[Chem. Pharm. Bull. 20(8)1805—1808(1972)]

UDC 547.455.08 : 543.51.543.544

Gas Chromatography and Its Combination with Mass Spectrometry of Urinary Sugar Alcohols

Hidehiko Haga, Toshio Imanari, Zenzo Tamura[1a] and Atsushi Momose[1b]

Faculty of Pharmaceutical Sciences, University of Tokyo[1a] and Laboratory of Racing Chemistry[1b]

(Received March 8, 1972)

A gas chromatographic method and GC-MS is described for the analysis of urinary sugar alcohols as their trifluoroacetyl derivatives. Nine urinary sugar alcohols were identified; erythritol, threitol, fucitol, ribitol, arabinitol, xylitol, mannitol, glucitol and galactitol. The occurrence of fucitol in urine is noteworthy.

Concerned with galactosemia,[2] pentosuria,[3] cataract[4] and the metabolism of sugars,[5] the analysis of urinary sugar alcohols is important. Urinary sugar alcohols have been measured by many different methods, among these, gas chromatographic methods have distinct advantage in terms of speed and sensitivity. Wells, *et al.*[6] discribed a method for the analysis of sugars and sugar alcohols in urine by gas chromatography of the corresponding trimethylsilyl (TMS) ethers, where separation of isomers of sugar alcohols was unsatisfactory.

Matsui, *et al.*[7] established the gas chromatography of sugar alcohols as their trifluoroacetyl (TFA) derivatives, where all of them, except D-glucitol and L-iditol, were separated from each other. For the application of this method, however, coexistence of sugars interferes with gas chromatography. We investigated removal of concominant sugars and combination of gas chromatography with mass spectrometry (GC-MS) to establish an analytical method of sugar alcohols in urine.

Experimental

Materials——Ethyl acetate (GR; Kanto Chemical Co., Ltd.), trifluoroacetic anhydride (TFAA) (GR), phenylhydrazine (EP), phenylhydrazine-*p*-sulfonic acid (EP; Tokyo Kasei Kogyo Co., Ltd.), sodium borohydride (GR; E. Merck AG) were used directly. Erythritol, D-arabinitol, D-xylitol were commercial samples. Fucitol and ribitol were prepared from the corresponding aldoses by $NaBH_4$ reduction.[8] L-Threitol was prepared from L-threonolactone by treatment with $NaBH_4$.[9]

QAE-Sephadex (50—100 mesh) was exhaustively washed with water and the fines were removed by decantation after the treatment with acid and alkali, the resin was converted into the borate form by passing a saturated solution of $K_2B_4O_7 \cdot 5H_2O$, washed with dist. water and stored moist.

Gas Chromatography——Gas chromatography was performed on a Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector. The glass tube (1.8 m × 4 mm i.d.) was packed with 2% XF-1105 on a support of Gas-Chrom P (80—100 mesh).

1) Location: *a*) *Hongo, Bunkyo-ku, Tokyo*; *b*) *Kamiyoga, Setagaya-ku, Tokyo.*
2) T.J. Egan and W.W. Wells, *Amer. J. Dis. Child.*, **111**, 400 (1966); R. Quan-Ma, H.J. Wells, W.W. Wells, F.E. Sherman and T.J. Egan, *ibid.*, **112**, 477 (1966).
3) O. Touster and S.O. Harwell, *J. Biol. Chem.*, **230**, 1031 (1958).
4) R. van Heyningen, *Biochem. J.*, **73**, 197 (1959); H.J. Wells and W.W. Wells, *Biochemistry*, **6**, 1168 (1967).
5) W.A. Wood, *Ann. Rev. Biochem.*, **35**, 538 (1966).
6) W.W. Wells, T. Chin and B. Weber, *Clin. Chim. Acta*, **10**, 352 (1964).
7) M. Matsui, M. Okada, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **10**, 1383 (1968).
8) M.L. Wolfrom and A. Thompson, "Methods in Carbohydrate Chemistry," Vol. II, ed. by R.L. Whistler and W.L. Wolfrom, Academic Press, New York, 1963, p. 65.
9) H.L. Frush and H.S. Isbell, *J. Am. Chem. Soc.*, **78**, 2844 (1956).

