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UV Spectrophotometric Development and HPLC Methods for Estimation of Paracetamol in Oral Syrup Formulation: Validation, Comparison and Application

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Abstract--- *In the present study, a high performance liquid chromatography (HPLC) and developed ultra-violet spectroscopic (UV) methods were validated and compared for detection the quantity of paracetamol forms that are taken orally . HPLC method was performed at ambient temperature using C18 column with a mobile step composed of water and methanol (70:30, v / v). The mobile phase was flow at a rate of 1 mL/min and UV-detector detection was achieved at 243 nm. UV method was completed with δmax at 256 nm. The results of the study were confirmed according to ICH guidelines, and the plotted standard curves show strong linearity for HPLC and UV-Vis with regression coefficients of 0.9978 and 0.9998 respectively. For both methods the detection limit (LOD) and the quantification limit (LOQ) were determined. Accuracy were 102.91, 100.00 and 99.78% for HPLC method, and 102.91, 100.00 and 99.78% for UV method. The methods were also found precise and robust (RSD< 2%). Assay of four oral syrup formulation was detected by both methods . There was no statistically important difference between the power obtained from both used methods by paired t-test and F-test at a meaning level of 5%. Thus the standard addition method used in UV-Vis was as safe as that used in RP-HPLC and; Therefore, there was no need to waste time, energy and money using HPLC unless it was justified by an emergency.*

Keywords--- *Paracetamol, UV Spectrophotometric Method, HPLC, Validation, Comparison.*

I. INTRODUCTION

Acetaminophen (Paracetamol) is a pain reliever and a fever lowering agent, utilized to cure many syndrome such as headache, muscleaches, arthritis, backache, toothaches, colds, and fevers (CAS registry number: 103-90-2). This is pure, odorless, crystalline powder with a slightly bitter taste, is freely soluble in alcohol, is soluble in hot water and sodium hydroxide 1 N [1]. Paracetamol PCM is the de-ethylated active metabolite of phenacetin, with molecular formula of C8H9NO2 and m.wt of 151.16. Its chemical structure is given in fig. 1. [2]

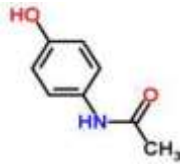

Fig. 1: Chemical Structure of Paracetamol

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Paracetamol drugs are sold in all pharmacies but also convenience stores, grocery stores, gas stations, gift shops in the hotel lobby, etc., with no limits on the amount that can be bought. Although the tremendous popularity of APAP as a pain reliever but a dose linked to toxin [3]. From the biochemical aspect, having absorbed hastily and entirely from the duodenum, acetaminophen's metabolism takes place within the hepatic microsomes. Ninety percent of acetaminophen in the liver is transformed to sulfate and glucuronide conjugates which are urinated. The rest is metabolized via oxidation by the hepatic cytochrome P450 into NAPQI which is a toxic, electrophilic intermediated substance with high reactivity [4]. The primary mechanism of action is thought to be cyclooxygenase inhibition (COX), with a predominant COX-2 effect. COX enzyme inhibition prevents the conversion of arachidonic acid to prostaglandin H2, a stable intermediate by-product that is converted into pro-inflammatory compounds. Across the central nervous system, COX-enzyme inhibition lowers prostaglandin E2 concentrations[5]. The difference between paracetamol and the "true" anti-inflammatory anti-steroidal drugs (NSAIDs) like ibuprofen or indomethacin. Since their interaction with the COX enzyme is not competitive, NSAIDs have more powerful peripheral anti-inflammatory effects, even more in an inflammatory (high hydroperoxides) [6]. In the United States Pharmacopeia (USP),[1] the study of acetaminophen is obviously stated with thorough clarification of the chromatographic conditions used. In the same way, RP-HPLC is a technique that is effective and recognized worldwide and is highly sensitive. This approach is widely used in study of drugs[7,8]. In several pharmaceutical drug tests a UV-visible spectrophotometer is known to be a critical tool. This is due to its extensive use in the identification, quantification and measurement of the purity of the Active Pharmaceutical Drug (API) in the raw materials, manufacturing processes and final formulation[9].

The literature survey reported that PAR was calculated by titrimetry[10], spectrophotometry[11,12], high performance thin layer chromatography (HPTLC)[13,14] GC – MS[15], HPLC – UV[16,17], and UPLC – MS / MS[18] in its single type or in combination with other medicines.

