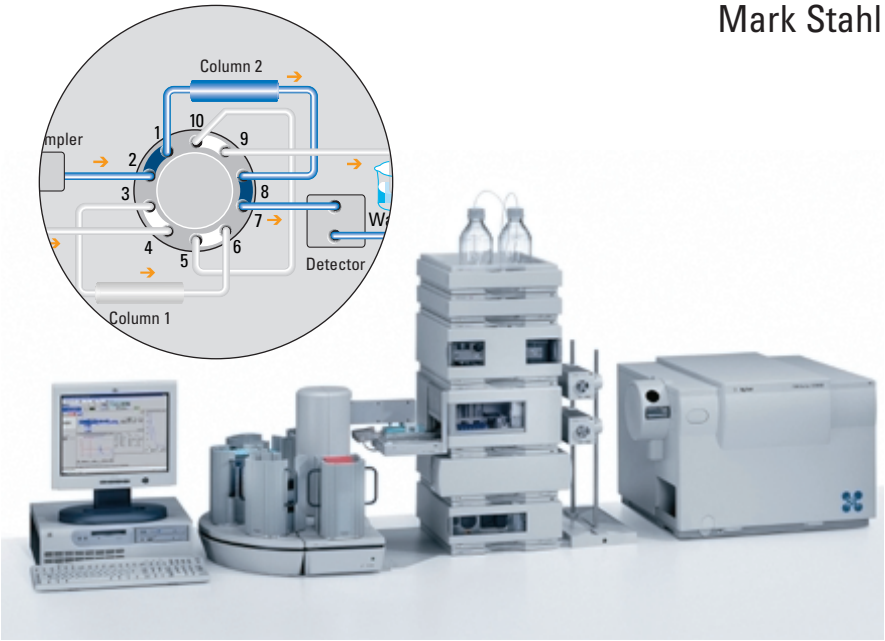
The Agilent 1100 Series high throughput system – instrumentation, software and applications

Application

Mark Stahl



Abstract

This Application Note describes the Agilent 1100 Series high throughput system, which is specially designed for the analysis of high sample numbers. Cycle times of less than one minute can be achieved using the high-speed well-plate autosampler with injection times less than 20 seconds in combination with short columns and high flow rates. Sample capacity is extended up to 30,720 samples using the Agilent 1100 Series sample capacity extension and the Agilent 1100 Series well-plate handler. Different instrument configurations and software packages make the system suitable for different environments. Speed in combination with high performance, highest robustness and flexibility makes the Agilent 1100 Series high throughput LC/MS system best suited for 24 x 7 operation.

Agilent Technologies

Introduction

Due to increasing automation and enormous progress in combinatorial chemistry, drug discovery and many other fields in organic and biological chemistry, the number of samples arising per day has been and still is growing. Especially central analytical labs of large enterprises have to handle sample numbers of several thousands daily. To ensure fast and smooth analysis of these samples the analysis system has to meet several criteria:

- high sample throughput
- highest robustness for high uptimes, and
- open access for experts and occasional users

Instrumentation

The Agilent 1100 Series high throughput system (figure 1) consists of a binary pump equipped with a micro-degasser, a column compartment, a well-plate autosampler equipped with an automation interface, a well-plate handler, which is the sample storing device, a diode-array detector (DAD) and a mass selective detector (MSD), based on quadrupole, Time of Flight or Ion Trap technology. The complete system can be controlled using different software packages, depending on the user's requirements[1, 2, 3].

High sample throughput

High sample throughput in HPLC is mainly determined by the following:

- instrument speed,
- instrument capacity,
- speed of the analytical method
- robustness (uptime) of the instrument, and
- efforts to maintain, verify performance and if needed to repair the instrument.

The speed of an LC instrument is determined by:

- the dead volume of the system,
- the speed of sample injection,
- the speed of the detector (fast enough to collect enough data points?), and
- the possibility to use valves to switch between columns or solvents.

