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# DETERMINATION OF NAPROXEN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE

**Abeer Sattar Abbas[1], Mohsen Hamzah Baker[1] and Iqbal Salman Muhammed[2]***

[1]Department of Chemistry, College of Education for Women, Tikrit University, Tikrit, Iraq.
[2]Department of Chemistry, College of Education for Pure Sciences, University of Diyala, Iraq.
*e-mail : ikbalsalman696@gmail.com

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**ABSTRACT : Naproxen was separated and estimated in its pure form and in pharmaceutical preparations from three different companies (Pioneer, Mb.c, Actiras) containing three concentrations (500, 500, 500) mcg/ml of the pharmaceutical preparation, using Rp-HPLC. (0.1000) g of standard substance was weighed with an appropriate amount of several solvents (water, methanol, acetonitrile or a water-acetonitrile mixture) in a volumetric vial (100 ml), to know the solubility and stability of the drug under study. The prepared solutions were placed in an ultrasound machine at room temperature for (20) minutes with continuous stirring until complete dissolution, then completing volume to mark using appropriate solvent. Then three different concentrations were taken within limits of calibration curve, which are (7, 22, 43) mcg/ml and the measurement was repeated three times, under optimal conditions that were found in study, which is (50% water + 50% methanol) at acid function pH= 6.6, with an average flow velocity of mobile phase (1.5) ml/min and a measurement time of (1.862) minutes using a separation column with dimensions (15 × 0.46 cm/5um) C18, as (10 µl) of solution of the drug understudy in appropriate solvent, using manual injection through injection system and it was measured using a UV spectrophotometer detector at a wavelength of (254) nanometers, which is best wavelength in terms of area, as the area under top at this wavelength is higher. This indicates that materials have high absorbance at this wavelength. The proposed method has high precision and accuracy, as percentage standard deviation RSD% was less than one, and the recovery rate for three standard concentrations of the drug (98.43) and deviation value (2.40%). And the values of detection limit concentrations of naproxen are (0.5) mcg/ml and the area value is (1391).**

***Key words :*** Naproxen, high-performance liquid chromatography (HPLC), separation, estimation.

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## INTRODUCTION

Naproxen is a white crystalline powder, the molecular formula of the drug is $C_{14}H_{14}O_3$ and its molecular weight is 230.3 g/mol and it is insoluble in water and it can dissolve in ethanol and methanol (British Pharmacopoeia, 2005 and Ali *et al*, 2021). Steroids are anti-inflammatory, which inhibit action of prostaglandin enzyme, which is responsible for making substances in the body that cause inflammation and pain. The treatment has an analgesic and antipyretic effect [Ahmed, 2011, Wang et al, 2018) and is found in pharmaceutical preparations in form of tablets, syrup, and suppositories (Jain *et al*, 2011). Chromatography was first discovered by Russian botanist Mikhail Tsvet (Hadacek, 2002 and Moradi *et al*, 2019). In this technique, the mobile phase is a liquid carrying with it the components of sample or mixture concerned with separation through a stationary phase consisting of a chromatographic column packed with very small solid particles with a granular size ranging between (1.5 - 5.0 µm) that can remove well materials. In the liquid phase, process of holding components of mixture on chromatographic column takes place due to physicochemical forces between components and two phases (stationary and mobile) including Van der Waals forces, polar effects, electrostatic effects and hydrogen bonds, and sum of these forces determines quality of separation (Fitzpatric, 1978; Zhu *et al*, 2021; Khalil *et al*, 2020; Türkan *et al*, 2020 and Moradi *et al*, 2019). The most important classes of high-performance liquid chromatography technology based on polarity of mobile

phase are normal-phase high-performance liquid chromatography (NP-HPLC) and reverse-phase high-performance liquid chromatography (RP-HPLC) (Horvath, 1986 and Nahar *et al*, 2020). Fieding and Crathorne separated six types of polycyclic aromatic hydrocarbons and diagnosed them using (NP-HPLC) technique on a silica column with a time of (15 min) using a mobile phase of (0.01%) (Acetone in Hexane) with a flow rate of (1.5 ml/min) and detector used is a UV detector at a wavelength of (287 nm) (Crathorne, 1978). As for Old and Lichthaler, they separated some pollutants from PAHs in the marine environment and diagnosed them using (NP-HPLC) technology on a silica column and a mobile phase of (n-pentane) at a flow rate. (1-2 ml/min) detector used is a refractive index detector (Lichtenthaler and Oreld, 1983). PAHs were separated from human blood serum exposed to these compounds by Sirimanne and his group using RP-HPLC technology on an (ODS-C18) column and a UV detector at a wavelength of (254 nm). And with a time of (35 min) and a mobile phase consisting of acetonitrile) - (Water) with a flow rate of (1 ml/min). The results were compared with serum of a normal human being and study gave best Value for recovery at 60°C (Sirimanne *et al*, 1996). Chowdhury *et al* (2020) has used RP-HPLC technology using fixed-phase liquid crystals in column, to separate a mixture of PAHs compounds using (Methanol- Water) as a mobile phase and at a flow rate of (1 ml/min). Given importance of pharmaceutical compounds in medical field and the pharmaceutical industry in general and naproxen in particular, our goal was to develop rapid, economical, and accurate methods for determination of drug in its pure state and pharmaceutical preparations, using extraction technique by high-performance liquid chromatography (HPLC).

