Agilent 1200 Series LC Method Development Solution for the analysis of degradation products of metoprolol tablets

Application Note

Drug Discovery

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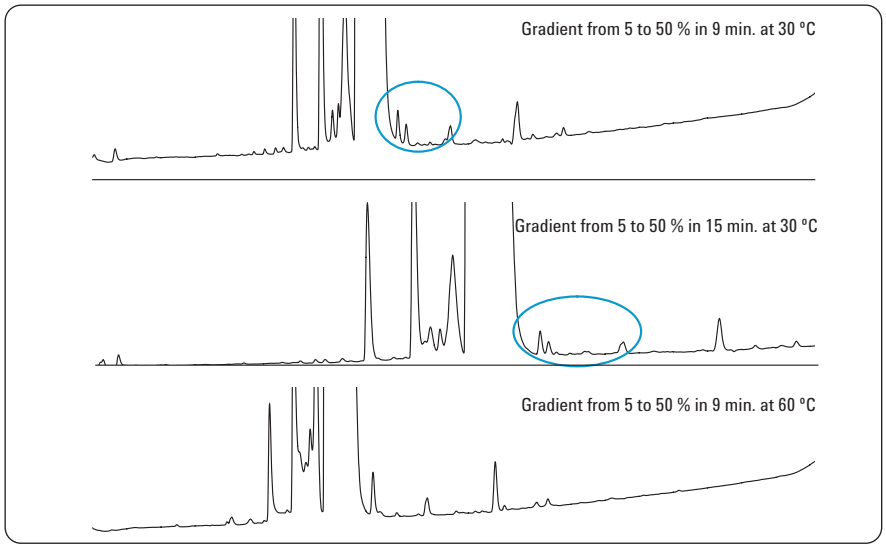

Abstract

The Agilent 1200 Series LC Method Development Solution is designed for the highest flexibility and automation. This system was used to find optimum separation conditions for the analysis of degradation products of drug compounds. As an example, metoprolol tablets were exposed to high temperature and the resulting products were analyzed on several columns using:

- Automated setup of methods and sequences using the Agilent ChemStation Method Scouting Wizard
- Six Agilent ZORBAX RRHT columns of different selectivities
- Different mobile phases
- Different temperatures and gradients

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Introduction

When developing new pharmaceutical drugs, it is important to check the shelf life and stability of the active pharmaceutical compound and the final tablet to be sure that no unexpected and potentially harmful degradants are formed. The tablets are stressed under different conditions and the products are analyzed, typically by HPLC. To be sure that no unexpected compounds co-elute with the main compound, one must be certain that the analytical method can resolve all sample compounds. Therefore, analytical LC method development is required. Typically, to get a quick overview and to get good starting conditions for the final fine-tuning of the method, an array of columns with a set of solvents is tested initially. This method-scouting process helps to find the best-suited column and mobile phase for the separation of a set of compounds. Often, the whole procedure is very time-consuming because automation of the complete process is not available.

The Agilent 1200 Series LC Method Development Solution offers a highly flexible system that can be used for as many as eight columns up to 100 mm in length, and as many as six columns with lengths of up to 300 mm. In addition, the Agilent ChemStation Method Scouting Wizard automates the setup of methods and sequences to screen the available combinations of columns, solvents, predefined gradients, and temperatures. Additionally, for fully automated method development and optimization, the system can be combined with highly sophisticated method development software from Agilent partners:

- ACD/AutoChrom for ChemStation from ACD/Labs
- ChromSword Auto for ChemStation from ChromSword Baltic

In this Application Note, we will describe:

- How to set up a sequence using the Agilent ChemStation Method Scouting Wizard, using as an example the degradation products of metoprolol tablets
- Analysis of the degradation products using six different columns and two different sets of mobile phases
- Fine-tuning using one column and two different gradients with two different temperatures

Experimental

Equipment

An Agilent 1200 Series Rapid Resolution LC system was incorporated into the Agilent 1200 Series LC Method Development Solution. The LC system was comprised of the following modules with firmware revisions A.06.01 or higher:

- Agilent 1200 Series binary pump SL with degasser
- Agilent 1200 Series high performance autosampler SL plus
- Two Agilent 1200 Series thermostatted column compartments SL plus (G1316C) with installed valve drives
- Agilent method development valve kit, high pressure
- Agilent method development capillary kit, low dispersion, for short columns
- Agilent 1200 Series diode-array detector (DAD) SL
- Several Agilent ZORBAX Rapid Resolution High Throughput (RRHT) columns with1.8 µm particle size
- Agilent ChemStation B04.01 with Agilent ChemStation Method Scouting Wizard add-on

Sample preparation

Two tablets containing 50 mg metoprolol each were pulverized and heated to 80 °C for three hours. The residue was dissolved in 4 mL of water. Two syringe filters were used to remove all nondissolved particles – first a Minisart 0.8 µm filter followed by an Agilent 0.45 µm filter. One microliter of the resulting liquid was injected into the LC system.

Results and discussion

Agilent ChemStation Method Scouting Wizard

The Agilent ChemStation Method Scouting Wizard is an add-on to the Agilent ChemStation software. It is used to generate the methods and the sequence table required to screen the complete matrix of selected columns, solvents, predefined gradients, and temperatures for several samples in an easy and logical way.

