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A Review: The Use of Chromatographic Methods in Caffeine Analysis during 2000-2020

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Abstract:
Background: *Caffeine is an alkaloid compound derived from xanthine that is naturally found in coffee beans. Caffeine has long been added to a variety of products, foods, beverages, and dietary supplements. Different concentrations of caffeine in raw materials and food products encourage researchers to develop more effective and accurate analytical methods in determining caffeine content. This study aims to provide an overview of the use of chromatographic methods in caffeine analysis.*
Methods: *The preparation of this article uses a literature study method from scientific books and international journals in the last 20 years (2000-2020) with the keywords Caffeine, Validation Methods, HPLC, and TLC.*
Results: *From the results that have been traced, it was found that the instrument used to determine caffeine levels can use liquid chromatography techniques, namely HPLC and TLC-Densitometry. Process validation is evidence that guarantees research results that meet the following requirements: specification, linearity, sensitivity, limit of detection (LOD) and limit of quantification (LOQ).*
Conclusion: *The results of reviews of several journals found that HPLC with various experimental conditions was the most used method in caffeine analysis*
Key Word*: Caffeine, Validation Method, HPLC, TLC*

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I. Introduction

Caffeine has long been added to various products, foods, beverages, and dietary supplements with a function as a central nervous system stimulant (1). The amount of caffeine consumed as well as consumer demographics have long been a concern worldwide(2). Caffeine is one of the most widely consumed drugs in the world, surpassing alcohol and nicotine to improve cognitive function and improve *mood* bad for consumers(3).

The moderate dose of caffeine is 400 mg/day. This dose can improve mood, alertness, and physical endurance, and can improve cognitive function. Caffeine is generally positive for moderate doses but can lead to a clinical diagnosis of anxiety, physical tremors, twitching, and cognitive impairment at a higher level(3).

Pharmacologically, caffeine is an adenosine receptor antagonist. Caffeine has a structure similar to adenosine which binds to receptors adenosine the cell surface wall without causing the receptor activation. This results in a decrease in adenosine activity increasing the activity of the neurotransmitter dopamine. This increase in dopamine activity is the basis for the stimulatory effect of caffeine. Caffeine is efficiently and quickly absorbed by the stomach and small intestine, with peak plasma levels occurring within the first 30 minutes(4).

Caffeine is an alkaloid compound derived from xanthine (purine base) which is naturally found in coffee beans. According to the literature, every 100 mg of coffee powder contains an average of 11.59 mg of caffeine(5). Caffeine with the chemical name 1,3,7-trimethylxantin or 1,3,7-trimethyl 2,6 purine dioxin and with the molecular formula $C_8H_{10}N_4O_2$ has a molecular weight of 194.19. Caffeine at room temperature is a colorless, odorless, and slightly bitter taste powder. Caffeine will dissolve in 50 parts of water, 6 parts of water at 80ºC; 1.5 parts of boiling water; 75 parts of alcohol; 25 parts of alcohol at 60ºC; 6 parts chloroform and 600 parts ether. Caffeine is soluble in boiling water but at room temperature the best solvent is chloroform. The structure of caffeine can be seen in Figure 1(6).

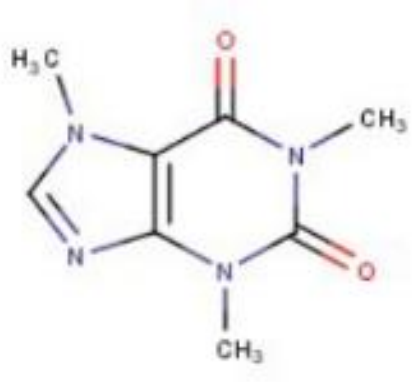

Figure 1. Chemical structure of caffeine(6)

The concentration of caffeine in food and beverage products varies. The large variety of ingredients added to a food or beverage product that contains caffeine has prompted researchers to develop effective and accurate chromatographic methods for caffeine analysis. Caffeine is usually considered safe when consumed moderately. Moderate caffeine consumption can improve freshness, concentration, fatigue, and athletic performance(7). Caffeine is one of the many elements in food that can have physiological effects. Scientific and historical evidence showing healthy adults can consume 400 mg/day of moderate caffeine which has no adverse effects on this population(8).

