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# Determination of Caffeine and Few Metals in Halabja Tea and Comparing with Various Tea Samples in Erbil Markets

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## Abstract:

This paper deals with two main parts: part (I) performed the caffeine levels evaluation in three commercial tea leaves (black, green, and herbal tea) in Erbil markets and Halabja city. The caffeine was extracted by liquid-liquid extraction using dichloromethane and chloroform solvents. The extracted caffeine was purified, and then identified by melting point (M.P), ultraviolet (UV) and Fourier transform infrared (FT-IR) spectroscopy that provides spectra identical to the standard caffeine crystals. The level of caffeine in black tea was found greater than in green tea, presumably because black tea are made of older tea leaves, in contrast, there is no caffeine obtained from Halabja tea. Part (II) mentioned the determination of metal ions (lithium, sodium, potassium, and calcium) in 5 brands of black and green tea (leaves and bags) in addition to Halabja tea; using flame atomic emission (FAES) and flame atomic absorption (FAAS) spectroscopy. The tea beverages were prepared by three different methods: (chemically, infusion, and continuous infusion). There was a moderate quantity of sodium, potassium, and calcium was determined in the 5 brands of tea, while lithium found in the trace. Halabja tea was free from caffeine while lithium, sodium, potassium, and calcium elements of the Halabja herbal tea are all higher than all the other types of tea leaves and bags.

**Keywords:** Caffeine; Extraction; Halabja tea; Tea infusion; Continuous infusion; Metals; Flame atomic emission spectroscopy; Flame atomic absorption spectroscopy, Li, Na, Ca, La.

## 1. Introduction:

Tea (*Camellia Sinensis*) is one of the most popular beverages consumed around the globe, it comes after water. It ranks second in terms of the most widely distributed beverages. Tea is regarded mostly for its pleasant scent and flavor, as well as its potential favorable influence on attitude, health advantages, and cultural activities [1]. Kenya, Sri Lanka, China, and India as well as Vietnam and Turkey, are indeed the leading tea producers [2], and these countries supply tea to a variety of nations, including Iraq. The most frequent categorization of tea is based on the type of leaf fermentation. A difference is established here between practically non-fermented green, yellow and white tea, partially fermented Pu-erh and oolong tea, and completely fermented black tea [3]. Based on its sensory properties, black tea has been the most popular; however, interest in green tea has been growing for several years [4].

Tea is one of the major sources of caffeine intake [5]. Caffeine is a naturally occurring important alkaloid present in tea that behaves as a stimulant and diuretic, delays weariness, and has an effect on the central nervous system [6], due to that, the determination of caffeine is an important factor in the quality control of tea. Caffeine levels in beverages have been assessed using several analytical techniques, including UV-vis spectrophotometry [7, 8], flow-injection analysis using sample pre-treatment [9], and mostly by HPLC [10, 11] supported membrane-modified piezoelectric flow sensor [12], Fourier transforms infrared (FT-IR) spectrometry [13].

Water extracted major and trace chemicals of tea leaves during the infusion process, in addition to water-soluble organic compounds. Many of these substances affect the flavor of the tea infusion, play an important part in metabolic processes, and are necessary for human well-being in general. A lack or excess of these components can cause sickness and/or be harmful to one's health [14, 15].

It is critical to monitor metal content in daily food and drink consumption. This might be done notably for widely consumed beverages such as tea because metals absorbed from plant materials during tea processing that might be useful or hazardous to human health [16]**.** Except for these organically, tea leaves and prepared tea is high in many elements, including major, minor, and trace elements. The element content is unique to tea kinds and is primarily attributable to how this tea is made as well as the geographical origin of tea plants, which includes soil composition, climate, local environmental circumstances, and agricultural techniques [17- 24]. Some elements, i.e., Na, K, and Ca, are necessary for human health and hence may contribute to the nutritional value of tea [17, 20, 23- 28].

The aim of this work is the determination of some essential elements such as Li, Na, K and Ca using FAES and FAAS as well as estimation of caffeine in tea brands (black and green (leaves and bags)) that are available in Erbil markets and violet herbal tea which produced for the first time in Halabja city.