Mass Spectrometry and GC-MS——Hitachi RMU-6 and RMU-7 mass spectrometers were used singly or being combined with Hitachi K-53 gas chromatograph.

A stainless steel tube (2 m×3 mm i.d.) or a glass tube (1 m×4 mm i.d.) packed with 2% XF-1105 on a support of Gas-Chrom P (80—100 mesh) was used.

Mass spectra were measured under the following conditions; ionizing voltage: 40 eV, accelerator voltage: 1200 V, T_i: 140°, T_{tub}: 170°, T_{sep}: 200°.

Sugar alcohols-TFA derivatives were prepared as described below, and the reaction solutions were concentrated to remove ethyl acetate and excess TFAA and applied to a mass spectrometer.

In the case of GC-MS, sugar alcohol-TFA derivatives were dissolved again in 50 μl of ethyl acetate and 0.5 to 2 μl of the solution was injected to GC-MS.

Mass numbers were measured by the use of perfluorokerosene as an internal standard.

Gas Chromatographic Separation and Determination of Sugar Alcohols in Urine

The neutral fraction of urine includes sugar alcohols, aldoses and ketoses. We have successfully removed sugars by a reaction with a mixture of phenylhydrazine and phenylhydrazine-*p*-sulfonic acid. The reactions and excess reagents were removed by ion exchange resins. Then sugar alcohols were selectively separated from the other neutral materials by a QAE-Sephadex column (broate form).

Procedure

Two ml of normal urine was percolated through columns of Amberlite CG-120 (H^+) (0.8×12 cm) and Amberlite CG-4B (AcO^-) (0.8×12 cm) at room temperature. The effluent was evaporated to dryness under reduced pressure at below 50°. To the residue, 1 ml of dist. water, 60 mg of phenylhydrazine-*p*-sulfonic acid and 0.025 ml of phenylhydrazine were added. The solution was heated at 100° for 2 hr and centrifuged, then the supernatant was percolated through columns of Amberlite CG-4B (AcO^-) (0.8×12 cm) and CG-120 (H^+) (0.8×12 cm) at room temperature. The effluent was evaporated to dryness under reduced pressure. The residue was dissolved in 2 ml of water and percolated through a QAE-Sephadex column (borate form) (0.8×3 cm) and the column was washed with 10 ml of water. The sugar alcohols were eluted with 3 ml of a mixture of conc. HCl and CH_3OH (1:24) and the effluent was evaporated to dryness. To the residue, 1 ml of CH_3OH was added and evaporated to dryness to remove boric acid as methyl borate. After the repetition of this treatment two or three times, the residue was dried *in vacuo*. The sample was treated with 0.1 ml of ethyl acetate and 0.1 ml of trifluoroacetic anhydride (TFAA) for 15 min at room temperature. One to three μl of the reaction mixture was injected to a gas chromatograph.

Quantities of sugar alcohols were determined by the peak height ratio method using galactitol as an internal standard.

Recoveries of added sugar alcohols to urine were almost quantitative (Table I).

Table I. Recovery of Added Glucitol and Mannitol to Urine

Glucitol		Mannitol	
Added amount (μg)	Recovery (%)	Added amount (μg)	Recovery (%)
100	97.9	50	103.6
200	97.5	100	103.2
300	97.2	150	101.8
400	99.3	200	104.7
500	97.2	300	98.4

Under the conditions described above we found nine discrete peaks corresponding to sugar alcohols on gas chromatograms of sugar alcohols from urines of apparently healthy individuals. A typical gas chromatogram is shown in Fig. 1.

