



Flash chromatography on cartridges for the separation of plant extracts - Rules for the selection of chromatographic conditions, and comparison with MPLC

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Introduction

During the last decade, several systems for rapid preparative chromatography with pre-packed cartridges have been commercialized. Pre-packed cartridges ensure rapid separation cycles and ease of use.

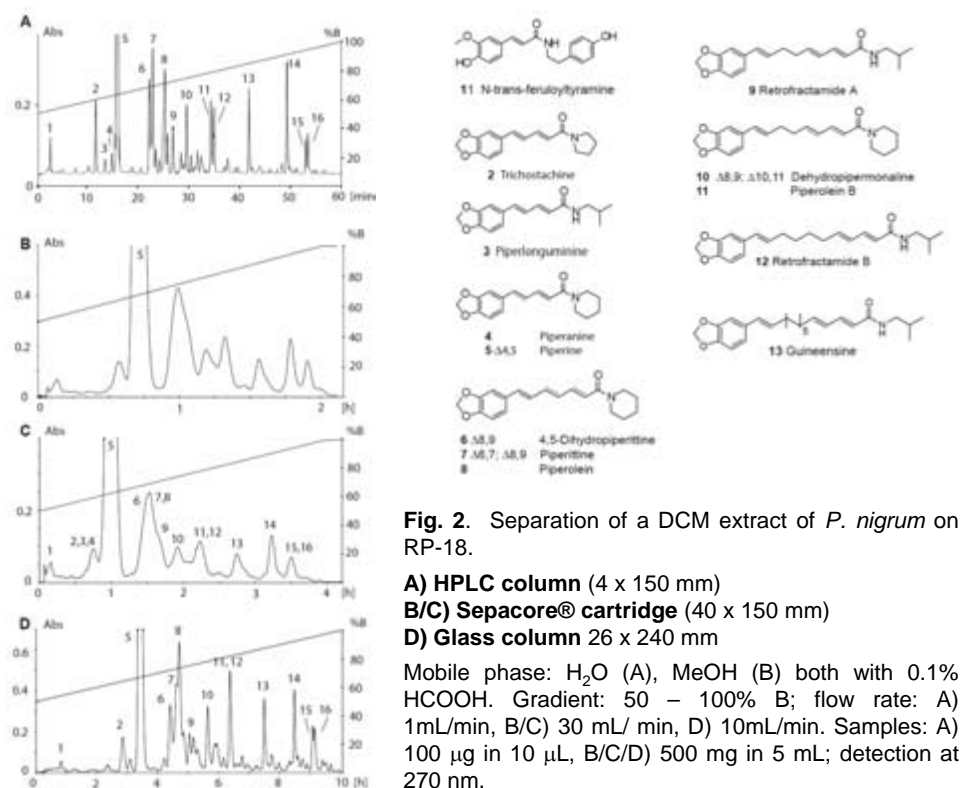
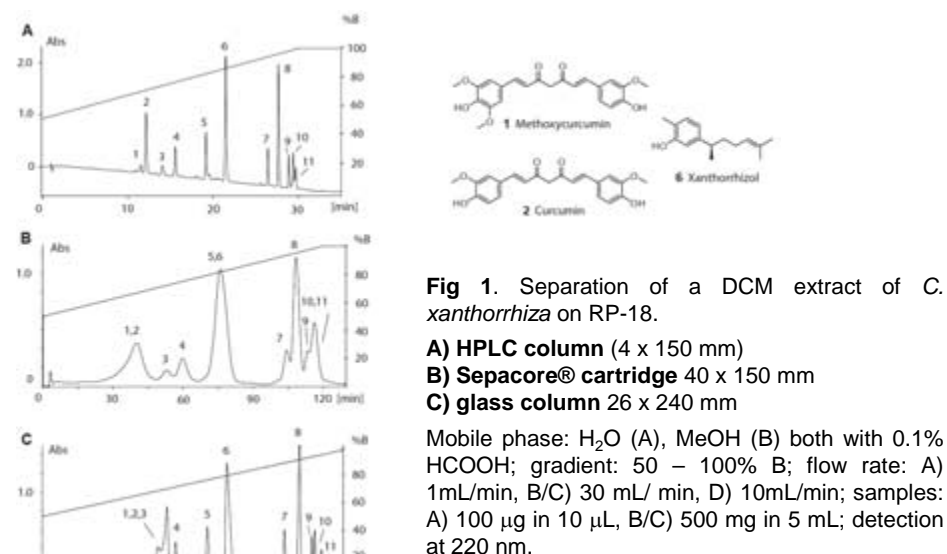
Flash chromatography systems were initially developed for rapid and easy purification of synthetic products, and numerous applications are documented in the experimental section of publications in synthetic chemistry. In contrast, application of such systems in the separation of complex natural product mixtures such as extracts has been neglected.

The aim of this work was to explore the potential and limitations of cartridges for the purification of natural product extracts. Empirical rules have been established for the determination of the separation conditions by preliminary TLC and HPLC analyses. The influence of solid introduction compared to liquid injection has been also investigated. The performance of the cartridges was compared to that of classical self-packed MPLC glass columns for the separation of complex plant extracts of medicinal importance.

