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# Automated normal-phase preparative high-performance liquid chromatography as a substitute for flash chromatography in the synthetic research laboratory

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**Abstract**

An automated normal-phase preparative HPLC system was developed in order to omit time-consuming flash column chromatography in the synthetic research laboratory. The system is equipped with steel columns packed with spherical 12 μm silica and is able to separate samples in a range of 0.1–10 g depending on the column diameter and chromatographic problem. It was designed to be used as an open access instrument in the research department. The general users select from binary gradient programs after running an analytical TLC with the raw product. The HPLC instrument was fully controlled by the Chromeleon software from Dionex. A Gilson 215 robot served as injector/collector. 

*Keywords:* Preparative chromatography; Automation

## 1. Introduction

Separation of reaction mixtures or purification of main products of chemical transformation is a challenging part of a chemists everyday work that is often more time-consuming than the previous setting up of the chemical reaction. The most common purification method in the research laboratory besides crystallisation and distillation is flash chromatography [1]. This absorption chromatography technique allows the purification of reaction products on silica with organic solvents as eluents. Flash chromatography is widely used but the procedure starting with setting up and packing of the column, choosing the appropriate solvent system, collecting fractions and tracking compounds in the collected fractions is time-consuming. Automated fraction collectors [2] or detection systems [3] help to save labour.

We report in this paper an alternative separation method based on a new generation of a fully automated preparative normal-phase HPLC system. Several automated HPLC systems for laboratory scale separation were described in the literature [4–6]. A wide range of equipment has been developed for the purification of libraries in combinatorial chemistry [7,8].

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The main goal of our development was to design a simple to use system which would allow every chemist in the department to purify reaction mixtures (0.1–10 g) on a hands-on instrument previously done by flash chromatography. After running an analytical TLC the user should be able to select an appropriate gradient program and to start the automated HPLC system in order to separate the reaction mixture.

## 2. Materials and methods

### *2.1. HPLC instrumentation*

Preparative HPLC separations were performed using a Varian Prostar 215 binary high-pressure gradient system controlled through the Dionex Chromeleon software package. A Varian Prostar 320 UV–Vis detector equipment with a dual pathlength flowcell (4 mm/0.15 mm) was used to acquire UV signals at 254 nm.

A Gilson 215 robotic system served as injector and fraction collector. The Gilson robotic system was fully controlled by the Dionex Chromeleon software. The Gilson 215 has been slightly modified by our mechanical engineering department to hold standard 50 mL tubes in removable racks for fraction collection and 8 mL or 20 mL vials in the injector block. Sample injection in a stainless steel loop (10 and 20 mL) was controlled by a Gilson 819 high-pressure valve actuator.

### *2.2. HPLC conditions*

Separation was achieved using silica LiChrospher Si60 12 μm from Merck. The silica was packed in stainless steel columns with a length of 125 and 25 mm or 50 mm I.D. Packing of the columns was performed with a silica slurry in methanol on a Merck packing stand at a pressure of 40 bar.

Hexane and ethyl acetate (Prepsolve, Merck) was used for the binary exponential gradients described in the discussion part of this paper. Solvent barrels (30 L) were directly connected to the pumping system via a gastight PTFE tubing. The flow-rate for the 25 mm I.D. column was 30 mL/min and for the 50 mm I.D. column 80 mL/min. The solvent waste was collected in a 70 L steel tank equipped with a level monitoring system.

### *2.3. Sample preparation*

Raw products from the research laboratories were dissolved in methylene chloride (100–600 mg in 8 mL and >600 mg in 20 mL) and filtered through a silica plug or a Chromafix snapcap (Macherey–Nagel) to remove polar compounds. The result of the prepurification was checked by TLC and if necessary the filtration was repeated. TLC was performed with Polygram SIL G/254 (Machery–Nagel) in ethyl acetate–hexane (1:4) as eluent.

Selection of the appropriate HPLC gradient method was done after characterisation of the polarity range of the separation problem. The polarity range was detected in comparison with a mixture of eight compounds with different retentions in the described TLC system. The organic compounds were acetanilide, benzyl alcohol, dimethyl phthalate, diethyl phthalate, acetophenone, dibutyl phthalate, ethyl benzoate and 1,2-diphenylethane. All compounds (analytical grade) were purchased from Fluka (Buchs, Switzerland). A solution in ethyl acetate containing 1.25% of each of the reference compounds was used for the analytical TLC.

