GLC Determination of Saccharin in Pharmaceutical Products

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Abstract □ A GLC method was developed for the assay of saccharin in pharmaceutical products. The procedure requires silylation with N,O- bis(trimethylsilyl)acetamide and subsequent chromatography on trifluoropropyl methyl silicone, using n- octacosane as an internal standard.

Keyphrases □ Saccharin—GLC determination in pharmaceutical products, silyl derivatives formed □ GLC—analysis, saccharin in pharmaceutical products, silyl derivatives formed

Subsequent to the ban on the use of cyclamates in pharmaceutical products, saccharin has been subjected to increased scrutiny and governmental regulations (1). Various methods for the quantitative and qualitative determination of saccharin have been reported, including TLC (2), IR (3), and a nonaqueous titration after separation by ion-exchange chromatography (4). One early reference to a GLC procedure involved the preparation of the N- methyl ester using diazomethane (5). Other investigators reported variations such as changes in the liquid phase and use of an internal standard (6, 7).

Conacher and O'Brien (8) reported a GLC method for the determination of saccharin in soft drinks, using diazomethane as the methylating agent and methyl silicone (JXR) as the liquid phase. According to their report, the methylation of saccharin consistently gave two peaks in a 17:3 ratio, which they postulated to be the N- methyl derivative of saccharin and the O- methyl derivative of pseudosaccharin, respectively. Their quantitative data were based on area measurements of the rapidly eluted, poorly shaped major peak (8).

This paper describes the determination of saccharin in pharmaceutical products utilizing silylation with N,O -bis(trimethylsilyl)acetamide[1]. This procedure offers several improvements upon previous methods. Diazomethane is toxic, potentially explosive, and rather difficult to prepare and use, while the silylation reaction is rapid and leads to a derivative needing no further treatment prior to chromatography.

A literature survey indicated a complete lack of assay methods for saccharin in complex pharmaceutical dosage forms. The ion-exchange chromatographic method is time consuming and suitable only for simple aqueous preparations, since any other acid or acid salt in the dosage form interferes with the determination.

The present method was found to be completely free of interferences from active and inactive ingredients in the products analyzed.

Table I—Saccharin Assay Results

Product	Claim	Found[a]
Tablet A	15.0 mg/tablet	Batch 1: 15.1
		Batch 2: 14.0
		Batch 3: 14.1
		Batch 4: 14.9
Tablet B	30 mg/tablet	Batch 1: 28.0
		Batch 2: 29.0
Tablet C	0.76 mg/tablet	Batch 1: 0.80
		Batch 2: 0.73
		Batch 3: 0.72
		Batch 4: 0.74
Elixir A[b]	5.0 mg/5 ml	4.9
Elixir B[b]	10.8 mg/5 ml	11.6
Elixir C[b]	14.4 mg/5 ml	14.4
Elixir D[b]	3.6 mg/5 ml	3.8
Syrup[b]	5.5 mg/5 ml	5.5
Suspension	5.0 mg/5 ml	Batch 1: 5.1
		Batch 2: 5.0
		Batch 3: 5.0

[a] Average of duplicate determinations calculated as sodium salt. [b] Synthetic formulations.

EXPERIMENTAL

Equipment—A gas chromatograph[2] equipped with a single hydrogen flame-ionization detector and interfaced with a digital computer[3] was used. The column was 0.9-m × 0.3-cm (3-ft × 0.125-in.) o.d., type 304 stainless steel tubing, packed with 100–120-mesh silanized diatomaceous earth[4] and coated with 5% trifluoropropyl methyl silicone (OV-210).

The operating temperatures were: column, 160°; detector, 250°; and injector port, 240°. Nitrogen was the carrier gas with a flow rate of 30 ml/min. The hydrogen gas flow was adjusted for maximum sensitivity at 30 ml/min, and the air flow was 300 ml/min. The *electrometer range* was 10^{-10} amp/mv.

N,O- Bis(trimethylsilyl)acetamide was used as the silylating reagent. The isopropyl ether (bp 67–69°) used was alcohol free[5]. All other chemicals were reagent grade with the exception of the hydrocarbon n- octacosane[6], the internal standard, which was practical grade.

Preparation of Internal Standard Stock Solution—One hundred milligrams of n- octacosane was accurately weighed into a 50-ml volumetric flask and dissolved in and diluted to volume with cyclohexane.

Preparation of Reference Standard Stock Solution—Seventy-six milligrams of reference saccharin was accurately weighed into a 100-ml volumetric flask and dissolved in and diluted to volume with methanol.

Preparation of Reference Standard—A 4.0-ml aliquot of the reference standard stock solution was pipetted into a 10-ml volumetric flask and evaporated to dryness on a steam bath under nitrogen. Then 1.0 ml of silyl reagent was pipetted into the residue and mixed well. The flask was stoppered and heated on a steam bath for 30 min. After cooling to room temperature, 1.0 ml of the internal standard stock solution was pipetted into the flask and thoroughly mixed.

