Spectrophotometric determination of Caffeine in Nescafe, Cacao, Cappuccino and Coffee samples collected from some Libyan Markets

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Abstract: Coffee is one of the most popular beverages that are widely consumed in the world. This study aimed to determine the caffeine in Nescafe, Cacao, Cappuccino, Coffee, using a UV-Visible spectrophotometer. In addition, studied caffeine stability in water. In the presented study, seventeen samples were collected from some Libyan markets. A simple, rapid, and, reliable extraction method for the determination of caffeine in the samples using dichloromethane, n-hexane, and methanol as the extracting solvents. The method is validated over a wide linear range of 2 – 10 µg/ml with correlation coefficients being consistently greater than 0.999. The minimum caffeine level was observed in the Nesquik (Cacao), Nestle Italian S.P.A sample (8.66 µg/ml), while the Nestle (Nescafe), Asbania sample showed the highest caffeine content (461.87ppm).The study of caffeine stability indicates that caffeine is stable in water for more than two weeks.

Keywords: Coffee derivatives; Caffeine; Extraction; solvents, and UV-Vis Spectrophotometer.

Introduction:

Coffee is one of the most important commodities in the global economy, and millions of tonnes of it are produced and consumed every year [1]. Coffee involves a variety of pharmacologically active constituents, and it has long been argued whether coffee drinking is beneficial or destructive for cardiovascular disease [2-3]. Caffeine is an important substance in coffee, responsible for its stimulating effects and bitter taste. Consuming too much caffeine can cause people to experience unpleasant short-term symptoms (such as restlessness, insomnia, and tachycardia), and there may be more serious long-term effects such as the increased risk of cardiovascular disease [4]. A naturally occurring alkaloid is found in varying quantities in the leaves, seeds, or fruits of plants. Caffeine is a bitter white crystalline xanthine' alkaloid that acts as a mild psychoactive stimulant drug. It also possesses a weak diuretic action [5-6].The most common sources of caffeine (chemically defined as 1,3,7-trimethylxanthine) are coffee, cacao beans, cola nuts, chocolate, tea leaves, and carbonated beverages. The worldwide consumption of products derived from these natural materials means caffeine is one of the most popular and commonly consumed drugs in the world [7,8]. The methylxanthine caffeine (1,3,7-trimethylxanthine) is the most commonly consumed drug globally [9]. Caffeine is a central nervous system stimulant and is used to reduce physical fatigue and to prevent or treat drowsiness [10]. Caffeine is also a common ingredient of soft drinks such as cola and energy drinks where it is deliberately added as a flavouring agent and to make the drinks addictive [11]. A wide variety of methods have been employed including UV-Visible spectrophotometer and Performance-High Liquid Chromatography (HPLC) being the method of choice by many researchers in determining the caffeine contents in tea, coffee, and beverages [12–20]. The aimed of this study used a rapid, and simple

extraction method that it is modification from other methods recommended isolating and determining caffeine in Nescafe, Cacao and Coffee and its derivatives using UV-Visible Spectrophotometer.

Material and Methods

Apparatus: The absorbance was measured by a DU 800 spectrophotometer (Beckman Coulter, USA) with one cm glass cells.

Chemicals and Reagents: All chemicals, analytical standards, reagents, and solvents used in this study were of analytical grade and highly pure. Caffeine was purchased from (BDH-Analor) with a purity of 99.2 %. Also, other chemicals and solvents were used including (Dichloromethane (CH_2Cl_2) (AlphaChemika™), with a purity of 99.7 %; n- Hexan (SCP), with a purity of 95 %; Methanol (Scharlau), with purity 99.8 %) as the extracting solvents; Tris (hydroxymethyl)-aminomethane BDH Laboratory Supplies; Hydrochloric acid 25% (Riedel-dehaen).

Preparation of standard solutions: A stock caffeine solution of (0.553 µg/ml) was prepared by dissolved 0.0553 g in 100 ml. The working standard solution was prepared by appropriate dilution of the stock. (2-10µg/ml). Tris (hydroxymethyl)-aminomethane: 5.60g of Tris, was dissolved in 500 ml distilled water used in extraction steps. The pH of the solution was adjusted to (pH ≈ 9).

