Method Development for Enantiomers Using the Agilent 1200 Infinity Series Method Development Solution

Application Note

Small Molecule Pharmaceuticals & Generics

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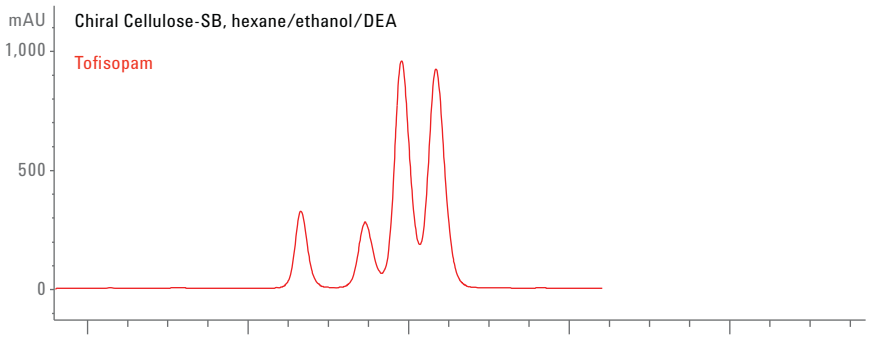
Abstract

Chiral compounds are present in many pharmaceutical products, and the determination of each enantiomer during drug development and production is mandatory. The Agilent 1200 Infinity Series Method Development Solution, in combination with the Agilent Method Scouting Wizard, is best suited for method development of chiral compounds. Scouting sequences are set up with a few mouse clicks. Up to six long, chiral columns can be installed. Different mobile phase combinations using binary, ternary, or quaternary isocratic solvent mixtures are applicable. The solvent composition is the same over the complete run.



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Introduction

The determination of enantiomers in pharmaceutical products is mandatory to avoid possible dangerous side effects. The US Food and Drug Administration has given official recommendations. The stereoisomeric composition of a drug with a chiral center must be known, and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies must be known. Specifications for the final product should assure identity, strength, quality, and purity from a stereochemical viewpoint[1].

Method development for chiral compounds requires chiral columns or modifiers; chiral columns are preferred. Chiral columns are available for reversed phase and normal phase chromatography. In this Application Note, chiral columns, which need normal phase solvents, are used. Trifluoracetic acid (TFA) and diethylamine (DEA) are used as modifiers for the analysis of tofisopam and vanillyl-mandelic acid.

Due to its stereogenic center at C(5)-atom, tofisopam exists as two enantiomers (R(+) and S(–)). Upon dissolution, its diazepine ring system will exist in two boat conformations, leading to two conformers for each enantiomer. The driving force for conformer transition is attributed to the steric repulsion effect between C(4)-methyl and C(5)-ethyl groups. Consequently, four isomers have to be separated for tofisopam[2].

Vanillyl-mandelic acid has one chiral center and, consequently, two enantiomers have to be separated.

The Agilent 1200 Infinity Series Method Development Solution, with its capability to connect up to six long columns, was used in combination with the Agilent Method Scouting Wizard. This additional software package allows setting up scouting sequences, including column, solvent, gradient, and temperature scouting, with a few mouse clicks. In addition, flushing, equilibration, and column storage steps are included.

Experimental

The following instruments were used:

Agilent 1200 Infinity Series Method Development Solution

- Agilent 1290 Infinity Quaternary Pump (G4204A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment 1 (G1316C) with valve drive installed (Option #058), equipped with 1200 bar valve head
- Agilent 1290 Infinity Thermostatted Column Compartment 2 (G1316C) with valve drive installed (Option #058), equipped with 600 bar valve head
- Agilent 1290 Infinity Diode Array Detector G4212A
- Agilent Method Development Valve Kit with two Agilent 1200 Infinity Series Quick-Change 8-position/9-port valves (G4230B), and with Agilent Capillary Kit (Option #003)

