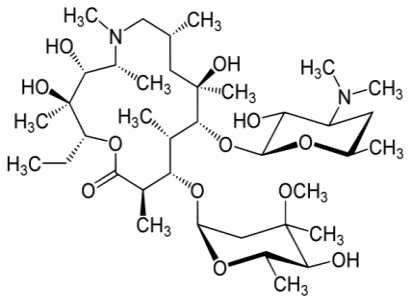
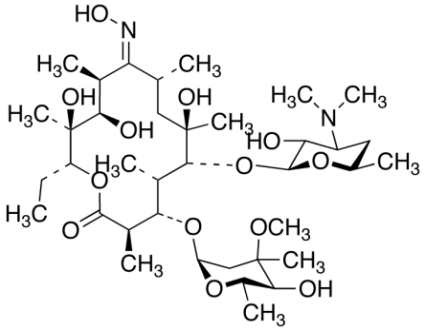
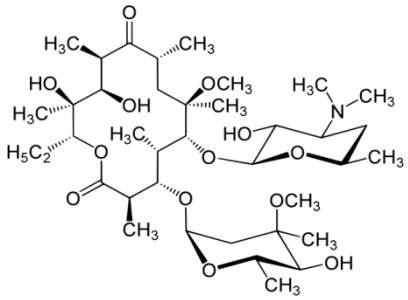
Determination of macrolide antibiotics, their intermediates and transformation products in solid environmental matrices







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ICCE, Oslo, 21 June 2017

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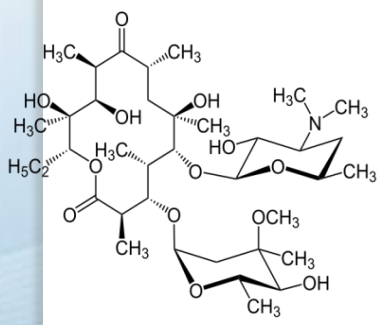
Introduction

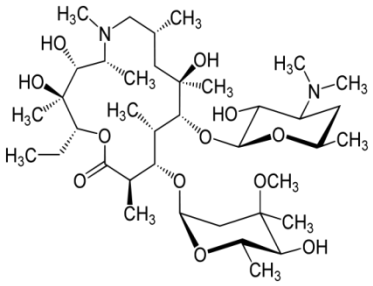
- Antibiotic residues in the environment – possible contribution to selection and spread of antibiotic resistance
- Numerous analytical methods for determination of antibiotics in environmental samples
- Focus on parent compounds
- The contribution of transformation products (TPs), synthesis intermediates and byproducts to the total mass loads mostly unknown

Macrolide antibiotics

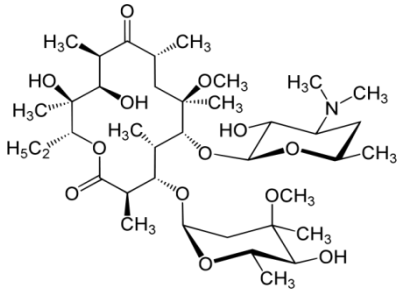
- Among the top 3 mostly consumed classes of antibiotics
- Incomplete removal during wastewater treatment and relatively high sorption affinity → occurrence in sludge and sediments



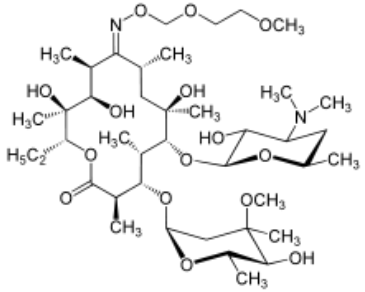
Erythromycin (ERY)



Azithromycin (AZI)

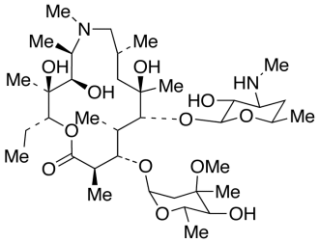


Clarithromycin (CLA)

