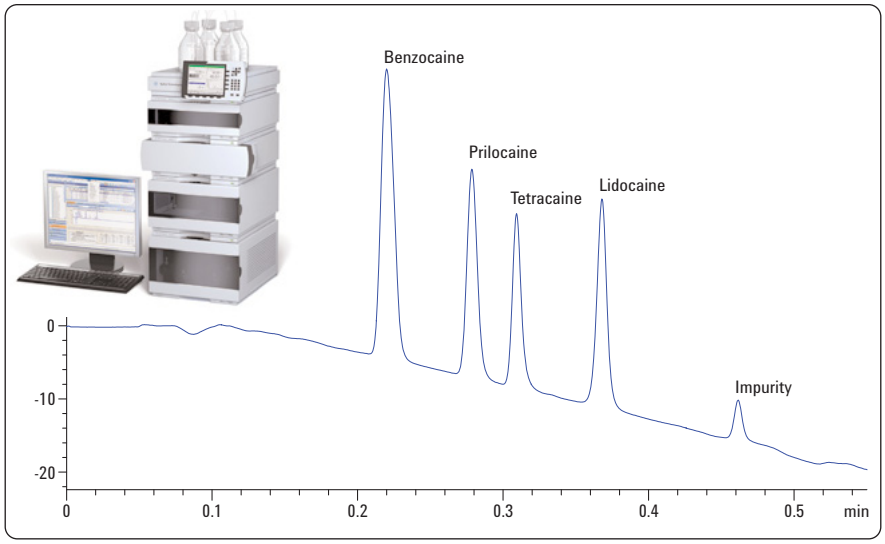
High speed separation of anesthetics on the Agilent 1290 Infinity LC system with different columns

Application Note



Authors

Detlef Wilhelm
ANATOX GmbH & Co. KG
Fuerstenwalde, Germany

Edgar Naegele
Agilent Technologies GmbH
Hewlett-Packard St. 8
Waldbronn, Germany

Abstract

The limits of resolution, peak capacity and pressure can be explicitly reduced when analyzing with the Agilent 1290 Infinity LC system. The power and flow design of the Agilent 1290 Infinity Binary Pump allows the use of various eluent compositions with any column type, and provides the high sensitivity of the new UV detection system.

The need to convert existing methods to fast or high resolution methods, causes difficulties such as the adaptation of delay volumes of the former HPLC system or the back pressure of the required column type to the new setup.

This Application Note shows the separation of local anesthetics with different column types. It demonstrates the transfer of parameters from a 5 µm column to columns with particles < 2 µm. The results show high resolution even under high throughput conditions. The best separation results (0.4 min) were achieved with the Agilent ZORBAX RRHD Eclipse Plus C18 HD 50 mm × 2.1 mm, 1.8 µm column with an overall runtime of 1 min, including regeneration. The results for the determination of the precision of areas and retention times (< 0.5 %) show that all criteria for qualified instruments are fulfilled. The correlation coefficients for linearity for all components are better than 0.999. No carryover was detected.

Agilent Technologies

Introduction

The development of the Agilent 1290 Infinity LC system resolved many issues around ultra-high performance, ultra-high pressure liquid chromatography. In addition, it has extended the limits of resolution, peak capacity, and pressure.

The power and flow design of the pump with reduced delay volumes, the elimination of an extra mechanical pulsation damper, and the new Jet Weaver for gradient mixing allows the use of any eluent composition, and any column type while still producing the highest sensitivity.

Many other HPLC systems need to be optimized to special column types, (such as columns with 4.6 mm diameter) because of their flow design. The Agilent 1290 Infinity LC system uses a small system volume, which has very little influence on dispersion and peak width. This allows the use of any column, with any diameter, or length, filled with any particle size packing, and still provides good results. This is especially true with 2.1 mm columns.

The recent trend to improve resolution, save time, and reduce solvent costs was to transfer methods from 4.6 mm columns with 5 µm particles to columns with smaller diameters and smaller particles. This also lowered the cost per analysis by shortening the analysis time. The transfer of methods by calculation to fast or high resolution methods provides the challenges of adaptating delay volumes of the former HPLC system, and adjusting the back pressure of the required column type to the new setup.