The setup for a method scouting campaign includes several steps:

- *Create a new screening campaign (figure 1)*
  This step includes naming and describing the campaign and its root folder, and is always the start for setting up a new experiment. Alternatively, a predefined screening campaign can be loaded and modified. This is very useful if standardized procedures for initial method scouting must be repeated with new samples.

- *Define the screening campaign (figure 2)*
  This step includes selection of master method and campaign range. All basic parameters that are not altered during the scouting campaign (for example, detector settings) are taken from the master method.

- *Set up column screening (figure 3)*
  In this screen, the columns that are to be evaluated are selected. The columns that show up here must be defined previously in the more column thermostat cluster > configure columns screen under Instruments, and are recognized automatically by the Agilent ChemStation Method Scouting Wizard.

- *Set up solvent selection (figure 4)*
  The Agilent ChemStation Method Scouting Wizard recognizes which kind of pump (binary or quaternary) is present in the system. It also recognizes whether an additional external solvent selection valve (G1160A) or internal solvent selection valve is present (only available with a binary pump). The solvents are shown in the software with their predefined names.

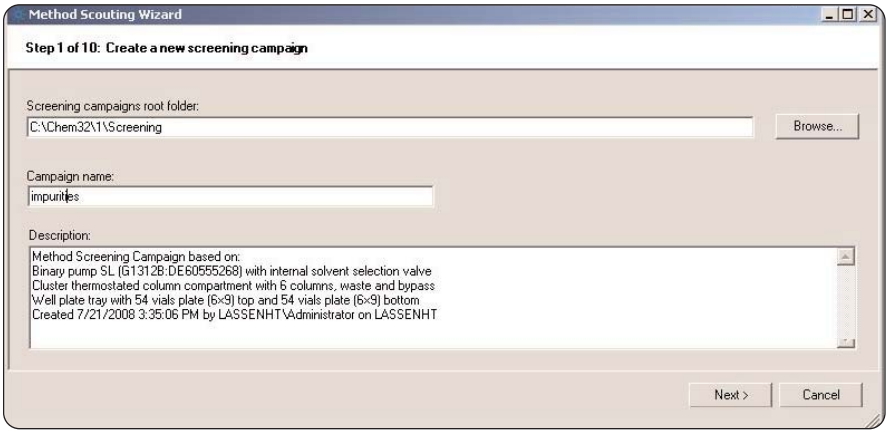

Figure 1
Starting a campaign.

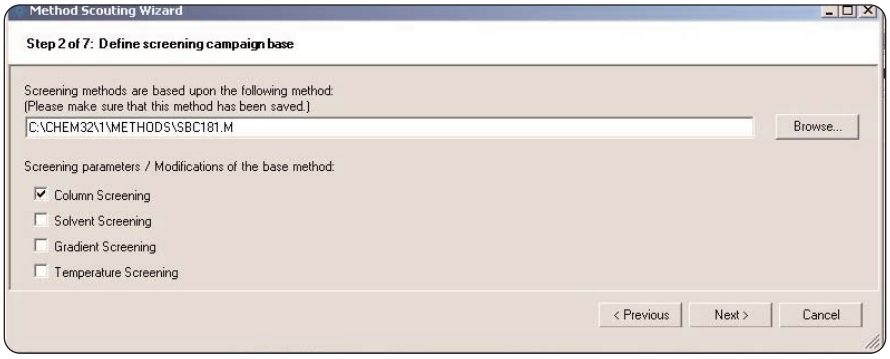

Figure 2
Defining the campaign range and selecting the master method.

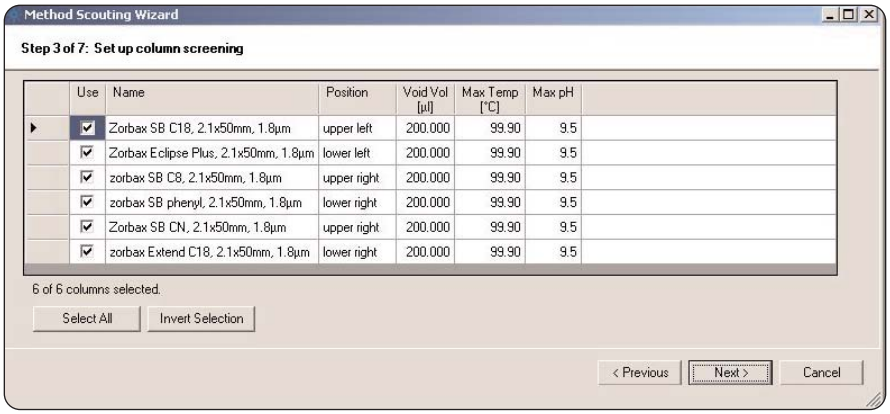

Figure 3
Selecting columns.

