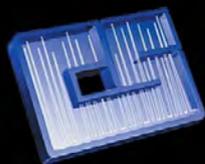




SYMBIOSIS™

LAB EFFICIENCY OF UNLIMITED DIMENSIONS

HOLLAND  
**Spark**  
LABORATORY



**CHROMATOGRAPHIC  
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## ABOUT SPARK

SPARK HOLLAND IS WORLD-CLASS PROVIDER OF INNOVATIVE SAMPLE INTRODUCTION, EXTRACTION AND SEPARATION TECHNOLOGY FOR HPLC, MS, GC AND NMR SYSTEMS. WITH 15,000 UNITS INSTALLED, SPARK PROVIDES SUPERIOR INSTRUMENTATION FOR MOST MAJOR HPLC SYSTEM SUPPLIERS. OVER 500 AUTOMATED ONLINE SPE SYSTEMS BY SPARK ARE NOW OPERATIONAL AROUND THE WORLD.

WITH OUR ABILITY TO LISTEN TO CUSTOMERS, OUR COMMITMENT TO REDUCE THEIR WORRY LIST AND WITH 25% OF OUR STAFF ASSIGNED TO R&D, SPARK IS DEVELOPING SOLUTIONS TODAY THAT CUSTOMERS WILL NEED TOMORROW.

HOLLAND  
**Spark**  
INSTRUMENTATION

## ACHIEVING THE LABORATORY EFFICIENCY YOU NEED

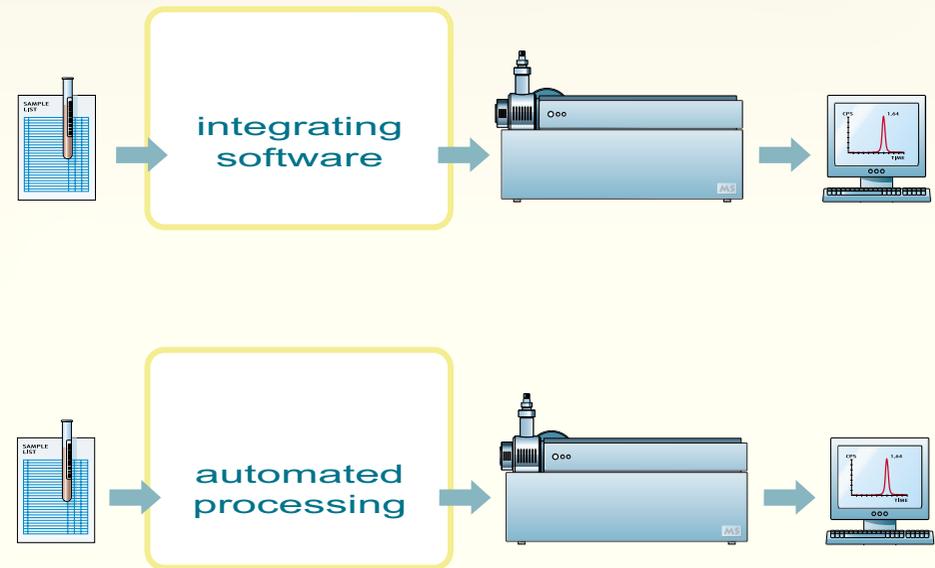
Ever more efficient drug discovery strategies and technologies generate rapidly increasing numbers of drug candidates. The universal applicability of LC-MS/MS as the predominant tool for bioanalysis has been a great help in streamlining the lab organization. However, no universal approach exists yet for sample preparation and traditional techniques like liquid-liquid extraction and protein precipitation are still widely used.

The aim of any lab manager for larger throughput therefore often results in expanding the number of MS systems and robotizing sample preparation. The one creates high depreciation levels, while the other often automates single steps of sample preparation but not the total process.

### A well defined solution

Spark can make an effective turnaround in lab efficiency possible by treating it as a project with well defined goals and time schedules for design and implementation. The solution should consist of:

- a proven technology that allows higher throughput in the laboratory
- a system that integrates both information flow and sample flow
- support aimed at turning the current situation into an implemented solution



## XLC-MS AN INTEGRATED FRONT-END TECHNOLOGY

Although 96-well SPE and online SPE have already replaced labor-intensive manual SPE, effective automation can only be achieved with a universal sample prep method. With XLC-MS (eXtraction Liquid Chromatography), Spark Holland introduces the first hyphenated technique that integrates sample extraction and LC separation. XLC-MS permits direct injection of raw biological samples such as plasma, serum or urine without prior filtration, centrifugation or protein precipitation.

