ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
**Research Article**
July 2020 Vol.:18, Issue:4


# RP-HPLC Method for the Estimation of Paracetamol and Parabens in Pediatric Syrup

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**Submission:** 20 June 2020
**Accepted:** 27 June 2020
**Published:** 30 July 2020

**Keywords:** Paracetamol, Parabens, RP-HPLC method, ICH guidelines

**ABSTRACT**

Simple, rapid stability indicating RP-HPLC method is developed and validated for the determination of Paracetamol and Parabens (Methyl, Ethyl, Propyl parahydroxybenzoate) in Pediatric Syrup. The HPLC method was developed by using Zorbax C18 column; (100×4.6×5µm) column at 254nm, the flow rate of 1ml/min., Injection volume of 20µl, column oven temperature of 25°C using an equal volume of Methanol and Water used as mobile phase (50:50v/v). The retention times were found to 1.329, 2.264, 3.088, and 4.696 minutes. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The correlation coefficient ($r^2$) were found to be 0.997, 0.995, 1.000 and 1.000 respectively, % recovery was 100.0%, 100.0%, 100.0% and 100.0%. %RSD for precision was found to be 1.1, 0.4, 0.5, and 0.9 respectively. The HPLC method was found to be linear, accurate, precise, economical, and reproducible. The method can be suggested for routine analysis and the method can be recommended that can be adopted in regular Quality control tests in Industries.

## INTRODUCTION

Paracetamol/Acetaminophen is one of the most popular and most commonly used analgesic and antipyretic drugs around the world, available without a prescription, both in mono- and multi-component preparations. It is the drug of choice in patients that cannot be treated with non-steroidal anti-inflammatory drugs (NSAID), such as people with bronchial asthma, peptic ulcer disease, hemophilia, salicylate-sensitized people, and children under 12 years of age, pregnant or breastfeeding women. It is recommended as a first-line treatment of pain associated with osteoarthritis. The mechanism of action is complex and includes the effects of both the peripheral (COX inhibition), and central (COX, serotonergic descending neuronal pathway, L-arginine/NO pathway, cannabinoid system) ant nociception processes and „redox "mechanism. Paracetamol is a well-tolerated drug and produces few side effects from the gastrointestinal tract; however, despite that, every year has seen a steadily increasing number of registered cases of paracetamol-induced liver intoxication all over the world. Given the growing problem of the safety of acetaminophen is questioned the validity of the sale of the drug without a prescription. Many additives and preservatives are considerably used in food, drugs, and cosmetics to prevent their aging and decomposition (10). Parahydroxybenzoate esters and their sodium salts, usually named parabens, have been used for many decades as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. Parabens are effective over a wide pH range with a broad spectrum of antimicrobial activity and are effective against yeasts and molds. Antimicrobial activity increases with increasing alkyl chain length for the commonly used methyl, ethyl, propyl, and butyl parabens, and synergy between parabens has been reported. In oral pharmaceutical formulations, combinations of methylparaben and propylparaben are applied with concentrations generally ranging from 0.015 to 0.2% for methylparaben and 0.02% to 0.06% for propylparaben. Other parabens are also used in pharmaceuticals to a lesser extent, such as ethylparaben and butylparaben (1, 2, 3). In this paper, we have been based on the study of paracetamol and preservative content in children's syrups using high-pressure liquid chromatography. Syrups are available on the market of Bosnia and Herzegovina and the Republic of Croatia (6, 7, 8).

## MATERIALS AND METHODS

**Table No. 1: Chemical specifications**

| Sr. No. | Name of Chemical/Reagent | Manufacturer/Suppliers | Grade |
|---|---|---|---|
| 1. | Paracetamol | USP | / |
| 2. | Methyl parahydroxybenzoate | USP | / |
| 3. | Ethyl parahydroxybenzoate | USP | / |
| 4. | Propyl parahydroxybenzoate | USP | / |
| 5. | Sample 1-A | NA | / |
| 6. | Sample 2-J | NA | / |
| 7. | Sample 3-B | NA | / |
| 8. | Sample 4-G | NA | / |
| 9. | Water | Millipore | HPLC |
| 10 | Methanol | Semikem | HPLC |
| 11. | 0.45 µm Nylon Filter | Agilent | / |

**Table No. 2: Instruments and specifications**

| Sr. No. | Name of Instrument | Manufacturer |
|---|---|---|
| 1. | Analytical Balance | Mettler Toledo |
| 2. | HPLC with DAD | Agilent |
| 3. | Column | Agilent |

**Optimized chromatographic conditions:**

Stationary phase: Zorbax C18 column (100 x 4.6 mm, 5µm)

Mobile phase: Methanol: Water Mobile phase ratio: 50:50 (v/v)

Flow rate: 1.0 ml / min

Detector wavelength: 254 nm

Column temperature: 25°C

Injection volume: 20 µl

Run time: 7 min

**Preparation of mobile phase:**

Mix a mixture of HPLC Grade Water 500 mL, 500 mL of Methanol HPLC, and degas in an ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration (5).

