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Selective determination of caffeine and trigonelline in aqueous extract of green coffee beans by FT-MIR-ATR spectroscopy

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ABSTRACT

A non-destructive, fast, simple and reliable Fourier transform mid-infrared attenuated total reflectance spectroscopy (FT-MIR-ATR) method for the selective determination of caffeine and trigonelline in the aqueous extract of green coffee beans was developed and validated. The calibration curves were linear in the range 2000 − 7000 mg/L for caffeine and trigonelline with $R^2 \geq 0.9997$. The limits of detection (LOD) were 140 and 100 mg/L and the limits of quantification (LOQ) were 470 and 330 mg/L for caffeine and trigonelline, respectively. The precision (% RSD) was 3.0% and 4.3% for caffeine and trigonelline, respectively. The developed method was applied to 20 samples of green coffee beans to determine the two alkaloids. The amount of caffeine and trigonelline in the green coffee beans were found in the range 0.84 − 1.15% (*w/w*) and 0.83 − 1.13% (*w/w*), respectively. The accuracy of the developed analytical method was evaluated by spiking standard caffeine and trigonelline to green coffee beans and the average recoveries were 93 ± 5% and 98 ± 4%, respectively. Therefore, the developed FT-MIR-ATR methods can be used for direct determination of the two alkaloids in the green coffee beans.

1. Introduction

Coffee is an evergreen plant, which belongs to the Rubiaceae family. It is one of the most widely consumed beverages, most commonly traded commodity throughout the globe because of its pleasant taste, aroma and physiological as well as psychoactive properties [1–3]. Out of the various species of the genus *Coffea*, two varieties are economically and commercially important: *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee) [4–6]. Arabica beans are more valued by the trade because they are assumed to have a lovely flavor as well as aroma so that they are highly appreciated by the consumers than Robusta beans [4,6]. Green coffee beans are rich in bioactive compounds, which basically consist of alkaloids and named as the methylated derivatives of xanthine (caffeine and theobromine) and trigonelline [2,7]. Methylxanthines (caffeine and theobromine) are present in many foods and beverages like coffee, tea, carbonated beverages, some chocolate products, caffeinated water, chewing gum [8], cacao beans, cola nuts and mate leaves [9]. Among these different possible sources, coffee is well known to have the highest caffeine concentration. Trigonelline is the second class of alkaloid present in coffee, barley, corn, onion, pea, soybean and tomato. However, it is the second most predominant alkaloid in coffee [7].

Caffeine causes stimulation of the central nervous system, heart rates, respiration [10], myocardial stimulation and peripheral vasoconstriction physiological effects [2]. Besides, it is used for the treatment of many diseases such as asthma, nasal congestion, headache. However, reports on human and animal studies showed that caffeine produces mental and behavioral effects that are similar to those of typical psychomotor stimulant drugs like amphetamine and cocaine [11]. Theobromine also shows similar stimulating effects as caffeine, but to a lower degree since it does not affect the central nervous system [12]. It is used for pharmaceutical purposes to prevent and treat shortness of breath caused by asthma and other respiratory disorders, such as bronchodilator, vasodilator and mild muscle relaxant [13]. Trigonelline which is the methylated alkaloid of nicotinic acid has the potency to improve memory retention and acts as anti-invasive against cancer cells [10]. The chemical structures of the alkaloids are depicted in Fig. 1.

The quantity of alkaloids (caffeine, theobromine and trigonelline) in green coffee beans is influenced by several factors such as, genetic properties of the cultivars, coffee variety, environmental factors (soil, altitude, sun exposure), climatic parameters (rainfall, temperature), maturity of the beans at harvest, harvesting method and agricultural practices (shade, pruning, fertilization) [7]. These factors provide different coffee characteristics and beverage attributes. Therefore, it is necessary to develop faster analytical methods that allow differentiation and determination of coffee composition [6]. This is because of the

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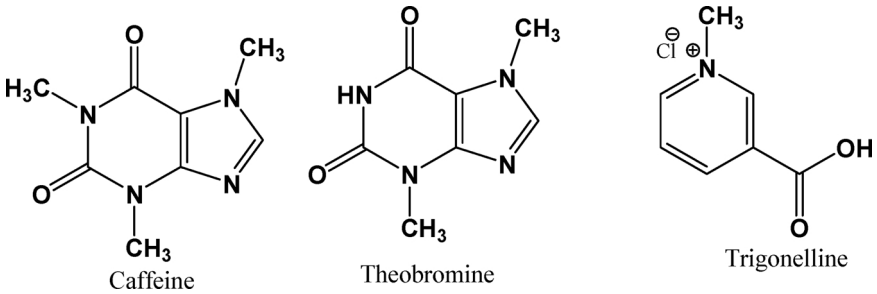

Fig. 1. Structures of the three coffee alkaloids studied.

