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A Validated Stability Indicating Reversed-Phase Hplc Method For Simultaneous Estimation For Assay And Preservatives Content Of Butalbital, Acetaminophen, Caffeine Oral Solution 50mg/325mg/40mg Per 15 Ml

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Abstract

A quick, simple and stability indicating reverse-phase HPLC method was developed and validated for estimation of Assay and Preservative content of Butalbital, Acetaminophen, Caffeine Oral Solution 50mg/325mg/40mg per 15 mL and Preservatives-Methylparaben and Propylparaben, in accordance with the ICH Q2R1 guidelines. The method demonstrated. The method demonstrated system precision, specificity, precision at the limit of quantitation (LOQ), accuracy, solution stability, linearity, ruggedness and robustness. The HPLC conditions consisted of a mobile phase composed of pH 4.50 buffer and methanol in a ratio of 70:30 (v/v)-mobile phase A and 35:65 (v/v)-mobile phase B with a flow rate of 1.2 mL/min and UV detection at 216 nm for Butalbital, Acetaminophen and Caffeine, 254 nm for Methylparaben and Propylparaben. The entire chromatography run time was 16 minutes. Linearity was established over a concentration range of 40%-160% with a correlation coefficient of more than 0.995. Accuracy was confirmed within the range between 50% and 120%. This validated HPLC method is suitable for the precise quantification of Butalbital, Acetaminophen, Caffeine Oral Solution 50mg/325mg/40mg per 15 mL and Preservatives-Methylparaben and Propylparaben, making it an effective method for quality control and assurance in pharmaceutical manufacturing.

Keywords: Butalbital, Acetaminophen, Caffeine, Methylparaben, Propylparaben, HPLC.

1. Introduction

Butalbital, acetaminophen and caffeine are supplied in liquid form for oral administration.
Each 15 mL contains:
Butalbital USP, 50 mg
Acetaminophen USP, 325 mg
Caffeine USP, 40 mg
Alcohol, 7.368%

In addition, this drug product contains the following inactive ingredients: citric acid, ethyl maltol, liquid glucose, glycerin, methylparaben, propylene glycol, propylparaben, purified water, saccharin sodium, sorbitol solution, sucrose, with FD&C yellow #6 as coloring, and natural and artificial flavoring.

Figure 1: Butalbital (5-allyl-5-isobutylbarbituric acid), is a short to intermediate-acting barbiturate. It has the following structural formula:

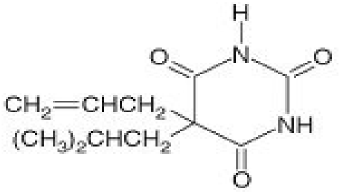


Figure 2: Acetaminophen (4'-hydroxyacetanilide) is a non-opiate, non-salicylate analgesic and antipyretic. It has the following structural formula:

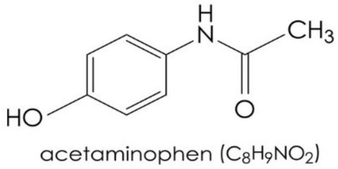


Figure 3: Caffeine (1,3,7-trimethylxanthine) is a central nervous system stimulant. It has the following structural formula:

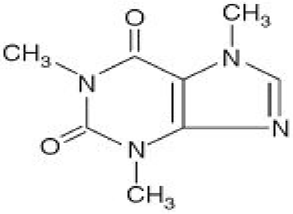


Figure 4: Methylparaben is a Preservative, help to increase the shelf life and avoid bacterial and fungal growth. It has the following structural formula:

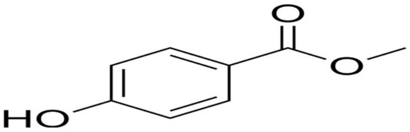
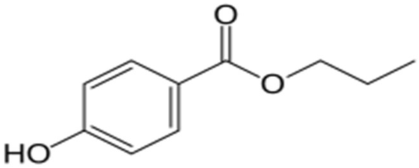


Figure 5: Propylparaben is a Preservative, help to increase the shelf life and avoid bacterial and fungal growth. It has the following structural formula:



Multidrug pharmaceutical preparations for the therapy of pain of weaker genesis contain the different components, usually butalbital, acetylsalicylic acid and acetaminophen with caffeine, codeine, derivates of pyrazolones, vitamins, phenacetine, pentazocine which can improve the pharmacological value of these preparations. Concerning the different mechanisms of action, they sometimes act as synergists which lead to a better efficiency. Since each component in the multicomponent preparation is in the fewer amounts than in the monocomponent of each one, the main metabolic organs are less loaded since any components engages the different subsystem of metabolism.

For the assay of butalbital, acetylsalicylic acid, acetaminophen and caffeine and phenobarbital in the mixtures, the different methods have been reported, including spectrophotometry [1], second derivative spectrophotometry [2],[3] and capillary chromatography [4].

Considering the properties of the compounds investigated, such as mid polarity, as well as thermolability and low volatility, HPLC methods have been the most explored. They include an assay of acetylsalicylic acid, acetaminophen, ascorbic acid [5] paracetamol, pseudoephedrine hydrochloride and triprolidine hydrochloride [6], acetaminophen-caffeine-butalbital [7].

Scholars have written about several methods of analysis, with liquid chromatography being the most common; liquid gas chromatography, capillary electrochromatography, capillary electrophoresis, gas chromatography with mass detection, HPLC (photodiode array spectroscopic), TLC, UV spectrophotometric generally used [8],[9],[10],[11].

A review of the literature reveals a limited number of reported analytical techniques for quantification of Butalbital, Acetaminophen, Caffeine using HPLC. However, no simple and single HPLC method is available to quantify the assay of Butalbital, Acetaminophen, Caffeine and Preservatives, Methylparaben, Propylparaben in liquid form for oral administration. The present study aims to develop a simple, precise, accurate, linear, rugged, robust and stability-indicating method using HPLC for the analysis of simultaneous estimation for Assay and Preservative Content of Butalbital, Acetaminophen, Caffeine Oral Solution 50mg/325mg/40mg per 15 mL and Preservatives-Methylparaben, Propylparaben in accordance with the ICH guideline Q2 R1.

The present paper describes a rapid, simple RP-HPLC method with UV detection for a direct simultaneous quantification of butalbital, acetaminophen and caffeine including preservatives: methylparaben and propylparaben in liquid formulation.

3. Materials and Methods

3.1. Drugs and Chemicals

3.1.1 Potassium Phosphate Monobasic : Reagent grade
3.1.2 Orthophosphoric Acid : Reagent grade
3.1.3 Water : USP purified or equivalent
3.1.4 Acetonitrile : HPLC grade
3.1.5 Methanol : HPLC grade

Butalbital Reference standard, Acetominophen Reference standard, Caffeine Reference standard, Methylparaben Reference standard, Propyparaben Reference standard bought from Sigma Aldrich. Butalbital drug substance, Acetominophen drug substance, Caffeine drug substance was obtained from Siegfried USA, Granules India, Seigfried Pharma Chemikalien, respectively. Methylparaben and Propylparben purchased from Spectrum Chemical MFG CORP.

3.2. Instrumentation

3.2.1 High Performance Liquid Chromatographic system consisting of a pump, an injector, PDA/UV-Visible detector and suitable data processing software
3.2.2 Digilab Ultrasonic bath
3.2.3 Mettler Analytical balance
3.2.4 Mettler Micro balance
3.2.5 Metrohm pH meter

3.3. Mobile Phase Preparation

- **Buffer Preparation**: Accurately weighed and transferred about 1.36g Potassium Phosphate Monobasic in a suitable flask containing 1000 mL of water and allowed to dissolve after the pH was adjusted to 4.50±0.05 with diluted Ortho-phosphoric acid (1.0 ml of Orthophosphoric acid in 100 ml of water). Filtered through 0.45 µm membrane filter.
- **Mobile phase A Preparation**: Transferred 700 mL of Buffer solution and 300 mL Methanol in a suitable container, mixed well and sonicated to degas.
- **Mobile phase B Preparation**: Transferred 350 mL of Buffer solution and 650 mL Methanol in a suitable container, mixed well and sonicated to degas.

3.4. Diluent Preparation

Transferred 700 mL of Methanol and 300 mL of Water in a suitable container and mixed well.

3.5. Preparation of Standard Solution:

- **Preparation of Stock Solution-1:** Weighed accurately and transferred about 25 mg of the Butalbital RS, 162.5mg of Acetaminophen and 20mg of Caffeine and transferred into a 100 mL volumetric flask. Added about 15.0 mL of diluent into the flask and sonicated to dissolve. Diluted the flask volume with diluent and mixed well (Concentration of about 250µg/ml of Butalbital, 1625µg/ml of Acetaminophen and 200 µg/ml of Caffeine).
- **Preparation of Stock Solution-2:** Weighed accurately about 37.5 mg of Methylparaben RS and transferred into a 50 ml volumetric flask. Added 15.0 mL of diluent into the flask and sonicated to dissolve. Diluted the flask volume with diluent and mixed well (Concentration of about 750 µg/ml of Methylparaben).
- **Preparation of Stock Solution-3:** Weighed accurately about 30 mg of Propylparaben RS and transferred into a 200 ml volumetric flask. Added 15.0 mL of diluent into the flask and sonicated to dissolve. Diluted the flask volume with diluent and mixed well (Concentration of about 150 µg/ml of Propylparaben).

- **Preparation of Working Standard Solution:** Transferred 10.0 ml of Stock solution-1, 8.0 ml of Stock solution-2 and 4.0 ml of Stock solution-3 into a 50ml volumetric flask. Diluted the flask volume with diluent and mixed well (Concentration of 50ug/ml of Butalbital, 325µg/ml of Acetaminophen, 40µg/ml of Caffeine, 120µg/ml of Methylparaben and 12µg/ml of Propylparaben).

3.6. Preparation of Sample Solution (For Preservatives):

3.6.1. Preparation of Sample Solution: Weighed accurately and transferred about 7.5 g of Butalbital, Acetaminophen and Caffein Oral solution into a 100 mL volumetric flask (equivalent to about 20 mg of Butalbital, 135 mg of Acetaminophen, 16 mg of Caffein, 12mg of Methylparaben and 1.2mg of Propylparaben). Added diluent about 3/4th of the volume of the flask and sonicated to dissolve. Diluted to volume with diluent and mixed well (Sample solution concentration of about 200 µg/mL of Butalbital, 1350 µg/mL of Acetaminophen, 160µg/ml of Caffeine, 120 µg/ml of Methylparaben and 12 µg/ml of Propylparaben). Filtered the sample solution using 0.45 µm Nylon filter by discarding the first 4mL of the filtrate.

