Method kit for chromatography on normal phase (SiOH)

Introduction

Chromatography on normal phase (NP) (stationary phase: non modified silica, SiOH) is a general method for purification and separation of organic compounds. Prior to purification of a sample on NP, however, a mobile phase (eluent) with an appropriate polarity has to be prepared in order that a target compound can be well separated and isolated in high purity. In general the isocratic chromatographic purification of a sample on normal phase focuses on a single component in a given mixture. The adjustment of the optimal polarity of a binary organic mobile phase is done mixing a polar (strong eluent) and a non polar solvent in a certain ratio in order that the R_f value of the spot of the target compound on a TLC is approximately 0.3.
For the separation of more than one components in a mixture a gradient elution may be applied increasing the ratio of the strong eluent (polar component). However, in contrast to chromatography on reversed phase (modified stationary phase) the elution force of an eluent is not linear in respect to its content of the strong solvent in a binary mixture such as hexane / ethyl acetate [1]. Thus a non linear gradient has to used in order to increase the elution force in a linear way.

The method kit

The method kit for purification of samples on normal phase of HPLConsult comprises 3 elements:

1. A predefined set of 12 non linear binary and ternary gradients with heptane (hexane or cyclohexane) / ethyl acetate and methanol
2. 3 Reference mixtures *Ref1*, *Ref2* and *Ref3*
3. A TLC assignment scheme

The set of 12 non linear binary /ternary gradients is specific for a certain column dimension and it has to be elaborated empirically using an exponential formula [2] containing the following parameters: a) the total gradient time **T**, b) the starting conditions **K** (%B and %C) of the gradient and c) an exponential factor **a** for the exponential curve. Once the set of 12 gradients is done, it can be applied for any neutral organic sample using the assignment scheme with the references *Ref2* and *Ref3* as follows:

1. The mixture to be separated is spotted on two TLC plates together with a spot of *Ref2* and *Ref3* (*Fig. 1*).
2. One TLC plate is developed in EA/Hex 1:4 and the second TLC plate is developed in EE 100%. (In case of a very polar compound a 3rd TLC may be developed in EA/MeOH 9:1).
3. With the assignment scheme the optimal gradient can be seen easily assigning the corresponding optimized exponential gradient method (*Fig. 2-5*).

Ref1 can be used for the simple and fast qualification of the column and the LC system in general: When the aspect of the test chromatogram is ok, then the gradient pump is working correctly and the column is ok as well.

