Determination of Caffeine in Arabic Coffee by HPLC and AAS for mineral elements

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Abstract: Coffee is one of the most famous beverages all over the world. In addition to its central nervous system stimulation effects, reports suggested that coffee consumption may help prevent several chronic diseases, including type 2 diabetes mellitus, Parkinson's disease and liver disease (cirrhosis and hepatocellular carcinoma). Due to many reports concerning the pros and cons effects of caffeine as a major component of coffee, a necessity to know the concentration of caffeine in coffee and other products containing it must be taken into account. Saudi Arabic coffee is the most important or the main beverage in Kingdom of Saudi Arabia (KSA). Different Arabic coffee origins available in markets in KSA and there is no or little information available about the concentration of caffeine and the mineral elements of them. In this study, qualitative and quantitative identification of caffeine was carried out for four different Arabic coffee origins commercially available in Taif governorate, KSA using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Pb, Fe and Cu elements were determined by atomic absorption spectrometer (AAS) whereas flame emission spectrometry (FES) used for estimation K and Na. The suitable digestion of the Arabic coffee carried out by following meanly the similar way of traditional cooking in the KSA. The LC-MS method optimized in term of the kind of column used, mobile phase, and the flow rate, in addition to the parameters for mass spectrometry. The results showed that the concentration of caffeine in the analyzed samples were between (573-960 µg/ml). On the other hand, the concentration of sodium and potassium were (199.1-200.4 µg/g) and (15.9-17.99 mg/g) respectively. The lead and iron were not detected whereas copper between 7.98-19.95 µg/g. In conclusion, this manuscript provides a simple and fast LC-MS method for qualitative and quantitative estimation of caffeine could be used in food and pharmaceutical industries and as a tool for liver function test in hepatitis patients. It must be increasing concern about labeling the concentration of caffeine on products containing it. Therefore, the authors recommend these methods for analysis of caffeine and minerals concerning the food and human health safety.

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Key words: Arabica coffee, Caffeine, Liquid chromatography-mass spectrometry, Atomic absorption spectrometry, Flame emission spectrometry, mineral elements

1. Introduction:

Coffee beverage was reported to be among the most widely consumed beverages in the world. It is prepared by infusion of ground roasted dried coffee beans. Among 80 different coffee species (Rubiaceae family) the most commercialized are: *Coffea arabica* and *Coffea canephora.* Beverage from *C. arabica* is considered nobler and has the most renowned taste qualities **(Farah, 2009; Heckman *et al.*, 2010; Farah, 2012).** Coffee has a complex chemical mixture containing more than a thousand different chemicals, including carbohydrates, lipids, alkaloids, vitamins, minerals, and phenolic compounds **(Macrane, 1985; Spiller, 1998; Daglia 1998; 2007; Higdon and Frei, 2006).** In addition to its central nervous system stimulation effects, results of epidemiological researches suggested that coffee consumption may help prevent several chronic diseases, including type 2 diabetes mellitus, Parkinson's disease and liver disease (cirrhosis and hepatocellular carcinoma). A significant inverse association between regular coffee intake and liver disease progression was found in a prospective study of individuals with hepatitis C and bridging fibrosis or cirrhosis at baseline **(Corrao *et al.*, 2001; Ruhl and Everhart, 2005; Higdon and Frei, 2006; Freedman *et al.*, 2009).**

Caffeine is the most known major coffee compounds, due to its physiological and pharmacological properties. Caffeine (1,3,7-trimethylxanthine), its structural formula as shown in figure (1) is an alkaloid of the xanthine group.

Caffeine is one of the most comprehensively studied ingredients in the food products. Its name was derived from the German word *kaffee* and the French word *caf´e*, each meaning coffee. The natural sources of caffeine include different varieties of coffee beans which are the world's primary source of caffeine, tea leaves (*Camellia sinensis*), guarana seeds (*Paullinia cupana*), mate leaves (*Ilex paraguariensis*), kola nut seeds (*Cola nitida, Cola acuminata*) and cocoa beans (*Theobroma cacao*) (**Barone and Roberts, 1996; Paradkar and Irudayaraj, 2002; Cornelis and El-Sohemy, 2007; Tello *et al.*, 2011; Victor, 2012**). Despite the fact that tea is globally consumed more widely than coffee, coffee is the main source of caffeine in daily consumption given its generally higher caffeine content **(James, 2003).** Caffeine is also a common ingredient of soft drinks such as cola and it is packed in the form of tablets with the claim that using caffeine of pharmaceutical quality improves mental alertness. It is used by students during the exams period and by peoples who work or drive for long hours **(Al-Faris, 2009).**

Many studies concluded that the healthy person can have up to 400 mg of caffeine a day without experiencing negative effect, such as anxiety or heart problems. The daily dose of pregnant women, elder persons, children and non-healthy persons should be given under physician supervision **(Massey, 1998; Matijasevich *et al.*, 2005; Temple, 2009; Heckman *et al.*, 2010).** So that many countries all over the world have begun to take regulatory action, including sales restrictions and product labeling for the products containing caffeine.

