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DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PRIMIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC METHOD

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Primidone, in its pure form as well as in tablet dosage form. Chromatography was carried out on a ODS C18 (4.6 x 250mm, 5µm) column using a mixture of Methanol: Ammonium acetate buffer, pH 3.5 (65:35) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 225nm. The retention time of the Primidone, was 2.252 ±0.02min respectively. The method produces linear responses in the concentration range of 10-50mg/ml of Primidone.The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEY WORDS

Primidone, RP-HPLC, validation

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also, quality is important in every product or service, but it is vital in medicines as it involves life.

CHEMICALS USED:

Table: chemicals used

S.No	Chemical	Brand names
1	Primidone	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Ammonium Acetate	Sura labs

RESULTS AND DISCUSSION

Trails

Trail 1:

Column : Symmetry C18 (4.6×250mm)5µ
Column temperature : 40ºC
Wavelength : 210nm
Mobile phase ratio : ACN: Water (50:50) V/V
Flow rate : 1ml/min
Injection volume : 12µl
Run time : 6min

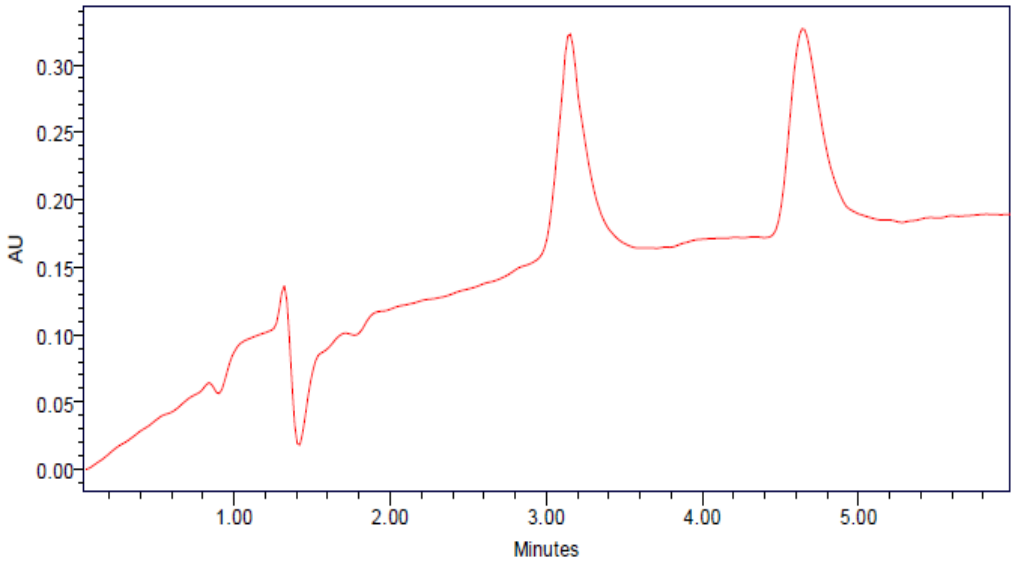


Figure: chromatogram for trail 1

Observation:
In this trial, it shows improper separation of peak in the chromatogram. so it requires more trials to obtain good peaks.

Trail 2:

Column : Symmetry C18 (4.6×250mm)5µ
Column temperature : 40°C
Wavelength : 210nm
Mobile phase ratio : Methanol: Water (55:45) V/V
Flow rate : 1.2ml/min
Injection volume : 8µl
Run time : 10min