XLC integrates sample eXtraction, LC separation and injection into the MS. Sample preparation takes place in parallel with the LC-MS run so that in effect sample processing time is reduced to zero.



**XLC-MS increases lab efficiency as it:**

### **revolutionizes sample throughput**

- More samples per day per instrument
- Reduced sample handling, still a 100% fresh sorbent for every sample
- Faster automated method development.

### **dramatically improves analytical performance**

- Total extract transfer into LC-MS, analyte enrichment and peak re-focusing ensure the highest assay sensitivity.

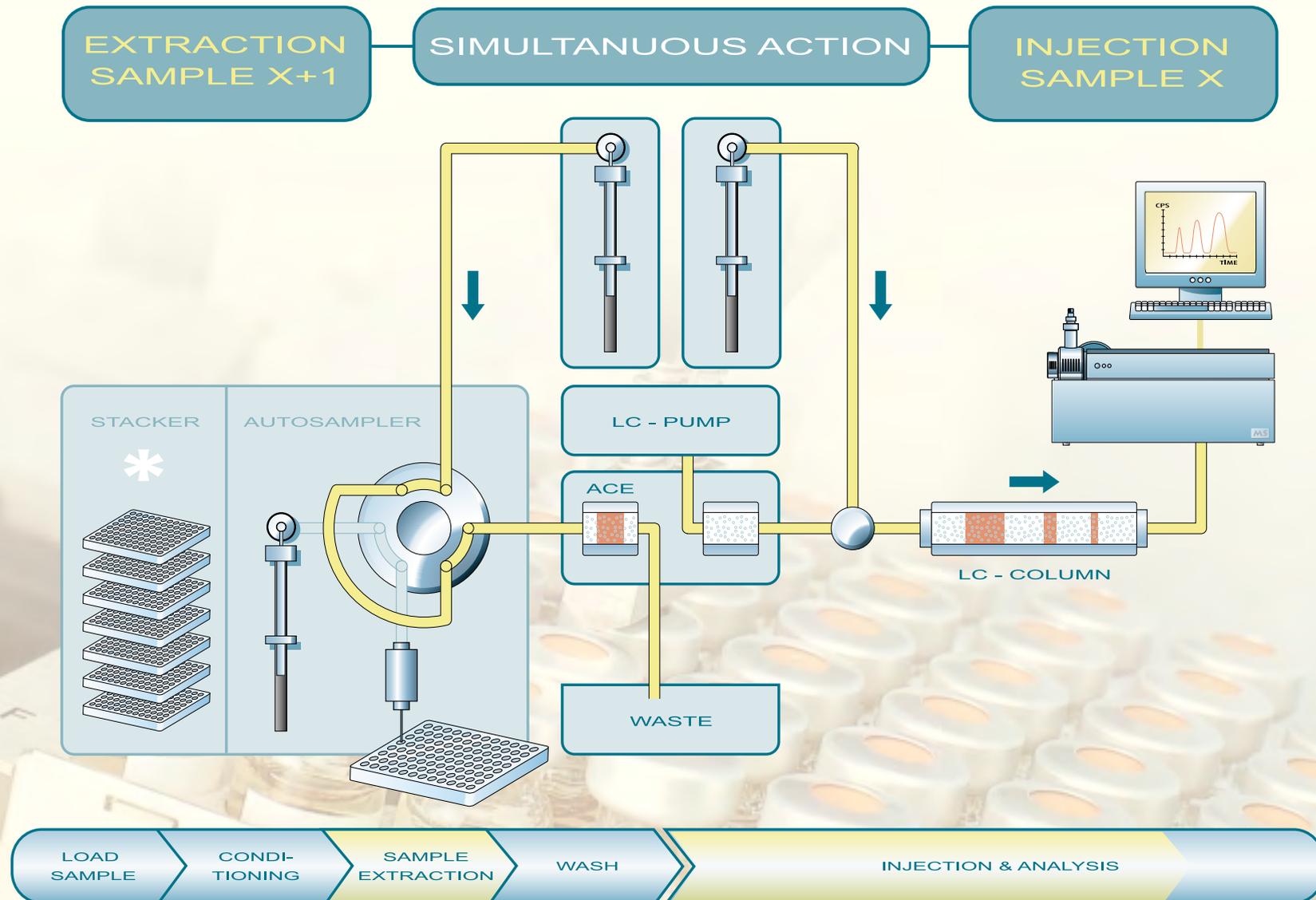
### **brings down costs**

- Reduced labor cost per sample
- Reduced method development time
- Reduced consumables cost
- Lower costs for outsourcing.

