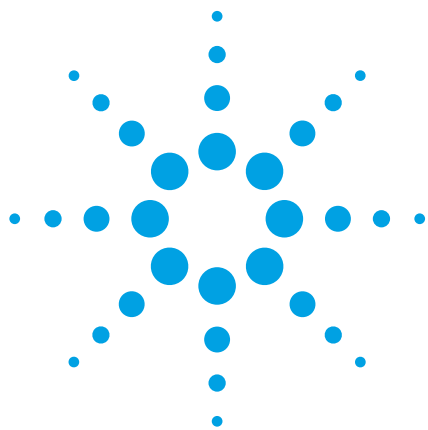




LC walk-up system using the Agilent 1200 Series LC Method Development Solution and Agilent Easy Access software

Test of reaction kinetics, column scouting, and impurity checks with one LC system and up to eight columns

Application Note

Drug Development

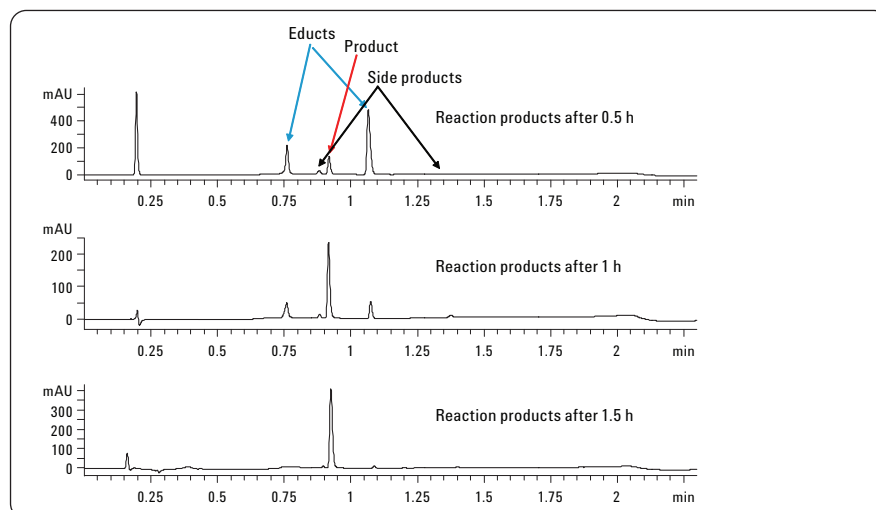
Authors

Angelika Gratzfeld-Hüsgen

Michael Frank

Agilent Technologies

Waldbronn, Germany



Abstract

In chemical synthesis laboratories different users are involved in the synthesis of new compounds. Typically, different projects are running for several groups or single users. The needs and analytical tasks for liquid chromatography in combination with mass spectrometers are:

- In-time reaction monitoring
- Control of reaction products
- Column scouting before preparative LC
- Purity analysis after preparative LC
- Fast and easy access to a running LC system
- Availability of columns of different selectivity
- Predefined chromatographic methods
- Fast reporting if sample has been analyzed



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Introduction

The main task of a chemical synthesis laboratory is to generate new compounds like new drug compounds, new pesticides, or new dyes that fulfill certain prerequisites given by the governing company. Typically, several attempts to reach a desired structure are required and often several modifications of such a structure are desired as well. The individual synthesis of compounds involves reaction monitoring, control of reaction products, and purity check of compounds that were cleaned by preparative LC. Traditionally, many of these tasks have been done by thin layer chromatography; however, with rapid LC/MS instrumentation, results can be achieved even faster, and most importantly, with much more information depth. That is the reason why more and more companies make LCMS systems available to their chemists.

Recently, walk-up LC/MS systems are used in these environments, which provide easy access for inexperienced users, analysis of the submitted samples with predefined columns and chromatographic methods, and information about the results by, for example, e-mail after completion of the run. In many cases these users are unfamiliar with LC/MS systems. Therefore, superusers take care of the LC/MS system, providing the chromatographic methods, keeping the LC/MS system running and, in case of errors, being able to restart the system.

Typically, several projects are done in parallel. Therefore, different user groups or single users need access to a running LC system. Most important, the different projects often require different separation selectivity because of different compound properties.

All these tasks can be performed on the Agilent 1200 Series LC Method Development Solution and Agilent Easy Access software. This LC system can be used with up to eight columns of different length and internal diameter. The superuser can define different analytical methods for the installed columns, including appropriate equilibration methods. The superuser can also define projects for different user groups or single users. Different permission levels can be set for users and groups. Priorities can be set; for example, reaction monitoring samples can have highest priority and are analyzed immediately. After completion of the analysis, reports can be sent via e-mail to the appropriate user.

