ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Zaid Mahdi Jaber Al-Obaidi et al /J. Pharm. Sci. & Res. Vol. 11(2), 2019, 339-342

A comparative study for the quantification of paracetamol in multicomponent oral solution employing standard addition method utilized in UV-Visible spectroscopy and RP-HPLC

Zaid Mahdi Jaber Al-Obaidi[1]*, Awatef A. Ebrahim Al Ani[2], Atheer Majid Rashid Al-Juhaishi[3], Ali Naim Hussein[4]
1 Department of Pharmaceutical Chemistry - College of Pharmacy - University of Kerbala- Kerbala – Iraq
2 Department of Pharmacy – Ibn Hayyan University College -Kerbala – Iraq
3 Department of Clinical Pharmacy - College of Pharmacy - University of Kerbala- Kerbala – Iraq
4 Department of Pharmacy – Asool Al-Deen University College -Baghdad – Iraq

Abstract:
The standard addition method is widely utilised in the drug analysis protocols. This method is utilized to compare between two of the most commonly used techniques namely, the Reversed Phase High Performance Liquid Chromatography (RP-HPLC) and the UV-Visible spectrophotometer. This study was conducted relying on a well-established USP analysis method for the quantification of paracetamol as an active pharmaceutical ingredient in a given oral solution. The RP-HPLC conditions were optimized for the analyses of the prepared samples inoculated with predetermined spikes of the standards. Consequently, these prepared samples were analysed employing both techniques. As a result, the plotted standard curves shows good linearity with regression coefficients of 0.9996 and 0.9995 for RP-HPLC and UV-Vis respectively. The Limit of detection (LOD = 3SD) and Limit of quantification (LOQ = 10SD) were calculated for both techniques. With the aid of the straight line equations, sample concentration was estimated for both techniques. The percent amount was found to be 97.74% and 96.39% for RP-HPLC and UV-Vis respectively. The authors conclude that the standard addition method utilised in UV-Vis was as accurate as that utilised in RP-HPLC and hence, no need to waste efforts, time and money with the usage of RP-HPLC unless clear need justifies its use.

Keywords: Standard addition, RP-HPLC, UV-Vis, Paracetamol quantification, Acetaminophen oral solution.

INTRODUCTION:

Paracetamol, also known as acetaminophen, is a frequently utilized drug worldwide for its analgesic and antipyretic properties. The chemical structure is shown in figure 1 below. The analysis of acetaminophen is revealed clearly in the United States Pharmacopeia (USP)[1] with full explanation of the chromatographic conditions utilized. In the same context RP-HPLC is a reliable and worldwide accepted, highly sensitive technique. This technique is utilized extensively in drug analyses[2,3]. A UV-Visible spectrophotometer is proven as a fundamental tool for many pharmaceutical drug analyses. This is due to its wide utilization in the identification, quantification, and purity measurement of the Active Pharmaceutical Product (API) in the raw materials, manufacturing processes, and final formulation[4]. Considering paracetamol, a comparative study was performed utilizing both above techniques[5]. However, standard addition method comparing both techniques for the quantitation of paracetamol has not been attempted yet. In this study, the quantity and percent assay of Paracetamol is determined with a standard addition method employed with UV-Visible spectrophotometer according to USP methods[6].

In this work, the authors aim to perform a comparative study for the quantitation of paracetamol in multicomponent oral solution utilizing standard addition method with the employment of both RP-HPLC and UV-Vis techniques. This study was conducted on January 2018 at laboratories of college of pharmacy- University of Kerbala.