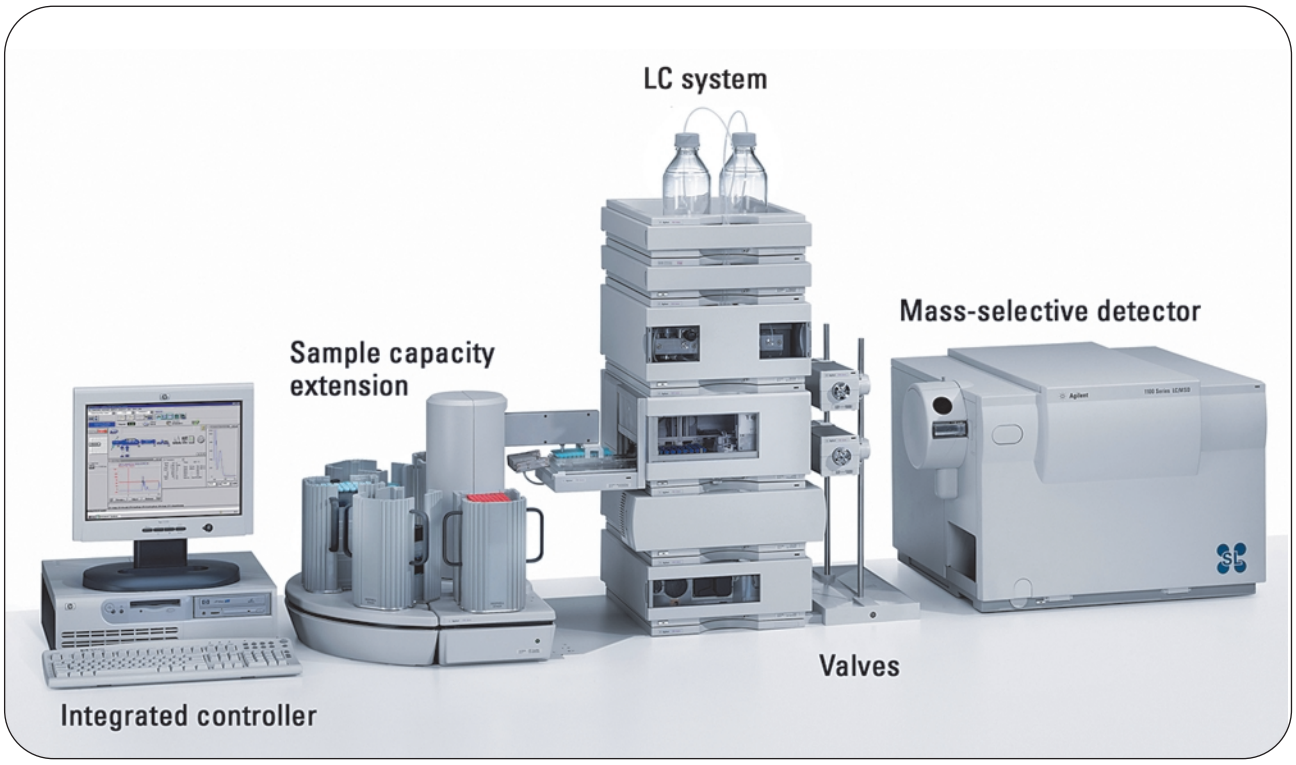


Figure 1
The Agilent 1100 Series high throughput LC/MS system

Columns: 4.6 x 30 mm Zorbax SB C-18
Flow rate: 3 mL/min
Gradient: 40 to 55 % organic phase in 0.3 min
DAD sampling rate: Peak width > 0.01 min
Injection volume: 1 µL in overlapped injection mode
Sample: Hydrocortisone, Beclomethasone, Hydrocortisone acetate

