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WATER-ASSISTED TRAP FOCUSING FOR ULTRA-LARGE VOLUME INJECTION IN REVERSED-PHASE NANO-LIQUID CHROMATOGRAPHY-ELECTRON IONIZATION MASS-SPECTROMETRY

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Nano HPLC gradient elution is the separation method of choice in many emerging LC-MS applications. This success is due to the synergistic effect of nano HPLC flow rates and ionization efficiency. However, the good mass sensitivity of nano HPLC is diminished by the severe injection volume limitation. Solvent-based solute focusing of aqueous samples in short trap columns, operating in switching mode, can overcome this constraint. Nevertheless, if the injection volume is too large or when the sample is in organic solvents, solutes are poorly retained by the trap during injection, and volume overload can occur, leading to altered peak shapes and signal loss. We present an efficient, instrumental method which relies on water dilution to assist trap solute focusing. An Agilent 1290 Infinity II UHPLC pump was used to deliver 20 μL/min of water to assist the dilution and trap focusing of a 20 μL sample in organic solvent (CH_3OH or CH_3CN). Trap elution (Agilent AdvanceBio-Mapping trap column 0.3 x 5 mm x 2.7 μm) and chromatographic separation (Agilent Zorbax SB-C18 0.0.75 x 150 x 3.5 μm) were carried out with an Agilent 1100 series nano pump at a flow rate of 400 nL/min. Gradient elution was from 100% of solvent A (97% water:3% ACN, v/v) to 100% of solvent B (ACN) in 10 min. An Agilent 7010B QQQ mass detector was equipped with a LEI LC-MS interface set at 400°C. MS data were acquired in MRM and SCAN modes. A 100% water flow delivered by the pump was directed to a first tee-union (T1) (Figure 1) where it was split into two stream channels. At 20 μL/min, the selected split ratio generated two streams: (A) 16.5 μL/min and (B) 3.5 μL/min. The higher flow stream was directed to a second tee-union (T2), while the lower one passed through a six-port valve (V1) before reaching T2. The valve was equipped with a 20 μL sample loop. A sample, in organic solvent, was injected into the loop. Trap loading: V1 was switched and the sample was carried at 3.5 μL/min to T2 mixing tee. The two streams mixed inside T2 carrying the sample at 20 μL/min into the trap in an aqueous environment for an optimized sample focusing. The trap was connected to a second six-port valve (V2) for back-flushing operation. To evaluate the T2 dilution at different distances from the T-junction exit, the transport phenomena equations, which express the conservation of mass, and momentum of chemical species were

numerically solved within the COMSOL Multiphysics® environment. Under the hypothesis of isothermal, incompressible Newtonian fluids and laminar mixing flow, preliminary investigations allowed quantifying the influence of the parameters that affect the flow behavior. Moreover, due to the laminar nature of the flow rate, a microfluidic chip (Dolomite Inc, Royston, UK) was used instead of T2 to improve mixing of the two solvents when in trapping mode.

Once the sample was fully transferred and loaded into the trap, V2 was switched allowing the trap to be back-flushed by the nano HPLC solvent gradient at 400 nL/min in 10 min.

To demonstrate the feasibility of this approach, pesticide mixtures in organic solvent extract from a soil matrix was considered. Good results in term of peak width and chromatographic resolution were obtained. Limit of detections of 10 and 100 μg/Kg in MRM and full scan modes were achieved, respectively.

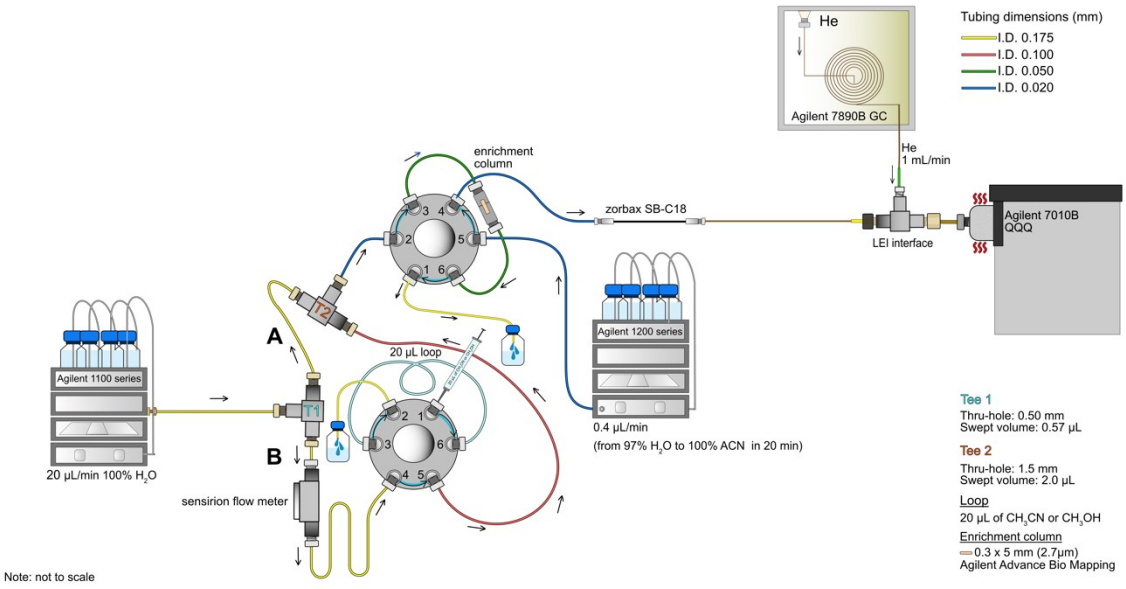

Figure 1.Overview of water assisted trap focusing system in loop loading position. A: higher flow rate; B: lower flow rate.