The aim of the current effort is to establish and validate a new analytical method used by UV-Vis techniques and comparative analysis with USP system (HPLC) for determining PCM in oral syrup dosage type. We have put an effort into developing a cost-effective, efficient, and robust method with enough validation parameter data in this proposed method. This research was performed at MDI Company-Baghdad Laboratories in January 2018.

II. MATERIALS AND METHODS

Reagents

Paracetamol working standard was gained from the MDI Company -Baghdad -Iraq. Two batches of oral syrup containing paracetamol 120mg/5ml were bought from MDI company store in Baghdad governorate after testing their license number, and another two brand were bought from market. The water and methanol utilized in the HPLC experiments were ultra pure and sodium hydroxide bought from Merck, Germany. All solution that utilized in the UV spectrophotometric experiments were dissolved and diluted via Distilled water.

Instrumentation and Analytical Conditions

HPLC Devices: A Shimadzu (Japan) HPLC system model LC labsolution, LC2030 3D plus was used.

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Ultraviolet detection was achieved at 243 nm. The drug analyses data were gained and repaired using LC solution (Version 1.3, Shimadzu, Japan) software running under Windows 10 on a PC. The mobile phase, water: methanol (3:1 v/v) pumped at a flow rate of 1.0 ml/min through the column (C18; 250 mm X 4.6 mm, 5µ Dr. Maisch, Germany) at 25^0C. The mobile phase was degassed prior to use under vacuum.

UV-Visible Spectrophotometer Devices

A double-beam UV-Visible spectrophotometer (Model UV-1800, Shimadzu, Japan) was utilized. Data from the drug analysis were collected and analyzed using UV Probe software (Shimadzu, Japan) running on a PC under the professional Windows XP.

Preparation of the Standard Solutions

For the HPLC process, stock solution prepared by paracetamol working standard weight accuracy of 50mg, transferred to a volumetric flask of 100 ml and dissolved in the mobile phase; 2ml of this solution was reduced by mobile process to 10ml. As needed, dilution was prepared at five different concentrations (0.02, 0.03, 0.04, 0.05 and 0.06mg / ml). For the UV spectrophotometric test, it reliably weighed 100 mg of paracetamol working standard, transferred to a 100 ml volumetric flask, and dissolved in a 0.01M sodium hydroxide solution. 5.0ml of this solution was diluted with some solvent to 50ml and 5ml of that solution was diluted in some solvent again to 50ml. There were also five different concentrations (0.005, 0.0075, 0.01, 0.0125 and 0.015) prepared by dilution as needed. The measurements were obtained as a blank, with 1cm quartz cells against 0.01N NaOH. Standard paracetamol solutions were tested separately within range 200-400nm.

Preparation of the Sample Solutions

HPLC Method

A Volume equivalent to 500 mg (V*δ ml) of oral solution paracetamol, was put in the volumetric flask of 250 ml and dissolution was happened in the mobile phase, then 5ml of prepared solution was taken and diluted to 250ml with mobile phase (0.04 mg/ml).

UV Spectrophotometric Method

An accurately volume equal to 250mg of paracetamol, was put in the volumetric flask250 ml and diluted in the 0.01N solution of Na(OH)2., then 5ml of this solution was diluted to 50 ml, and again 5ml from end solution was diluted to 50 ml by some diluent (0.01mg/ml).

Method Validation

The methods have been tested for linearity, accuracy, recovery and suitability to the device. The goal of this method is to explain that the method is suitable for immediate use, as stated in the instructions of the International Conference on Harmonization (ICH) [19].

Linearity

The standardization curve was achieved with five standard solution concentrations (0.02-0.06mg / ml for the HPLC method and 0.005-0.015mg / ml for the UV spectrophotometric method). The solutions were prepared in

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three-fold, it was estimated by using linear regression analysis, calculated using the least square regression method. LOD and LOQ were determined using the slope and intercept values from the regression equation.

Precision

The repeatability (within-day) and intermediate precision (inter-day) were used to achieve precision of the assay. The repeatability was assessed by comparing samples at the same concentration and on the same day. The intermediate precision was analyzed through three separate days of analyzing the assays. Six sample solutions were prepared (0.04 mg / ml for HPLC method and 0.01 mg / ml for UV spectrophotometric method) and each of these solution was read in triplicate.

