Journal of Chromatography A, 1436 (2016) 19–27

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Frontally eluted components procedure with thin layer chromatography as a mode of sample preparation for high performance liquid chromatography quantitation of acetaminophen in biological matrix

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ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 20 January 2016
Accepted 20 January 2016
Available online 24 January 2016

Keywords:
Sample preparation
Frontally eluted components
Matrix effects

ABSTRACT

A new concept of using thin-layer chromatography to sample preparation for the quantitative determination of solute/s followed by instrumental techniques is presented Thin-layer chromatography (TLC) is used to completely separate acetaminophen and its internal standard from other components (matrix) and to form a single spot/zone containing them at the solvent front position (after the final stage of the thin-layer chromatogram development). The location of the analytes and internal standard in the solvent front zone allows their easy extraction followed by quantitation by HPLC. The exctraction procedure of the solute/s and internal standard can proceed from whole solute frontal zone or its part without lowering in accuracy of quantitative analysis.

1. Introduction

The sample preparation is an essential part of instrumental analysis. The majority of samples encountered in a laboratory practice is not in a form to be directly placed into the analytical instruments. The preparation process is intended to provide a representative, reproducible, and homogenous solution that is suitable for injection into an equipment for high performance liquid chromatography (HPLC) and/or mass spectrometry (MS) instruments. The primary goal of sample preparation is to isolate one or several target analytes from the other components of the sample mixture (matrix). A sample pre-treatment procedure should provide quantitative recovery of analytes, involve a minimum number of steps, and (if possible) is susceptible to automation [1]. The use of internal standard and standard addition method aids in better quantitation, when recovery of analyte is not complete [2]. Several analytical techniques have been established for the purifications and quantification of compounds in samples. Nowadays commonly used sample-preparation methods are liquid–liquid extraction (LLE) [3,4] and solid-phase extraction (SPE) [5,6]. Related procedures have been also described such a fast, solventless solid phase microextration (SPME) [7,8], microextraction in packed syringe (MEPS) [9,10]. The popular method of blood sample preparation for evaluating newborn screening test, preceding HPLC analysis, is dried blood spot (DBS) [11–13]. The one group of sample preparation methods is the planar chromatography technique. In the past, after the sample separation on the chromatographic plates, the spot containing substances of interest were scraped off from the thin-layer chromatography/high-performance thin-layer chromatography (TLC/HPTLC) plate, extracted into vials and transferred to further instrumental analysis [14–16]. This sample preparation process was time and labor consuming and characterized by low repeatability because it was not possible to completely scrap the whole solute zone from the chromatographic plate. The popular method for measuring thin-layer chromatograms without destroying them based on the transmission and/or reflectance of light by the layer is the scanning densitometry, involves plotting the absorbance or fluorescence of light from a scanned lane of the TLC plate [17,18]. The intensity of the light reaching the detector depends on the analyte amount in the scanned zone. The method is fast and simple but not without limitations. The most common errors in the quantitative analysis are associated with a scanning in a direction perpendicular to the direction of development of the chromatogram and with a gradient of layer thickness or various size of the adsorbent particles. In addition densitometry offers considerably higher detection limit than online detectors do e.g., in high performance liquid chromatography (HPLC).

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http://dx.doi.org/10.1016/j.chroma.2016.01.053
0021-9673/

The introduction of TLC–MS interface into laboratory practice has simplified the extraction procedure of the substance from the chromatographic plates. Application of this equipment to qualitative analysis has been well documented in scientific literature [19,20], however, in the case of quantitative analysis a very few papers have been published [21]. In order to obtain satisfactory results in the latter an internal standard is still necessary. An important issue of this mode is that analyte/s and internal standard form single zone, from which these compounds could be transferred to further analysis stage.

