VARIABLES INFLUENCING SEPARATION EFFICIENCY IN PRESSURIZED PLANAR ELECTROCHROMATOGRAPHY

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Introduction

Variables influencing separation

- mobile phase - stationary phase equilibration,
- pressure applied to the chromatographic plate,
- temperature,
- polarization voltage
- buffer concentration and pH,
- modifier concentration
- mode of sample application

Examples of separation

Conclusions

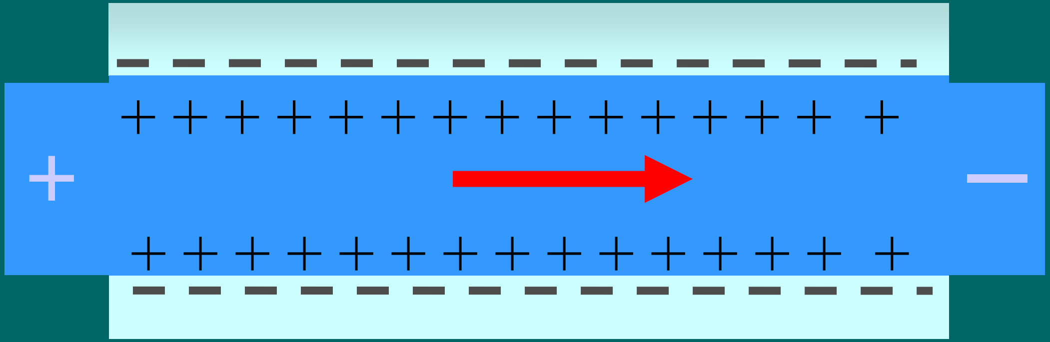
The idea of using electroosmosis as a mechanism for development of a planar medium can be traced back to:

Martin (R. Consden, A.H. Gordon, A.J.P. Martin, Biochem. J. 40 (1946) 33)

and Synge (D.L. Mould, R.L.M. Synge, Analyst 77 (1952) 964).

Electroosmotic flow arises from the formation of an electrical double layer at the solid – liquid interface

$$\equiv \mathrm{SiOH} \leftrightarrow \equiv \mathrm{SiO}^- + \mathrm{H}^+$$



Mobile phase velocity for pressure driven system:

$$u_p = \frac{d_p^2 \Delta P}{L \theta \eta}$$

u_p - mobile phase velocity
θ - flow resistance parameter
η - mobile phase viscosity
d_p - particle size
L - column length

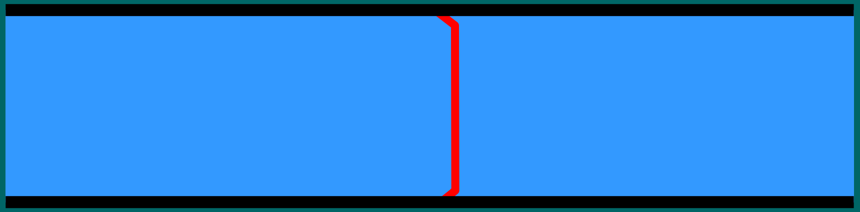
Mobile phase velocity for electroosmotically driven system:

$$u_{eo} = \frac{\varepsilon_r \varepsilon_o E \zeta}{\eta}$$

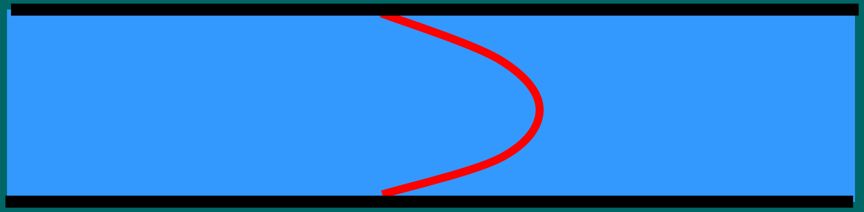
u_{eo} - electroosmotic flow
ε_o - permittivity of vacuum
ε_r - dielectric constant
ζ - zeta potential
E - electric field

Flow profiles

electroosmotical flow



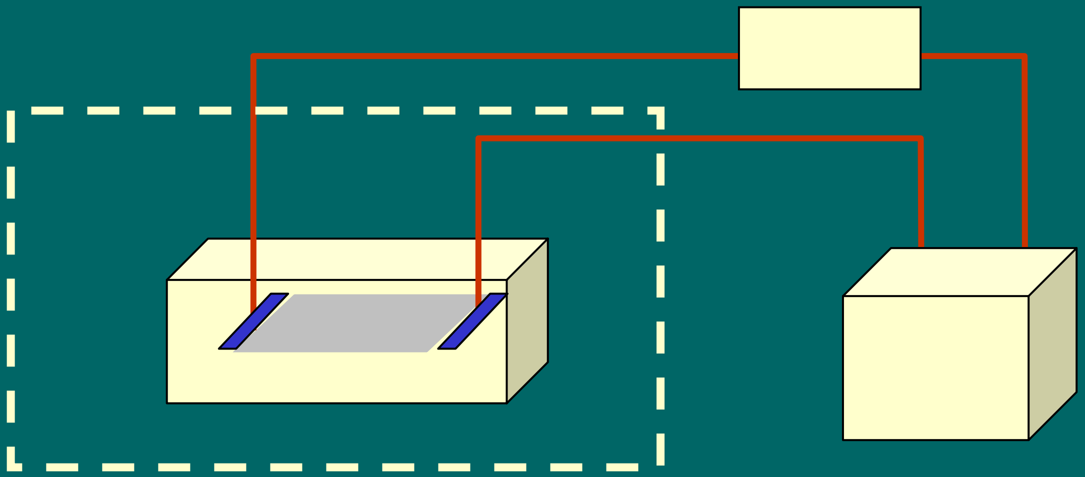
pneumatical flow



V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23

M. Pukl, M. Prosek, R.E. Kaiser, Chromatographia 38 (1994) 83

C. F. Poole, I. D. Wilson, J. Planar Chromatogr. 10 (1997) 332

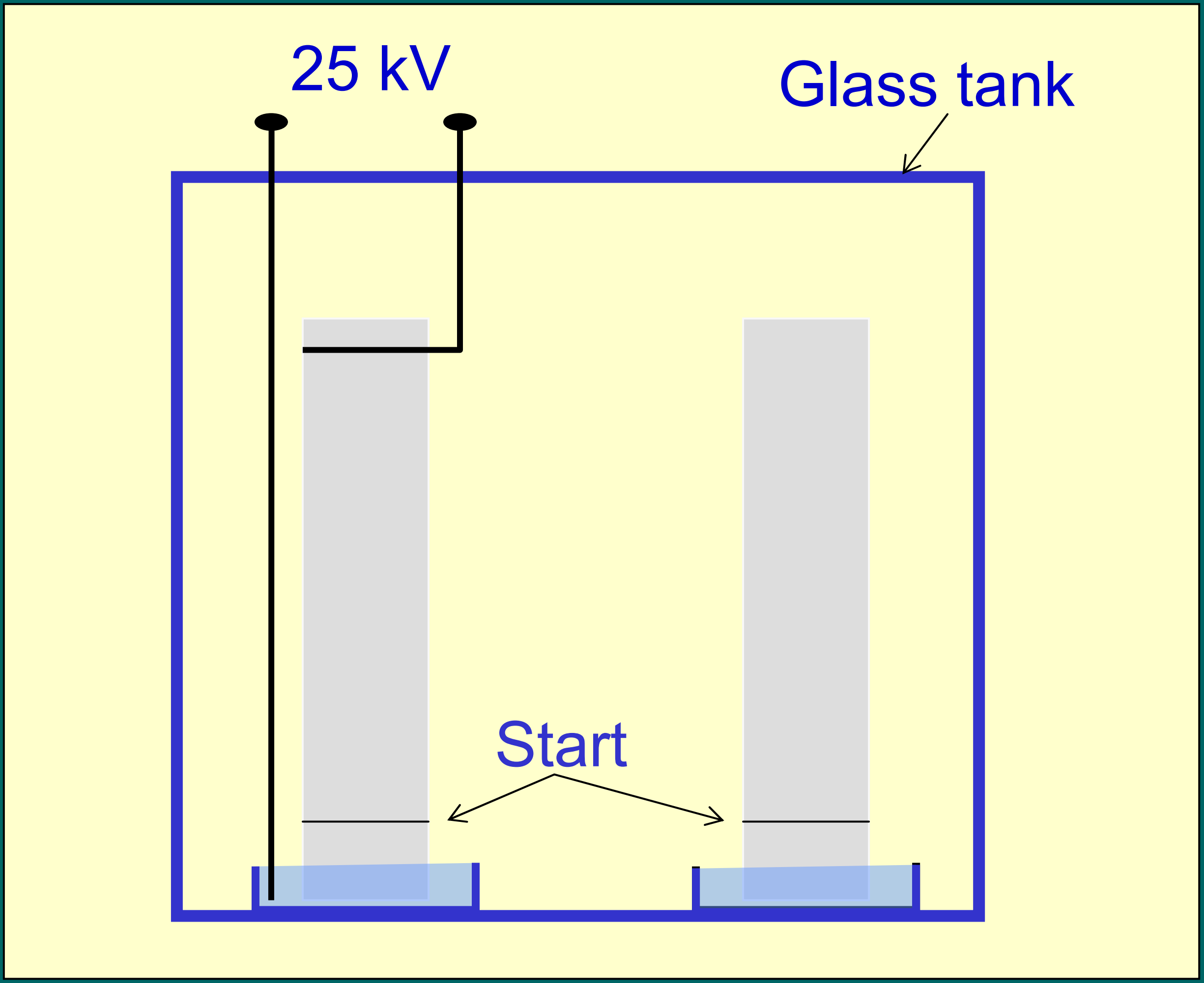


AMMETER

POWER SUPPLY

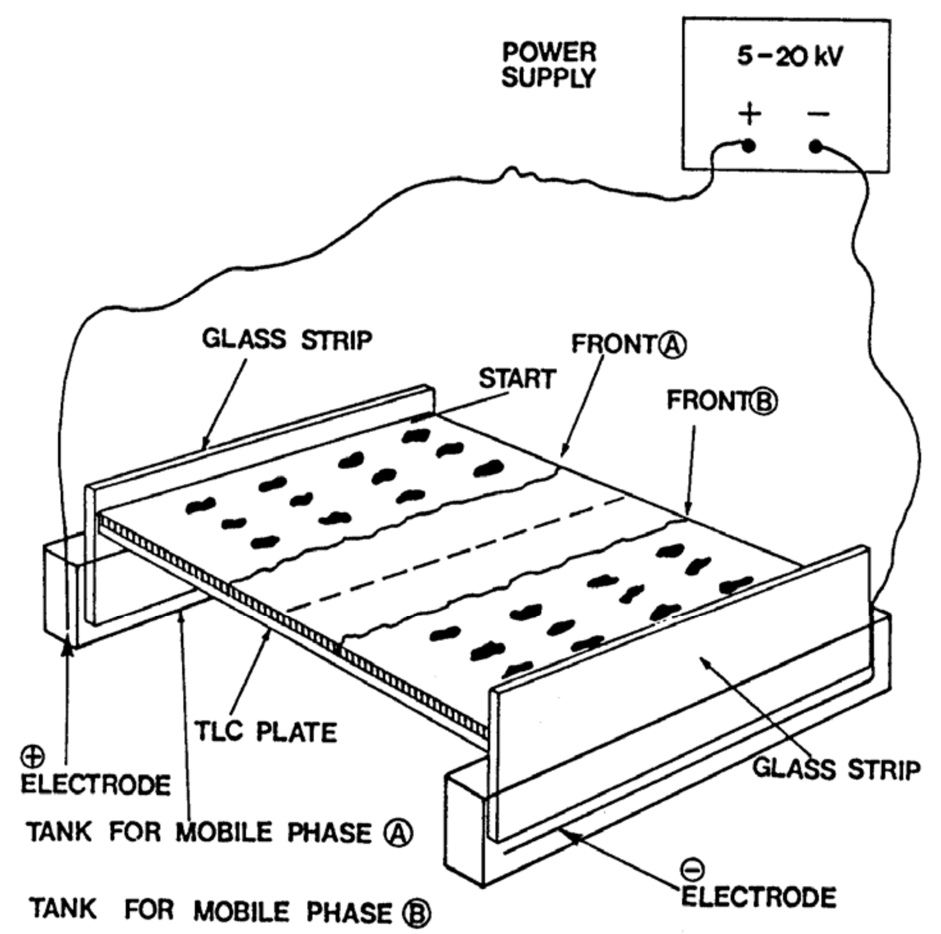
CHAMBER FOR PLANAR ELECTROCHROMATOGRAPHY

Apparatus for high-speed thin-layer chromatography



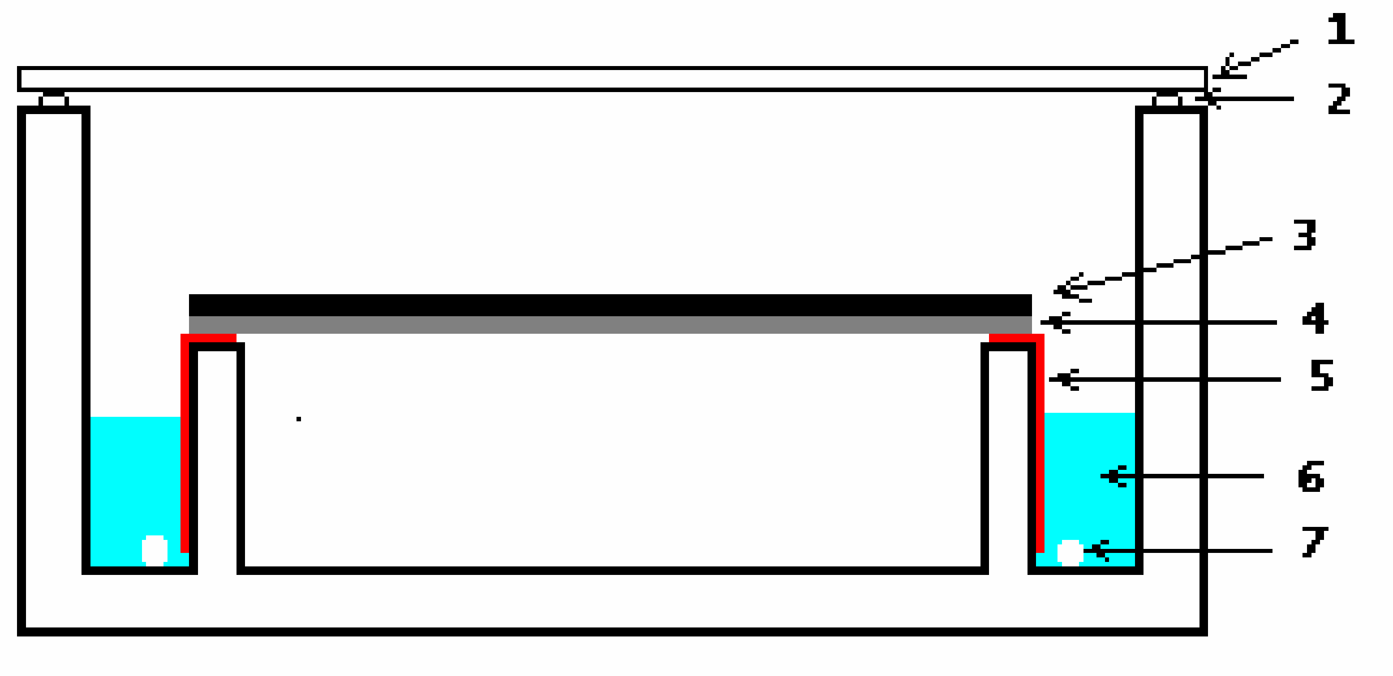
V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23

