Nitration processes of acetaminophen in nitrifying activated sludges

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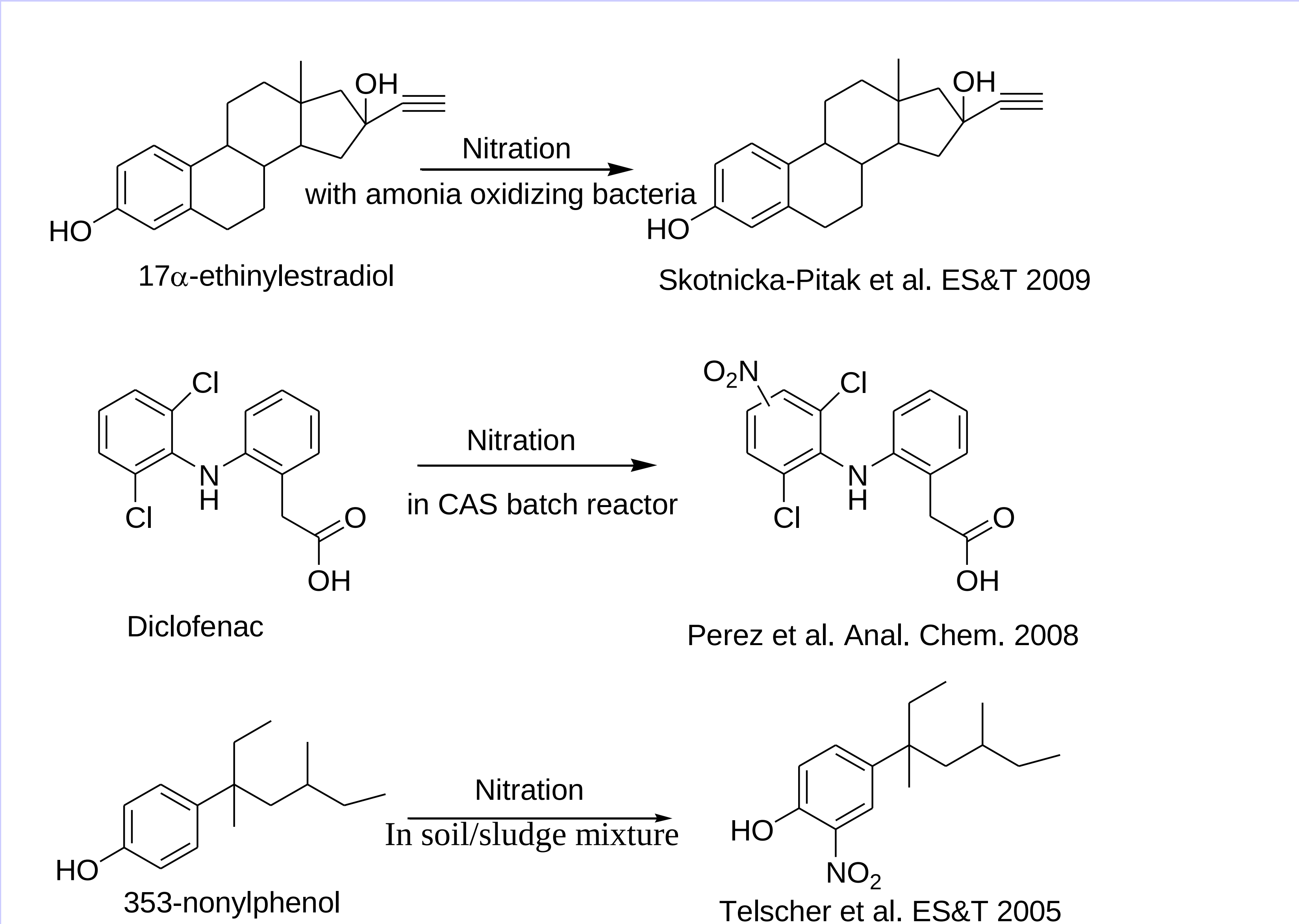
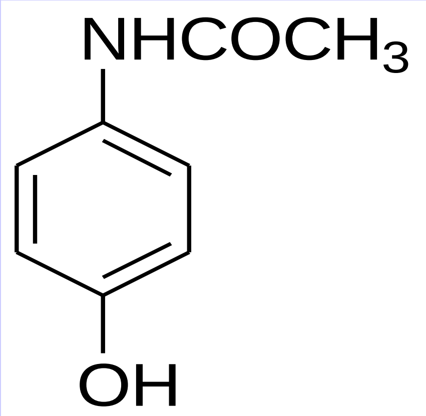
An unexpected biotransformation pathway: nitration



Objectives

- **To investigate the nitration mechanisms of phenolic compounds in nitrifying activated sludge.**
- **At different scales: field-, batch- and molecular-scale experiments.**
- **Acetaminophen (paracetamol) as a probe compound**.

Why paracetamol ?