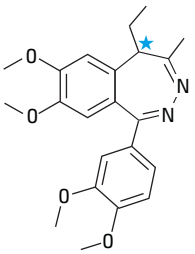
Acquisition and evaluation software

Agilent OpenLAB CDS, ChemStation Edition Rev. C.01.05

Agilent ChemStation Method Scouting Wizard A2.03

Compounds analyzed

Purchased from Sigma-Aldrich, Germany



Tofisopam Vanillyl-mandelic acid

Tofisopam isomers
R-(+) Major, R-(–), S-(+), S-(–) Major

Chromatographic conditions

Parameter	Value
Columns	YMC CHIRAL Amylose-C, YMC CHIRAL Cellulose-C, YMC CHIRAL Cellulose-SB (all columns supplied by customer)
Mobile phases	A) Hexane B) Ethanol C) 1 % TFA* in ethanol D) 1 % DEA** in ethanol
Flow rate	1 mL/min
Injection volume	5 µL
Column temperature	25 °C
Sample temperature	10 °C
DAD	230/10 nm, Ref: 360/100 nm, 10 Hz, 10-mm cell

*TFA =trifluoroacetic acid
**DEA = diethylamine

Results and Discussion

Method development for chiral compounds can be done using the Agilent 1200 Infinity Series Method Development Solution in combination with the Agilent Method Scouting Wizard. At the beginning, the user has to create a basic method, which is used as the start method in the Method Scouting Wizard. The wizard guides through a procedure ending in the creation of a scouting sequence. The sequence includes gradient, solvent, or temperature scouting methods for column scouting. For additional information, methods for flushing, column equilibration, and column storage are automatically integrated[3]. Typically, chromatography gradients are applied in reversed phase. In the case of chiral separations, isocratic conditions are used. This creates some small changes in the setup procedure.

In the setup procedure, all screening parameters had to be selected (Figure 1), even though, in our start sequence, we used no gradients and employed only one temperature.

A ternary gradient was selected for the analysis of tofisopam, using hexane as Solvent A, ethanol as Solvent B, and 1 % DEA in ethanol as Solvent D (Figure 2). To be able to analyze vanillyl-mandelic acid in the same sequence using acidic conditions, 1 % TFA in ethanol was used as Solvent C. Using this setup, neutral, acidic, and basic compounds could be analyzed in one or separated sequences, without the need to change solvents and perform time-consuming flush procedures. Tofisopam can be analyzed using neutral and basic conditions, whereas vanillyl-mandelic acid needs acidic conditions.

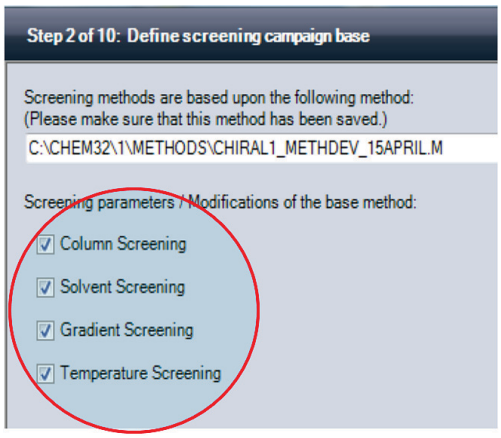


Figure 1. Selecting scouting parameters.

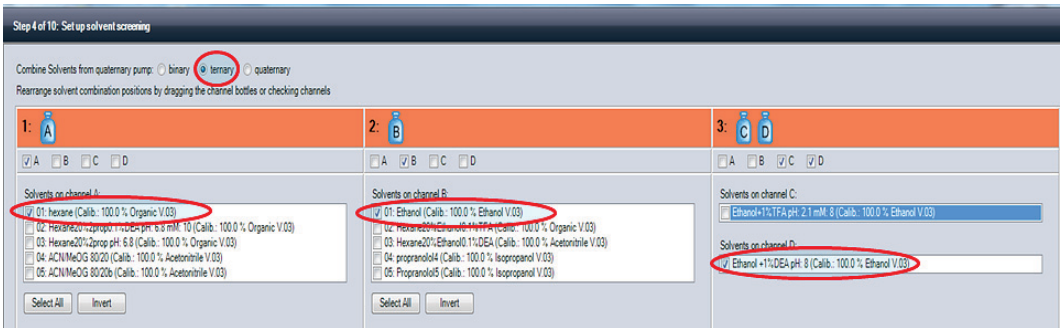


Figure 2. Selection of gradient type and mobile phases.