Schematic diagram of experimental horizontal chamber for high voltage planar electrochromatography



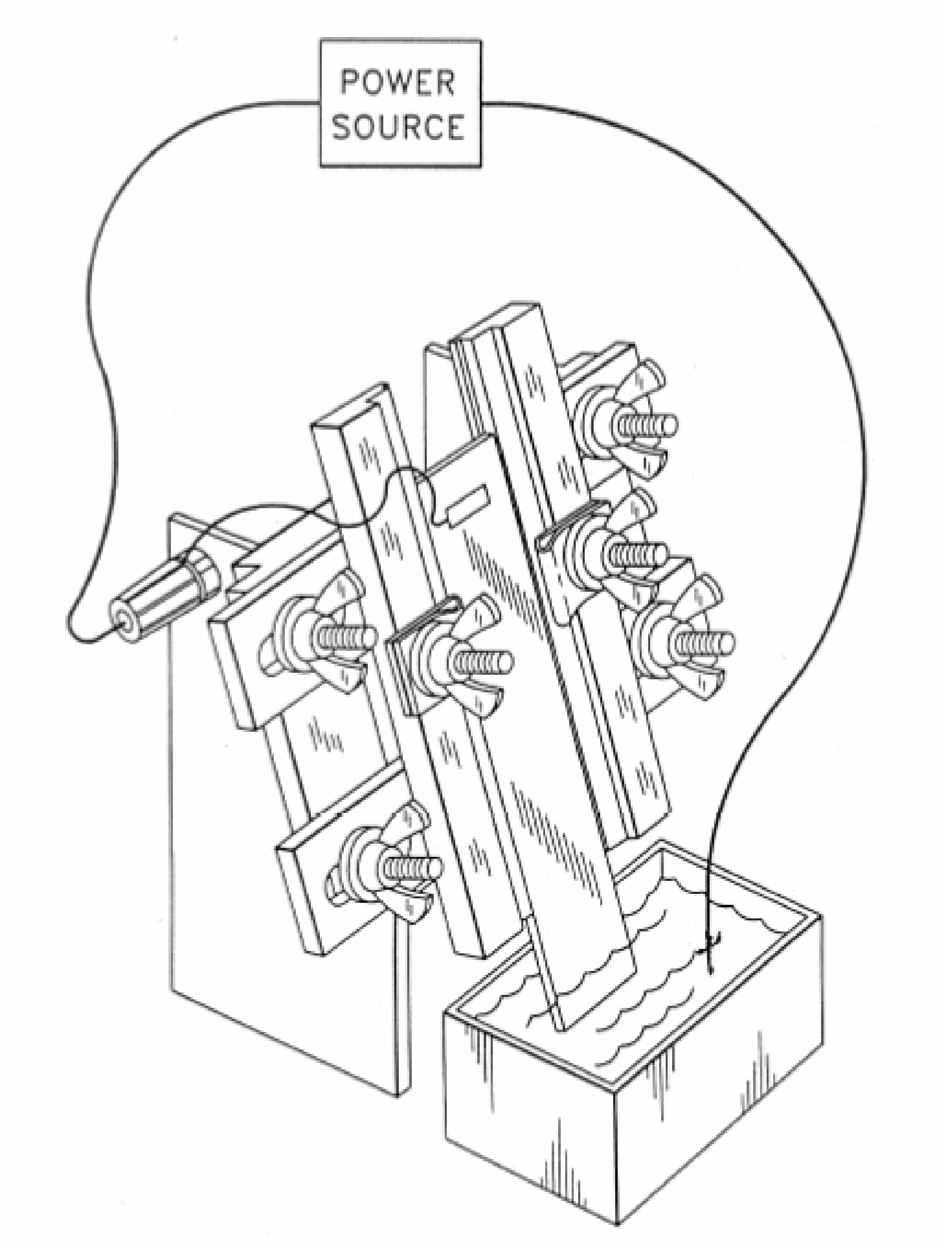
M. Pukl, M. Prosek, R.E. Kaiser, Chromatographia 38 (1994) 83

Planar chromatography in horizontal chamber



1) cover plate, 2) silicone seal, 3) chromatographic carrier plate, 4) adsorbent layer, 5) paper wick, 6) mobile phase, 7) electrode

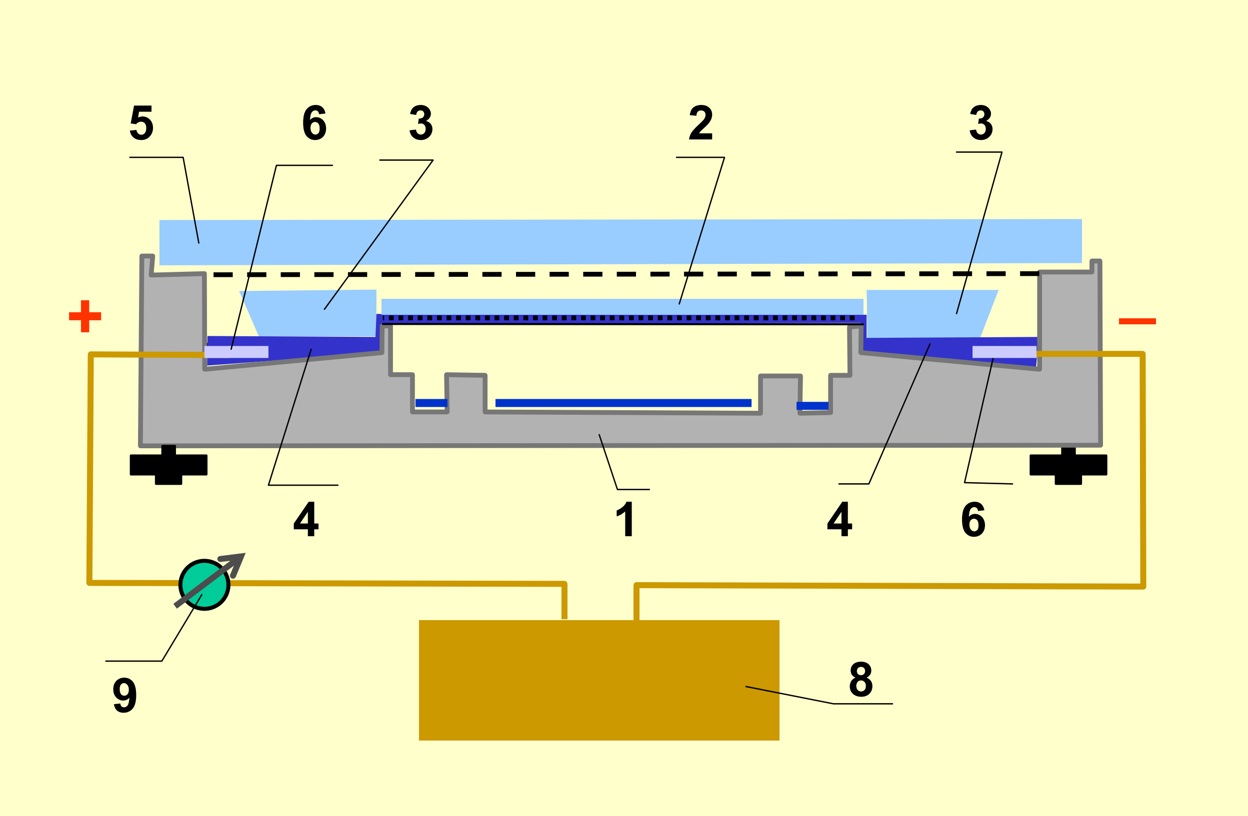
A. G. Howard, T. Shafik, F. Moffat, I. D. Wilson, J. Chromatogr A, 844 (1999) 333



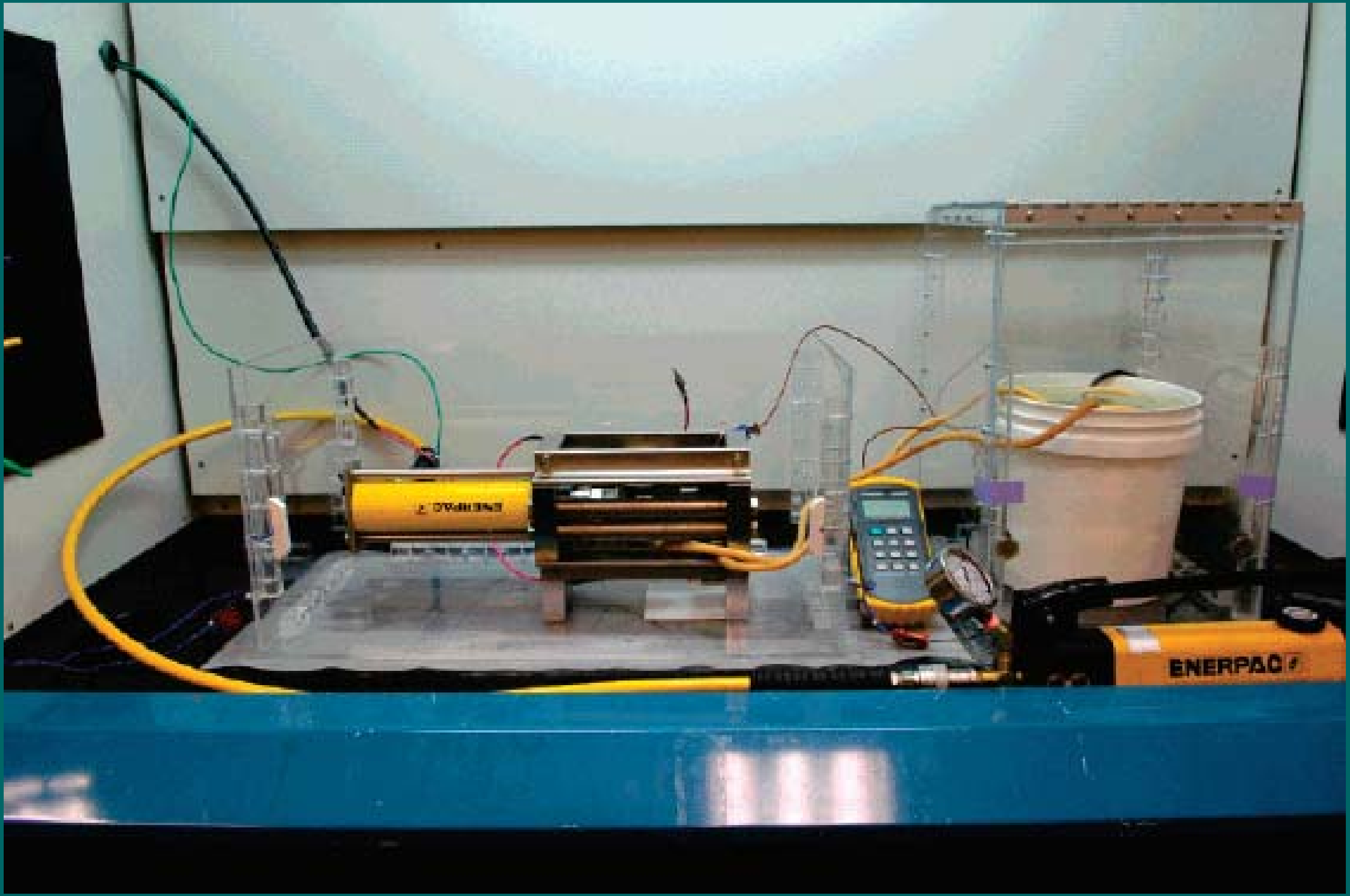
Apparatus for performing planar electrochromatography

D. Nurok, M.C. Frost, D.M. Chenoweth, J. Chromatogr. A 903 (2000) 211

Horizontal DS chamber adapted for planar electrochromatography (PEC)

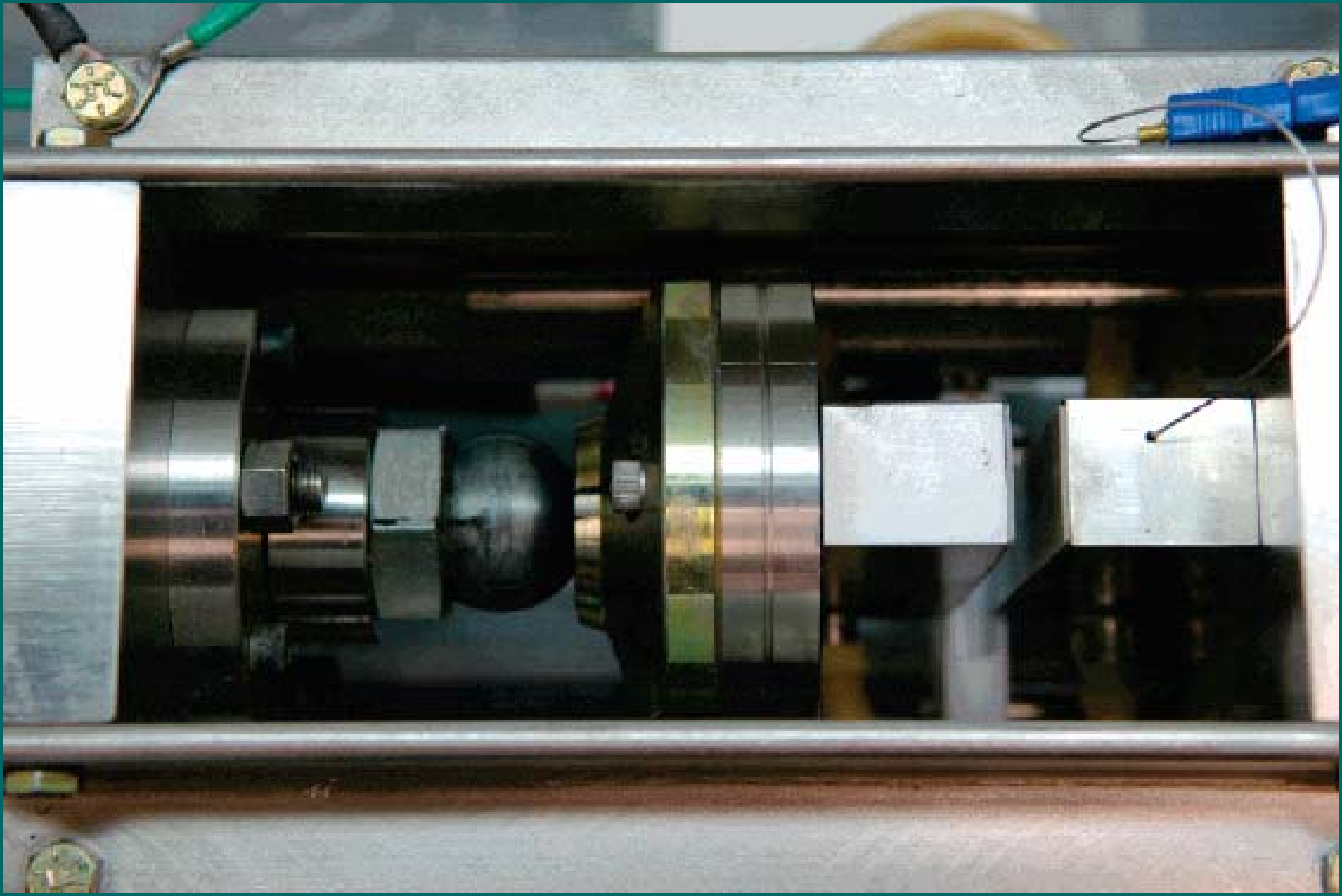


T.H. Dzido, R. Majewski, B. Polak, W. Gołkiewicz, E. Soczewiński, J. Planar Chromatogr. , 16 (2003) 176



Apparatus for performing PPEC.