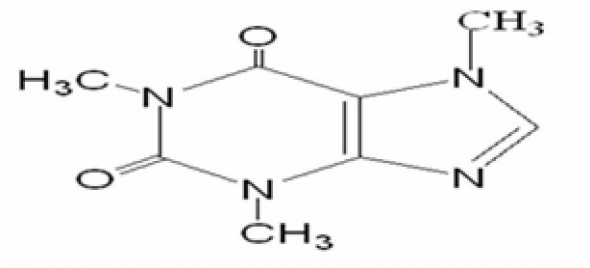

Figure (1) Caffeine (1,3,7- trimethylxanthine)

Many modern analytical methods for caffeine determination in the most varied matrices have been used different techniques such as, gas chromatography-mass spectrometry, high performance liquid chromatography with UV detector and high performance liquid chromatography-mass spectrometry **(Aresta *et al.*, 2005; Brunetto *et al.*, 2007; Perrone *et al.*, 2008; Srdjenovic *et al.*, 2008; Li *et al.*, 2010; Lin et *al.*, 2010)**. Each of the analytical techniques for determination alkylxanthines has advantages and limitations. The liquid chromatography-mass spectrometry (LC-MS) is increasingly recognized as important separation and determination tool because it is easy to use, speed of analysis small sample volume and good reproducibility **(Shrivas and Wu, 2007).**

Minerals are inorganic nutrients, play an important role in human nutrition. These minerals are critical for the growth and formation of bones, enzymes and hormones, synthesis of vitamins, as well as for the healthy functioning of the nervous system, blood circulation and cellular integrity, if maintained at required levels **(McDowell, 2003; Nabrzyski, 2007; Marta *et al.*, 2012).**

Several papers reported the amount daily mineral intake of elements in coffee powder available in Indian market by atomic absorption spectrometry and in some Brazilian coffee by inductively coupled plasma atomic emission spectrometry **(Santos *et al.*, 2001; Suseela *et al.*, 2001; Marta *et al.*, 2012)**. Coffee beverage could be a good source of essential elements such as sodium and potassium and for the minor elements such as copper, iron and zinc. The daily intakes of the elements are primarily depended on the concentrations in coffee beverage and the amounts of coffee consumed through the day (**Pohl *et al.*, 2013**).

Coffee and tea are the most two popular drinks in Kingdom of Saudi Arabia (KSA). The present study aimed to characterize the quantity of caffeine and some mineral elements in different types of Arabic coffee purchased from the local markets in Taif governorate, KSA. Qualitative and quantitative analysis of caffeine were done using reversed phase liquid chromatography - electrospray ionization-positive-selective ion recording-mass spectrometry (LC-ESI(+ve)-SIR-MS). The sodium and potassium were determined using flame emission spectrometry (FES) whereas atomic absorption spectrometry (AAS) was used for determination copper, iron and lead.

2. Experimental

2.1. Chemicals

All solvents, standards and reagents were of high quality. Caffeine standard was ordered from Fluka Analytical. Methanol and formic acid were HPLC grade from Sigma-Aldrich Chemicals. For the flame atomic absorption spectrometer and flame emission spectrometer studies copper, iron, lead, potassium and sodium stock standards solutions obtained from Buck Scientific. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA).

2.2. Collection and preparation of coffee samples.

There are different brands of Arabic coffee samples available in KSA markets which are

imported from different coffee producing counties in the world. For our project we select four famous kinds of Arabic coffee which were ready for cooked. The commercial names were Harry form Ethiopia, Indian from India, Vietnam from Vietnam and Kolanyi from Yemen. In this study, for each sample about 2.5 grams of grounded Arabic coffee as it purchased from the local market were mixed and shaken with 100 ml of boiling water at room temperature for two minutes. Heat treatment was applied to keep the solution in gently boiling condition for 20 minutes and adjusted to 100 ml volume with pure water (this way is equivalent to the traditional method the people used in KSA). Before injection and further analysis, solution was filtered and diluted if necessary with deionized water. The suspension solution was passed through a stainless steel mesh sieve before filtering by the Whitman filter paper using a Buchner funnel. The sample made up to a final volume of solution sample 100 ml. Centrifuged and stored in refrigerator. An aliquot of the samples was diluted with purified water to bring the concentration of the samples within the ranges of the calibration curves. All standards and solutions ready for analysis were passed through the 0.45 µm disc filter.

