Analytica Chimica Acta xxx (2017) 1–10

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Green-modified micellar liquid chromatography for isocratic isolation of some cardiovascular drugs with different polarities through experimental design approach

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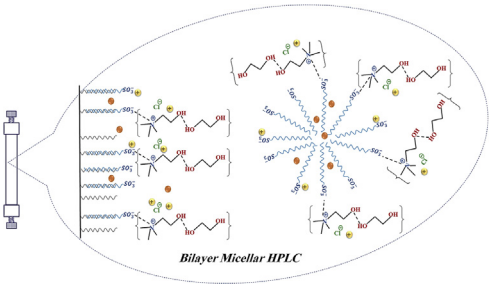
HIGHLIGHTS

- Innovating of bilayer pseudo-phase stationary phase in micellar liquid chromatography.
- Employing a new green modifier in micellar liquid chromatography.
- Simultaneous isocratic isolation of hydrophilic and hydrophobic compounds in human plasma samples.
- Introducing a reliable chromatographic system for retaining polar species.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 August 2017
Received in revised form
16 November 2017
Accepted 16 December 2017
Available online xxx

Keywords:
Cardiovascular drugs
Deep eutectic solvents
Green liquid chromatography
Hydrochlorothiazide
Losartan potassium
Triamterene

ABSTRACT

Bilayer pseudo-stationary phase micellar liquid chromatography (MLC) was developed for simultaneous isocratic isolation of hydrochlorothiazide, as a basic-polar (hydrophilic) cardiovascular drug, as well as triamterene and losartan potassium, as acidic-nonpolar (hydrophobic) cardiovascular drugs. Utilizing a deep eutectic solvent (DES), as a novel green mobile phase additive in combination with acetonitrile (ACN) and acetic acid (ACA), drastically improved the chromatographic behavior of the drugs. Concentration of sodium dodecyl sulphate (SDS), as well as volume percentages of ACN, DES, and ACA were optimized by using a central composite design. The optimal composition of the mobile phase (0.12 mol L^{-1} SDS, 5% ACN, 4% DES, and 2% ACA) was chosen through the desirability function. The chromatographic peaks of both hydrophilic and hydrophobic drugs, respectively, emerged at high and low retention time values in the shortest total analysis time of 20 min (at a flow rate of 2 mL min^{-1}). Analytical characterization of the developed approach was investigated through Food and Drug Administration (FDA) guidelines. Applicability of the method was evaluated by analysing of human plasma samples which were directly injected into the system.

1. Introduction

Cardiovascular diseases (CVDs) are one of the most widespread mortal diseases all over the world. As stated in World Health Organization (WHO) report, the biggest worldwide cause of death is CVDs [1]. CVDs are typically recognized by some disorder symptoms including high cholesterol level, heart arrhythmia and hypertension [2] among which hypertension is a key sign and risk factor [1]. A global research has predicted that about 1.56 billion people will be suffering from hypertension by 2025 [3]. Clinical findings have revealed that one of the greatest tactics for protection

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https://doi.org/10.1016/j.aca.2017.12.021
0003-2670/

against CVDs is the reduction of blood pressure through the use of antihypertensive drugs [4]. According to medical-related studies and reports, compared to monotherapy, combined therapy [5] has attained highly effective results with a lower degree of negative effects and heath treatment costs [4–6]. One of the most effective fix-dosage combinations (FDC) that is mainly used in the treatment of CVDs and reduction of blood pressure is hydrochlorothiazide-triamterene (HCT-TRI) combination. This combination is the oldest FDC in antihypertensive drugs [7] after the first introduction of thiazide-based drugs as highly efficient antihypertensive agents [8]. Recently, pharmaceutical studies have demonstrated that triamterene (TRI) plays two positive roles in HCT-TRI combination pills. These pills not only compensate the side effect of hydrochlorothiazide (HCT) and prevent hypokalemia but also significantly improve the efficiency of HCT versus blood pressure [9]. The synergic effect of this combined drugs has produced a higher selectivity in performance function, efficiency, and safety [10,11].

As mentioned above, the life quality of millions of people in the world is affected by hypertension, hence, its diagnosis and treatment are crucial from the medical standpoint as stated in WHO report [1]. Although various analytical techniques such as electrochemical methods [12], spectrophotometric techniques [13], sensitized fluorescence approach [14], chemometrics protocols [15] and thin layer chromatography (TLC) [16,17], have been reported for simultaneous diagnosis of antihypertensive drugs, high performance liquid chromatography (HPLC) approach can undeniably provide higher precision and reliable results [18–20]. One of the main problems of HPLC techniques is matrix effect due to direct injection of biological samples [2]. Hence, introducing a reliable method which can directly measure the blood pressure-controlling drugs such as HCT, TRI and LOS (losartan potassium) in body fluid is still on high demand.