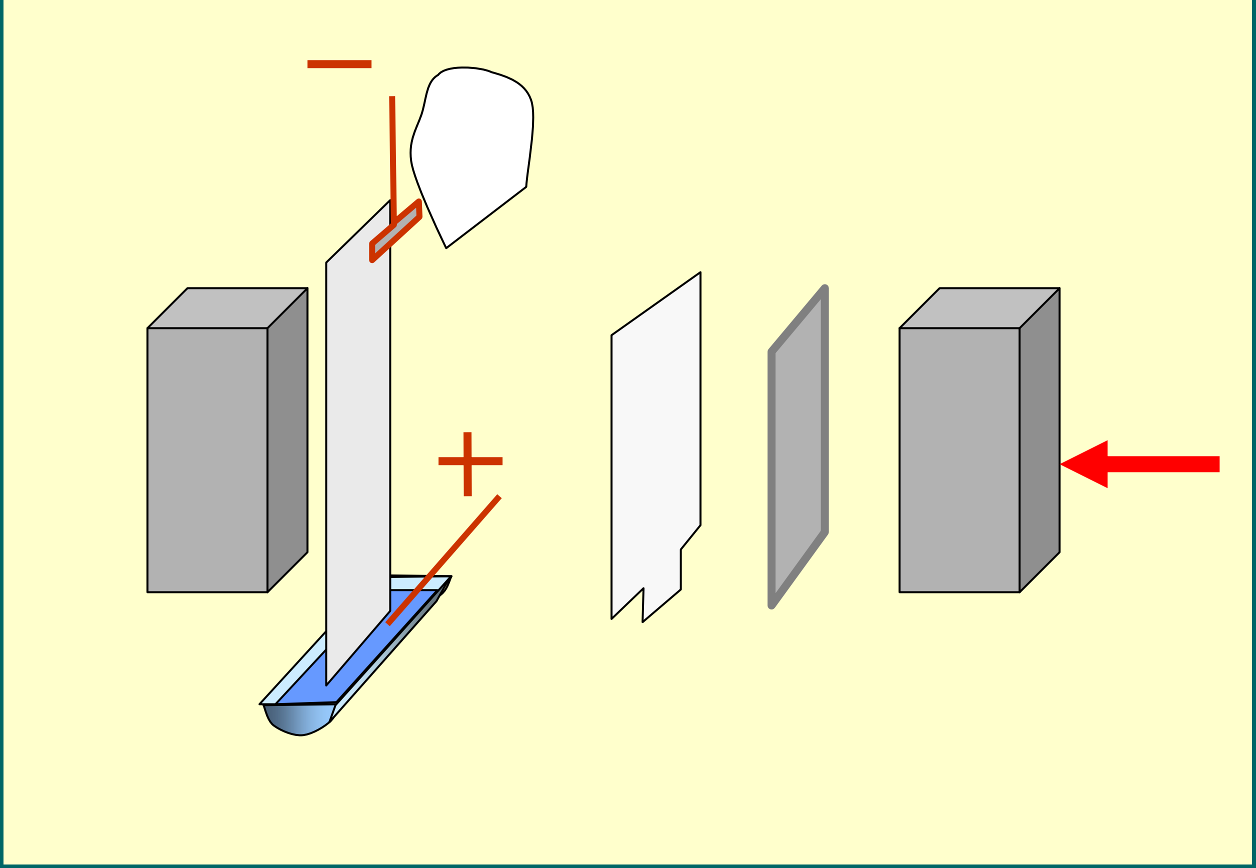
D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.



Open ball joint and corresponding socket, and die blocks with aluminum nitride sheets. The picture shows the thermocouple on the right and the safety lead and lead to the anode on the upper left,

D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.

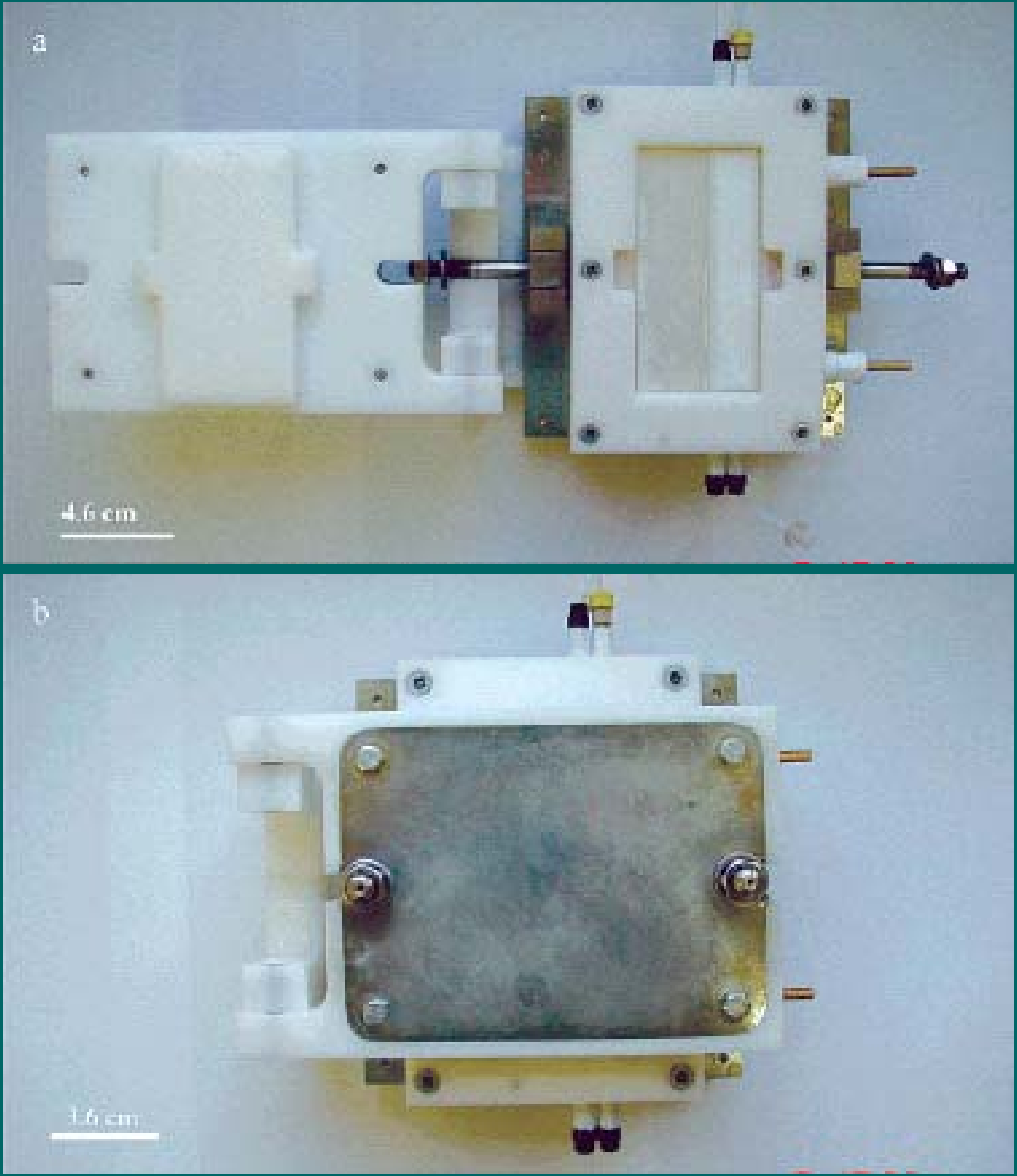
Principle of action of the apparatus for PPEC by Nurok and coworkes (Anal. Chem. 2004, 76,1690-1695)



D. Nurok,* J. M. Koers, A. L. Novotny, M. A. Carmichael, J. J. Kosiba, R. Santini,*,† G. L. Hawkins, and R. W. Replogle, Anal. Chem.**2004,** 76,1690-1695

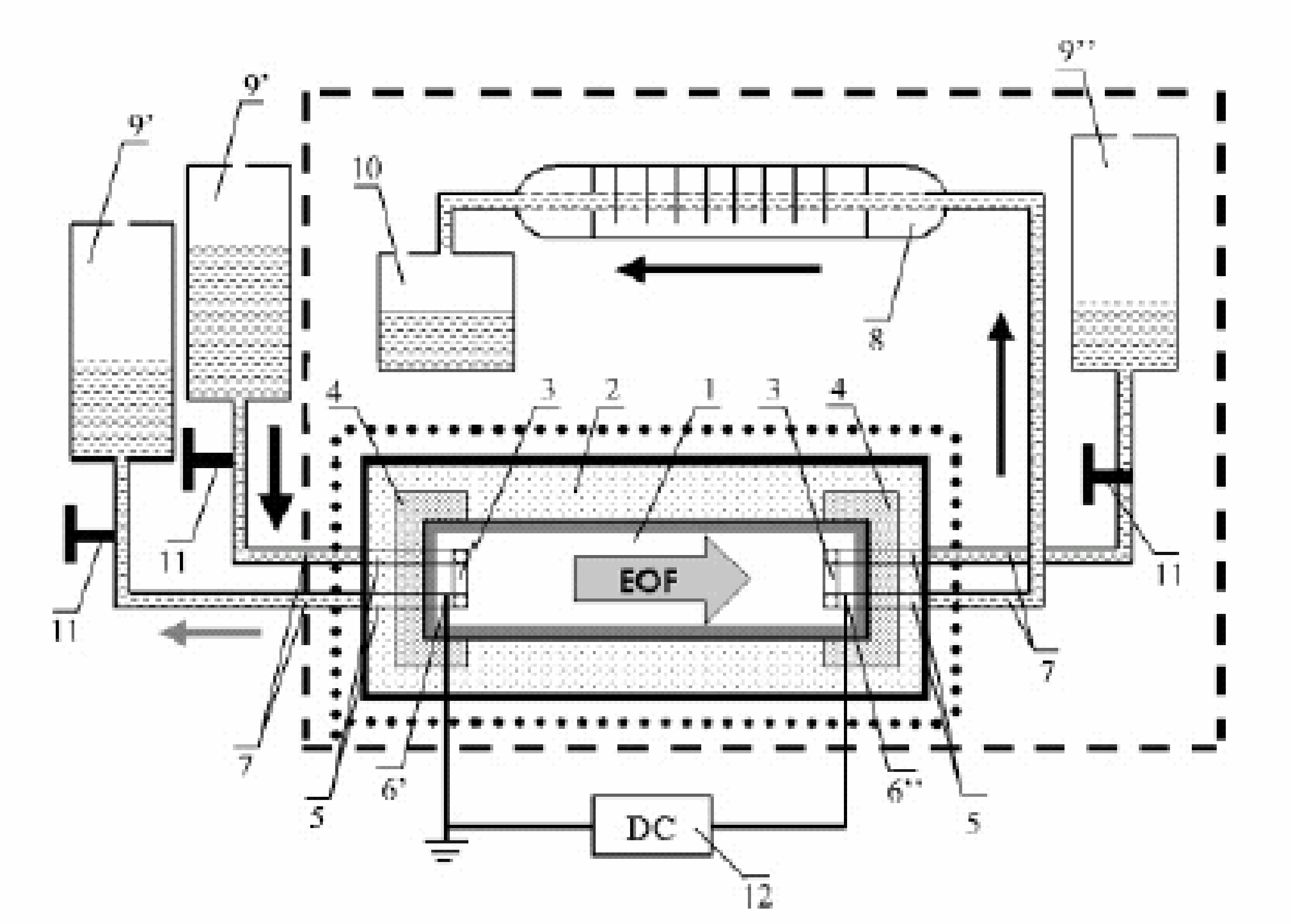
Picture of the PPEC instrument. The chromatographic base lies in the throat of the press.

P. A. Tate, J. G. Dorsey, J. Chromatogr. A, 1103 (2006) 150–157.



The chamber for planar electrochromatgraphy: (a) with opened lid (2 x 10 cm chromatographic plate with separated sample mixture is placed inside the chamber) and (b) with closed lid.

T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.

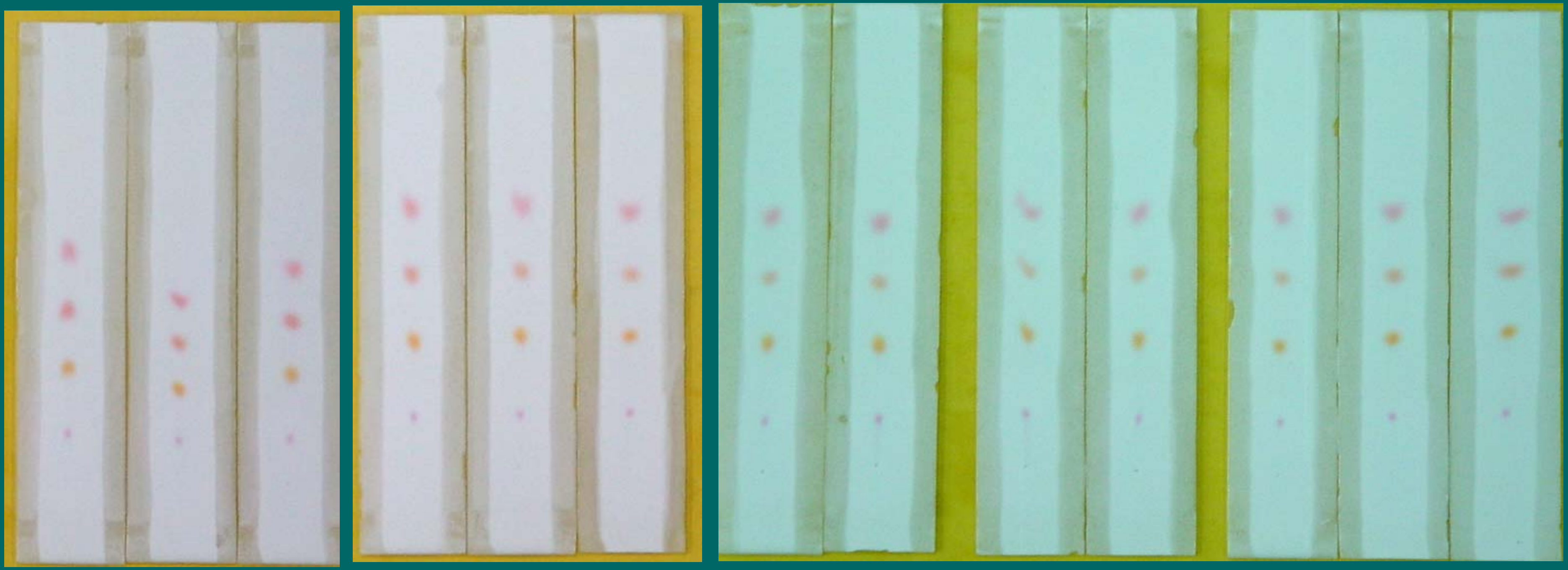


Conceptual view of the device for planar electrochromatography in a completely closed system.

