Journal of Chromatography A, 1364 (2014) 271–275

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Thin-layer chromatography combined with diode laser thermal vaporization inductively coupled plasma mass spectrometry

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ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 18 August 2014
Accepted 21 August 2014
Available online 27 August 2014

Keywords:
Thin-layer chromatography
Inductively coupled plasma mass spectrometry
Diode laser thermal vaporization
Hyphenated techniques
Speciation
Cobalamin

ABSTRACT

Here we present a novel coupling of thin-layer chromatography (TLC) to diode laser thermal vaporization inductively coupled plasma mass spectrometry (DLTV ICP MS). DLTV is a new technique of aerosol generation which uses a diode laser to induce pyrolysis of a substrate. In this case the cellulose stationary phase on aluminum-backed TLC sheets overprinted with black ink to absorb laser light. The experimental arrangement relies on economic instrumentation: an 808-nm 1.2-W continuous-wave infrared diode laser attached to a syringe pump serving as the movable stage. Using a glass tubular cell, the entire length of a TLC separation channel is scanned. The 8-cm long lanes were scanned in ~35 s. The TLC – DLTV ICP MS coupling is demonstrated on the separation of four cobalamins (hydroxo-; adenosyl-; cyano-; and methylcobalamin) with limits of detection ~2 pg and repeatability ~15 % for each individual species.

1. Introduction

Individual chemical forms of an element, species, often differ in their chemical properties, biological activity or bioavailability, making differentiation among the species a must. Inductively coupled plasma mass spectrometry (ICP MS) offers sensitive and specific detection of many metals and nonmetals, but it lacks the ability of providing molecule-specific information since this information is inevitably lost during the atomization process. Hence, there is an obvious need for coupling ICP MS with a separation technique to differentiate the individual species. Typically, separation techniques with high separation efficiency, such as high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) [1], are preferred and are coupled on-line using a nebulizing system interface [2–4]. Alternatively, off-line coupling with a laser ablation (LA) [5,6] system or substrate-assisted laser desorption (SALD) [7,8] can be employed. However, the ICP MS speciation does not necessarily require high resolution separation and development of faster, simpler or less expensive approaches is still appealing. In this regard, coupling thin layer chromatography (TLC) to LA ICP MS for elemental speciation has received certain attention in past years, as TLC (1) provides a convenient and simple separation technique with negligible investment in instrumentation and materials; (2) allows screening of even crude samples with minimal sample preparation requirements; (3) there are no memory effects as a new stationary phase is always used; (4) allows high-throughput screening by performing multiple separations at the same time and (5) offers archiving of the separations [9,10]. Moreover, the method enables combining samples and mobile phases which would not be compatible with an HPLC column in certain applications [11]. These advantages led to the development of many TLC – MS methods, an expanding area with great future growth still expected [12].

An elemental analysis of TLC by the means of LA ICP MS was introduced by Resano et al. in 2007, who used cellulose plates for qualitative arsenic screening of biological samples [10]. The original technique required cutting the TLC plates into fragments of an appropriate size defined by the dimensions of a commercial ablation cell. The development of a dedicated chamber, better suited for direct ablation from the TLC plates, and the usage of a laser with a larger effective spot area to improve the LODs were suggested. The technique was later used with silica gel plates for fast and quantitative speciation of Cr^{III}/Cr^{VI} in waste waters using narrow separation channels and silicon present in the stationary phase as the internal standard [13]. Other reports of TLC – LA ICP MS dealt with the speciation of S, Ni and V [11] or Ni and V [14] in crude oils

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http://dx.doi.org/10.1016/j.chroma.2014.08.069
0021-9673/

and their fractions. Alternatives to LA sampling from TLC for elemental speciation are presented by utilization of extraction device demonstrated on speciation of iodine [15], or plasma jet desorption atomization (PJDA) coupled to atomic fluorescence spectrometry for speciation of mercury [16].

Diode laser thermal vaporization (DLTV) ICP MS was recently introduced by our group as a sensitive and low-cost technique of submicroliter dried droplet analysis [17]. The technique employs an 808-nm continuous-wave diode laser to scan a paper substrate and induce its pyrolysis. Black ink is printed on the surface of the substrate by a common ink-jet printer in order to facilitate absorption of laser power. The aerosol generated by pyrolysis is carried into the ICP MS. The technique provided a quantitative determination of common metals with low pg limits of detection. More recently, a simple glass tubular cell which permitted DLTV analysis of up to 24 samples on a single paper strip was presented [18]. Advantages of DLTV include optional use of prearranged multielemental calibration sets, convenient sample handling, archiving and transportation.

To our knowledge, the infrared diode laser was reported only for thermal desorption of small organic molecules, such as phospholipids from TLC plates coupled to the MS with atmospheric pressure chemical ionization [19,20]. In this case, the TLC plates were covered with graphite suspension of a graphite absorber which decreased the necessary laser power for desorption.

Here, we present a novel coupling of DLTV ICP MS with TLC for sensitive elemental analysis; an approach which represents an alternative to more demanding speciation techniques, such as HPLC or CE coupled to the ICP MS or LA ICP MS systems. Unlike conventional ablation chambers with limited dimensions commonly used for LA, employing a 17-cm long glass tubular cell allowed analysis of the entire TLC lanes. As a model system for demonstrating the TLC – DLTV ICP MS performance, cobalamins (CAs), cobalt bearing complex molecules were chosen.

