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Analysis of Aldoses and Alditols by Capillary Gas Chromatography as Alditol Trifluoroacetates

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Analysis of aldoses and alditols by capillary gas chromatography as alditol trifluoroacetates was carried out by using a fused silica capillary column (cyanopropyl-bonded phase) and a hydrogen flame ionization detector. Seventeen alditols were completely resolved within 18 min. The detection limits were about 1—4 ng/injection which are one hundred times smaller than as those of a packed column. Special care was necessary in the use of internal standards for the simultaneous determination of multiple components, and good reproducibility was obtained by using double internal standards.

Keywords——aldose; alditol; alditol trifluoroacetate; capillary gas chromatography

Quantitative analysis of sugar and sugar alcohols is of increasing importance in biochemistry and other fields and has been reported by many authors.

In high-performance liquid chromatography (HPLC), a ultraviolet (UV) detector is not applicable since sugars and sugar alcohols lack UV sensitive chromophores. The differential refractometer usually employed is relatively insensitive and precludes the use of gradient elution, leading to serious limitations in microanalysis by HPLC. Thus, some recent studies have been reported on precolumn[1] or postcolumn[2] derivatization in order to detect these compounds either photometrically or fluorimetrically.

In gas chromatography, as sugars and sugar alcohols are non-volatile compounds they should be converted into volatile derivatives. Many derivatization procedures have been studied which include methylation, acetylation, trimethylsilylation, and trifluoroacetylation.[3] Among them, trifluoroacetylation has some advantages: 1) preparation of trifluoroacetyl (TFA) derivatives is simple, rapid and quantitative; 2) the presence of halogen atoms in TFA derivatives enables highly selective and sensitive determination by using an electron capture detector (ECD).

Recent progress in high-resolution capillary gas chromatography has been enormous, and some studies have also been described on acetyl,[4] and trimethylsilyl (TMS)[5] derivatives of sugar alcohols. However, capillary gas chromatography of TFA derivatives has not been reported yet.

We have examined capillary gas chromatography of TFA derivatives of sugars and sugar alcohols applicable to various biological materials in order to develop a method for further investigation of their precise clinical profiles in pathological conditions. The present report describes a procedure for the analysis of aldoses and alditols, which involves reduction of aldoses, trifluoroacetylation of alditols, and capillary gas chromatography of the TFA derivatives. Special care was necessary in the use of internal standards for the simultaneous determination of multiple components.

Experimental

Materials——Erythritol, xylitol, ribitol, galactitol, glucitol, arabinitol, fucose, rhamnose, and trifluoroacetic

anhydride were obtained from Nakarai Chemicals, Ltd., Kyoto. Erythrose, ribose, arabinose, xylose, galactose, glucose, mannose, and *myo*-inositol were purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo. 2-Deoxygalactose, 2-deoxyglucose, 6-deoxyglucose, allose, talose, threose, idose, threitol, and perseitol were supplied by Sigma Chemical Co., St. Louis, U.S.A. Ethyl acetate was obtained from Kanto Chemical Co., Ltd., Tokyo, and sodium borohydride from E. Merck AG, Darmstadt, West Germany.

Gas Chromatography——A Shimadzu GC-9A gas chromatograph equipped with an SPL-G9 inlet system (Shimadzu) and a hydrogen flame ionization detector, and interfaced with a Shimadzu C-R3A Chromatopac data system, was employed. The column was a Shimadzu CBP10-M25-025 fused silica capillary column (cyanopropyl-bonded phase, 25 m × 0.2 mm (i.d.), film thickness 0.25 μm). Gas flow rate (helium) was 2 ml/min, and the split ratio was 1 : 20. The injector and detector temperatures were 200 °C and the column oven was maintained at 150 °C.

Preparation of Derivatives——Alditols were prepared from the corresponding aldoses by $NaBH_4$ reduction,[6b] where 10—50 μg of aldoses was applied instead of 100—500 μg. Alditols were treated with 0.1 ml of ethyl acetate and 0.1 ml of trifluoroacetic anhydride for 30 min at room temperature and 1—2 μl of the reaction mixture was injected directly into the gas chromatograph.

