Communications to the Editor

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF HEXOSAMINES AND HEXOSAMINOLS DERIVED BY TREATMENT WITH 7-FLUORO-4-NITROBENZO-2-OXA-1,3-DIAZOLE

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Hexosamines or hexosaminols (products of hexosamines produced by reduction with sodium borohydrate) were converted to fluorescent derivatives by treatment with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole. They were separated by high-performance liquid chromatography with a RADIAL PAK C_{18}® column (Waters) using aqueous 8% (v/v) acetonitrile solution as the mobile phase. The detection limits were within a few pmol.

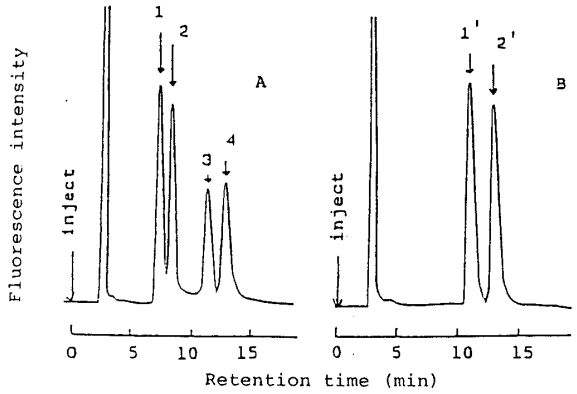
KEYWORDS ——— hexosamine; hexosaminol; glucosamine; galactosamine; high-performance liquid chromatography; 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole

High-performance liquid chromatography (HPLC) has been widely used to determine glucosamine and galactosamine in biological materials. Recently, Hjerpe et al.[1)] developed a sensitive determination method by HPLC using a precolumn derivation method. In their method, hexosamines were converted to the corresponding 5-dimethylaminonaphthalene-1-sulfonyl (DNS) derivatives and submitted to HPLC equipped with a fluorimetric detector. However, this method was very complicated and time-consuming in the derivation and separation.

In this communication, we describe chromatographic separation of hexosamines and hexosaminols using 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) as a prelabeling reagent, which was proposed by Imai et al.[2)] for the determination of amino acids. We have applied our technique in the identification and quantification of glucosamine and galactosamine.

The reaction conditions for the preparation of the NBD-derivatives of hexosamines were as follows. To a 10 µl sample solution, 10 µl of 0.1 M phosphate buffer (pH 6.7) and 20 µl of 50 mM NBD-F in ethanol were added, and the mixture was heated to 60°C for 30 min; The reaction was stopped with the addition of 60 µl of 0.1 M HCl and the reaction mixture was submitted to HPLC. A typical chromatogram of glucosamine and galactosamine and the conditions of HPLC are shown in Fig. 1 A. Glucosamine and galactosamine gave two peaks which may correspond to α- and β-anomer respectively, but chromatographic patterns were convenient for the identification of those hexosamines.

Alternatively, hexosamines were converted to corresponding hexosaminols such as glucosaminol and galactosaminol by reduction with $NaBH_4$, following the procedure reported previously for the gas chromatographic method,$^{3)}$ and treated with NBD-F.



A. 1,3 : NBD-galactosamine, 2,4 : NBD-glucosamine
B. 1' : NBD-glucosaminol, 2' : NBD-galactosaminol

Conditions
Column: RADIAL PAK C_{18} (8 mm i.d. x 100 mm)
Mobile phase: 8% (v/v) acetonitrile
Flow rate: 1 ml/min
Detector: Shimadzu RF-530, Ex 470 nm, Em 545 nm
Sample size: 10 µl
Sample: Glucosamine and galactosamine
(1 nmol each)

Fig. 1. Chromatograms of NBD-Derivatives of Hexosamines (A) and Hexosaminols (B)

The reduction procedure was as follows: 0.5 ml of sample solution containing 5 nmol to 5 µmol of each hexosamine and 0.5 ml of aqueous 1% (w/v) $NaBH_4$ solution were mixed and kept for 30 min at room temperature. The excess $NaBH_4$ was destroyed by adding 1 M HCl and the solution was evaporated to dryness in vacuo after adding one drop of 1-propanol to prevent bumping. To the residue, 0.5 ml of methanol was added and the mixture was evaporated to dryness to remove boric acid as methyl borate. The residue was dissolved in 0.5 ml of distilled water and an aliquot of the sample solution containing hexosaminols was submitted to the derivation procedure described above. The NBD-derivatives of glucosaminol and galactosaminol were submitted to HPLC under the same conditions used with the hexosamines. In this reduction method, glucosamine and galactosamine each gave one peak as shown in Fig. 1 B. Calibration curves for both hexosamines were linear in the range of 10 µM to 1 mM with a sample size of 10 µl. The detection limit (S/N=2) was 5 pmol.

The retention times of the NBD-derivatives of hexosamines and hexosaminols are summarized in Table I.

Table I. Retention Times of NBD-Hexosamines and NBD-Hexosaminols

Compound	Retention time (min)		Compound	Retention time (min)
Glucosamine	8.4	13.2	Glucosaminol	11.6[a)]
Galactosamine	7.2	11.2	Galactosaminol	13.6
Mannosamine	8.1	14.3	Mannosaminol	10.4[a)]

a) Rs of NBD-glucosaminol and NBD-mannosaminol is 0.85 under the conditions given in Fig. 1.

The proposed methods may be useful for the routine analysis of hexosamines and/or hexosaminols.

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