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METHOD FOR QUANTITATIVE DETERMINATION CAFFEINE FROM COFFEE

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Abstract

Caffeine is an alkaloid in the purines group, found in coffee beans. It is slightly soluble in water, partially soluble in ethanol and soluble in chloroform. To quantify the caffeine content, it should be extracted into a suitable solvent and the extract analyzed by HPLC. Two extraction techniques have been experimented: by boiling and solid-liquid extraction. The first method separated 0.2 mg caffein per gram of green coffee.

Key words: caffeine, HPLC, assay, extraction

INTRODUCTION

Caffeine (Fig.1) is a compound widely studied today. Intensive research is being conducted on its effects on the human body, on its consumption. Today we can say that we are addicted to caffeine. It is found in many other products, not only in the coffee. Thus, they found traces of caffeine in sodas, in some teas, chocolate and it is also used in pharmaceutical and cosmetic industry (Alghamdi A.H. et all, 2005).

Caffeine is found in coffee beans, which are the fruits of the plants belonging to the botanical genus *Cofeea*. The main coffee trees are *Coffea Arabica* and *Coffea canephora*, known as Robusta (Khan K. et all., 2006).

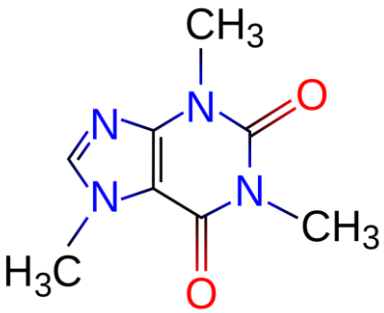

Fig. 1 Caffeine

The oldest written mention of coffee is in ”*Al-Ganum fit-Tebb*” (”The Cannon of Medicine”) written by Avicenna (980-1037). The last part of the book, the 5th, is a pharmacopoeia containing 760 recipes for remedies, including " *Buncham* ". He describes the drink, then known as *Buncham*, as hot and dry while very good for the stomach, it strengthens the limbs, cleans the skin and gives a pleasant aroma to the whole body (Alghamdi A.H. et all, 2005).

Caffeine is an alkaloid of the purine group. It is part of the defense system of the plant because of the antifungal effect, it is a selective and chemosterilant phytotoxine for certain insects. The caffeine content of green beans is 0.58% -1.7% for the Arabica variety and 1.16% -3.27% for the Robusta variety (Andrews K.. et all., 2007). For the roasted beans, the quantity drops to around 1% in Arabica and and 2% in Robusta. In coffee, caffeine is mostly associated with chlorogenic acids at a 1: 1 ratio complex π. Other alkaloids found in the coffee beverage are: xanthine, hypoxanthine, adenine and guanine.

Caffeine is presented as prismal, hexagonal, colorless, odorless, crystals, with a bitter taste. A methylated xanthine derivative, it is an alkaloid of vegetable origin, so it is a weak base (pK a = 0.6). The basic core is xanthine-2,6-dioxipurine formed by condensing a pyrimidine ring with an imidazole ring. Its melting point is at 235-238 ° C. The solubility of caffeine is moderate (2 g/100 ml water) at room temperature, but its solubility increases in boiling water (66g/100ml). It is partially soluble in ethanol (1.5 g/100 ml). The best solvent for caffeine is chloroform. By combining caffeine with various sodium salts of organic acids, its solubility increases (for example, combining caffeine with sodium benzoate or sodium salicylate can increase its solubility in water). Because it does not form salts, caffeine can be extracted from alkaline solutions using an organic solvent. This property is used for its separation from theophylline and theobromine.

It is a stimulant of the central nervous system, increases blood pressure, has a bronchodilator effect, diuretic and stimulant of intestinal peristalsis. Caffeine is considered a psychoactive drug that causes dependence. Tolerance for some of the effects of caffeine increases with time, for example its tolerance for the effect on blood pressure or the diuretic effect. Caffeine has a weak protective effect against certain cancers, heart disease or Parkinson’s. Some people however, have trouble sleeping after caffeine consumption. It also increases the number of blood neurotransmitters (adrenaline, acetylcholine, dopamine, serotonin, epinephrine, glutamic acid) and the metabolic rate. Caffeine reduces the risk of Type 2 diabetes, it is an antioxidant that reduces free radicals in the body.