2.3. Preparation of standard caffeine solution

Caffeine standard stock solution prepared by precisely weighed caffeine (100 mg) was dissolved by sonication for 30 min in 100 ml volumetric flask with 50% aqueous methanol resulting a caffeine concentration of 1000 µg/ml. The intermediate caffeine stock solution and working caffeine solutions were prepared before using in the injection to the instrument.

2.4. LC-ESI-MS analysis

2.4.1 Optimization of ESI-MS parameters for caffeine by direct infusion

Direct infusion method using ESI-MS (Waters 3100) in positive ion mode was performed to get optimum condition for detecting caffeine ion. The analytical conditions for injection include the injection of caffeine standard (1 µg/ml) directly to the ion source by means of a syringe pump at a flow rate (0.02 ml/min) for ten minutes. The analytical conditions for mass spectrometry were; desolvation temperature (350 ^{o}C), desolvation gas flow (500 L/h), cone gas flow (50 L/h) and source temperature (150 ^{o}C). The two basic parameters capillary voltage and cone voltage were changed till reach an excellent caffeine ion. Mass spectrum was scanned in the ESI positive ion mode in the range between 20-220 *m/z*. Maslynx 4.1 software was used for data analysis.

2.4.2 LC-ESI-MS conditions

Chromatographic separation was performed on a Water Alliance HPLC 2695 (Waters Associates Inc., Milford, MA) consisting of a quaternary pump, auto-sampler, column thermostat, reversed phase column X-Terra 18 (3.9 x 150 mm, 5 µm) and a guard pre-column module with a Bondapak C_{18}, 4 µm Guard insert. Isocratic elution was optimized and carried out in a mobile phase consisting of methanol: water (75: 25; v/v) ml with 0.1% formic acid. The mobile phases were prepared daily by filtering through 0.45 µm membrane disc filter and degassed by sonication before use. The column was kept at a temperature of 25 ± 1 °C. The total flow from the column was 0.3 ml/ min. Water 3100 mass spectrometer equipped with electrospray ionization (ESI) source. Nitrogen gas generator unit (NM30LA-MS Gas Generator from Peak Scientific Instruments Ltd., MA, USA) used for supplying nitrogen gas. The positive ion mode parameters were as follows: capillary voltage 3 kV, cone voltage 15 V, extractor voltage 2V, RF lens 0.3 V, source temperature 110 $^{\circ}$C; desolvation temperature 450 $^{\circ}$C, desolvation gas flow 500 L/hr, cone gas flow 50 L/hr and source temperature 150 $^{\circ}$C. In addition to these parameters, the instrument was monitored in selective ion recording (SIR) scan mode in which the analyzer isolates only the protonated caffeine ion 195.2 $[M+H]^{+}$ to the detector. The data were collected by a computer using Masslynx 4.1 software to control all the variables and data collection.

2.4.3 Calibration curve and sample analysis

Eight different concentrations of caffeine standard were chromatography using LC-ESI (+ve) -SIR-MS after optimizing the analytical conditions. A calibration curve was obtained by plotting the peak area versus the concentration of each standard. Samples were injected at the same conditions of standard analysis. Chromatograms of samples obtained were analyzed using Maslynx 4.1 software based on the comparison of retention times of the samples with those of the standard for qualitative analysis and calibration curve for quantitative analysis.

2.5. Determination of mineral elements by AAS and FES

AAS (Buck scientific model 210 VGP, equipped with a deuterium background corrector and automatic hollow cathode lamp switch) was used for absorbance measurement and digital concentration readout device to analyze samples of copper, iron and lead by direct aspiration into the burner. Table 1 shows the optimum operation conditions for the elements under study. Different concentrations of each standard; Cu, Fe and Pb; were analyzed using the optimum condition. A calibration curve for each element under study was obtained by plotting the concentration against the reading of absorbance. At the same conditions the samples were aspired into the

instrument and the absorbance read. From the calibration curve of standard, the concentration of each element was known. The experiment was done triplicates and the value obtained was the mean± standard deviation.

Table 1: Recommended analysis conditions of AAS for Cu, Fe and Pb analysis

Element	Cu	Fe	Pb
Wavelength	327.4 nm	372.0 nm	217.0 nm
Current	1.5 mA	3 mA	3 mA
Slit	0.7 nm	0.2 nm	0.2
Air flow meter reading	5	5	5
Acetylene flow meter reading	4	4	4
Background correction deuterium	ON	ON	ON

FES (Jenway clinical PFP7) is a low-temperature (air/natural gas) flame atomic emission photometer, designed for the routine determination of sodium and potassium in aqueous solutions, food industry and physiological fluids. The low-temperature (about 1700 °C) generates strong emission only from the most easily excited elements. Wavelength isolation is by use of a simple narrow-band pass interference filter that is designed to transmit only the interest line. After optimizing the analytical conditions, serial concentrations of each standard; sodium and potassium; were analyzed. A calibration curve for each element under study was obtained by plotting the concentration against the reading of emission signal. At the same conditions the samples were analyzed. From the calibration curve of standard, the concentration of each element was known. The experiment was done triplicates and the value obtained was the mean± standard deviation.

