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HPLC Method for Qualitative Analysis of Acetaminophen in Pharmaceutical Painkillers (Paracetamol, Panadol, and Saridon) in Tablet Formulations

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ABSTRACT

This study aimed to verify the therapeutic components of the most common commercial pharmaceutical painkillers (Paracetamol, Panadol, and Saridon) and determine their degree of purity and thus protect the consumer using the high-performance liquid chromatography (HPLC) method. All ingredients were quantified in tablet formulations. The chromatographic separation was carried out on a slim C18 column by gradient elution using a mixture of acetonitrile and water (75:25 v/v) as the solvents. The flow rate was 1.0 mL min^{-1} and the injection volume was 10 µL. Detection was performed at 280 nm using a photodiode array detector. The retention times (t_R) of acetaminophen were observed to be 0.55 min (paracetamol), 0.53 min (panadol), and 0.53 min (saridon). The findings revealed that the retention time (t_R) values for acetaminophen in all three medications were very similar. In addition, the HPLC chromatogram revealed that saridon contains two other active ingredients (saridon and propyphenazone). The t_R of propyphenazone was detected to be 0.80 min (saridon). The t_R of caffeine was remarked to be 1.20 min (saridon).

طريقة HPLC للتحليل النوعي للأسيتامينوفين في مسكنات الألم الصيدلانية (الباراسيتامول والبانادول والساريدون)

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الكلمات المفتاحية:
HPLC
روبيفينازون
باراسيتامول
كافيين
ساريدون

الملخص

هدفت هذه الدراسة إلى التحقق من المكونات العلاجية لأكثر مسكنات الألم الصيدلانية التجارية شيوعاً (الباراسيتامول والبنادول والساريدون) وتحديد درجة نقاوتها وبالتالي حماية المستهلك باستخدام طريقة كروماتوغرافيا السائل عالية الأداء (HPLC). تم قياس جميع المكونات كمياً في تركيبات الأقراص. تم إجراء الفصل الكروماتوجرافي على عمود C18 رفيع بواسطة شطف متدرج باستخدام خليط من الأسيتونتريل والماء (25:75 حجم / حجم) كمذيبات. كان معدل التدفق 1.0 مل دقيقة $^{-1}$ وكان حجم الحقن 10 ميكرولتر. تم إجراء الكشف عند 280 نانومتر باستخدام كاشف مجموعة الثنائي الضوئي. وقد لوحظ أن أوقات الاحتفاظ (t_R) للأسيتامينوفين هي 0.55 دقيقة (الباراسيتامول)، 0.53 دقيقة (البانادول)، و0.53 دقيقة (ساريدون). كشفت النتائج أن قيم وقت الاحتفاظ (t_R) للأسيتامينوفين في جميع الأدوية الثلاثة كانت متشابهة جدًا. بالإضافة إلى ذلك، كشف التحليل اللوني HPLC أن الساريدون يحتوي على مكونين نشطين آخرين (الساريدون والبروبيفينازون). تم اكتشاف أن t_R للبروبيفينازون يبلغ 0.80 دقيقة (ساريدون). لوحظ أن t_R للكافيين يبلغ 1.20 دقيقة (ساريدون).

1. Introduction

One of the most important events in chromatography technology was the development of liquid chromatography into what is now known as high-performance liquid chromatography (HPLC) [1]. The pharmaceutical industry has widely used HPLC to separate, identify, and quantify the active ingredients and the excipients in the formulation [2]. The importance of chromatography in the pharmaceutical industry is to ensure that the quality of the drugs produced is excellent [3]. High pressure or high efficiency, high

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performance, where the development was done by introducing a new injection system (injection by valve) and introducing modern detectors to detect materials the moment they leave the column, and thus separation and detection are carried out in a few minutes [4].

This method is considered the most important development in liquid chromatographic separation methods, in which the stationary phase is in the form of fine-sized particles and the mobile phase (liquid) is pushed through the column owned by the stationary phase using a pump at pressures of up to 8000 Psi [5]. The pump is used to facilitate the flow of the medium moves through the column at speeds ranging from 0.50-5 ml/min, although the particles of the stationary phase reach a diameter of only several micrometers. It has already been possible to obtain flow speeds ranging from 1-4 ml/min using columns filled with particles of up to half a diameter [6].