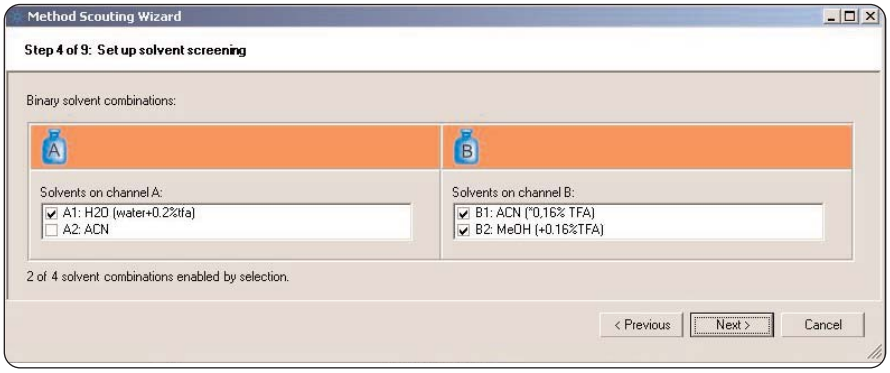

Figure 4
Selecting the solvent sets.

- *Set up gradient selection (figure 5)*
  If a variation between different pre-defined gradients was selected for the scouting campaign, the screen shown in figure 5 appears. Here, the gradients can be defined either graphically or by the appropriate entries into a gradient table. Similarly, pre-defined temperatures can be selected and added to the screening matrix (not shown).

- *Review and select method (figure 6)*
  In the next screen, the required combinations of columns and methods that will be created can be reviewed. If the full screening matrix is not needed, unwanted combinations can be deselected by the user. Also, incompatible combinations of temperatures and solvents with the selected columns can be automatically excluded. This is very useful to increase the column lifetime.

- *Set up equilibration and flushing (figures 7 and 8)*
  Here the "flush and column care" parameters and the equilibration times are defined. The flush setup requires that a waste and/or bypass position be defined. This setup has many different options that cover cases like miscible and immiscible solvents that might mix at a solvent change, different positions of the degasser, and various procedures to handle the column after usage.

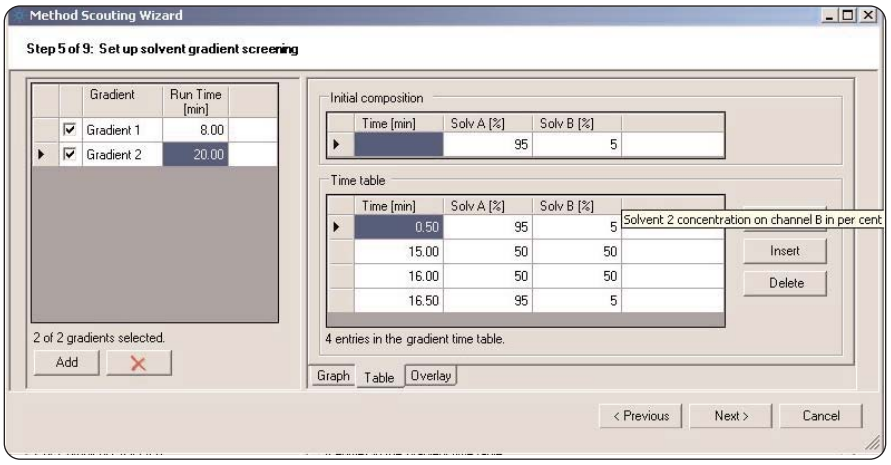

Figure 5
Setting different gradients.

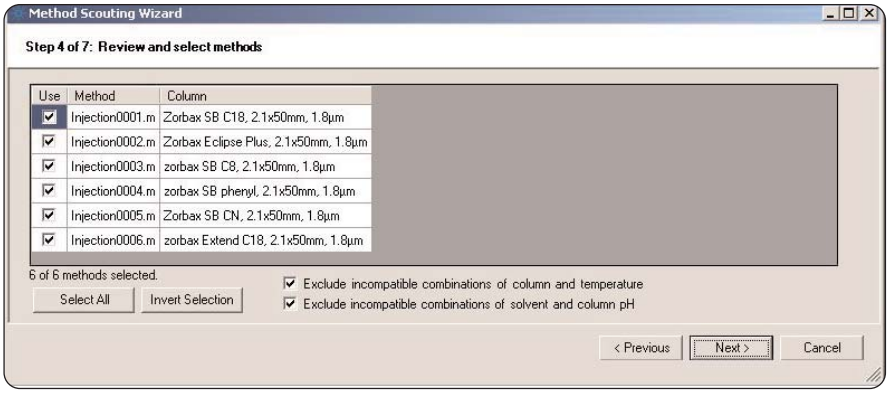

Figure 6
Reviewing and selecting methods.