To improve your laboratory efficiency with XLC-MS, Spark will assess current practices and flow in your laboratory, define the desired automation solution and actively support implementation.



# XLC-MS INTEGRATES SAMPLE EXTRACTION AND LC SEPARATION

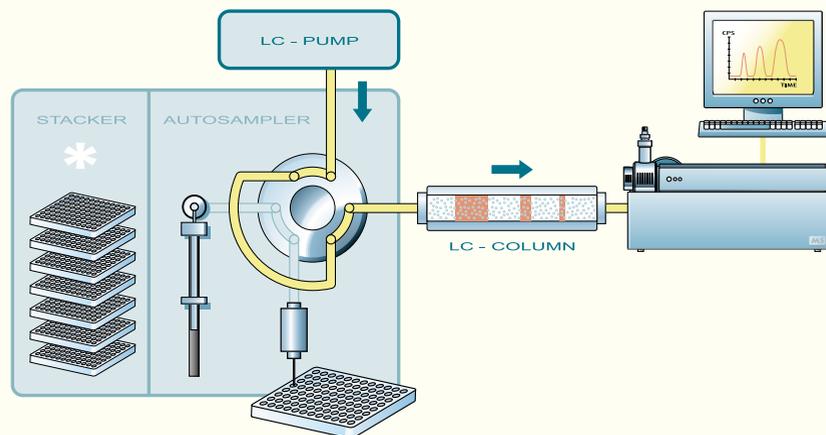


## XLC-MS AND PROCESSING MODES

Spark has used XLC technology to create a fully integrated system that allows use of different operating modes without changing a single connection: Symbiosis™. Three main operating modes are incorporated in the system. These are (Fast)-LC, XLC High Throughput and XLC Method Development, assuring total flexibility and efficiency for existing and future assays.

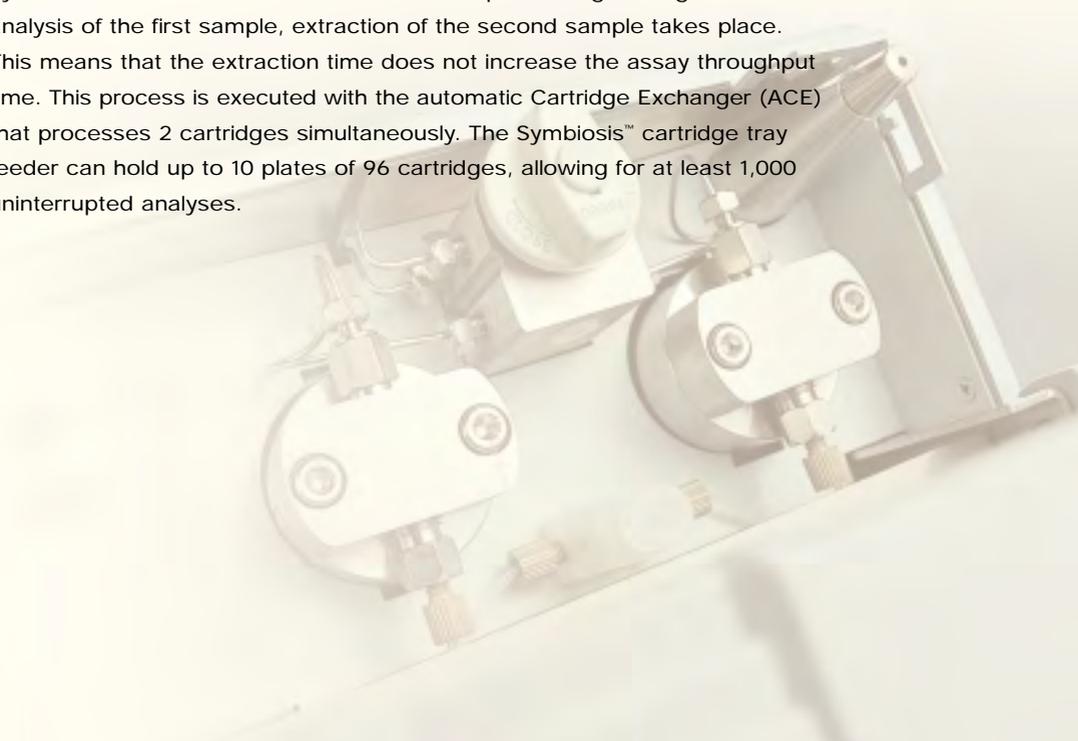
### (Fast)-LC mode

The LC or, if required, Fast-LC mode of Symbiosis™ provides full LC functionality for running existing methods. Samples that have been prepared using existing sample prep techniques such as 96-well SPE, Liquid-Liquid extraction or protein precipitation can instantly be run in the (Fast-) LC mode. This allows you to gradually implement XLC-MS as the automation platform for bioanalysis without the need to change all existing assays at once.