**Table 1:** Used equipment.

| Appliances | Origin and Manufacturer |
|---|---|
| High Performance Liquid Chromatography | SHMADZU, LC-20AD, Japan |
| Electric balance (0.0001 gm) | Italy |
| pH meter | Mettle Toledo, Japan |
| Ultrasonic water bath sensitive | APEI, PD-303 UV, Japan |

## EXPERIMENTAL

### Instruments used

Table 1 shows the devices used in the measurement.

### Chemical materials used

The standard naproxen was obtained from the State Company for the Manufacturing of Pharmaceuticals and Medical Appliances, Samarra, Iraq. The chemicals that were used in the research under study were obtained from the local markets and were of high purity, as shown in Table 2.

### Pharmaceuticals commercial formulated used

In this study, some pharmaceutical preparations available in the local markets and from different origins and factories were used for the purpose of estimating active substances of the drugs under study, as shown in Table 3.

### Working principle

Naproxen was separated and quantified in its pure form and in pharmaceutical preparations, using Reversible Phase High Performance Liquid Chromatography (Rp-HPLC) (Dhandapani *et al*, 2010).

### Select the appropriate solvent

0.1000 g of the standard substance was weighed with an appropriate amount of several solvents (water, methanol, acetonitrile, or a water-acetonitrile mixture) in a volumetric vial (100 ml), for the purpose of knowing the solubility and stability of the drug under study. The prepared solutions were prepared in an ultrasound device at room temperature for 20 minutes with continuous stirring until completion of dissolution, then completing the volume to mark by using the appropriate solvent (Gómez *et al*, 2020) and the obtained results are shown in Tables 4-5.

### Preparation of a Standard Solution for (NAP) (1000 ug/ml)

This solution was prepared by dissolving (0.1000 g) of the standard substance of naproxen with an amount of (water - methanol) in a volumetric bottle of 100 ml, and placing the solution in an ultrasound device at room temperature for 20 minutes with continuous stirring. Dissolving process Complete the volume to the mark using

**Table 2 :** The chemicals used in the experiment.

| Chemical compounds | Chemical formula | Purity% | Molar mass g/mol | Prevenance |
|---|---|---|---|---|
| Naproxen | $C_{14}H_{14}O_3$ | 99.99 | 230.26 | SDI – Iraq |
| Deionized water | $H_2O$ | 100 | 18 | Switzerland |
| Methanol, HPLC grade (MeOH) | $CH_3OH$ | 99.9 | 32.04 | CHEM-LAB Belgium |
| Acetic acid | $C_2H_4O_2$ | 99.5 | 60.052 | ALPHA CHEMIKA- India |
| Acetonitrile HPLC grade (ACN) | $C_2H_3N$ | 98.8 | 41.053 | CHEM-LAB Belgium |

**Table 3 :** The commercial pharmaceuticals.

| Drug Samples | Contenting (mg/ Does) | Provenance |
|---|---|---|
| **Naproxen** | 500 | Actavis |
| **Naproxen** | 500 | Mb-c |
| **Naproxen** | 500 | Pioneer |

**Table 4 :** The type of solutions used as mobile phases.

| No. | Solutions used | | | |
|---|---|---|---|---|
| | $H_2O$ | $CH_3OH$ | $CH_3COOH$ | $CH_3CN$ |
| 1 | 50% | 50% | | |
| 2 | 49% | | 1% | 50 |
| 3 | 60% | | | 40% |

the appropriate solvent, as this solution was used as a storage solution (Vittal *et al*, 2019).

