Separation and Detection of Caffeine, Theophylline and Theobromine from Coffee Varieties, Carbonated Soft Drinks and Alcoholic Beverages

DELIA NICA BADEA[1]*, CODRINA LEVAI[2]

[1] Constantin Brancusi University of Targu Jiu, Faculty of Technical, Medical and Behavioral Sciences, 30 Calea Eroilor, 210135, Targu Jiu, Romania

[2] Victor Babes University of Medicine and Pharmacy, 9 Revolutiei 1989 Blvd., 300041, Timisoara, Romania

The paper evaluates the presence of methyl xanthine compounds: caffeine, theophylline, theobromine used as ingredients in carbonated soft drinks or as color and flavor ingredients in alcoholic beverages. The active components extracted from the selected products (coffee, tea, drinks) was separated and identified chromatographically using plates with silica nano -Sil NH_2 / UV-254, mobile phase ethanol - water (50: 1, 50: 3, 50: 5; 50: 7; v / v) and 60 F254 plates, mobile phase acetone-toluene-chloroform (40:30:30 v / v). Separated caffeine and identified by TLC was analyzed using a HelWet Packard 5890 Gas Chromatograph equipped with MS 5972 mass detector and spectral library to confirm identification. This simple and rapid TLC, GC / MS instrumental method is useful in controlling traces of methyl xanthine compounds in food as a food safety measure.is useful in controlling traces compound of food products containing methylxanthines as a food safety measure.

Keyword: TLC / GC / MS, Caffeine, coffee extracts, soft drinks, cogniac falsification

The main organic compounds that determine the central stimulation of coffee are purine bases. Of these, caffeine is predominant, the amount of which varies by species and variety: *Coffea Arabica* (0.9-2%), *Coffea Robusta* (up to 3%). Caffeine is accompanied by small amounts of adenine (2.2 mg %), guanine, xanthine and hypoxanthine (all less than 2mg %), as well as theophylline (0.62mg %) and theobromine (0.15-0.25% and even 1.85 mg %). The organic compounds that impart *Thea sinensi* tea, the stimulating action are purine bases whose main representative is caffeine (theina) found in teas of different origins, in the quantity of 1.86 - 4.91% in dry leaves.

Caffeine is the most consumed alkaloid, in the form of infusion of tea and coffee, soft drinks, as a medicine or functional food. Caffeine is thought to reduce fatigue, increase physical strength, support mental alertness and concentration [1]. Caffeine is a methyl derivative of xanthine, a natural product with pharmacological actions in the human body and with toxic effects in high doses. Daily doses of caffeine that are considered normal are below 250 mg ([2]. Caffeine consumption can add addiction and side effects, a dose greater than 1g can lead to death [3]. Using caffeine in cola drinks can help boost performance [4] or reduce the incidence of type 2 diabetes [5]. The International Olympic Committee (IOC) considers caffeine as a drug, the level of 12μg / mL in urine at athletes being considered an abuse. Excess caffeine produces a feeling of nervousness, insomnia, heart rate acceleration, especially in children and adolescents, and pregnant women can cause birth defects, weight-loss children [2].

Thin Layer Chromatography (TLC) is a simple, rapid, widely applicable method in food chemistry of plant origin for the extraction, isolation and identification of biologically active components, including methyl xanthine compounds of coffee, tea, soft drinks based on extracts caffeine, [6-9]. The methyl xanthine components: caffeine, theobromine, theophylline, active principles characterizing coffee, teas, foodstuffs or pharmaceuticals, were examined using chromatographic separation and identification methods coupled with mass spectrometry as a confirmatory method [10-12].

Coffee and tea drinks naturally contain methyl xanthine compounds. Caffeine, theophylline and theobromine are used for commercial purposes as ingredients in many carbonated soft drinks, including cola, pepper and citrus beverages, or as color and flavor ingredients for alcoholic beverages. The achievement of food safety, in general, soft and alcoholic beverages, on which the falsification of possible preparations as a rule are subjected to alcoholic beverages: wine, natural spirits, and spirits industry, etc. [13], require qualitative and quantitative analysis of the components of the preparations and products of commerce.

