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Quantitation of Sugars in Street Drug Samples

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ABSTRACT: A gas chromatography method for the quantitation of sugars in street drug samples is presented. After isolation of the sugars from interfering adulterants, and derivatization, they were determined on a 3% OV-17 system.

KEYWORDS: toxicology, abuse drugs, chromatographic analysis, sugars

The use of trimethylsilyl derivatives for gas chromatography quantitation of monosaccharides and disaccharides was reported as early as 1963 [*1*]. Other studies have shown that the use of hydroxylamine hydrochloride to form oximes before derivatization prevents anomer peaks [*2,3*]. In the above studies, a variety of food products were examined, along with measured quantities of dextrose, sucrose, fructose, maltose, and lactose. Of these sugars, only lactose and dextrose are usually seen in samples of interest to forensic science.

The present study was undertaken to determine whether the polyalcohols mannitol and inositol could be simultaneously quantitated with the ketoses and whether common street adulterants would interfere with either a diluent of interest or the internal standard.

Materials and Methods

Reagents

The following reagents were used:

1. STOX reagent (No. 49805)—Pierce Chemical Co., Rockford, Illinois (contains hydroxylamine hydrochloride and phenyl-B-D-glucopyranoside internal standard in pyridine solution).
2. STOX working solution—1 part STOX reagent diluted with 9 parts pyridine which has been dried over sodium hydroxide (NaOH) pellets.
3. Tri-Sil Z reagent (No. 49231)—Pierce Chemical Co. (trimethylsilylimidazole in dry pyridine).
4. Ammoniacal chloroform ($CHCl_3$)—prepared by shaking 200 mL of $CHCl_3$ with 20 mL of concentrated ammonium hydroxide (NH_4OH), passing the $CHCl_3$ layer through dry filter paper.

Note that Reagents 1, 2, and 3 must be stored in a refrigerator. They should be discarded if yellowing occurs.

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Equipment

The following equipment was used:

1. 3% OV-17 on Gas-Chrom Q 100/120 mesh—Applied Science Laboratories, State College, Pennsylvania.
2. A gas chromatograph with 6-ft by 4-mm glass column packed with 3% OV-17. The oven must be programmable at 10°C per minute. The instrument used in this study was a Hewlett-Packard 5840 configured for on-column injection and equipped with a flame ionization detector.
3. A heating block to hold small test tubes or vials at 70 to 75°C.

Operating Parameters

The operating parameters included the following:

1. The column was oven programmed from 180°C (1-min initial hold) to 280°C to 10°C/min.
2. The nitrogen carrier gas flow rate was 60 mL/min.

Procedure

A standard stock solution was prepared by dissolving accurately weighed amounts of standard sugars to give approximately the following amounts in 100 mL of distilled water: mannitol, 275 mg; inositol, 375 mg; lactose, 575 mg; dextrose, 275 mg; and sucrose, 575 mg.

Also, accurately weighed individual standards were prepared with the approximate concentrations listed above. Working standards were prepared by diluting 10.0 mL of each of the solutions to 100.0 mL with methanol (MeOH). An amount of 1.0 mL of each of the working standards was pipetted into separate 2-mL autosampler vials or small test tubes and evaporated to dryness at 70 to 75°C in the heating block with the aid of an air current. STOX working solution, 1.0 mL, was added and the mixture was heated at 70 to 75°C in the heating block for 60 min with the container uncapped.

It is important to note that this operation should be carried out in a hood.

Approximately 1 mL of TRI-SIL Z was added, and the tube was capped and heated at 70 to 75°C for an additional 30 min. The solution was cooled and analyzed on the gas chromatograph using a 3-μL injection and adjusting attenuation so that the internal standard gave 50 to 80% scale deflection. Figure 1 shows a typical chromatogram. Those samples containing interfering compounds were first treated by the following extraction (See Table 1 for the list; the interfering compounds are in all capital letters.)

A weighed sample equivalent to 250 mg of sugars was mixed with 2 g of acid-washed Celite 545, and the mix was moistened with a small amount of $CHCl_3$. This mixture was packed into a chromatographic column, approximately 22 by 250 mm, which contained a plug of 2 g of acid-washed Celite 545 moistened with $CHCl_3$. Glass wool plugs were used at the top and the bottom of the column.

The column was washed with 200 mL of NH_4OH-saturated $CHCl_3$, and the material in the column was extruded into a 100-mL beaker and dried thoroughly at 105°C. A volume of 50.0 mL of water was pipetted into the beaker containing the extruded column contents and mixed well (sonication works nicely, if available). The solution was heated for 10 min on a steam bath. A 5.0 mL aliquot was cooled, filtered, and diluted to 50.0 mL with MeOH.

A 1.0-mL aliquot of the MeOH dilution was evaporated to dryness and treated by the derivatization procedure.

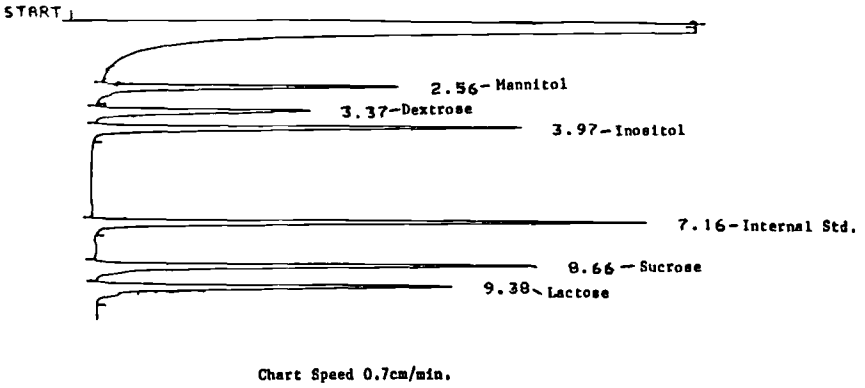

FIG. 1—*Typical chromatogram.*

Results

Reproducibility of the gas chromatography (GC) system was first checked by duplicate injections of the mixed standard. After one injection of the standard mix, the built-in integrator/calculator was calibrated, and subsequent runs were compared with those response factors. Results are given in Table 2.

