# Development and Validation of A Reverse-Phase High Performance Liquid Chromatography Analysis for Paracetamol in Tablets Formulations

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**Abstract:** *A simple yet sensitive high performance liquid chromatography method was developed and validated for the determination of paracetamol (PCM) content in different tablets dosage form. The mobile phase consisted of a mixture of methanol: water (80:20 v/v). The HPLC analysis was performed at a flow rate of 1 mL/min using a Thermo Synchronise C18 (150 × 4.6mm, 5μM) and an UV detection wavelength of 254 nm was used. The method was validated for selectivity, linearity, precision, accuracy, limit of quantification (LOQ), limit of detection (LOD), robustness and solution stability. The calibration curve was linear over a concentration range of 20 to 70 μg/mL ($r^2$ = 0.9997) with LOD and LOQ of 0.01 and 0.03 μg/mL, respectively. The retention time for paracetamol was 1.5 min. The relative standard deviation percent (%RSD) and the relative error percent (%RE) of calibration curve were between 0.03-1.03% and 0.05-1.11%, respectively. The intra- and inter-day precision and accuracy were between 0.02-1.55% and 0.10-0.78%, respectively. In addition, the %RSD of short term*

*stability and the method robustness were between 0.4-1.20% and 0.09-1.43%, respectively. All results followed the ICH guideline Q2 (R1) which indicates the precision and accuracy of the developed method. The method was successfully applied to assess PCM content in different marketed tablets in Iraq.*

***Keywords: Drug content, HPLC, Paracetamol.***

## 1. Introduction

The quality of post-marketing drug products have been addressed in several WHO publications [1-3]. In Iraq, although the rigorous legal mandate for licensing of manufacture/import of drugs, but the complex transactions involving many intermediaries, lack of regulation in many boarder sites make the market for substandard drugs more lucrative one. Moreover, in quality control analysis, time and cost effective analysis are of important issues, which need to be addressed [4-6].

Hence, developing a single analysis method for any active pharmaceutical ingredient (API) will replace the need for two or three separate methods and thereby saving the time and cost. In addition, this single method can be easily used to evaluate the quality of post-marketing pharmaceutical products.

Paracetamol (PCM, acetaminophen) is a nonsteroidal anti-inflammatory drug which highly used in Iraq for its antipyretic and analgesic properties. It is available in different dosage forms (Tablets, syrup and suppositories). A review of the literature revealed that no single method was used to evaluate PCM content in tablets dosage forms from different brands. Most of the published articles evaluate PCM in single brand [7-10]. Therefore, the aims of this study are to develop a simple yet sensitive HPLC method for PCM and to use the developed method in the quality control analysis.

## 2. Materials and Methods

### 2.1 Purchasing samples

Four marketed PCM tablets were commonly available in the community pharmacies Ten strips for each brand were purchased (1 strip from each pharmacy) and named as Batch 1, 2, 3 and 4 (B1, B2, B3 and B4). The samples were collected by local collaborators so that the vendor had no indication as to the purpose of the purchases.

### 2.2 Apparatus and Chromatographic Conditions

The HPLC system consisted of a Shimadzu LC-20AD delivery pump (Shimadzu, Japan) equipped with the SIL-20A HT prominence autosampler, (Shimadzu, Japan) fitted with 100 µL sample loop, UV/Vis detector (SPD-20A, Shimadzu, Japan), DGU-20A3 prominence degasser (Shimadzu, Japan) and the chromatointegrator (CBM-20A prominence communications bus model, Shimadzu, Japan).

The chromatographic separation of the analyte was achieved at 40°C (CTO-10AS VP, Shimadzu column oven) using a Synchronise C18 analytical column, $150 \times 4.6$mm ID, 5µm (Thermo, USA) The mobile phase which was consisted of methanol: water (80:20 v/v) was filtered through a 0.45 µm nylon membrane filter (Whatman, UK) under vacuum and degassed prior to use. The analysis was carried out at a flow rate of 1.0 mL/min. The detector wavelength was set at 254 nm. The injection volume was 5 µL controlled by autosampler. Methanol was HPLC grade (J. T. Baker Analyzed, China). PCM was obtained from Zulat Pharmacy SDN BHD (Kuala Lumpur, Malaysia).

### 2.3 Solutions for the validation study

A stock solution of PCM was prepared by dissolving 50 mg of PCM in 50 mL of methanol in order to give the concentration of 1 mg/mL. Working solutions containing 20 to 70 µg/mL of PCM were prepared by serial dilutions of aliquots of the stock solution

with the mobile phase. Five µL aliquots were injected (six times) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area versus the concentration of PCM in µg/mL was plotted and the corresponding regression equation was obtained.

