Optimizing the Agilent 1100 Series high throughput LC/MS system

Application

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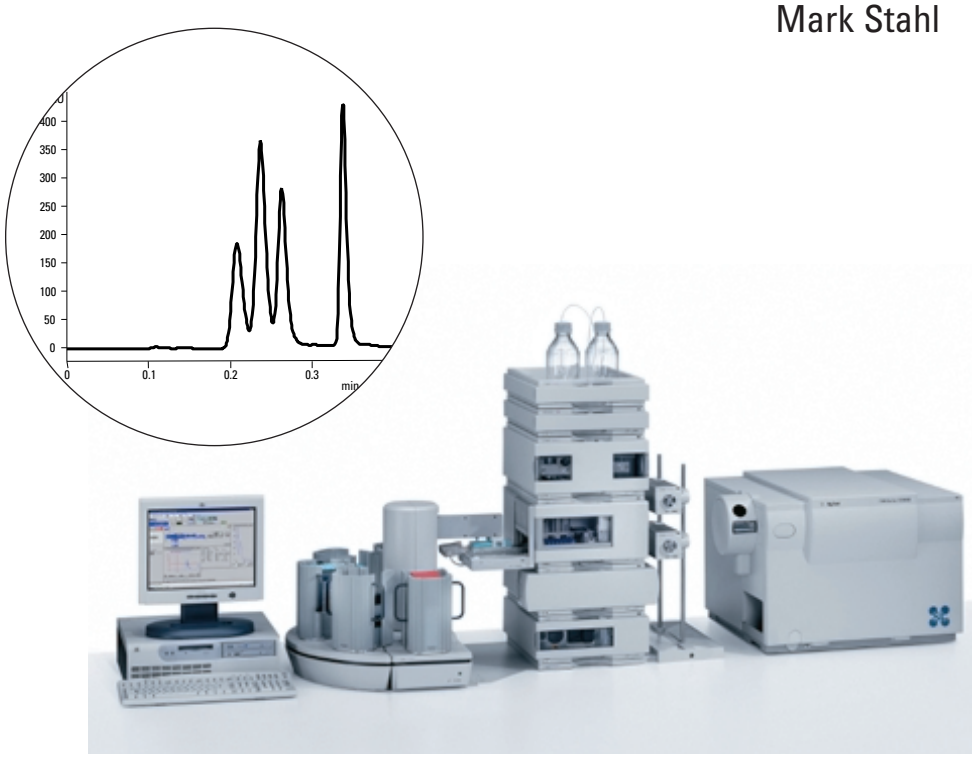

Abstract

This Application Note describes the optimization of the Agilent 1100 Series high throughput LC/MS system for different analytical tasks. We describe in detail how to optimize chromatography, diode-array and MSD detection to archieve highest sample throughput in combination with substance identification, how to acquire quantitative information while maintaining very high sample throughput and how to achieve fast analysis of very low sample amounts.

Agilent Technologies

Introduction

Due to increasing automation and the enormous progress in combinatorial chemistry, drug discovery and many other fields in organic and biological chemistry, the amount of samples arising per day has been and still is growing enormously. Especially central analytical labs of large enterprises have to analyze sample numbers of up to several thousands per day. These samples result from totally different origins, thus leading to totally different demands for the analysis. In many cases, substance identification is the goal, in others an exact quantification may be required. For most issues, sensitivity is not a limiting factor, but for many biochemical analysis it is one of the major requirements. In this Application Note analyses have been grouped into three different categories depending on the information required:

1. Highest sample throughput in combination with substance identification
2. Best quantification while maintaining a high sample throughput
3. Highest sensitivity in combination with fast analyses

Theoretical background

To speed up analysis, several factors have to be taken in consideration. To create a minimal dead volume the column length should be minimized but this results in poorer chromatographic separations. To retain as much performance as possible, particle diameters are lowered, but here the created backpressure is limiting. Smaller particle diameters also offer the advantage that with increased flow rates theoretical plate heights do not increase as much as with larger particles (figure 1). Therefore the use of small particle diameters below 2 µm is highly recommended for high throughput analysis.

To convert a common separation into a fast separation, chromatographic parameters have to be adjusted too. Therefore, isocratic and gradient separations have to be distinguished. In isocratic separations the chromatographic separation is more or less independent of the flow. With increasing flow rate dead time (t_0) and retention time (t_R) are lowered proportionally which results in a constant retention factor k and thus also selectivity α. Therefore higher flow rates result in lower run times with roughly the same resolution. In gradient separations this is different. Here the retention factor k changes continuously with the gradient. Thus k is not a good parameter to characterize gradient separations and it is replaced by k*:

$$k^* = 0.87 t_G \left(\frac{F}{V_M \Delta\%B S} \right)$$

with V_M = column void volume, $\Delta\%B$ = gradient percent range, S = constant for a given solute/solvent combination, F = flow rate and t_G = gradient time. Due to this the formula for the selectivity α changes to

$$\alpha = \frac{k_2^*}{k_1^*} .$$

These formulas show that in gradient elution changing flow rates has the same effect as changing the gradient. For example, at half flow rate the same resolution is achieved with a doubled gradient length. Thus to achieve comparable gradient separations the gradient volumes (gradient time multiplied with flow rate) have to be held constant. As high flow rates are used for high throughput analysis to achieve lowest run times this in this case leads to the advantage that shorter gradients can be used without loosing much chromatographic resolution. As the flow rate is closely related to the column diameter, this has also to be taken into consideration for up- and downscaling of methods, e.g. from a 4.6 mm column to a 1 or a 0.3 mm capillary column.

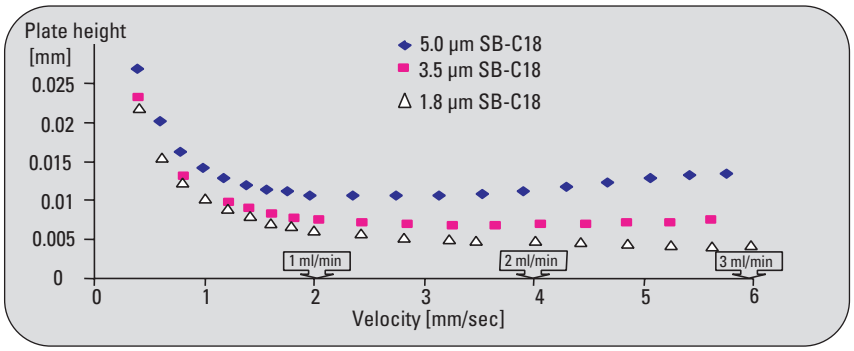

Figure 1
Column particle size comparison - Van Deemter curve for acetonitrile/water

1. Highest sample throughput in combination with substance identification

To achieve fastest cycle times, also called injection-to-injection times, sensitivity and resolution have to be sacrificed. Whereas identification of substances is achieved easily, the resulting resolution often does not allow accurate quantification, however, an estimation of the proportions can be done. In many cases this is sufficient and allows to achieve the desired high sample throughput. The Agilent 1100 Series high throughput LC/MS system shown in figure 2 is ideally suited for these analyses. The system consists of a degasser for online degassing the solvents, two binary pumps, a well-plate autosampler equipped with an automation interface, a well-plate handler which is the plate storing device, an internal or an external 10-port valve, a thermostatted column compartment, a diode-array detector (DAD), a mass selective detector (MSD) which can be based on quadrupole or on ion trap technology and a data evaluation unit. To reduce the dead volume of the system (figure 3) as far as possible, the mixer of the binary pump can be removed and capillaries with low internal diameter (180 µm or below) should be used. Removing the mixer increases baseline noise somewhat but still leads to acceptable performance. To reduce the dead volume further, short