### Preparation and application of various pharmaceutical preparations

This study was conducted by preparing different solutions of commercial drug models from several companies. Ten pills of pharmaceutical preparations (Pioneer, Mb.c, Activas) containing (500, 500, 500) mg of naproxen were weighed after being well crushed in for each grain, the total weight of the ten grains of the three companies was (5.380, 5.374, 5.378) g, respectively and the average weight of one grain (0.5380, 0.5374, 0.5378) g, respectively, after which the powder was well homogenized for the purpose of estimating the active substance. The solution was prepared by taking (0.001) g of the weight of pill with an equivalent of active substance and diluting the solution to (10) milliliters using mobile phase, and then 10 µl of the solution under study and dissolved in the appropriate solvent was injected using manual injection through the injection system. The measurement was carried out by applying optimal conditions using a UV spectrophotometric detector at a wavelength of (254) nm and a mobile phase (50% water + 50% methanol) at pH = 6.6 and at a rate of flow rate of the mobile phase (1.5) ml / minute and a measurement time of 1.8 minutes using a separation column with dimensions (15×0.46 cm/5um) C18 (Ziyaadini *et al*, 2021) as shown in Fig. 7.

## RESULTS AND DISCUSSION

### High Performance Liquid Chromatography Method

### Optimum conditions selection for the drug

This method was used to determine the naproxen drug under study, as this method was implemented through the injection of (10 µl) of the standard naproxen solution using manual injection through the injection system, as the solution of the drug dissolved in the solvent (50% methanol and 50% water) was injected. Suitable for filtering using special filters of type 0.45 µl in a stream of liquid mobile phase on a column of type (15 × 0.46 cm/ 5um) C18 by reversible phase high-performance liquid chromatography technique (Rp-HPLC), the drug was detected and the signal was recorded for the used ultraviolet detector (Hassan and Nam, 2021) and a number of factors were studied for the purpose of obtaining a regular peak of the estimated substance with a short retention time, as the following were studied:

### First: Selection the Mobile Phase Type

This study was conducted using different types of solutions as mobile phases in the form of a mixture, as in Table 5, for the purpose of obtaining the best shape and separation of the naproxen bit, as (10 µl) of the solution of the substance under study dissolved in the solvent was injected, using manual injection through the injection system, and the measurement was carried out using an ultraviolet spectrophotometer detector at a wavelength of 254 nm and at a flow rate of 1.5 ml/min using a separation column with dimensions (15 × 0.46 cm/5um). C18 and the Table 4 represents the type of solutions used as mobile phases and the Fig. 1 represent the shape of the drug peaks under study, the shape of the peak, area and time of detention were adopted for the best phase, and the best mobile phase was (50% methanol + 50% water).

### Second: Study of the Mobile Phase Flow Rate

For the purpose of obtaining the best separation time for a short retention time, different velocities of the solution were studied, which are (0.7, 1, 1.5) ml/min, to know the effect of the flow velocity on the separation process, as a mobile phase (50% methanol and 50% water) and a function was used. Acidic (pH= 6.6). The measurement was carried out using a UV spectroscopy detector at a wavelength of (254 nm), using a separation

**Table 5 :** Results of the mobile phase study.

| Type of mobile phase | Area | Retention time tR. min |
|---|---|---|
| $H_2O$% + $CH_3OH$ % | 1572525 | 1.862 |
| 49% $H_2O$ + 1% $CH_3COOH$ + 40% $CH_3CN$ | 3676760 | 2.812 |
| 60% $H_2O$ + 40% $CH_3CN$ | 1627155 | 6.540 |

**Table 6 :** Results of the study of mobile phase flow velocity change.

| Flow rate ml/min. | Retention time tR. min. | Area |
|---|---|---|
| **0.7** | 3.599 | 3321408 |
| **1** | 2.558 | 2080171 |
| **1.5** | 1.862 | 1572525 |