The samples under study (samples collection): Seventeen samples were collected from Libyan markets. Nine of these samples were coffee samples, two were cacao samples, one was Cappuccino and five were Nescafe samples. As shown in table (1).

The caffeine extraction procedure: The extraction procedures were carried out with a slight modification based on the other study [18-22]. 10 g of the samples were weighed and dissolved in distilled water and the volumes were made up to 30 ml with distilled water (sample solution). After 15 min, the samples were filtered, 1 ml was taken and the caffeine and other drugs were extracted by the addition of 1 ml of Tris, then 8 ml of a mixture of (15 ml of dichloromethane, and 35 ml of Hexane). The solutions were mixed for 15 min, and after being centrifuged at 3700 rpm for 15 min, then transferred toa rotary evaporator at speed 185 and the temperature kept at 50 ºC. Evaporated the organic phase by rotary at 50 ºC, until dryness and reconstituted to 10 ml with methanol. The absorbance of the diluted solutions measured at 271 nm.

Caffeine stability procedure: For the stability study of caffeine in water, the absorbance of the standard caffeine solutions (2-10 µg/ml) was measured on UV-VIS spectrophotometer at 271 nm on the same day (fresh solutions), an at different times: 2, 3, 7, and 15 days

Results and Discussion:

Calibration curve of caffeine: The absorbance values are plotted against concentrations to generate a standard calibration curve as shown in Figures (1) and (2), where the spectrum and a calibration curve of different concentrations of caffeine (2 - 10 µg/ml) were measured at λ_{max}. Which showed a good linear relationship between the absorbance and concentrations of the standard solutions at 271 nm.

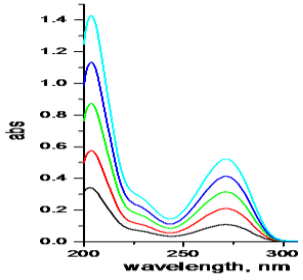

Figure (1): Spectrum of different concentrations of Caffeine at 271 nm

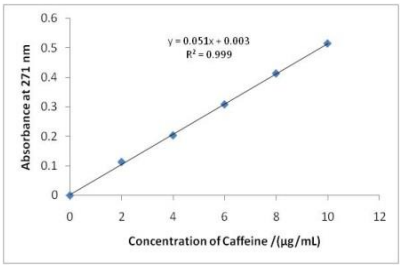

Figure (2): Calibration curve for caffeine.

The Spectrophotometric method results recorded that, as shown in Figure (3), the absorbance and the spectrum of caffeine in all the samples under study, respectively. Where it is clear that there is only one peak at 271 nm, to indicate a perfect extraction method for the caffeine. The caffeine contents in the coffee samples were in the range between (20.19 – 400.31 µg/ml). While in Nescafe samples the contents fluctuated in the range of (14.50 – 461.87 µg/ml), on the other side, the contents of caffeine in cacao and cappuccino samples were ranged between (8.66 – 167.74 µg/ml). The results of the samples were shown in table (2). The minimum caffeine level was observed in Cacao - Nesquik sample (NESTLE Italiana S.P.A) (8.66 µg/mL), while the highest caffeine level was observed in the Nescafe - Nestla sample, Asbania, (461.87 µg/ml). Caffeine concentrations in samples under the study were in the range of 8.67 - 461.87µg/ml with an average of 211.46 µg/ml.

Stability of caffeine in water: We have studied the stability of caffeine in water by UV-VIS Spectrophotometer at 271 nm, to make sure the stability of the slope in the calibration curve during our measurements and calculations. The table (3) and the figure (4) show the absorbance at 271 nm of standards solutions of caffeine in water for five different series of concentrations at different times, from these results we can confirm that the caffeine is stable in water during two weeks.

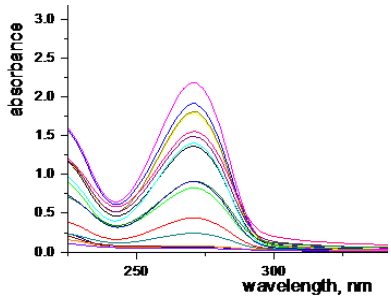

Figure (3): Spectrums of caffeine in all the samples under study.

Validation method: There are different factors used for the validation of the analytical methods including Linearity, Accuracy, Precision, RSD%, Recovery, LOD, and LOQ.