Example

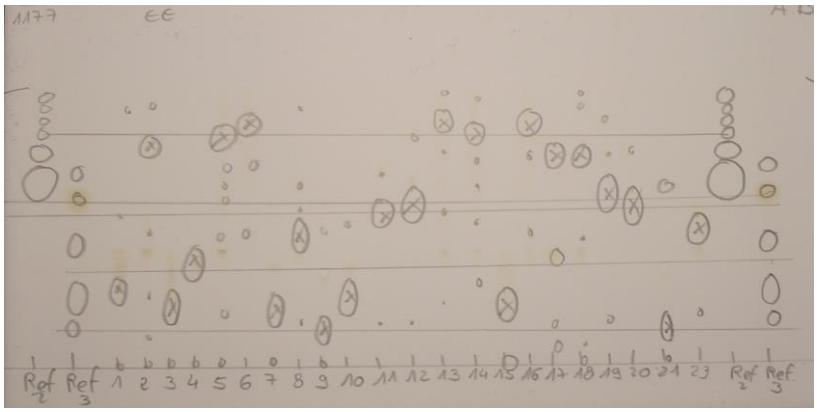

Fig. 1 *TLC: parallel method assignment for 23 samples*

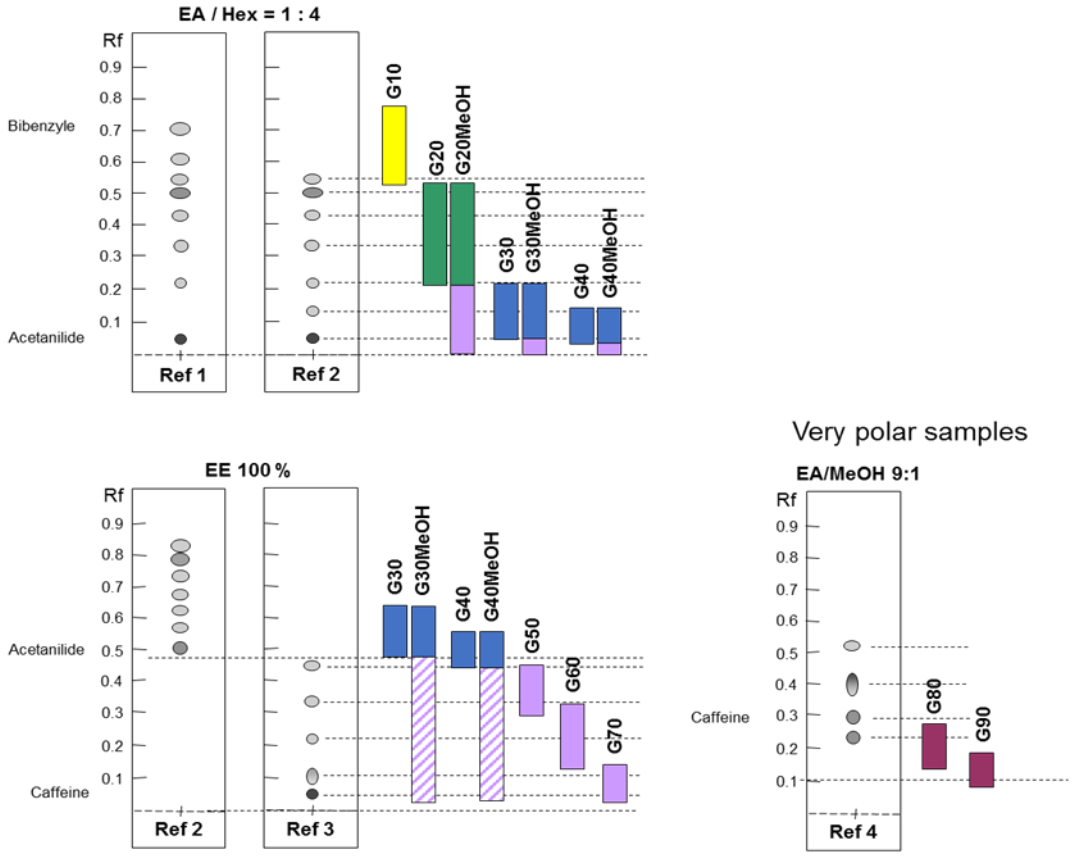

Fig. 2 *TLC Assignment scheme*

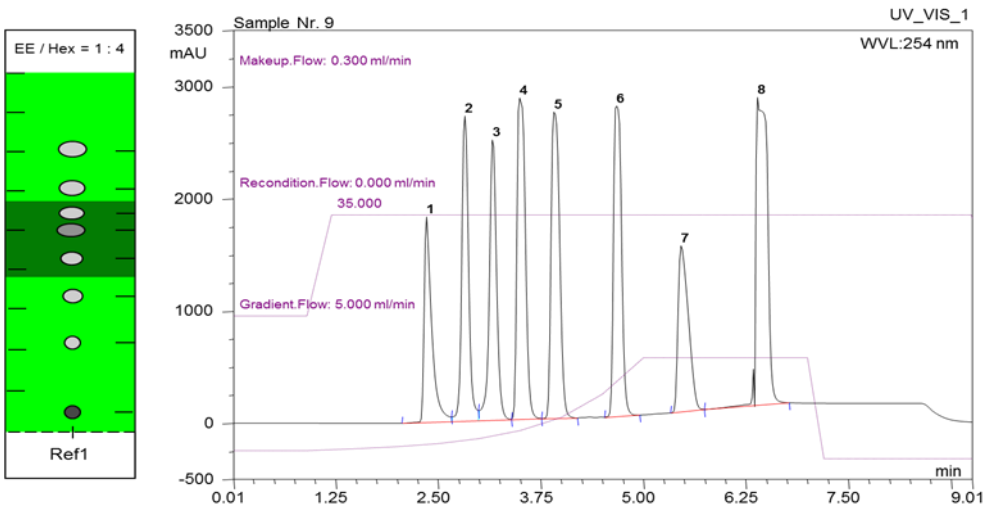

Fig. 3 *Separation with medium polar focus for Ref1: gradient G20*

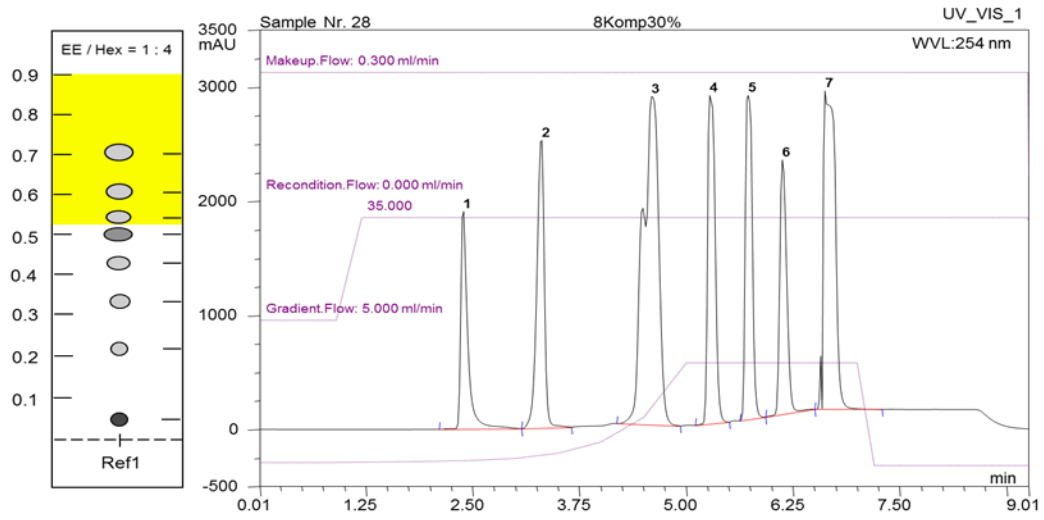

