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Enantiomeric separating study of D – and L – carnitine with capillary zone electrophoresis

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Abstract: A new capillary zone electrophoretic method has been developed for the enantiomeric separation and quantification of enantiomers of carnitine. D – andL – carnitine were derivatized with 4′ – bromophenacyl bromide producing a derivatization that can be detected by UV absorbance detection. The separation was performedby using a selective chiral containing sulfated – β – cyclodextrin and (S, S) – 1,7 – bis(4 – benzyl – 5 – hydroxy – 2 – oxo – 3 – azapentyl) – 1,7 – diaza – 12 – crown – 4. Acetonitrile was used as electroosmotic modifier and separation was carried out in an uncoated capillary. Under the optimal conditions D – and L – carnitine were separated completely and the limits of detection for both isomers were about 80 ng/mL.

Key words: Derivatization; Enantiomeric separation; electrophoresis; carnitine
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Carnitine [β – hydroxy – γ – trimethylaminobutyric acid] is a chiral biological substance found in different tissues of animals, some plants and microorganisms. Its enantiomers show different biological activities. L – carnitine is found to play an important role in the transport of fatty acids across the mitochondrial membrane by the transferase and translocase enzyme system [1,2]. Recently, carnitine and its ramifications have been proved to possess interesting pharmacological and nutritional properties [3]; On the other hand, D – carnitine has been shown to have a considerable toxic influence on biochemical processes [4]. In the past, patients have been treated with D, L – carnitine for anorexia, dyspepsia and other pathologies. However, D – carnitine is an emulative depressor to the L – carnitine acetyltransferase, leading to a depletion of body L – carnitine storage [5], the use of the pure L – carnitine and its ester is recommended. An uncoated capillary, sulfated – β – cyclodextrin and (S, S) – 1,7 – bis (4 – benzyl – 5 – hydroxy – 2 – oxo – 3 – azapentyl) – 1,7 – diaza – 12 – crown – 4 as chiral additive and acetonitrile as electroosmotic flow (EOF) modifier were used for the separation. The optimized method has beencharacterized and appropriately validated for the determination of these compounds.

1 Experiment

1.1 Instrumentation

A Beckman capillary electrophoresis instrument P/ACE 2000 (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector operating at 260 nm was used and a 32 Karat software station was used to perform the data collection and controlling the operational variables of the system.

Fused – silica capillary material (yongnian Hebei, China) of 60 or 40 cm length (60 or 40 cm to the detection

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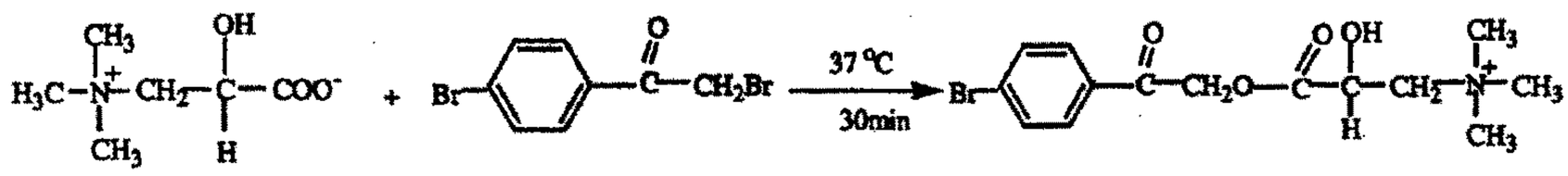
window) × 75 μm I.D. was used. Operation voltages were 30 kV. Injection was performed in the electrokinetic mode. Injection time was 10 seconds using a voltage of 10 kV.

1.2 Operating conditions

A positive power supply of 30 kV was used with a constant current mode of operation. Capillaries of effective length of 60cm × 75μm I.D were used and the temperature was 25℃. The capillaries were washed for 5 min with 0.1 mol/L sodium hydroxide solution, 10 min with water and 5 min with running buffer. The separation buffer was 50 mmol/L phosphoric acid, 1.25% (w/v) sulfated – β – cyclodextrin, 0.94% (w/v) (S, S) – 1,7 – bis (4 – benzyl – 5 – hydroxy – 2 – oxo – 3 – azapentyl) – 1,7 – diaza – 12 – crown – 4 (in acetonitrile) and the detection was carried out at 260 nm.

2.3 Derivatization

The derivatization reaction is described as follow.



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2 Results and discussion

2.1 Optimization of the CE separation

2.1.1 Influence of pH

PH effects were studied over the range of 3 ~ 9.6 for the model substance using phosphate 50 mmol/L as background electrolyte and 1.25% (w/v) sulfated – β – cyclodextrin and 0.94% (w/v) (S, S) – 1,7 – bis (4 – benzyl – 5 – hydroxy – 2 – oxo – 3 – azapentyl) – 1,7 – diaza – 12 – crown – 4 (in acetonitrile) as chiral selector. As a result, all separations showed enhanced resolution at pH3 ~ 7, the best separation was pH = 3.0.

2.1.2 Effect of applied voltage

The effect of the applied voltage in the range of 10 ~ 30 kV on the migration time as well as on the separation was studied at a phosphate buffer system (50 mmol/L) with 1.25% (w/v) sulfated – β – cyclodextrin and 0.94% (w/v) (S, S) – 1,7 – bis (4 – benzyl – 5 – hydroxy – 2 – oxo – 3 – azapentyl) – 1,7 – diaza – 12 – crown – 4 (in acetonitrile) as chiral selector. Good enantiomeric separations were achieved in the range of 10 ~ 30 kV. At a high voltage (30 kV) which was also the limit of the devices, the higher Joule heating of the capillary made the resolution a 21% decrease, however, at a low voltage, the diffusion of band was broadened and the separated time was longed. So the voltage of 20 kV seemed to be a good compromise of sufficient resolution with acceptable speed of analysis.