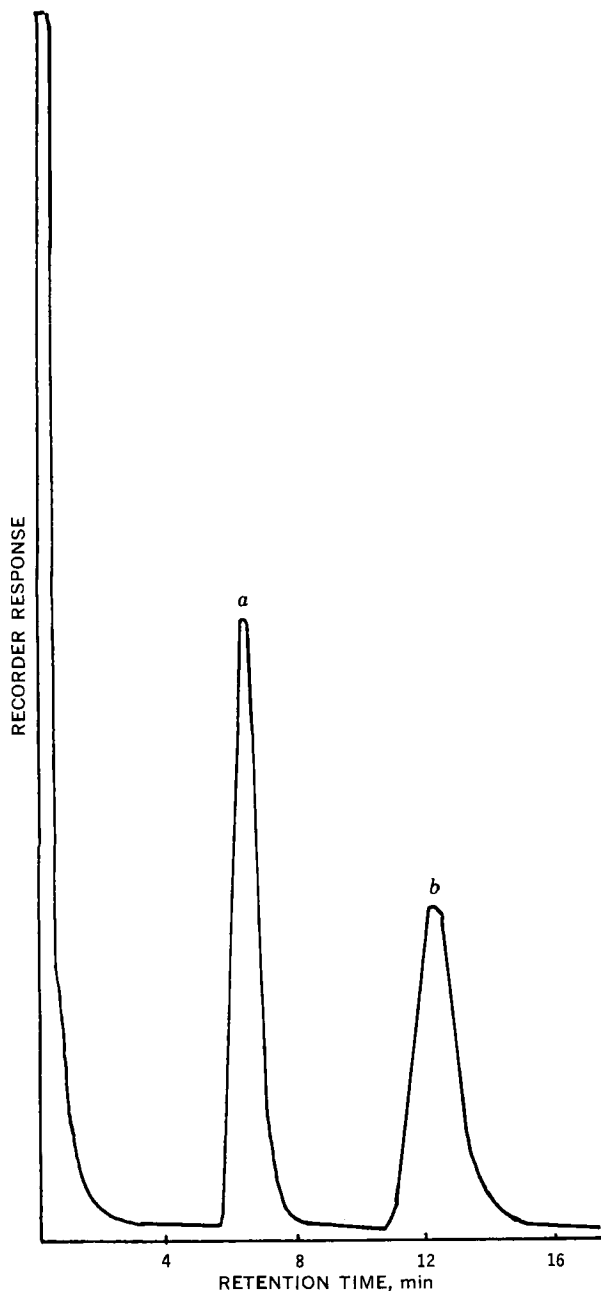**Preparation of Samples**—A suitable quantity of the syrup, suspension, or pulverized tablet mass equivalent to approximately

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Figure 1—*Gas chromatogram of a sample preparation containing* (a) *saccharin as the trimethylsilyl derivative and* (b) *n-octacosane.*

Table II—Statistical Evaluation

Determinations	Saccharin, mg/Tablet
1	0.85
2	0.78
3	0.80
4	0.82
5	0.83
6	0.80
Mean	0.81
SD	±0.025
95% Confidence limit	±0.0262

made to establish the response factor and to calculate the saccharin content of the samples.

Calculations—The response factor for saccharin is determined as follows:

$$RF_S = \frac{A_S(\text{std}) \times C_{IS}}{A_{IS}(\text{std}) \times C_S} \qquad (\text{Eq. 1})$$

where:

A_S(std) = peak area of saccharin in the working standard solution
A_{IS}(std) = peak area of internal standard in the working standard solution
C_{IS} = concentration of internal standard (milligrams per milliliter) in the working standard solution
C_S = concentration of saccharin (milligrams per milliliter) in the working standard solution

The saccharin content in pharmaceutical products can be calculated as follows:

mg saccharin/dosage unit =

$$\frac{A_S(\text{spl}) \times C_{IS} \times \text{dilution factor}}{A_{IS}(\text{spl}) \times RF_S \times \text{sample weight (g or ml)}} \qquad (\text{Eq. 2})$$

where A is the area, C is the concentration, and RF is the response factor (Eq. 1). The conversion factor is milligrams saccharin × 1.317 = sodium saccharin.

RESULTS AND DISCUSSION

Peak Characterization—GLC–mass spectrometry data indicated the molecular ion of the derivative to be at m/e 255, which corresponds to the monotrimethylsilyl derivative of saccharin. IR and NMR studies of the silylated product showed the loss of the imino proton, indicating that the reaction probably produced N-trimethylsilyl derivative.

An IR scan of the peak eluting from the GLC column, using an interfaced IR spectrophotometer, indicated the presence of the expected carbonyl band at 1700 cm^{-1} in addition to a strong band at 1250 cm^{-1} due to the substitution of a trimethylsilyl group. A more conclusive characterization of the derivative or the effects of enolization of saccharin, if any, would be beyond the scope of this paper.

