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## SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

### SELECTIVE SEPARATION SYSTEM FOR THE QUANTITATIVE DETERMINATION OF ISOMERIC BENZOPYRENES AND OF CORONENE

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#### SUMMARY

Thin-layer chromatographic investigations on cellulose acetate sheets and polyamide plastic sheets show that both systems permit the selective separation of some polycyclic aromatic hydrocarbons. These results could be confirmed on columns under conditions of high-pressure liquid chromatography. The selective determination of coronene was successful on cellulose acetate columns. Isomeric benz(*a*)pyrene and benz(*e*)pyrene could be separated on polyamide columns.

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#### INTRODUCTION

Recently, the advantages of high-pressure liquid chromatography (HPLC) have been discussed with regard to the separation and quantitative determination of substances with a high melting-point<sup>1,2</sup>. The use of this method could facilitate the quantitative determination of polycyclic aromatic hydrocarbons (PAHs). However, the separation efficiency of the columns applied is not yet adequate<sup>3</sup> in HPLC to achieve the almost complete separation of all PAHs from a complex mixture.

Therefore, the development of selective separation systems seems to be more promising at the present time. A group separation of PAH compounds of about the same ring size on Sephadex columns, as described previously<sup>4</sup>, could enable the quantitative determination of the individual substances of this group to be achieved following a precise separation. First experiments which enabled the separation of isomeric benzpyrenes were carried out with cellulose acetate columns<sup>5</sup>. In screening tests, we used thin-layer chromatography (TLC) on cellulose acetate and polyamide sheets, and these systems were subsequently transferred to HPLC. The selective separation of coronene on a cellulose acetate column as well as the separation of isomeric PAHs on polyamides could be developed.

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## MATERIALS AND METHODS

TLC separations were carried out on polygram sheets of polyamide-DC 6 and 30% acetylated cellulose MN 300 (Macherey, Nagel & Co., Düren, G.F.R.). Cellulose acetate MN 300 (30%) was subjected to a wet sieving procedure, and one fraction with sieve mesh sizes of 50–60  $\mu\text{m}$  was isolated. Polyamide-6 HPLC-Sorb (Macherey, Nagel & Co., Düren, G.F.R.), 20–32  $\mu\text{m}$ , was used as the column support.

All TLC investigations were carried out in saturated N-chambers. A 0.2- $\mu\text{l}$  volume of an acetone solution containing 0.3  $\mu\text{g}/\mu\text{l}$  of one PAH was placed on polyamide or cellulose acetate sheets. For HPLC, separations were carried out in a Universal liquid chromatograph, UFC 1000 (Hupe & Busch, Groetzingen, G.F.R.), with a PMQ II UV detector (Zeiss, Oberkochen, G.F.R.) and an 8- $\mu\text{l}$  micro-flow cell. Columns were packed according to the balanced-suspension procedure<sup>6</sup> by means of an Orlita 3  $\times$  dosage-membrane pump, Type M3S 4/4-4-4 (Orlita, Giessen, G.F.R.). Polyamide powder suspended in methanol and cellulose acetate material suspended in methanol-carbon tetrachloride was then packed into the columns under high pressure. The columns were then immediately washed with the respective solvents as stated in the tables.

## RESULTS AND DISCUSSION

*Determination of coronene on cellulose acetate columns*

The TLC investigations of some PAHs on cellulose acetate sheets showed (Table I) that coronene has a relatively high  $R_F$  value. The  $R_F$  value is near to that of PAHs with 3–4 rings. As coronene could be separated from these lower condensed PAHs on alumina or silica gel columns without any problems, there is the possibility of a subsequent precise separation of more highly condensed PAHs.

TABLE I

$R_F$  VALUES OF SOME POLYCYCLIC AROMATIC HYDROCARBONS ON 30% CELLULOSE ACETATE SHEETS

Solvent: ethanol-dichloromethane (2:1).

<i>Substance</i>	<i>R<sub>F</sub> value</i>
Anthracene	0.79
Pyrene	0.71
Benz(b)fluoranthene	0.54
Benz(a)pyrene	0.37
Dibenz(a,h)pyrene	0.21
Coronene	0.75

We tried to transfer these TLC results to HPLC. The elution values (Fig. 1) confirmed the TLC results. Accordingly, this separation method permits the highly selective and sensitive determination of coronene, which is normally very difficult because of its ring size. At a measuring wavelength of 304 nm on the UV detector, 10 ng of coronene in a volume of 5  $\mu\text{l}$  can still be detected.

