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Accomplishing near real-time HPLC for bioprocess control

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PD strategies are moving toward performing large numbers of in-parallel experiments, with the collection of much real-time process and product data. Such initiatives as personalized medicine and biosimilars also drive the need to know more about entity CQA as early as possible. In PC, more near-real time CPP and even product attribute values are being considered as control parameters. This all leads to the demand for rapid and comprehensive process parameter and product attribute measurements. Modern chromatographic columns and systems now provide rapid information on aggregation by SeC, charge variants by IeX, intact mass, primary structure, many post-translational modifications by reversed-phase and cleaved glycan analysis by HIC. Finally, required HPLC validation parameters (specificity, linearity, accuracy, precision, limits of detection and quantification) are being established supporting complient, absolute quantification methods for recombinant proteins. Two recent initiatives are enabling both the development and practical application of such heightened bioprocess knowledge to process control. Commercialized validated systems support the cell-free auto sampling, flowpath and destination control of in-process bioreactor samples to any number of analytical instrumentation. And, such hard- and soft-ware computer advances as Solid State Drives (SSD), cloud computing, and Big Data methods now support the processing of any number of input data through advanced statistics, mathematics or logic-enabled enterprise control algorithms.

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Possibility of fast separation with simultaneously high resolution of small solutes under gradient elution in RPLC

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Fast separation of substances under isocratic elution in HPLC always accompanies sacrificing the substance resolution by HPLC. It was recently found that the retention of intact proteins in HIC and that of peptides in RPLC under gradient elution are totally governed by two variables of steady-region (SR) which is dominated by multiple molecular interactions (MMI) and migration region (MR) which is dominated by partition coefficient. In this study, when the small solute of n-alcohol benzene homologue was separated on a RPLC column under gradient elution by non-synchronous sampling, the retention of the homologue was found to have the character of SR and MR. As chain length N of the homologue is greater than a certain value, and the longer the chain length is, the longer the retention time in the SR will be. However, the ratio of the retention time in the SR to that of the MR decreases with increasing N. The same result was also found to be valid as mobile phase consisting of water-five organic solvents of acetonitrile, methanol, ethyl alcohol, n-propanol, and iso-propanol. This fact indicates that the formation of the SR here sources mainly from one type of molecular interaction of dispersion force of the homologue to the RPLC stationary phase, but rather the MMI, molecular size, and the type of mobile phase which occur in the circumstance of the separation of proteins and peptides. In other words, so long as the interaction force between solute and stationary phase is high enough i.e., N value here is large enough, the two variables governing solute retention under gradient elutionin LC is a universal existed rule. For further intensive investigation of the SR feature by stoichiometric displacement theory (SDT), the energy (affinity) of the homologue in SR was found to be 5.22 folds of that in MR, or the stoichiometric displacement equilibrium constant of the former is 105.2 folds of the latter. Thus, small solutes, have large enough non-polarity here, like biopolymers, can be also employed for fast separation with simultaneously high resolution under gradient elution with a very short column. The SR may be also as an operation space for carrying out fast on-line two dimensional LC separation by means of on-line manner. A short RPLC column (25 x 4.6 mm I.D) and mobile phase of methanol-water were employed to separate the mixture solution of the homologue under one minute gradient elution with flow rate 10.0 mL/min, each of the existed homologues of N from 1 to 10 was totally and completely separated with only 1.5 min.

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