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Autor(en): **Lunder, T.L. / Nielsen, C.**

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Determination of Glycyrrhizin in Liquorice Roots and Extracts by High Performance Liquid Chromatography

T. L. Lunder and *C. Nielsen*

Nestlé Products Technical Assistance Co. Ltd., La Tour-de-Peilz

Introduction

Liquorice, an extract from the roots of the liquorice plant, Glycyrrhiza glabra, has been manufactured and used by man for many centuries.

References to liquorice are found in many of man's earliest medical records, including those of the Greek physician Hippocrates. Glycyrrhizin*, 50 times sweeter than sucrose, is the component of liquorice extract which imparts its characteristic flavour, and this property explains the use of liquorice in some branches of the food industry as for instance in sweets and drinks.

Botany

Plant liquorice comes from the dried rhizome and roots of Glycyrrhiza glabra L. variety *typica* (Spanish liquorice), or of G. glabra L., variety *glandulifera* (Russian liquorice).

Liquorice may also be extracted from the roots of Abrus precatorius, Periandra dulcis, Periandra mediterranea, and from the bark of the trees Lucuma glycyphylla, Achras sapota and Sideroxylon richardii.

Analytical methods

Several analytical methods have been proposed for the determination of glycyrrhizin.

Nieman (1) gives, in his study, the means of assay and the chemical structure of glycyrrhizin.

The most widely used method is the acid precipitation process elaborated by *Houseman* (2) which lacks nevertheless of accuracy. *Wiest* (3) describes a colorimetric method and *Onrust* (4) proposes a polarographic technique.

* Synonims: Glycyrrhizic acid, glycyrrhizinic acid, glycyrrhetinic acid glycoside.

A synthesis of the methods of *Wiest* and *Onrust* has been carried out by *Cundiff* (5) which proposed his colorimetric method after the hydrolysis of glycyrrhizin.

A review of analytical methods for glycyrrhizin has been published by *Steinegger* and *Marty (6)*.

But with the development of the technique known as high pressure, or high performance liquid chromatography a great impetus has been given to the food components analysis and several methods have been published for glycyrrhizin determination (7, 8, 9).

We have elaborated a new method which uses the separating power of a reversed phase system associated with a gradient profile employing acid potassium phosphate and isopropylalcohol.

Experimental

Apparatus and reagents

— Siemens liquid chromatograph S 102, equipped with a gradient programme
— Spectrophotometer Zeiss PM2 DLC
— Philips PM 8252 dual pen recorder
— MSE mini minor centrifuge
— Solution of 0.1 N potassium acid phosphate (KH_2PO_4)
— Isopropylalcohol 50% in distilled water.

Procedure

Sample preparation

2 g of powdered liquorice root are weighed out exactly into a 100 ml round bottomed flask and refluxed with about 60 ml distilled water during 1 hour.

The mixture is cooled down and centrifuged.

Clear overflowing liquid is poured off into a 100 ml volumetric flask. Residue is transferred with a few ml distilled water into the round bottomed flask and refluxed for further 30 min.

Content is centrifuged and the overflowing liquid is poured into the 100 ml volumetric flask and filled to the mark with distilled water.

Before injection, samples are filtered on Schleicher and Schull disposable filter holders FP 030 to avoid clogging of the column. For powdered instant extracts of liquorice, about 100 mg are weighed out and the same procedure is followed.

For deglycyrrhizinated samples, about 500 mg must be weighed. Depending on the quality of the extract, the centrifugation step may be sometimes suppressed (extracts giving clear solutions).

Standard ammonium glycyrrhizinate

About 70 mg of ammonium glycyrrhizinate (Fluka No. 50531) are weighed out exactly into a 100 ml volumetric flask, dissolved with distilled water and filled to the mark.

Chromatographic analysis

Column:	Bondapak C_{18}, 25 x 0.4 cm, stainless steel
Sample loop:	50 µl
Flowrate:	1.5 ml/mn
Pressure:	ca. 100 bar
Detector:	254 nm
Recorder chart speed:	300 mm/h
Gradient:	convexe No. 7
Solvents:	A. KH_2PO_4 0.1 N B. isopropylylalcohol 50%
Mode:	2 minutes isocratic with 20% B/A, followed by 5 min convexe gradient to 85% B/A, 10 min isocratic in the position 85%, 10 min to come back to 20% and, finally, 10 min for the conditioning of the column with the initial solvent.

