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High-Performance Liquid Chromatographic Determination of Glycyrrhizin in Licorice Products

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A high-performance liquid chromatographic (HPLC) method is described for the analysis of glycyrrhizin in licorice products, determined as glycyrrhizic acid. The samples are extracted in a solution of NH_4OH with final HPLC determination using a reversed-phase column with detection at 254 nm. Precision studies indicate a % Cv of less than 2.5. Recoveries of added glycyrrhizic acid range from 93 to 105%, with data linear (r = 0.99) for a 20-fold range. The HPLC method was compared to a wet chemical spectrophotometric method, and good agreement was observed. The HPLC method is precise, accurate, and time conservative and was successfully applied to a wide variety of licorice-containing products.

The extract of the licorice root has many uses. It is used extensively in the tobacco, confectionery, and pharmaceutical industry (Hall, 1973). The root contains about 5–10% of a glycoside which contains two glucuronic acid units and glycyrrhetinic acid (Hall, 1973). The glycyrrhetinic acid has a structure (see Figure 1) similar to other steroids and a similar potential pharmacological effect (Strecher, 1976; Koster and David, 1968; Chamberlain, 1970). This glycoside usually occurs in a combined calcium and potassium salt form as glycyrrhizic acid. For a more detailed survey of the compound, one should refer to a treatise by Nieman et al. (1957).

The analysis of glycyrrhizin as glycyrrhizic acid has been accomplished by gas–liquid chromatography (GLC) (Larry, 1972), thin-layer chromatography (TLC) (Nour et al., 1976), and various wet chemical methods (Cundiff, 1964; Habib et al. 1979). High-performance liquid chromatography (HPLC) has been used for the analysis of glycyrrhizin (Chamberlain, 1970; Lunder and Neilsen, 1980; Beasley et al., 1979; Bricout, 1978). Additionally, it has been used for the determination of glycyrrhetinic acid after acid hydrolysis. The HPLC assay is accurate, precise, and time conservative. Other HPLC methods have analyzed licorice root while this method reports the analysis of glycyrrhizin in finished confectionery items containing licorice. In this method, HPLC is used for the analysis of glycyrrhizin in a wide variety of licorice products.

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MATERIALS AND METHODS

High-Performance Liquid Chromatograph. The HPLC used in this study consisted of a Waters Model ALC/GPC 201. The unit consisted of a Model 440 absorbance detector with a 254-nm filter and an M6000A solvent delivery system. The injection system was a Rheodyne Model 7120 syringe injector. Data acquisition was accomplished with a Shimadzu E-1A data unit. The HPLC column used was an EM Laboratories RP-18 (25 cm × 4.5 mm i.d.). The HPLC mobile phase consisted of

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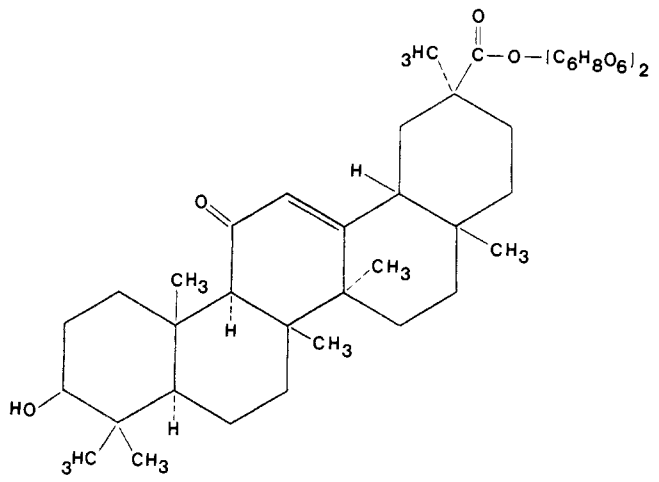

Figure 1. Glycyrrhizic acid.