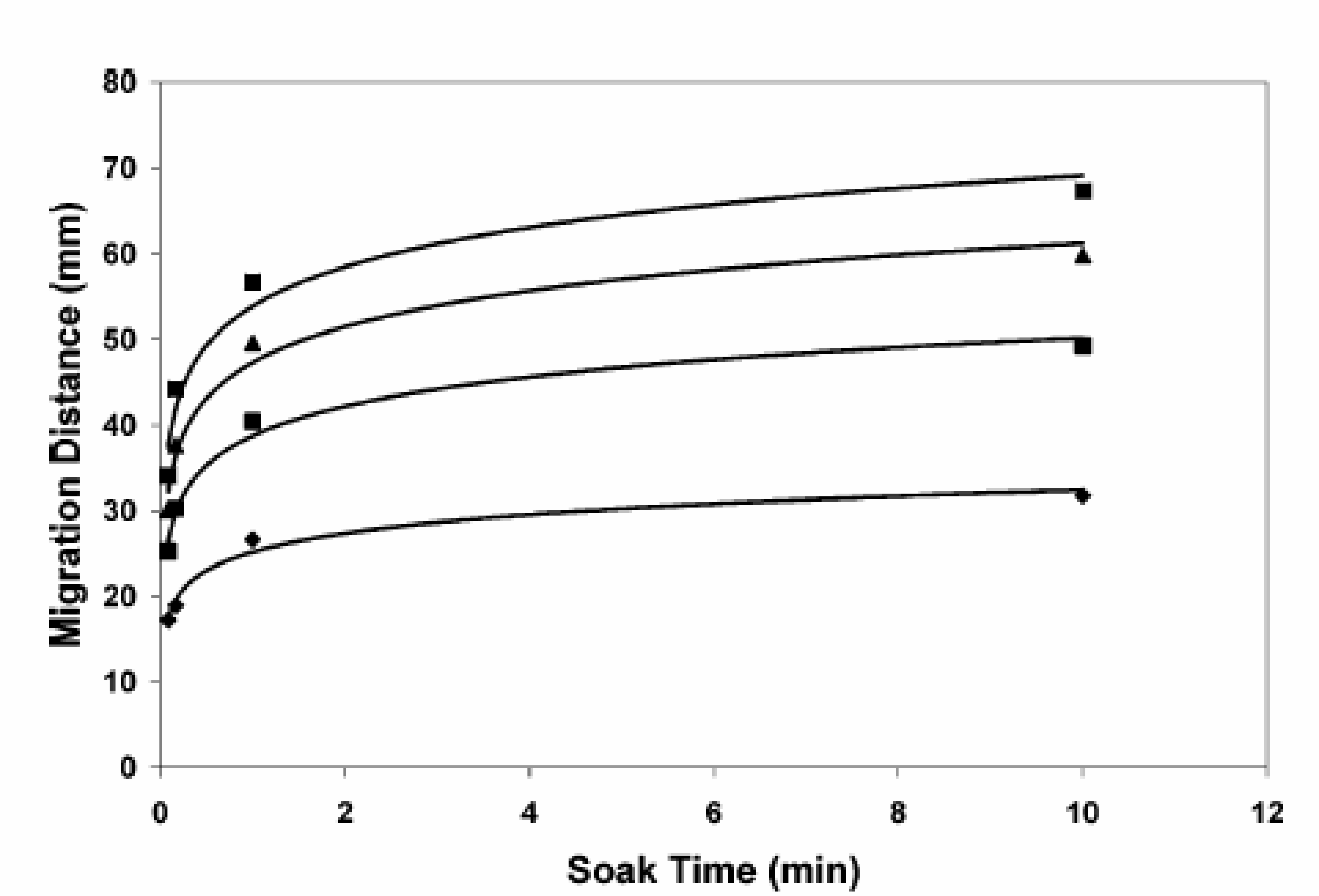
T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.

Mobile phase - stationary phase equilibriation,

Different time of stationary phase saturation with mobile phase



T. H. Dzido, J. Mróz, G. Jóźwiak, J. Planar Chromatogr. 17 (2004) 404 – 410.



Migration distance vs. soak time on HPTLC plates conditioned at 160°C.

A.L. Nowotny, D. Nurok, R.W. Replogle, G.L. Hawkins, mR.E. Santini, Anal. Chem., 78 (2006) 2823 – 2831.

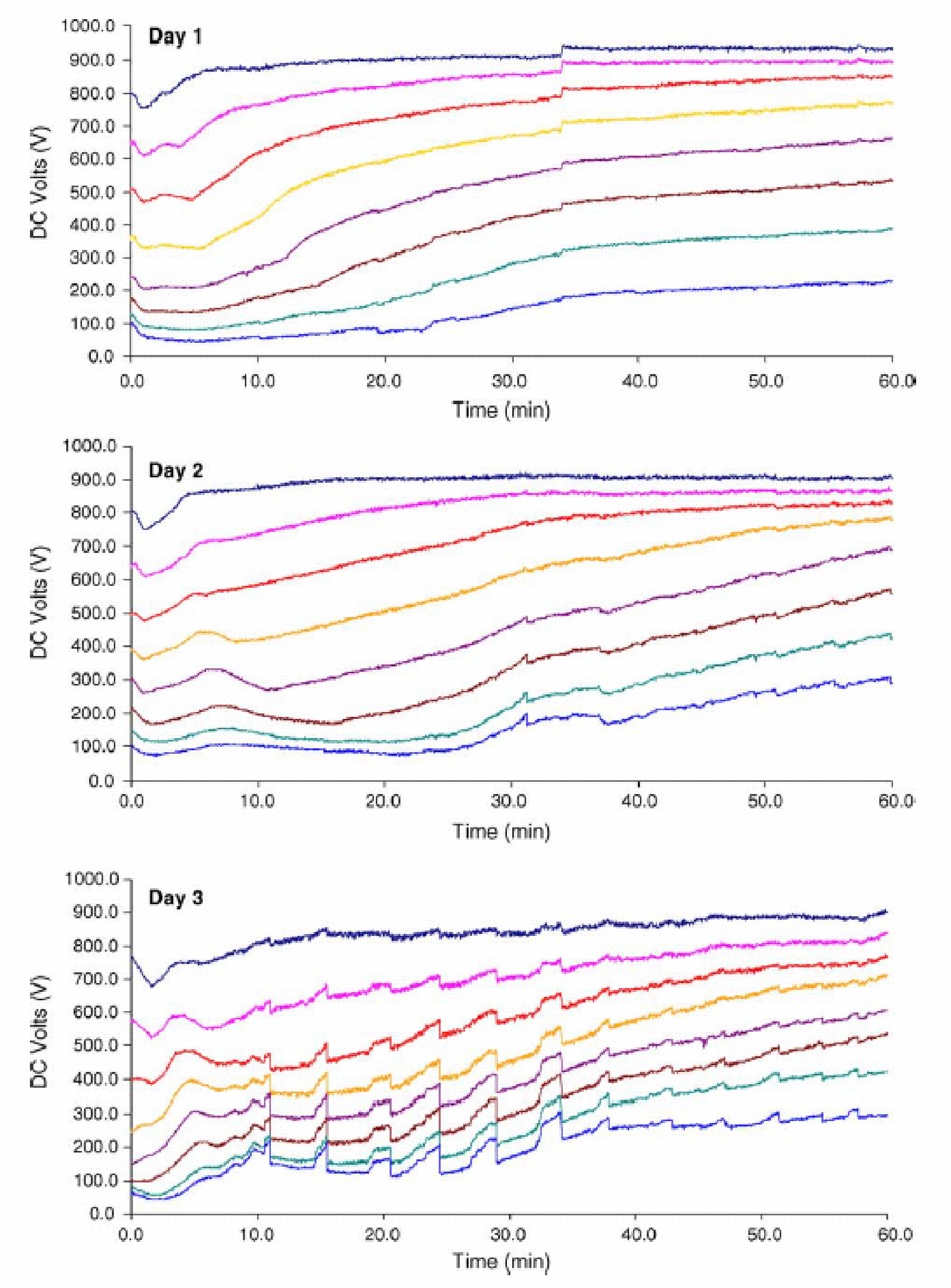
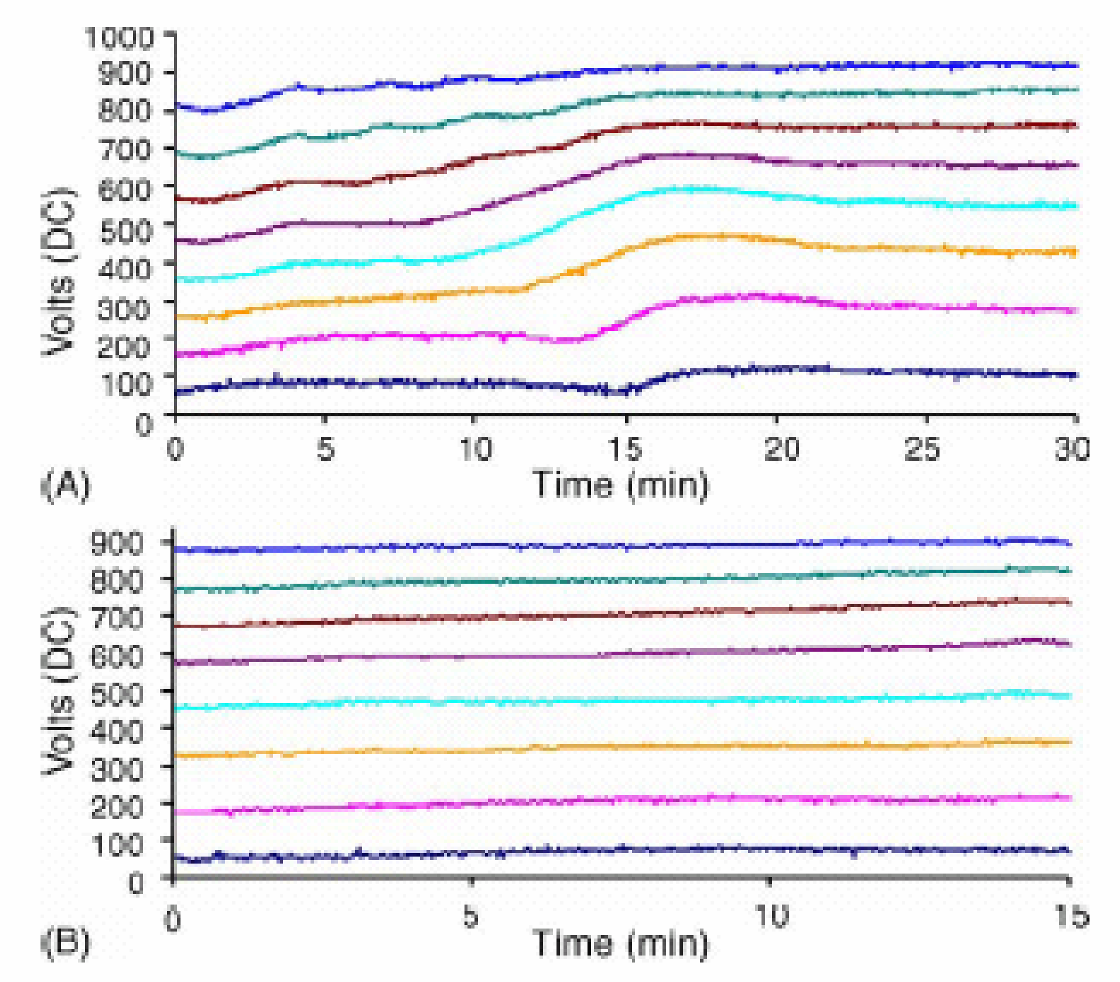


Plate 1 equilibration effects with 1000VDC applied. Each data series corresponds to an individual reader electrode. The data series are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir.

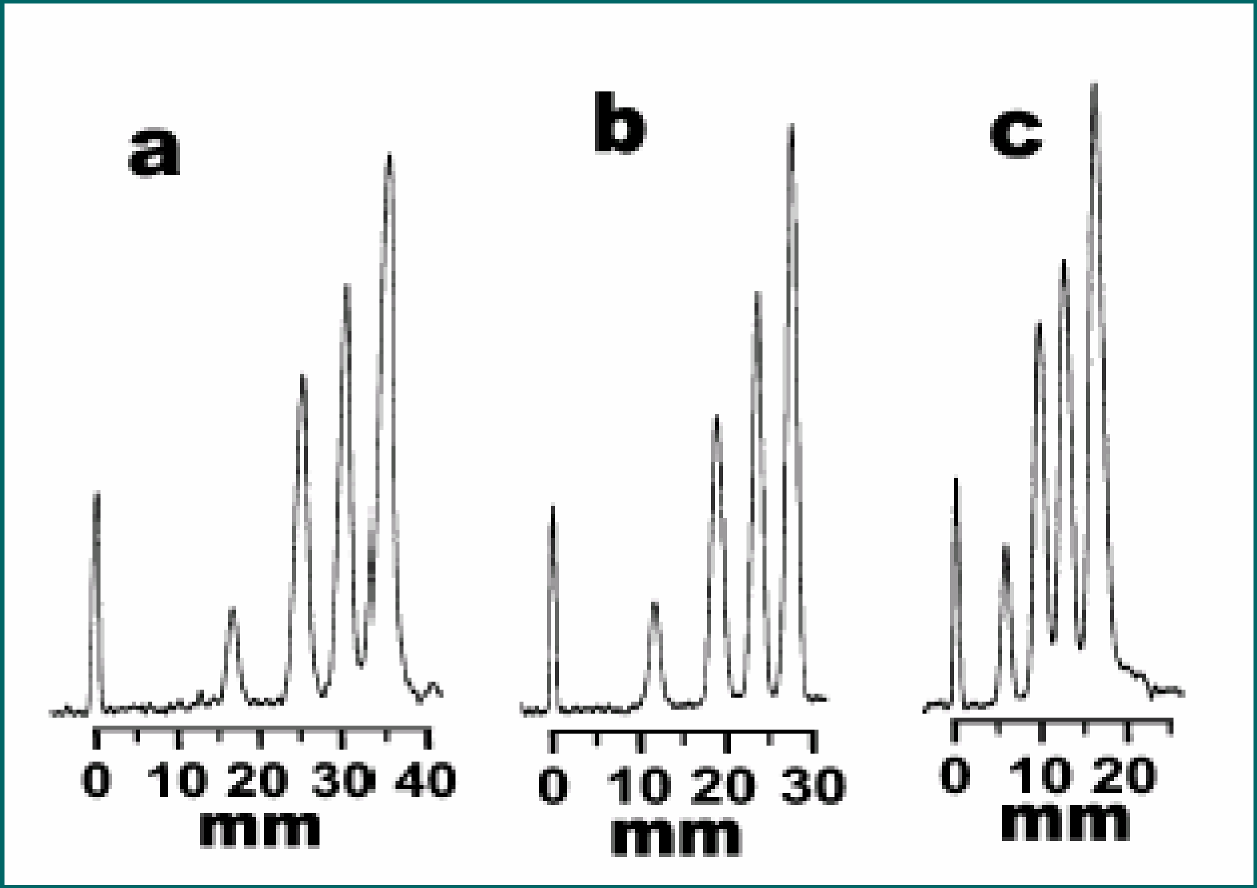
P.A. Tate, J.G. Dorsey / J. Chromatogr. A, 1079 (2005) 317–327



(A) Graph of voltage vs. time, illustrating the new TLC plate sealing method does not change the flowprofile or equilibration time. (B)Voltage profile after EOF equilibration, plate removal (simulate spotting) and replacement into apparatus.

