

## Chiral HPLC-FD Method Validation for Determination of Several *Beta*-Blockers and Fluoxetine in Biodegradation Assays

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Chiral pharmaceuticals and the fate and effects of their enantiomers in the environment are still largely unknown [1, 2]. Enantiomers have different interactions with enzymes, receptors and any chiral molecules leading to different biological activities and affecting organisms in a different manner. Thus, biodegradation tends to be enantioselective in contrast to abiotic degradation. The methods developed to quantify the enantiomeric fraction in the environment and to follow biodegradation are scarce [3]. Thus in this work we describe the development and validation of HPLC methods that allow the enantiomeric separation of widely used drugs namely four *beta*-blockers: alprenolol (ALP), propranolol (PHO), metoprolol (MET) and atenolol (ATE) and the antidepressant fluoxetine (FX). The macrocyclic antibiotic vancomycin CSP (ASTEC Chirobiotic V 5  $\mu$ m) was used under polar organic mode (methanol:ethanol:triethylamine:acetic acid.50:50 v/v) and fluorescence detection for enantiomeric fraction quantification. The developed methods were established using a minimal medium inoculated with activated sludge as a matrix which is the condition used in the biodegradation studies. The vancomycin CSP was able to resolve ALP and PHO as well as MET, ATE and FX in two chromatographic runs. The chromatographic parameters obtained have shown the separation factor ( $\alpha$ ) between 1.12 and 1.34 and resolution ( $R_s$ ) between 1.30 and 4.35. The methods demonstrated to be selective and linearity with  $r^2$  higher than 0.999 for the range selected. The method detection limits were between 2.5 to 10 ng/mL. These methods were applied to follow the biodegradation of the target chiral compounds during 15 days. The biodegradation assays were performed using activated sludge from a WWTP and the results indicate the higher degradation extents for the *S*-enantiomer forms.

1. Kümmerer, K., J Environ Manage 90, 2354–2366 (2009).
2. Stanley, J. K and Brooks, B. W., Integr Environ Assess Manag 5: 3, 364–373 (2009).
3. Hashim, N.H., Shafie, S. and Khan, S.J., EnvironTechnol 31:12, 1349–1370 (2010).

**Acknowledgments:** A.R. Ribeiro acknowledges FCT, Portugal for the grant (SFRH/BD/64999/2009), QREN-POPH, European Social Fund and MCTES.

This work was also financially supported by CESPU (09-GCQF-CICS-09) and PTDC/EBBEBI/111699/2009



36th International Symposium on  
High Performance Liquid Phase  
Separations and Related Techniques  
Budapest Congress & World Trade Centre  
19–23, June, 2011 • Budapest, Hungary