The Qualification and Quantification of Caffeine in Two Different Caffeinated Pharmaceutical Formulas Employing RP-HPLC Technique.

Zaid Al-Obaidi

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Kerbala, Iraq

Received Date: 20/Aug/2015

Accepted Date: 12/Oct/2015

الخلاصة

خلفية: تكامل طريقة للتحليل الدوائي هونهج متصاعد بين مجالات العلوم متعددة التخصصات. ان تقنية الكروماتوغرافيا السائلة عالية الأداء (HPLC) قد اثبتت لتكون حجر الزاوية في التحليل الدوائي. خلال العقد المنصرم تقرر ان يكون (HPLC) الطريقة التحليلية المختارة للعديد من المركبات. ان نظام (HPLC) تم استخدامه في تطبيقات متشعبة منها: التحليلات الكمية / النوعية للعينات البايلوجية، الحساب الكمي للمحتوى الصيدلاني الفعال، عزل المركبات من مخاليطها، واستخدامات اخرى عديدة. هذا العمل يهدف للقياس النوعي والكمي للكافيين في مستحضرين صيدلانيين هما بالاسم؛ اقراص "بنادول اكسترا" و"ثكر فلر" شامبوبتوظيف تقنية (RP-HPLC).

طرق العمل: تم استخدام طريقة الطور الثابت مع نسبة ثابتة للناقل كما في الميثانول: الماء (40:60). وكان معدل تدفق 0.8 مل / دقيقة بينما كان الطول الموجي 273 نانومتر. وقد رسم منحنى المعايرة عن طريق استخدام تركيزات التالية (80 و60 ,40 ,20 ,0.0) ميكروغرام / مل. تم فحص عينات ستة وثلاثين حبة دواء تحتوي على الكافيين كما اذيبت في الميثانول وتم تصفيتها بوساطة 0.22 ميكرومتر فلتر غشاء النايلون ذي الحقنة. من ناحية أخرى تم فحص ثلاث عينات مستقلة للشامبووالتي تحتوي على الكافيين. وخففت العينات، رجت، وتم تصفيتها مع مرشحات حقنة 0.22 ميكرومتر ذات غشاء النايلون. وأخيرا، تم حقن المحاليل المخففة إلى (HPLC) وقد لوحظت البيانات وتم تسجيلها.

نتائج: وقد لوحظ المجالات المطلوبة لرسم منحنى المعايرة. وقد تم تحديد ذروة الكافيين في كل عينة صيدلانية. وبناء على ذلك تم تحديد كمية القمم التي تم تحديدها. تم العثور على محتوى الكافيين في أقراص تحتوي على مادة الكافيين لتكون 64,96 ملغ. في حين لوحظ تركيز الكافيين في الشامبو٪0.0047.

المناقشة والاستنتاج: في هذا العمل تم الاستفادة من تقنية الـ HPLC الطور العكوس للقياس النوعي والكمي لمادة الكافيين في مستحضرين صيدلانيين مختلفين هما بالاسم؛ اقراص الـ Panadol® Extra وشامبوالـ Thicker Fuller ®Hair. قد نضجت تقنية الكروماتوغرافيا السائلة عالية الأداء (HPLC) لدرجة أن أي دواء موجود تقريبا يمكن تحليله باستخدام الطرق القائمة التي يمكن العثور عليها في الادبيات التحليلية. للعمل المستقبلي يمكن فحص أدوية أخرى أويمكن فحص حتى التركيبات ذات الأدوية المتعددة مع هذه التقنية الدقيقة والمضبوطة.

الكلمات المفتاحية

كافيين، الكروماتوغرافيا السائلة عالية الاداء–الطور العكوس، أقراص بانادول اكسترا، شامبوثكر فلر المحتوي على الكافيين.

Abstract

Background: Method optimization of drug analysis is an arising approach among multidisciplinary science fields. The High performance liquid chromatography (HPLC) has been approved to be the cornerstone of drug analysis. Over the last ten years, HPLC has been figured as the analysis method of choice for many compounds. HPLC system has been utilized in diversified applications such as: the quantitative/qualitative analyses of biological samples, calculating the amounts of active pharmaceutical ingredient (API), isolating specific components from their mixtures, and many

others. This work aims to qualify and quantify the caffeine in two different pharmaceutical formulas namely; Panadol® Extra tablets and Thicker Fuller Hair® shampoo employing RP-HPLC.

Methods: Isocratic method was utilized with fixed mobile phase ratio as methanol: water (40:60). The flow rate was 0.8 ml/min while the wavelength was 273nm. Calibration curve was plotted via the utilization of the following concentrations (0.0, 20, 40, 60, and 80) µg/ml. Thirty six caffeinated tablet samples were examined as dissolved by methanol and filtered with 0.22 µm nylon-membrane syringe filter and diluted. On the other hand three independent caffeinated shampoo samples were examined. The samples were diluted, shacked, and filtered with 0.22 µm nylon-membrane syringe filters. Finally, the diluted solutions were injected to the RP-HPLC and the data was observed and recorded.

Results: The areas required to plot the calibration curve were observed. The caffeine peak was identified within each pharmaceutical sample. Consequently the identified peaks were also quantified. The mean content of caffeine in caffeinated tablets was found to be 64.96 mg. Moreover, the caffeine concentration in the shampoo was observed as 0.0047%.

