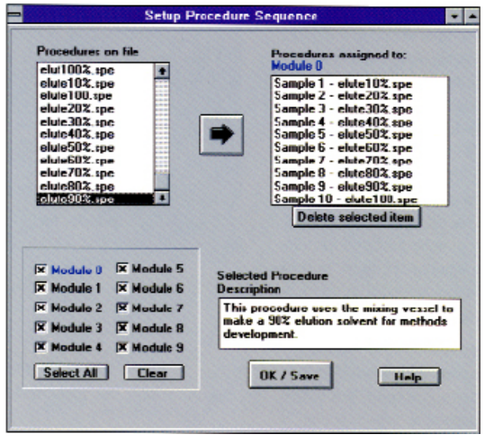
Rational Methods Development

Optimizing Extraction Strategies

Automated SPE Methods Speed Assay Development

Developing an HPLC assay for an investigational peptide, a high-throughput drug metabolism laboratory used SPE techniques to isolate the peptide and an internal standard from rat plasma. The RapidTrace Workstation allowed them to develop an optimized extraction strategy in a fraction of the time required by conventional bench methods.

By applying stored methods to a series of samples, and selectively varying extraction conditions, the lab was able to achieve analyte recovery of about 85% (compared with the previous 60-65%), with no loss of precision. Based on these results, the RapidTrace methods were later extended to human samples, and showed excellent accuracy and precision over a standard curve range of 2.5-2500 ng/mL.



Recovery Data for RapidTrace SPE Extraction Method of Investigational Peptide in Human Plasma

Concentration Range of Standard (ng/mL)	Mean Concentration Found (n=5) (ng/mL)	Percent Recovery
2.5	2.47	99%
5.0	5.07	101%
10.0	10.2	102%
25.0	25.4	102%
50.0	50.7	101%
100.0	95.3	95%
250.0	248.0	99%
500.0	519.5	104%
1000.0	1042.5	104%
1750.0	1735.7	99%
2500.0	2448.5	98%

Validation Manual Included

Every RapidTrace SPE Workstation comes with its own Validation Manual. This is a comprehensive document that includes detailed, step-by-step instructions to help you meet GLP, GMP and GOP requirements. Experienced Caliper Life Sciences consultants are available on a contract basis to assist with any validation assignment.

TurboVap LV Workstation Is the Ideal Productivity Partner

Biotage's TurboVap LV Evaporation Workstation offers "load it and leave it" high throughput, controlled evaporation for up to 50 samples simultaneously. Patented "gas vortex shearing" is 3-10 times faster than conventional nitrogen blow-down techniques.

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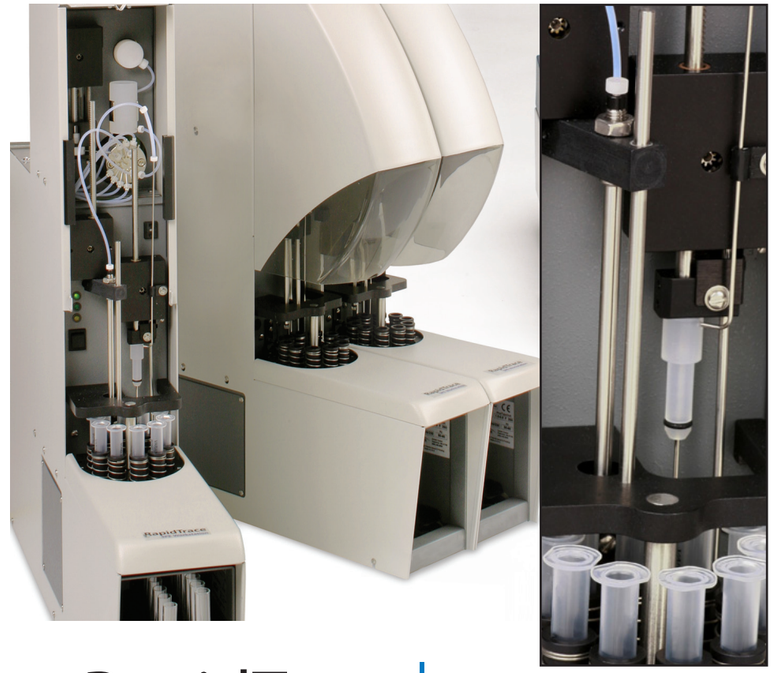


RapidTrace | SPE Workstation

Automated High Throughput SPE Extraction

Advanced bioanalytical techniques like LC/MS/MS and GC/MS are meeting the increasing demand for greater speed and specificity in toxicological screening. But conventional sample preparation methods have not kept pace. In particular, solid phase extraction (SPE), an essential step in the analysis of many biomolecules, has become a through-put limiting step in many laboratories.

