Application of ChromSword Software for Automatic HPLC Method Development and Robustness Studies.

Separation of Terbinafine and Impurities

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The presentation describes application of automated HPLC method development for separation of a mixture of terbinafine and impurities. In our approach the system was specified to start with rapid optimisation steps automatically exploring the entire design space through software intelligence to find the best analysis conditions. Screening of different columns, solvents and buffers, instrument parameters as well as rapid optimisation, fine-tuning, robustness studies, and documentation have been implemented automatically in one software platform.

The system was used effectively for optimisation of separation of terbinafine and impurities in the reversed-phase mode with the Quality by Design (QbD) principles. ChromSwordAuto® and AutoRobust software can significantly reduce method development time.

Terbinafine hydrochloride, (E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphtalene methanamine hydrochloride (Lamisil) is an active pharmaceutical ingredient with antifungal activity used in different drug formulations. The remaining patent or exclusivity for Lamisil expired on 30 June, 2007 and the US Food and Drug Administration has approved the first generic versions of prescription Lamisil (terbinafine hydrochloride) tablets. There are only a limited number of methods described for the determination of terbinafine, its impurities and degradation products by reversed-phase HPLC. Published methods are either long-term or require ion-pair additives or aggressive conditions that reduce lifetime of reversed-phase columns [1-3].

HPLC method development is frequently a time consuming process which requires tests of different columns, mobile phases and other conditions to provide a reliable and robust method to be used for impurities profiling or quality control analyses. In this article we describe how time for method development can be reduced substantially utilising specialised HPLC (UHPLC) method development system controlled by automated method development and robustness tests software.

Instruments

Agilent Technologies 1290 Method Development Systems with DAD, two thermostated column compartments, internal 8 pos. column selector and external 12 pos. solvent selector

Method Development Software

ChromSwordAuto® software for computer-assisted and automatic HPLC method development (ChromSword).

AutoRobust software for automatic robustness studies of HPLC methods (ChromSword).

Both software are compatible with Empower (Waters), ChemStation, OpenLab (Agilent Technologies), Chromeleon (Thermo Fisher) chromatography data systems.

Columns Set (100 x 4.6 cm):

1. SynergiFusionRP, 4µ (Phenomenex)
2. X-TerraRP18.3.5µ (Waters)
3. Kinetex C18, 2.6µ (Phenomenex)
4. HyperSelectHiPurityC18, 5µ (ES Industries)
5. Gemini NXC18, 5µ (Phenomenex)
6. Lichrospher100RP18, 5µ (Merck KGaA)

Mobile phases:

0.1%TEA+HAc pH 7.3 with Acetonitrile and Methanol

Results and Discussion

Automated method development process included the following steps:

1. Columns set selection
2. Rapid optimisation
3. Fine optimisation
4. Retention models building, fine tuning
5. Robustness studies

Step 1. Columns set selection.

The columns set was selected form ChromSword column characteristics database. The column database software module builds selectivity maps of more than 100 commercially available reversed-phase columns. These maps allow selection of columns with different selectivity for a different concentration of acetonitrile and methanol in mobile phases. The columns set described in the experimental part of the article is one of many method development column combinations which can be chosen from the column selectivity maps.

Step 2. Rapid Optimisation.

The rapid optimisation automated algorithm performs only few runs to find optimal conditions for every column/solvent/buffer combination. The rapid optimisation algorithm starts without any preliminary

information about a sample or initial retention data. The first gradient run is a scouting and the second and following runs are results of optimisation. As a results good isocratic, linear or multi-step gradient conditions can be found. In this step 6 columns and 2 organic solvents (ACN and MeOH) were automatically tested with totally 36 runs during 18 hours.

Kinetex C18, 2.6µ column with methanol as an organic solvent provided the best separation with a minimal time. In Figure 1 a chromatogram and a gradient profile that the system found automatically are shown for the critical pair of isomers.

Step 3. Fine optimisation.

The fine optimisation step was done to find optimal and alternative methods for separation of the mixture with the best combination of a column\solvent which was found after the Step 2. During the fine optimisation process the system performs detailed study of a sample for impurities profiling, peak tracking, building retention models and searches for isocratic and gradient conditions to separate all components of a mixture. In this step the 4 alternative gradient methods were proposed and retention models constructed for all sample components.