Linearity—The detector response for saccharin was found to be linear in the range of 0.24–2.33 mg/ml at a constant internal standard concentration. The slope of the linearity curve was calculated to be 0.646 with a Y intercept of −0.006.

A study of silylation time was carried out using intervals of 10–60 min on the steam bath. The results indicated that the reaction is essentially complete in 20 min.

Precision and Accuracy—Analytical data obtained for several pharmaceutical products indicate that the procedure is applicable to a wide range of dosage forms without any modifications in analytical techniques (Fig. 1 and Table I). A statistical evaluation of the precision of the method was carried out for an antacid tablet formulation (Table II).

Recovery—A study was also undertaken to estimate the recovery of saccharin from the dosage forms. Formulations to which known amounts of sodium saccharin were added prior to acidification and extraction were analyzed and indicated essentially complete recovery (Table III).

6.0 mg of saccharin was accurately transferred into a 50-ml centrifuge tube. Twenty-five milliliters of 10% aqueous hydrochloric acid was added, mixed well, and allowed to stand for 15 min. This mixture was extracted with five 20-ml portions of isopropyl ether and shaken mechanically for 15 min each time. All extracts were passed through anhydrous sodium sulfate and collected in a 125-ml erlenmeyer flask.

The extract was evaporated to dryness on a steam bath under nitrogen, and the residue was dissolved in 4.0 ml of methanol. A 2.0-ml portion of this solution was transferred into a 10-ml volumetric flask and evaporated to dryness. Then 1.0 ml of silyl reagent was added to the residue and mixed well. The flask was stoppered and heated on a steam bath for 30 min. After cooling to room temperature, 1.0 ml of the internal standard stock solution was pipetted into the flask and thoroughly mixed.

Procedure—The chromatographic column was conditioned by making a few 10-μl injections of the working standard solution. Several 5-μl injections of the standard and sample solutions were

Table III—Recovery Data

Product	Initial Assay	Saccharin Added, mg	Final Assay	Recovery, %
Tablet	16.9 mg/tablet	4.8	21.7 mg/tablet	100
Tablet	32.6 mg/tablet	9.3	42.0 mg/tablet	100
Suspension	5.1 mg/tablet	5.1	10.5 mg/5 ml	103
Suspension	5.1 mg/5 ml	10.5	16.3 mg/5 ml	105
Elixir	3.8 mg/5 ml	5.5	9.1 mg/5 ml	98
Elixir	3.8 mg/5 ml	10.2	13.6 mg/5 ml	97

Interferences—To establish the applicability of this procedure to various pharmaceutical dosage forms, synthetic formulations were made by adding sodium saccharin and were analyzed in addition to commercially available products. By the use of saccharin-free placebo formulations, it was found that pharmaceutically active ingredients such as ascorbic acid, nicotinyl alcohol, aprobarbital, dextromethorphan hydrobromide, chlorpheniramine maleate, acetaminophen, and sulfamethoxazole did not interfere with the analysis.

One pharmaceutical product contained a vitamin B-complex mixture made up of thiamine mononitrate, riboflavin phosphate, pyridoxine hydrochloride, niacinamide, and calcium pantothenate. The ingredients in the mixture did not cause any interference. Commonly used pharmaceutical excipients such as corn syrup, sucrose, citric acid, sodium citrate, sodium benzoate, propylene glycol, stearic acid, magnesium stearate, and mannitol also did not affect the utility of this method.

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PHARMACEUTICAL TECHNOLOGY

Drying Rates of Tablet Granulations I: Effect of Certain Granulating Adjuvants on Drying Rates

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Abstract ☐ The design and construction of a laboratory size dryer and other accessories suitable for investigating the drying rate kinetics of granules under controlled external conditions are described. Granulations of lactose and sulfathiazole, representing water-soluble and insoluble materials, were prepared using various commonly used binders, and their drying rates were determined. The binders and diluents affected the drying rate curves for these granulations both qualitatively and quantitatively. Granules made with starch paste and gelatin solution required maximum time and energy for drying and those made with simple syrup USP required the least among the binders studied. Generally, three linear slopes were observed when the drying rate was plotted against the moisture remaining, indicating that granulation drying may be considered as occurring through three distinct phases.

Keyphrases ☐ Tablet granulations—effect of granulating adjuvants on drying rates ☐ Granulating adjuvants—effect on drying rates of tablet granulations ☐ Drying rates, tablet granulations—effect of granulating adjuvants ☐ Excipients—effect of granulating adjuvants on drying rates of tablet granulations

The manufacture of pharmaceutical tablets requires the preparation of granular material for compression. One common method of preparing these suitable granules is by wet granulation. Although the industrial pharmacist is well aware of the cost savings in the choice of excipients, size of tablet, and speed of