This Application Note describes the separation of four local anesthetics using different column types from different vendors.[1] It will also describe the transfer of parameters from a 5-µm column to columns with particles < 2 µm from different vendors. The results show high resolution even under the high throughput conditions. The best results were achieved with the Agilent ZORBAX RRHD Eclipse Plus C18 HD 50 mm × 2.1 mm, 1.8 µm column. The criteria for precision of retention times and areas are fulfilled, and demonstrate the versatility of high speed applications.

Experimental

Instrumentation

An Agilent 1290 Infinity LC system with the following configuration was used:

G4220A	1290 Infinity Binary pump with integrated vacuum degasser and 35 µL Jet Weaver as mixing device
G4226A	1290 Infinity Autosampler
G1316C	1290 Infinity Thermostatted Column Compartment
G4212A	1290 Infinity Diode Array Detector
Software:	ChemStation B.04.02

Configuration of the Agilent 1290 Infinity LC system

Preparation of samples

Reference samples

The stock solution of each anesthetic was prepared by dissolving 10 mg of each compound in water in a 100 mL volumetric flask yielding a concentration of 100 µg/mL (Figure 1). Samples were prepared by mixing aliquots of each component to yield the final concentration. The reference sample used to check the separation was prepared by mixing 2.5 mL of each stock solution in a 10-mL flusk to yield a ready-to-use solution. As an example for the calibration samples: the solution used for calibration of the 10 µg/ml point was prepared by mixing 1 mL of each stock solution in a 10-mL volumetric flask and diluting it to the final volume with water. Calibration points used to evaluate the correlation were: 1, 2.5, 10, 25, 50, 100 µg/mL with the Agilent ZORBAX RRHD Eclipse Plus C18 50 mm × 2.1 mm, 1.8 µm column at 1.9 mL/min.

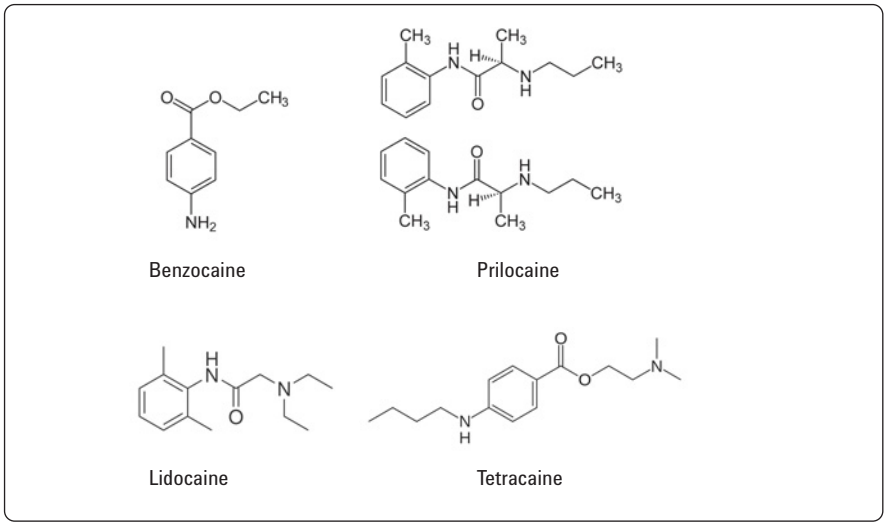


Figure 1
Chemical structures.

Chromatographic conditions

Columns	Agilent ZORBAX Eclipse Plus C18, 150 × 2.1 mm, 5 µm
	Agilent ZORBAX Eclipse Plus C18, 50 × 2.1mm, 3.5 µm
	Agilent ZORBAX RRHD Eclipse Plus C18, 50 × 2.1 mm, 1.8 µm
	Waters BEH C18, 50 × 2.1 mm, 1.7 µm
Mobile Phase	A: 50 mM Ammonium formate, pH=8.2
	B: Acetonitrile

Detailed chromatographic conditions are listed in Table 1.

