DETERMINATION OF CAFFEINE IN SOFT DRINKS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

ALKOLSÜZ GAZLI İÇECEKLERDE YÜKSEK PERFORMANSLI SIVI KROMATOGRAFİSİ İLE KAFEİN TAYİNİ

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SUMMARY: Some popular brands of soft drinks analyzed for caffeine content by high-performance liquid chromatography. Decarbonated soft drinks are injected directly into the chromatograph without any sample preparation. Caffeine was separated as a single peak with a retention time of 3.5 min. and acetanilide with 7.9 min. It is found that the caffeine content of several soft drinks produced in Turkey range from 23.1 to 44.2mg per 330ml can. The method is also applied for caffeine determination in caffeine-free soft drinks and found that the caffeine-free soft drinks are less than one miligram per 12-oz. can, except the caffeine-free Sample 7 which has 1.20mg in a 12-oz. can.

ÖZET: Çok tüketilen bazı alkolsüz gazlı içeceklerin kafein içeriği yüksek performanslı sıvı kromatografisi ile analiz edilmiştir. Gazı uzaklaştırılan alkolsüz gazlı içecekler, örnek hazırlama safhası olmaksızın kolona direk olarak enjekte edilmektedir. Kafein alıkonma süresi 3,5 dak. olan tek bir pik halinde ayrılmıştır. Asetanalitin alıkonma süresi ise 7,9 dakikadır. Türkiye'de üretilen bazı alkolsüz gazlı içeceklerin kafein içeriği 330ml teneke kutu için 23,1 ile 44,2mg arasında bulunmuştur. Bu metod aynı zamanda kafeinsiz alkolsüz gazlı içeceklerin kafein tayini için de uygundur. Kafeinsiz alkolsüz gazlı içeceklerde kafein oranı 12oz. teneke kutu için bir miligramdan az bulunmuştur. Sadece Örnek 7'nin 12oz. teneke kutusundaki kafein oranı 1,20 mg olarak bulunmuştur.

INTRODUCTION

Caffeine is an alkaloid, structuraly identified as 1, 3, 7-trimethylxanthine. Caffeine containing beverages are consumed daily in nearly all countries. Coffee, tea, soft drinks and cocoa are the main sources of caffeine. Moreover various pharmaceutical products contain caffeine in combination with other drugs.

Caffeine is generally classified as a central nervous system stimulant and can be addictive. Regularly used enough caffeine may cause physical harm, notably heart disease, bladder cancer in males and behavioral disorder. Of the special concern is the possibility that caffeine used during pregnancy cause or contribute to birth defects and difficulties (GILBERT, 1986).Some of the consumers have turned to decaffeinated coffee and tea and caffeine-free soft drinks.

The determination of the level of caffeine in foodstuffs is becoming increasingly important in the light of recent concern about the health effects of this compound and its widespread consumption by the public. Several analytical methods exist for the quantitation of caffeine in coffee, tea and soft drinks. These methods include Kjeldahl method (ANONYMOUS, 1975), UV spectrophotometry (NEWTON, 1979), gas chromatography (STRAHL, 1977) and high-performance liquid chromatography (HPLC) (KREISER and MARTIN, 1978; BLAUCH and TARKA, 1983).

It was reported, however, that most of the methods devised for the analysis of formulations with full caffeine content, can not be reliably applied for the determination of caffeine in decaffeinated or caffeine-free products due to their much lower caffeine content (MUHTADI. et. al. 1990). ASHOOR et. al. (1983) reported an HPLC method for the caffeine content in decaffeinated coffee, tea and beverage products. MUHTADI et. al.(1990) developed an HPLC method for all types of coffee, tea and soft drinks. The HPLC methods have the advantages of high sensitivity, shorter analysis time and improved separation. Most of the above methods require some degree of sample preparation, usually consisting of a liquid-liquid extraction with a solvent such as benzene or chloroform. SMYLY et. al. (1976) reported that caffeine in soft drinks could be determined by direct injection into the chromatograph.

This paper reports an application of a reverse phase HPLC system for the caffeine contents of popular brand soft drinks in Turkey. Samples are directly injected into the chromatograph. The caffeine contents are also determined for caffeine-free soft drinks with the same method. The method utilized in

this study was based on a modified version of the method of ALKAYSI et. al. (1988) for determination of caffeine in plasma and saliva.

MATERIALS AND METHOD

Apparatus and Reagents

Analysis was carried out by liquid chromatography a Beckman pump Model l26, with a variable wavelength detector Model l66. The injector head was fitted with a 50μ sample loop. An ultrashere (5μm) ODS column (4.5xl50mm, Beckman) was used for the separation. The mobile phase for this isocratic separation of caffeine consisted of 0.05 M ammonium acetate buffer, acetonitrile and methanol (82:l5:3). The final pH was adjusted to 4.0 using glacial acetic acid. All solvents were HPLC grade (Fisher Scientific). The flow rate was 1 ml/min. The column eluate was monitored at a detection wavelength of 254nm. Acetanilide, analytical grade (Aldrich), was used as an internal standard at a concentration of l0μ g/ml.

Sample Preparation

Popular brands of soft drinks were purchased in Ankara. Caffeine-free soft drinks were purchased in Tallahassee, USA.Soft drinks were decarbonated by agitation and ultrasonic treatment. Then 400μl of each sample were taken and added 200μl acetanilide as an internal standard. The samples were vortex mixed for one minute and 50μl sample were immediately injected into the HPLC system. Analysis were performed in triplicate.