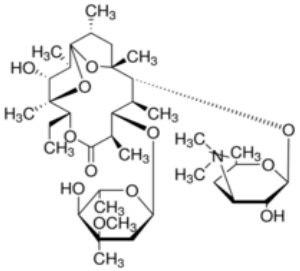


Roxithromycin (ROX)

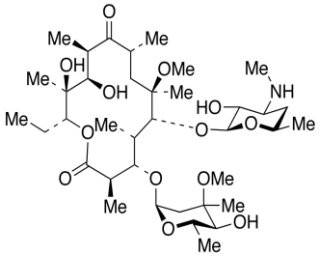
Metabolites and TPs



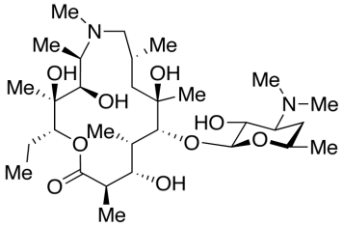
N'-desmethyl AZI (*N*'-DMA)



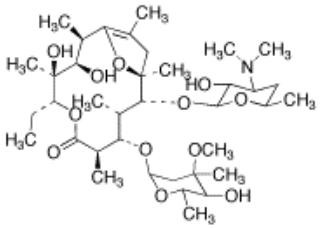
Anhydro ERY (ERY-H_2O)



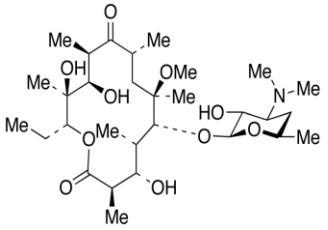
N-desmethyl CLA (DMC)



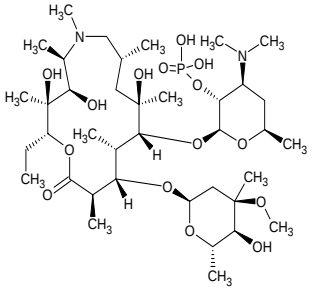
Descladinosyl AZI (DCA)



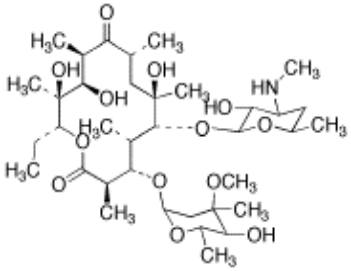
ERY enol ether (EEE)



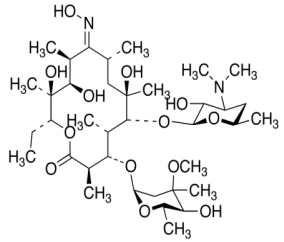
Descladinosyl CLA (DCC)



Phosphorylated AZI (AZI-PO4)

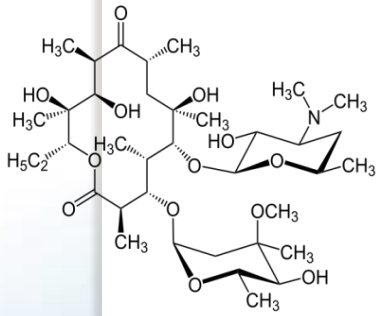


N-demethyl ERY (DME)

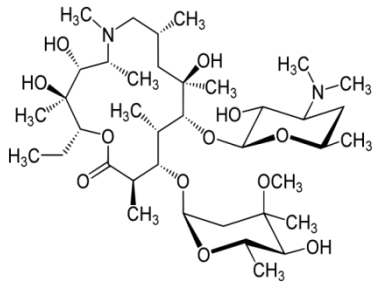


ERY oxime (EOX)