## 2. Experimental:

### 2.1. Apparatus and Instruments:

Gallen Kamp digital flame atomic emission spectrophotometer, PYE UNICAM SP9 (Philips) flame atomic absorption spectrophotometer, Toshiba melting point apparatus, SHIMADZU FT-IR spectrophotometer, SHIMADZU UV-1900i spectrophotometer model (2018), (HERBERT ARNOLD 629 Weil BURG/LAHN W- Germany) muffle furnace.

### 2.2. Materials:

The following analytical grade materials were used without any further purification: lithium sulfate monohydrate, sodium chloride, potassium chloride, calcium chloride, lanthanum chloride heptahydrate, pure caffeine, dichloromethane, chloroform, nitric acid, calcium carbonate, and sodium sulfate.

### 2.3. Preparation of Stock Solutions:

All glassware was washed with de-ionized water, and then dried in an oven at 105 ºC before use. 100 mg/L stock solution of lithium ions was prepared by dissolving 0.9218 g of ($Li_2SO_4.H_2O$) in 100 mL of de-ionized water and then completing it to 1 L. 100 mg/L stock solution of sodium ions was prepared by dissolving 0.2542 g of (NaCl) in 100 mL of de-ionized water and then completing it to 1 L. 100 mg/L stock solution of calcium ions were prepared by dissolving 0.27693 g of ($CaCl_2$) in 100 mL of de-ionized water then completing it to 1 L. 1000 mg/L stock solution potassium ions were prepared by dissolving of 1.9070 g of (KCl) in 100 mL of de-ionized water then completing it to 1 L. 10% (w/v) $LaCl_3$ was prepared by dissolving 29.45 g of ($LaCl_3.7H_2O$) in 100 mL of de-ionized water.

### 2.4. Preparation of Standard Solutions:

### 2.4.1. Lithium, Sodium, and Calcium Standard Solutions:

Working standard solutions (S. Sol.) (25 mg/L) was prepared by diluting 6.25 mL of stock solutions to 25 mL, then from this solution series, standard solutions in the range of (0.5-18.0) mg/L were prepared by serial dilutions. The estimation of each solution was measured at maximum sensitivity. All the standard and sample solutions of Ca were containing 0.2% (w/v) lanthanum chloride.

### 2.4.2. Potassium Standard Solutions:

A series of potassium standard solutions in the range of (50-750) mg/L were prepared directly by diluting of potassium stock solution by serial dilution. The estimation of each solution was measured at maximum sensitivity.

## 2.5. Caffeine Extraction Procedure:

### 2.5.1. Extraction by ($CH_2Cl_2$):

A sample of 5 g tea leaves was carefully weighed and placed in a conical flask. The tea sample was treated with 4.0 g of calcium carbonate ($CaCO_3$) and 100 mL of de-ionized water. The mixture was boiled for 15 min on a hot plate with stirring, cooled to roughly 50 $^oC$, and then filtered using vacuum filtration through Whatman filter paper. Then the precipitate is washed with a known amount of deionized water. The filtrate was kept at room temperature before being placed into a separating funnel. The funnel was filled with 15 mL of dichloromethane and gently shaken. After the layers were separated, the organic layer was drained into a 50 mL conical flask. The extraction was carried out 5 times with fresh 15 mL dichloromethane. Anhydrous sodium sulfate was used to dry the dichloromethane solution. Swirling the mixture for several minutes allowed the sodium sulfate to dehydrate the solution; it was then filtered into a 100 mL dry pre-weighed conical flask. The conical flask was eventually placed in a steam bath, and the contents were evaporated to dryness at a relatively low heat of the water bath. The container was weighed to determine the crude caffeine amount [29].

### 2.5.2. Extraction with ($CHCl_3$):

10 g of tea leaves were placed in a 250 mL conical flask, along with 100 mL of de-ionized water and 10 g of $CaCO_3$. The mixture was then brought to boiling for 25 min while stirring. The mixture was filtered using a Buchner funnel, and the filtrate was taken to a 250 mL separating funnel. 80 mL

chloroform was poured into the separating funnel and carefully swirled for 5 min. The mixture was then allowed to settle such that the chloroform layer could be seen. The separating funnel stopper was opened, and the chloroform was collected in a 100 mL beaker and evaporated to dryness in the hood on a water bath. The flask was weighed to determine the crude caffeine amount [29].