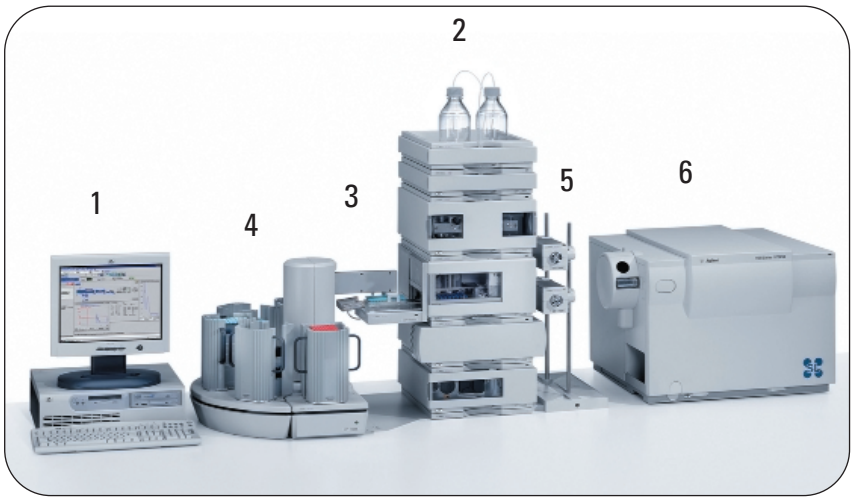

Figure 2
The Agilent 1100 Series high throughput LC/MS system
1. Data evaluation unit 2. Agilent 1100 Series LC system with degasser, binary pumps, well-plate sampler, thermostatted column compartment and DAD 3. Automation interface of the well-plate sampler, 4. Well-plate handler 5. External valves 6. Mass selective detector based on quadropole or ion trap technology

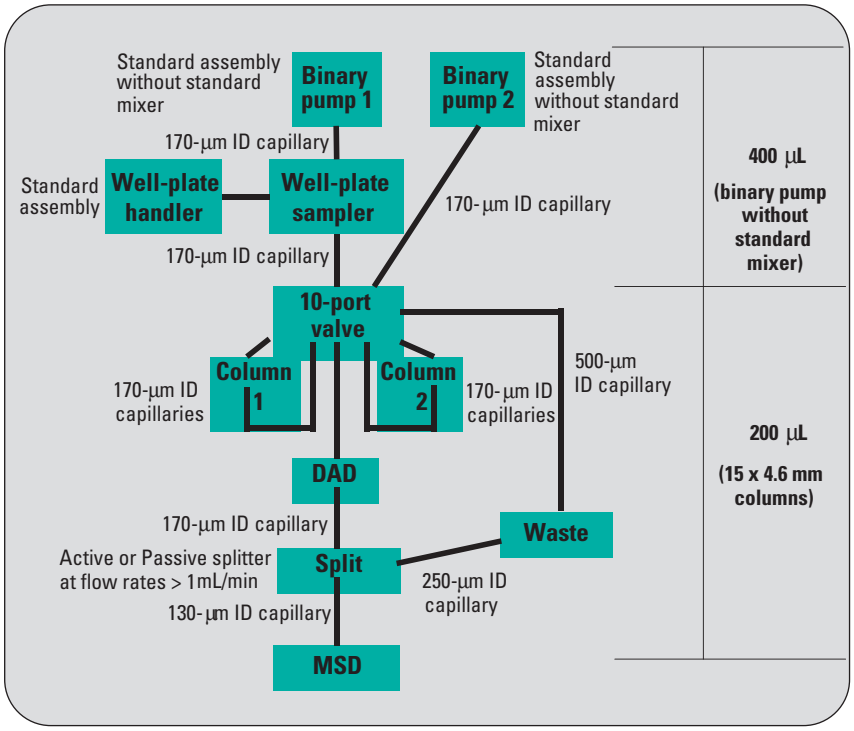

Figure 3
Schematic diagram of the Agilent 1100 Series high throughput LC/MS system

columns below the length of 30 mm are recommended. The internal diameter of these columns should be relatively high (> 3.9 mm) in order not to generate too much backpressure, thus allowing to perform separations with high flow rates of 4 or 5 mL/min. These combinations ensure that the delay time of the gradient (time gradient needs to reach the detector) is kept to a minimum. Some possible column - flow combinations are shown in table 1. To maintain resolution smaller particle size materials should be used, but again the resulting backpressure is the limitation. A good solution is to use the Agilent 1100 sub two micron particles (particle size of 1.8 µm), however 2.5-µm or 3.5-µm particles may also be used. In general, all column types can be used but the most common, the most generally applicable and the ones with the highest peak capacity are reversed phase columns.

To achieve short cycle times, short gradients have to be used. For this purpose a 0.5 or a 1-minute gradient can be regarded as standard gradients. To keep cycle times low only a very short, if any, isocratic step should be used before the gradient. To ensure the use of a generic method the gradient should include both extreme regions (e.g. 5 to 95 % ACN). Using ESI as ionization method the addition of ion pairing reagents should be kept as low as possible and only volatile buffers (for example formic acid, ammonium hydroxide or ammonium carbonate) should be used. Two typical gradients are shown in table 2.

Column Dimension	Flow Rate	Cycle Time for a 0.5 min gradient
50 x 4.6 mm	1 mL/min	2.5 min
50 x 4.6 mm	4 mL/min	1.4 min
30 x 4.6 mm	1 mL/min	2.2 min
15 x 4.6 mm	1 mL/min	2.0 min
15 x 2.1 mm	1 mL/min	2.0 min
15 x 4.6 mm	5 mL/min	1.1 min

Table 1
Injection to injection times that can be achieved with different column dimensions and flow rates.

Short high throughput gradient		Standard gradient	
0 min:	5 % ACN	0 min:	5 % ACN
0.5 min:	95 % ACN	3 min:	5 % ACN
0.51 min:	5 % ACN	17 min:	95 % ACN
0.7 min:	5 % ACN	19 min:	5 % ACN
		25 min:	5 % ACN
Flow:	5 mL/min	Flow:	1 mL/min

Table 2
Comparison of a standard gradient and a high throughput gradient.

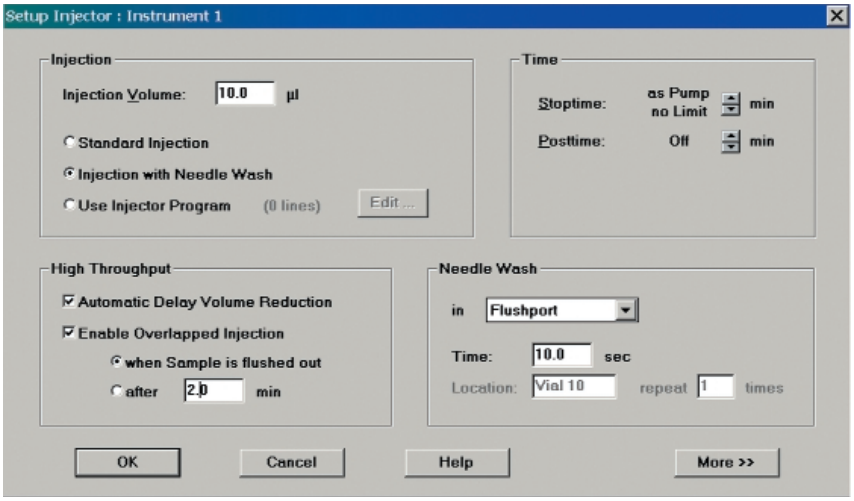

Figure 4
Injector setup for high throughput analysis.

To achieve shortest cycle times two columns can be used in parallel. This allows to analyze the sample on one column while the other one is regenerated. The control of the columns is done by a valve switching to the next position after having finished the first analysis. This reduces the cycle time of the amount necessary for column regeneration. You only have to ensure that the gradient has already reached the detector and is followed by starting conditions before switching the valve to the next column.
For sample storage and delivery the well-plate autosampler in combination with the well-plate handler is used. All different kinds of vial- and well-plates can be used with this system and you are also able to define your own ones. Using 384 well-plates the well-platehandler has a sample capacity of up to 30,720 samples thus allowing, the analysis of thousands of samples per day, in combination with the high robustness of the complete system and fully unattended analysis with very short cycle times. To achieve fast analysis automatic delay volume reduction and overlapped injection should be enabled. Even during these very short cycle times you still have enough time to perform a 10 s needle wash with an appropriate solvent prior to injection to ensure a low carry over of the well-plate sampler (figure 4). The carry over of sticky compounds can be reduced further by switching the injection valve at high organic concentrations to clean the valve grooves.

