利用管柱切換之螢光高效液相層析法偵測大白鼠尿液中之 D-乳酸

Fluorimetric Determination of D-Lactate in Rat Urine Using a Column-Switching High-Performance Liquid Chromatography

中文摘要

D-乳酸的濃度在微生物感染程度上是一個有用的指標，其他一些人類的疾病，像腦病、糖尿病和酸中毒的情況，也均可發現血漿中 D-乳酸的濃度有上升的情形。爲了更有效的定量 D-乳酸，故發展管柱前螢光衍生化之高效液相層析法。選擇 4-Nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ)和 4-(N,N-dimethylaminosulfonyl)-7-piperazino-2,1,3-benzoxadiazoles (DBD-PZ)作爲衍生化試劑，掌性管柱則選用 Chiralcel OD-RH，Chiralcel OJ-R 和 Chiralpak AD-RH，以不同組成的移動相來作容量係數、分離係數和解析度的研究。根據結果顯示，以 NBD-PZ 作爲螢光衍生化試劑，使用 Chiralpak AD-RH 在移動相氰甲烷：水＝60：40 時爲最好的掌性分離條件。之前的文獻報導其沖提順序皆爲 L 在前 D 在後，這樣會產生的問題是當小量的 D-乳酸存在於大量的 L-乳酸中時，巨大的 L-乳酸波峰可能會掩蓋到隨後出現的少量 D-乳酸波峰，在我們的方法中沖提順序反轉，可以解決這樣的問題。

在分析檢品上，以高效液相層析儀將 NBD-D,L-乳酸在碳 18 管柱以激發光 491 nm 及發射光 547 nm 下分離出，(D + L)-乳酸衍生物的部分藉由轉換閥的作用直接轉至 amylose 掌性管柱，這樣便可將 D-乳酸衍生物和 L-乳酸衍生物作鏡像分離，鏡像比例也可從此得知。此分析方法的準確度在 96.93%到 104.85%間，精密度之相對變異數在 0.80%到 14.44%間，表示此爲可接受之分析方法。

上述的方法應用於以 streptozotocin 誘導之糖尿病鼠尿液的分析，可以發現糖尿病鼠尿液中 D-乳酸和肌酸酐的比例相對於正常大白鼠有極爲顯著的增加。D-乳酸的濃度於此病理狀態下的變化仍需長期追蹤。

英文摘要

D-Lactate level is an useful indicator for the degree of the microbial contamination, and it has some relations to the pathophysiology of human diseases such as encephalopathy, diabetes and acidosis, in which plasma D-lactate levels were increased.

For more effective quantitative determination of D-lactate, an HPLC method with pre-column fluorescent derivatization was developed. D,L-Lactate were fluorimetrically derivatized by 4-nitro-7-piperazino- 2,1,3-benzoxadiazole (NBD-PZ) and 4-(N,N- dimethylaminosulfonyl)-7- piperazino-2,1,3-benzoxadiazole (DBD-PZ). The following chiral columns were selected: Chiralcel OD-RH, Chiralcel OJ-R and Chiralpak AD-RH. The capacity factor, the separation factor and the resolution were

investigated by modifying the compositions of the mobile phases. According to the results, a highly sensitive method which includes fluorescent derivatization of D,L-lactate with NBD-PZ was established. The elution order of D,L-lactate derivatives was L and D by recent literatures, but the elution order in our method was inverse. The inversion of the elution order can solve the problem of large peak of L-lactate covers over the peak of D-lactate when a small amount of D-lactate with a large excess of L-lactate in the biological samples.

The NBD-D,L-lactate is separated by HPLC on an octadecylsilica (ODS) column and determined fluorimetrically at 547 nm with 491 nm of excitation wavelength. During the separation step on the ODS, the peak fraction of (D + L)-lactate derivatives was introduced directly to an amylose chiral column by switching the flow of the elute via a six-port valve. Then, D-lactate derivative was separated enantiomerically from that of L-lactate, and the enantiomeric ratio was determined from the chromatogram. The inter-day and intra-day levels of precision were acceptable, with coefficients of variation from 0.80% to 14.44%.

The proposed method was applied to the urine of diabetic rats induced by intraperitoneal administration of streptozotocin, and the significant increases of D-lactate related to creatinine were observed in the diabetic rats as compared to the normal rats. It should be necessary to pursue the changes of D-lactate concentrations under the pathological conditions.ec .