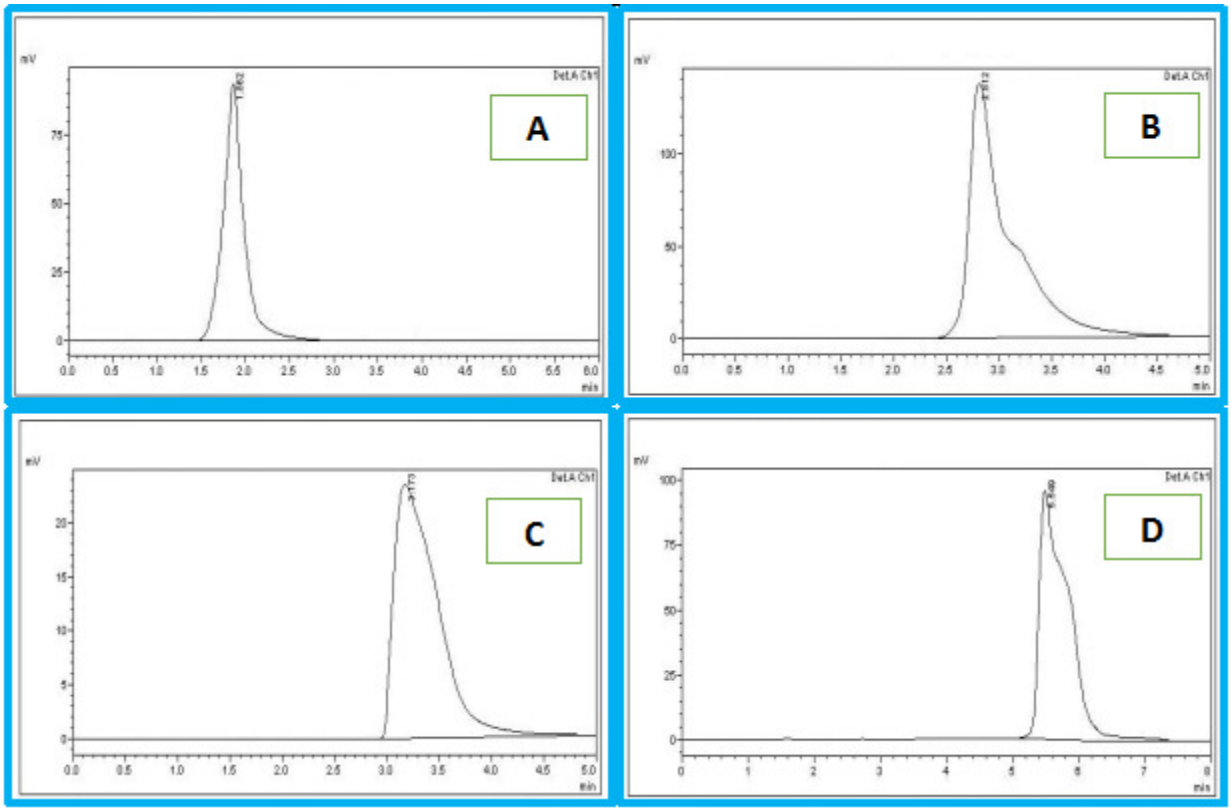


**Fig. 1 :** Mobile phase A: 50%methanol + 50%water (which is ideal), B: 50% acetonitrile+ 49% water + 1% acetic acid, C:50% acetonitrile + 50% water, D:40% acetonitrile + 60% water.

