Journal of Chromatography A, 1344 (2014) 31–41

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Implementation of gradients of organic solvent in micellar liquid chromatography using DryLab®: Separation of basic compounds in urine samples

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 25 March 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
Micellar liquid chromatography
Gradient elution
Sodium dodecyl sulphate
1-Propanol
Basic compounds
Urine samples

ABSTRACT

In micellar liquid chromatography (MLC), chromatographic peaks are more evenly distributed compared to conventional reversed-phase liquid chromatography (RPLC). This is the reason that most procedures are implemented using isocratic elution. However, gradient elution may be still useful in MLC to analyse mixtures of compounds within a wide range of polarities, decreasing the analysis time. Also, it benefits the determination of moderately to low polar compounds in physiological fluids performing direct injection: an initial micellar eluent with a low organic solvent content, or a pure micellar (without surfactant) solution, will provide better protection of the column against the proteins in the physiological fluid, and once the proteins are swept away, the elution strength can be increased using a positive linear gradient of organic solvent to reduce the analysis time. This work aims to encourage analysts to implement gradients of organic solvent in MLC, which is rather simple and allows rapid analytical procedures without pretreatment or the need of re-equilibration. The implementation of gradient elution is illustrated through the separation of eight basic compounds (β-blockers) in urine samples directly injected into the chromatograph, the most hydrophobic showing large retention in both conventional RPLC and MLC. The use of the DryLab® software to optimise gradients of organic solvent with eluents containing a fixed amount of surfactant above the critical micellar concentration is shown to provide satisfactory predictions, and can facilitate greatly the implementation of gradient protocols.

1. Introduction

Micellar liquid chromatography (MLC) is a reversed-phase liquid chromatographic (RPLC) mode, where the mobile phase is basically composed of an aqueous solution of an ionic or neutral surfactant above the critical micellar concentration (CMC). This RPLC mode has evolved from laboratory curiosity in Ref. [1], to its current practical utility. There are more than three decades of MLC experience with several hundreds of reports [2,3]. One of the most outstanding features of MLC is its unique selectivity, often attributed to the ability of micelles to organise solutes at the molecular level. However, the association of surfactant monomers to the bonded phase, forming a structure similar to the exterior of open micelles [4], has deep implications in the chromatographic behaviour (analysis time, selectivity and efficiency). The adsorption of an approximately fixed amount of surfactant monomers on the stationary phase [5] gives rise to a stable modified packing, with properties remarkably different from those of the underlying bonded phase [6,7].

MLC was first proposed as a chromatographic mode with a mobile phase containing only water, buffer, micelles and a small amount of surfactant monomers [1]. Therefore, micelles played the role of the organic modifier. However, solutions containing only surfactant are too weak and yield poor peak shape. To remediate these problems, Dorsey et al. suggested the addition of a small amount of organic solvent to the mobile phase [8]. Since then, most reported analytical procedures in MLC make use of aqueous solutions of surfactant, mixed with organic solvent (mainly 1-propanol, 1-butanol, 1-pentanol and acetonitrile) [2,3], where the concentration of organic solvent is maintained low enough to allow the formation of micelles. The advantage of increasing the concentration of organic solvent to values producing micelle breakdown has been also addressed in terms of reduced analysis time, larger selectivity and improved peak shape, with respect to MLC

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http://dx.doi.org/10.1016/j.chroma.2014.03.073
0021-9673/

and conventional RPLC [9–11]. The technique has been called high submicellar liquid chromatography (HSLC). In HSLC, hydrophobic interaction may be dominant due to the reduction of the ionic surfactant coating on the stationary phase, the disaggregation of micelles and the decrease in mobile phase polarity.

The unique selectivity of MLC, together with the smaller consumption of organic solvent and lower toxicity, with respect to conventional hydro-organic RPLC, may not be compelling reasons for a shift to the use of this chromatographic mode. Perhaps, the major reason of the increasing interest in MLC is the possibility of performing the direct injection of physiological samples through the solubilisation of the protein components by interaction with the micelles in the mobile phase, and the protection of the stationary phase by coating with surfactant monomers to avoid clogging [12–14]. The proteins are thus swept away, eluting with or shortly after the solvent front. Micelles also release protein-bound drugs, which results in higher concentrations in the mobile phase for partitioning to the stationary phase and detection. These features simplify the procedures and increase the sample throughput.

In conventional RPLC without additive, there is a linear dependence between the logarithm of the retention factor ($\log k$) and the solute polarity, usually measured as the logarithm of the octanol–water partition coefficient ($P_{o/w}$) [15]. This relationship gives rise to one of the main problems of the technique, called the "general elution problem of chromatography": if the polarity range is too wide, it will be difficult to find a set of chromatographic conditions able to balance satisfactory resolution for the least retained solutes and reasonable retention time for the most retained ones [16]. A logical solution to overcome such a situation is gradient elution, where the elution strength is increased gradually as the analysis progresses, by altering at least one experimental factor (usually the organic solvent content).