Biotage's RapidTrace SPE Workstation eliminates SPE bottlenecks, so that your lab can realize the full benefits of today's powerful and costly analytical instruments.

RapidTrace is the perfect complement to high-speed analytical techniques, a powerful high-throughput workstation dedicated specifically to SPE bioextraction. In its full modular configuration, a single workstation can process 40-60, even 100, samples per hour!

RapidTrace SPE Workstation is designed especially for the regulated pharmaceutical, clinical and forensic environment, RapidTrace is also a robust automated platform for quickly developing rugged, reliable SPE methods. These procedures can be easily transferred from workstation to workstation from site to site, or from the pharmaceutical to an outside Contract Research Organization.

Automated High Throughput SPE Extraction

Increase Throughput for Routine Sample Handling - Your SPE processes can keep up with your analytical capabilities. Plus, the modular design of the RapidTrace workstation lets you add capacity as required; for example, as a compound moves from initial toxicology screening into more formal pre-clinical studies and then into human clinical trials.

Accelerate Methods Through Development and Optimization - Using a friendly, familiar Windows interface, you can easily implement a structured, "rational" strategy for SPE experiments to determine optimum SPE conditions for each of your samples.

Readily Transferable Methods - Methods stored in the RapidTrace controller can be instantly recalled, and can be transferred to other RapidTrace workstations with 100% confidence.

Parallel Processing Assures Maximum Productivity - Each module operates independently. So if one is shut down for operator attention, the others stay on-line.

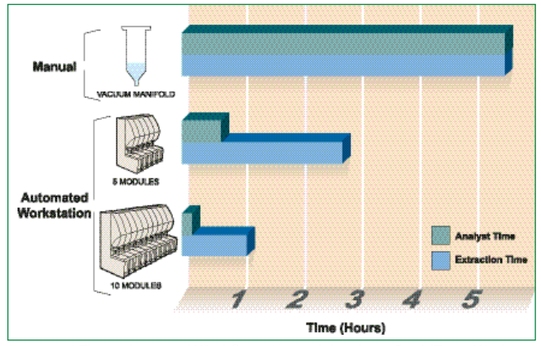
Improve Operator Safety - Biotage's automated approach improves lab safety by minimizing operator contact with hazardous samples and chemicals.

Validation Documentation Included with Each Workstation *Better Analytical Results* - Finally, RapidTrace provides the consistently clean extracts that sophisticated analytical instruments like LC/MS/MS and GC/MS require for optimum performance.

Unmatched Performance and Throughput

RapidTrace eliminates tedious and time-consuming manual bioextraction methods. Every step of the SPE process is automated and rigorously controlled, to give you a level of reproducibility that simply can't be obtained with conventional techniques. Capable of handling up to ten samples/module, a 10 module RapidTrace Workstation delivers the throughput a busy lab needs to keep up with 2-5 minute LC/MS/MS cycle times.

- Modular design lets you add throughput capability as required-up to 100 samples per run at up to 100 SPE samples per hour! Modules within a group can run different methods. Or, modules can be separated and even used in different labs.
- All sample and solvent flow rates are individually controlled under positive pressure. You'll improve results by optimizing each step of the SPE process for maximum accuracy and precision (something you can't do with a conventional vacuum manifold).
- You can program individual modules and even individual samples with different conditions to speed development and test methods for ruggedness and reliability.



Improved Throughput and Workflow - In one lab using a RapidTrace Workstation versus a vacuum manifold to extract 75 samples, there was a 93 % reduction in analyst time and a 72 % reduction in extraction time.