Linearity: Computing a linear least-squares regression analysis on the plot of the absorbance of caffeine to the external standard versus concentrations demonstrated a linear relation over the range of 2 - 10 µg/ml(using five concentration levels) with correlation coefficients (R^2) being consistently greater than 0.999.The calibration curve (figure 2) was obtained using Microsoft office excel and it illustrates a positive linear relationship between the instrumental signal and the concentration of the caffeine standards for the UV/ Vis spectrophotometric methods.

Limit of detection (LOD): The LOD is defined as the concentration of analyte required to give a signal equal to three times the standard deviation of the blank. The LOD was calculated using the following equation:

$$LOD = \frac{3 \cdot s_{y/x}}{b}$$

Where s is the average of the standard deviation SD_{yx} of the peak ratio (peak area of analyte/ peak area of external standard), and bis the average of the slope of a calibration curve.

In this study, the limit of detection. (LOD) value for caffeine in the samples using a UV-Visible Spectrophotometer was 0.3199 µg/ml.

Limit of quantitation (LOQ):is defined as the concentration of analyte required to give a signal equal to ten times the standard deviation of the blank. The LOD was calculated using the following equation:

$$LOQ = \frac{10 \cdot s_{y/x}}{b}$$

The limit of quantitation (LOQ) value for caffeine in the samples by UV-Visible spectrophotometer was determined to be 0.96 µg/ml.

Accuracy and precision: Accuracy is expressed as percent relative error (% R.E.), from the statistic equation:

RE % = (measured – real)/real * 100,

Or RE % = (Slope – Average)/ Slope * 100.

In this study the Accuracy (% R.E.) was 0.49%. Precision is expressed as percent relative standard deviation (% RSD), which in this study was 0.0506 %. The precision of the method (within-day variations of replicate determinations) was checked by measuring caffeine, 5 times at the LOQ level. The precision of the method, expressed as the RSD % at the LOQ level, was 0.05 for caffeine by UV-Vis Spectrophotometer. For accuracy, a standard working solution of caffeine was prepared. The prepared standards were measured 5 times as a test sample. From the respective absorbance counts, the concentrations of the caffeine were calculated using the detector responses. The accuracy is defined in terms of the % deviation of the calculated concentrations from the actual concentrations calculated from the regression equation standard solution.

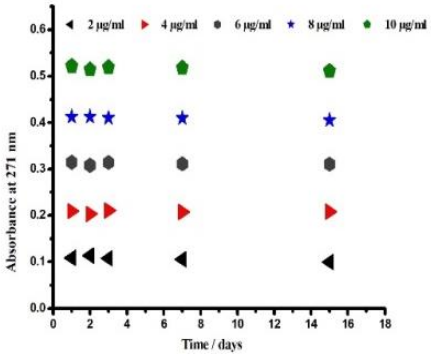

Figure (4): Stability of caffeine in water by UV-VIS Spectrophotometer.

Table (1) : Samples under study collected from Libyan markets

No.,	Name of Samples	Sources
Coffee Samples		
S1	Bun powder	Albyda- libya
S2	Alkalij	Zlitn city
S3	Turkish	Produced in Turkey
S4	Yamen	Alzawya- libya
S5	Al karasta coffee with coriander	Derna- Libya
S6	Khaled coffee with habahan	Benghazi - Libya
S7	Bala	Benghazi - Libya
S8	Qahwatna	Albyda- libya
S9	NwatAltamer	Tokra- libya
Nescafe Samples		
S10	Caffee Break	MC In Egypt
S11	Caffee Max	Turkya
S12	Gold	Poland
S13	Orga Mix	Egypt
S14	Nestla	Asbania
Cacao Samples		
S15	Cacaoo Saied	Tunisie
S16	Nesquik	NESTLE Italiana S.P. A
Cappuccino Samples		
S17	Clever	Czech rebublic

Table (2): Concentrations of caffeine in the studied samples (n=3).