## 2.6. Recrystallization:

For the purification process, three different solvents were tested in which they were: (methanol, ethanol, and butanol), it appeared that ethanol is better than the other solvents because it gives a higher yield as they were tested, so it was chosen as the best solvent. The produced crude crystals were recrystallized by heating with ethanol (95 %) in which caffeine is soluble in the heated ethanol and then the heated solution was filtered for removing insoluble impurities and the caffeine crystals were in the filtrate portion and the crystals were separated by cooling the content of the flask in ice-bath and filtering using Buchner funnel. The crystals were washed with 5 mL of cold ethanol. The caffeine crystals were on the filter paper that was previously weight and left in a desiccator for one day for drying and weighing.

## 2.7. Elemental Analysis by FAES and FAAS:

### 2.7.1. Chemically Preparation of Tea Samples:

For both techniques, all the samples of tea which were purchased from the Erbil local markets and Halabja city for analysis are dried at 105 $^{o}$C in an oven then 10 g from each type of tea leaves and the bag were weighed in a porcelain crucible and ignited in a muffle furnace at 250 $^{o}$C for two hours. The crucible with its contents was cooled in a desiccator and weighed. The crucible residue was transferred to a beaker and dissolved in 200 mL of 0.1 M $HNO_3$ for all types of tea leaves and bags before being filtered, the filtrate was used for the analysis of lithium, sodium, potassium, and calcium elements. The blank solution is 0.1 M $HNO_3$ [29].

### 2.7.2. Tea Infusion (Homemade Preparation) Procedure:

ALWAZAH black tea was used for this purpose, the tea infusions were prepared with tap water because many people are preparing tea by using it, (1-5) g of the tea leaves were immersed into 100 mL of boiled deionized water for 5 min for preparation of (1-5) % (w/v) tea solution and then filtered through a filter paper, the filtrates were collected and the contents were determined. The blank solution was prepared by using boiled deionized water after cooling.

### 2.7.3. Continuous Tea Infusion Procedure:

Some people are interested a tea in which it was prepared by heating the tea with cold water, for this reason, a 1% ALWAZAH black tea to water ratio was prepared by heating for about 15 min and then filtered, this work is done for comparison between the elements that are releasing into the tea solution when hot water was used for preparation or when cold water was used and then heated for a known time.

## 3. Results and Discussion

### 3.1. Estimation of Caffeine:

The liquid-liquid extraction of caffeine was performed from three tea leaves (Halabja, AHMAD black, and DOGHZAL green tea) by using two different solvents (dichloromethane and chloroform), these two solvents were utilized for comparison. From the results, it was indicated that the percentage yield of extraction was higher when chloroform was used as extracting solvent than dichloromethane. The extraction efficiency of chloroform is due to the polarity of the compound and also the caffeine itself is a polar compound so chloroform was caused the extraction of more caffeine, results shown in Table (1). The results were compared with Kurdistan Standardization and Quality Control Authority (KSQCA) for the year 2012, (cited from Iraqi standards) with the range of (1.9 - 3.6) %, while the amount of caffeine in samples was greater than the standards.

**Table (1): The identification characteristics of caffeine:**

| Type of tea leaves | Yield% Caffeine ($CH_2Cl_2$) | M.P. ($^oC$) | λ (max) | Yield% Caffeine ($CHCl_3$) | M.P. ($^oC$) | λ (max) |
|---|---|---|---|---|---|---|
| **Halabja Herbal tea** | - | - | - | - | - | - |
| **AHMAD Black tea** | 6.38 | 230-232 | 273 | 19.86 | 230-231 | 273.5 |
| **DOGHAZAL Green tea** | 4.96 | 229-231 | 273.5 | 11.75 | 229-231 | 273 |

M.P. of Standard Caffeine: (230-233) $^oC$.