**Diluent Preparation:**

Use the mobile phase as diluents.

**Preparation of the Paracetamol and Parabens Standard Solution Preparation:**

Accurately weigh and transfer 10 mg of Paracetamol and 10 mg of Parabens standard into a 50mL clean dry volumetric flask add about 30mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

**Standard solution for calibration curve:**

Further, pipette the above stock solution to the concentrations 0.06mg/ml; 0.08mg/ml; 0.13mg/ml; 0.16mg/ml and 0.19mg/ml.

**Sample Solution Preparation:**

Accurately weigh and transfer about 0.5g of each sample into a 50mL clean dry volumetric flask add about 30mL of Diluent and sonicate for 5 minutes and make volume up to the mark with the same solvent.

**Stock solution:**

Further pipette 8.0ml of the above Sample solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

Inject 20 µL of the standards and sample solutions into the chromatographic system and measure the areas for Paracetamol and Parabens peaks and calculate the % Assay by using the calibration curve.

## RESULTS

**1. System suitability:** All the system suitability parameters are within range and satisfactory as per ICH guidelines.

**Table No. 3: System suitability studies of Paracetamol and Parabens method**

| Property | Paracetamol | Methyl parahydroxybenzoate | Ethyl parahydroxybenzoate | Propyl parahydroxybenzoate |
|---|---|---|---|---|
| Retention time (min) | 1.329 | 2.264 | 3.088 | 4.696 |
| Theorotical plates | 2394 | 4104 | 4567 | 4863 |
| Tailing factor | 1.145 | 1.157 | 0.740 | 1.143 |

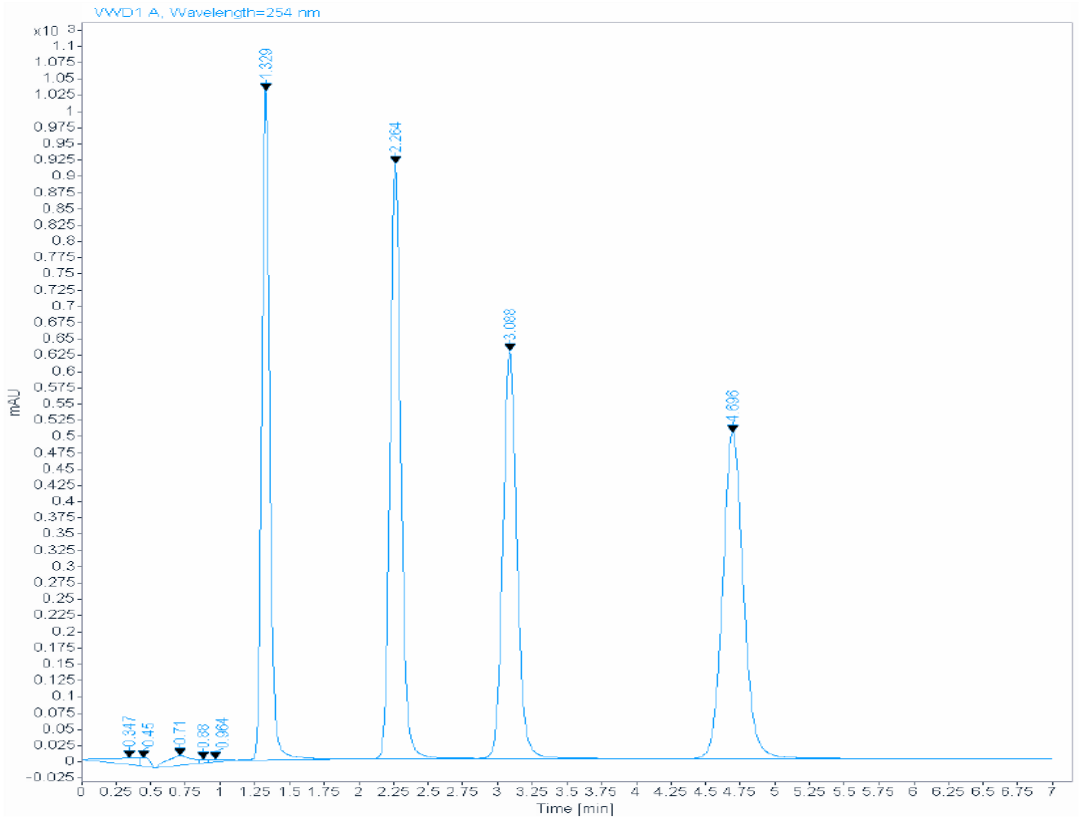


**Figure No. 1: Typical chromatogram of Paracetamol and Parabens**

**2. Linearity:**

Five Linear concentrations of Paracetamol (0.06-0.19mg/ml) and Parabens (0.06-0.19mg/ml) are prepared and injected. Regression equation of the Paracetamol and Parabens are found to

be, y = 950.55x + 1393, y = 1397.1x + 2198, y = 1575.6x + 602 and y = 1552.1x + 219. And regression coefficient were 0.997; 0.995; 1.000 and 1.000.