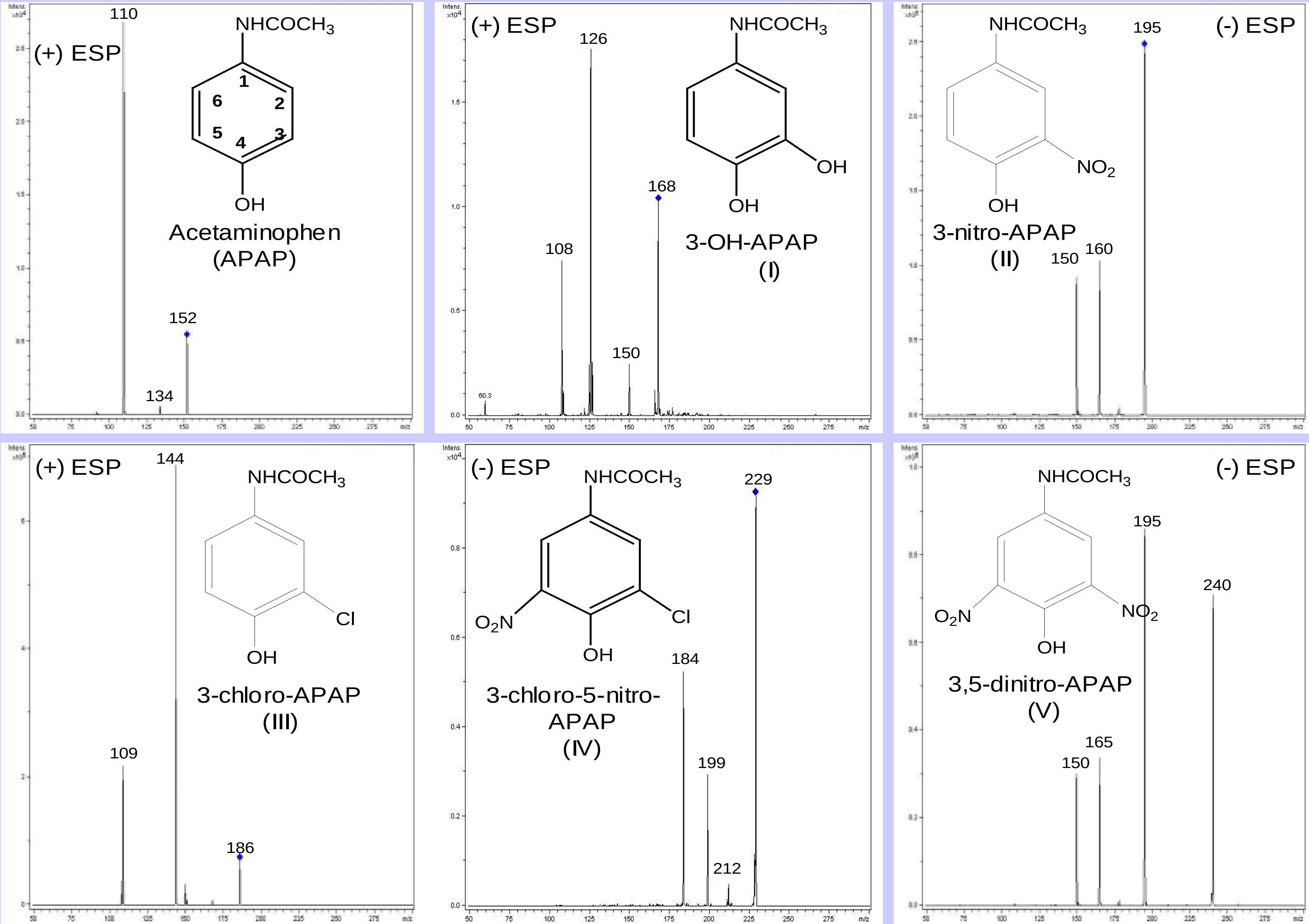
pKa = 9.4
logKow = 0.45
Sw > 1g/L

- Readily prone to nitration

- High occurrence in WWTPs (1-10 µg/L range)

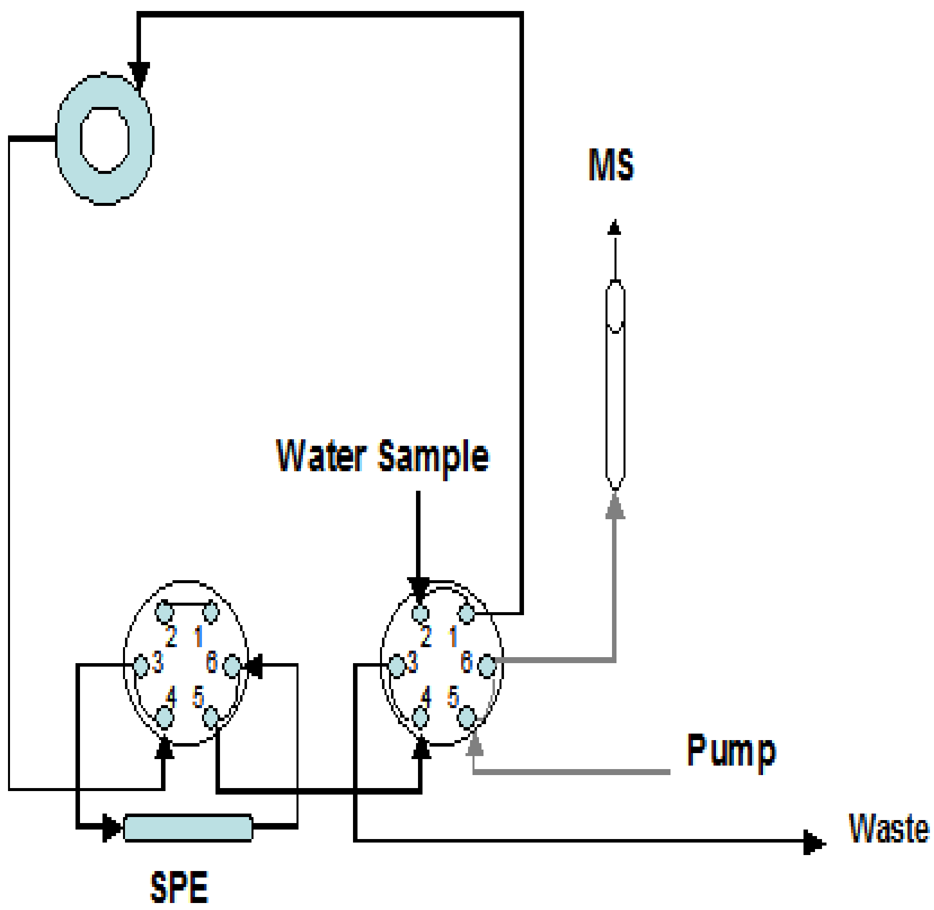
- Nitrated derivatives can be easily synthetized