3. Results and Discussion

Arabic coffee is a general name that refers to the two main ways coffee is prepared in many Arab countries; Turkish-style and Saudi-style. Saudi Arabic coffee is made from coffee beans roasted very lightly with cardamom and sometimes other spices like saffron (to give it a golden color), cloves and cinnamon. Serving coffee (gahwa) in KSA is a sign of hospitality and generosity. In Ramadan month, the peoples in Saudi Arabia break their fast with dates, water and Arabic coffee which gave them energy to perform the extended prayer held on the evening of Ramadan. Many Arabic coffee from different origin found in Saudi local markets without any labeling concerning its ingredients and the way of preparation. Regarding to many reports showing positive and negative effects (according to the dose taken) on caffeine as a major and famous biologically active compound in coffee **(Smith, 2002; Thompson and Keene, 2004; Van Dam and Hu, 2005; Higdon and Frei 2006; Schrader *et al.*, 2013),** four different origins of Arabic coffee obtained from the markets of Saudi Arabia were prepared according to traditional procedure and analyzed for their content of caffeine. Also, five mineral elements were determined.

3.1. Determination of caffeine

The qualitative and quantitative estimation of caffeine has been attempted using several chromatographic methods. These include high-performance liquid chromatography (HPLC)**,** ultraviolet (UV) spectroscopy and thin layer chromatography-mass spectroscopy (TLC-MS) **(Lau *et al.*, 1992; Naik and Nagalakshmi 1997; Prosek *et al.*, 2000).** LC-MS is the method of choice for determining caffeine at low levels and in very complex matrices. The three main advantages of LC-MS over conventional HPLC method can be represented by 3 "S" sensitivity, selectivity and speed **(Daniel *et al.*, 2008; Adam *et al.*, 2010).** Despite coffee being the major food source of caffeine, LC-MS method have been largely used for the analysis in association with other components, such as catechins **(Victor, 2012)**. In this study the authors used optimized and developed simple LC-MS method for caffeine determination.

3.1.1. Optimization of ionization conditions for caffeine standard

The direct infusion of the caffeine standard (1 µg/ml, flow rate 20 µl/min for ten minutes) to mass spectrometer was carried out using the full scan in positive ion mode. The intensity of protonated caffeine ion 195 $[M+H]^+$ was observed by varying the most important two interrelated parameters; cone voltage and capillary voltage. As shown in figures (2 - 4), at a constant capillary voltage (3 kV) with increasing the cone voltage from 0-50 V, the intensity of protonated caffeine ion 195 $[M+H]^+$ increased with maximum at 15 V followed by decreasing the intensity till reach a minimum value at 50 V. On the other hand by using three different capillary voltages 1-3 kV (at constant cone voltage 15 V), highest signal at capillary voltage 3 kV was appeared. It was seemed that, by adjusting the capillary cone voltage at 3 kV and 15 V respectively, highly intense caffeine ion was obtained while above or bottom of these values the intensity of ion decrease due to the fragmentation of ion or less energy for ionization the caffeine molecule. So, the following tuning parameters were found to be optimal conditions for the ionization caffeine compound: capillary voltage 3.0 kV, cone voltage 15 V, extractor voltage 2V, RF lens 0.3 V, source temperature 110 °C; desolvation temperature 450 °C , desolvation gas flow 500 L/hr

and cone gas flow 50 L/hr. The overall ionization efficiency to form $[M+H]^+$ ion for the caffeine ion molecular can be seen basis peak at mass 195.08 m/z.

