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Comparison of high-performance liquid chromatography and supercritical fluid chromatography using evaporative light scattering detection for the determination of plasticizers in medical devices

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ABSTRACT

Recently, interest in supercritical fluid chromatography (SFC) has increased due to its high throughput and the development of new system improving chromatographic performances. However, most papers dealt with fundamental studies and chiral applications and only few works described validation process of SFC method. Likewise, evaporative light scattering detection (ELSD) has been widely employed in liquid chromatography but only a few recent works presented its quantitative performances hyphenated with SFC apparatus. The present paper discusses about the quantitative performances of SFC-ELSD compared to HPLC-ELSD, for the determination of plasticizers (ATBC, DEHA, DEHT and TOTM) in PVC tubing used as medical devices.

After the development of HPLC-ELSD, both methods were evaluated based on the total error approach using accuracy profile. The results show that HPLC-ELSD was more precise than SFC-ELSD but lower limits of quantitation were obtained by SFC. Hence, HPLC was validated in the ±10% acceptance limits whereas SFC lacks of accuracy to quantify plasticizers. Finally, both methods were used to determine the composition of plasticized-PVC medical devices. Results demonstrated that SFC and HPLC both hyphenated with ELSD provided similar results.

1. Introduction

Supercritical fluid chromatography (SFC) is currently experiencing a renewed interest in the field of separation science. It is obvious that new generation of instruments [1] has widely contributed to the reemergence of this technology both at the analytical and the preparative scales. Nowadays, the advantages of SFC including high efficiency, short runtime, fast column equilibration and ecological interests are worldwide approved.

The majority of recently published works described the fundamental aspects of SFC in the field of separation science [2–4] and chiral applications [5,6]. Only few publications evaluated the quantitative performances of SFC system through validation method and when it was done, only UV and mass spectrometry (MS) detections were implemented. In the literature, method validation was described for chiral separations to assess the enantiomeric purity of potential drugs in aqueous formulation [7] and after asymmetric synthesis [8]. SFC/UV was also convenient for achiral applications such as quality control of medicines [9] and analysis of trace level pharmaceutical impurity [10]. More recently, Nováková et al. demonstrated the ability of SFC-MS for screening of doping agents in biological fluids [11]. For these various applications, validation method was based on the determination of response function and the evaluation of both precision and trueness. More recently, a full validation considering total error approach was applied in SFC for the quality control of antibiotics in dosage forms [12] and for the enantiomeric purity control after preparative purification [13]. The authors concluded that SFC-UV was able to give accurate results in spite of lower precision and sensitivity than HPLC-UV.

Evaporative light scattering detection (ELSD) is extensively used in liquid chromatography as a quasi-universal detector

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and is particularly appreciated for the detection of molecules with poor absorption properties [14,15]. Regarding validation method, only a few studies achieved to validate HPLC-ELSD method using the accuracy profile approach. Respaud et al. [16] succeeded the validation of L-glutamic acid over the 5–25 $\mu g\,mL^{-1}$ concentration range with acceptance limits set at ±15%. Likewise, LC-HILIC-ELSD was a suitable method to quantify sodium residuals with an acceptable accuracy (±20%) [17]. However, for concentrations lower than 62 $\mu g\,mL^{-1}$, this method was desperately short of trueness and precision.

The advantages of coupling ELSD to SFC have long been described [18–21] and were appreciated for pharmaceutical [22] and environmental [23] applications in the field of polymeric sciences [24] and for the analysis of natural products [25,26]. However, to the best of our knowledge, a full validation considering total error approach was not yet reported.

Plasticized-PVC is widely employed as medical devices (tubing used for blood transfusion, drugs infusion and extracorporeal circulation as dialysis). Since the classification of the famous plasticizer, di(2-ethylhexyl phthalate) (DEHP), as a potential carcinogen, mutagen and reprotoxic for reproduction chemical compound (CMR1B), non-phthalate plasticizers, such as citrates, adipates and trimellitates, have emerged on the plasticizer market. The massive use of plasticizer in medical devices (until 40%, w/w) had let the regulatory authority to pay attention to the harmful effect of plasticizers on human health [27,28]. Trying to answer this question, the first work was to identify and quantify these plasticizers in medical devices and to search trace amount of DEHP [29].

In the literature, the analysis of phthalates has been widely investigated either by gas [30,31] or by liquid chromatography [32–34]. Few developed methods dealt with the analysis of non-phthalate plasticizers. Those publications mainly described the interest of mass spectrometer hyphenated with GC [30,35] or LC [36] for the determination of the migration of plasticizers from PVC toys or tubing. Gimeno et al. [37] propose a GC/MS method for the quantitation of fourteen phthalates and five non-phthalates (ATBC, DEHA, DEHT, TOTM, DINCH) plasticizers in PVC medical devices.

