Separation of Sedative – Hypnotic Drugs with Mixed Micellar Liquid Chromatography

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ABSTRACT: *Separation of ten sedative- hypnotic drugs was performed by RP-HPLC using mixed micellar mobile phase. Effect of temperature, type and amount of organic modifier in mobile phase on efficiency (N) and asymmetry factor (B/A) showed that, the appropriate conditions for a good separation were 35°C and 7% (V/V) butanol in mobile phase. Variations of selectivity factor versus butanol concentration, Mixture of SDS/ Brij- 35 concentrations and pH of mobile phase showed that the appropriate conditions were 7%(V/V), 15 mM SDS +1.0mM Brij-35 and pH=3 in mobile phase respectively.*

KEY WORDS: *Sedative-hypnotic drugs, High performance liquid chromatography, Micellar liquid chromatography, Mixed micellar liquid chromatography, Separation of sedative-hypnotic drugs.*

INTRODUCTION

Assignment of particular compound to the sedative - hypnotic class of drugs indicates that major therapeutic use is to sedation with concomitant relief of anxiety or to encourage sleep. The sedative - hypnotic drugs are also used by the community as anesthesia, relaxants and anticonvulsants. They are often abused by the young illicit drug user in large doses causing profound behavioral effects. Their continued abuse lead to dependence. The older population are not immune to using sedative-hypnotic drugs and are also often dependent on their effects [1-6]. Sedative - hypnotic drugs may also cause to sudden death if misused [7]. Since , these kinds of drugs are widely seen in clinical and forensic cases, their measurement in specimens are widely practiced [8,9]. Chromatographic techniques, particularly HPLC are the most commonly used to identify specific sedative-hypnotic drugs [9-11]. Micellar liquid chromatography (MLC) is a mode of reversed phase liquid chromatography (RPLC), which uses a surfactant solution above the critical micellar concentration (CMC) in mobile phase [12]. In some situations, MLC can offer many advantages over conventional RPLC. For instance, cationic, anionic and neutral species can be separated simultaneously in MLC. The other advantages of MLC technique are the low cost, non-flammability, non-toxicity and easy disposal of the mobile phases. In this paper, appropiate conditions (including: temperature, type and concentration of organic modifier, micelle concentration and mobile phase pH) for separation of ten sedative-hypnotic drugs were found using aqueous solution of SDS and Brij -35.

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EXPERIMENTAL

Materials

Sodium dodecyl sulfate (SDS), polyoxyethylene (23) dodecanol (Brij-35) and Inorganic chemicals such as $NaH_2PO_4.2H_2O$ were obtained from Fluka company (Buchs, switzerland). Ethosuximide, primidone, phenobarbital, oxazepam, carbamazepin, clonazepam, lurazepam, diazepam ,chlordiazepoxide and flurazepam were obtanied from Sigma (St.louis.MO, USA). All other chemicals were HPLC and analytical grade. The drugs name with the corresponding code is represented in Table 1.

Procedure

All mobile phases were vaccum- filtered through 0.45 μm filters (Millipore, Milford, MA, USA). Stock solution of 1 mg/mL of samples were prepared separately in methanol. A 0.5 M stock solutions of SDS and 0.1 M of Brij-35 were prepared and diluted with deionized doubly distilled water, organic solvent and phosphate buffer when they were needed.

Apparatus

The chromatographic measurments were carried out with HPLC system equiped with a liquid chromatographic pump series 10 and a model LC-95 UV/Visible spectrophotometer Detector (Perkin- Elmer. Norwalk, CT, USA). A Reodyne Injector Model 7125(Cotati , CA ,USA) with 10 μL loop. The analytical column used was C_{18} (250* 4.6 mm, 10 μm) from waters(Milford, MA, USA).The UV detector was set at 254 nm to monitor the compounds.

Definition 0f chromatographic parameters

Chromatographic parameters used in this paper were capacity factor (K') , selectivity factor (α), Asymmetric factor (B/A) and number of theoretical plates (N). Definition and determination methods for these parameters are as the following.

$$K' = (t_R - t_o) / t_o$$

Where t_R and t_o are retention times for retained and nonretained compounds.

$$\alpha = K'_2 / K'_1$$

Table 1: The sedative - hypnotic drugs name with their Corresponding code.

