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HPLC-MS/MS METHOD FOR QUANTIFICATION OF THE NEUROPEPTIDE Y Y1 RECEPTOR ANTAGONIST BIBP 3226 IN CELL EXTRACTS

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Neuropeptide Y (NPY) is involved in various physiological processes, including the regulation of feeding behavior and energy homeostasis. NPY activates different receptors in several brain regions. Recently, Y1 receptor (Y1R) has arisen as a potential regulator in the local control of bone turnover suggesting that an anti-receptor strategy may be a useful therapeutic approach to prevent and/or reverse bone loss. BIBP 3226 is a potent Y1R selective antagonist that has been successfully used in *in vitro* studies showing a positive impact in bone turnover and thus providing good perspectives towards its application as a pharmacological tool for bone regeneration. Hence, the major aim of the present work was to implement a method based on high performance liquid chromatography coupled to triple quadrupole-tandem mass spectrometry for quantification of BIBP 3226 in cellular internalization assays.

Chromatographic separation was achieved using a reversed phase Kinetex® core-shell C8 column at 30 °C and elution in isocratic mode using a mixture of acetonitrile and water (30:70, v/v), containing 0.1% (v/v) formic acid, at 0.25 mL min^{-1}. Total run time was 5.0 min, with retention time of 3.7 min for the target compound. The MS/MS was operated in positive ionization mode (ESI+) and data were acquired in multiple reaction monitoring (MRM) mode (*m/z* 474>167 for quantification and *m/z* 474>107 for identity confirmation). Calibration curves were linear for concentrations ranging from 0.5 to 30 ng mL^{-1}. BIBP 3226 was quantified in cell extracts obtained from internalization assays performed with bone marrow and breast cancer cells, after solvent evaporation and resuspension in mobile phase. LOD and LOQ were 0.04 and 0.1 ng mL^{-1}, respectively, corresponding to values as low as 0.3 and 0.8 pg per well.

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