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Simple chamber for temperature-controlled planar chromatography

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Abstract

This article describes a construction of a simple developing device designed for temperature control of thin-layer chromatographic plates. The plates can be developed by the ascending technique under temperature gradient or non-gradient conditions. Saturated or unsaturated chamber conditions can be easily selected. The effects that give rise to pseudo-non-linear Van't Hoff plots, e.g. a temperature irregularity inside the chamber or heat evolving during solvent adsorption near the migrating front of the mobile phase are minimized. The preliminary temperature–retention studies show that the device is suitable for temperatures ranging from −20 to 60 °C. Using a binary mobile phase mixture (methanol–water, 70:30, v/v) the velocity of the mobile phase front on the HPTLC RP-18W plates at different temperatures was investigated. Under these conditions the retention profiles of four natural estrogens (estetrol, estriol, 17β-estradiol and estrone) were examined. The application of the described device for temperature–retention studies is also discussed.

Keywords: Instrumentation; Temperature effects; Steroids; Estrogens

1. Introduction

It is well documented that temperature can be used as a critical parameter for controlling the selectivity and the efficiency of normal and reversed-phase chromatographic systems [1,2]. Temperature–retention profiles can provide valuable information about solute conformation changes, the stationary phase transitions as well as the chromatographic retention mechanism [3]. Furthermore, the influence of temperature on the selectivity in liquid chromatographic systems increases if the mobile phase is modified with an inclusion additive [4]. However, relatively few studies have dealt with the temperature effects in planar chromatography and most of them have been performed within a narrow temperature range [5]. In contrast with the great progress in construction of the developing devices toward low eluent consumption, the problem of highly precise and reproducible plate temperature has not been successfully resolved and this topic is still poorly studied [6,7].

Using classical non-thermostated developing tanks or horizontal TLC chambers, a constant and reproducible plate temperature is obtained when the developing device is placed into the thermostatically controlled ovens or cooling cabinets. Generally, the developing chambers available on the market have a high heat capacity (e.g. glass containers) or are made of low heat transfer materials (e.g. horizontal PTFE chambers). Hence, to ensure proper plate temperature equilibrium the developing chambers must be ther-

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mostated for a long time before beginning the chromatographic experiment. Therefore, the total analysis time substantially increases when the low or high temperature regions are studied, which is a major disadvantage to this technique. The method is also not suitable for longitudinal temperature gradient separations, especially when fast temperature changes of the chromatographic plate are required. For these reasons home-made devices are still constructed and the technical problems associated with temperature-controlled thin-layer chromatography are reported [5,6,8–11].

The objective of this work is to describe a relatively simple, inexpensive, adaptable and robust developing chamber for temperature-controlled thin-layer chromatography. Some attempts have been made to determine the chromatogram developing times at different temperatures using methanol–water (70:30, v/v) mixture as a mobile phase. The capability of the described equipment for separation and thermodynamic studies has been investigated.

2. Experimental

2.1. Chemicals

Estetrol was a product of Steraloids (Newport, RI, USA). Estriol, 17β-estradiol and estrone were purchased from Sigma (St. Louis, MO, USA). Methanol (HPLC grade, 99.8%) was a product of BDH (Poole, UK) and was used without further purification. Water was purified by double distillation. The detection mixture components, cupric sulfate ($5H_2O$) and 85% orthophosphoric acid, were obtained from commercial suppliers.

2.2. Chromatography

Chromatographic experiments were performed on water-wettable RP-18W high-performance thin-layer chromatographic plates purchased from Merck (Darmstadt, Germany). The mobile phase was a methanol–water (70:30, v/v) mixture. Steroid stock solutions were prepared in pure methanol at a concentration of 1 mg ml^{-1}. The chromatographic plates were spotted with 2 μg (2 μl) of steroid standards. After methanol evaporation, the plates were placed into the developing chambers fitted with filter paper. Before each run the plates were thermostated for 10 min in a dry chamber. Then 5 ml of developing solvent were applied to the filter paper saturating the chamber with mobile phase vapor. The chamber was left to stand for 10 min to ensure the proper temperature and saturation equilibrium.

The plate temperature was controlled with an accuracy of ±0.01 °C using a battery of modified Dewar flasks (Fig. 1) connected to a Polystat digital circulating thermostat (Model 12101-15; Cole Parmer, Chicago, IL, USA). As a circulating fluid a mixture of ethylene glycol–water (1:1, v/v) was applied. Experiments were performed at temperatures from −20 to +60 °C in 10 °C increments.