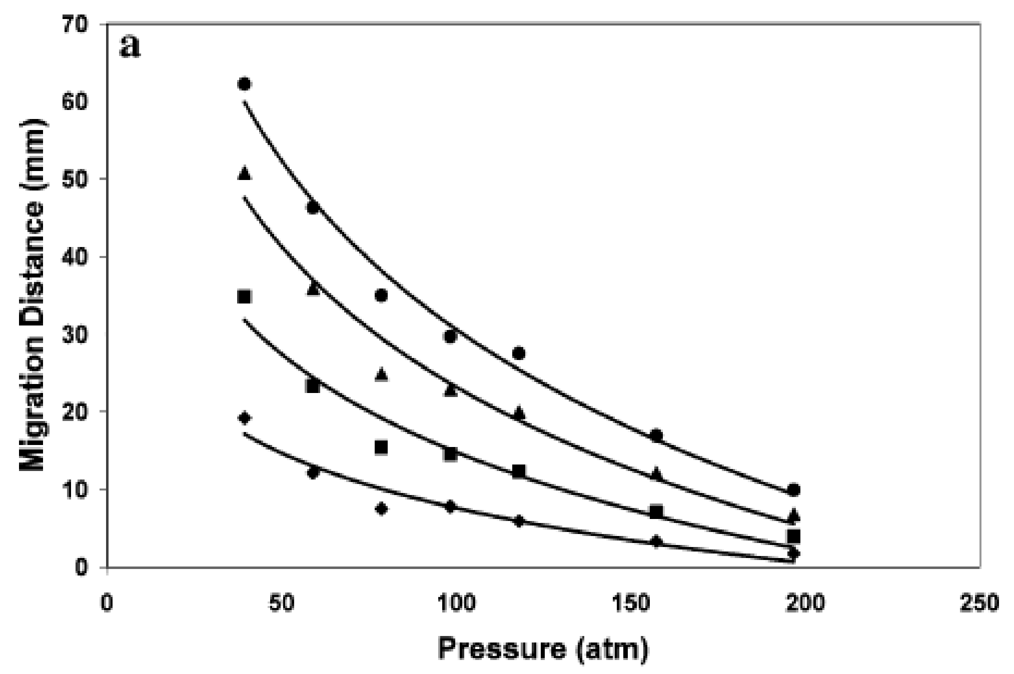
P. A. Tate, J. G. Dorsey, J. Chromatogr. A, 1103 (2006) 150–157.

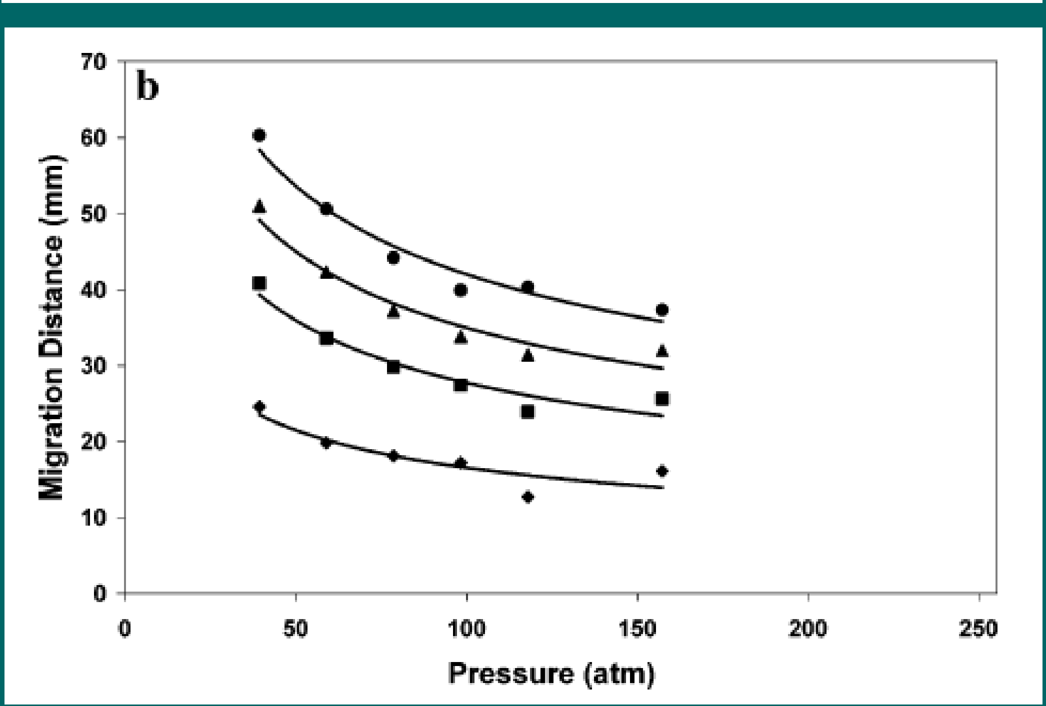
Pressure applied to the chromatographic plate



A 1-min separation on a LiChrospher C18 plate at 9 kV and a pressure of (a) 11.8, (b) 19.7, or (c) 118 atm; 55% ACN in acetate buffer pH 4.7.

D. Nurok,* J. M. Koers, A. L. Novotny, M. A. Carmichael, J. J. Kosiba, R. Santini,*,† G. L. Hawkins, and R. W. Replogle, Anal. Chem.2004, 76,1690-1695

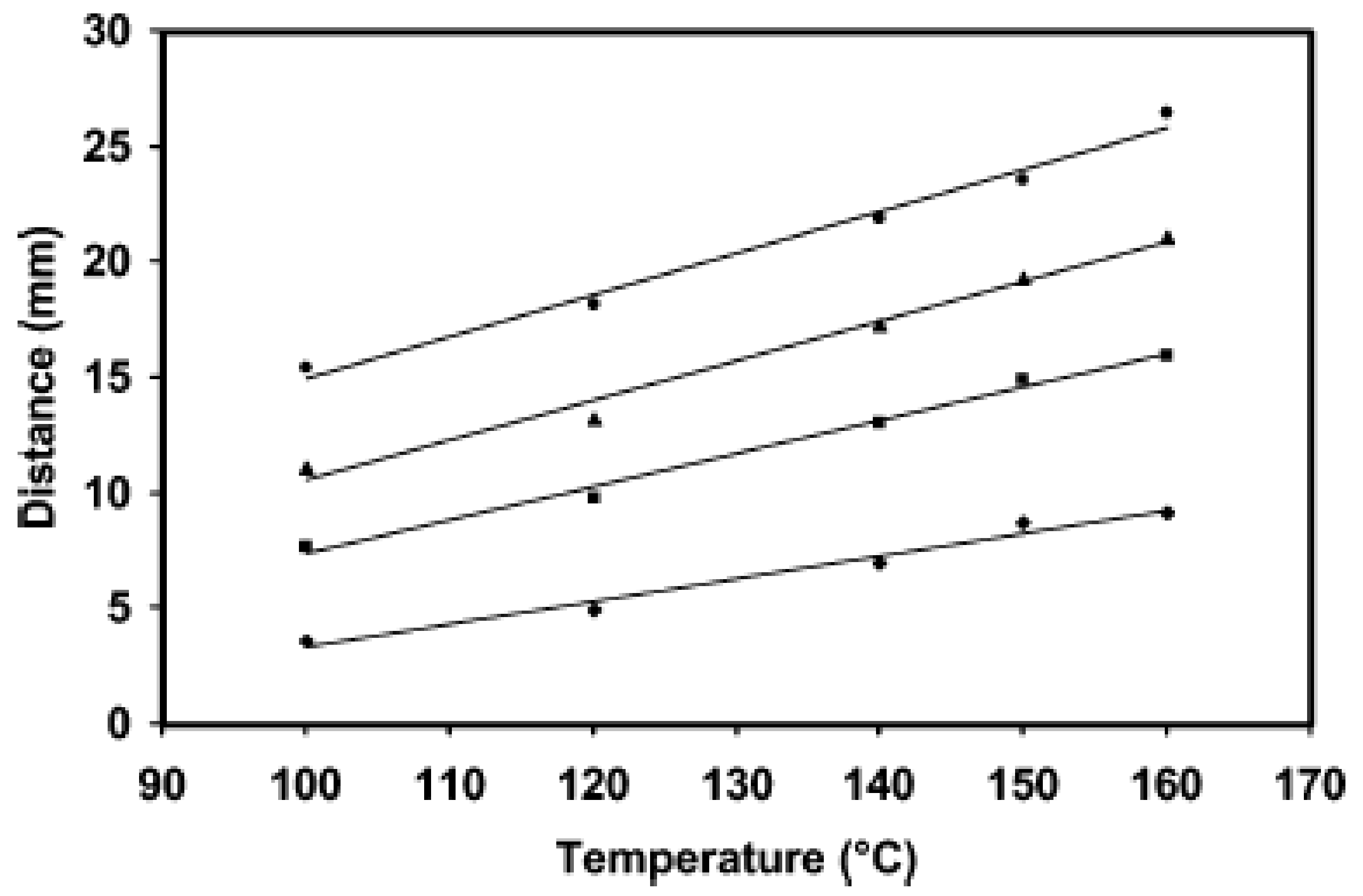




Migration distance versus pressure drop; (a) regular plates, (b) high – performance plates.

D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.

Temperature influence



Migration distance versus oven temperature for LiChrospher plates conditioned for 20 min. Separation 3 min, 59 atm, 6 kV.

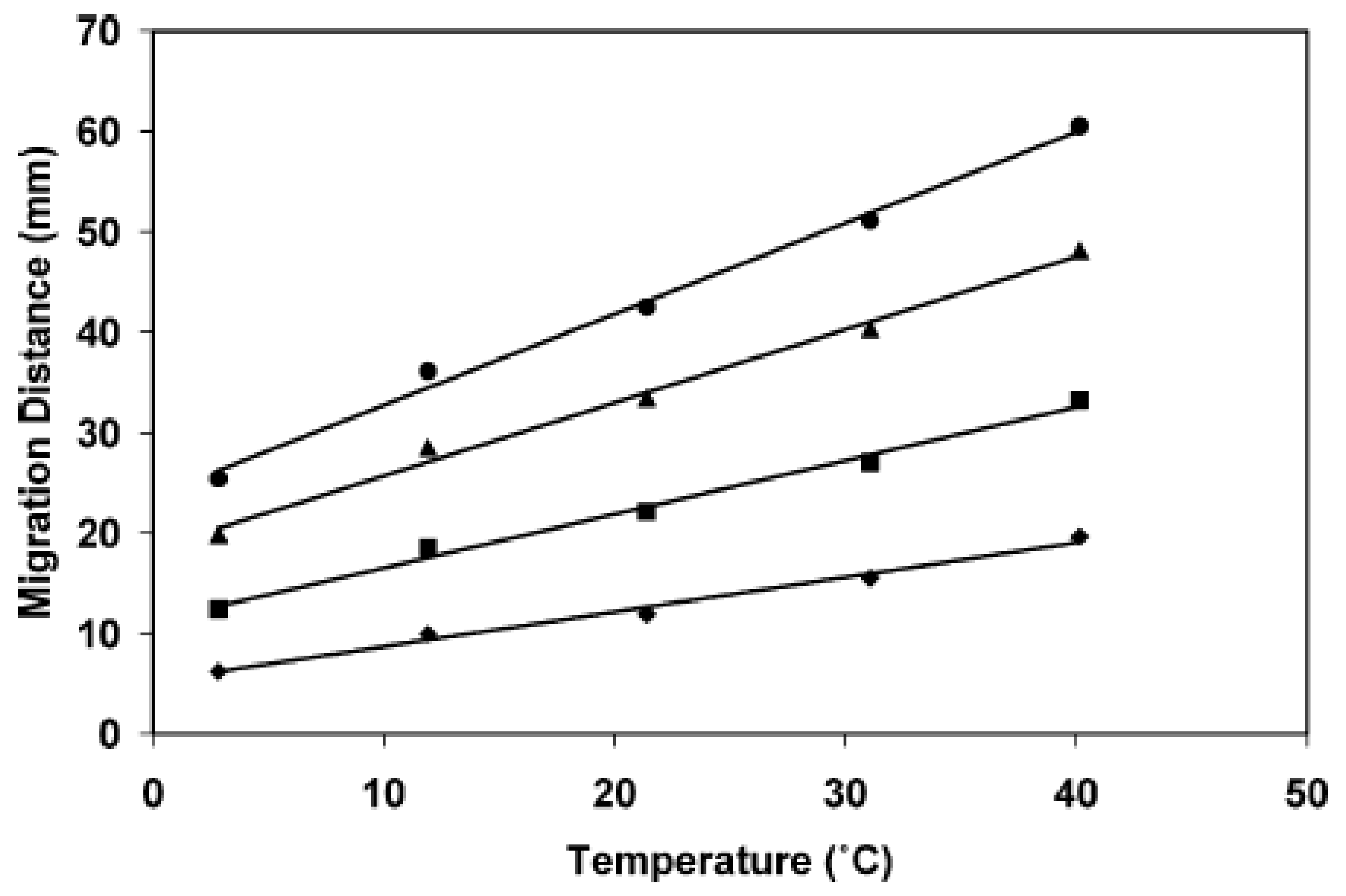
D. Nurok,et al., Anal. Chem. 2004, 76,1690-1695

$$\eta \propto \exp(const / RT)$$

$$\zeta = \frac{\sigma\delta}{\varepsilon_0 \varepsilon_r}$$

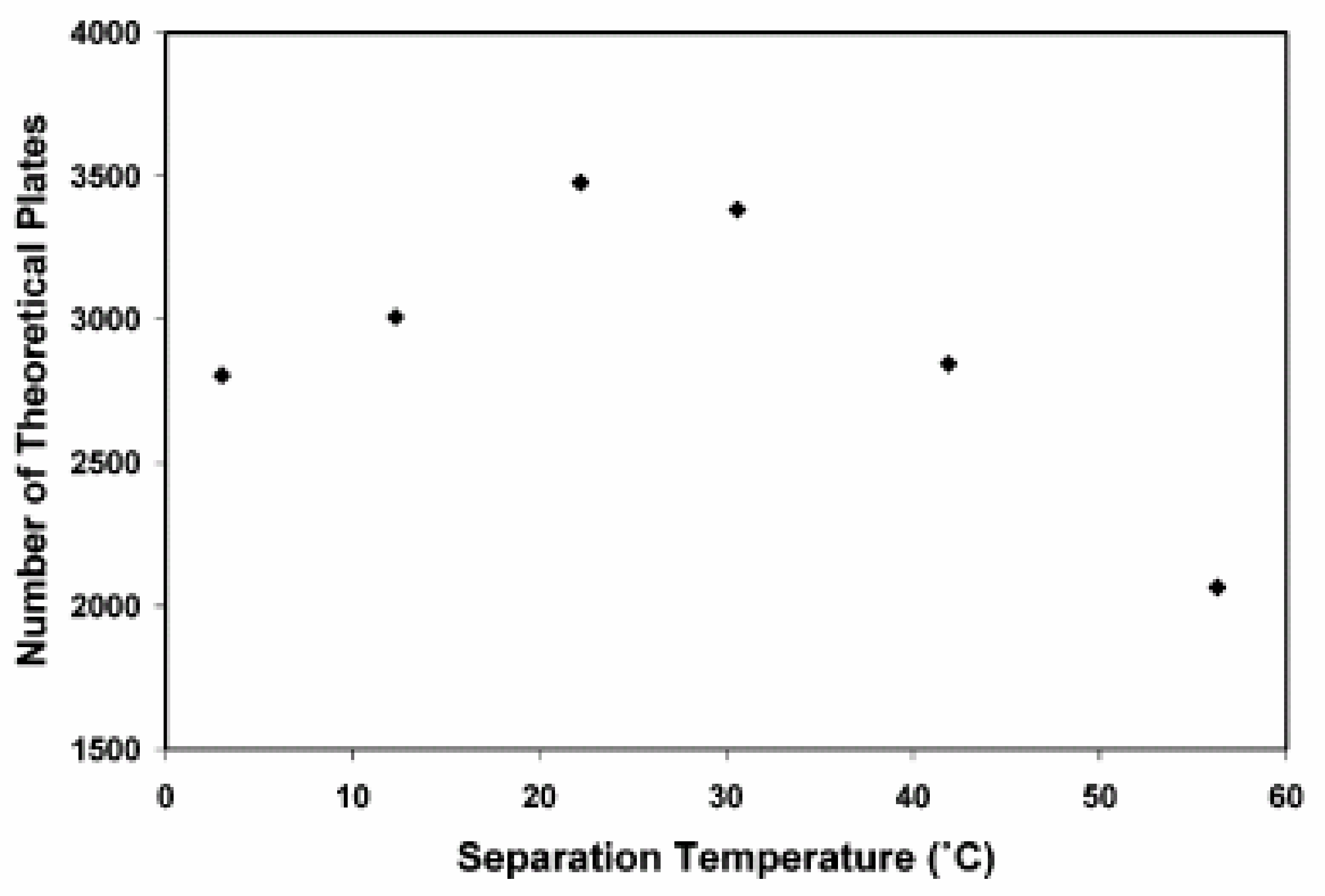
$$\delta = \sqrt{\frac{\varepsilon_0 \varepsilon_r RT}{2cF^2}}$$

$$u_{eo} = \frac{\varepsilon_r \varepsilon_o E \zeta}{\eta}$$



Migration distance versus separation temperature on regular plates conditioned at 120 °C for 20 min.

