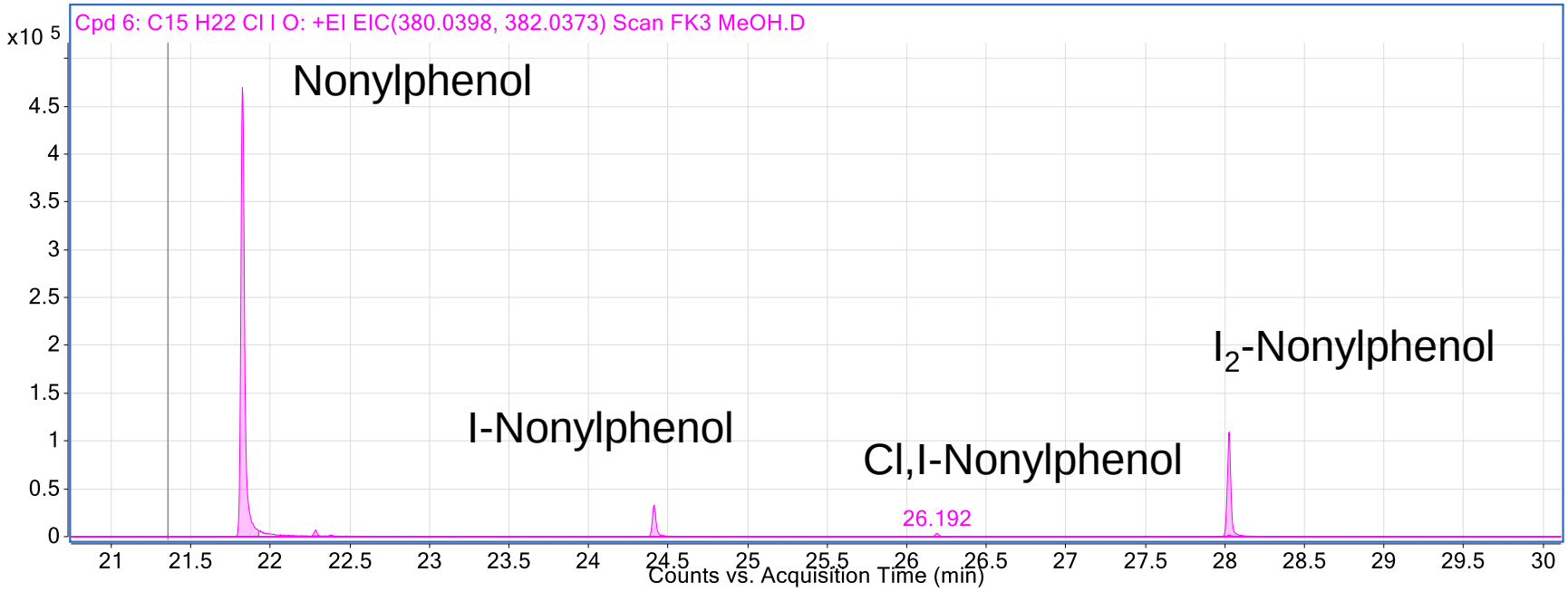
- 10min
- 30min
- 1h
- 2h
- 5h



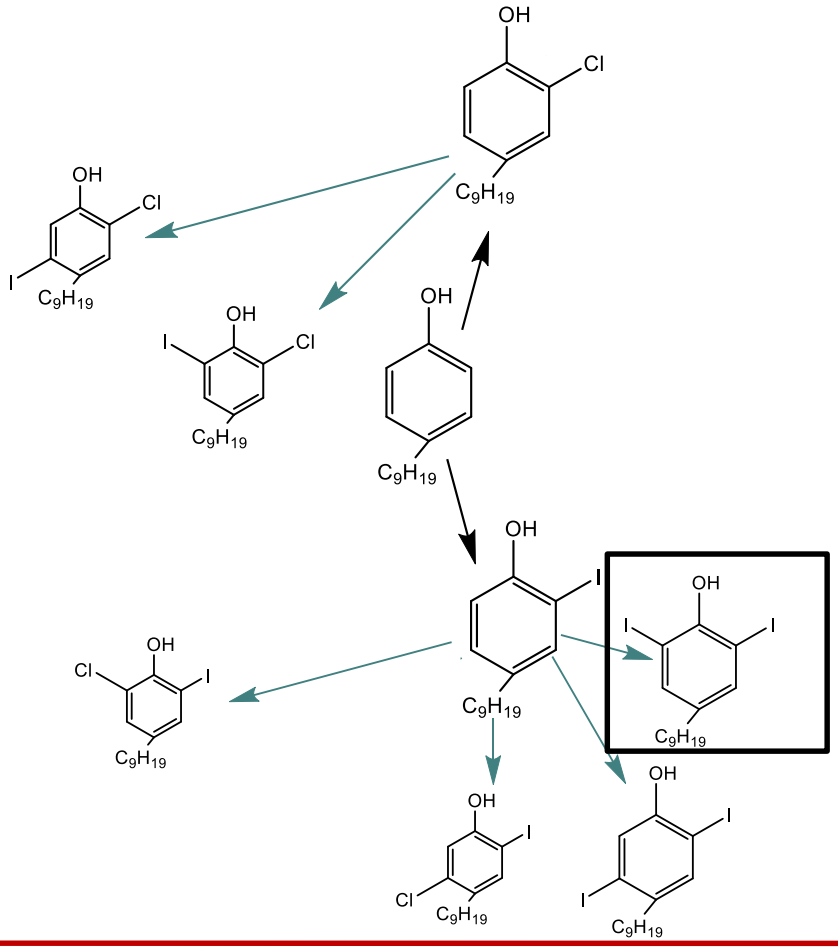


Oxidation with Chlorine Dioxide

$NP+Cl_2+Br^-+I^-$



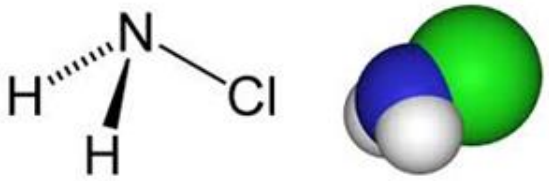
Identified DBPs after Oxidation with Chlorine Dioxide

