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Quantitative Determination of Saccharin in Food Products by Ultraviolet Spectrophotometry

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A procedure for the determination of saccharin in chewing gum and other food products has been developed. Saccharin is isolated by extracting the acidified sample solution with chloroform. The residue, after evaporation of the chloroform, is dissolved in 1% Na_2CO_3 solution and absorbance is determined at 235 and 244 nm; the latter wavelength absorbance is to correct for any possible interference. Recoveries ranged from 83 to 113%. Analyses of commercial products of various brands gave 90 to 105% of label claim.

Saccharin is the major noncaloric sweetener currently in use on a sucrose sweetness equivalent basis (Walter, 1973). It has been used as a sweetener in a wide variety of food products for more than 60 years. In spite of the limitation on the use of saccharin by the FDA and its removal from the GRAS list its annual consumption in 1972 exceeded 4 million pounds (National Academy of Sciences–National Research Council, 1974).

The literature contains numerous reports on the determination of saccharin in foods and beverages. Various techniques were used; colorimetry (Fernandez-Flores et al., 1973), gas–liquid chromatography (Conacher and O'Brien, 1970; Ratchik and Viswanathen, 1975), infrared spectrophotometry (Coppini and Albasini, 1968), polarography (Lasheen, 1966), gravimetry (Oakley, 1947), and quite recently an ion-selective electrode method (Hazemoto et al., 1974). Basile (1966) reported an ultraviolet procedure for the determination of saccharin in wine, whereby saccharin was isolated as the free acid and interfering materials were eliminated by oxidation with alkaline permanganate solution. The saccharin was extracted from the sample with chloroform, and determined from its absorbance at 278 nm. In this procedure, interfering materials that are extractable with chloroform and possess an aromatic structure after the permanganate treatment will still interfere in the determination. This is true in many food products which contain flavoring materials and benzoates. De Garmo et al. (1952) used a uv procedure to determine the stability of saccharin solution on heating whereby absorbance of saccharin in sodium hydroxide solution was determined at 268 nm.

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The method reported in this paper utilized the solubility of saccharin and its salts in sodium carbonate solution and its unique absorption characteristic in this solution; two distinct absorption maxima are exhibited at 235 and 229 nm in addition to the broad absorption band exhibited in the 265–275-nm range. The double absorption maxima can serve as an identity test for saccharin. High sensitivity is achieved by this method; the molar extinction coefficient of saccharin in 1% Na_2CO_3 solution is 6260 at 235 nm and 8170 at 229 nm, calculated from absorbance of a solution containing 0.03 mg/ml. The coefficient at 268–275 nm in both aqueous and organic matrix is approximately 1550. In addition to the absorption characteristics, the method is simple and accurate requiring minimal preparation to remove interfering substances.

EXPERIMENTAL SECTION

Method. *Reagents and Apparatus.* All reagents used were either ACS reagent grade, U.S.P., or N.F. A UNICAM SP 800 recording spectrophotometer was used, with the following settings: fast scan, energy programme control set at normal energy E, slit width indicator set at 0, the deuterium lamp is on, 190–450-nm wavelength range. Quartz cells, 10 mm thickness, were used.

General Procedure. An aliquot of the specified sample was transferred to a 125-ml separatory funnel. Distilled water was added to make the volume 50 ml. Concentrated HCl, 7.5 ml, was added, and the solution was extracted three times with 35 ml of chloroform. The separatory funnel was shaken 1–2 min each time and the layers were allowed to equilibrate for at least 5 min. The chloroform layer (bottom) was decanted through a funnel fitted with a chloroform-wetted plug of glass wool or cotton (0.5-in. thickness) into a 250-ml beaker. The separatory funnel,

funnel tip, the filter funnel, and its tip were rinsed with a few milliliters of chloroform. The filter plug was gently squeezed with a spatula, care being taken that no entrapped aqueous or emulsified material is squeezed out into the beaker. The chloroform was evaporated on a steam bath aided by a gentle stream of air or nitrogen. The beaker was removed from the steam bath when the residue was free of chloroform. To the residue was added 20 ml of 1% Na_2CO_3 solution, and the beaker was placed on the steam bath for 5 min. Residue clumps, if any, were broken with a glass rod. The solution was cooled and transferred to a 100-ml volumetric flask. In cases where undissolved particles remained, the solution was filtered through a Whatman No. 4 filter paper.