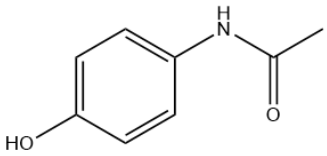

Figure 1: Chemical structure of *N*-(4-hydroxyphenyl)acetamide (acetaminophen, $C_8H_9NO_2$)[7]

MATERIALS AND METHODS

Chemicals and reagents:

1. Paracetamol was gifted from Samarra Drug Industry (SDI), Iraq
2. Colden® oral solution (Batch No. 1, Expiry Date 1-2018, SDI) was purchased from local pharmacy. Colden® oral solution contains the following active ingredients in each 5 mL of the solution:
   a. Paracetamol BP 120 mg
   b. Pseudo-ephedrine HCl 15 mg
   c. Chlorpheniramine Maleate 2 mg
   d. Vitamin C 50 mg
3. NYLON Syringe Filters Polypropylene housing diameter: 25mm pore size: 0.22µm non-sterilized purchased from Giorgio11185's store Jiangsu, Mainland, China.
4. 3-mL and 5-mL disposable syringes (Changzhou, Kangfulia, China) were purchased from local pharmacy.
5. Methanol (HPLC grade), and Micropipettes (White: 1µL -10 µL, Yellow: 10µL -100 µL and Blue: 100µL-

1000µL) all were purchased from Himedia Laboratories, Mumbai, India.

Instrumentation:

1. HPLC system (Shimadzu, Japan):
   i. C18 column (5 µm, 4.6 mm X 250 mm)
   ii. Mobile phase (methanol: water (1:3)).
   iii. 100 µL syringe.
   iv. 10 µL loop.
   v. Photodiode array detector was set at 243 nm.
   vi. Built-in degasser.
2. UV-Visible spectrophotometer (Model SPUV-26, Sco Tech, Germany)
3. 4-digit sensitive balance (Model Radwang- Wagi Elektroniczne, Poland)
4. Ultrasonic cleanser with heater (Model SRI, Copley scientific, UK)

HPLC System and Chromatographic Conditions:

Isocratic method was utilized with fixed Mobile phase ratio as methanol: water (1:3). The time of the run was optimized and set to 5 minutes. The flow rate was set as 1 ml/min while the wavelength was assigned as 243nm.

Paracetamol Standard stock solution preparation:

A 100-mg of anhydrous paracetamol powder was accurately weighed employing a 4-digit sensitive balance and then was dissolved within 10 ml Mobile phase placed within a 100-mL volumetric flask. The solution was sonicated for 10 minutes to assure full dissolution. Then, the solution was further diluted with Mobile phase to volume, and was mixed well to obtain a stock solution of 1 mg/mL or 1 µg/µL.

Lambda Max Selection:

A tube filled with exactly measured 10-mL Mobile phase was spiked with 200 µL of the paracetamol standard and mixed well. This solution was scanned to get the maximum absorbance. The lambda max was labelled and recorded.

Pharmaceutical Sample Preparation: Colden® Oral Solution:

An accurately measured volume of 20.833 ml of the Colden® Oral Solution (equivalent to 500 mg of paracetamol) was transferred to a 250-mL volumetric flask, where it was diluted with Mobile phase to volume, and mixed well. Then a 5.0 mL of this solution was transferred to a 1000-mL volumetric flask and was diluted with the Mobile phase up to volume and mixed well. Afterward, the resultant final solution was filtered as 10.0-mL aliquots with 0.22µm nylon-membrane syringe filter. The clear filtrate was utilized for the standard addition preparation.

Standard Addition Calibration Curve Preparation:

RP-HPLC Standard Addition Calibration Curve Preparation:

A series of five 10-mL tubes were prepared. Each tube was filled with 10.0 mL filtrate of the sample preparation final solution as revealed earlier. For a 5-tube series a distinct filter was utilized and the first 10 mL filtrate was discarded. The 5 tubes were labelled and spiked with (8, 16, 24, 32, and 40) µL of paracetamol standard. The obtained concentrations were (0.8, 1.6, 2.4, 3.2, and 4) µg/mL respectively. Finally, three replicates (three series with total 15 tubes) were prepared and run in the chromatographic system and the resulted peaks were recorded.