2. Materials and methods

2.1. Chemicals and materials

Analytical standards of methylcobalamin (Me-CA), adenosylcobalamin (Ado-CA), hydroxycobalamin (OH-CA) and cyanocobalamin (CN-CA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (≥99.8%, p.a.) was obtained from Bárta a Cihlář (Rožnov p. R., Czech Republic), 1-butanol (≥99.5%, p.a.) from Merck (Darmstadt, Germany) and concentrated NH_3 (26 ± 2%, p.a.) solution from Mikrochem (Pezinok, Slovakia). The stock solutions of individual CA standards were prepared in the concentration 4 g L^{-1}

Single element aqueous calibration stock solution Astasol® (Analytika, Prague, Czech Republic) containing 1 g L^{-1} Co in 2% nitric acid was used as a standard for nebulizer ICP MS.

TLC cellulose plates with aluminum (HX267159) and plastic (HX137212) supports were purchased from Merck (Darmstadt, Germany).

2.2. Vitamin supplement extraction

The cobalamin extraction procedure from dietary supplement tablets was adapted from the literature [21]. Ten tablets of a dietary vitamin supplement (NBTY, Inc., Vitamin B-12, Bohemia, NY, USA) were accurately weighed and ground to a uniform powder. Approximately 20 mg of the powder was weighed in a brown-color 2-mL glass vial; 1 mL of water was added and the vial was sonicated in the dark for 30 min. The solution was decanted into a 2-mL plastic vial, centrifuged at 5000 g for 5 min and finally the supernatant was filtered with a 0.45-μm syringe filter.

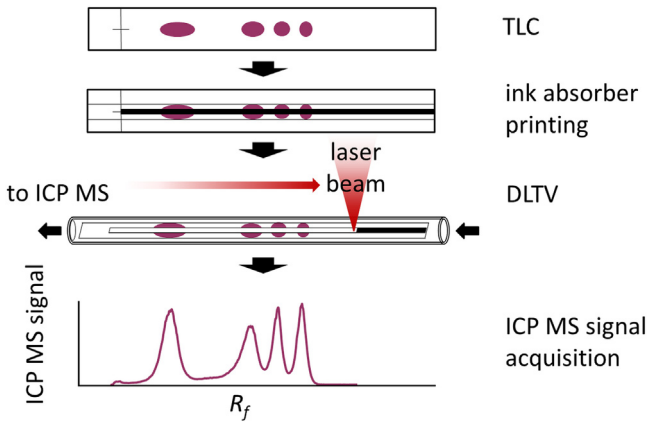

Fig. 1. TLC – DLTV ICP MS schematic diagram.

2.3. Thin-layer chromatography

Aluminum-backed TLC cellulose plates were developed for 2.5 h with a mixture of 1-butanol: ethanol: water = 10:3:7 (v/v/v) containing 0.5% (v/v) of concentrated NH_3 solution [22]. The TLC was performed in the ascending setup in a chamber saturated with the mobile phase vapors under a mild red light to prevent CA photo-degradation. The position of the solvent front was marked by scoring a line into the stationary phase. The 0.5 μL of the vitamin supplement extract and the individual CA standards (10 μg L^{-1}–4 g L^{-1}) were spotted, using a conventional micropipette, onto the TLC start marked with a lead pencil, approximately 1.0 cm above the mobile phase level, 8 mm apart.

2.4. Diode laser thermal vaporization

To facilitate the laser pyrolysis of cellulose, the black ink absorber (HP CB316E, Hewlett Packard) was printed onto the TLC sheets using a commercial inkjet printer (HP Photosmart C5380). Two templates for printing onto the TLC sheets were used; for the detailed template designs, see Fig. S1 in the supplementary material. The template consisting of 2.5 mm × 1.0 mm rectangles spaced 2.0 mm apart was used for the initial substrate and cobalamine characterization and optimization of laser focusing.

The second template composed of 1.0 mm × 8.0 mm lines and was used to overprint the developed TLC separations. The printing was done in two steps: First, the template was printed in the center of a common office paper sheet. The dried developed TLC plate was precisely aligned over the printed template so that the centers of the separation lanes overlaid the black ink central lines on the paper and was attached to the paper by adhesive tape. Next, the template was printed directly onto the TLC plate using the office sheet of paper for alignment. The overprinted plate was cut into 3-mm wide strips containing the entire separation lanes. The strips were flattened, inserted into the 17-cm long tubular vaporization cell with i.d. 3.8 mm and the center of the overprinted area was scanned by the 1.2-W 808-nm continuous-wave diode laser (RLDH808-1200-5, Roithner LaserTechnik, Austria). The schematic of the procedure is shown in Fig. 1. For additional details on the instrumental setup, see reference [18].

2.5. ICP MS

Using the DLTV technique, the generated aerosol was carried by a carrier gas (helium) at a flow rate of 1.0 L min^{-1} from the cell to an ICP MS, model 7500CE (Agilent Technologies, Inc., Santa Clara, CA, USA). A flow of argon (0.6 L min^{-1}) was mixed with the helium carrier gas flow subsequent to the cell. The ion signals of ^{59}Co and ^{13}C