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Original Article

Evaluation of Acetonitrile Deproteinisation of the Serum Samples for the Analysis of Drugs in Serum Using Capillary Zone Electrophoresis

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Absrtact

Deproteinisation with acetonitrile (along with methanol or other reagents) is a useful and rapid technique in analysis of drugs or their metabolites in human serum. In this paper application of this simple technique in biopharmaceutical analysis using capillary electrophoresis (CE) is evaluated. Some drugs with different ionic and protein binding properties were selected and dissolved in human serum. The efficiency of deproteinisation of spiked serum samples with acetonitrile and further analysis with CE was evaluated for each sample with special interest on the neutral drugs. The results showed that deproteinisation method is more efficient for charged molecules with low protein bindings. For neutral, relatively non-polar compounds (such as praziquantel), a MEKC method is preferred. For neutral, highly polar molecules (such as methimazole), other means of sample preparation must be considered.

Keywords: Capillary Electrophoresis; Serum; Acetonitrile Deproteinisation.

Introduction

Measuring drug levels in human serum is of fundamental importance in pharmacokinetic studies and reveals intoxication and is essential for therapeutic drug monitoring of a number of different classes of drugs, such as cardiac agents, certain antibiotics, and antineoplastics (1). Capillary electrophoresis (CE) with the ability to separate the components of complex aqueous samples with very high resolution is an attractive technique for biomedical and biopharmaceutical analysis.

Analysis of serum samples for endogenous substances and drugs presents special problems because of the high and variable components of proteins and other ions. Different approaches for sample preparation in CE include direct sample injection or after ultrafiltration using a micellar electerokinetic (MEKC) method (2), direct injection of diluted serum into a capillary zone electrophoresis (CZE) medium (3), acetonitrile deproteinisation (4) and the enhancement of the sensitivity by analyte derivatisation (5) and solid-phase extraction. In this work, acetonitrile deproteinisation will be evaluated with special interest on the neutral drugs. Drugs selected in this study (Figure 1) were methimazole and praziquantel (neutral molecules), atenolol and chlorpromazine (basic, cationic drugs) and theophylline (acidic, anionic drug). Human serum samples spiked with each of these drugs were deproteinised by acetonitrile and analyzed using a CZE method. Peak shape and efficiency of the separation were evaluated in each case.

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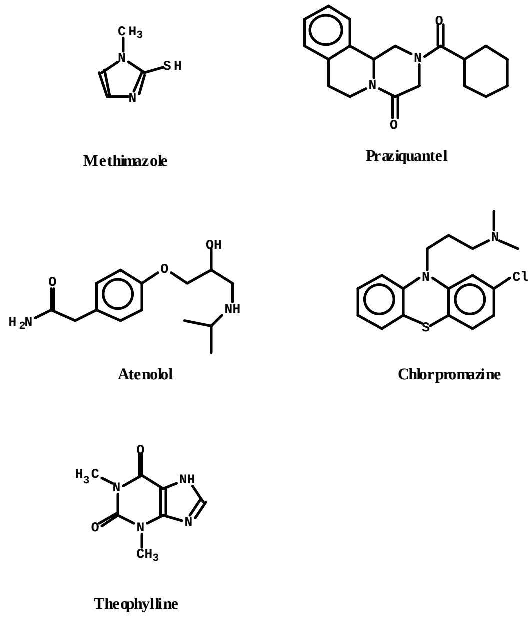

Figure 1. Chemical structures of model drugs

Experimental

CE Conditions

A model P/ACE 2210 Beckman (P/ACE) capillary electrophoresis instrument connected to Beckman System Gold Chromatography Software on a PS/2 IBM PC, and using uncoated fused silica capillary of 570 mm total length (500 mm to the detector) and 0.05 mm I.D. was employed. The capillary was kept at constant temperature using a thermostated liquid coolant. For the CZE experiments sodium tetraborate 20 mM at pH 8.5 was used as the running buffer and for the MEKC experiments the same buffer with added 50 mM SDS was used. All samples were introduced into the capillary using hydrodynamic injection at 5 seconds. Other conditions included: detection wavelength of 254 nm, voltage of 20 KV, and a temperature of 25° C.

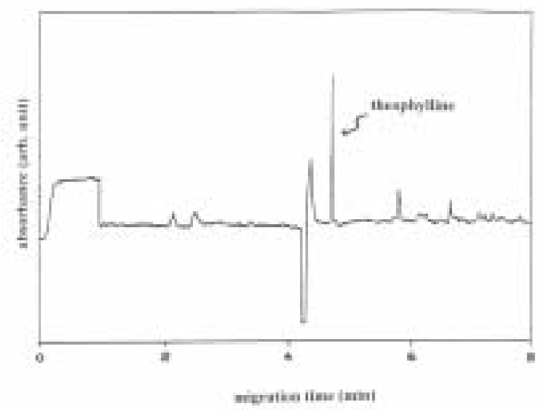

Figure 2. Electropherogram of supernatant of a sample serum spiked with theophylline (0.02 mg/ml) and deproteinised with acetonitrile 60%. Conditions: sodium borate buffer 20 mM pH 8.5 voltage 20 KV 214 nm wavelength 25° C temperature.