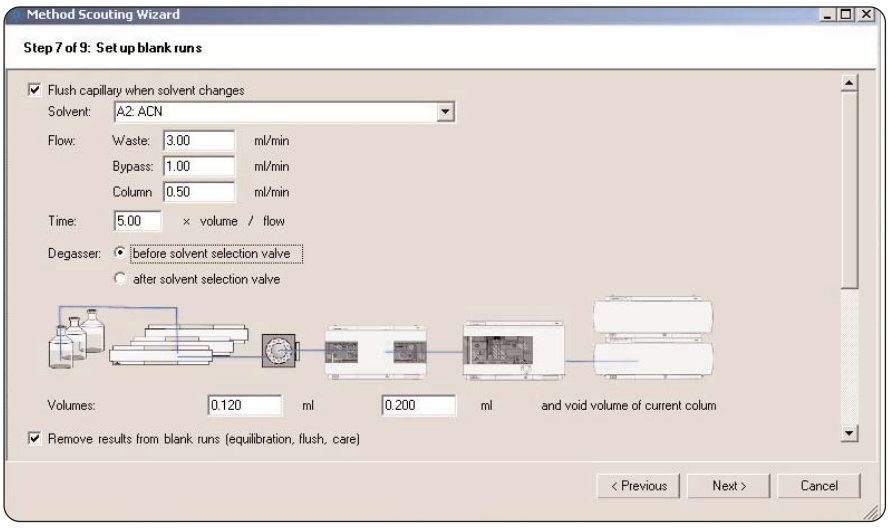

Figure 7
Flush condition when solvent is changed.

• *Set up the samples (figure 9)*
In this screen, the samples are defined. Further, the number of injections per vial, the injection volume, and the vial size are defined. For the convenience of the user, the required sample amount for the complete sequence is calculated. More sample lines can be added just by clicking the "Add" button.

• *Summary (figure 10)*
Finally, a summary screen informs the user about the complete screening campaign, including a description of the campaign, a sequence summary, and an estimate of the solvent usage and waste production. After clicking the "Finish" button, all methods are generated and saved in the project folder, the sequence is saved, and the screening campaign definitions are saved.

After completing the campaign setup, the sequence can be loaded and started.

Practical example

For the following practical example, a sample was chosen that contained the main compound metoprolol and degradation products.

The following workflow was selected:
- Creation of a first test chromatogram on a C-18 phase
- Test of six different columns with two sets of mobile phases
- Selection of the column and mobile phase set with the most peaks
- Variation of gradient and temperature for the selected column
- Selection of final conditions

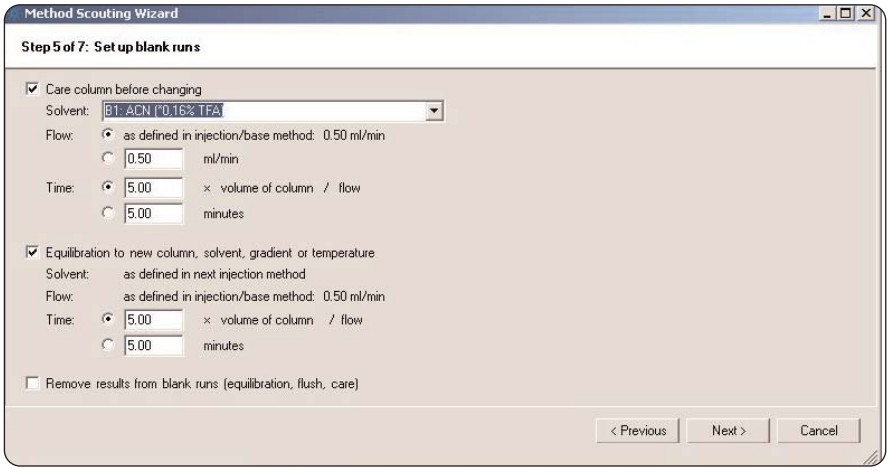

Figure 8
Setting the column care and equilibration runs.

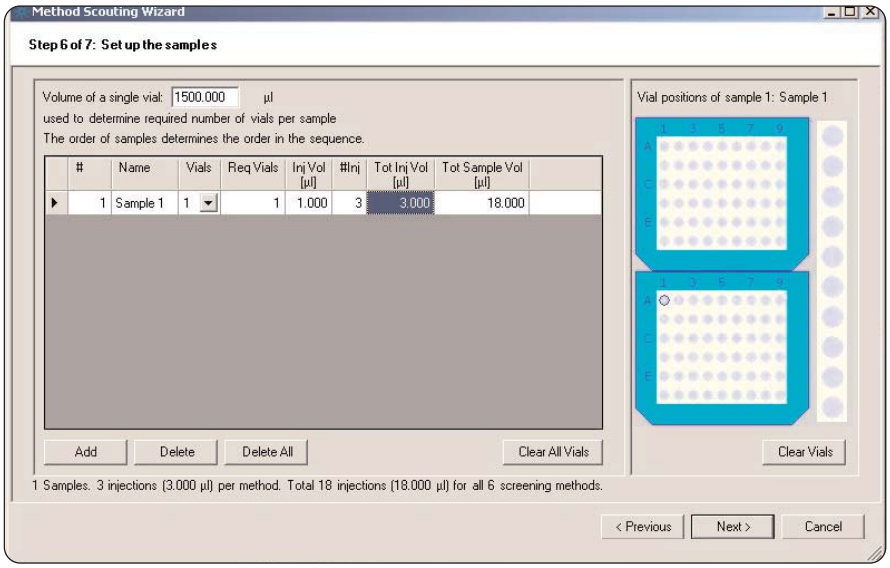

Figure 9
Setting up the samples.