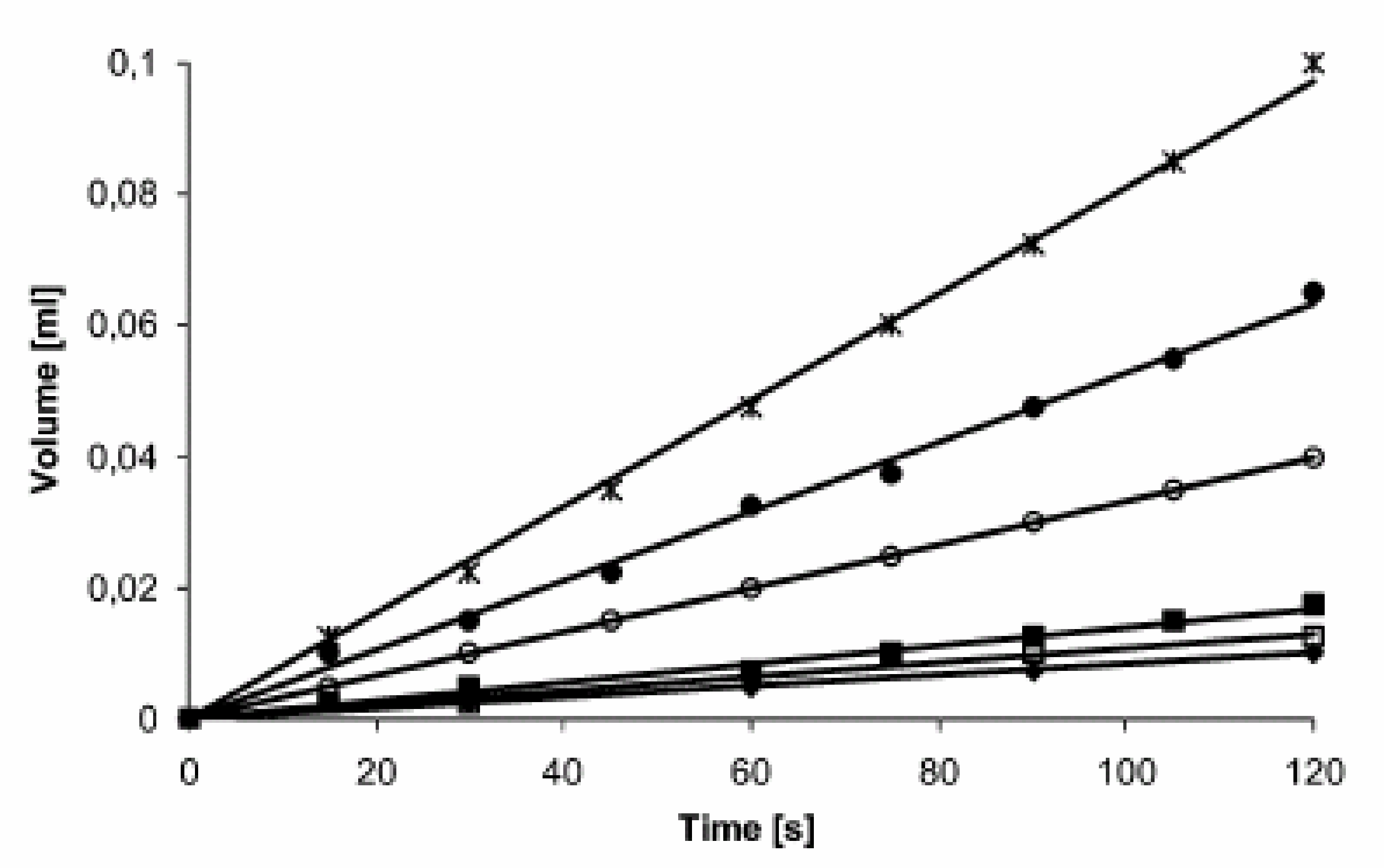
D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.



Number of theoretical plates for o-nitroaniline versus separation temperature on high-performance plates conditioned at 160 °C for 20 min.

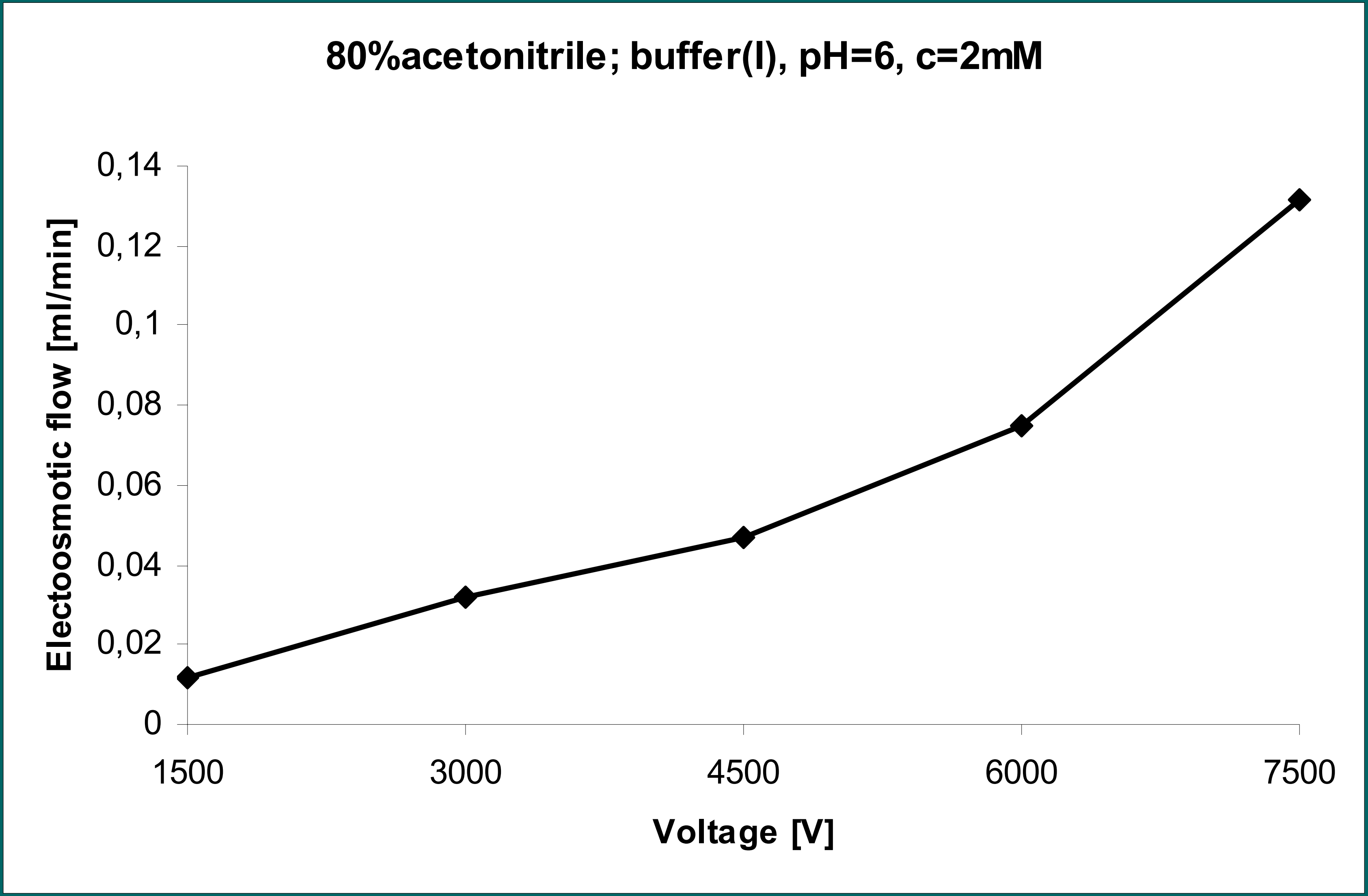
D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.

Polarization voltage



Plots of the mobile phase volume passed through thechromatographic plate vs time of PPEC experiment for various voltages: 0.75, 1, 1.5, 2.5, 3.5, and 4.5 kV. TLC RP18 F254s plate (Merck, Darmstadt), 80% acetonitrile in buffer (3.74 mMcitric acid, 12.52 mM disodium hydrogen phosphate, pH) 6).

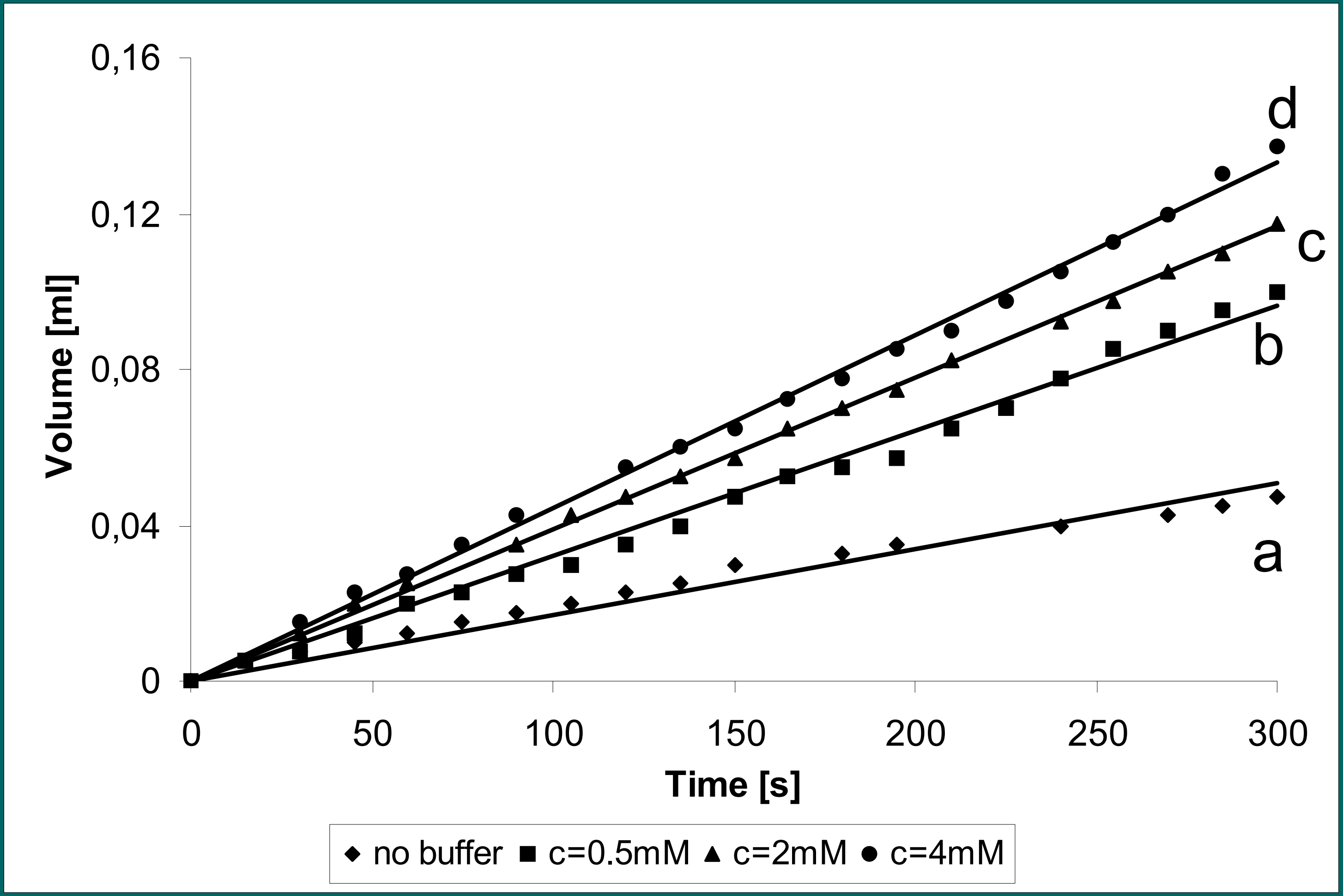
T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.



Plot of mobile phase flow rate vs. polarization voltage.

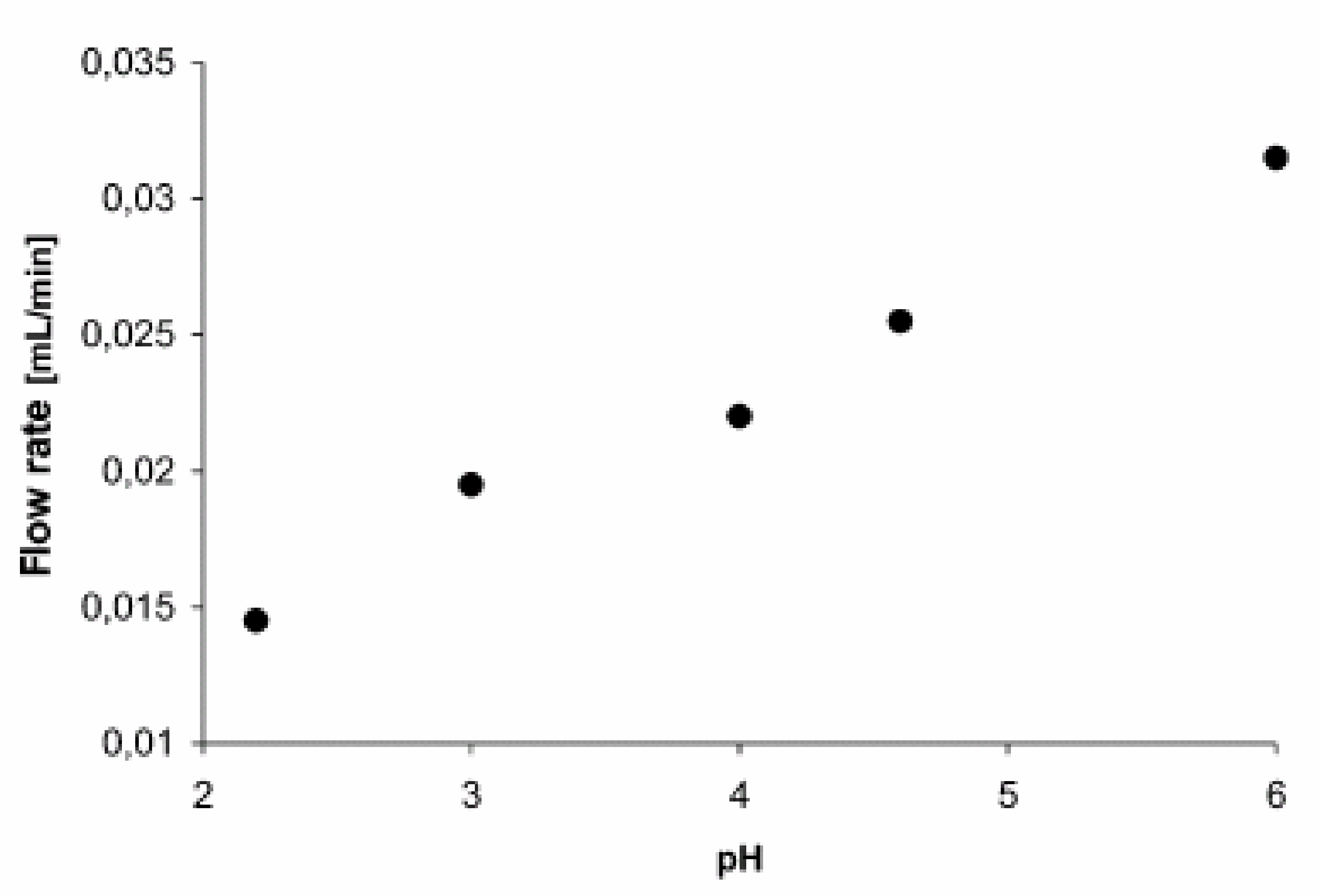
T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.