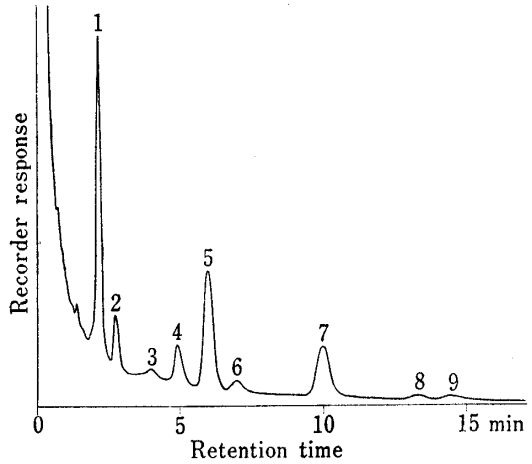

Fig. 1. Gas Chromatogram of Urinary Sugar Alcohols

peak: 1: erythritol; 2: threitol; 3: fucitol; 4: ribitol; 5: arabinitol; 6: xylitol; 7: mannitol; 8: glucitol; 9: galactitol
condition: 2%XF-1105 (on Gas Chrom P), glass tube, 1.8m×4mm *i.d.* column 140°, HFID 200°, N_2 90 ml/min

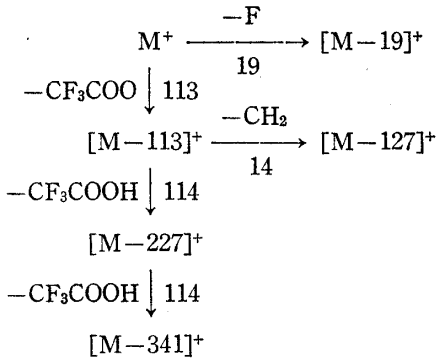

Chart 1

The quantities of the various sugar alcohols, excreted in urine collected in 2 hours after overnight fasting of apparently healthy individuasl, are shown in Table II.

Table II. Excretion of Sugar Alcohols in Urine (μg/hour)

Sample	Sex	Age	Ery.	Thr.	Fuc.	Rib.	Ara.	Xyl.	Man.	Glu.	Gal.
1	M	32	2805	303	217	358	4860	329	495	312	476
2	M	28	3330	419	195	414	4200	392	375	343	332
3	M	24	3300	906	291	456	4950	384	345	228	417
4	M	31	1650	305	178	278	1700	218	560	267	210
5	M	32	1640	197	153	342	1480	298	331	214	268

Ery.: erythritol, Thr.: threitol, Fuc.: fucitol, Rib.: ribitol, Ara.: arabinitol, Xyl: xylitol, Man.: mannitol, Glu.: glucitol, Gal.: galactitol

GC-MS for Identification of Urinary Sugar Alcohols

The coincidence of the retention time, however, is not sufficient evidence for identification. Therefore we attempted to identify these nine peaks in Fig. 1. by GC-MS method. At first we measured mass spectra of authentic sugar alcohols-TFA derivatives by means of direct and indirect (heated) inlet systems and by GC-MS method. There was little difference among the spectra measured by these methods. The mass spectra of TFA derivatives of the isomeric sugar alcohols were quite similar and the fragmentation pattern was common to all the homologs of sugar alcohols as shown in Chart 1. Molecular peaks were not detected in any mass spectra but $[M-19]^+$ peaks were detected in all mass spectra. Recently O.S. Chizhov, *et al.*[10] reported that molecular peaks instead of $[M-19]^+$ peaks had been detected in all spectra of sugar alcohol-TFA derivatives, however even by using the same reaction condition they described, we did not detect molecular peaks.

Secondly we measured mass spectra of sugar alcohol-TFA derivatives in urine using GC-MS method. These spectra completely coincided with the corresponding authentic spectra (Fig. 2,3,4,5), demonstrating that the nine peaks consisted of pure TFA derivatives

10) O.S. Chizhov, B.A. Dmitriev, B.M. Zolotarev, A. Ya Chernyak and N.K. Kochetkov, *Org. Mass Spectrom.*, **2**, 947 (1969).