3.6.2. Preparation of the Sample Solution (For Assay): Pipetted out 6.0 ml of Sample preparation for Preservatives (from Section 3.6.1) into a 25 mL volumetric flask. Diluted the flask volume with diluent and mixed well (Sample solution concentration of about 48 µg/ml of Butalbital, 324 µg/mL of Acetaminophen and 38 µg/mL of Caffeine). Filtered the sample solution using 0.45 µm Nylon filter by discarding the first 4mL of the filtrate.

3.7. Chromatographic Parameters

For chromatographic separation, a Agilent Eclipse XDB-C18, 250x4.6mm, 5µ Part# 990967-902 HPLC column was used. The HPLC conditions consisted of a mobile phase composed of pH 4.50 buffer and methanol in a ratio of 70:30 (v/v)-mobile phase A and 35:65 (v/v)-mobile phase B with a flow rate of 1.2 mL/min (gradient)* and UV detection at 216 nm for Butalbital, Acetaminophen and Caffeine, 254 nm for Methylparaben and Propylparaben. The total chromatography run time is 16 minutes. The column oven temperature is 40°C, the sampler temperature is maintained at 5°C, and the injection volume is 10 µL.

***Pump gradient Program:**

Time (Min)	Mobile Phase-A	Mobile Phase-B
0.00	100	0
6.00	100	0
7.00	0	100
14.00	0	100
14.50	100	0
16.00	100	0

3.8. Equilibration and test injections:

- Pump the mobile phase through the system until the column is equilibrated and the baseline is stabilized. Minimum 30 Minutes recommended for HPLC system equilibration.
- Injected diluent (blank) preparation.
- Injected Standard preparation. The Retention Time (RT) for Acetaminophen is about 2.9 minutes, Butalbital is about 10.8 minutes, Caffeine is about 4.6 minutes, Methylparaben is about 9.8 minutes and Propylparaben is about 12.4 minutes (Retention time is for information purpose only).

3.9. Chromatographic Procedure:

- Perform injection of diluent preparation.
- Perform five (5) replicate injections of Working Standard solution. The % RSD of peak area of Butalbital, Acetaminophen and Caffeine should be NMT 2.0 and Propylparaben, Methylparaben should be NMT 5.0
- Perform two (2) replicate injections of check standard preparation.
- Calculate the check standard accuracy. Check standard accuracy should be within 98.0%-102.0% for Butalbital, Acetaminophen and Caffeine and 95.0%-105.0% for Propylparaben, Methylparaben.
- Check Standard Accuracy Calculation,

$$= \frac{\text{Check Std Area}}{\text{Std Area}} \text{ X } \frac{Weight\ of\ Std(mg)}{Weight\ of\ \ Chec\ \ Std(mg)} \text{ X } 100$$

- Perform injection of sample preparation.
- Record the chromatograms and calculate the content of Butalbital, Acetaminophen, Caffeine, Propyl paraben and Methylparaben as shown in the formulae:

3.10 Calculation:

Calculate the amount of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben found in each sample as follows:

3.10.1 %LC (Butalbital):

$$\frac{Spl\ Area}{Std\ Area} \times \frac{Wstd\ (mg)}{100\ (mL)} \times \frac{10.0\ (mL)}{50\ (mL)} \times \frac{P}{100} \times \frac{100\ (mL)}{Wspl\ (g)} \times \frac{25\ (mL)}{6.0\ (mL)} \times \frac{Density\ (g/mL)}{LC(50)} \times 15 \times 100$$

3.10.2 %LC (Acetaminophen):

$$\frac{Spl\ Area}{Std\ Area} \times \frac{Wstd\ (mg)}{100\ (mL)} \times \frac{10.0\ (mL)}{50\ (mL)} \times \frac{P}{100} \times \frac{100\ (mL)}{Wspl\ (g)} \times \frac{25\ (mL)}{6.0\ (mL)} \times \frac{Density\ (g/mL)}{LC(325)} \times 15 \times 100$$

3.10.3 %LC (Caffeine):

$$\frac{Spl\ Area}{Std\ Area} \times \frac{Wstd\ (mg)}{100\ (mL)} \times \frac{10.0\ (mL)}{50\ (mL)} \times \frac{P}{100} \times \frac{100\ (mL)}{Wspl\ (g)} \times \frac{25\ (mL)}{6.0\ (mL)} \times \frac{Density\ (g/mL)}{LC(40)} \times 15 \times 100$$

3.10.4 %LC (Methylparaben):

$$\frac{Spl\ Area}{Std\ Area} \times \frac{Wstd\ (mg)}{50\ (mL)} \times \frac{8.0(mL)}{50\ (mL)} \times \frac{P}{100} \times \frac{100\ (mL)}{Wspl\ (g)} \times \frac{Density\ (g/mL)}{LC(30)} \times 15 \times 100$$

3.10.5 %LC (Propylparaben):

$$\frac{Spl\ Area}{Std\ Area} \times \frac{Wstd\ (mg)}{200\ (mL)} \times \frac{4.0(mL)}{50\ (mL)} \times \frac{P}{100} \times \frac{100\ (mL)}{Wspl\ (g)} \times \frac{Density\ (g/mL)}{LC\ (3)} \times 15 \times 100$$

Spl Area = Peak area of Acetaminophen, Butalbital, Caffeine, Methylparaben and Propylparaben from the Sample solution
Std Area = Average Peak area of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben from the Standard solution
Wstd = Weight of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben RS in mg
Wspl = Sample weight in g
P = Purity of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propyl paraben RS in percent, based on label instruction
LC = Label Claim

4.0 Method Validation and Results

The following validation parameters were determined for the developed method: Precision, Accuracy, Linearity, Specificity, Ruggedness (Intermediate Precision) and Robustness, as per the ICH guidelines.

4.1 System Precision

To explain the system precision, A standard solution was prepared as per the method and injected. The relative standard deviation for peak responses of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben from six (6)-replicate injections of the standard solution was calculated and reported. USP tailing factors for Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben were reported (Table 1,2,3,4,5). The % RSD from six (6)-replicate injections of standard peak response of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben is not more than 5.0. Acceptance criteria for the USP tailing factor for Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben are not more than 2.0 from the working standard solution, which met all specified acceptance criteria and proved the system is precise.

4.2 Linearity and Range

To determine the solutions of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben from 40% to 160% levels were injected into HPLC system. The linearity graph was plotted from 40% to 160% (Table 6,7,8,9,10,11) and (Figure 6,7,8,9,10). The correlation coefficient (r^2) for Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben met the acceptance criteria of not less than 0.99. The linear regression data shows that the method is linear over the entire concentration range 40% to 160%, and it is adequate for its intended concentration range.

4.3 Method Precision

The precision of the assay method was determined by injecting six (6)-individual sample solutions of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben at test concentration level (Table 12,13,14,15,16). The samples were prepared as per the method. The % RSD from six (6)-individual sample preparations met the acceptance criteria of not more than 5.0 which proved the method is precise. Typical chromatograms (Figure 11,12,13,14,15).

4.4 Ruggedness (Intermediate Precision)

The Intermediate Precision (Ruggedness) of the assay method was determined by injecting six (6)-individual sample solutions of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben at test concentration levels by a second analyst using a different HPLC system and a different column on a different day. The samples were prepared as per the method. The % RSD for six (6)-individual sample preparations found to be less than 10.0, which showed the method is rugged (Table 17,18,19,20,21,22).

4.5 Solution Stability

To establish the standard, sample solutions were prepared and injected. Standard and sample solutions stored at 5°C were injected at the following time intervals: Initial (0 hours), 24 hours and 48 hours for standard. Initial (0 hours), 24 hours and 48 hours for sample. The relative difference in response from the initial and time point for standard and the relative difference in % Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben sample solutions were generated. The relative difference in response between initial and time points found to be less than 10% for Standard and Sample as well. Based on this data, it is assessed that standard and sample solutions are stable up to 48 hours when stored at 5°C (Table 23,24).

4.6 Specificity

Blank, Standard, Control, and Stress Sample Preparation.

To obtain the blank, standard, control and stress sample solutions were prepared and injected into the chromatographic system for identification and degradants interference with the Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben peak. Any secondary peak arising from the forced degradation study should not interfere with the Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben peak. No interference should be observed from the diluent and degradants at the retention time of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben peak. The peak purity analysis using a Photo Diode Array (PDA) detector demonstrated the peak homogeneity, which showed the method is specific.

4.7 Specificity by Forced Degradation

A forced degradation study is performed in order to prove that the method is stability indicating. The drug product was stressed under the following conditions.

1. Acid Stress
2. Base Stress
3. Peroxide Stress
4. UV light Stress
5. Heat Stress

Prepared solutions of acid, base, peroxide, heat and UV stress samples and diluted further with diluent for peak purity analysis. All the stock and diluted solutions of control and stress samples were injected into HPLC for the determination of % assay and peak purity, respectively.

- No interference was observed from diluent, placebo and all known impurities at the retention time of Acetaminophen, Butalbital, Caffeine, Methylparaben and Propylparaben in spiked sample.
- Peak purity analysis using the photodiode array detector (PDA) demonstrated the Analyte peak homogeneity which proved the method is specific (Table 25,26,27). Typical

Spiked chromatograms and Peak purity plots (Figure 16,17,18,19,20,21,22).

4.8 Method Accuracy (Recovery)

The recovery of Butalbital, Acetaminophen, Caffeine, Methylparaben and Propylparaben were performed in placebo from 50%-120%. The samples were prepared as per the method in triplicate and injected. Recovery found between 80% and 120%. The %RSD for recovery of triplicate preparations was found to be less than 10.0, which proved the method is accurate (Table 28,29,30,31,32).