Field studies: Targeted compounds



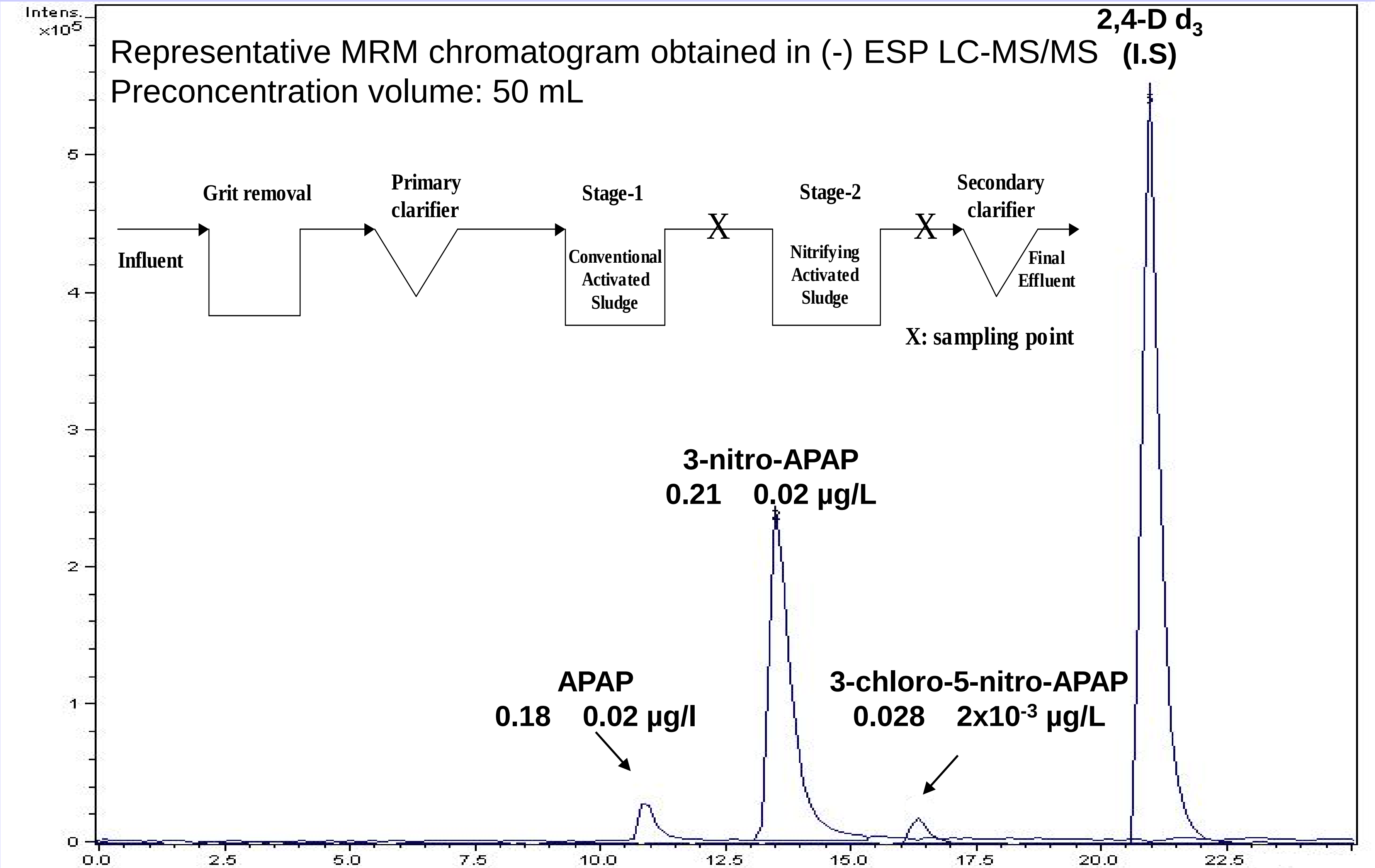
Analytical methodology

On-line SPE-LC-MS/MS



Compound	Recoveries (%)
3-OH-APAP	55 (± 12)
APAP	75 (± 8)
3-nitro-APAP	78 (± 8)
3-chloro-APAP	86 (± 6)
3-chloro-5-nitro-APAP	93 (± 4)
3,5-dinitro-APAP	95 (± 5)

Aix-en-Provence WWTP effluent analysis



Time (min)

Field data (24 h composite samples)

	Sampling month	APAP	3-OH-APAP	3-chloro-APAP	3-nitro-APAP	3-chloro-5-nitro-APAP
Stage-2 influent	Oct. 2008	3.45 ± 0.21	0.96 ± 0.11	0.24 ± 0.02	n.d	n.d
	Nov. 2008	5.35 ± 0.32	1.44 ± 0.17	0.85 ± 0.04	n.d	n.d
	Dec. 2008	6.75 ± 0.41	1.86 ± 0.22	0.76 ± 0.04	n.d	n.d
Stage-2 effluent	Oct. 2008	0.19 ± 0.02	n.d	n.d	0.18 ± 0.02	$0.03 \pm 1x10^{-3}$
	Nov. 2008	0.35 ± 0.03	n.d	n.d	0.26 ± 0.03	$0.11 \pm 5x10^{-3}$
	Dec. 2008	0.64 ± 0.05	n.d	n.d	0.32 ± 0.03	$0.09 \pm 3x10^{-3}$

Batch experiments

Experimental conditions:

[MLSS] = 2.5 g/L

pH = 7-7.5

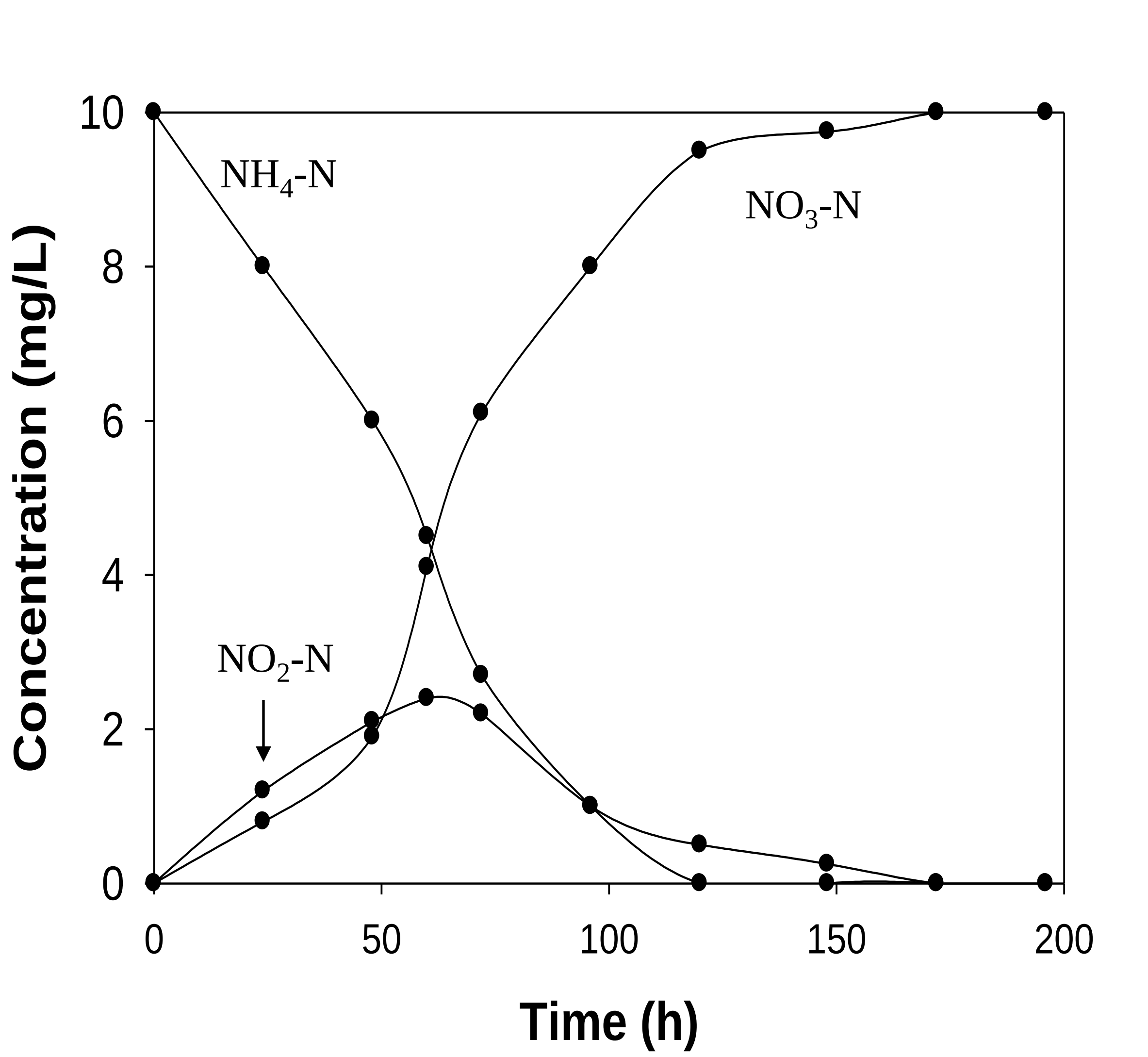
T = 25 C

$[O_2] > 3$ mg/L

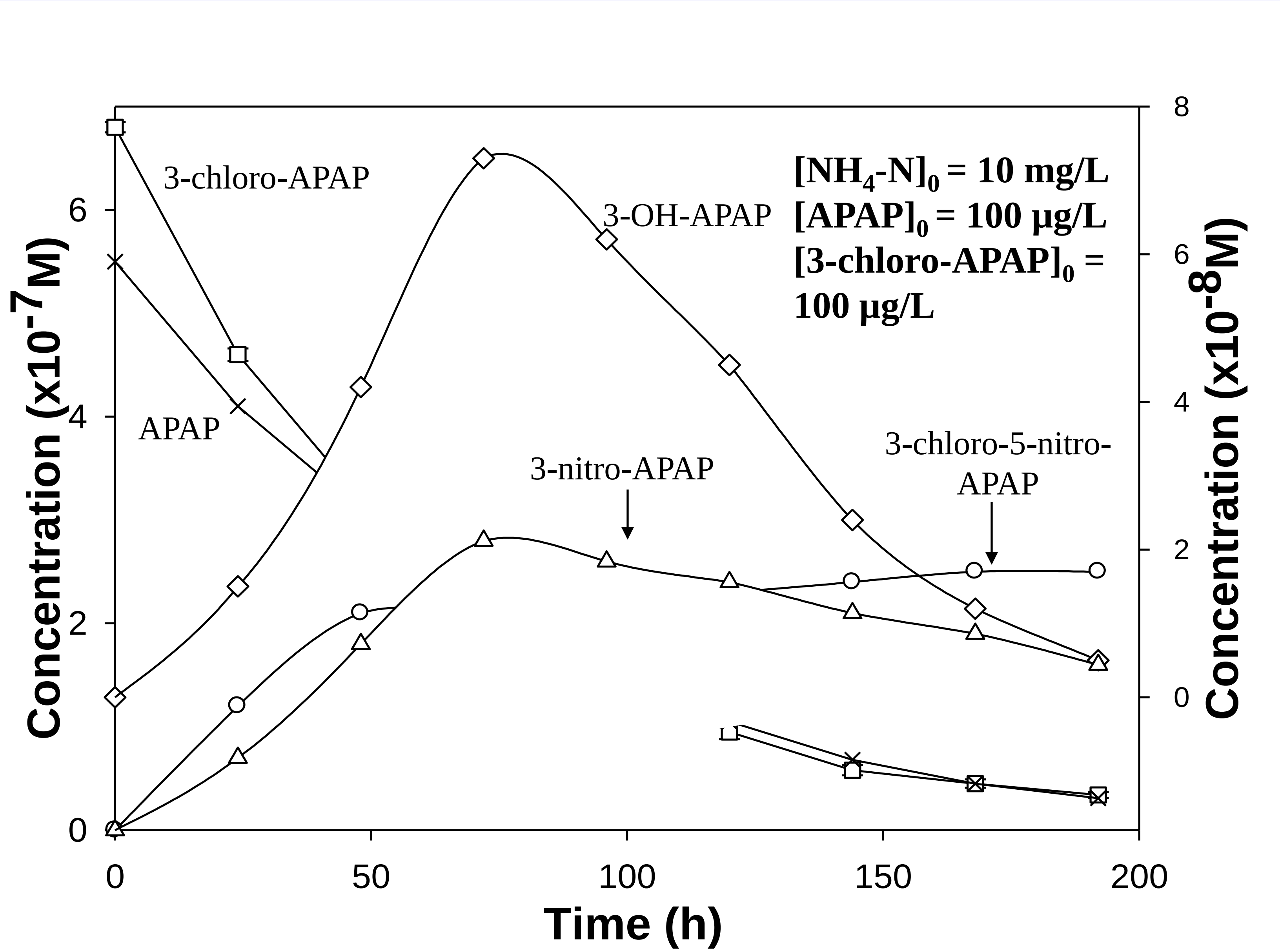
$[NH_4+]$ = 10 mg/L

$[APAP]_0$ = 100 µg/L