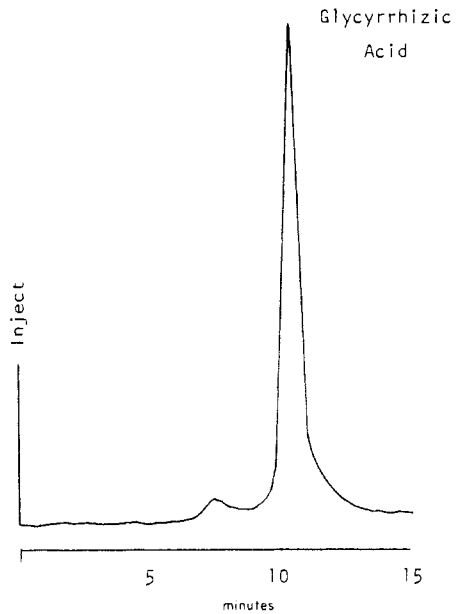

Figure 2. Chromatogram of glycyrrhizic acid standard. Column: RP-18 (EM Laboratories). Mobile phase: 60/34/6 $CH_3OH/H_2O/CH_3CO_2H$. Flow rate: 1.5 mL/min. Detector: Model 440 UV at 254 nm (0.02 AUFS). Sample: glycyrrhizic acid standard.

methanol, water, and acetic acid in the ratio of 60/34/6 (v/v/v). The mobile phase was filtered and degassed prior to use. The HPLC operated at a flow rate of 1.5 mL/min. The concentration of glycyrrhizin in a sample was obtained by comparing the peak area of the standard to that of the sample.

Standard. Five milligrams of glycyrrhizic acid ammonium salt (Tridom Fluka) was weighed into a 100-mL volumetric flask and diluted to volume with LC water for a final concentration of 0.05 mg/mL.

Samples. All licorice samples were commerically available and had national distribution.

Sample Preparation. All samples were ground in a mill or blender and extracted according to the following procedure. Two grams of sample was weighed to the nearest 0.01 g into a 100-mL blender cup of a Sorvall Omni-mixer. Approximately 30 mL. Of water (90–95 °C) and 2 mL of concentrated NH_4OH (37%) were added, and the contents were blended for 3 min. The resulting solution was quantitatively transferred to a 150-mL beaker. The pH of the solution was adjusted to a pH of 7.0 with H_3PO_4 and 1 mL of 10% diastase was added. This was incubated at 37 °C for 30 min, cooled to room temperature,

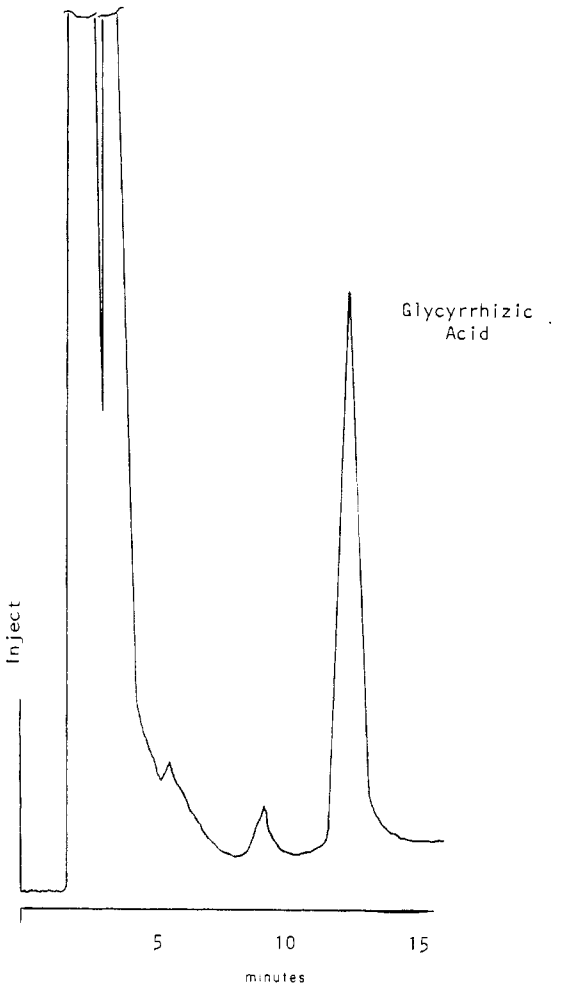

Figure 3. Chromatogram of licorice extract. Column: RP-18 (EM Laboratories). Mobile phase: 60/34/6 $CH_3OH/H_2O/CH_3CO_2H$. Flow rate: 1.5 mL/min. Detector: Model 440 UV at 254 nm (0.02 AUFS). Sample: licorice extract.