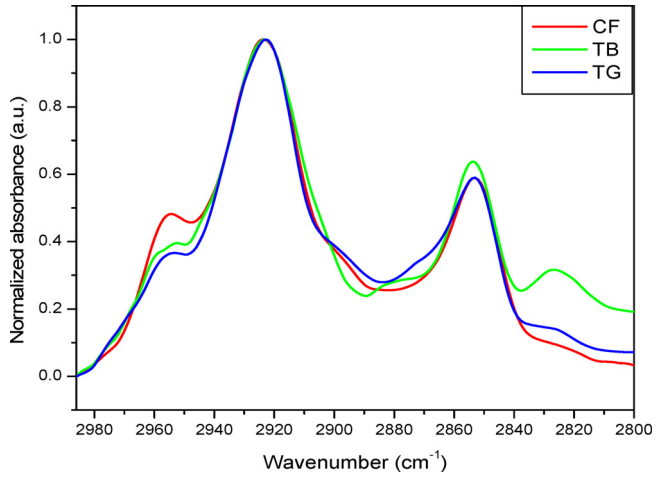

Fig. 2. FT-MIR-ATR absorption spectra of the three coffee alkaloid standards dissolved in water in the region 2986 – 2800 cm^{-1} (CF – caffeine, TB – theobromine, TG – trigonelline).

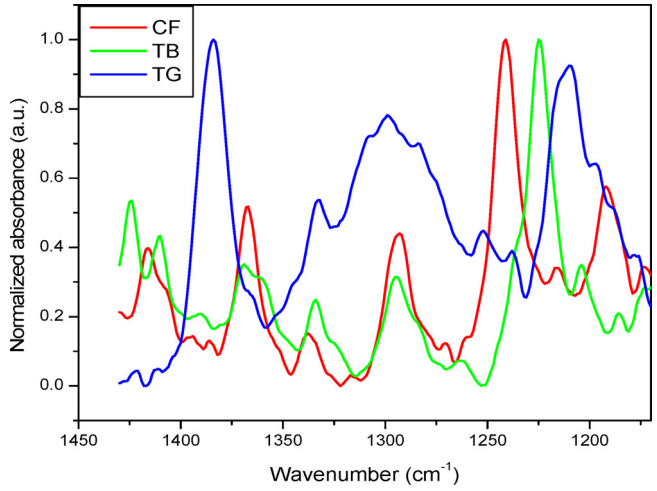

Fig. 3. FT-MIR-ATR absorption spectra of the three coffee alkaloid standards dissolved in water in the region 1430 – 1170 cm^{-1} (CF – caffeine, TB – theobromine, TG – trigonelline).

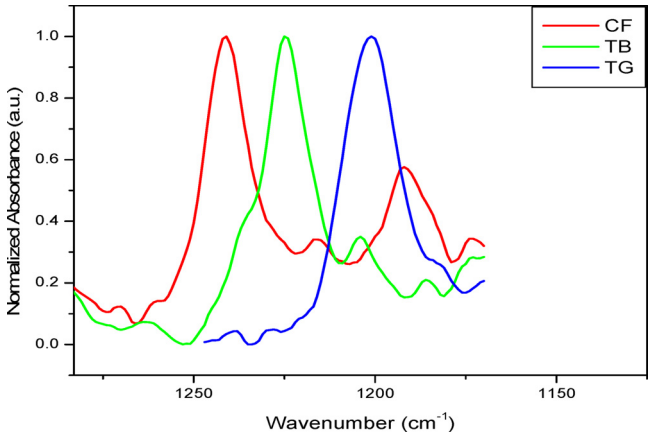

Fig. 4. FT-MIR-ATR absorption spectra of the three coffee alkaloid standards dissolved in water in the selected range (1270 – 1170 cm^{-1}) (CF – caffeine, TB – theobromine, TG – trigonelline).