Consumption of caffeine in low doses provides several benefits. In a study conducted by Smit and Rogers, they explained that consumption of 100 mg of caffeine can have positive effects on cognitive function, such as restoring one's awareness and attention and maintaining reduced cognitive function due to lack of sleep. Excess caffeine consumption can also negatively impact sleep patterns, attention, and daytime sleepiness(9). The study by Ahluwalia and Herrick using NHANES data reported that approximately 75% of US children between the ages of 6 and 19 consume caffeine, with the average consumption of 25 mg/day in children 2–11 years of age and 50 mg/day in children 12–17 years(10). Another study that also used the NHANES dataset reported average caffeine consumption in children and adolescents taking 35 mg/day, with ages 4–8 years taking 15 mg/day, ages 9–13 years taking 26 mg/day, and ages 14–19 years taking 61 mg/day(11). The purpose of this *review article* is to provide an overview of caffeine analysis, especially the chromatographic method that has been carried out from 2000-2020. With this review article, it is hoped that readers will be easier to understand and choose the right and accurate chromatographic method for caffeine analysis.

II. Method

The preparation of this article uses techniques in the form of literature studies by searching for sources in the form of officially published books, national and international journals in the last 20 years (2000-2020) data searches were carried out online using the keywords "*analysis*", "*chromatography*" and "*caffeine*". The search was carried out through several official and trusted websites in the form of *Scient Direct*, NCBI, *Researchgate*, Pubmed, *Google Scholar*, and *E-books* published and reliable. This study was prepared by conducting a literature review of two methods commonly used to analyze caffeine in food and beverage products, namely High-Performance Liquid Chromatography (HPLC), Densitometric Thin Layer Chromatography (TLC-Densitometry / HPTLC).

III. Result

Many caffeine analysis methods have been developed and researchers also make it easy to determine the presence or absence of caffeine in the product and determine how much it is in the product in question. Of the many analytical methods that have been developed, chromatographic methods are quite popular. The chromatography method that is often found in determining caffeine content in food, beverages, and pharmaceutical preparations is high-performance liquid chromatography (HPLC), which is an extension of conventional column chromatography and thin-layer chromatography equipped with densitometry. The following are the search results for literature studies related to the validation method using chromatography :

Table 1. Caffeine analysis methods from several research results

No	Method	Mobile Phase	Column	Linearity	Precision	Flow Rate	LOD	LOQ	Specificity	Reference
1.	HPLC–(DAD)	THF(tetrahidrofuran) : Acetonitrile (90: 10, v/v)	Zorbax Eclipse XDB-5µm C8 column 150x4.6 mm	0,2 $x10^{-3}$– 0,1 mg/mL	0,11 -0,78 %	0.8 mL/min	0.07 mg/mL	0.2 mg/mL	273 nm	(12)
2.	RP-HPLC	Buffer dibasic phosphate solution : acetonitrile (93:07, v/v)	ODS C18 (250 mm×4mm i.d., 5 µm)	0,024-0,036 mg/mL	0,04 %	1.5 mL/min	2.4 mg/mL	3.3 mg/mL	215 nm	(13)
3.	RP-HPLC	Consists of buffer Methanol	Cosmosil C-18 (250×4.6mm , 5.0µm)	0,03-0,15 mg/mL	0,2621 %	1.0 mL/min	14.51 mg/mL	43.98 mg/mL	220 nm	(14)
4.	RP-HPLC/UV detection	A (1% acetic acid: acetonitrile) B (1% acetic acid : water)	SS-C18 (150 mm 4.6 mm i.d., 3 µm particle size	0,21 $x10^{-3}$-5,25 $x10^{-3}$ mg/mL	2,6 - 4,3 %	0,4 mL/min	0,06 $x10^{-3}$ mg/mL	0,21 $x10^{-3}$ mg/mL	270 nm	(15)
5.	HPLC–(DAD)	Acetonitrile : 0.1% H3PO4 (30:70)	reverse-phase C18 column (4.6 × 250 mm, 5 µm; Thermo Scientific)	0,001 – 0,02 mg/mL	1,8 %	1.5 mL/min	0,05 $x10^{-3}$ mg/mL	0,16 $x10^{-3}$ mg/mL	220 nm	(16)
6.	HPLC	methanol: distilled water (30:70)% (v/v)	Shim-pack VP-ODS with internal diameter 4.6 mm and length 250 mm	0,01-0,1 mg/mL	0,5%	1.3 mL/min	0,023 $x10^{-3}$ mg/mL	0,07 $x10^{-3}$ mg/mL	270 nm	(17)
7.	HPLC-UV	deionized water : acetonitrile (9:1, v/v).	a Zorbax Bonus-RP column(Agilent, 2500 × 2.1 mm, 3.5 µm).	0,1 $x10^{-3}$ – 0,2 mg/mL	3-5 %	1ml/min	0,03 $x10^{-3}$ mg/mL	0,1 $x10^{-3}$ mg/mL	220 nm	(18)
8.	RP-HPLC	Methanol : glacial acetic acid (50:50 % v/v)	HiQSilC18 Column (250 x 4.5mm, i.d. 5 ìm)	2,5 $x10^{-3}$-0,015 mg/mL	0,505-1,427 %	1 mL/min	3.3 σ/Sand	10 σ/S	224 nm	(19)
9.	HPLC-DAD	water acidified with 0.1% formic acid as the weak phase (phase A) and methanol acidified with0.1% formic acid as the strong phase (phase B)	Kinetex C18 100A column (5 µm particles, 4.6 mm internal diameter and 25 cm length; Phenomenex, Torrance, CA)	-	-	1 mL/min	3,3 $x10^{-3}$ mg/mL	4,6 $x10^{-3}$ mg/mL	280 nm	(20)
10.	RP-HPLC	Destilate water : methanol (60:40)	C18column (4.5 mm x 250 mm; 5 µm particle size)	0,012 - 0,028 mg/mL	0,24-0,87 %	1 mL/min	0.152 x 10^{-3} mg/mL	0.461 x 10^{-3} mg/mL	272 nm	(21)
11.	HPLC	100%	Chromolith®	0,015$x10^{-3}$	-	-	0.066x	0.2x	270 nm	(22)