In this application, we used the Agilent 1200 Series LC Method Development Solution with attached Agilent 6410 Single Quadrupole MS in combination with the Easy Access software for the following laboratory environment:

- Three user groups need access to the LC/MS system
 - Four different projects are running
- All groups have the following analytical tasks:
- Reaction monitoring
 - MS identification of the reaction product
 - Column scouting of reaction mixtures to find the most suitable column for preparative purification
 - Purity control after preparative LC

Experimental

Equipment

The Agilent 1200 Series Rapid Resolution LC system comprised the following modules with firmware revisions A.06.10 or higher:

Agilent 1200 Series binary pump SL with degasser

Agilent 1200 Series high performance autosampler SL Plus

Two Agilent thermostatted Column Compartment SL Plus with installed valve drives

Method development valve kit (G4230B): high pressure with method development capillary kit, low dispersion, for short columns

Agilent 1200 Series diode array detector (DAD) SL

Agilent 6410 single quadrupole MS with multimode source

Several Agilent ZORBAX Rapid Resolution High Throughput (RRHT) 1.8 μ m columns

Agilent ChemStation B04.01 with Agilent Easy Access software

Results and discussion

Description of the LC/MS system

The Agilent 1200 Series LC Method Development Solution consists of two or three clustered thermostatted column compartments SL Plus integrated into an Agilent 1200 Series standard LC or RRLC system.¹ One column compartment contains the valve that is connected to the pump and delivers the flow to the different columns. The second column compartment contains the valve that is connected to the detector and delivers the flow coming from the active column to the detector. A maximum of eight columns up to 100 mm in length can be installed in two clustered column compartments using the low-dispersion heat exchangers (Figure 1).

The system can be equipped with an additional clustered solvent selection valve, installed on one pump channel, which offers the possibility to have up to 15 different solvents. The two clus-

tered column compartments offer four independent heating zones. This allows setting columns at different temperatures, which is of advantage if, for example, higher temperatures are needed for special applications.

Description of the Easy Access software

The Agilent Easy Access software allows the users to "walk up" with their samples, input sample information, choose from the list of methods, position the samples as directed by the system, and then return to their labs and wait for an e-mail containing the results.^{2,3}

Depending on the permission level of the users, they are allowed to change the selected method to a certain extent, like injection volume or number of injections.

The system configuration and administration is done by a superuser, who is responsible for keeping the system running for all the other users.

Application example

In the example presented in this application note, the system was set up with four short 50 × 2.1 mm columns of different stationary phases:

- one 100 × 2.1 mm column
- one 150 × 2.1 mm column

All columns were packed with 1.8- μ m particles.

Configuration and administration by the superuser

A chromatographic method was set up for each column in the ChemStation software. This has to be done by the superuser, who is the only one who has access to the screen in Figure 2. The ChemStation methods are used in the method setup screen in the Easy Access software as CS method (Figure 2). To facilitate the usage for the end user, the naming of the easy access method was kept in a logical context with the usage, in this case,

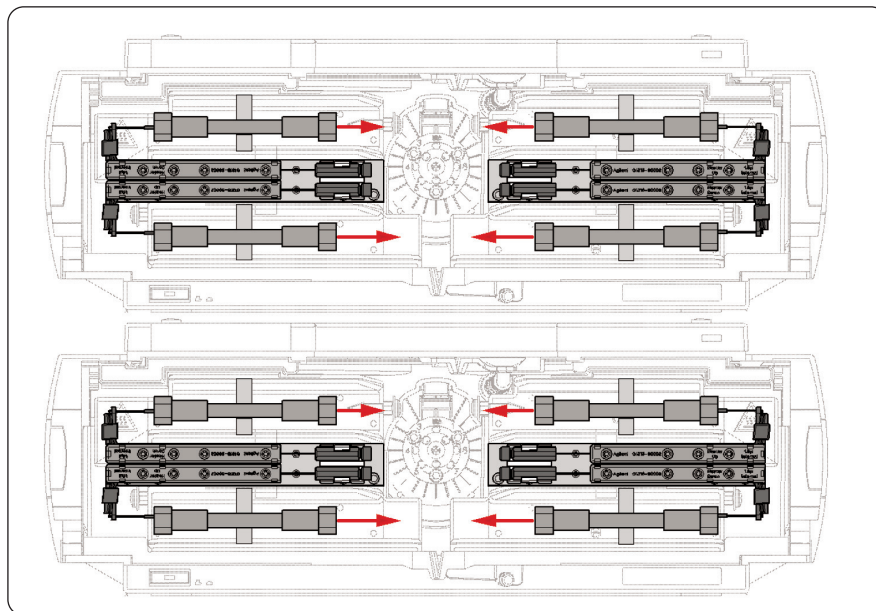


Figure 1
Clustered Agilent 1200 Series thermostatted column compartments SL plus with up to eight columns.