Discussion and conclusion: In this work the RP-HPLC has been utilized in the qualification and quantification of caffeine in two different pharmaceutical formulas namely; Panadol® Extra tablets and Thicker Fuller Hair® shampoo. HPLC technology has matured to the extent that almost any existing drug can be analyzed by an existing method that can be found in the analytical literatures. For future work other drugs can be examined or even multidrug formulas can be tried with such accurate and precise RP-HPLC technique.

Keywords

Caffeine, RP-HPLC, Panadol Extra Tablets, Thicker Fuller Caffeinated Shampoo.

1. Introduction

High performance liquid chromatography (HPLC) has been proven to be the cornerstone in the separation and purification techniques. HPLC can be outlined as a separation of variety of compounds depending on the differences in their distribution equilibrium. This equilibrium stands between two phases, the mobile phase pumped with high pressure pumps and the stationary phase that packed inside columns that the mobile phase is delivered through. The prolonged retention of a compound is revealed as a higher distribution into the stationary phase. This compound would be separated from those with lower distribution into the stationary phase. Between the late 1960s separation and/or purification in multidiscipline industries involving pharmaceuticals, biotechnological, environmental, food and others [1].

Over the last ten years, HPLC has been figured as the analysis method of choice for many compounds. HPLC is achieved with the insertion of a tiny volume of usually diluted liquid sample into a continuous stream of a moving liquid named as mobile phase. This mobile phase crosses a column which contains the packed particles of the stationary phase. The separation of a mixture of variety of compounds into each single compound relies on differences between the retentions of each specified compound. Inside the column, the retention depends on the partioning of the compound between the mobile and the stationary phases. Accordingly, different compounds possess different mobilities. This resulted in different retention times, tR. Simply, the retention time is defined as the time required for the detection of the target compound after its injection. [2]

For any compound to be determined, the detector must sense and differentiate this compound from the mobile phase and convert this data into an electrical signal. For a stable system and fixed operation conditions the two compounds with exactly the same retention times are said to be the same compound. Therefore, for qualitative identification it is mandatory to match the retention times of known compounds (basically named standard) with the retention times of components in the unknown mixture (named sample). [3,4]

HPLC is just one type of liquid chromatography (LC), meaning the mobile phase is a liquid. In general when the term “HPLC” is utilized then it means the normal phase HPLC. In this study, another type of LC has been utilized which is called reversed phase HPLC (RP-HPLC). RP-HPLC is the most common type of HPLC. The term “reversed phase” reveals that the mobile phase is relatively polar, while the stationary phase is relatively non-polar. So that the more the non-polar the compound is, the greater the retention time will be. In other words, in RP-HPLC hydrophobic compounds have longer retention times than hydrophilic compounds. Partition, adsorption, ion-exchange, size-exclusion, and thin-layer chromatography are considered as other more general types of HPLC [5,6].

HPLC system has been utilized in many worldwide applications such as: (1) the quantitative/qualitative analyses of physiological samples to determine amino acids, nucleic

acids, and proteins. [2] Calculating the amounts of active pharmaceutical ingredient (API), synthetic side products, the resulted products of the degradation process in pharmaceuticals. [3] Evaluating the amounts of harmful materials like insecticides and pesticides. [4] Inspecting environmental samples. [5] Isolating specific components from their mixtures [7].

HPLC can be operated in many modes. The way by which the solutes interact with the stationary phases necessitates the sorting of the mode of the chromatography [8]. The variety of modes of liquid chromatography is summarized in the following table (see Table 1 below). Moreover, as in this work the RP-HPLC technique has been performed, and then a focus on the chemical nature of the stationary phase is illustrated in Fig.(1) below.

In this work the RP-HPLC has been utilized in the qualification and quantification of caffeine in two different caffeinated pharmaceutical formulas. The aim of this work is to qualify and quantify the caffeine in two different pharmaceutical formulas namely; Panadol® Extra tablets and Thicker Fuller Hair® shampoo employing RP-HPLC.

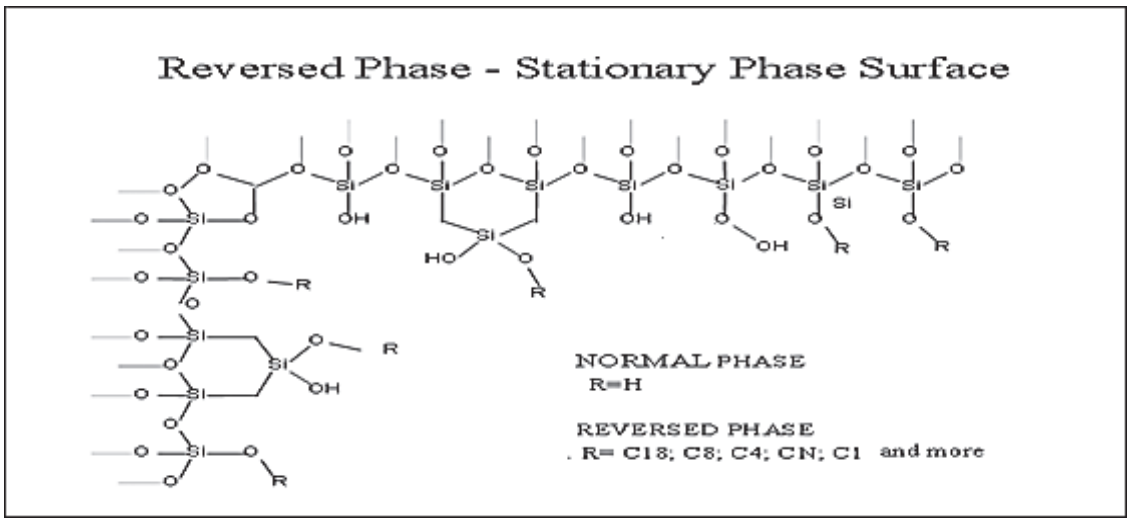

Fig.(1): The surface of Reversed Phase stationary phases [9].