The beaker and glass rod were rinsed with small portions of 1% Na_2CO_3 solution and added to the volumetric flask. The solution was made up to volume with 1% Na_2CO_3 solution. When filtration was necessary funnel and filter paper were thoroughly rinsed with the carbonate solution into the volumetric flask before diluting the contents to volume.

A standard containing an approximately equal amount of saccharin to that of the sample was processed along with the sample.

The sample and standard solutions were scanned from 325 to 220 nm on a recording spectrophotometer against 1% Na_2CO_3 solution as a blank. In cases where a recording spectrophotometer was unavailable the absorbance of the solutions was determined at 235 and 244 nm. The saccharin concentration was determined in the sample as follows:

net absorbance

$$A = A_{235} - A_{244}$$

$$[\text{saccharin}] = (A_x/A_s)C_s(V_t/V_s)(100/W)$$

where C_s = concentration of working standard, mg/100 ml; V_t = total volume of sample solution; V_s = volume of aliquot taken for analysis; W = weight of original sample in milligrams; A_x = net absorbance of sample solution; A_s = net absorbance of standard solution. If the original sample was a liquid and the sample taken for analysis was measured in volume rather than weight, the term W in the above equation can be replaced by "volume of original sample × 1000". The final concentration will be in percent (weight/volume).

The absorbance at 244 nm was introduced to correct for any interference; however, if there was no apparent interference (this was determined by comparing the absorbance of the sample to the standard of equivalent concentration at 244 nm) no correction was needed. The absorbance at 235 nm corrected for the baseline was used in the calculation. In cases of no interference the use of $A_{235} - A_{244}$ in both sample and standard did not alter the results. The tangents of the sample and standard spectra from 260 to 240 nm and extended to 235 nm may also be used for correction instead of the absorbance at 244 nm. Results obtained using both methods of correction gave very good agreement.

Sample Preparation. (a) *Chewing Gums.* An accurately weighed sample of gum (9–15 g) was placed in a freezer (−5°C) for 15–20 min. The gum was removed from the freezer and placed in a Waring blender where it was pulverized by blending at high speed for 1–2 min. An aliquot of 150 ml of distilled water was added to the blender, all the material clinging to the glass or lid was scraped into the liquid, and the mixture was blended for 3–4 min. The liquid was filtered into a 200-ml volumetric flask through a funnel containing a plug of glass wool or cotton. The blender and residue were rinsed twice with 15 ml of distilled water and the washings were decanted into the funnel. The residue was squeezed with a spatula and the liquid transferred to the funnel. The blender and residue were rinsed with 15 ml of distilled water, the rinsing was decanted into the filter, and the solution was diluted to the mark with distilled water.

An aliquot of 75 ml of the sample solution was transferred into a 125-ml separatory funnel and extracted twice with 35 ml of chloroform. The chloroform layer (bottom) and the emulsion phase, if any, were discarded. From the remaining aqueous layer a 50-ml aliquot was transferred into a 125-ml separatory funnel and the analysis was continued as described under General Procedure.

(b) *Low Calorie Iced Tea Mix.* An accurately weighed amount (1–2 g) was transferred to a 50-ml volumetric flask, dissolved with distilled water, and diluted to volume. An aliquot of 20 ml was transferred to a 125-ml separatory funnel and extracted twice with 25 ml of chloroform. The layers were allowed to equilibrate and the chloroform layer and any emulsion that formed were discarded. A 5-ml aliquot of the emulsion-free aqueous layer was transferred to a 125-ml separatory funnel. To the funnel were added 45 ml of distilled water and 7.5 ml of concentrated HCl. The mixture was extracted three times with 35 ml of chloroform. The layers were allowed to equilibrate for at least 5 min after each extraction. The bottom layer, including the emulsion, was transferred to a 250-ml beaker via a funnel containing a glass wool plug (1–1.5 in. thick) which has been pre-wetted with chloroform. The analysis was continued as described in the General Procedure section starting with "The separatory funnel, funnel tip, the filter funnel, and its tip were rinsed with a few milliliters of chloroform".

The 5-ml aliquot of the pre-extracted sample represents one-tenth of the original iced tea mix sample solution.

(c) *Low Calorie Beverages Containing No Benzoates or Sorbates.* A 20-ml sample was transferred to a 125-ml separatory funnel and extracted with 30 ml of chloroform. The layers were allowed to equilibrate and the chloroform layer plus any emulsion that was formed were discarded. A 10-ml aliquot of the aqueous layer was transferred to a 125-ml separatory funnel, and the analysis was continued as described in the General Procedure section.

