Branched PEG analysis

About 15 years ago, pharm-analyt specialised in PEG analysis, be it small linear PEGs of only 2 - 3 kDa or larger PEGs up to 100 kDa. The matrices we have analysed over the years have been complex such as plasma, urine and even human and animal tissues. However, cerebrospinal fluid and final drug product or bulk samples were also part of the repertoire.
Now a different PEG has been considered, a randomly branched PEG, which is said not to trigger an immune response in metabolism and is therefore probably safer than the linear PEGs commonly used in the past. As a refresher, over the last few decades, many APIs, especially proteins, have been pegylated to extend their half-life in the metabolism and thus make it much easier to treat patients, especially in the area of life-long treatments.

Both, HPLC-MS/MS analysis with either reversed-phase chromatography or size-exclusion chromatography (SEC) were used.
Branched PEGs in human plasma were investigated for linearity, recovery and possible LLOQ in the range of 3 to 30 kDa.

SEC showed difficulties with strong matrix effects when protein precipitation alone was used due to the presence of many peptides in the respective molecular weight range. However, when protein precipitation followed by liquid-liquid extraction was used, the results were quite good: The achievable LLOQs for the two smaller branched PEGs were around 30-50 ng/mL, for the larger branched PEGs around 15 ng/mL. Selectivity is slightly better compared to regular linear PEGs, because the fragment ions used in MRM detection are only from branched PEGs, not linear ones.

RP chromatography works well for both, protein precipitation only for sample preparation, or protein precipitation followed by liquid-liquid extraction. In the latter case, selectivity may be slightly improved. The achievable LLOQs for the two smaller branched PEGs were around 50-100 ng/mL, for the larger branched PEGs around 15 ng/mL were achievable.