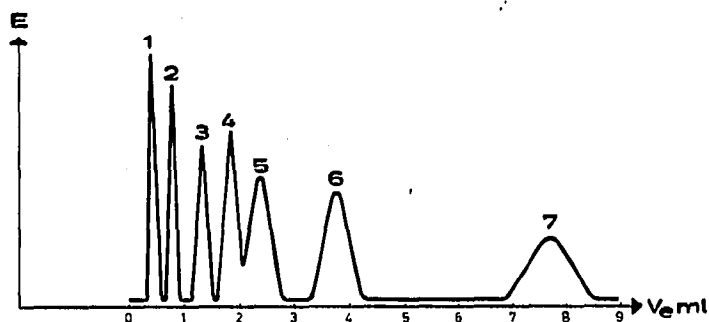


Fig. 1. HPLC separation of some PAHs on 30% cellulose acetate.  $d_p = 50\text{--}60\ \mu\text{m}$ ;  $\Delta p = 25\ \text{atm.}$ ; flow-rate = 30 ml/h; 2 columns of 50 cm, I.D. = 2 mm. Solvent: ethanol-dichloromethane (2:1). 1 = Solvent; 2 = naphthalene; 3 = anthracene; 4 = coronene; 5 = pyrene; 6 = benz(b)fluoranthene; 7 = benz(a)pyrene.

#### Separation of PAHs on polyamide

There is growing interest in the use of polyamide on account of the medium value of its polarity. The steric structure of this synthetic material proved to be suitable for the separation of isomeric compounds<sup>7</sup>.

The separation characteristics of PAHs on polyamides had not been investigated before. Here too, we first used TLC on polyamide sheets as a screening test for the selection of suitable solvent systems. The use of alcohols as solvents showed very interesting separation possibilities; it was possible, for example, to separate the isomeric benzpyrenes completely from each other (Table II). The presence of a small amount of a non-polar solvent as additive prevents the formation of unsymmetrical spots (tailing effect). The higher is the amount of non-polar solvent added to the alcohol (systems III-IV, Table II), the smaller are the spots at decreased separation efficiency. The separation efficiency of systems can be controlled by the suitable selection of solvents.

Following these TLC pilot tests, we investigated the efficiency of separation

TABLE II

#### $R_F$ VALUES OF SOME POLYCYCLIC AROMATIC HYDROCARBONS ON POLYAMIDE-6 PLASTIC SHEETS

Solvent systems: I = ethanol; II = methanol; III = ethanol-dichloromethane (8:12); IV = ethanol-toluene (13:7); V = methanol-chloroform (1:1); VI = isopropanol-benzene (15:5).

Substance	$R_F$ value					
	I	II	III	IV	V	VI
Anthracene	0.57	0.70	0.96	—	—	—
Phenanthrene	0.55	—	0.93	—	—	—
Pyrene	0.52	0.62	0.88	0.86	0.81	—
Benz(e)pyrene	0.45	0.40	0.80	0.75	0.66	0.71
Benz(b)fluoranthene	0.26	0.36	0.67	0.72	0.50	0.57
Perylene	0.24	0.27	0.66	0.70	0.48	0.55
Benz(a)pyrene	0.21	0.29	0.62	0.66	0.44	0.47

of PAHs on polyamide-6 columns under conditions of HPLC. We were particularly interested in the separation of isomeric PAHs.

In comparing silica gel columns with columns packed according to the balanced suspension method, it was found that these show similar low-pressure drops ( $\Delta p = 50$  atm) at a flow-rate of 42 ml/h (Table III). As on TLC plates, there is the possibility of separating anthracene and phenanthrene on HPLC columns. Columns that are narrower and longer could improve this separation.

TABLE III

## SEPARATION OF SOME PAHs ON POLYAMIDE COLUMNS

HPLC-Sorb polyamide-6;  $d_p = 20\text{--}32 \mu\text{m}$ ; column = 1 m  $\times$  4 mm I.D.;  $n(\text{benzene}) = 1300$ ;  $\Delta p = 50$  atm; flow-rate = 42 ml/h.

Substance	$V_{o_{max.}}$ (ml)
Benzene	8.8
Naphthalene	9.7
Phenanthrene	12.2
Anthracene	12.6
Pyrene	17.5
Benz(e)pyrene	33.5
Benz(a)pyrene	44.0

As in TLC, isomeric benzpyrenes are separated completely. This means that polyamide-6 can be used for the selective separation of isomeric benz(a)pyrene and benz(e)pyrene, which are known as pilot substances. The reproducibility of this procedure can be compared with that of methods using silica gel columns. With increasing elution volume, the peaks showed a small degree of asymmetry. In this case, the influence of small amounts of lipid solvents as additives or alterations to the polyamide support should be investigated in order to prevent this tailing effect.

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