Code	Drug name	Code	Drug name
1	Ethosucximide	6	Clonazepam
2	Primidone	7	Lorazepam
3	Phenobarbital	8	Diazepam
4	Carbamazepin	9	Chlordiazepoxide
5	Oxazepam	10	Flurazepam

where K'_1 and K'_2 are capacity factors for neighboring compounds 1 and 2

($k'_2 > k'_1$).

To determine asymmetric factor(B/A)For a chromatographic peak, a perpendicular line was drawn from peak vertex to x axis (or time axis). This makes peak width in two parts. The ratio of width part on the right hand side to the left hand side is considered as B/A.

The number of theretical plates (N), was calculated from following equations.

$$N = 5.54 (t_R / W_{0.5})^2 \quad (1)$$

(for symmetric peak)

and

$$N = 41.7 (t_R / W_{0.1})^2 / (B/A + 1.25) \quad (2)$$

(for asymmetric peak)

where t_R and $W_{0.5}$ and $W_{0.1}$ are retention time for compound, peak width at 50 percent and 10 percent from base line respectively.

RESULTS AND DISCUSSION

Effect of temperature on efficiency and asymmetry factor

The temperature of the column affect pressure, analysis time and separation. The lower efficiency of MLC as compared to conventional RP-LC is due to higher viscosity of micellar mobile phase. Efficiency of column could be improved at higher temperature due to faster mass transfer of solute between mobile and stationary phases [13]. The basic parameters for detemination of proper temperature were the number of theoretical plates, N, and asymmetry factor. B/A. From data listed in Table 2, it was found that The most appropriate temperature was 35 °C for separation of these compounds.

Table 2: Effect of temperature on chromatographic parameters for Oxazepam.

Temp. (^{0}C)	t_R(min)	B/A	N
25	29.6	1.466	246
30	28.8	1.461	311
35	28.0	1.416	364
40	26.8	1.454	379

Conditions: Mobile phase, 30 mM SDS + 1.5 mM Brij- 35 Column C_{18} (250× 4.6 mm, 10 μm), Sample: 10 μl, 50 μg/mL Oxazepam, Flow rate= 1.2 mL/min, pH=3.

Table 3: Effect of organic modifier on chromatographic parameters for Oxazepam.

Organic Solvent	t_R (min.)	N	B/A
None	28/0	364	1.416
Acetonitrile	22.4	475	1.500
Methanol	23.2	592	1.375
Propanol	18.4	750	1.143
Butanol	11.6	817	1.125
2- Propanol	21.6	600	1.250

Conditions: Mobile phase, 30 mM SDS + 1.5 mM Brij – 35+5% (v/v) organic modifier. Column C_{18} (250×4.6 mm, 10 μm), Sample: 10 μl 50 μg/mL Oxazepam,, Flow rate = 1.2 mL/min, pH=3, T= 35 ^{0}C.

Effect of organic modifier in sepatation of sedative-hypnotic drugs Choice of orginc modifier

The addition of short-chain alcohols to micellar mobile phase reduces the thickness of the film of surfactant molecules covering the stationary phase and thus produces an enhancement in efficiency [14]. Here again two basic parameters N and B/A were used for selection of the best organic modifier. Results showed that a considerable improvement in chromatographic efficiency was obtained by the addition of organic solvent to micellar mobile phase(Table 3). The efficieny was increased in the following order for different organic modifiers.

Butanol> Propanol> Isopropanol> Methanol>Acetonitrile

As the polarity of organic solvent decreases the efficiency increases, and this could be related to improvement brought about by wetting the stationary phase with organic modifier.

Effect of butanol concentration on N, B / A , K' and α

To determine the proper amount of the butanol in moloile phase to affect an improvement in separation, the paremeters N and B/A were studied. Results showed that addition 1-10 % of butanol improved peaks shape but the best efficiency was observed at 7 % of butanol. Effect of butanol concentration on N, B/A is shown in Table 3.

Effect of butanol concentration on capacity factor was studied and a nonlinear relationship was obsorved between lnk' and butanol concentration (v/v). Effect of butanol concentration on selectivity is shown in Fig. 1. Variations of selectivity factor showed that 7 % butanol was the best conentration for separation of these compounds.