## 3. Results and discussion

### *3.1. Equipment*

Before setting up a hands-on preparative HPLC system that handled the separations previously done by flash column chromatography it was necessary to study the needs of the research department and to set the scope and limitations of such an instrument. It was very clear that only a robust and easy to use system would lead to general acceptance. Using the Chromeleon software package from Dionex we were able to combine the best suited HPLC hardware from different manufacturers for our needs. Chromeleon allows the control of every step of the automated system. On the other hand, the easy to handle sample table of the software made the HPLC system accessible to every user only after a short briefing. The chemist supplies the system with sample information

and total volume of the sample solution. He chooses in a pull down menu the appropriate gradient program and then places the sample vials in a designed position in the injector rack. He leaves the system and comes back when the separation is finished to take out his fraction tubes from the collector as stated in a print out providing him with all the chromatographic details of the separation. The system operator is responsible for the instrument conditions and checks the columns by running a test mixture from time to time.

The fraction collector is placed in a fume hood. All tubing is stainless steel and grounded to prevent electrostatic charge. The software controls the pump pressure online. A sudden drop of pressure indicating a leak in the system or the rise of pressure above a defined value stops the run immediately.

### 3.2. Choosing the gradient system

In organic synthesis reactions were mostly monitored by TLC. This fast and very easy method gives information on the grade of transformation of a reaction and shows the amount of side products or remaining starting materials in the reaction mixture as a rough estimate. It also gives the chemist information on the polarity range of the chromatographic separation he has to deal with after working up the reaction.

We used the information from the TLC system to decide which gradient program on the automated preparative HPLC system has to be chosen to purify the raw material from the reaction.

A mixture of eight organic compounds with different polarity and $R_F$ values (0.05–0.75) in a defined TLC system was our reference system and helped to select the appropriate HPLC method.

It has been distinguished between three polarity ranges. Separation problems in a TLC $R_F$ range between 0.05 and 0.25 were solved by a polar HPLC method. For $R_F$ values from 0.25 to 0.6 a standard and from 0.6 to 0.85 a apolar HPLC gradient