Serum Spiking and Deproteinisation Procedures

Drug Solutions- Each drug was prepared at a concentration of 0.2 mg/ml. In the case of methimazole and chlorpromazine HCl, the drug was dissolved in water. Theophylline was dissolved in 1 portion of NaOH 0.02 M and then the solution was diluted with 9 portions of water up to a volume of 10 ml. Atenolol and praziquantel were dissolved in 1 portion methanol and then 9 portions of water were added. These solutions were used for spiking the serum. Further dilute solutions of each drug at concentrations of 0.02 mg/ml were prepared using the running buffer as diluent and subjected to CE.

Serum Spiking- For each drug, 0.1 ml of the concentrated drug solution (0.2 mg/ml) was

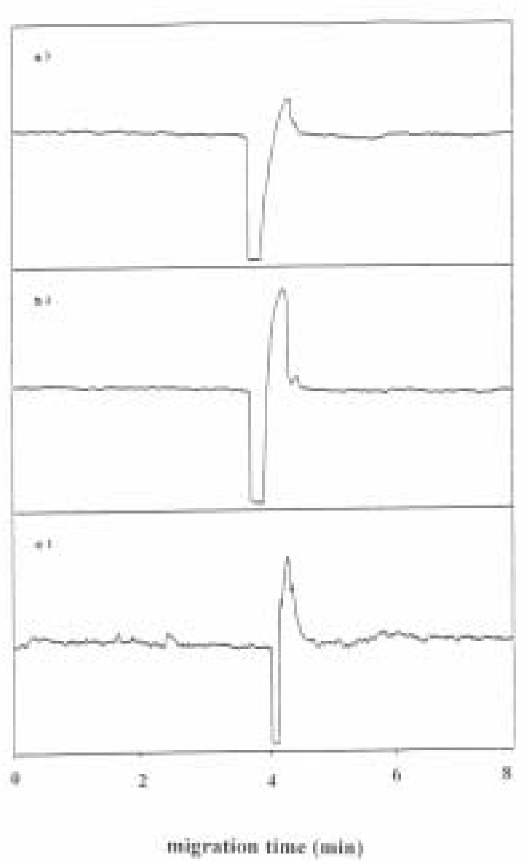

Figure 3. Electropherograms of supernatants of serum samples deproteinised with acetonitrile 60%. (a) Blank serum (b) serum spiked with methimazole (0.02 mg/ml) using a CZE method with the conditions as described in Figure 2 and (c) the same as (b) but using a MEKC method (50 mM SDS was added to the CZE buffer).

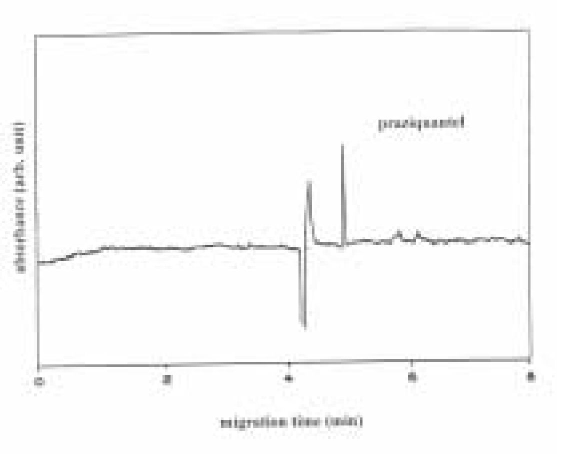

Figure 4. Electropherogram of supernatant of a sample serum spiked with Praziquantel (0.02 mg/ml) and deproteinised with acetonitrile 60%. Conditions as described in Figure3c

mixed with 0.9 ml human serum to obtain a final concentration of 0.02 mg/ml of the drug in serum.

Serum Deproteinisation- 0.4 ml of the spiked serum was added to 0.6 ml acetonitrile in a vial. The mixture was shaken in a sonic bath for 5 minutes and then centrifuged for a further 5 min at 1500 rpm. The supernatant was subjected to CE.