### XLC High Throughput

Many assays can instantly be run using Spark's Generic Method. Symbiosis™ default mode is concurrent serial processing. During LC-MS analysis of the first sample, extraction of the second sample takes place. This means that the extraction time does not increase the assay throughput time. This process is executed with the automatic Cartridge Exchanger (ACE) that processes 2 cartridges simultaneously. The Symbiosis™ cartridge tray feeder can hold up to 10 plates of 96 cartridges, allowing for at least 1,000 uninterrupted analyses.

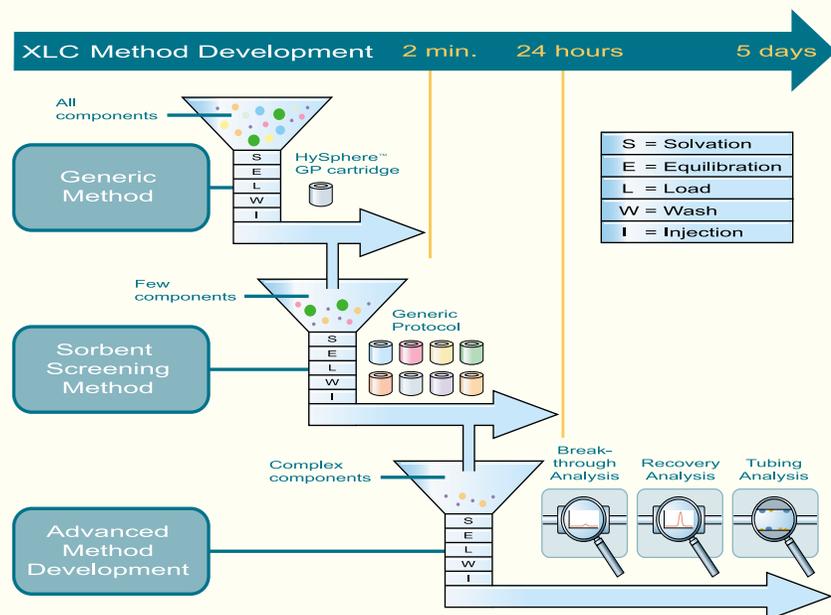
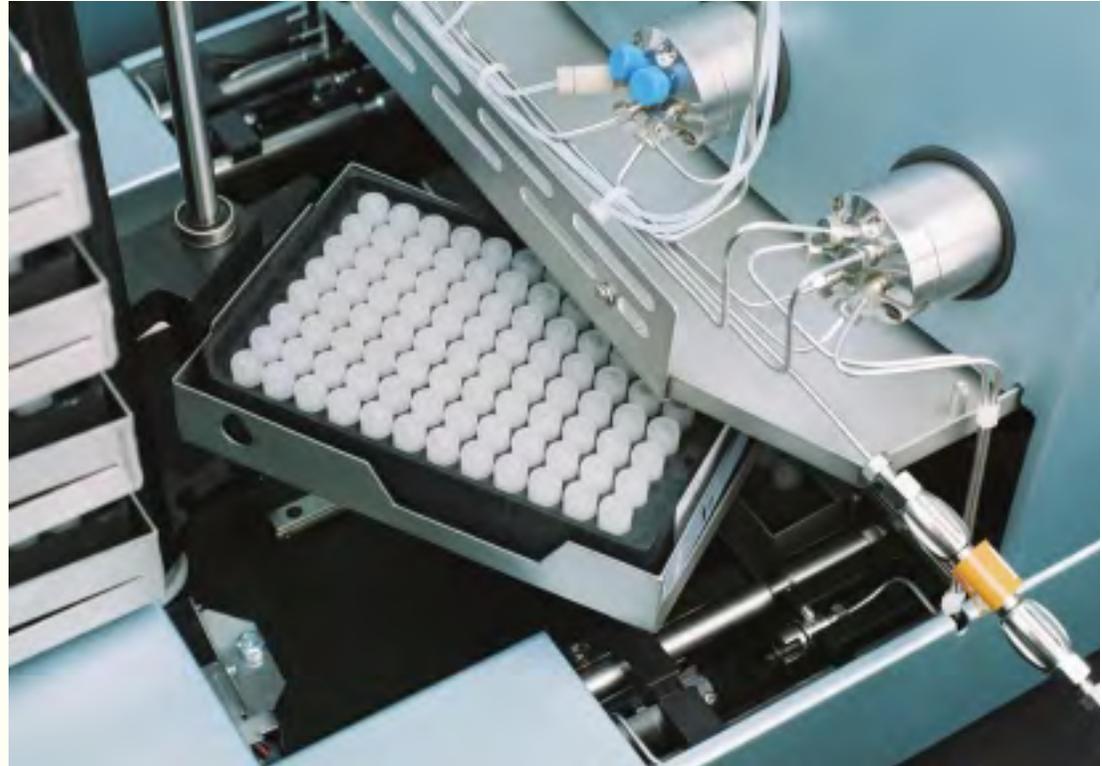