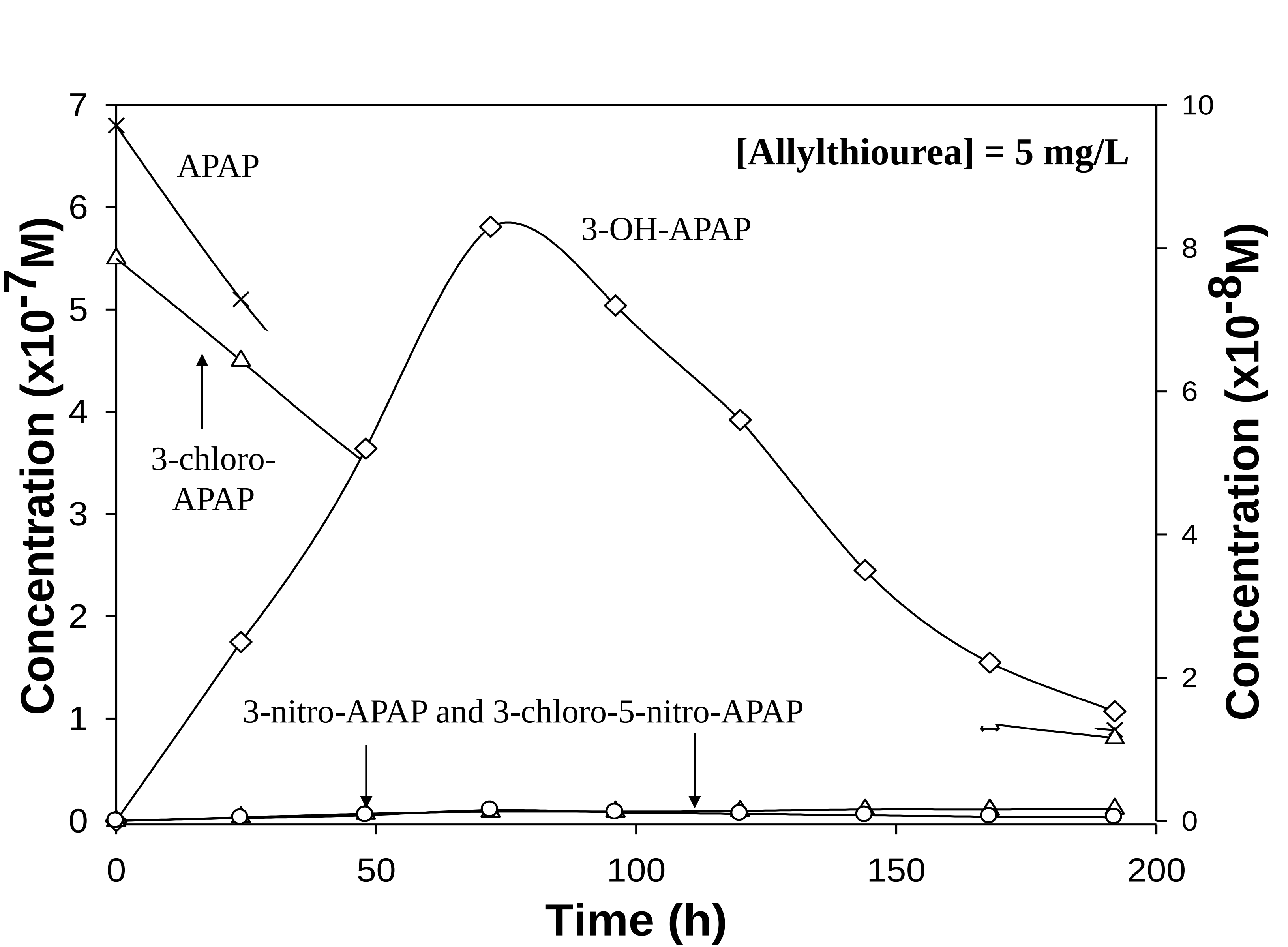
$[3\text{-chloro-APAP}]_0$ = 100 µg/L



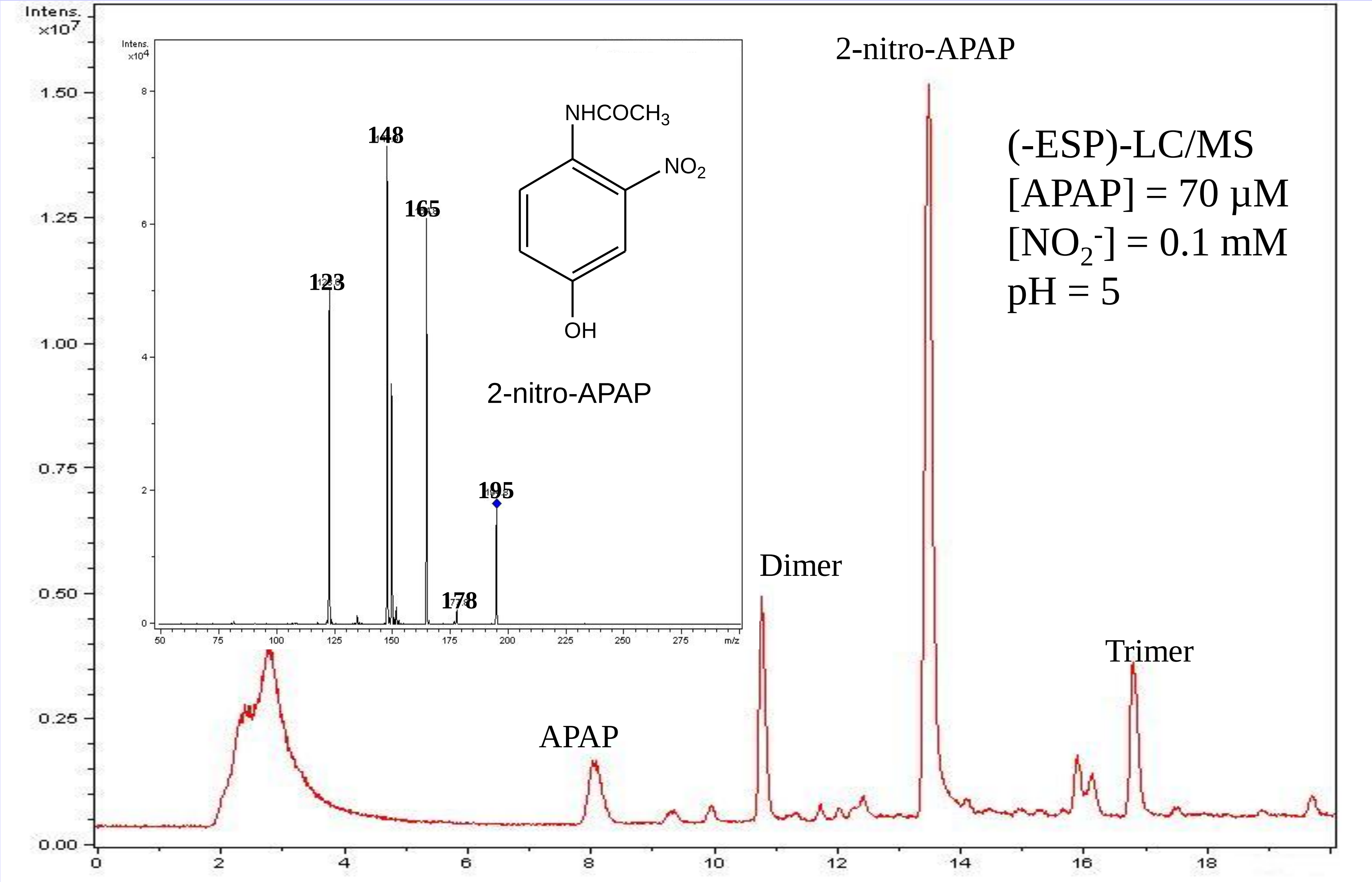
Batch 1 experiment: no nitrification inhibition