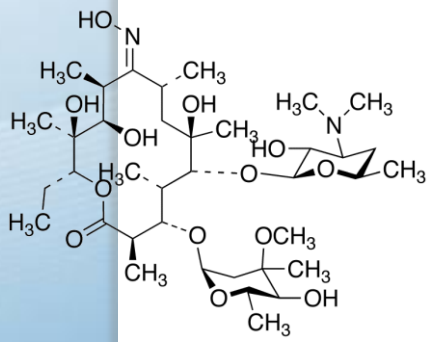
Intermediates in AZI synthesis



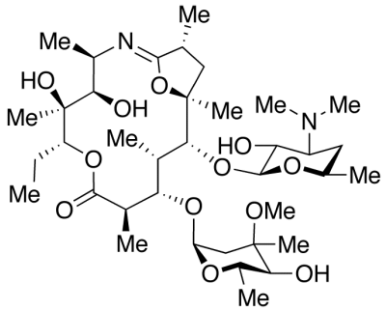
Erythromycin (ERY)



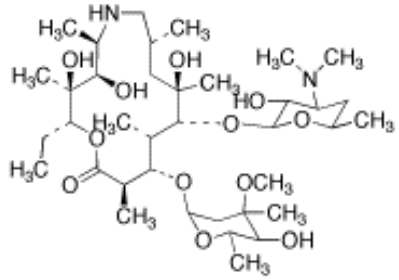
Azithromycin (AZI)



Erythromycin oxime (EOX)