**Table No. 4: Calibration data of Paracetamol**

| Sr. No. | Concentration Paracetamol (%) | Response Paracetamol |
|---|---|---|
| 1 | 40 | 5057.54085 |
| 2 | 50 | 6074.67106 |
| 3 | 80 | 9278.00260 |
| 4 | 100 | 11085.03060 |
| 5 | 120 | 12506.50420 |

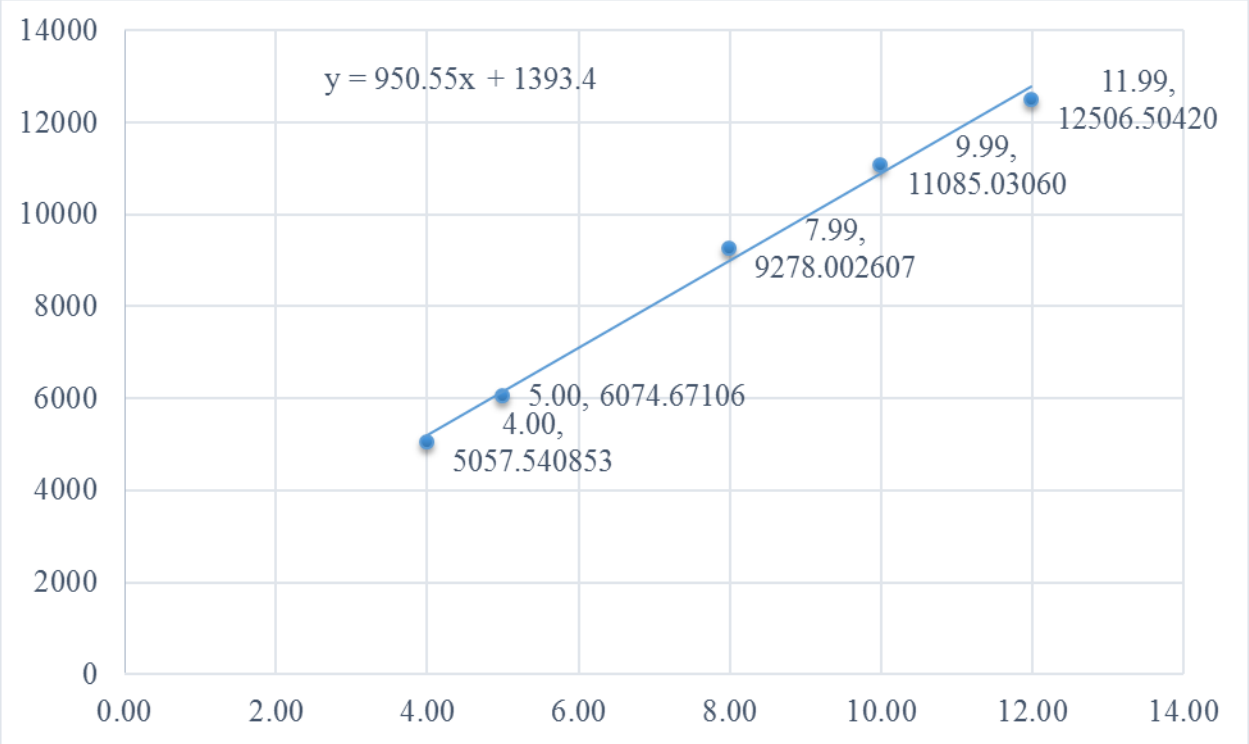


**Figure No. 2: Calibration curve of Paracetamol**

**Table No. 5: Calibration data of Methyl parahydroxybenzoate**

| Sr. No. | Concentration Methyl parahydroxybenzoate (%) | Response Methyl parahydroxybenzoate |
|---|---|---|
| 1 | 40 | 7664.29264 |
| 2 | 50 | 9429.10677 |
| 3 | 80 | 14511.95606 |
| 4 | 100 | 17091.71224 |
| 5 | 120 | 19119.23503 |