Batch 2 experiment : ammonia oxygenase inhibition

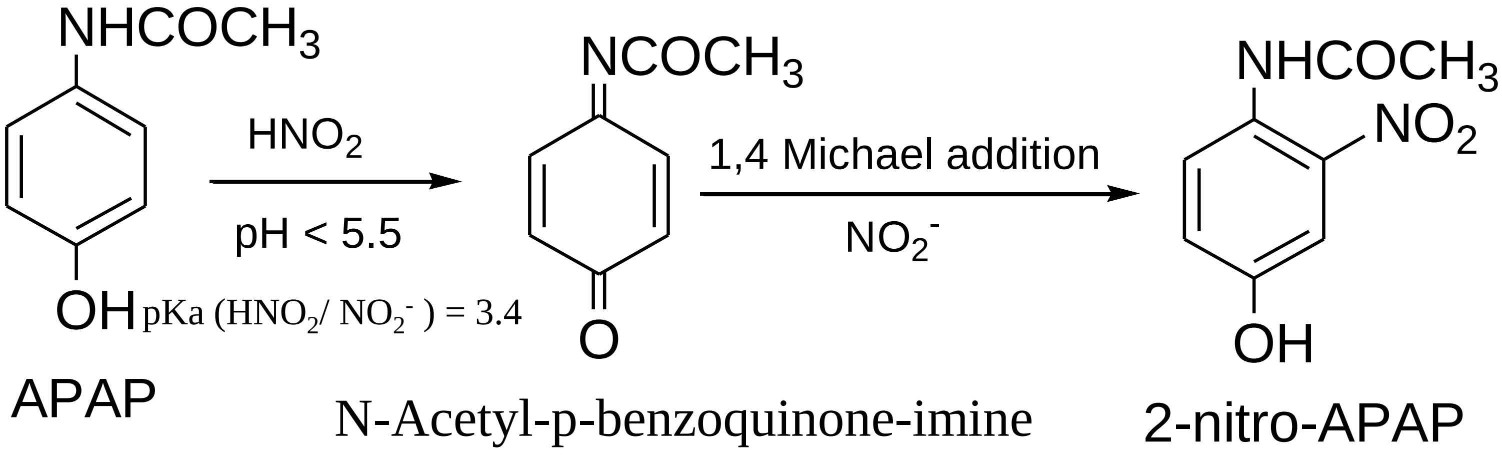


Reaction of APAP with HNO_2



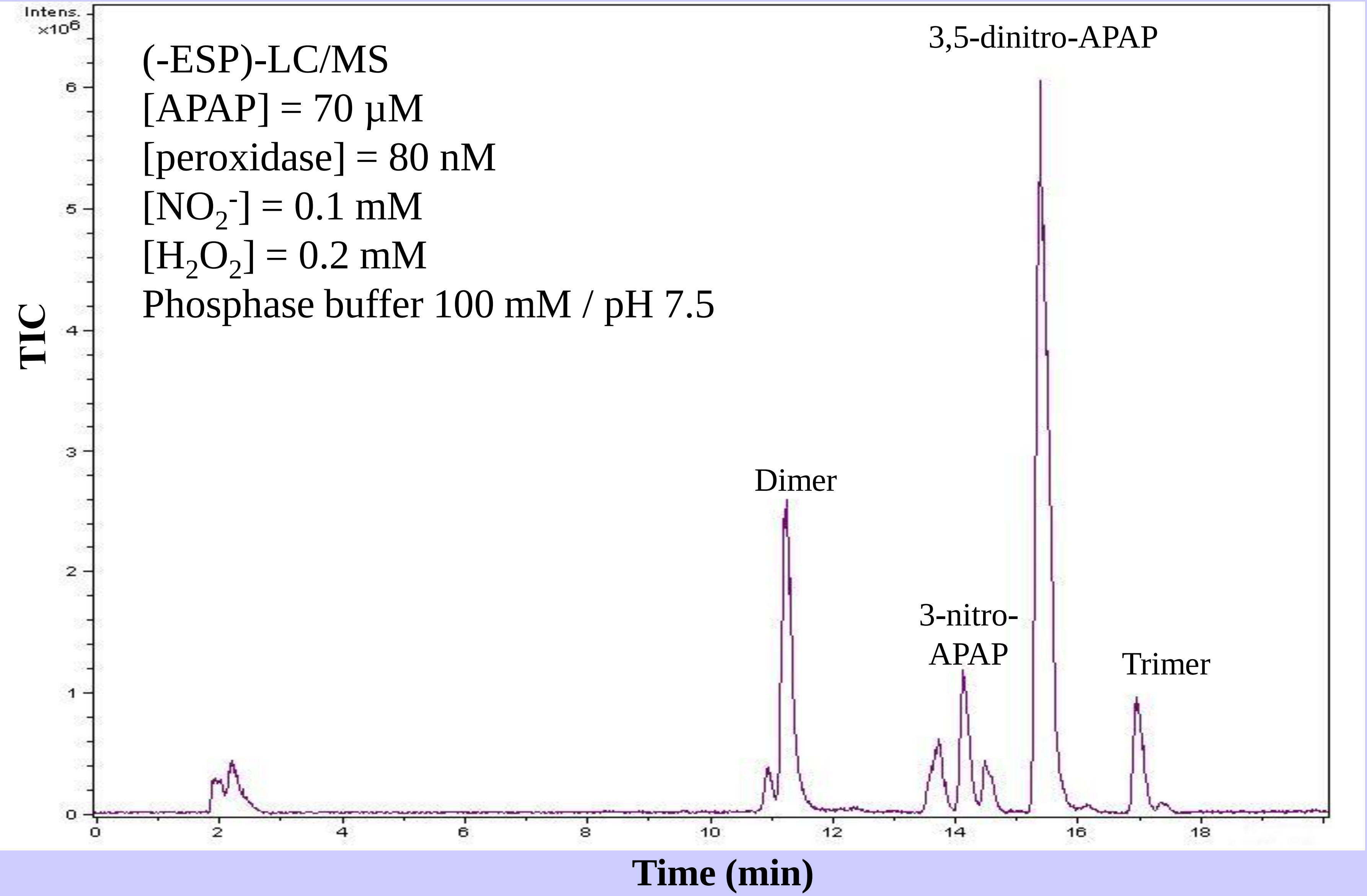
Time (min)