## 2.4 Preparation of the sample solution

One PCM tablet of each batch was crushed to a homogeneous powder and transferred to 100 mL volumetric flask, then dissolved in 50 mL of methanol by shaking the flask for 15 min with the help of sonicator, and volume was made up to mark with methanol. Then, the solution was filtered through a 0.45 µm nylon membrane filter (Whatman, UK) under vacuum and a filtrate was further diluted with water to obtain sample solutions of concentrations within the calibration curve range.

## 2.5 Selectivity

The selectivity of the developed method was determined in the presence of excipients present in pharmaceutical products. The interference between drug and tablet excipients were evaluated from the comparison of spectral purity obtained from the analysis for the standard solutions and sample solutions.

## 2.6 Linearity

To evaluate the linearity of the method, six calibration curves in the concentration range of 20 to 70 µg/mL were prepared. The calibration curves were plotted for a peak area of the analyte against the corresponding concentration, which is obtained by using the linear regression analysis.

## 2.7 Precision

The intra-day and inter-day precision were evaluated by analyzing quality control samples at low, medium and high

concentrations of 20, 50 and 70 µg/mL, respectively. For the intra-day variation, sets of six replicates of quality control samples were analyzed on the same day and for the inter-day validation, six replicates of quality control samples were analyzed on three different days [11, 12].

## 2.8 Accuracy

A recovery study was carried out by standard additions method. Known amount of standard drug was added to the pre-analyzed tablet formulation in 80, 100, and 120% of the target test concentrations. At each level of amount, three determinations were performed [11, 12].

## 2.9 Limit of detection and limit of quantification

The sensitivity of the method was determined based on the standard deviation of the response and the slope as described in ICH guidelines Q2 (R1) [13]. The limit of detection (LOD) and quantification (LOQ) were calculated according to the following equations [13]:

**LOD = 3.3 σ/ S; LOQ =10 σ/S**
Where σ = the standard deviation of the response; S = the slope of the calibration curve.

## 2.10 Solution stability

Reference solutions were stored in the refrigerator for 14 days and re-analyzed in an injection sequence by employing freshly prepared standard solutions for a short-term stability. The above experiments were performed by using low, medium and high quality control samples [12].

## 2.11 Method Robustness

The method robustness was assessed as a function of altering methanol and water volume ratio; the changes were over a range of

±5% of the target experimental condition. The concentration of
solution analyzed was 20 µg/ml (n=6).

# 3. Results and Discussion
## 3.1 Method optimization

Many Trails were performed to select a suitable mobile
phase. The mobile phase (methanol: water) was chosen due to its
availability, low cost and gave good peak resolution. In addition,
using water can increase the lifespan of the column if compared to
buffer solution [14].

## 3.2 Method validation

The newly developed HPLC method was validated to
confirm that the present method was suitable for its intended
purpose as described in ICH guideline Q2 (R1) [13]. The described
method was validated in terms of selectivity, linearity, precision,
accuracy, limit of detection, limit of quantification, solution
stability and robustness.

### 3.2.1 Selectivity

The method was shown to be selective for PCM. Figure 1a
shows a typical separation of PCM (30 µg/mL). Analysis of mobile
phase confirmed that there were no interfering peaks. In addition,
the effect of excipients from sample solution on the specificity of
the developed HPLC method was also examined. No significant
interfering peaks from the excipients were found at the retention
time of PCM (1.5 min) (Figure 1b). Hence, the developed analytical
method was suitable for the analysis of PCM tables.

### 3.2.2 Linearity

The mean linear regression equation was, y = 23530 (± 69) x
+ 36608 (± 3076) with a correlation coefficient of 0.9997. The
results showed that an excellent correlation existed between the

peak area and concentration of the analyte. The result of linearity is presented in Table 1.

*Table 1 Summery of Calibration Curve Results for Paracetamol (PCM), n = 6*

| Theoretical PCM concentration (µg/mL) | RSD (%)[*] | RE (%)[**] |
|---|---|---|
| 70 | 0.03 | 0.05 |
| 50 | 0.03 | 0.13 |
| 30 | 0.72 | 0.93 |
| 20 | 1.03 | 1.11 |

[*] **RSD%' relative standard deviation percent**
[**] **RE%, relative error percent**

### 3.2.3 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [13]. The intra- and inter-day precision (relative standard deviation percent, % RSD) ranged from 0.02 to 1.55. The RSD % of peak area of six replicates was found to be <2% All the results were within the acceptable limits which indicates that the proposed method is precise [15]. The results are shown in Table 2.