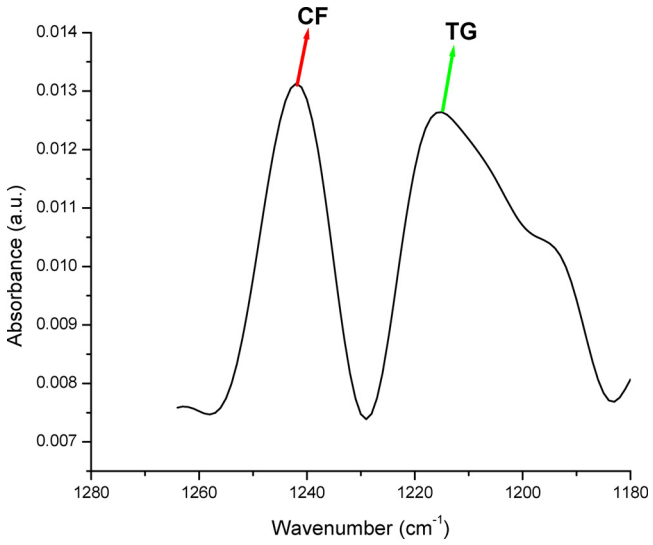

Fig. 5. FT-MIR-ATR absorption spectrum (frequency filtered, low pass = 0.08 Hz) for caffeine and trigonelline in green coffee beans dissolved in water (CF – caffeine, TG – trigonelline).

fact that interest in coffee quality assessment is provoked by the need to supply the consumer with a consistently high quality product at a reasonable price to win competitive market [4,6].

Many analytical methods were applied for characterization and determination of alkaloids in coffee. Some of them are ultraviolet visible spectroscopy [14–16], fluorescence spectroscopy [17], Fourier transform near infrared spectroscopy [17,18], high performance liquid chromatography [7,10,19], liquid chromatography combined with mass spectrometry [20] and electroanalytical methods such as voltammetry [11,21,22]. Most of the reported methods require carcinogenic organic solvents for extraction, time-consuming sample preparation procedures, more than one chromatographic step, high skilled technician and high price which make them inconvenient. In contrast to these, spectroscopic methods have got more attention due to their rapidity, cost effectiveness, simplicity, accuracy, precision reliability and ability to measure multiple components without tedious sample preparation. These methods are also highly applicable to determine food composition like coffee and are suitable for routine analysis [4,23].

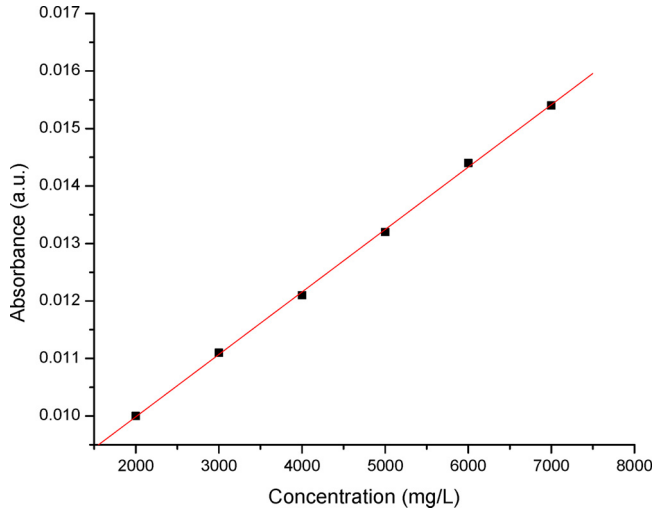

Fig. 6. Plot of concentration vs absorption of caffeine standard dissolved in water (calibration curve for caffeine). The standard solutions were scanned in triplicate in the free spectral range (1430 − 1170 cm^{-1}). The absorption spectral data were collected at 1243 cm^{-1}.