Among the negative effects of caffeine we can mention increasing blood pressure that can lead to an increased risk of stroke, reduce fine control mobility, increases cortisol secretion and sleep disorders.

The purpose of this paper is to determine the caffeine content in coffee beans trough chromatographic methods.

MATERIALS AND METHODS

Caffeine was extracted from green coffee beans, of commercial Arabica variety. For the extraction, 1,2-dichloroethane, Na_2CO_3, chloroform and Na_2SO_4 p.a. quality from Merck were used. For the elution was used acetonitrile HPLC grade, sodium monophosphate, hexylamine and phosphoric acid, purchased from Merck.

Two methods have been tested for the extraction of caffeine: solid-liquid extraction using the Soxhlet apparatus and the reflux boiling water and liquid-liquid extraction using a suitable solvent. Both methods used the same amount of coffee beans.

Green coffee beans were crushed, were introduced to the extraction thimble. 100 ml of dichloroethane was placed in the flask. The extraction lasted for 30 minutes, after which the organic solution was left to cool to room temperature and filtered. The filtrate was distilled in vacuo at a bath temperature of 60 ° C in order to remove the solvent used for the extraction. The distillation residue was taken up in 6 ml of chloroform. The obtained solution was diluted to a fixed volume and subjected to HPLC analysis.

For the extraction by boiling in distilled water: in a beaker containing 100 ml of distilled water there were placed 20g of crushed green coffee beans and simmered for 10 minutes. The resulting mixture was cooled to room temperature, filtered and the filtrate obtained was made alkaline with 2 g Na_2CO_3. If the pH is alkaline, the extraction of caffeine is carried out with 3x30 ml of dichloroethane. The combined organic layer was dried over anhydrous Na_2SO_4. The solvent was distilled in vacuo, crystals of caffeine formed in the flask and were dissolved in 6 ml of chloroform. The chloroform solution was diluted and analyzed by HPLC.

For the HPLC analysis an ABL&E JASCO chromatograph was used, consisting of the pump module PU-1580, LG-980-025 ternary gradient module, DG-980-50 degasser module, UV detector/VIS UV-1575, Rheodyne manual injector, separation column NUCLEODUR C18 250x4,6mm and data processing program BORWIN 5.1. Isocratic elution was performed at room temperature with a mobile phase consisting of: acetonitrile, hexylamine, potassium phosphate and phosphoric acid. UV detection was performed at 254 nm.

For quantification the external standard method was used. For this, a standard solution was prepared, containing a known quantity of standard caffeine dissolved in a known volume of solvent. This standard solution was used to determine retention time and caffeine content of the extracts.

RESULTS AND CONCLUSIONS

Standard solutions and chloroformic obtained by extraction were subjected to chromatographic analysis. For the main peak of caffeine the retention time determined was 22.7 minutes.

Following the analyzes there were obtained the following chromatograms for caffeine extraction with the Soxhlet apparatus (Fig. 2) and caffeine extraction by boiling. (Fig.3):