λ $_{(max)}$ of Standard Caffeine: 272.5 nm.

The estimation of caffeine was done as follows: **(I) Finding Melting Point (M.P.):** for the standard caffeine and the pure extracted caffeine from the three samples, the M.P. for standard caffeine was (230-233) $^{o}$C and for the samples extracted by dichloromethane were (230-232 and 229-231) $^{o}$C and chloroform extract were (230-231, 229-231) $^{o}$C for AHMAD black and DOGHAZAL green tea respectively but Halabja tea shows no yield of caffeine.

**(II) UV- Spectra Analysis:** for standard caffeine was (272.5 nm) and for caffeine extracted by the two extraction solvents were (273, 273.5 nm) for AHMAD black tea as shown in (Fig. 1-A) and (273.5, 273 nm) for DOGHAZAL green tea as shown in (Fig. 1-B) using dichloromethane and chloroform respectively, but Halabja tea showed no signal (Fig. 1-C), the $\lambda_{(max)}$ for the AHMAD black and DOGHAZAL green tea are very close to the standard caffeine but Halabja tea far from the standard, that means it does not contain caffeine.

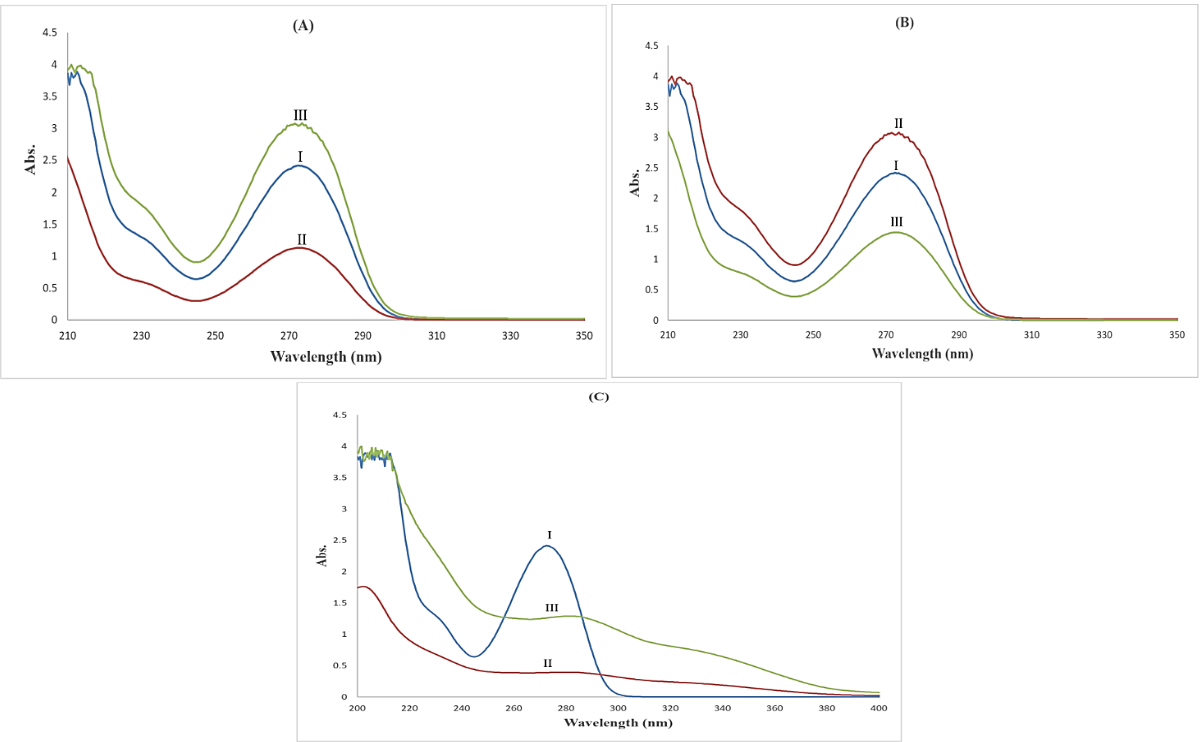

**Figure (1): UV– spectra for: (A) AHMAD tea; (B) DOGHAZAL tea; (C) Halabja tea; Curve (I): Standard caffeine; (II): Caffeine extracted by $CH_2Cl_2$; (III): Caffeine extracted by $CHCl_3$.**

