Journal of Chromatography A, 1510 (2017) 73–81

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development of a supercritical fluid chromatography method with ultraviolet and mass spectrometry detection for the characterization of biomass fast pyrolysis bio oils

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ARTICLE INFO

Article history:
Received 16 March 2017
Received in revised form 30 May 2017
Accepted 1 June 2017
Available online 3 June 2017

Keywords:
Supercritical fluid chromatography
Mass spectrometry
Bio-oil
Fast pyrolysis
Complex sample

ABSTRACT

The characterization of complex mixtures is a challenging issue for the development of innovative processes dedicated to biofuels and bio-products production. The huge number of compounds present in biomass fast pyrolysis oils combined with the large diversity of chemical functions represent a bottleneck as regards analytical technique development. For the extensive characterization of complex samples, supercritical fluid chromatography (SFC) can be alternative to usual separation techniques such as gas (GC) or liquid chromatography (LC). In this study, an approach is proposed to define the best conditions for the SFC separation of a fast pyrolysis bio-oil. This approach was based on SFC data obtained directly from the bio-oil itself instead of selecting model compounds as usually done. SFC conditions were optimized by using three specific, easy-to-use and quantitative criteria aiming at maximizing the separation power. Polar stationary phases (ethylpyridine bonded silica) associated to a mix of acetonitrile and water as polarity modifier provided the best results, with more than 120 peaks detected in SFC-UV.

1. Introduction

Because of the necessity to develop new sources of energy for the future, the production of second generation (2G) biofuels from lignocellulosic biomass seems to be a promising option, implying different ways of conversion [1]. Fast pyrolysis is a thermochemical process operated within 400–450 °C range, enabling biomass liquefaction. The resulting product, also called pyrolysis oil or bio-oil, is very rich in oxygen compounds, corrosive and thermally unstable. It therefore needs to be upgraded to be used as biofuels [1]. To characterize bio-oils chemical composition at a molecular level, several analytical techniques were investigated [2], in particular chromatographic techniques. Gas chromatography (GC) permits to characterize a part of bio-oil composition (estimated at about 40%) [3–5]. However GC is currently unsuitable for compounds with high polarity and/or low volatility and/or poor thermal stability. In those cases where GC cannot be applied, liquid chromatography (LC) can be an alternative for the characterization of bio-oils [6,7]. On-line comprehensive two-dimensional liquid chromatography (LCxLC) was successfully applied to the aqueous fraction of bio-oils [6–8] and compared more recently to on-line LC × SFC [8]. SFC may be a promising approach to analyze bio-oils as it combines the advantages of GC (low fluid viscosity and high diffusivity of solutes) with those of LC including (i) the possibility of separating polar and/or low volatile compounds and (ii) the availability of a large panel of stationary phases providing very different selectivities. Furthermore, SFC conditions are expected to be soft with a usual temperature range between 30 and 60 °C, compatible with most of the compounds present in bio-oils.

Over the last decade, a new generation of SFC devices has been commercialized and the interest for SFC has grown in various fields [9] (pharmaceuticals, bioanalysis, agrochemicals, food products). The addition of an active backpressure regulator associated with a new generation of pumps, able to generate low flow-rates in a reproducible way, leads currently to robust and reliable analyses. However, in view of published results, it seems that SFC has been mainly used for the separation of relatively simple samples. As regards bio-oils, a major issue is to find out well representative compounds able to mimic their great chemical complexity in terms of functional groups and molecular weights. To take into account the matrix complexity in the early stages of method development, we chose in this study to investigate SFC conditions by directly

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http://dx.doi.org/10.1016/j.chroma.2017.06.003
0021-9673/

analyzing a biomass fast pyrolysis oil. The ultimate objective of our work was to achieve a detailed molecular description of such a bio-oil. To do so, several steps were required, starting from the optimization of SFC-UV conditions (stationary phase, mobile phase, temperature, etc. . .) and going to an in-depth analysis based on hyphenation between SFC and high resolution mass spectrometry. The present work corresponds to the first step of our methodology and aims to get a better understanding on how SFC retention is influenced by key experimental parameters such as temperature, pressure or mobile phase composition. As a result, the purpose of this paper was not to provide bio-oil molecular data but rather to propose a relevant methodology, based on quantitative and objective criteria, allowing to select the best chromatographic conditions in order to separate the highest number of compounds that are present in complex matrices such as biomass fast pyrolysis oils. Accordingly, a novel approach was proposed with three optimization criteria directed towards maximizing peak capacity. As an outlook, preliminary results based on the resulting optimized SFC separation and hyphenation to mass spectrometry detection are illustrated in order to point out the potential of this technique for bio-oil analysis.