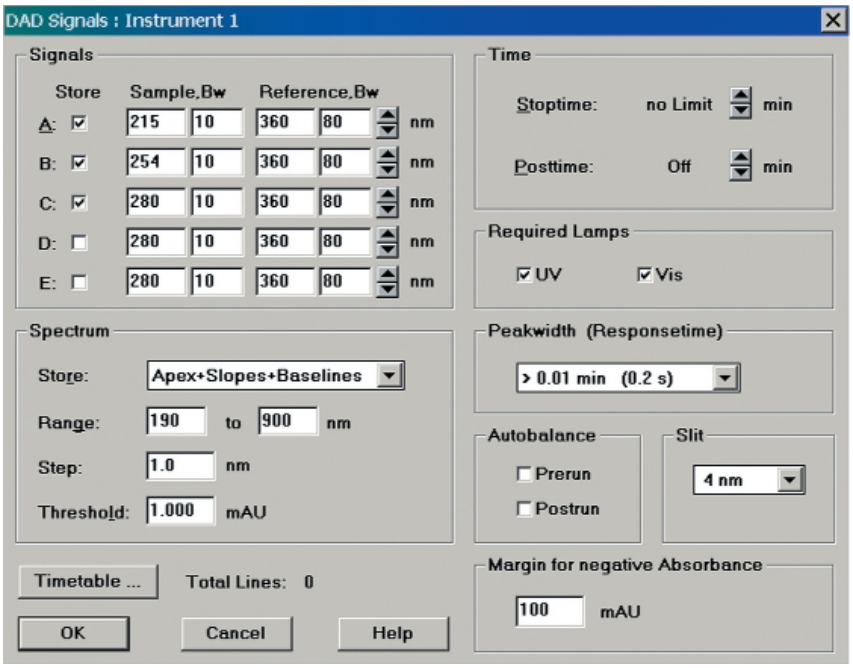

Figure 5
DAD settings for ultrafast high throughput analysis.

For detection a DAD and an MSD can be used in combination or as stand-alone detectors. After having passed the DAD, the flow is split to 1 mL/min for an ESI source by a splitter. For an APCI source an even lower split ration may be used. The flow that is not directed to the MSD may be collected with a fraction collector but for this purpose an active splitter should be used to obtain good peak shapes. This issue will be discussed in more detail in the section “Optimization of your system for quantification purposes”.

For the DAD the following settings in figure 5 are recommended to ensure fast, sensitive and reliable detection. After having chosen an appropriate detection wavelength (e.g. 215 and 254 nm) and a bandwidth of 10 nm to ensure fast detection in combination with adequate sensitivity, an appropriate reference wavelength (e.g. 360 nm) in combination with a wide bandwidth (e.g. 80 nm) should be defined. If you want to save spectra, you should save all spectra in peak or apex, slope and baseline spectra to get spectral information in combination without creating large file sizes. To obtain good spectral resolution (e.g. for library searches) the step size should be set to 1 nm. To obtain good chromatographic peak shapes, the Agilent ChemStation tries to acquire at least 20 spectra during a chromatographic peak. Therefore, the expected chromatographic peak width (at half height) has to be entered in the DAD screen. Due to the high flow rates, the expected

peak width is very low. A typical value for the narrowest peak is 0.01 min. For the slit width a good combination of spectral resolution and sensitivity is achieved at 4 nm. Whereas for normal analysis an autobalance is performed prior to each run, this is skipped to further reduce the cycle time. As the detector drift is very low, for qualitative analysis only, it is sufficient to do an autobalance only once before starting an analysis. This saves additional 5 s per run. For the MSD the settings in figure 6 are recommended to ensure sensitive and reliable detection. In the MSD screen a peak width of 0.01 min should also be entered. Here fast scan, data reconstruction and time filter should be enabled to ensure optimal performance. The fast scan option optimizes the scan speed of the quadrupole. Therefore, some resolution and sensitivity are sacrificed. Data reconstruction uses a moving average filter to reconstruct the spectra from the recorded data. With this mathematical operation the resolution sacrificed with the fast scan option is gained back, thus resulting in a comparable spectral resolution to a normal scan, however, the data reconstruction algorithm only works for singly charged ions. The time filter box activates that the specified peak width is used for the Gaussian filter used within the ChemStation. This should always be activated assuming that the correct peak width was entered. To reduce the file size condensed data storage may be activated, but if detailed isotopic spectral information is required it is better to

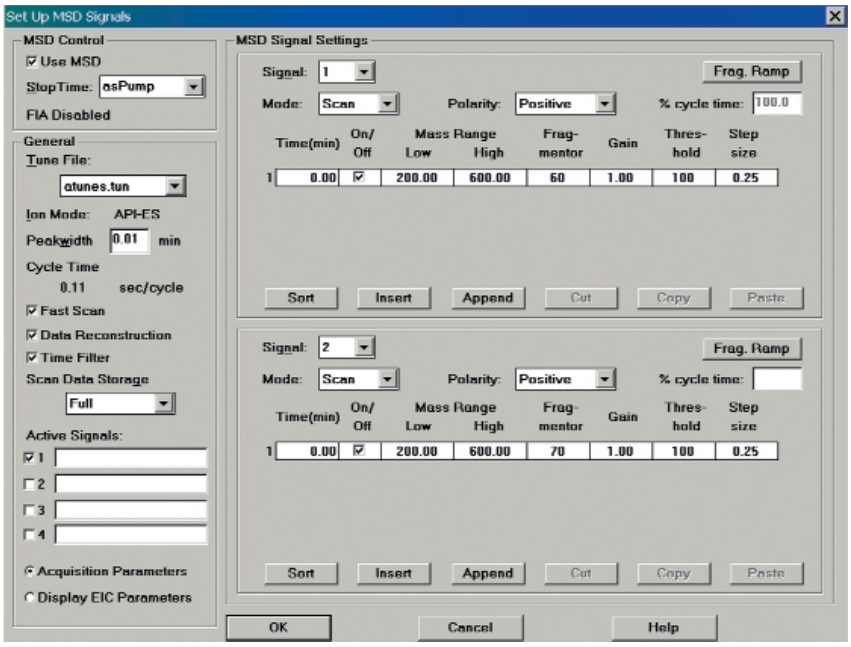

Figure 6
MSD settings for ultrafast high throughput analysis.

activate *full data storage* to store all data acquired. To obtain fast quadrupolar scan cycles in combination with high sensitivity the scan range should be narrowed as much as possible and a relatively large step size, for example, 0.25 Da should be used, but the stepsize used depends on the spectral resolution needed. To reduce injection-to-injection times as much as possible, the outlet capillary of the DAD should be connected to the ESI source directly rather than to the valve inside the MSD. Using an ion trap as detector allows to perform automated MS/MS experiments.
In contrast to the single quad this allows to select a single parent ion and perform MS/MS experiments thus generating a daughter ion spectrum for this compound only, even when other substances coelute. To maintain chromatographic resolution the number of precursors should be set to a low value and the threshold should be set to a relative high value. As concentration is not an issue accumulation time and space charge density target should also be set to relatively low values to obtain a good chromatographic resolution. To further support this the averaging values should be set to low values too. The adjustments of scan range and step size are similar to those described above for the quadrupole instrument.
With such a system cycle times of 1.1 min can be achieved in