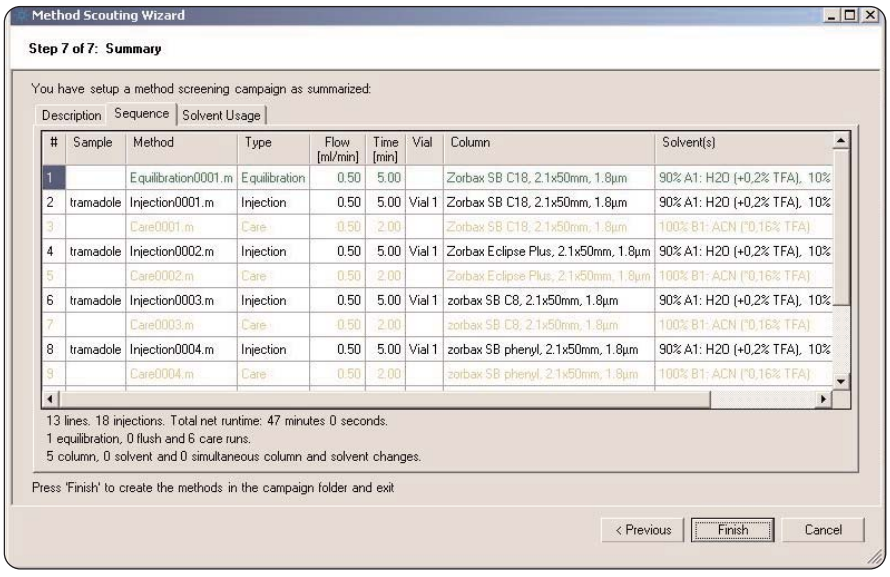

Figure 10
Sequence that is automatically generated.

Chromatographic conditions for initial tests

Sample:	Metoprolol + decomposition products
Columns:	2.1 x 50 mm columns packed with 1.8 µm particles
Mobile phase:	Water + 0.2 % TFA / ACN + 0.16 % TFA
Flow rate:	0.5 mL/min
Gradient:	5 to 50 % in 5 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	210, 230, 254, 280/10 nm, Ref 360/100 nm, 3 mm detector cell, 20 Hz
Configuration:	Low-delay-volume configuration with 0.12 mm capillaries and low-dispersion heat exchanger

Some initial experiments were done on an Agilent ZORBAX SB-C18 column (see chromatogram and conditions in figure 11). Trifluoroacetic acid (TFA) was chosen as the modifier for the mobile phases. The first experiments were done on a 2.1 x 50 mm column. They indicated that a gradient from 5 to 50 % organic solvent was a good start. These preliminary results showed that heating the metoprolol tablets produced degradation products.

For the method scouting experiments, six different columns were chosen, as shown in figure 12. The Agilent ChemStation Method Scouting Wizard was used to set up the sequence. The selected solvent combinations were acetonitrile (ACN) and water, as well as methanol and water. All had TFA as modifier. After setting up a master method, the Agilent ChemStation Method Scouting Wizard was started and an appropriate sequence was set up by the wizard. The resulting chromatograms for the six columns using acetonitrile and water as mobile phase are shown in figure 12. The combination of methanol and water did not lead to better performance.

Chromatographic conditions for scouting experiments

Sample:	Metoprolol decomposition products
Columns:	2.1 x 50 mm columns packed with 1.8 µm particles
Mobile phase:	Water + 0.2 % TFA / ACN + 0.16 % TFA or water + 0.2 % TFA / methanol + 0.16 % TFA
Flow rate:	0.5 mL/min
Gradient:	5 to 50 % in 5 min
Injection volume:	1 µL
Column temperature:	all 30 °C
DAD:	210, 230, 254, 280/10 nm, Ref 360/100 nm, 3 mm detector cell, 20 Hz
Configuration:	Low-delay-volume configuration with 0.12 mm capillaries and 1.6 µl heat exchanger