Using short columns, high flow rates and the high-speed Agilent 1100 Series well-plate sampler, cycle times less than one minute can be achieved[4, 5, 6]. Figure 2 shows the influence of run time, equilibration time and time needed for the injection on the total cycle time. As shown in figures 2 and 4, very short cycle times can be achieved by using a 10-port/2-position column switching valve (figure 3), allowing two columns to be used in parallel. The advantage is that the analysis can be done on one column while the other one is regenerated. In this case, a second pump is needed to provide the appropriate mobile phase mixture for proper regeneration[7, 8]. The detection system can be a UV detector and/or a mass spectrometer. Using this instrument set-up cycle times of about one minute are easily achieved as shown in figure 4. In the application example, four sulfonamides were analyzed using the LC system described previously, and an Agilent 1100 Series DAD in series with an Agilent 1100 Series quadrupole MSD. After the DAD the column flow of 4 mL/min was split to 1 mL/min for the MSD. A steep gradient was used from 30 to 90 percent organic phase in 0.6 minutes. The total cycle time, i.e. the time from injection to injection, was as short as 1 minute 10 seconds[9].

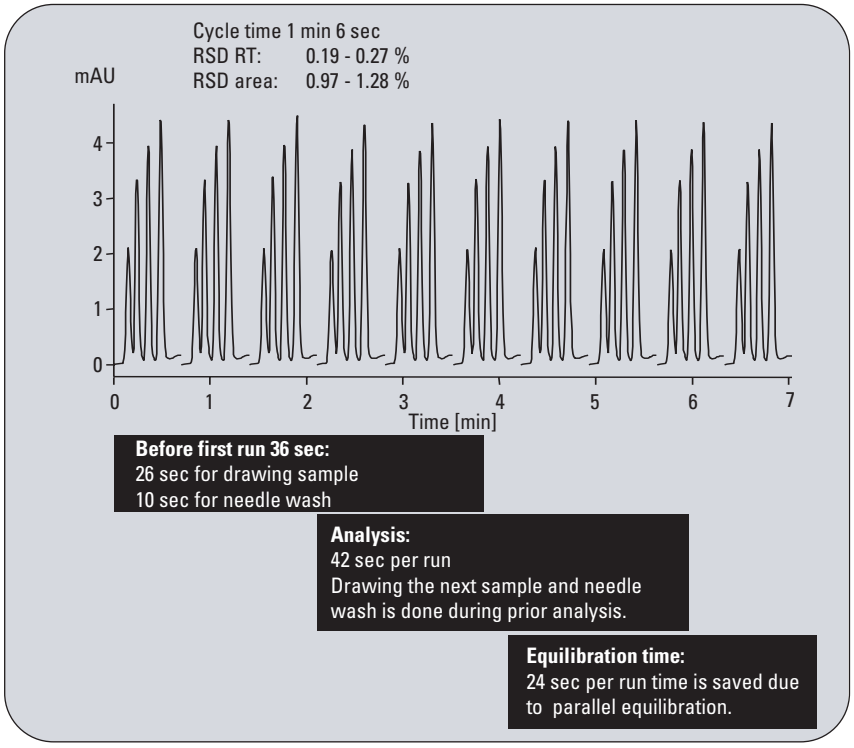


Figure 2
Analysis of drugs in 1 min 6 seconds on two 4.6-mm id columns operated in parallel allowing parallel column equilibration

