Easy solution for distorted peaks in HPLC methods of the European Pharmacopoeia (Ph. Eur.)

When working with stipulated methods sometimes issues with peak shape, efficiency and resolution may arise, especially for early eluting compounds. The most common reason for this is the use of a sample solvent of higher elution strength as the initial composition of the mobile phase, like pure methanol or DMF in a reversed phase separation. When these methods were developed or introduced to the Pharmacopoeia this solvent mismatch wasn't as important as today. Bigger dwell volumes of early HPLC systems have led to sufficient dilution of injection solvent before entering the column. Nowadays HPLC instruments have a minimized system volume and thus made way for insufficient mixing with the initial mobile phase leading to issues with irreproducibility and distorted peak shape.
But there is still a way around this without violating Pharmacopoeia guidelines.

If all sample constituents are soluble in your initial eluent composition you can use a custom sandwich injection method:

- draw a 2-5-fold excess of weak solvent (e.g. water or buffer in RP-LC) or initial mobile phase into the injector from an additional vial
- draw in your sample as specified in the Pharmacopoeia method
- draw another 2-5-fold excess of weak solvent or initial mobile phase
- wait for about 10-15 seconds before injecting everything

This adjustment doesn't change any chromatographic parameters and leads to a pre-injection dilution of sample and to an increase of the on-column focusing effect for the analytes due to isocratic compression.

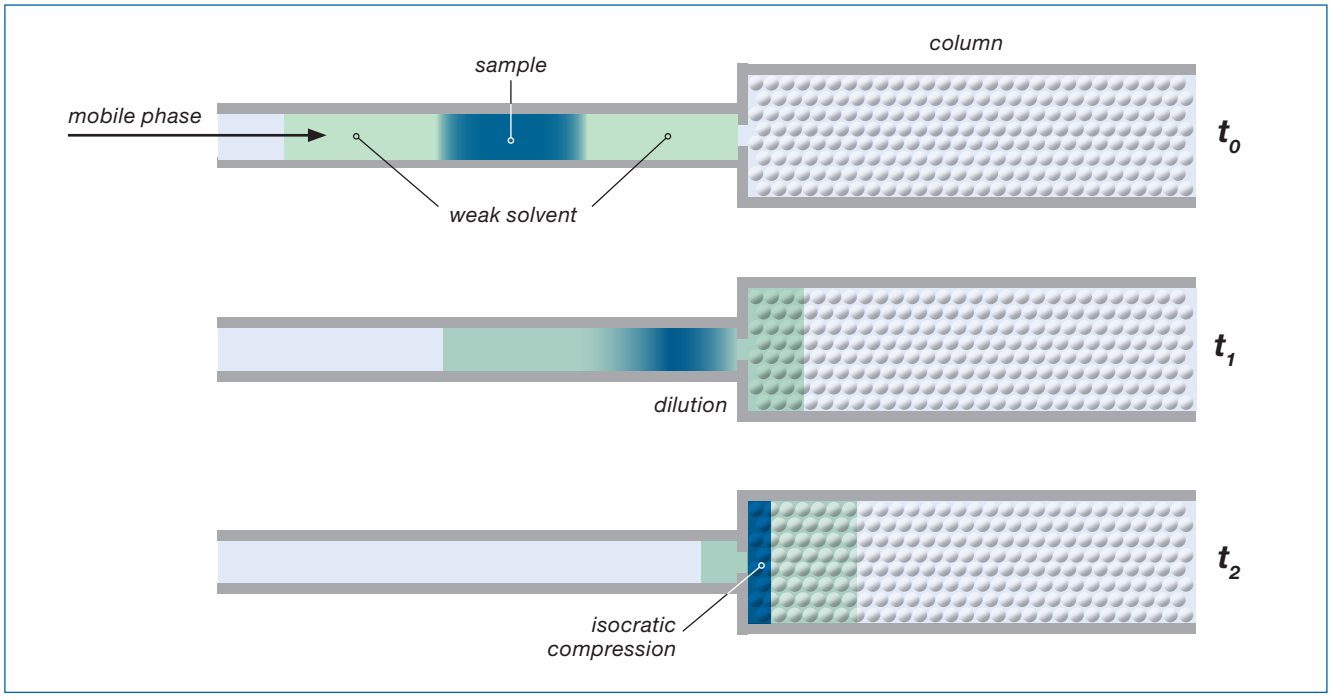

Figure 1: example of pre-injection dilution and isocratic compression of strong sample solvent in a sandwich injection method

In most software for modern HPLC instruments you either have the ability to easily program your sandwich injection method yourself or choose it from a list of preprogrammed methods already provided by the manufacturer.

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