2.1.3 Effect of the temperature

Since the host – guest complexation mechanism is a kinetically driven process and there is potential Joule heating within the capillary under usual voltage settings of 10 ~ 30 kV, temperature effects are supposed to be a crucial parameter for chiral separation in capillary zone electro – phoresis. Joule heating mainly depending on the power, capillary dimensions, conductivity of the running buffer and applied voltage was limited by using an active temperature control.

Accordingly our temperature study ranging from 15 to 50℃ on enantiomeric separations revealed a significant but not Van 't Hoff – type relationship of increased migration and resolution versus. The resolution is decreasing as the temperature is rising due to the potential Joule heating.

2.1.4 The effect of sulfated – β – cyclodextrin and (S, S) – 1,7 – bis (4 – benzyl – 5 – hydroxy – 2 – oxo – 3 – azapentyl) – 1,7 – diaza – 12 – crown – 4 concentration

The effect of CD concentration on the separation, reported as an essential parameter in CD – modified CZE by several authors. Optimum separations for the model substance 4′ – bromo phenacyllcarnitine were achieved at the concentration of 1.25% (w/v) sulfated – β – cyclodextrin and 0.94% (w/v) (S, S) – 1,7 – bis (4 – benzyl – 5 – hydroxy – 2 – oxo

-3-azapentyl)-1,7-diaza-12-crown-4 (in acetonitrile). When rising or decreasing the concentration of CD or (S, S)-1,7-bis (4-benzyl- 5-hydroxy-2-oxo-3-azapentyl)-1,7-diaza-12-crown-4, it was an obvious decreasing of resolution. Under the optimum conditions, the model substances constructed a steady substance with CD and (S, S)-1,7-bis (4-benzyl- 5-hydroxy-2-oxo-3-azapentyl)-1,7-diaza-12-crown-4 by hydrogen bond and static and so on.

2.1.5 Effect of modifier on separation

Organic modifiers added to the electrolyte buffer system are in many cases reported to facilitate a finetuning of enantiomeric separations. In our texts, acetonitrile was added to the electrolyte buffer system not only as a solvent but also as a modifier. We have studied this effect on the enantiomeric separation of BPB-carnitine adding acetonitrile at the range of 10% ~ 30% to a sodium phosphate(50 mmol/L, pH3.0) background electrolyte system with 1.25% (w/v) sulfated-β- cyclodextrin and 0.94% (w/v) (S, S)-1,7-bis (4-benzyl- 5-hydroxy-2-oxo-3-azapentyl)-1,7-diaza-12-crown-4 (in acetonitrile) as chiral selector at a capillary temperature of 25℃.

The effect on EOF was negligible due to the lower pH revealing a prior low EOF conditions. But there was a significant decrease in resolution. Accordingly, the modifier effect cannot be simply reduced to the influence on EOF. An additional effect on chiral recognition mechanism via interaction with the selector and/or the analyte can be assumed. Also, the change in bulk liquid viscosity and consequently diffusion velocity and/or interaction kinetics should not be neglected. In general, the prediction of modifier effects seems to be hampered by the high complexity of the variously equilibrium involved.

2.2 Calibration Curve, Linearity, Reproducibility and Quantification Limits

By using the optimized conditions described above, standard solutions of L-carnitine and D-carnitine were prepared and derivatized and injected. A good linearity of the peak area to L-carnitine and D-carnitine concentration were obtained from 0.4 to 6.4 μg/mL, the R were 0.999 of L-carnitine and 0.998 of D-carnitine. The detection limit determined for the method were 0.08ug/ml and were taken as the minimum peak area that gave a response six times higher than the noise signal.

The precision of the method was determined by consecutive analyses of a 8ug/ml D/L-carnitine standard sample. The relative standard deviation of derivatization was 3.2% (n = 6) and the relative standard deviation of the migration time was 2.4%. The good linearity and precision of the method were thus confirmed.

3 Conclusion

D-carnitine and L-carnitine can be separated by capillary electrophoresis using sulfated-β- cyclodextrin and chiral crown as chiral additive with 4′-bromophenacylbromide derivatization. The method is rapid, simple and sensitive enough for the D/L-carnitine. The advantages of the present method are the good resolution, sensitivity and short separation times.

All the conditions of the assay were carefully verified through optimized validation. This method is adequate for routine assay of the enantiomeric excess of carnitine in raw material and in pharmaceuticals.

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D - 和 L - 肉碱对映体的毛细管电泳分离研究

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摘 要:提出了用毛细管区带电泳分离和检测肉碱对映体的方法。肉碱消旋体用 4 - 溴苯甲酰甲基溴衍生化,并经选择性手性试剂——磺化 β - 环糊精和手性冠醚[(S,S) - 1,7 - bis (4 - 苯基 - 5 - 羟基 - 2 - 氧 - 3 - 氮苄基) - 1,7 - 二氮杂 - 12 - 冠 - 4]协同作用后,可得到良好分离,并用紫外检测器检测,两异构体的检测下限约为 80 ng/mL。

关键词:衍生化;对映体分离;毛细管电泳;肉碱

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The new advance of ion chromatography

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Abstract: The new advance of ion chromatography was reviewed, and the some other techniques, such as electrochemistry andnew type stationary phases, are also discussed.

Key words: IC; advance; electrochemistry