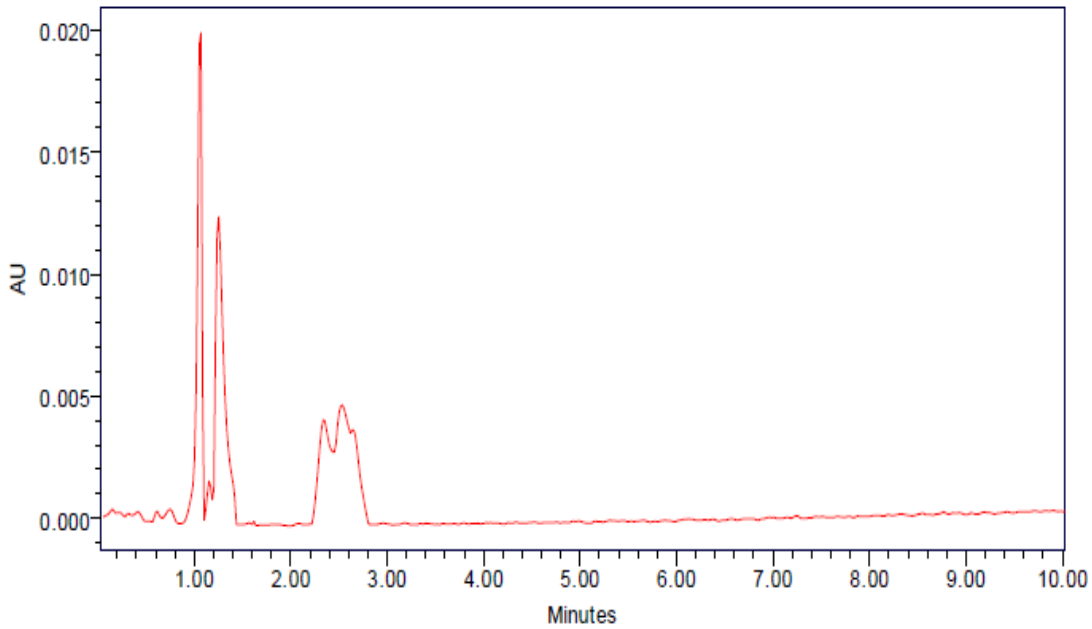


Figure: chromatogram for trail 2

Observation: In this trial, it shows improper separation of peak in the chromatogram. so it requires more trials to obtain good peaks.

Trail 3:

Column : ODS C18 (4.6×250mm, 5µ)
Column temperature : 40ºC
Wavelength : 210nm
Mobile phase ratio : Methanol: Phosphate buffer (10:90) V/V
Flow rate : 0.7ml/min
Injection volume : 15µl
Run time : 10min

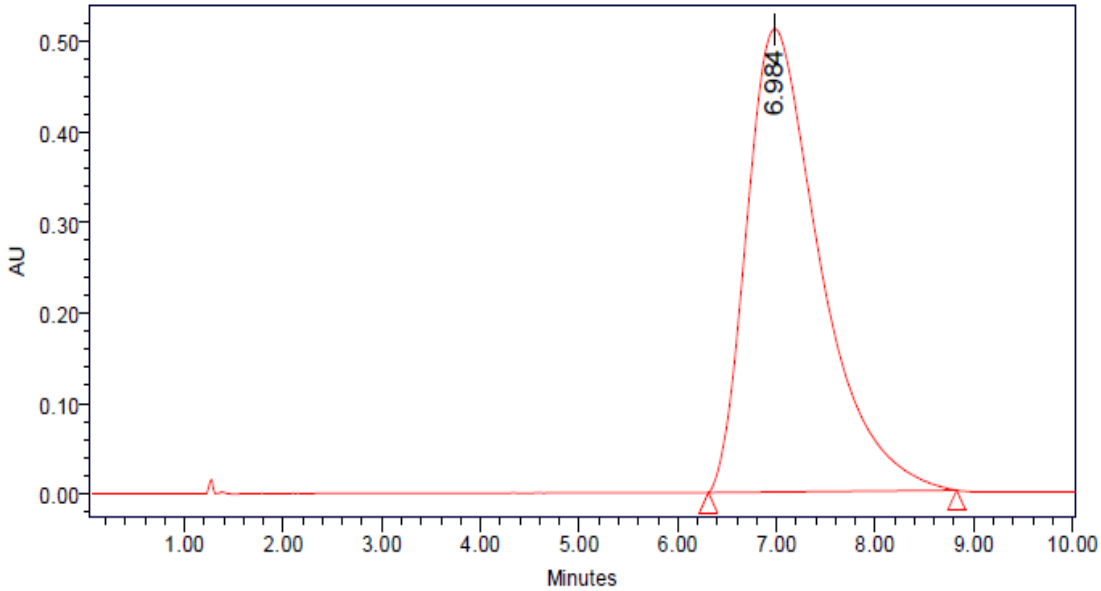


Figure: chromatogram for trail 3

Table: peak results for trail 3

S.No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Primidone	6.984	26396862	512105	1.61	1532

