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Evaluation of hydrophilic interaction liquid chromatography stationary phases for analysis of opium alkaloids

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ABSTRACT

The separation of a mixture containing five major opium alkaloids, namely morphine, codeine, thebaine, noscapine and papaverine has been investigated in hydrophilic interaction liquid chromatography (HILIC) mode using five different stationary phases: bare silica, zwitterion, aminopropyl, diol and cyanopropyl. In order to propose the appropriate column for separation and purification, retention behaviors of the five natural opioids have been studied on mentioned HILIC stationary phases. The mechanism of separation in diverse HILIC media, based on the formation of water-rich layer on surface of the HILIC stationary phases and the physicochemical properties of opium alkaloids, such as pKa (acidic pK) and the octanol-water distribution coefficient (log Do/w) are discussed. Chromatographic responses including modified limit of detection LOD_m, signal to noise ratio $(S/N)_m$, and defined modified R_{Sm} have considered for suggestion of the suitable column for quantitative/qualitative and preparative purposes. According to the obtained results, diol stationary phase is best suited for analytical chromatography, whereas bare silica and zwitterionic stationary phases are appropriate for preparative applications.

1. Introduction

Opium poppy (*Papaver somniferum* L., *Papaveracea*) is an annual plant containing benzylisoquinoline alkaloid papaverine; the phthalideisoquinoline alkaloid noscapine and the morphinan alkaloids morphine, codeine and thebaine [1–3]. The importance of this medicinal plant is derived from its five major alkaloids including morphine, codeine, thebaine, noscapine and papaverine which are vastly produced and consumed by pharmaceutical industries. They have been used directly and indirectly (via semi-synthesis) as valuable drugs due to potent pharmacological activities, for example analgesic properties of codeine and morphine, the coronary vasodilator function of papaverine, the potentially anti-cancer drug and cough suppressant features of noscapine [1–3]. An overview on the reported separation approaches indicated that there are numerous appropriate techniques available for qualification and quantitative analysis of opium alkaloids such as thin-layer chromatography [4], capillary electrophoresis [5,6], gas chromatography [7] and high-performance liquid chromatography (HPLC), with a vast diversity of detection methods [8–10], particularly mass spectrometry (MS) [11–14], UV absorbance and/or fluorescence detections [8], and chemiluminescence [9]. Analysis of opium alkaloids is typically performed by applying reversed-phase high-performance liquid chromatography (RP-HPLC), accompanied by ion pairing reagents. Although ion-pairing agents such as fluorinated compounds could meaningfully promote peak shape, in the presence of this reagents, separations are very susceptible to minor variations in chromatographic circumstances [15]. Besides, application of ion-pairing agents causes increased analysis time, decreased column endurance, and interference in operation of the liquid chromatography–mass spectrometry (LC–MS), due to low volatility and ion suppression effect [10]. HILIC, a variation of normal-phase chromatography, is an efficient alternative separation technique to RP-HPLC for separating polar, weakly acidic, and basic compounds on polar stationary phases such as bare silica gel, diol, cyano, zwitterionic, etc. Suitable retention of highly polar constituents, the possibility of applying higher flow rates thanks to the

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larger amount of organic modifier in the mobile phase, higher loading capacity, improved mass sensitivity because of the impressive spraying and/or desolvation process, separation efficiency and good orthogonality to RP-HPLC are major reasons for resurgence of interest in HILIC mode [16–25]. It has been perceived that conventional RP-LC encountered with difficulties in analysis of basic samples like alkaloids due to residual unreacted silanol groups, and even after end capping by smaller silylating reagents for inclusion of more reactive silanols in common RP columns, the problem still persists. Mixed mode separation as well as anion-exchange and hydrophobic interactions occur as a result of remaining silanol groups on silica surface which may cause peak tailing and decrease in column efficiency [26]. Moreover, highly water-rich mobile phase in RP-HPLC provokes the collapse of water-repellent stationary phase and reduces the column lifetime [27]. Unlike RP-HPLC, structural diversity of HILIC-type stationary phases are broader and can be categorized into neutral, polar and ionic surfaces [19]. In HILIC, a miscible binary mobile phase, rich in organic content, causes elution of the sample from the column. Previous hypothesis indicated that semi-stagnant aqueous-rich solvent on HILIC media and aqueous-depleted solvent nearby it, construct a biphasic system in a way that analyte components tend to partition from the eluent to hydrophilic surrounding. Nowadays, chromatographers believe that HILIC is an intricate, multi-parametric retention procedure comprising envisaged partition, polar, and ion-exchange interactions which might be manifested in altered selectivities for various HILIC stationary phases [15].