*Table 2 Experimental Values of Mean Concentration of*
*Paracetamol (PCM), RSD (%) Presented for Validation*
*Parameters of Paracetamol*

| Parameters | Theoretical PCM concentration (µg/mL) | % RSD |
|---|---|---|
| Intra-day | 70 | 0.02 |
| | 50 | 0.14 |
| | 20 | 1.22 |
| Inter-day | 70 | 0.06 |
| | 50 | 0.44 |
| | 20 | 1.55 |
| Short term stability | 70 | 0.40 |
| | 50 | 1.04 |
| | 20 | 1.20 |
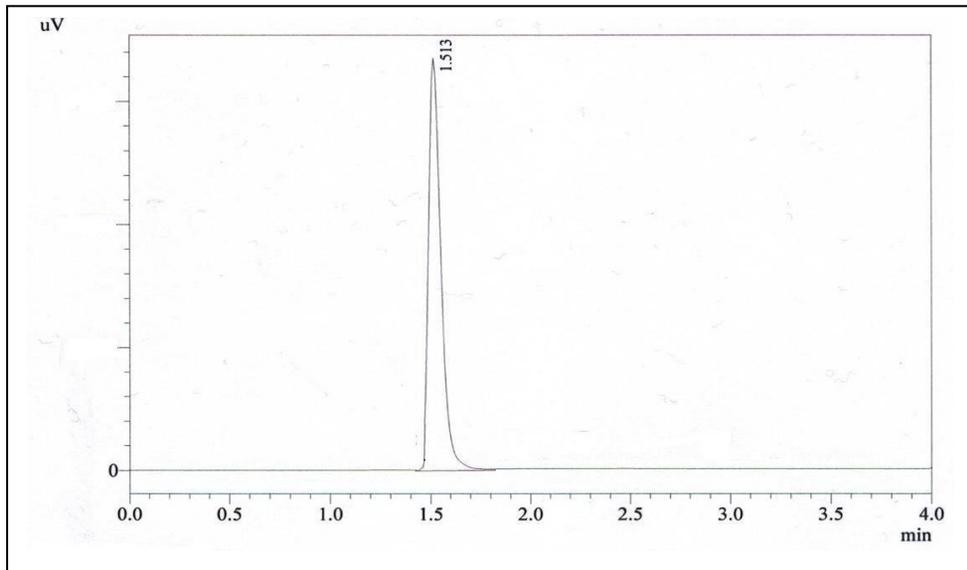
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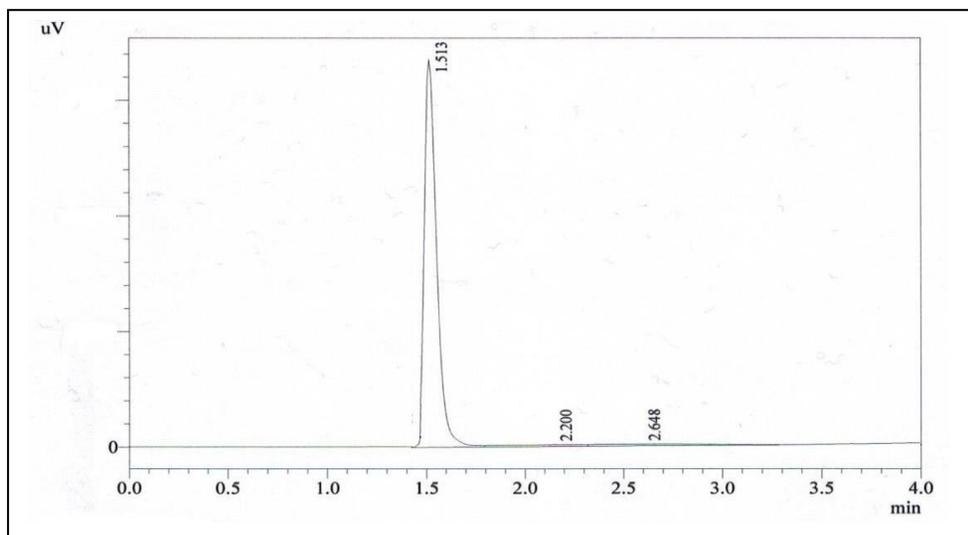


*(b)*

*Figure 1 Characteristic HPLC-UV chromatograms of paracetamol (PCM). (a) paracetamol standard solution, (b) Sample solution; the retention time of PCM is 1.5 min.*

### 3.2.4 Accuracy

The results of the recovery study of the physical mixtures are shown in Table 3. All the results of accuracy were within the acceptable limits [15] which indicate the accuracy of the validated method to detect PCM in tablet formulation.