Recently, we demonstrated the suitability of supercritical fluid chromatography hyphenated with evaporative light scattering detector for the simultaneous analysis of three plasticizers (ATBC, DEHT, TOTM) added with DEHP [38]. In particular, ELSD was appreciated for the detection of non aromatic plasticizer (ATBC) which was not detected by UV. Later, porous graphitic carbon (PGC) stationary phase was used to resolve more complex mixture of plasticizers in a short runtime using $CHCl_3$/heptane mixture as co-solvent [39].

In the present paper, we investigate the interest of SFC-ELSD as a quantitative method to determine the weight content of plasticizer in PVC tubing. First, an HPLC-ELSD method using PGC support was developed and then considered as reference method to compare their performances with SFC-ELSD. Attention was paid on the choice of the mobile phase composition and of the ELSD parameters (nebulizer temperature, nebulizer gas pressure and drift tube temperature) to attain high ELSD response. Then, HPLC and SFC methods were validated based on the total error approach using accuracy profile and their respective performances were compared in the field of quantitative analysis (limits of quantitation, sensitivity, trueness, precision).

An SFC apparatus from PIC solution coupled with a Sedex 85 and an HPLC-ELSD from Waters were used in this study. All results and conclusions are relevant to these particular systems and may not apply to others.

2. Materials and methods

2.1. Chemicals

Acetyl tri-n-butyl citrate (ATBC), benzylbutylphthalate (BBP), di(2-ethylhexyl)adipate (DEHA), di(2-ethylhexyl) phthalate (DEHP), di(2-ethylhexyl) terephthalate (DEHT), diisononyl phthalate (DINP), trioctyltrimellitate (TOTM) were purchased from Sigma Aldrich (Steinheim, Germany) and 1,2-cyclohexanedicarboxylic acid diisononylester (DINCH) from BASF (Levallois, France). Structures of these plasticizers were presented in Fig. 1. Acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), n-propranol (n-PrOH), 2-propanol (i-PrOH), methylene chloride (CH_2Cl_2) and chloroform ($CHCl_3$) 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).

2.2. Chromatographic apparatus

2.2.1. SFC-ELSD

Chromatographic separations were carried out using a SFC-PICLAB hybrid 10–20 apparatus equipped with an autosampler, three 40P pumps, a column oven with a 10-columns selection valve and a 6-solvents switching valve (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 20 μL sample loop (corresponding to the injection volume). The unit was also composed of a Smartline 2600 PDA detector (Knauer, Berlin, Germany). Detection wavelength was set at 240 nm. After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR) and the outlet tube was heated at 55 °C to avoid ice formation during the carbon dioxide depressurization. An ELSD model Sedex 85 (Sedere, Alfortville, France) was also used. It was plumbed between the PDA and the back pressure regulator using a 0.010 inch i.d. stainless steel tee and a 65 μm × 160 cm Peek tubing. The pressure of the nebulizer gas (N_2) was set at 3 bar, the drift tube temperature and the gain were 31 °C and 7, respectively. All data were recorded with SFC PicLab Analytic Online 3.1.2 and processed with Analytic Offline 3.2.0.

Plasticizers were separated on porous graphitic carbon (100 × 4.6 mm i.d., 5 μm) from Thermo-Scientific (Waltham, USA) preceded with a guard column (4 × 4 mm i.d., 5 μm). The column temperature was 35 °C and the outlet pressure was 20 MPa. The mobile phase was composed of CO_2 and a mixture of heptanes–$CHCl_3$ (35/65: v/v) used as co-solvent. The mobile phase was delivered at a flow rate of 2 $mL\,min^{-1}$ under gradient mode from 15 to 60% of co-solvent in 4.6 min.

2.2.2. HPLC-ELSD

Chromatographic analyses were performed on a Waters system equipped with a gradient quaternary 600E pump model, an on-line degasser apparatus, a 7125 Rheodyne injector (20 μL loop), a 996 PDA and a 2420 ELSD (Milford, MA, USA). The ELSD was plumbed in series after the PDA. After optimization, the pressure of the nebulizer gas (N_2) was set at 0.2 MPa, the nebulizer and the drift tube temperature were set at 36 °C and 35 °C, respectively and the gain was set at 20. Data were collected and processed on a computer running with Empower software (version 2) from Waters. Separations were carried out on a PGC (100 × 4.6 mm i.d., 5 μm) column from Thermo-Scientific preceded with a guard column (4 × 4 mm i.d., 5 μm) both kept at 35 °C. In the optimized conditions, the analytes were eluted using a mobile phase