

# ANALYSIS OF GUARANA (*PAULLINIA CUPANA* VAR. *SORBILIS*). III. IDENTIFICATION AND DETERMINATION OF GUARANA BEVERAGES BY HPLC ANALYSIS OF CAFFEINE AND THEOPHYLLINE

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A simple and rapid HPLC method is presented to verify if the addition of guarana seeds (resp. its extracts) to Guarana soft drinks is in accordance with the Brazilian Juice Law. The method includes a solid phase extraction of the methylxanthine derivatives and a reversed phase HPLC separation of caffeine and theophylline. The evaluations base upon the total amount of caffeine and the caffeine/theophylline ratio in the beverage.

## INTRODUCTION

Guarana seed (*Paullinia cupana* var. *sorbilis*) [resp. its extract] is the significant ingredient of soft drinks called Guarana which are of great importance on the beverage market in Brazil. The content of guarana in the soft drinks is regulated by the Juice Law (6/12/73). Accordingly Guarana has to content between 0.02 and 0.2 g of guarana seeds or the equivalent of the extract in 100 ml of the beverage. Up to now the observance of these legal regulations is normally controlled by the analysis of the caffeine content only. The high content of caffeine in guarana seeds (up to 5,8% dry matter) is well known and confirmed by modern analytical methods<sup>1,2</sup>.

Since other sources of caffeine are much more inexpensive than guarana seeds (mainly the synthetically produced pure drug) it is imaginable that the guarana content is simulated by caffeine of other origin.

Maravalhas<sup>3</sup> already showed by a paper chromatographic method that guarana seeds contain theobromine and theophylline besides caffeine. He suggested to use the detectibility of theophylline as a proof for the presence of guarana in a beverage.

On the other hand a HPLC method for the quantitative determination of the above mentioned purine bases in guarana seeds was described recently by us<sup>2</sup>. We found the ratio of the caffeine/theophylline content to be within 100:0.5 and 100:1.6, significantly different from the respective ratios in coffee, cola and mate.

In this report we present an easily to perform application of that HPLC method<sup>2</sup> for the identification and quantitative determination of guarana in soft drinks, considering not only the total amount of caffeine, but also the caffeine/theophylline ratio.

## EXPERIMENTAL

### Sample Cleanup

As the beverage was enriched with carbonic acid, ca. 50 ml of the sample were degassed in an supersonic apparatus. Thereupon a disposable solid phase cartridge CHROMABOND C18 1 ml was succidingly washed with 2 ml of methanol and

5 ml of water. Hence 10 ml of the degassed sample were given onto the disposable cartridge (fig. 1). Then the stationary phase was washed with 2 ml of water and finally the methylxanthine derivatives were eluted with exactly 1 ml of methanol. An aliquot of 20  $\mu$ l of the methanolic solution was injected onto a HPLC C-18-column.

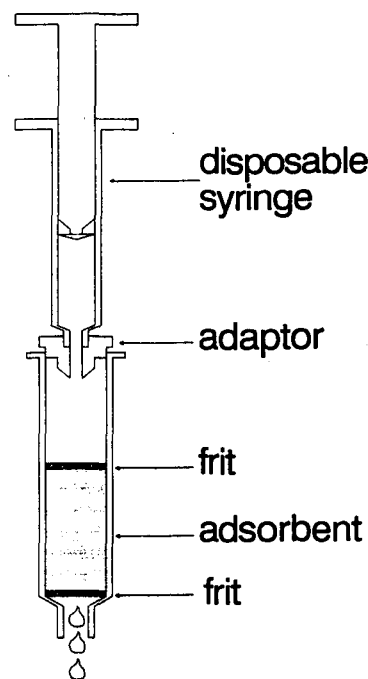


Fig. 1. Arrangement for solid phase extraction

### Chromatographic System

The HPLC instrument was a VARIAN model 5000 equipped with a 20  $\mu$ l loop injector (RHEODYNE 7125) and a UV-Vis detector PERKIN-ELMER LC 55B set to 280 nm. The HPLC cartridge column (25 cm x 4 mm I. D.) was filled with LiChrospher C18, 5  $\mu$ m, equipped with a precolumn (1 cm x 4 mm I. D.) filled with the same material. The mobile phase consisted of a gradient of 0.02 M sodiumdihydrogenphosphate buffer, adjusted to pH 7 and methanol. The time schedule is listed in the following scheme.

### Gradient program

time (min)	% methanol	time (min)	% methanol
0	15	18	100
5	15	19	0
9	50	22	0
13	100	23	15

The chromatograms were monitored and quantitatively evaluated with a Sigma 10 integrator (PERKIN-ELMER) by the external standard method.

### RESULTS AND DISCUSSION

Exemplary the analyses were performed with a sample of the beverage "Guarana" of the Brazilian firm SKOL. Regularly the peaks for caffeine and theophylline were unequivocal and well separated whereas theobromine (retention time approx. 4 min) was overlapped by coextracted matrix substances (fig. 2). No further trials were undertaken to improve this separation because the quantitative determination of theobromine was not considered.