4.9 Filter Study

To explain filter effect the sample solution of Butalbital, Acetaminophen, Caffeine Oral Solution 50mg/325mg/40mg per 15 mL were filtered by discarding 1 mL, 4 mL, 6 mL and 8 mL of the filtrate by using 0.45 µ Nylon filter and the samples were injected. An unfiltered centrifuged solution was also prepared and injected. Compare the results of the filtered sample with that of the centrifuged sample results (Table 33,34). The relative % impurity difference between the centrifuged sample and the filtered sample is found to be less than 25%, therefore the sample solution should be filtered through a 0.45 µm Nylon filter by discarding the first four (4 mL) of filtrate.

4.10 Robustness

Robustness is a measure of the method's capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of method reliability during normal use. To establish the Standard solution was prepared and injected into the chromatographic system as per the conditions specified in the method. The same standard solution was re-injected by changing one parameter at a time, keeping other parameters constant. A set of system suitability data was calculated for standards injected under altered method conditions and compared against the values generated under actual method conditions (Table 35,36,37,38,39).

Method Parameters:

1. Column Operating Temperature by ±5°C (Procedural temperature is 40°C)
   a. Temperature Plus→45°C
   b. Temperature Minus→35°C
2. Flow Rate variation ± 0.2 mL/min (Procedural Flow rate is 1.2 mL/min)
   a. Flow Rate Plus→1.4 mL/min
   b. Flow Rate Minus→1.0 mL/min

3. pH of Buffer by ±0.2 (Procedural Buffer pH 4.50)
   a. pH Plus → 4.70
   b. pH Minus → 4.30

All the system suitability requirements were met with small deliberate variations in column temperature, flow rate and pH of the buffer. Slight changes in retention time; however, system suitability met the acceptance criteria. Calculations for all other system suitability parameters met the acceptance criteria and the data generated are comparable with the normal conditions. Based on the above result, it is concluded that the method is unaffected by small, deliberate variations in column temperature, flow rate and pH of the buffer in the mobile phase and therefore the method is proved to be robust.

5.0 Results and Discussion

Table 1. Precision Data for Acetaminophen

Injection#	*Sample Name*	*RT (Min.)*	*Response (AU)*	**USP Tailing factor**
1	*Standard*	*2.963*	*4250333*	*1.2*
2	*Standard*	2.955	*4267611*	*1.2*
3	*Standard*	2.980	*4285804*	*1.2*
4	*Standard*	2.980	*4276881*	*1.2*
5	*Standard*	2.958	*4288021*	*1.2*
Mean		2.960	4273730	
%RSD		0.4	0.4	

Table 2. Precision Data for Butalbital

Injection#	*Sample Name*	*RT (Min.)*	*Response (AU)*	**USP Tailing factor**
1	*Standard*	*10.969*	*812271*	*1.2*
2	*Standard*	10.975	*811319*	*1.2*
3	*Standard*	10.973	*817134*	*1.2*
4	*Standard*	10.958	*813236*	*1.2*
5	*Standard*	10.964	*817952*	*1.2*
Mean		10.955	814382	
%RSD		0.4	0.4	

Table 3. Precision Data for Caffeine

Injection#	*Sample Name*	*RT (Min.)*	*Response (AU)*	**USP Tailing factor**
1	*Standard*	*4.644*	*1381593*	*1.2*

2	*Standard*	4.633	*1383113*	*1.2*
3	*Standard*	4.638	*1391904*	*1.2*
4	*Standard*	4.643	*1390237*	*1.2*
5	*Standard*	4.634	*1399957*	*1.2*
Mean			1391361	
%RSD			0.5	

Table 4. Precision Data for Methylparaben

Injection#	*Sample Name*	*RT (Min.)*	*Response (AU)*	**USP Tailing factor**
1	*Standard*	*9.926*	*5943878*	*1.2*
2	*Standard*	9.930	*5957907*	*1.2*
3	*Standard*	9.929	*5982157*	*1.2*
4	*Standard*	9.914	*5972138*	*1.2*
5	*Standard*	9.919	*5990242*	*1.2*
Mean			5969264	
%RSD			0.3	

Table 5. Precision Data for Propylparaben

Injection#	*Sample Name*	*RT (Min.)*	*Response (AU)*	**USP Tailing factor**
1	*Standard*	*12.851*	*529995*	*1.2*
2	*Standard*	12.857	*531324*	*1.2*
3	*Standard*	12.856	*533760*	*1.2*
4	*Standard*	12.840	*532485*	*1.2*
5	*Standard*	12.846	*534031*	*1.2*
Mean			532319	
%RSD			0.3	

Table 6. Linearity Data for Acetaminophen

S. No	*Sample Name*	*Name*	*Amount (µg/mL)*	**Peak Response**
1	*40%_ Linearity*	*Acetaminophen*	*129.9100*	*1717856*
2	*80%_Linearity*	Acetaminophen	*259.8199*	*3398589*
3	*100%_ Linearity*	Acetaminophen	*324.7749*	*4216427*
4	*120%_ Linearity*	Acetaminophen	*389.7299*	*5073292*
5	*160%_ Linearity*	Acetaminophen	*519.6398*	*6718412*

Table 7. Linearity Data for Butalbital

S. No	*Sample Name*	*Name*	*Amount (µg/mL)*	**Peak Response**
1	*40%_ Linearity*	*Butalbital*	*20.0120*	*351280*
2	*80%_Linearity*	Butalbital	*40.0239*	*651377*
3	*100%_ Linearity*	Butalbital	*50.0299*	*806770*
4	*120%_ Linearity*	Butalbital	*60.0359*	*975085*
5	*160%_ Linearity*	Butalbital	*80.0479*	*1284858*

Table 8. Linearity Data for Caffeine

S. No	*Sample Name*	*Name*	*Amount (µg/mL)*	**Peak Response**
1	*40%_ Linearity*	*Caffeine*	*16.3838*	*562511*
2	*80%_Linearity*	Caffeine	*32.7676*	*1109393*
3	*100%_ Linearity*	Caffeine	*40.9595*	*1378242*
4	*120%_ Linearity*	Caffeine	*49.1514*	*1659677*
5	*160%_ Linearity*	Caffeine	*65.5362*	*2200780*

Table 9. Linearity Data for Methylparaben

S. No	*Sample Name*	*Name*	*Amount (µg/mL)*	**Peak Response**
1	*40%_ Linearity*	*Methylparaben*	*45.1244*	*2259857*
2	*80%_Linearity*	Methylparaben	*90.2488*	*4491514*
3	*100%_ Linearity*	Methylparaben	*120.3317*	*5913663*
4	*120%_ Linearity*	Methylparaben	*150.4146*	*7304676*
5	*160%_ Linearity*	Methylparaben	*210.5805*	*9813537*

Table 10. Linearity Data for Propylparaben

S. No	*Sample Name*	*Name*	*Amount (µg/mL)*	**Peak Response**
1	*40%_ Linearity*	*Propylparaben*	*6.0738*	*269728*
2	*80%_Linearity*	Propylparaben	*9.1107*	*397385*
3	*100%_ Linearity*	Propylparaben	*12.1477*	*527363*
4	*120%_ Linearity*	Propylparaben	*15.1846*	*659919*
5	*160%_ Linearity*	Propylparaben	*24.2953*	*1051604*

Table 11. Correlation Coefficient (r^2) Values

Name	**Correlation Coefficient (r^2)**
Acetaminophen	0.9998
Butalbital	0.9982
Caffeine	0.9999

Methylparaben	0.9993
Propylparaben	1.0000

Table 12. Method Precision of Acetaminophen – Analyst 1

Injection#	Name	RT (Min.)	Response (AU)	Percent_LC
1	Preparation-1	2.960	4178227	97.6
2	Preparation-2	2.955	4166742	97.4
3	Preparation-3	2.954	4188017	98.0
4	Preparation-4	2.954	4178499	97.8
5	Preparation-5	2.958	4184501	97.6
6	Preparation-6	2.949	4194406	98.0
Mean			4181732	97.7
%RSD			0.2	0.2

Table 13. Method Precision of Butalbital – Analyst 1

Injection#	Name	RT (Min.)	Response (AU)	Percent_LC
1	Preparation-1	10.972	809127	99.3
2	Preparation-2	10.975	811312	99.7
3	Preparation-3	10.976	812011	99.9
4	Preparation-4	10.976	810245	99.6
5	Preparation-5	10.973	810348	99.3
6	Preparation-6	10.970	812606	99.7
Mean			810942	99.6
%RSD			0.2	0.2

Table 14. Method Precision of Caffeine – Analyst 1

Injection#	Name	RT (Min.)	Response (AU)	Percent_LC
1	Preparation-1	4.637	1345596	98.9
2	Preparation-2	4.632	1348431	99.2
3	Preparation-3	4.631	1355846	99.9
4	Preparation-4	4.631	1351926	99.6
5	Preparation-5	4.634	1354708	99.4
6	Preparation-6	4.626	1356342	99.7
Mean			1352141	99.5

%RSD			0.3	0.4

Table 15. Method Precision of Methylparaben – Analyst 1

Injection#	*Name*	*RT (Min.)*	*Response (AU)*	**Percent_LC**
1	*Preparation-1*	*9.925*	*6042268*	*97.3*
2	*Preparation-2*	9.928	*6063094*	*97.8*
3	*Preparation-3*	9.929	*6069272*	*98.0*
4	*Preparation-4*	9.933	*6063131*	*97.8*
5	*Preparation-5*	9.930	*6059155*	*97.5*
6	Preparation-6	9.923	6054623	97.5
Mean			6058591	97.6
%RSD			0.2	0.3

Table 16. Method Precision of Propylparaben – Analyst 1

Injection#	*Name*	*RT (Min.)*	*Response (AU)*	**Percent_LC**
1	*Preparation-1*	*12.853*	*532283*	*97.1*
2	*Preparation-2*	12.857	*534180*	*97.5*
3	*Preparation-3*	12.857	*534784*	*97.7*
4	*Preparation-4*	12.862	*534407*	*97.6*
5	*Preparation-5*	12.859	*533579*	*97.2*
6	Preparation-6	12.852	533095	97.2
Mean			533718	97.4
%RSD			0.2	0.3