Experimental part

Given the fact that the compounds methyl xanthine be found in various products and preparations of coffee and the assumption that the number of drinks, in particular the spirits of the type cognac colored of chinese teas (green or black), was used to detect caffeine, theophylline, theobromine developing a method of analysis consisting of thin layer chromatography and gas chromatography (GC) coupled with mass spectrometry (MS) [14, 15].

The extraction of the three active substances is based on the basic character of these alkaloids. Alkaloids are generally extracted from the salts with dilute hydrochloric or sulfuric acid and re-extracted, being prepared for analysis. Commercial dry coffee and tea samples, dried to a maximum of 40 ° C in powder form, are mixed with 15 mL of 0.05 M H_2SO_4, allowed to stand at room temperature for 20 min , filtered and the residue re-extracted with 5 mL 0.05 M H_2SO_4. The aqueous phase contains the compounds that alkalize with ammonia (4 mL) and extract with 100 mL of CH_2Cl_2. The extract is dried with anhydrous Na_2SO_4, then the solvent is evaporated to give the alkaloids as an oil or crystals.

From the caffeine, theophylline, theobromine, xanthine and hypoxanthine solutions at 60 mg / L solutions, 5μL / well Brandt micropipettes were applied onto silica gel plates. The fake brandy samples taken in the study were 200 mL which, by distillation in the rot vapor, were concentrated to 10-15 mL. This volume was transfused

* email: nicabadeadelia@yahoo.com

into a 20 mL flask and shaken. The volumes applied on the plate were 5mL / spot using a Brandt micro pipe. At work were taken four cognac samples containing caffeine and 2 samples of theophylline. Along with standards, samples of coffee extracts, tea, Pepsi and brandy were applied. Chromatographic plates used for TLC analysis are silica gel plates: Nano-Sil NH_2 / UV 254 and 60 F254, 10x20 cm, Merck. Developments were performed in the N-saturated chamber by ascending technique using ethanol-water mixtures (50: 1; 50: 3; 50: 5; 50: 7; v / v) as mobile phase for Nano-Sil NH_2 plates and acetone-toluene-chloroform mixture (40:30:30 v / v), for silica gel plate coated with fluorescent indicator F254, 10x20 cm. After development, the plates are dried in the oven at 100°C and examined under UV light at 254 nm. For this purpose, a Camag UV lamp was used.

Spots corresponding to caffeine and theophylline in fake beverages were extracted from the chloroform plate 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 60°C, 3°C / min, transfer temperature 240°C. The quantitative assessment of caffeine concentrations in false cognacs and theophylline can be achieved by recording the spots with a Desaga Cd densitometer at 254 nm [14, 16].

Results and discussions

The characteristics of the chemical compounds that were analyzed in the samples of products using coffee and tea extracts are: xanthine, hypoxanthine, caffeine, theobromine, theophylline and theobromine are shown in Table 1. These components were extracted, separated and identified using chromatography on thin layer, gas chromatography and confirmed by mass spectrometry.

In the first part of the experiment, there was a separation and identification of the target compounds by TLC with two types of chromatographic plates and two types of mobile phases: water-ethanol and acetone-toluene-chloroform, in optimal volumetric ratios for separation (fig. 1 and fig. 2). In Part II, we confirmed the identifications made in the first part (TLC), using the GC / MS analytical technique and the computer spectra library (fig. 3).

In order to ensure an optimal separation of the compounds from the samples applied on the Nano-Sil NH2 / UV 254 chromatographic plates, the methanol-water mixture was used in various volumetric ratios (fig. 1). Using the methanol-water mixture (50: 7 v / v) as the mobile phase, the methyl xanthine derivatives were not well separated on the chromatographic plates, they were brought forward as shown in fig. 1 (D). By reducing the volume of water, the results are better, (fig. 1) (A, B and even C). In the coinage sample you can see the traces of caffeine present in the material with which the cognac (tea or coffee) is colored.