Observation: In this trial, it shows improper separation of peak and less plate count in the chromatogram. So, it requires more trials to obtain good peaks.

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Ammonium acetate buffer, p^H 3.5(65:35)
Column : ODS C18 (4.6×250mm, 5µ)
Column temperature : 40ºC
Wavelength : 210nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 8min

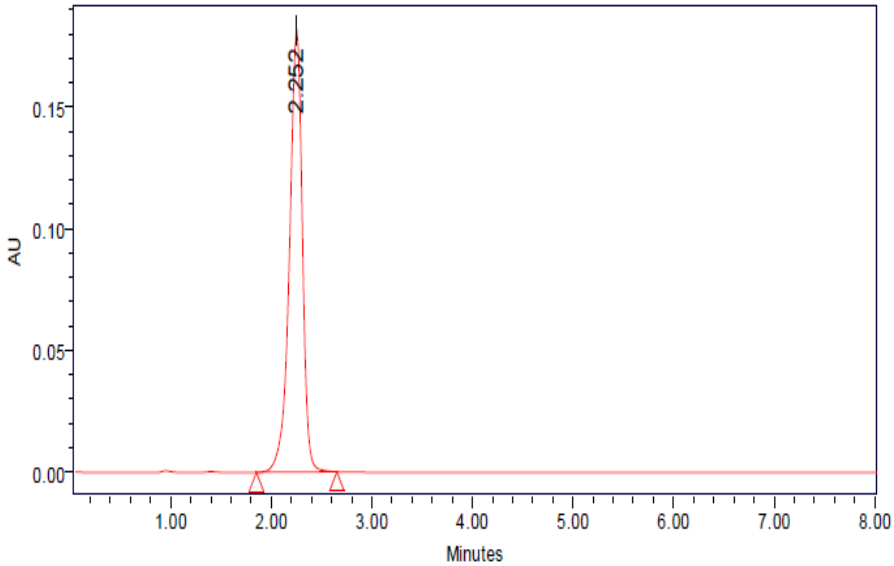


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Primidone	2.252	1417834	187270	0.83	6291

Observation: In this trial, it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So, it is optimized.

Optimized Chromatogram (Sample)

Figure: Optimized Chromatogram (Sample)