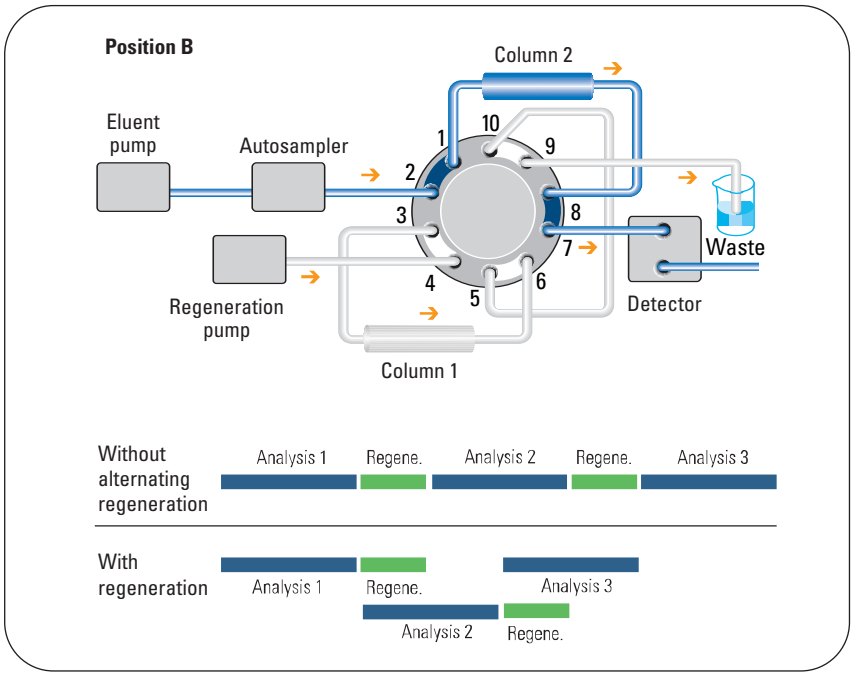


Figure 3
Shortest cycle times by alternating column regeneration for the analysis of sulfonamides

Sample:	1 Sulfamethizole 2 Sulfamethazine 3 Sulfachloropyridazine 4 Sulfadimethoxime
Flow:	4 mL/min
Gradient:	0.6 min gradient from 30-90 % ACN (0.1 % formic acid)
Columns:	4.6 x 15 mm Zorbax SB C-18, 1.8 µm
MSD:	Peak width 0.01 min

If multiple solvents are needed a solvent selection valve can be added. Similar valves may also be inserted to automatically choose between different columns (figure 5). This extends the number of possible methods/analysis, which can be carried out fully automated. Application Notes dealing with these issues in more detail are available from Agilent Technologies[7, 10].

Detector consideration for high throughput analysis

For high throughput analysis, where peaks can have peak widths below 1 second UV and MS detection have to be fast enough to provide enough data points for proper quantitation and identification[6].

The Agilent 1100 Series diode array UV detector provides fast 20 Hz full spectra and multi-signal data acquisition. If the appropriate peak width setting is chosen, the Agilent 1100 Series DAD selects 20 data points per chromatographic peak, even for fast and ultra-fast applications. The Agilent 1100 Series MSD offers a wide variety of mass spectrometric detectors. The Agilent quadrupole MSD allows not only to achieve highest sensitivity in combination with good accuracy in SIM mode but also an extremely high chromatographic resolution in combination

