Automated HPLC Method Development and Robustness Tests for a Mixture of Hair Dyes Employing Two Different Instruments

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A computer-assisted and automated HPLC method development procedure has been described. The procedure involves columns scouting, gradient optimisation, robustness studies, and method improvement stages to provide a method compatible with HPLC and UHPLC instruments. The method development of a mixture of nine hair dyes using ChromSwordAuto method development software and Thermo Scientific and Agilent HPLC/UHPLC instruments was used as an example of the procedure.

Introduction

HPLC method development (MD) is a process for finding conditions to separate a mixture of compounds. Methods for quality control of products should fulfil prerequisites for robust, accurate, and precise HPLC analysis. Optimisation of an HPLC method requires time and effort, even for highly skilled analysts. Method development (MD) can start as a 'trial and error' process with one variable at a time strategy (OVAT) or applying experimental designs (ED) with changing several variables at a time. The most common ED approaches are central composite, Placket-Burman, and Factorial designs [1,2]. Such approaches aim to reduce the number of experimental runs, resulting in less time, solvents, and money spent on method development [3]. Any method development approach can provide reasonable results only in the case of columns that provide a suitable mechanism for the separation to occur. A screening segment is frequently used to find promising stationary (SP) and mobile phase (MP) combinations that provide good efficiency and selectivity of separation of target solutes. Combining this stationary and/or mobile phase screening with computer-assisted simulation and optimisation enhances the pace of the process. In this way, the optimum conditions can be reached based on two or more initial chromatographic runs utilising retention behaviour modelling [4,5]. The optimal method to do this approach is for the software to take control of the whole process and allow for the modelling and optimisation to be performed automatically by the software. This includes the peak tracking, determination of retention behaviour, peak purity, application of the Monte Carlo stochastic simulations, and built-in MD guidelines for prediction and optimisation of chromatographic conditions [6]. In this way, the time an analyst spends controlling the instrument, analysing the results, and building retention models is much less than using screening and then off-line simulation and optimisation.

This article will explain a straightforward and time-saving automated method development procedure using the ChromSwordAuto software package. To demonstrate the process a mixture of hair dyes was used as a case study. The modern trend with hair dyes is to use several colours to produce streaks or gradations. Therefore, quality control methods should be able to separate several dyes in one composition. LC instruments from different manufacturers can be used to reproduce the same method, and both HPLC and UHPLC instruments are included in the method development. The procedure includes screening, optimisation and robustness test steps. Optimisation results are improved with the statistical design of experiments (DOE) and 1, 2 and 3D resolution maps. This type of approach can efficiently be applied to develop analytical methods for different samples across all industries.

Experimental

Instrumentation

The HPLC method development for the mixture of nine dyes was performed using Agilent 1290 Infinity II instrument (Waldbronn, Germany) consisting of a quaternary pump, diode array detector, auto-sampler and column compartment with a build-in 8 positions column switching valve and Thermo Scientific Ultimate 3000 instrument (Germering, Germany) consisted of DAD-3000RS diode array detector, TCC-3000RS column compartment with 6 positions column switching valve, WPS-3000RS auto-sampler and HPG-3400 high-pressure gradient pump.

Chromatography data system

- Agilent Instrument Control Framework (ICF) A.02.05 package with LC drivers A.02.18.
- ChromSwordAuto 5.1 method development CDS, containing ChromSwordAuto Scout, Developer, AutoRobust, and ReportViewer modules which support different method development tasks.
- Chromeleon 7.2. SR 4 to control Ultimate 3000 instrument.

Columns

The method development screening procedure was carried out using ACE ChromSword advanced and extended method development kits (Theale, UK). The kits contain six columns, all 3 microns in particle diameter, with different

silica properties and selectivity: ACE Excel3 C18, ACE Excel3C18-PFP, ACE Excel3 C18-amide, ACE Excel3 C18AR, and ACE Excel3 SuperC18. All 10 0mm x 4.6 mm columns were installed into the column compartment with 6- and 8-positions column switching valve.

Mobile phases

Fresh ultrapure water (MilliQ) with 0.1% Trifluoroacetic acid (TFA) (v/v) was used in the channel B and acetonitrile (ACN) with 0.1% TFA in channel A to create a binary gradient condition for the separation of the dye samples

Sample

The mixture included the following dyes: HCRed, HCBlue 11, Moon Light Blue, Arian Mad Red, Stroh Gelb, Arrian Sien Brown, Basic Violet 2, Disp Violet 1, Acid Violet 43. Compounds contain aromatic rings with other functional acidic and basic groups (Figure 1). The chemical structure of some dyes is confidential.