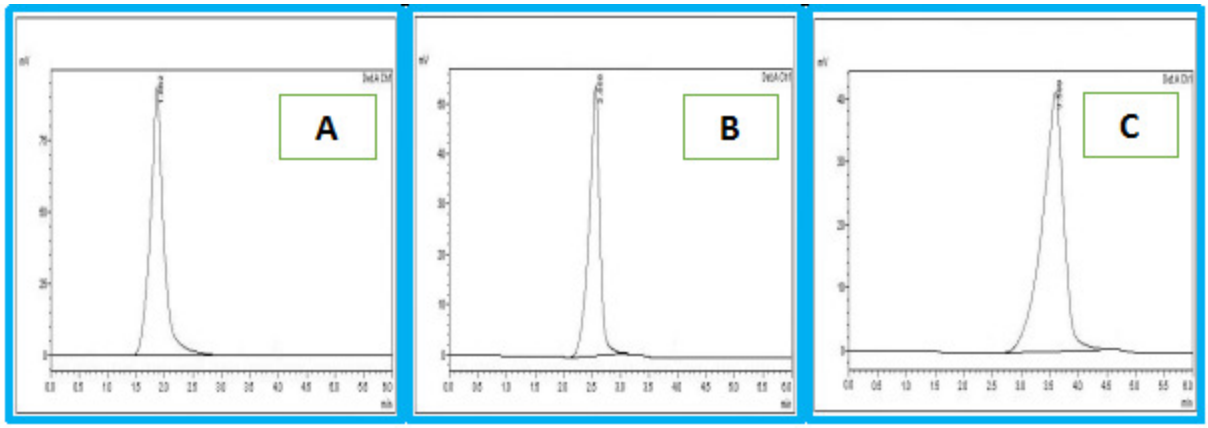


**Fig. 2 A :** Flow rate of the mobile phase A: 1.5 (which is ideal), B: 1, C: 0.7.

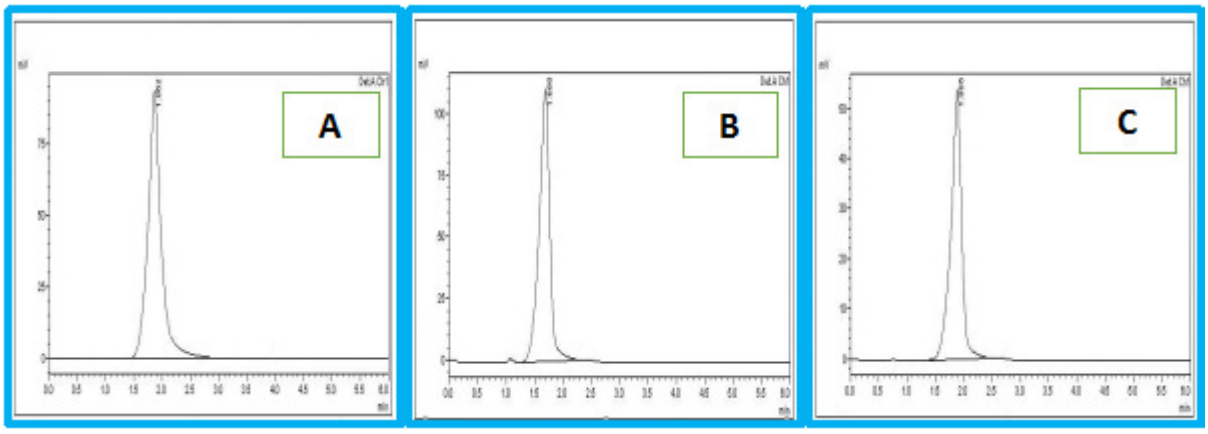


**Fig. 3 :** The peak of the drug when using a wavelength of A: 254 nm (which is the ideal), B: f 235 nm, C: 270 nm.

**Table 7 :** Results obtained from the wavelength study.

| Wavelength range | Retention time tR. min | Area |
|---|---|---|
| **235** | 1.888 | 1321098 |
| **254** | 1.869 | 1572525 |
| **270** | 1.985 | 176539 |

**Table 8 :** Results obtained for working conditions and the best conditions.

| Parameter | Use Factor | Area | Rt | Optimum value |
|---|---|---|---|---|
| Mobile phase | 50% MeOH + 50% $H_2O$ | 1572525 | 1.862 | 50% MeOH + 50% $H_2O$ |
| | 50% Acetonitrile + 49% $H_2O$ + 1% $CH_3COOH$ | 3676760 | 2.812 | |
| | 40% Acetonitrile + 60% $H_2O$ | 1627155 | 6.540 | |
| Flow rate (ml/min) | 0.7 | 3321408 | 3.599 | 1.5 |
| | 1 | 2080171 | 2.558 | |
| | 1.5 | 1572525 | 1.862 | |
| Wavelength (nm) | 235 | 1321098 | 1.888 | 254 |
| | 254 | 1572525 | 1.862 | |
| | 270 | 176539 | 1.985 | |

**Table 9 :** Analytical statistics obtained from calibration curves.

| Statistical factors | Value/ NAP |
|---|---|
| **Linear equation** | $Y=17744_x + 8382.1$ |
| **Slope (m)** | 17744 |
| **Intercept** | 8382.1 |
| **Correlation of Linearity $R^2$** | 0.9995 |
| **Percentage linearity ($R^2$%)** | % 99.95 |
| **Correlation Coefficient (r)** | 0.9997 |
| **R.S.D.** | 0.13 |
| **LOD" µg/mL** | 0.5 |
| **LOQ" µg/mL** | 1.65 |
| **Linearity range µg/mL** | 1-60 |

column (15 × 0.46 cm/ 5 um) C18 and a measurement time (10 minutes). The ideal speed was 1.5 ml/min, as in Table 6 and Fig. 2.

### Third: Study of Wavelength Change

This study was conducted using a number of wavelengths (235, 254, 270) nm using the mobile phase consisting of (50% methanol + 50% water) with pH = 6.6, as (10 µl) of the drug solution was injected. Under study in the appropriate solvent, using manual injection through the injection system. The measurement was carried out using a UV spectrophotometer detector at a flow rate of the mobile phase (1.5 ml/min) and a measurement time of (1.869 minutes) using a separation column with dimensions (15 × 0.46 cm/5um) C18, from Through the obtained results, we conclude that the wavelength (254) nm is the best wavelength in terms of area, as the area under the top at this wavelength was the highest peak, which indicates that the materials have high absorbance at the wavelength (254) nm and as shown in Table 7 and as in Fig. 3.

