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Solvent Front Position Extraction procedure with thin-layer chromatography as a mode of multicomponent sample preparation for quantitative analysis by instrumental technique

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ABSTRACT

A concept of using thin-layer chromatography to multicomponent sample preparation for quantitative determination of solutes followed by instrumental technique is presented. Thin-layer chromatography (TLC) is used to separate chosen substances and their internal standard from other components (matrix) and to form a single spot/zone containing them at the solvent front position. The location of the analytes and internal standard in the solvent front zone allows their easy extraction followed by quantitation by HPLC.

1. Introduction

Analysis of samples containing numerous components presents a major challenge in modern, especially quantitative analysis and simultaneously has become one of the most interesting topics for analytical chemists in the last years in clinical chemistry, environmental or clinical analysis [1,2]. Different analytical techniques are designed to quantitatively determine specific components in a mixture including: chromatography, electrophoresis, electrochromatography, spectrophotometry [3–8]. The majority of samples encountered in laboratory practice cannot be directly placed into these analytical instruments. The analyses are often only possible after time-consuming sample preparation consisting of a number of operations depending on the type of sample and identification technique used that are applied to (a) release of the drug from biological matrix, (b) improvement in sensitivity and/or (c) removal of sample components that can damage sensitive instrument hardware such as HPLC/CEC columns and mass spectrometer ion sources [9–11].

In our previous paper [12] we proposed a novel concept of sample preparation, Frontally Eluted Components (FEC), which is based on liquid-solid phase extraction of analytes from an adsorbent layer of the chromatographic plate, followed by its analysis with instrumental techniques [13,14]. Frontally Eluted Components procedure retains SPE advantages, however, it is cheaper and leads to considerably enhanced purification of a sample than by conventional SPE. This is due to the fact that thin-layer chromatography plate is used to completely separate substances and its internal standard from other components of a sample contrary to other sample preparation techniques e.g. SPE [15–17], QuEChERS [18], liquid-liquid extraction [19–21]. The main idea of the FEC technique is that after the final stage of the thin-layer chromatogram development the target substances and the internal standard form a single zone at the solvent front position. The concept of the FEC mode is based on the assumption that substances of interest show equal solute dispersion in the band at solvent front if their retardation is comparable. This concept has been confirmed for very simple serum sample, which comprised analyte (acetaminophen) and internal standard (acetanilide) [12]. The precise location of the analyte and the internal standard band in the solvent front position allows their easy extraction followed by quantitation by the HPLC. No marker and no UV lamp is necessary in this stage of the FEC procedure what is contrary to that proposed by Oellig et al. [13,14]. The quantitative results and the validation parameters of acetaminophen determination obtained using the FEC procedure followed by the HPLC were comparable to the direct HPLC analysis. Relative standard deviations was below 5%.

Therefore, based on theoretical considerations and the preliminary results presented in the paper mentioned it is worth of investigation if the method can be applied to quantitative determination of a more complex sample composed of substances showing

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different properties including retardation. In this paper we present our last results for simultaneous determination of substances in the multicomponent (acetaminophen, aminophenazone, theophylline, caffeine, acetanilide, ciprofloxacin, tramadol, acetylsalicylic acid, acebutolol) sample, which was prepared with thin-layer chromatography procedure to quantitation by instrumental technique (HPLC).

In the light of the results presented in former article one can observe that analyzed substance and internal standard migrates with the front of the mobile phase. It was the reason that we named the method Frontally Eluted Components. Further research has shown that in the case of samples containing a large number of substances it is not possible to achieve migration of all substances with the front of the mobile phase. Their presence on the solvent front zone was achieved by developing the chromatogram several times. In light of these facts we think that the more appropriate name of the presented method could be Solvent Front Position Extraction (SFPE). This name will be used in the next part of the text.