Agilent Easy-Access - Edit Existing Method

Method Name: fast kinetic CS Method: COLSCOUT SBC18.M

Description: 5 to 85% B in 3 min

Est. run time: 3 min. Time to equilibrate after change: 1 min.

Method priority (1 is highest): 1 CS Equil. Method: COLSCOUT SBC

UV wavelength (Channel A):

Sample,Bw	Reference,Bw	
254	10	380 80 nm

Method Type:

☐ Loop (no column)

☒ Chromatography

☐ Use Target Masses

Injection volume:

Minimum: 1 µl

Nominal: 3 µl

Maximum: 40 µl

LC/MSD Method Parameters

Method is in POSITIVE ion mode

Default Target Masses:

Update Cancel

Figure 2
Agilent Easy Access software screen to set up chromatographic parameters by the superuser.

"fast kinetic." Information about the gradient is given in the description section. Under CS EquilibrationMethod, the equilibration of the column is determined. In our case, each column was equilibrated using the chromatographic method that should also be used for the analytical run. This ensures that the column has seen the actual gradient

once already and that possible remaining peaks on the column from the previous analysis are not falsifying the actual chromatogram. But any other predefined method using the same column can be used to perform the equilibration. The equilibration method will only be executed if the analytical method is changed.

Having made all needed entries, the method setup in the Easy Access software is finished. In the next screen, the superuser has to define the user groups and users who should have access to the system and to what extent they are allowed to modify methods and other parameters.

Having configured the system, the user can now walk up and submit his sample. Depending on his permissions he fills in the necessary information, selects a method, modifies the method, puts one or several vials in the positions directed by the system, and walks away (Figure 3).

Group 1 submits samples for prep purification work

A chemist from group 1 has submitted a sample for column scouting. He has selected four columns with different selectivity to find the best column for a preparative cleanup of the sample. He puts the vial into the vial tray and the analysis can start using the selected columns with appropriate equilibration and run parameters. At the end the user gets for each run a report and can decide which column is the best (Figure 4).

Chromatographic conditions

Columns: 2.1 mm id × 50 mm, 1.8 µm, ZORBAX SB-C18, ZORBAX SB-CN, ZORBAX Bonus RP-C18, ZORBAX Eclipse PhenylHexyl
 Gradient: 5 to 85%B in 0.8 min, hold at 85%B to 1.5 min, re-equilibration at 5%B until 2.4 min at 0.8 mL/min flow
 DAD and MS detection

In this case, the Agilent ZORBAX SB-C18 column is the best for the prep analysis because resolution before and after the main peak is better than on the other three columns. For the prep analysis, the same column chemistry was chosen, using a 21.2 mm id × 150 mm ZORBAX SB-C18, 5 µm, PrepHT870150-902. The preparative LC/MS system is also operated as a walk-up system controlled by the Agilent Easy Access software.

Figure 3
User "Arno" submits a sample for column scouting.