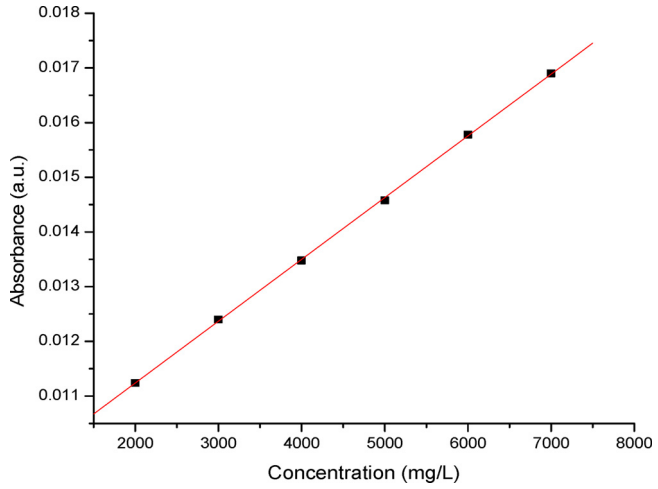

Fig. 7. Plot of concentration vs absorbance for trigonelline standard dissolved in water (calibration curve for trigonelline). The standard solutions were scanned in triplicate in the free spectral range (1430 − 1170 cm^{-1}). The absorption spectral data were collected at 1212 cm^{-1}.

Very recently, a non-destructive analysis of caffeine and trigonelline on single green coffee beans by hyperspectral imaging (HSI) has been reported [24]. The intra-bean distribution of coffee constituents was analysed in Arabica and Robusta coffees on a large sample set from 12 countries, using a total of 260 samples. Individual green coffee beans were scanned by reflectance HSI and then the concentration of caffeine and trigonelline analysed with a reference method (HPLC-MS). Large variations in caffeine and trigonelline were found between different species and origin, but also within beans from the same batch [24].

Table 2
Percentage of caffeine and trigonelline found in green coffee beans by the proposed FT-MIR-ATR method.

Origin of coffee sample	Caffeine mean ± SD (% w/w, n = 3)	Trigonelline mean ± SD (% w/w, n = 3)
Abosto Area	0.97 ± 0.04	0.95 ± 0.04
Gedeo zone	1.04 ± 0.05	0.96 ± 0.01
Wendogenet	1.01 ± 0.03	0.93 ± 0.06
Kefa Zone Bonga	1.13 ± 0.05	1.10 ± 0.03
Wolaita Sodo	0.86 ± 0.02	0.83 ± 0.04
Jimma Zone	0.88 ± 0.03	0.89 ± 0.02
Silte Kafa	1.04 ± 0.02	1.03 ± 0.03
Jimma Ber	1.00 ± 0.02	0.96 ± 0.03
Malokoza Laha	0.94 ± 0.04	0.96 ± 0.05
Asendabo	0.87 ± 0.03	0.88 ± 0.04
Dekulilu Tayarea Koza	0.86 ± 0.02	0.87 ± 0.03
Malokoza Masharo	0.90 ± 0.03	0.89 ± 0.04
Koisha-Konta	0.94 ± 0.04	0.95 ± 0.05
Wato Asendabo	0.84 ± 0.02	0.83 ± 0.04
Kasha-Chida	0.88 ± 0.03	0.89 ± 0.02
Kaffa	1.09 ± 0.04	1.07 ± 0.03
Benishangul	1.15 ± 0.03	1.13 ± 0.04
Harar	0.99 ± 0.03	1.03 ± 0.04
Wollega	1.11 ± 0.01	1.08 ± 0.02
Yirgachefe	1.05 ± 0.03	1.07 ± 0.04

Among the spectroscopic methods UV-VIS spectroscopy is the most widely used for the determination of caffeine in different types of coffee samples. But UV-VIS spectroscopy is not applicable for the direct determination of caffeine in aqueous extract of coffee beans because this method uses organic solvents like dichloromethane [14,16]. Furthermore, there is no any report for the determination of trigonelline and theobromine in green coffee beans by UV-VIS spectroscopy. The literature surveys also revealed that there is no study reported for the determination of trigonelline and theobromine in green coffee beans by FT-NIR or FT-MIR spectroscopy. Fourier transform infrared attenuated total reflectance (FT-MIR-ATR) spectroscopy has got an acceptance for its non-destructive and rapid profiling of chemical compounds and was used in quality control [25]. It has also reflected an effective performance in the identification of coffee varieties and blends [23]. However, there is no report for the determination of caffeine, theobromine and trigonelline in green coffee beans by FT-MIR-ATR spectroscopy. Hence the objective of the present study was to develop a selective FT-MIR-ATR method for the determination of alkaloids in green coffee beans.