Fast and Easy Development of "Bullet-Proof" Methods

Because you can write methods in minutes (and call them up in seconds), you can optimize your SPE conditions in a fraction of the time required using conventional techniques. Column types, reagents, concentrations and flow rates can be thoroughly explored; for example, you can vary a single parameter (pH, percent solvent, cartridge type, flow rate, etc.) incrementally to see its affect on recovery and precision. This unique "rational SPE design" capability dramatically speeds the development of rugged, reliable methods.

Editing methods is just as easy. You can insert, delete or change steps, or adjust any of the parameters. Methods can be assigned to an individual sample or by module. They can be saved and run together for true "multi-method" SPE, in an unattended run.

Improved Productivity

New Drug Candidate Plasma Studies

Pharmaceutical Lab Improves Productivity and Reduces Assay Costs

A large pharmaceutical company required analytical data on plasma levels for a new drug candidate in early phase clinical trials. Considered a "routine" analysis, the sample load was outsourced to a contract lab.

In an effort to improve turnaround time and reduce costs, the lab turned to RapidTrace SPE automation. The original method, developed using 3 mL SPE cartridges on a vacuum box, was transferred to the automated unit, further optimized, and quickly validated. Within days, the 10-module RapidTrace workstation was on-line, delivering a faster, more reliable assay and considerable savings in time and labor.

Performance/Productivity Comparision

Drug In Plasma Extraction

Item	RapidTrace (10 Modules)	Vacuum Manifold
No. of manual Steps to Run a Sample	5	17
Time to Run 75 Samples	1 Hour	4.5 Hours
Failure Rate	0%	15%
No. of Runs Required for 75 Sample Batch	1	3
No. of Standards and Controls for 75 Sample Batch	5	15
Operator Time	0.3 Hours	4.5 Hours

High Sample Throughput Assay

Screening for Cocaine Metabolite (BZE)

Drug Testing Lab Processes 56 Samples/ Hour with High Reliability

A common test for drugs of abuse is screening urine for the presence of Benzoylecgonine (BZE) for confirmation by GC/MS. The SPE cleanup is labor intensive, and, using conventional vacuum manifold methods, is highly susceptible to operator variations.

To bring the process under control, to improve lab throughput and to reduce analysis costs, a contract testing lab automated the procedure using a RapidTrace Workstation. The automatic procedure can be run in just 11 minutes; on the lab's 10-module Workstation, it now takes less than two hours to process 100 samples!

RapidTrace BZE Method Using 3 mL Varian Bond Elut® Certify SPE Cartridge

Step	Source	Destination	Volume (mL)	Flow (mL/sec)	
1 Condition	MeOH	Waste 2	3.0	.30	
2 Condition	diH_2O	Waste 1	3.0	.30	
3 Condition	pH6	Waste 1	3.0	.30	
4 Load	Sample	Waste 1	4.0	.04	
5 Pause	—	Time = 0.1 minute			
6 Purge-Cannula	diH_2O	Cannula	4.0	.40	
7 Rinse	diH_2O	Waste 1	3.0	.15	
8 Rinse	H_3PO_4	Waste 1	3.0	.15	
9 Rinse	MeOH	Waste 2	3.0	.15	
10 Rinse	Vent	Waste 1	5.0	.30	
11 Collect	Mixed	Fraction 1	3.0	.20	
12 Rinse	Mixed	Waste 2	2.5	.20	
13 Rinse	MeOH	Waste 2	2.5	.20	
14 Purge-Cannula	MeOH	Cannula	3.0	.40	
15 Purge-Cannula	diH_2O	Cannula	3.0	.40	

RapidTrace BZE Method Results

Within Run

Control Target Value (ng/mL)	Sample Number 1	2	3	4	5	Mean	C.V. (Percent)	Average Recovery
120	113	118	120	119	113	117	2.9	97.5%
180	182	176	186	176	174	179	2.8	99.4%

Run-to-Run

Control Target Value (ng/mL)	Run Number 1	2	3	4	5	Mean	C.V. (Percent)	Average Recovery
113	117	117	119	122	124	120	2.4	106.2%

Linearity - R^2=0.9999 (75-1000 ng/mL)

Throughput - 56 Samples/Hour (10 Module RapidTrace Workstation)