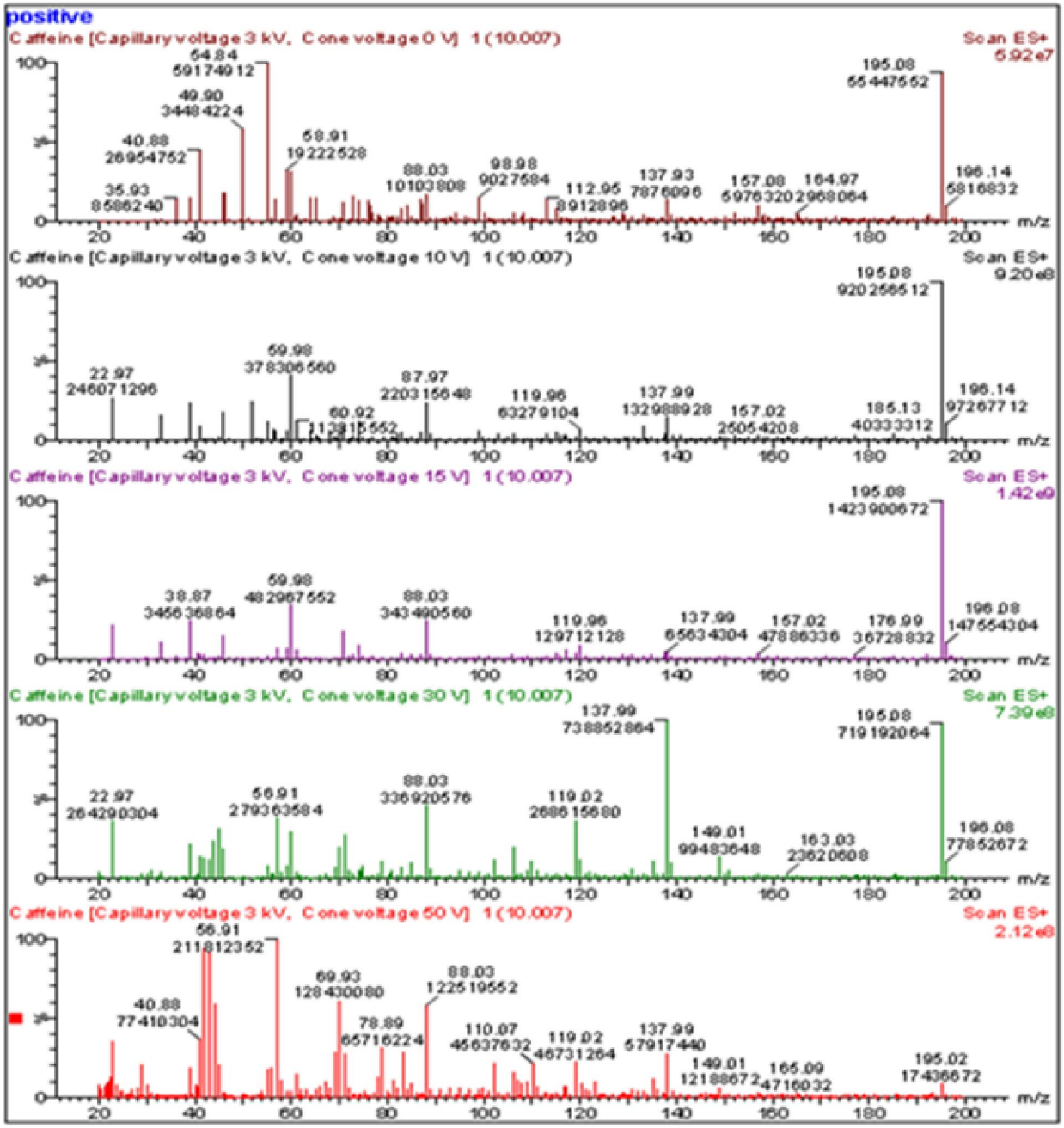

Figure 2: Full scan (20-220 *m/z*) mass spectra generated from a direct infusion of caffeine standard solution to ESI(+ve) MS at five different cone voltage values.

