CHROMTECH

PrepBase

Application Note PB 401

**Improve HPLC Sample Preparation in an Analytical Laboratory –
A New Automated Sample Preparation Process**

By

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LP Raman* Technical Marketing Manager Whatman, Inc. 9 Bridewell Place Clifton, NJ 07013	Ingo Christ General Manager Chromsys LLC, PO Box 15131 Alexandria, VA 22309

Introduction

Autosamplers have improved productivity of HPLC systems by allowing them to work for 24 hours per day. By combining filtration and storage into one simple device that can be integrated with the autosampler, Whatman has developed a produced called Mini-UniPrep™ that improves productivity of HPLC systems further by enhancing the efficiency of the HPLC sample preparation process.

Preparing an analytical sample for injection into HPLC equipment can involve several steps. Figure 1 below shows the typical protocol for sample preparation to remove protein precipitates prior to HPLC analysis.
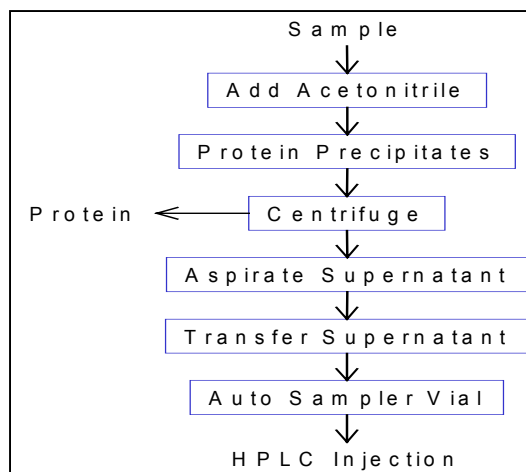
If large numbers of samples are to be analyzed, sample preparation protocols tend to be tedious. For example, an individual in a lab using the above protein precipitation protocol has to follow six different steps before getting the sample ready for injection into HPLC. When sample preparation is done manually, our anecdotal experience suggests an operator can prepare about 50 samples in a working day.

Figure 1 – Typical HPLC sample preparation protocol

This article explores a new just-in-time automated sample preparation method that is increasingly being accepted in the industry. It uses Mini-UniPrep (MUP) (from Whatman® Inc., Clifton, NJ) as a sample preparation device and a 'PrepBase™' add-on to a PAL™ autosampler (CHROMSYS, Alexandria, VA and CHROMTECH, Idstein, Germany).

What is Mini-UniPrep?

Whatman Mini-UniPrep is an innovative product that is a filtration device and storage-vial all combined into one (Figure 2). Mini-UniPrep is designed as sample preparation device used for clarification, particle removal or purification of liquids. Besides its ease-of-use and convenience, MUP users can cut down the sample processing time generally by one-third, reduce the need of disposable lab supplies (e.g., pipettes, transfer vials) and reduce chemical usage.

Figure 2 – Mini-UniPrep Sample Preparation Device

How does Mini-UniPrep work?

Mini-UniPrep consists of two parts – Chamber that can hold up to 500 µL of sample and plunger that holds the filtration media. To prepare a sample (Figure 3):

- Place unfiltered sample in chamber
- Compress filter plunger
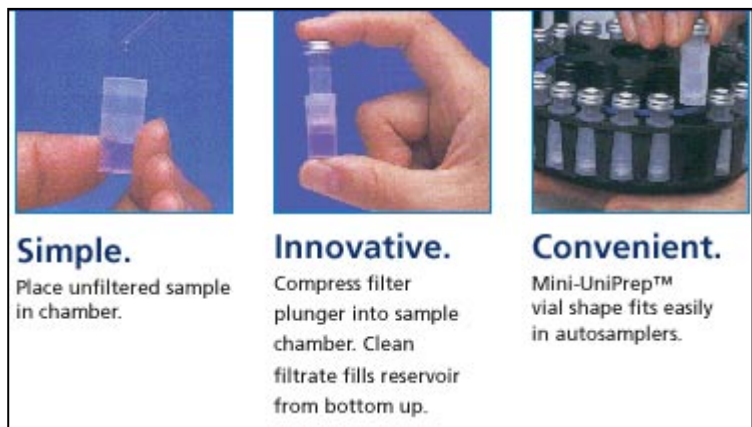- Clean filtrate fills reservoir from bottom up

Simple.
Place unfiltered sample in chamber.