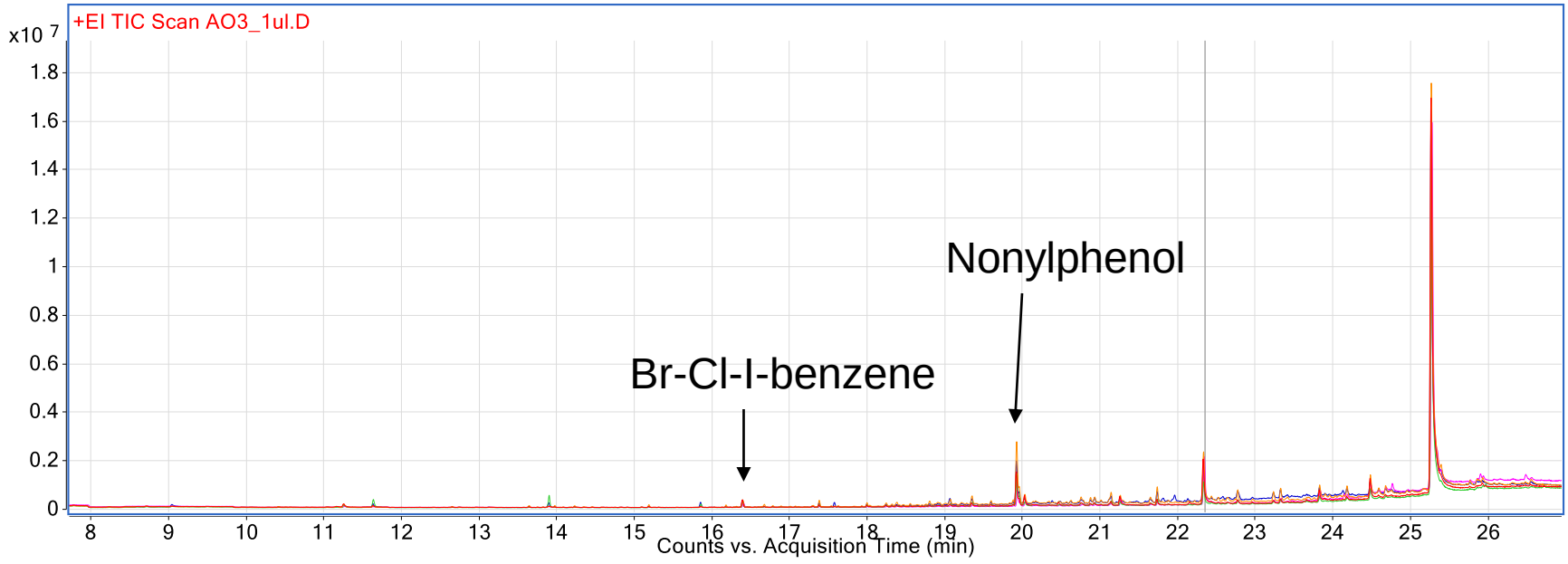


Oxidation with Monochloramine

DBP formation after oxidation with monochloramine:

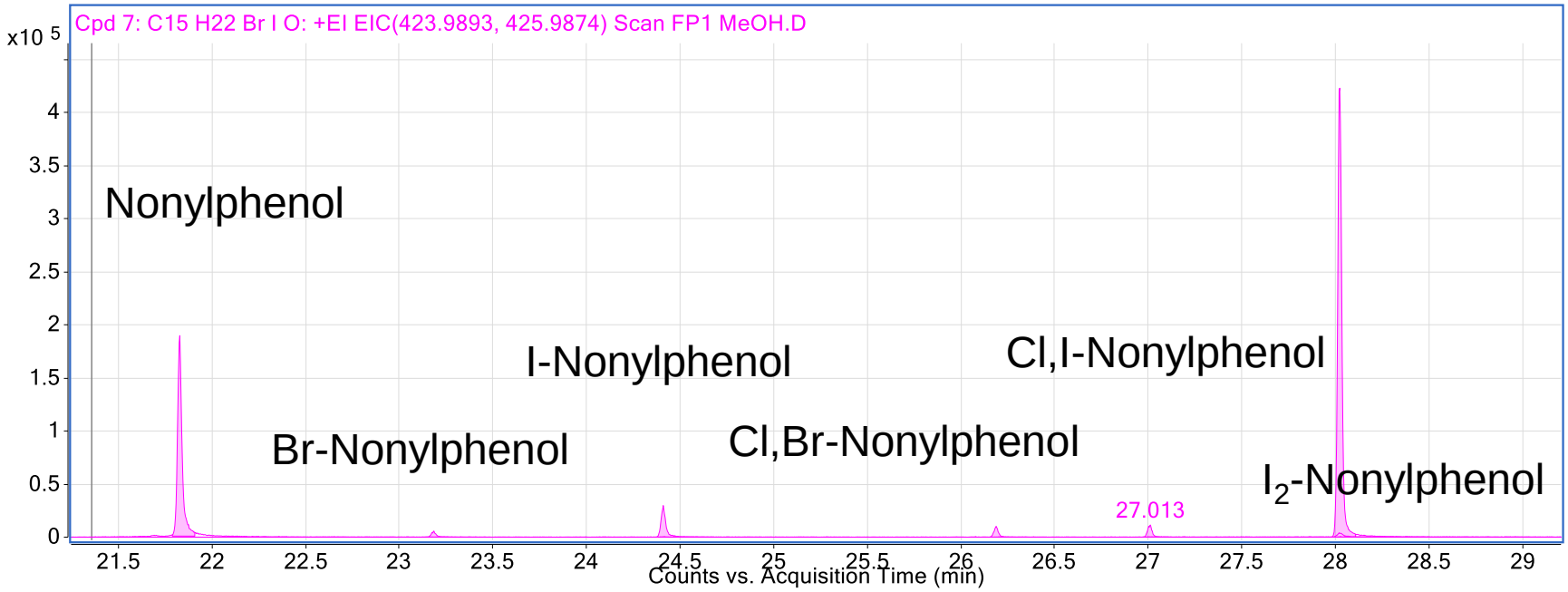
- 10min
- 30min
- 1h
- 2h
- 5h



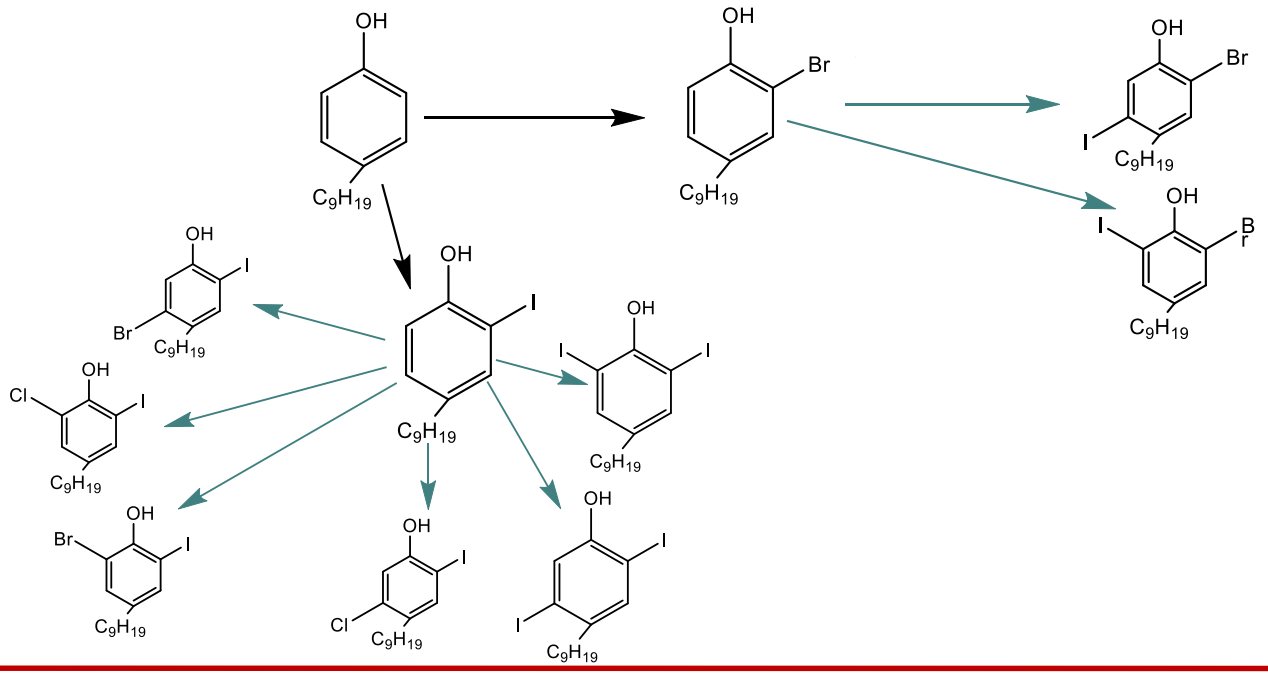


Oxidation with Monochloramine

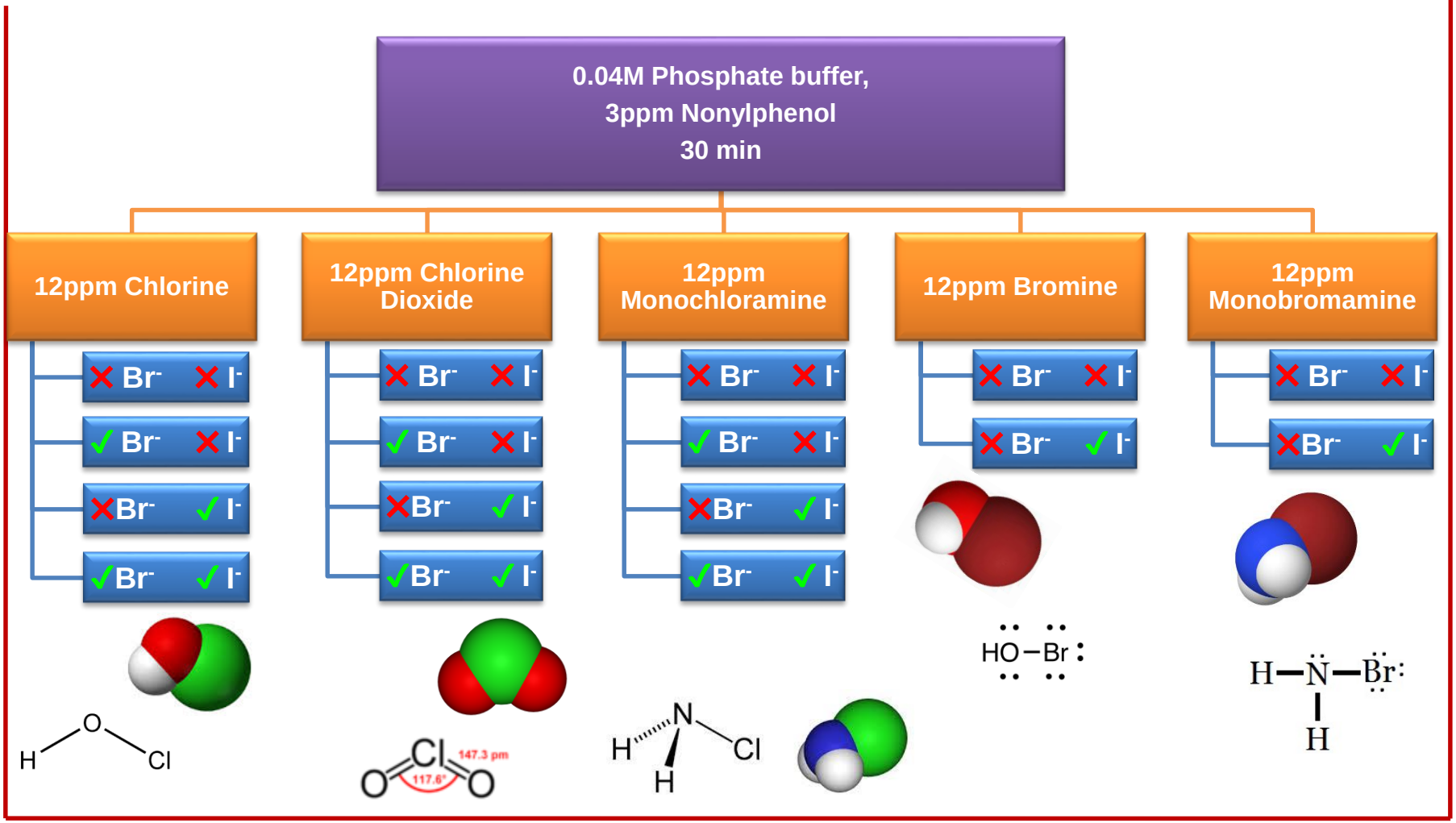
NP+NH_2Cl+Br^-+I^-



Identified DBPs after Monochloramine

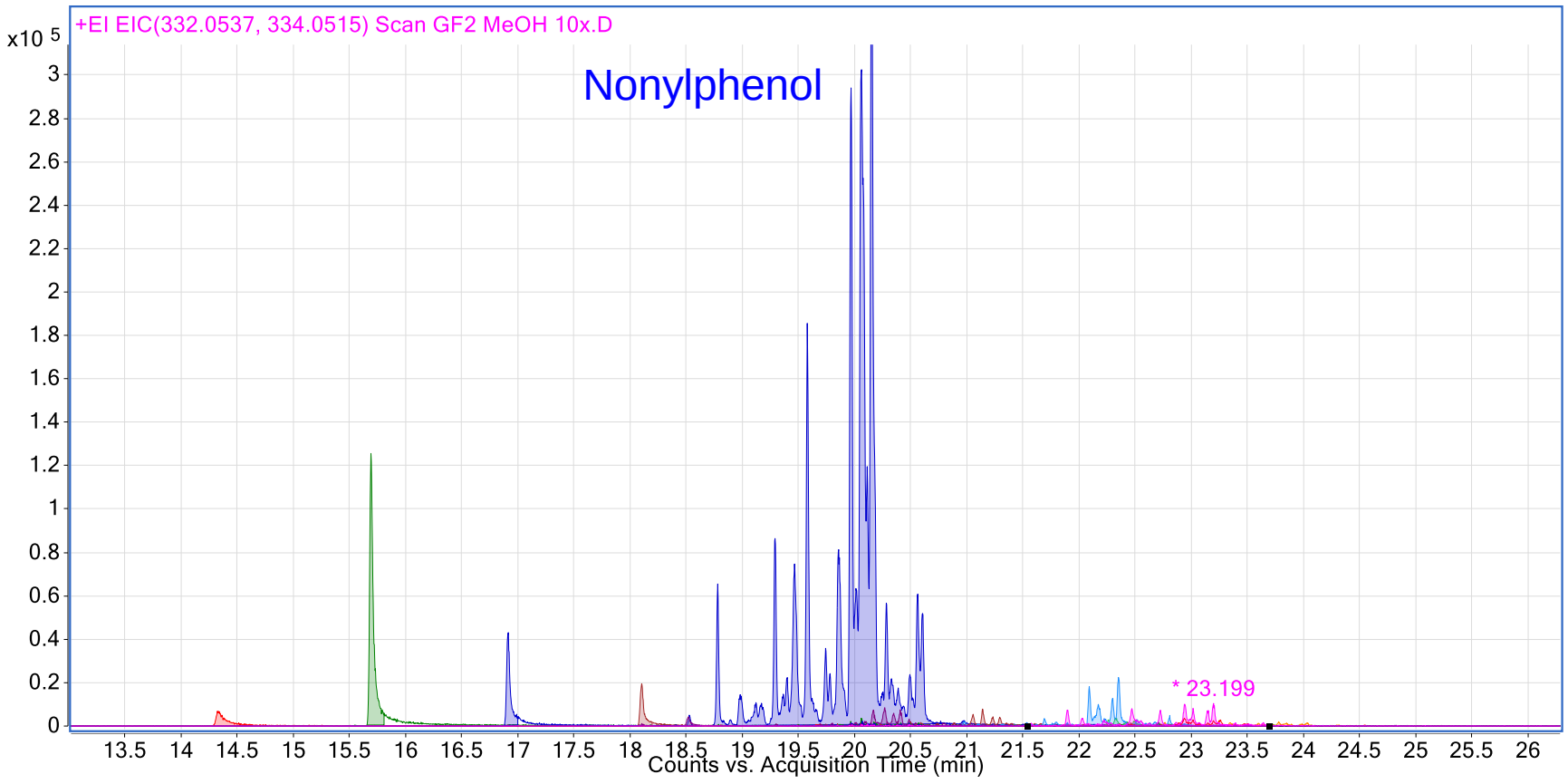


Nonylphenol (technical mixture)