The interesting approach of sample preparation to mass spectrometry analysis, wherein the analyte and the internal standard formed one/single zone in planar medium, was proposed by Yun-Qing et al. to chlorphenamine quantitation [22]. In his method the filter paper was cut into isosceles triangle with a base of 1.0 cm and a height of 4.0 cm. The triangle paper sheet was pre-marked with two parallel lines. The first one (1.0 cm from the base line) for origin of sample solution, which was spotted on it, and the second one, the finish line, for the end of developing (0.3 cm from the V-shaped tip). The developing solvent was contacted with the triangle 0.3 cm below of the origin line. When the mobile phase moved to the finish line, the paper sheet was removed from the bath. The solvent front moved through the marked line with sample and approached just at the V-shaped tip. After being dried under airflow, the V-shaped tip was placed under the visible plasma beam of desorption corona beam ionization. The method improved the signal intensity of chlorphenamine by 30-fold due to the analyte concentration at the tip and the reduction of the background suppression. Another method of sample preparation using planar technique, which seeks to that analysed solute and internal standard form a single zone on adsorbent layer of chromatographic plate, was recently presented by Oellig and Schwack [23–25]. They proposed the new clean-up concept in multi residue analysis of substances (mainly pesticides) by liquid chromatography. In this method, named planar solid phase extraction, the sample solution was applied on chromatography plate as a band or spot, next the chromatogram was developed in the chromatographic chamber. After chromatographic plate drying in a stream of cold air, second development with another solvent was carried out in the backwards direction. The distance of second development was shorter compared to the development in the first direction. The target analyte zone (analyte and internal standard), visualized by appropriate dye, was extracted using TLC–MS interface. The authors underlined that the planar solid phase extraction is effective, reliable, rapid and economic technique to clean-up plants extracts for pesticide residue analysis. Average recovery of substances is near 100% with relative standard deviations below 4% [23–25].

The methods described above seem to be very promising, however, are characterized by some limitations, see discussion below. In this paper we intend to propose a procedure, which in our opinion is free of the limitations described below, however, retains theirs advantages. In the paper we present a quantitative analysis of acetaminophen in biological matrix as an exemplification of our procedures and the proof that its use leads to repeatable and accurate results.

2. Experimental

2.1. Materials and reagents

Chromatographic plates, Si 60 254 F, 10 × 20 cm, were supplied by Merck (Darmstadt, Germany). Methanol, acetonitrile, acetone, ether, ethyl acetate, toluene of analytical grade and methanol for HPLC were purchased from POCh (Gliwice, Poland). The deionized water was produced in the laboratory with HydroLab system (Gdańsk, Poland). Citric acid monohydrate was supplied by Merck (Dermstadt, 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 was purchased from Sigma–Aldrich. Acetanilide and syringe PTFE membrane filter, pore size 0.22 μm, diameter 130 mm, were purchased from POCh (Gliwice, Poland). Bovine serum was purchased from Biomed, Lublin. Tablets: Paracetamol (Pabianickie Zakłady Farmaceutyczne Polfa S.A), Excedrin Migrastop (Novartis Consumer Health GmbH, Munich, Germany).

2.2. Preparation of internal standard and analyte solutions

A combined solutions of acetaminophen and acetanilide were prepared by dissolving appropriate amounts of each substance in methanol. The stock solutions were stored in a refrigerator at 4 °C.

To quantitation ten tablets Excedrin Migrastop (250 mg acidum acetylsalicylicum, 250 mg paracetamolum, 65 mg coffeinum) were weighed and their average weight was calculated (0.666 g). The tablets were finely powdered, 1.332 g was weighed and transferred to 50 mL volumetric flask containing 10.0 mL of methanol. The 0.500 g of acetanilide was accurately weighed and transferred to the flask containing acetaminophen. The suspension was shaken for 30 min then diluted to the volume with methanol and filtered using Whatman no. 21 filter paper. The solution was diluted ten times. The 25 μL of this solution was added to 1 mL of serum, serum solution and methanol. The sample was appropriately diluted when applied to HPLC experiments.

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 to vials. The Agilent 1290 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. TLC plate preparation

TLC Si 60 254 F 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 samples on TLC plate

Samples were applied as a small single drops on the chromatographic plate by using U-100 insulin syringes (1 mL), BD Medical (Franklin Lakes, New Jersey). The volume of drop was about 10–15 μL.

2.6. Planar chromatogram development

The chromatograms were developed with methanol to a distance 30 or 50 mm with chromatographic plate face up. Several chromatogram development strategies using the horizontal DS chamber were examined: single development (P1), double development in the same direction (P2), single development without the