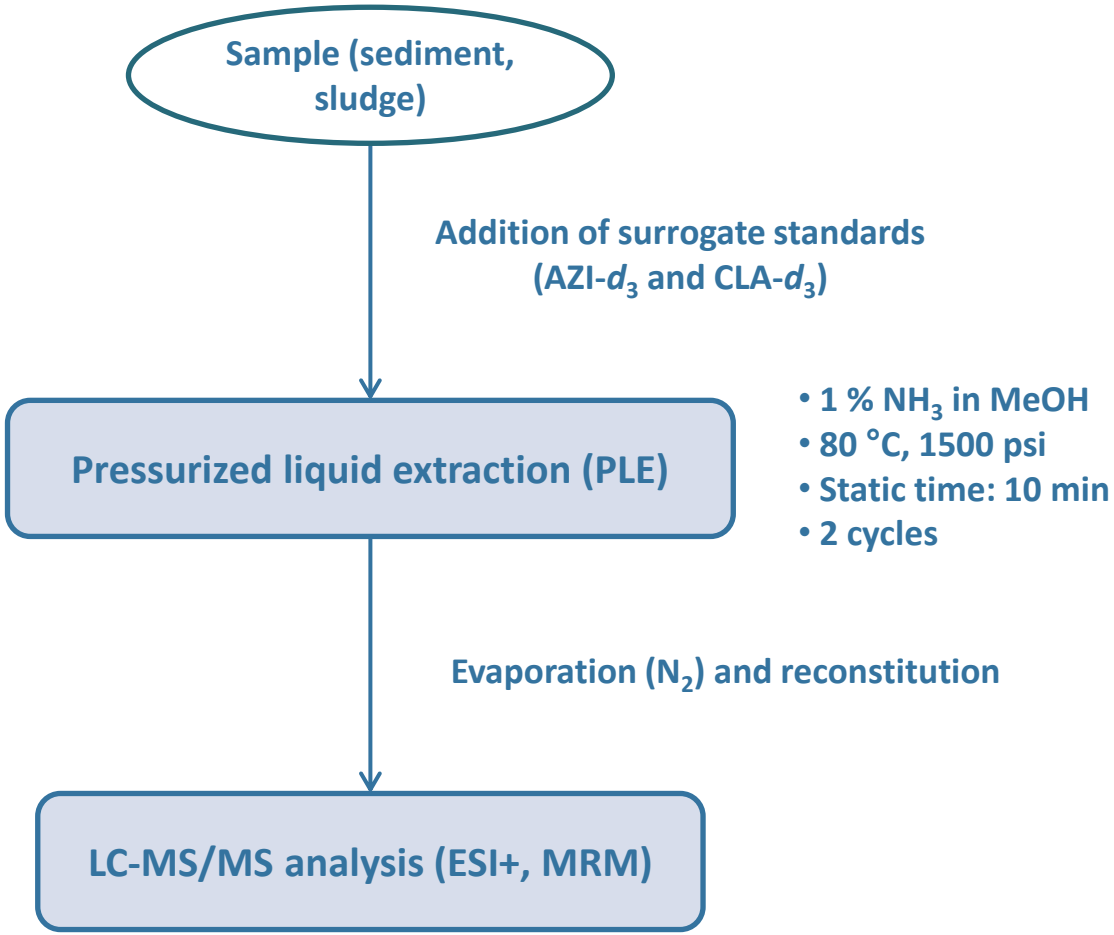


Erythromycin imino ether (EIE)



N-desmethyl azithromycin (*N*-DMA)

Analytical procedure



MS/MS parameters (MRM)

COMPOUND	ABBREVIATION	PRECURSOR ION (*m/z*)	PRODUCT ION 1 (*m/z*)	CE 1 / V	PRODUCT ION 2 (*m/z*)	CE 2 / V
DESCLADINOSYL AZITHROMYCIN	DCA	296.2	116.0	15	158.0	12
PHOSPHORYLATED AZITHROMYCIN	AZI-PO_4	829.5	671.4	30	573.4	35
N- DESMETHYL AZITHROMYCIN	N-DMA	368.3	158.0	19	420.1	15
N'- DESMETHYL AZITHROMYCIN	N'-DMA	368.3	434.1	16	144.0	17
AZITHROMYCIN	AZI	375.2	591.3	16	83.1	31
DESCLADINOSYL CLARITHROMYCIN	DCC	590.4	158.0	18	116.0	31
ERYTHROMYCIN IMINO ETHER	EIE	366.2	573.3	8	83.0	11
N-DEMETHYL ERYTHROMYCIN	DME	720.5	144.0	24	562.2	15
ERYTHROMYCIN	ERY	734.5	158.0	25	576.3	15
ERYTHROMYCIN OXIME	EOX	749.5	591.3	16	158.0	29
ANHYDROERYTHROMYCIN	ERY-H_2O	716.5	558.2	19	158.1	41
N'-DEMETHYL CLARITHROMYCIN	DMC	734.5	144.0	23	576.2	12
CLARITHROMYCIN	CLA	748.5	158.0	34	590.3	26
ROXITHROMYCIN	ROX	837.5	158.0	42	679.3	27
ERYTHROMYCIN ENOL ETHER	ERY-EE	716.5	558.2	19	158.1	41
AZITHROMYCIN -d_3	AZI-d_3	376.8	594.3	16	83.0	28
CLARITHROMYCIN -d_3	CLA-d_3	731.5	593.3	17	161.0	24