Table 17. Intermediate Precision of Acetaminophen - Analyst 2

Injection#	*Name*	*RT (Min.)*	*Response (AU)*	**Percent_LC**
1	*Preparation-1*	*2.935*	*4459226*	*98.4*
2	*Preparation-2*	2.933	*4514839*	*98.8*
3	*Preparation-3*	2.933	*4463908*	*99.1*
4	*Preparation-4*	2.933	*4448068*	*98.0*
5	*Preparation-5*	2.931	*4488477*	*98.5*
6	Preparation-6	2.934	4446668	98.5
Mean			4470198	98.6
%RSD			0.6	0.4

Table 18. Intermediate Precision of Butalbital - Analyst 2

Injection#	*Name*	*RT (Min.)*	*Response (AU)*	**Percent_LC**
1	*Preparation-1*	*10.780*	*834949*	*100.4*
2	*Preparation-2*	10.786	*839553*	*100.2*
3	*Preparation-3*	10.775	*833119*	*100.8*
4	*Preparation-4*	10.781	*835543*	*100.4*
5	*Preparation-5*	10.778	*835887*	*100.1*
6	Preparation-6	10.777	828277	100.0
Mean			834555	100.3
%RSD			0.4	0.3

Table 19. Intermediate Precision of Caffeine - Analyst 2

Injection#	*Name*	*RT (Min.)*	*Response (AU)*	**Percent_LC**
1	*Preparation-1*	*4.621*	*1429584*	*99.8*
2	*Preparation-2*	4.617	*1450604*	*100.4*
3	*Preparation-3*	4.618	*1431637*	*100.5*
4	*Preparation-4*	4.619	*1426169*	*99.4*
5	*Preparation-5*	4.615	*1439969*	*100.0*
6	Preparation-6	4.618	1426732	100.0
Mean			1434116	100.0
%RSD			0.7	0.4

Table 10. Intermediate Precision of Methylparaben - Analyst 2

Injection#	*Name*	*RT (Min.)*	*Response (AU)*	**Percent_LC**
1	*Preparation-1*	*9.842*	*6073287*	*99.5*
2	*Preparation-2*	9.825	*6149496*	*100.0*
3	*Preparation-3*	9.831	*6042115*	*99.6*
4	*Preparation-4*	9.829	*6078185*	*99.5*
5	*Preparation-5*	9.827	*6100426*	*99.5*
6	Preparation-6	9.831	6066047	99.8
Mean			6084926	99.6
%RSD			0.6	0.2

Table 21. Intermediate Precision of Propylparaben - Analyst 2

Injection#	*Name*	*RT*	*Response*	**Percent_LC**

		(Min.)	*(AU)*	
1	*Preparation-1*	*12.446*	*548809*	*100.1*
2	*Preparation-2*	12.428	*557192*	*100.8*
3	*Preparation-3*	12.433	*546138*	*100.2*
4	*Preparation-4*	12.431	*549251*	*100.1*
5	*Preparation-5*	12.437	*551653*	*100.1*
6	Preparation-6	12.431	548865	100.5
Mean			550318	100.3
%RSD			0.7	0.3

Table 22. Result Comparison (Intermediate Precision/Ruggedness)

Name	**Mean Percent_LC**		**% Difference**
	Analyst-1	**Analyst-2**	
Acetaminophen	97.7	98.6	0.9
Butalbital	99.6	100.3	0.7
Caffeine	99.5	100.0	0.5
Methylparaben	97.6	99.6	2.0
Propylparaben	97.4	100.3	2.9

Table 23. Solution Stability of Standard at 5°C

Standard Name	**Response (AU)**			**% Difference**	
	Initial	**24 Hrs.**	**48 Hrs.**	**24 Hrs.**	**48 Hrs.**
Acetaminophen	4273730	4261547	4265223	0.3	0.2
Butalbital	814382	822923	826523	1.0	1.5
Caffeine	1391361	1382848	1385866	0.6	0.4
Methylparaben	5948204	5969264	5960478	0.4	0.2
Propylparaben	528110	532319	528917	0.8	0.2

Table 24. Solution Stability of Sample at 5°C

Standard Name	**Percent_LC**			**% Difference**	
	Initial	**24 Hrs.**	**48 Hrs.**	**24 Hrs.**	**48 Hrs.**
Acetaminophen	97.7	99.7	99.8	0.0	2.1
Butalbital	99.6	101.0	101.5	1.4	1.9
Caffeine	99.5	99.4	99.6	0.1	0.1
Methylparaben	97.6	99.6	99.9	2.0	2.3
Propylparaben	97.4	99.2	99.4	1.8	2.0

Table 25. Forced Degradation-Peak Purity (at 216 nm)

S. No	Sample Name	Acetaminophen		Butalbital		Caffeine	
		Purity Angle	Purity Threshold	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
1	Control Sample	0.587	1.003	0.309	1.136	0.767	1.051
2	Acid Stress Sample	0.550	1.003	0.344	1.145	0.795	1.059
3	Base Stress Sample	0.560	1.003	0.385	1.138	0.785	1.052
4	H2O2 Stress Sample	0.598	1.003	0.316	1.140	0.807	1.054
5	Heat Stress Sample	0.586	1.003	0.338	1.146	0.768	1.063
6	UV Stress Sample	0.604	1.003	0.413	1.142	0.801	1.057

H2O2: Hydrogen Peroxide/Oxidative Stress

Table 26. Forced Degradation-Peak Purity (at 254 nm)

S. No	Sample Name	Methylparaben		Propylparaben	
		Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
1	Control Sample	0.559	1.003	0.438	1.192
2	Acid Stress Sample	0.615	1.002	0.723	1.778
3	Base Stress Sample	0.613	1.003	0.384	1.172
4	H2O2 Stress Sample	0.558	1.003	0.730	1.311
5	Heat Stress Sample	0.428	1.150	0.583	1.002
6	UV Stress Sample	0.458	1.181	0.557	1.003

H2O2: Hydrogen Peroxide/Oxidative Stress

Table 27: Specificity

Name	RT (Min)	RRT
Acetaminophen	2.9	n/a (w.r.t. Acetaminophen)
Butalbital	10.8	3.7 (w.r.t. Acetaminophen)
Caffeine	4.6	1.6 (w.r.t. Acetaminophen)
Methylparaben	9.8	3.4 (w.r.t. Acetaminophen)
Propylparaben	12.4	4.3 (w.r.t. Acetaminophen)

n/a: not applicable

Table 28. Recovery for Acetaminophen (at 50%, 100% and 120%)

Accuracy/ Parameter	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean	% RSD
Accuracy-50%-1	158.9565	160.5918	101.0		
Accuracy-50%-2	158.9565	158.9392	100.0	105	0.5
Accuracy-50%-3	158.9565	159.5889	100.4		
Accuracy-100%-1	317.9131	312.6512	98.3		
Accuracy-100%-2	317.9131	312.9347	98.4	98.5	0.3
Accuracy-100%-3	317.9131	314.3009	98.9		
Accuracy-120%-1	397.3913	391.9130	98.6		
Accuracy-120%-2	379.3913	390.7369	98.3	98.7	0.4
Accuracy-120%-3	379.3913	393.6564	99.1		

Table 29. Recovery for Butalbital (at 50%, 100% and 120%)

Accuracy/ Parameter	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean	% RSD
Accuracy-50%-1	24.0401	24.4178	101.6		
Accuracy-50%-2	24.0401	24.1727	100.6	101.0	0.5
Accuracy-50%-3	24.0401	24.2311	100.8		
Accuracy-100%-1	48.0803	47.2938	98.2		
Accuracy-100%-2	48.0803	47.3038	98.4	98.4	0.3
Accuracy-100%-3	48.0803	47.4826	98.8		
Accuracy-120%-1	60.1004	59.1975	98.5		
Accuracy-120%-2	60.1004	58.9229	98.0	98.5	0.4
Accuracy-120%-3	60.1004	59.4152	98.9		

Table 30. Recovery for Caffeine (at 50%, 100% and 120%)

Accuracy/ Parameter	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean	% RSD
Accuracy-50%-1	19.2112	19.3528	100.7		
Accuracy-50%-2	19.2112	19.1099	99.5	100.1	0.6
Accuracy-50%-3	19.2112	19.2536	100.2		
Accuracy-100%-1	38.4223	37.9704	98.8		
Accuracy-100%-2	38.4223	37.9830	98.9	99.0	0.3
Accuracy-100%-3	38.4223	38.1645	99.3		
Accuracy-120%-1	48.0279	47.7356	99.4		
Accuracy-120%-2	48.0279	47.6378	99.2	99.5	0.3
Accuracy-120%-3	48.0279	47.9249	99.8		

Table 31. Recovery for Methylparaben (at 50%, 100% and 120%)

Accuracy/ Parameter	*Amount Added (µg/mL)*	**Amount Found (µg/mL)**	**% Recovery**	**Mean**	**% RSD**
Accuracy-LOQ-1	*59.6902*	*62.2835*	*104.3*		
Accuracy-LOQ-2	*59.6902*	*62.5085*	*104.7*	*104.6*	*0.2*
Accuracy-LOQ-3	59.6902	62.5000	104.7		
Accuracy-100%-1	*119.3804*	*120.1942*	100.7		
Accuracy-100%-2	*119.3804*	*121.0038*	*101.4*	*101.1*	*0.4*
Accuracy-100%-3	119.3804	*120.8562*	101.2		
Accuracy-120%-1	149.2256	148.9999	99.8		
Accuracy-120%-2	149.2256	149.3225	100.1	99.9	0.1
Accuracy-120%-3	149.2256	148.9931	99.8		

Table 32. Recovery for Propylparaben (at 50%, 100% and 120%)

Accuracy/ Parameter	*Amount Added (µg/mL)*	**Amount Found (µg/mL)**	**% Recovery**	**Mean**	**% RSD**
Accuracy-50%-1	*6.2215*	*5.9756*	*96.0*		
Accuracy-50%-2	*6.2215*	*5.9744*	*96.0*	*96.0*	*0.0*
Accuracy-50%-3	6.2215	5.9722	96.0		
Accuracy-100%-1	*12.4431*	*11.8574*	95.3		
Accuracy-100%-2	*12.4431*	*11.9301*	*95.9*	*95.7*	*0.3*
Accuracy-100%-3	12.4431	*11.9285*	95.9		
Accuracy-120%-1	15.5538	14.8963	95.8		
Accuracy-120%-2	15.5538	14.9370	96.0	95.9	0.1
Accuracy-120%-3	15.5538	14.8981	95.8		

