

ChromSword 

**Most Intelligent
Automated**

HPPLC

**Method Development
Solutions**

Genore
chromatografia

ul. Dynarska 1/23, 01-493 Warszawa
tel. 22 40 107 34 do 35, fax: -36
e-mail: info@genore.pl
www.genore.pl

Used by top pharmaceutical companies

Automated Development of a Fast HPLC Method for Reaction Monitoring

Michael Pfeffer, Chemical Development, Bayer Schering Pharma, Berlin, Germany.

For the production of drug substances, chemical processes are monitored at each chemical step, to guarantee optimum quality and yield. To be able to act as soon as possible and to finish the chemical process successfully, analytical methods are required, yielding reliable results rapidly. Method development software integrated with HPLC instruments and short columns enable a rapid and unattended separation of components of reaction mixtures.

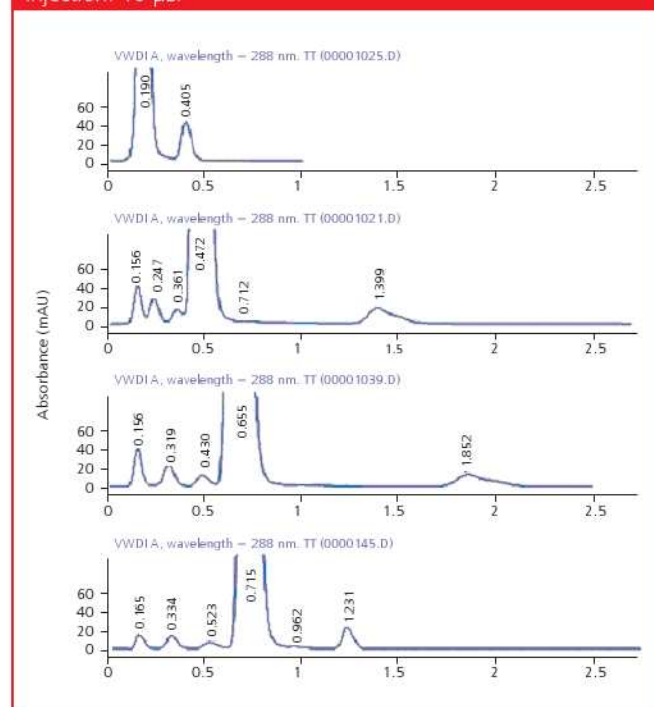
This application note describes one example how fast methods can be developed for monitoring chemical processes. The goal was to obtain an acceptable chromatographic resolution of the relevant peaks (i.e. the educt) by means of a conventional HPLC instrument and, furthermore, chromatographic separations lasting no longer than 5 min. The mobile phase was going to be optimized using ChromSwordAuto method development software.¹ A very short column (e.g. YMC-Pack ProC18 RS) was used in combination with a quite high flow-rate. The development should be operated overnight unattended.

The result obtained the following day was respectable. The method development report has already been assembled automatically. The HPLC system controlled by ChromSwordAuto only needed 4 hours for the development. The sample solution was injected for isocratic and gradient elution. Some of the chromatograms are shown in Figure 1. The system proceeded the optimization of the retention and to improve the resolution of peaks by changing the composition of the mobile phase step-by-step. The chromatograms picked out of the whole ChromSword sequence demonstrate how the compounds were separated by the short column little by little. Finally, educt (RT = 1.231 min) and product (RT = 0.715 min) were separated excellently. The peak shape was improved by applying gradient elutions. Three other polar compounds were separated very well lasting, strictly speaking, less than 2 min. The YMC-Pack ProC18 RS short column turned out to be rather stable and robust enough to withstand the high flow-rate continuously.

This approach of automatic method development using very short columns combined with a high flow-rate provided by a conventional HPLC instrument proved to be very effective. The equilibration time was minimal, the dead volume of the instrument did not play any role. The method met the requirements and could be allocated with minimal efforts in time.

At the very end the most important thing: The customers are satisfied. They get the analytical results without inadmissible time delay. Decision making for the conduction of the production process can be made rapidly and with minimal risk for quality and yield of the drug.

Figure 1: A couple of chromatograms picked out of a ChromSword sequence for unattended HPLC method development. (The lowest chromatogram represents the final HPLC method.) Column: YMC Pro C18 RS 3 µm, 20 × 4.6 mm. Mobile phase A: Water containing 0.1% (v/v) formic acid, B: Acetonitrile; Gradient: 10% B to 20% B in 2 min; 25 °C; Flow-rate: 2.0 mL/min. Detection: 286 nm; C: 1 mg/mL; Injection: 10 µL.



Reference

1. www.chromsword.de.



Bayer Schering Pharma

D-13342 Berlin, Germany

tel. +49 30 468 1433 2

E-mail: michael.pfeffer@schering.de