Quantification

Five different caffeine concentrations were used as standards: l.0, 5.0, 50.0, l00.0 and l50.0μg/ml. Peak area ratios (caffeine / internal standard) were measured and standard curves were constructed by plotting concentration versus peak area ratios.

Recovery Study

For the recovery study, a new product caffeine-free Sample l0 was chosen. Caffeine was added to the samples at three different concentrations (50, 75, l25μ/ml). Determination of the caffeine content of spiked samples was done as described above and the percent recovery calculated.

RESULTS AND DISCUSSION

Table 1 displays the caffeine content of several soft drinks produced in Turkey. The values range from 23.1 to 44.2 mg per 330 ml can. They are all within the range quoted on the cans by their manufacturers. When the results compared with the FDA Consumer (LECOS, 1984) and Consumer Reports (ANONYMOUS, 1991) results, it appears that the caffeine contents of Sample 1 and Sample 5 studied here are approximately 10 mg less than the values given in the above mentioned reports. Other results are in very close agreement with the reported values for the same brands of soft drinks. Lower caffeine contents for Sample 1 was also reported in earlier studies by GLASKO et. al. (1989) and by HANKIN and McLEAN (1988).

Table 2 shows the caffeine content of some of the caffeine-free soft drinks consumed in USA. Due to the fact that caffeine-free soft drinks are not yet produced in Turkey, this part of the present study was carried out with the soft drinks produced in USA. It is stated in Consumer Reports (ANONYMOUS, 1991) that the caffeine-free colas are found less than one mg per 12-oz can. In this study , the caffeine contents of the soft drinks are less than one mg per 12-oz can , except the caffeine-free Sample 7 which contains caffeine more than one mg per 12-oz can. The new products Sample 9 and Sample 10 marketed in 1993 in USA have the lowest caffeine contents within all the soft drinks tested.

Table 1. Caffeine content of selected soft drinks

Soft drink	Caffeine content (mg/330ml serving)
Sample 1	34.1
Sample 2	44.2
Sample 3	33.0
Sample 4	33.2
Sample 5	23.1
Sample 6	41.4

Table 2. Caffeine content of selected caffeine-free soft drinks

Soft drink	Caffeine content (mg/12-oz. serving)
Sample 7	1.20
Sample 8	0.49
Sample 9	0.08
Sample 10	0.06
Sample 11	0.46
Sample 12	0.19
Sample 13	0.19

Table 3. Recovery of caffeine from caffeine-free Sample 10

Concentrations (μg/ml)	Recovery (mean±SD%)	CV(%)[a]
50	96.2±0.17	0.18
75	99.3±0.17	0.17
125	99.4±0.21	0.21

a. CV = Coefficient of variation.

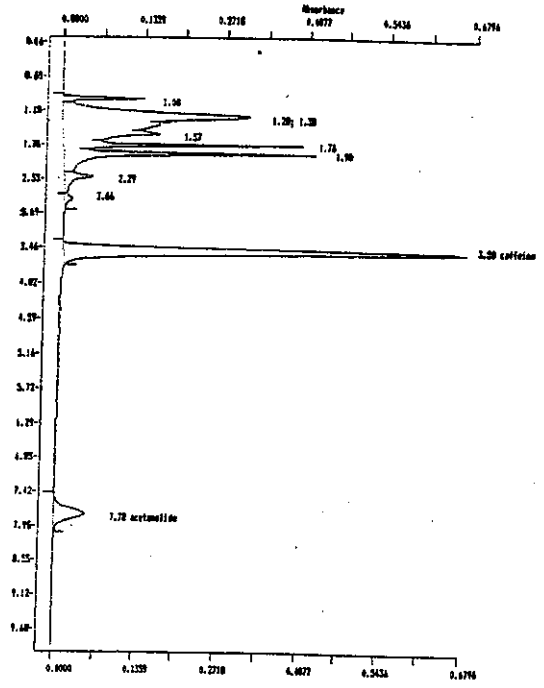

Figure 1. Typical chromatogram of caffeine and the internal standart (acetanilide) for Sample 3.

Some pediatricians have expressed concern over caffeinism in children. Restlessness, irritability, sleeplessness and nervousness are some of the syptoms found in children and teen-agers resulting from consumption of large amounts of cola beverages and chocolate. A nursing mother should be aware that caffeine passes into her milk and that this could have a stimulating effect on her infant, following her use of beverages, food or other products containing caffeine (BUNKER and McWILLIAMS, 1979). Consumption of soft drinks by young children and teen-agers is enormously increasing nowadays in Turkey. Since the caffeine is added to soft drinks during manufacturer processes, it would be a wise decision if the manufacturers produce caffeine-free soft drinks as well. This will serve as to lower the caffeine intake of children, as well as for adults who choose to take lower level of caffeine.

A typical separation profile of soft drinks are shown in Fig. 1. The caffeine peak was well resolved with no interference in all the chromatograms obtained. Under the HPLC conditions used in this study, caffeine was separated as a single peak with a retention time of 3,5 min. and acetanilide with 7.9 min. The recovery of caffeine from the spiked samples obtained by the HPLC method ranged from 96.2±0.17 to 99.4±0.21 as shown in Table 3. These results indicate that the HPLC method has a high degree of accuracy. Another advantage of this method is that the sample was injected directly into the liquid chromatograph. There is no sample preparation required. The method is suitable for caffeine-free soft drinks as well as for products with caffeine.

In conclusion, the HPLC method presented here is a simple, fast, accurate and sensitive technique for determining the caffeine contents of soft drinks.

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