### 3.2.5 Limit of detection and limit of quantification

The LOD and LOQ were found to be 0.01 μg/mL and 0.03 μg/mL, respectively. The results of LOD and LOQ were indicating a high sensitivity of the method.

*Table 3 Recovery Data for Paracetamol (PCM) of Four Different Batches.*

| % of target concentration | Mean % recovery | RSD % |
|:---:|:---:|:---:|
| 80 | 96.44 (0. | 0.78 |
| 100 | 101.11 | 0.04 |
| 120 | 98.76 | 0.10 |

### 3.2.6 Solution stability

The drug was found to be stable in the above mentioned condition which was sufficient to complete the whole analytical process. The solution stability results are shown in the Table 2.

### 3.2.7 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [13]. No marked changes in the chromatograms demonstrated with % RSD range from 0.09 to 1.43

%. The low values of the %RSD indicated the robustness of the method [13].

### 3.3 Application of the method

Four marketed PCM tablets were analyzed for drug content (B1, B2, B3 and B4). According to European Pharmacopoeia [16], the criteria for drug content states that PCM tablets must contain not less than 85% and not more than 115% of PCM stated on the label. The results of analysis of the four brands of PCM tablets are represented in Table 4. From the results of assay, all batches passed the EP specification.

*Table 4 Assay Results of Paracetamol Tablets.*

| Batch | Brand Name | Average content (%), N=100 |
|-------|------------|----------------------------|
| B1    | Panadol®   | 99.44±1.04                 |
| B2    | Paracetol® | 97.19±1.56                 |
| B3    | Paracetamol® | 96.06±2.22               |
| B4    | Strimol®   | 97.79±2.75                 |

### 4. Conclusion

A new simple yet sensitive reverse phase liquid chromatography method was developed for the determination of PCM in tablet dosage forms. The validated method showed satisfactory results for all the validation parameters tested. The short retention time allowed the analysis of a large number of

samples in a short period of time and it was therefore less costly. The developed method was successfully applied for drug content analysis of commercially available PCM tablets. All batches passed the drug content criteria.

## Disclosure

The author reports no conflicts of interest in this work.

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# تحليل البراسيتامول الموجود في نماذج متعددة من الحبوب عن طريق تطوير طريقة الطور المعكوس بجهاز الكروماتوكرفي ذو الضغط العالي للسائل

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**المستخلص**

في هذه الدراسة، محتوى مادة البراسيتامول في انواع عدة من الحبوب قد حدد باتباع طريقة بسيطة وحساسة بأستخدام جهاز الكروماتوكرفي. استخدم في هذه الطريقة مزيج من الميثانول والماء وبنسبة (80:20). بالاضافة الى ذلك،ان سرعة المزيج في عمود الكروماتوكرفي (كاربون-18) حددت ب 1 ميليليتر في الدقيقة و قابلة الامتصاص الطيفي ضبطت على درجة 254 نانو متر. ان التحقق من فعالية هذه الطريقة قد كان من خلال معرفة وحساب الانتقائية، الدقة، اقل كمية قابلة للقياس، اقل كمية مقاسة، ومتانة واستقرارية المادة وغيرها من العوامل. اظهرة النتائج ان منحني المعايير كان بخط مستقيم على نطاق التراكيز من 20 الى 70 ميكروغرام للمليتر الواحد واقل كمية قابة للقياس واقل كمية مقاسة كانت 0.01 و 0.03 ميكروغرام للمليتر الواحد، بالتتابع. ان وقت الاحتفاظ للبراسيتامول داخل عمود الكروماتوكرفي 1.5 دقيقة. علاوة على ما سبق، ان النسبة المئوية للانحراف المعياري النسبي والخطأ النسبي لمنحنى المعايير كانت بين 0.03-1.01 و 0.05-1.11، بالتتابع. اضافة الى ما سبق، كانت دقة القياس في اليوم الواحد وما بين الايام ودقة الاستخلاص بين 0.02-1.55 % و0.01-0.78 %، بالتتابع. بالاضافة الى ذلك، كانت النسبة المئوية للانحراف المعياري النسبي لمتانة واستقرارية المادة ما بين 0.4-1.20 % و 0.09-1.43 %، بالتتابع. وقد كانت النتائج باجمعها مطابقة لما هو منصوص عليه في الدليل الدستوري مما يدل على دقة ومتانة الطريقة

المطورة.  وقد تم تطبيق هذه الطريقة بنجاح لتقييم محتوى نماذج متعددة من حبوب البراسيتامول.

**الكلمات الرئيسية: المحتوى الدوائي، براسيتامول، HPLC.**