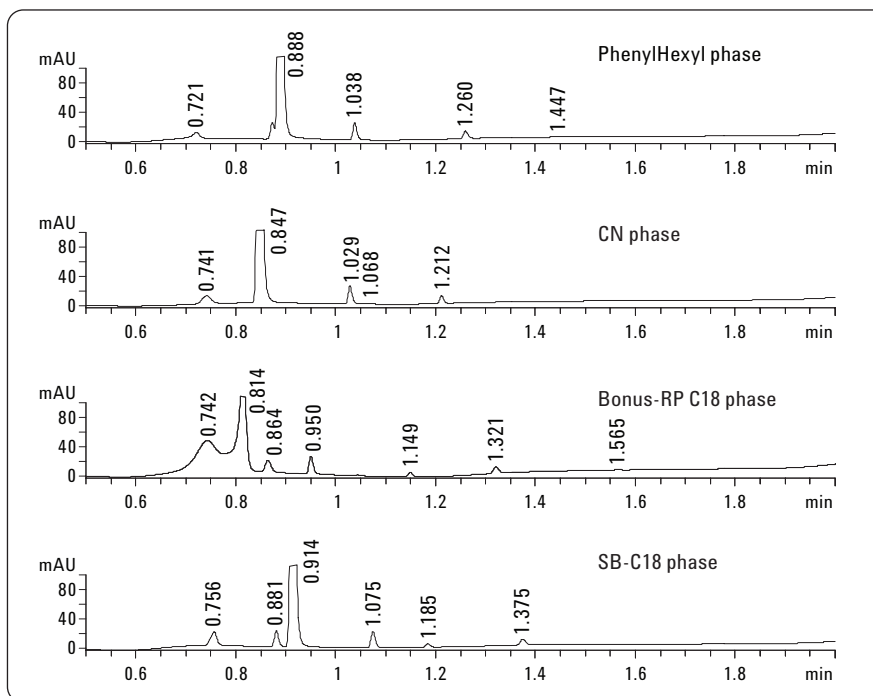


Figure 4
Column scouting for optimum prep analysis conditions.

After purification

Having purified the compound and evaporated the solvent, the resulting fraction was again submitted by a co-worker of group 1 and was analyzed on a 150-mm long column to see whether the fraction was clean, that no thermal degradation occurred during solvent evaporation and, if not, to see as many impurities as possible.

The left part of Figure 5 shows the sample plates with positions filled with vials. On the right side the done sample and the waiting samples are listed. In this case, the purity check sample will be the next sample injected. At the top of this screen is the estimated time the system will be occupied by the remaining runs. The user can now estimate how long it will take to get the results. After the analysis, the user gets the results via e-mail.

The fraction was pure enough for further analytical evaluations (Figure 6).

Chromatographic conditions

Column: 2.1 mm id x 150 mm ZORBAX SB-C18, 1.8 μ m
Gradient: 10 to 85%B in 8 min, hold until 9 min at 85%B, re-equilibration with 10%B until 11 min
Flow rate: 0.3 mL/min
DAD and MS detection

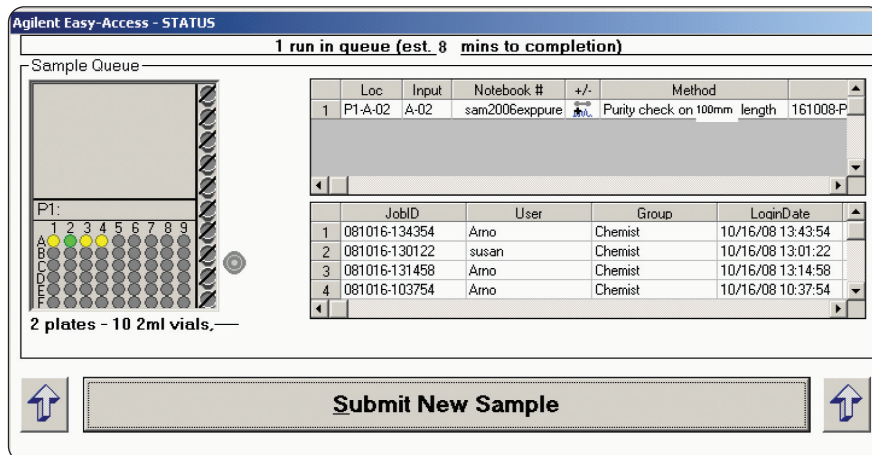


Figure 5
Monitoring screen of Agilent Easy Access software.

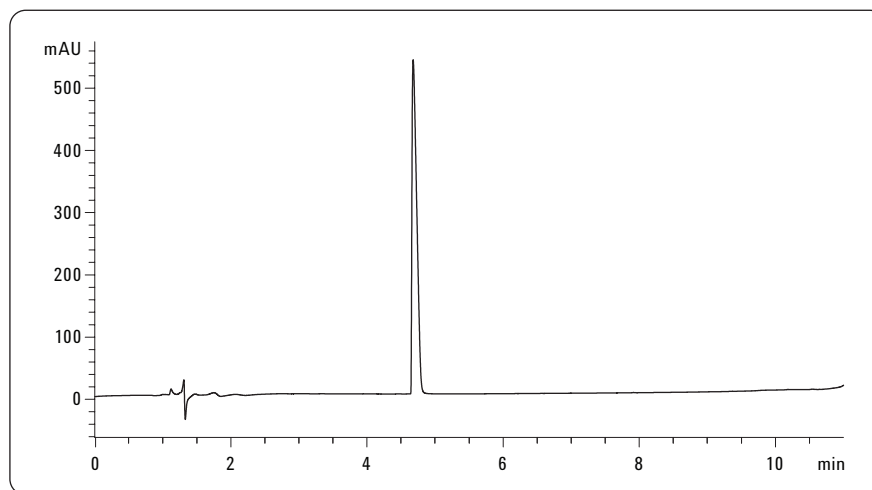


Figure 6
Purity check after preparative purification.