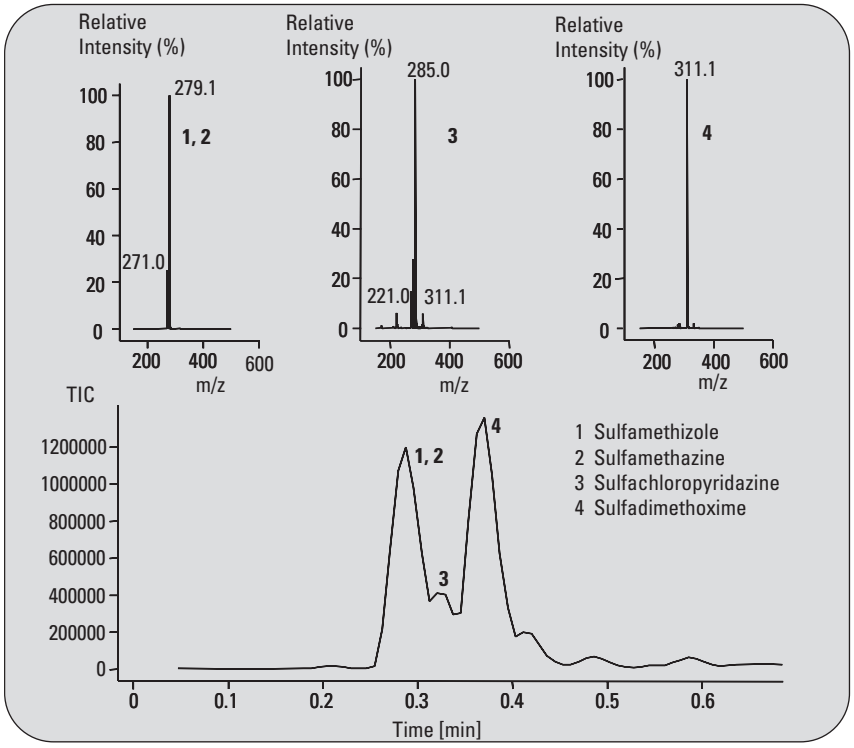


Figure 4
Analysis of drugs in 1 min 6 seconds on two 4.6 mm id columns operated in parallel allowing parallel column equilibration

with secure substance identification due to very fast scan cycles and high mass accuracy. If even better chromatographic resolution is needed, the ESI-TOF instrument with its very fast duty cycle times may be an alternative. In addition, it offers the advantage of very high resolution and accuracy (3 ppm) allowing unambiguous substance identification and determination of totals formulas. For further characterization of analytes and for secure and sensitive identification and quantification in the MRM mode, the Agilent Ion Trap can also be used as mass spectrometric detector. Its capability to perform MS^n analysis makes it a very valuable tool especially for structural analysis. In combination with high throughput analysis, easy control of ion accumulation is also very valuable for quantification, analysis sensitivity and chromatographic resolution[6]. The different mass spectrometric detectors can be equipped with an ESI, APCI or an APPI source. If necessary, changing to an atmospheric pressure Maldi source within minutes is also possible. Therefore, highest sensitivity and flexibility for all purposes is guaranteed.

Adding sample capacity to the Agilent 1100 Series LC system

The vial/well-plate handler converts the normal Agilent 1100 LC system into an open high capacity system. Figure 6 shows the start configuration of the vial/well-plate handler. It demonstrates how a

vial or well plate is transported between the holding racks and the well-plate autosampler. A robotics arm placed in the middle of the well-plate handler moves the plates from the racks to the platform. This platform represents the interface between well-plate handler and sampler. From the platform the plate is then pushed into the appropriate position of the well-plate sampler. The start configuration of the well-plate handler (figure 7) can be extended with further racks to a maximum of 30,720 samples using 384 well-plates. Different types of well-plates can be used, such as shallow well-plates, deep well-plates and vial well-plates for 2 mL or 1.5 mL vials. The system can be equipped with a handheld and a stationary barcode reader. This enables safe identification of samples, raw-data and results belonging together.

Robustness

To obtain high sample throughput, one of the most important factors is instrument robustness. To ensure robustness, Agilent implemented the Early Maintenance Feedback (EMF) feature for timely preventive maintenance. This allows to plan most maintenance activities and thus guarantees smooth operation. In case of an instrument failure the diagnostic screen allows fast localization of the defect, which also results in fewer down times. All system components used in the 1100 Series modules are of highest quality, ensuring highest reliability and robustness. For example, the