Setup for testing

With the following setup for the reference sample the transferred methods can be checked:

- Establishment of a chromatographic separation to compare the performance of different column types (Resolution > 2)
- Precision of areas must be < 1 % RSD.
- Precision of retention times must be < 0.5 % RSD.
- Linearity should be given at least with $R^2 > 0.999$
- With these limits and settings for testing the following samples were prepared and analyzed (Table 2).

	Agilent ZORBAX Eclipse Plus C18, 150 × 2.1 mm, 5 µm	Agilent ZORBAX Eclipse Plus C18, 50 × 2.1 mm, 3.5 µm	Agilent ZORBAX RRHD Eclipse Plus C18, 50 × 2.1 mm, 1.8 µm	Waters BEH C18, 50 × 2.1 mm, 1.7 µm
Flow rate	0.8 ml/min	0.5 ml/min	1.9 ml/min	1.5 ml/min
Gradient	0-1 min 0-28% B 1-7 min 28-70% B	0-4 min 0-70% B	0-0.45 min 0-70% B	0-0.45 min 0-70% B
Temperature	40 °C	40 °C	40 °C	40 °C
Injection volume	5 µl	5 µl	1 µl	1 µl
Detection	DAD, Signal 225/4, Reference 400/80, standard Cell (1 µl, 10 mm)			
Data rate	2 Hz	10 Hz	80 Hz	80 Hz
Maximum pressure	98 bar	65 bar	945 bar	865 bar

Table 1
Instrument conditions.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	3
Reference sample	Verify precision of areas and retention times for reference solution	10
Calibration	Verify linearity	3 for each level
Highest concentration and Blanc solution	Verify carryover	3 of each sample

Table 2
Sample setup for testing.

Results and discussion

Due to the varied pharmacological properties of local anesthetics, they are used in many different anesthesia applications.

The chromatographic properties result from the chemical structure; many of them are aminoesters or aminoamides. These primary or secondary amines (Figure 1) tend to tail on RP-columns at low pH-values. Separations in the mid or high pH-range (pH=8-10) are preferred to avoid asymmetric peaks. Therefore, RP materials with high stability such as the ZORBAX Eclipse Plus C18 are needed. A typical chromatogram for a separation of four local anesthetics, with the impurity originated from tetracaine, at pH = 8.2 is shown in Figure 2. The instrument conditions are listed in Table 1. A simple mixture for the eluents without attention to the baseline was chosen.

