# High-Pressure Liquid Chromatographic Determination of Saccharin in Artificial Sweeteners and Pharmaceuticals

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**Abstract** □ A high-pressure liquid chromatographic procedure is presented for determining saccharin in various formulations. The method is fast, precise, and accurate and is specific for saccharin in the presence of its most likely impurities and degradation products. Reversed-phase chromatography on a micro-$C_{18}$ column is utilized with an internal standard, and detection is by UV absorption at 280 nm.

**Keyphrases** □ High-pressure liquid chromatography—analysis, saccharin in various artificial sweeteners and pharmaceuticals, stability indicating □ Saccharin—analysis, high-pressure liquid chromatographic, in various artificial sweeteners and pharmaceuticals, stability indicating □ Stability—saccharin, various artificial sweeteners and pharmaceuticals, high-pressure liquid chromatography

Current literature is abundant in techniques for saccharin determination. Spectrophotometry (1, 2), gravimetry (3, 4), polarography (5), partitioning (6), and specific ion electrode (7, 8) techniques are available but lack the specificity needed for a stability-indicating assay. TLC techniques (9, 10) have a limited and unknown specificity and accuracy. GLC techniques (11–15) require isolation of the saccharin in the dry state and derivatization as either the methyl or trimethylsilyl derivative, and their specificity usually is not stated.

A liquid chromatographic procedure using an ion-exchange column has been described (16). This method was applied to soft drinks and foods, but its specificity for saccharin is unknown. Another liquid chromatographic procedure (17) using a μBondapak $C_{18}$ column and aqueous acetic acid was not applied to pharmaceuticals. Its specificity for saccharin and, therefore, its use as a stability-indicating assay are questionable.

This paper describes the development of a high-pressure liquid chromatographic procedure for saccharin or saccharin sodium determination in artificial sweeteners. With modifications in sample handling, this procedure is applicable to a wide range of pharmaceutical preparations containing saccharin. The specificity and speed of this method allow its use as both a stability-indicating and a quality control assay.

## EXPERIMENTAL

**Apparatus**—The high-pressure liquid chromatograph was equipped with a 280-nm absorbance detector. A 30-cm × 3.9-mm μBondapak $C_{18}$ column of 10-μm average particle size was used with an eluent flow rate of 1.5 ml/min. Peak areas were determined by a computerized laboratory data system, but calculations may be performed using peak heights if desired.

**Materials and Reagents**—Saccharin and saccharin sodium equivalent to USP specifications and theophylline[1] of USP purity were used. All other reagents and chemicals were ACS reagent grade or equivalent.

**Mobile Phase Preparation**—Ten milliliters of acetic acid was added to 600 ml of methanol and diluted to 2 liters with distilled water. The eluent was filtered through a 0.5-μm cellulose acetate filter and degassed prior to use.

**Internal Standard Solution**—Theophylline, 0.35 ± 0.05 g, was dissolved in ~250 ml of eluent.

**Saccharin Standard Solution**—About 0.7 g of reference standard saccharin sodium or 0.6 g of reference standard saccharin was weighed accurately and diluted to 100.0 ml with 50% methanol–water.

**Standard Solution for Injection**—Ten milliliters of internal standard solution was combined with 10.0 ml of saccharin solution, and about 30 ml of eluent was added.

**Procedure for Artificial Sweetener**—Depending on the dosage level, the amount of ground sample equivalent to ~50 mg of saccharin was weighed into a flask. Ten milliliters of internal standard and ~40 ml of eluent were added. After dissolution and filtration, the sample was ready for injection.

**General Procedure for Other Pharmaceutical Preparations**—A sample size equivalent to 1–10 mg of saccharin was reacted with 50 ml of 5% HCl. The solution was extracted with a total of 100 ml of ether in several portions. The ether portions were combined and taken to near dryness with heat and a nitrogen stream. An aliquot of internal standard solution was added commensurate to the saccharin level in the sample. This solution was injected without further dilution.

**TLC**—Solution volumes of standards and samples corresponding to ~200 μg of saccharin were spotted onto 20 × 20-cm fluorescent silica gel (0.25 mm) plates. After drying, the plates were developed in chloroform–methanol–ammonia (70:30:0.5 v/v) to about a 15-cm solvent migration. The saccharin spots were excised with the aid of UV light and taken up in 4 ml of 2% ammonia in methanol. After centrifugation, the UV absorbances were measured at 269 nm.