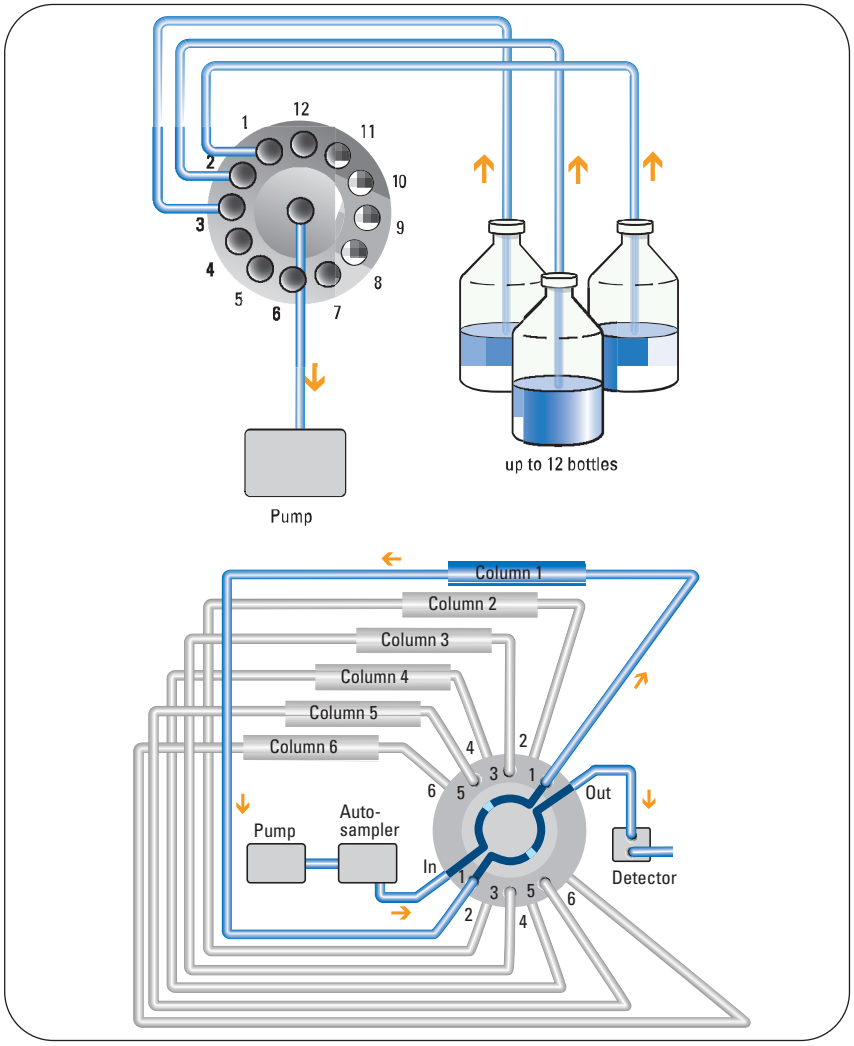


Figure 5
Column and solvent selection valves

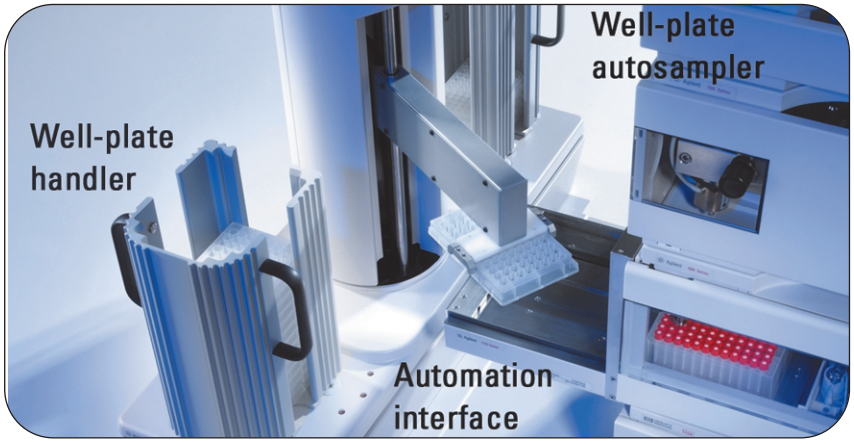


Figure 6
Agilent 1100 Series well-plate sampler equipped with the automation interface and the well-plate handler converts the Agilent 1100 Series LC into an open system.