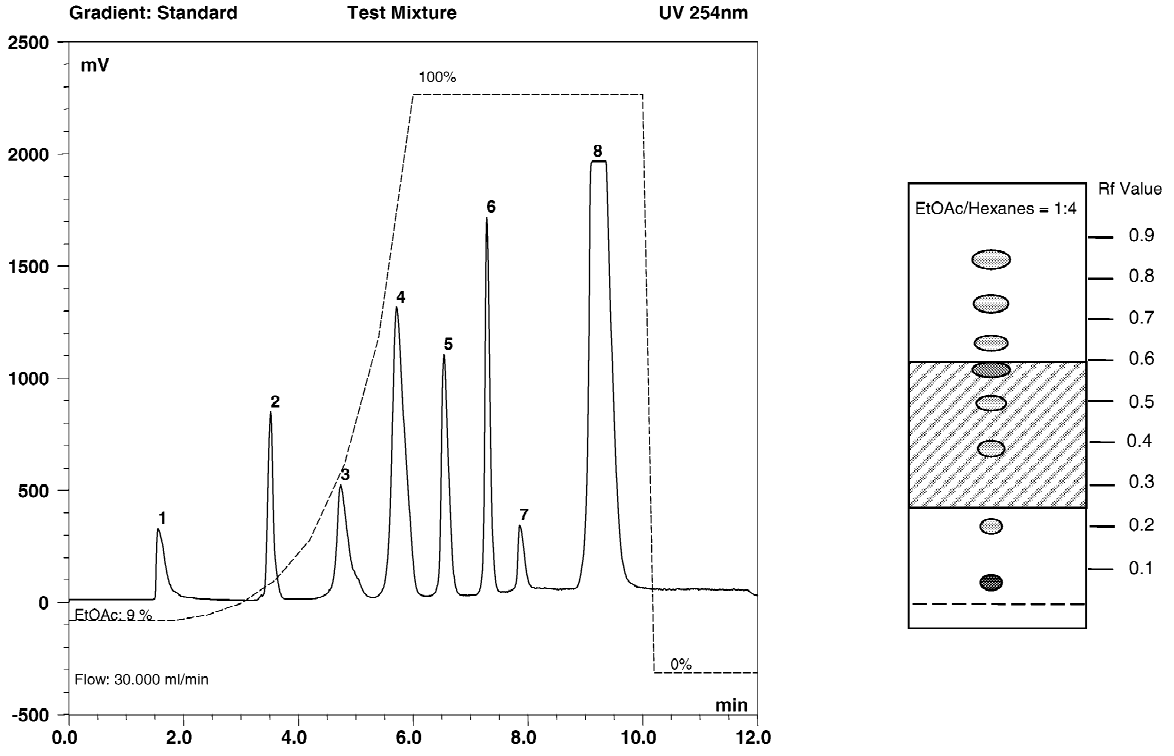


Fig. 1. Standard gradient used for separation problems in the $R_F$ range of 0.25 and 0.6 in the analytical TLC. Gradient (-----). $a=6$; $K=0.09$; $T=6$,(see definitions in caption to Fig. 4.) Chromatogram of the preparative HPLC separation of 400 mg of test mixture.

program was used (Figs. 1–3). If the separation problem of the chemist was in a $R_F$ range >0.85 or <0.05 in the defined TLC system the separation could not be performed by the hands-on HPLC instrument.

The HPLC programs beyond the three methods were exponential binary gradients of ethyl acetate and hexane (see Fig. 4) specially developed to spread peaks in the focused separation range. In Figs. 1–3 the chromatograms of a preparative separation of the eight component reference mixture is shown. The ethyl acetate concentration during the gradient programs was plotted on the chromatograms. The calculation of the time dependent ethyl acetate concentration of the gradients was done according to the equation shown in Fig. 4. The start concentration and the time at 100% ethyl acetate of the polar gradient were selected in a way to ensure that polar compounds have been eluted completely from the column.

### 3.3. Sample preparation and chromatography

We run the preparative HPLC system with organic solvent gradients on silica. The evaporation of the solvents in the collected fractions is much easier compared to acetonitile water mixtures used in reversed-phase chromatography. The disadvantage of normal-phase chromatography is the necessary pretreatment of the samples being purified on a HPLC system. Reaction mixtures tend to contain very polar impurities that would destroy columns if not removed prior to injection. We therefore filter the mixtures through small silica plugs (Figs. 5 and 6) containing 200, 400 or 900 mg of silica to remove polar impurities. Larger samples and samples containing larger amounts of polar substances were filtered through frits containing standard flash chromatography silica. The filtered samples were diluted with methylene chloride to a total volume of 8 mL for samples with a total mass lower than 600 mg