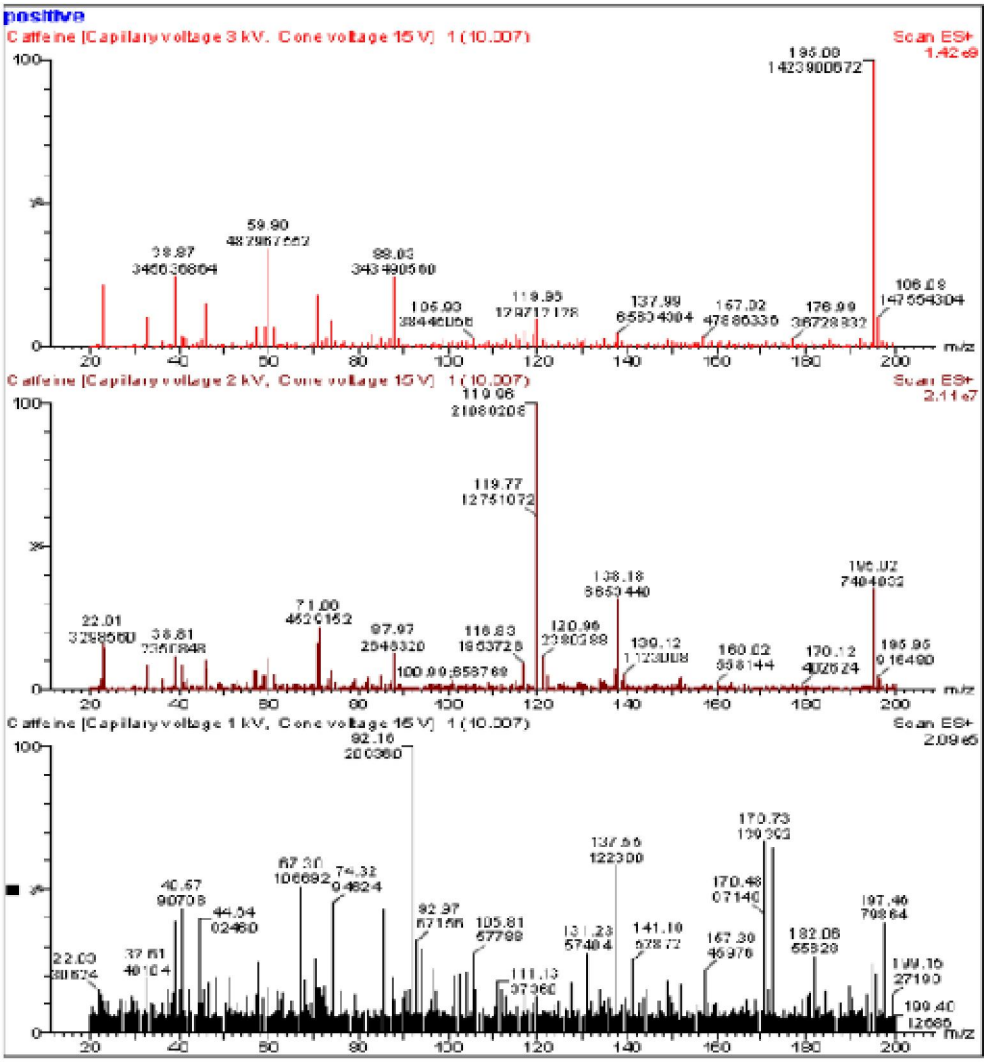

Figure 3: Full scan (20-220 *m/z*) mass spectra generated from a direct infusion of caffeine standard solution to ESI(+ve) MS at three different capillary voltage values.

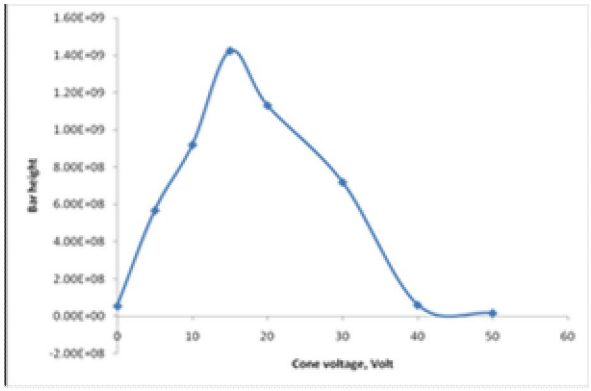

Figure 4: A line plot of the average bar height (intensity) of protonated caffeine ion 195 $[M+H]^+$ at different cone voltage.

4.2 Optimization of chromatographic conditions and standard calibration

Several trials were done to get good separated caffeine peak by liquid chromatography under the previously adjusted ionization conditions for caffeine. Due to the co-existence of hundreds of other compounds in samples of our study, interference and minimization of our target peak were appeared. To overcome this problem, selective ion recording (SIR) scan mode was used. The SIR mode used to maximize sensitivity and can give a thousand-fold increase in detector response over the full scan mode. SIR enhances sensitivity because only the target ions of interest are monitored so that no time is spent recording non-target ions. Mass spectrometry in the SIR mode has been widely applied especially in pharmaceutical analysis (**Enz *et al.*, 2004; Wang *et***

***al.*, 2009; Yogeshwar *et al.*, 2010; Choea *et al.*, 2012; Raju *et al.*, 2012; Bhatt *et al.*, 2013).**

By using the LC-ESI(+ve)-SIR-MS technique, different concentrations of caffeine standard were analyzed. Figure 5 showed the SIR spectrum of caffeine in positive ion mode (m/z = 195). From standard caffeine chromatograms (Figure 6), a calibration curve was obtained (Figure 7) by plotting peak area versus the mass loading of the caffeine at the optimum conditions. Calibration linearity for caffeine was investigated by making the replicate injections of each standard prepared at eight different concentrations. The average of three for each sample was taken. As shown in figure 7 excellent linearity was achieved throughout the range from 0.6 to 20 µg mass load. Regression equation (Y=24615x+41132) and correlation coefficient [(r) is 0.999] revealed a good linearity response for the method.