standard valves allow 50,000 to 60,000 switches and the micro valves 120,000 switches.
The pump seals and pistons are designed to offer high robustness for demanding solvents and the UV lamps have a lifetime of 2,000 hours and more. To test the reliability of the well-plate handler 120,000 movements were carried out without any failure, resulting in highly reliable plate exchanges. The orthogonal nebulizer spray of the mass spectrometers allows highest tolerance against non-volatile salts in combination with high sensitivity, whereas the robust skimmer increases the tolerance towards biological samples. The combination of these features makes the Agilent 1100 Series high throughput LC/MS system extremely robust, allowing reliable 24 x 7 operation which results in highest sample throughput.

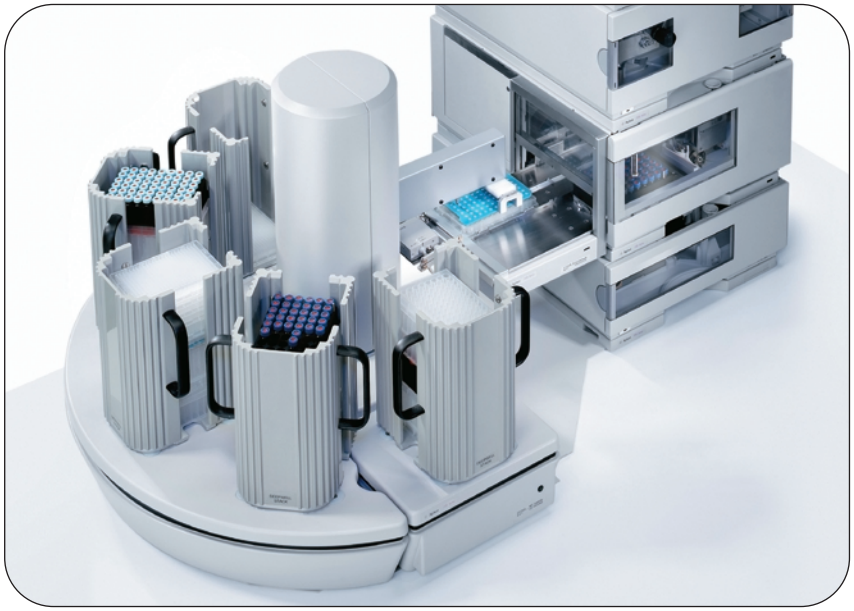


Figure 7
Sample capacity extension and well-plate handler of the Agilent 1100 Series high throughput LC/MS system leading to a sample capacity of up to 30,720 samples.

Software for different environments in high throughput analysis

The Agilent ChemStation controls the complete system, including well-plate handler, valves and mass spectrometric detectors. For the analysis of a large number of plates, a new execution level – the so-called hypersequence – has been developed. While a sequence controls the execution of single runs, the hypersequence controls the execution of different sequences (figure 8).

