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The bridge between thin layer chromatography-mass spectrometry and high-performance liquid chromatography-mass spectrometry: The realization of liquid thin layer chromatography-mass spectrometry☆

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ABSTRACT

The combination of thin layer chromatography (TLC) and mass spectrometry (MS) has been studied for decades, but for most cases MS detection is done after TLC separation is finished. Here, an online simultaneous TLC–MS analysis system, liquid thin layer chromatography–mass spectrometry (LTLC–MS), is developed which successfully synchronize TLC separation process and MS detection process like GC–MS and HPLC–MS do. And there's no need to use specially designed TLC, just regular TLC plates are enough. LTLC–MS method is composed of a newly developed ambient ionization method, glow discharge—matrix assisted infrared desorption ionization (GD-MAIRDI), and forced-flow TLC (FFTLC) technique, which guarantees the MS detection process does not disturb the TLC separation process throughout the whole analysis. The whole LTLC–MS analysis only need two steps and less than 15 min. Mixtures as well as the two main components of a pain relief pills have been successfully analyzed by LTLC–MS. This proof of concept study opens up new possibilities of combining TLC with MS, and will further broaden the application abilities of TLC.

1. Introduction

As one of the important members in chromatography family, thin-layer chromatography (TLC) is widely used in many areas like food analysis, pharmaceutical research, forensic science, as well as chemical reaction monitoring, as it confers some important advantages, such as convenience, low solvent consumption, high throughput, low memory effect, and so on [1]. But there are two major drawbacks that limit the application abilities of TLC: 1) the qualitative difficulty (the traditional R_f value is variable, and does not provide compound identification); and 2) it is time-consuming (although it is easier and more convenient than GC or LC, the total process, including solvents equilibration, development, UV lamp observation and detection, always costs 20–60 min). Although the development of high performance TLC (HPTLC) increased the speed and throughput of TLC development [1], the qualitative problem is still not fully solved because adsorption or fluorescence spectrometry used in HPTLC still cannot give out specific information about what the fraction is. To solve the qualitative difficulty, MS is the best choice because of its high specificity, sensitivity, and speed. Just like in GC–MS and LC–MS, the key part in combining TLC with MS is also the interface. Thanks to the fast development in MS ionization methods, researchers have developed many methods to combine TLC with MS [2–5], such as TLC/SIMS [6] (second ion mass spectrometry), TLC/MALDI-MS [7–12], TLC/APMALDI-MS [13], TLC/ESI-MS [14,15], TLC/DESI-MS [16–18], TLC/APCI-MS [19,20], TLC/EASI-MS (easy ambient sonic spray ionization mass spectrometry) [21], TLC/PAMLDI-MS (plasma

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assisted multi-wavelength laser desorption ionization mass spectrometry) [22], other laser related TLC–MS methods [23–26] and so on [27–30]. They are applied not only to qualitative analysis, but also to quantifications and MS imaging of analytes. All these make TLC more versatile. However, most of these methods do not circumvent the time-consuming problem of TLC analysis, because the MS detection process is mostly carried out after the TLC separation process.

To solve the qualitative difficulty and the time-consuming problems simultaneously, synchronize TLC separation with MS detection is the best approach. To guarantee the successful processing of the continuous separation and detection, the TLC plate must keep intact from the beginning to the end, and the detection process (i.e. desorption and ionization) shouldn't interfere the separation process. MALDI typically operates in vacuum environment, so it is infeasible to combine the separation process and detection process together; moreover, MALDI requires a matrix, which is also true of APMALDI; laser ablation related methods would cause damage to plates. ESI and APCI has been used to detect the fractions of the sample after they eluted from the TLC plate [19,31,32]. Compared with off-line TLC–MS methods, these on-line methods are more convenient. To make on-line TLC–MS interface more integrated and at the same time, suitable for regular TLC plates, new interface must be developed, which wouldn't disturb TLC separation process and damage the plate. Ambient ionization method [33] could be a very good candidate.

GD is a kind of ambient ionization method which can be used for direct analysis of kinds of compounds [34–36]. Herein we developed a new GD-MAIRDI method, and integrated it with FFTLC [37,38] method. Thus the TLC separation process and MS detection process were coupled for real-time analysis, allowing the realization of LTLC–MS. Pencil marks as well as a desorption chamber were used to help increase the desorption efficiency and protect the TLC plate from damage. A pulsed diode laser and FFTLC were used to guarantee that the separation process would not be disturbed by the detection process. A prepared mixture and the main active ingredients in pain relief pills have been successfully separated and detected in less than 15 min. LTLC–MS gives detection signals at the same time of TLC separation, that is to say TLC separation process and MS detection process are synchronized. This is quite similar to GC–MS and LC–MS, and also makes LTLC–MS analysis more time-saving and labor-saving than traditional TLC–MS. All these characteristics will make TLC more versatile and useful in many areas especially reaction monitoring.