Proposed mechanism of 2-nitro-APAP formation

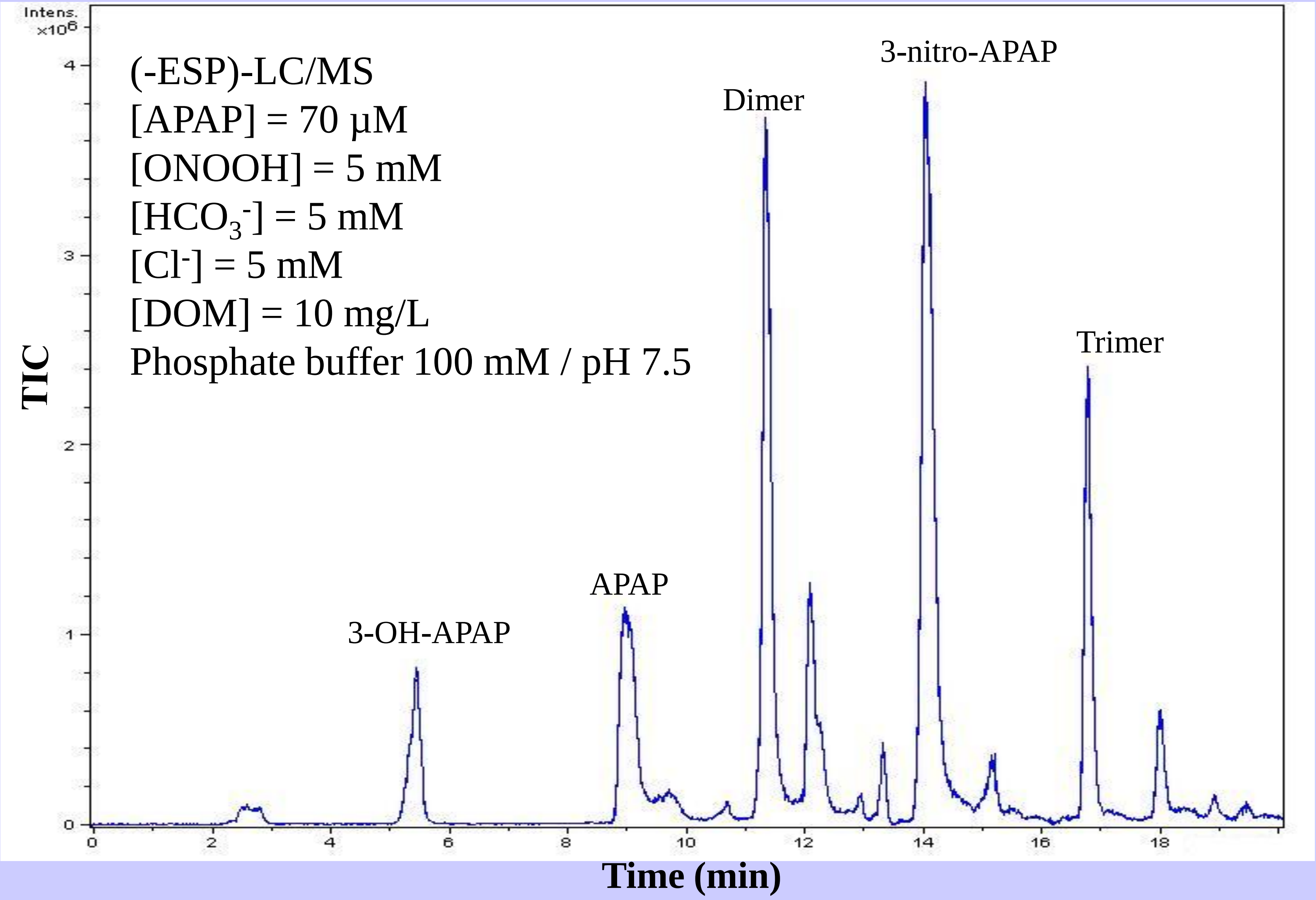


Matsumo et al. Chem. Pharm. Bull. 1989

Reactivity of APAP with horseradish peroxidase



Reactivity of APAP with peroxynitrite

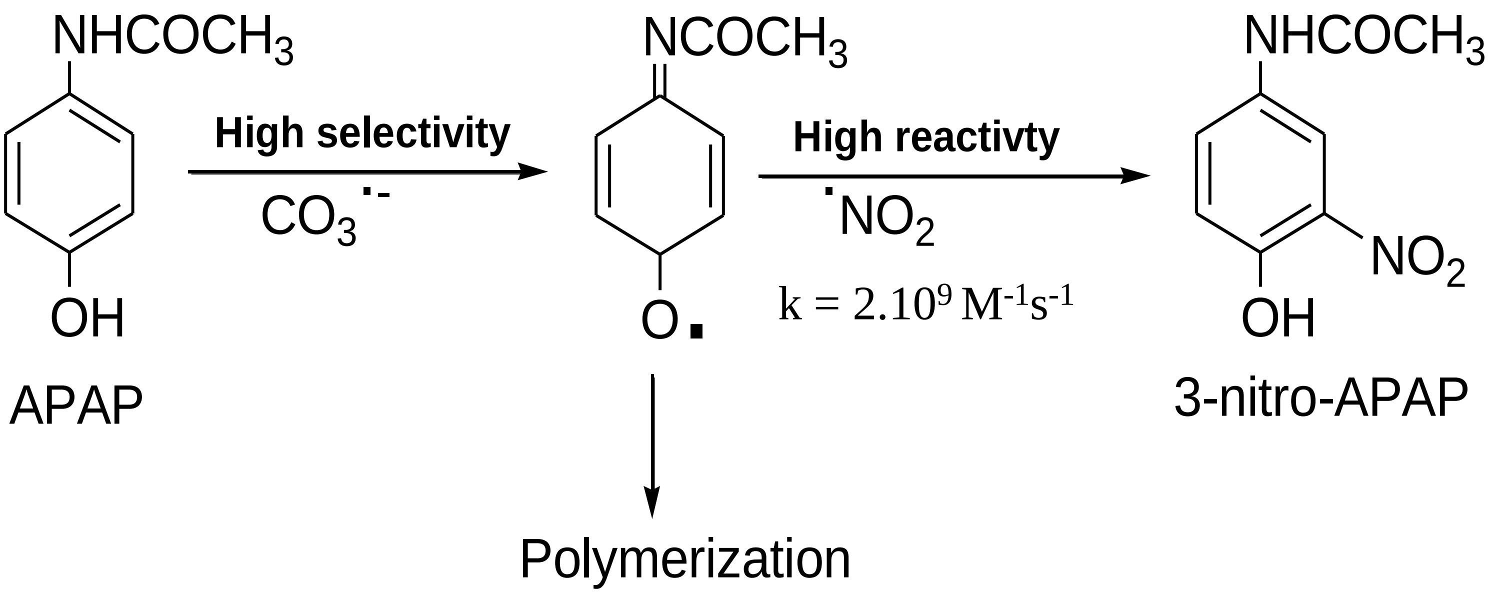


Proposed mechanism of 3-nitro-APAP formation

$$ONOO^- + H^+ \rightleftharpoons ONOOH \qquad pKa = 6.8$$

$$ONOOH \longrightarrow {}^{\bullet}NO_2 + {}^{\bullet}OH \qquad k = 5.3 \times 10^9\ M^{-1}s^{-1}$$

$$ONOO^- + CO_2 \longrightarrow {}^{\bullet}NO_2 + CO_3^{\bullet -} \qquad k = 1 \times 10^4\ M^{-1}s^{-1}$$



Where is peroxynitrite coming from?