Tofisopam and vanillyl-mandelic acid were analyzed using isocratic conditions, therefore, the solvent composition had to be the same over the complete run time. To achieve this, at 1 minute, the solvent composition was set equal to the start conditions.

For tofisopam, the first gradient contained hexane and ethanol only, and Solvent C was set to zero. For the second gradient, DEA was used, solvent B was set to 10 %, and Solvent C, which contained 1 % DEA in ethanol, to 10 %. In total, 20 % of ethanol was applied over the complete run time and 0.1 % DEA (Figure 3).

The Agilent Method Scouting Wizard was used for the following experiment:

- Column scouting with three columns and gradient scouting with two mobile phase compositions for tofisopam
- Column scouting with three columns and gradient scouting with one mobile phase composition for vanillyl-mandelic acid

Table 1 shows the combined test conditions.

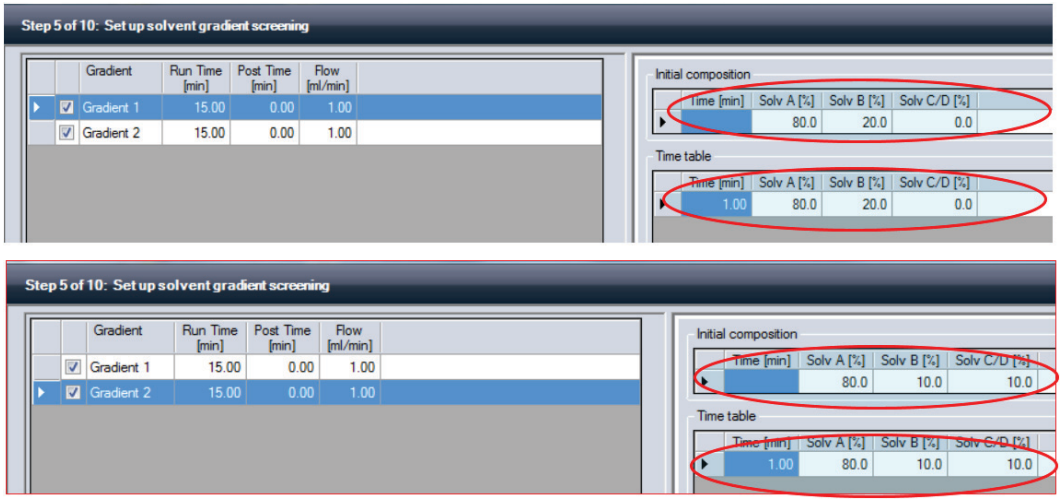


Figure 3. Selection of gradients.

Table 1. Test conditions.

Mobile phase	Hexane	Ethanol	1 % TFA	1 % DEA	Separated chiral compounds	Column YMC chiral polysaccaride
Composition 1	80 %	20 %			Tofisopam	Cellulose SB, C and Amylose
Composition 2	80 %	10 %		10 %	Tofisopam	Cellulose SB, C and Amylose
Composition 3	80 %	10 %	10 %		Vanillyl-mandelic acid	Cellulose SB, C and Amylose

The results of Experiment 1 are combined in Figure 4. The resulting chromatograms of column scouting and gradient scouting runs are overlaid. Separation for all four peaks was obtained on the Chiral Cellulose SB. Both mobile phase compositions provided similar resolution.

These results were presented in special method development reports, one for maximum peaks (Figures 5 and 6) and another for maximum resolution (Figures 7 and 8). In the Maximum Peak report, the number of found peaks was given. To ensure that only the relevant peaks were counted for the analysis, the integration parameters had to be selected accordingly. In the first part, four peaks were found for the Chiral Cellulose-SB and Column C, and only two for the Chiral Amylose-C.

