Identification and Quantification of Active Principles of Products and Preparations for Tea and Coffee. Counterfeiting Detection of Beverages by Coupling with Extracts Xanthine TLC/GC/MS

DELIA NICA BADEA *
Universitiy of Constantin Brancusi Targu-Jiu, 33 Eroilor Str., 210135, Targul Jiu, Romania

This paper addresses the experimental separation, identification and quantification of active principles from products and preparations of coffee and tea as well as detecting counterfeits to which may be subject a number of soft drinks, alcoholic extracts using a basis of tea and coffee, chromatographic thin layer, gas chromatography coupled with mass spectrometry.

Keyword: xanthine compounds, detect fakes, TLC / GC / MS

Tea (Camellia sinensis), coffee (Coffea arabica), cocoa (Teobroma cacao), cola (Cola nitida) are products containing the active ingredient plant alkaloids: caffeine, theophylline, theobromine. In terms of food they are processed and used for preparing tea, soft drinks and alcoholic beverages, cuisine, chocolate.

Analysis of the three active ingredients caffeine, theobromine, theophylline by instrumental techniques is dictated by the necessity of analytical control of foodstuffs and pharmaceuticals which are due to pharmaco-dynamic effects important for the human body.

A range of refreshments or Type brandy, are painted with Chinese tea (black or green) [1]. These fakes can be easily identified by detecting the drinks caffeine, theophylline and theobromine.

These active principles present in various beverages may be identified by gas chromatography [2], column chromatography and high performance thin layer [3-9].

Separation and Identification of Compounds Xanthine

Experimental part

The study proposes a simple model for the identification of caffeine, theophylline, theobromine, in products and preparations of coffee and tea type drinks some brandy by thin layer chromatography and gas chromatography [10.11].

Experimentally, solutions of caffeine, theophylline, theobromine, xanthine and hypoxanthine at concentrations of 60 mg / L were prepared. From these solutions were applied volumes of 5 μL / with Brandt micropipette spot on silica gel plates Durasil - 25 UV 254 (Macherey - Nagel), HPTLC plates, Nano - NH2 Sil / UV 254 plates and Poligram - Sil G / UV 254 (Macherey - Nagel). Alongside standards were applied to extract samples of coffee, English tea, Pepsi and vodka.

Development was conducted in room N - saturated by ascending technique. After development the plates are dried in an oven at 1000 C and examined under UV light at 254 nm UV lamp Camag type. The spots corresponding to caffeine and theophylline in spirit forged (fig. 1), was extracted on the plate with chloroform and the extract was analyzed using a gas chromatograph - HelWet Packard 5890 equipped with mass detector MS 5972.

Working conditions: MP5-MS column (30 x 0.32 x 0.25), helium carrier gas, temperature program of 60, 30^{0}C / min, transfer temperature 240^{0}C.

Results and discussions

Separation and identification xanthine compounds from produs and preparations were made by many probing of which were selected those from figures 1-3.

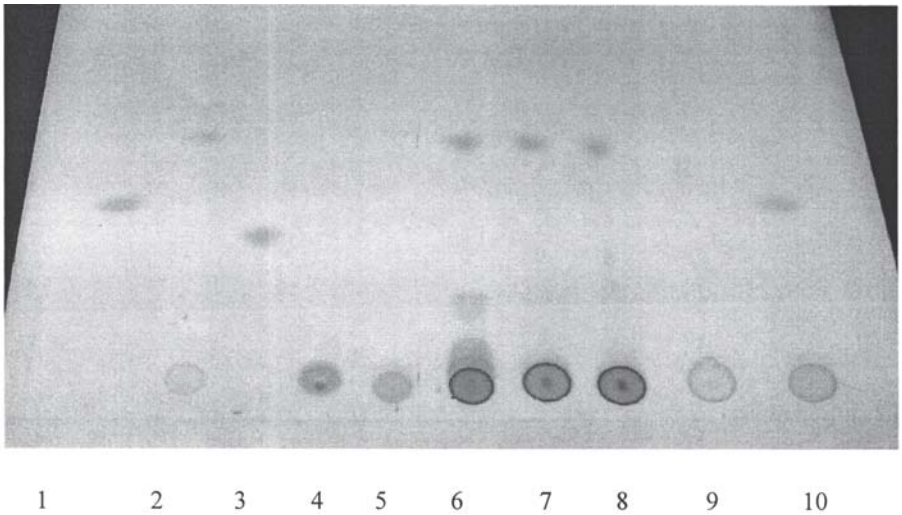


Fig. 1. Chromatogram of compounds separated on silica gel plates with UV 254 Durasil 25 as mobile phase mixture using acetone - toluene - chloroform (40:30:30 v / v). 1 - theophylline, 2 - caffeine, 3 - theobromine, 4 - hypoxanthine, 5-xanthine, 6 - Ahmad tea extract, 7 - Alvorado coffee extract, 8 - Elite coffee extract, 9 - Pepsi Twist, 10 - fake brandy decaffeinated tea.