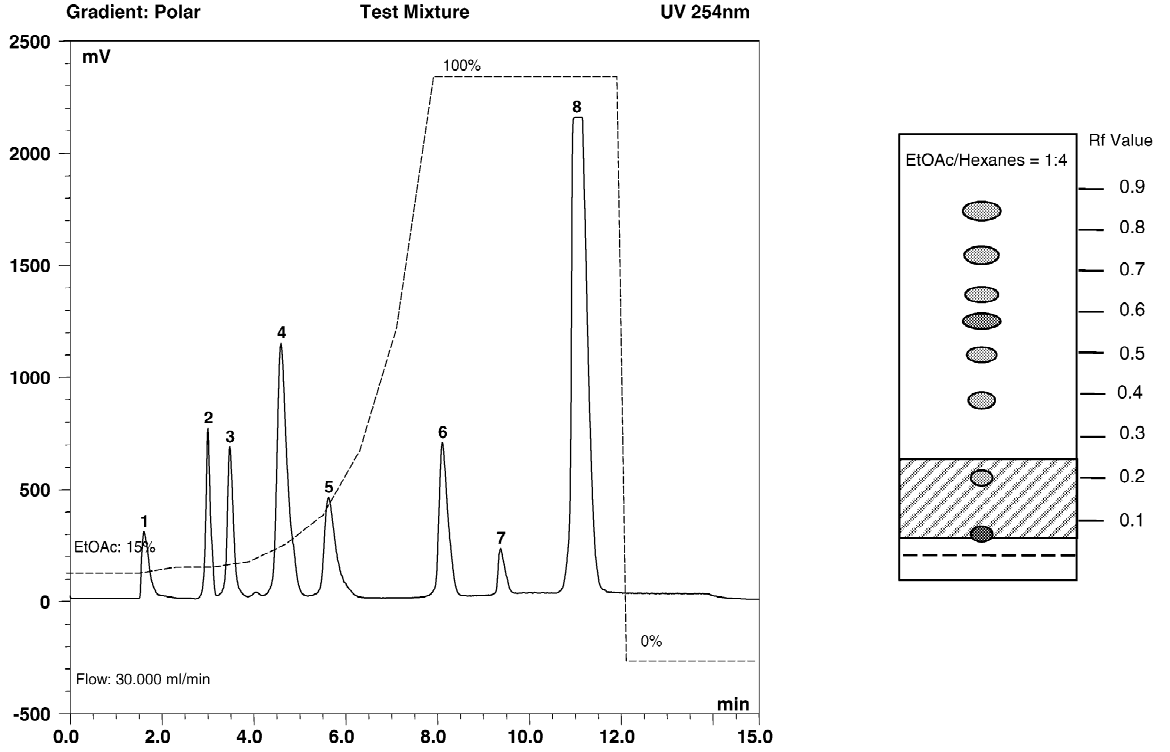


Fig. 2. Polar gradient used for separation problems in the $R_F$ range of 0.05 and 0.25 in the analytical TLC. Gradient (-----). $a=7$; $K=0.15$; $T=8$. Chromatogram of the preparative HPLC separation of 400 mg of test mixture.

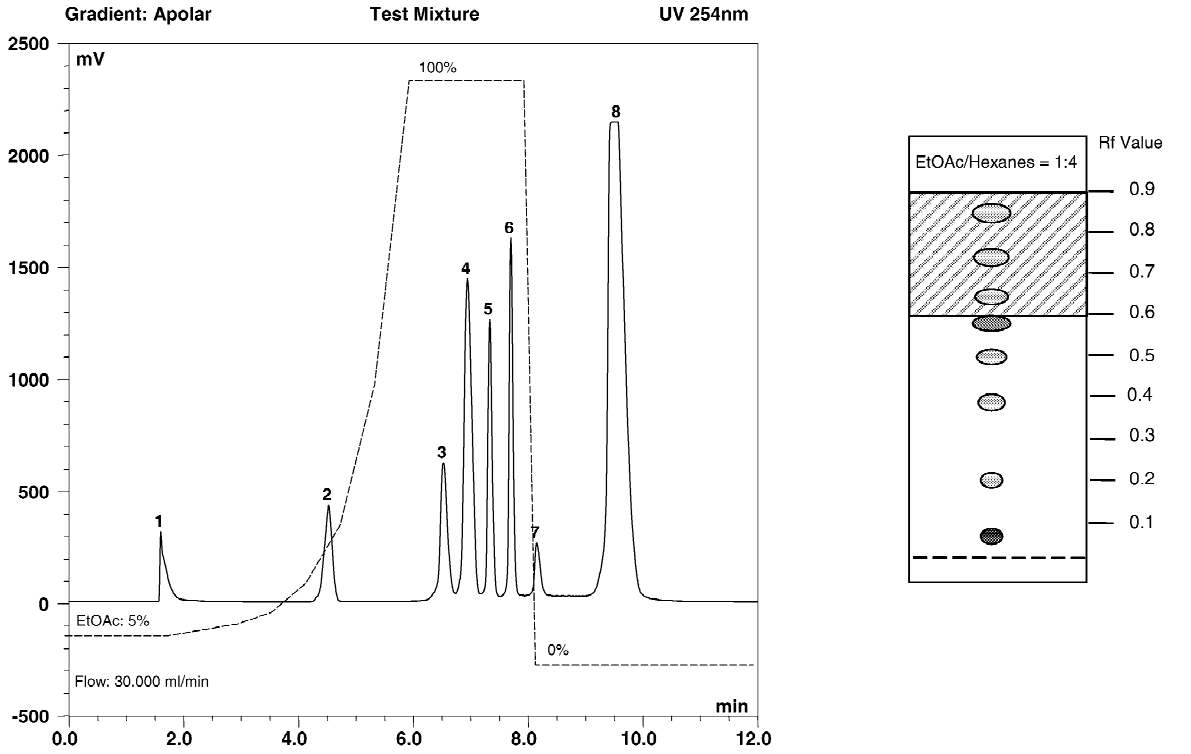


Fig. 3. Apolar gradient used for separation problems in the $R_F$ range of 0.6–0.9 in the analytical TLC. Gradient (-----). $a=8$; $K=0.05$; $T=6$. Chromatogram of the preparative HPLC separation of 400 mg of test mixture.

using the 25 mm I.D. column or to a total volume of 20 mL if separating larger amounts on a 50 mm I.D. column.

