

Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am interested in separating molecules on the basis of their unsaturation, but the compounds do not lend themselves to GC. A co-worker suggested that I use a technique called *argentation chromatography*, but I have not been able to find a commercial source of a column. Can you give me some advice?

Answer:

Argentation chromatography has been used since the early days of chromatography to separate molecules according to their double bond content. Application of this technique was useful for separating isomers with double bonds and for segmenting *cis/trans* isomers. The mechanism of this classic separation technique is based on the association of silver ions, which are coated onto a stationary phase with the double bonds of the molecule, probably through interactions of their respective electron clouds.

Argentation columns (and TLC plates) were made by impregnating the silica particles with silver ions by slurring the packing (or soaking the TLC plate) in an aqueous solution of silver nitrate and removing the water by evaporation. The coated particles were then dried before use. Often, the first coating level that was prepared for use was targeted to be 25%; if retention and/or selectivity adjustment of the compounds was desired, newer columns were prepared using packings coated at either a lower (for less retention) or a higher (for increased retention) level. Obviously, the following variables must be taken into consideration: the coating level; the reproducibility of the coating level from batch to batch; and the age of the coated packing, which would change the separation characteristics. Because of these issues, the preparation of the columns was left to the user, and few, if any, commercial manufacturers sold an "off the shelf" product.

Without commercially available columns, there has been limited activity in modern HPLC using the argentation technique. One study involved impregnating a prepacked silica gel column with silver and successfully separated olefins using HPLC (1). Another innovative report used a commercially available reversed-phase column with an eluent of silver nitrate dissolved in a methanol-isopropanol solution (2). By having silver nitrate continuously in the mobile phase, they accomplished separations of triacylglycerols on the basis of carbon number, number of double bonds, and geometrical isomerization (*cis/trans*). The separation depended upon the concentration of silver nitrate, with 85mM being the most acceptable concentration to attain the desired resolution. The following precautions were mentioned in the paper: not exceeding the solubility limits of the salt; keeping the mobile phase in dark bottles to protect it from a light reaction; minimizing the amount of light that enters the detector cell when analyses were not being run; keeping the temperature constant during analyses; and flushing the system with a mobile phase containing no silver nitrate when not doing analyses.

If you do argentation HPLC, you must prepare your own column and system. Consult the references below and do a keyword search for recent reports. Also, contact the various column manufacturers for any suggestion that they might have.

References

1. H.D. Friedel and T. Matusch. Separation of non-polar sesquiterpene olefins from tolu balsam by HPLC: silver perchlorate impregnation of a prepacked preparative silica gel column. *J. Chromatogr.* **407**: 343-50 (1987).
2. B. Petersson, O. Podlaha, and B. Jirskog-Hed. Triacylglycerol analysis of partially hydrogenated fats using HPLC. *J. Chromatogr.* **653**: 25-35 (1993).

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC-MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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