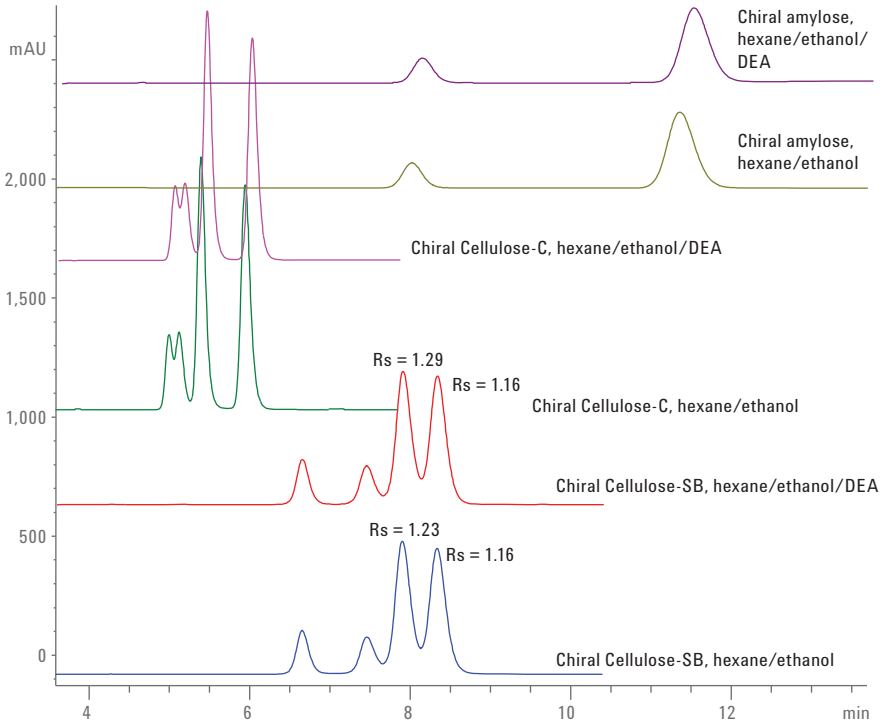


Figure 4. Column scouting and gradient scouting results for tofisopam.

Sample: **tofisapam**

Name DAD1A

Peaks	#	Datafile	Column	Solvent 1	Solvent 2	Solvent 3	Temp.	Gradient
4	2-2	1CB-0202.D	Cell-SB	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1% DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 1
4	4-2	1CB-0402.D	Cell-SB	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1% DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 2
4	6-2	1CB-0602.D	Cell C	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1% DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 1
4	8-2	1CB-0802.D	Cell C	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1% DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 2
2	10-2	1CB-1002.D	Amylose	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1% DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 1
2	12-2	1CB-1202.D	Amylose	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1% DEA pH: 8 (Calib.: 100.0	25.0 °C	Gradient 2

Figure 5. Part 1 of of Maximum Peak report.

All resolution values for all integrated peaks were totaled for the maximum resolution report. The Maximum Resolution report showed that the best separation was found for the Chiral Cellulose-SB column with DEA as the modifier, followed by a slightly lower value without a modifier on the same column.

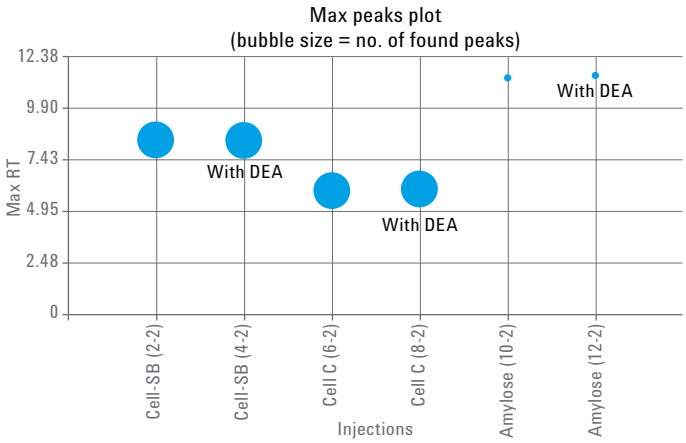


Figure 6. Part 2 of a maximum peak report.