(d) *Low Calorie Soft Drinks and Beverages Containing Benzoates and Sorbates.* A 20-ml aliquot was transferred into a 250-ml separatory funnel. Sixty-five milliliters of distilled water and 15 ml of concentrated HCl were added. The mixture was extracted twice with 50 ml of CCl_4. The layers were allowed to equilibrate and the CCl_4 layer (bottom) and any emulsion formed were discarded. A 50-ml aliquot of the aqueous layer was transferred to a 125-ml separatory funnel and analysis was continued as in the General Procedure section starting with "...was extracted three times with 35 ml of chloroform...".

The 50-ml aliquot of the aqueous phase represents half of the beverage sample taken for analysis, i.e. 10 ml.

(e) *Artificial Sweetener Tablets, Liquids, and Raw Material (Saccharin).* An accurately weighed amount of 0.5–0.7 g of the dry material (10–15 pulverized tablets) or a 2-ml aliquot of the liquid concentrate, if it contained no benzoates, parabens, or sorbates, was put into a 500-ml volumetric flask. The sample was dissolved and diluted to volume with 1% Na_2CO_3 solution. An aliquot containing 3–4 mg of saccharin was transferred to a 100-ml volumetric flask and diluted to volume with 1% Na_2CO_3 solution. The solution was scanned from 350 to 220 nm using 1% Na_2CO_3 as a blank. A standard solution con-

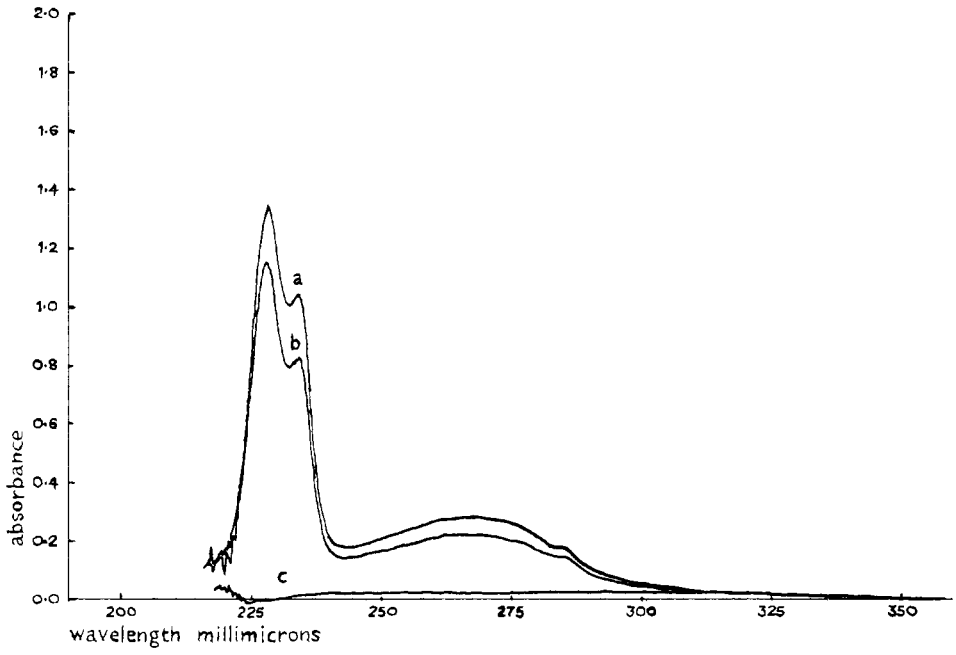

Figure 1. Spectra of saccharin and sodium saccharin in 1% Na_2CO_3 solution. Concentration of each is 0.03 mg/ml (reference is 1% Na_2CO_3 and path length is 10 mm): (a) saccharin solution; (b) sodium saccharin solution; (c) baseline (1% Na_2CO_3 solution).

taining 3–4 mg of saccharin/100 ml that was diluted directly from the stock standard was scanned like the sample and used to determine the concentration. See the General Procedure section for calculation of the amount of saccharin.