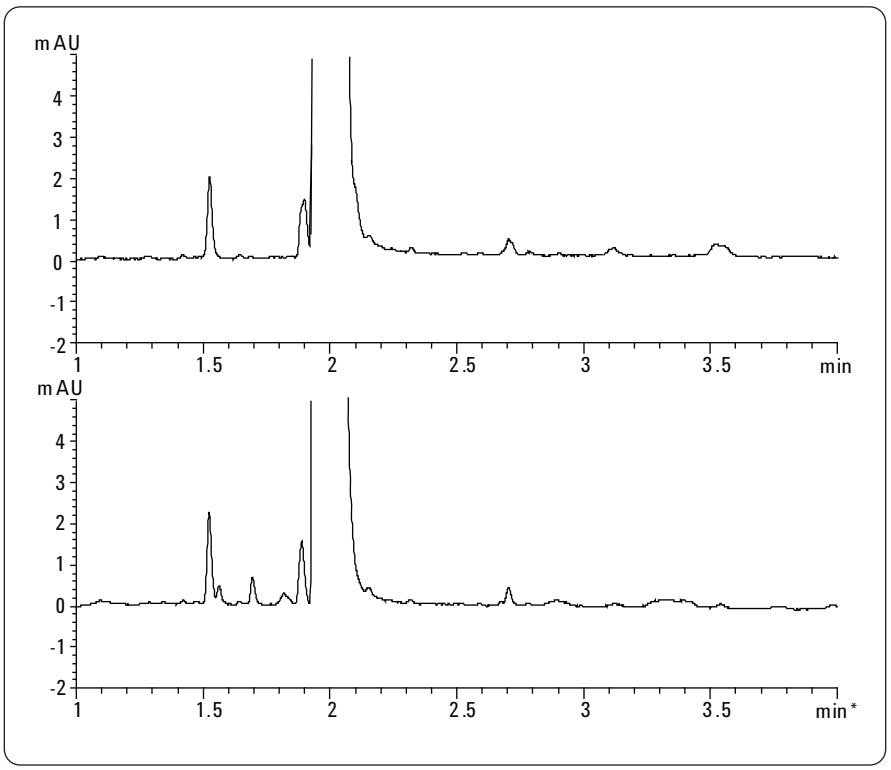

Figure 11
First test chromatograms. Top chromatogram: New tablet. Bottom chromatogram: Tablet after heat degradation.

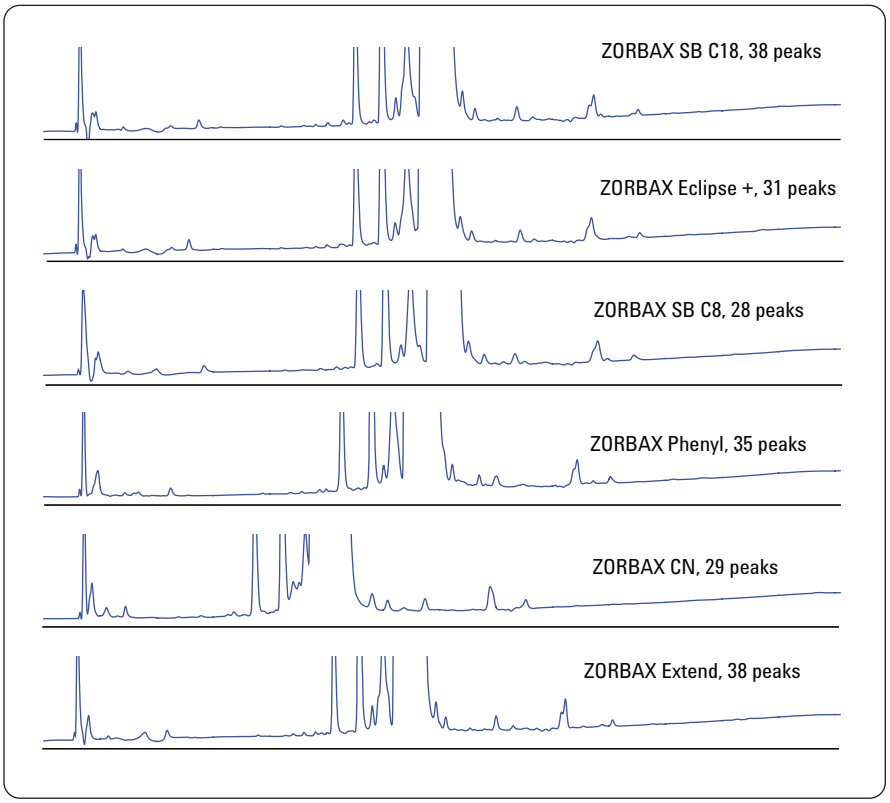

Figure 12
Results obtained from method scouting experiments.

The Agilent ZORBAX SB-C18 column was selected as the column with the most appropriate performance. The experiments showed that two wavelengths are necessary to see more peaks (figure 13).

The entire column scouting procedure using six columns and two sets of solvents took about six hours under the specified chromatographic conditions – and was accomplished in a fully unattended fashion.

In a further experiment, the selected column was used with two different acetonitrile gradients and two different temperatures; see figures 14 and 15. It is obvious that the less-steep gradient at 30 °C gives the best separation, especially for the peaks that are eluted after the main peak.

The fine-tuning process took about 2.5 hours for completing the evaluation of two gradients with two different temperatures using the selected chromatographic conditions.

The following conditions were selected for the final method (see middle chromatograms in figures 14 and 15):

- Column: Agilent ZORBAX SB-C18 RRHT, 2.1 x 50 mm, 1.8 µm particles
- Mobile phase: Water + 0.2 % TFA / ACN + 0.16 % TFA
- Flow rate: 0.5 mL/min
- Gradient: 5 to 50 % in 15 min
- Injection volume: 1 µL
- Column temperature: 30 °C
- DAD: 210, 280/10 nm, Ref 360/100 nm, 3 mm detector cell, 20 Hz
- Configuration: Low-delay-volume configuration with 0.12 mm capillaries and low-dispersion heat exchanger

In figure 16, spectra are compared from metoprolol and its degradation products. Some of the spectra are quite similar to the one obtained from the main compound. To evaluate the structure even further, the method could be transferred to an appropriate LC/MS system like an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight LC/MS system.