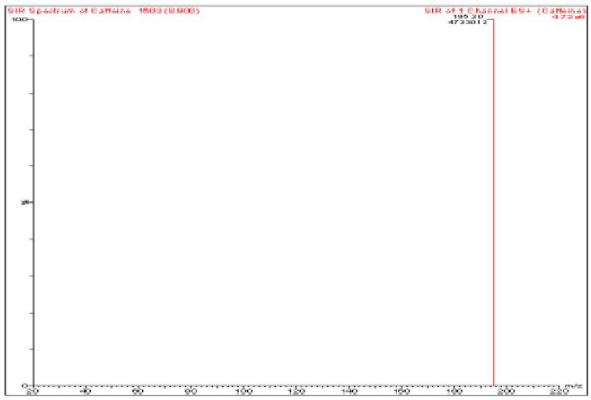

Figure 5: Selective ion recording (SIR) spectrum of caffeine standard using LC-ESI(+ve)-SIR-MS technique.

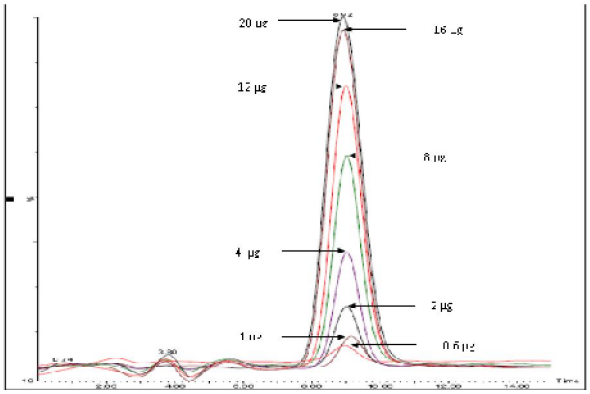

Figure 6: Overlay chromatograms of eight different concentrations of caffeine standard using LC-ESI (+ve)-SIR-MS technique.

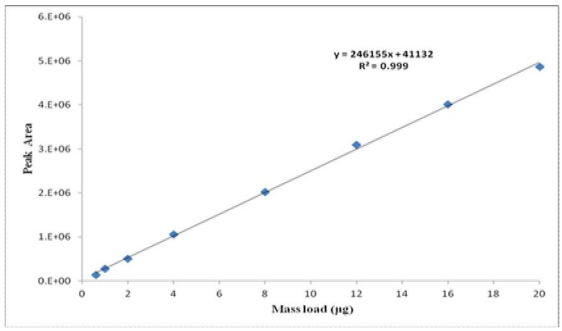

Figure 7: Calibration curve of caffeine standard.

4.4 Analysis of caffeine in Arabic coffee samples:

After optimizing the analytical conditions for the method using caffeine standard, the four different origin Arabic coffee samples were analyzed at the same conditions. Figure 8 shows typical SIR chromatograms of the caffeine in the different samples. Caffeine compound was found in all the four samples with different quantity. By knowing the area under the curve and from the standard calibration curve, the quantity of caffeine in all samples was calculated. Figure (9) showing the mean of the concentrations of caffeine in four Arabic coffee beverage prepared from different brands according to the Saudi traditional method. The total caffeine content was found to be between 573.1- 960.8 µg/ml. The lowest caffeine content (573.1 µg/ml) was found in Harry coffee from Ethiopia while the highest (960.8 µg/ml) was found in India brand. It can see that the concentration of caffeine in the Arabic coffee vary from country to country, then it must be needed to make labeling for caffeine content on products containing it. It was reported that the variation in caffeine content in plants containing it widely depending on many factors such as the genotype, the agronomic management, the soil factors, the climatic conditions, post-harvest technology and the method of preparation **(Stavric *et al.*, 1988; Brunetto *et al.*, 2007)**.

5.1 Estimation of mineral elements in Arabic coffee samples

Food minerals play a vital role in restoring and maintaining optimal health. Each day our bodies rely on minerals and trace minerals to maintain our heartbeat, send and receive signals to and from our nervous system, aid in the utilization of other important minerals, and assist in numerous enzymes reactions in the body. Micronutrient deficiencies are a major public health problem **(Thilly *et al.*, 1992; Batra and Seth, 2002; Soetan *et al.*, 2010).**

The three elements copper, iron and lead were analyzed using AAS. The correlation coefficient [r > 0.99] obtained from calibration curves of the three elements; Cu, Fe and Pb; showed a good linearity

response for method (Table 2). By analyzing the samples under the same conditions of standard, the results as shown in table (2) revealed the absence of lead and iron in all samples whereas copper not detected in Indian origin while the other brands showed the ranged value between 7.98-19.95 µg/g. Lead is considered as toxic mineral. It was not detected in this study using AAS and this coincide with reported results using ICP-AES as a tool of analysis in different kinds of coffee (**Santos and Olivera, 2001; Pohl *et al.*, 2013).**