Table (1): A summary of a variety of the operation modes of HPLC [10].

Typical solutes	Typical mobile phase	Typical Stationary Phases	Stationary Phases chemistry	Mode
Fatty and oily	Hexane; isopropanol; methylene chloride	Silica, Alumina	Polar-hydrophilic	Normal Phase
Almost all organic compounds	Water; methanol; acetonitrile; buffers; ion pairing agents	Alkylated silica, mostly C18	Non-polar-lipophilic	Reversed Phase
Any ion-charged compounds	Water; buffers; acid; base	Ionic functional groups on silica or polymer	Ion-bonding	Ion exchange
Enantiomers small and large molecules	Two modes: aqueous and non-aqueous	Chiral groups on silica surfaces	Chiral recognition	Chiral
Biomolecules or their substrates	Water; buffers	Either substrates or biomolecules,	Bioaffinity	Affinity
Polymers: synthetic or biological	Two modes: aqueous and non-aqueous	Gel type polymers	Sieving by size	Size Exclusion

2. Materials and Methods

2. 1. Materials

1. HPLC system (Shimadzu, USA):
   b. C18 column (5 µm, 4.6 mm X 250 mm)
   c. 100 mL syringe.
   d. Photodiode array detector.

2. Ultrasonic cleanser with heater (Scientific Lab, Italy)

3. 4-digit sensitive balance (Radwang-2013, Poland)

4. Methanol (HPLC grade), Acetonitrile (HPLC grade), and Micropipettes (Yellow: 10mL -100 mL and Blue: 100mL- 1000mL) all were purchased from Himedia Laboratories, Mumbai, India.

5. Water for injection (pure, no additives)

6. Chrom Tech® nylon membrane syringe filters; pore size 0.22 µmall from Himedia Laboratories, Mumbai, India

7. 3-mL and 5-mL disposable syringes (Changzhou, Kangfulia, China) were purchased from local pharmacy.

8. Caffeine standard anhydrous powder (Merck, UK)

9. Panadol® Extra Tablets (Glasgow Smith Kline, Ireland) and Thicker Fuller Hair® Caffeinated shampoo (Schwarzkopf, USA) were purchased from local pharmacy

2. 2. Methods

1. HPLC system and chromatographic conditions:

Isocratic method was utilized with fixed mobile phase ratio as methanol: water (40:60). The time of the run was set for 10 (minutes) with an extra 5 (minutes) with pure methanol to wash the column each run before the other run. The flow rate was set as 0.8 (ml/min) while the wavelength was assigned as 273 (nm).

2. Calibration curve acquisition:

A 10 (mg) of anhydrous caffeine powder was carefully and accurately weighed employing a 4-digit sensitive balance and then was dissolved within 100 (ml) methanol to obtain a stock solution of 100 (µg/mL). The stock solution was diluted with suitable volumes of methanol to obtain triplicates of the following concentrations (0.0, 20, 40, 60, and 80) µg/mL. The triplicates of each concentration were examined with the RP-HPLC and the areas related to each concentration were recorded. Then the mean (of the area units) was calculated for each concentration and the calibration curve was plotted.

3. Pharmaceutical sample preparation:

a. Panadol® Extra:

Three sheets of twelve-tablet each were examined. For each the following procedure was performed. The tablet was transferred into a 10 (ml) volumetric flask with stopper. The methanol then was added till the 10 (ml) was reached. Then the volumetric flask was sonicated for 10 (minutes). Afterward, the resultant was filtered with 0.22 (µm) nylon-membrane syringe filter and diluted as 100 (µL) aliquots were taken and completed to 10 (mL). Then this solution was injected to the RP-HPLC and the data were observed.

b. Thicker Fuller Hair® Caffeinated Shampoo:

Three different (Thicker Fuller Hair®) Caffeinated Shampoo samples were examined. For each sample the following steps are accurately and precisely performed.

Three of 100 (µL) aliquots were separately diluted to 10 (ml). Then each 10 (ml) tube were shacked well and filtered with (0.22) µm nylon-membrane syringe filters. Finally, these triplicated solutions were injected to the RP-HPLC and the data were observed.

3. Results

3. 1. Caffeine lambda max:

The lambda max was found to be 273 (nm) as shown in the fig.(2) below:

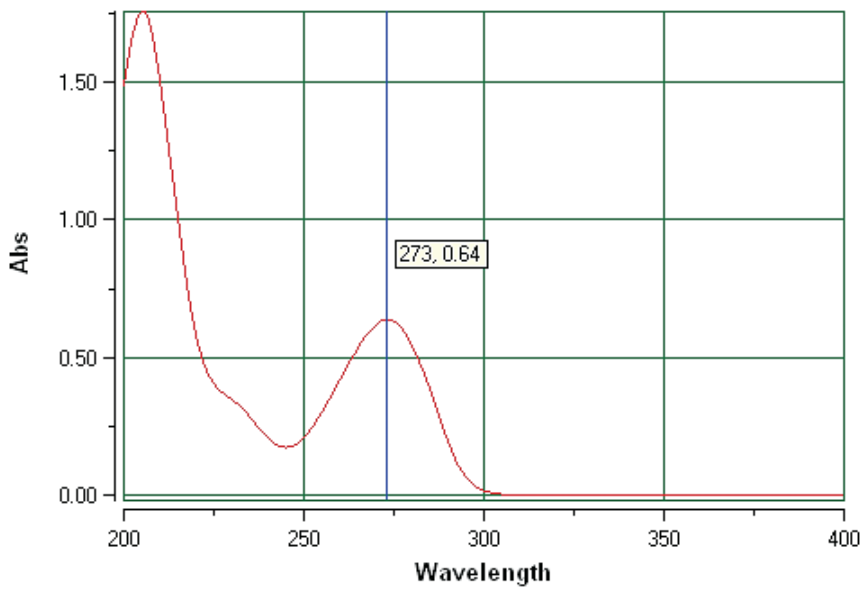

Fig.(2): the maximum wavelength of caffeine as assigned as 273 (nm).

3. 2. Caffeine calibration curve

The calibration curve was observed from the AUs for each concentration as shown in Fig.(3).

Fig.(3): The peaks observed with each standard caffeine concentration. The y-axis shows the intensity in (µv) and therefore data was taken manually by peak picking process.

Table (2): The intensity of the peaks (AUs) observed with each standard caffeine concentration.

Caffeine Conc. µg/mL	Intensity by Area Units (AUs)
0	0
20	189.1
40	350.1
60	526.72
80	682.53
100	851.1

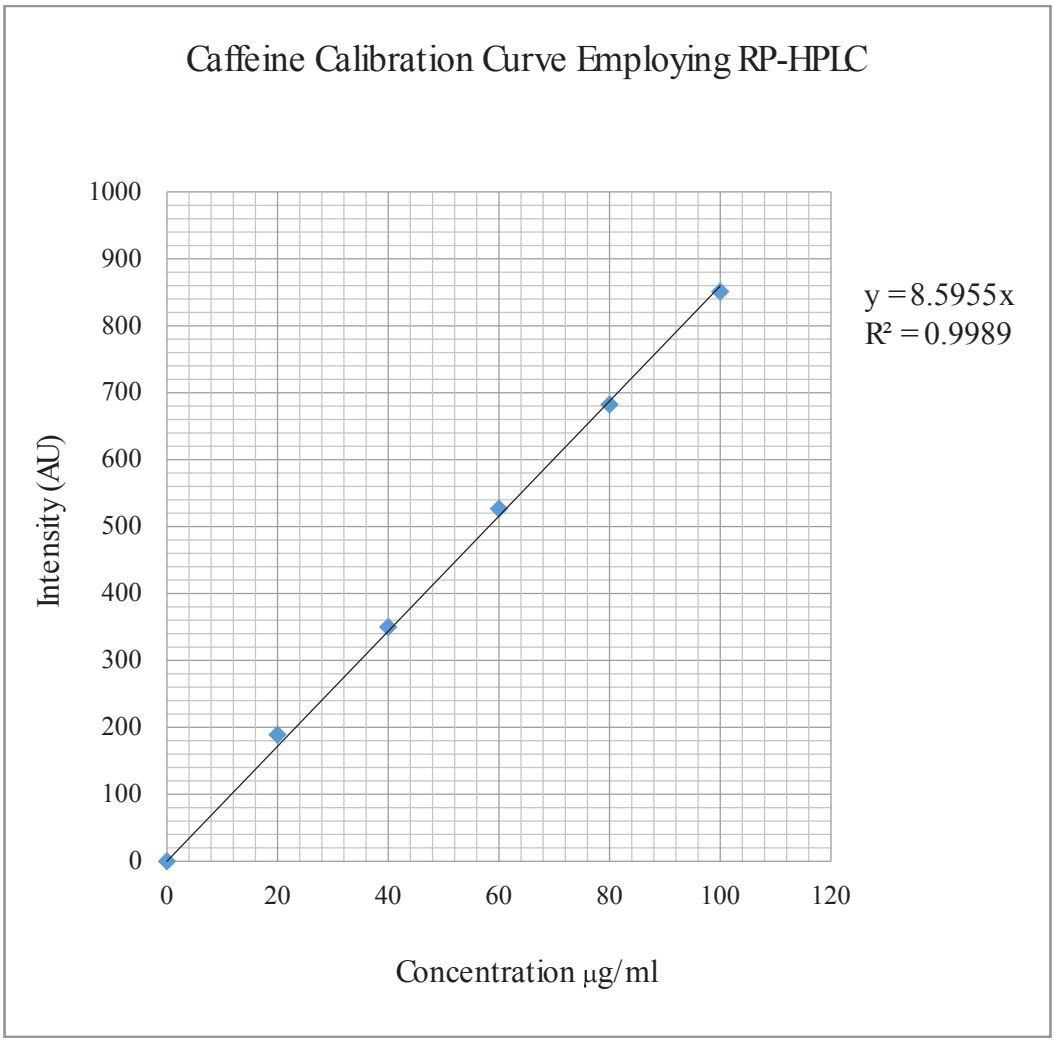

Fig.(4): Caffeine Calibration Curve Employing RP-HPLC. The R^2 was found to be 0.9989 while the slope was 8.5955. The solvent was HPLC-grade methanol.