		methanol	Performance RP-18e (4.6 x 10 mm, 5 µm) column	– 0,4 mg/mL			10^{-6} mg/mL	10^{-6} mg/mL		
12.	HPLC	Acetonitrile : water (25:75 v/v)	Bio SiL HL C18, 5 mm, 250 x 4.6 mm column	0,01-0,08 mg/mL	1,21 %	2,0 mL /min	$9x10^{-5}$ - $1,7x10^{-4}$ mg/mL	$2,5x10^{-4}$ – $5,6x10^{-4}$ mg/mL	207 nm	(23)
13.	HPLC	Water : acetonitrile : methanol (83:6:11)	Monolithic Rp-18 e 100 – 4.6 mm (Merck KGaA, Germany) and BDS Hypersil gold C-18 (4.6 mm I.D. x250 mm) columns	$0,1x10^{-3}$ – 0,08 mg/mL	1,8 %	1,4 mL/min	0,17 $x10^{-3}$ mg/mL	0,51 $x10^{-3}$ mg/mL	280 nm	(24)
14.	HPLC	-	Zobax-SB-C_8 reversed-phase packed column, German, Agilent Technology (4.6 mm x 150 nm: 5 µm)	0,005-0,025 mg/mL	1,15-1,28 %	1 mL/min	$0,63x10^{-3}$ mg/mL	1,9 $x10^{-3}$ mg/mL	272 nm	(25)
15.	TLC-Densitometry	Methanol : ethyl acetate : ammonia 25% 13:77: 10 (v/v/v)	silica gel 60 F254	0,18–0,48 mg/mL	< 5,7 %	-	5,43 $x10^{-3}$ mg/mL	18,11 $x10^{-3}$ mg/mL	274 nm	(26)
16.	(HPTLC)	Chloroform : Acetone (8.8:1.2)	Silica gel 60 F254	-	< 1 %	-	0,011 mg/mL	0,042 mg/mL	254 nm	(27)

IV. Discussion

High-Performance Liquid Chromatography (HPLC) is used in drug quality control because of its sensitivity, reproducibility, and specificity. In a chromatographic analysis, the main problem of this method involves optimization of experimental conditions such as column type selection, column temperature, variation and composition of mobile phases as well as analysis of wavelength selection (28). In a method of analysis, it is necessary to validate the method as an act of assessment of a parameter to prove that the parameter meets the requirements for use. The analysis parameters that are often considered invalidating a method include linearity and concentration range, precision, specificity, the limit of detection, and limit of quantification (LOD and LOQ)(29).