Sample: Sulfamethizole (1), Sulfamethazine (2), Sulfachloropyridazine (3), Sulfadimethoxine (4), 500 ng each
Gradient: 0.5 min from 5 – 95 % ACN
Flow: 4 mL/min
Column: Zorbax STM SB C18 column 4.6 x 15 mm, particle size 1.8 µm

combination with a generic gradient from 5 to 95 % acetonitrile in 0.5 min. As an example a separation of four sulfonamides is shown in figure 7 and compared to a "normal" analysis in figure 8. To further reduce cycle times, you may acquire different injections in one data file. This skips the initialization times of the instrument. To do this you will have to use an injector program for the injections and complex timetables for pumps and valves. Data analysis may be complemented by a data analysis macro cutting this multiple injection file into a single file per injection. The drawback of this approach is that method generation is no longer simple and that the different times can no longer be regarded as single timing events but are integrated

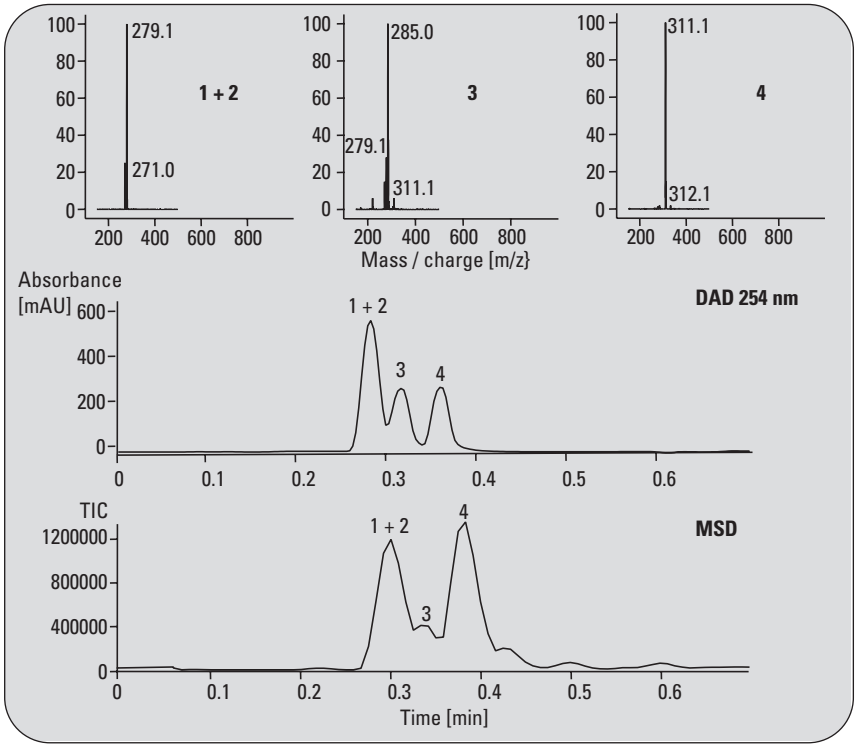

Figure 7
High throughput analysis of 4 sulfonamides

HT - Analysis:
Sample: Sulfamethizole (1), Sulfamethazine (2), Sulfachloropyridazine (3), Sulfadimethoxine (4), 500 ng each
Gradient: 0.5 min from 5 – 95 % ACN
Flow: 4 mL/min
Column: Zorbax STM SB C18 column 4.6 x 15 mm, particle size 1.8 µm
Normal Analysis:
Sample: Sulfamethizole (1), Sulfamethazine (2), Sulfachloropyridazine (3), Sulfadimethoxine (4), 500 ng each
Gradient: 15 min from 5 – 95 % ACN
Flow: 1 mL/min
Column: Extend C18 column 4.6 x 50 mm, 3.5 µm particle size

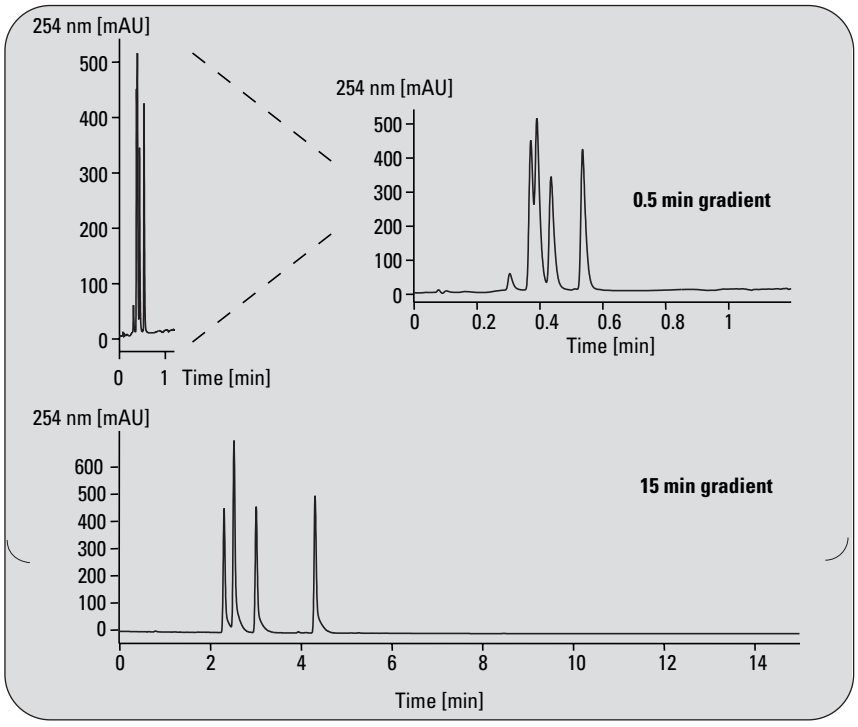

Figure 8
Comparison of the UV chromatograms at 254 nm of a 0.5-min gradient and a 15-min gradient.

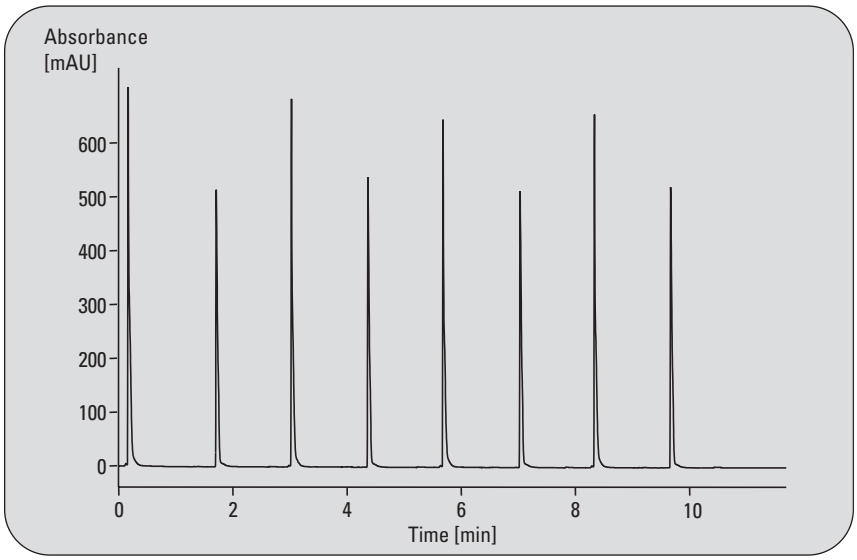

Figure 9
UV chromatogram (280 nm) of 8 Caffeine injections acquired into a single data file.

Sample:	Caffeine, 500 ng
Gradient:	1 min from 5 – 95 % ACN
Flow:	4 mL/min
Column:	Zorbax STM SB C18 column 4.6 x 15 mm, particle size 1.8 µm

Injector	1. Pump	Valve	Detectors
Draw sample	0 min: 5 % ACN	1.3 min: Next position	0 min: Start
20 sec needle wash	1 min 95 % ACN	2.6 min: Next position	10.6 min: Stop
Inject	1.01 min: 5 % ACN	3.9 min: Next position	
Wait 0.7 min	1.3 min: 5 % ACN	5.2 min: Next position	
Valve bypass	2.3 min: 95% ACN	6.5 min: Next position	
Draw sample	2.31 min: 95 % ACN	7.8 min: Next position	
20 sec needle wash	2.6 min: 5 % ACN	9.1 min: Next position	
Needle into seat		10.4 min: Next position	
Valve mainpass	*Continue to 8 cycles*		
Eject sample	*up to 10.6 min*		
Wait 0.45 min			
Valve bypass	Flow 4 mL/min		
Draw sample	1 min gradient, but can also		
20 sec needle wash	be performed with shorter		
Needle into seat	gradients		
Valve mainpass			
Eject sample	2. Pump isocratic		
Wait 0.45 min	reequilibration with 4 ml/min		
Continue to 8	at 5 % ACN		
injection cycles			

Table 3
Exemplary timetables for the acquisition of 8 injections into 1 data file

within a complex action-timing network of the overall instrument. This makes it quite complicated to change and adopt the method to a specific problem. Table 3 shows timetables for a method with 8 injections into one data file and figure 9 shows the corresponding chromatogram.