Accuracy

The accuracy of an analytical procedure is the similarity of the test results to the true value obtained by the procedure. At the beginning of the process, it was calculated by recovery of known quantities of paracetamol working standard applied to the samples. Five replicates of three concentration levels were determined: 50%, 100%, and 150%. The findings were explain the percentage of recuperated paracetamol in the study and the percentage of RSD.

Specificity

The Specificity of HPLC system was tested by comparing the sample preparations that obtained from excipients which engage with the commercial oral syrup preparation with the chromatograms that gained from standard.

Robustness

The robustness of the HPLC system was calculated by evaluating the samples under different conditions, such as changes in the organic phase percentage (±10 percent) in the mobile phase and changes in the flow rate (0.9-1.1 mL / min). It observed the effect of parameters of retention time and system suitability. The drug content was evaluated for UV under the experimental variables such as shifts in sodium hydroxide concentration, sample distance, wavelength, and sample solution stability (Ambient and Refrigerator).

Statistical Analysis

A statistical procedure was carried out to find statistical difference among these developed methods. The statistical tests, i.e. analysis of variance (ANOVA) and paired t-test were applied to statistically compare these two analytical methods at 95 % confidence interval level ($p<0.05$).

III. RESULTS AND DISCUSSION

HPLC Method

This method was sophisticated to analyze paracetamol as an appropriate method in the pharmaceutical preparation. The conditions that used in chromatographic were changed to produce a better assay test. The selection of the mobile phase was optimized from the peak parameters (symmetry, tailing), time , run, cost and easy preparation. Figure(2) shows a typical chromatogram that was acquired from a standard analysis and a paracetamol sample solution. As shown in figure (2), paracetamol was eluted to form a symmetric edge. The retention time

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observed (7.4 min) permitted a rapid determination of the drug with run time of 1 ml / min, which is critical for routine analyses.

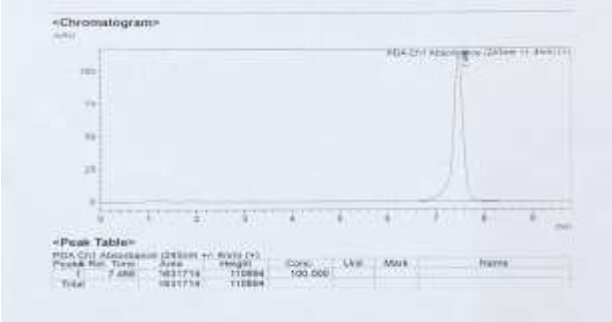

Fig. 2: Representative Peaks of Paracetamol Reference Standard 0.04 mg/ml

The paracetamol calibration curves were built by plotting peak area vs. concentration (Figure 3), paracetamol showed good linearity in the range of 0.02-0.06 mg / ml, with correlation coefficient of r2= 0.9978. The LOD and LOQ were 2.597 and 8.657 µg / ml, respectively, suggesting high sensitivity to the process. (Table 1).

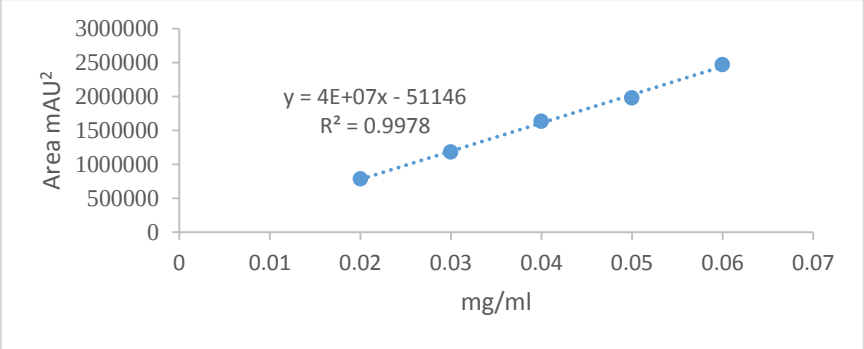

Fig. 3: Linear Response of Peak Area against Paracetamol Concentration (HPLC Method)

The precision of the method was detected and the mean recovery of the drug was done by taking different of the drug standard and analyzing with rate(50%, 100% and 150%) in the same method. The results was found to be 97.64, 100.00 and 100.66% respectively, with RSD 0.27, 0.26 and 0.48% respectively, evidencing an agreement values (Table 3). The system suitability factors of paracetamol was not change when the composition of M.ph, flow rate and wavelength were changed (Table 4a). The sample solution (Ambient and refrigerator) was stabled (Table 4b). The low values of the RSD% refer to the method was powerful enough.