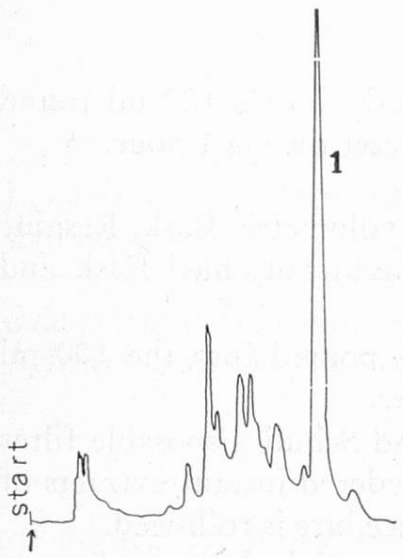

Fig. 1. Liquorice root from Siegfried

Gradient profile:
A. KH_2PO_4 0.1 N,
B. i-PrOH 50%
from 20% B/A to 85% B/A
in 5 min
Peak 1 = glycyrrhizin

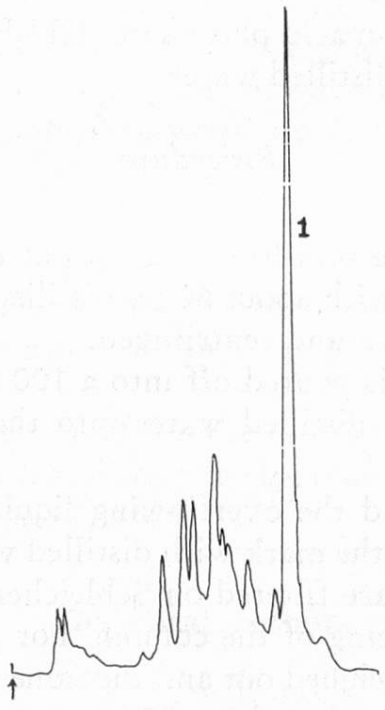

Fig. 2. Liquorice root from Dixa

Gradient profile:
A. KH_2PO_4 0.1 N,
B. i-PrOH 50%
from 20% B/A to 85% B/A
in 5 min
Peak 1 = glycyrrhizin

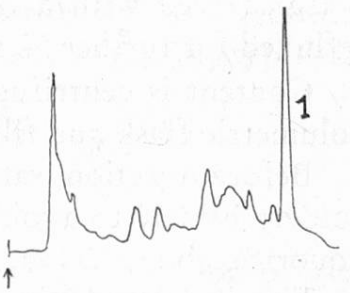

Fig. 3. Soluble extract from McAndrews

Gradient profile:
A. KH_2PO_4 0.1 N,
B. i-PrOH 50%
from 20% B/A to 85% B/A
in 5 min
Peak 1 = glycyrrhizin

Results and discussion

The chromatography of glycyrrhizin in liquorice by HPLC with a gradient system presents the advantage to give not only glycyrrhizin but to define, in the same time, the profile of the extract.

A liquorice root from Siegfried (Zofingen, Switzerland) (fig. 1), from Dixa (St-Gall, Switzerland) (fig. 2) and a partially deglycyrrhizinated extract from McAndrews (USA) (fig. 3) give different profiles which may be used as «finger prints» characteristic of liquorices from different origin.

As the aim of this work is the determination of glycyrrhizin, peaks which appear with a gradient elution have not been identified. The peak of glycyrrhizin has been conformed by doping the sample with a standard of ammonium glycyrrhizinate and by carrying on an isocratic run (8) with an alternative solvent system (figures 4 and 5).

The ammoniacal liquorice extract from Merck (No. 4203) has been also employed as reference (fig. 6).