No.,	Samples	Conc. of Caffeine (µg/ml)
Coffee Samples		
S1	Bun powder	250.72
S2	Alkalij	80.90
S3	Turkish	152.32
S4	Yamen	350.74
S5	Al karasta coffee with coriander	256.73
S6	Khaled coffee with habahan	400.31
S7	Bala	326.79
S8	Qahwatna	332.19
S9	NwatAltamer	20.19
Nescafe Samples		
S10	Caffee Break	440.45
S11	Caffee Max	14.50
S12	Gold	271.49
S13	Orga Mix	44.46
S14	Nestla	461.87
Cacao Samples		
S15	Saied	14.77
S16	Nesquik	8.66
S17	Cappuccino -Clever	167.74
Average of caffeine concentration		211.4647

Table (3): Absorbance at 271 nm of standards solutions of caffeine for different serials of concentrations at different times.

Time / days	**Absorbance of caffeine at 271 nm**				
	2 µg/ml	**4** µg/ml	**6** µg/ml	**8** µg/ml	**10** µg/ml
1	0.1083	0.2093	0.3148	0.413	0.522
2	0.1133	0.2033	0.3081	0.4129	0.5143
3	0.1077	0.2106	0.3141	0.4106	0.5192
7	0.1057	0.2071	0.3113	0.4101	0.5181
15	0.0995	0.2082	0.3107	0.4057	0.5115

Mihalčíková developed a highthrough-ghput sequential injection analysis (SIA) spectrophotometric assay for the determination of caffeine in coffee drinks samples including soluble coffee, coffee from espresso machines, and brewed coffee drinks were perfo-rmed by. Where the sample was treated with Carrez reagent for matrix suppression followed by filtration ther-eafter analyte was isolated from organic acids by a short C18 monolithic column (10x4.6mm). Caffeine was detected at 274 nm.

Where he determined the caffeine at the linear range was 1 - 15 mg L-1, and the determination coefficient (r2) was 0.9969. The limit of detection (LOD) and limit of quantification (LOQ) were 0.128 and 0.425 mg L-1, respectively. The relative standard deviation (RSD) was 3.58 % (n = 12, 10 mg L-1) [23]. Some studies indicated that the results of the analysis showed that the amount of caffeine in some coffee samples were ranged ranges from 501.97 to 564.07 (mg/L), were in an average amounts compared to the literature value of different varieties of coffee (473.33 - 13.33mg/L) and which is very good because mild amounts caffeine is advised for health and even the amounts of caffeine is in excess or extra-large it may needs decaffeination

to avoid the excess caffeine from the coffee [24]

Conclusion:

The amount of caffeine in the samples under study was in order (from the higher to lower amount): Nescafe > Coffee > Cappuccino > Cacaoo (by type). The highest amount of caffeine in analyzed samples was found in Nescafe sample (Nestla), while the lowest was recorded in cocaoo sample (Nesquik). The extraction method used in this study provided a high efficiency. The results obtained for analysis of caffeine in the samples under study using UV-Visible Spectrophotometer showed that there are differrences in the concentrations of caffeine in these samples. The average concentrations of caffeine in Nestla (Nescafe) is greater than all the other samples (coffee, cappuccino and cocaoo). The caffeine content of the analyzed samples was not found to be alarming since it correlated well with documented values. The results obtained by UV-Visible Spectrophotometer for the Study of stability of caffeine in water (table 4 and figure 5) showed that the caffeine was stable in water during more than two weeks. The FDA has mentioned for healthy adults, it could take about 400 milligrams a day—that's about four or five cups of coffee—as an amount not generally associated with dangerous, negative effects. However, there is wide variation in both how sensitive people are to the effects of caffeine and how fast they metabolize it (break it down) [25]. EFSA used a survey conducted in the UK to calculate caffeine levels in different food products. This survey contained information on caffeine concentrations from 400 samples of teas – loose leaves, bags, vending machines, and instant tea – and coffees – filter coffee, vending machines, espresso, and instant coffee – prepared at home, in workplaces, or bought in cafes and other retail outlets. For foods for which the UK survey did not report caffeine levels, an average of mean values reported in other representative surveys was used, except for "energy drinks", for which the caffeine concentration (320 mg per liter) of the most popular brand was chosen. [25].
Also most of the caffeine in the samples analyzed in this study were within (less than) the guidelines given by the Libyan National Centre for Standardization and Metrology (LNCSM) for coffee and Nescafe (1% = 10000 mg/L) [26].

We advise the other researchers to study the determination of caffeine concentrations or other substances and their metabolisms in other samples using several techniques to complete our study.