### Fourth: The acidic function for the Mobile Phase

As for the acid function, it was stable because the drug alone does not need to adjust the acid function and Table 8 shows the results obtained for the optimal working conditions.

### Standard Series for Drug

The drug solution that was used for the purpose of preparing the standard series of the drug with a concentration (100 mcg/ml) was prepared. The concentrations of the series were prepared by making the appropriate determination using the solvent (50% methanol and 50% water) by applying the dilution law of the solutions and the concentrations ranged between (1-60) µg/ml, to determine the range of concentrations subject to the Lambert-Beer law, (10 µl) of the drug solution was injected using manual injection through the injection system, and the area under the top of the series of drug concentrations was measured and recorded by applying the optimal conditions (Ramya *et al*, 2020) and as in the Fig. 4 and Table 9.

### Calibration Curve for Naproxen

A series of solutions whose concentrations ranged from (1-60) mcg/ml of the solution of the drug under study were prepared for the purpose of preparing the standard calibration curve for estimating the substance under study. The standard curve was obtained by plotting the area values on the (Y) axis against the known concentration on the (X) axis and Fig. 4 shows the calibration curve for naproxen and it was shown that the range of concentrations above subject to the Lambert-Beer law, a deviation occurs on the straight line when this concentration is exceeded and the obtained correlation coefficient ($R^2$) values were (0.9995) for naproxen and this indicates a strong correlation between the measured area and concentration of the drug within the curve range, and that the values of The LOD limit and LOQ quantification limit are (0.5) and (1.65), respectively