Innovative.
Compress filter plunger into sample chamber. Clean filtrate fills reservoir from bottom up.

Convenient.
Mini-UniPrep™ vial shape fits easily in autosamplers.

Figure 3 – Mini-UniPrep Sample Preparation Device
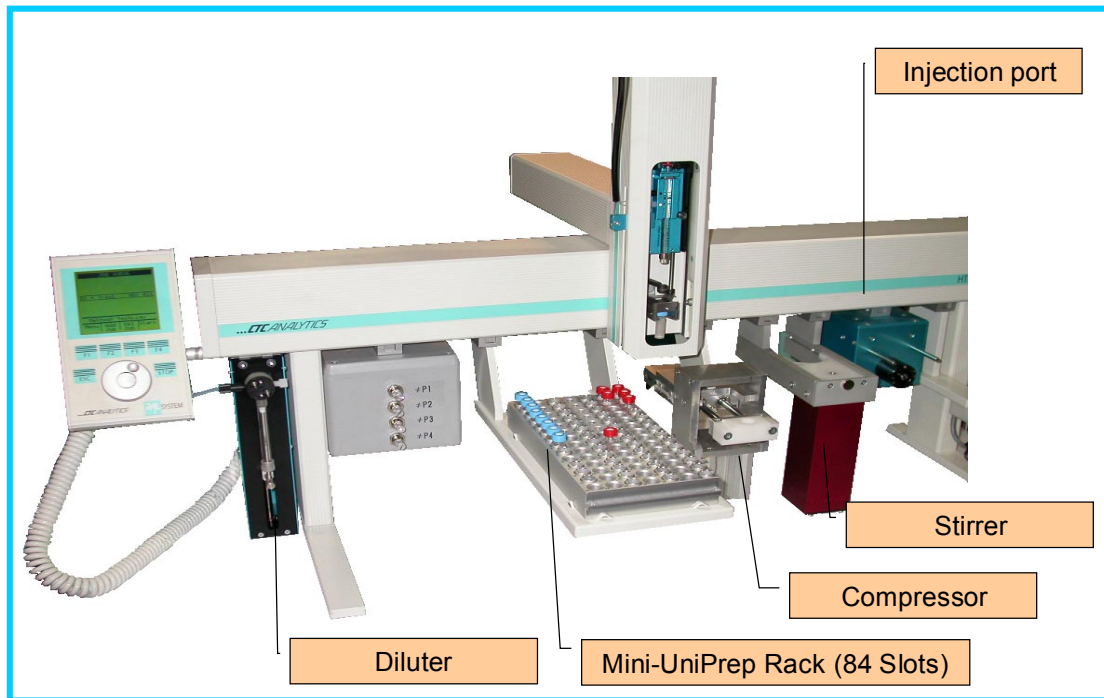
PrepBase/ PAL Automation Platform

Figure 4 – PrepBase sample preparation robot

Chromsys's PrepBase/PAL is an XYZ robot for "online filtration" automation, including sample preparation, transport and injection into an analyzer. Figure 4 above shows a picture of the automation platform. It is designed to extract, heat, cool, shake or stir samples. It also can be used for diluting samples and preparing a dilution series. It can automate almost all the steps required for HPLC sample preparation. The PrepBase uses Whatman's Mini-Uniprep vials.

How does automated sample preparation system work?

Sample is taken from a vial and injected into the sample chamber of the Mini-UniPrep Syringeless Filter. The autosampler's arm then inserts the filter plunger into the opening of the sample chamber. The assembled syringeless filter is then transported into the PrepBase, which presses the filter into the sample chamber, forcing sample through the filter. The autosampler syringe then pierces the septa, picks up the sample and injects it into the user's chromatographic system.