2. Methodology

2.1. Chemicals and samples

The chemicals used were standard caffeine (J.T. Baker Chemical Company, Phillipsburg, NJ, USA), standard theobromine (Sigma-

Table 1
Recovery results of caffeine and trigonelline by the developed FT-MIR-ATR method.

Origin of coffee sample	Type of alkaloid in coffee sample	Amount of alkaloid before spiking (mg/L)	Amount of added (mg/L)	Amount of alkaloid found (mg/L)	Recovery (%) (n = 3)
Abosto (Sidama)	Caffeine	9.04×10^3	1000	9.98×10^3	94 ± 7
	Trigonelline	8.10×10^3	1000	9.03×10^3	93 ± 3
Gedeo zone	Caffeine	9.15×10^3	1000	10.10×10^3	95 ± 5
	Trigonelline	8.07×10^3	1000	9.07×10^3	100 ± 6
Wendogenet (Sidama)	Caffiene	8.99×10^3	1000	9.92×10^3	93 ± 2
	Trigonelline	8.00×10^3	1000	9.01 × 103	101 ± 2

Table 3
Comparison of LOD, LOQ, RSD and percentage recovery of the developed method with the literature methods.

Compound	LOD (mg/L)	LOQ (mg/L)	RSD (%)	Recovery (%)	Method	References
Caffeine	3.6	10.9	1.7	95 ± 7	HPLC-DAD-MS	[19]
Trigonelline	3.8	11.4	1.0	102 ± 2		
Caffeine	53	-	4.2	-	^{1}H-NMR	[28]
Trigonelline	23	-	2.4	-		
Caffeine	0.85	1.52	< 0.05	-	UV-VIS	[29]
Trigonelline	-	-	-	-		
Caffeine	0.17	-	0.20 - 6.2	99 ± 3	HPLC-UV	[7]
Trigonelline	0.26	-	1.1 - 5.0	99 ± 5		
Caffeine	1.1	-	-	-	Voltammetry	[30]
Trigonelline	-	-	-	-		
Caffeine	140	470	3.0	93 ± 5	FT-MIR-ATR	This study
Trigonelline	100	330	4.3	98 ± 4		

Aldrich, Italy) and standard trigonelline hydrochloride (Sigma-Aldrich, Switzerland). Twenty Arabica green coffee bean samples were collected from the main coffee growing areas (East, South and West) of Ethiopia. Distilled water was used as a solvent throughout the study.

2.2. Instrument and apparatus

Standards and samples were weighed using electronic balance (ARA520, OHAUS CORP., China). Magnetic stirrer with a hot plate (Model 04803-02, Cole Parmer Instrument Company, 230 V, 50 Hz, and 2 Amp, USA) was used to dissolve the standards and samples. Blending device mortar and pestle was used for grinding green coffee bean samples and 300 μm siever (Chicago, ILL. 60656 USA) was used for sieving the ground sample powder. Fourier transform infrared spectrometer (Perkin Elmer, spectrum 65 Spectrophotometer, USA) with a sample holder of zinc selenide crystal in the attenuated total reflectance (ATR) mode was used in the study.

2.3. Preparation of standard alkaloid solutions

Standard solutions of caffeine and trigonelline were prepared by dissolving 1 g of the standards separately in 80 mL distilled water in 100 mL separate volumetric flasks. Solubility was facilitated by magnetic stirrer with hot plate (~ 40 °C) for caffeine since it is slightly soluble in water but trigonelline is completely soluble in water without applying stirrer on hot plate. The caffeine solution was allowed to cool to the room temperature (22 °C) and diluted with distilled water up to the mark. The concentration of the stock solutions was 10,000 mg/L. Standard solution of theobromine was prepared by dissolving 1 g of the standard in 300 mL distilled water in a 500 mL beaker by stirring on a magnetic stirrer with hot plate (~ 40 °C) since it is not sufficiently soluble in water.

The working standard solutions for calibration curve were prepared by diluting 5.0, 7.5, 10, 12.5, 15, 17.5 mL of caffeine and trigonelline stock solutions to 25 mL with distilled water in separate volumetric flasks to get concentrations of 2000, 3000, 4000, 5000, 6000 and 7000 mg/L of caffeine and trigonelline. About 1 mL of the standard solution was placed in the zinc selenide crystal to fully cover its surface for the spectral measurements. The working standard solutions were scanned in triplicate in the free spectral range (1430 − 1170 cm^{-1}) selected for this study. The absorption spectral data were collected from their typical absorption peak maximum obtained at around 1243 and 1212 cm^{-1} for caffeine and trigonelline, respectively for plotting the calibration curves.

