A systematic approach to chiral screening and method development

Chromatographic separations occur when a combination of stationary phase and mobile phase provide an environment where selectivity between analytes with different chemical properties can be expressed. In reversed phase chromatography for example analytes are retained hydrophobically by the stationary phase, and then eluted in order of increasing hydrophobicity. When looking at enantiomers the chemical and physical properties are identical and therefore some form of chiral recognition is required in order to achieve a separation.

The mechanism for enacting chiral separations involves the formation of a diastereomeric complex, either between a mobile phase additive and the analyte of interest, or between the stationary phase and the analyte. Once formed it is possible to separate the enantiomers, either because the diastereomeric complex has different properties, or due to the stability of the complex formed, with one enantiomer being preferentially retained over the other. The use of chiral modifiers in the mobile phase, whilst possible is generally not adopted in HPLC (although is used in capillary electrophoresis) as the cost of such additives renders this option economically inviable in most cases. The types of chiral stationary phase utilised over the years include:

• **Pirkle phases.** Fully synthetic phases bases on functionalised amino acids, they provide chiral recognition by offering 3 points of interaction. Materials are generally bonded, so robustness is good. This type of phase generally exhibits low hit rates but offer good selectivity when they work.

• **Cyclodextrin based phases.** Fully synthetic bonded phases working on an exclusion principle with one enantiomer able to fit more precisely within the “chiral bucket” structure than the other.

• **Protein based phases.** Proteins such as BSA are coated onto silica, offering a broader range of chiral interactions, resolution perhaps not as good as for Pirkle or cyclodextrin phases but these columns offer a broader range of selectivity. Care needs to be taken with protein bases phases not to poison the phase, or to wash it off the silica support during use.

• **Ligand exchange.** The mechanism of ligand exchange uses penicillamine in combination with a mobile phase which contains copper II ions in a fairly specific way. The ions form complexes with amino acids allowing for them to be separated.

• **Polysaccharide bases stationary phases.** These are based on the naturally occurring chiral polymers cellulose or amylose, the polymers are derivatised to provide additional interactions. Depending on the solvents used the polymers swell to different degrees which creates “chiral pockets” within the structure allowing analytes partition in and out of effecting a separation. Recent developments to these have included the immobilisation of such phases allowing for a broader range of solvents to be used than could be previously. Solvents such as DMSO and DMF can be utilised which can be helpful when considering preparative separations where analyte solubility may be challenging.

Given the flexibility that polysaccharide phases offer by allowing normal phase, reversed phase, polar organic and SFC separations to be conducted with high efficiency, these phases have become the “go to” column choice for many users conducting chiral separations. The phases offer high hit rates which in turn increases the probability of finding a set of conditions what will provide an effective separation. Due to the complex nature of interactions between enantiomers and a chiral stationary phase it is not possible to accurately predict which stationary phase will provide a good separation. It is necessary therefore to screen different stationary phase and mobile phase compositions in order to test out the best combination for use in a separation. When screening our range of polysaccharide phases, Lux™, we screen all 3 modes normal phase, reversed phase and polar organic mode in tandem. Lux columns can also be successfully used under SFC conditions if users have access to this technology. We screen the following phases:

- Lux Cellulose 1
- Lux Cellulose 2
- Lux Cellulose 3
- Lux i-Amylose 3
- Lux i-Cellulose 5
- Lux Amylose 1
- Lux i-Amylose 1

Screening can also be conducted in LC-MS friendly conditions only if required. Precise details of screening can be found by viewing the chiral screening poster below.

Once screening has been completed it is then necessary to review the results obtained, taking the most promising one or two forwards for optimisation if required. This has been found to be the most effective way to develop chiral separations with good resolution and robustness.