2. Best quantification while maintaining a very high sample throughput

The instrument described above can also be used for quantification. Only minor adjustments have to be done to sacrifice some time to get the chromatographic separation required for accurate quantification. A slightly longer column (e.g. 50 mm) may be used in combination with a lower flow rate (1 or 2 mL/min) and a shallower gradient. This results in better separations and thus enables the possibility for quantification. Additionally, a lower noise level is generated thus resulting in more accurate integrations and a higher sensitivity. This can be supported by using the standard mixer within the binary pumps, again sacrificing some time. The well-plate autosampler can be used as stated above, with some slight changes. The time until the injection valve is switched into bypass can be elongated somewhat,

thus cleaning of the needle interior is done more thoroughly. Also the needle wash should be extended to 20 seconds to ensure very low carry over. For this purpose the valve switching procedure for cleaning the injector valve grooves may also be applied. The use of flow rates of 1 mL/min only has the advantage, that no split is necessary prior to the MSD which results in good peak shapes for quantification. For higher flow rates the use of an active splitter is recommended for quantitative analysis since much better peak shapes are obtained than with a passive splitter. Additionally, a fraction collector may be added to collect the purified substances. In general, quantification with a UV or a DAD detector leads to much better precisions than those with an MSD but the MSD is more sensitive. Therefore, wherever possible quantification should be based on UV rather than on MSD. For the DAD the following settings are recommended to ensure most accurate detection (figure 10). After having chosen an appropriate detection wavelength (e.g. 215 and 254 nm) and a bandwidth of 10 nm to ensure specific detection an appropriate reference wavelength (e.g. 360 nm) in combination with a wide bandwidth (e.g. 80 nm) should be defined. To save spectra, you should save all spectra in *peak* or *apex*, *slope* and *baseline spectra* to get spectral information in combination without creating large file sizes. To obtain good spectral resolution (e.g. for library searches) the step size should be set to 1 nm. To obtain good

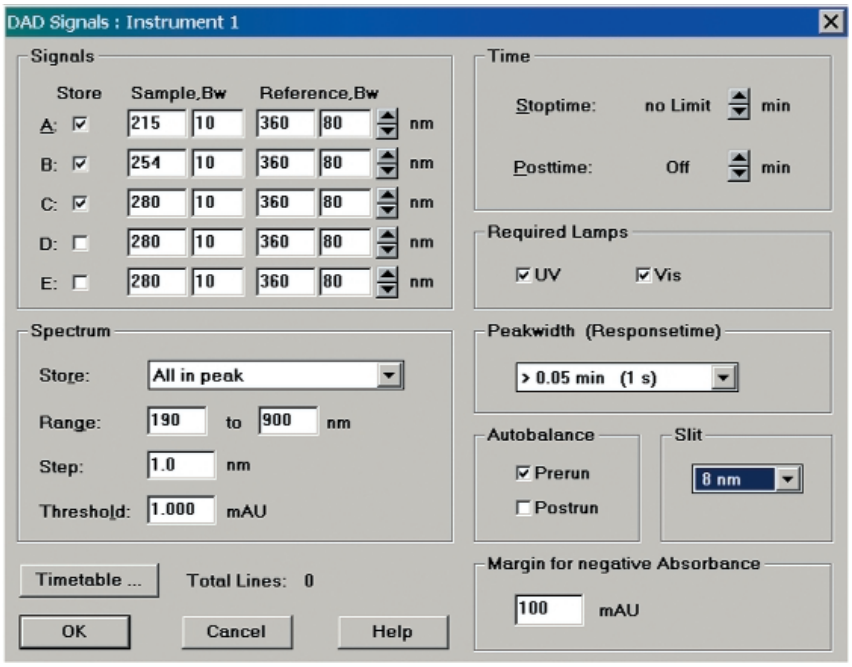

Figure 10
DAD settings for exact quantification.

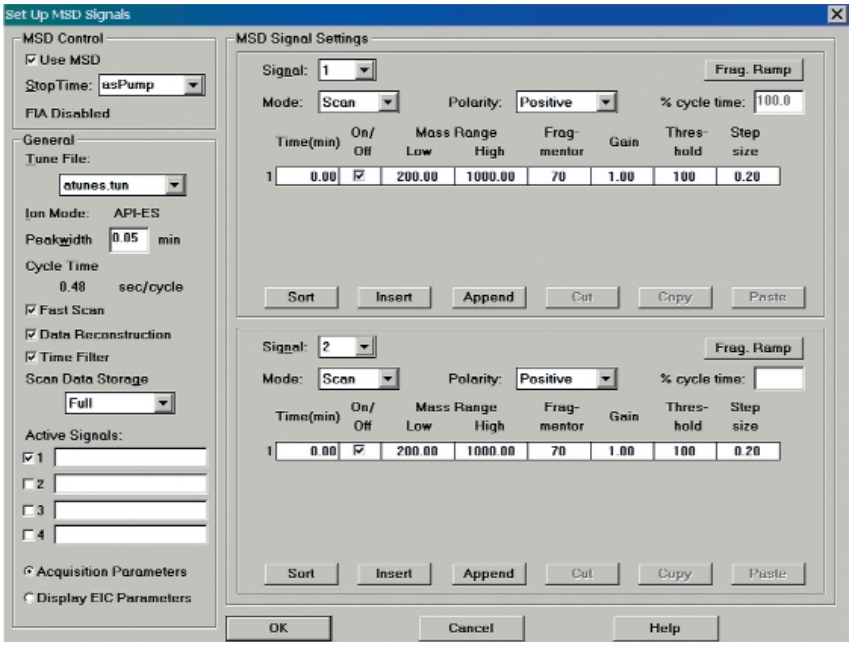

Figure 11
MSD settings for exact quantification.

Sample: Sulfamethizole (1), Sulfamethazine (2), Sulfachloropyridazine (3), Sulfadimethoxine (4), 500 ng each
Gradient: 10 min from 5 – 95 % ACN
Flow: 1 ml/min
Column: Zorbax STM SB C18 column 4.6 x 30 mm, particle size 1.8 µm

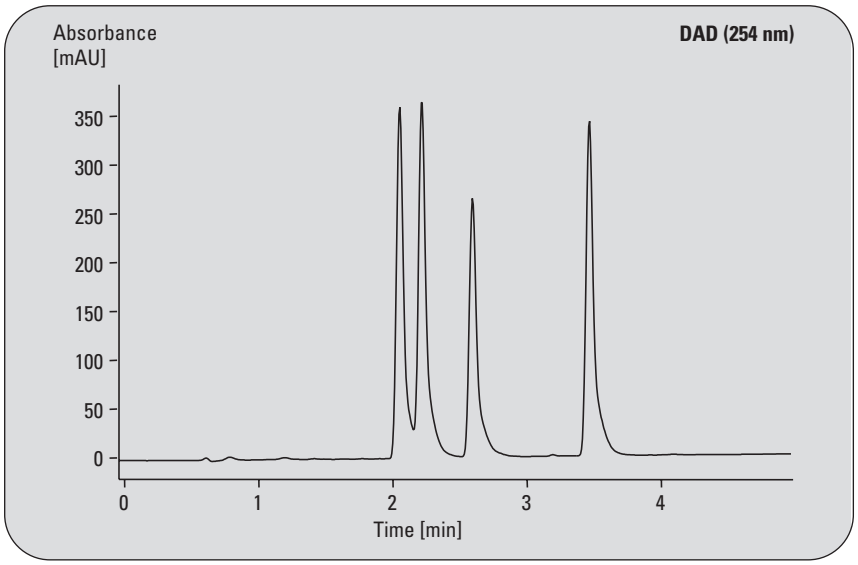

Figure 12
Analysis of 4 sulfonamides optimized for quantitative purposes.