3. 3. Panadol® Extra sample

3.3.1. Panadol® Extra sample identification:

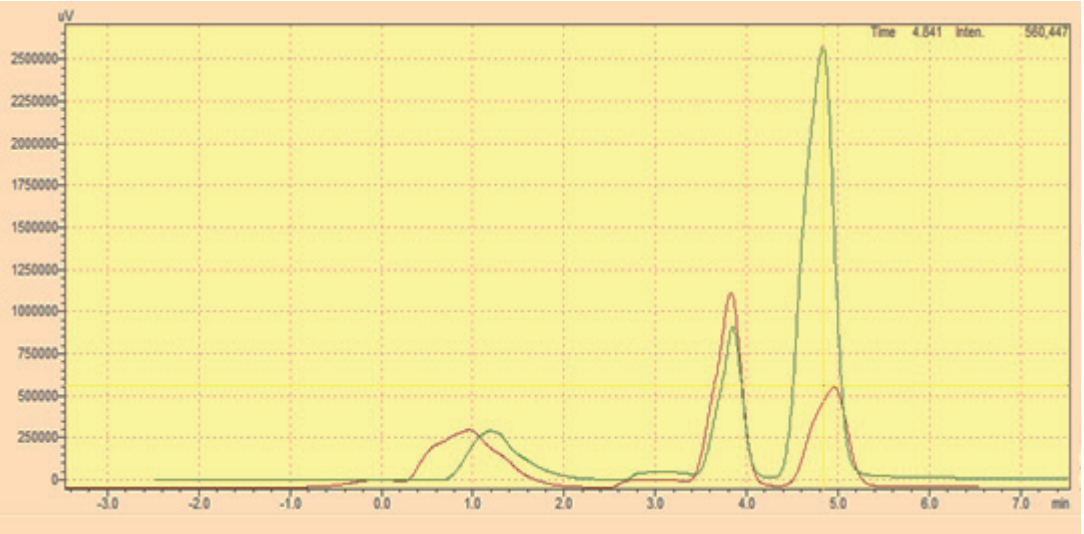

Fig.(5): Panadol® Extra sample identification utilizing caffeine standard addition. The peak with the retention time 4.84 minute was identified as caffeine peak as it is increased when caffeine standard was added.

3.3.2. Panadol® Extra sample quantification

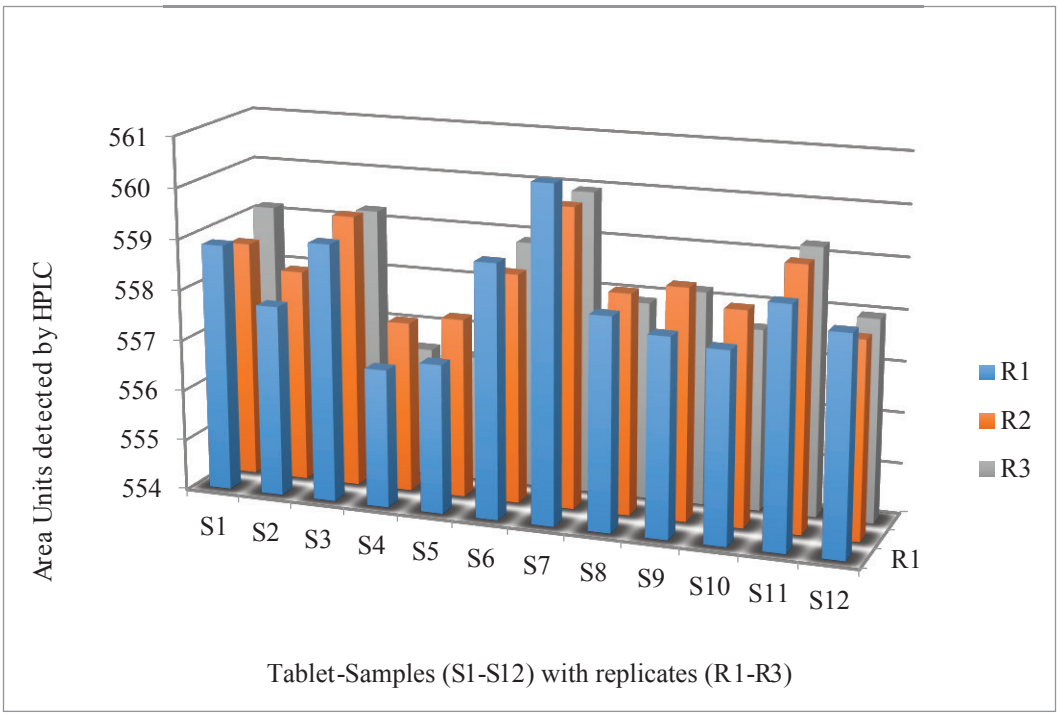

Fig.(6): The area units in correspondence with the Panadol® Extra tablet-sample (S1-S12) with replicates (R1,R2, and R3). The range was from minimum (556.48 AU) detected with (S5, R3) to maximum (560.55 AU) detected with (S7, R1).

The amount of caffeine was calculated as shown below:

Caffeine Concentration µg/mL = AU/slope

Amount of caffeine in each tablet = caffeine concentration (µg/mL) × 1000 (mL) (dilution factor).

Table (3): The caffeine content in milligrams ± RSD per each Panadol® Extra tablet-sample.