The linearity of a method is a measure of how well the response vs concentration calibration plot approaches a straight line. Linearity can be assessed by taking a single measurement at multiple concentrations of the analyte. The data is then processed using a linear least squares regression. The resulting plot slope, intercept, and correlation coefficient provides desired information about linearity(30). Good linearity is indicated by a value of $r^2 > 0.999$ and an intercept value of less than 2%(13). Scrupulosity *(precision)* is a measure that indicates the degree of fit between the individual test results, measured by the spread of individual results from the average if the procedure is applied repeatedly in samples taken from a homogeneous mixture(29). RSD is a measure of precision. RSD allowable range <2%(13). specificity (*detection*) of a method is its ability to only measure certain substances carefully and precisely in the presence of other components that may be present in the sample matrix(29). Measurement of the maximum wavelength of caffeine was carried out using an ultraviolet spectrophotometer at a wavelength of 200-400 nm(31). The limit of detection (LOD) is the smallest number of analytes in the sample that can be detected which still gives a significant response compared to blanks. The detection limit is a limit test parameter. The limit of quantitation (LOQ) is a parameter in microscopic analysis and is defined as the smallest quantity of analyte in a sample that can still meet the criteria of being careful and careful(29).

In a literature study conducted on the effect of flow rate and mobile phase composition on retention time (tR), peak width (W50), and some theoretical plates for caffeine were studied using standard solutions, Bransivla obtained measurements of various samples of food, beverage, and natural products. with the results shown, namely a strong correlation to the value of caffeine which indicates the effectiveness and high selectivity of the method used. The use of the C8 column resulted in better resolution, intensity, shape, and peak symmetry. The lowest calculated concentration (LOQ) with acceptable accuracy and precision was 0.2 mg/L. Furthermore, the LOD defined as S /N> 3 was 0.07 mg caffeine/L. Intermediate precision was evaluated over three days (repetition inter-day) using standard solutions. This solution (0.2-10.0 µL) was injected daily under the same conditions and the results were used for a repeat study. The solution was stored at room temperature (25 ± 2 ° C), reducing the recovery value from 100.42% to 96.6% for all compounds. When refrigerated in the dark, recovery ranged from 100.42% to 98.7% over three days for all compounds. The RSD value in this study shows that precision is acceptable with a retention time of 0.11% -0.78% with a peak time of 0.80% -2.06% (12).

The determination of the linearity test shows the response of the detector system measurement which is linear over the range used in the method. Linearity is determined using a calibration chart with an increase in the number of standard solutions. The linearity of the method is observed within the range of the expected concentration indicating its suitability for analysis. The linearity reduction of caffeine is shown in the HPLC method, which is in the range of 26-34 µg/mL, for the sensitivity of the method to the LOD and LOQ values of caffeine, namely 2.4 mg/mL and 3.3 mg/mL. The precision of this method is determined by intra-day and inter-day precision. It is expressed as% RSD of a series of measurements. Experimental values were obtained for caffeine repetition in the sample. The results obtained showed% RSD <2, which means that the precision is acceptable(13).

In a study conducted by Madhusudan and the HPLC method using a Cosmosil C-18 column (250 × 4.6mm, 5.0µm), a mobile phase consisting of a buffer and methanol at a flow rate of 1.00 mL/minute and gradient determination with a UV detector at 220 nm. At a flow rate of 1.00 mL/min gave acceptable retention times, and good resolution for caffeine at 13.85 min and 9.21 min. Precision is measured in repeated measurements, carried out by injecting the standard solution six times (n = 6) and measuring the peak. The RSD obtained in this study was 0.706 for caffeine (CAF). This shows that the accuracy of the method is acceptable because the RSD is not more than 2%(14).

Linearity is evaluated by constructing an external calibration curve for each compound. The calibration curve is obtained by plotting the peak area of the analyte versus its concentration for different concentrations. Each concentration of the mixed standard solution was injected in triplicate and the regression parameters were calculated. These results suggest that external standard calibration can be applied for quantitative purposes. In the study conducted by Bae, the caffeine linearity test was shown in the HPLC method, which was in the range 0.21-5.25mg / L, for the sensitivity method developed, was assessed by determining the detection limit (LOD) and quantification (LOQ). LOD and LOQ under the current chromatographic conditions were calculated based on the response and slope of each regression equation at 3:1 and 10:1 signal-to-noise (S / N) ratios, respectively. Selectivity was evaluated in the absence of interference in the same chromatographic window as that examined in the mixed standard solution and analysis method blank (solvent extraction). No annoying peaks were observed in the blank chromatogram at the quantification wavelength. The accuracy of the developed method is determined by measuring Intra and inter-day precision. For intra-day precision, the mixed standard solution was analyzed for six replications within 1 day, whereas for inter-day precision, solutions were examined in triplicate for 3 consecutive days. Precision is expressed as the percentage of relative standard deviation (% RSD). The overall% RSD score for Intra and inter-day is less than 4.3%(15).