Group 2 submits samples for reaction monitoring

In the meantime a chemist from group 2 has submitted within 1.5 hours several samples to monitor a reaction for completion. To get a result in time, the samples were analyzed with highest priority. Selecting the method Fast Kinetics sets these samples on first priority and analysis start immediately after the previous run has ended. The results of the kinetic measurements are shown in Figure 7.

Chromatographic conditions

Column: 2.1 mm id × 50 mm ZORBAX SB-C18
 Gradient: 5 to 95%B in 1.5 min, hold at 95%B until 2.5 min, re-equilibration at 5%B until 3.6 min
 Flow rate: 0.8 mL/min
 DAD and MS detection

In addition to the UV chromatogram, the MS signal in scan mode was used to control whether the expected product was produced and also to get some rough ideas about the masses of the side products. In Figure 8 the obtained mass spectra are combined.

The expected product was formed with in 1.5 hours, with high yield. Two side products were formed that have to undergo further structure evaluations.

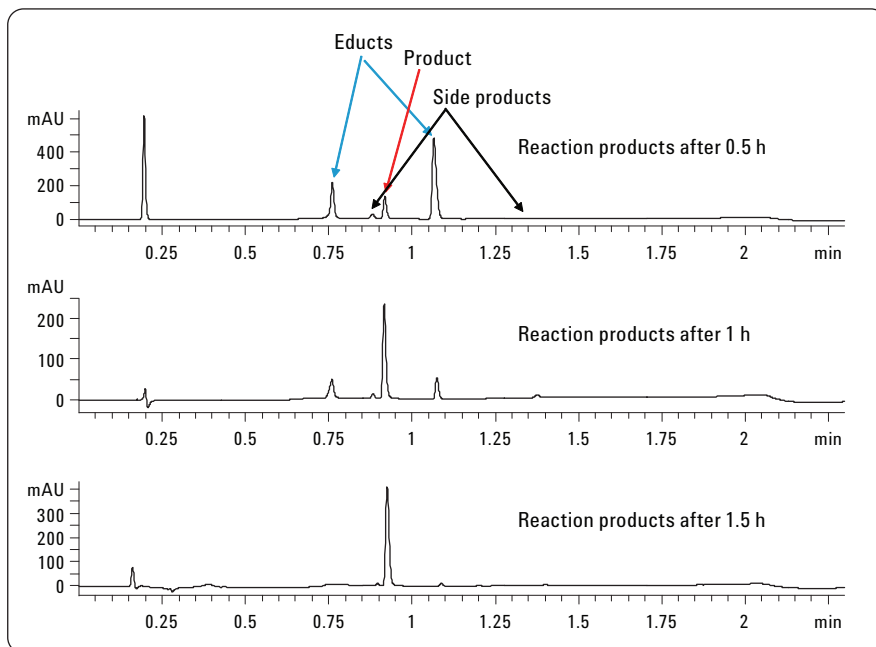


Figure 7
Study of reaction kinetics.