Table 33. Filter Study

S. No	**Parameter/ Condition**	**Percent_LC**		
		Acetaminophen	**Butabital**	**Coffeine**
1	Without filtration (As is)	97.8	100.2	99.5

2	0.45µm Nylon filter 1 mL	97.6	100.1	99.5
3	0.45µm Nylon filter 4 mL	97.5	99.3	99.3
4	0.45µm Nylon filter 6 mL	97.2	99.5	99.0
5	0.45µm Nylon filter 8 mL	97.5	99.8	99.8

Table 34. Filter Study

Parameter/Condition	**Percent_LC**	
	Methylparaben	**Propylparaben**
Without filtration (As is)	97.8	97.4
0.45µm Nylon filter 1 mL	97.8	97.4
0.45µm Nylon filter 4 mL	97.9	97.5
0.45µm Nylon filter 6 mL	97.7	97.3
0.45µm Nylon filter 8 mL	97.8	97.3

Table 35. Robustness (Acetaminophen)

Parameter/Condition	*RT (Min.)*	**Response (AU)**	**% RSD**	**USP Tailing**
Normal Conditions	*3.0*	*4250333*	*0.4*	*1.2*
Column Temp. Plus 5°C (45°C)	*2.9*	*4236179*	*0.1*	*1.2*
Column Temp. Minus 5°C (35°C)	3.1	4231829	0.4	1.2
Flow rate Plus 0.2 mL (1.4 mL)	*2.6*	*3639544*	*0.3*	*1.2*
Flow rate Minus 0.2 mL (1.0 mL)	3.6	*5095053*	0.4	1.2
pH Plus 0.2 (4.70)	*3.0*	*4281445*	*0.3*	*1.2*
pH Minus 0.2 (4.30)	3.1	*4331721*	0.4	1.2

Table 36. Robustness (Butalbital)

Parameter/Condition	*RT (Min.)*	**Response (AU)**	**% RSD**	**USP Tailing**
Normal Conditions	*11.0*	*812271*	*0.4*	*1.2*
Column Temp. Plus 5°C (45°C)	*10.9*	*821374*	*0.2*	*1.2*
Column Temp. Minus 5°C (35°C)	11.3	806335	0.4	1.2
Flow rate Plus 0.2 mL (1.4 mL)	*10.4*	*706082*	*0.2*	*1.2*
Flow rate Minus 0.2 mL (1.0 mL)	12.0	*989718*	0.4	1.3
pH Plus 0.2 (4.70)	*10.7*	*826174*	*0.3*	*1.2*
pH Minus 0.2 (4.30)	11.4	*818432*	0.4	1.3

Table 37. Robustness (Caffeine)

Parameter/Condition	*RT (Min.)*	**Response (AU)**	**% RSD**	**USP Tailing**
Normal Conditions	*4.6*	*1381593*	*0.5*	*1.2*
Column Temp. Plus 5°C (45°C)	*4.4*	*1376357*	*0.1*	*1.2*

Column Temp. Minus 5°C (35°C)	5.0	1381337	0.4	1.2
Flow rate Plus 0.2 mL (1.4 mL)	*4.0*	*1177420*	*0.3*	*1.2*
Flow rate Minus 0.2 mL (1.0 mL)	5.6	*1661308*	0.3	1.2
pH Plus 0.2 (4.70)	*4.6*	*1390998*	*0.3*	*1.2*
pH Minus 0.2 (4.30)	5.1	*1391324*	0.4	1.3

Table 38. Robustness (Methylparaben)

Parameter/Condition	*RT (Min.)*	**Response (AU)**	**% RSD**	**USP Tailing**
Normal Conditions	*9.9*	*5943878*	*0.3*	*1.2*
Column Temp. Plus 5°C (45°C)	*9.8*	*5873756*	*0.1*	*1.2*
Column Temp. Minus 5°C (35°C)	10.1	5944544	0.3	1.2
Flow rate Plus 0.2 mL (1.4 mL)	*9.4*	*5035055*	*0.3*	*1.2*
Flow rate Minus 0.2 mL (1.0 mL)	10.8	*7142774*	0.3	1.3
pH Plus 0.2 (4.70)	*9.9*	*5941671*	*0.2*	*1.2*
pH Minus 0.2 (4.30)	10.2	*5945618*	0.4	1.3

Table 39. Robustness (Propylparaben)

Parameter/Condition	*RT (Min.)*	**Response (AU)**	**% RSD**	**USP Tailing**
Normal Conditions	*12.8*	*529995*	*0.3*	*1.2*
Column Temp. Plus 5°C (45°C)	*12.7*	*521369*	*0.1*	*1.2*
Column Temp. Minus 5°C (35°C)	13.5	525986	0.3	1.2
Flow rate Plus 0.2 mL (1.4 mL)	*12.1*	*443221*	*0.2*	*1.2*
Flow rate Minus 0.2 mL (1.0 mL)	14.4	*634564*	0.3	1.2
pH Plus 0.2 (4.70)	*12.3*	*534448*	*0.2*	*1.2*
pH Minus 0.2 (4.30)	13.4	*538432*	0.4	1.3