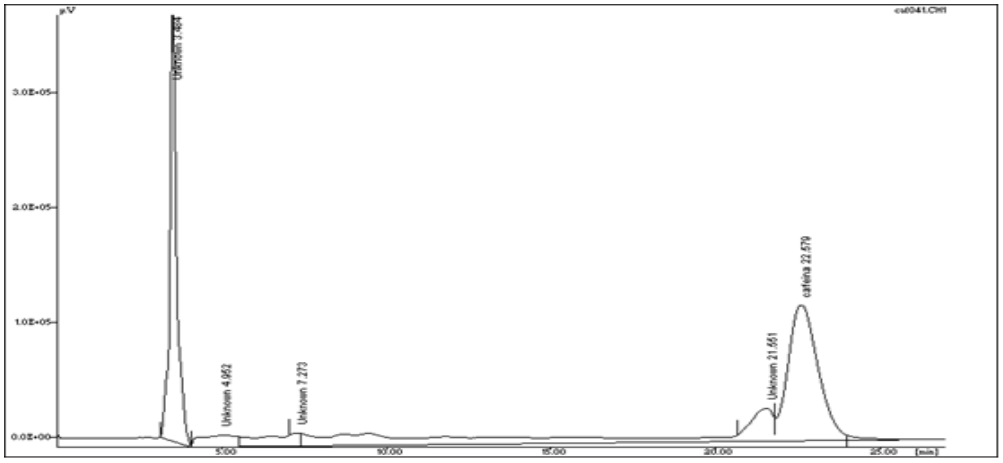

Fig. 2. Chromatogram of caffeine extraction with the Soxhlet apparatus

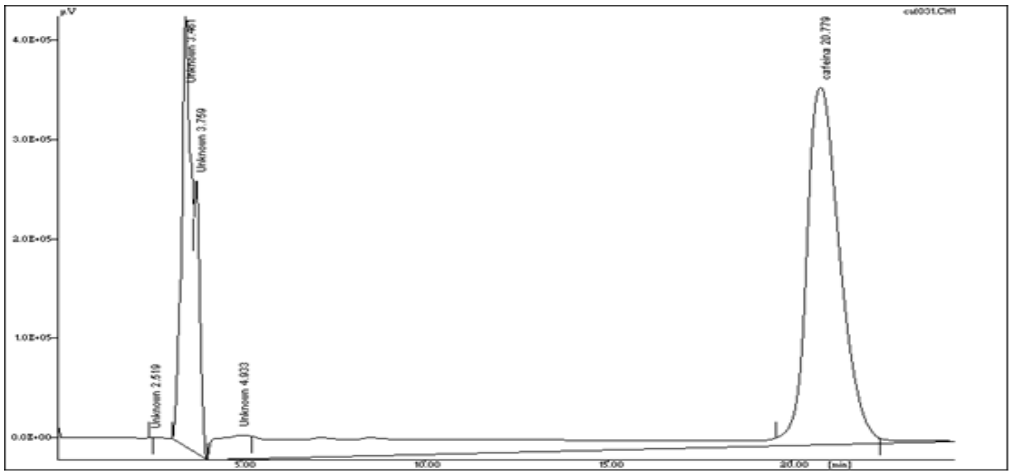

Fig. 3. Chromatogram of caffeine extraction by boiling

The results showed that the extraction carried out using Soxhlet apparatus obtained a concentration of 0,212mg/ml of caffeine for the sample, this means that the extract obtained from 20 g of green coffee beans contained 1.276 mg of caffeine. In the case of extraction by boiling a concentration of 0,635mg/ml of caffeine was obtained from the sample meaning that the extract obtained from the same amount of green coffee beans contained was 3,811 mg caffeine.

In the case of extraction with a Soxhlet apparatus in addition to the caffeine other compounds were obtained (for example fats, aldehydes, esters, alcohols, etc.) and the extraction by boiling allowed obtaining a greater amount of caffeine.

CONCLUSION

Following extraction of caffeine from coffee and quantification by chromatographic HPLC analysis was obtained the following results:

- the sample obtained after the extraction with the Soxhlet apparatus had a concentration of 0.212 mg / mL caffeine, and the sample obtained by liquid-liquid extraction a concentration of 1.6351 mg / mL caffeine, demonstrating that these values after the extraction of caffeine in aqueous solution results are better, the greater the amount extracted .
- the sample obtained from green coffee beans by boiling them has a concentration of 1.6351 mg / ml caffeine and caffeine extraction from roasted coffee by the same method to obtain a concentration of 1.51 mg / ml caffeine. These values demonstrate that following roasting coffee

beans lose an amount of caffeine from the beans about 7.65 % of caffeine crude- lost

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