Using TLC 60 F254, 10x20 cm (Merck) high performance silica gel chromatographic plates, separation, identification and quantification of selected active principles were performed, the result being shown in figure 2. In figures 2 (A and B) show the five caffeine concentrations and five theophylline spots with the remaining theobromine, xanthine and hypoxanthine (spot 8, 9) respectively. The chromatogram of figure 2C, the presence of caffeine in: the commercial coffee varieties (spot 4-6) is evidenced;

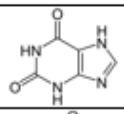
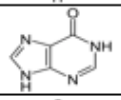
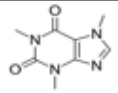
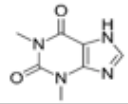
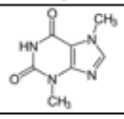
Name	Formula/Molar mass	Structural formula	IUPAC nomenclature	Spread
Xanthine	$C_5H_4N_4O_2$ 152.11 g/mol		3,7-Dihydro-purine-2,6-dione	Plants, animals
Hypoxanthine	$C_5H_4N_4O$, 136.112g/mol		1H-purin-6(9H)-one	Plant and animal enzime
Caffeine	$C_8H_{10}N_4O_2$, 194.19 g/mol		3,7-Dihydro-purine-2,6-dione	Coffee, guarana, yerba mate, tea, kola, guayusa, holly
Theophylline	$C_7H_8N_4O_2$, 180.164 g/mol		1,3-Dimethyl-7H-purine-2,6-dione	Tea, cacao, yerba mate, kola
Theobromine	$C_7H_8N_4O_2$ 180.164 g/mol		3,7-dimethyl-1H-purine-2,6-dione	Cacao (chocolate), yerba mate, kola, guayusa, holly

Table 1
COMPOUNDS OF THE XANTHINE CATEGORY

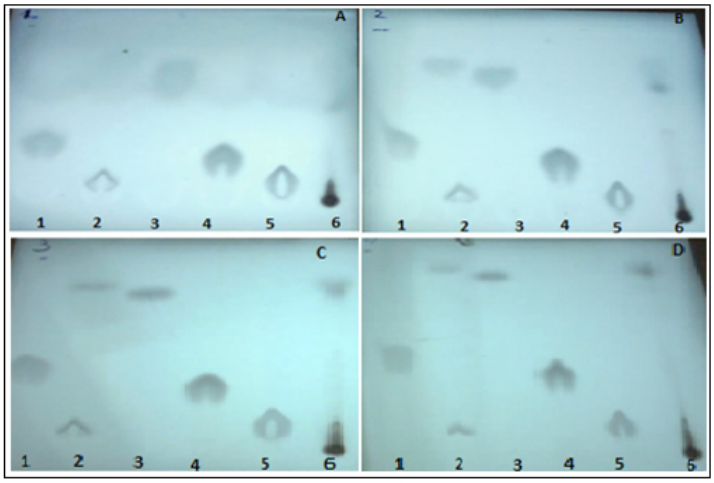


Fig. 1. Chromatogram of compounds separated on Nano-Sil NH2 / UV 254 plates A) methanol-water (50: 1 v / v); B) methanol-water (50: 3 v / v); C) methanol-water (50: 5 v / v); D) methanol-water (50: 7 v / v). 1-theophylline; 2 - caffeine; 3-theobromine; 4-hypoxanthine; 5-xanthine; 6 - fake brandy extract.

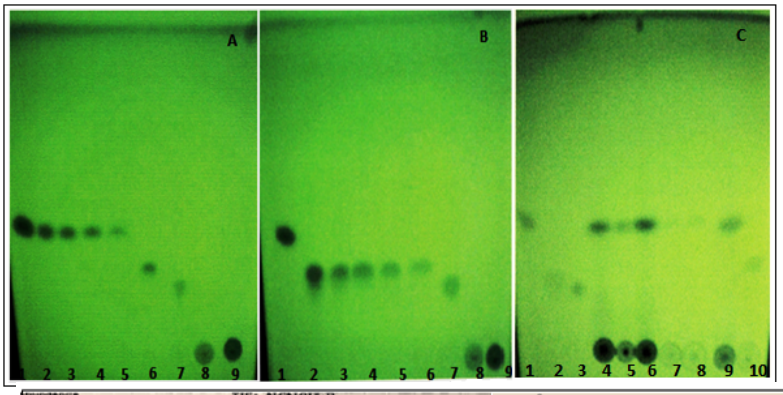


Fig. 2. Chromatograms of the compounds separated by TLC. Plates 60 F254, 10x20 cm. Mobile phase: acetone-toluene-chloroform (40:30:30 v / v); (A) 1-5 caffeine in different concentrations, 6-theophylline, 7 – theobromine, 8-xanthine, 9 – hypoxanthine; (B) 1-caffeine, 2 - 6 theophylline, different concentrations, 7 - theobromine; 8-xanthine; 9 – hypoxanthine; (C) 1 – caffeine, 2-theophylline, 3-theobromine, 4 - Alvorado coffee, 5 - Amory Extra coffee, 6 - instant coffee (Elite), 7 – Pepsi, 8 - Coca Cola, 9 ,10 fake colored cognac with decaffeinated tea.