Experiment design for Optimisation of Chromatographic conditions

The ChromSwordAuto Developer module supports different method development tasks. When a user chooses a task, the module automatically creates sequences, executes them, and processes results and spectra, when spectra are available. To perform column screening and gradient optimisation, the "Rapid Optimisation" task was chosen in the ChromSwordAuto Developer. The task was specified to screen and search for optimal conditions in the full range (0-100%) of concentration of an organic modified for each column with flow rate 1 ml/min and temperature 30°C as initial conditions.

For method improvement and robustness testing, the ChromSwordAuto AutoRobust software module was used. The module automatically creates a design of experiments with OFAT or multi-variate design and executes it as a sequence of runs and processes the results to determine critical method attributes and create 1,2, and 3D resolution maps.

The ChromSwordAuto Scout module was used to determine the peak elution order.

Results and discussion

The method development using the ChromSwordAuto Developer rapid optimisation task provides two essential modes - screening and method optimisation [6]. Dyes have chemical structures including aromatic and heterocycles with basic and

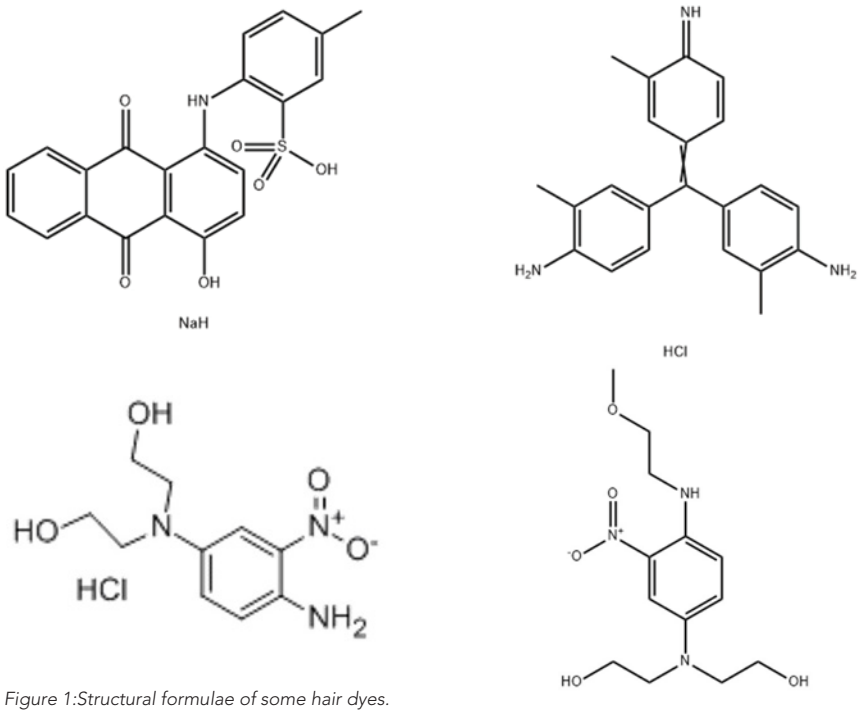

Figure 1:Structural formulae of some hair dyes.