References:

[1] J. S. Bonita, M. Mandarano, D. Shuta, and J. Vinson, "Coffee and cardiovascular disease: In vitro, cellular, animal, and human studies," Pharmacol. Res., vol. 55, no. 3, pp. 187–198, 2007, doi: 10.1016/j.phrs.2007.01.006.

[2] M. C. Cornelis and A. El-sohemy, "Coffee , caffeine , and coronary heart disease," no. Table 1, pp. 29–31, 2007.

[3] A. Di Castelnuovo, R. Di Giuseppe, L. Iacoviello, and G. De Gaetano, "Consumption of cocoa, tea and coffee and risk of cardiovascular disease," Eur. J. Intern. Med., vol. 23, no. 1, pp. 15–25, 2012, doi: 10.1016/j.ejim.2011.07.014.

[4] K. Ramalakshmi and B. Raghavan, "Caffeine in coffee: Its removal. Why and how?," Crit. Rev. Food Sci. Nutr., vol. 39, no. 5, pp. 441–456, 1999, doi: 10.1080/10408699991279231.

[5] K. W. Andrews et al., "The caffeine contents of dietary supplements commonly purchased in the US: Analysis of 53 products with caffeine-containing ingredients," Anal. Bioanal. Chem., vol. 389, no. 1, pp. 231–239, 2007, doi: 10.1007/s00216-007-1437-2.

[6] P. M. L. Teo et al., "Early tumour response and treatment toxicity after hyper fractionated radiotherapy in nasopharyngeal carcinoma," Br. J. Radiol.,

vol. 69, no. 819, pp. 241–248, 1996, doi: 10.1259/0007-1285-69-819-241.

[7] B. F. Harland, "Caffeine and nutrition, " Nutrition, vol. 16, no. 7–8, pp. 522–526, 2000.

[8] R. C. Malenka, E. J. Nestler, S. E. Hyman, A. Sydor, and R. Y. Brown, "Molecular neuropharmacology: a foundation for clinical neuroscience," NY McGraw-Hill Med., 2009.

[9] B. B. Fredholm, K. Bättig, J. Holmén, A. Nehlig, and E. E. Zvartau, "Actions of caffeine in the brain with special reference to factors that contribute to its widespread use," Pharmacol. Rev., vol. 51, no. 1, pp. 83–133, 1999.

[10] A. Nehlig, J.-L. Daval, and G. Debry, "Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects," Brain Res. Rev., vol. 17, no. 2, pp. 139–170, 1992.

[11] F. D. A. Food, "Drug Administration (2014) Food additive status list." 2016.

[12] M. C. R. De Camargo and M. C. F. Toledo, "HPLC determination of caffeine in tea, chocolate products and carbonated beverages," J. Sci. Food Agric., vol. 79, no. 13, pp. 1861–1864, 1999.

[13] M. S. Bispo, M. C. C. Veloso, H. L. C. Pinheiro, R. F. S. De Oliveira, J. O. N. Reis, and J. B. D. Andrade, "Simultaneous determination of caffeine, theobromine, and theophylline by high-performance liquid chromatography," J. Chromatogr. Sci., vol. 40, no. 1, pp. 45–48, 2002, doi: 10.1093/chromsci/40.1.45.

[14] M. Abdul Mumin, K. Farida Akhter, M. Zainal Abedin, and M. Zakir Hossain, "Determination and Characterization of Caffeine in Tea , Coffee and Soft Drinks by Solid Phase Extraction and High Performance Liquid Chromatography (SPE–HPLC)," Malaysian J. Chem., vol. 8, no. 1, pp. 045–051, 2006.

[15] N. Violeta, T. I, and M. Elena, "Quantitative determination of caffeine in carbonated beverages by an HPLC method," J. Agroaliment. Process. Technol., vol. 14, pp. 123–127, 2008, [Online]. Available: www.tpa-timisoara.ro.

[16] V. Nour, I. Trandafir, and M. Ionica, "Chromatographic Determination of Caffeine Contents in Soft and Energy Drinks Available on The Romanian Market," Sci. Study Res. Chem. Chem. Eng. Biotechnol. Food Ind., vol. 11, no. 3, pp. 351–358, 2010, [Online]. Available: http://pubs.ub.ro/?pg=revues&rev=cscc6&num=201011&vol=3&aid=3107.