UV Spectrophotometric Method

The suggested spectrophotometric method allowed a fast and accessible quantitation of paracetamol in oral solutions without any consuming for time in preparation of the samples. The absorption spectra of paracetamol showed λmax at 257nm, which was the wavelength used (Fig 4). was observed over the concentration range of 0.005- 0.02 mg / ml on standard paracetamol solutions, with the correlation coefficient of r2= 0.9998. LOD and LOQ were found to be respectively 0.261 and 0.868 mg / ml (Table 1), suggesting a high method sensitivity .

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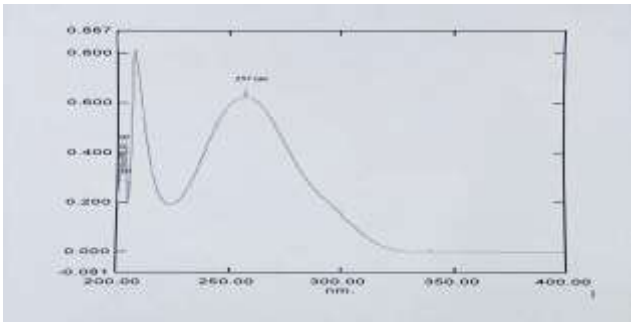

Fig. 4: UV Spectrum of Paracetamol

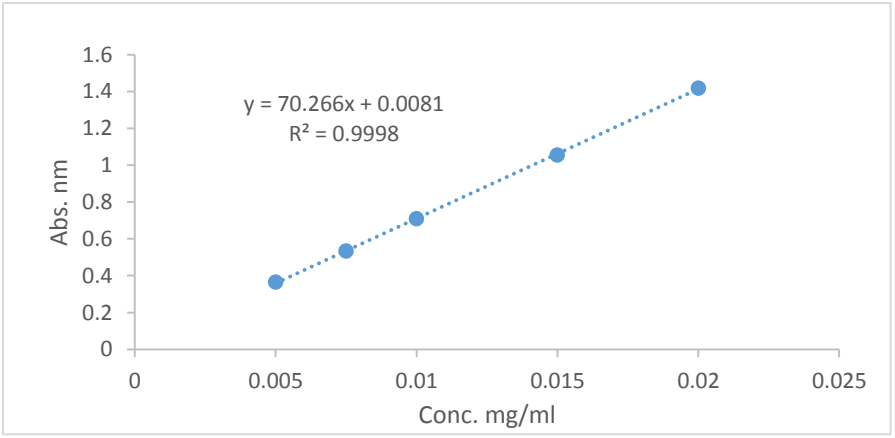

Fig. 5: Linear Response of Peak Area against Paracetamol Concentration (UV Method)

Table1: Results for Linearity Study

Parameters	HPLC method	UV method
Regression equation	Y=41747720*X-51146	Y=70.266*X+0.0081
Linearity Range (mg/mL)	0.02-0.06	0.005-0.02
Standard Deviation (SD)	35967.38	0.006102
LOD (µg/mL)	2.597	0.000261
LOQ (µg/mL)	8.657	0.000868
Slope	41548347	70.26552
Standard Error on Slope	205480.5	0.209069
Confidence interval of slope	41337260-41747720	70.14138-70.5069
Intercept	-51146	0.008147
Standard Error on Intercept	3361.1	0.005283
Confidence interval of Intercept	(-47259.6)-(-53121.2)	(-0.00216)-(0.008371)
Correlation Co-efficient	0.9978	0.9998

The method's accuracy was measured with RSD percentage values of 0.73% for repeatability and 0.53 and 0.73 for intermediate accuracy, which were observed for six replicates at 0.01mg / mL (Table 2). The accuracy was calculated by triplicate testing of three sample solution concentrations (50,100 and 150%), A good process accuracy was checked with a mean recovery of 102.91, 100.00 and 99.78 respectively, with RSD percentage of 0.96, 0.50 and 0.30 respectively (Table 3). The method's robustness was evaluated by assessing the effect of small variations in

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experimental variables, such as changes in the sodium hydroxide composition, sample thickness, wavelength and sample solution stability (Ambient and refrigerator). The small differences in each of the variables did not significantly impact outcomes. This gave an indicator of the reliability of the proposed method during periodic analysis (Table4a&b).