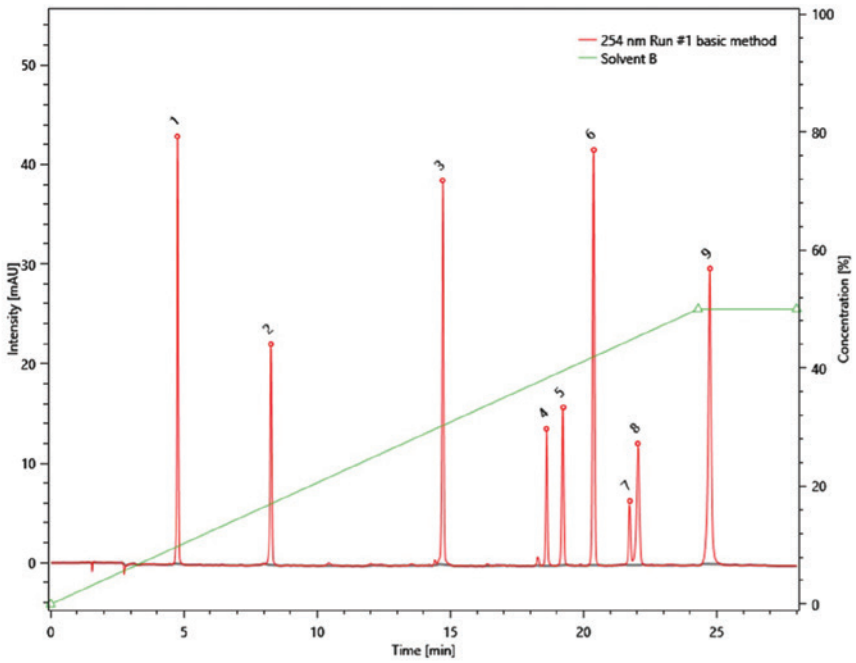

Figure 2: Chromatogram of a mixture of 9 hair dyes after the rapid optimisation. Instrument: Ultimate 3000 RS. Column: ACE Excel3 C18-amide, temperature 30°C, flow rate 1.0.ml/min, gradient: 0 min-0%, 24.3 min – 50%, 28 min – 50% of acetonitrile in a MP.

acidic functionalities and stationary phases with different properties should be tested to find a practically useful column. Each selected column was screened by the software automatically applying the initial optimisation run, which depends on the column volume and the flow rate used. The Ultimate 3000 instrument was used for the screening and rapid optimisation experiments. The screening results yielded a clear view of the column efficiency and selectivity. Excel3 C18-AR had shown broad peaks present, while ACE Excel3 C18-amide and SuperC18 columns gave the most promising results. Further, the software automatically evaluated the peak spectral purity, identified the peaks, and built the primary retention models that were used for further optimisation. To build a retention model of compounds the software applies the linear-solvent-strength and more complex non-linear models. While the unattended rapid optimisation performs three to five runs, the retention behaviour model is improved, and the resulted runs conclude to the potentially optimum gradient separation at selected conditions. The column ACE Excel3 C18-amide was selected for further experiments for method transfer, method improvement, and robustness testing (Figure 2.). The choice was based on the peak width, the number of peaks separated, near baseline resolution for separation of a critical pair (Rs = 1.5 - 1.6),

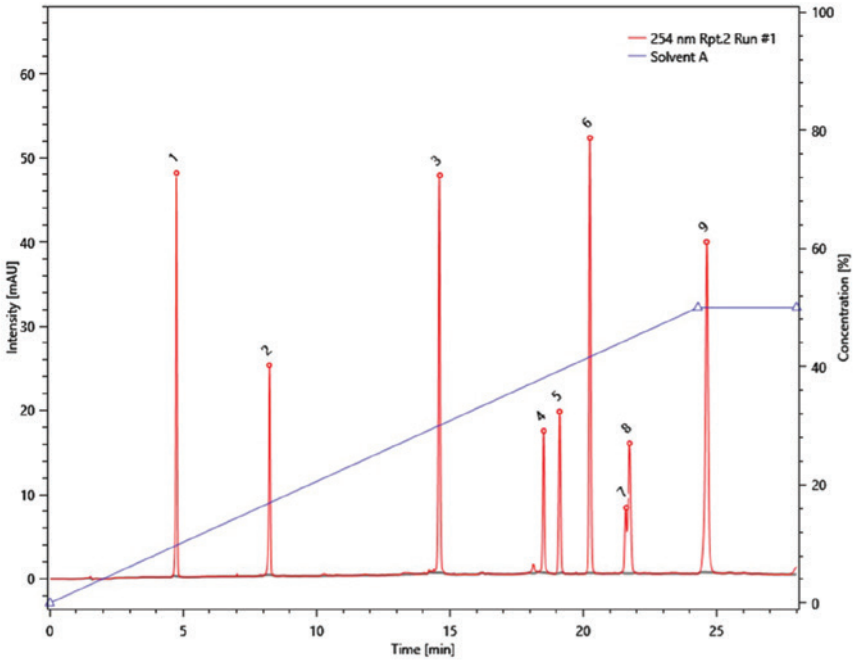

Figure 3: Chromatogram of a mixture of 9 hair dyes after the rapid optimisation.