Sample No. (Panadol® Extra tablet)	Caffeine content mg/tablet ± RSD
S1	65.02 ± 0.0449
S2	64.91 ± 0.0395
S3	65.06 ± 0.0270
S4	64.79 ± 0.0767
S5	64.80 ± 0.0946
S6	65.01 ± 0.0440
S7	65.17 ± 0.0652
S8	64.93 ± 0.0409
S9	64.94 ± 0.0583
S10	64.90 ± 0.0602
S11	65.04 ± 0.0532
S12	64.92 ± 0.0370
Mean	64.96

The accuracy (the absence of error) = 1-((stated-measured)/stated) =1- ((65-64.96)/65) = 0.9994

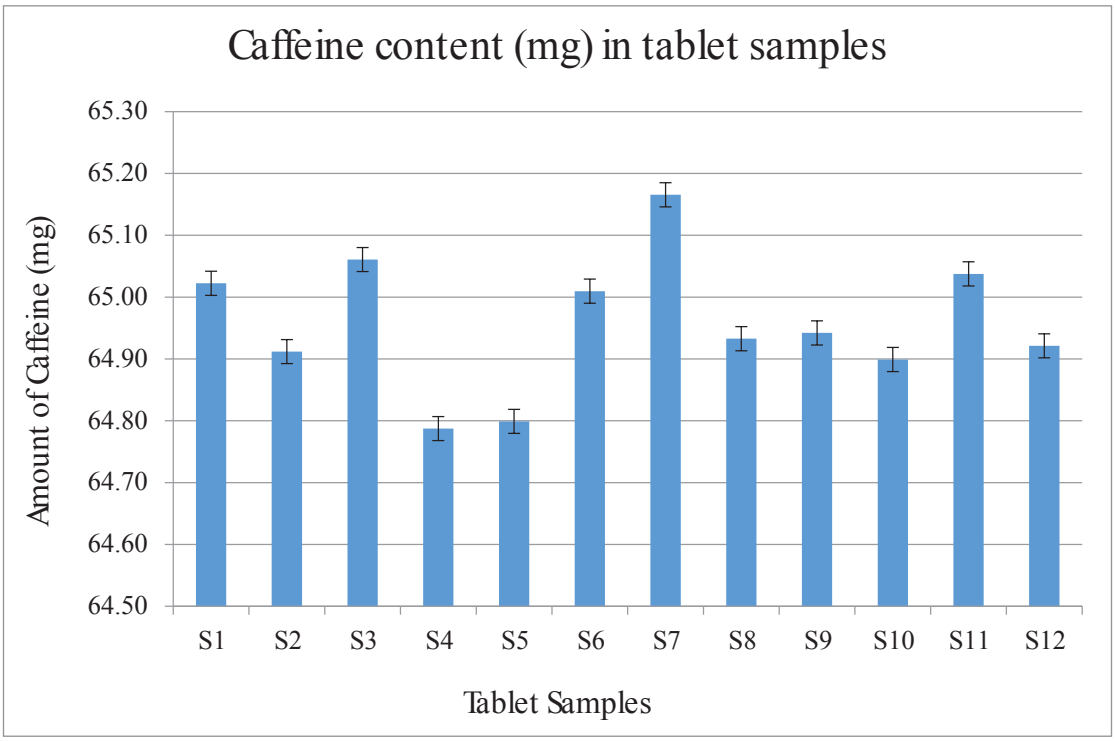

Fig.(7): The amount of caffeine in milligrams vs. each Panadol® Extra tablet-sample (S1-S12). The data is expressed as Mean±RSD.