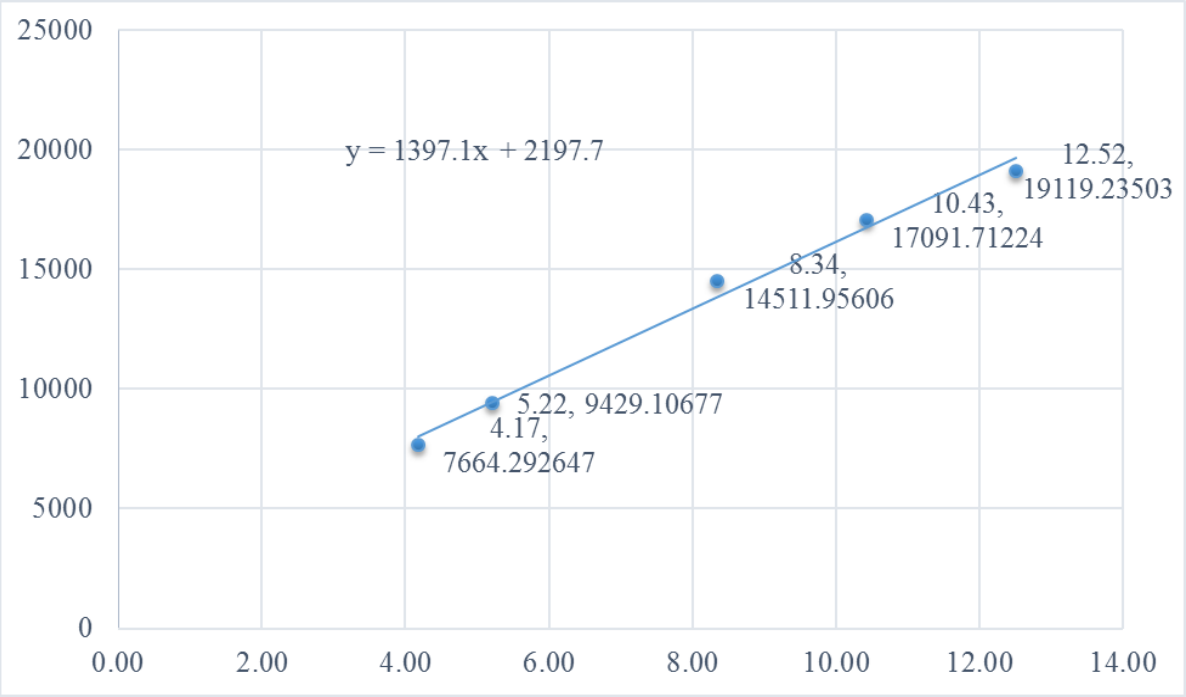


**Figure No. 3: Calibration curve of Methyl parahydroxybenzoate**

**Table No. 6: Calibration data of Ethyl parahydroxybenzoate**

| Sr. No. | Concentration Ethyl Parahydroxybenzoate (%) | Response Ethyl parahydroxybenzoate |
|---|---|---|
| 1 | 40 | 6783.37434 |
| 2 | 50 | 8429.667317 |
| 3 | 80 | 13378.10352 |
| 4 | 100 | 16400.00326 |
| 5 | 120 | 19286.09050 |