[17] A. B. M. A. Maidon, A. O. Mansoer, and H. Sulistyarti, "Study of various solvents for caffeine determination using UV spectrophotometeric.," J. Appl. Sci. Res., no. May, pp. 2439–2442, 2012.

[18] Amos-Tautua, W. Bamidele Martin, and E. R. E. Diepreye, "Ultra-violet spectrophotometric determination of caffeine in soft and energy drinks available in Yenagoa, Nigeria," Adv. J. Food Sci. Technol., vol. 6, no. 2, pp. 155–158, 2014, doi: 10.19026/ajfst.6.2.

[19] G. Elmanfe and K. Ali, "Abd Al zain, R.,(2017)." Spectrophotometric Determination of Caffeine in Juices, Soft Drinks and Energy Drinks collected from some Local Markets in El-Bieda City–Libya," in 2nd Libyan Conference on Chemistry and its Applications (LCCA-2), 2017, pp. 9–11.

[20] G. M. Elmanfe, H. O. Khalifa, O. Khreit, and O. Abduljalil, "Determination of Caffeine and some other Drugs in Cappuccino , Nescafe , Cacao , Coffee samples collected from some Libyan Markets using High Performance Liquid Chromatography (RP-HPLC)", JOPAS, VOL.20, NO. 4, pp. 149-155, August, 2021, DOI: .51984/JOPAS.V20I4.1739

[21] F. Lo Coco, F. Lanuzza, G. Micali, and G. Cappellano, "Determination of theobromine, theophylline, and caffeine in by-products of cupuacu and cacao seeds by high-performance liquid chromatography," J. Chromatogr. Sci., vol. 45, no. 5, pp. 273–275, 2007, doi: 10.1093/chromsci/45.5.273.

[22] A. M. Massadeh, A. A. Gharaibeh, and K. W. Omari, "A single-step

extraction method for the determination of nicotine and cotinine in jordanian smokers' blood and urine samples by RP-HPLC and GC-MS," J. Chromatogr. Sci., vol. 47, no. 2, pp. 170–177, 2009, doi: 10.1093/chromsci/47.2.170.

[23] L. Mihalčíková, "High throughput method for determination of caffeine in coffee drinks," analytical chemistry, Faculty of pharmacy in Hradec Králové, Charles university in Prague, 2016.

[24] Z. Tadesse Wondimkun, "The Determination of Caffeine Level of Wolaita Zone, Ethiopia Coffee Using UV-visible Spectrophotometer," Am. J. Appl. Chem., vol. 4, no. 2, p. 59, 2016, doi: 10.11648/j.ajac.20160402.14.

[25] U. S. F. and D. Administration, "Food Additive Status List.(2005)," Trtrieved from http//www. cfsan. fda. gov/~ dms/opa-appa. html, 2009.

[26] Libyan National Center for Standardization and Metrology, Coffee. LNS 395, "LNCSM," 2005.

الملخص: تعتبر القهوة من أكثر المشروبات الشعبية التي يتم استهلاكها على نطاق واسع في العالم، تهدف هذه الدراسة إلى تقدير مادة الكافيين في عدة عينات من النسكافيه والكاكاو والكابتشينو والقهوة باستخدام تقنية (UV-Visible spectrophotometer). ايضا دراسة ثباتية الكافيين في الماء. حيث تم جمع سبعة عشر عينة المتاحة في السوق الليبي، هذه الطريقة اعتمدت على طريقة استخلاص بسيطة وسريعة وموثوقة في العينات باستخدام ثنائي كلورو ميثان، ن-هكسان، وميثانول كمذيبات استخلاص. تم تقدير الكافيين على مدى من التراكيز10-2 μg/ml ، معامل الارتباط أكبر من 0.999. قد لوحظ الحد الأدنى لمستوى الكافيين في عينات(Nesquik (Cacao، Nestle Italian S.P.Aبتركيز .8.66 μg/ml واعلى محتوى من الكافيين كان في العينة Nestle (Nescafe),Asbania بتركيز .462.87 μg/ml وجدت الدراسة الثباتية الكافيين في الماء تصل لأكثر من أسبوعين.

الكلمات الافتتاحية: مشتقات القهوة، الكافيين، استخلاص، مذيبات، الامتصاص الطيفي