The steroid spots were visualized by spraying the plates with a mixture composed of cupric sulfate, orthophosphoric acid and water (10 g, 5 ml and 95 ml, respectively). After heating the plates at 130 °C for at least 5 min the estrogens were apparent as red or gray spots on a white background. The

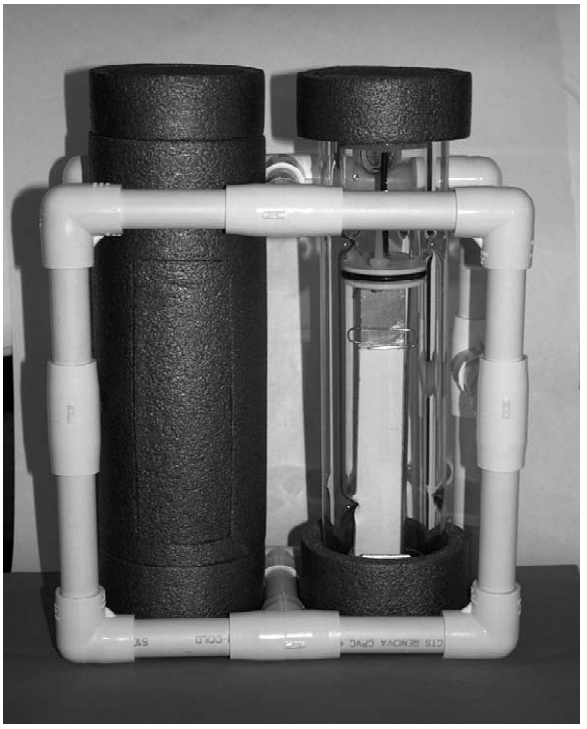

Fig. 1. The battery of the thermostated developing chambers for conventional TLC.

retardation factor (R_F) and the rate of mobility factor (R_M) values were calculated in the usual manner and were based on the average of at least five independent determinations of each solute.

3. Results and discussion

3.1. General description

The temperature-controlled developing system is based on the Dewar flasks (Fig. 1). Chromatographic plates are placed into the chambers with vertical position. Chamber temperature is controlled using an external circulating thermostat. The circulating fluid maintains a constant temperature inside the chamber. Typically, the system can be arranged to work with up to 10 modules. However, the temperature stability is better for the system composed of five or less units and depends strongly on length and insulation quality of the connection pipes and on cooling/heating rate of the circulating thermostat.

Each chamber module consists of external insulation, glass Dewar flask, chromatographic plate holder, mobile phase container and mobile phase injection pipes. The last three components (plate holder, eluent container and injection pipes) are integrated as one removable unit. Fig. 2 illustrates a schematic diagram of the chamber module. The external insulating jacket is made of polyurethane foam tube. This component increases temperature stability and prevents the external chamber surfaces from water and hoarfrost condensation at low temperatures. The chamber is equipped with two lids. The external cover is part of the external foam jacket. The internal lid sealed by a silicone o-ring is made of PTFE and regulates the working volume of the chamber. The internal lid sits below the external cover at ~25% of the total chamber height. This minimizes the temperature differences inside the working volume of the chamber. Fig. 3 shows construction details of the chromatographic plate-supporting structure, which is mounted directly to the internal lid. The plate holder is made of aluminum and is equipped with a glass eluent container. Additionally, the lid contains two stainless-steel injection pipes, which are used for chamber saturation prior to chromatographic development

