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# Thin layer chromatography coupled with electrospray ionization mass spectrometry for direct analysis of raw samples

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ABSTRACT

Conventional mass spectrometric analysis of raw samples commonly requires sample pretreatment and chromatographic separation using high performance liquid chromatography or gas chromatography, which could be time-consuming and laborious. In this study, thin layer chromatography (TLC) coupled with electrospray ionization mass spectrometry (ESI-MS) was developed for direct analysis of raw samples. The sorbent material of the TLC plate was found to be able to retain the interfering compounds and allow interested analytes to be extracted, ionized and detected by ESI-MS with much reduced matrix interference. Our results showed that this method could be effectively applied in direct analysis of samples containing common interfering compounds, e.g., salts and detergents, and rapid detection and quantitation of target analytes in raw samples. Offline and online separation and detection of different components in mixture samples, e.g., plant extracts, using TLC-ESI-MS were also demonstrated. Overall, this study revealed that TLC-ESI-MS could be a simple, rapid and efficient method for analysis of raw samples.


## 1. Introduction

Mass spectrometry (MS) is an important analytical technique in chemical and biological fields because of its high speed, sensitivity and specificity. However, analysis of raw samples by MS usually requires extensive sample pretreatment and chromatographic separation using high performance liquid chromatography (HPLC) or gas chromatography (GC), which could be laborious and time-consuming. In the past decades, development of sampling and ionization methods for direct analysis of raw samples has been an important research area in MS. For instance, the development of various ambient ionization techniques, e.g., direct analysis in real time (DART) [1], desorption electrospray ionization (DESI) [2] and electrospray-assisted laser desorption ionization (ELDI) [3], which are characterized by that samples are ionized under atmospheric pressure and no or only little sample preparation is involved, significantly reduces the time and cost required for analysis of raw samples [4–6]. In addition, the techniques of solid-substrate ESI, in which sample solution is loaded and ionized on the open surface of a solid substrate, e.g., paper [7], wooden tips [8,9], aluminium foil [10], and metal needles [11–13], were also developed to facilitate mass spectrometric analysis of complex samples by avoiding the problem of clogging potentially encountered in conventional capillary-based ESI, allowing more convenient sampling and reducing matrix interferences [11,14,15]. The techniques of ESI with paper (paper spray) and wooden tips (WT-ESI) were successfully applied in direct detection of analytes in raw samples, e.g., blood, urine and oral fluid samples, and the separation effects of filter paper and wooden-tip surface were believed to be related to their capabilities for direct sample analysis [8,9,16–19].

Thin layer chromatography (TLC) is a simple, rapid and efficient separation method, and thus coupling of TLC with MS has good potential for direct analysis of raw samples. Coupling of TLC with MS has attracted much attention [20,21], and various ionization methods, including fast atom bombardment (FAB) [22,23], matrix-assisted laser desorption/ionization (MALDI) [24], and ambient

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ionization methods such as liquid extraction junction [25–27], desorption electrospray ionization (DESI) [28,29], direct analysis in real time (DART) [30–32], easy ambient sonic-spray ionization (EASI) [33] and electrospray laser desorption ionization (ELDI) [34], have been employed for the coupling. In addition, direct coupling of TLC with ESI, in which separated sample spots were cut out for directly ESI or samples were separated online and then directly sprayed out, was also demonstrated [35–37]. However, the major purposes of these coupling studies were to facilitate identification of the separated TLC spots by MS or online separation and detection of compounds in simple mixtures. In this study, we mainly aimed to employ TLC plate as a solid substrate medium to reduce matrix interference and enhance detection of target analytes in raw samples by ESI-MS. Separation and identification of components in mixture samples were also investigated in this study. Our results indicated that TLC coupled with ESI-MS (TLC-ESI-MS) could be a simple, rapid and efficient method for detection, including quantitative measurements, of analytes in raw samples.

## 2. Material and methods

### 2.1. Materials

Acetone, ligroin, sodium chloride (NaCl), sodium dodecyl sulfonate (SDS), octyl β-D-glucopyranside (OG), myoglobin and lysozyme were purchased from Sigma (St. Louis, MO). Gramincidin D (from *bacillus breris*) was purchased from International Lab (USA). Ketamine was purchased from Alfasan (Woerden, Holland). Methanol, acetonitrile and chloroform were purchased from Tedia (Fairfield, OH). Ketamine-D4 was purchased from Cerilliant (Round Rock, Texas). Petroleum was purchased from Fisher Scientific (Pittsburgh, PA). Formic acid was purchased from Fluka (Buchs, Germany). Ethyl acetate was purchased from Acros (Fairlawn, NJ). Ginsenoside Rc was purchased from Shanghai Tauto Biotech (Shanghai, China). The urine sample was collected from a healthy male volunteer. Herbal medicines Fruit of *Schisandra sphenanthera* (FSS) and Fruit of *Schisandra chinensis* (FSC) were purchased from pharmacy stores in Hong Kong. Fresh spinach leaves were purchased from a supermarket in Hong Kong. Extracts of spinach leaves, FSS and FSC, were prepared as described previously [38]. TLC plates (Model: Alugram Sil G/UV254) were purchased from Macherey-Nagel (Düren, Germany).