Instrument: Agilent 1290 Conditions as described in Figure 2

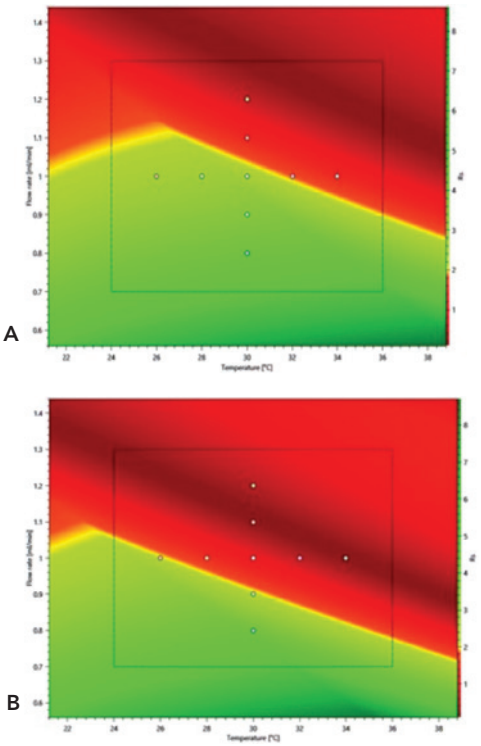

Figure 4: Resolution maps for effects of the temperature and the flow rate .

(A). Ultimate 3000 RS instrument. (B) – Agilent 1290 Infinity II. Conditions at the central squares are described in Figure 2.

The large blue square positions: the flow rate 0.8 ml/min and the temperature 26°C

method run time, and the gradient profile. However, reproducing the method on the Agilent 1290 Infinity II instrument shows that these conditions are not practically acceptable for this instrument where the resolution of the critical pair does not exceed 0.85 (Figure 3). Various reasons can be for the low reproducibility of a method on systems from different manufacturers. The effective gradient profile can be different for different types of pumps (low- or high pressure mixing systems) and different solvent mixers. The effective temperature inside of a column can be different due to the difference in the construction of compartments. To further improve the method selected after using the rapid optimisation algorithm, the ChromSwordAuto AutoRobust program was used to determine the robustness of the method. The AutoRobust is a direct robustness testing tool that automatically creates and executes full, fractional, and custom designs of experiments. The tests were set with four levels with a central point for the temperature (°C), (-4, -2, +2, +4) concentration organic modifier (%), (-4, -2, +2, +4) gradient time (min) (-2, -1, +1, +2), and flow rate (ml/min) (-0.2, -0.1, +0.1, +0.2). This DOE was executed for two instruments: UHPLC Agilent 1290 Infinity II and HPLC Ultimate 3000. Further results were processed to build one- two- and three-dimensional (1D, 2D, 3D) resolution maps. The different test operation factors and levels of method variables can be selected to build temperature – gradient time, temperature-gradient time-flow rate, and other combinations of method parameters. These results enable building a design space of a method where a change in operating conditions will not affect the quality of a method. Resolution maps (Figure 4.) have the square at the centre, which is the nominal method, and the circles appearing in the 2D map are the real experiments performed by the system. Analysis of the resolution maps and simulation of chromatograms for a combination of three different variables enables a chromatographer to determine critical method variables and to find conditions where resolution and run time can be increased or decreased. The results showed that the flow rate and the temperature are the most critical method attributes and relatively small temperature changes (to 26°C from 30°C) and flow rate (to 0.8 ml/min from 1.0 ml/min) enable us to improve the resolution up of the