6.0 Representative Linearity graphs, Chromatograms of Blank, Standard and Sample are depicted below:

Figure 6. Linearity Plot for Acetaminophen

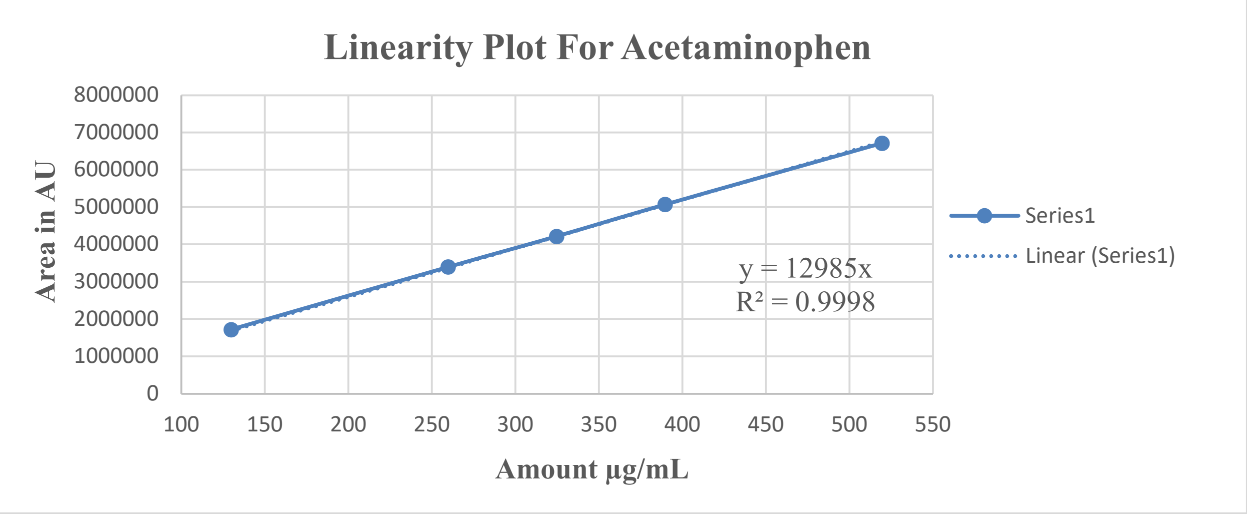


Figure 7. Linearity Plot for Butalbital

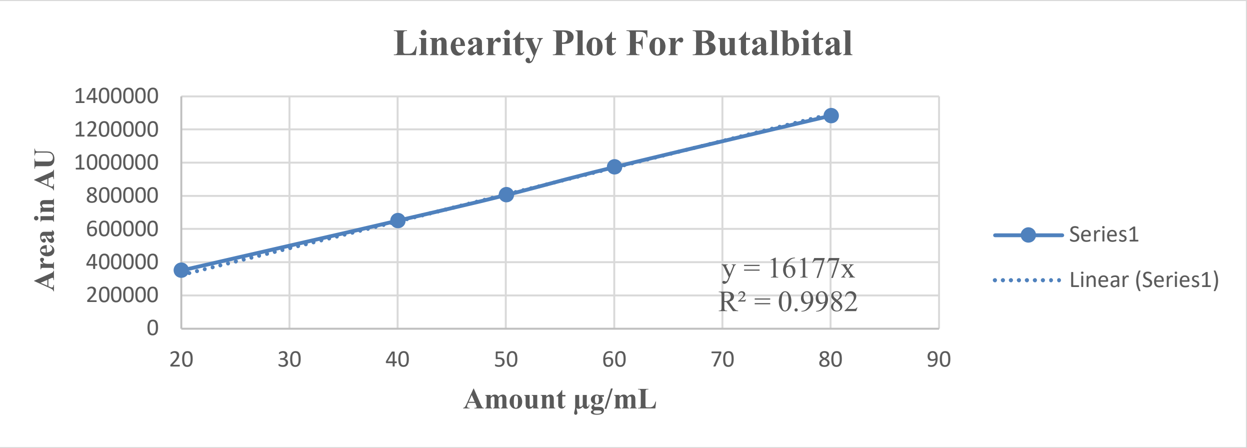


Figure 8. Linearity Plot for Caffeine

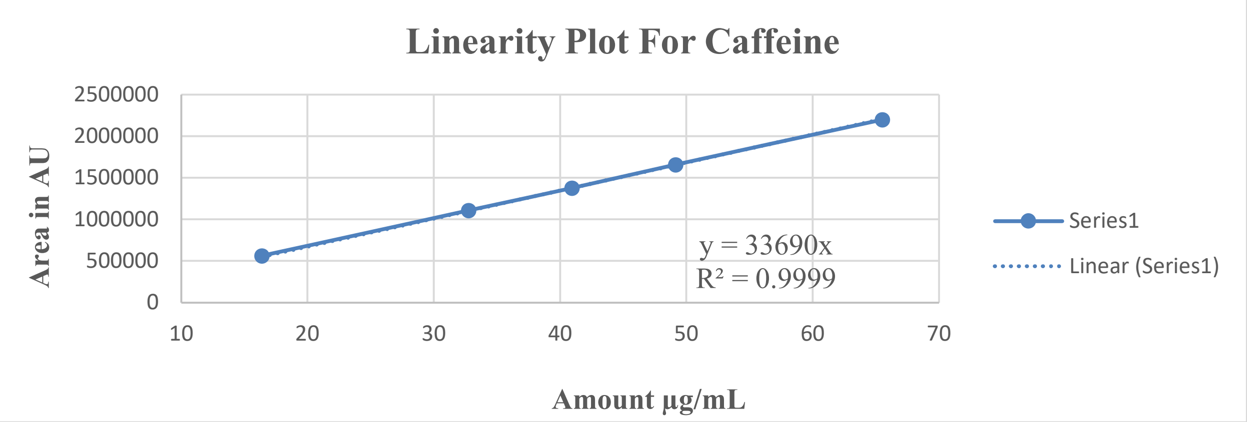


Figure 9. Linearity Plot for Methylparaben

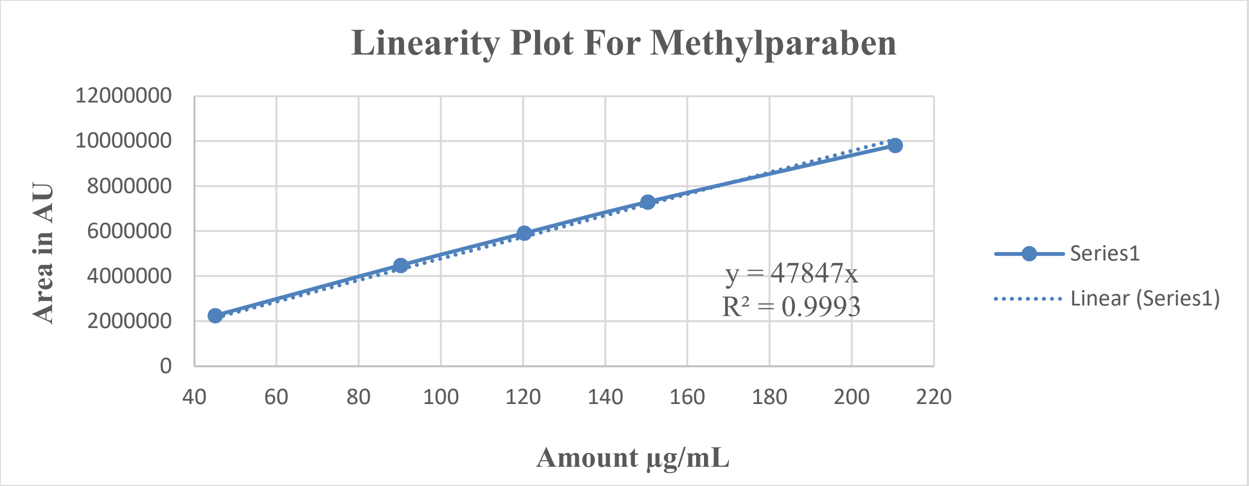


Figure 10. Linearity Plot for Propylparaben

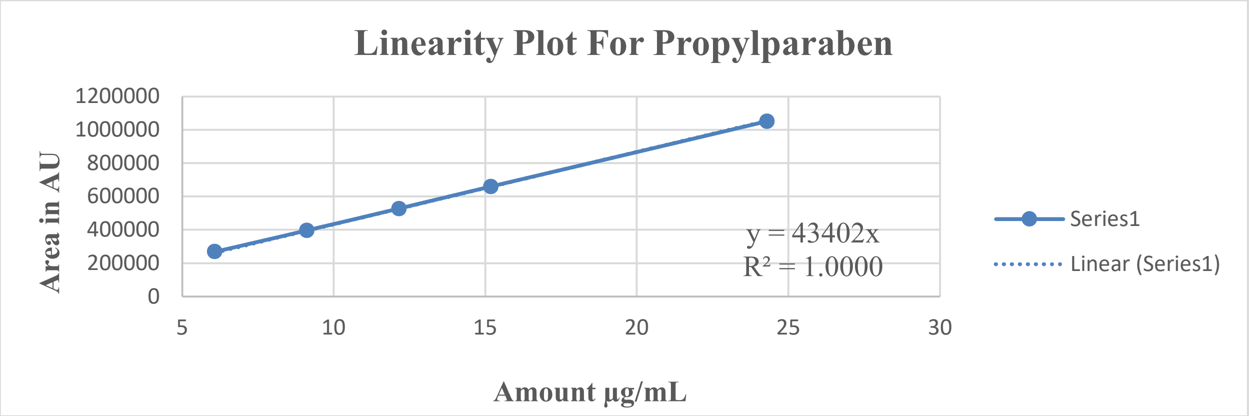


Figure 11. Typical Chromatogram of Blank at 216 nm (Acetaminophen, Butalbital, Caffeine)

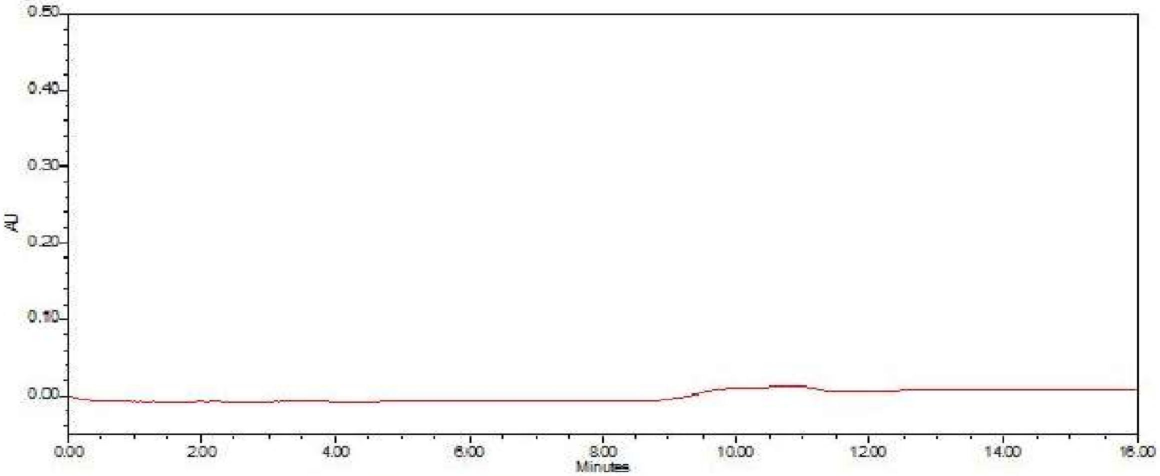


Figure 12. Typical Chromatogram of Blank at 254 nm (Methylparaben and Propylparaben)

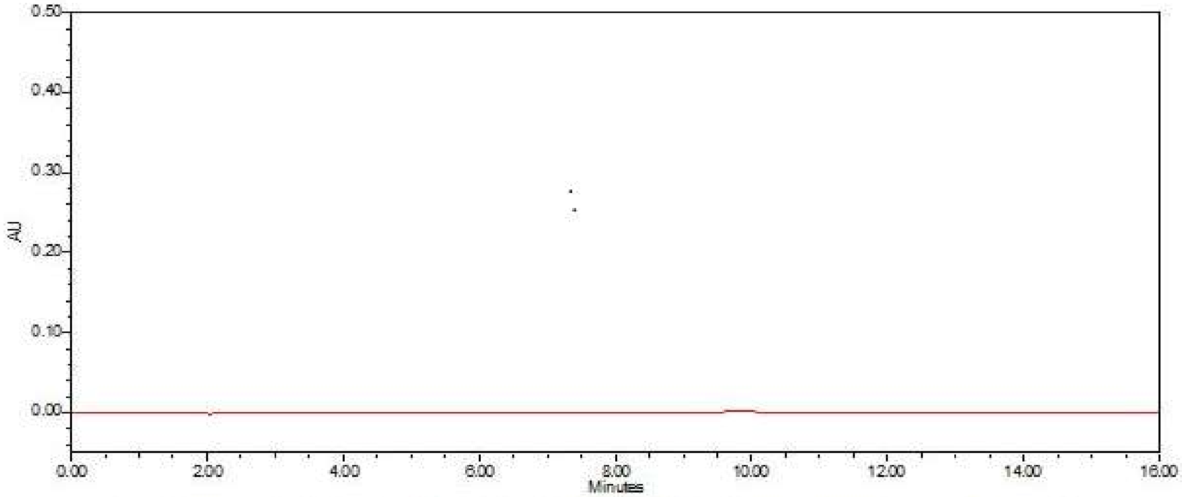


Figure 13. Typical Chromatogram of Standard at 216 nm (Acetaminophen, Butalbital, Caffeine)

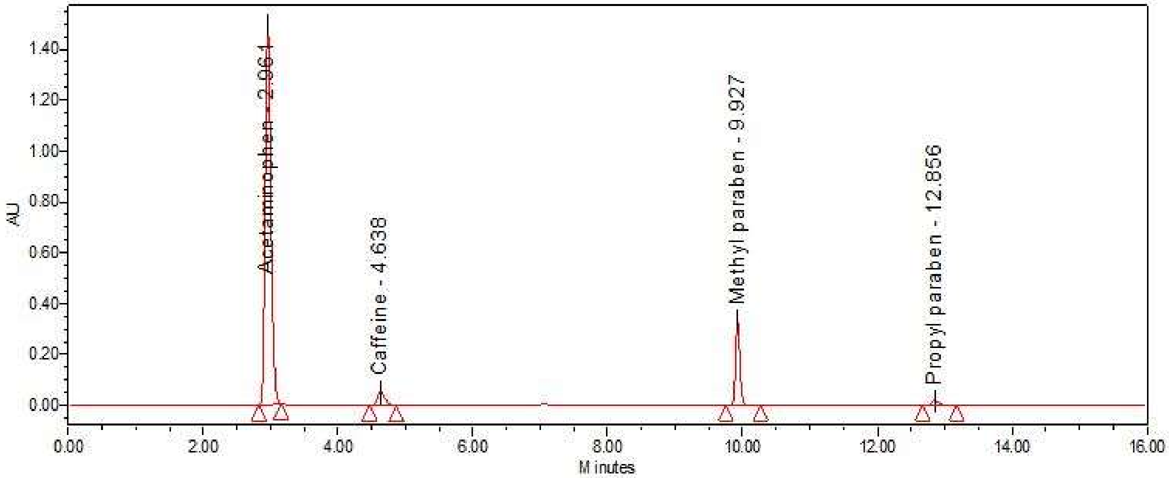


Figure 14. Typical Chromatogram of Standard at 254 nm (Methylparaben and Propylparaben)