** email: nicabadeadelia@yahoo.com.; Tel. 0762684144, 02532/273216*

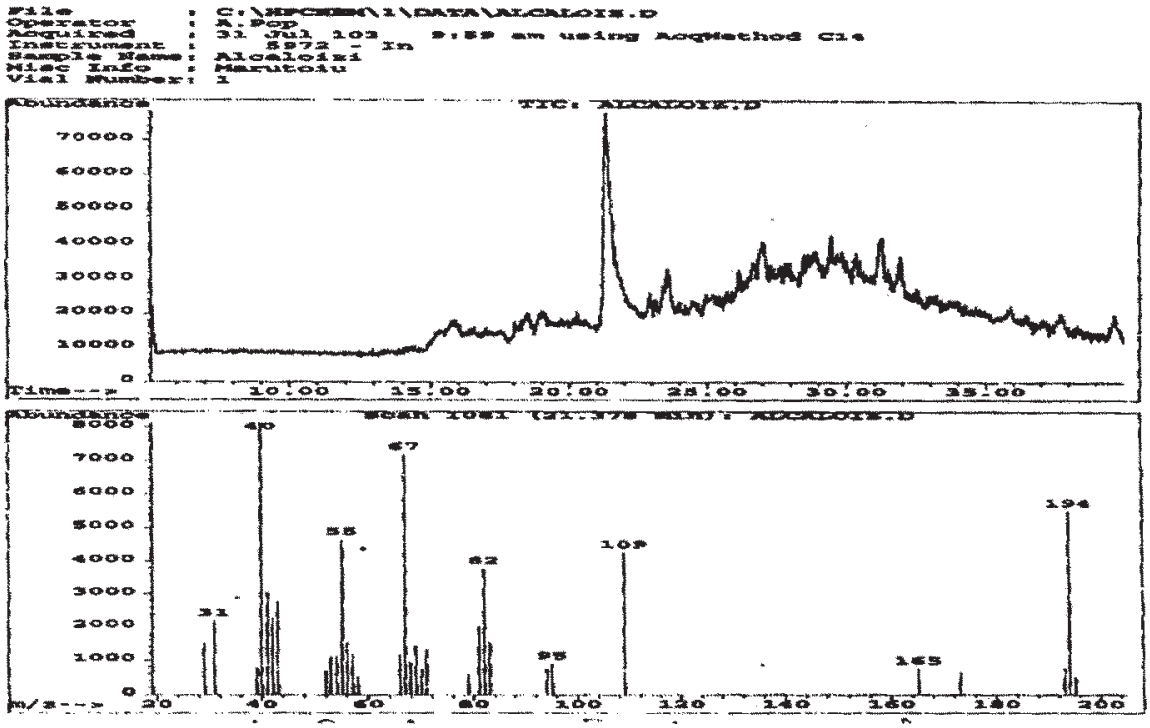


Fig. 2.Cromatogram caffeine separated by gas chromatography (a) and mass spectrum of the compound with retention time 21.5 (b)