Improving productivity of HPLC sample preparation

The Mini-UniPrep based method for HPLC sample preparation improves productivity by simplifying and integrating different steps into one. Because the process is simple, samples can be prepared faster. The chamber of MUP can be used as a reaction/mixing vessel. This allows for end-to-end automation of sample preparation process. Often HPLC requires only approximately 50-µL injection. Due to its size, Mini-UniPrep uses only just the right amount of sample.

Reduce number of steps needed for HPLC Sample preparation

The Mini-UniPrep simplifies sample preparation. Figure 5 below shows a comparison between a simple filtration protocol that is followed universally for HPLC sample preparation.
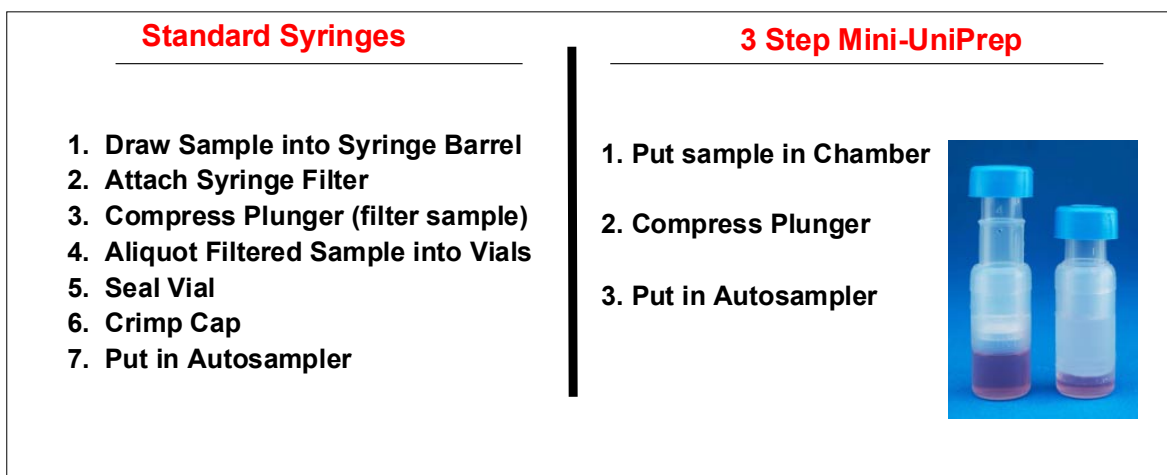

Standard Syringes	3 Step Mini-UniPrep
1. Draw Sample into Syringe Barrel 2. Attach Syringe Filter 3. Compress Plunger (filter sample) 4. Aliquot Filtered Sample into Vials 5. Seal Vial 6. Crimp Cap 7. Put in Autosampler	1. Put sample in Chamber 2. Compress Plunger 3. Put in Autosampler

Figure 5 - Comparison between syringe filtration and Min-UniPrep Filtration

Compared to seven steps required to filter a sample using a syringe filter, it can be done in just three steps with a Min-UniPrep. This reduces the complexity of the process by avoiding having to transfer the fluid from one vessel to another as shown in Figure 5. Reducing the number of processing steps directly translates into improved efficiency of the sample preparation process.

End-to-end automation of sample preparation

The process of HPLC sample preparation can be automated end-to-end using Mini-UniPrep. To demonstrate the benefits of automation, we have validated automated sample preparation for assay of Cyclobenzaprine HCl Tablets.