3.4. Thicker Fuller Hair® Caffeinated shampoo sample:

3.4.1. Thicker Fuller Hair® Caffeinated shampoo identification:

The caffeine peak was identified as shown in Fig.(8) below

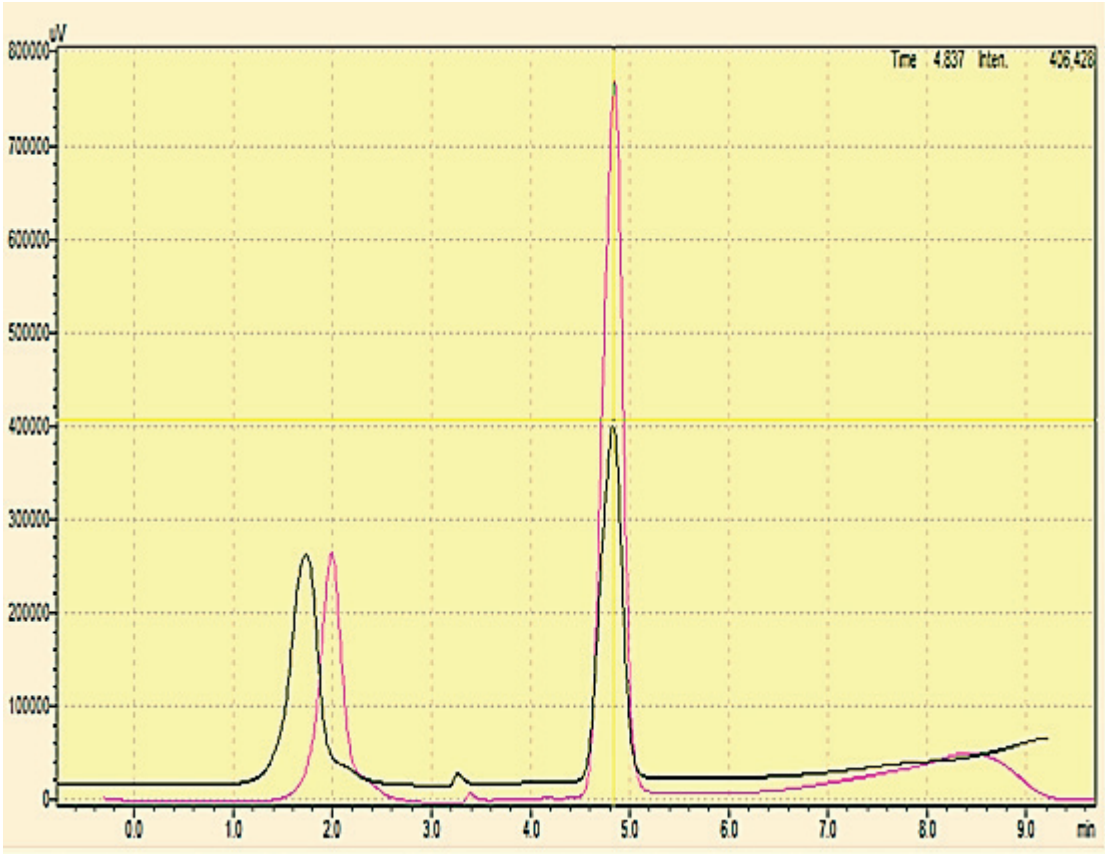

Fig.(8): Thicker Fuller Hair® Caffeinated shampoo sample identification utilizing caffeine standard addition. The peak with the retention time 4.837 minute was identified as caffeine peak as it is increased when caffeine standard was added.

3.4.2. Thicker Fuller Hair® Caffeinated shampoo sample quantification:

The concentration of caffeine was calculated as shown in fig.(9).

Caffeine Concentration µg/mL = AU/slope

Concentration of caffeine in Thicker Fuller Hair® Caffeinated shampoo sample = caffeine concentration (µg/mL) × 100 (mL) (dilution factor).

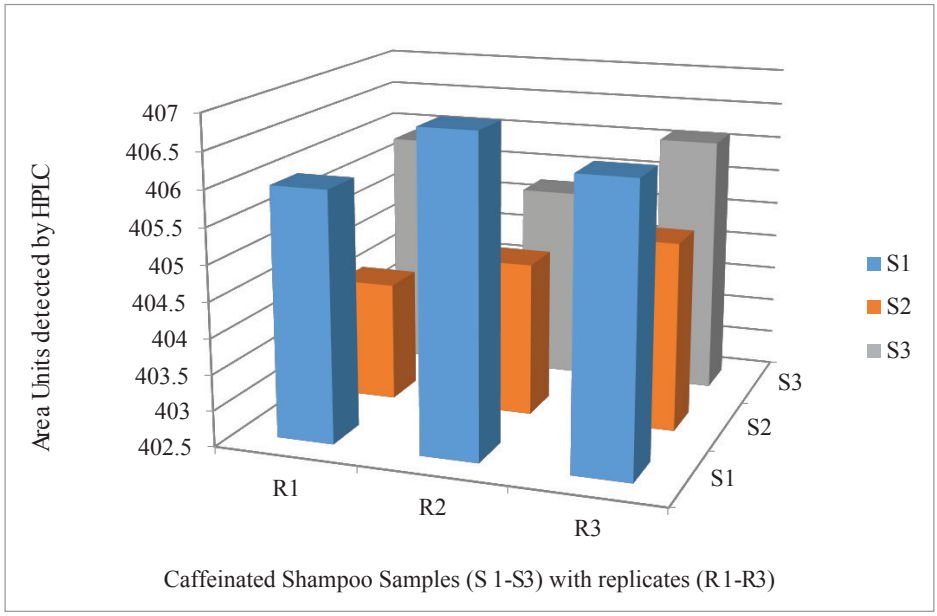

Fig.(9): The area units in correspondence with the Thicker Fuller Hair® Caffeinated shampoo samples (S1, S2, and S3) with replicates (R1, R2, and R3). The range was from minimum (404.17 AU) detected with (S2, R1) to maximum (406.88 AU) detected with (S1, R2).

Table (4): the caffeine conc. w/v % ± RSD for each caffeinated shampoo sample.

Caffeinated shampoo Sample No.	Conc. w/v % ± RSD
S1	0.004728 ± 0.001107
S2	0.004707 ± 0.001186
S3	0.004720 ± 0.001131

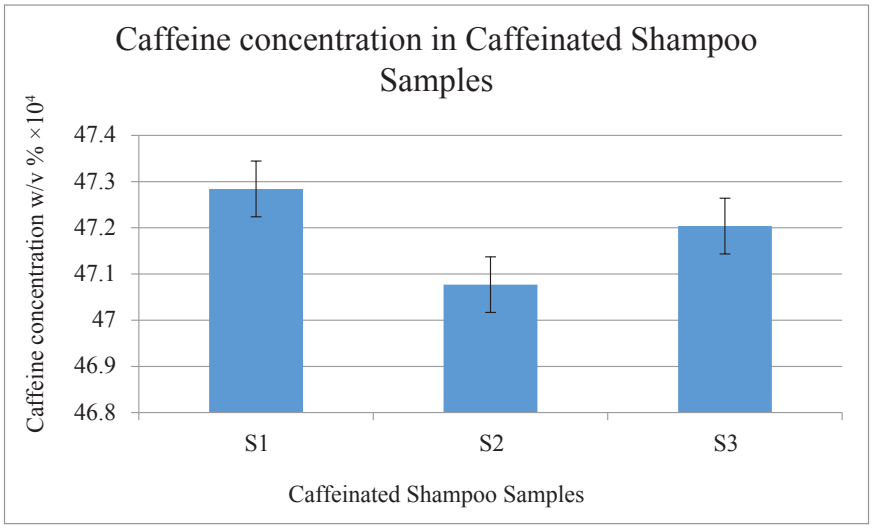

Fig.(10): Caffeine concentration w/v % × 10^4 in correspondence to three independent Thicker Fuller Hair® Caffeinated Shampoo. The data is expressed as Mean±RSD.

