**Enantiomeric fractionation of metoprolol and its major transformation products for quantitative biodegradation measurement and elucidation of biodegradation mechanisms**

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The occurrence of pharmaceutical compounds residues and their transformation products in surface water as a result of their incomplete removal during sewage treatment is well documented. This is contrasted by the still limited knowledge on their fate in river downstream of wastewater treatment plant discharges. Due to the absence of quantitative tools, information remains scarcely available regarding the capacity of receiving rivers to self-purify pharmaceutical residues.

Consequently, this work aims to contribute to the development of enantiomeric fractionation as quantitative marker of biodegradation by using metoprolol, a widely dispersed chiral compound, as a probe compound. For this purpose, on the basis of a newly developed chiral analytical method, the stereochemistry of R,S-metoprolol and its major metabolites O-desmethylmetoprolol, α-hydroxymetoprolol and metoprolol acid was studied in biological wastewater treatment plants and in laboratory biodegradation batch experiments including nitrifying sludge and water / sediment system.

Stereoselective degradation of metoprolol was exclusively observed under biotic conditions, confirming the specificity of enantiomeric fraction variations to biodegradation processes. In laboratory and field studies, metoprolol was always transformed into metoprolol acid with a significant (S)-enantiomer enrichment of metoprolol. The results of enantiomeric enrichment pointed the way for a quantitative assessment of biodegradation processes due to a good fit (R2 > 0.98) of the aerobic degradation of metoprolol to the Rayleigh dependency in the different investigated degradation processes. This could be contemplated probably due to identical transformation mechanisms and kinetics of both metoprolol enantiomers. In particular, the linear relationship trend observed between metoprolol removal in wastewater treatment plants and enantiomeric variations of metoprolol between influent and effluent suggests that enantiomeric fractionation could be used as a relevant indicator of wastewater treatment plants biodegradation efficiency.

The stereochemistry of metoprolol and its metabolites could also teach on the biodegradation mechanisms of metoprolol. In contrast to human metabolism, the benzylic hydroxylation pathway of metoprolol did not occur upon environmental conditions and the O-dealkylation pathway was probably limited. Instead, a novel degradation route was suspected involving the formation of an unstable benzylic radical and vinyl ether intermediates which might account for the significant and specific stereoselective biotransformation mechanisms of metoprolol once entering in the environment.