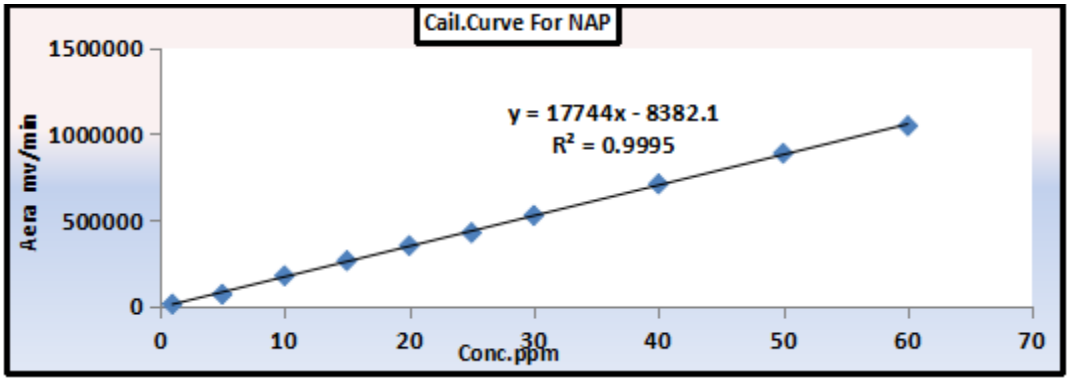


**Fig. 4 :** Calibration curve (NAP) using high-performance liquid chromatography.

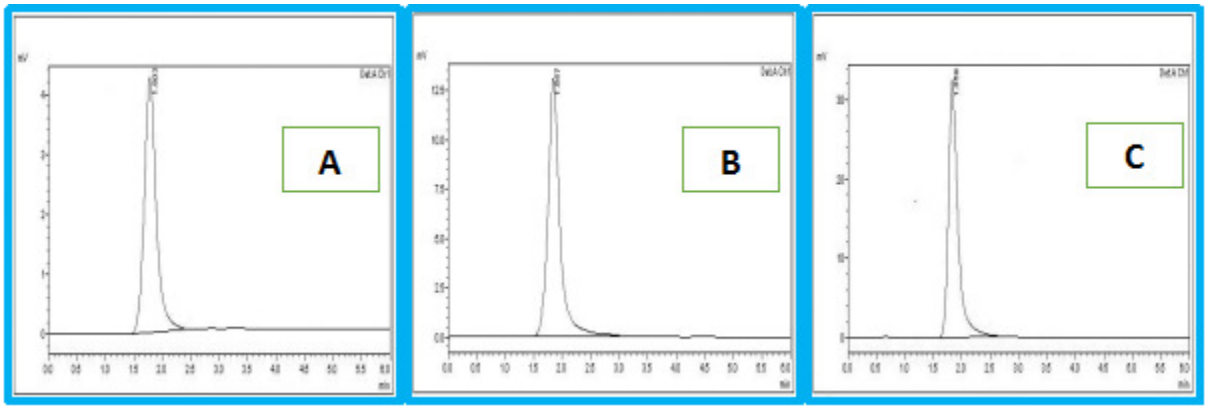


**Fig. 5 :** The peak of drug when concentration of A: 5mg/mL, B: 15mg/mL, C: 25mg/mL.

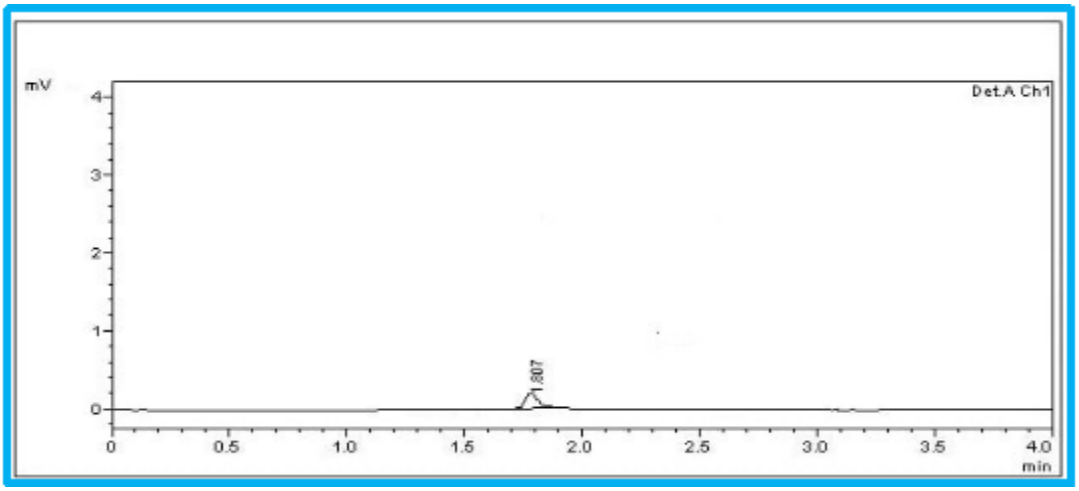


**Fig. 6 :** Detection limit values of naproxen.

(Tartaglia *et al*, 2020). Fig. 4 shows the calibration curve for naproxen and Tables 4-9 shows the statistics obtained from the standard calibration curve for naproxen and Fig. 5 shows the concentrations used in the study.

## Method accuracy and precision

This study was conducted by taking three different concentrations within the limits of the calibration curve, which are (7, 22, 43) mcg/ml and repeating the measurement three times, as the optimal conditions were applied (50% water + 50% methanol) at the acid function pH = 6.6. Whereas, 10 µl of the solution of the drug under study was injected into the appropriate solvent, using manual injection through the injection system. The measurement was carried out using a UV spectrophotometer detector at a wavelength of (254) nm, with a flow rate of (1.5) ml/min, and a measurement time of (1.862) minutes using a separation column with dimensions (15 × 0.46 cm/ 5um) C18. The obtained results are summarized in Table 10, which clearly shows that the proposed method has high precision and accuracy, as it is expressed as a percentage standard deviation (RSD%), whose value is less than one. As for accuracy, it is expressed as a percentage of relative error, and the