Fig. 4 *Separation with apolar focus for Ref1: gradient G10*

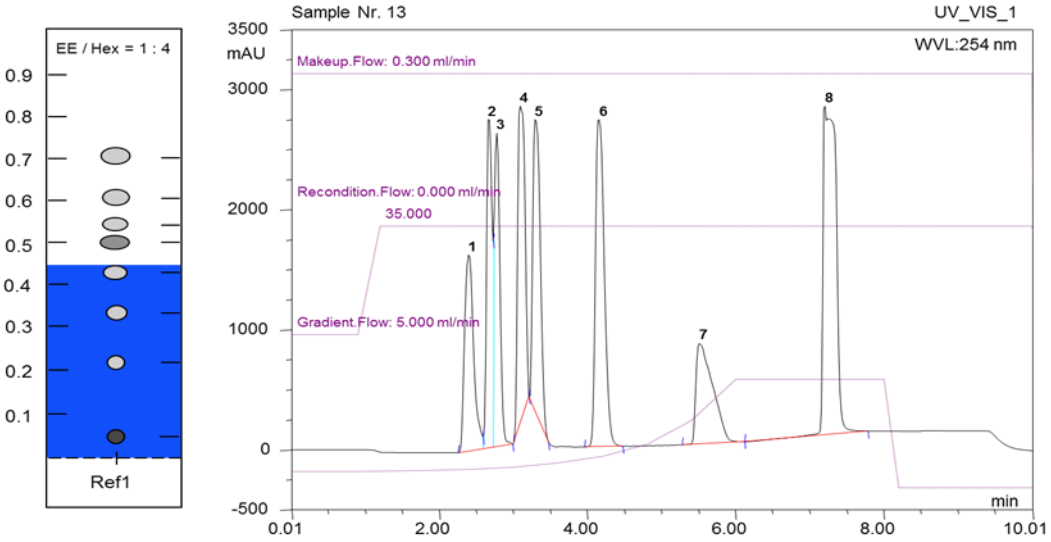

Fig. 5 *Separation with polar focus for Ref1: gradient g30*

Literature:

[1] V. R. Meyer, 'Praxis der Hochleistungs-Flüssigchromatographie', Sauerländer, Aarau, 1999.
[2] P. Renold, E. Madero, T. Maetzke, *J. Chromatography A*, 908, (2001), 143.

November 2007