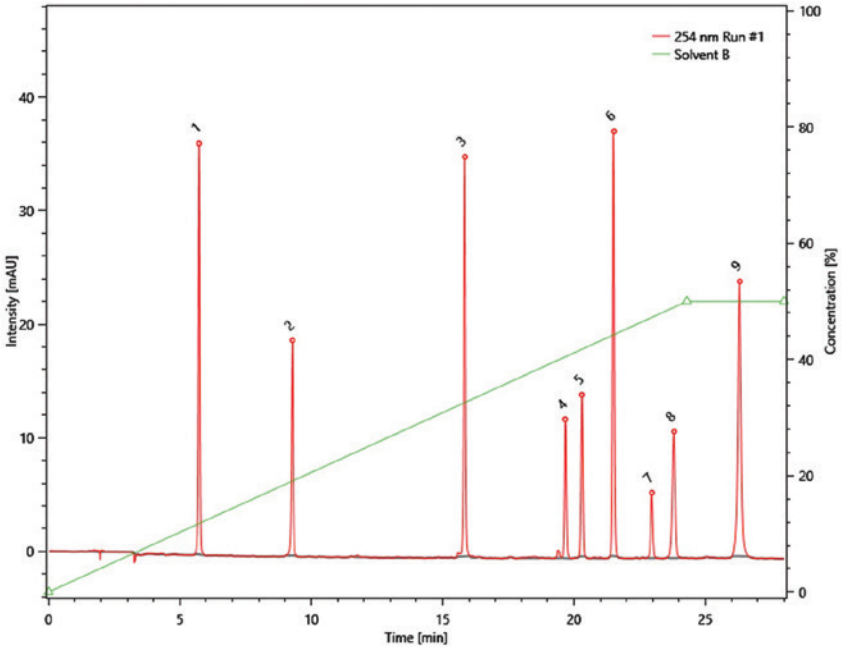

Figure 5: Chromatogram of a mixture of 9 hair dyes. Instrument: Ultimate 3000 RS.

Column: ACE Excel3 C18-amide, temperature 26°C, flow rate 0.8.ml/min, gradient : 0 min-0%, 24.3 min – 50%, 28 min – 50% of acetonitrile in a MP.

to 2.85 for both instruments and robustness of the method (Figures 5 and 6). This condition of the separation appears to be the most robust, where method parameter variations (5-10%) do not significantly impact the resolution of running samples with different types of instruments. For separation of the critical pair with less resolution (Rs = 1.9 -2.0) conditions that provide substantially less run time can be found (Figures 7 and 8). The robustness studies can therefore be considered as an additional tool to improve the performance of the method. It should be mentioned that robustness study is a mandatory part for development of quality control methods in chemical and pharmaceutical industry.

The fully unattended screening and optimisation procedure lasted 18 hours resulting in good separation of target peaks. The whole method development process was completed within two days with minimum input from the operator. This process shows how powerful automated method development is when automatically detecting peaks and building the retention optimisation models to achieve optimum separation resulting from robustness testing. Additionally, the extensive part of the analytical quality by design (QbD) approach is to obtain maximum information from the minimum number of experiments performed and select the parameters for optimum separation [7]. This concept, in part, is what ChromSword automation provides.

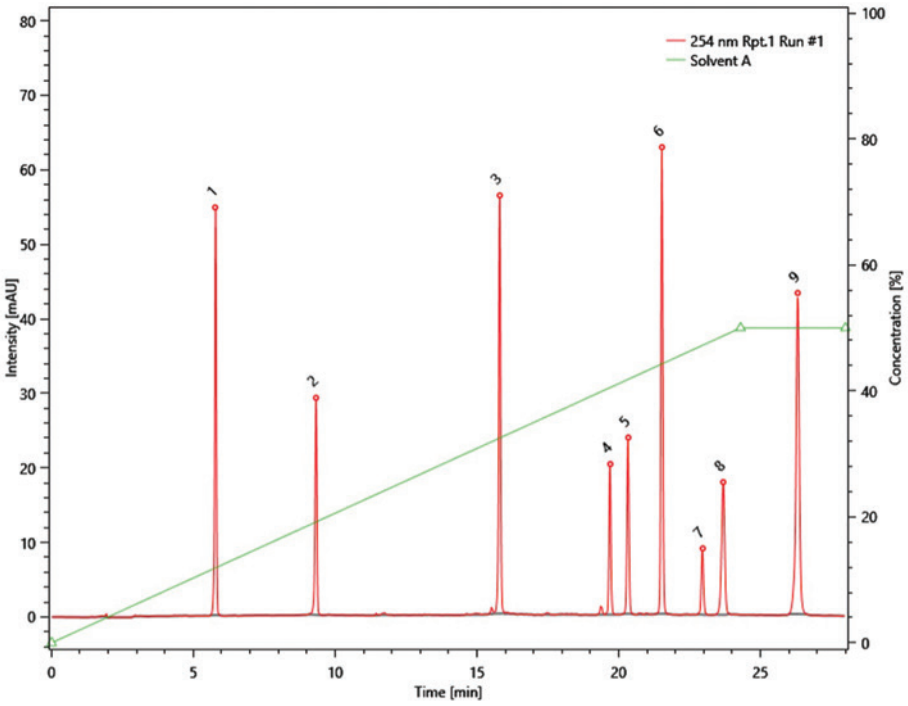

Figure 6: Chromatogram of a mixture of 9 hair dyes. Instrument: Agilent 1290. Conditions as described in Fig. 5.