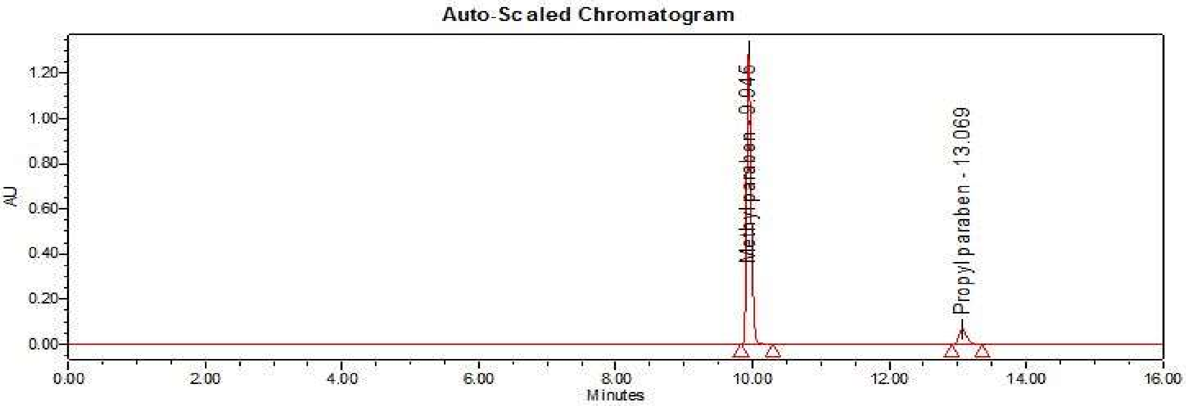
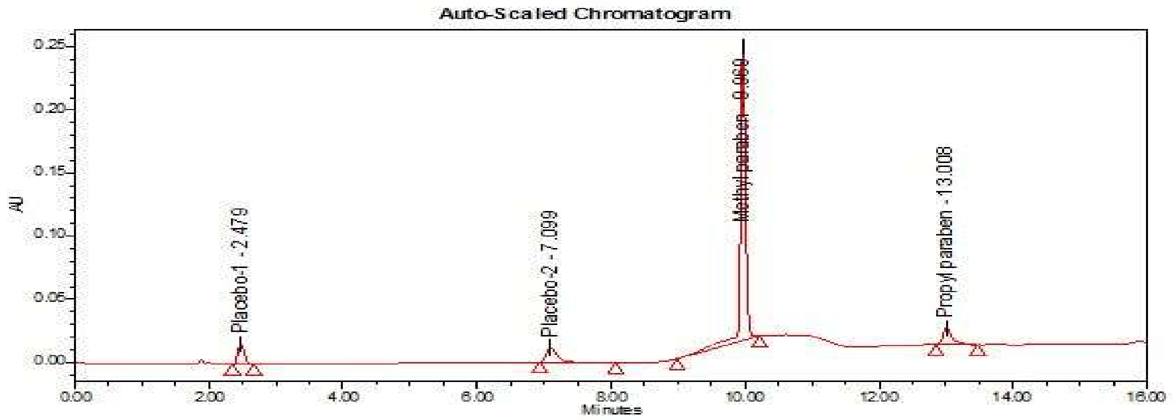


Figure 15. Typical Chromatogram of Placebo



7.0 Typical Chromatograms for Spiked samples and Peak purity plots are depicted below.

Figure 16. Typical Chromatogram of Spiked Sample at 216 nm (Acetaminophen, Butalbital,

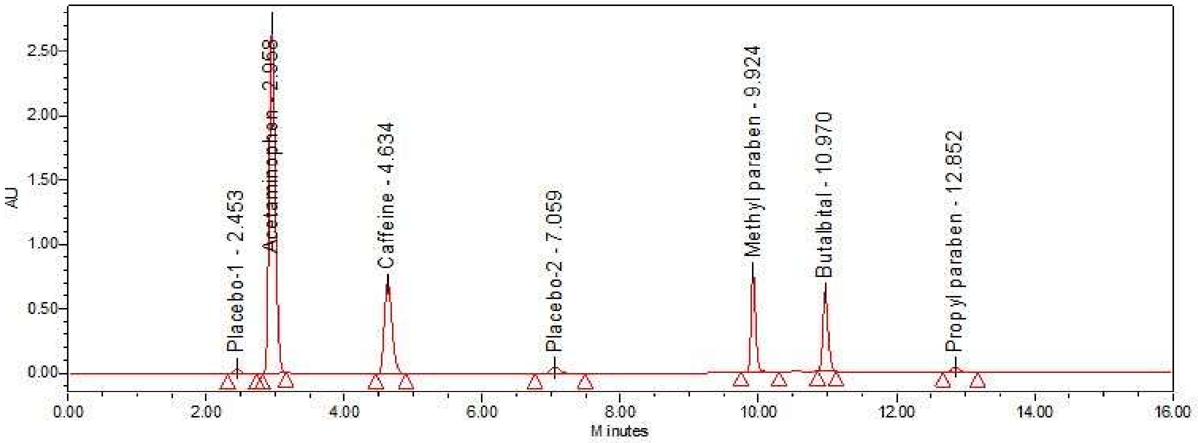


Figure 17. Peak Purity Plot for Spiked Sample at 216 nm (Acetaminophen)

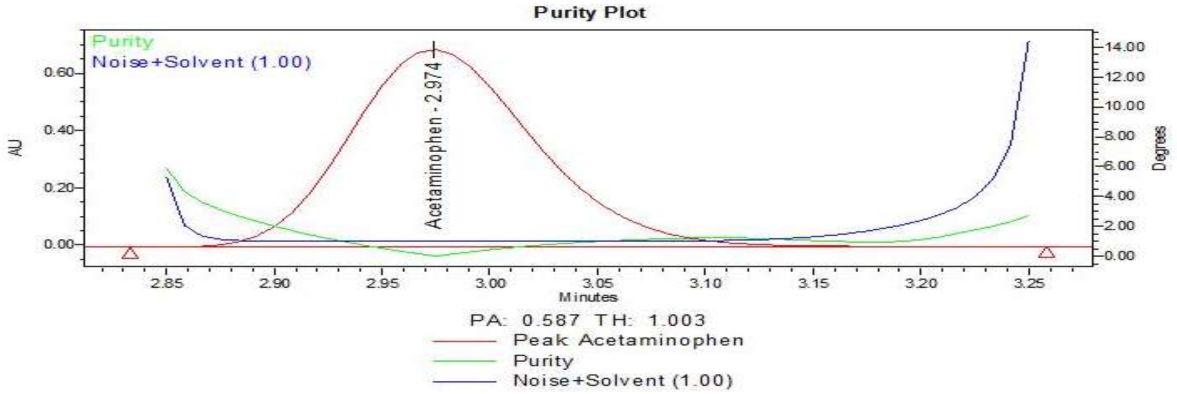


Figure 18. Peak Purity Plot for Spiked Sample at 216 nm (Butalbital)

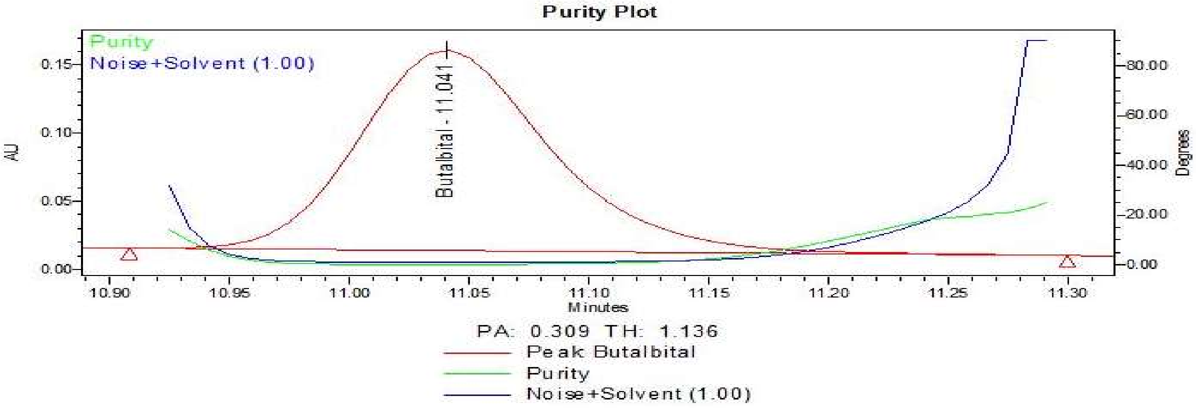


Figure 19. Peak Purity Plot for Spiked Sample at 216 nm (Caffeine)

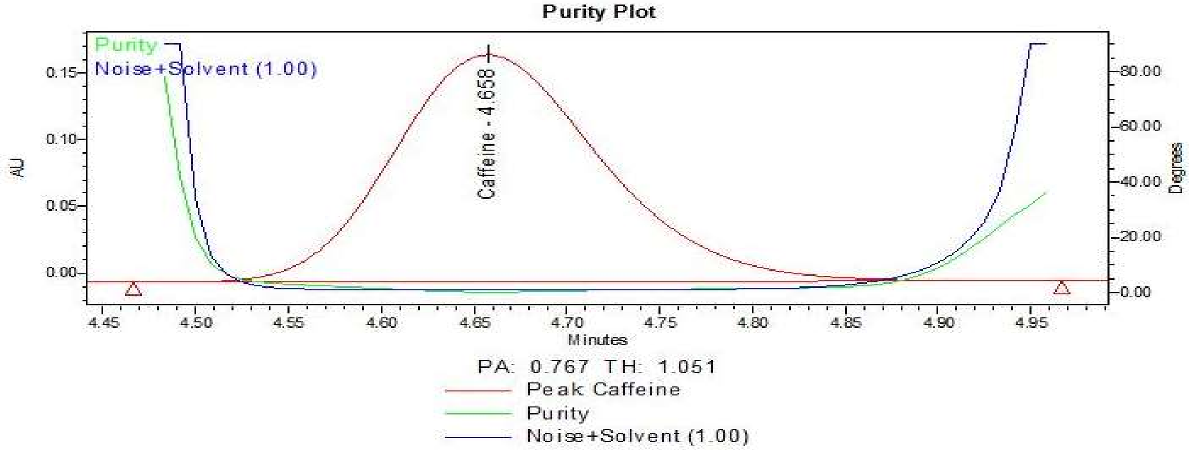


Figure 20. Typical Chromatogram of Spiked Sample at 254 nm (Methylparaben, Propylparaben)

