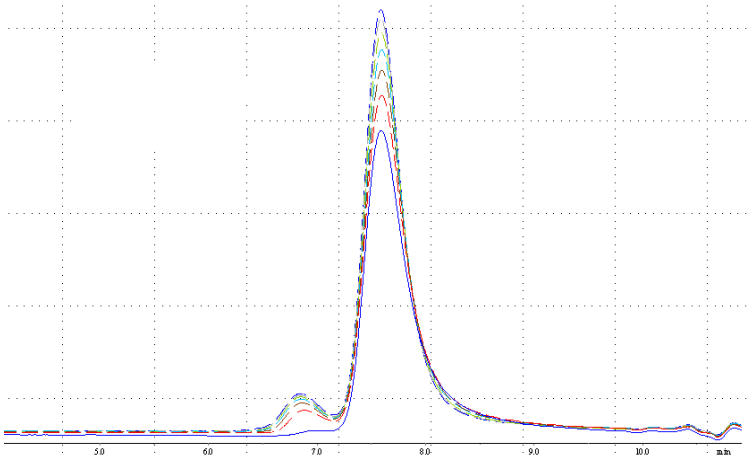
Column Conditioning

Column conditioning is a step apart from general column equilibration where a property of the column itself is modified, whereas in column equilibration, the solvent composition throughout the column is the primary consideration.

The use of ion-pair reagents is a common example that can fall in a grey area between column equilibration and column conditioning. For example, alkylsulfonate ion-pair reagents on C18 columns are very common, and since they are in the mobile phase, often considered as part of general equilibration. However, as they may be difficult or impossible to completely remove from the column once introduced and embedded into the stationary phase, the selectivity can be irreversibly altered. This is not necessarily a problem if it is controlled and consistent, and is why dedicating particular columns for use with such ion-pair conditions is recommended. Otherwise it can often be identified as a root cause to some irreproducibility troubleshooting cases on columns where the history of usage is unclear.

Generally, column conditioning is not recommended, as such changes to the column are not necessarily considered by the column manufacturer. Some common cases, such as the implementation of ion-pair reagents, are usually not a problem as long as their conditioning properties are thoroughly examined and implemented consistently on new columns.

Another common case could be with commonly adsorbed compounds, such as proteins. Best is to avoid the necessity to condition a column for such by minimizing the possible adsorption with appropriate pHs and buffers in the mobile phase, however when otherwise unavoidable, conditioning injections can often be an easy practice to implement to overcome adsorptive recovery loss (especially for non-specific or uncharacterized mechanisms).



By sequentially injecting samples as part of column conditioning (before system suitability is initiated), the binding sites can be saturated so as to avoid adsorption on subsequent injections. The level of saturation of the binding sites can be monitored by the peak responses of the conditioning injections, and considered saturated once the response (of the same sample preparation/vial) increases to a consistent level.

It will also be important to confirm that subsequent blank injections do not exhibit carryover effects (not illustrated here).

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Author: Scott Krepich