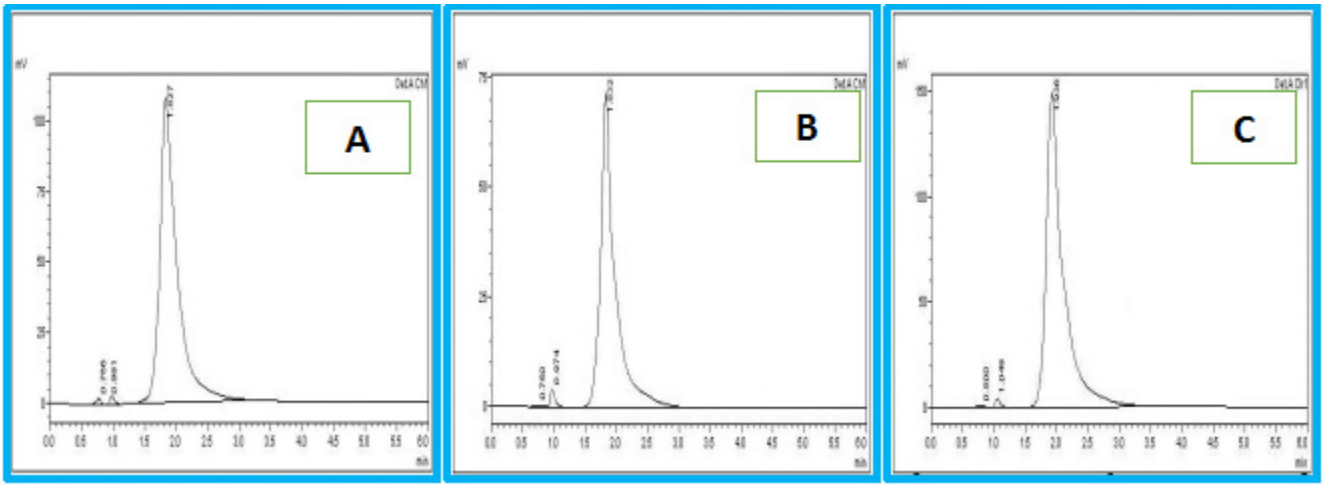


**Fig. 7 : A-** Actavis drug application, **B-** Mb-c drug application, **C-** Pioneer drug application.

**Table 10:** Method accuracy and control for the standard drug naproxen.

| NAP µg/mL | | % Recovery | | %Error | R.S.D n =3 |
|---|---|---|---|---|---|
| Taken | Found | | | | |
| 7 | 6.97 | 99.57 | Mean =98.43 | -0.43 | 0.15 |
| 22 | 21.05 | 95.68 | | -4.32 | 0.27 |
| 43 | 43.02 | 100.05 | S.D.=2.40 | 0.05 | 0.19 |

**Table 11:** Values of the detection limit concentrations of naproxen.

| Conc. um/ml | Area Naproxen |
|---|---|
| 1 | 10914 |
| 0.5 | 1391 |

and pre-prepared operational conditions, as (10 µl) of pharmaceutical solution of naproxen was injected to three different companies (Pioneer, Mb.c, Actiras) containing three concentrations (500, 500, 500) mcg/ml of pharmaceutical preparation. The separation was carried out using a mobile phase consisting of 50% methanol and 50% water at an acidity function (pH = 6.6), a flow rate (1.5 ml/min) and the results are shown in Fig. 7, which represents shape of the peaks of pharmaceutical preparation for three companies under study and Table 12 shows the results obtained.

**Table 12 :** Determination of the drug in commercial pharmaceutical preparations.

| Drugs name | Drug Type | Label Claim mg/ tab. | Mean amount found mg/tab. | % Mean amount found | R.S.D n=3 |
|---|---|---|---|---|---|
| 0.22 | 99.996 | 499.98 | 500 | NAP | **Actavis** |
| 0.31 | 100.002 | 500.01 | 500 | NAP | **Mb-c** |
| 0.28 | 99.99 | 499.95 | 500 | NAP | **Pioneer** |

recovery rate for three standard drug concentrations is (98.43) and the deviation value is (2.40%).

### Determination Limit for the Naproxen

This study was conducted by gradual dilution of the lowest concentration in the calibration curve and area measurement for the purpose of calculating detection limit for the proposed method, as (10 µl) of the drug solution under study was injected, and optimal conditions were applied, and the results were summarized in Table 11 and it was shown: Clearly, detection limit values for naproxen are (0.5) mcg/ml and the area value is (1391), as shown in Fig. 6, which shows the peak of detection limit for naproxen (Camilo and Foley, 2021).

### Application on pharmaceutical preparations

This study was conducted according to the optimal

## CONCLUSION

The study conducted for determination of naproxen in its pure form and in pharmaceutical preparations by high-performance liquid chromatography method showed that method is highly accurate and easy. When comparing the results obtained from the high-performance liquid chromatography method, it was shown that method is highly compatible and that it is identical within international constitutional ratios. This method was worked on in the laboratory temperature, so you do not need to control the temperatures and also use cheap organic solvents without need to use expensive organic solvents. The results obtained from the high-performance liquid chromatography method showed that they are identical and within acceptable limits when compared with other

analytical methods found in the references.

## Recommendations

Applying the results that were reached in the study and adopting it as a method for estimating and controlling pharmaceutical preparations containing naproxen and in its pure state. The possibility of using high-performance liquid chromatography method as an accurate and sensitive method for determination of other drugs. The possibility of applying the results of proposed method for determination of naproxen on biological models such as blood serum. Benefiting from the results obtained by applying them by conducting research using different analytical techniques to estimate naproxen.

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