Purines give sharp absorption at 270 nm, whereas caffeine absorbs at 272.5 nm due to the replacement of three methyl groups on three nitrogen atoms. The development of absorption band 272.5 nm for caffeine is related to the electronic transition of n—σ* and π—π* where unpaired electrons are abundant on nitrogen atoms and carbonyl groups in addition to double bonds [29].

**(III) FT-IR Spectroscopy**: The FT-IR absorption peaks for standard (Fig. 2-D) and extracted caffeine from AHMAD black tea and DOGHAZAL green tea (Fig. 2) using chloroform indicate that they are near each other and so confirm the exact structure of the extracted caffeine as shown in Table (2). A very tiny shift that is done by some absorption peaks is due to the errors in the measuring instrument. But FT-IR spectra for Halabja tea have not coincided with the standard caffeine spectra it also shows that it does not contain caffeine as it is indicated in (Fig. 2-C).

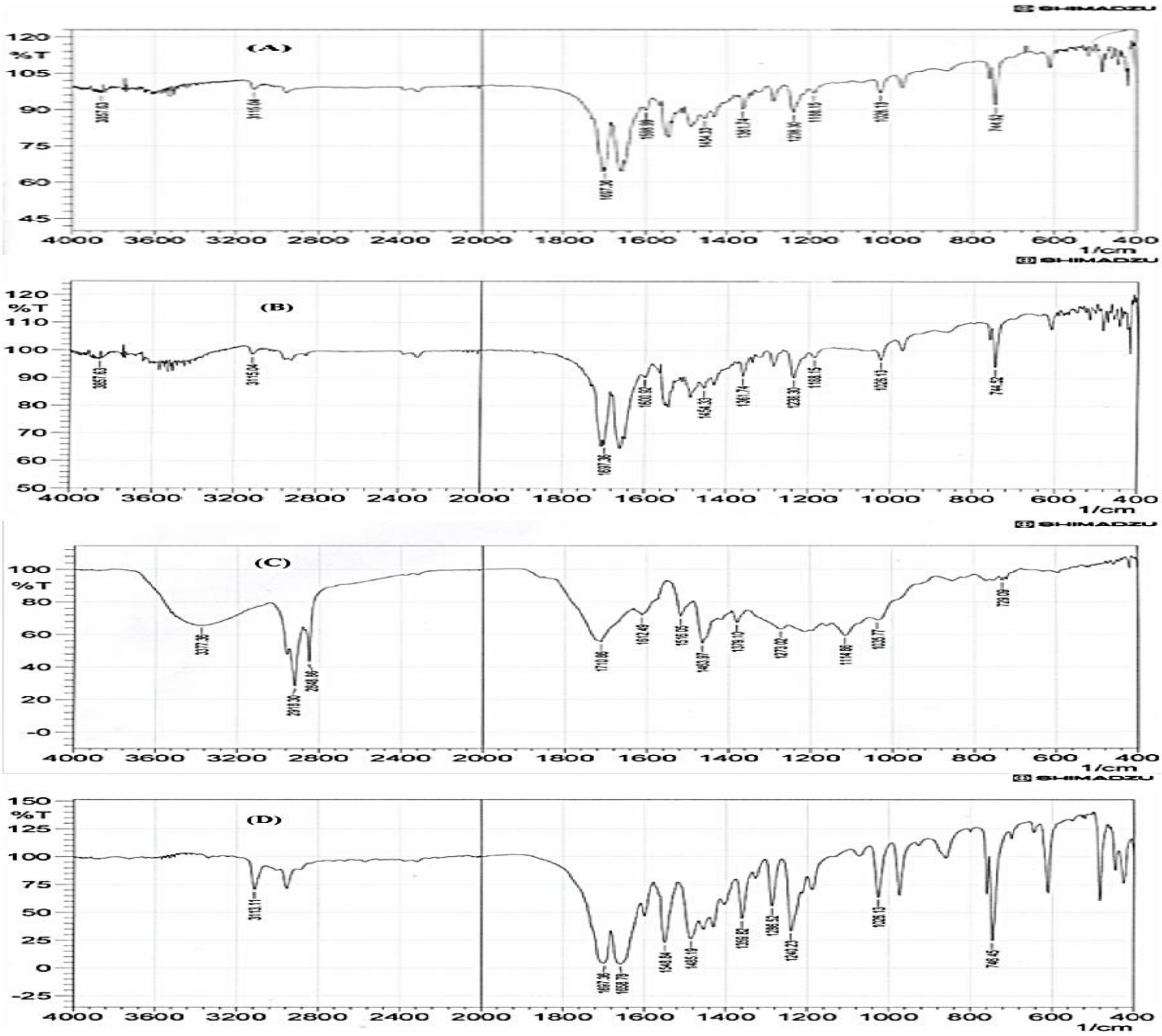

Figure (2): FT-IR spectra for caffeine: (A) AHMAD black tea; (B) DOGHAZAL green tea; (C) Halabja tea; (D) Standard caffeine.

**Table (2): FT-IR absorption peaks for standard and extracted caffeine from AHMAD black and DOGHAZAL green tea:**

| Functional groups | Wave number ($cm^{-1}$) for standard caffeine | Wave number ($cm^{-1}$) for AHMAD black tea | Wave number ($cm^{-1}$) for DOGHAZAL green tea |
|---|---|---|---|
| $\upsilon_{C-CH3}$ | 3885 | 3857.63 | 3857.63 |
| $\upsilon_{C-H}$ | 3113.11 | 3115.04 | 3115.04 |