UV-Vis Standard Addition Calibration Curve Preparation:

A series of 6 10-mL tubes were prepared. Each tube was filled with 10.0 mL filtrate of the sample preparation final solution as revealed earlier. For a 5-tube series a distinct filter was utilized and the first 10 mL filtrate was discarded. The 6 tubes were labelled and spiked with (20, 40, 60, 80, 100, and 120) µL of paracetamol standard. The resultant standard concentrations were (2, 4, 6, 8, 10, and 12) µg/mL respectively. Finally, three replicates (three series with total 18 tubes) were prepared and tested within 1-mL quartz cell at 243 nm and the data was saved. All experiments were conducted at room temperature.

RESULTS:

Paracetamol lambda max:

The lambda max was found to be 243nm as shown the figure below:

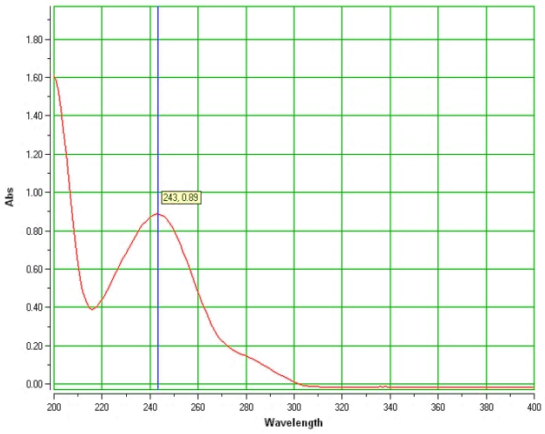

Figure 2. Reveals the observed lambda max for Paracetamol dissolved in distilled water employing UV-instrument. The wavelength of 243 nm was accredited.

Paracetamol Standard Addition Calibration Curve Observed with RP-HPLC:

The calibration curve was observed from the intensities of the peaks for each standard concentration as shown in figure 3.

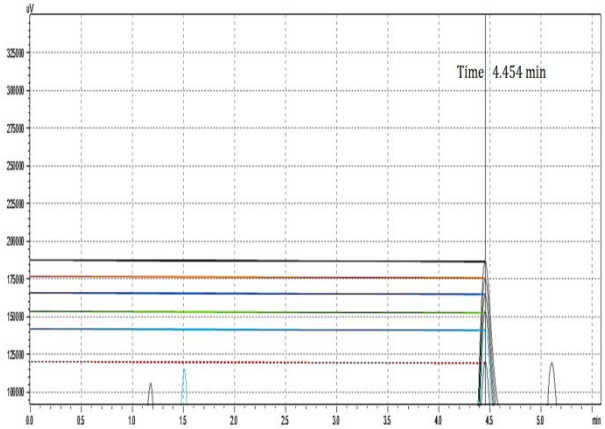

Figure 3: The peaks observed with standard addition for each paracetamol concentration. The dash line is for the paracetamol oral solution alone whereas the others are with the standard addition. The x-axis represents the time in minutes where the retention time was 4.454 min. The y-axis shows the intensity in µV and therefore data was taken manually by peak picking process.

Thereafter, the calibration curve was plotted as shown below:

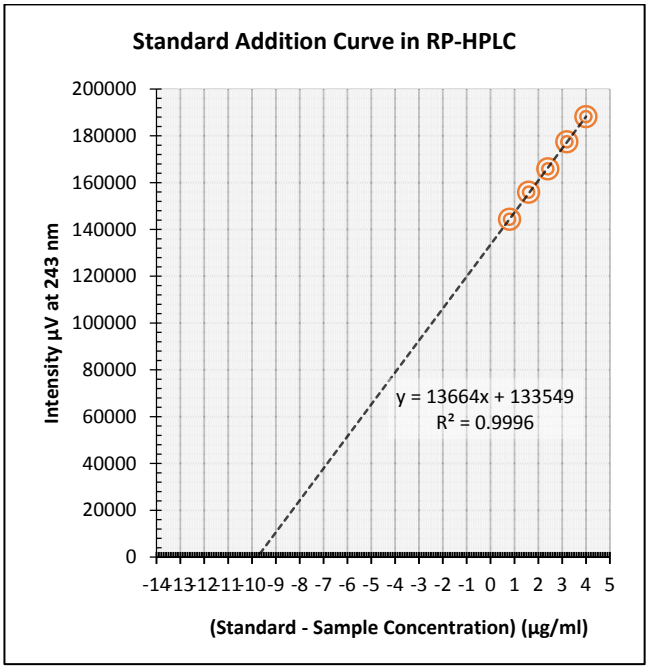

Figure 4: The standard addition calibration curve for paracetamol observed with RP-HPLC technique. The intercept of the extrapolated straight line represents the concentration of the diluted sample without the concentration of the standard.

Paracetamol Standard Addition Calibration Curve Observed with UV-Vis:

The data required for the standard addition calibration curve was observed from the UV-Vis machine. Consequently, the absorbance versus the concentration of the peaks for each standard addition concentration was plotted and shown in figure 5 below.

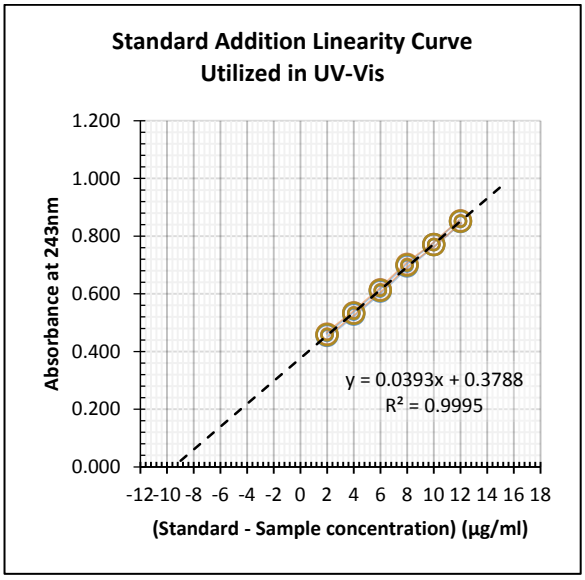

Figure 5: The standard addition calibration curve for paracetamol observed with UV-Vis technique. The intercept of the extrapolated straight line represents the concentration of the diluted sample alone.

Estimation of paracetamol content in the oral solution sample:

For the RP-HPLC the Straight line equation is y = 13664x +133549 and hence, when y = 0 then x = -9.774.

With the employment of the equation:

Standard concentration - Sample concentration = 0 - Sample concentration = x

- sample concentration = -9.774 then the sample concentration = 9.774 µg/mL

So, when x is multiplied by the dilution factor (i.e. 50,000 mL) then: 9.774 µg/mL × 50,000 mL = 488.69 × 10^3 µg = 488.69 mg.

While for the UV-Vis, the Straight line equation is y = 0.0393x +0.3788 so, when y = 0 then x = -9.639.

With the employment of the equation:

Standard concentration - Sample concentration = 0 - Sample concentration = x

- sample concentration = -9.639 then the sample concentration = 9.639 µg/mL

So, when x is multiplied by the dilution factor (i.e. 50,000 mL) then: 9.639 µg/mL × 50,000 mL = 481.93 × 10^3 µg = 481.93 mg.

After gaining such data, the comparison between the parameters of the RP-HPLC and the UV-Vis instruments can be tabulated below.

Table 1: A comparative data between the RP-HPLC and the UV-Vis instruments in the observation of the standard addition technique for the quantitation of paracetamol in oral solution formula.