### Sorbent screening method

A rapid automated “trial-and-error” approach can be applied using a HySphere™ Method Development cartridge tray in combination with a sorbent screening protocol. The tray contains 8 different sorbents varying from very polar to strongly hydrophobic. It enables programming of parameters such as solvent volume and type, mixing percentage and flow rate. After programming, these special cartridge trays allow automated assessment of the optimum SPE parameters for a variety of SPE sorbents.

### Advanced method development

If maximum optimization is required, Symbiosis™ offers an advanced method development mode. Using 2 HySphere™ cartridges in series, every run will provide information on analyte recovery, breakthrough and adsorptive losses in the system tubing. These data provide the vital input for a most efficient method optimization. Once developed, Symbiosis™ can instantly start processing using this method, without making any changes to the hardware.



### XLC Method Development mode

The XLC method development mode gives freedom to choose from different approaches, depending on available development time, assay complexity and required assay performance.

### Generic method

For instant processing of small batches of samples from pre-clinical studies, a generic method has been developed. This method is applicable for a wide range of compounds in terms of hydrophobicity. Just add internal standard and place the sample in the autosampler, no protein precipitation required, nor filtration or centrifugation. The protocol includes use of a HySphere™ GP cartridge and recommendations for the LC Column.

## THE FULL SYSTEM SOLUTION

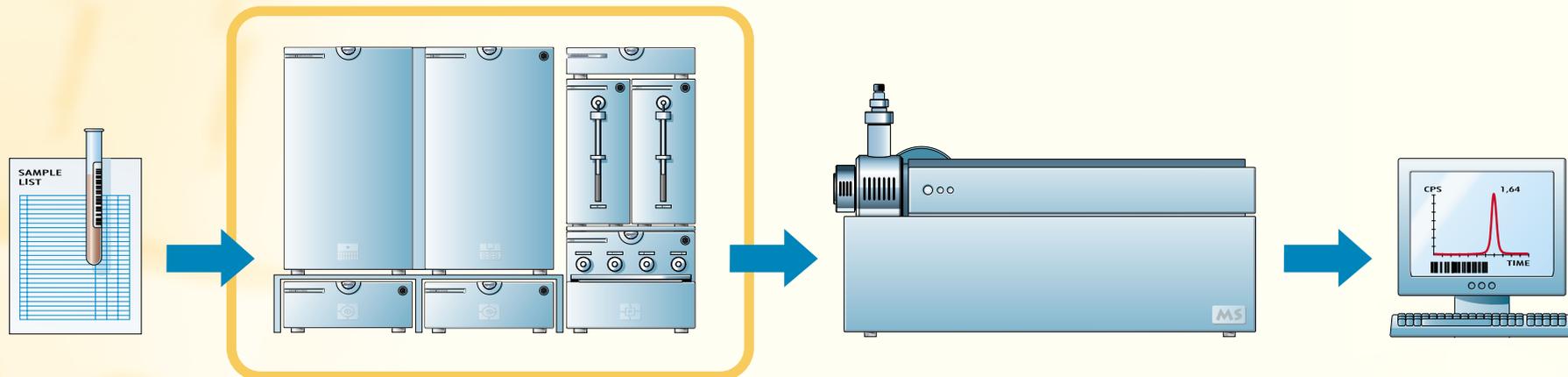
**Symbiosis™** is Spark's integrated front-end system based on XLC technology. The combination of XLC and Symbiosis™ provides the laboratory with an extremely versatile system with integrated processes that otherwise require a variety of non-integrated instrumentation. Its development is based on 15 years of experience in online SPE sample prep (Prospekt 1™ and Prospekt 2™) from 500 installed units worldwide. The system includes a new generation high throughput autosampler, a conditioned stacker to store sample trays, and online extraction with full isocratic or gradient LC-functionality.