| $\upsilon_{C-N}$ | 1026.13,1190,1240.23 | 1026.13,1188.15,1238.3 | 1026.13,1188.15,1238.3 |
| $\upsilon_{C=O}$ | 1697.36 | 1697.36 | 1697.36 |
| $\upsilon_{C=N}$ | 1590 | 1598.99 | 1600.92 |
| C-H deformation | 746.45 | 744.52 | 744.52 |
| Sym. and asym. $CH_3$ bending | 1359.82 -1462.0 | 1361.74-1454.33 | 1361.75-1454.33 |

### 3.2. Elemental Analysis:

Determination of lithium, sodium, and potassium ions was done using FAES, while calcium ions were measured using FAAS. Moderate concentrations of sodium, potassium, and calcium were determined while lithium had a trace amount. Table (3) shows the concentrations (mg/L) of lithium, sodium, and potassium elements extracted in tea leaves and bags (black and green) and herbal tea using direct calibration curves for each element alone. It was indicated that the concentration of these elements in black tea leaves was in the range (0.7054 - 0.8703) mg/L, (2.8116 - 4.0264) mg/L, (219.9818 - 267.3406) mg/L; black tea bags in the range (1.2000 - 1.5297) mg/L, ( 2.9852 - 5.9354) mg/L, (252.7687 - 434.9180) and for green tea leaves in the range (0.7054 - 0.8703) mg/L, (2.8116 - 6.4561) mg/L, (263.6976 - 205.4098) mg/L and for green tea bags in the range (1.6946 - 1.8595) mg/L, (3.3323 - 3.8529) mg/L, (307.4135 - 318.3424) mg/L for lithium, sodium and potassium respectively from the 5 brands of tea, but Halabja herbal tea was contains (2.5189) mg/L lithium, (16.6613) mg/L sodium and (678.9982) mg/L potassium. It is clear that the concentration of those three elements was higher in Halabja herbal tea than in the other 5 brands of tea.