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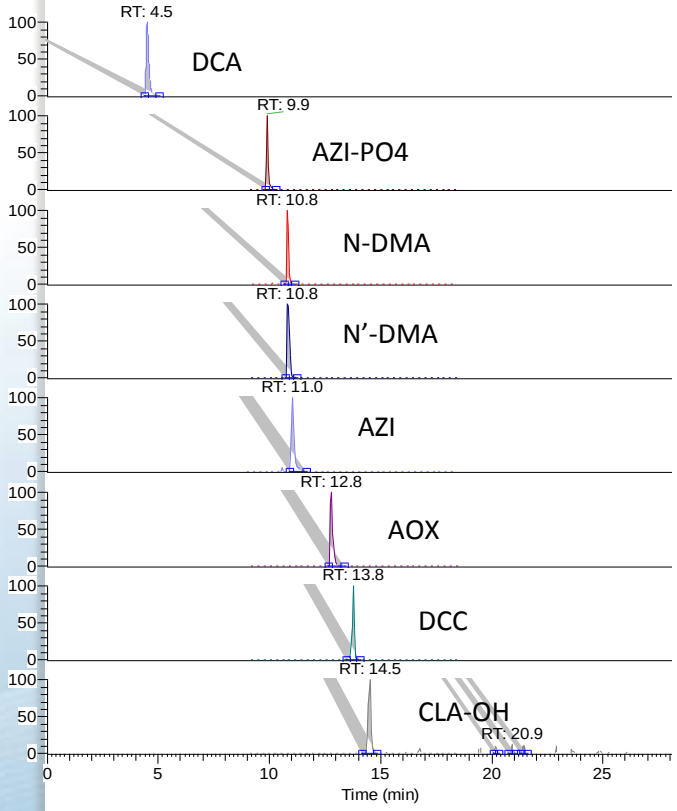
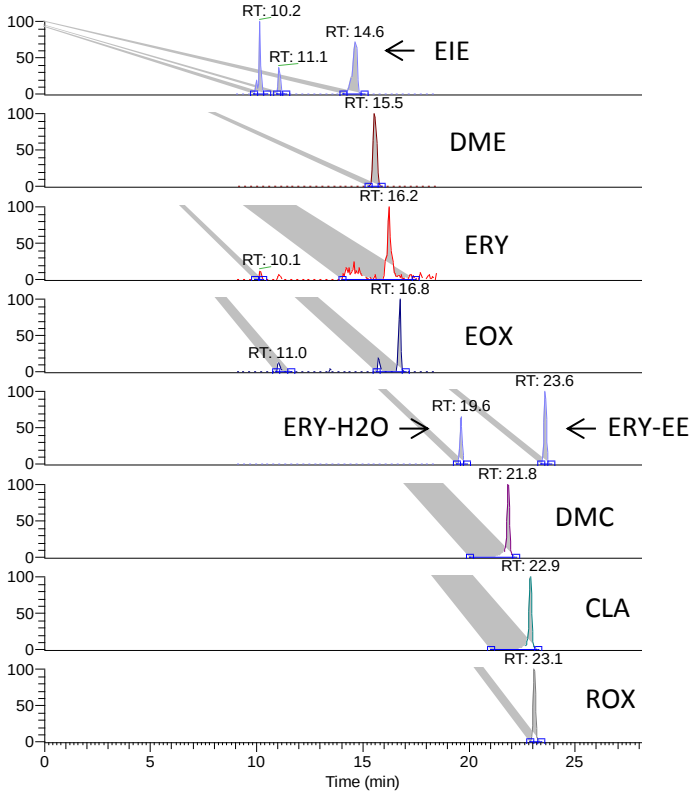
Chromatographic separation





- Column: ACE C18 PFP (150 x 3 mm; 3 µm)
- Eleunt A: 0.1 % HCOOH in water
- Eluent B: ACN