In order to check the efficiency of the solid phase extraction with the CHROMABOND cartridges the recoveries of caffeine were determined. For pure caffeine solutions (20 ppm in water) the recovery yielded to  $99.5 \pm 3.6\%$ . In samples spiked with 16 ppm caffeine and 2.6 ppm theophylline, 89.4% of the added caffeine and 81.3% of the added theophylline were recovered. Obviously matrix effects are the reason for the less recoveries in the beverage sample.

Considering the latter recoveries the contents of caffeine and theophylline in the examined beverage amount to 74.9 ppm resp. 0.62 ppm. Taking as a basis an average content of 4.7% caffeine in guarana seeds the addition of guarana (extract) to the beverage is calculated to approx. 1.6 g/L. This is within the legal limits from 0.2 to 2.0 g/L. The caffeine/theophylline ratio in the sample amounts to 100:0.83. This is also within the natural limits from 100:0.5 and 100:1.6. Thus the following conclusions are allowed:

The proposed method is qualified for the simple and rapid identification and quantitative determination of guarana (extracts) in beverages. Obviously the content of guarana in the examined example is in accordance with the Brazilian Juice law.

An additional method to confirm the correct content of guarana in soft drinks could be based upon (+)-catechin and (-)-epicatechin, compounds which recently were found to be significant ingredients of guarana seeds<sup>4</sup>.

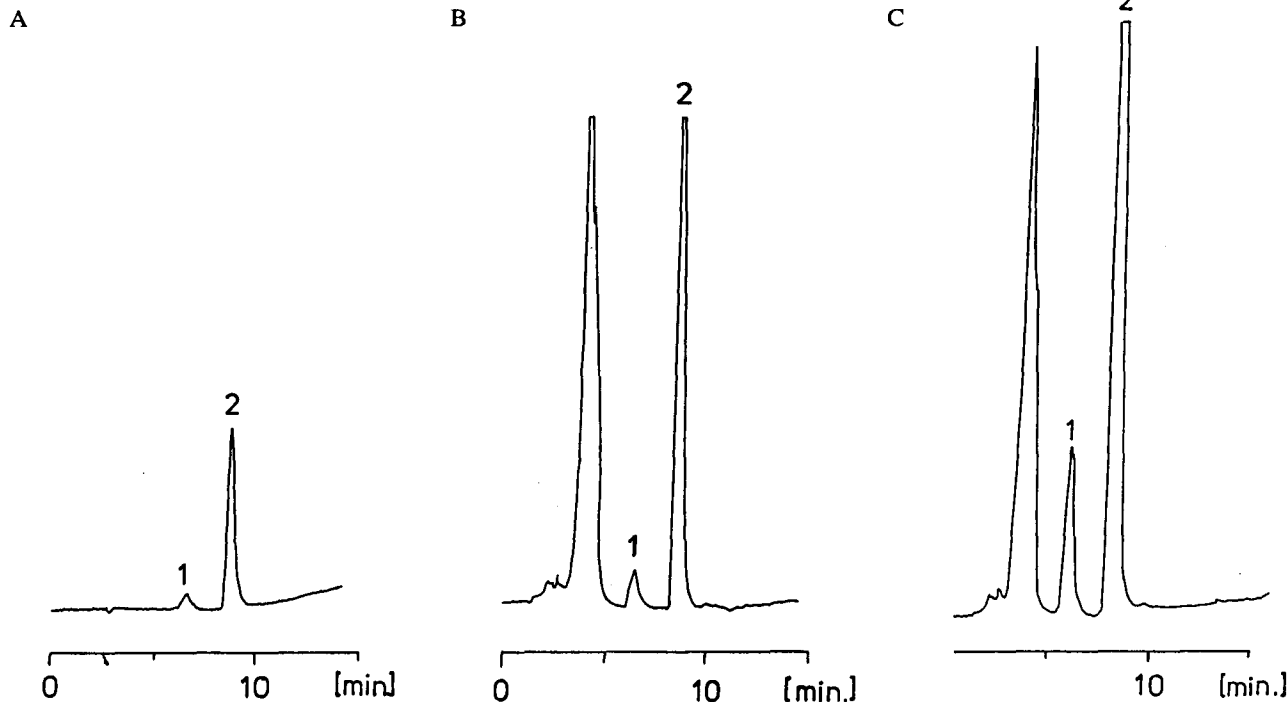


Fig. 2 a) HPLC standard solution separation of 1.3 ppm theophylline (1) and 20.0 ppm caffeine (2). b) HPLC beverage sample separation of theophylline (1) and caffeine (2) after solid phase extraction. c) HPLC beverage sample spiked with 2.56 ppm theophylline (1) and 16.0 ppm caffeine (2) after solid phase extraction.

### ACKNOWLEDGMENT

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