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A chemometric approach to elucidate the parameter impact in the hyphenation of evaporative light scattering detector to supercritical fluid chromatography

Marie Lecoeur[a,*], Nicolas Simon[a,b], Valérie Sautou[c,d], Bertrand Decaudin[a,b], Claude Vaccher[a], for the ARMED study group

[a] Univ Lille Nord de France, UDSL, EA 4481, UFR Pharmacy, 59006 Lille, France
[b] Department of Pharmacy, University Hospital, Lille, France
[c] Department of Pharmacy, University Hospital, Clermont-Ferrand, France
[d] EA 4676 C-Biosenss, Auvergne University, Clermont-Ferrand, France

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ABSTRACT

The aim of this work was to elucidate the effects of parameters influencing the evaporative light scattering detector (ELSD) response when it was coupled to supercritical fluid chromatography (SFC). Phthalates, currently used as plasticizers in medical devices, were selected as model compounds. The configuration of the hyphenation setup was firstly optimized and shown that both peak efficiency and sensitivity were improved by connecting the ELSD to the SFC before the back pressure regulator (BPR). By using a tee-junction which splits the flow after the PDA towards the collect fraction (or waste) and the ELSD, this instrument configuration has the advantage to be applicable for small-scale preparative SFC. The impacts of other parameters such as mobile phase composition and flow rate, outlet pressure, column oven temperature and ELSD drift tube temperature on the ELSD signal were evaluated using a chemometric approach. First, it was demonstrated that a classical mobile phase composed of CO_2–methanol 90:10 (v/v) was suitable to obtain great nebulization efficiency. The flow rate of the eluent was the second main effect factor. The setting must be as low as possible to avoid the loss of large particle size in the drift tube resulting in a loss of signal intensity. Concerning the outlet pressure, the configuration of the setup between SFC and ELSD requires a setting as high as possible to limit the partial liquid–vapor separation of the mobile phase in the restrictor tube. Finally, due to the low quantity of solvent which must be evaporated in the detector, a drift tube temperature of 25 °C is suitable for the hyphenation of ELSD to SFC. In the optimized conditions, the proposed SFC/ELSD method could be suitable to quantify plasticizers in medical devices.

1. Introduction

For several years, supercritical fluid chromatography (SFC) is gaining interest because of the typical properties of the mobile phase, the low cost of carbon dioxide (CO_2) compared to organic solvents, the fast analysis speed, the wide polarity compatibility and also the current development in SFC instrumentation, both for analytical [1,2] and preparative scale [3] applications. Due to its versatility, SFC could be considered either as an alternative to normal or reverse phase liquid chromatography, depending on the nature of the stationary phase used [4,5]. Several recent reviews reported various applications in the field of enantiomeric separations [6], drug discovery [7] and food analysis [8]. SFC system is commonly equipped with a UV–visible detector located before the back pressure regulator (BPR). However, for several classes of molecules (for example: fatty acids, saccharides, PEG-based polymers), the use of UV detection is very limited. That is why the development of SFC methods using evaporative light scattering detector (ELSD), which do not require heavy instrument modification, is gaining interest. Although the ELSD suffers from a dramatic lack of sensitivity, it presents the advantages to be robust, inexpensive [9] and ability to be used under solvent–gradient conditions [10]. ELSD is considered as a mass detector with a poor range of linearity which requires the use of double logarithm coordinates for calibration [11].

The hyphenation of ELSD to liquid chromatography has been largely investigated [9,12–14]. Regarding SFC, only few studies

* Corresponding author. Tel.: +33 3 62 28 30 27; fax: +33 3 20 95 90 09.
E-mail address: marie.lecoeur@univ-lille2.fr (M. Lecoeur).

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were achieved to describe the effect of various parameters acting on ELSD detection [15–19]. So far, optimization was systematically conducted by varying one experimental parameter at a time, all the others being fixed, whereas they could present interaction effects. Moreover, when a chemometric approach is implemented [17], only ELSD settings (nitrogen flow rate, drift tube temperature, orifice size) was studied without taking into account the influence of SFC parameters. Indeed, both outlet pressure and eluent flow rate are often optimized during the method development but only two papers deals about their effect on the ELSD signal [15,19]. In regard to the published papers, authors did not agree with the setting of detection parameters. The first discrepancy is about the configuration of the SFC-ELSD interface. For example, some authors connected the ELSD after the back pressure regulator (BPR) [15], whereas in some other papers, the flow rate was split by using a tee-junction between the detector and the BPR [20]. Likewise, the choice of the ELSD drift tube temperature was not systematically explained. Hence, in very similar chromatographic conditions (outlet pressure, eluent flow rate and composition), drift tube temperature was set at 30–40 °C [20,21] or at 60–70 °C [17,22–25] without any discussion about the impaction on the ELSD response. To our knowledge, only E. Lesellier et al. showed that the increase in the drift tube temperature leads to a decrease in response of a semivolatile compound [19].

Consequently, there is a real need for understanding the impact of the experimental parameters on the SFC/ELSD performances. For this purpose, a chemometric approach based on a fractional factorial design was used for the first time, to our knowledge, for the hyphenation of ELSD to SFC.