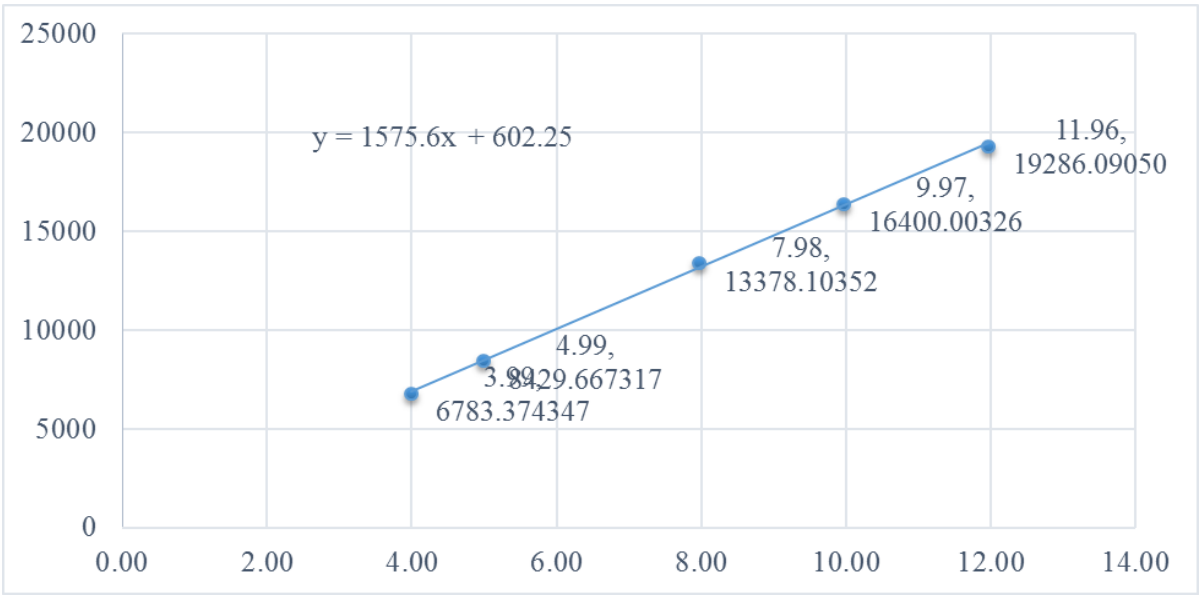


**Figure No. 4: Calibration curve of Ethyl Parahydroxybenzoate**

**Table No. 7: Calibration data of Propyl Parahydroxybenzoate**

| Sr. No. | Concentration Propyl Parahydroxybenzoate (%) | Response Propyl Parahydroxybenzoate |
|---|---|---|
| 1 | 40 | 6564.50016 |
| 2 | 50 | 8151.55924 |
| 3 | 80 | 13013.62695 |
| 4 | 100 | 16143.75749 |
| 5 | 120 | 19267.33529 |

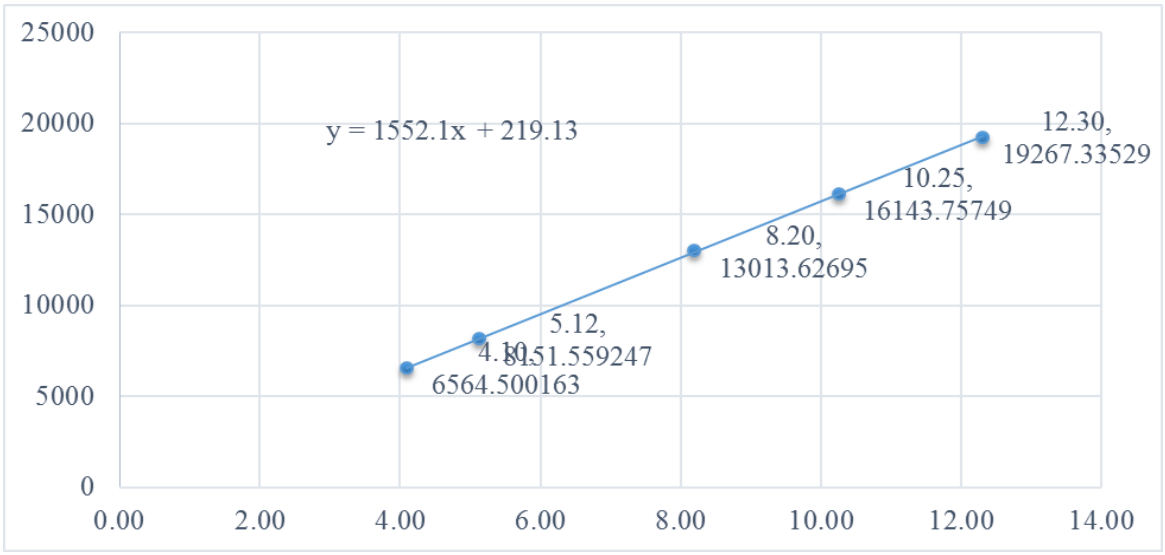


**Figure No. 5: Calibration curve of Propyl parahydroxybenzoate**

### 3. Precision:

Intraday precision (Repeatability) was performed and % RSD for Paracetamol and Parabens were found to be 1.1%, 0.4%, 0.5% and 0.9% respectively.

**Table No. 8: Repeatability results for Paracetamol and Parabens**

| Sr. No. | Paracetamol | Methyl parahydroxybenzoate | Ethyl Parahydroxybenzoate | Propyl Parahydroxybenzoate |
|---|---|---|---|---|
| 1 | 99.04 | 99.60 | 99.43 | 99.01 |
| 2 | 99.82 | 100.11 | 100.26 | 100.43 |
| 3 | 101.14 | 100.29 | 100.31 | 100.56 |
| Mean | 100.00 | 100.00 | 100.00 | 100.00 |
| SD | 1.1 | 0.4 | 0.5 | 0.9 |
| RSD | 1.1 | 0.4 | 0.5 | 0.9 |

### 4. Accuracy:

Three concentrations 80%, 100%, 120%, were injected in a triplicate manner and the amount Recovered and % Recovery was displayed in Table 9.

**Table No. 9: Peak results for Paracetamol and Parabens**

| Drug | The concentration of the sample (%) | Area | RSD (%) | Mean Recovery |
|---|---|---|---|---|
| Paracetamol | 80 | 6074.67106 | 0.1 | 100.00 |
| | 100 | 9278.00260 | 1.1 | |
| | 120 | 11085.03060 | 0.1 | |
| Methyl Parahydroxybenzoate | 80 | 14511.95606 | 0.1 | 100.00 |
| | 100 | 17091.71224 | 0.4 | |
| | 120 | 19119.23503 | 0.1 | |
| Ethyl Parahydroxybenzoate | 80 | 13378.10352 | 0.1 | 100.00 |
| | 100 | 16400.00326 | 0.5 | |
| | 120 | 19286.09050 | 0.1 | |
| Propyl Parahydroxybenzoate | 80 | 13013.62695 | 0.3 | 100.00 |
| | 100 | 16143.75749 | 0.9 | |
| | 120 | 19267.33529 | 0.1 | |

## 5. Assay:

Standard preparations are made from the API and Syrup Sample Preparations.