The sample components are injected and then separated from each other based on the difference in the distribution equilibrium of each component between the liquid still phase and the liquid mobile phase [7]. The relative concentration of the component in the stationary phase Cs to the mobile phase Cm is expressed by the distribution coefficient K:

$$K = \frac{C_s}{C_m}$$

The equation shows that the movement of the component is inversely proportional to the distribution coefficient, that is, the component with the highest distribution coefficient moves slowly and vice versa [8]. Separation cannot take place except in the presence of a mixed difference in the distribution coefficient of the components of the mixture.

The HPLC is used to separate the active substance from the drug components, and determine its concentration [9]. It depends on the physical separation of the active substance through two phases, and this is represented by the appearance of a peak where the area inside it is calculated. Then, it is compared to the peak area of the standard solution of known concentration, and the concentration of the sample is equal to the substances' concentration.

The device consists of the following main parts: mobile phase reservoir, pump, injection station, column, detector, and means of recording the chromatogram (recorder or computer). Reagents used in high-performance liquid chromatography (HPLC): the UV detector is considered the most common among detectors; photodiode; fluorescence detector which is highly selective and sensitive, usually used to detect compounds formed during chain reactions; refractive index detector which is a general detector that has limited sensitivity; conductivity detector which has good selectivity and sensitivity [10].

The basis of the work of the HPLC device is that the material to be analyzed is introduced into the mobilizing flow, where the speed of movement of the material inside the column is determined by the following: the number of physical or chemical bonds between them and the stationary substance, and the amount of trapped material depends on the composition of the stationary material and the solvent system. The time it takes for the material trapped inside the column to reach the other end of the column and appear on the detector is called the retention time. HPLC devices are readily available because they are used in multiple fields, including the field of biotechnology, clinical medical technology, and pharmaceutical analysis, in addition to their use in chemistry, cosmetics, energy, environment, and food industries [11, 12].

Acetaminophen is one of the most extensively used medications for pain and fever relief; it holds a unique place among analgesic medicines [13]. Propyphenazone has the same analgesic and antipyretic properties as Acetaminophen; both of them are used for treatment of pain and infection [14]. Caffeine is the most widely used central nervous system stimulant, which inhibits the phosphodiesterase enzyme and has an antagonistic effect at central adenosine receptors [15]. The combination of the three drugs is given for the cure of mild fever and severe pain [14].

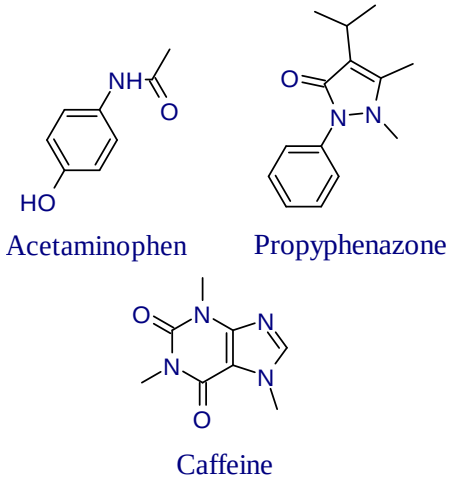


Fig. 1: Chemical structures of the analytes.

Saridon contains acetaminophen, propyphenazone, and caffeine (Figure 1) [16].

2. Materials and Methods

2.1 Materials: In January 2023, acetaminophen, Panadol, and Saridon were purchased from local pharmacies in Sirte, Libya. All chemicals were bought from authorized sources.

2.2 Instrumentation and chromatographic conditions: Analytical HPLC was achieved on a KNAUER Azura (Knauer, Berlin, Germany) system with a photodiode array detector. LC separations were performed on a C18 column. The mobile phase consisted of 75: 25 (v/v). The flow rate was set to 1.0 mL min−1 and UV detection was carried out at 280 nm at 27 °C.