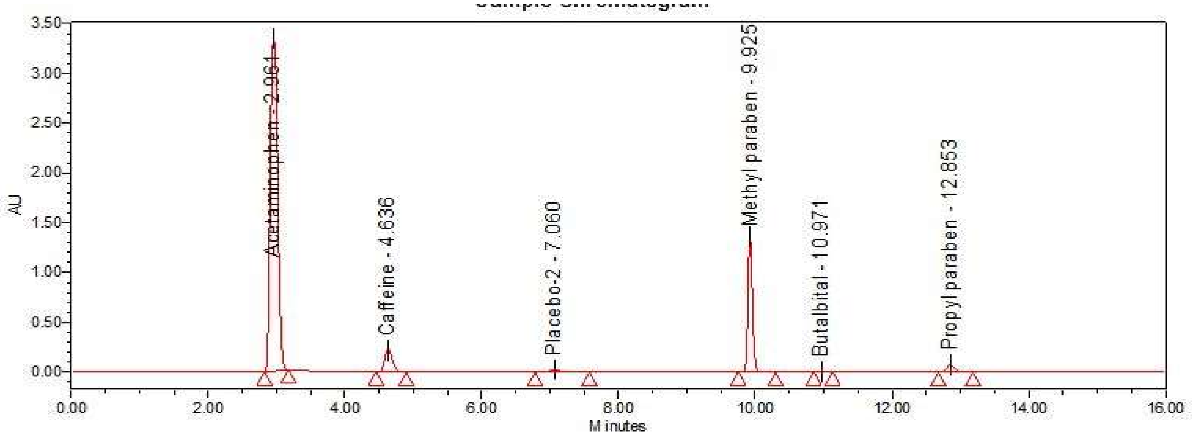


Figure 21. Peak Purity Plot for Spiked Sample at 254 nm (Methylparaben)

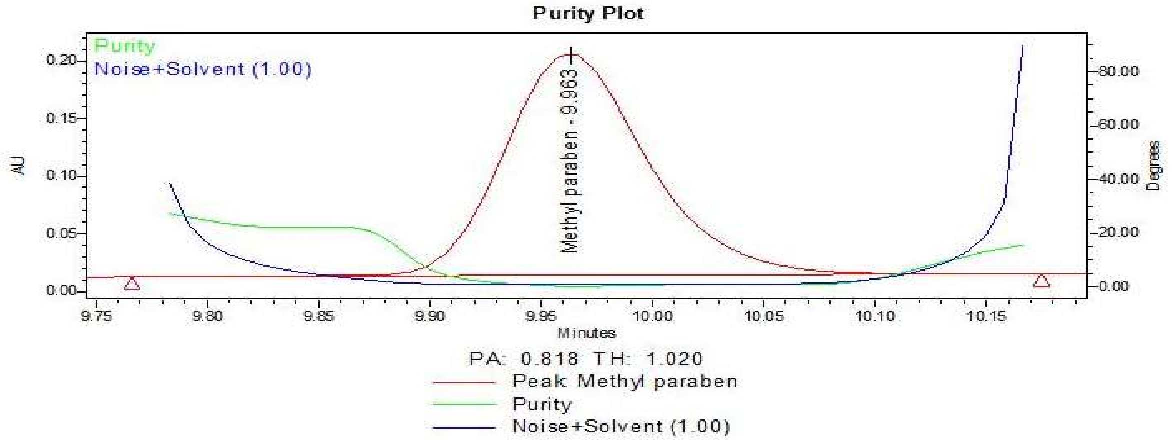
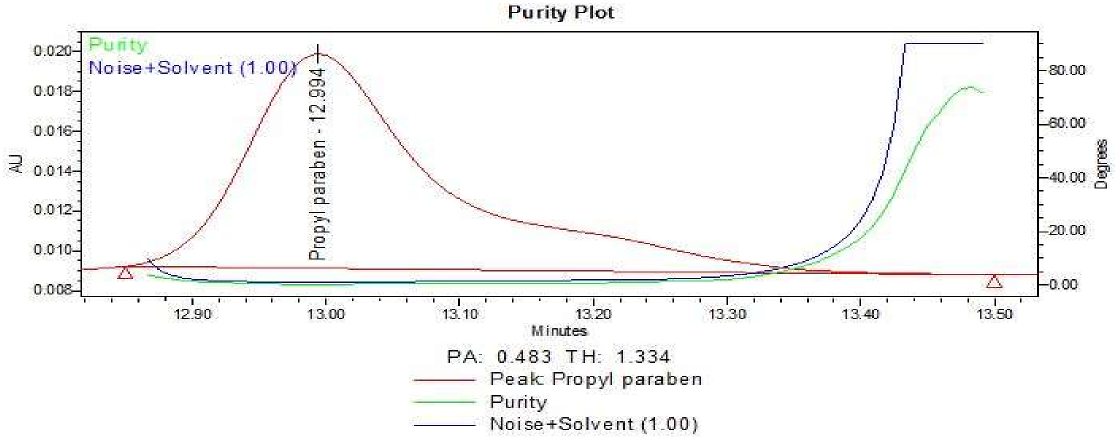


Figure 22. Peak Purity Plot for Spiked Sample at 254 nm (Propylparaben)



8.0 Conclusion

Based on the results of the above method validation studies, it is concluded that the method for determination of assay of Butalbital, Acetaminophen and Caffeine along with Preservatives-Methylparaben and Propylparaben is specific, precise, accurate, rugged, robust and linear over the concentration range from 40% to 160%. Standard and Sample solutions are stable for at least 48 hours when stored at 5°C. The filter study demonstrated that the samples can be filtered through a 0.45 µm Nylon filter by discarding at least the first four (4) mL of the filtrate. According to ICH standards, the method developed satisfies regulatory requirements. This makes it simple to use this method for the identification and simultaneous quantification of Butalbital, Acetaminophen, Caffeine and Methylparaben, Propylparaben using detector at dual wavelengths (216 nm and 254 nm, respectively) for routine analysis of production and as well stability samples.

9.0 References

1. Al-Rimawi, F. (2010). Normal-phase LC method for simultaneous analysis of Pseudophedrine hydrochloride, dextromethorphan hydrobromide, chlorpheniramine maleate and paracetamol in tablet formulations. *Saudi Pharmaceutical Journal*, *18*(2), 103–106. doi:10.1016/j.jsps.2010.02.006
2. Murtha, J. L., Julian, T. N., & Radebaugh, G. W. (1988). Simultaneous determination of pseudoephedrine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide by second-derivative photodiode array spectroscopy. *Journal of Pharmaceutical Sciences*, *77*(8), 715–718.

doi:10.1002/jps.2600770815

3. Zenon Kokot., Kinga Burda. (1998). Simultaneous determination of salicylic acid and
    a. acetylsalicylic acid in aspirin delayed-release tablet formulations by second-derivative UV
    b. spectrophotometry. Journal of Pharmaceutical and Biomedical Analysis, 18(4), 871-875.
    c. doi:10.1016/S0731-7085(98)00225-8

4. Deniz Emre., Nuran Ozaltin. (2007). Simultaneous determination of paracetamol, caffeine and
5. propyphenazone in ternary mixtures by micellar electrokinetic capillary chromatography.
6. *Journal of Chromatography B, 847(2), 126-132.*
7. doi: 10.1016/j.jchromb.2006.09.036
8. A Villari., N Micali., MFresta., G Puglisi. (1994). Simultaneous spectrophotometric
9. determination in solid phase of aspirin and its impurity salicylic acid in pharmaceutical
10. formulations. *J Pharm Sci, 81(19):895-8*.
11. doi: 10.1002/jps.2600810911

12. M. Jamil Akhtar., Sharfuddin Khan., Mushtaq Hafiz. (1994). High-performance liquid
13. chromatographic assay for the determination of paracetamol, pseudoephedrine
14. hydrochloride and triprolidine hydrochloride. *Journal of Pharmaceutical and Biomedical*
15. *Analysis, 12(3), Pages 379-382.*
16. doi: 10.1016/0731-7085(94)90014-0

17. C. Pistos., J.T. Stewart. (2004). Assay for the simultaneous determination of acetaminophen–
18. caffeine–butalbital in human serum using a monolithic column. *Journal of Pharmaceutical*
19. *and Biomedical Analysis, 36(4), Pages 737-741*
20. doi: 10.1016/j.jpba.2004.07.042

21. Jakkan, K., Singh, N., & Lokhande, R. S. (2019). Identification isolation and structural characterization of unknown impurities in cefdinir drug substance. *Int. J. Chem. & PharmSci*, *10*.
22. Kasa, S., Pippalla, S., Naidu, M. N., & Goyal, D. (2024). Development and validation of a UPLC method for the determination of docetaxel and its related substances in pharmaceutical dosage forms, an antineoplastic agent. *American Journal of Analytical Chemistry*, *15*(10), 333–346. doi:10.4236/ajac.2024.1510021
23. Dina S. El-Kafrawy., Tarek S. Belal. (2016). Validated HPTLC method for the simultaneous
24. determination of cinnarizine and dimenhydrinate in their combined dosage form. *Journal*
25. *of the Association of Arab Universities for Basic and Applied Sciences, 19, 15-22.*
26. doi: 10.1016/j.jaubas.2014.06.004

27. Z. Bouhsain., Salvador Garrigues., Miguel de la Guardia. (1997). PLS-UV spectrophotometric
28. method for the simultaneous determination of paracetamol, acetylsalicylic acid and
29. caffeine in pharmaceutical formulations. *Fresenius Journal of Analytical Chemistry,*
30. *357(7):973-976.*