**Table No. 10: Assay of Paracetamol and Parabens in Syrups**

| Sr. No. | Paracetamol assay (mg/5ml) | Methyl Parahydroxybenzoate assay (mg/5ml) | Ethyl Parahydroxybenzoate assay (mg/5ml) | Propyl Parahydroxybenzoate assay (mg/5ml) |
|---|---|---|---|---|
| Sample 1-A | 127.4 | 11.0 | Not detected | 1.2 |
| Sample 2-J | 128.9 | 4.7 | Not detected | 2.0 |
| Sample 3-B | 129.7 | 4.6 | Not detected | 1.9 |
| Sample 4-G | 136.3 | 7.6 | 1.3 | 0.8 |

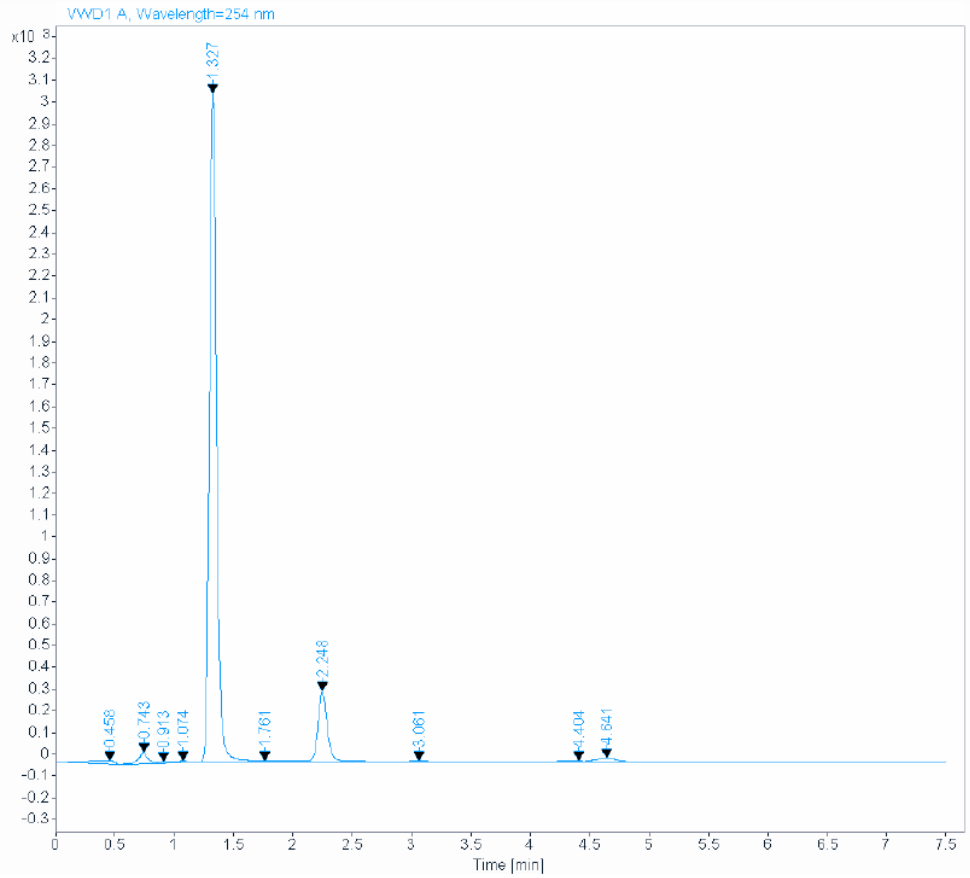


**Figure No. 6: Assay of Paracetamol and Parabens, Sample 1-A**

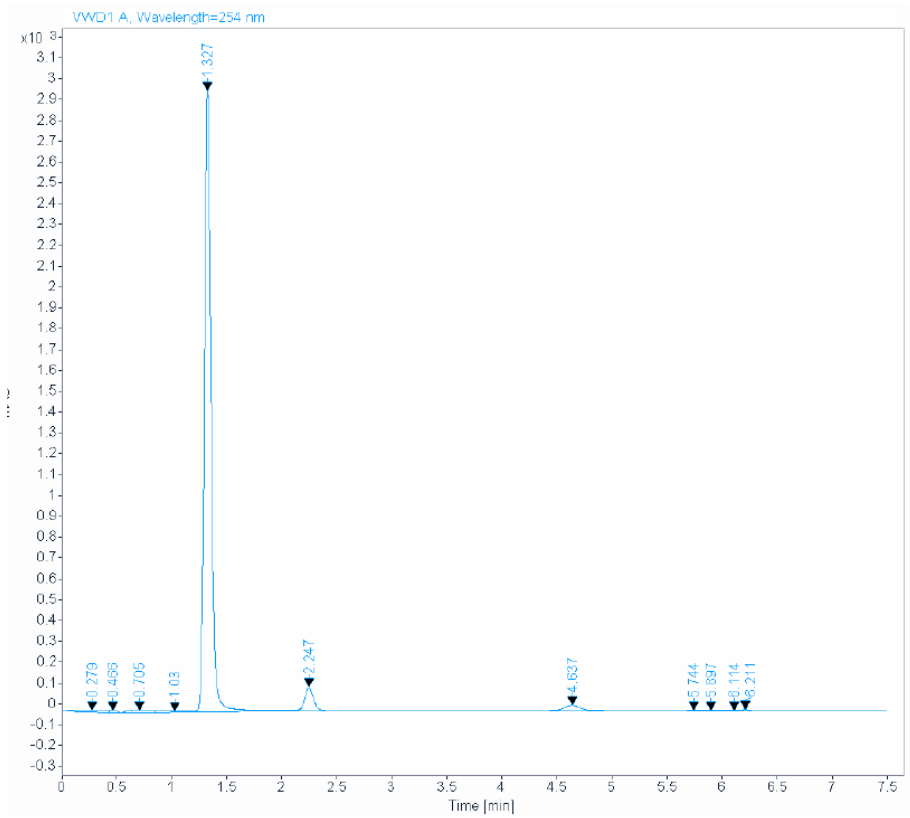


**Figure No. 7: Assay of Paracetamol and Parabens, Sample 2-J**

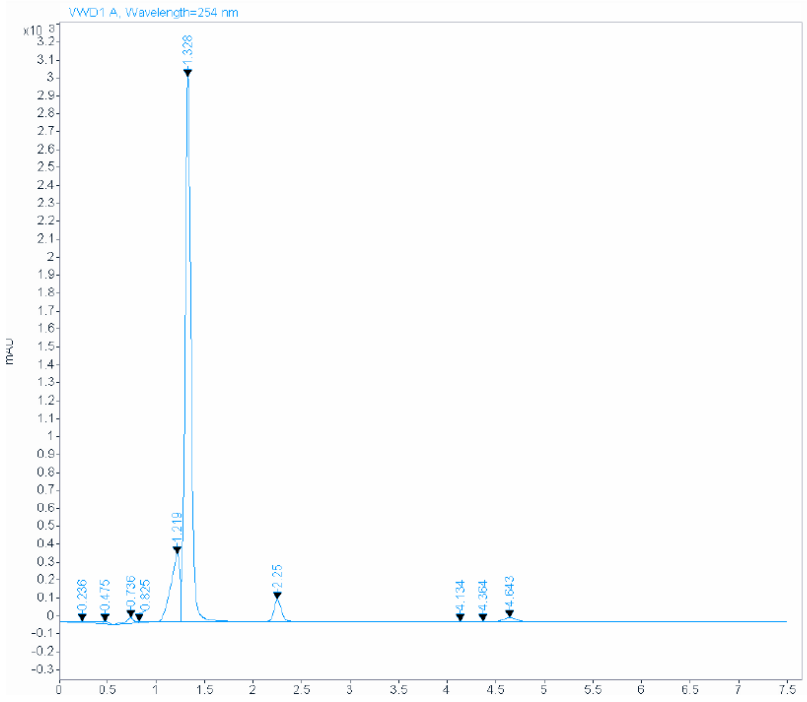


**Figure No. 8: Assay of Paracetamol and Parabens, Sample 3-B**

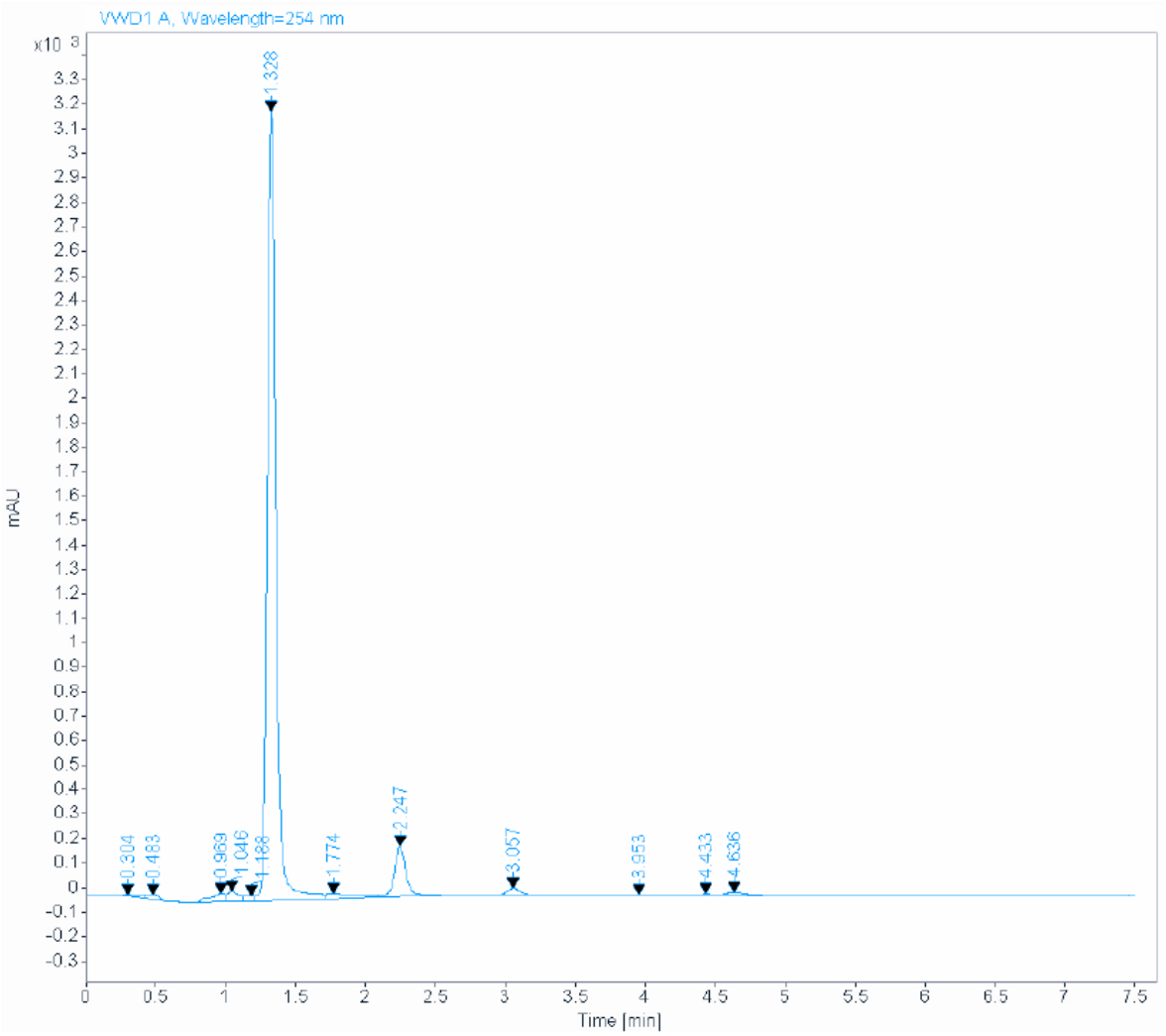


**Figure No. 9: Assay of Paracetamol and Parabens, Sample 4-G**

## DISCUSSION

The system with the mobile phase using Methanol: Water in the ratio of 50:50 (v/v) and a flow rate of 1 ml /min was found to be robust. The optimum wavelength for detection was 254 nm and a run time of 7 min at which better detector for the drug along with no interference was obtained.