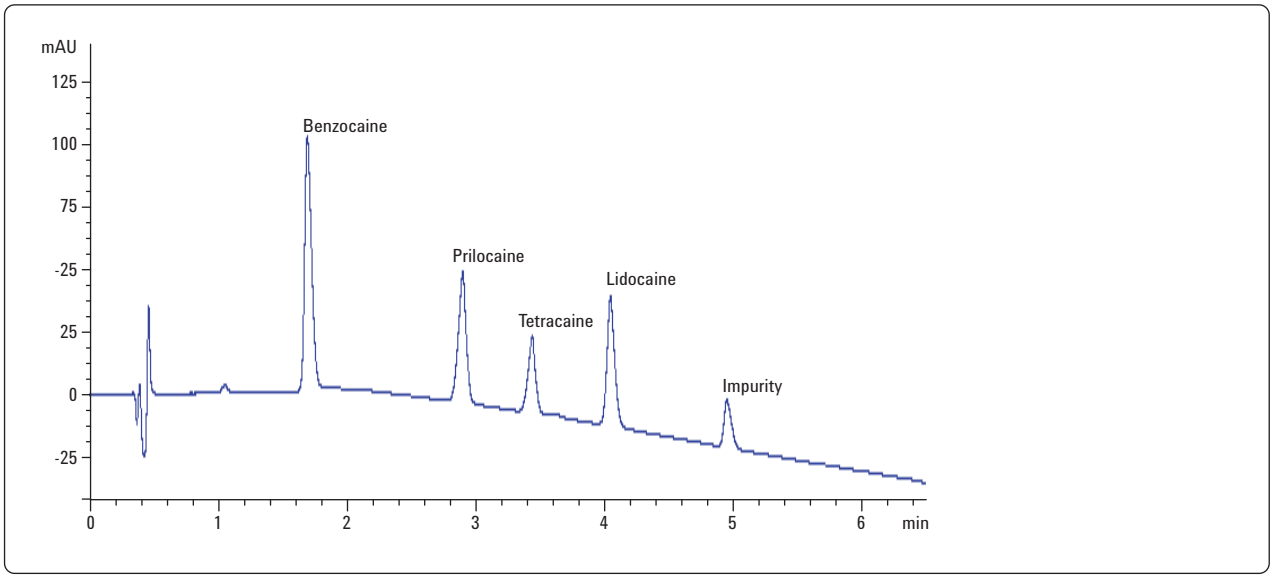


Figure 2
Separation of local anesthetics on Agilent ZORBAX Eclipse Plus C18, 150 × 2.1 mm, 5 µm.

When using 3.5 µm material to shorten analysis time, the parameters of the separation with the ZORBAX Eclipse Plus C18, 150 × 2.1 mm column with 5 µm-material can be used. With the Method Translator the new parameters can easily be calculated (Figures 3 and 4).

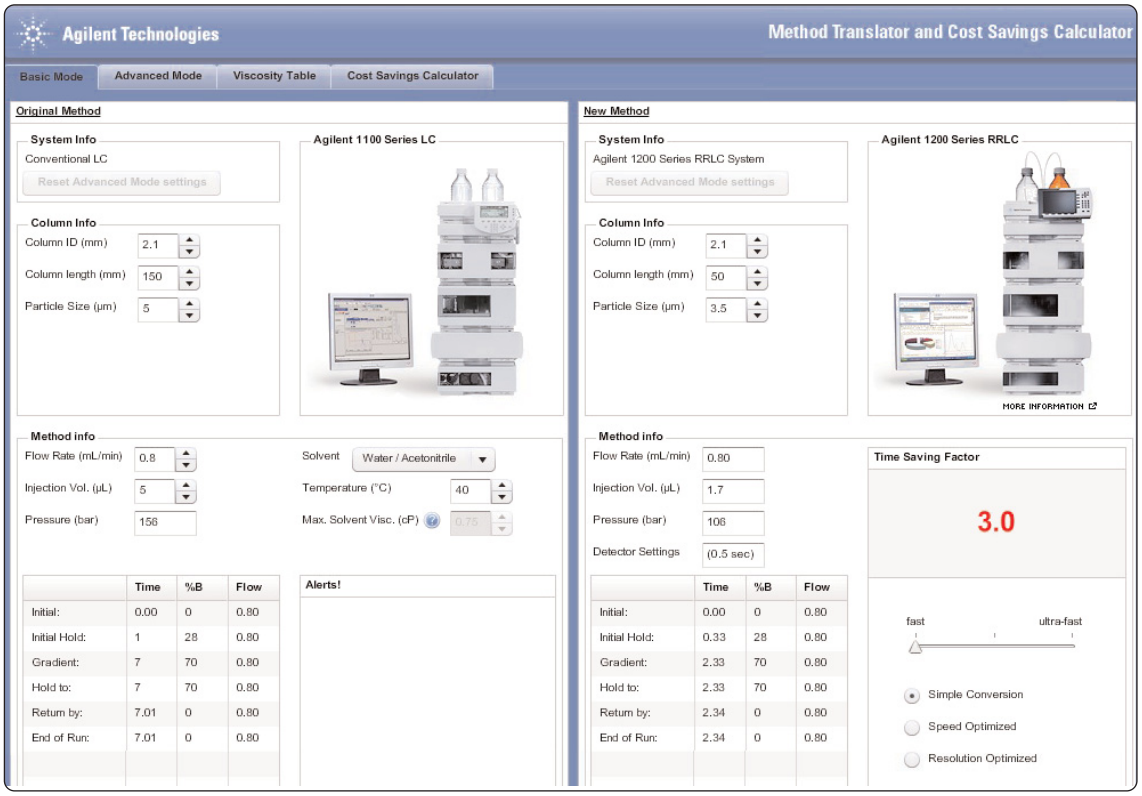


Figure 3
Calculating the new parameters for the 3.5 µm column with the Method Translator Software.