In cases where the liquid concentrate contained preservatives such as benzoates, parabens, or sorbates an aliquot containing 3–4 mg of saccharin was transferred to a 125-ml separatory funnel. Fifty milliliters of distilled water and 7.5 ml of concentrated HCl were added. The mixture was extracted with 50 ml of CCl_4. The layers were allowed to equilibrate and the CCl_4 layer was discarded. Analysis of the sample was continued as in the General Procedure section starting with "...was extracted three times with 35 ml of chloroform...". A saccharin sample of known concentration and equivalent to the unknown sample was processed in the same manner including the CCl_4 extraction.

Preparation of Standard Solutions. *Stock Solution.* A saccharin salt sample (100 mg) was weighed accurately and transferred to a 100-ml volumetric flask. The sample was dissolved and diluted to volume with distilled water.

Working Standard Solution. An aliquot of 4 ml of the stock standard was transferred to a 125-ml separatory funnel. Distilled water (46 ml) and 7.5 ml of concentrated HCl were added. The analysis was continued as in the General Procedure section starting with "...was extracted three times with 35 ml of chloroform...". The standards were treated exactly as the samples. When the sample was preextracted with chloroform before acidification or with CCl_4 after acidification, the standard was likewise preextracted.

In the case where samples required no acidification but were simply diluted and read directly, as in artificial sweetener tablets and bulk saccharin, the standard was likewise diluted from a stock standard which was prepared in 1% Na_2CO_3 solution instead of distilled water.

RESULTS AND DISCUSSION

This method was originally developed and used extensively in the analysis of chewing gums; however, to check its applicability to other products containing saccharin, various commercial products were analyzed: carbonated and noncarbonated beverages, iced tea mix, sweetener tablets, and concentrate. Extensive recovery studies were done in chewing gums of various flavors. Samples spiked with known amounts of saccharin, as well as chewing gums prepared with various levels of saccharin, were analyzed. Recovery of added saccharin was also determined in soft drinks samples.

Table I. Recovery of Saccharin from Food Products

Type of sample	Spiked or theor. amt of saccharin	Saccharin found	% recovery
Chewing gum (prepared with saccharin)[a]	0.0045%	0.0051%	113.3
	0.0182%	0.015%	83.3
	0.027%	0.028%	103.7
	0.036%	0.036%	100.0
Chewing gum (saccharin added)[b]	2.00 mg	1.91 mg	95.5
	2.00 mg	1.74 mg	87.0
	2.00 mg	2.16 mg	108.0
Iced tea mix (saccharin added)[b]	0.50 mg	0.51 mg	102.0
	0.50 mg	0.56 mg	112.0
	0.50 mg	0.53 mg	106.0
	0.50 mg	0.52 mg	104.0
Carbonated soft drink (saccharin added)[b]	2.00 mg	2.16 mg	108.0
	2.00 mg	2.05 mg	102.5
	2.00 mg	2.19 mg	109.5
	2.00 mg	2.15 mg	107.5
Saccharin tablets[b]	2.00 mg	1.82 mg	91.0
	2.00 mg	1.85 mg	92.5
Concentrated sweetener liquid[b]	2.00 mg	1.95 mg	97.5
	2.00 mg	2.08 mg	104.0

[a] The samples are manufactured with the various amounts of saccharin indicated. [b] Saccharin was added to the analysis samples as an aliquot of a stock standard.

Table I shows the recoveries obtained. In chewing gums the recoveries range from 83 to 113%. The lowest recovery was obtained on a chewing gum containing 0.018% saccharin. In addition to the actual recovery, the correlation between the amount of saccharin found and the amount