Figure 6 below shows a comparison of regular sample preparation procedure and automated sample preparation for analysis of Cyclobenzaprine HCl Tablets.
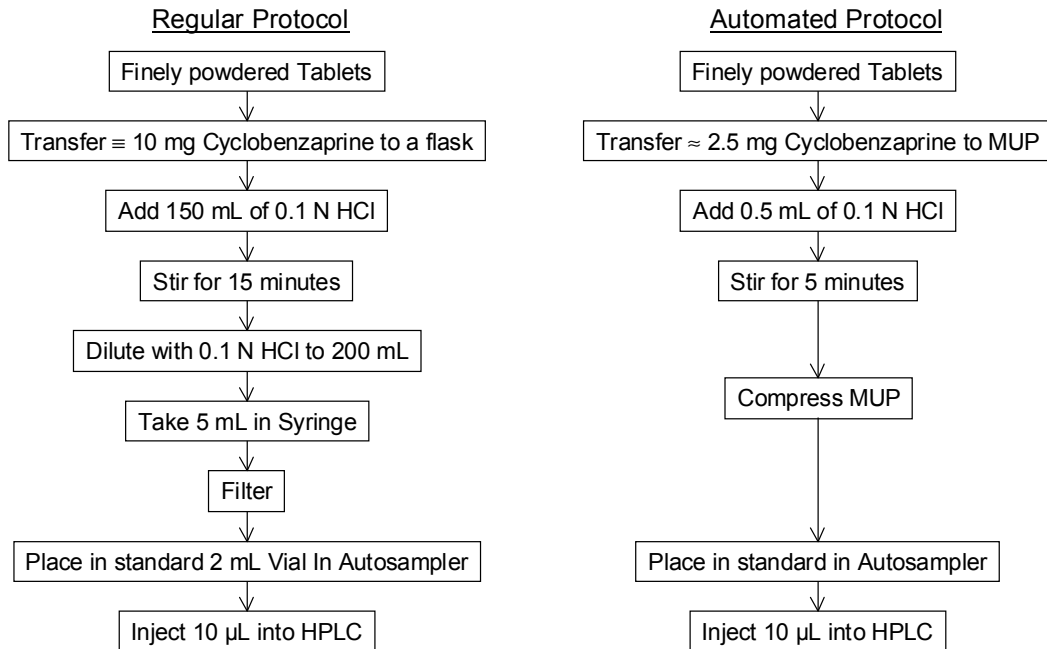
Regular Protocol

```
Finely powdered Tablets
        ↓
Transfer ≡ 10 mg Cyclobenzaprine to a flask
        ↓
Add 150 mL of 0.1 N HCl
        ↓
Stir for 15 minutes
        ↓
Dilute with 0.1 N HCl to 200 mL
        ↓
Take 5 mL in Syringe
        ↓
Filter
        ↓
Place in standard 2 mL Vial In Autosampler
        ↓
Inject 10 µL into HPLC
```

Automated Protocol

```
Finely powdered Tablets
        ↓
Transfer ≈ 2.5 mg Cyclobenzaprine to MUP
        ↓
Add 0.5 mL of 0.1 N HCl
        ↓
Stir for 5 minutes
        ↓
Compress MUP
        ↓
Place in standard in Autosampler
        ↓
Inject 10 µL into HPLC
```

Figure 6 - Comparison of regular and automated protocol for Cyclobenzaprine HCl tablets.

The regular protocol for analysis of Cyclobenzaprine HCl is specified under United States Pharmacopeia (USP) - 27. All the steps in the automated sample preparation protocol are done right in the Mini-UniPrep itself and the process is automated from start to finish.

Cyclobenzaprine was used because we had prior experience with this compound. Similar sample preparation methods are used in quality control of pharmaceutical tablets for:

- ❖ Content uniformity
- ❖ Dissolution testing
- ❖ Blending of table
- ❖ QC of Raw materials

A pharmaceutical company can run up to 100 assays similar to the above for each manufacturing batch.

Compared to the regular protocol, the automated protocol is much faster. Figure 7 below shows the comparison of time savings between the regular protocol and the automated protocol.