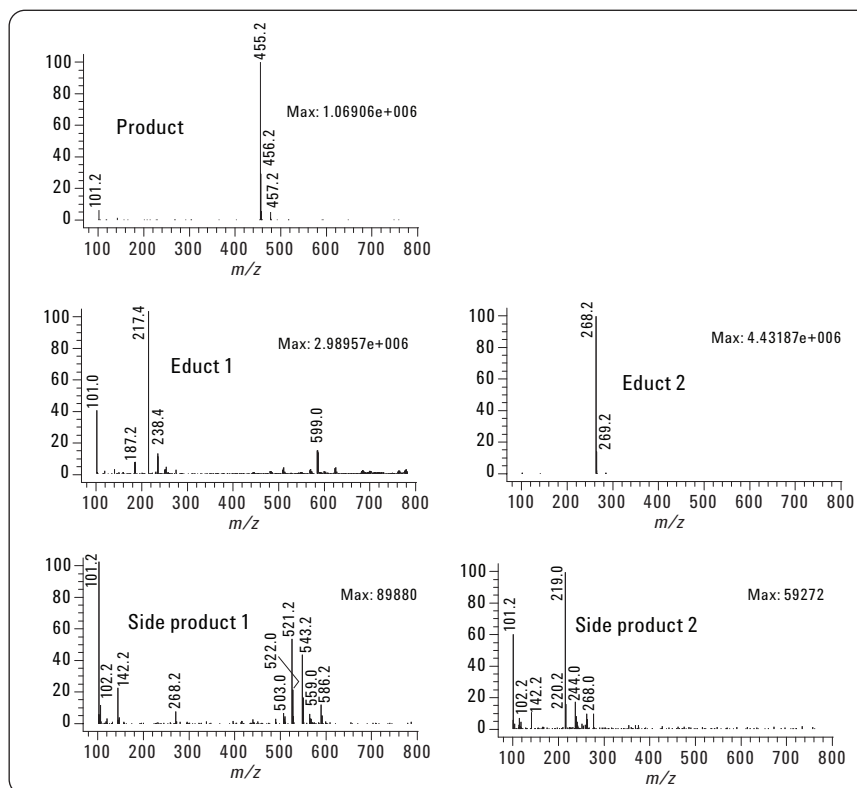


Figure 8
Mass spectra of submitted sample.

Group 3 submits a sample to control a reaction product

The third group that has access to the LC/MS system is submitting a sample that has been obtained from a reaction of two compounds. The resulting chromatogram shows main peaks with very low resolution. The LC/MS analysis was used to give some information about the reaction products. In Figure 9 the UV chromatogram is shown with three or four main peaks and four small peaks. Also, the MS (scan mode) total ion chromatogram and the resulting extracted ion chromatograms for the main peaks are shown. The expected product with the mass 450.4 at RT 1.087 min was formed after 2 hours.

The reaction kinetic seems to be rather slow or the reaction parameters, like temperature, etc., are not appropriate. Therefore, the yield of the expected product is low.

Conclusion

Setting up an open access LC/MS environment with analytical and preparative LC/MS systems increases the productivity of chemists so that they get the fastest access to the analytical results. This allows the chemists to perform the next required step in their workflow without waiting for a remote analytical laboratory.

Adding multiple-column and multiple-solvent availability to such a system by using the Agilent 1200 Series LC

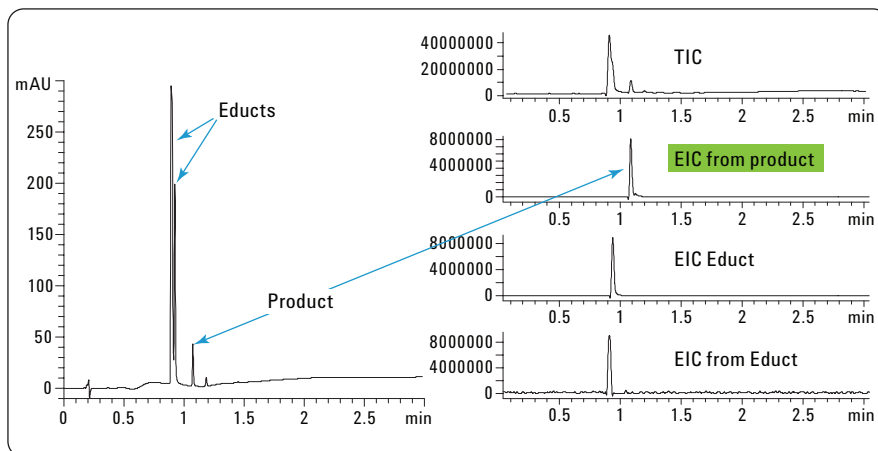


Figure 9
UV chromatogram, TIC, and EIC of a reaction mixture.

Method Development Solution together with the Agilent Easy Access software tailors the system even more to the needs of the chemists and allows them to become even more productive. Different user groups can use up to eight columns with different selectivity or separation efficiencies, always matching the properties of the compounds and the analytical task, without the need to change any hardware.

This provides optimum conditions for column scouting, reaction monitoring, and purity checks after preparative cleanup. Different detectors can be attached to the system, including UV and mass spectrometer. The Easy Access software allows samples with different priorities to be run in a logical and convenient way. Reporting can be done via e-mail.

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