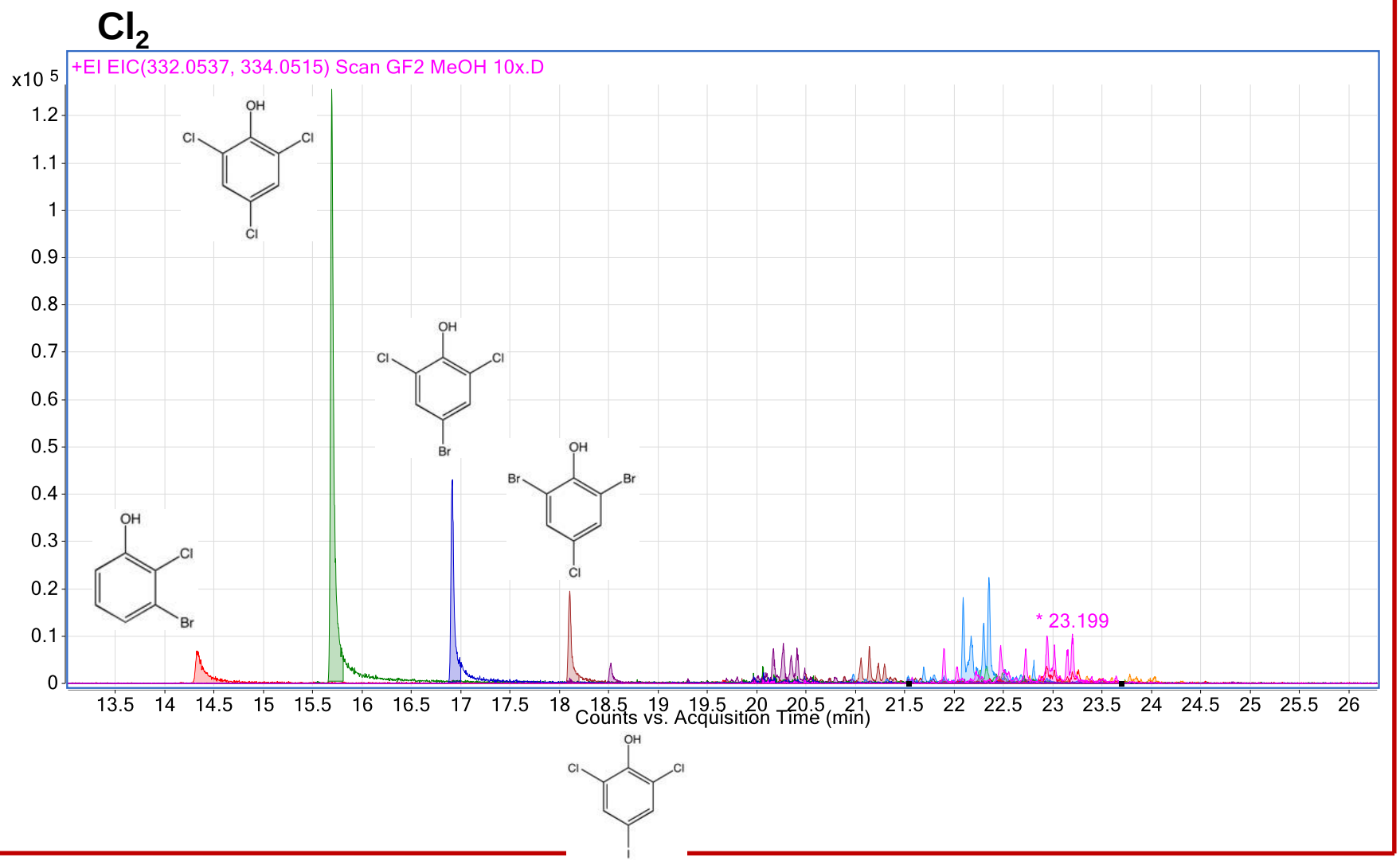


Nonylphenol – technical mixture

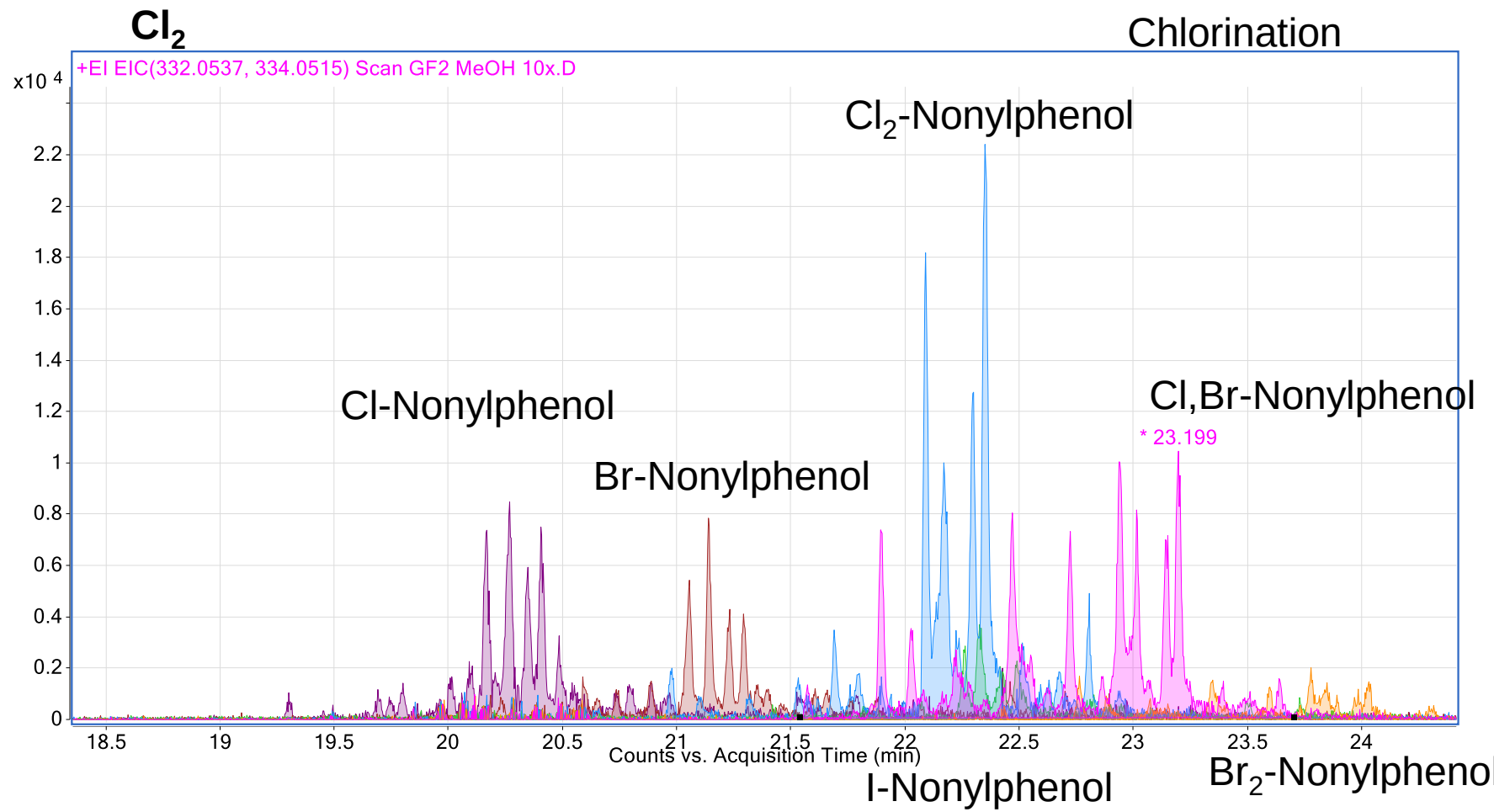
Cl_2



Nonylphenol – technical mixture

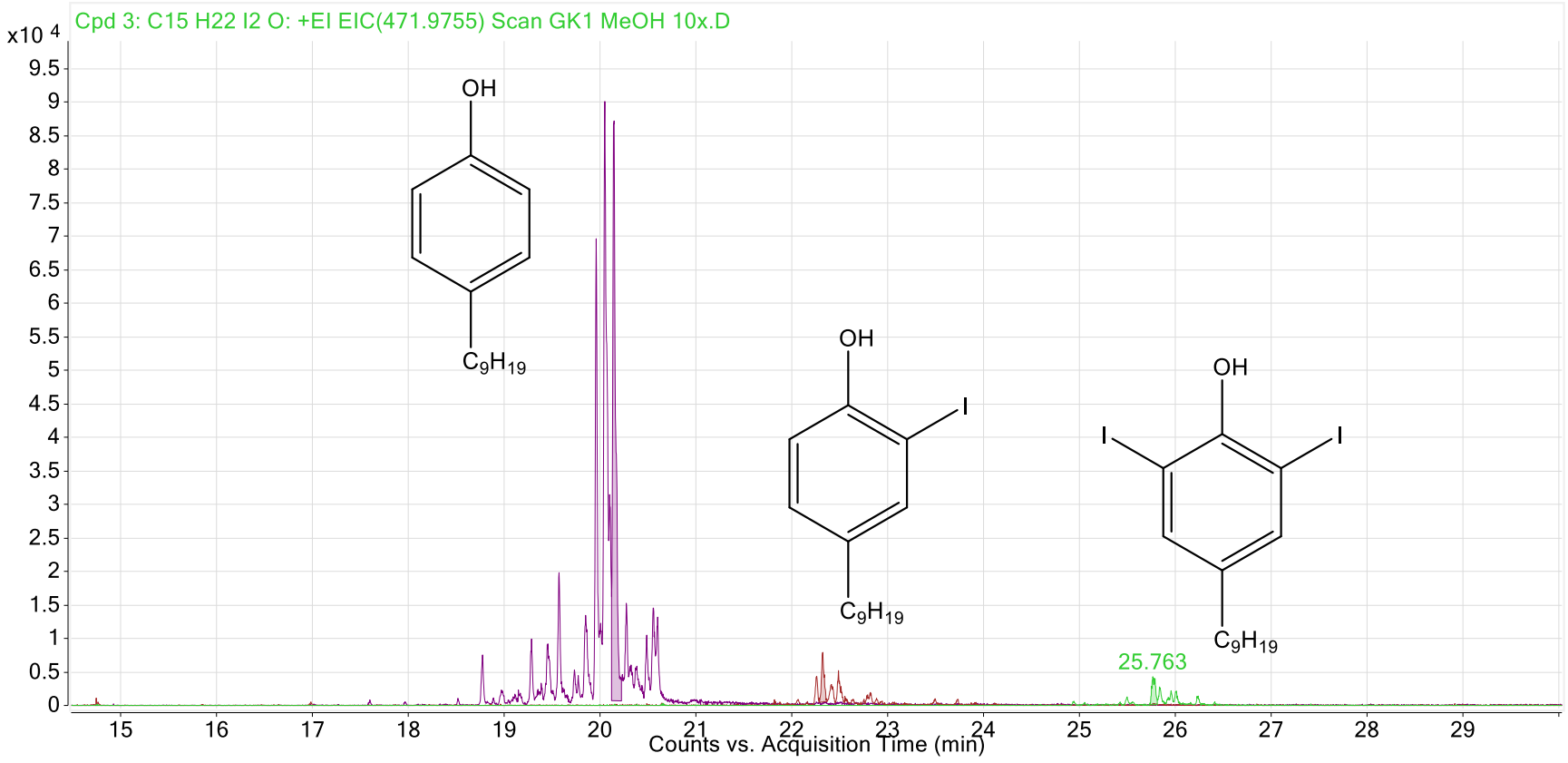


Nonylphenol – technical mixture



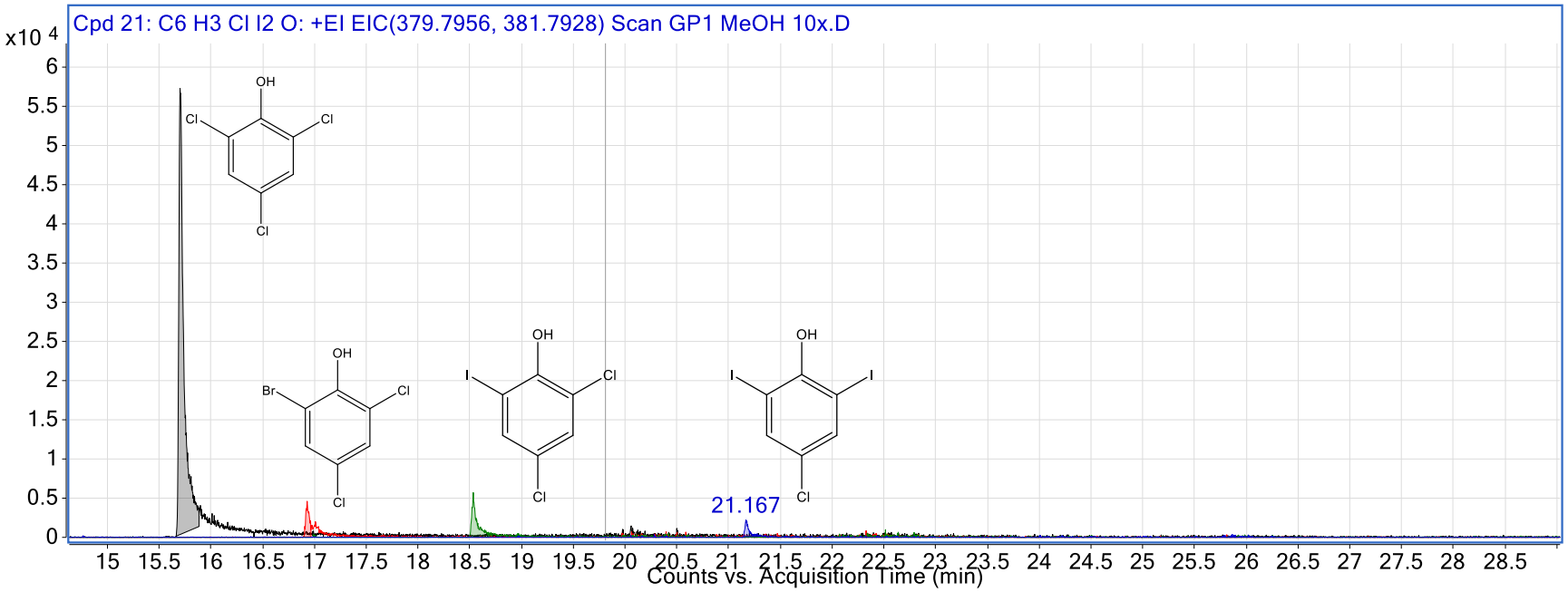
Nonylphenol – technical mixture