## RESULTS

Individual stock solutions of saccharin and theophylline were prepared, and aliquots of each were combined in different ratios for injection to determine the response linearity. Thus, for this experiment, both the saccharin and theophylline contents were varied simultaneously. The response ratios (both areas and peak heights) were plotted *versus* the weight ratios injected over 0–4 μg of saccharin and 0–1.6 μg of theophylline injected. The results of the least-squares linear regression analysis (Table I) indicated very good linearity by both peak height and area measurements. A typical chromatogram is shown in Fig. 1. The amounts injected were 2.9 μg of saccharin and 0.6 μg of theophylline.

Laboratory-prepared synthetic samples of the artificial sweetener were assayed to determine the accuracy of the method. Three groups of five samples each in which the placebo was held constant while the sodium saccharin was varied at 80, 100, and 125% of label were assayed by the procedure (Table II). The data indicated no bias in the method and statistically no difference in the groups by $F$ and $t$ tests. The calculation is:

$$\text{mg of saccharin sodium/g of sample} = \frac{R_{\text{sample}}}{R_{\text{standard}}} \times \frac{\text{mg of saccharin sodium standard}}{\text{sample weight}} \quad \text{(Eq. 1)}$$

where the $R$'s are the saccharin to theophylline response ratios. The method was validated for peak height and area ratios.

A manufactured sample was assayed 15 times, varying the sample weights from 80 to 120% recommended. The relative standard deviation of the assay was ±0.3%. The solutions for injection were stable for many weeks as demonstrated by a lack of change in the response ratios.

The method was challenged directly by the presence of seven possible impurities and degradation products documented in the literature (1, 18–20). Figure 2 shows a chromatogram of a typical saccharin and theophylline sample solution after quantities of seven of these compounds, each equal to the amount of saccharin present, were added. The identities of the peaks are: 1, 1,5-naphthalenedisulfonic acid; 2, ammonium-*o*-sulfobenzoate; 3, 2-(aminosulfonyl)benzoic acid; 4, *p*-carboxyben-

[1] Mallinckrodt.



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**Table I—Linear Regression of Response Ratio *versus* Weight Ratio, Saccharin to Theophylline**

| Weight Ratio | Area Ratio | Peak Height Ratio |
|---|---|---|
| 2.0 | 0.319 | 0.519 |
| 3.2 | 0.518 | 0.843 |
| 4.0 | 0.648 | 1.018 |
| 5.0 | 0.804 | 1.343 |
| 6.0 | 0.966 | 1.569 |
| 8.0 | 1.300 | 2.065 |
| Slope | 0.163 | 0.258 |
| Correlation coefficient | 0.999 | 0.999 |
| Standard error of estimate | 0.02 | 0.01 |
| Percent variation | 2.0 | 0.7 |

zenesulfonamide; 5, salicylic acid; and 6, *o*- and *p*-toluenesulfonamides. All of the peaks were well resolved from both saccharin and theophylline, and none of these compounds at reasonable levels interfered in the method.

The artificial sweetener was subjected to stress conditions for an attempted forced degradation study. The samples showed no measurable degradation at 100° for 1 month and also were unaffected by 2 weeks of exposure to strong fluorescent and incandescent light. However, a sample stored at 75° at very high humidity gave a low assay and the formation of a new peak in the chromatogram. The assay value by the liquid chromatographic procedure was 44.9% of initial. This new peak had a retention time corresponding to 2-(aminosulfonyl)benzoic acid, the alkaline hydrolysis product, which was suspected to be the most likely first degradation product. This sample was assayed by TLC, and the result was 42.8% of initial.

**Table II—Synthetic Samples ($n$ = 5)**

| Group, % | Recovery, % | *RSD*, % |
|---|---|---|
| 80 | 100.5 | 0.51 |
| 100 | 100.1 | 0.46 |
| 125 | 100.1 | 0.30 |