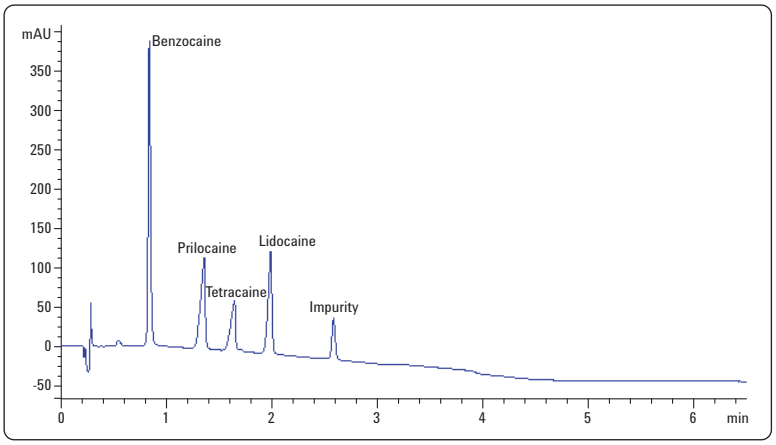


Figure 4
Separation of local anesthetics on Agilent ZORBAX Eclipse Plus C18, 50 × 2.1 mm, 3.5 µm.

To further reduce the analysis time the parameters can be transferred to columns with particles < 2 µm. Leaving the column dimension constant (50 mm × 2.1 mm) will improve the separation power because of the increased number of plates. When the system is independent of back pressure like the 1290 Infinity LC system the flow and the gradient shape can be increased, which dramatically decreases the run time. The results can be seen with separations in Figures 5 and 6. Both the Waters BEH C18 and the Agilent ZORBAX RRHD Eclipse Plus columns with particles < 2 µm provide a full separation of all peaks.

Table 3 lists the results of resolution calculations for all anesthetics separated with the different columns. For all peaks the resolution is greater than 2.5, even at highest flows and highest back pressures. With the BEH column the back pressure is remarkably higher resulting in lower flow rates and the peak shape shows some tailing, which is probably reduced at higher pH values. With the ZORBAX RRHD Eclipse Plus column no peak tailing at pH = 8.2 is seen as a result of good endcapping. An overall run time of 1.00 min is achieved with a flow of 1.9 mL/min. This is because reequilibration is done in 30 s, due to the small system and delay volume of the column.

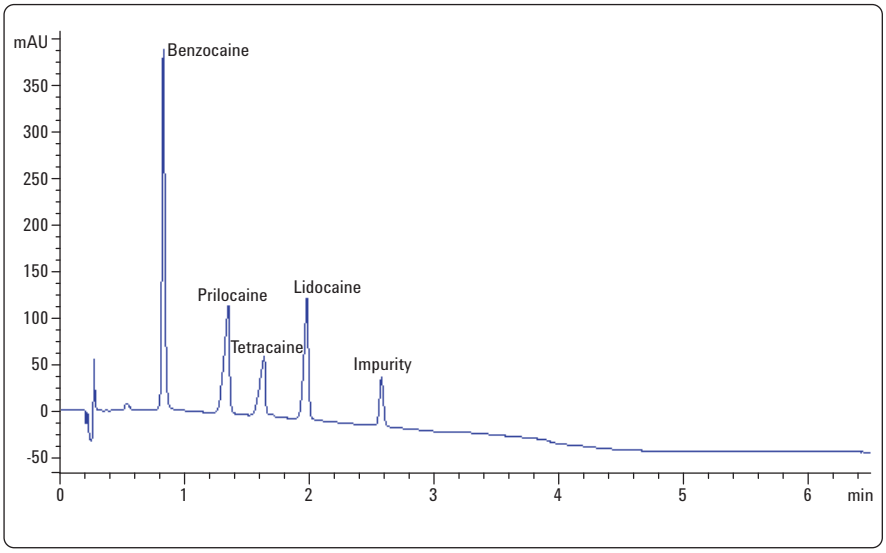


Figure 5
Separation of local anesthetics on Waters BEH C18, 50 × 2.1 mm, 1.7 µm, Flow: 1.5 ml/min.