	Sample Preparation Time for Assay					
	Automated Method (Mini-UniPrep)			Regular Method (Syringe Filter)		
	Weighing (minutes)	Dissolving /Diluting (minutes)	Total Time (minutes)	Weighing (minutes)	Dissolving /Diluting (minutes)	Total Time (minutes)
ASSAY	2.62	6.95	9.58	5.27	15.00	20.27

Figure 7- Comparison between regular and automated HPLC sample preparation

On an average the HPLC sample can be prepared in half the time compared to the regular protocol. While the regular method took 20 minutes to prepare the sample, with the automated method, it took only 10 minutes, including 5 minutes of stirring time. When large numbers of samples are to be processed, this translates into significant savings of operator time.

Optimizing chemical usage

Chemical usage can be minimized when a Mini-UniPrep based sample preparation process is used. In the protein precipitation protocol described in Figure 1, an excess amount of acetonitrile is used to precipitate the protein. By using a Mini-UniPrep based sample preparation protocol, the amount of acetonitrile can be optimized. Figure 8, below shows the removal of protein with increasing ratio of actonitrile: plasma.

AcN/Plasma	Plasma (µL)	AcN (µL)	Total (µL)	Protein Removal (%)	Comments	Excess AcN than optimum
1x	250	250	500	N/A	Filter blockage	
2x	167	333	500	99.35%	Turbid Filtrate	
3x	125	375	500	99.34%	None	0%
4x	100	400	500	99.46%	None	33%
5x	83	417	500	99.47%	None	67%
9x	50	450	500	99.68%	None	200%

Figure 8 - Protein removal as a function of increasing acetonitrile concentration.

By using a Mini-UniPrep for protein precipitation, complete protein removal is attained at 3:1 ratio of acetonitrile to plasma. In comparison, up to 200% more acetonitrile can be used in the regular protein precipitation protocol.

Examples of Mini-UniPrep Automated Sample Preparation System

Automated Mini-UniPrep is increasingly being accepted in the industry as a sample preparation method. Earlier we described how this automated system could be used for analysis of Cyclobenzaprine HCl tablets. We discuss here how this automated system can be used in other settings.

Cosmetics

The automated Mini-UniPrep system has been used to analyze the contents of cosmetic products. Typically, 0.2g - 0.5g sample of lipstick, foundation or mascara is added to a 20mL vial in the PAL/PrepBase robot. The diluter then adds about 20 mL of solvent to the vial. The vial is then transported to a mixing unit. There, it was shaken or stirred in a temperature controlled environment. After the mixing it completely, the robot arm draws 50 µL six times and transfers the liquid to the sample chamber of the Mini-UniPrep vial. The autosampler's arm then inserts the filter plunger into the opening of the sample chamber. The assembled syringeless filter is then transported into the PrepBase, which presses the filter into the sample chamber, forcing sample through the filter. The autosampler syringe pierces the septa, picks up the sample and injects it into the user's chromatographic system. The Mini-UniPrep vials are then discarded in a container. The automated system significantly improved the productivity of analytical lab services at this customer.

Food analysis - verification of label claim

The automated PrepBase/Mini-UniPrep system is used by a food manufacturer to verify label claim made on the package. Because this was a manufacturing plant, hundreds of samples were analyzed each day and the sample preparation process tended to be tedious and repetitive. In the automated system, liquid food product is added to the sample chamber of the Mini-UniPrep
Vial. The autosampler's arm is then inserted the filter plunger into the opening of the sample chamber. The assembled syringeless filter is then transported into the PrepBase, which presses the filter into the sample chamber, forcing sample through the filter. The autosampler syringe then pierces the septa, picks up the sample and injects it into the user's chromatographic system. The Mini-UniPrep vial was returned to the tray.

Conclusions

Mini-UniPrep allows for quick and easy, all-in-one HPLC sample preparation. Compared to conventional methods, samples can be prepared up to three times faster. The MUP uses a standard design and is compatible with most major auto-samplers. An automated Mini-UniPrep system can improve the efficiency of the analytical lab. There is less need for acquisition of supplies, storage of samples, and disposal of chemicals and lab supplies.

* With courtesy of Whatman and Chromsys LLC.