Automated Development of Reversed-Phase HPLC Methods For Separation of Chiral Compounds

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Software for automated method development was applied for optimisation of chiral separations in reversed-phase HPLC. The strategy includes automated screening of column/eluent combinations, rapid optimisation and robustness studies to determine design space of the method according to the quality by design concept.

Introduction

Determination of enantiomeric purity of lead compounds, their starting materials and intermediates are important tasks in drug discovery and drug development. HPLC is the most widely used technique for determination of enantiomeric purity of compounds but method development for chiral separations can be a resource intensive process. Since scientists often deal with novel molecules, historical information is often of limited use. Typically chiral method development involves screening a group of columns and a set of mobile phases to identify promising starting conditions and then optimisation of mobile phase compositions and gradients to meet the requirements for the analytical or preparative separation of isomers. The Quality by Design (QbD) approach for method development requires additional knowledge collection about a sample to provide increased robustness of methods (sample and method profiling). Implementation of an automated HPLC method development strategy with ChromSwordAuto® software enabled chromatographers to significantly reduced the time required to find optimal separation conditions for complex mixtures of pharmaceutical drug candidates [1] and stereoisomers samples [2]. ChromSwordAuto® software has been reported previously for chiral method development in normal phase mode [3]. Normal-phase liquid chromatography is widely used for the separation of enantiomers on different chiral columns [4].

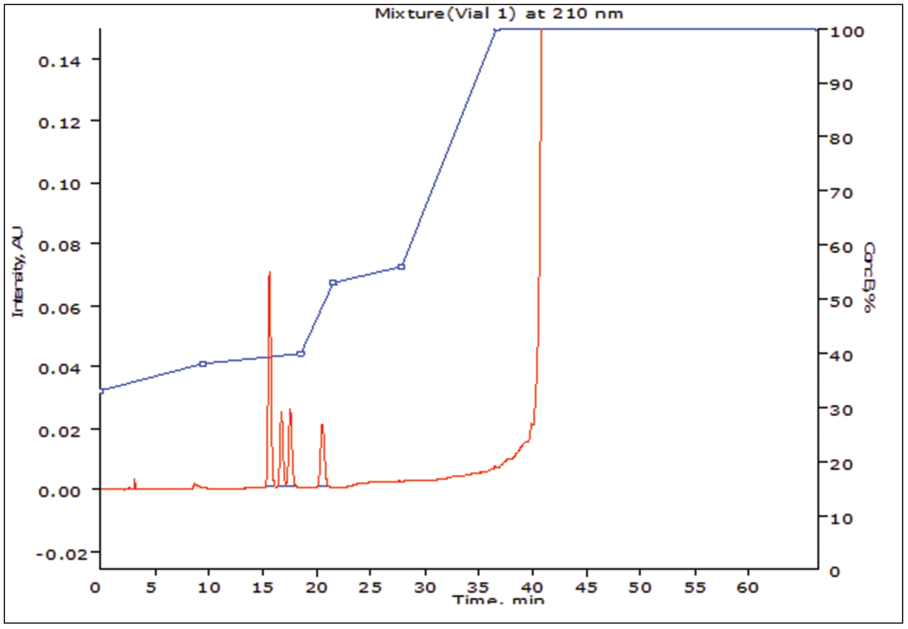

Figure 1. Separation of four isomers that ChromSwordAuto optimised automatically with Agilent 1200 LC system. The resolution (Rs) is 2.2, 1.6 and 4.6 for three peak pairs correspondently

However, for highly polar and ionic samples, normal phase chromatography has only limited application [5]. For aqueous-organic mobile phases separations, especially when pH flexibility is required, reversed-phase liquid chromatography is usually used. In this article we describe the application of automated method development technology for the optimisation of enantiomer separation in reversed-phase liquid chromatography. An integrated solution based on ChromSwordAuto method development software and HPLC instrumentation has been used for separation of 10 different racemic mixtures.

Experimental

Instruments

Agilent 1100 and 1200 HPLC systems with quaternary pump, diode array detector, autosampler, two thermostated column compartments, external 6 columns selector and external 12 positions solvent selector were used in this work.