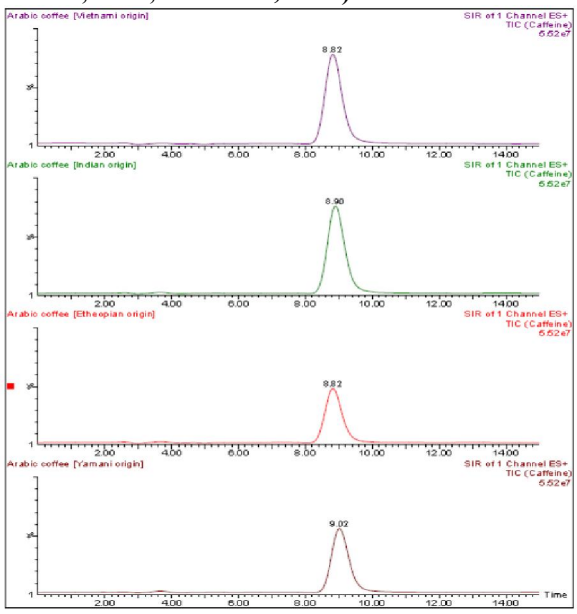

Figure 8: SIR chromatograms for the four Arabic coffee samples.

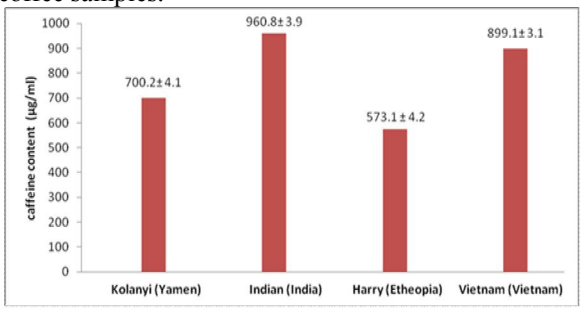

Figure 9: Quantity of caffeine (µg/ml) in the coffee beverage prepared from different Arabic coffee origin.

Sodium and potassium were analyzed using FES. After setting the FES for the best emission signal of the elements under study, the standard and samples were analyzed at the same conditions. The correlation coefficient (r) was higher than 0.99 for the two elements which revealed the presence of a good linearity response for method (Table 2). All coffee beverages prepared from different Arabic coffee origins showed nearly the same similar concentration of sodium and potassium (Table 2). The level of minerals found in coffee would be dependent on the levels of minerals present in the soil.

5. Conclusion

Saudi Arabic coffee is the most important beverage in KSA. Due to the positive and negative effect of caffeine (according to the dose taken) which is the major and mainly responsible component for the physiological properties of coffee, it must be need to know the concentration of caffeine in coffee and other products containing it and consequently know the amount of beverage taken and containing the recommended daily intake of caffeine. This study provides clearly demonstrated chromatographic method containing a combination of high performance liquid chromatography coupled with mass spectrometry for determination of caffeine in Arabic coffee or other products containing caffeine in less than nine minutes with high reproducibility and accuracy. This method was more rapid and simple than traditional procedures. The LC-ESI(+ve)-SIR-MS technique appears to be suitable for routine analysis and quality control in the food and pharmaceutical industries. Also, this method can be used as a tool for practical assessment of hepatic function in cirrhotic patients. The atomic emission and atomic absorption spectrometry are a fast, simple, and sensitive method for the determination of sodium, potassium, iron, lead and copper in solution. The method is free from interferences from other elements. The authors recommend these methods for analysis of caffeine and minerals concerning the food and human health safety.

Table 2: Composition of mineral elements in four Arabic coffee from different origin using AAS and FES.

Composition	Kolanyi (Yemen)	Indian (India)	Harry (Ethiopia)	Vietnam (Vietnam)	Linearity (µg/ml)	r
Cu (µg/g)	19.95±0.11	ND	7.98±0.29	10.15±0.19	0-5	0.998
Fe (µg/g)	ND	ND	ND	ND	0-10	0.997
Pb (µg/g)	ND	ND	ND	ND	5-50	0.997
K (mg/g)	15.96±1.01	17.99±1.23	15.96±1.03	16.90±0.98	0-1.2	0.995
Na (µg/g)	199.1± 2.02	199.1±1.09	200.42±2.03	198.80±1.99	0-2.5	0.995

The values were expressed as mean of triplicate determinations ± standard deviation.
r: Correlation coefficient ND: not detected

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Conflict of interest

Authors have declared that there is no conflict of interest in this manuscript.

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