**Introduction**

There is an increasing focus on the beyond Rule of 5 (bRo5) compounds [2] which are expected to modulate difficult-to-drug targets with a huge relevance in immunosuppression, treatment of infectious and viral diseases and cancer. Reliable determination of physicochemical properties is required for a successful design of bRo5 drugs, being lipophilicity one of the most important parameter [3]. HPLC is a widely used analytical technique to measure lipophilicity descriptors.

**Aims of the study**

Setting up a chromatographic strategy for the measurement of log P for bRo5 drugs. Determination of a chameleonicity index.

**Experimental method**

The RP-HPLC analyses were performed at 30°C with 20 mM ammonium/acetate at pH 7.0 and acetonitrile at 60%. The flow rate was 1.0 mL/min. We tested two columns: Supelcosil LC-ABZ column (Supelco, 5 cm × 4.6 mm, 5 μm particle size, 120Å pore size) and XBridge™ Shield RP18 (Waters, 5 cm × 4.6 mm, 5 μm particle size, 130Å pore size). We identified log k’60 as the best chromatographic index and named BRlogP our lipophilicity descriptor. We validate BRlogP with a dataset of 36 known neutral Ro5 compliant compounds [4]. Finally we determined BRlogP and ElogP [4] of nine bRo5 drugs.

**Results**

We verified that log k’60 X-Bridge endowed better pH stability than log k’60 LC-ABZ. We related log k’60 X-Bridge with published log P values and found a strong correlation (R²=0.93, Fig. 1) We refer to the value generated via this method as BRlogP. We used partial least squares regression (PLSR) coupled with block relevance (BR) analysis and multiblock PLSR (MB-PLSR) to verify that the balance of intermolecular interactions expressed by published logP values is about the same that than that expressed by BRlogP. (Fig. 3)

For nine bRo5 drugs we measured BRlogP and ElogP, which is one of the most known lipophilicity indices implemented in drug discovery. The relationship between BRlogP and ElogP for bRo5 drugs is very good but different from that found for the 36 Ro5 compounds (Fig. 2).

BRlogP and ElogP provide two diverse environments with different polarity due to the mobile phase composition and the extrapolation process required to obtain ElogP. This could impact the conformers’ population and thus log P value of bRo5 compounds which can behave as molecular chameleons (molecules which adapt their conformation to the environment).

Finally we found an excellent correlation (R² = 0.80) between the lipophilicity range (ElogP-BRlogP) and a potential chameleonicity index obtained as the difference in Polar Surface Area (PSA) calculated on the crystallographic conformers of the compounds [6].

**Conclusion**

BRlogP is a valuable and fast tool to experimentally access lipophilicity of neutral Ro5 compliant compounds. Combination of BRlogP with ElogP provides an experimental logP range that could be implemented in bRo5 drug discovery programs as a chameleonicity index.

**References**