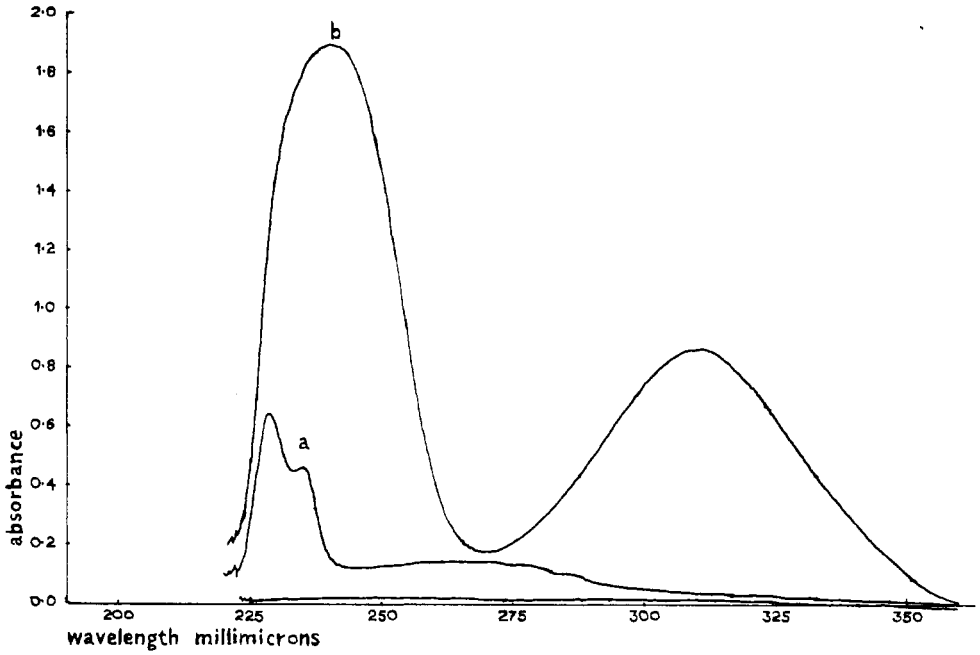

Figure 2. Spectra of methyl anthranilate and sodium saccharin extracted from 10% sugar solution according to procedure c with and without chloroform preextraction (sample solution contained 4 mg of methyl anthranilate and 0.15 mg of sodium saccharin per ml): (a) preextraction with chloroform prior to acidification; (b) no preextraction with chloroform prior to acidification; spectrum is of 1:5 dilution of the final solution; represents 8 mg of methyl anthranilate + 0.3 mg of sodium saccharin per 100 ml.

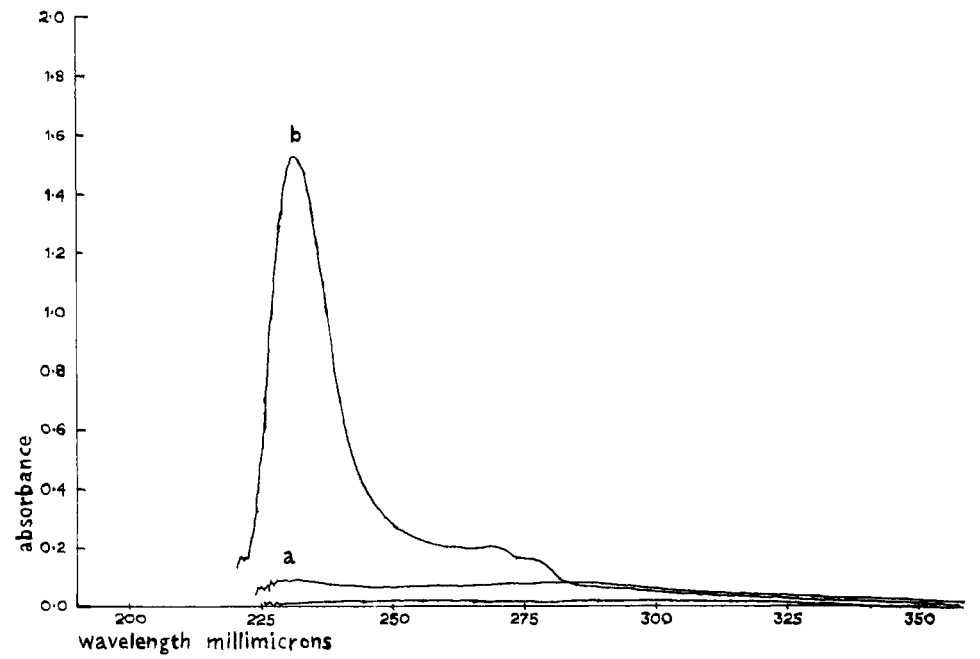

Figure 3. Spectra of sodium benzoate extracted from carbonated beverage according to procedure c (no CCl_4 preextraction) and procedure d (CCl_4 preextraction): (a) procedure d (CCl_4 preextraction); (b) procedure c (no CCl_4 preextraction).

declared on the label served as an additional guide to the applicability of the method.

Table II shows the correlation between the amount of saccharin found and the amount declared on the product label. Good recoveries and good correlation between analytical data and the label claim indicate that the method is applicable to many saccharin containing products after removal of interfering materials.