chromatographic peak shapes the Agilent ChemStation tries to acquire at least 20 spectra during a chromatographic peak. Therefore, the expected chromatographic peak width has to be entered in the DAD screen. To obtain as many single measurements as possible during a chromatographic peak the expected peak width (at half height) should be set to a value slightly lower than that of the narrowest peak. A typical value may be 0.05 min. For the slit width it is important to ensure spectral resolution. The lower the slit width, the better the spectral resolution, however your analyses are less sensitive. For quantitative purposes a width of 8 nm may be used as starting point. To obtain the best integrations possible an autobalance should be performed prior to each run. For the MSD the settings in figure 11 are recommended to ensure accurate detection. In the MSD screen a peak width of 0.05 min should also be defined. Also here *fast scan*, *data reconstruction* and *time filter* should be enabled. These functions are used only when they are needed and the Agilent ChemStation automatically decides whether to use or not to use them. Due to the data reconstruction algorithm no spectral resolution is lost. To obtain fast quadrupolar scan cycles in combination with high sensitivity and accurate quantification the scan range should be narrowed as much as possible. For quantitative measurements a compromise of spectral resolution and MS cycle time has to be drawn. On the one hand a lower step size leads to a better spectral resolution, on the other hand a higher stepsize

		Mean Peak 1	Standard deviation Peak 1 (%)	Mean Peak 2	Standard deviation Peak 2 (%)
UV (254 nm)	**RT (min)**	2.051	0.13	2.212	0.10
	Area	1392	0.09	1562	0.14
MSD (EIC)	**RT (min)**	2.081	0.13	2.245	0.13
	Area	15750000	2.76	6415000	2.43
		Mean Peak 3	**Standard deviation Peak 3 (%)**	**Mean Peak 4**	**Standard deviation Peak 4 (%)**
UV (254 nm)	**RT (min)**	2.591	0.07	2.591	0.13
	Area	1132	0.78	0.07	0.26
MSD (EIC)	**RT (min)**	2.623	0.07	3.49	0.05
	Area	5555000	2.25	0.13	2.55

Table 4
Precisions of high throughput analysis on two parallel columns

leads to more measurement time per single step thus resulting in high sensitivity and accuracy. A stepsize around 0.2 Da may be an acceptable compromise for quantitative analysis. When using scan mode for quantification the creation of extracted ion chromatograms for the substances of interest is necessary. An even better way resulting in much higher sensitivity and accuracy is the use of the SIM (selected ion monitoring) mode. Here the quadrupole is not scanned but locked at the masses of interest thus leading to higher sensitivity and exacter quantification. For an ion trap MS accumulation time and space charge density also have to be controlled. Here a compromise between ion accumulation and amount of data points per chromatographic peak has to be drawn. Additionally, you have to ensure that not too many ions are loaded into the trap thus resulting in space charge effects leading to poorer resolution, mass defects and less exact quantification. To ensure a reliable and specific quantification the MRM mode can be used. This allows sensitive determination of an intensive quantifier ion in combination with qualifier ions thus leading to an accurate and sensitive quantification in combination with secure identification. Figure 12 shows the separation of the substances previously shown in figure 7 analyzed for quantitative purposes. The separation is much better allowing quantification of the substances, however, a longer analysis time is necessary. In table 4 precisions of the measurements are shown. When using two columns in parallel for quantitative analysis it must be considered that methods have to be validated either on both columns together (thus leading to a slightly reduced precision of the analysis) or separately for each column. However, then it has to be controlled which sample is injected onto which column. This can easily be achieved with Agilent ChemStation and Agilent ChemStore.

3. Highest sensitivity in combination with fast analyses

The system used for high throughput analysis of lowest sample amounts is similar to the one described above only miniaturization of the flow path was achieved to increase the sensitivity. For this purpose, two capillary pumps are used instead of the binary pumps, a micro well-plate autosampler equipped with an 8 µL or alternatively a 40 µL sample loop instead of the standard well-plate autosampler and a micro valve instead of the normal 10-port valve. Additionally, all tubing material diameters should be adjusted to an internal diameter of 50 µm and 0.3 x 50 mm or even shorter capillary columns may be used. These changes reduce the dead volume of the system to roughly 20 µL. Depending on the information required the system can be equipped with a DAD and an MSD or an MSD only. Depending on the requirements a low flow rate of 2 or 3 µL/min may be used for very sensitive analysis but this will result in quite long cycle times. To keep the required time as low as possible, fast solvent exchange should be used at

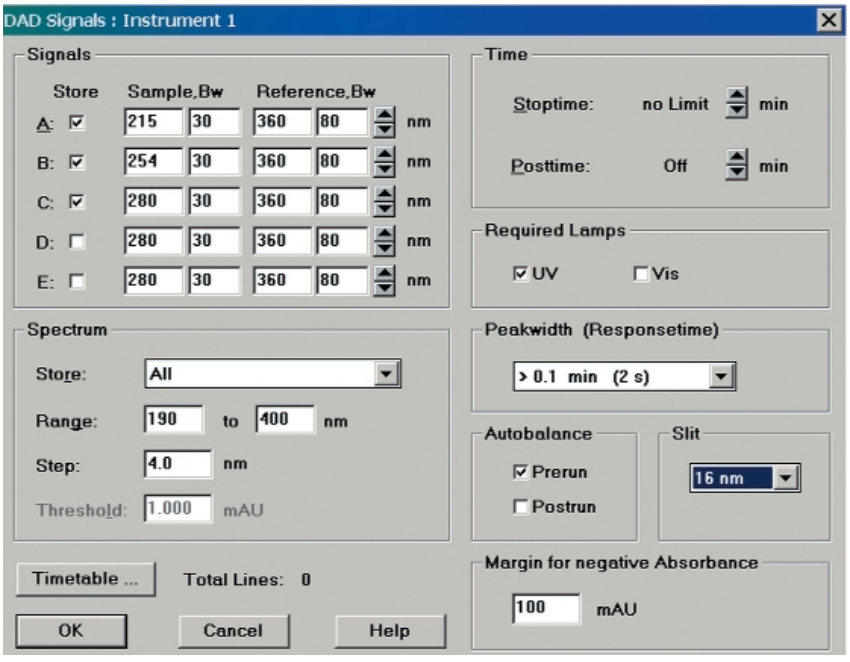

Figure 13
DAD settings for the detection of lowest sample amounts.

the end of the analysis, but this cannot be combined with overlapped injection. To reduce the analysis time a high flow rate of 20 µL/min can be used. Due to the high flow rates the fast solvent exchange feature is not needed thus overlapped injections can be used to save time. To ensure a complete delivery of the gradient onto the column the sample flush out factor should be reduced to 2 because otherwise a part of the gradient may be trapped in the sample loop. This reduces injection-to-injection time to 2.2 min and only little sensitivity is sacrificed due to the relatively high flow rate and the MS being a concentration-sensitive detector rather than an amount-sensitive detector.