The optimized method is validated based on the main analytic validation of the parameters. Linearity data, detection, and limit quantification to determine caffeine by the developed HPLC-DAD method are quite concise. In the study conducted by Musa, no annoying peaks were found in the chromatogram due to the sample excipients. Linearity data were validated using ANOVA which showed significant linear regression ($p < 0.05$) and no significant deviation from linearity ($p < 0.10$). Meanwhile, the sensitivity of the chromatography system used was assessed by determining the limits of detection and quantification; the result is considered low and indicates good sensitivity of the method. The detection limit values and quantification limits obtained by the researchers were 0.05 mg/L and 0.16 mg /L with linearity in the range 1.0 - 20.0 mg/L and the precision at each 1 µg/ml, % RSD obtained was 1.8%. This shows that the precision is acceptable(16).

For the sensitivity testing of the Limit Detection Method (LOD) and the Limit Quantification Method (LOQ), the LOD was estimated as Standard Deviation (SD). LOQ was calculated by multiplying the SD with the chromatography system used assessed, by determining the detection limit and quantification of the results considered low and showing good method sensitivity, detection limit values , and quantification limits were at 0.023 mg/L and 0.07 mg/L. In the linearity of the method, the calibration graph was made using 20 µl injection. Six different caffeine concentrations from 10 ppm to 100 ppm were analyzed according to the experimental conditions. Then the calibration curve is set according to the response obtained (peak area) and the caffeine

concentration in the standard solution. The results show a good linear relationship, namely at 10-100 mg/L. The analytical accuracy of this method was assessed by the repeatability of 6 determinations of the 40 ppm caffeine solution and a relative standard deviation of 1.25% calculated for the peak area. The retention time of caffeine is 7.347 minutes, with a relative standard deviation of the RSD 0.5% therefore, in standard solutions, the HPLC method provides a stable retention time.(17).

The analytical performance of this method is under optimal conditions in the linear calibration range, namely in the range 0.1-200 mg/L, and LOD was found to be 0.03 mg/L for caffeine with a determination coefficient of more than 0.995. With a LOQ value of 0.1 mg/L, it is calculated based on the peak of the analyte with 10 times the signal-to-noise ratio. The accuracy of this method was investigated in standard solutions at concentration levels of 0.1 and 200 mg/L caffeine, by performing three replications daily. The same solution was analyzed three times each day, for five days, for the day to day evaluation precision. The duration of the HPLC-UV analysis was 10 minutes. The day-to-day accuracy of this method was investigated in standard solutions at concentration levels of 0.1 and 200 mg/L caffeine, by performing three replications daily. The same solution is analyzed three times each day, for five days, for a day-to-day precision evaluation. The relative standard deviation (RSD) values obtained ranged from 3% to 5% and from 4% to 6%, respectively(18).

In the linearity test carried out by Akshay, the standard solution was diluted to prepare a linearity standard solution in the concentration range of 2.5-15 µg/mL of caffeine. Each solution will be analyzed to plot a calibration curve. The standard deviation (SD), slope, intercept and correlation coefficient of the calibration curve can be calculated to ensure the linearity of this method. For the inter-day variation study, 3 different concentrations (5, 7.5, and 10 µg/mL) were analyzed on 3 consecutive days for the drug and% RSD was calculated. The RSD value obtained was 0.505% -1,427%(19).

In the research conducted by Silvia et al, using the HPLC-DAD analysis method with a mobile phase in the form of water acidified with acid, 0.1% formic as the weak phase (phase A), and methanol acidified with 0.1% formic acid as the strong phase. (phase B). This HPLC-DAD measurement uses a wavelength of 280 nm. The results obtained from the validation method were values of the flow rate of 1 ml/min, LOD, and LOQ 3.3 µg/mL, 4.6 µg/mL(20). For the RP-HPLC method using a mobile phase of water (distilled and demineralized): methanol (60:40) and using a C18 column (4.5 mm x 250 mm; particle size 5 µm). The validation results obtained are as follows. For linearity, it was obtained 12-28 µg/mL, the flow rate of 1 mL/min, LOD and LOQ were 0.152 µg/mL and 0.461 µg/mL, respectively. From the research, it was found that the RSD value was 0.24% -0.87% as a precision parameter(21).