Step 4. Retention models building and off-line optimisation (optional)

This step is optional because the software finds conditions automatically. However it was interesting to build retention models of retention behaviour of all components to simulate chromatograms and to find alternative conditions for separation of a mixture. A useful option is prediction of retention from chemical structures and column properties. For terbinafine the software predicted retention factor of 1.7 at 90% and 11 at 76% of methanol in a mobile phase for the Kinetex C18 column. Those values were experimentally obtained at 91% and 78% of methanol in a mobile phase. Such predictability only from structural formulae and column properties can save time to find practically good separation conditions. Fine-tuning procedure applied after input data of only one run allows prediction of retention for practically acceptable level.

ChromSword can automatically optimise gradient profiles with more than three variables. Typically method development software can build resolution maps for 2D or 3D dimensions - gradient time, temperature, pH or other variable. In this case initial (C_0) and final concentration (C_t) of a solvent in a mobile phase are constant and specified by a user and only gradient time (Gt) of a linear gradient and temperature (T) can be optimised. ChromSword uses the Monte-Carlo procedure of optimisation and optimises simultaneously C_0, C_t, Gt and T for a linear gradients (4D) and also position of every gradient node (concentration and time) and temperature for the whole gradient profile for multistep gradients with up to 100 nodes. Automated optimisation proposed a method with full separation of all compounds and run time less than 10 min (Figure 2). This method was selected for the next step to test robustness.

	Step	Analyst (hours)	ChromSword (hours)
1	Columns set selection	0.5	----------
2	Rapid optimisation	1	18
3	Fine Optimisation	1	11
4	Fine Tuning	1	3
5	Robustness studies	1	4

Table 1

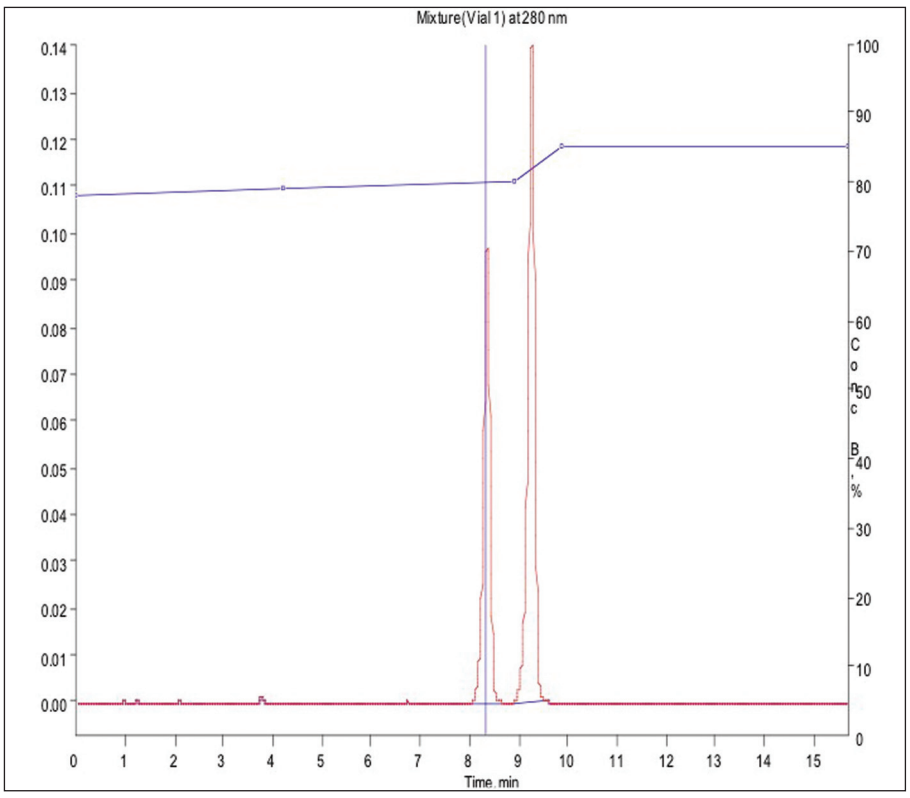

Figure 1. Separation of a critical pair of terbinafine impurities after rapid optimisation.