Figure 1 shows spectra of both saccharin and sodium saccharin in 1% Na_2CO_3. The double absorption maxima at 235 and 229 nm are characteristic of saccharin. Absorbance at 235 nm is linear in the range of 0–4.5 mg/100 ml, while at 229 nm linearity holds only to 3.3 mg/100 ml. For standard solutions of saccharin and sodium saccharin up to 2 mg/100 ml, the ratio of the absorbance at 235 nm to that at 229 nm ranged from 0.66 to 0.68.

The absorbance at 235 rather than 229 nm is used for the analysis because it is more linear and is not as close to the cut-off point of 1% Na_2CO_3 at 224 nm.

Interfering materials that are encountered in food products are benzoates, sorbates, parabens, and flavorants such as anthranilates. Interference from anthranilates and other flavorants is eliminated by preextraction with chloroform prior to acidification of the sample solution, as in procedures a, b, c, and e, while interference from preservatives (benzoates, parabens, and sorbates) is

Table II. Determination of Saccharin in Various Products

Sample	Theor. amt (label declaration)	Saccharin found	% of claim
Saccharin tablets	1/4 grain/ tablet	16.0 mg	98.6
Concentrated sweetener liquid	22.7%[a]	22.5%[a]	99.1
Iced tea mix	3.47%	3.40%	98.0
Chewing gum			
spearmint	0.1%	0.105%	105.0
fruit	0.1%	0.102%	102.0
fruit	0.1%	0.095%	95.0
cinnamon	0.1%	0.090%	90.0
Noncarbonated isotonic drink	2.1 mg/oz	2.2 mg/oz	105.0
Carbonated soft drink	10 mg/oz	10.09 mg/oz	101.0
(lemon type flavor)	7.3 mg/oz	7.5 mg/oz	103.0
Cola flavored carbonated soft drink	12 mg/oz	12.2 mg/oz	101.7

[a] These values are on weight/volume basis.

Table III. Results Obtained at Various Wavelengths

Sample	Theor. amt of saccharin in the product	% saccharin found		
		Using A235-A244	Using A235	Using A268
Fruit gum (sugarless)	0.10	0.11	0.14	0.12
Spearmint gum (sugarless)	0.10	0.11	0.12	0.16
Cinnamon gum (sugarless)	0.10	0.09	0.12	0.36
Iced tea mix (low calorie)	3.47	3.40	3.88	5.13
Concentrated liquid sweetener	22.7	22.50	22.17	22.59
Carbonated soft drink (no saccharin + no benzoates)	0	0	0.004	0.001
Regular iced tea mix (no saccharin)	0	0	0.036	0.036
Regular spearmint gum (no saccharin)	0	0.002	0.014	0.036
Regular peppermint gum (no saccharin)	0	0	0.003	0.013
Regular cinnamon gum (no saccharin)	0	0.004	0.023	0.078

eliminated by CCl_4 preextraction as in procedure d.

Figure 2 demonstrates that methyl anthranilate was completely removed although it was present at higher concentration than saccharin in the sample solution, while Figure 3 shows that only traces of benzoate remain after a carbonated beverage containing the preservative, but no sodium saccharin, was preextracted with CCl_4. The amount of sodium benzoate in the beverage was estimated at 0.035% by comparison with a sodium benzoate standard. The remaining traces of benzoate after CCl_4 preextraction will result in an error of 0.002% in the determination of saccharin in this beverage if it were to contain 10 mg of sodium saccharin per fluid ounce.

Table III shows the results which were obtained for various sample solutions when the calculations were based on absorbance at 268 and at 235 nm with and without correcting for the absorbance at 244 nm. The best results are obtained when calculations are based on the absorbance at 235 nm corrected for that at 244 nm. It is also apparent from the table that when no interference is encountered comparable results are obtained with and without the correction for 244-nm absorbance, as is the case in the concentrated liquid sweetener sample. The values for nonsaccharin products indicate the extent of error which may be encountered.

Since the standard is carried through the same procedures as the samples to be analyzed there is no error due to possibility of incomplete extraction of saccharin. To determine the extent of recovery of saccharin from a sample, 2.05 mg of sodium saccharin was extracted by following procedure d, and the procedure for a, b, c, and e, and comparing the absorbances to that of a straight dilution standard. Procedure d resulted in 90% recovery, while the procedure for a, b, c, and e resulted in 92% recovery of the saccharin present in the sample.

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