2.4. Preparation of coffee samples

The twenty green coffee bean samples were crushed and powdered using mortar and pestle. The powder was screened via 300 μm sieve to have homogeneous texture. A 2.5 g of the ground coffee powder was transferred in to a 500 mL beaker and 100 mL of distilled water was added to it. The mixture was stirred for one and half hour using magnetic stirrer with hot plate to get dissolved the alkaloids in the solution. The solution was filtered by Whatman filter papers to separate the insoluble particles from the solution. The filtrate (clear solution) was used for quantitative determination of coffee alkaloids by the developed method (FT-MIR-ATR).

2.5. Determination of caffeine and trigonelline in green coffee beans

About 1 mL of the sample solution was placed in the zinc selenide crystal to fully cover its surface for the spectral measurements. The sample solutions were scanned in triplicate in the free spectral range (1430 − 1170 cm^{-1}) which clearly showed well separated absorption maximum peak for caffeine and trigonelline. The absorption spectral data were collected from their typical absorption peak maximum obtained at around 1243 and 1212 cm^{-1} for caffeine and trigonelline, respectively, for their quantification.

2.6. Determination of LOD and LOQ

The detection and quantification limits of the developed method (FT-MIR-ATR) were determined for the caffeine and trigonelline by preparing 150 mg/L of standard solutions of each compound and scanned ten times in the selected spectral range (1430 − 1170 cm^{-1}) by adjusting the accumulation scan at forty (40). Then the LOD and LOQ of the FT-MIR-ATR method were computed three times and ten times, respectively, of the standard deviation of the background signal from ten (10) measurements divided by the slope of the calibration equation.

3. Results and discussions

3.1. Investigation of FT-MIR-ATR spectra of coffee alkaloid standards

The FT-MIR-ATR spectroscopy was used to investigate the coffee alkaloid standards (caffeine, theobromine and trigonelline) by using water as a solvent. The compounds were scanned in two spectral ranges (2986 − 2800 cm^{-1} and 1430 − 1170 cm^{-1}) to find the region of their distinctive absorption (Figs. 2–4). In the region 2986 − 2800 cm^{-1}, the compounds of interest absorbed at around 2924 cm^{-1} and 2854 cm^{-1} due to -C-H asymmetrical and symmetrical vibrational stretching, respectively and overlapped with each other in the region. Hence, it was not possible to differentiate the three compounds and this region cannot be used for quantification of caffeine, theobromine and trigonelline in the green coffee beans using distilled water as a solvent. Therefore, the spectral range 2986 − 2800 cm^{-1} was not used in this study. The three compounds showed different absorption peaks in the range 1430 − 1170 cm^{-1} and can be determined individually without interference from each other. Hence, the spectral range 1430 − 1170 cm^{-1} was selected for quantification of the three alkaloids in this study. The

three coffee alkaloids exhibited an absorption peak at around 1243 cm^{-1}, 1225 cm^{-1} and 1211 cm^{-1} for caffeine, theobromine and trigonelline, respectively. The absorption was due to the -C-N stretching. The strong absorption peak shown at around 1385 cm^{-1} (Fig. 3) is the typical characteristic $–CH_3$ umbrella vibrational stretching of the two alkaloids (caffeine and trigonelline). Besides, 1243 cm^{-1} and 1212 cm^{-1} absorption peaks were found in the spectrum of aqueous solution of green coffee beans for caffeine and trigonelline (Fig. 5).

3.2. Evaluation of analytical parameters of FT-MI'R-ATR method

Analytical parameters of FT-MIR-ATR method for the determination of the coffee alkaloids was evaluated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy (recovery). The calibration curves (Fig. 6 and 7) were linear in the range 2000 − 7000 mg/L for caffeine and trigonelline, respectively, with calibration equations of $y = 1 \times 10^{-6}x + 0.007$ and $y = 1 \times 10^{-6}x + 0.009$, where, y indicates absorption and x designates concentration in mg/L. The magnitudes of the correlation coefficient (R^2) were 0.9997 and 0.9999, respectively, for caffeine and trigonelline which show strong relationship between the concentration and absorbance in the linear ranges.