Step 4. Automatic robustness tests

Robustness studies are important stage of method development process to build design space and determine operating range of method variables according the quality by design (QbD) approach.

The following design for automatic robustness studies with AutoRobust module was used:

1. Temperature, gradient slope, flow rate – the full factorial design when effect of all possible combinations of method variables are studied
2. Temperature, gradient slope, flow rate, wave length, injection volume, equilibration time, three column batches – Plackett–Burman design when a statistical design of experiments is created automatically for 12 runs and 7 two-level factors.

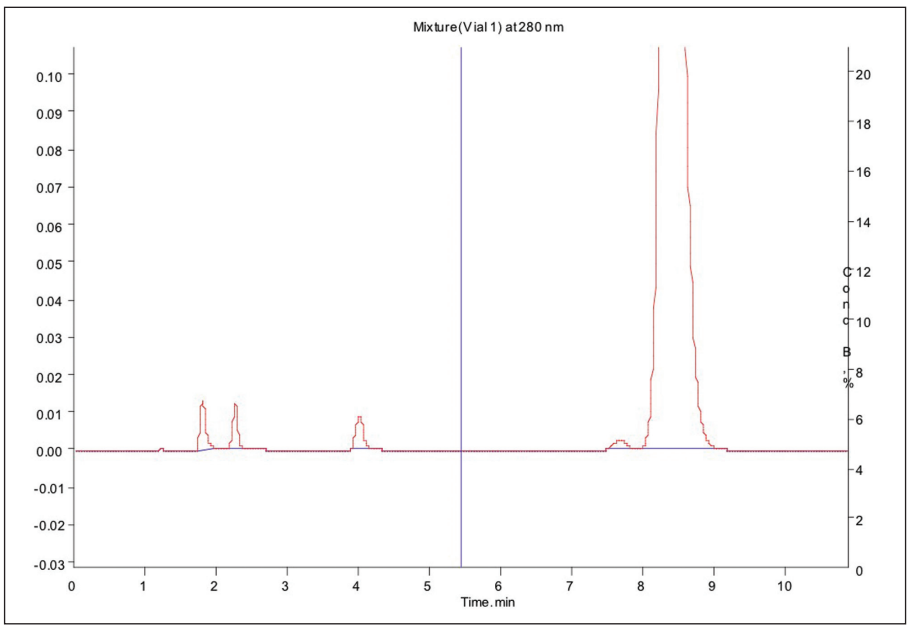

Figure 2. Separation of terbinafine and impurities.
Gradient. 0 min. – 50% B, 1.0 min. - 80%, 11.0 – 80%. T = 30°C; flow rate 1.0 ml/min, WL 280nm.

The design plans are created fully automatically that allows to avoid human factor for possible errors. Results of studies confirmed robustness of the method and possibility to transfer it to other laboratories.

An important point for method development in regulated laboratories are detailed reporting of all steps. ChromSwordAuto and AutoRobust generate reports automatically and this enables an analyst save time for preparation of necessary project documentation.

Automated method development. How long does it take?

Time contributions for automated method development are shown in the Table 1.

It is necessary to note that the experiments were performed with UHPLC system and it takes 30-50% more time for automated work with HPLC instrument.

Conclusion

The system for automated HPLC method development was used effectively for optimisation of separation of terbinafine and impurities in the reversed-phase mode with the Quality by Design (QbD) principles.

The robust rapid method with analysis time less than 10 min. has been developed

Screening of different columns, solvents and buffers, instrument parameters as well as rapid optimisation, fine tuning, robustness studies, and documentation have been implemented automatically in one software platform.

The use of automatic procedures implemented in ChromSwordAuto® and AutoRobust software can significantly reduce method development time.

The technology allows to test automatically many different conditions in a short period of time. It allows to obtain comprehensive and important information about a sample, critical parameters for peak shape and resolution and to find the best separation conditions.

References

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