2.3 Application to pharmaceutical formulation procedures: 20 tablets were finely ground. 2.0 g was taken from the ground powder of each type. This weight was put into a 250 mL volumetric flask, 100 mL of CH_3CN or methanol was added, and the flask was sonicated for 15 minutes before being filtered into a 250 mL volumetric flask. The residue was washed three times with the before-described solvent, followed by a final volume of the same solvent to get a stock solution with 0.053 M concentration. 1.9 mL of stock solution is transferred to a 100 mL volumetric flask and diluted with the same solvent to 100 mL to get the concentration of 10^{-3} M.

2.4 HPLC method: Using the mobile phase, the previously mentioned solution was diluted before being injected into the device with an injection volume of 10 µL. The separation was carried out under the aforementioned chromatographic conditions.

Table 1: retention time (t_R) values of acetaminophen, propyphenazone and caffeine in the samples that dissolved in CH_3CN.

Active compound	t_R values (min)		
	Paracetamol	Panadol	Saridon
Acetaminophen	**0.55**	**0.53**	**0.53**
Propyphenazone	---	---	**0.78**
Caffeine	---	---	**1.20**

Table 2: retention time (t_R) values of acetaminophen, propyphenazone and caffeine in the samples that dissolved in methanol

Active compound	t_R values (min)		
	Paracetamol	Panadol	Saridon
Acetaminophen	**0.55**	**0.53**	**0.53**
Propyphenazone	---	---	**0.80**
Caffeine	---	---	**1.20**

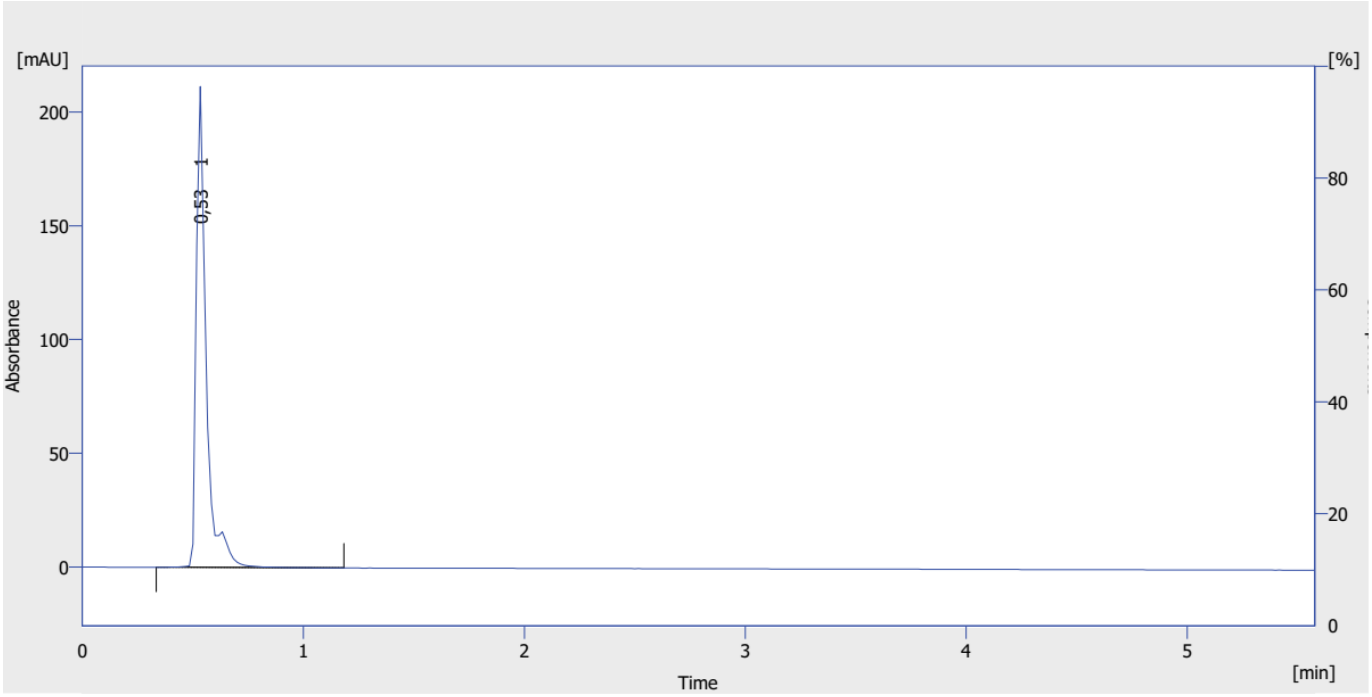
3. Discussion:

The Selection of the drugs was based on their frequent use among us in Libya by most people because they are most commonly used as pain relievers and to relieve fever. As shown in Figures 1, 2, and 3 there are three peaks with similar retention times that refer to the retention time of the active substance acetaminophen in three commercial drugs. The retention time (t_R) values for acetaminophen, are written down in Table 1. These t_R values

were 0.53 min (Paracetamol), 0.55 min (Panadol), and 0.53 min (Saridon).

On the other hand, there are three peaks for the saridon, at 0.55, 0.80, and 1.20 min. Peak 1 (paracetamol) with a retention time of 0.55 min, peak 2 (propyphenazone) with a t_R of 0.80 min, and peak 3 (caffeine) with a t_R of 1.20 min.

There are a bit differences between the t_R values that are shown in Tables 1 and 2. These differences may be attributed to numerous factors that affect the t_R, including the analysis conditions, column type, column dimension, column deterioration, and the presence of active sites like contamination and so forth [17]. To use a well-known example, when you cut off a portion of the column, all of the peaks show at shorter durations.



45986tghb Fig. 2: HPLC chromatogram of acetaminophen in Paracetamol using specified chromatographic conditions.

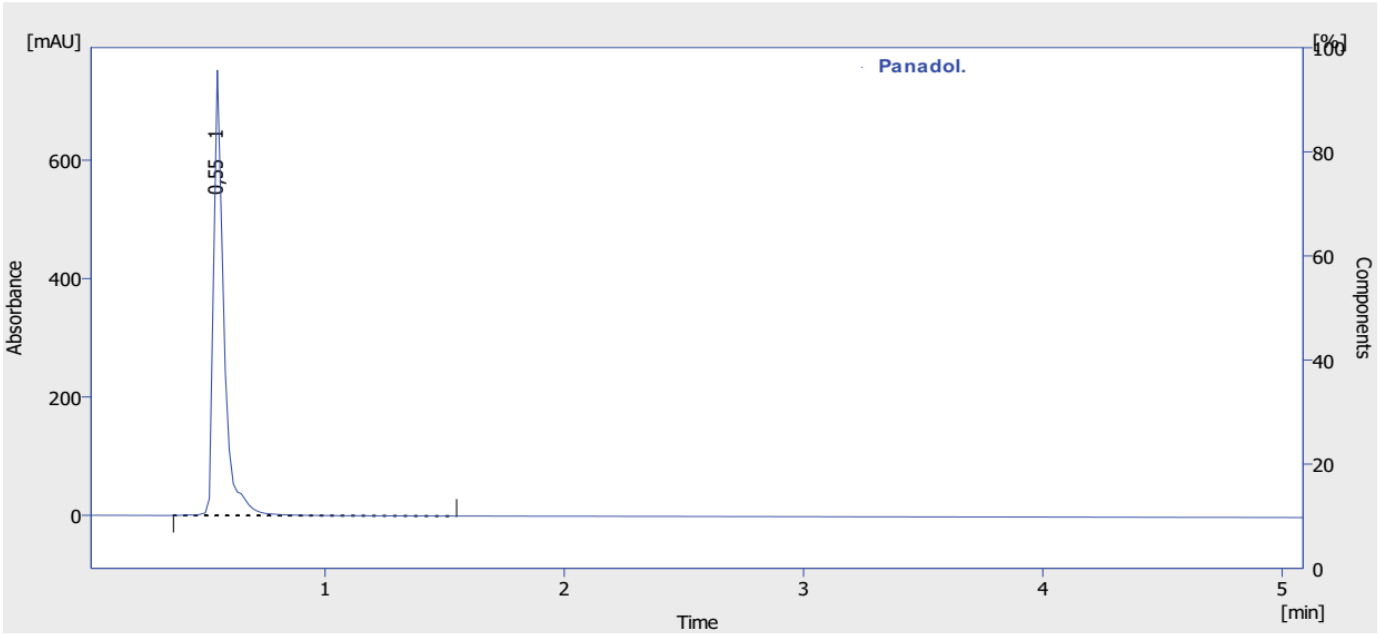


Fig. 3: HPLC chromatogram of acetaminophen in Panadol using specified chromatographic conditions.

