Chemical Stability Assay

Background: Stability in aqueous solutions is a fundamental requirement to a successful drug candidate. Degradation may be caused by a variety of mechanisms: hydrolysis, oxidation, light-catalyzed degradation and others. In early stages of drug discovery, screening for stability in buffer solutions at acidic, neutral and basic pH is desirable for eliminating potentially troublesome candidates. Chemical stability analyses are often performed by HPLC with UV-Vis detection at three wavelengths. Yet, in some cases degradation products may be poorly separated from parent compounds, causing inaccuracy in analysis. For this reason, we recommend to perform this analysis by LC-MS. The assay is performed in a reusable 96-well Teflon plate (Millipore) to avoid possible artifacts caused by adsorption of certain compounds to polypropylene surfaces. Upon request, polypropylene plates can be used in the same assay setup for the non-specific binding assessment, which can be conveniently combined with the chemical stability assay, as PTFE (Teflon®) and polypropylene have different binding characteristics.

Service Details: Glycine buffer (pH 8 – 11), PBS (pH 7 – 8) and acetate buffer (pH 4-6) are used in this assay to cover main pH ranges used in ADME. Stock solutions at a concentration of 10 mM of the test compounds are prepared in DMSO and stored at -20°C. Working concentration of test compounds is 1-5 µM solution in DMSO and buffers. The compound solutions are incubated in experimental buffers at 37°C for specified time intervals. The sample aliquots are taken at 6 time points: 0, 60, 120, 180, 240 and 300 min. To stabilize the samples prior to HPLC-MS analysis, they are stored at -25°C in 66% methanol/33% buffer, covered with adhesive sealing film. All samples are analyzed by HPLC-MS in a single batch within 8 hours after collection. The HPLC-MS measurements are performed using Shimadzu VP HPLC system coupled with tandem mass spectrometer API3000 (PE Sciex). Data acquisition and analysis are performed using Analyst software (PE Sciex). In this assay, propantheline is used as a quality control. This compound is stable at acidic pH, slightly unstable at pH 7.4 and unstable at pH 9.4.

Deliverable: Stability is calculated as % test compound remaining relative to the T=0 peak area. Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 µmol) or 50 µL of 10-20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, depending on the particular project. We do not need to know structures of the molecules for ADME testing. However, brutto formulas have to be provided for all studies involving MS detection.