### 2.2. Thin layer chromatography-electrospray ionization mass spectrometry (TLC-ESI-MS)

#### 2.2.1. TLC plates

The TLC plates used in this study have an aluminum base coated with silica gel stationary phase particles. Prior to sample loading, the TLC plates were cut into a dimension of 20 × 15 mm (L × W) and one end of each plate was cut into V-shape with a tip angle of ~60°.

#### 2.2.2. TLC-ESI-MS coupling

TLC-ESI-MS experiments were performed on a quadrupole-time-of-flight mass spectrometer (Q-TOF2, Waters-Micromass) or a triple quadrupole mass spectrometer (Quattro Ultima, Waters-Micromass). The experimental setup was similar to the direct coupling method reported previously [35]. Briefly, a TLC plate, which has an aluminum base for conduction of electric current, was cut into triangle shape and positioned ~8 mm from the inlet of the mass spectrometer with a clip connected with the high voltage supply of the mass spectrometer (Fig. 1). During data acquisition, the capillary voltage and cone voltage were set at 3.5 kV and 30 V, respectively. The ion source temperature was set at 80 °C and all

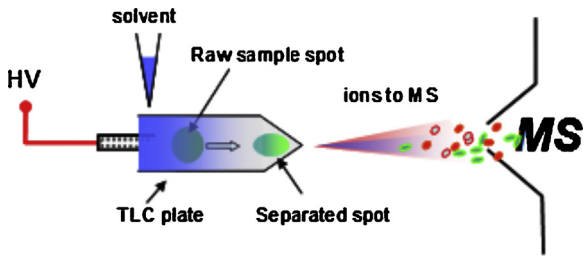


**Fig. 1.** Schematic diagram of the TLC-ESI-MS setup.

desolvation gases were turned off. Nano-ESI experiments were carried as similar to our previous study [9].

### 2.3. Sampling methods

#### 2.3.1. Sampling for analysis of samples with interfering contaminants and raw urine

Sample solution, typically 2 μl, was applied onto the TLC plate at a position ~8.0 mm away from the sharp tip end. The sample solution appeared to dry quickly after the sample loading. The TLC plate with loaded sample was first mounted in front of the MS inlet and high voltage was applied to the plate. Ten microliter of elution solvent was then applied around the sample spot position for extraction of the sample. Electrospray could be generated when the elution solvent, i.e., methanol for small organic molecules and ACN/$H_2O$ 50:50 with 0.5% formic acid for peptides and proteins, reached the tip end of the plate.

#### 2.3.2. Analysis of plant extracts: offline and online samplings

For offline sampling, the plant extract was pre-separated on a TLC plate and the sample spots were located with an UV lamp (Spectronics Corp, NY). The plate regions with sample spots of interest were cut into a triangle shape with a size covering the entire sample spot, typically with ~6 mm base and tip angel of ~45°, which were then mounted in front of the MS inlet with a clip connected with high voltage. Ten microliter of elution solvent, i.e., methanol, was added onto the plate for elution of the sample for MS analysis.

For online sampling, sample solution was loaded ~20 mm from the tip end of the TLC plate and stood until dryness. The plate with sample was then mounted in front of the MS inlet and connected with high voltage. Ten microliters of developing solvent (acetone/ligroin, 1/9, v/v) was applied onto the sample spot for several repeated cycles until the pigments were seen to travel and separate along the TLC plate to the tip end.

## 3. Results and discussion

### 3.1. Analysis of samples containing salts and detergents

Salts and detergents are common interference compounds that can significantly suppress signals of analytes in ESI-MS. The capability of TLC-ESI-MS in analysis of samples containing salts and detergents was investigated in this study. First, methanoic solutions of 1 μM of ginsenoside Rc (GRc), an active component of ginseng herbs [39], containing different concentrations of NaCl were analyzed with TLC-ESI-MS. For samples containing 1–100 mM of NaCl, sodiated ion of GRc at *m/z* 1101 was predominately observed and no interfering ion signal from cluster ions of NaCl was detected (Fig. 2a–c). When the concentration of NaCl was increased to as high as 1 M, the interfering signals from NaCl cluster ions were observed, yet the sodiated ion of GRc remained the major detected peak (Fig. 2d). As a comparison, when NaCl-containing samples were analyzed by nano-ESI, which has a higher salt tolerance than normal ESI, interfering signals of NaCl cluster ions were detected