Phthalic acid esters usually called phthalates are widely used in industrial applications (vinyl flooring, ink, glue, cosmetics, textile...). In polymer industry, phthalates act as plasticizers to improve flexibility and workability of polyvinylchloride (PVC). Among applications, di(2-ethylhexyl)phthalate (DEHP) was very useful for producing medical devices such as blood bags and tubing used for blood transfusion, drugs infusion, dialysis, feeding, cardiopulmonary bypass and endotracheal intubation. As phthalates are not chemically bonded in the PVC, they can be released from the medical device during contact with blood, enteral or total parenteral nutrition admixtures or lipophilic drugs, or drugs that contain surfactant then penetrate into the human fluid. DEHP is considered as class-1B carcinogen, mutagen or toxic for reproducing chemical compound due to its reproductive toxicity in animal studies [26]. Hence, since 2010, European authorities have challenged the use of DEHP in medical devices destined to the administration or removal of drugs, biological liquids or other substances into or from the human body. This action has forced the manufacturers to replace DEHP by alternative plasticizers (ATBC, DEHA, DEHT, DINP, DINCH, TOTM) whose impact on health has not yet been reported.

HPLC-UV and GC–MS are both techniques usually developed to quantify phthalates in environmental matrices or intravenous injection solutions [27]. However, UV detection is not suitable for non UV-absorbent plasticizers such as ATBC, DEHA and DINCH and the implementation of GC seems to be difficult for the less volatile compound (TOTM). Hence, SFC–ELSD could be a good alternative technique to analyze new plasticizers added to medical devices.

The aim of this study was to better understand the impact of factors influencing the ELSD response in the hyphenation to SFC. For this purpose, a series of five UV detectable and undetectable plasticizers with a wide range of log *P* values (from 4.3 to 11.6) and boiling points (from 173 to 414 °C) were selected as model compounds. The results obtained contribute to elucidate the impact of experimental parameters on ELSD responses and allow reduction of the number of parameters to be optimized.

2. Materials and methods

2.1. Chromatographic apparatus

Chromatographic separations were carried out using an SFC–PICLAB hybrid 10–20 apparatus equipped with an autosampler, three 40P pumps, a column oven with a 10-column selection valve and a 6-solvent switching valves (PIC solution, Avignon, France). The proportion of the co-solvent in the mobile phase was adjusted by a piston pump. It was then directly added in the carbon dioxide feeding, and the mixture of co-solvent and carbon dioxide was pumped by another piston pump at the total flow-rate. The pump head used for CO_2 was cooled to −8 °C by a cryostat (Huber Minichiller, Offenburg, Germany). The injection valve was supplied with a 10 μL sample loop. The unit was also composed of a Smartline2600 DAD detector with a high-pressure resistant cell (Knauer, Berlin, Germany). Detection wavelength was set at 225 nm. After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR) with a void volume of 250 μL. The outlet tube was heated at 55 °C to avoid ice formation during the carbon dioxide depressurization. Data were recorded with SFC PicLab Analytic Online 3.1.2 and processed with Analytic Offline 3.2.0.

An ELSD model Sedex 85 (Sedere, Alfortville, France) was used in this study. It was either plumbed between the PDA and the back pressure regulator using a 0.010 in. i.d. stainless steel tee-junction and a 65 μm × 160 cm Peek tubing or to the outlet capillary after the BPR using a 100 μm × 160 cm Peek tubing. During the separation optimization, the pressure of the nebulizer gas (N_2) was set at 3 bar, the drift tube temperature and the gain were 30 °C and 7, respectively.

The columns tested were a Viridis 2-Ethylpyridine (250 mm × 4.6 mm, 5 μm) and a Viridis C18 (same dimensions, carbon load: 16%) from Waters Europe (Guyancourt, France), a Synergi Polar RP (250 mm × 4.6 mm, 5 μm) from Phenomenex (Le Pecq, France), an Uptishere Strategy C18-3 (carbon load: 22%) with the dimensions (250 mm × 4.6 mm, 3 μm) from Interchim (Montluçon, France). During the separation optimization, the column temperature was 35 °C and the outlet pressure was 150 bar. The mobile phase was delivered at a flow rate of 3 ml/min. The flow rate of the fluid entering into the nebulizer of ELSD was measured with an ADM 1000 flowmeter (Agilent, Waldbronn, Germany).

2.2. Chemicals and reagents

Acetyl tri-*n*-butyl citrate (ATBC), benzylbutylphthalate (BBP), di(2-ethylhexyl phthalate) (DEHP), di(2-ethylhexyl) terephthalate (DEHT), trioctyltrimellilate (TOTM) were purchased from Sigma–Aldrich (Steinheim, Germany). All chemical structures were presented in Fig. 1. All solvents used (2-PrOH, EtOH, MeOH, ACN) were of analytical grade and were provided by VWR (Val de Fontenay, France). The carbon dioxide of N45 quality was purchased from Air Liquide (Puteaux, France).

2.3. Sample preparation

Stock solutions of each plasticizer (1000 μg/ml) were prepared in ACN. Experiments were carried out using a test solution composed of each plasticizer at a concentration of 100 μg/ml. BBP was added to the test solution as a potential internal standard for quantitative analysis.

2.4. Design of experiments

2.4.1. Choice of factors, factors levels and responses

Considering previous works reported in the literature [14,15,19,28] and our preliminary experiments, five potentially