Compound	Agilent ZORBAX Eclipse Plus, 150 × 2.1 mm, 5 µm, 0.8 ml/min	Agilent ZORBAX Eclipse Plus, 50 × 2.1 mm, 3.5 µm, 0.5 ml/min	Agilent ZORBAX RRHD Eclipse Plus, 50 × 2.1 mm, 1.8 µm, 1.9 ml/min	Waters BEH C18, 50 × 2.1 mm, 1.7 µm, 1.3 ml/min
Benzocaine	-	-	-	-
Prilocaine	11.57	8.52	3.91	4.23
Tetracaine	5.15	3.57	2.56	2.64
Lidocaine	5.94	4.65	5.13	3.88
Stop time	6 min	3 min	0.5 min	0.6 min

Table 3
Resolution of the anesthetics depending on column types (see Figures 2, 4-6).

Table 4 shows the data for the precision of the method applied to the separation with the Agilent ZORBAX RRHD Eclipse Plus C18, 50 mm × 2.1 mm column, and the high flow rate of 1.9 mL/min (Figure 6).

The data for precision of the retention times prove the high precision and stability of the flow, even at high pressure and high flow rates. The data also reflect the high efficiency of the new low volume jet weaver as a gradient mixing tool. The data for precision of areas show the good performance of the Autosampler. This is also illustrated by correlation coefficients for all components greater than 0.999 (Figure 7) with lidocaine as a reference.

	Retention times		Areas		Linearity
	Mean	RSD	Mean	RSD	R^2
Benzocaine	0.214	0.214	2,885,499.30	0.485	0.9998
Prilocaine	0.286	0.289	1,930,676.50	0.366	0.9999
Tetracaine	0.318	0.207	1,424,720.20	0.451	0.9998
Lidocaine	0.373	0.144	1,882,887.60	0.371	1.0000

Table 4
Determination of the precision of areas and retention times for the reference sample (chromatogram see Figure 6), linearity for 1-100 µg/ml calibration.

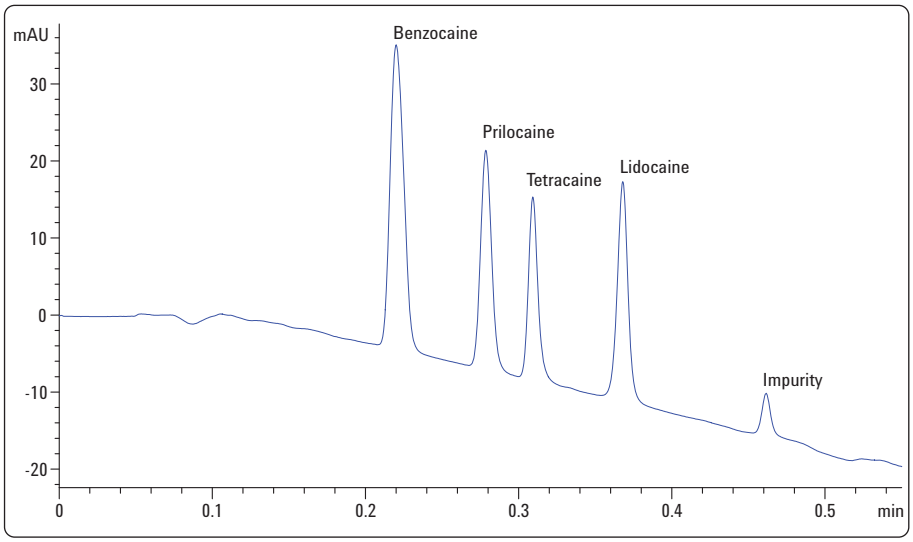


Figure 6
Separation of local anesthetics on Agilent ZORBAX Eclipse Plus RRHD C18, 50 × 2.1 mm, 1.8 µm, Flow: 1.9 ml/min.