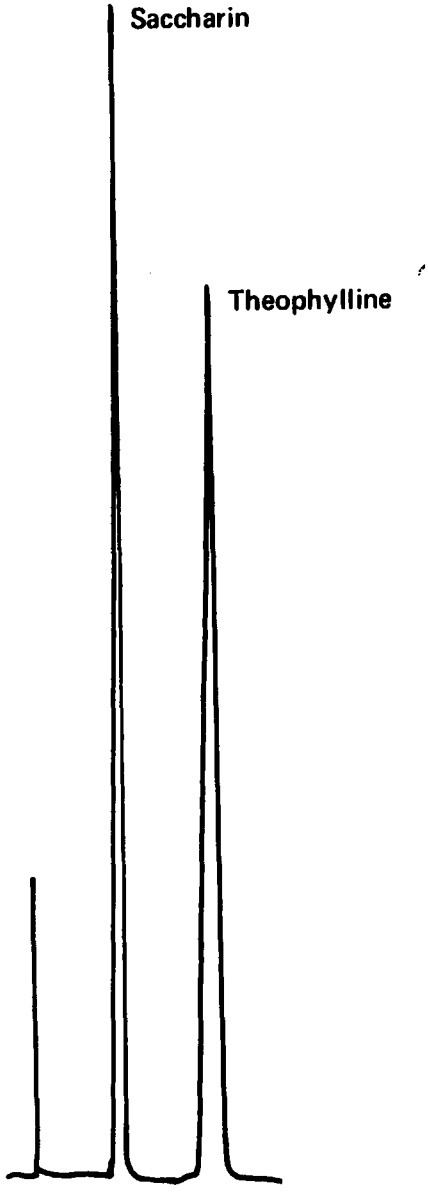


**Figure 1**—*Chromatogram of saccharin and theophylline.*

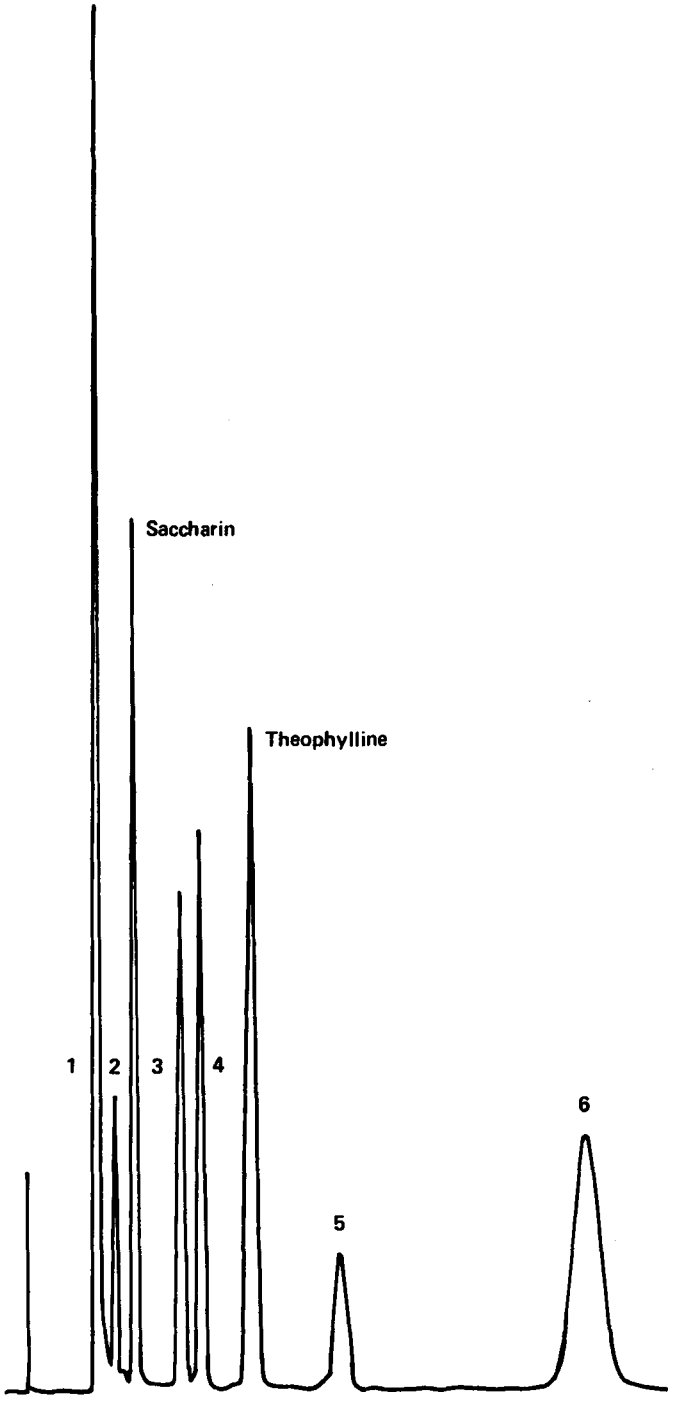


**Figure 2**—*Chromatogram of saccharin, theophylline, and postulated impurities and degradation products. (See text.)*

## DISCUSSION

The artificial sweetener consists of saccharin sodium and sodium bicarbonate. Acetic acid in the eluent neutralizes both. Acetic acid in the eluent increases the retention (capacity factor) of saccharin by suppressing ionization and also results in a much sharper peak. If saccharin is allowed to elute in its ionic form, very little retention occurs and little use is made of the separating capabilities of the column.