To overcome these problems and limitations, a practical mode of reverse phase HPLC (RP-HPLC) can be used which is referred to as micellar liquid chromatography (MLC). In this mode, not only a biodegradable surfactant has been used as the mobile phase but also the direct injection of plasma samples has been attained [21]. Utilization of an ionic liquid (IL) as the modifier [22] is another approach for improving the HPLC criteria such as achieving simultaneous separation of polar and non-polar components, modifying the retention performance, and reaching higher efficient peaks. However, the practical applications of ILs as mobile phase additives have been restricted due to having tedious synthesis and toxic effects as well as having high viscosity as has been reported for a large number of them [22–25].

Recently, a new generation of solvents, known as deep eutectic solvents (DESs), have been popularly utilized as green solvents in separation sciences [26–29]. Some advantages of DESs, compared to ILs, which give them high potentials for chromatographic applications are as follows: they are affordable, are usually prepared with a facile and fast synthesis procedure, are mainly obtained from natural primary metabolites, have adjustable viscosity and high miscibility with frequently used solvents in liquid chromatography such as water, have a high solubilization capacity for both polar and non-polar compounds, have a high biodegradability, do not have any interference in the UV-Vis detection regions in most cases, and have environmentally friendly properties [30–32]. Some applications of DESs, as modifying agent in mobile phase at the common hydro-organic mode of RP-HPLC, have been reported [23,33]. Since surfactants and DESs are biodegradable and have high solubilizing abilities for many compounds such as proteins in plasma samples, the integration of both can introduce a new practical strategy in bioanalytical chromatography.

The aim of the current work is to design an isocratic mobile phase based on MLC along with using DES as the modifier agent through the use of chemometric protocol for simultaneous isolation and quantification of HCT, TRI, and LOS which have different polarities and acidic-basic properties. To the best of our knowledge, there is no report about utilizing DES in MLC system. In the current work, simultaneous isocratic isolation of hydrochlorothiazide (a hydrophilic drug) as well as triamterene and losartan potassium (as hydrophobic drugs) was performed by isocratic elution due to the following reasons: isocratic elution has simple optimization protocol, has facile method transfer among different laboratories, has stable baseline, is more suitable for trace analysis, and is more practical from the validation purposes as mentioned in the literature [34]. By taking assistance from the recommended guidelines of the United States Food and Drug Administration (US-FDA), the developed procedure has been validated [35,36]. To investigate the practical ability of the procedure for analysis of real samples, calibration curves have been constructed in both standard and plasma samples and the results have been compared with each other through the matrix effect factor.

2. Experimental

2.1. Chemicals and solutions

Acetonitrile (ACN) (99.9%), methanol (MeOH) (>99%) and glacial acetic acid (ACA) (>99%) were obtained from Merck. Choline chloride (ChCl) (>98%) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Ethylene glycol (EG) (99.5%) was supplied by Panreac (Barcelona, Spania). All reagents were applied without any further refining. Pure powder of hydrochlorothiazide (HCT), triamterene (TRI) and losartan potassium (LOS) were kindly donated by Daroo-Pakhsh, Iran-Daroo, and PurSina pharmaceutical companies (Iran), respectively. Standard stock solutions of HCT (1000 μg mL^{-1}), LOS (1000 μg mL^{-1}), and TRI (250 μg mL^{-1}) were prepared in MeOH and kept at 4 °C up to use. The working standard solutions (5 μg mL^{-1}) which contained a mixture of the drugs were obtained daily by sufficient dilution of the stock solutions using the double-distilled water.

2.2. Preparation of deep eutectic solvent

The deep eutectic solvent based on choline chloride and ethylene glycol (ChCl-EG DES) was synthesized through a facile, one-step, thermal-assisted procedure as described previously [37]. Briefly, a proper amount of ChCl as the hydrogen-band acceptor and EG as the hydrogen-band donor (at molar ratio of 1: 3) were mixed under a heating magnetic stirrer with an oil bath temperature fixed at 80 °C. The mixture was agitated for 2 h to acquire a completely homogeneous colourless liquid. The obtained DES was stored at room temperature until use.

2.3. Plasma sample preparation

Drug-free human plasma samples were donated by Shiraz Blood Transfusion Centre (Shiraz, Fars, Iran) to follow the research. Hence, the blood-donor persons are unknown to our research group. These samples were aliquoted and introduced into polypropylene microtubes and stored at −20 °C and were thawed prior to use. Spiked human plasma samples with known amounts of the studied drugs were daily prepared through dilution of the stock solution with drug-free human plasma. The spiked plasma samples were vortexed and then equilibrated stagnantly for 30 s and 5 min, respectively. The samples were diluted to a ratio of 1:3 (i.e. 4-fold) with the developed mobile phase (this dilution stage was performed to avoid possible damaging of the column in MLC system) and were vortexed for 5 min to obtain homogeneous solutions. The

Please cite this article in press as: A.M. Ramezani, et al., Green-modified micellar liquid chromatography for isocratic isolation of some cardiovascular drugs with different polarities through experimental design approach, Analytica Chimica Acta (2017), https://doi.org/10.1016/j.aca.2017.12.021