Increasing the amount of water for the mobile phase can cause low peak height and poor peak area in separation using HPLC. Wavelength is another parameter that is considered when using an HPLC instrument equipped with an ultraviolet (UV) detector. Optimization of wavelength is very important because the target compound has the optimum absorbance at its wavelength. The optimum chromatographic signal response was obtained at 270 nm. At this wavelength, a caffeine signal response was obtained with the maximum observed peak area at 1.94 V/s. The wavelength does not affect the elution of caffeine from the column, but only affects the absorbance(22).

The linearity testing method is prepared by a series of solutions prepared by diluting the stock solution with the mobile phase for the final concentration. Each concentration was injected in triplicate and the mean value of the peak area was taken for the calibration curve. A linear response to the peak area ratio was observed in the concentration range of 0.01-0.08 mg/ml for caffeine. The limits of detection (LOD) and quantification (LOQ) were determined by adjusting the standard deviation which was recalculated between days from each calibration standard. LOD is defined as the lowest determinable quantity indicating the presence of the analyte at a given statistical confidence level (3 SD), and LOQ is defined as the lowest measurable quantity over which the analyte can be measured at a certain statistical confidence level (10 SD). Limit of detection, statistically calculated $1.7x10^{-4}$ mg/mL for caffeine. The quantity limit was found to be $5.6\ x10^{-4}$ mg/mL for caffeine. The accuracy of the intra-day method was determined by preparing caffeine standards at different concentrations and the value for each compound was determined by 10 repeated analyzes. The inter-day precision was checked with the same concentration as the intra-day test, and the determination of each compound was repeated day after day for 5 days. The RSD value obtained is 1.21%(23).

Rahim et al conducted a study using tea samples using the HPLC method and using a monolithic column to determine the levels of catechins and caffeine in tea. The detection used is a wavelength of 280 nm. The validation results obtained were linearity of 0.1–80 mg/L, the flow rate of 1.4 mL/min for LOD values of 0.17 mg/L, and LOQ of 0.51 mg/L. Good reproducibility of the peak area (RSD 1.8%) was found for all trials(24).

The caffeine solution was scanned using HPLC against the mobile phase as a blank. It was found that the linearity of the samples between the range 5-25 mg/mL indicated an acceptable linear regression coefficient ($R = 0.9983$). The calibration graph was created using a 20 µL injection loop. Then, a calibration curve is created according to the response obtained (peak area) and the caffeine concentration in the standard solution.

The results show a good linear relationship. precision must be exercised at two distinct levels; repeatability and reproducibility. Reproducible precision results from variations such as different days, analysts, and equipment. The precision criterion for the test method was that the instrument precision and intraassay precision (RSD) would be ≤ 2%. From the quantitative analysis obtained an acceptable relative standard deviation of 1.15% and 1.28% with a stable retention time of 1.84 ± 0.0066 minutes(25).

Florentinus et al validated and determined caffeine levels using the TLC-Densitometry method in energy drinks. The validation results obtained were Linearity of 180–480 µg/mL, LOD 5.43 µg/mL, and LOQ 18.11 µg/mL. The percentage of RSD as a precision parameter of the three levels of caffeine concentration in the two samples was below the maximum limit of the Horwitz RSD, namely 5.7% for high levels and 8% for low and medium levels. These results suggest that this method provides high precision and accuracy for determining caffeine at all concentration levels(26). Another study was also conducted by Sharma et al using the HPTLC method. The mobile phase used is Chloroform: Acetone (8.8: 1.2) and uses a 60 F254 Silica gel column. The wavelength used for detection in this study is 254 nm. The LOD obtained was 11 mg/L and LOQ was 42 mg/L. Intra- and inter-day precision were determined in triplicate on the same day (intra-day) and three different days and the resulting solutions were analyzed in triplicate. % RSD (Intra – inter-day precision) for caffeinated products <1%(27).

V. Conclusion

Caffeine is a chemical compound that is widely consumed in medicinal dosage forms or found in food and beverages. Caffeine is found in coffee and tea. The presence of caffeine in a product can be determined using validated analytical methods. The parameters of the validity of an analysis method can be determined from the parameters of linearity, accuracy, precision, sensitivity, the limit of detection (LOD), and limit of quantification (LOQ). *A review* that has been carried out on several articles listed in research journals as well as from other official books can be concluded that HPLC with various experimental conditions is a widely used method for caffeine analysis.

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