2. Experimental

2.1. Materials and reagents

Chromatographic plates, TLC RP-18 F254 and HPTLC Silica gel 60 F_{254}, 10 × 20 cm, were supplied by Merck (Darmstadt, Germany). Methanol of analytical grade, methanol and acetone for HPLC were purchased from POCh (Gliwice, Poland). The deionized water was produced in the laboratory with Hydro-Lab demineralizer (Gdansk, Poland). Citric acid monohydrate was supplied by Merck (Darmstadt, Germany), and disodium hydrogen phosphate by Standard (Lublin, Poland). Buffer solutions were prepared by mixing 0.1 M solution of citric acid and 0.2 M solution of disodium hydrogen phosphate in the 3.86:1 volume ratio and then diluted with deionized water. Acetaminophen, acetanilide, aminophenazone, caffeine, acetylsalicylic acid, theophylline, bromocresol green, azorubin, 1-aminoanthraquinone were purchased from Sigma–Aldrich. Acetobutolol was purchased from Biomedicals, Ohio, ciprofloxacin from Sreepathi Pharmaceutical Ldt. India, Tramadol from Inogent Laboratories, India. Syringe PTFE membrane filter, pore size 0.22 μm, diameter 13.0 mm, were purchased from POCh (Gliwice, Poland). Bovine serum was purchased from Biomed, Lublin.

2.2. Preparation of internal standard and analyte solutions

The stock solutions were prepared by dissolving appropriate amounts of each substance in methanol and stored in a refrigerator at 4 °C. Solutions of the substances mentioned were prepared by adding 10 μL of each stock solution to 1 mL of serum and methanol and finally were in the range of substances concentrations typically found in real blood/serum samples (Table 1).

Table 1
The values of substance concentration in the samples.

	Concentration in samples (mg/L)
Acebutolol	53.7
Aminophenazone	62.8
Acetaminophen	62.0
Caffeine	82.6
Theophylline	82.6
Tramadol	40
Ciprofloxacin	8.3
Acetylsalicylic acid	289.3

2.3. Instrumentation

Thin-layer chromatography experiments were performed using the horizontal DS chamber for TLC (model DS-II–5 × 10 from Chromdes, Lublin, Poland). For plate image documentation, the TLC Visualizer, CAMAG (Muttenz, Switzerland), was used. The CAMAG TLC–MS interface, connected with Agilent 1260 Infinity Isocratic pump, was used for extraction the substances. The Agilent1290 Infinity LC System (Santa Clara, United States) equipped with DAD detector was used for the HPLC experiments. The chromatography was performed with the Zorbax Eclipse Plus-C18 column (4.6 × 100 mm, 3.5 μm).

2.4. HPTLC plate preparation

HPTLC Silica gel 60 F_{254} 10 × 20 cm plates were cut into 2.5 × 10 or 2.5 × 5 cm pieces using TLC plate cutter (CAMAG). Before chromatogram development the plates were washed by immersion in methanol for 1 min. Afterwards the plates were dried in the air and activated in an oven at 105–110° C for 15 min.

2.5. Application of the samples on the HPTLC plate

The samples were applied as a small single drops on the chromatographic plate by using automatic pipette (PZ HTL S.A., Poland). The volume of drop was about 10 μL. In some experiments the autosampler applicator Linomat 5 (CAMAG, Muttenz) was used – the volume of sample solution was 10 μL.

2.6. Planar chromatogram development

On chromatographic plates face up the chromatograms were developed with methanol to a fixed distance in the 40–45 mm range. Several chromatogram development strategies using the horizontal DS chamber were examined, see also Fig. 1: unidimensional four times developments along longer edge of the chromatographic plate (P4), unidimensional five times developments along longer edge of the chromatographic plate (P5), two consecutive developments from two opposite sides along the shorter edge and to the middle of the plate, next unidimensional four times developments along longer edge of the chromatographic plate (SP4), two consecutive developments from two opposite sides along the shorter edge and to the middle of the plate, next uni-

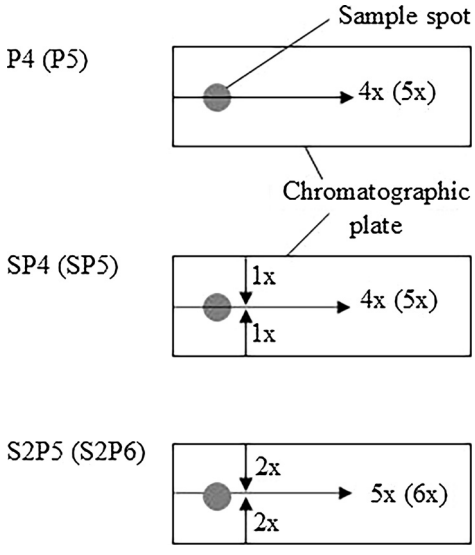

Fig. 1. Scheme of chromatograms development procedures.

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