2. Experiments

2.1. Material

Deionized water used in this experiment was provided by a Mili-Q Integral water purification system (Millipore, Billerica, MA, USA). The pencil used throughout this work was CHUNG HUA 2B pencil (made in China). The silica TLC plates used here were provided by Texin Biotechnoloby (China). All the chemicals used here were purchased from Sigma (St. Louis, MO, USA). Pain relief pills, were bought from Shuguang pharmacy company (Beijing). Each pill contains aminopyrine 0.15 g (41.1%), phenacetin 0.15 g (41.1%), caffeine 0.05 g (13.7%) and phenobarbitone 0.015 g (4.1%). All samples were dissolved in ethanol, unless where noted.

2.2. Preparation of pain relief pill sample

Two pills were grounded and 800 mg of the powder was ultrasonic-extracted for 20 min with 8 mL ethanol. Then the extract was filtered and the filtrate was stored for detection. The concentrations of each component in the extraction were: aminopyrine 0.12 M, phenacetin 0.16 M, caffeine 0.05 M and phenobarbitone 0.01 M (with the assumption that all the components were fully dissolved).

2.3. Laser diode driver

Here we used a diode laser as a desorption laser similar to the reported one [39] because of its many advantages: first of all, it is compact and cost effective, while at the same time easy to use. The laser modes can be adjusted according to specific experimental requirements, continuous mode and various pulsed mode, and the laser on and off time can be adjusted discretionarily. All these above advantages make the diode laser an ideal laser source for our LTLC–MS process.

The laser diode driver used was DS3-21312-112-K940F06M N-50.00 W (BWT Beijing LTD), 940 nm. Its detailed current-power comparison was shown in Table S1. When LTLC–MS was carried out, pulsed IR diode laser was used. Model DG535 four channel digital delay/pulse generator (Stanford Research System, Inc.) was used to control the on and off state of IR laser.

2.4. Fabrications of LTLC–MS system

One of the key element for the realization of LTLC–MS is the GD-MAIRDI source. As is shown in Fig. 1, it is constituted by three main parts: a glow discharge part, a desorption chamber and a developing chamber (Fig. 1a and b). The glow discharge part is a pin-to-ring construction with argon as working gas. The flow rate of argon is 40 L/h. The inner diameter of the glow discharge part and the desorption chamber are both about 1 cm, so the linear velocity of argon is about 0.14 m/s. By using such a high argon flow rate, most of the desorbed analyte molecules were carried away by the Argon gas, and there were no obvious desorbed molecules condense on the chamber wall. A voltage of −3.5 kV was applied to the pin (made by a piece of tungsten wire), and the ring (made by aluminum) was grounded. The desorption chamber connects with the glow discharge part tightly. On the two sides of the desorption chamber, there are two holes (diameter about 0.6 cm) directly facing to each other, one is for the laser, the other for the detection point of the TLC plate. The laser side has a piece of quartz glass on it to just allow laser light in. The use of the desorption chamber makes desorption happen in an enclosed space, avoiding the influences of air, which dramatically improves sensitivity and signal/noise (details are shown in Results and Discussion part). The developing chamber guarantees the LTLC separation process happen in a relatively independent space away from the influences of the outside environment. FFTLC was realized by using a syringe pump to provide the developing agents. The syringe pump was connected to the developing chamber by a convertor, which converts the syringe pump's single stream into three paralleled streams, thus the development agent can flow on the TLC plate in a block style and the reproducibility and the separation performance is improved (Fig. 1c). As for the TLC plates, the type of the TLC plates we used was high performance silica HF254, the particle size was 8 ± 2 μm (≥80%), the layer thickness was 0.15 mm–0.2 mm. The size of the TLC plates we used in LTLC–MS process was 12 mm × 100 mm. A gentle layer of 2B pencil mark was applied as matrix, the average grayscale of the final marks on the TLC plates was about 89 (R: 89, G: 89, B: 89).

2.5. LTLC–MS process

The developing agent used here was a mixture of carbon tetrachloride, ethyl acetate and methyl alcohol in the proportion of 3:3:1. Timing was started when the TLC plate was immobilized and the pump was turned on. As is illustrated in Fig. S1, the distance