Predefined XLC methods in combination with a fixed tubing configuration guarantee a

simple operation of the system without the need to change the configuration or tubing when switching between operating modes. Carry-over of the system is minimal due to practice proven wash routines, superior autosampler carry-over performance and of course use of the disposable HySphere™ extraction cartridges.

Both physical sample flow and sample data flow based on a single sample list, remain uninterrupted throughout the entire process. Thus, SPE, LC and MS are truly automated.



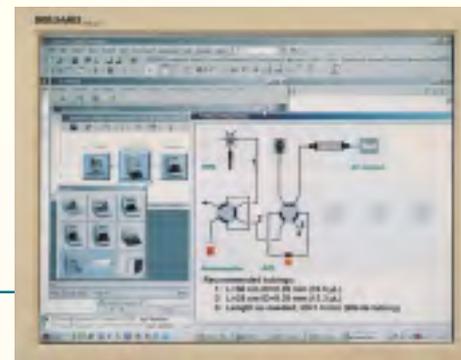
Symbiosis is an all round system

- Fully integrated and automated with SparkLink™ instrument control software
- Operates with a single sample run table throughout the process
- Compatible with Analyst™, Masslynx™, Xcalibur™ in a CFR 21 part 11 compliant environment
- Generic method for quick analysis of most common samples
- Integrated Gradient or Isocratic pumps give full LC capabilities with online sample extraction
- Allows for 3 levels of Method Development
- Software selectable operating modes
- Fixed tubing configuration allows free switching between (Fast-) LC/ XLC High Throughput and XLC Method Development
- High throughput, parallel sample handling brings sample prep time within LC time
- Superior analytical performance, lowest possible carry-over
- A fresh SPE sorbent for each sample assuring optimal sample cleanup
- No regeneration of columns required

#### SparkLink™ Instrument control software

The operating software for Symbiosis™ is the latest version of SparkLink™, allowing full integration with LIMS systems and MS control software. Sample lists from LIMS systems can be imported for immediate start of your analyses and sample runtables can be exported to MS instrument control software for complete data control.

SparkLink™ is compatible with Analyst™, Masslynx™, Xcalibur™ and Millennium™. SparkLink™ also includes predefined protocols for instant sample processing. An integrated run table for LC/ Autosampler and Extraction methods offers total control from a single window.



Spark

## THE HEART OF SYMBIOSIS™

### High Throughput temperature-conditioned autosampler

With 15 years of experience in autosampling, Spark developed a 5th generation autosampler that offers maximum throughput and flexibility. A special speed mode reduces cycle time with more than 50% for partial loopfill injections including needle wash. A conditioned stacker allows for cooled storage of biological samples under 100% controlled conditions up to 9216 samples (in 12 deep well or 24 shallow well plates, or any user-defined well plate or vial format). User selectable sample viscosity levels can be used to optimize between cycle time and performance.



### Online Extraction with HySphere™ Cartridges

The key to XLC technology is direct extraction of the sample onto a disposable HySphere™ SPE cartridge, using a high-pressure dispenser (HPD). Proven technology directly taken from Prospekt 2™ is integrated in Symbiosis™. Now you can achieve extractions in less than a minute routinely. The disposable format gives freedom of choice in sorbent or re-use for each single sample. The use of small particle SPE sorbents results in higher sensitivity and better resolution. Spark provides a complete program of HySphere™ cartridges. HySphere™ cartridges are delivered in 96-position trays that are placed directly into the Symbiosis™ Automatic Cartridge Exchanger (ACE). Each cartridge tray contains an RF chip with information on type, batch, and sample number. This information is displayed in the PC and incorporated in the final analysis report.

### Integrated LC-functionality

Symbiosis™ includes Isocratic or Gradient LC pumps. LC pump functionality is fully integrated in the SparkLink™ instrument control software for Symbiosis™. Apart from being used in LC elution, the LC pumps are also used for peak re-focusing in XLC mode, leading to maximum column efficiency.

As Symbiosis™ instantly runs with a generic protocol for most common samples, it facilitates automated high throughput analysis from day one. The required turnaround in lab efficiency will be achieved through a well-defined program that integrates the solution in the organization. Spark will diagnose your applications and processes and advise on improvements in your lab. A mutually agreed project plan will focus on introducing the equipment, training, support and phased implementation.