To the best of our knowledge, there is no comprehensive study regarding the application of HILIC mode for separation and isolation of narcotic analgesics. In the present work, special concentration is dedicated to the study of interactions between the HILIC stationary phase and opioid compounds. Moreover, performances of the five different HILIC stationary phases for separation of five major opium alkaloids were compared in order to select the best columns for analytical purposes. While selection of the best analysis is not difficult according to analytical data, decision for preparative scale is more approximate and commonly based on experience. However there are some parameters to help us for more accurate estimation such as number of theoretical plates (N), retention factor (k), selectivity factor (α), and resolution factor (Rs). *N* and *k* inform about single peaks, α and *Rs* are concerned for separation quality. While α doesn't inform about elution time, its direct effect on loading factor and productivity had made it more interesting for industrial chromatographists. However more attention should be paid for Rs involving N, k and α, to make a more comprehensive decision. Probably most accurate prediction would be achievable with simultaneous considering α and Rs. With this background some suggestions for preparative scale were assumed.

2. Experimental

2.1. Chemicals and reagents

Opium and the five major alkaloids were received from Temad Co. Darou Pakhsh (Tehran, Iran). Chemical structures of the opium narcotics are represented in Fig. 1 Acetonitrile (ACN) of HPLC gradient grade was purchased from Chem-Lab NV (Zedelgem, Belgium). Ultra-pure water produced by Millipore Milli-Q system (Billerica, MA, USA) was used throughout the experiments.

2.2. Solutions

2.2.1. HILIC mobile phase

Ammonium acetate buffer (60 mM) was prepared in purified water, and the pH was subsequently adjusted to 3 using glacial acetic acid. The applied mobile phase for simultaneous separation of opium narcotics on different HILIC columns was comprised of ammonium acetate buffer (solvent A) and ACN (solvent B).

2.2.2. HILIC conditions

Chromatography assessments were performed on five stainless steel columns (250 × 4.6 mm and 150 × 4.6 mm). Characteristics of all the columns used in this study are listed in Table 1. The same mobile phase was used during the entire experiments for comparison of column selectivities. The separation of five major opium alkaloids was achieved using the following gradient elution0 to 5% solvent A in 10 min which reaches to 15% within 15 min and 10–25 min 85% solvent B, a flow rate of 1.5 ml/ min or 0.9 ml/ min (according to column diameters), and 20 μl injection volume.

2.2.3. Preparation of standards

Initial stock solutions were prepared at concentrations of 5 mg/mL for morphine, codeine, thebaine and papaverine, and 2.5 mg/ mL for noscapine in acetonitrile/deionized water solvent mixture (95:5 v/v). These stock solutions were stored at 4 °C. Working solutions for analytical HPLC were prepared from suitable amount of preliminary stock solutions and were diluted to lower concentrations by acetonitrile/deionized water solvent mixture.

2.3. Data analysis

Physicochemical properties of opium narcotics such as pKa (acidic pK) and the octanol-water distribution coefficient at pH = 3 were calculated by means of the online ACD/I-Lab Web service. Log Do/w (an indicator of the equilibrium concentrations of the neutral species of a sample in octanol to the un-ionized and ionized types in water segment) and log Po/w (the partition coefficient) are used for neutral analytes or where the molecules exist in a single form. Since the compounds under investigation were ionized at the employed pH, log Do/w was used.

2.4. Instrumentation

A Knauer HPLC system (Berlin, Germany) with the EZChrom Elite software was used to monitor and acquire chromatographic data. The column was maintained at room temperature and sample components were detected at a wavelength of 280 nm.

3. Results and discussion

The phenomenon of 'retention' in HPLC occurs as the result of different synergistic and antagonistic intermolecular interactions between sample, stationary phase and the mobile phase. Diverse HILIC stationary phases such as bare silica, zwitterion, aminopropyl, diol and cyanopropyl, with various characteristics, display different physicochemical behaviors and hence, demonstrate different retentions, selectivities and resolutions for diversified opium alkaloids under HILIC conditions.

3.1. Selectivities of HILIC stationary phases

Structures of the HILIC media and bonded ligands are shown in Fig. 2 Typically, HILIC mobile phase contains more than 70% of acetonitrile and at least 3% of a polar solvent such as water to form a water-rich layer on the surface of the stationary phase. Due to the basic characteristic of opium alkaloids and consequently, their longer retention on HILIC media, strongly acidic pH was selected. Typical separation pattern of a mixture containing standards of narcotics on five different HILIC columns (mobile phases consisted of ACN-water containing 60 mM ammonium acetate, pH = 3.0) is illustrated in Fig. 3 As it is obvious, HILIC stationary phases with