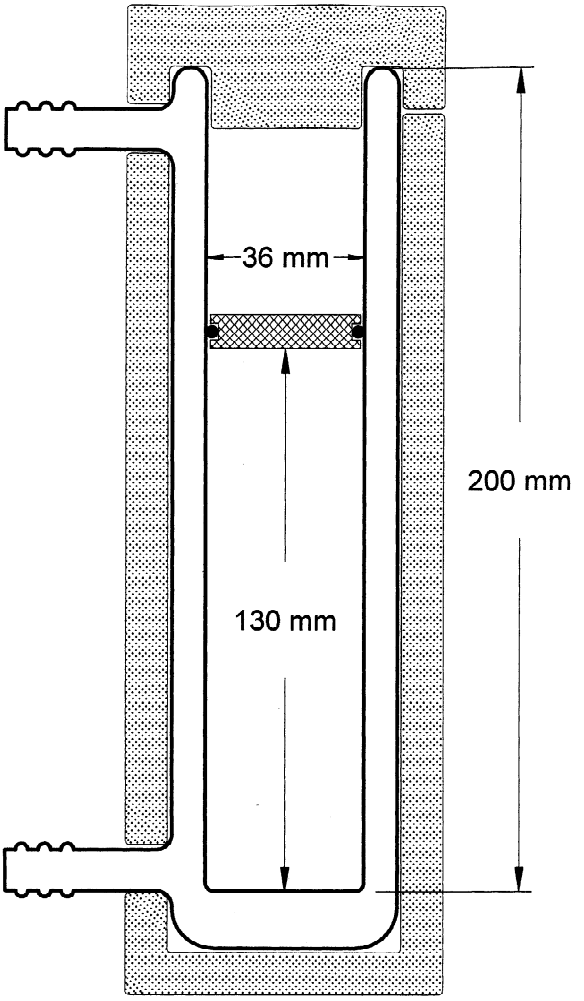

Fig. 2. Section drawing of the chamber unit.

(short pipe) and for mobile phase injection (long pipe). Using this simple capillary system, the running solvent (typically 1 or 2 ml) can be injected after the chamber is saturated with mobile phase vapors. To minimize temperature differences inside the chamber it is recommended that the mobile phase should be thermostated before injection.

3.2. Temperature–retention studies

To demonstrate the equipment capability for retention–temperature studies a mixture of estetrol, estriol, 17β-estradiol and estrone was chromatographed across a wide range of temperatures

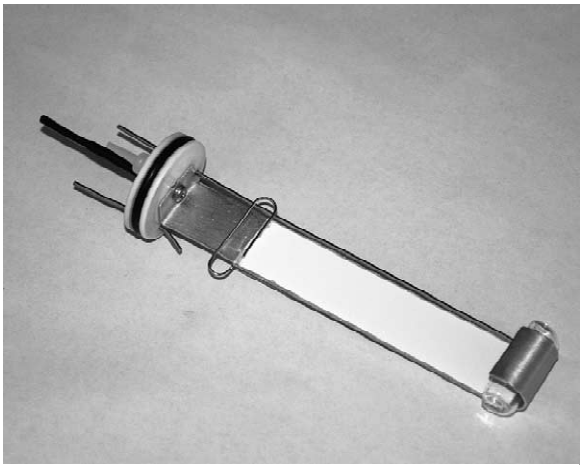

Fig. 3. Perspective view of the chromatographic plates' support.

(−20 °C to +60 °C). The mobile phase was composed of 70% (v/v) methanol in water. The components of interest were selected because of their linear retention–temperature behavior under column reversed-phase chromatography conditions [3]. The data presented in Fig. 4 indicate that a decrease in temperature causes a substantial increase to the retention of steroids. The relationship, between the retention parameter of solutes (R_M) and the reciprocal of absolute temperature ($1/T$), is linear. This observation confirms that the problems of pseudo-non-linear Van't Hoff plots, mainly associated with temperature irregularities inside the chamber (''distillation process'') or with a heat evolved during

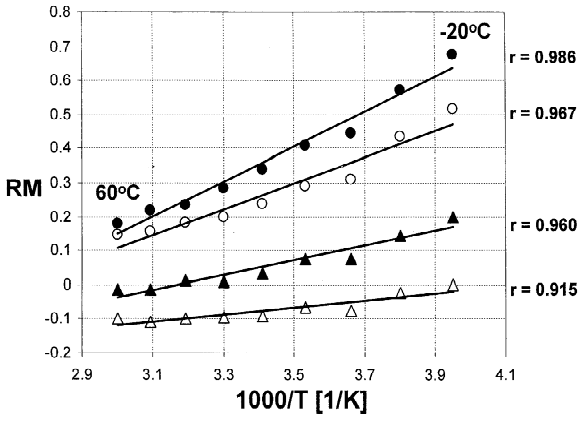

Fig. 4. The Van't Hoff plots of the investigated steroids; estetrol (△); estriol (▲); estrone (○); 17β-estradiol (●).

solvent adsorption near the migrating front of the mobile phase are considerably reduced [8]. It is noteworthy that the selectivity of the chromatographic system increases consistently as temperature decreases. Moreover, the spot sizes of the solutes are the same at the whole range of temperatures investigated and below 20 °C complete and baseline separation of the steroid mixture is observed.