Effect of SDS and Brij-35 concentration on capacity and selectivity factor

In MLC, plot of 1/k′ against mixed micelle concentration is an straight line [12]. Variation of 1/k′ against SDS concentration (at Brij-35 constant concentration) for sedative- hypnotics drugs were linear. The solute-micelle binding-constant, K_{AM}, value were obtained from the slope to intercept ratio (Table 4). Increasing the concentreation of SDS and Brij- 35 in the mobile phase increase the power or elution strengh of mobil phase and decrease the analysis time [15]. The separation of sedative-hypnotic drugs, decreases at higher concentrations of SDS and Brij-35. with consideration of selectivity factor values, the appropriate SDS and Brij-35 concentration in mobile phase were 15 mM and 1.0 mM respectively at pH=3.

Effect of mobile phase pH on retention and selectivity factor

Retention of weak organic acids and bases is affected

Table 4: Binding constant of sedative - hypnotic drugs, K_{AM}, derived from the plot 1/k' VS [SDS] at constant concentration of Brij-35 (1.0mM).

Sedative-hypnotic drugs	Corr.Coeff	Slope	Intercept	K_{AM}
Ethosuximid	0.9909	0.0380	1.3096	0.0290
Primidone	0.9792	0.0155	0.6198	0.0250
Phenobarbital	0.9801	0.0122	0.3383	0.0361
Carbamazepin	0.9874	0.0083	0.1520	0.0546
Oxazepam	0.9785	0.0081	0.0797	0.1016
Clonazepam	0.9823	0.0082	0.0664	0.1235
Lorazepam	0.9923	0.0081	0.0500	0.1620
Diazepam	0.9906	0.0063	0.0348	0.1810
Chlordiazepoxide	0.9903	0.0047	0.0276	0.1703
Flurazepam	0.9872	0.0038	0.0168	0.2262

*Conditions: Column C_{18} (250 * 4.6 mm, 10 µm),7% (V/V) butanol, Flow rate =1.2 mL/min, pH=3, T=35 ^{0}C, [SDS] = 20 – 80 mM.*

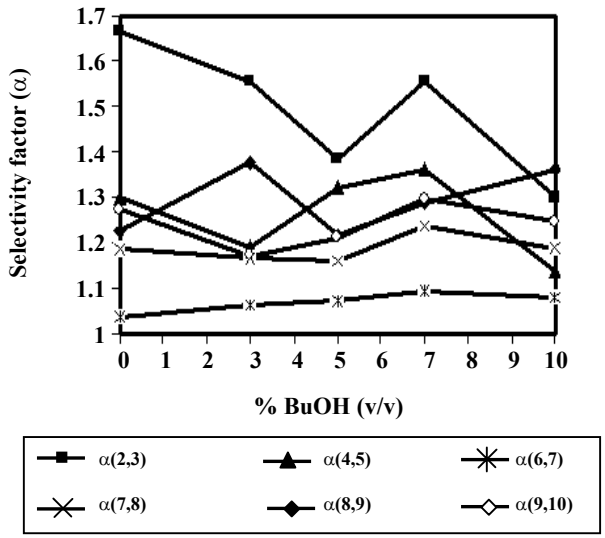

Fig. 1: Effect of butanol concentration on selectivity factor of sedative-hypnotic drugs.
Conditions: mobile phase, 30mM SDS + 1.5mM Brij-35, Column: C_{18} (250 * 4.6 mm, 10 µm), Flow rate= 1.2 mL/min, pH=3, T =35 ^{0}C.