The LOD of the developed method was 140 mg/L for caffeine and 100 mg/L for trigonelline. The limit of quantification (LOQ) was 470 mg/L and 330 mg/L for caffeine and trigonelline, respectively. Besides, the precision of the method was evaluated by scanning the lower end of calibration curve's concentration ten times and calculating the relative standard deviation (RSD) and the results found were 3.0 and 4.3% for caffeine and trigonelline, respectively. Furthermore, the accuracy of the developed analytical method was also evaluated by spiking 4 and 5 mL of 1000 mg/L caffeine and trigonelline standard solutions, respectively, to 10 mL solution of the sample solution. The percentage recoveries were found in the range 93 − 101% for the two alkaloids. The results of recoveries are given in Table 1.

3.3. Application of newly developed FT-MIR-ATR method for determination of caffeine and trigonelline in green coffee beans

The amount of caffeine and trigonelline in aqueous extract of twenty different real samples of green coffee beans were determined by scanning the spectra of sample solution in the range 1430 − 1170 cm^{-1} (Fig. 5) at 40 accumulation scans and calculated the concentration using the calibration equations. The percentage of caffeine and trigonelline were found to be 0.84 − 1.15% (*w/w*) and 0.83 − 1.13% (*w/w*), respectively. The results of the present study are given in Table 2. The results are comparable with the results reported in the literature 0.87 − 1.38% (*w/w*) for caffeine and 0.98 − 1.32% (*w/w*) for trigonelline [7], 0.8 − 1.4% (*w/w*) for caffeine and 0.6 − 1.2% (*w/w*) for trigonelline [26], 0.80 − 1.40% (*w/w*) for caffeine and 0.60 − 1.20% (*w/w*) for trigonelline [27], 0.90 − 1.10% (*w/w*) for caffeine [14] and 0.62 − 1.16% (*w/w*) for caffeine [16].

Determining the amount of the third coffee alkaloid (theobromine) in FT-MIR-ATR method by using distilled water as a solvent was not possible since it is not sufficiently soluble in water. Not only this but also, the amount of theobromine is very small (0.0048 − 0.0094% (*w/w*)) in the green coffee beans as reported by Mehari et al. [7]. This concentration is below the detection limit of the developed FT-MIR-ATR method. Hence, theobromine was not quantified in the green coffee beans because its concentration is below the detection limit of the developed method (FT-MIR-ATR). But, it is possible to quantify theobromine in other real samples like chocolate in which its amount is high.

3.4. Comparison of analytical parameters of proposed FT-MIR-ATR method with the methods reported in literature

The comparison of analytical parameters of the proposed FT-MIR-ATR method with the methods reported in the literature is summarized in Table 3. As can be seen in Table 3, the analytical parameters such as RSD and percentage recovery of caffeine and trigonelline of the developed method are comparable with most of the reported methods. However, LOD and LOQ of the present method for caffeine and trigonelline are much higher than most of the reported methods which indicates that the developed FT-MIR-ATR is less sensitive than most of the reported methods. Despite of this, since the contents of caffeine and trigonelline in the green coffee beans are generally in the range 0.6 − 1.4%, the two compounds can easily be determined by the proposed method. Furthermore, the developed method has paramount significance over most of the other methods. (1) It is rapid, cost effective, non-destructive, accurate, precise, reliable and able to measure multiple components in simultaneous manner without any sample preparation. (2) The proposed method is applicable for the direct determination of alkaloids in aqueous extract of green coffee beans which matches with direct consumption of coffee beverage. In contrast to this, most of the reported methods require carcinogenic organic solvents for extraction of coffee alkaloids, time consuming sample preparation procedures, high skilled technician and high price which make them inconvenient.

4. Conclusions

A non-destructive, fast, simple, inexpensive and reliable FT-MIR-ATR method was developed for the simultaneous and direct determination of alkaloids in the aqueous extract of green coffee beans. Caffeine and trigonelline were quantified in green coffee beans but not theobromine due to its lower amount in the green coffee beans which is below the detection limit of the developed method. The developed method revealed comparable recoveries and reproducibility with the reported results of other methods. Though LOD and LOQ of the present method for caffeine and trigonelline are much higher than the reported methods, the proposed method has the advantage over the other methods that it does not involve use of any organic solvent and any sample preparation except dissolution of powdered green coffee beans in water.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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