

Advanced Separation Technologies Inc. (Astec)  
37 Leslie Court, P. O. Box 297, Whippany, NJ 07981 USA  
Tel: (973) 428-9080 Fax: (973) 428-0152  
E-mail: astecusa@aol.com Website: www.astecusa.com

## Astec SILICA AND REVERSED PHASE HPLC COLUMN OPERATING INSTRUCTIONS

### **INTRODUCTION**

---

Astec columns have been designed to perform with state-of-the-art characteristics. Care in choosing each component, critically monitoring all chemistry and then bringing these together in a newly designed packing system gives you, the customer, the anticipated performance you seek.

### **CHEMISTRY**

---

The methodologies for all Astec reversed phase columns have been based upon established chemistries for 5 micron spherical media. Monofunctional silanes are used that yield the greater consistency of retention characteristics. The percentage of alkyl loading is chosen to give optimum resolution in high water mobile phase compositions. A second end capping step provides maximum possible coverage on all reversed phases and is monitored by FTIR for batch process and nitrobenzene retention for columns under anhydrous solvent conditions. The resulting silanol coverage of greater than 98% assures consistent selectivity and greatly enhances column stability.

### **COLUMN HARDWARE**

---

Astec columns are based on a stainless steel modular system. The tubing has a 4.6mm internal diameter ( $\pm 0.02$ ) and is polished by a patented process to an internal glossy finish of less than 8 microinches. The tubing is then ultrasonically cleaned. Threaded column end fittings are of low dead volume design with a 0.007 laser bore that centers to a 9° conical cavity for ideal sample dispersion. Connection to LC equipment requires a compression screw. The threaded modular design prevents column end distortion delivery consistent, reproducible performance with good peak symmetry.

### **COLUMN ASSESSMENT PARAMETERS**

---

The data provided on each QC sheet that accompanies the column is a means of establishing the consistent performance of the HPLC column. Before using the column, the test should be repeated to establish these characteristics within your own system's variance. Testing periodically will help to establish the condition of the column and the possible need for regeneration, repair or replacement.

### **COLUMN SERIAL NUMBER**

---

The number printed on the column you receive is on record with your purchase order at Astec and it is our means of tracing all column components should you experience

any difficulty or require historical information on the columns for an NDA. Be sure the number is recorded in your notebook for future reference.

### **QUALITY CONTROL TEST**

---

Every column is tested for individual performance. A void volume test is run to indicate the packing density which is a useful measure of the reproducibility of the column, other things being equal. Sodium iodide is used as a marker while it is not an absolute measure, statistical analysis has shown it to be predictable and meaningful. Two solutes are generally used to determine column symmetry and efficiency, one near the elution volume and one at an extended  $k'$  to measure overall column performance. For the reversed phase line, nitrobenzene retention under anhydrous mobile phase conditions is used to monitor silanol coverage while FTIR is used for the media itself.

### **REGENERATION AND LONG LIFE**

---

All HPLC columns deteriorate with use but several operating steps can help in increasing the life of a column. Impurities in the mobile phase and deterioration of the silica base can be circumvented by placing a silica guard column between the pump and injector. The purpose of this column is to preadsorb any polar contaminants from the mobile phase, to filter out dust and debris which can build up on the head of the primary column from the solvent, and finally, to saturate the mobile phase with silica for high aqueous phases to prevent attack of the silica base of the primary column. No effect on the results of a separation has ever been observed since the concentration of dissolved silica is extremely low. Second and most important is the use of guard columns between the injector and primary column. Its main function is to remove impurities from the sample that would normally adsorb at the inlet.

Silica column regeneration can be accomplished by pumping several column volumes of ethanol and finally, water. The column can now be re-activated by passing 50 mL of each of the following solvents through the column: ethanol; acetone, ethyl acetate; 1,1,1-trichloroethane; or, heptane. Solvents used for this procedure should be thoroughly dried using proper molecular sieves and ultrafiltration.

Reversed phase columns are to be regenerated by first passing methanol, water, 0.1M H<sub>2</sub>SO<sub>4</sub>, water and finally, methanol. Columns should be stored in methanol whenever possible.

Columns are supplied with compression nuts to reseal the column for storage in cellulose tubes with non-roll caps.

Advanced Separation Technologies Inc. presents these instructions as an aid to successful use of these products. Exceptions are possible. All statements herein are expressions of opinion which we believe to be accurate and reliable, but are presented without guarantee or responsibility on our part. While Advanced Separation Technologies Inc. has used its best efforts to present useful instructions, no warranty expressed or implied is given with respect to this material or products recommended herein.

©2003 Advanced Separation Technologies Inc.  
All rights reserved. Printed in USA.