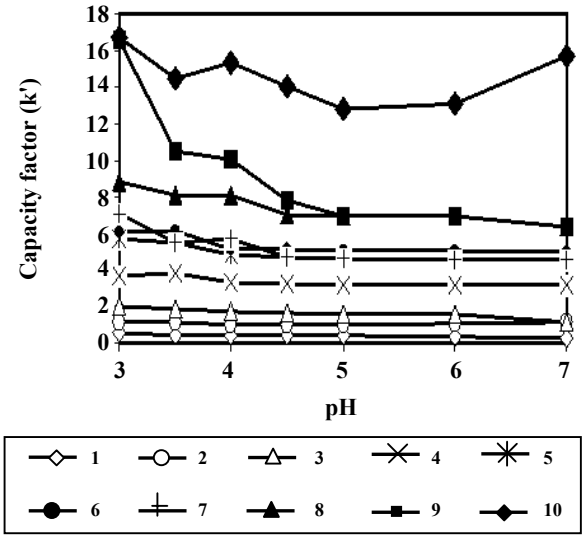

Fig. 2: Effect of mobile phase pH on capacity Factor (k') of sedative-hypnotic drugs.
Conditions: mobile phase, 15mM SDS +1.0 mM Brij-35 +7% (V/V) butanol,
Column: C_{18} (250 *4.6 mm, 10 µm), Flow rate= 1.2 mL/min, T =35 ^{0}C.

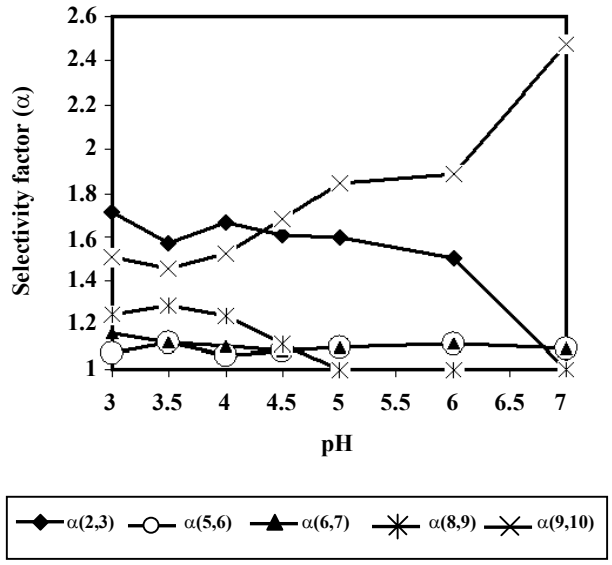

Fig. 3: Effect of mobile phase pH on Selectivity Factor (α) of sedative-hypnotic drugs.
Conditions: mobile phase, 15mM SDS + 1.0 mM Brij-35 + 7% (V/V) butanol, Column: C_{18} (250* 4.6 mm, 10 µm), Flow rate = 1.2 mL/min, T = 35 ^{0}C.

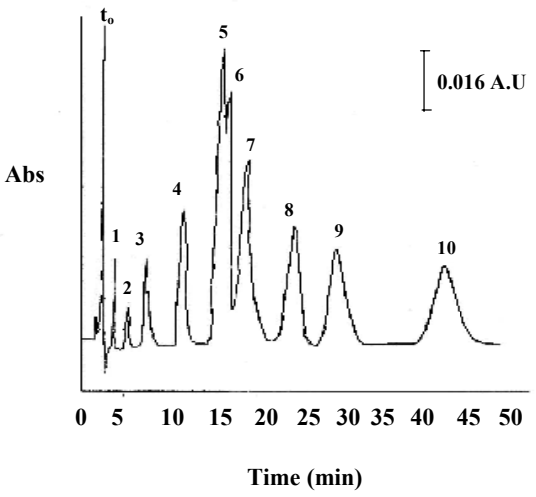

Fig. 4: Typical chromatogram for separation of sedative-hypnotic drugs atappropriate condition.
Conditions: mobile phase, 15mM SDS + 1.0mM Brij-35+ 7% (V/V) butanol, λ =254 nm, Column: C_{18} (250 * 4.6 mm, 10 µm), Flow rate =1.2 mL/min, pH=3, T = 35 ^{0}C.

by the pH of the micellar mobile phase. Since Solute-micelle partition coefficents of the dissociated and undissociated forms of a compound are different, Small changes in pH can significantly alter chromatographic retention, particularly when the mobile phase pH is ralated to the pKa value [16]. Plots of k´ against mobile phase pH (Figure 2) containing 15mM SDS + 1 mMBrij-35 + 7% (v/v) butanol show that k' values decrease at higher pH. Also, the variation of selectivity factor,α , against mobile phase pH showed that the , α , values decrease for all pairs at higher pH except for α 9,10 (Fig. 3) and the best separation of drugs was obtained at pH=3.The corresponding chromatogram are shown by Fig. 4.

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