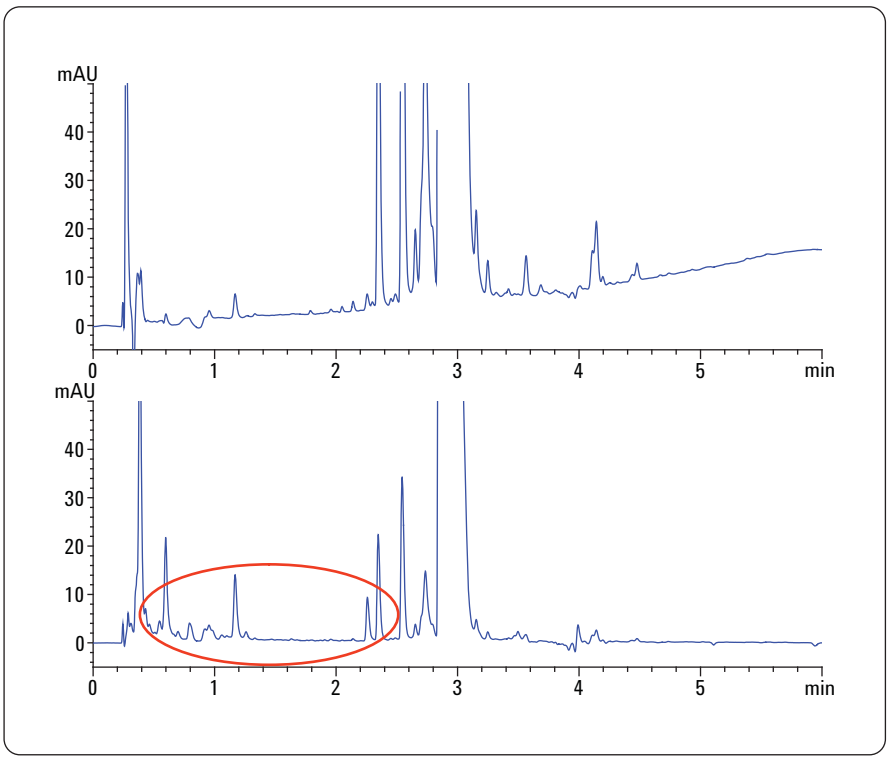

Figure 13
Two wavelengths gave more peaks.

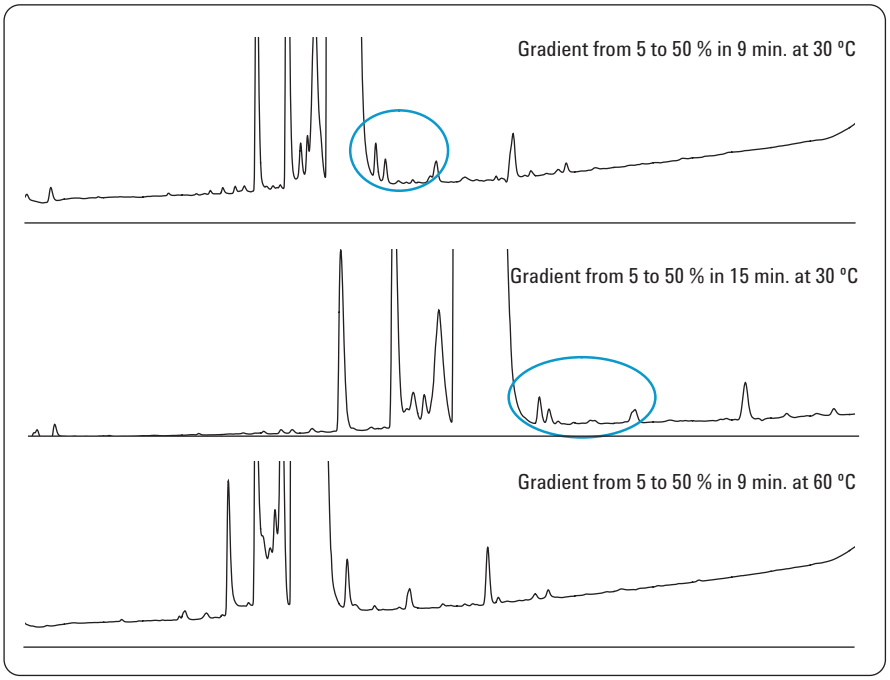

Figure 14
Fine-tuning of the method at 230 nm detection wavelength.

Conclusion

The Agilent 1200 Series Method Development Solution offers the highest flexibility together with the highest performance. The scouting experiments are set up in a very easy and logical way using the Agilent ChemStation Method Scouting Wizard. Even complex sequences with experiments involving several columns and solvents are set up automatically without the need to create methods manually. In addition, column care, flush, and equilibration methods are set up automatically with just a few clicks.

The Agilent ChemStation Method Scouting Wizard was used for the development of a separation method for degradation products of metoprolol tablets. Screening of all combinations of six columns and two sets of solvents, as well as subsequent fine-tuning with two different gradients and two different temperatures, was done within less than one working day. The system ran unattended most of the time, liberating the operator to perform other tasks.