## SUCCESSFUL AUTOMATION



### Return on investment

The first pay-off will be the increased throughput per LC-MS system, and the previous pages have shown how to achieve this. On an organizational level, such increase may well prevent new investments in MS systems. Also, the improved analytical performance will reduce method development time and the cost per sample will be reduced.

Then there are aspects that first seem of secondary importance: think of human error, the reduction of paperwork, and the allocation of your valued scientist to more important tasks than pipetting. All these will lead to higher efficiency and a significant reduction in cost, and, consequently, an increasing margin of profit. The result of all improvements in efficiency often means a return on your investment after only one year.

### Changing the organization

People tend to resist change. For that reason Spark considers training and support essential with first introduction of a new system. Transferring an existing SPE method to the new system and cross-validation of the method will be the next step to build up confidence. Continuous support from Spark's application engineers in each phase of the implementation project will lead to further efficiency and reduced costs.



# PHARMACEUTICAL LABORATORY OF

## Conditioned Stacker

Accurate temperature conditioned stacker that holds up to 24 shallow or 12 deepwell plates.



## Well Plate Waiter

Automatically transports the required tray to the autosampler.



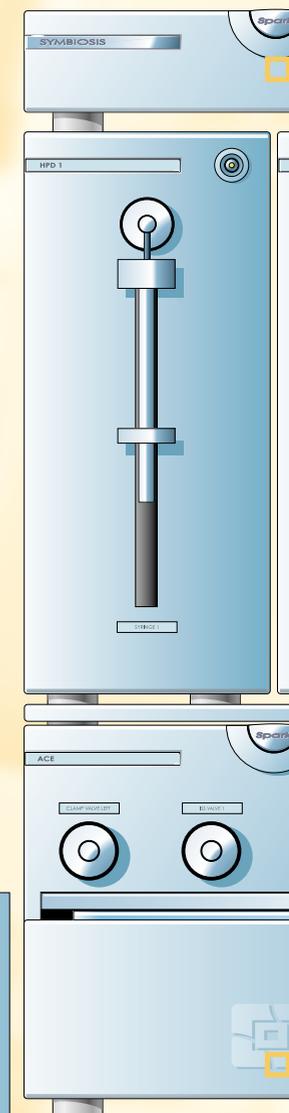
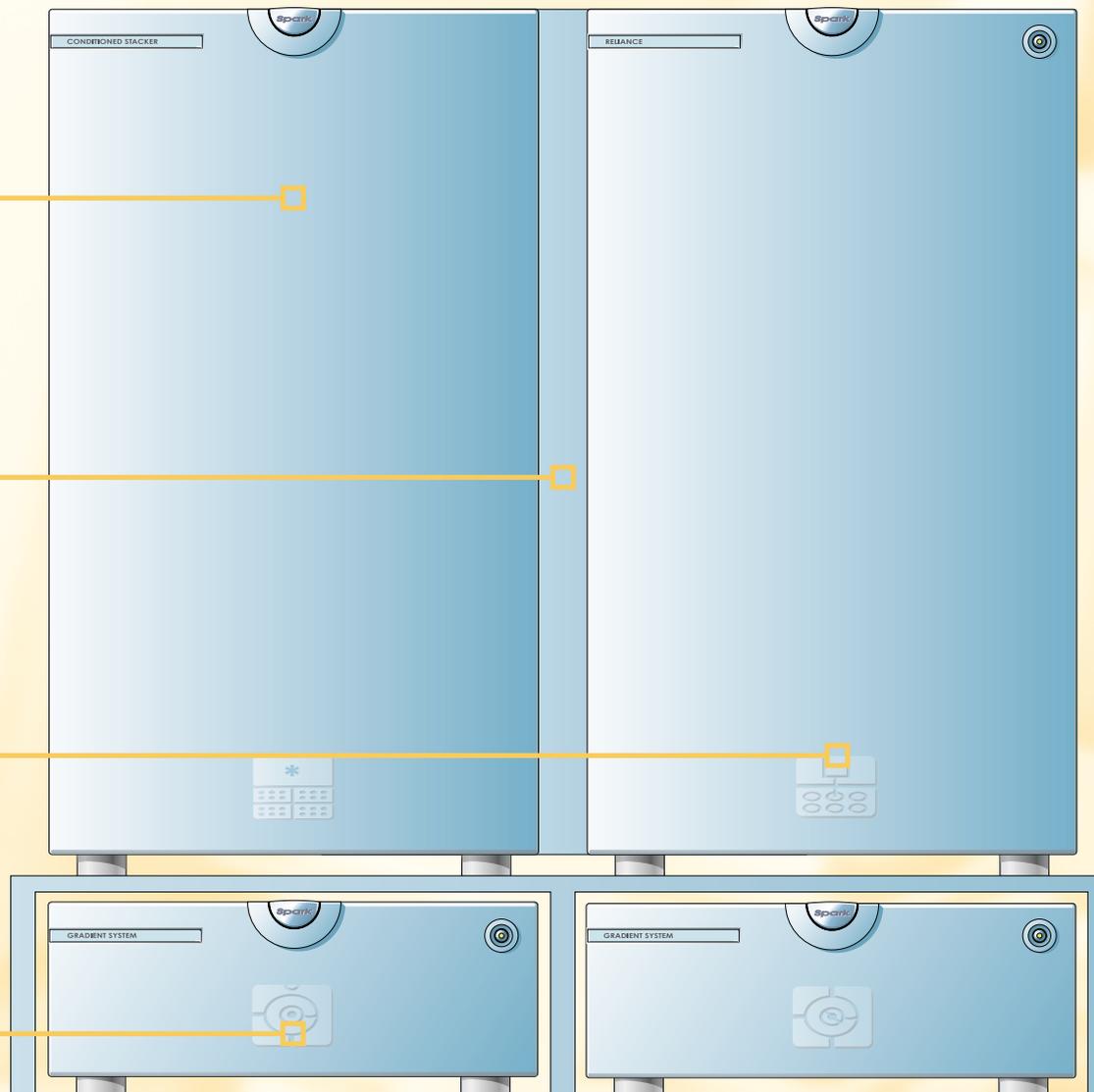
## Autosampler

