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Enantioselective fractionation of fluoroquinolones in the aqueous environment using chiral liquid chromatography coupled with tandem mass spectrometry

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3 Erika Castrignanò¹, Andrew M. Kannan¹, Edward J. Feil² and Barbara Kasprzyk-Hordern^{1*}4 ¹ Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, United Kingdom5 ² Department of Biology and Biochemistry, University of Bath, Bath, BA27AY, United Kingdom6 * Corresponding author: E-mail: b.kasprzyk-hordern@bath.ac.uk; Fax: +44(0) 1225 386231; Tel:
7 +44 (0) 1225 3850138 **Abstract**

9 This paper aims to examine the multiresidue enantiomeric profiling of (fluoro)quinolones and their
10 metabolites in solid and liquid environmental matrices using chiral HPLC-MS/MS method and a
11 CHIRALCEL[®] OZ-RH column. Simultaneous chiral separation was obtained for chiral ofloxacin
12 and its main metabolites ofloxacin-*N*-oxide and desmethyl-ofloxacin; moxifloxacin; the prodrug
13 prulifloxacin and its active compound ulifloxacin; flumequine; nadifloxacin and *R*-(+)-besifloxacin.
14 Achiral antibiotics (ciprofloxacin, norfloxacin and nalidixic acid) were also included in the method
15 to enable the analysis of all targeted quinolones within one analytical run. Satisfactory enantiomeric
16 resolution ($R_s \geq 1$) was obtained for five out of eight chiral drugs enabling quantitative analysis.
17 The overall performance of the method was satisfactory with a method precision <20%, relative
18 recoveries >70% for most of the analytes and method detection limits (MDL) at low ng L⁻¹ levels
19 ($0.1 < \text{MDL (ng L}^{-1}) < 6.4$, $0.1 < \text{MDL (ng L}^{-1}) < 6.6$ and $0.1 < \text{MDL (ng L}^{-1}) < 7.0$ in influent,
20 effluent and river waters for 83% compounds, $0.01 < \text{MDL (ng g}^{-1}) < 4.9$ in solids for 91%
21 compounds). Enantiomeric profiling from a week-long monitoring campaign in the UK showed that
22 (\pm)-ofloxacin was found to be racemic in upstream waters but it was enriched with *S*-(-)-enantiomer
23 in wastewater and in receiving waters. This could be due to the fact that ofloxacin can be used both
24 as a racemate and as a *S*-(-)-enantiomer. Its consumption was further confirmed by the chiral
25 signature of the investigated ofloxacin metabolites. As a result, alterations in the enantiomeric
26 composition of antibiotics could influence not only their activity and toxicity in the environment,
27 but also could induce changes in the microbial communities constantly exposed to them.

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