Table I. Precision Study (n = 8)

sample	concn	% Cv
black licorice	2.67 mg	2.35
glycyrrhizic acid standard	1.25 μg	0.78

Table II. Recovery Study of Glycyrrhizic Acid from Licorice[a] (n = 2)

mg added	mg Recovered	% recovery
0.5	0.53	105.0
1.0	1.03	103.0
1.5	1.53	102.0
2.0	1.86	93.0
5.0	4.83	96.6
		X = 99.9

[a] The licorice used contained 1.30 mg/g glycyrrhizic acid.

and transferred to a 100-mL volumetric flask with CH_3OH. The final extract was diluted to volume with additional CH_3OH and filtered through Whatman No. 42 paper or equivalent prior to analysis. Figure 2 shows a chromatogram of glycyrrhizic acid standards while Figure 3 is that of licorice extract.

RESULTS

Precision studies were performed on both standard and sample extracts with a % Cv of less than 2.5 in both cases. These data are presented in Table I.

Table III. Methods of Addition: Spectrophotometric Determination of Glycyrrhizic Acid in Licorice (n = 2)

amount of glycyrrhizic acid added, mg/g	amount of glycyrrhizic acid obtained, mg/g	% recovery
none	1.30	
0.2	1.50	100.0
0.4	1.70	100.0

Table IV. Product Survey (n = 2)

product description	amount of glycyrrhizic acid, mg/g
candy wafers (black)	0.11
jelly rings (black)	0.19
licorice toffees	0.88
licorice candies (France)	1.65
jelly candy (black)	0.25
hard-coated licorice candies	0.06
licroice gumdrops	1.39
licorice bits	0.94

The previously described HPLC method was tested on a black licorice with additions at five different levels. Additions were made prior to extraction. Table II summarizes this data. Concentrations of glycyrrhizic acid over a 20-fold range, from 0.5 to 10 mg/mL, were injected onto the LC and found to be linear with a regression coefficient of 0.99.

Additionally, the spectrophotometric method of Cundiff (1964) was evaluated with recovery checked at two levels and an average recovery of 100%. These data are summarized in Table III. A comparison of the HPLC and spectrophotometric methods shows excellent agreement.

Table IV outlines the results from a product survey consisting of eight licorice-containing products which covered the range of licorice candies from gumdrops to hard candies.

SUMMARY

The HPLC technique now allows the routine analysis of glycyrrhizin in licorice products. The technique is rapid, accurate, and precise and can be applied to a variety of licorice-containing confectionery items. Additionally, for those laboratories that do not possess an HPLC, the spectrophotometric method, while not as fast, serves as a good alternative for the analysis of glycyrrhizin in licorice confectionery items.

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Registry No. Glycyrrhizic acid, 1405-86-3.

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Caloric Utilization and Disposition of [^{14}C]Polydextrose in Man

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Polydextrose is a tasteless, nonsweet, low-caloric bulking agent formed by the random polymerization of glucose with lesser amounts of sorbitol and citric acid. It is not absorbed after oral administration, and the major portion of polydextrose is excreted in the feces. A fraction of fed polydextrose is fermented in the lower gut by the intestinal microflora to products such as volatile fatty acids (VFA) and CO_2; the VFA are caloric to the host, but the CO_2 is not. The metabolism and disposition of polydextrose in man is the same as that in the rat. Metabolic studies show that polydextrose has approximately 1 cal/g, or about 25% the value of glucose. Polydextrose can serve as a total or partial replacement for sugar and as a partial replacement for fat and flour in a variety of commmon processed foods with accompanying caloric reduction of those foods.

A substantial portion of the U.S. adult population is overweight (Bray, 1979), and methods and diets designed to normalize the weight of this population are legion. Among the more pleasant and alternative approaches to weight reduction would be the availability of a broad spectrum of prepared foods with reduced caloric density. The ingestion of such foods would result in a normal volume intake that contains significantly fewer calories when compared to standard food items.

Polydextrose, which was recently approved by the Food and Drug Administration, was developed to fulfill this purpose in foods. It is a substance which contains only 1 cal/g, or approximately 25% the value of food carbohydrate, and has broad utility as a bulking agent in a

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