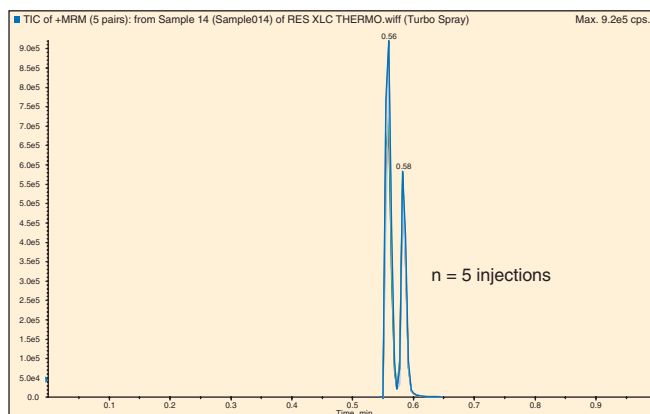


Optimal High-Speed LC-MS Separations Using JASCO χ -LC[®] Extreme Pressure HPLC system

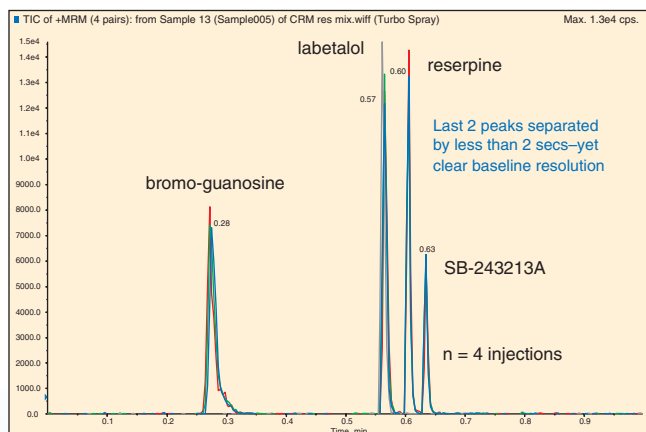
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As part of an evaluation of ultra-high flow liquid chromatography (UHPLC) a number of chromatographs (Agilent 1200, Flux Rheos Allegro, Waters Acquity and JASCO χ -LC) and small particle stationary phase columns (Waters Acquity BEH-C18 1.7 μ m, Zorbax Eclipse XDB-C18 Rapid Resolution HT 1.8 μ m and Thermo Hypersil Gold C18 1.9 μ m) were investigated in the Drug Metabolism and Pharmacokinetics department, Respiratory and Inflammation Centre of Excellence for Drug Discovery, GlaxoSmith-Kline, Stevenage, Herts, UK. Among the key parameters in the evaluation process were resolution and reproducibility of separation. These were investigated by repeated injections of (i) a mixture of two closely structurally-related analogues from a discovery programme which for proprietary reasons can only be identified as GSK A and GSK B (resolution test A), and (ii) of a test mix containing 4 components of differing chemical and physicochemical properties (resolution test B).

The greatest success, in terms of peak shape, separation and reproducibility of retention times was achieved using the JASCO χ -LC and a Thermo Hypersil Gold 1.9 μ m column. For resolution test A, the two compounds can be virtually baseline separated using a flow rate of 1mL/min and a gradient starting at 80% 0.1% w/v (aq) formic acid : 20% 0.1% w/v formic acid in acetonitrile. This mobile phase composition is held for 0.1 minutes before rising linearly to 95% 0.1% w/v formic acid in acetonitrile by 0.4 minutes. This composition is held until 0.7 minutes before returning to the initial composition by 0.8 minutes and re-equilibrating until a total of 1 minute. The two components GSK A and GSK B were separated by only 0.02 minutes yet were virtually baseline resolved due to the very narrow peak widths (<2 seconds at base) and high peak symmetry. Retention times were extremely consistent, coefficient of variation < 0.8% (equivalent to <0.3 seconds) indicating that a) the re-equilibration time was sufficient, and b) that the solvent delivery system was delivering highly accurate and reproducible gradients. See Figure 1.



Reproducibility of separation of 2 GSK compounds separated by only 1.2 secs using JASCO χ -LC and 5- x 2.1mm 1.9 μ m Thermo Hypersil Gold C18



Reproducibility of separation of a drug-like test mix using JASCO χ -LC and 5- x 2.1mm 1.9 μ m Thermo Hypersil Gold C18

For resolution test B, the four compounds are baseline separated using a flow rate of 1mL/min and a gradient starting at 97% 0.1% w/v (aq) formic acid : 3% 0.1% w/v formic acid in acetonitrile. This mobile phase composition is held for 0.1 minutes before rising linearly to 95% 0.1% w/v formic acid in acetonitrile by 0.4 minutes. This composition is held until 0.7 minutes before returning to the initial composition by 0.8 minutes and re-equilibrating until a total of 1 minute. The last two components, reserpine and a proprietary GSK compound, were separated at the peak apexes by only 2 seconds yet were baseline resolved due to the very narrow peak widths (<2 seconds at base) and high peak symmetry. Retention times were extremely consistent, coefficient of variation < 0.8% (equivalent to <0.3 seconds) indicating that a) the re-equilibration time was sufficient, and b) that the solvent delivery system was delivering highly accurate and reproducible gradients. Repeated injections yielded virtually superimposable chromatograms. See Figure 2.

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