The validated HPLC method was used for the simultaneous determination of Paracetamol and three Parabens in their combined dosage form. In the assay experiment, four samples were weighed separately and analyzed. The results indicate that the amount of Paracetamol in the Syrups is within the requirements of 90-110% of the label claim. For parabens, the composition was confirmed following the composition specified in the patient instructions.

The retention times were found to 1.329, 2.264, 3.088, and 4.696 minutes.

The calibration curve was constructed for Paracetamol and Parabens standard by plotting the concentration of compound versus peak area response. Standard solutions containing 0.06, 0.08, 0.13, 0.16, 0.19 mg/ ml of Paracetamol and Parabens with respectively were prepared and 20µl was injected into the HPLC column the linearity was evaluated by linear regression analysis, which was calculated by the least square regression method on the ordinate. The correlation coefficient ($r^2$) was found to be 0.997, 0.995, 1.000, and 1.000 respectively.

The accuracy study was performed for 80%, 100%, and 120 % for Paracetamol and Parabens. Each level was injected in triplicate into a chromatographic system. The area of each level was used for calculation of % recovery.

The reproducibility of the method was estimated by analyzing samples. Three injections of the standard mixture were analyzed.

## CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Paracetamol and Parabens in Pediatric Syrups. An efficient high performance liquid chromatographic method was developed for Paracetamol and Parabens. The HPLC method was developed by using Zorbax C18 column; (100×4.6×5µm) column at 254nm, the flow rate of 1ml/min., Injection volume of 20µl, column oven temperature of 25°C using an equal volume of Methanol and Water used as mobile phase (50:50v/v). The HPLC method was found to be accurate, precise, economical, and reproducible. The method can be suggested for routine analysis and the method can be recommended for the determination of Paracetamol and Parabens in Pediatric Syrups (3, 4, 9).

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