Parameter	RP-HPLC	UV-Vis
Slope	13664	0.0393
Intercept	133549	0.3788
Regression Coefficient (R^2)	0.9996	0.9995
Number of points	5	6
Standard Deviation (SD)	1782.8 uV	0.002 Au
Relative Standard Deviation (RSD)	1.11%	0.36%
Limit of detection (LOD = 3SD)	5348.4 uV	0.006 Au
Limit of quantification (LOQ = 10SD)	17828 uV	0.020 Au
Stated amount (mg)	500	500
Amount found (mg)	488.69	481.93
Amount found (% of stated)	97.74	96.39

DISCUSSION:

The lambda max of 243 nm was selected to be as far as possible from the solvent effect. The cut-off value for methanol was recorded as 205 nm[8]. This has been totally agreed for many researchers to employ the 243 nm as the lambda max for paracetamol[9],[10],[11]. The standard addition method utilized with the RP-HPLC showed a good linearity with a regression coefficient of 0.9996. This is true for the UV-Vis methods also. The UV-Vis quantification method was very close to that of the RP-HPLC and hence, the authors conclude that the standard addition method can be used with UV-Vis even with multicomponent oral solution.

Acknowledgement:
The authors would like to thank Samarra Drug Industry (SDI), Iraq for their collaboration and offering of standard. The authors also provide their appreciation to the College of Pharmacy- University of Kerbala for offering their laboratories and all other facilities.

Conflict of interest:
The authors declare no conflict of interest is encountered.

REFERENCES:

1 U.S. Pharmacopeia for Acetaminophen. United States Pharmacopoeial Convention, Rockfield, MD, USA. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m150.html
2 P. Govardhan Reddy, V. Kiran Kumar, VVSS. Appala Raju, J. Raghu Ram , N. Appala Raju. The Estimation of Ceftaroline Fosamil in Lyophillized Powder for Injection by RP-HPLC. Research J. Pharm. and Tech 2018; 11(2): 455-458.
3 Zaid Al-Obaidi. The qualification and quantification of Caffeine in two different caffeinated pharmaceutical formulas employing RP-HPLC. ALBAHIR 2015; 2(4): 76-91.
4 Sk.Mastanamma, P. Saidulu. B. Srilakshmi, N. Ramadevi, D. Prathyusha, M. Vidya Rani. Analytical Quality by Design Approach for the Development of UV-Spectophotometric method in the Estimation of Tenofovir Alafenamide in bulk and its Laboratory Synthetic Mixture. Research J. Pharm. and Tech 2018; 11(2): 499-503.
5 Murtaza Sayed, M. Ismail, Sanaullah Khan and Hasan M. Khan. A comparative study for the quantitative determination of paracetamol in tablets using UV-Visible spectrophotometry and high performance liquid chromatography. Physical Chemistry 17(1) (2015) 1-5
6 U.S. Pharmacopeia for Acetaminophen Oral Solution. United States Pharmacopoeial Convention, Rockfield, MD, USA. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m180.html
7 National Center for Biotechnology Information. PubChem Compound Database; CID=1983, https://pubchem.ncbi.nlm.nih.gov/compound/1983 (accessed May 4, 2018).
8 Moussa Yabré, Ludivine Ferey , Issa Touridomon Somé and Karen Gaudin. Greening Reversed-Phase Liquid Chromatography Methods Using Alternative Solvents for Pharmaceutical Analysis. Molecules 2018; 23 (5): 2-25
9 Keppy et al. (2009).Analysis of Acetaminophen with the Evolution Array UV-Visible Spectrophotometer. Thermo Fisher Scientific, Madison, WI, USA.
10 Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. J Anal Bioanal Tech. 2012; 7 (3): 2175 – 2187.
11 Patel DM, Sardhara BM, Thumbadiya DH, Patel CN. Development and validation of spectrophotometric method for simultaneous estimation of paracetamol and lornoxicam in different dissolution media. Pharmaceutical Methods. 2012;3(2):98-101. doi:10.4103/2229-4708.103885.