The sample vials were placed at a designated position in the injector part of the Gilson 215 robot (Fig. 7). The software then started the injection process and the separation took place. If a defined level of the absorption signal and a certain slope was reached the Gilson started to collect. If no peak was detected the eluent was not collected and transferred directly to the solvent waste container. The pumping system was controlled by the Chromeleon software as it was running the selected gradient program. After finishing the program the HPLC system stopped or worked out the next sample from the sample table.

$$c(\text{EtOAc}) = \frac{(e^{(a*t/T)}-1)*(1-K)}{e^{a}-1} + K$$

$$c(\text{hexanes}) = 1 - c(\text{EtOAc})$$

Fig. 4. Definition of the binary gradient: $T$=length of gradient (min); $K$=c(EtOAc) at $t=0$; $a$=exponential gradient parameter; $t$=runtime.

## 4. Conclusions

The replacement of flash chromatography separations by preparative HPLC in the synthetic research laboratories lead to an increase of productivity. The easy to use hands-on instruments are well established in the department. Nevertheless control of the pre-treatment of the samples and of the column condition in general needed some supervision of the instruments.

In addition to the six fully equipped normal-phase

Fig. 5. Reaction mixture dissolved in methylene chloride.

Fig. 7. Vial placed in designated sample position in the injector.

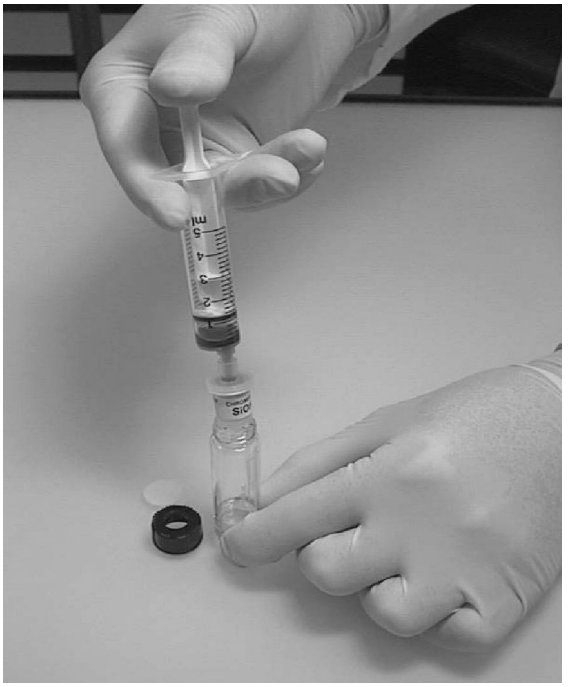

Fig. 6. Filtration of the reaction mixture through a silica plug into a standard vial.

HPLC systems of the department it is planned to run preparative separation with the same instrumentation but on modified phases to fulfil special needs of the chemists.

## References

[1] W.C. Still, M. Kahn, A. Mitra, J. Org. Chem. 43 (1978) 2923.
[2] J. Krohn, F. Verillon, Am. Lab. 18 (1986) 49.
[3] S.G. Westerbuhr, K.L. Rowlen, J. Chromatogr. A 886 (2000) 9.
[4] F. Vérillon, M.-F. Claverie, Lab. Rob. Autom. 3 (1991) 241.
[5] J.L. DiCesare, F.L. Vandemark, Ind. Res. Dev. 24 (1982) 138.
[6] F. Mellor, R. God, K.U. Bindseil, H. Gumm, GIT Spez. Chromatogr. 17 (1997) 19.
[7] L. Zeng, X. Wang, T. Wang, D.B. Kassel, Comb. Chem. High Throughput Screening 1 (1998) 101.
[8] L. Zeng, L. Burton, K. Yung, B. Shushan, D.B. Kassel, J. Chromatogr. A 794 (1998) 3.