$ClO_2 + Br^- + I^-$



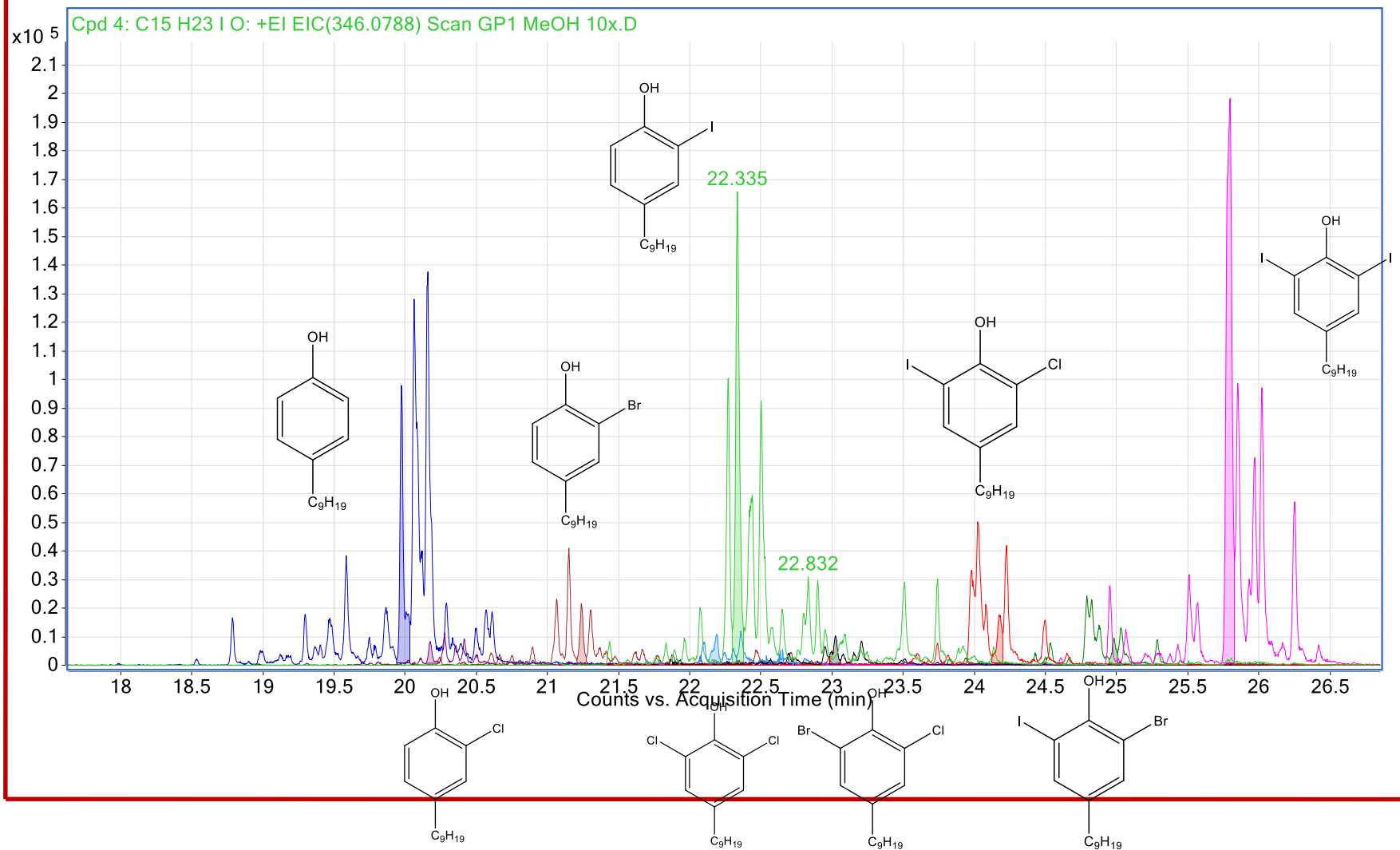
Nonylphenol – technical mixture

$NH_2Cl + Br^- + I^-$

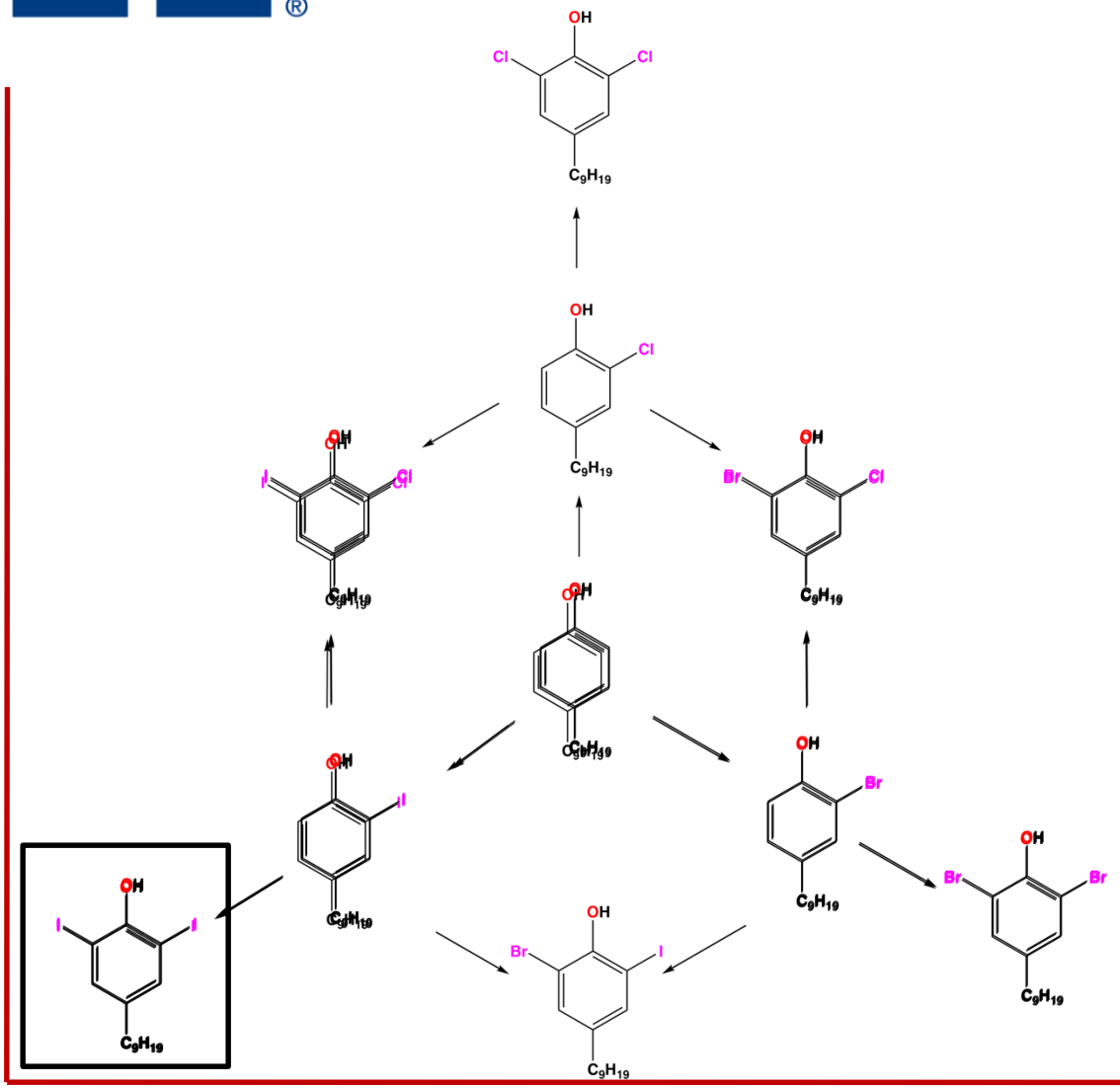


Nonylphenol – technical mixture

$NH_2Cl + Br^- + I^-$

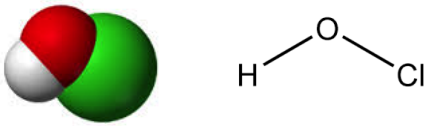


Identified Mono- and Dihalogenated DBPs after Chemical Oxidation