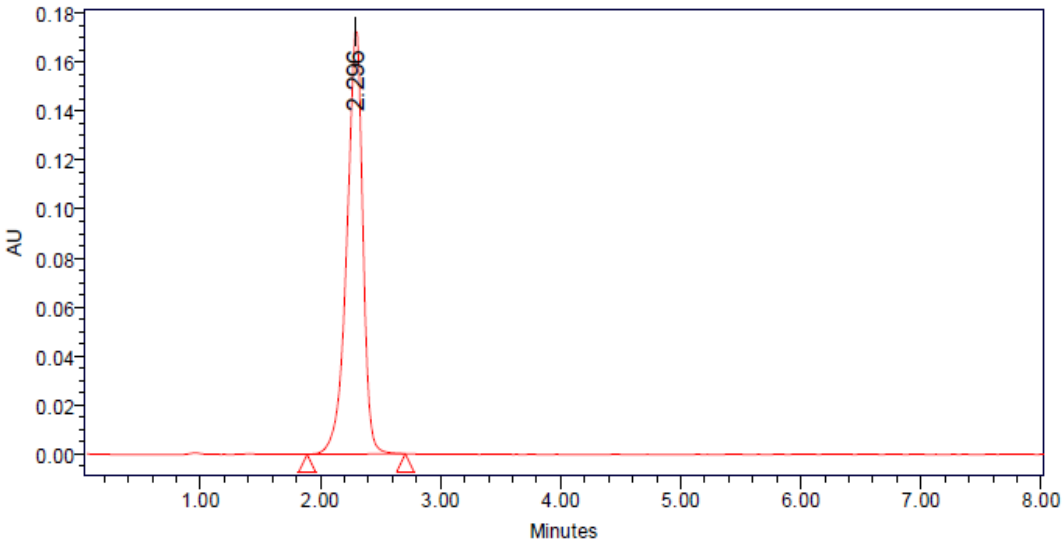


Table: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Primidone	2.296	1429918	173023	0.85	6450

