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Simple horizontal chamber for thermostated micro-thin-layer chromatography

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Abstract

The aim of presented work is to describe simple, fast and robust temperature-controlled system for non-forced-flow micro-planar chromatography. With this separation system the micro-TLC plates can be developed in horizontal position under temperature gradient or non-gradient as well as saturated or unsaturated chamber conditions using low amount of mobile phase ranging from 0.3 to 1.0 mL. The device may work at wide range of temperatures from −20 to 80 °C. Under such conditions the plate temperature equilibration can be obtained within 5–12 min and a typical non-forced flow run can be finished within short period of time ranging from 5 to 20 min. It has been revealed that micro-plate is capable to separate more than 10 spots in one direction or up to 180 spots per plate for two-dimensional and multi-development runs. Particularly, fast and efficient separation of number of analytes including fullerenes, cyclodextrins and steroids as well as complex samples obtained from natural products and pharmaceutical formulations was demonstrated. Moreover, the application of thermostated micro-planar chromatography for the retention and quantification studies is also discussed.

Keywords: Temperature; Thermostated chamber; Planar chromatography; Two-dimensional chromatography; Solid phase extraction; Fullerenes; Steroids; Estrogens; Testosterone; Macrocycles; Cyclodextrins; Herbs extracts

1. Introduction

In recent years, the role of planar chromatography, particularly, high-performance thin-layer chromatography (HPTLC) is systematically increasing [1–4]. The main advantage of HPTLC over other specific liquid mobile phase separation techniques including high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) is that the plate is able to separate a number of samples concurrently within a relatively short analytical run. Moreover, unlike column chromatography, the planar counterpart can work easily as fast and non-expensive two-dimensional method (2D-TLC). This usually significantly increases the plate capability toward efficient separation of complex samples. In practice typical separation power of non-forced flow rate HPTLC systems lies between 10 and 20 spots per plate in one direction. However, working under 2D-TLC mode the number of spots separated can significantly increase even one factor more. Depends on the plate size, solvent viscosity and temperature the results of HPTLC separation can be obtained within short period of time even less than 5 min. It is noteworthy to mention that recently, number of new detection methods like direct analysis in real time (DART) involving mass spectrometry (MS) techniques was introduced [5]. Such MS-based sophisticated analytical tools including matrix-assisted laser desorption/ionization mass spectrometry (TLC–MALDI-MS) or electron impact ionization mass spectrometry (TLC–EI-MS) were successfully applied for analysis of complex biological samples allowing the use of planar chromatography in metabolomic studies [6–8]. Using modern HPTLC plates the eluent developing distance can be reduced to less than 50 mm. This idea is based on the observation that minimum values of the plate height parameter (H) can be achieved if the solvent migration distance along the HPTLC plate is ranging from 30 to 40 mm [3]. Therefore, it can be expected that within and close to this migration distance range more dense spots can be

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obtained. In practice, under such conditions efficient separation for number types of compounds were observed, whereas total analysis time was dramatically reduced in comparison to chromatographic separation performed on 10 or 20 cm long TLC plates [3,9–11].

It should be noted that from its nature, isocratic planar chromatography has also a great capability to separate mixtures composed of low retarded components of interest. This problem can be simply explained taking into account non-linear relationship that exists between column (k) and planar (R_F) retention parameters and is described by the equation:

$$\log k = \log\left(\frac{1}{R_F - 1}\right) = R_M \quad (1)$$

where k = (analyte retention time − column dead time)/column dead time; R_F = spot migration distance from the origin/eluent front migration distance from the origin, and R_M is called mobility factor and this chromatographic retention parameter is frequently used for the structure–retention studies based on TLC data [12].

One of the interesting consequence of such non-linear interdependence between raw k and R_F retention parameters is high capability of planar systems to separate complex mixtures composed of relatively weakly retarded analytes ($k < 10$). This can be easily demonstrated through *e.g.* graphical representation of Eq. (1) [13]. Particularly, within low retarded group of analytes chromatographed under similar experimental conditions (the same type of stationary phase and mobile phase composition) a regular distribution of spots on the plate corresponds to strong irregular dispersion of peaks on the chromatogram generated by the column method. Such approach was recently used for efficient separation of series of natural estrogenic compounds separated on octadecylsilica coated and water tolerable HPTLC layers [13].

From practical point of view, one of the important and critical parameters for controlling the selectivity, efficiency and reproducibility of any separation system is temperature. The relationship between retention and temperature is known as the Van 't Hoff plot and is characterized by Eq. (2).

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{RT} + \ln \phi \quad (2)$$

where k, ΔH°, ΔS° and ϕ corresponds to retention factor, enthalpy change, entropy change and phase ratio of the separation system, respectively. The temperature retention profiles can provide valuable information about solute conformation changes, the stationary transitions and the chromatographic retention mechanism. Most recently, temperature driven separation based on liquid chromatographic systems seems to be an interesting alternative to more complicated gradient methods designed for separation of complex biosamples [13–18].

Interestingly, in term of practical application the TLC systems are very suitable for the separation at different temperatures. This is mainly because of the low flow rate and small amount of the mobile phase that is necessary to perform the separation process. Moreover, there is no Joule's heat evolved due to electric current flow as in planar or column electrophoresis systems [19,20]. Hence, to ensure proper plate temperature the cooling/heating devices can work with great precision and consume much less energy. For the majority of applications a constant and reproducible plate temperature may be easy obtained when the developing unit is placed directly into the thermostatically controlled ovens or the developing system based on the Dewar flask that is connected to the external circulating thermostat [21,22].

Generally, the glass containers or horizontal PTFE chambers available on the market have a high heat capacity or are made of low heat transfer materials. Hence, to ensure the proper plates temperature equilibrium the developing units must be thermostated for a long time before beginning of 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 the fast temperature changes of the chromatographic plate are required. For these reasons laboratory-made devices are still constructed and the technical problems associated with analytical and preparative temperature controlled thin-layer chromatography are subsequently reported [23–31].

This work is a continuation of the earlier contribution concerning temperature controlled planar chromatographic device [29]. The main goal of the present work is to describe the construction, operational properties and practical applications of simple and non-expensive thermostated horizontal microchamber unit working within wide range of sub-ambient and elevated temperatures. The capability of the described device for fast, robust and effective separation and quantification of number class of compounds using one and two-dimensional micro-planar chromatography has been demonstrated.

2. Experimental

2.1. Chemicals

Pure analytical standards of fullerenes (C60 > 99% and C70 > 98%) were obtained from TCI (Tokyo Kasei Kogyo, Japan). Native β-cyclodextrin was product of Fluka (Buchs, Switzerland). β-Cyclodextrin derivatives including methyl-β-cyclodextrin, heptakis(2,6-DI-*O*-methyl)-β-cyclodextrin and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin were purchased from Sigma (St. Louis, MO, USA). Steroids standards including testosterone, methyltestosterone as well as testosterone propionate, isobutyrate, phenylpropionate, isocaproate, enanthate and caprate were obtained from Polfa (Jelenia Góra, Poland). Estetrol was a product of Steraloids, USA, whilst estriol, β-estradiol and estrone were obtained from Aldrich. Spirulina 450 mg capsules (*Spirulina Maxima*) was product of A–Z Medica, Gdańsk, Poland. Herbs extracts including Intractum Visci (Visci herbae intractum) and Azucalen (Chamomillae extractum fluidum and Calendulae extractum fluidum, 1:1) were obtained from PhytoPharm (Klęka, Poland) and Herbapol (Wrocław, Poland), respectively.