Results

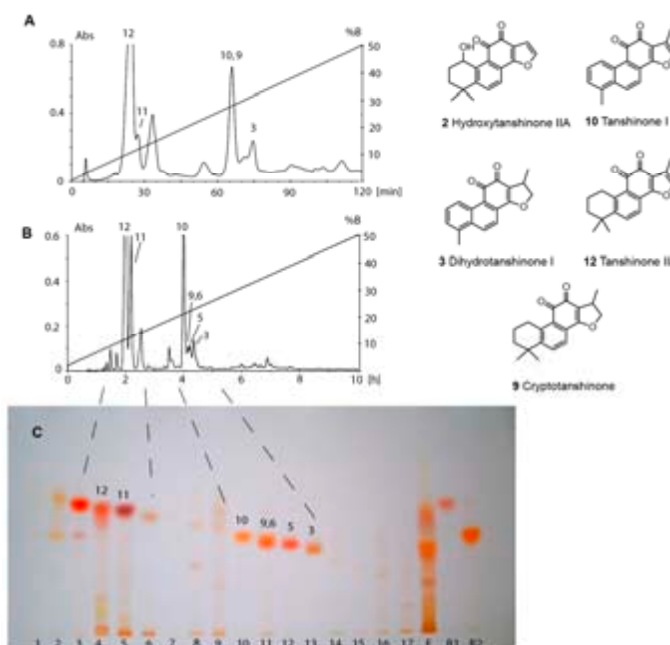
Correlation between HPLC and reversed phase flash chromatography

In preliminary experiments with reference compounds, a correlation was found between the capacity factors k' on the HPLC column and Sepacore cartridges ($k'_{\text{Sepacore}} = 1.15 \times k'_{\text{HPLC}}$; $R^2 = 0.97$). HPLC separations can be transposed by increasing the gradient time by a factor 2-4. These rules have been applied to the separation of dichloromethane extracts of *Curcuma xanthorrhiza* and *Piper nigrum*.

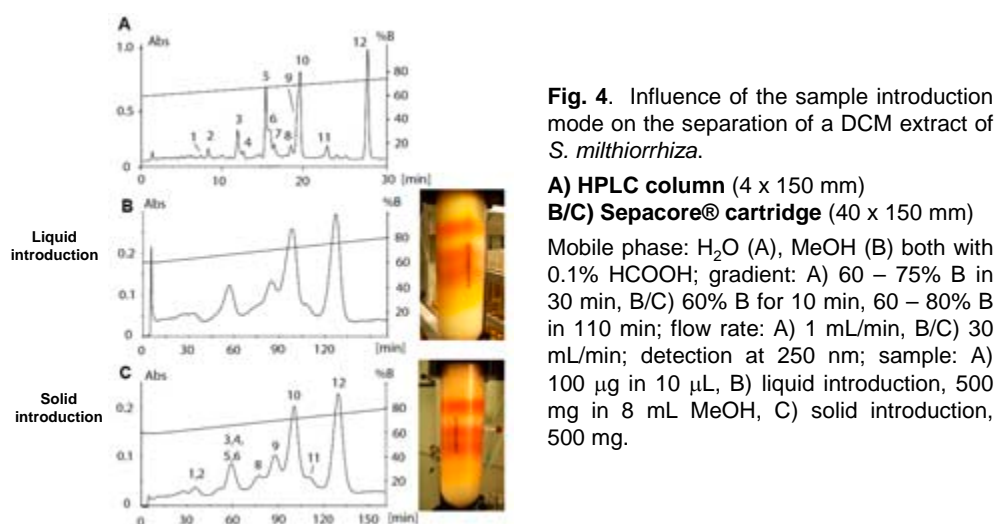


Transposition from TLC to flash chromatography on silica gel

In preliminary experiments, a correlation was found between $1/R_f$ and k' values ($k' = 1.1 \times 1/R_f$; $R^2 = 0.90$). Mobile phase compositions for starting and end point of a gradient should be selected such as to obtain R_f values of 0.15-0.2 for the most lipophilic component and the most hydrophilic constituent, respectively. This principle has been applied to the separation of a dichloromethane extract of *Salvia miltiorrhiza*.



Influence of the mode of sample introduction



Conclusions

- Reversed phase HPLC separations can be transposed by increasing the gradient time by a factor 2-4.
- For normal phase separations, solvent compositions resulting in R_f values of 0.15-0.2 on TLC for the most lipophilic and the most hydrophilic constituents, respectively, should be selected as gradient endpoints.
- Sepacore® cartridges enabled a good separation of compounds with a broad range of polarity, as typically found in plant extracts. The chromatographic resolution remained, however, lower than that achieved by MPLC on columns packed with material of smaller particle size. For poorly soluble extracts, solid introduction gave better results than liquid injection.
- Despite lower resolution as compared to MPLC, pre-packed cartridges are an attractive alternative for the purification of extracts and crude fractions due to their ease of use and speed of separation.

Instrumentation

Preparative separations were performed on a Sepacore® chromatography system (Büchi Labortechnik) consisting of two C 605 pump modules, a C 620 control unit, a C 635 UV detector and a C 660 fraction collector. The system was controlled by the software SepacoreControl 1.0. Flash chromatography separations were performed on pre-packed silica gel (40 – 63 µm) and RP18ec (40 – 63 µm) polypropylene cartridges (12 x 150 or 40 x 150 mm, Büchi) at a flow rate of 10 mL (12 x 150 mm cartridges) and 30 mL/min (40 x 150 mm cartridges), resp. Medium pressure liquid chromatography (MPLC) separations were carried out on a glass column (26 x 460 mm) packed with silica gel Si60 (15 – 40 µm) or LiChroprep RP18 (25 – 40 µm) (Merck), at a flow rate of 10 mL/min. Liquid injection was carried out through a 6-way valve with a 20 ml loop. Solid introduction was performed by means of a PrepElut cartridge (Flash chromatography) or a glass precolumn (MPLC) connected to the top of the cartridge or the column, resp. The samples were adsorbed to silica gel Si60 or LiChroprep RP-18, resp., prior to introduction.