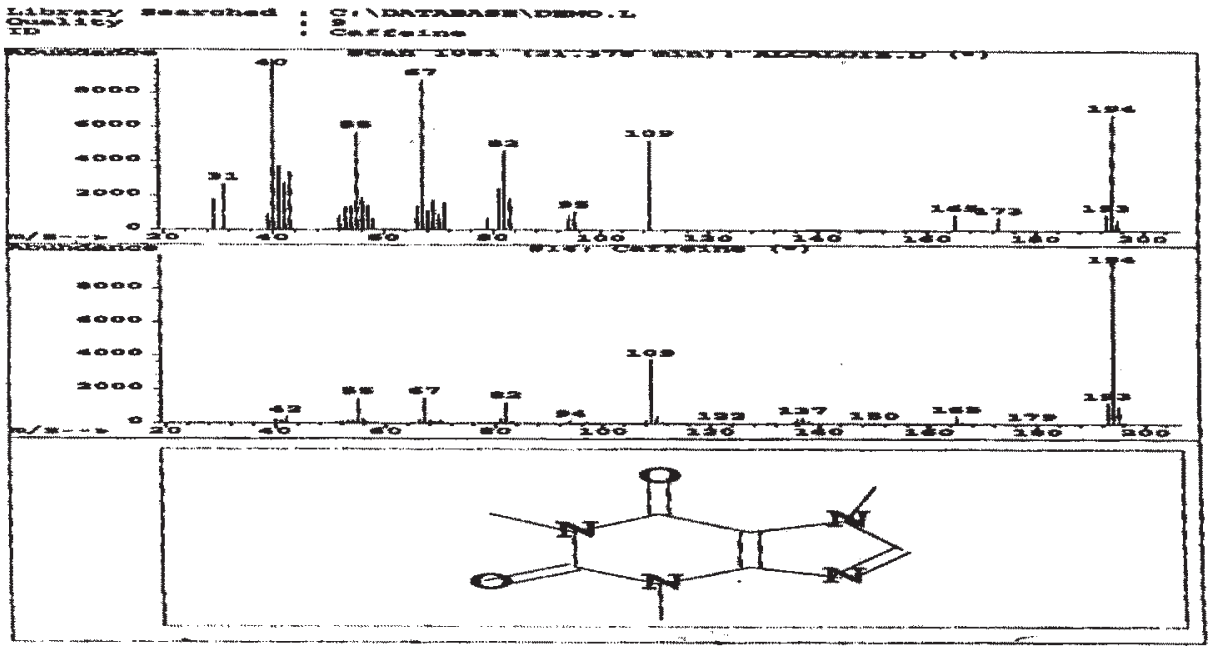


Fig. 3. Mass spectrum of caffeine, the substance separated by gas chromatography (a) from data base

Caffeine and theophylline quantification of products

Experimental part

Qualitative and quantity study of caffeine, theophylline, theobromine in different food products, coffee, tea, soft drinks, counterfait brandy , shows the separation of the active components on chromatographic plates with high performance silica gel (HPTLC) 60 F254, 10 x 20 cm (Merck) using as mobile phase a mixture of acetone - toluene - chloroform (40:30:30 v / v).

Selected chromatograms of the experimental set are shown in figure 4 and 5. For the calibration curve were used concentrations of 60, 50, 30, 20, 10, mg. / L. Sample of fake brandy was taken in the study of 200 mL by distillation rotovapor focused on 10 to 15 mL. This volume was decanted into a 20 mL flask and brought to the mark. Volumes were applied to the plate with a micropipette 5µL/spot Brandt. Quantitative assessment of caffeine and theophylline concentrations in brandy was done by spot registration using a CD wallet densitometer at wavelength 254 nm (fig. 6 and table 1).

Results and discussions

Xanthine derivatives were not well separated on plates with Nano - Sil NH2, using as mobile phase methanol - water (50:7 v / v), they were taken to the front. Reducing the volume of water, the results are better as can be seen from the chromatograms.

On silica gel plates with UV 254 Durasil 25 were separately standards xanthine derivatives and Ahmad tea extracts, coffee Alvorado, Elite coffee, lemon-flavored