Table 2: Results for Precision Study

Parameters	HPLC method	UV method
Regression equation	Y=41747720*X-51146	Y=70.266*X+0.0081
Linearity Range (mg/mL)	0.02-0.06	0.005-0.02
Standard Deviation (SD)	35967.38	0.006102
LOD (µg/mL)	2.597	0.000261
LOQ (µg/mL)	8.657	0.000868
Slope	41548347	70.26552
Standard Error on Slope	205480.5	0.209069
Confidence interval of slope	41337260-41747720	70.14138-70.5069
Intercept	-51146	0.008147
Standard Error on Intercept	3361.1	0.005283
Confidence interval of Intercept	(-47259.6)-(-53121.2)	(-0.00216)-(0.008371)
Correlation Co-efficient	0.9978	0.9998

Table 3: Results of the Study by Recovery

Instrument	**HPLC**			**UV spectrophotometer**		
Recovery Level %	**50**	**100**	**150**	**50**	**100**	**150**
Mean Recovery %	97.64	100.00	100.66	102.91	100.00	99.78
SD	0.27	0.26	0.49	0.99	0.50	0.30
RSD %	0.27	0.26	0.48	0.96	0.50	0.30

Table 4: Robustness Study for Analytical Variation

Method	Parameter	Range	R. time ml/min	Mean of assay%	SD	RSD%
HPLC	M.ph Water- methanol%	62-38% 67-33% 72-28%	7.9 8 8	100.09 100.1 100.05	0.02 0.03 0.09	0.02 0.03 0.09
	F. rate ml/min.	0.9 1.0 1.1	9.9 8.9 8.1	100.22 100.05 100.11	0.09 0.17 0.08	0.04 0.08 0.08
	W. length nm	242 243 244	8.9 8.9 8.9	99.84 100.02 100.42	0.04 0.08 0.78	0.04 0.08 0.78
UV	Conc. Na OH mg/ml	0.01 0.02		100.29 100.09	0.87 0.55	0.87 0.55
	W. length	256 257 258		100.09 100.05 100.09	0.22 0.5 0.35	0.22 0.35 0.35

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Table 5: Robustness Study for Stability Solution

Storage condition	Method	Day1	Day2	Day3	Mean±SD	RSD%
Refrigerated	HPLC	100.21	99.74	99.41	99.91±0.44	0.44
	UV	101.91	101.18	101.49	101.53±0.37	0.36
Ambient	HPLC	100.15	99.74	99.52	99.80±0.32	0.32
	UV	101.71	101.31	101.08	101.37±0.32	0.31

IV. CONCLUSION

The comparison between analytical methods (spectrophotometric and the official method) were done by using statistical analysis. ANOVA was implemented and did not discover any important difference between the experimental values produced by the two methods in the sample. The measured t-value and F-value at a confidence interval of 95% were found to be less than the tabulated values of both methods. The HPLC method and the UV method sophisticated and achieved in oral solutions for the study of paracetamol were applied to different lots and brands (Table 5). The HPLC method is unique to oral solutions for testing paracetamol. No interfering peaks were noted when simulated excipient samples were applied to the sample solutions. UV spectrophotometric results showed no important difference from those obtained with HPLC method. Finally, the suggested method has been found to be effective, simple, fast, precise, accurate and sensitive for the routine of quality control in pharmaceuticals.

Table 6: Statistical Data for Comparison Assay of UV and HPLC Methods

Name &Company	Paracetamol syrup MDI	Antipyrol syrup SDI	Brand 1	Brand 2
HPLC method Mean%±SD	100.83±0.086	101.65±0.097	98.75±0.098	100.34±0.146
UV method Mean%±SD	101.01±0.116	101.59±0.163	98.93±0.085	100.82±0.265
F test*	1.81	2.25	0.75	3.27
t-value*	2.24	0.55	2.35	2.73

***Limits of 95 % confidence Interval**

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