In MLC, the situation is different due to the more effective removal of highly hydrophobic compounds from the stationary phase transported by the micelles. This gives rise to linear k versus $\log P_{o/w}$ relationships [17], which is translated in a larger number of compounds being eluted per time unit in the isocratic mode with regard to conventional RPLC. Chromatographic peaks are thus more evenly distributed, with longer retention times for the least retained compounds [18]. This behaviour has been called "gradient effect" and is the main reason that almost all reported MLC procedures use isocratic conditions. In spite of this, gradient elution can be useful in MLC to expedite some analyses, or even its application may enhance the separation capability of the technique.

However, after the seminal reports by Dorsey, Khaledi and coworkers [19–22], describing the capability and usefulness of gradient MLC to speed up the elution of strongly retained compounds, only very few authors have been concerned with the development of gradient elution procedures in MLC. Paleologos et al. determined biogenic amines in fish, chicken and wine samples using an eluent with fixed 0.40 M sodium dodecyl sulphate (SDS) and increasing acetonitrile (from 30 to 50% v/v) [23,24]. Ghorbani et al. determined water-soluble vitamins in multi-vitamin tablets with fixed 0.016 M SDS and increasing 1-butanol (from 3.5 to 10%) [25]. Bryant and Altria determined basic drugs and neutral compounds using gradients with increasing SDS and 1-pentanol (a simultaneous increase in both surfactant and organic solvent from 0.006 M SDS/0.7% 1-pentanol to 0.08 M/9.8%, and from 0.011 M/1.4% to 0.11 M/14%, respectively) [26]. Cao et al. separated phenolic compounds with fixed 0.05 M SDS and increasing acetonitrile (from 0 to 30%) [27].

Particularly interesting is the work of Nakao et al., who determined several radio-metabolites in plasma samples during positron emission tomography (PET) to evaluate the pharmacokinetics of the PET ligands using gradients with fixed SDS and increased 1-butanol or acetonitrile [11,28,29]. The high-speed analysis of short-lived radioligands is essential, together with the possibility of processing a large amount of samples to derive correct metabolite pharmacokinetic functions. The authors demonstrated that MLC overcomes the limitation of conventional RPLC, which only allows metabolite analysis for a limited number of plasma samples. That work was developed guided by the principle of direct injection of the physiological sample in the chromatographic column, in conditions where it is protected (MLC conditions), and the rapid elution of highly retained analytes (after the protein separation, a rapid increase of organic solvent reaching HSLC conditions).

In our opinion, in spite of the advantage of using isocratic elution in MLC, more analysts should consider the improvement of some procedures through the application of gradient elution. To encourage the implementation of gradient MLC, in this work, we show in detail the development of a gradient procedure for the separation of a set of basic compounds (β-blockers) in urine samples with direct injection, using a fixed amount of SDS and a linear increase of organic solvent. The use of the DryLab® software to predict the optimal separation conditions is described.

2. Experimental

2.1. Reagents and columns

The following basic compounds (β-blockers) were analysed (see Table 1): acebutolol, atenolol, carteolol, labetolol, metoprolol and propranolol from Sigma (St. Louis, MO), celiprolol from Rhône-Poulenc Rorer (Alcorcón, Spain), and oxprenolol from Ciba-Geigy (Barcelona, Spain). The drugs were dissolved in a small amount of the organic solvent added to the eluent, and diluted with water. The concentration of the injected solutions was 20 μg/ml for the aqueous mixtures, and 5 μg/ml for the fortified urine samples, which were obtained from human volunteers.

The mobile phases contained sodium dodecyl sulphate from Merck (99% purity, Darmstadt, Germany), and acetonitrile or 1-propanol from Scharlau (Sentmenat, Barcelona). The pH was buffered at 3 with 0.01 M anhydrous sodium dihydrogen phosphate (Fluka, Steinheim, Germany). All experiences were performed with nanopure water, obtained with a Barnstead ultrapure water purification system from Thermo Scientific (Dubuque, IA). The drug solutions and mobile phases were filtered through 0.45 μm Nylon membranes from Micron Separations (Westboro, MA).

Two chromatographic columns were used: Zorbax Eclipse XDB-C8 and Zorbax Eclipse XDB-C18 (both 150 mm × 4.6 mm i.d., 5 μm particle size) from Agilent (Waldbronn, Germany).

2.2. Apparatus and software

An Agilent liquid chromatographic system was used, which was equipped with the following modules: a quaternary pump (HP 1200) run at 1 ml/min, an autosampler (HP 1100) with 2 ml vials, and a UV–visible detector (HP 1100) set at 225 nm. The temperature was controlled at 25 °C with a thermostated column compartment (HP 1100). The maximal operating pump pressure was 400 bar.

The system was controlled by an OpenLAB CDS LC ChemStation (Agilent B.04.03). The separation conditions were optimised with the assistance of the DryLab® software (Molnár Institute, Berlin, Germany). The chromatograms were exported into DryLab® with the PeakMatch® software from Agilent in AIA format (*.cdf) for "peak tracking".

The dwell volume (total volume of plumbing in a gradient system between the point where the gradient is formed and the column inlet) was determined by removing the column from the system and connecting the injector directly to the detector. The midpoint of the signal obtained with a 0–100% linear gradient, formed by mixing water and 0.1% acetone, indicated the dwell