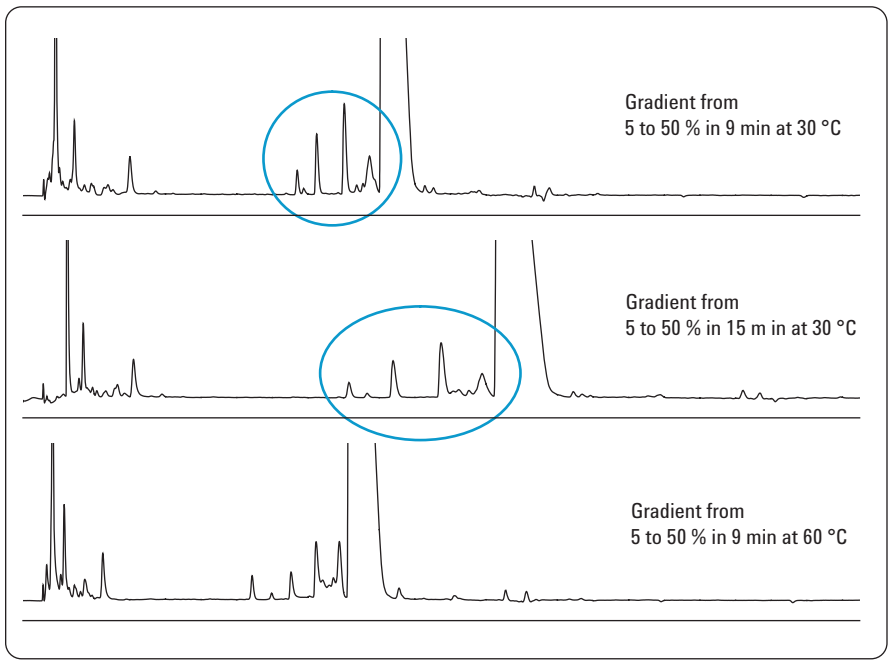

Figure 15
Fine-tuning at 280 nm detection wavelength.

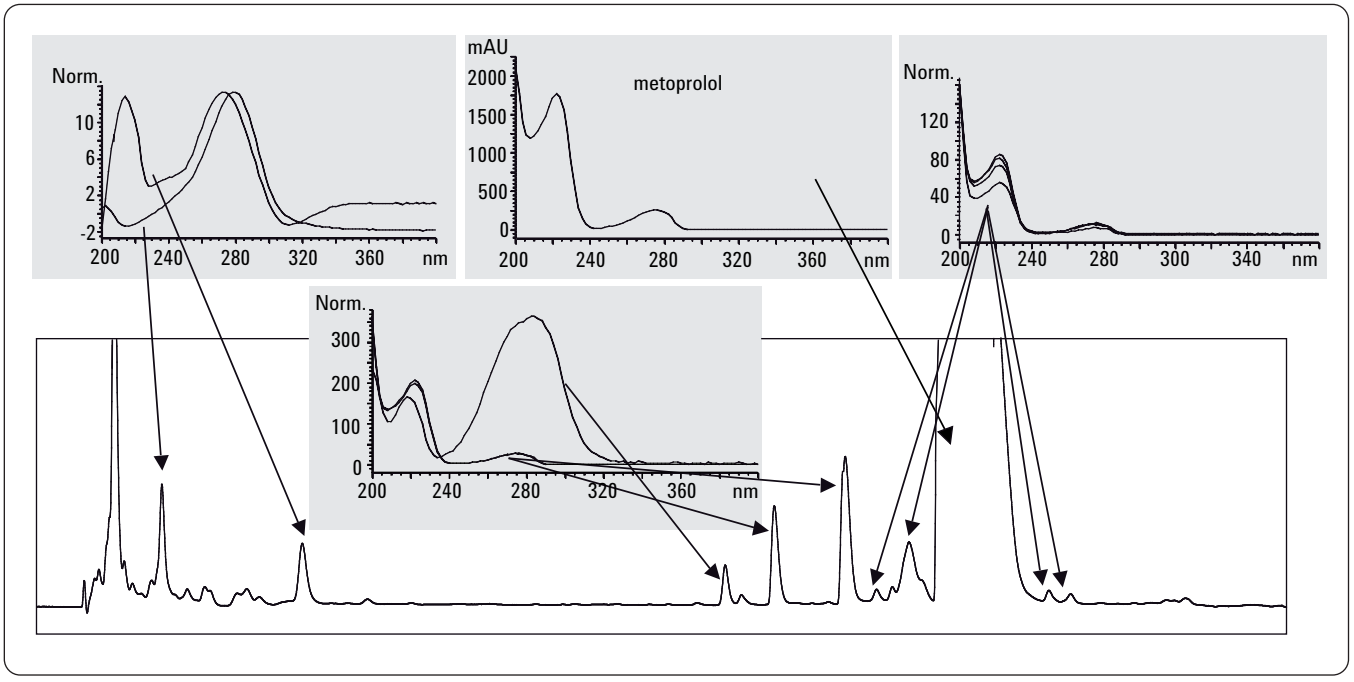

Figure 16
Spectra of metoprolol and degradation products.

www.agilent.com/chem/1200mds

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