Experimental Polar Surface Area: EPSA

In order for an orally administered drug to be efficacious it needs to have good absorption. One challenge in achieving this is getting the drug across the lipid bilayer membrane – as such, a method of determining this permeability is required as part of any design – make – test cycle.

During early-stage drug discovery programmes, permeability has traditionally been measured by artificial membrane permeability assays or by in vitro permeation using cell monolayers (Caco-2).

While these methods work well for molecules following Lipinski's Rule of five (Ro5)[1] they do not provide robust and consistent results for a variety of non-classical drug candidates, such as PROTACs or cyclic peptides.

Guimarães et al[2] showed passive permeability to be essentially driven by polarity, size, and lipophilicity and, for peptides, it is thought that the main obstacle for permeability is polarity. As such, having a way to accurately calculate the Polar Surface Area (PSA) of molecules that are 'beyond Ro5' in their properties is vital.

Existing methods for determining PSA, such as Topological Polar Surface Area (TPSA) calculation, doesn't take into account Intramolecular Hydrogen Bonds (IMHBs) and as such is not ideal for 'beyond Ro5' compounds which often contain them.

2014, Goetz et al[3], introduced a Supercritical Fluid Chromatography (SFC) based methodology to provide experimental results that could be directly related to permeability, referred to as Experimental Polar Surface Area (EPSA) which, unlike TPSA, does account for a IMBHs.

To confirm this, Goetz took 42 pairs of analogous compounds, one of which could form an IMHB and the other, not, and showed that in the low dielectric constant environment of SFC (which is conducive to IMHB formation) coupled with a stationary phase capable of forming hydrogen bonds such as Chirex 3014 (figure 1), molecules with an IMHB eluted earlier than the analogue without a IMHB (figure 2).

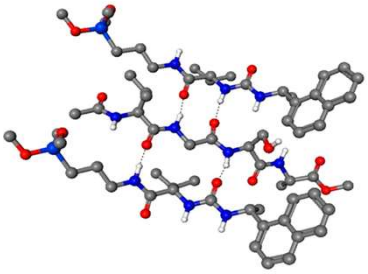

Figure 1 : Interaction model of an example tetrapeptide NAc-Val-Gly-Ser-Ala-OMe with two EPSA stationary phase molecules (Phenomenex Chirex 3014)[4]

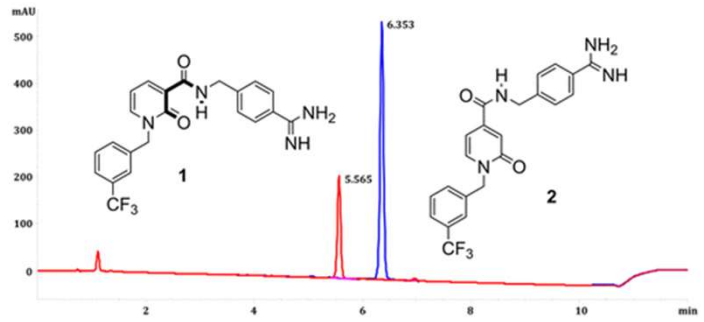

Figure 2 : Superimposed chromatograms of compounds 1 capable of IMHB formation (red trace) and 2 incapable of IMHB formation (blue trace)[5]

Consequently, EPSA is assumed to be a much better approximation of PSA than TPSA for molecules with IMHBs.

Reach Separations have now implemented an EPSA assay based on the SFC retention time of the target molecule and a calibration curve of known standards and their EPSA values.

To ensure robustness of our results, the calibration was performed again multiple times across three days, ensuring fresh preparation of solvents and standards in each case, the calibration curve (figure 3) shows the reliability of both the instrumentation and the data and we are confident that we can provide reliable data quickly and efficiently.

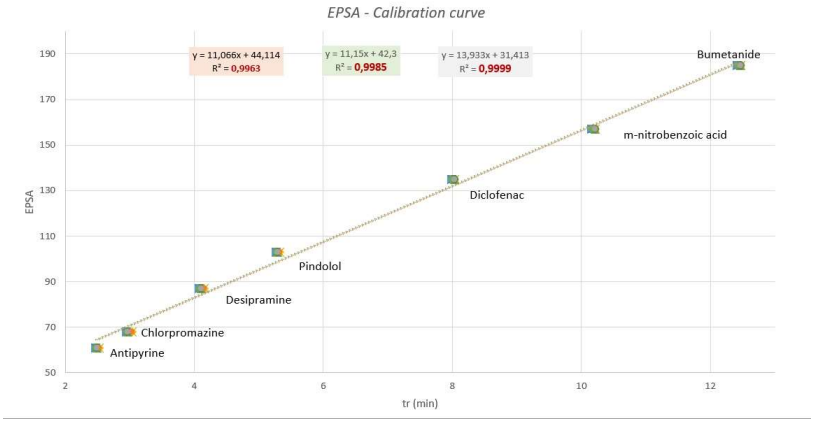

Figure 3 : Typical daily calibration curve of reference standards

1 Drug Discovery Today: Technologies. 2004, 1, 4, 337-341,
2 Journal of Chemical Information and Modeling (2012), 52 (4)
3 J. Med. Chem. 2014, 57, 7, 2920−2929
4 ACS Med. Chem. Lett. 2014, 5, 10, 1167−1172
5 J. Med. Chem. 2014, 57, 7, 2920−2929

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