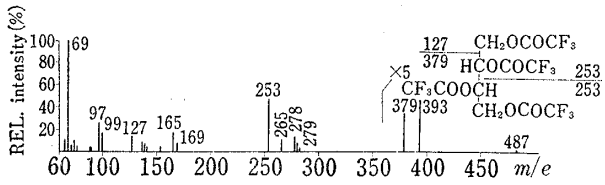

Fig. 2. Mass Spectrum of the TFA Derivative of Threitol from Urine (M.W. =506)

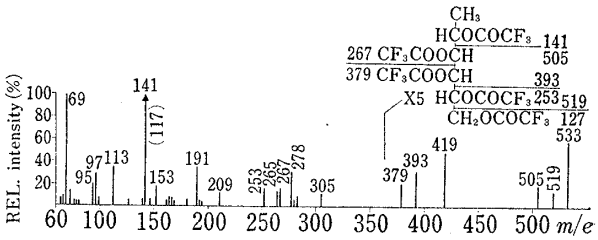

Fig. 3. Mass Spectrum of the TFA Derivative of Fucitol from Urine (M.W.=646)

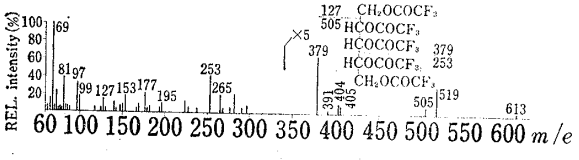

Fig. 4. Mass Spectrum of the TFA Derivative of Ribitol from Urine (M.W.=632)

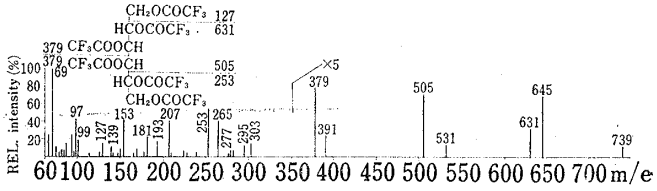

Fig. 5. Mass Spectrum of the TFA Derivative of Galactitol from Urine (M.W.=758)

of sugar alcohols, erythritol,[11] threitol,[12] fucitol, ribitol,[13] arabinitol,[14] xylitol,[15] mannitol,[6] glucitol (sorbitol),[15] galactitol (dulcitol).[2,13]

Discussion

The occurrance of fucitol in urine is noteworthy as this deoxy sugar alcohol has not been detected in nature, while L-fucose has a fairly widespread distribution. L-Fucose occurs in seaweeds[16] and a few gums, and found as a constituent of blood group substances from both animal and human souces,[17] human milk oligosaccharides,[18] urinary glycoprotein[19] and other glycoproteins. L-Fucose derived from these origins might be reduced *in vivo* by the action of aldose reductase which was proved to exist in the seminal vesicles and placenta of sheep and rat liver,[20] and rat lens.[4]

The mass spectra of isomeric sugar alcohol-TFA derivatives are very similar, though they are barely different in relative intensities of some peaks. Therefore it will be almost impossible to identify sugar alcohol-TFA derivatives by means of mere mass spectrometry, and the satisfactory resolution of isomeric sugar alcohol-TFA derivatives by gas chromatography is quite essential for their identification and separatory determination.

As the peaks obtained by our gas chromatographic method fully consist of TFA derivatives of urinary sugar alcohols, the method should be sufficiently reliable.

Acknowledgement The authors wish to thank Mr. K. Kamei for his technical assistance.

11) O. Touster, S.O. Hecht and W.M. Todd, *J. Biol. Chem.*, **235**, 951 (1960).
12) N. Spencer, *J. Chromatog.*, **30**, 566 (1967).
13) E. Pitkänen and K. Sahlström, *Ann. Med. Exp. Fenn.*, **46**, 151 (1968).
14) O. Touster and S.O. Harwell, *J. Biol. Chem.*, **230**, 1031 (1958).
15) E. Pitkänen and A. Pitkänen, *Ann. Med. Exp. Fenn.*, **42**, 113 (1964).
16) R.H. Côté, *J. Chem. Soc.*, **1959**, 2248 (1959).
17) E.A. Kabat, H. Bear, A.E. Bezer and V. Knaub, *J. Exptl. Med.*, **88**, 43 (1948); H.G. Bray, H. Henry and M. Stacey, *Biochem. J.*, **40**, 124 (1946).
18) A. Kobata, *Seikagaku*, **42**, 829 (1970).
19) A. Gottschalk, *Nature*, **170**, 662 (1952).
20) H.G. Hers, *Biochem. Biophys. Acta*, **37**, 120 (1960).