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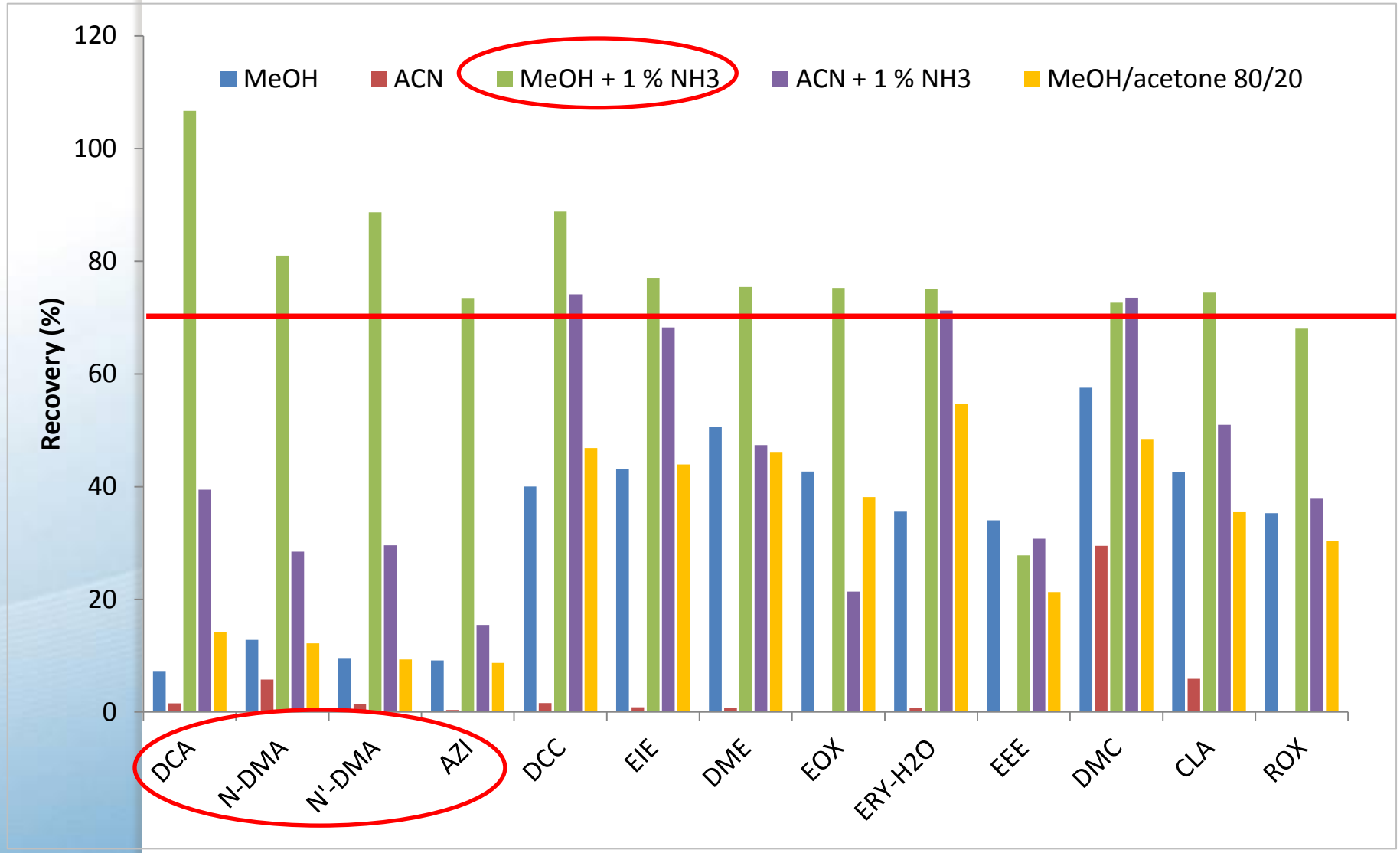
Extraction recovery (preliminary experiments)