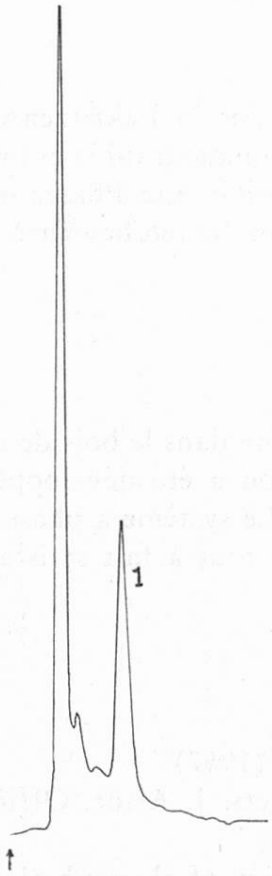

Fig. 4. Liquorice root from Siegfried
Isocratic with MeOH(600) : H_2O(340) : CH_3COOH(50)
Peak 1 = glycyrrhizin

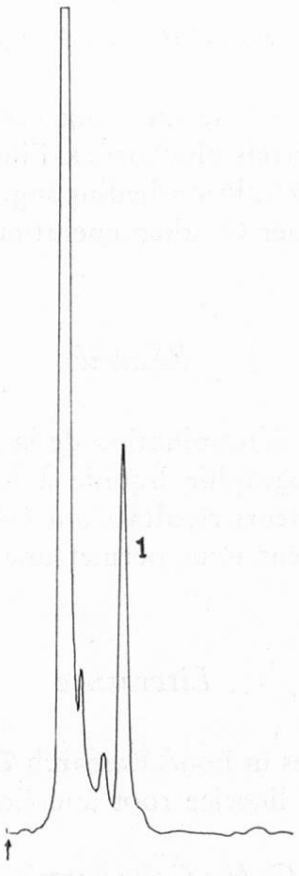

Fig. 5. Liquorice root from Dixa
Isocratic with MeOH(600) : H_2O(340) : CH_3COOH(50)
Peak 1 = glycyrrhizin

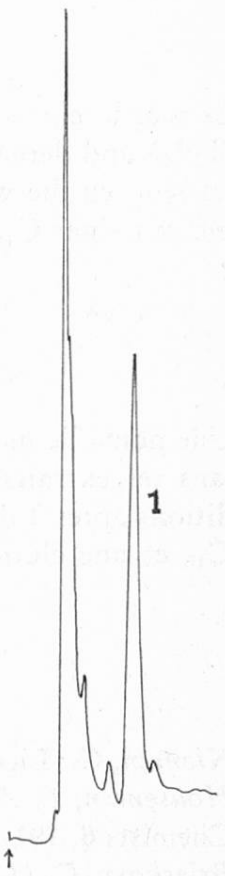

Fig. 6. Ammoniacal extract of liquorice Merck
Isocratic with MeOH(600) : H_2O(340) : CH_3COOH(50)
Peak 1 = glycyrrhizin

Following results have been obtained on the mentioned samples:

	% glycyrrhizin	
Liquorice Siegfried	4.95	
Liquorice Dixa	5.55	
Extract from USA	6.60	
Ammoniacal extract Merck	45.81	(labelled without guarantee: 50%)

The normal glycyrrhizin content of a liquorice root is about 5% and soluble extracts contain normally about 20—24% glycyrrhizin.

Summary

A new method for detection and separation of glycyrrhizin in licorice roots and soluble extracts with high performance liquid chromatography (HPLC) is described. The best conditions for extraction and quantitative determination of glycyrrhizin have been elaborated. The reversed phase system with a C_{18} column and a gradient elution has revealed to be the best one.

Zusammenfassung

Es wurde eine neue Methode zur Bestimmung des Glycyrrhizins in Lakritzenwurzeln (Süßholz) und deren Extrakten mittels Hochdruck-Flüssigkeitschromatographie entwickelt. Dabei wurden die wirksamsten Extraktionsbedingungen ausgearbeitet. Ein Phasenumkehrsystem mit einer C_{18}-Säule und einer Gradientenelution hat sich am besten bewährt.

Résumé

Une nouvelle méthode pour la détermination de la glycyrrhizine dans le bois de réglisse et dans ses extraits par chromatographie liquide à haute pression a été développée. Les conditions aptes à donner les meilleurs résultats ont été étudiées. Le système à phase inversée C_{18} et une élution avec gradient nous permet une séparation tout à fait satisfaisante.

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T. L. Lunder
C. Nielsen
Nestlé Products Technical
Assistance Co. Ltd.
Research Laboratories
P. O. Box 88
CH-1814 La Tour-de-Peilz