Little is known about the consumption of lithium and its content in food. In the light of available knowledge, it should be emphasized that the role of Li in life processes is still not fully understood [30]. Sodium is a minor component of tea minerals that has very little impact on tea quality, it is important for maintaining a cation-anion balance. The loss of sodium can lead to a water loss. On the other hand, high sodium intake can be fatal for patients suffering from hypertension [31, 32]. Herbal teas are rich in sodium in all cities [33]. Potassium is a mineral that is essential for all plant and animal organisms. An adult man's body has (100– 200) g of potassium. Its level in blood is relatively constant (regulated by the adrenal cortex), and has an impact on the functioning of the cardiac muscle as well as nervous system excitability. Low potassium content in the blood (hypokalemia) is the cause of familial periodic paralysis, an increase in the concentration in blood (Hyperkalemia) damages the work of the heart muscle work [34].

**Table (3): Concentration (mg/L) of Lithium, Sodium, and Potassium in tea leaves and bags (black and green) and herbal tea:**

| No. | Tea leaves/ bags | Lithium (mg/L) | | Sodium (mg/L) | | Potassium (mg/L) | |
|---|---|---|---|---|---|---|---|
| | | Leaves | bags | Leaves | Bags | Leaves | Bags |
| 1 | ALWAZAH Black | 0.7054 | 1.5297 | 2.9852 | 5.935 | 219.982 | 434.918 |
| 2 | CIHAN Black | 0.8703 | 1.2 | 3.5058 | 4.026 | 267.341 | 329.271 |
| 3 | MAHMOOD Black | 0.8703 | 1.2 | 3.8529 | 4.721 | 252.769 | 311.057 |
| 4 | AHMAD Black | 0.8703 | 1.3649 | 4.0264 | 2.985 | 238.197 | 252.769 |
| 5 | DOGHAZAL Black | 0.8703 | 1.3649 | 2.8116 | 3.506 | 238.197 | 285.556 |
| 6 | ALWAZAH Green | 0.7054 | 1.6946 | 2.8116 | 3.332 | 216.339 | 318.342 |
| 7 | MAHMOOD Green | 0.8703 | 1.8595 | 6.4561 | 3.853 | 263.698 | 307.414 |
| 8 | DOGHAZAL Green | 0.7054 | - | 2.8116 | - | 205.41 | - |
| 9 | Halabja Herbal | 2.5189 | - | 16.6613 | - | 678.998 | - |

All the samples and calibration standard solutions used for calcium (Ca) determination containing 0.2 % (w/v) lanthanum chloride (except homemade and continuous infusion samples, due to the formation of a precipitate when lanthanum chloride was added and so the formation of two layers), because elements such as aluminum or phosphate that may be present in tea samples depress the response in the air/ acetylene flame so the addition of a 0.2 % (w/v) lanthanum chloride as releasing agent will reduce this effect. From the results, it was shown that the calcium content of black tea leaves was between (7.8323 - 10.1278) mg/L, but for green tea leaves was in the range of (9.5540 - 11.4396) mg/L, so it was clear that green tea leaves contained more calcium than black tea leaves. Calcium in black tea bags was in the range (15.0469 - 84.2802) mg/L but in green tea bags was (13.6532 - 38.7950) mg/L containing less calcium than in black tea bags. According to Halabja herbal tea (408.5350) mg/L Table (4), the calcium content in Halabja herbal tea was much higher than in other tea brands both for green and black teas. Calcium participates in a variety of key physiological processes, including cardiac muscle rhythm maintenance and neuron and muscle excitability reduction [35]. Hypercalcemia is defined as an elevated Ca concentration (particularly above 2.6 mM), which may be linked to the development of myeloma, hyperparathyroidism, and vitamin D intoxication [36].

**Table (4): Concentration (mg/L) of Calcium in tea leaves and bags (black and green) and herbal tea:**

| No. | Tea leaves/ bags | Calcium (mg/L) | |
|---|---|---|---|
| | | Leaves | Bags |
| 1 | ALWAZAH Black | 7.9962 | 84.2802 |
| 2 | CIHAN Black | 10.1278 | 18.8182 |
| 3 | MAHMOOD Black | 9.144 | 17.0146 |
| 4 | AHMAD Black | 8.2422 | 15.8668 |
| 5 | DOGHAZAL Black | 7.8323 | 15.0469 |
| 6 | ALWAZAH Green | 9.5539 | 13.6532 |
| 7 | MAHMOOD Green | 10.4558 | 38.795 |
| 8 | DOGHAZAL Green | 11.4396 | - |
| 9 | Halabja Herbal | 408.535 | - |