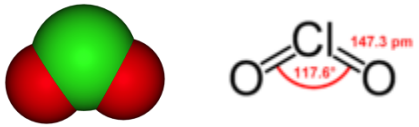


Most abundant

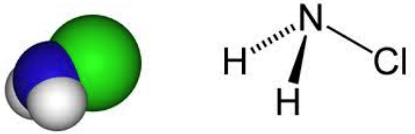
Chlorine + Br^- + I^-



Chlorine dioxide + Br^- + I^-

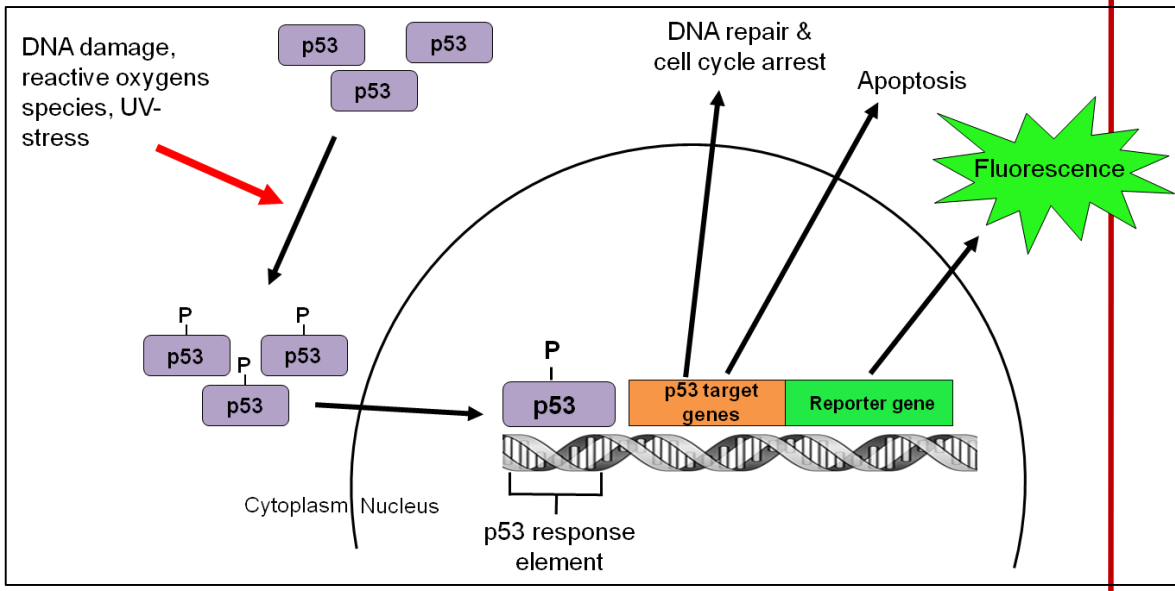


Monochloramine + Br^- + I^-

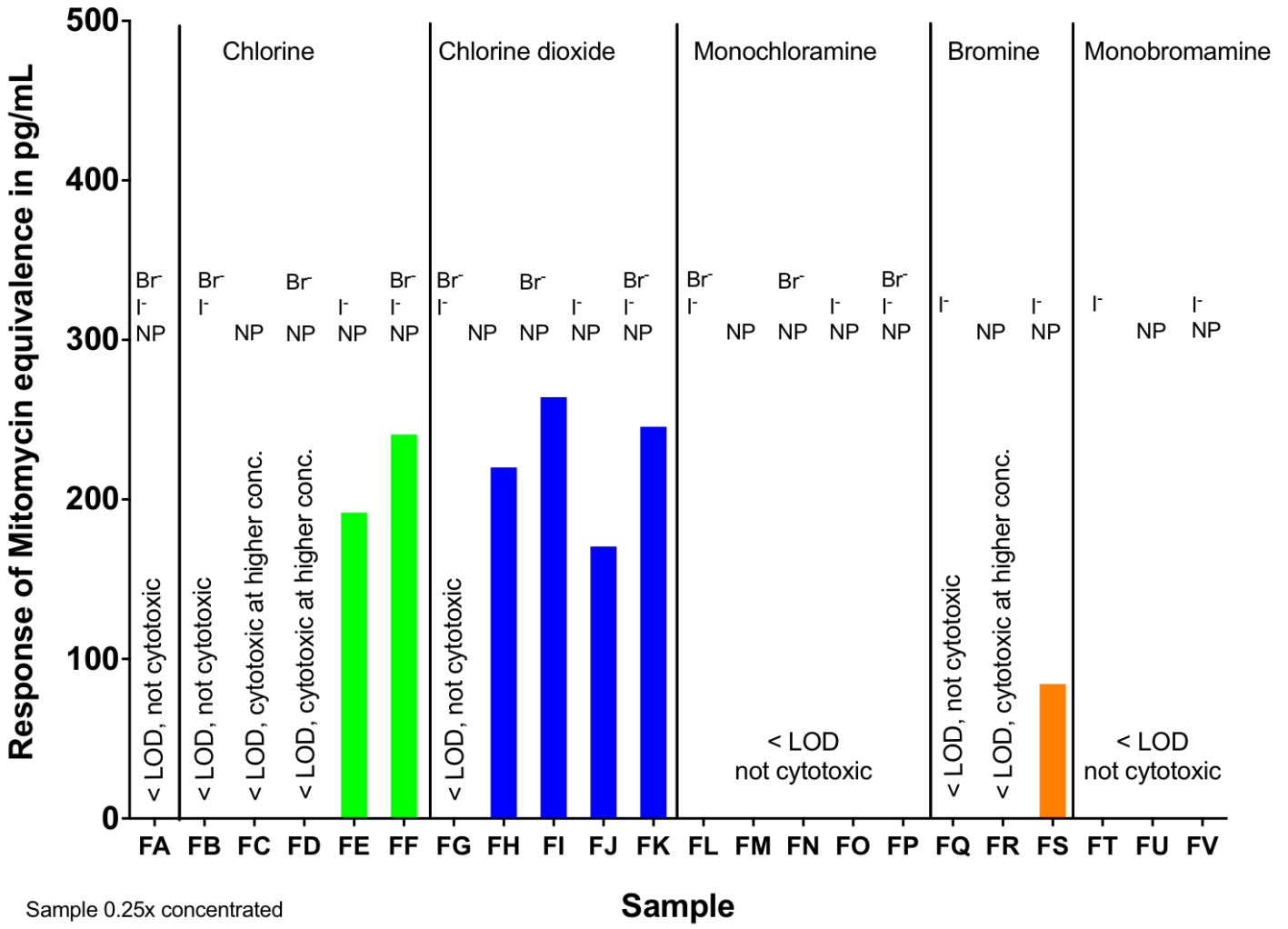


p53 *in vitro* Bioassay

- Uses CellSensor™ p53-bla HTC-116 cell based assay (Life Technologies)
- This is a cell line that has a fluorescence based reporter gene spliced into the cell to allow the identification of agonists/antagonists of the p53 pathway.
- This assay can be used as an indicator of **DNA damage**.