Chromatography data system.

ChromSwordAuto® 4 enables control of Agilent LC instruments via Agilent ChemStation, EZChrom, Waters Empower and Thermo Scientific Chromeleon chromatography data systems (CDS).

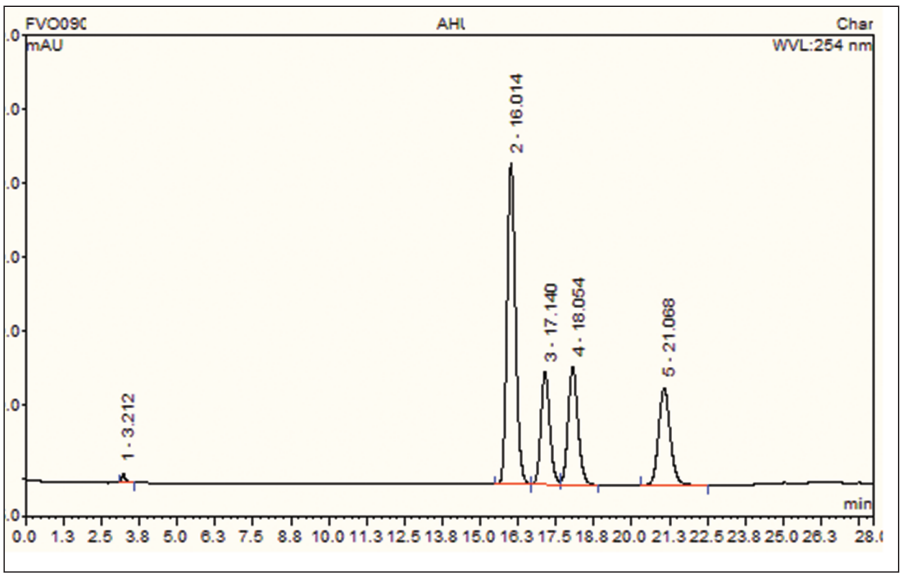

Figure 2. Reproducing a method optimised automatically with Agilent 1100 instrument with Agilent 1100 system. Rs is 2.2, 1.7 and 4.6 for three peak pairs correspondently

However non-Agilent CDS have limitations in controlling different combinations of Agilent internal and external column or solvent switching valves and thermostatted column clusters. ChromSwordAuto® 5 controls the Agilent instruments via the Agilent Instrument Control Framework (ICF) and has no such limitations. ChromSwordAuto® 5 is an integrated method development data system and does not require any other CDS to control instruments and acquire data.

Method Development Software

ChromSwordAuto® software for computer-assisted and automated HPLC method development.

AutoRobust – software for automatic robustness studies of HPLC methods

Columns and mobile phases

Derivatised polysaccharide columns are the most widely used phases for chiral HPLC separations due to their broad range of application. In our work the following Daicel columns for RP-LC were used: CHIRALPAK® AD-RH, CHIRALPAK®AS-RH, CHIRALCEL® OD-RH and CHIRALPAK® OJ-RH. These phases have the same coated chiral selector as the normal-phase columns but are coated to a hydrophobic high quality silica support. They are suited for applications with samples that require flexibility in terms of pH range.

Mobile phases

For neutral and acidic analytes:

Water / acetonitrile (ACN) or 0.1% H3PO4 pH 2 in water / acetonitrile

For basic analytes:

Potassium hexafluorophosphate as an additive to mobile phases is used in HPLC to improve peak shape for basic analytes [6]. It is also recommended by the columns manufacturer for these chiral columns [7]. In this application the following mobile phases were used:

25 mM KPF_6 adjusted to pH 5.5 with H_3PO_4 / acetonitrile or 25 mM KPF_6; 20 mM $(NH_4)_2HPO_4$ pH 7.7/ acetonitrile and the borate buffer pH 9.0/ acetonitrile (for Chiralpack columns).