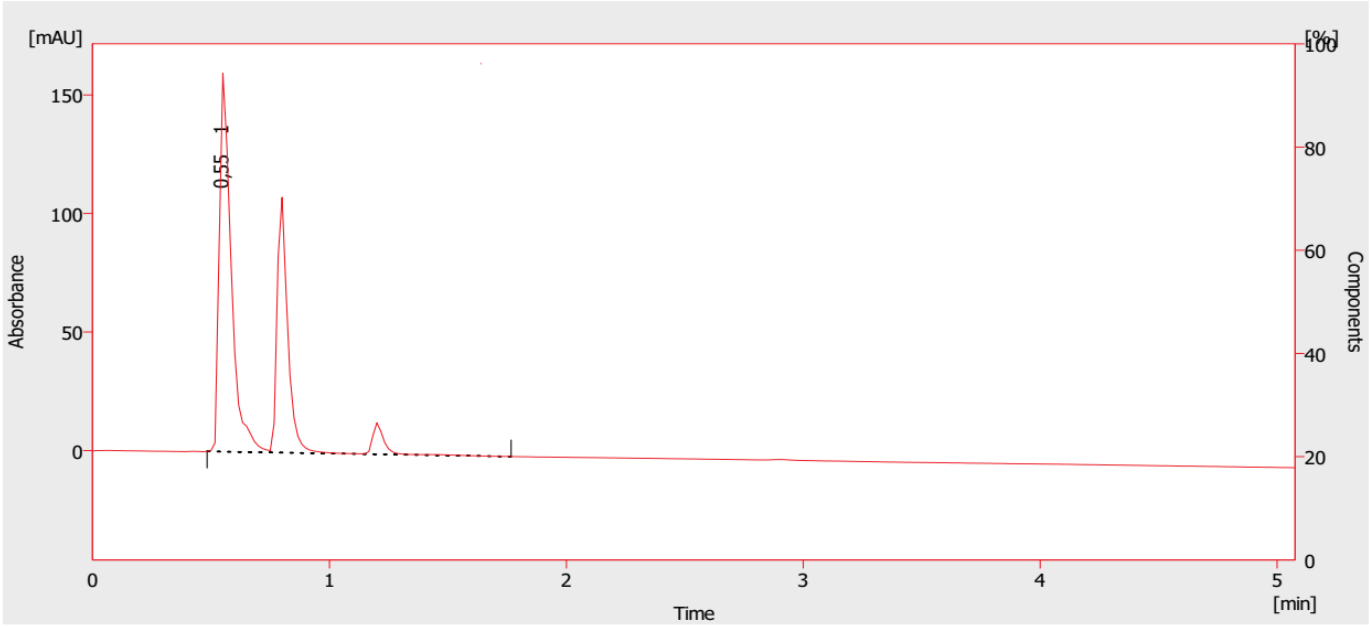


Fig. 4: HPLC chromatogram of acetaminophen in Saridon using specified chromatographic conditions.

3. Conclusion

The objective of this study was to ascertain the retention time (tR) values of the active functional groups of acetaminophen in the painkillers Paracetamol, Panadol, and Saridon using the high-performance liquid chromatography (HPLC) method. The findings revealed that the retention time (t_R) values for acetaminophen in all three medications were similar. This method is easy to use and can be used in the routine control of combined pharmaceutical preparations containing simultaneously acetaminophen, propyphenazone and caffeine.

4. Conflicts of Interest

There are no conflicts of interest declared by the authors.

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