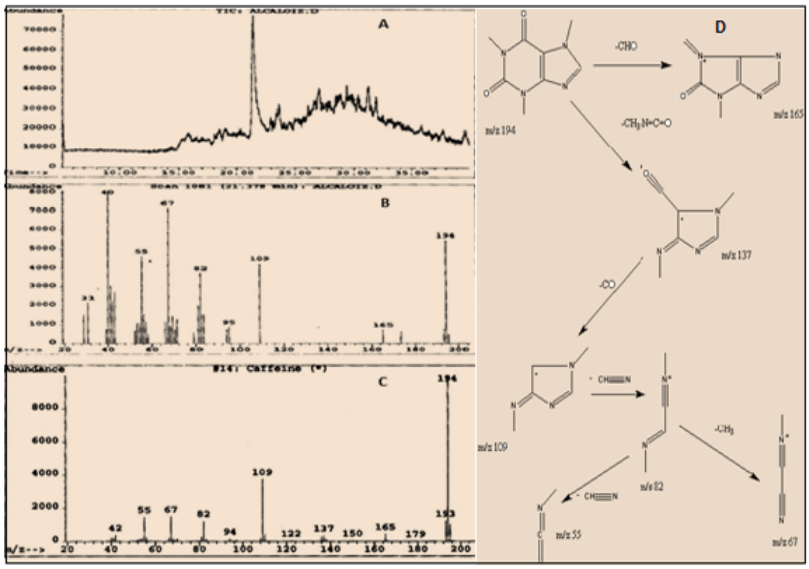


Fig. 3. Characteristics of gas chromatogram and mass spectra of caffeine.
(A) Gas chromatogram separated caffeine; (B) Mass spectrum of the compound with retention time 21.5; (C) Caffeine mass spectrum in the data library; (D) caffeine-specific ions resulting from MS

in small quantities in soft drinks (spot 7, 8); in fake cognac (spot 9). Theophylline presence is observed in fake colored cognac with coffee and tea extracts, (fig. 2) (C, spot 10).

For better identification and confirmation, the spot having the same Rf value as the caffeine standard was cut from the chromatographic plate and the chloroform extracted, and the extract was analyzed using a HelWet Packard 5890 gas chromatograph equipped with a mass MS5972.

From figure 3 (A), it can be seen that for the peak with the retention time 21.5min., corresponding to caffeine whose experimental mass spectrum is shown in figure 3 (B), is compared to the caffeine mass spectrum in the computer spectrum data library (fig. 3C) as a confirmation method described in the literature[17, 18]. The mass spectra of the separated compound (fig. 3B) is similar to the mass spectrum existing in the computer spectra library (fig. 3, D) which recognizes it and confirms it. Figure 3 (C) shows the correspondence between the fragment profile (m/z) by MS and the fragmentation pattern reported in the literature (m/z 195, m/z 165, m/z 137, m/z 109, m/z 67, and m/z55) [19]. Based on the correlation between densitometric peak peaks and the concentration of caffeine and theophylline in the spots, the calibration curves are plotted, allowing the quantification of the two caffeine and theophylline active principles in counterfeit cognacs with coffee and tea extracts (14.7 mg / L, 11. 9 mg / L caffeine and 5.24 mg / L, 8.35 mg / L theophylline) [16].

In a previous paper was studied the Concomitent quantification of Caffeine, cotinine and N-methyl uric acid in urine. Application for athlete monitoring and pharmaceutical screening [20].

Conclusions

The development of a simple method of separating, identifying and confirming by coupling thin layer chromatography with GC / MS, coproduced confirmation of spectra from the data library can be used to detect methyl xanthine compounds (caffeine, theophylline, theobromine) from nonalcoholic soft drinks, and in counterfeiting of alcoholic beverages by the addition of extracts as flavor and color enhancers. This method can be used for the qualitative and quantitative control of these methyl xanthine compounds in various preparations and foodstuffs as a measure to achieve food safety supervision.

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