5th generation high throughput autosampler with high minimal carry-over.

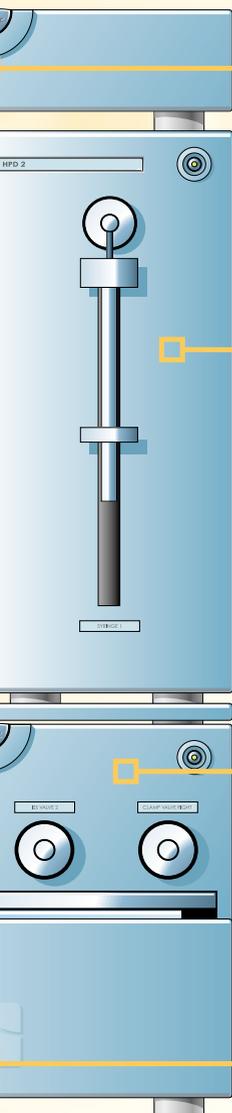


## Gradient & Isocratic Pump

Integrate full LC capabilities with online SPE sample clean-up.



# UNLIMITED DIMENSIONS



**Organizer**  
Holds LC-solvent bottles.



**Mix HPD**  
Constant flow High Pressure Dispensers for conditioning, sample loading and cleanup.



**Fixed Tubing configuration**  
Allows for easy switching between operating modes.



**ACE**  
Automatic Cartridge Exchanger with double cartridge clamp and valve switching.

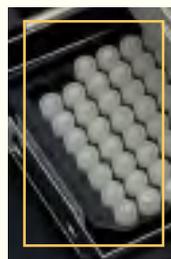
## UNIVERSAL



**XLC Technology**  
Integrated extraction and LC separation with 3 operating modes allow use of existing assays and new Symbiosis™ methods.



**SparkLink™ Software**  
Fully integrated instrument control software with communication features for LIMS, MS, HPLC software in a 21CFR part 11 compliant environment.



**SPE Cartridges**  
Sorbent size of <math><10 \mu\text{m}</math> of HySphere™ SPE cartridges speeds up sample loading and reduces cycle time.

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## THE SMART SOLUTION

### Increased throughput

- More samples per day per analyst
- Reduced sample handling
- 100% fresh sorbent for each sample
- A single sample list for entire LC-MS
- Faster method development

### Improved analytical performance

- Total extract transfer into MS
- Less sample needed for LLOQ
- Analyte enrichment and peak focusing for highest assay sensitivity

### Cost reductions

- Labor cost per sample is reduced
- Reduced Method Development time
- Reduced costs for consumables

### Phased implementation

- Diagnosis of current situation
- Automation project planning
- Agreed implementation trajectory
- Customized product training and support

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