

SPE PROTEIN RETENTION, SPS vs ODS

The following study was conceived and carried out by T. J. Szczerba in the Regis Technologies laboratories.

In the solid phase extraction (SPE) format, a small amount of packing--perhaps 100 mg--is packed onto a filter held at the bottom of a short, squat "column". In theory, when a sample is passed through the SPE packing, the packing not only retains the analyte of interest but also separates it from undesired components such as serum proteins, which are passed to waste. A popular bonded phase for this purpose has been the octadecylsilyl (ODS).

Although restricted access media (RAM) were originally developed to allow direct injection analysis (1)--thus obviating need for other measures--nevertheless the use of RAM packings in SPE format could be not only feasible but also advantageous. For such use, The SemiPermeable Surface (SPS) RAM would be a prime candidate (1-4).

In SPS packings, any of several functional groups is selectable. One of these is the octadecylsilyl ODS: the ODS group is carried under the SPS outer surface (1-3; see especially, 3).

This study was conducted to show that when SPS-ODS rather than conventional ODS packings are used, SPE cleanup of serum becomes far more effective; the resultant extracted samples, much less contaminated by residual serum proteins.

Experimental:

Overview:

Throughout, SPS-ODS was compared with conventional ODS. All other parameters were held invariant.

SPE:

Preparation: 100 mg of 40 μ bonded silica particles were packed into SPE columns. The SPE columns were then pretreated by two successive 2-mL washes, methanol first, then water.

Serum: Human, as is (Figures 1 and 2); and spiked with 200 ng carbamazepine/mL serum (Figure 3).

Sample: 2 mL serum/water 1/1.

Wash: 1 mL water (Figure 1); 3 mL water (Figure 2).

Eluent: 1 mL methanol/water 1/1.

HPLC:

Column: C8, 15 cm x 4.6 mm I.D.

Sample: 500 μ L SPE eluate.

Mobile phase: Methanol/water 1/1, 1 mL/min.

Detection: UV, 254 nm, 0.1 AUFS.



Figure 1. These 1-mL-wash chromatograms show the compositions of methanol/water eluants from the two SPE tubes: the left, from the one packed with conventional ODS; the right, from the SPS-ODS-packed tube. Compared to the SPS-ODS-packed tube, the tube that had been packed with conventional ODS is shown to have performed poorly, that is, to have retained far more components at far greater concentration.

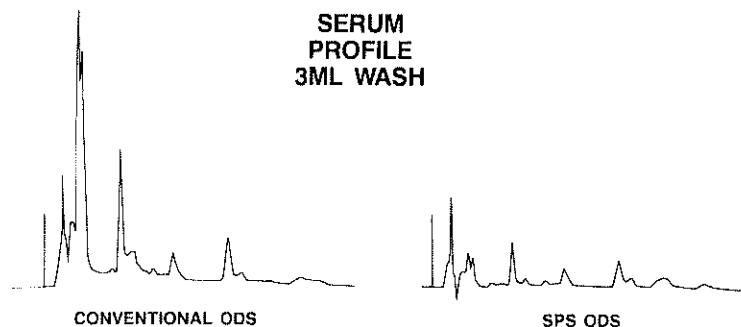


Figure 2. For the chromatograms shown in Figure 1, the SPE tubes had after sample loading received 1-mL. water washes. For the chromatograms shown here, the wash volumes were increased from 1 mL to 3 mL. The extra wash volume helped, diminishing the number, concentration, and distribution of the components that had been retained on the conventional ODS packing. Nevertheless, in comparison with the SPS-ODS, and whether the comparison is against the 1-mL wash (Fig. 1, right) or the 3-mL wash (Fig. 2, right), the conventional ODS suffers, retaining in each case much more material than the SPS-ODS.

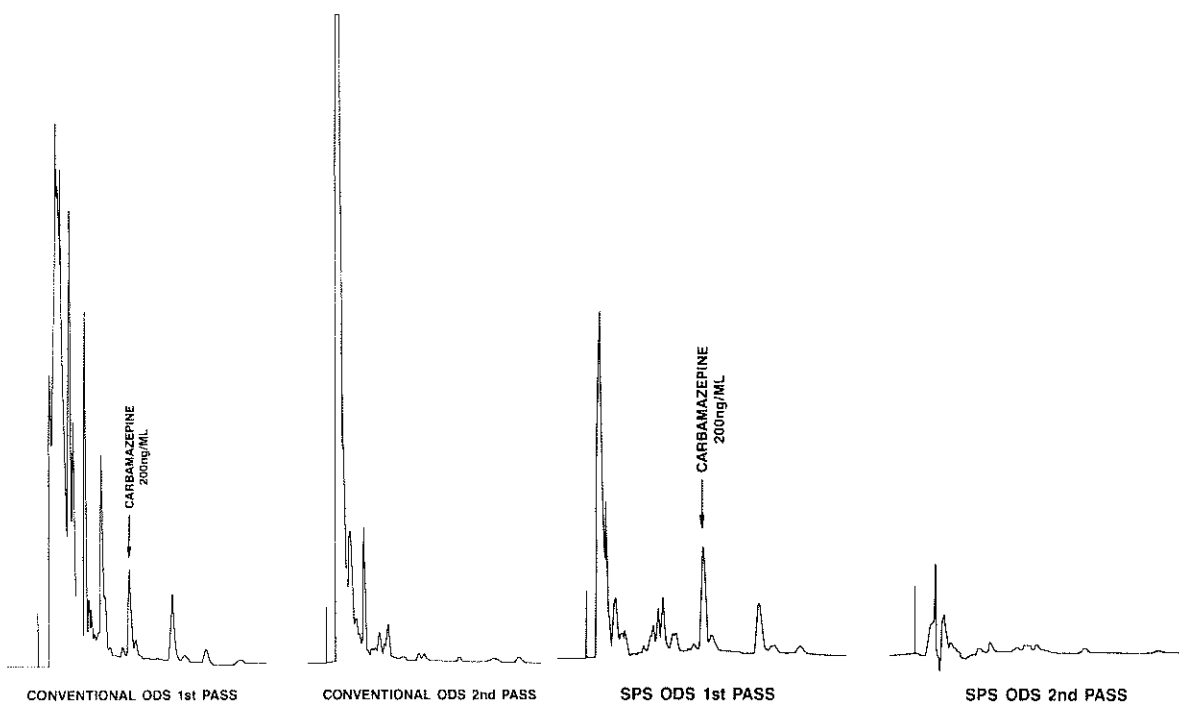


Figure 3. As was pointed out earlier, SPE tubes are supposed to extract some component of interest--here, carbamazepine, 200 ng/mL serum--and pass the undesirable components to waste. That was done here. Although the conventional ODS tube is shown to have extracted the carbamazepine (left chromatogram: CONVENTIONAL ODS 1st PASS), it did not separate the carbamazepine well from the accompanying serum proteins. Even when the SPE effluent was passed through a second SPE tube, many undesirables were still retained by the ODS, but could be then eluted (right chromatogram: CONVENTIONAL ODS 2nd PASS).

In welcome contrast, the SPS-ODS material not only extracted the carbamazepine but also retained far less of the undesirable serum proteins (left chromatogram : SPS ODS 1st PASS). Then, relatively few of the undesirables were retained when the SPS-ODS tube was washed a second time (right chromatogram: SPS ODS 2nd PASS).

Clearly, the uses of RAM packings can be expected to widen markedly.

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