Supplementary Notes

FlashPack: Fast and simple preparation of ultra-high performance capillary columns for LC-MS

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Supplementary note A

Alternative destabilization approaches

On par with the magnet bar assisted destabilization, cupola can be destroyed by simple tapping of the pressure bomb from outside. However, since the destabilization must be done continuously for the whole packing time (~2-3 taps/sec), manual destabilization is difficult and impractical for packing of long colums. The advantage of the manual destabilization is that it does not damage the sorbent and the sorbent volume can be reduced. Mechanical cupola destabilization can be also achieved by sonication (either of the sorbent vial or just the capillary), which is also likely to further improve the reproducibility of column packing [1].

A different approach is based on the reduction of interactions between sorbent particles, similar to reducing the strength of the mortar binding the bricks in the dome. This is achieved by using less polar solvents (than methanol) in the sorbent slurry, such as acetone or isopropanol/methanol mixtures.

These two alternative approaches can be easily implemented to increase the column packing rate. However, their efficiency and utility may be limited by sorbent chemistry and they both interfere

with self-assembling frit formation in pulled ESI emitter columns. Manual tapping of the pressure bomb and magnet-bar assisted mechanical destabilization of the capillary end do not have such limitations.

Supplementary Note B

High concentration sorbent suspension

The capillary column packing rate is proportional to the concentration of the sorbent slurry. Thus, maximum packing rate will be achieved at maximum sorbent concentrations. Sorbent suspension in methanol can be maintained only up to ~200 mg/ml with a small rotating magnet bar. At higher concentrations the sorbent is partially settled and there is a sorbent concentration gradient from the bottom of the vial to the top. Very high concentration packing is commonly achieved using a dense paste-like sorbent slurry. However it requires large amount of sorbent material and, more important, is of limited compatibility with the pressure bomb setup (**Manuscript Fig. 1a**): the mechanism of the packing process implies faster solvent than sorbent consumption which will lead to the sorbent slurry going dry during the packing. To address these issues we suggest to use a gravity-settled sorbent layer. It requires several times less material than the dense sorbent slurry: 50-100 mg is enough to produce the layer of super-concentrated (500-1000 mg/ml) sorbent suspension deep enough to keep the proximal capillary column end fully immersed and to allow magnet-bar assisted cupola destabilization. The sorbent layer is prepared by letting the sorbent particles passively sediment for 20-30 min after resuspension in an excessive volume of methanol. Only one of the tested sorbents - Triart C18 from YMC - did not settle from methanol, but stayed in the form of neutral buoyance flakes. For this sorbent we suggest other solvents than methanol for the suspension preparation, e.g. methanol/isopropanol mixture or acetone. The slowest magnet bar rotation speed is required to avoid sorbent resuspension through the solvent volume, which will

reduce local sorbent concentration next to the column entrance and, consequently, the packing rate.

The *FlashPack* method uses several times more sorbent material than the standard low concentration column packing method. To reduce the sorbent consumption we suggest reusing the sorbent suspension. A new batch of sorbent must be introduced regularly due to gradual sorbent grinding by the rotating magnet bar. In our experience a 50-100 mg sorbent vial is sufficient for packing dozens of capillary columns. Conical or round bottom vials can be used to reduce sorbent volume (**Supplementary Fig. S3c**). An added advantage is that the conical shape vial keeps the magnet bar at the center of the vial and in close contact with the capillary.

Reference

1. Godinho, J.M., et al., *Implementation of high slurry concentration and sonication to pack high-efficiency, meter-long capillary ultrahigh pressure liquid chromatography columns.* J Chromatogr A, 2016. **1462**: p. 165-9.