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

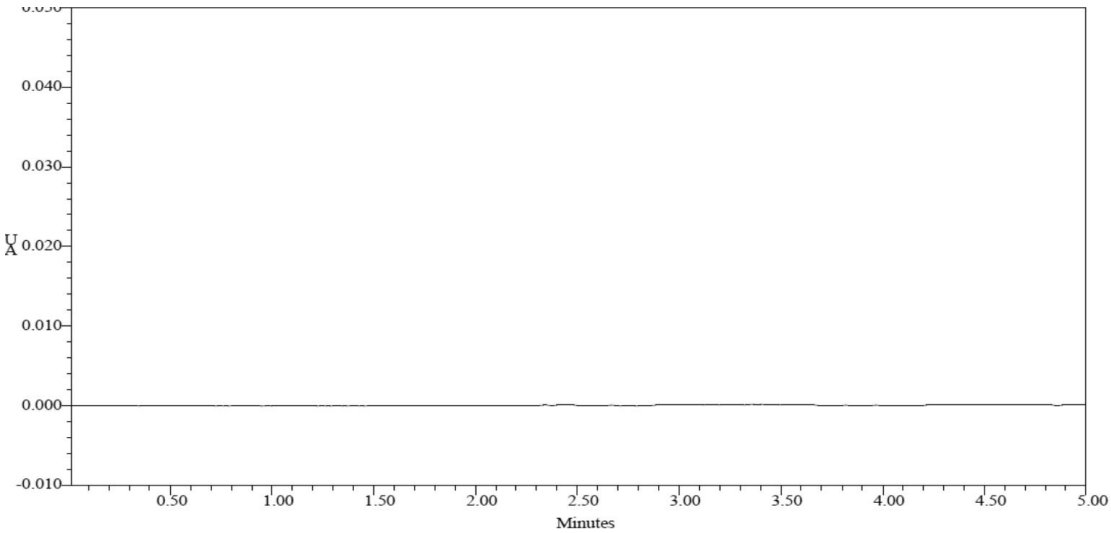


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

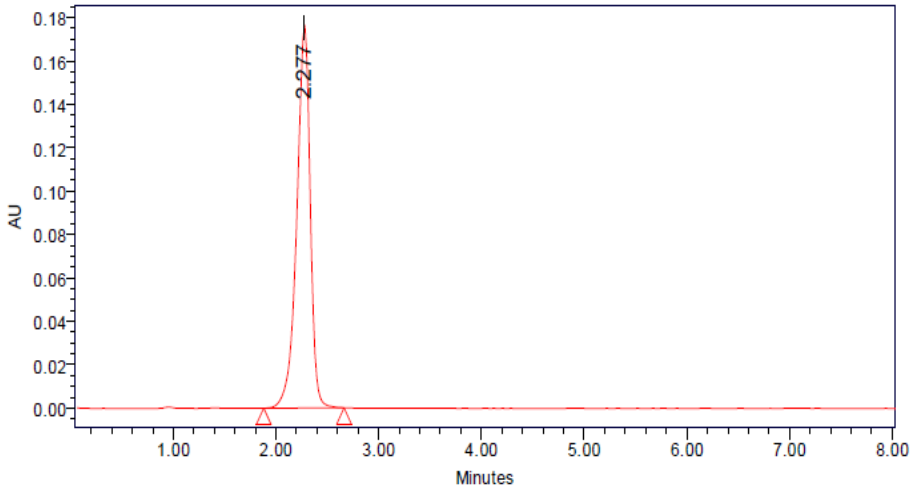


Fig: Chromatogram showing injection -1

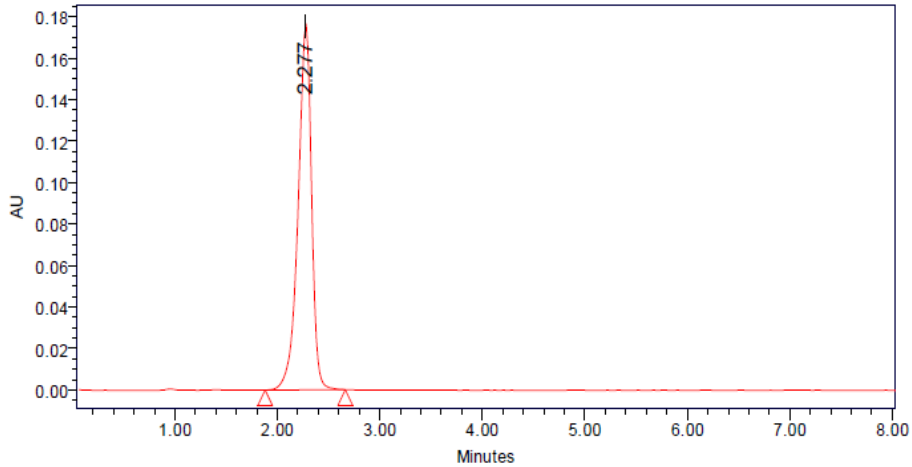


Fig: Chromatogram showing injection -2

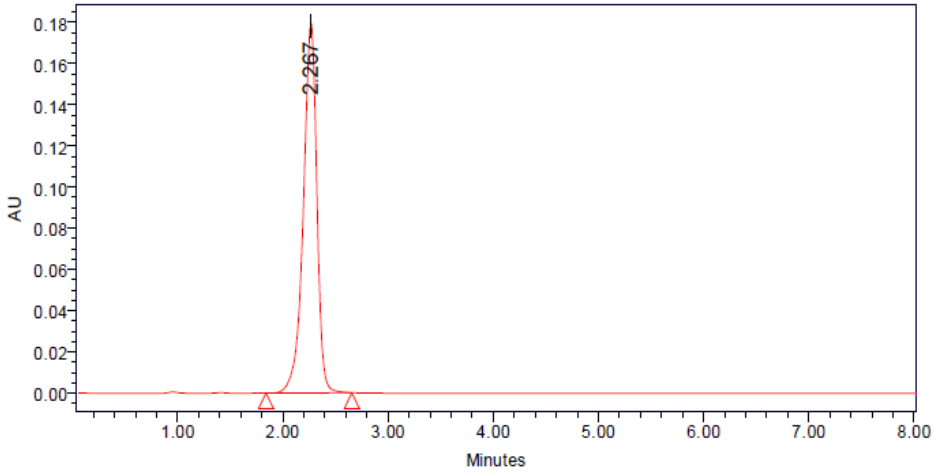


Fig: Chromatogram showing injection -3

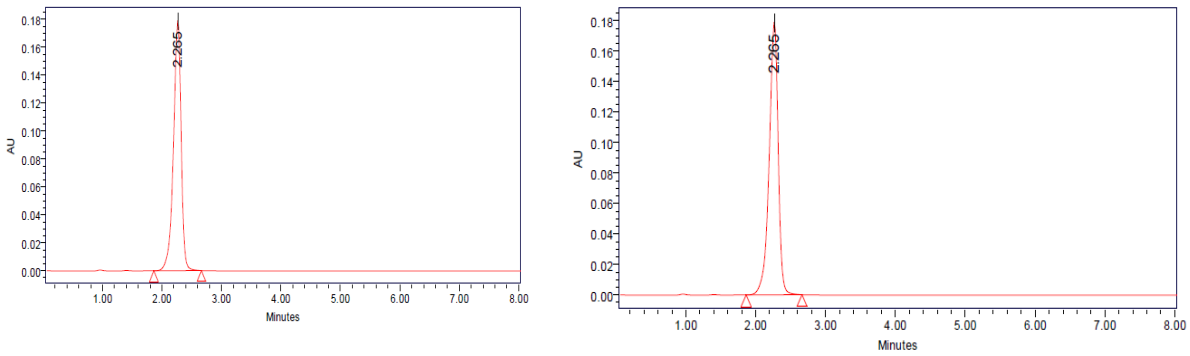


Fig: Chromatogram showing injection -4

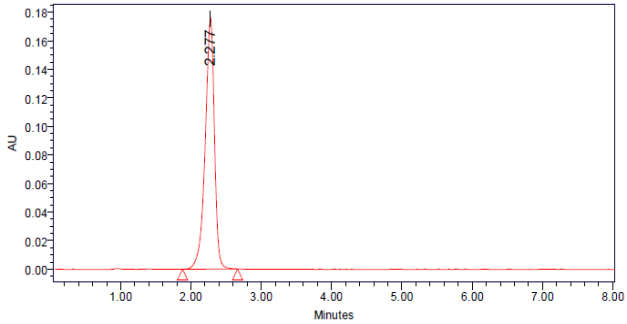


Fig: Chromatogram showing injection -5

Table: Results of system suitability for Primidone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Primidone	2.277	1419842	198640	6291	0.85
2	Primidone	2.277	1429006	170366	6346	0.85
3	Primidone	2.267	1418422	169256	6985	0.86
4	Primidone	2.265	1408754	1532657	6584	0.85
5	Primidone	2.277	1425462	180021	6325	0.86
Mean			1420297			
Std. Dev.			7737.69			
% RSD			0.54			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate **Primidone** in drug product.

Assay (Standard):