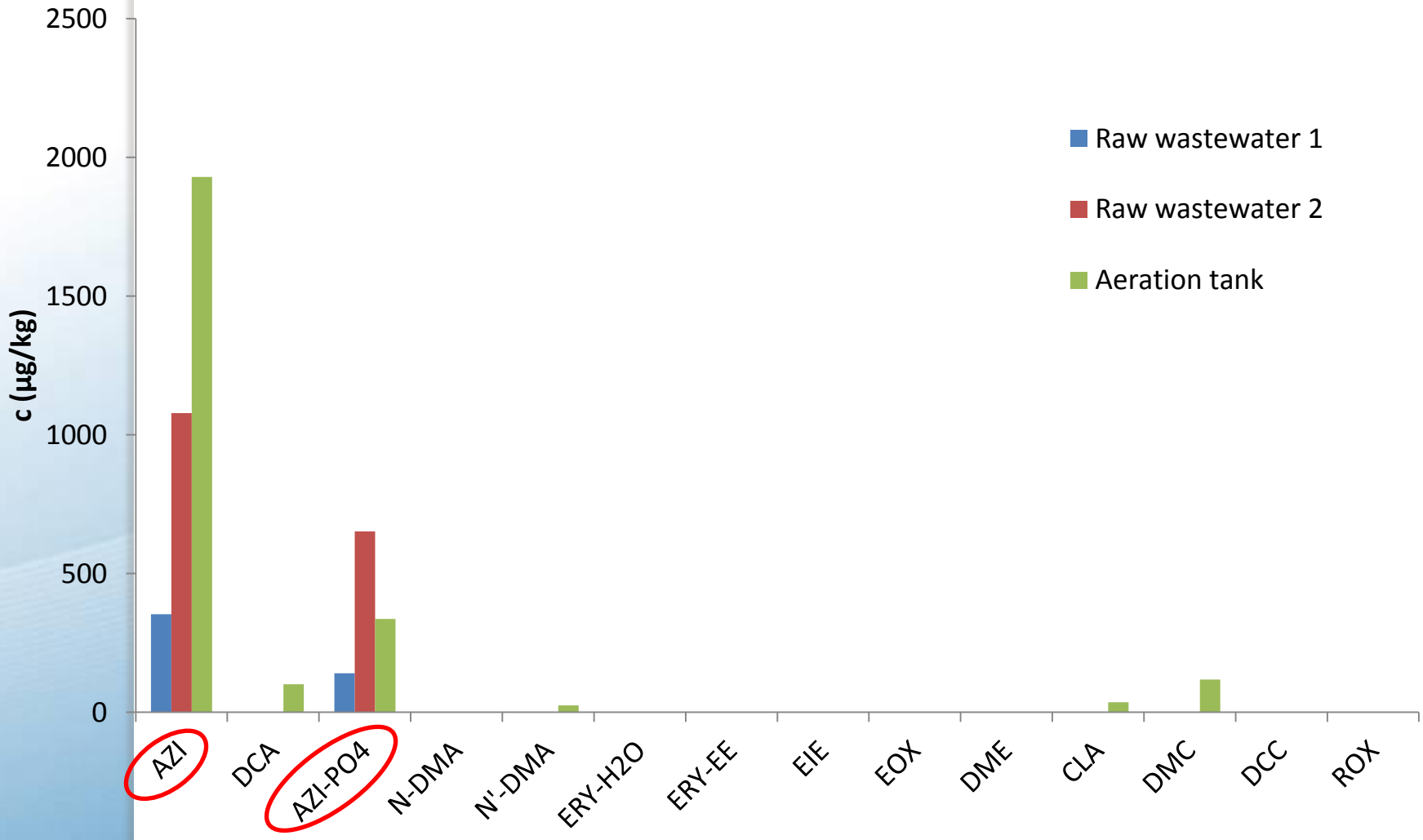


Method validation – river sediment

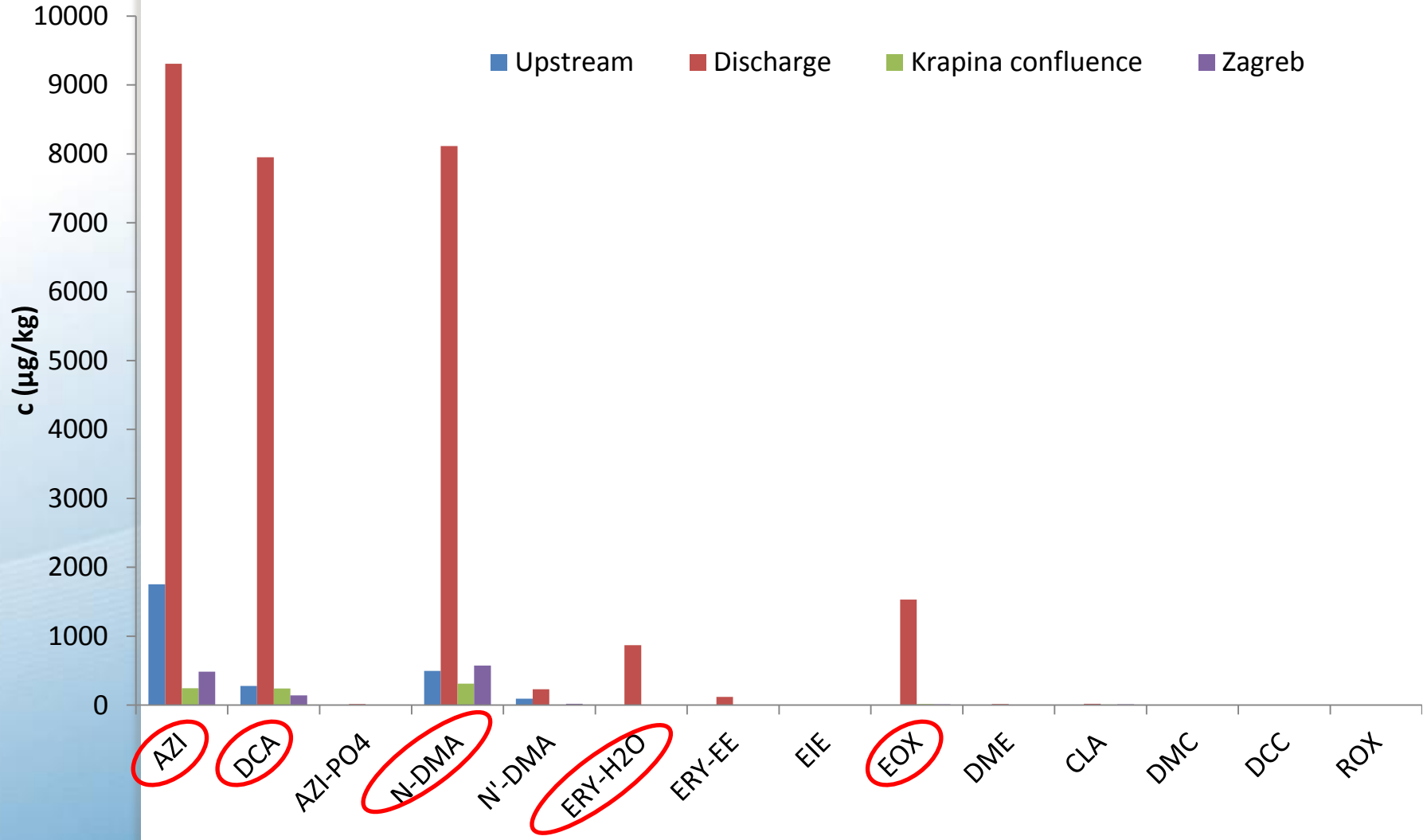
Compound	Recovery (%)	Matrix effect (%)	Accuracy (%)	MDL (µg/kg)	MQL (µg/kg)
DCA	91	0	111	2.1	6.4
AZI-PO4	11	5	15	0.7	2.2
N-DMA	81	3	94	2.0	6.0
N'-DMA	82	3	103	1.5	4.5
AZI	82	7	97	1.7	5.1
DCC	99	3	107	0.3	0.9
EIE	92	1	98	1.5	4.6
EOX	88	6	83	0.7	2.1
ERY-H2O	94	4	105	1.7	5.2
ERY-EE	54	1	55	0.3	0.8
DMC	91	-6	93	1.5	4.5
CLA	89	3	98	0.4	1.2
ROX	87	5	97	0.5	1.6
DME	99	6	93	0.3	0.8

Spiking level: 200 ng/g, n=4
MDL = method detection limit; MQL = method quantification limit

Application to real samples – suspended solids (WWTP of the city of Zagreb)



Application to real samples – sediments (Sava river)



Conclusions

- The developed method allows quantitative determination of an extended range of macrolide compounds in solid environmental samples
- Several synthesis intermediates and TPs were determined for the first time
- Some of them significantly contributes to the overall mass balance of macrolides
- Pharmaceutical industry facilities can be important source of sediment pollution with antibiotics and related compounds

Acknowledgements

- Croatian Science Foundation:
  - Project COMPASS (grant number IP2014-09-7031)
  - Project WINAR (grant number UIP-2014-09-9350)

- The staff of the wastewater treatment plant of the city of Zagreb

Thank you for your attention!

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