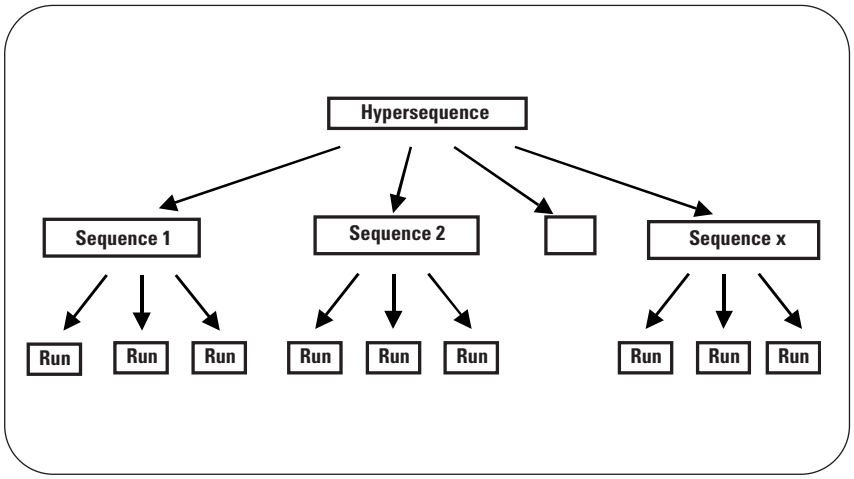


Figure 8
Hypersequence – a new level of automation

Different sequences can be used either on the same plate or on different plates. Also, all different plate formats can be used within one hypersequence. In addition, on-the-fly editing, pausing and resuming of the hypersequence is possible. This level of control is built into the Agilent ChemStation similar to the sequence level. Hypersequence parameters control data storage, barcode reader, hypersequence shut-down commands and so on, whereas the hypersequence table aligns the plate's barcode with the plate-type used and the sequence. Thus, the barcode reader identifies the plate, the corresponding sequence is loaded and the single runs are carried out as specified in the sequence table. The hypersequence table and the sequence table can be imported as a CSV file. To review the large amount of data created, a browser software, specially developed for this took, can be used together with the Agilent ChemStation.

Special software makes the system fully compliant with regulatory needs. Therefore, adding Agilent ChemStation Security Pack and Agilent ChemStore to the Agilent ChemStation base software may be useful. The Agilent 1100 Series high through-put system fully supports the requirements of 21 CFR Part 11 and therefore can be used in regulated environments.

Conclusion

The Agilent 1100 Series high throughput system is able to analyze samples with injection-to-injection times of less than 1 minute. The sample capacity can be extended up to 30,720 samples and the instrument can handle nearly all different types of vials and well plates. The high robustness of the instrument and the EMF feature of the Agilent ChemStation allow 24 x 7 operation. To increase the instrument's high flexibility different software packages are available for regulated and non-regulated environments. The Agilent 1100 Series high throughput LC/MS system is therefore an extremely reliable and flexible system for the analysis of high sample numbers per day.

References

1.
Unattended operation, excellent results, Agilent 1100 Series high-throughput LC/MS system, *Agilent Technologies Brochure*, *publication number 5988-6706EN*, **2002**.

2.
Performance characteristics of the Agilent 1100 Series modules and systems for HPLC, *Agilent Technologies Technical Note*, *publication number 5965-1352E*, **1996**.

3.
High-throughput quantitation with the Agilent 1100 Series LC/MSD and analyst software, *Agilent Technologies Technical Note*, *publication number 5988-5205EN*, **2001**.

4.
Optimizing the Agilent 1100 Series LC system, *Agilent Technologies Solution Guide*, *publication number 5988-2682EN*, **2001**.

5.
Optimizing the Agilent 1100 Series system for higher sample throughput on columns with internal diameters of 1 and 2 mm, *Agilent Technologies Technical Note*, *publication number 5968-3166EN*, **1998**.

6.
Optimizing your high throughput system, *Agilent Technologies Application Note*, *publication number 5988-9863EN*, **2003**.

7.
New dimensions for HPLC applications, *Agilent 1100 Series valve solutions*, *Agilent Technologies Brochure*, *publication number 5988-6707EN*, **2002**.

8.
High throughput HPLC – Alternating column regeneration with the Agilent 1100 Series valve solutions, *Agilent Technologies Application Note*, *publication number 5988-7831EN*, **2002**.

9.
High-throughput analysis with the Agilent 1100 Series high-throughput LC/MS system, *Agilent Technologies Application Note*, *publication number 5988-9638EN*, **2003**.

10.
Method development – solvent and column selection with the Agilent 110 Series valve solutions, *Agilent Technologies Application Note*, *publication number 5988-8347EN*, **2002**.

Mark Stahl is Application Chemist at Agilent Technologies, Waldbronn, Germany.

www.agilent.com/chem

Published September 1, 2003
Publication number: 5988-9914EN

Agilent Technologies