From a practical point of view, the optimum chromatographic separation is a compromise between maximum resolution and minimum analysis time. In classical planar chromatography the total analysis time is the same for all solutes and the solutes' mobility is driven by non-forced flow of the eluent through capillary action. Principally, the running time is strongly affected by the developing distance, the degree of saturation of the vapor phase, the particle size of the stationary phase and the viscosity of the mobile phase. The eluent viscosity depends on the mobile phase composition and the temperature. Usually, higher temperatures significantly reduce the time needed for an analysis, due to changes in viscosity of the mobile phase. However, it is not easy to predict how the movement of the mobile phase will slow down for a given temperature and chromatographic system.

Fig. 5 illustrates the changes in the eluent front migration times at different temperatures and front distances. The developing distance ranges from 2 to 7 cm and each contour line on the map corresponds to 30 min of the developing time. As can be seen, developing times below 1 h are observed for the wide range of temperatures investigated. Considering that high separation power of modern TLC plates enables an effective separation at 5-cm developing distance, the sample can be analyzed at relatively short developing times, even at low temperatures. Furthermore, it is expected that developing times can be substantially reduced for a number of less viscous organic solvents such as acetonitrile, acetone, tetrahydrofuran and their binary mixtures with water.

4. Conclusions

The chamber and the plate holder design allow highly accurate and stable chromatographic plate temperature control. The system provides reproduc-

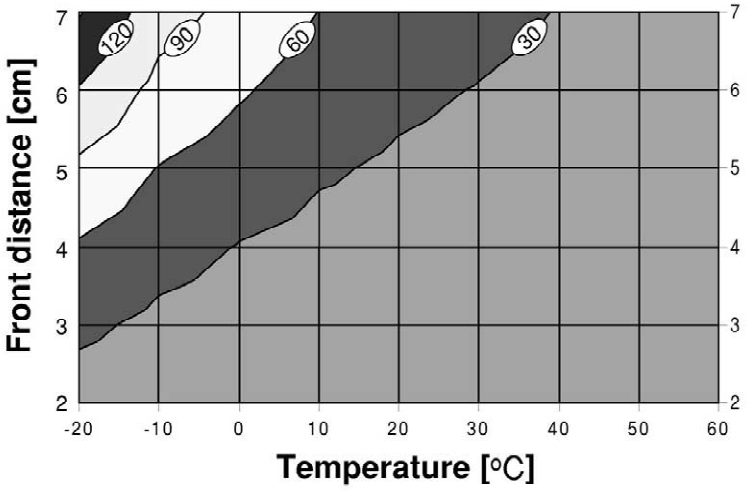

Fig. 5. The contour map of the solvent front migration times at different solvent front distances and temperatures using methanol–water (70:30, v/v) mobile phase and HPTLC RP-18W plates. The spaces between contour lines correspond to 30 min.

ible results at low and high temperature regions and can be a reliable alternative to other developing systems. It is noteworthy, that to ensure the proper internal temperature equilibrium the device should be thermostated for a short time before beginning the chromatographic experiment, therefore the total analysis time can be significantly reduced. Moreover, the injecting system allows minimal solvent consumption, similar to horizontal developing devices. The dimensions of the chamber module can be adapted to different plate sizes, depending upon particular needs. In terms of the practical application, the described developing system is easy to use, adaptable and can be applied as an effective tool for temperature–retention studies using the planar chromatography technique. The system has been successfully applied in improving the separation of a natural estrogen mixture.

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References

[1] J.W. Dolan, L.R. Snyder, N.M. Djordjevic, D.W. Hill, T.J. Waeghe, J. Chromatogr. A 857 (1999) 1.
[2] G. Herbut, J.S. Kowalczyk, J. High Resolut. Chromatogr. 4 (1981) 27.
[3] L.A. Cole, J.G. Dorsey, K.A. Dill, Anal. Chem. 64 (1992) 1324.
[4] P.K. Zarzycki, R. Smith, J. Chromatogr. A 912 (2001) 45.
[5] P.K. Zarzycki, H. Lamparczyk, Chem. Anal. (Warsaw) 46 (2001) 469.
[6] J. Sherma, Anal. Chem. 72 (2000) 9R.
[7] Camag Bibliography Service, Planar Chromatography, Camag, 1997.
[8] P.K. Zarzycki, J. Planar Chromatogr. 14 (2001) 63.
[9] L. Lepri, V. Coas, P.G. Desideri, L. Checchini, J. Planar Chromatogr. 3 (1990) 311.
[10] H.T.K. Xuan, M. Lederer, J. Chromatogr. A. 659 (1994) 191.
[11] T.H. Dzido, J. Planar Chromatogr. 14 (2001) 237.