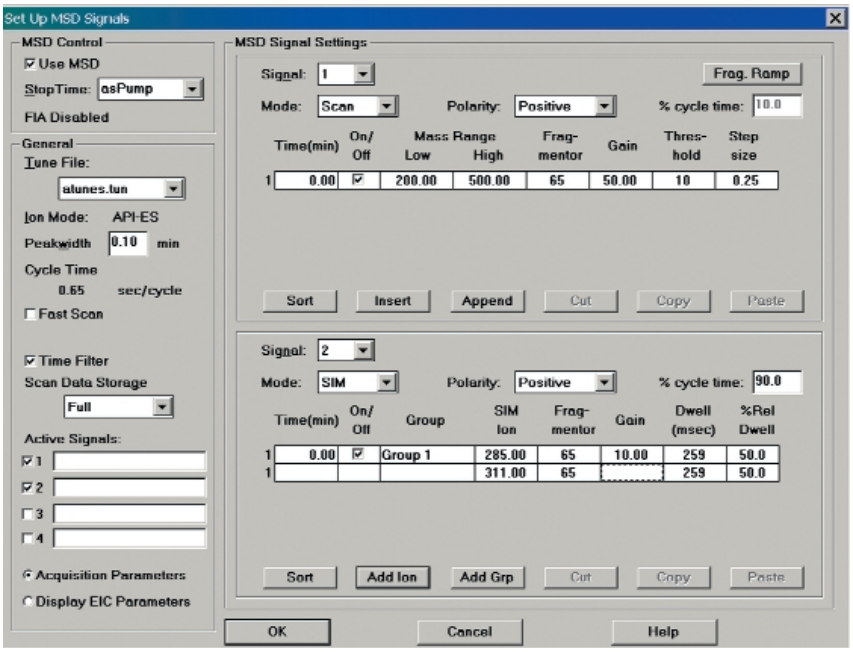

Figure 14
MSD settings for the detection of lowest sample amounts.

For the DAD the settings in figure 13 are recommended to ensure highest detection sensitivity. After having chosen an appropriate detection wavelength (e.g. 215 and 254 nm) and a bandwidth of 30 nm to ensure most sensitive detection an appropriate reference wavelength (e.g. 360 nm) in combination with a wide bandwidth (e.g. 80 nm) should be defined. If you want to save spectra, you should save all spectra to acquire all spectra during a chromatographic run thus allowing manual averaging of the spectra recorded which leads to a better sensitivity for spectra. To obtain not too large file sizes in combination with still relatively good spectral resolution (e.g. for library searches) the step size should be set to 4 nm and the range should be as narrow as possible. To obtain good chromatographic peak shapes the Agilent ChemStation tries to acquire at least 20 spectra during a chromatographic peak. Therefore, the expected chromatographic peak width has to be entered in the DAD screen. To obtain high sensitivity in combination with acceptable chromatographic peak shapes the expected peak width (at half height) should be set to a typical value for the majority of the peaks and not to that of the narrowest ones. Typical values are 0.1 min. The slit width should be set to 16 nm to obtain the highest sensitivity possible. To also ensure sensitive measurements here, an autobalance should be performed prior to each run. For the ESI source of the MSD the micro sprayer is used to ensure optimized ionization conditions and thus highest sensitivity. The settings in figure 14 are recommended to ensure most sensitive detection. In the MSD screen a peak width similar to that used for the DAD is recommended. To ensure highest sensitivity *fast scan* and *data reconstruction* should be disabled. To further support this, the scan range should be narrowed as much as possible and the step size should be increased thus allowing to obtain higher sensitivity while sacrificing spectral resolution. In many cases a step size of 0.3 Da may be an acceptable compromise between sensitivity and mass spectral accuracy. To obtain highest sensitivity, the use of SIM mode is recommended. Here the quadrupole is locked at specified masses thus resulting in highest sensitivity and best chromatographic peak shapes. For an ion trap accumulation time and space charge density also have to be controlled. Here, relatively high values should be

A:

Sample:	Sulfamethizole (1), Sulfamethazine (2), Sulfachloropyridazine (3), Sulfadimethoxine (4), 10 ng each
Gradient:	0.5 min from 5 – 95 % ACN
Flow:	20 µL/min
Column:	Zorbax Extend C18 capillary column 0.3 x 50 mm, 3.5 µm, particle size

B:

Sample:	Sulfamethizole (1), Sulfamethazine (2), Sulfachloropyridazine (3), Sulfadimethoxine (4), 500 ng each
Gradient:	0.5 min from 5 – 95 % ACN
Flow:	4 mL/min
Column:	Zorbax STM SB C18 column 4.6 x 15 mm, particle size 1.8 µm

used to store as many ions as possible and thus generate highest sensitivity. This to some extent sacrifices chromatographic and mass spectrometric resolution and accuracy but optimizes sensitivity. For the detection of known compounds the MRM mode can be used. This allows choosing an intensive quantifier ion in combination with qualifier ions thus resulting in high sensitivity in combination with secure identification and quantification.
A separation of the same substances shown in figure 7 and 12 analyzed for sensitivity purposes is shown in figure 15. Although no limit of detection has been determined signal intensities (Y – axes) and injected amounts clearly demonstrate the high sensitivity of the capillary LC/MS system.

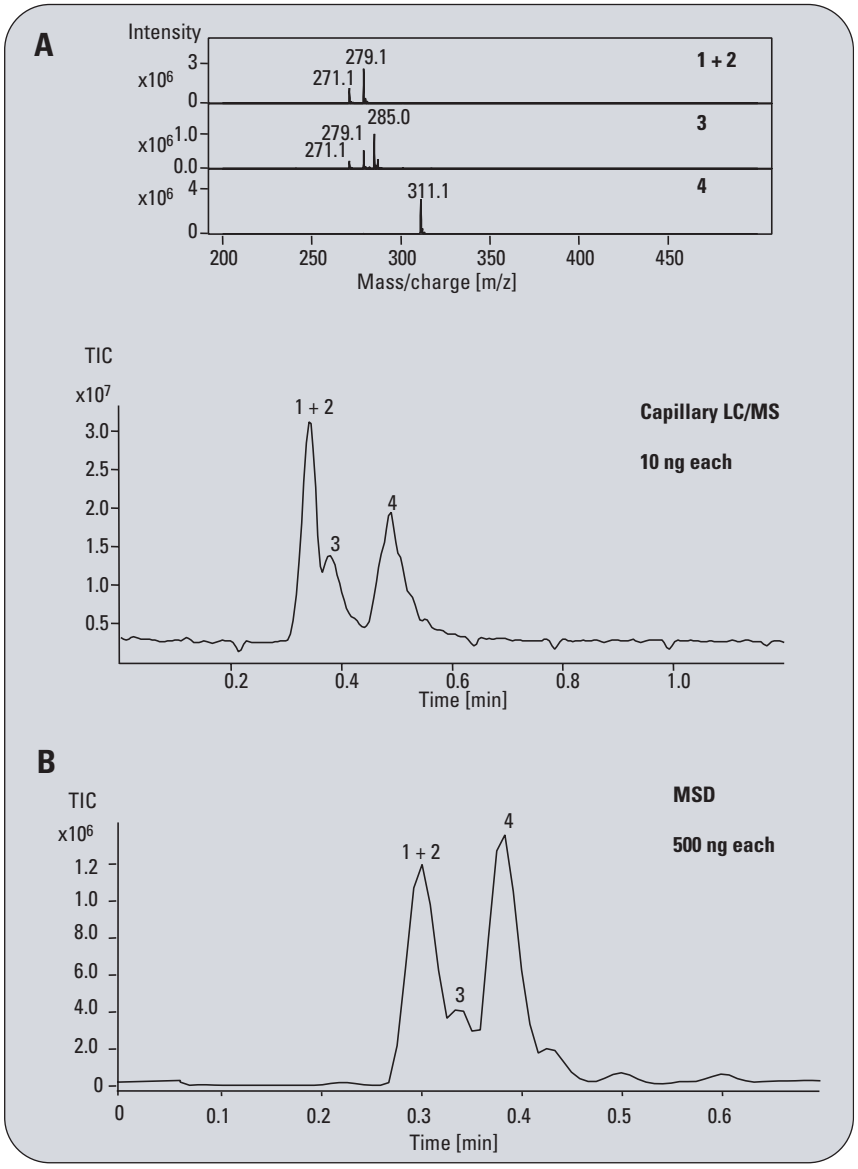

Figure 15
A; Capillary LC/Ion trap high throughput separation of 4 sulfonamides
B: LC/MSD high throughput analysis of 4 sulfonamides. Comparison of the y-axes and the injected sample amounts of A and B clearly demonstrates the high sensitivity of the capillary LC/MS system.