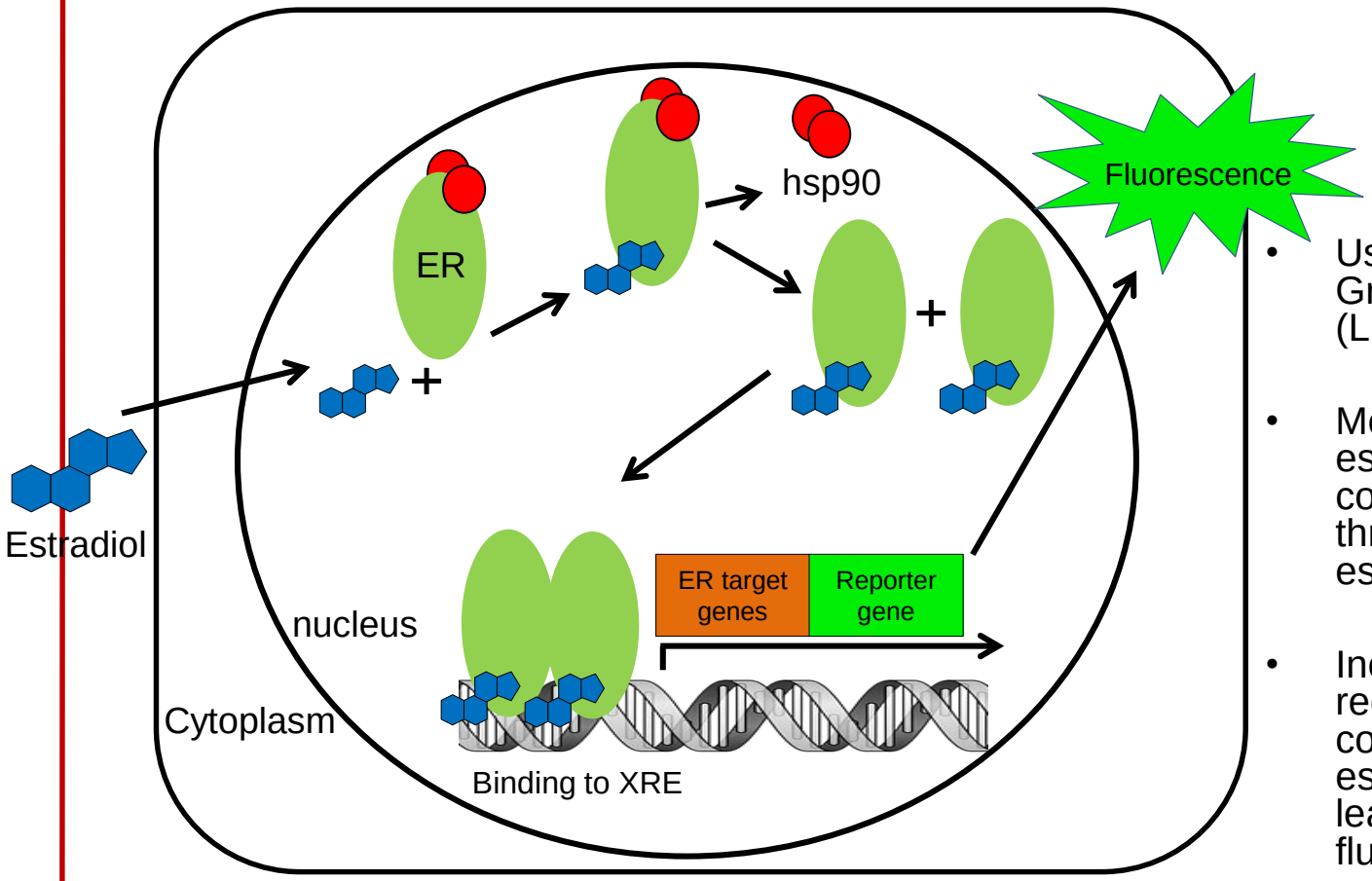


Linear NP – p53 bioassay



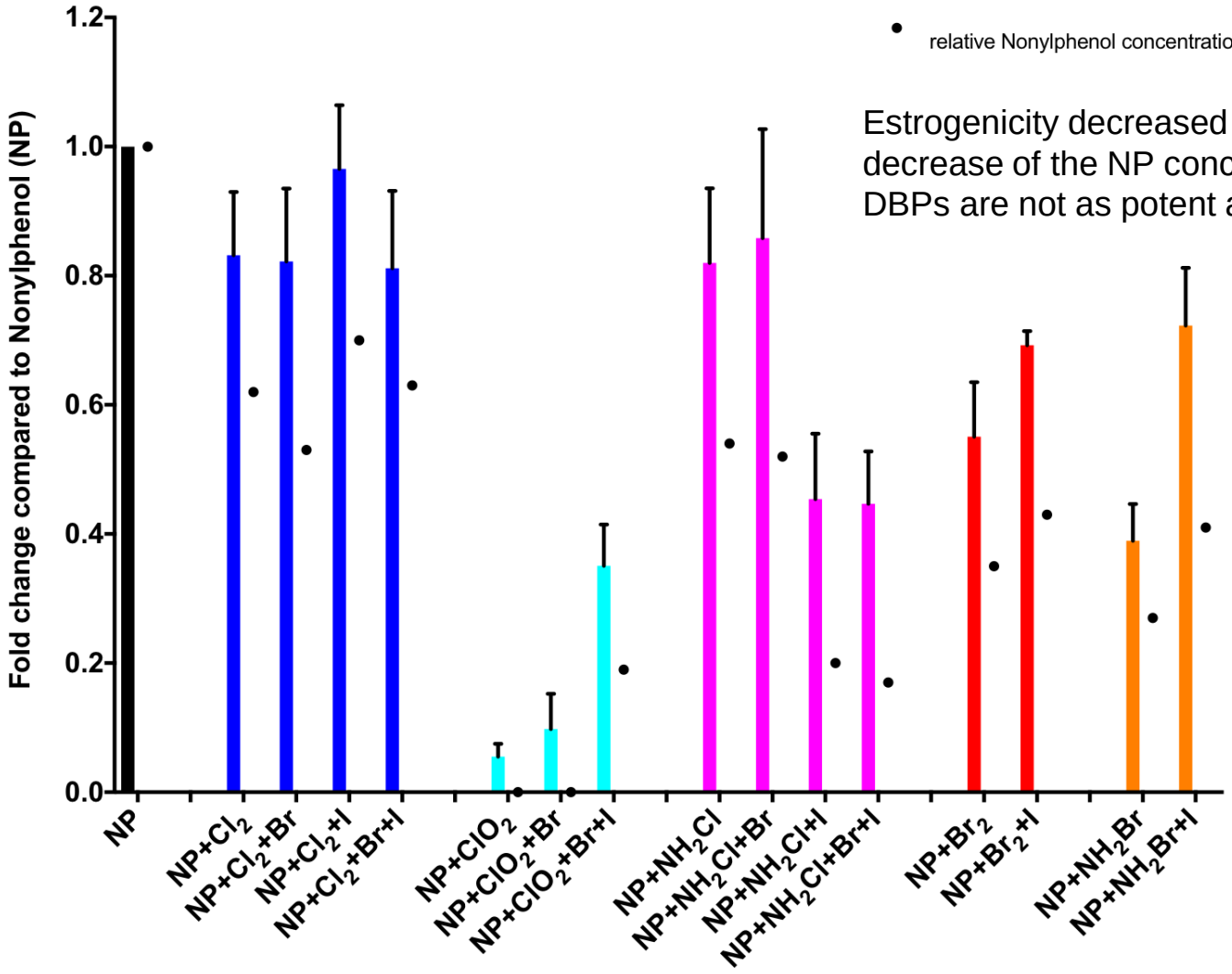
Sample 0.25x concentrated

Estrogen Receptor alpha (ERalpha) *in vitro* Bioassay



- Uses ER alpha-UAS-*bla* GripTite cell based assay (Life Technologies)
- Measures the relative estrogenic response of a compound or mixture through binding of the estrogen receptor alpha
- Increase affinity to the receptor and/or increased concentration of estrogenic compounds lead to increase in fluorescence

Tech. NP – ER bioassay



Estrogenicity decreased proportionally with the decrease of the NP concentration
DBPs are not as potent as NP

Summary/Next Steps

- Chlorinated, brominated and iodinated DBPs were identified in batch experiments using Cl_2.
- I_2-NP was the most prominent DBP in experiments using ClO_2 or NH_2Cl.
- Smaller amounts of brominated DBPs occurred in NH_2Cl experiments.

- P53 assay showed
  - no activity for NH2Cl or NH2Br
  - Toxicity in samples containing I^- when using Cl_2 and Br_2
  - Toxicity in all samples when using ClO_2 in the presence of Br^- and I^-
- Estrogenicity of all samples decreased proportionally with decreasing NP concentration.

- Measuring NP-DBPs in wastewater effluents
  - ppt level found in tertiary effluent (NP-Cl,NP-Cl_2, NP-Br, NP-Br_2)
  - Combining results from other analyses (GC-ICP-MS, LC-qTOF) to verify and identify other potential DBPs.

Questions?

Thank you for your attention!