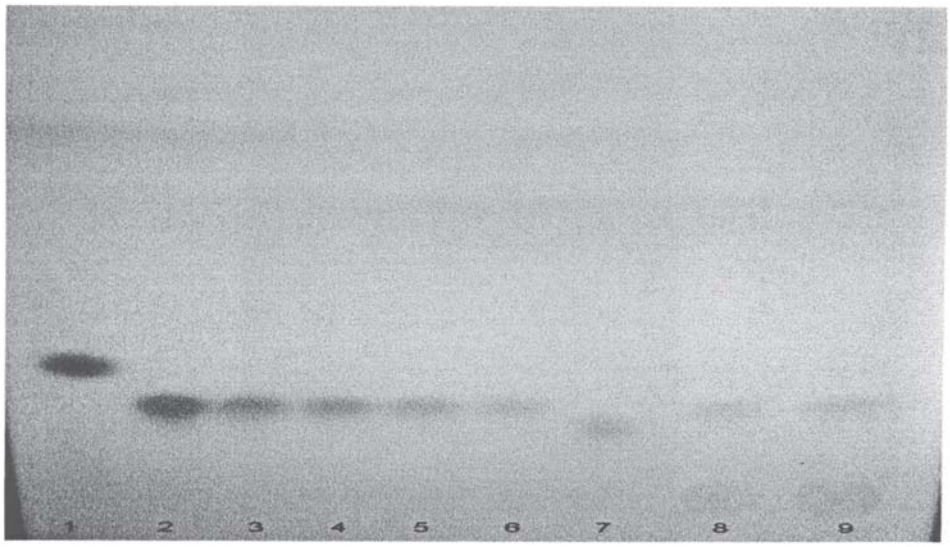


Fig. 4. Chromatogram of mixture: 1 - caffeine, 2-6 different concentrations of theophylline, 7 - theobromine, 8-9 two different concentrations of theophylline counterfeit drinks.

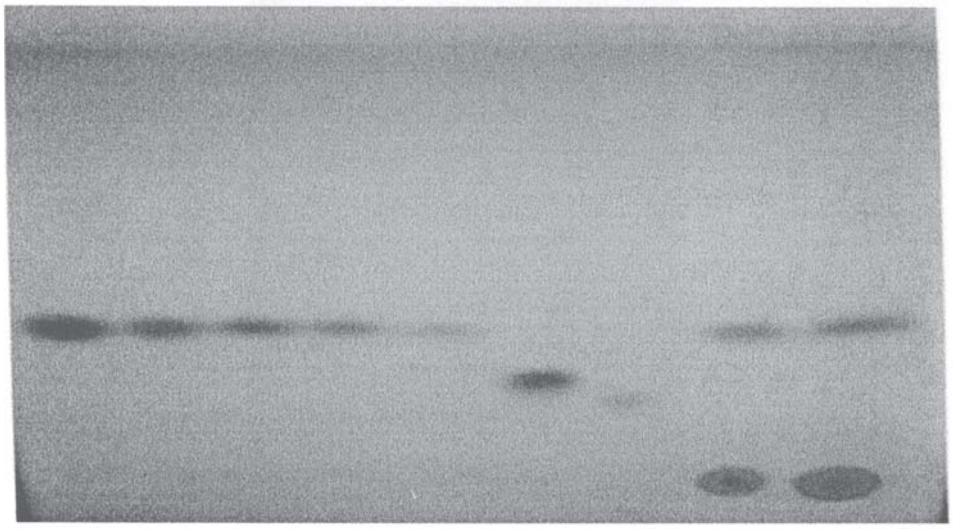
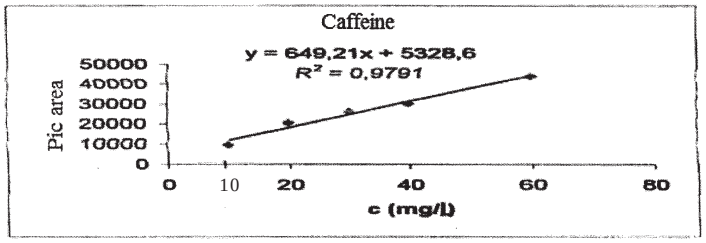


Fig. 5. Chromatogram of mixture: 1-5 caffeine, different concentrations, 6 - theophylline; 7 - theobromine, 8-9 drinks with caffeine forged with different concentrations (natural tea that has coloured brandy)



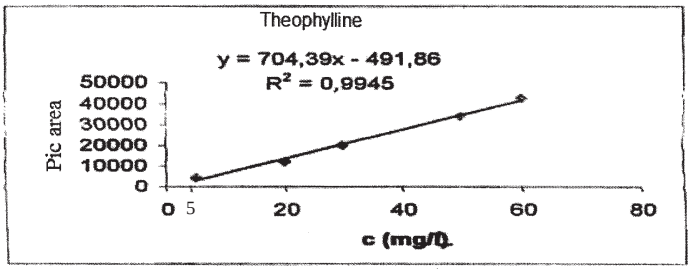


Fig. 6. Curves assessing the content of caffeine and theophylline.

Pepsi Twist, and a brandy counterfeit with decaffeinated tea (fig. 1) . In extracts of tea and coffee can be seen the presence of caffeine, and in tea large amounts of xanthine and hypoxanthine left at start. Also shown in brandy is the presence of traces of caffeine and theophylline. The same results are also obtained on plates with reversed phase separation of the three basic components of tea and coffee (caffeine, theophylline and theobromine) that can be detected in beverages with coffee extracts, tea, Pepsi and counterfeit cognac.