### 3.3. Tea infusion (Homemade Preparation) and continuous infusion:

It was indicated that by increasing the concentration of the tea solution from 1 to 5 % more lithium, sodium, and potassium elements were released into the water ranging from (1.2000 - 3.1784) mg/L, (21.1746 - 31.5872) mg/L, and (190.8379 - 686.2842) mg/L for lithium, sodium, and potassium respectively, in contrast, the calcium concentration decreasing into the water ranging from (7.4223 - 6.1926 mg/L) as shown in Table (5). The continuous tea infusions as shown in Table (5 sample No. 6), while 1% tea was prepared using cold water and heated for 15 min, the concentration of releasing elements was increased especially for potassium when it is compared with 1 % tea infusion. So, it is better to prepare tea by continuous infusion in order to increase the concentration of potassium which has an impact on the functioning of the cardiac muscle as well as nervous system excitability [34]. Table (6) consists of the statistical information for the calibration curves, for ions understudies.

**Table (5): Concentration of Li, Na, K, and Ca using homemade prepared and continuous tea infusion:**

| No. | ALWAZAH Black tea | Lithium (mg/L) | Sodium (mg/L) | Potassium (mg/L) | Calcium (mg/L) |
|---|---|---|---|---|---|
| 1 | 1% | 1.2000 | 21.1746 | 190.8379 | 7.4223 |
| 2 | 2% | 2.1892 | 21.1746 | 369.3443 | 6.8485 |
| 3 | 3% | 2.5189 | 24.6455 | 460.4189 | 6.8485 |
| 4 | 4% | 2.8486 | 26.3809 | 595.2095 | 6.1926 |
| 5 | 5% | 3.1784 | 31.5872 | 686.2842 | 6.6845 |
| 6 | Cold water* (1%) | 1.5297 | 21.1746 | 212.6958 | 9.7179 |

* Heating for 15 min.

**Table (6): Statistical information for Li, Na, and K using FAES and Ca using FAAS:**

| Parameters | Lithium | Sodium | Potassium | Calcium |
|---|---|---|---|---|
| Slope | 6.0656 | 5.7622 | 0.2745 | 20.329 |
| Intercept | 1.7213 | 0.2012 | 30.615 | 3.5333 |
| Linear range | 0.5-4.0 | 0.5-8.0 | 50.0-750.0 | 1.0-18.0 |
| Limit of detection (LOD) (mg/L) | 0.2203 | 0.3215 | 54.324 | 0.937 |
| Limit of quantification (LOQ) (mg/L) | 0.6675 | 0.9741 | 164.62 | 2.8395 |
| Correlation coefficient ($R^2$) | 0.9975 | 0.9991 | 0.9954 | 0.9954 |
| % SD | 3.671 | 2.8151 | 0.6214 | 0.5023 |

**The limit of detection and limit of quantification was determined by the following equations:**

$$LOD = 3.3 \times (Residual\ SD\ of\ regression\ line/Slope)$$

$$LOQ = 10 \times (Residual\ SD\ of\ regression\ line/Slope)$$

## 4. Conclusion:

Chloroform has higher efficiency for caffeine extraction due to its polarity. The two different brands of black and green tea contain caffeine, black tea contains more caffeine than green teas, but Halabja tea was free from caffeine. Ethanol is a very good solvent for caffeine purification. The concentration of releasing elements was increased especially for potassium while using continuous tea infusions and homemade preparation, but the concentration of calcium was decreased when using homemade preparation and sodium concentration remains constant while using continuous infusion. It was indicated that by the analysis of the four elements they all had a higher concentration in tea bags than tea leaves, and also, they were all have a higher concentration in Halabja tea in comparison to the other 5 brands of black and green tea (leaves and bags), so that we can say Halabja tea is herbal tea rather than natural tea due to the reasons above and also its violet color and acidic taste.

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