Buffer concentration and pH



Volume of the mobile phase passed through the RP18 F254s TLC plate vs time of experiment for various buffer concentrations: (a) no buffer; (b) 1.34 mM citric acid, 2.32 mM disodium hydrogen phosphate; (c) 5.37 mM citric acid, 9.26 mM disodium hydrogen phosphate; (d) 10.74 mM citric acid, 18.52 mM disodium hydrogen phosphate. pH of all buffer solutions is 4.6, 80% acetonitrile.

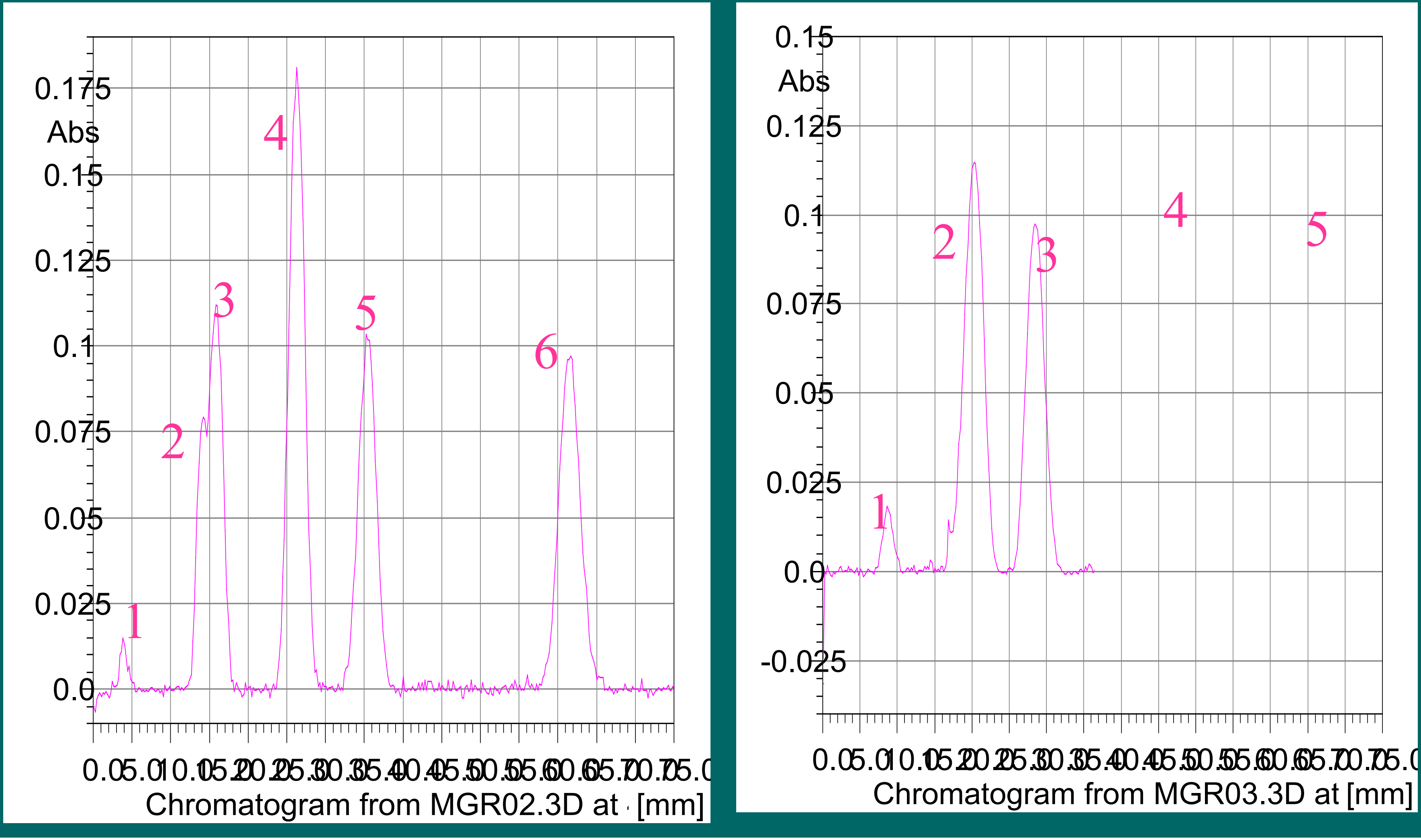
T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.



The influence of pH value of buffer solution in the mobile phase on flow rate of the mobile phase. TLC RP18 F254s plate Merck, Darmstadt). 80% acetonitrile in buffer; 2.5 kV.

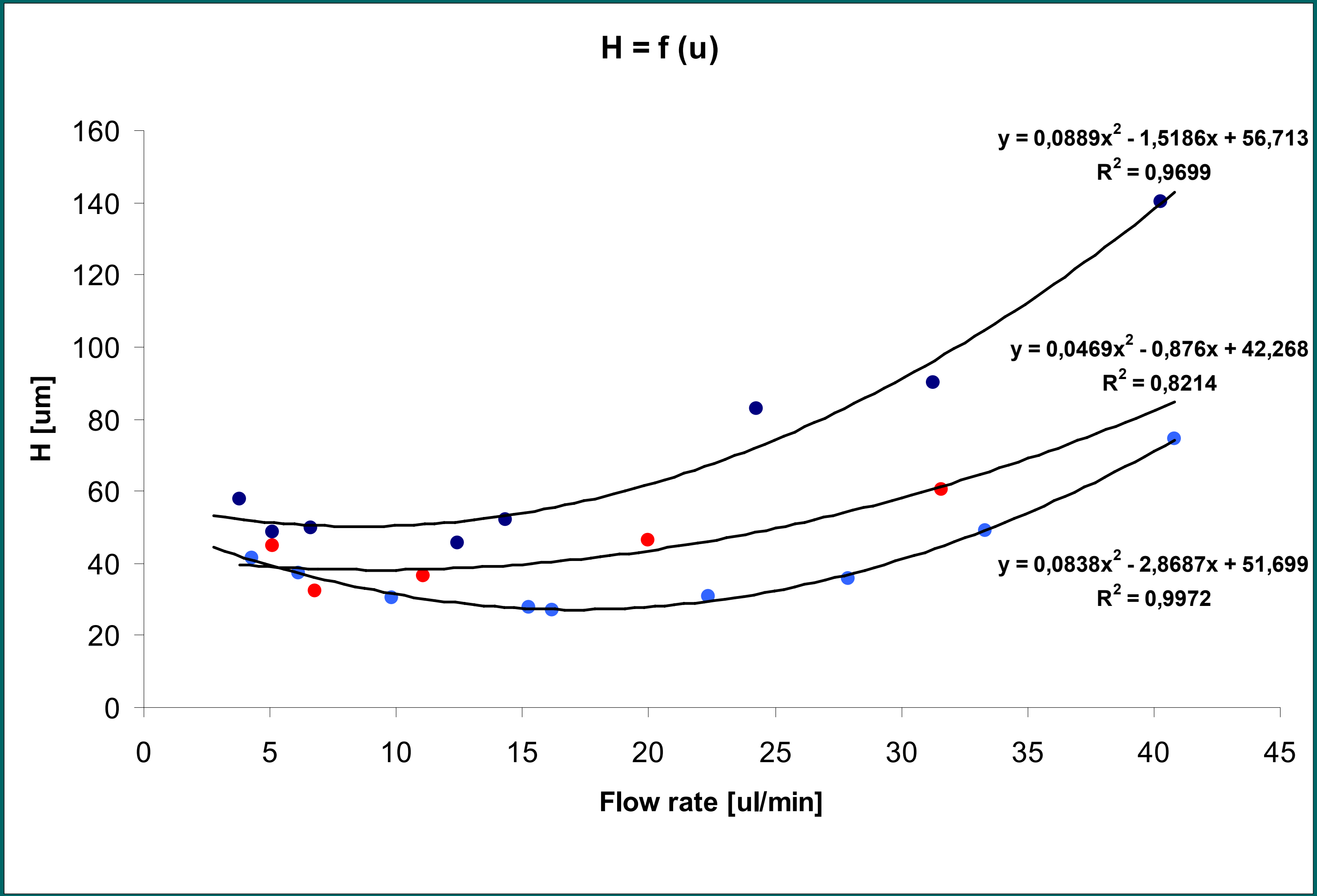
T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.

Modifier concentration

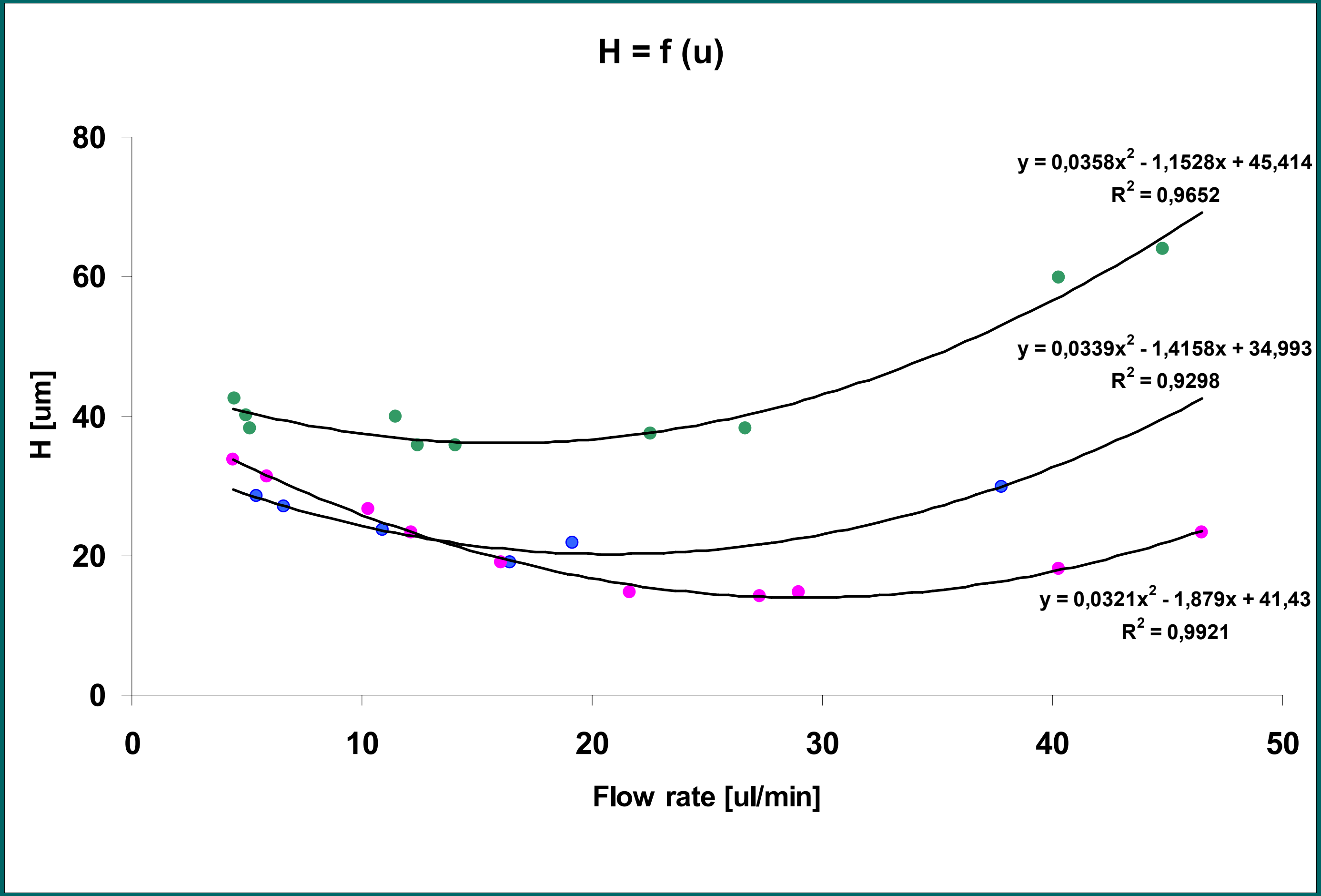


2.5 kV, (a) 80%, (b) 90 % ACN, RP-18 TLC plate (E. Merck), separation time 5 min; (1) Sudan IV, (2) 4-chlorophenylazo-2-naphthol, (3) 1-(3-pyridylazo)-2-naphthol, (4) 4-(diethylamino)azobenzene, (5) 1-(4-hydroxyphenylazo)2-naphthol, (7) 4-nitroaniline; scanned with DAD Scanner (J&M).