2. Materials and methods

2.1. Chemicals and samples preparation

Solvents (acetonitrile, methanol, water) were MS grade from Sigma Aldrich (Steinheim, Germany). Carbon dioxide SFC grade (99,97%) was purchased from Air Liquide (B50 bottle under pressure). Tetrahydrofuran (THF) was purchased from VWR (Fontenay sous bois, France). The fast pyrolysis bio-oil was obtained from conifer sawdust, provided by IFP Energies nouvelles. The sample was diluted in THF (1/5 w/w) before analysis.

2.2. Instrumentation and chromatographic conditions

All experiments were performed on an Acquity UPC^2 instrument (Waters, Milford, MA, USA). Key parameters (stationary and mobile phases, back pressure, column temperature and gradient conditions) were optimized according to a procedure developed in the 'Results and discussion' section. The type of stationary phase was optimized according to the procedure described in the next section. The studied columns and their characteristics are reported in Table 1. The mobile phase flow-rate was 1.4 mL/min for columns #1, 2, 3, 5 and 7, except for column #4 and 6 whose geometry characteristics were taken into account by using a flow-rate at 3.5 mL/min.

The injection volume was 1 μL for all experiments. The injector needle was washed with 600 μL of methanol after each injection. The variance due to extra column band broadening was measured under liquid chromatographic conditions by the method of statistical second moment [10] and estimated at 85 μL^2. The measured dwell volume, corresponding to the instrument volume between the meeting point of the solvents and the column inlet, was 425 μL. This value is higher than UHPLC ones (typically about 100 μL) which is related to the large mixing chamber (approximately 300 μL) used in this study to avoid any demixing phenomenon. The column outlet was connected to a photo-diode array detector (PDA) equipped with a high pressure UV cell (400 bar) with a volume of 8 μL and a path length of 10 mm. The detection wavelengths varied between 210 and 400 nm with a bandwidth of 1.2 nm. The sampling rate was set at 40 Hz. The instrument was controlled by Empower 3 software (Waters).

Mass spectra were obtained using a LCMS 2020 instrument (Shimadzu, Kyoto, Japan) equipped with either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources both working in negative and positive modes. A simple quadrupole ensured the mass measurement in the range 80–800 *m/z*. MS parameters were optimized to favor the detection of pseudo-molecular ions. Such an optimization was based on model compounds detection. Nebulizing gas flow and dry gas flow were 0.5 and 10 L/min respectively. The interface temperature was set at 400 °C and the desolvation line temperature was 250 °C. For ESI source, the interface voltage was +3.5 kV and −5 kV in positive and negative ion modes respectively. For APCI source, the corona current in negative mode and positive modes were respectively 80 μA and 70 μA.

3. Results and discussion

3.1. Methodology for optimizing key SFC parameters

This work aims at developing an efficient method for the SFC separation of fast pyrolysis bio-oils. The lack of extensive knowledge on the components of such very complex mixtures prevented us from considering model compounds to develop the separation method as it was done in the past for closely related samples [6]. The development of the separation method was therefore carried out on the bio-oil. In this way, a strategy was implemented to optimize key parameters from a few preliminary experiments. Key parameters for any SFC separation, include the type of stationary phase, the type of co-solvent, the gradient conditions, the column geometry, the column temperature and the backpressure of the SFC system. The flow-rate was selected so that the maximum pressure and hence the minimum analysis time were attained. The choice of optimization criteria was based on sample peak capacity. This latter is known to be a powerful descriptor to assess the separation power of a given chromatographic system for a given sample under given gradient conditions and furthermore to compare the ability of different chromatographic systems to separate the components of this sample. The sample peak capacity, n_{grad}, in gradient elution was defined by Dolan et al. [11] as

$$n_{grad} = \frac{t_n - t_1}{w} \quad (1)$$

where t_n and t_1 are the retention times of the most and the least retained solutes under gradient conditions and w, the average 4σ peak width. Eq. (1) can be useful to compare different chromatographic systems provided that the number of compounds in the sample is limited. However, gradient separations of very complex samples such as bio-oils lead to multiple peak coelutions which make it impossible to determine the average peak width and hence the sample peak capacity from Eq. (1). Furthermore, an additional peak capacity, due to the isocratic initial step, has to be taken into account for the determination of the total sample peak capacity. Under isocratic conditions (corresponding to the initial composition), the sample peak capacity, n_{iso}, is given by Eq. (2) defined as [11]

$$n_{iso} = \frac{\sqrt{N}}{4} \times \ln \frac{1 + k_{n,iso}}{1 + k_{1,iso}} \quad (2)$$

where N is the column plate number, $k_{1,iso}$ and $k_{n,iso}$ are the retention factors of the least and most retained compounds under initial isocratic step. N is related to the column length, L_c and the particle diameter, d_p, by Eq. (3):

$$N = \frac{L_c}{h \times d_p} \quad (3)$$

where h is the reduced column plate height.

35 years ago, Snyder and co-workers developed the semi-empirical Linear Solvent Strength Theory (LSST) based on a linear