Results and Discussion

The baseline resolution of alditol TFA derivatives was achieved on a CBP 10 column

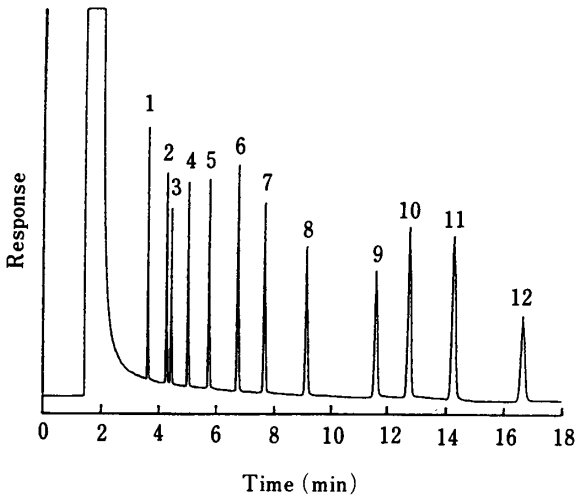

Fig. 1. Gas Chromatogram of Alditol TFA Derivatives

Peaks: 1, erythritol; 2, threitol; 3, rhamnitol; 4, fucitol; 5, ribitol; 6, arabinitol; 7, xylitol; 8, mannitol; 9, glucitol; 10, galactitol; 11, *myo*-inositol.

TABLE I. Relative Retention Times for Alditol Trifluoroacetates

Parent aldose	Corresponding alditol	Relative retention time
Erythrose	Erythritol	0.473
Threose	Threitol	0.568
Rhamnose	Rhamnitol (6-deoxymannitol)	0.577
Fucose	Fucitol (6-deoxygalactitol)	0.654
6-Deoxyglucose	6-Deoxyglucitol	0.746
Ribose	Ribitol	0.750
Arabinose (lyxose)	Arabinitol (lyxitol)	0.879
Xylose	Xylitol	1.000 (7.61 min)
Allose	Allitol	1.063
Mannose	Mannitol	1.193
Talose (altrose)	Talitol (altritol)	1.311
2-Deoxyglucose	2-Deoxyglucitol	1.346
Glucose	Glucitol	1.516
Idose	Iditol	1.572
2-Deoxygalactose	2-Deoxygalactitol	1.666
Galactose	Galactitol	1.668
—	*myo*-Inositol	1.820
—	Perseitol	2.162

TABLE II. Reproducibilities in Determination of Alditols (C.V. %)

Exp. No.	Ery	Thr	Rham	Fuc	Rib	Ara	Xyl	Man	Glu	Gal	Ino	Per
1	8.54	7.90	—	—	I.S.	1.76	0.96	4.28	3.75	4.75	—	—
2	12.3	11.4	—	—	4.87	4.24	4.27	2.61	2.30	I.S.	—	—
3	0.77[a]	0.35[a]	I.S.-1	0.67[a]	1.26[a]	0.78[a]	1.88[b]	1.70[b]	1.08[b]	1.46[b]	1.11[b]	I.S.-2

Ery, erythritol; Thr, threitol; Rham, rhamnitol; Rib, ribitol; Ara, arabinitol: Xyl, xylitol; Man, mannitol; Glu, glucitol; Ino, *myo*-inositol; Per, perseitol; C.V., coefficient of variation; exp. No. 1, $n=10$; exp. No. 2, $n=10$; exp. No. 3, $n=5$; I.S., internal standard. *a*) Calculation was based on I.S.-1. *b*) Calculation was based on I.S.-2.

under isothermal conditions (Fig. 1). Relative retention times are shown in Table I. All of the theoretically possible tetritols, pentitols, and hexitols were separated from each other within 13 min, including glucitol and iditol, which could not be separated by the packed column.[6a] With regard to analysis time, while our method using TFA derivatives takes 13 min under isothermal conditions, the method by Jansen *et al.*[5] using TMS derivatives takes about 20 min and that by Klok *et al.*[4] takes about 40 min, both with programmed temperature changes.

As shown in Table I, all of the compounds tested could be separated from each other, except 2-deoxygalactitol and galactitol. Therefore, it is now very easy to select internal standards for qualitative analysis, depending on the purposes and targets of measurement. However, because of the use of a split injector, special care is necessary for the accurate determination of multiple sugar components, which have a wide range of volatility. Table II shows the reproducibilities in determination of alditols when different internal standards were used and calculation was based on the peak area ratio. In exp. No. 1 and No. 2, a single internal standard was used and the results show that the remoter the peak of the compound from that of the internal standard, the larger the coefficient of variation (C.V.) value. In exp. No. 3, two internal standards were used and the C.V. values were within a satisfactory range.

As regards sensitivity, the detection limits of alditols were 1—4 μg/injection which were about one-hundredth of those (0.1—0.5 ng/injection) when a packed column was used. It is likely that higher sensitivity can be achieved when an electron-capture detector, which is sensitive to halogen atoms, is applied.

In conclusion, the capillary gas chromatography of aldoses and alditols developed here shows high resolution, great sensitivity and satisfactory reproducibility within a short time, and therefore, seems to be applicable to microanalysis of sugars and sugar alcohols in various fields.

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