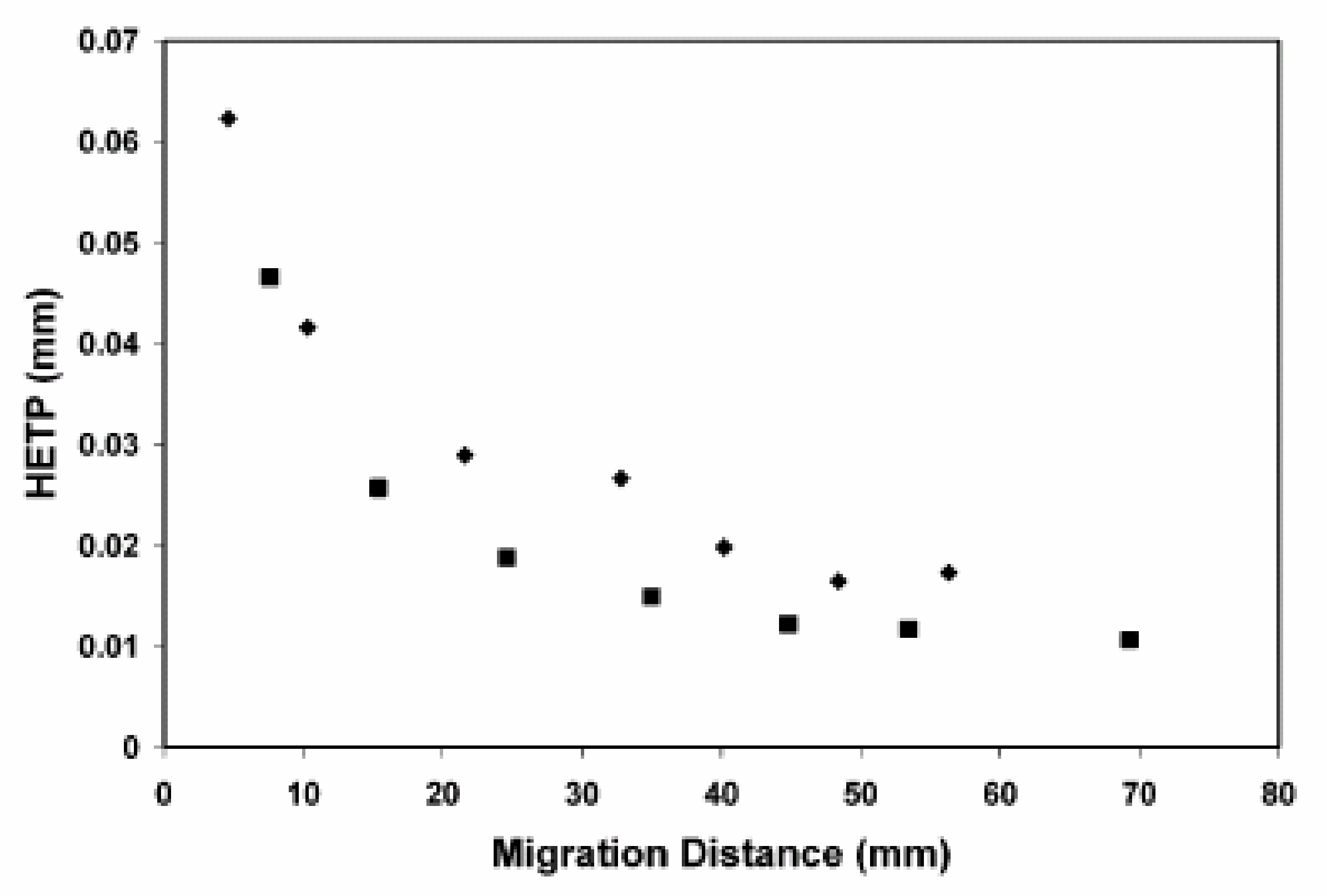
Mode of sample application



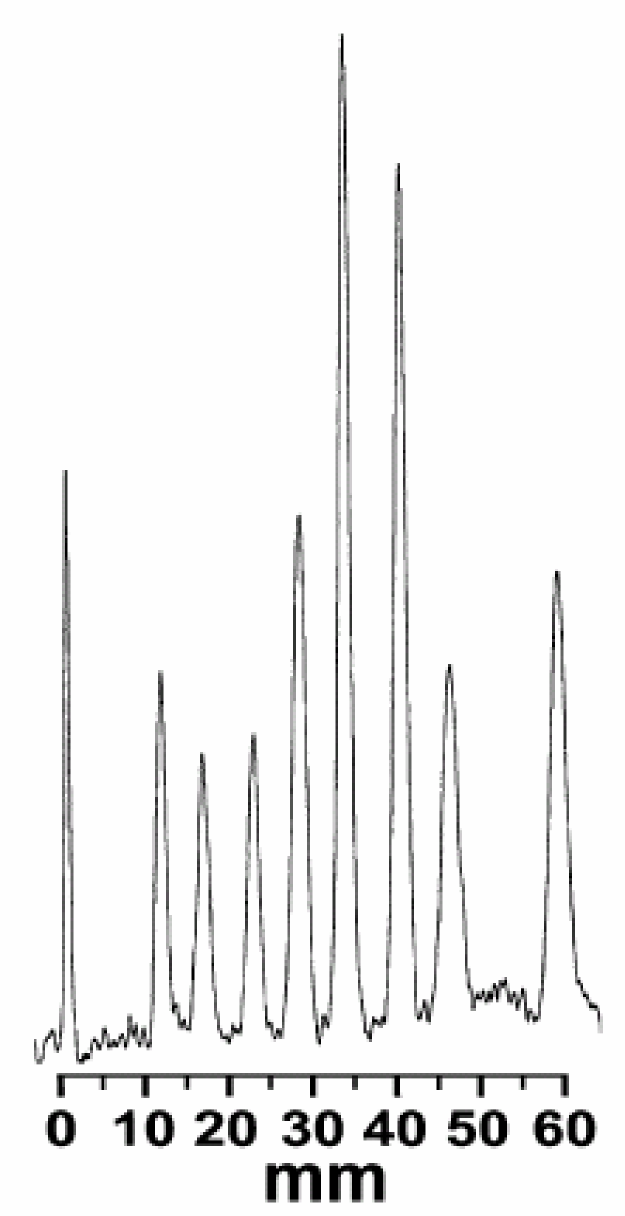
Influence of mobile phase flow rate on plate height for various modes of sample application; 80% acetonitrile in buffer, TLC RP18 F254s plate (Merck, Darmstadt).



Influence of mobile phase flow rate on plate height for various modes of sample application; 80% acetonitrile in buffer, HPTLC RP18 F254s plate (Merck, Darmstadt).

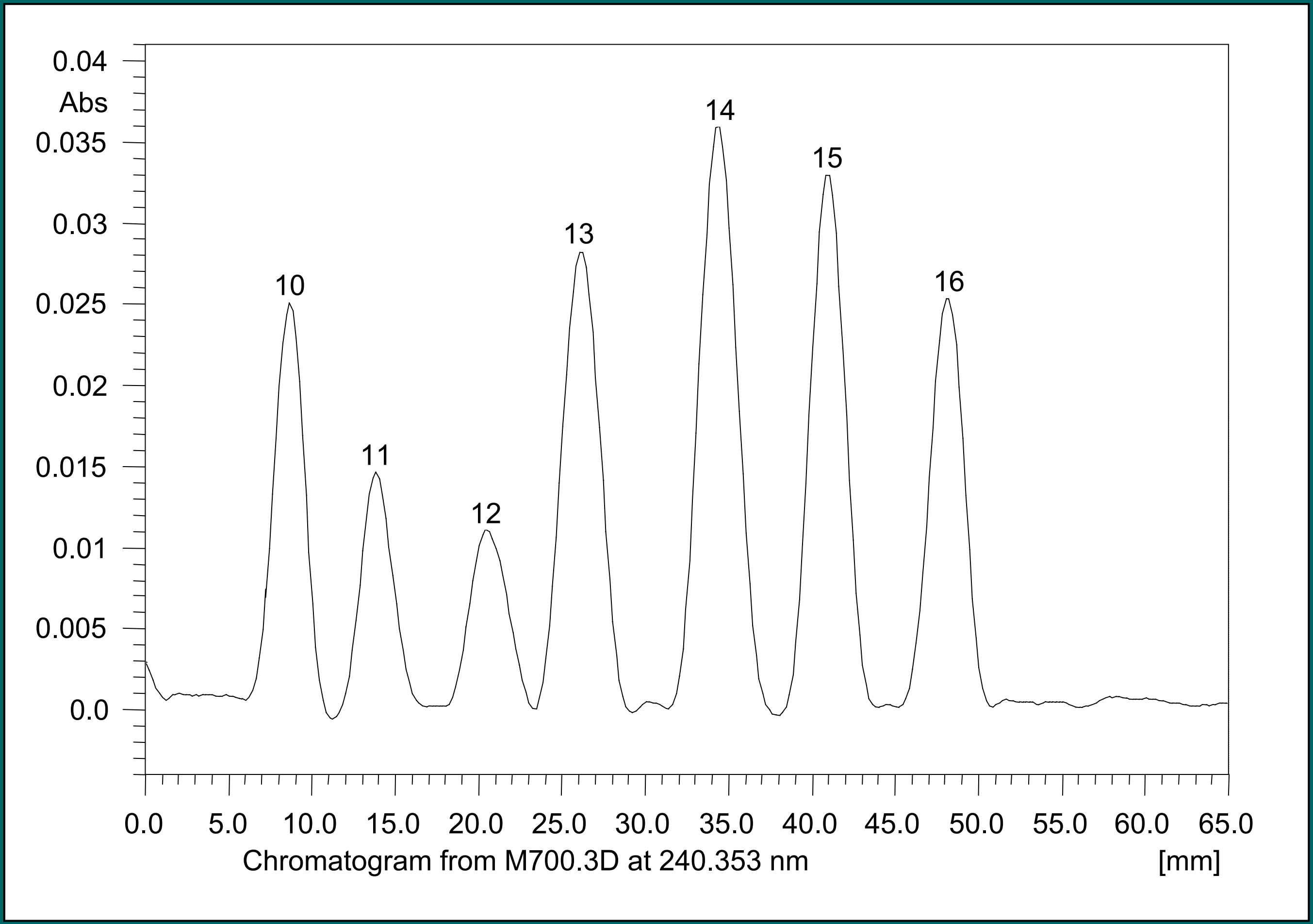


Height equivalent of a theoretical plate (HETP) for o-nitroaniline versus migration distance. The upper plot is for the regular plates and the lower plot is for the high-performance plates. D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.

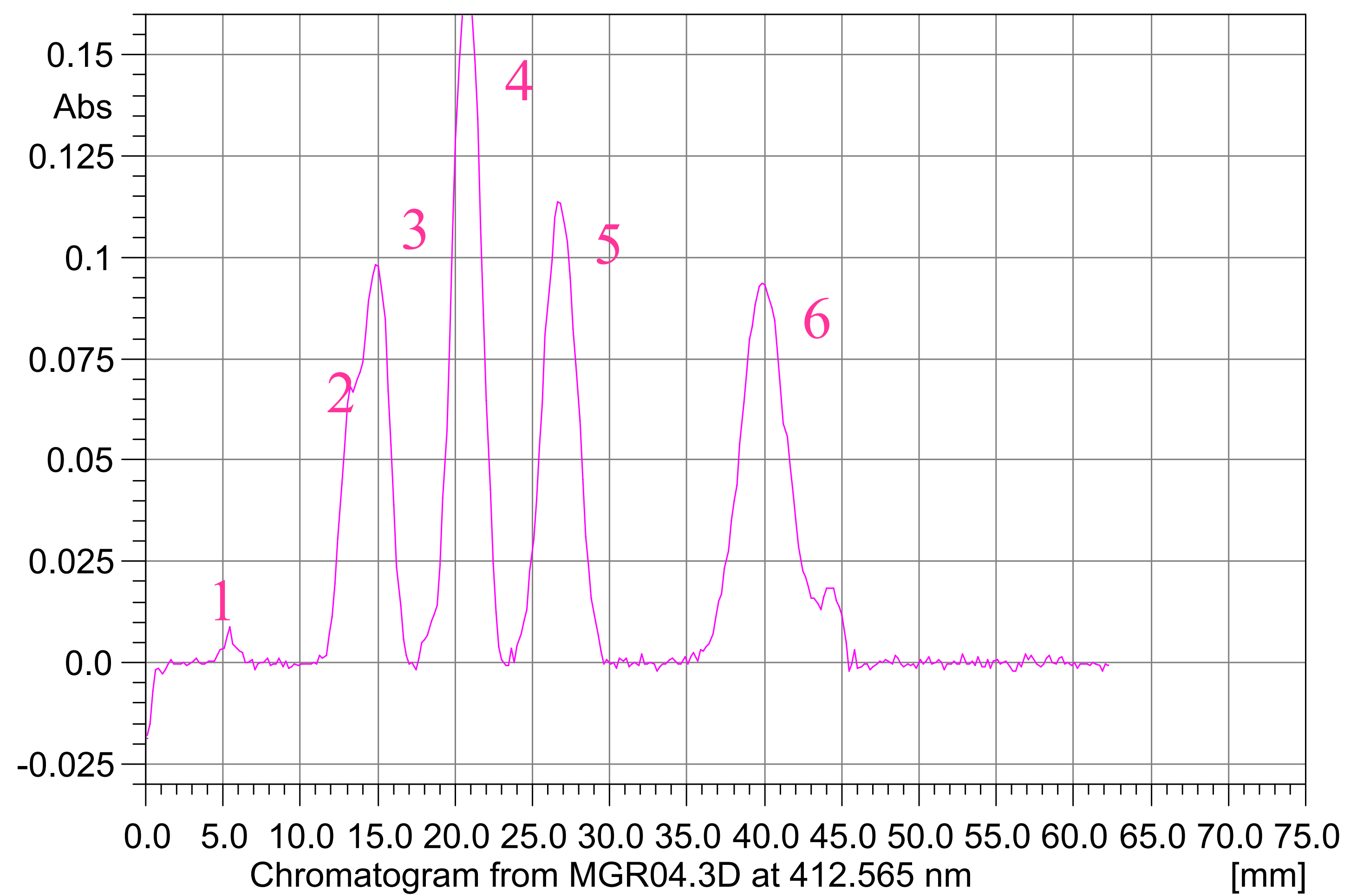


Separation of a nine-component mixture in 2 min. PPECwas performed at 9 kV and 63 atm using of 55% aqueous acetonitrile containing 5 mM acetate buffer at a pH of4.7 on a high-performance layer.

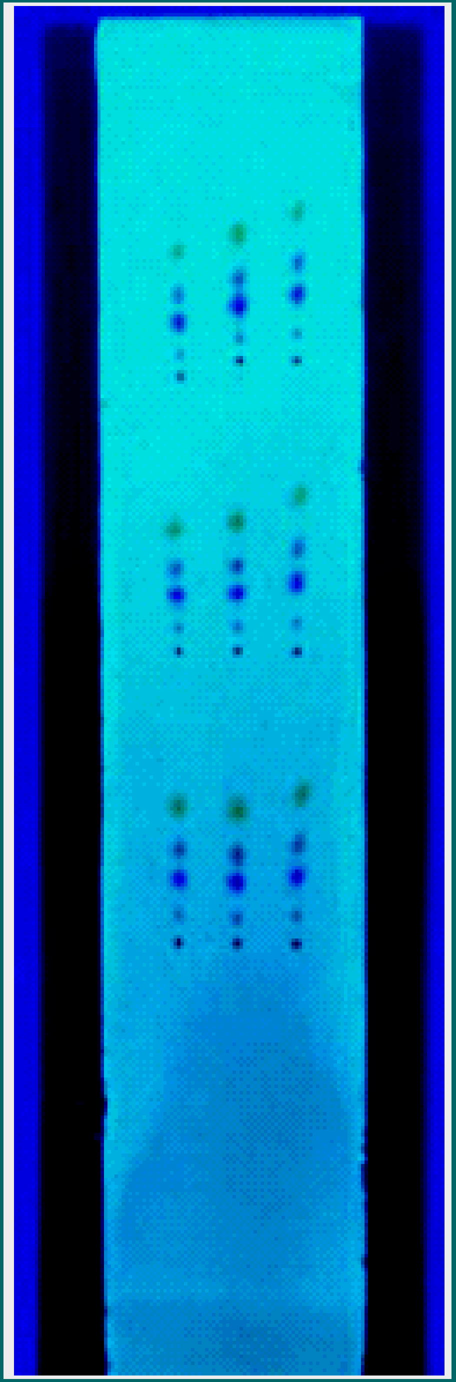
D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.



Electrochromatogram of pesticide mixture, 60% acetonitrile in water, HPTLC RP18W F254s Plate (Merck, Darmstadt)



7.5 kV, 80% ACN, RP-18 TLC plate (E. Merck), separation time 45sec; (1) Sudan IV, (2) 4-chlorophenylazo-2-naphthol, (3) 1-(3-pyridylazo)-2-naphthol, (4) 4-(diethylamino)azobenzene, (5) 1-(4-hydroxyphenylazo)-2-naphthol, (7) 4-nitroaniline, scanned with DAD Scanner (J&M).



Separation of nine samples by PPEC in 1 min on a LiChrospher C18 plate at 59 atm and 7 kV. The solutes and mobile phase are the same as those in Fig. 17

D. Nurok,* J. M. Koers, A. L. Novotny, M. A. Carmichael, J. J. Kosiba, R. Santini,*,† G. L. Hawkins, and R. W. Replogle, Anal. Chem.2004, 76,1690-1695

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