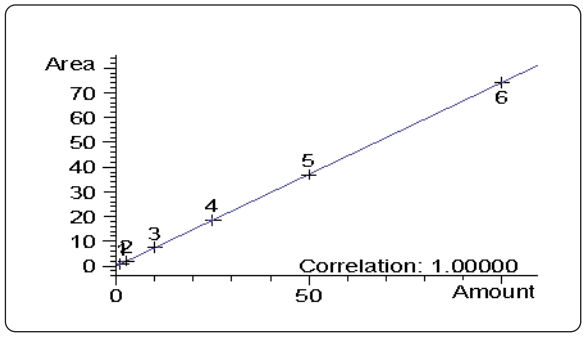


Figure 7
Calibration curve for lidocaine as example for all anesthetics.

A further test to evaluate the sampler performance is the determination of carryover. Figure 8 shows the chromatogram after an injection of the mixture. No carryover can be seen.

Conclusion

The new Agilent 1290 Infinity LC is designed to provide the highest speed, resolution and sensitivity. A new power range allows you to operate with columns filled with any particle type, any column dimensions, or any mobile and stationary phase. The 1290 Infinity LC is the first system to allow method transfer from any Agilent HPLC System to a new system.

The example separation of four local anesthetics has also shown that applications with conventional columns will run with high performance. The Method Translator is a helpful tool to make these methods faster. The good results of method transfer show that the selectivity and performance of the Agilent ZORBAX Eclipse Plus C18 material is independent of the particle size. The overall run time of the final method of 1.00 min, including reequilibration shows the infinite number of opportunities for establishing high resolution and ultrafast liquid chromatography.

With the new low volume jet weaver, effective gradient mixing provides high precision of gradient times.

The results shown in Tables 3 and 4 illustrate that all criteria for the precision of determination: areas, retention times, and resolution are fulfilled. Also the coefficients for linearity for all components are better than 0.999. All results explicitly show the applicability of the 1290 Infinity LC system for quality control testing as well as for high resolution and ultrafast liquid chromatography.

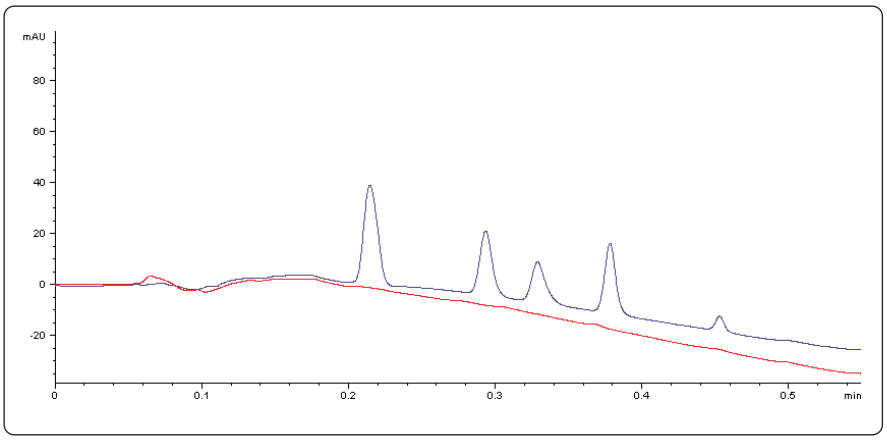


Figure 8
Blank injection to detect any carryover (blue-mixture, red-blank).

The infinite possibilities of the system are best shown by the overall run time including reequilibration of 1.00 min. The good flow design of the 1290 Infinity LC system assures the user that no band broadening or peak distortion will occur to hinder the separation power. In addition the "system pressure" will not limit the possible high operating flow rates.

In summary, the data obtained in this Application Note demonstrates the versatility and reliability of the Agilent 1290 Infinity LC system. It shows fast method transfer from or to any column and particle size and therefore, is applicable for any desired application. The Agilent 1290 Infinity LC system meets highest requirements for every LC function.

References

1. R. Ricker, "Bonded-phase selectivity, separation of local anesthetics", Agilent Publication 5988-6424EN, 2002

www.agilent.com/chem/1290

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