4. Discussion

The selection of lambda max of the absorption wavelength of caffeine was quite dependent on the cutoff values of the solvent system. As clearly shown in Fig.(2) the lambda max is 205 (nm) and the other peak is 273 (nm). Currently, the 273 (nm) is widely accepted as the lambda max for caffeine in many scientific literatures [11]. The 205 (nm) wavelength was excluded as it lies within the methanol cutoff solvent value which is 210 (nm) as shown in Table (5) below:

Table (5): Properties of HPLC Mobile Phases (12).

Mobile Phase	Polarity Index (P′)	UV cutoff (nm)
Ethanol	4.3	210
Methanol	5.1	210
Acetonitrile	5.8	190
Water	10.2	—

The calibration curve was plotted depending on the areas of the peaks assigned for each injected concentration. The curve shows linearity (y=8.5955x) and a good r-squared value (R2=0.9989) as shown in Fig.(4).

The caffeine standard addition method was utilized to identify the caffeine peak among other peaks within the pharmaceutical products utilized in this project (i.e. Panadol extra tablets and caffeinated shampoo). The caffeine standard addition method was successfully employed as shown in Fig.(5) and Fig.(8).

The reliability of any analyses is highly dependent on a number of samples tested to reduce the random error. Accordingly, 36 tablet samples were utilized to perform the required test [13].

Moreover, Fig.(6) shows that the range of the minimum and maximum AUs was from 556.48AU and 560.55AU respectively. This reveals a very good precision as confirmed with the data showed in Table (3) with the relative standard deviation values. Thus the deviation in the tablets contents is tolerated as shown in Fig.(7) and Fig.(9) The accuracy (the absence of error) was also found to be 0.9994 which is required in such analysis.

Scientists estimate up to 60 cups a day would be needed for significant amounts to reach follicles in the scalp. So that's only about 6000 (mg) of caffeine, which might regrow your hair after stopping your heart. Instead of drinking it – it seems you need to rub a caffeine-rich solution into the scalp. Fortunately there are some caffeinated shampoos on the market [14].

5. Conclusion

In this work the RP-HPLC has been utilized in the determination of caffeine in two different caffeinated pharmaceutical formulas. HPLC technology has matured to the extent that almost any existing drug can be analyzed by an existing method that can be found in the analytical literatures.. For future work other drugs can be examined or even multidrug formulas can be tried with such accurate and precise RP-HPLC technique.

References

[1] Isenberg HD: Clinical microbiology procedures handbook; 3rd ed. Chapter 12,19 and chapter 14. updates. American Society for Microbiology, Washington, DC, (2007).

[2] O'Gara, J. E., Alden, B. A., Walter, T. H., Petersen, J. S., Niederlaender, C. L., and Neue, U. D. Analytical Chemistry 67, 3809-3813, (1995).

[3] Cheng, Y. F., Walter, T. H., Lu, Z., Iraneta, P., Alden, B. A., Gendreau, C., Neue, U. D., Grassi, J. M., Carmody, J. L., and O Gara, J. E. LC GC 18, 1162-1173, (2000).

[4] Neue, U. D., Phoebe, C. H., Tran, K., Cheng, Y. F., and Lu, Z. Journal of Chromatography A 925, 49-67, (2001).

[5] Neue, U. D., Walter, T. H., Alden, B. A., Jiang, Z., Fisk, R. P., Cook, J. T., Glose, K. H., Carmody, J. L., Grassi, J. M., and Cheng, Y. AMERICAN LABORATORY 31, 36-39, (1999).

[6] Neue, U. D., Mendez, A., Iran, K. V., and Diehl, D. M. HPLC Made to Measure: A Practical Handbook for Optimization.WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, (2006).

[7] Velagaleti, R., Burns, P., and Gill, M. Drug Information Journal 37, 407-438, (2003).

[8] Ahuja, S., and Scypinski, S. Handbook of Modern Pharmaceutical Analysis, Academic Press.A Harcourt Science and Technology Company. 3, (2001).

[9] Gilar, M., Fountain, K. J., Budman, Y., Neue, U. D., Yardley, K. R., Rainville, P. D., Russell Ii, R. J., and Gebler, J. C. Journal of Chromatography A 958, 167-182, (2002).

[10] Marchand, D. H., Snyder, L. R., and Dolan, J. W. Journal of Chromatography A 1191, 2-20, (2008).

[11] National Center for Biotechnology Information. PubChem Compound Database; CID=2519, https://pubchem.ncbi.nlm.nih.gov/compound/2519 (accessed Oct. 5) (2015).

[12] ChemWiki The dynamic Chemistry E-textbook. High-Performance Liquid Chromatography. [online]. Last accessed on 14-March-(2015) at: http://chemwiki.ucdavis.edu/Analytical_Chemistry/Analytical_Chemistry_2.0/12_Chromatographic_and_Electrophoretic_Methods/12E%3A_High-Performance_Liquid_Chromatography.

[13] Taylor J. R. An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements. University Science Books. p. 94 (1997).

[14] Hagan P. Coffee could hold the cure for baldness. Daily mail health article. [online]. (2007). Last accessed on 14-March-2015 at: http://www.dailymail.co.uk/health/article-430669/Coffee-hold-cure-baldness.html