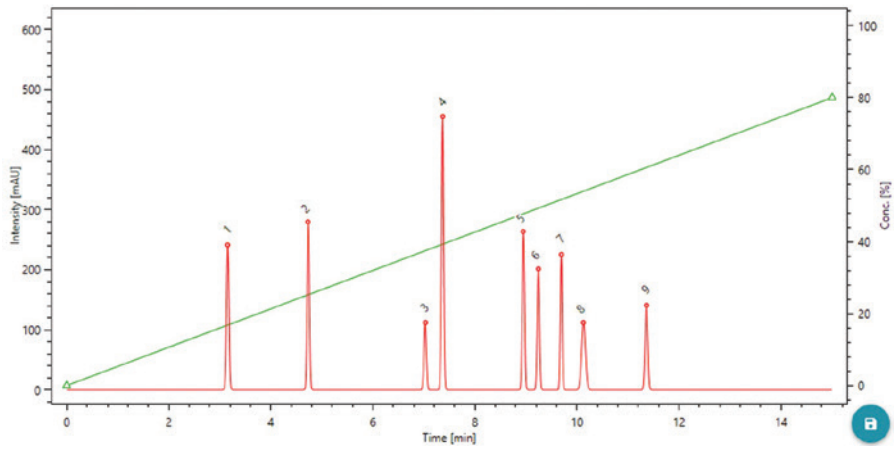

Figure 7: Simulated chromatogram

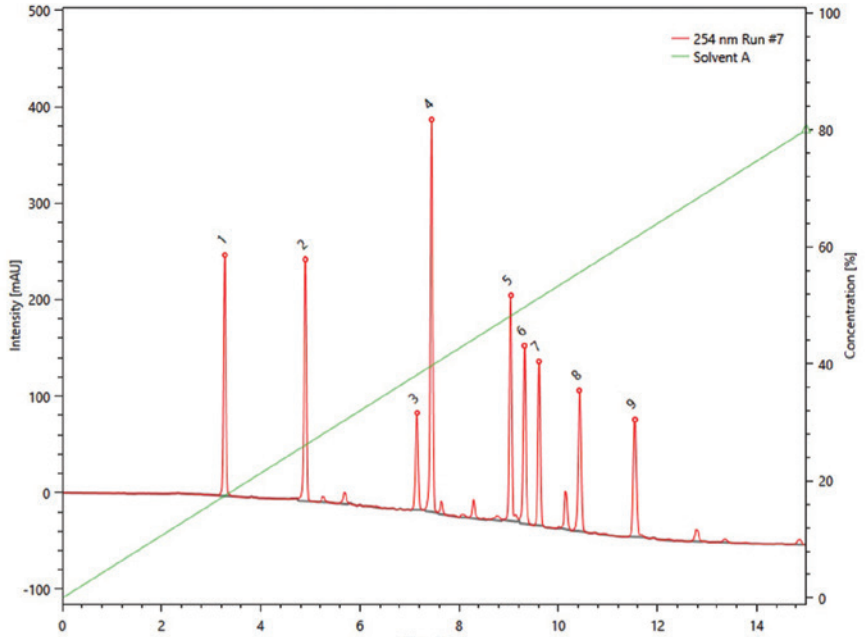

Figure 8: Experimental chromatogram.
Instrument: Agilent 1290. Column: ACE Excel3 C18-amide, temperature 40°C, flow rate 1.0 ml/min, gradient : 0 min-0%, 15.0 min – 80% acetonitrile in a MP.

Conclusions

Automated method development with ChromSwordAuto Developer and AutoRobust increases the efficiency of the process and provides extensive information about the sample and critical method parameters. A straightforward strategy for automated method development includes column/solvent screening, unattended optimisation to determine a practically acceptable method, and robustness study to improve separation and determine a range of operating conditions to reproduce a method with different LC instruments.

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