Conclusion

Three different high throughput scenarios can be distinguished. Analysis with shortest cycle times for the highest sample throughput possible, quantitative analysis still offering a relatively high sample throughput and the scenario when sample amount is the limiting factor. In the first case resolution and sensitivity is sacrificed for speed. In the second one exact quantification conditions are established for the sake of sensitivity and speed and in the last case highest sensitivity is achieved at medium resolution and speed. Whereas the first two scenarios can be covered with the same instrument, for extremely sensitive analysis an instrument optimized for low flow rates and sample amounts has to be used. Choosing the instrument which fits your purposes and setting the correct parameters in combination with high robustness and reliability of the Agilent 1100 Series modules allows to achieve a very high sample throughput in combination with qualitative and quantitative information of even very low sample amounts. A summary of the detector settings (MSD and DAD) for the three different scenarios is shown in tables 5 and 6.

Generic settings, as starting conditions DAD

Application	Peakwidth	Signal Wavelength (SW) and Signal Bandwidth (BW)	Reference Wavelength (RW) and Reference Bandwidth (RB)	Slit	Step	Store	Auto balance
Identification in combination with minimal analysis time	> 0.01	SW: 205, 215, 254, 280 nm BW: 10 nm	RW: 360 nm RB: 80 nm	4 nm	1 nm	All in peak	off
Identification and most accurate quantification	> 0.05	SW: 205, 215, 254, 280 nm BW: 10 nm	RW: 360 nm RB: 80 nm	8 nm	1 nm	Apex, Slope and Baseline	on
Identification of lowest sample amounts	> 0.1	SW: 205, 215, 254, 280 nm BW: 30 nm	RW: 360 nm RB: 80 nm	16 nm	4 nm	All or every second[1]	on

[1] To reduce the high data amount the step size is set to 4 nm still leading to good identification results.

MSD

Application	Peakwidth	Fast Scan (FS) Data Reconstruction (DR)[1] Time Filter (TF)	Scan Range	Scan Step Size	SIM	Relative Dwell time for SIM
Identification in combination with minimal analysis time	> 0.01	FS: enabled DR: enabled TF: enabled	< 600 Da	= 0.25 Da (for a lower step size you have to decrease the scan range further)	Use the most prominent ion as quantifier ion. To ensure the analysis you may fragment ions and add other intense and specific m/z as qualifier ions	50 % quantifier ion 50 % qualifier ion
Identification and most accurate quantification	> 0.05	FS: enabled DR: enabled TF: enabled	< 2000 Da (extended scan range possible due to larger peak width)	= 0.2 Da	Use the most prominent ion as quantifier ion. To ensure the analysis you may fragment ions and add other intense and specific m/z as qualifier ions	50 % quantifier ion 50 % qualifier ion
Identification of lowest sample amounts	> 0.1	FS: disabled DR: disabled TF: enabled	< 2000 Da (extended scan range possible due to larger peak width)	= 0.3 Da	Use the most prominent ion as quantifier ion. For maximum sensitivity use only one SIM ion at a time and separate fragmentor values for each ion	100 % quantifier ion

[1] Data reconstruction can be used for singly charged ions only.

Table 5
Generic settings as starting conditions for DAD and MSD.
Comparison of the DAD and MSD settings for ultra high throughput analysis, for high throughput analysis in combination with quantification and for highest detection sensitivity during high throughput analysis.

Detector setup – For known separations
DAD

Application	Peakwidth	Signal Wavelength (SW) and Signal Bandwidth (BW)	Reference Wavelength (RW) and Reference Bandwidth (RB)	Slit	Step	Store	Auto balance
Identification in combination with minimal analysis time	Narrowest peak width at inflection points	SW: Choose an appropriate wavelength for each compound BW: Choose a narrow bandwidth to allow rough quantification in case of coeluting compounds	RW: Choose a reference wavelength outside but also as close as possible to the expected absorbance range RB: Use a wide reference bandwidth of 80 or 100 nm	For optimum spectral conformation choose a slit such that FWHM[1]/ Slit ≥ 10	1 nm	All in peak (to reduce data amount)	off[3]
Identification and most accurate quantification	Narrowest peak width at inflection points	SW: Choose an appropriate wavelength for each compound BW: a) No coelution: For each compound choose BW = peak width at inflection points b) Coelution: For quantification choose a bandwidth with no absorbance of other compounds	RW: Choose a reference wavelength outside but also as close as possible to the expected absorbance range RB: Use a wide reference bandwidth of 80 or 100 nm	For optimum spectral conformation choose a slit such that FWHM[1]/ Slit ≥ 10 In case FWHM/Slit > max slit width, then set slit to 8 nm	1 nm	Apex, Slope and Baseline (to reduce data amount)	on
Identification of lowest sample amounts	Typical peak width at inflection points	SW: Choose an appropriate wavelength for each compound BW: For each compound choose BW = peak width at inflection points	RW: Choose a reference wavelength outside but also as close as possible to the expected absorbance range RB: Use a wide reference bandwidth of 80 or 100 nm	For optimum spectral conformation choose a slit such that FWHM[1]/ Slit ≥ 10 In case FWHM/Slit > max slit width, then set slit to 16 nm	4 nm	All or every second[2] (allows summation of spectra in the ChemStation, thus leading to higher sensitivity for the spectra of a peak)	on

[1] FWHM: full width at half maximum of the absorbance band.
[2] To reduce the high data amount the step size is set to 4 nm still leading to good identification results.
[3] To save the time necessary for balancing and due to the DAD drift being minimal balancing is not necessary prior to each run.

MSD

Application	Peakwidth	Fast Scan (FS) Data Reconstruction (DR)[1] Time Filter (TF)	Scan Range	Scan Step Size	SIM	Relative Dwell time for SIM
Identification in combination with minimal analysis time	Narrowest peak width at inflection points	Activate FS, DR and TF to sacrifice some sensitivity for fast scan cycles in combination with high spectral resolution and acceptable sensitivity	A narrow scan range is recommended to ensure increased spectral resolution and sensitivity	The lower the step size, the higher the resolution, but less analysis time can be spent per single step thus resulting in a decreased sensitivity and vice versa	Fewer ions means more analysis time is spent for a single ion thus resulting in higher sensitivity	The higher the dwell time percentage the more analysis time is spent on this ion resulting in higher sensitivity
Identification and most accurate quantification	Narrowest peak width at inflection points	Activate FS, DR and TF to sacrifice some sensitivity for fast scan cycles in combination with high spectral resolution and acceptable sensitivity	A narrow scan range is recommended to ensure increased spectral resolution and sensitivity. For quantification extracted ion chromatograms have to be generated	The lower the step size, the higher the resolution, but less analysis time can be spent per single step thus resulting in a decreased sensitivity and vice versa.	The fewer ions being monitored, the more analysis time is spent for a single ion thus resulting in higher sensitivity. These signals can be integrated directly for quantification	The higher the dwell time percentage the more analysis time is spent on this ion resulting in higher sensitivity
Identification of lowest sample amounts	Typical peak width at inflection points	Activate TF only and deactivate FS and DR to obtain highest sensitivity.	Narrow the scan range as much as possible to gain as much time per single quadrupole step resulting in highest sensitivity	The higher the step size, the less steps have to be made per quadrupole scan cycle thus more time can be spent per single step resulting in increased sensitivity	The less ions, the more analysis time is spent for a single ion thus resulting in higher sensitivity. Using the SIM mode provides much higher sensitivity than the scan mode does	The higher the dwell time percentage the more analysis time is spent on this ion resulting in higher sensitivity

[1] Data reconstruction can be used for singly charged ions only.

Table 6
Detector setup for known separations.
Comparison of the DAD and MS settings for ultra high throughput analysis, for high throughput analysos in combination with quantification and for highest detection sensitivity during high throughput analysis.

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