For a better identification, the spot that has the same Rf value with the caffeine standard was cut on chromatographic plate and the substance extracted with chloroform and the extract was analyzed using a gas chromatograph. From figure 2 (a) one can see that the peak with retention time 21.5 corresponds to a mass spectrum that was identified as belonging to caffeine (fig. 3).

Table 1
CONCENTRATION OF CAFFEINE AND THEOPHYLLINE PEAKS DEPENDING ON THE AREA.

Caffeine- brandy		Theophylline - brandy	
Area (ua)	C (mg/L)	Area (ua)	C (mg /L)
43991,9	60	42798.7	60
30126,2	40	34226.2	40
26124,7	30	20123,8	30
2063,8	20	12193,0	20
9641.9	10	4422.7	10

Using silica gel plates high performance chromatography (HPTLC) 60 F254, 10x20 cm (Merck), has developed a method for separation, identification and quantification of active principles of the exciting food, coffee, tea, alcoholic beverages and soft drinks. In selected figures from the tested set (fig. 4 and 5) are presented spots and of the five concentrations of caffeine, theophylline with theobromine, the xanthine and hypoxanthine remaining at start. Chromatograms made reveal the presence of caffeine, theophylline, the drinks counterfeit with coffee, tea or soft drinks and other coffee and tea products. Based on the relationship between peak area and concentration of caffeine and theophylline recorded in the spots were developed calibration curves figure 6, allowing quantification of the weight of the two active ingredients caffeine and theophylline in counterfeit brandy with coffee and tea extracts. The experimental results reveal the presence of caffeine in beverages brandy type in a concentration of 14.7 mg / L and 11.9 mg / L.

Share of theophylline in brandy-type products under study, is at levels of 2.54 mg / L and 8.35 mg / L.

Conclusions

Thin layer cromatography can be used for separation, identification and quantification of some xanthine compounds (caffeine, theobromine, theophylline), active ingredients and from prepared food products, coupled with other GC, MS, IR techniques .

A range of beverages, especially brandy spirit type, are coloured with Chinese tea (black or green). These fakes can be easily identified by detecting the caffeine, theophylline and theobromine in drinks by thin layer chromatography and gas chromatography.

References

1. BULANCEA, M., Authentication, appraisal and identification of counterfeit food products, Academic Publishing House, Manchester, 2002, p. 99-129, 186-187.
2. HSIU - JUNG LIN, MEI-LING WANG, CHUNG-WEN CHEN, BAO-SHUNG HWANG, LEE MIN-HSIUNG, AND YOUK-MENG CHOONG, A Gas Chromatographic Method for Determination Of Nicotamide, esters and Caffeine in Commercial Parabeam Heath Drinks, Tonic Drinks and Cold Formulas, Journal of Food and Drugs Analysis, 2000, 8 (3), p 113-183, 180-186.
3.CAMAG A - 70.1: Notes Aplication - Instrumental Thin Layer Chromatography - Veterinary Drugs Tierarzneimittel Institute Berlin GmbH (lfTA), Private communication, 1996.
4.LEHMAN, G., HAUG, I., SCHLOESSER, RZ, LEBENSM - Unters - Forsc., MERCK Extrelut Aplication NT Guide. SLP Chromatography, 1981, 172 (2), p 87-89.
5. DONER, LW, J. Assoc. Off. Anal. Chem., 1991, 74, 14.
6.OMARI, M., OKOMOTO, T., YAMADA, F., J. High Resolute. Chromatogr., 1983. P 6, 47.
7. SPANGENBERG, R., BROMER, SEIGEL, KILEIN, KF., GIT Spezial Separation I, 2001, p 37.
8. KUNUGI, A., TOBE, K., J. Mgh Resolute. Chromatogr., 1997, p 28, 456
9. VAUK, I., GALCA-WANDRA, A., PRAŞEK, M., J. Planar Chromatogr., 1997, p 10, 416.
10. MARUŢOIU, C., TOFAN, M., NICA-BADEA, D., POPESCU, A., Thin layer chromatography, Food analysis, Etnograph Publishing House, Cluj-Napoca, 2005, p 177.
11. NICA-BADEA, D., Control of active principles of food exciting, Brancusi Academic Publishing, 2011, ISBN 978-973-144-425-3,p 136

Manuscript received: 4.04.2011