

Comparing retention in xanthenes separation between a silica and titania stationary phase in HPLC

Agnieszka PIWIEN

Introduction

The Objective of analytical chemistry is to determine chemical compositions.

Nowadays, it mostly relies on instrumental methods. Research

focuses on discovering new materials and chromatography principles.

Columns with a silica layer are currently being used as a stationary phase in high performance liquid chromatography (HPLC). Owing to their low mechanical, thermal and pH stability the scientists are forced to investigate the different column supports. Therefore, the study on titanium support column was performed on the grounds of its strong Lewis acid sites probably resistant to changes of pH.

The aim of these experiments was to compare the effectiveness of these two columns.

Experimental methods

First of all the xanthenes and their derivatives were analyzed in the silica column Aquasil (50mm x 2,1mm I.D., 1,9 μ m) in RPLC mode then in the titanium dioxide column Sachtopore-NP (100mm x 2,1mm I.D., 5 μ m) in HILIC mode. The Titania column was also studied under the influence of a mobile phase with and without a buffer solution.

All solutions were prepared using deionized water (Elga LAB WATER) and acetonitrile in a 1:1 (v/v) ratio. Uracile and naphthalene were respectively used as dead time markers, the former in the RP mode and the latter in HILIC mode (Hydrophilic interaction liquid chromatography). Seven derivatives of xanthine: caffeine, theophylline, theobromine, 7 β - hydroxypropyltheophylline, β - hydroxyethyltheophylline, 1,7- dimethylxanthine and diprophylline were tested.

Xanthenes were separated on an Agilent chromatography system with an autosampler. Injections were made in different volumes (1-7 μ L) and the mobile phase flow rate was set to 200 μ L/min. UV detection was carried at 254nm. Separation parameters such as: the retention time tR, the number of theoretical plates- N and the asymmetry- As were recorded.

Results and discussion

The difference between xanthine separation on the Aquasil column compared to that on the Sachtopore column is illustrated in fig.1 and 2. The silica Aquasil column did not separate theophylline from 1,7- dimethylxanthine (Fig1.).

HILIC method used with a Sachtopore titanium column turned round the succession of the xanthenes' retention. Fig.2. represents the capability of the titania column to separate all analyzed compounds.

Adsorption phenomenon can be observed on Fig.1, otherwise Fig.2 describes the partition phenomenon.

Conclusion

The different parameters were studied for these two columns and then compared. TiO₂ seems to be a better stationary phase than silica for this application: the separation on titania stationary phase was more effective.

Fig.1. Dependence of retention factor $\ln k$ to % of ACN in mobile phase in Aquasil column, conditions: flow rate 200 μ L/min, ACN-Water (v/v)

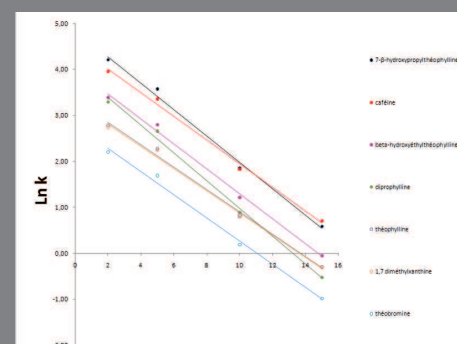
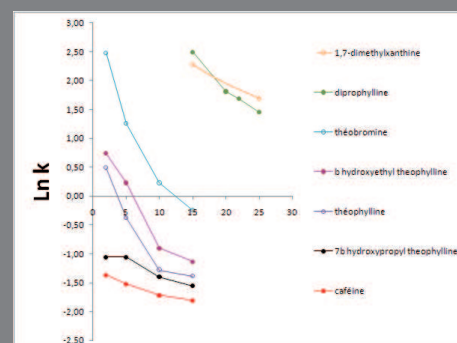


Fig.2. Dependence of retention factor $\ln k$ to % of water in mobile phase in Sachtopore column, conditions: flow rate 200 μ L/min, ACN-Water (v/v)



LSA – Bâtiment CPE
Master Analyse et Contrôle
Université Claude Bernard – Lyon 1