The maximum deviation of 0.7% for the Run 1 lactose result was consistent with expectations for multiple determinations of the same solution performed on a well-tuned gas chromatograph.

To check the reproducibility of the method further, triplicate derivatizations were performed on the individual sugar standard solutions, comparing them with the standard mix. Results are given in Table 3, and show the precision and accuracy expected for triplicate GC quantitations.

The method was then given to a second analyst for collaboration. This analyst's initial results exhibited variations double and triple those shown in Table 3, even though pre-

TABLE 1—*Interfering compounds and their retention times.*[a]

Compound	Retention Time, min	Relative Retention Time, min
Mannitol	2.55	0.36
Dextrose	3.35	0.47
Inositol	3.95	0.55
PHENCYCLIDINE	4.79	0.67
LIDOCAINE	5.24	0.73
BENZOYLTROPEINE	5.85	0.82
CAFFEINE	5.98	0.84
METHAPYRILENE	6.44	0.90
Internal standard	7.16	1.00
PROCAINE	7.16	1.00
Sucrose	8.65	1.21
COCAINE	8.75	1.22
PYRILAMINE	8.80	1.23
Lactose	9.37	1.31
HEROIN	11.30	1.58
QUININE	11.48	1.60

[a]The interfering compounds are in all capital letters.

TABLE 2—*Reproducibility—theoretical percentage (average of two determinations).*

Sugar	Theoretical % (Average of Two Determinations)
Mannitol	99.8
Dextrose	100.0
Inositol	100.1
Sucrose	100.1
Lactose	100.3

cision comparable to the results shown in Table 2 was obtained for multiple runs of the same derivatized standard mix solution. This pointed towards a procedural problem rather than an instrumental one. The variations were traced to the second analyst's use of a commercial pipetting device with disposable plastic tips. This device is not intended to perform precision aliquoting. A second run using Class A pipettes for all aliquoting brought the results into the ranges shown in Table 3.

Mixed samples of known composition were made, and were given as blind samples to three analysts who made duplicate determinations with the aggregate results shown in Table 4.

Dextrose was not included in this portion of the study because of its relatively low incidence in street samples.

To determine whether common controlled substances and adulterants might interfere in the sugar quantitations, these compounds were separately added to vials containing approximately 1 mL of the standard mix, and evaporation and derivatization were carried out according to the method. The retention times obtained are listed in Table 1.

Cocaine and pyrilamine both interfere with the sucrose peak, but since sucrose is seldom found in street samples, this is not as serious a problem as that presented by procaine, which co-elutes with the internal standard and is a popular adulterant in street samples.

To eliminate the co-elution problems, a number of extraction techniques were studied. The one giving the best results is detailed in the Procedure section above. Recoveries from three synthetic mixtures are shown in Table 5.

Discussion

Although the method presented is straightforward and uses proven techniques, care in sample preparation, especially in the pipetting of the 1.0-mL aliquots, is necessary to achieve precision and accuracy. Furthermore, since oximes are formed, some information may be lost, particularly when beta lactose is present. In the few instances in which the ratio of alpha to beta lactose is desired, the quantitation may be rerun, eliminating the use of the STOX reagent. Note, however, that commercial alpha lactose contains 5 to 10% of the beta anomer.

TABLE 3—*Reproducibility—theoretical percentage by run.*

Sugar	Run 1	Run 2	Run 3	Average
Mannitol	98.0	99.3	96.8	98.0
Dextrose	96.8	97.2	99.7	97.9
Inositol	102.9	100.4	99.2	100.8
Sucrose	95.8	96.6	97.3	96.6
Lactose	96.9	97.2	98.3	97.5

TABLE 4—*Results of blind determinations.*[a]

Sample	Mannitol	Inositol	Sucrose	Lactose
1	18.0 (17.9)	34.9 (32.7)	26.6 (25.3)	24.9 (24.1)
2	18.7 (20.3)	13.9 (14.3)	53.9 (54.3)	10.0 (11.2)
3	20.7 (21.7)	26.3 (24.6)	0 (0)	52.4 (53.7)
4	13.1 (14.0)	27.7 (26.1)	12.9 (11.7)	49.8 (48.2)
5	22.4 (22.8)	29.7 (29.2)	9.3 (9.3)	38.8 (38.2)
6	25.1 (24.5)	21.3 (20.1)	16.3 (15.2)	40.9 (40.2)

[a]Values are in percentages; values in parentheses are theoretical percentages. Because of rounding, the totals for each column may not equal 100.00.

TABLE 5—*Recoveries from synthetic mixtures.*

Sample	Composition	Theoretical	Found
1	cocaine, procaine, sucrose	31.6% sucrose	31.3% sucrose
2	brown heroin, procaine, lactose	33.2% lactose	33.3% lactose
3	brown heroin, procaine, mannitol, inositol	24.5% mannitol 25.4% inositol	24.4% mannitol 24.9% inositol

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