Sample: tofisapam

Name DAD1A

Resolution Sum	#	Datafile	Column	Solvent 1	Solvent 2	Solvent 3	Temp.	Gradient
5.02	4-2	1CB-0402.D	Cell-SB	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1%DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 2
4.92	2-2	1CB-0202.D	Cell-SB	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1%DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 1
4.79	8-2	1CB-0802.D	Cell C	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1%DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 2
4.74	6-2	1CB-0602.D	Cell C	A01: hexane (Calib.: 100.0 % Organic V.03)	B01: Ethanol (Calib.: 100.0 % Ethanol V.03)	D: Ethanol +1%DEA pH: 8 (Calib.: 100.0 % Ethanol V.03)	25.0 °C	Gradient 1

Figure 7. Part 1 of a maximum resolution report.

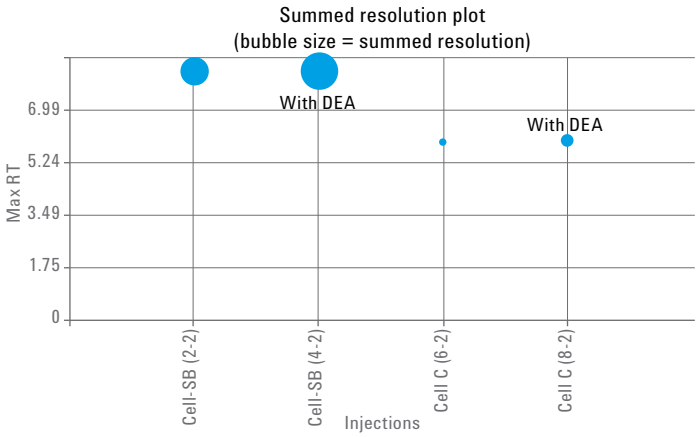


Figure 8. Part 2 of a maximum resolution report.

A second compound, vanillyl-mandelic acid, was analyzed using Composition 3 (Table 1). The best separation was obtained on the Chiral Amylose-C column with hexane/ethanol/1 % TFA in ethanol = 80/10/10 (Figure 9).

Conclusion

The Agilent 1200 Infinity Series Method Development Solution, in combination with the Agilent Method Scouting Wizard software, is best suited for method development using chiral columns and conditions. Scouting sequences were set up with a few mouse clicks, testing two compounds with three columns and two mobile phase compositions.

Separation of the four tofisopam isomers and the two vanillyl-mandelic acid enantiomers could be achieved using the columns and solvent compositions under isocratic conditions as shown in Table 2.

Table 2. Test results.

Mobile phase	Hexane	Ethanol	1 % TFA	1 % DEA	Separated chiral compounds	Column YMC chiral polysaccaride
Composition 2	80 %	10 %		10 %	Tofisopam	Cellulose SB
Composition 3	80 %	10 %	10 %		Vanillyl-mandelic acid	Amylose

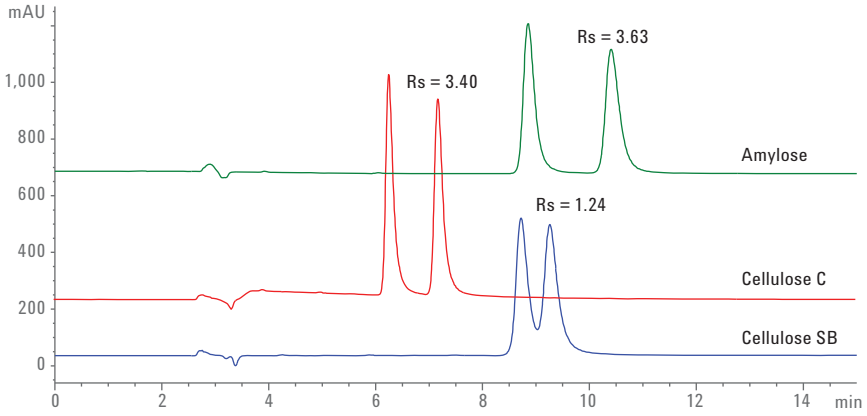


Figure 9. Analysis of vanillyl-mandelic acid on three chiral columns.

References

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