Results and Discussion

For rapid analysis, minimum sample preparation is desirable. To achieve this in HPLC, acetonitrile deproteinisation is quite commonly used for sample preparation. This technique has been applied to CZE (4). Different organic solvents have been used for serum deproteinisation (e.g. acetonitrile, methanol, ethanol, and 1-propanol). Acetonitrile affects the electroosmotic flow less

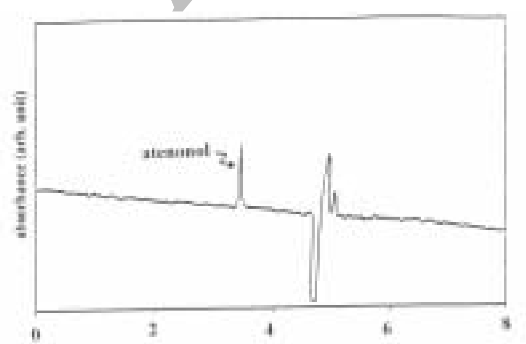

Figure 5 Electropherogram of a serum sample spiked with the drug atenolol (0.02 mg/ml) and deproteinised with acetonitrile 60%. Conditions as described in Figure 2.

than the alcohols. However, in CE, it does not conduct current. Shihabi (4) reported that mixtures of acetonitrile and water increase the plate number of theophylline and he explained this effect on the basis of stacking effect due to lower conductivity of the acetonitrile-water zone in the capillary. Theophylline is negatively charged at the buffer pH reported by Shihabi (pH = 8.5) and therefore, migrates after the solvent zone. This is shown in Figure 2. In the case of a neutral drug, such as methimazole, the pattern is different. Under CZE conditions methimazole is carried towards the detector by the electroosmotic flow. Thus, it remains in the original sample zone. Acetonitrile is also a neutral compound and does not leave the original sample zone and changes the UV absorptivity of the zone (Figure 3a). Therefore, in a mixture, both methimazole and acetonitrile will be carried together (Figure 3b) and no separation will be obtained. One way to overcome this problem is to use MEKC method instead of CZE. In the case of methimazole, adding 50 mM SDS to the running buffer resulted in a partial separation (Figure 3c), because methimazole is a relatively small, polar molecule with low tendency to interact with SDS micelles. In contrast, praziquantel which is also a neutral compound, separates from the original sample zone using MEKC (Figure 4). An explanation for this

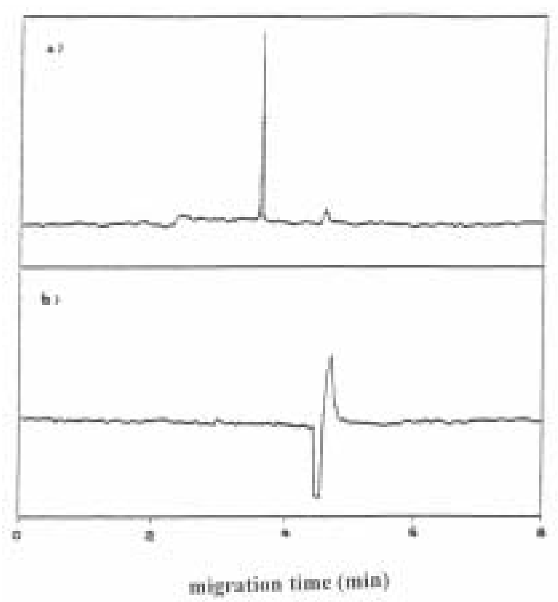

Figure 6. Electropherograms of (a) chlorpromazine HCl in buffer and (b) supernatant of serum spiked with chlorpromazine HCl (0.02 mg/ml) and deproteinised with acetonitrile 60%. Conditions as described in Figure 2.

phenomenon is that praziquantel is a non-polar compound which interacts with SDS micelles and migrates slower than the acetonitrile zone.

A similar experiment was carried out for the drugs atenolol and chlorpromazine. These two drugs are positively charged at pH 8.5. Therefore, it is expected that both drugs migrate faster than acetonitrile and leave the original sample zone. Deproteinised serum, which had been spiked with atenolol, was subjected to CZE and the result is shown in Figure 5. The result for chlorpromazine was different (Figure 6a), surprisingly, no peak relevant to chlorpromazine was observed in the electropherogram, although it was shown that this drug migrates within 4 minutes under the proposed CZE conditions (Figure 6b). The difference between atenolol and chlorpromazine is in their ability to bind to the plasma proteins. Chlorpromazine highly binds to the plasma proteins whilst atenolol partially binds to them (7). It seems that acetonitrile failed to extract chlorpromazine from the proteins.

Conclusions

Deproteinisation of the serum with acetonitrile is a simple and fast method for preparation of serum samples in CZE. However, the method is more efficient for charged molecules with low protein bindings. For neutral, relatively non-polar compounds, a MEKC method is preferred. For neutral, highly polar molecules (such as methimazole) other means of sample preparation must be considered.

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