Before an assay can be considered qualified for a stability-indicating program, its specificity characteristics must be proven. Specificity in this method is provided mainly by the liquid chromatographic column. It is clearly not proper to assume specificity simply because a chromatographic process is involved. For this study, the proposed method was challenged in three ways.

First, a literature search was conducted to determine the documented impurities and degradation products of the active drug saccharin. These compounds were subjected to the column under the assay conditions and were all innocuous to the assay. These compounds must be added directly to the sample solution so that possible matrix effects are eliminated.

Second, a forced degraded sample of the actual product was assayed for two reasons: (*a*) the actual decomposition products in the drug matrix may be different from those postulated in the literature and (*b*) interferences by degradation products of the excipients are possible. When the assay passes these conditions, it is more likely to prevail during the shelflife of the product.

Third, the forced degraded sample assay, whenever possible, should be compared with a completely independent assay based on different analytical principles. If possible, this reference method should be of demonstrated accuracy. When the results of the two methods agree within the expressed objectives, the method developer can be confident of the reliability of the new method. These three approaches are by no means completely rigorous but can be regarded as minimum criteria for a proposed stability-indicating method.

The general procedure given for other pharmaceutical preparations was applied to mouthwashes, analgesic liquids, and antidiarrhetics. Occasionally, such samples can be injected after dilution, addition of the internal standard, and clarification. Generally, however, ether extraction is required because of the low saccharin levels in the formulations. The ether extraction technique also is preferable because of the added selectivity of the extraction step and the extended column life resulting from injection of a cleaned-up sample solution.

Because of pharmaceutical formulation diversity, components other than saccharin are frequently extracted and the resulting chromatograms are less simple than those of the artificial sweetener. However, interferences often may be eliminated by proper pH and methanol adjustments in the eluent. The selectivity of the chromatographic process provides enough resolution so that the saccharin assay can be carried out in the presence of other ingredients such as salicylic acid and alkyl parabens. Injections can be made every 5 min, and about 30 samples of artificial sweetener can be analyzed per 8-hr day. Fewer samples of the other pharmaceutical formulations can be analyzed because of the lengthier sample-handling procedure, but this procedure is necessary for almost any technique.

In summary, this reversed-phase liquid chromatographic system provides a precise, accurate, and specific saccharin assay with high sample throughput. The procedure is applicable to a wide range of pharmaceuticals, especially for a stability-indicating program.

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# High-Performance Liquid Chromatographic Assay for the Anthelmintic Agent Mebendazole in Human Plasma


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Received June 30, 1976, from the *Drug Disposition Section, Ortho Pharmaceutical Corporation, Raritan, NJ 08869.* Accepted for publication January 16, 1979.



**Abstract** □ A rapid and specific high-performance liquid chromatographic (HPLC) assay for quantitative plasma mebendazole determination is described. After a simple extraction, the compound was analyzed by HPLC using a reversed-phase column and a UV detector (313 nm). Quantitation was accomplished using an internal standard; peak area ratios were determined with an integrating computer. The average mebendazole recovery over a concentration range of 0.01–0.20 μg/ml was 75.9 ± 3.8% *SD*, and the maximum assay sensitivity was ~10 ng/ml.



**Keyphrases** □ High-performance liquid chromatography—analysis, mebendazole in human plasma □ Mebendazole—analysis, high-performance liquid chromatography, in human plasma □ Anthelmintic agents—mebendazole, high-performance liquid chromatographic analysis in human plasma


Mebendazole[1] (I), methyl 5-benzoyl-2-benzimidazolecarbamate, is a broad spectrum anthelmintic agent (1–3). Initial absorption studies in humans (1) suggested that 6–10% of the radioactivity was recovered in the urine following 5–7 mg po of $^{14}$C-mebendazole (18.5–22.7 μCi). Approximately 2.5% of this urinary radioactivity was unconjugated mebendazole, about 20% was the decarbamated metabolite, and 75% was of undetermined identity. Plasma radioactivity levels remained low throughout the study. There are no published methods sensitive enough to

[1] Vermox tablets, Ortho Pharmaceutical Corp.




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