Results and discussions

Strategy of Automatic Method Development

ChromSwordAuto® and AutoRobust support four modes of method development – screening, rapid optimisation, fine optimisation and robustness tests [1-3, 8]. In the screening mode the program performs a plan testing different columns and mobile phases with user predefined methods. In the optimisation modes ChromSwordAuto® communicates with the HPLC instrument to make real-time decisions based on resulting chromatograms, and can continue to create and execute methods until the desired separation is achieved, without operator supervision. The program collects and evaluates data from finished runs, builds appropriate retention models of analytes and searches for optimal isocratic or gradient methods automatically. In the robustness test mode the program starts from a method to be tested and automatically creates and performs a series of runs to study the effect of method variables on separation. Combinations of these modes enable a chromatographer to apply different strategies for method development and optimisation. The strategy described in [3] includes rapid optimisation for different combinations of column and eluent with the following fine optimisation of the best combination. Authors [1,2] have applied another strategy which involves: (1) the automated screening of various column and mobile phase combinations, (2) rational selection of the best starting conditions; and (3) subsequent automated fine optimisation to generate optimised separation methods. In our approach the system was specified to start with a user defined number of initial screening experiments with the following rapid optimisation steps using the built-in software intelligence to find good analysis conditions rapidly. For final methods automatic robustness studies were performed exploring effect of method variables on resolution of target compounds to determine the applicability range (method profiling) according to the quality by design concept.

The approach was applied for the enantioseparation of ten racemic mixtures. Results for the AHU377 compound (structure is confidential) with two chiral atoms in the molecule as an example are described below.

The automated method development procedure contained three steps – screening, rapid optimisation and robustness tests with automated report generation for the every step.

Step 1. In this screening step, experiments were automatically planned and performed with a total screening time of 1.5 hours. In the screening mode 16 combinations of column and mobile phases were tested with one gradient run (10-80% ACN, gradient time 15 min., 30°C). After this stage the Chiralcel OJ-RH column with 0.1% H_3PO_4, pH2 was found as the most promising condition for the following optimisation.

Step 2. In this step the ChromSword algorithm for Rapid Optimization was performed with 4.0 hours total optimisation time. In this automatic optimisation mode ChromSwordAuto® performs three runs. From this, suitable isocratic, linear or multi-step gradient conditions can be rapidly found. Two organic solvents (ACN and methanol, MeOH) were automatically tested in a total of 6 runs. In Figure 1 the chromatogram and the gradient profile that the system found are shown.

Step 3. Automatic robustness tests. AutoRobust supports different designs of experiments (DOE) including one parameter at a time, full factorial design

and statistical DOE [9]. Two types of design of experiments for automatic robustness studies with AutoRobust were used. The first included, a one parameter at a time design, to test effects of temperature (T), flow rate (F), concentration of gradient profile nodes (C), time of gradient profile nodes (Gt), injection volume, equilibration time and wavelength. After performing these tests, several critical method parameters were identified – temperature, flow rate, concentration and time of gradient profile nodes. All possible combinations of these method variables were tested with a full factorial design in 2 levels with +/- of 10 % of the basic method variable variation. The design plans are created automatically and avoid human factors as a possible source of error. As a result 2D (C-F, C-T, C-F, T-F, Gt-T, Gt-F) and 3D (C-F-T; Gt-F-T) resolution maps were built to find conditions for the stable method. For an additional test, the method was reproduced with another type of instrument – an Agilent 1100. The results (Figure 2) illustrate that the method has good selectivity and resolution to separate the target compounds with different type of HPLC instruments.

Conclusions

Reversed-phase HPLC is a good alternative of the normal-phase mode for chiral separations. Chiral reversed-phase LC can be utilised for impurity profiling where short method development time and flexibility of methods are required during the drug development process. ChromSwordAuto® screening with rapid optimisation are powerful approaches for development of chiral methods. The technology allows a user to screen and optimise automatically many different conditions in a short period of time. It also allows a user to obtain important information about a sample, critical parameters for peak shape and resolution, and to find the best available separation conditions. Automated method development with ChromSwordAuto® software and method development instruments significantly reduces the time and effort to find optimal separation conditions.

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