NH_4^+ oxidation by ammonia oxidizing bacteria (AOB)

– First step

$$NH_3 + O_2 + 2H^+ \ 2e^- \xrightarrow[\text{(AMO)}]{\text{Ammonia mono-oxygenase}} NH_2OH + H_2O$$

$\rightarrow$ $^{\bullet}NO$ (side-product)

- Second step

$$NH_2OH + H_2O \xrightarrow{\text{Hydroxylamine oxidoreductase}} HNO_2 + 4H^+ + 4e^-$$

- Third step

$$O_2 + e^- \longrightarrow O_2^{\bullet -}$$

$$^{\bullet}NO + O_2^{\bullet -} \longrightarrow ONOO^- \qquad k = 1.9 \times 10^{10}\ M^{-1}s^{-1}$$

$$ONOO^- + H^+ \longrightarrow ONOOH \qquad pKa = 6.8$$

Conclusions

- **Formation of nitro-APAP derivatives with a environmental profile more worrisome than APAP.**
- **Nitration is probably linked to $^{\cdot}$NO generation by nitrifying bacteria.**
- **This result must be validated with other phenolic pollutants (i.e. bisphenol A, nonylphenol).**

Nitration processes in the environment

- Photonitration

$$NO_3^- + h\nu + H_2O \rightarrow {}^{\bullet}OH + {}^{\bullet}NO_2 + OH^- \qquad [\Phi_1 = 0.01]$$

$$NO_2^- + h\nu + H_2O \rightarrow {}^{\bullet}OH + {}^{\bullet}NO + OH^- \qquad [\Phi_2 = 0.02\text{-}0.07]$$

$$NO_2^- + {}^{\bullet}OH \rightarrow {}^{\bullet}NO_2 + OH^- \qquad [k_3 = 1.0\times10^{10}\ M^{-1}\ s^{-1}]$$

- Thermal nitration by HNO_2 (in the dark)

pKa (HNO_2/ NO_2^-) = 3.4 relevant at pH< 5.5

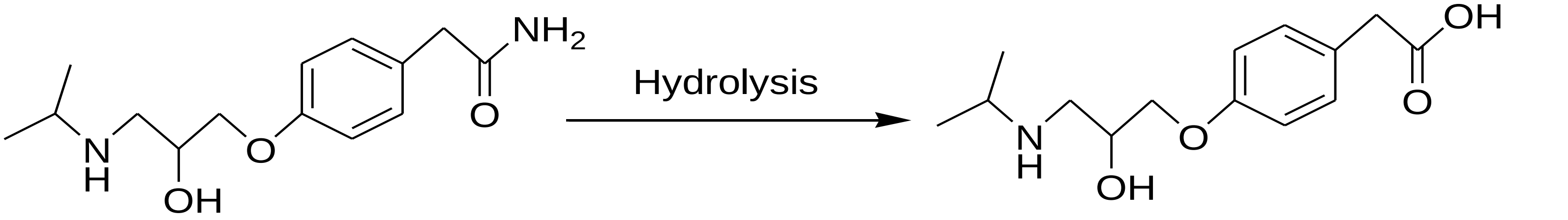
- Bionitration by peroxidases in presence of NO_2^- and H_2O_2
- Bionitration by ONOOH (peroxynitrite)

$${}^{\cdot}NO + O_2^{\cdot -} \longrightarrow ONOO^- \qquad k = 1.9\ x10^{10}\ M^{-1}s^{-1}$$

$$ONOO^- + H^+ \rightleftharpoons ONOOH \qquad pKa = 6.8$$

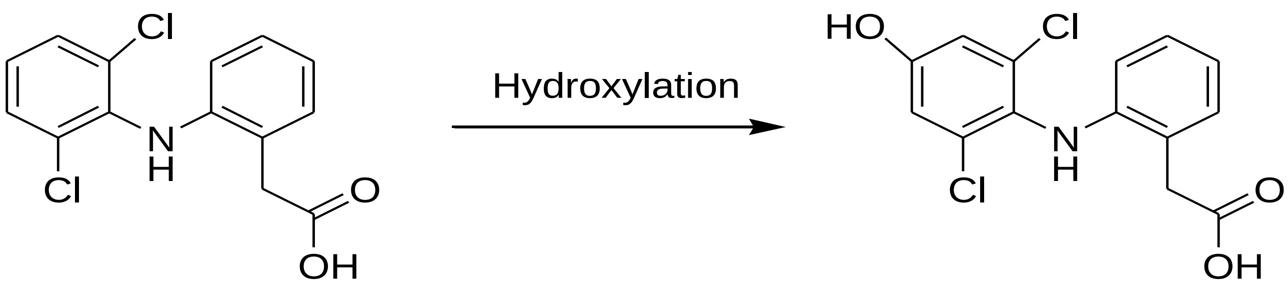
$$ONOO^- + H^+ \longrightarrow {}^{\cdot}NO_2 + OH^- \qquad k = 5.3\ x\ 10^9\ M^{-1}s^{-1}$$

Expected biotransformation pathways



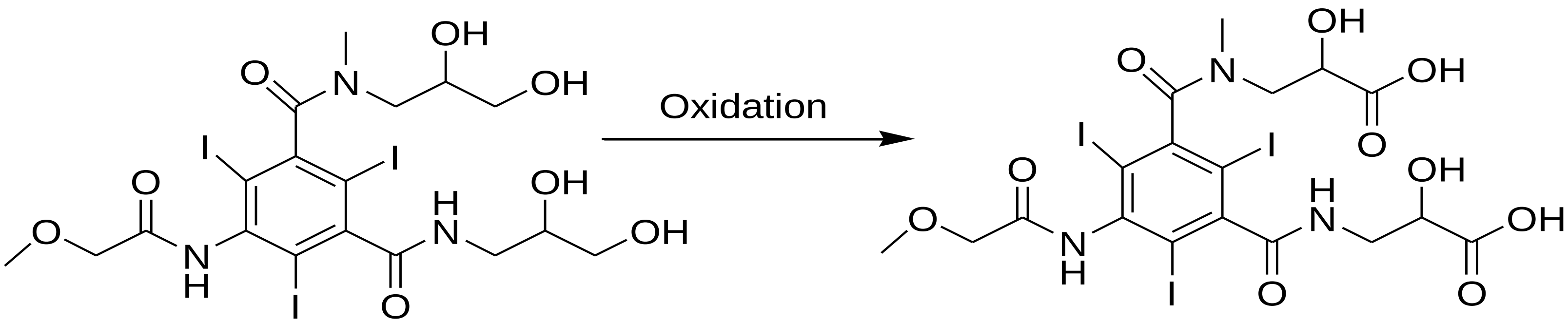
Atenolol

Radjenovic et al. J. Chromatogr. A. 2008



Diclofenac

Perez et al. Anal. Chem. 2008



Iopromide

Schulz et al. ES&T 2008