Fig. Chromatogram showing assay of standard injection -1

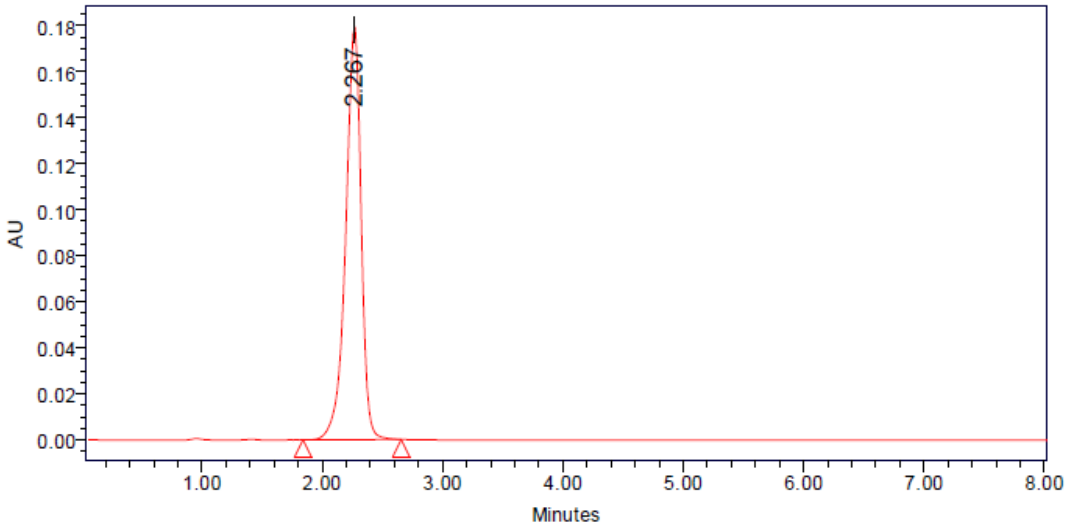


Fig. Chromatogram showing assay of standard injection -2

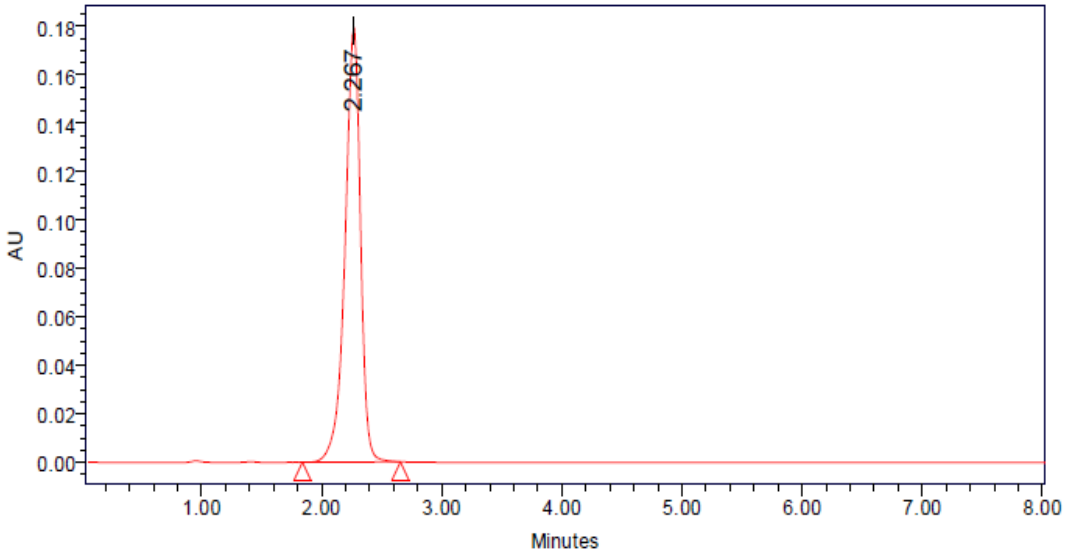


Fig. Chromatogram showing assay of standard injection -3

Table: Peak results for assay standard

S.No	Name		RT	Area	Height	USP Tailing	USP Plate	Injection
1	Primidon		2.265	1417834	167270	0.85	6291	1
2	Primidon		2.267	1421060	168871	0.85	6549	2
3	Primidon		2.267	1419161	168999	0.85	6982	3

Assay (Sample):

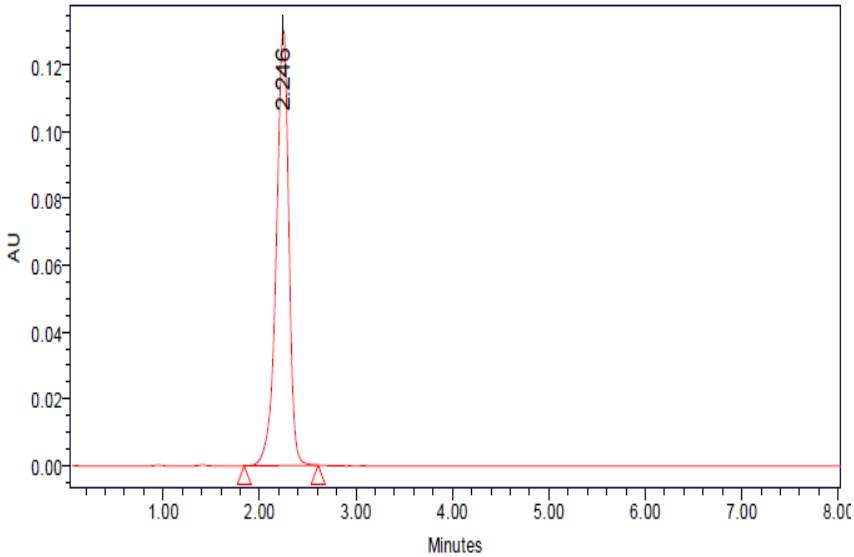


Fig: Chromatogram showing assay of sample injection-1

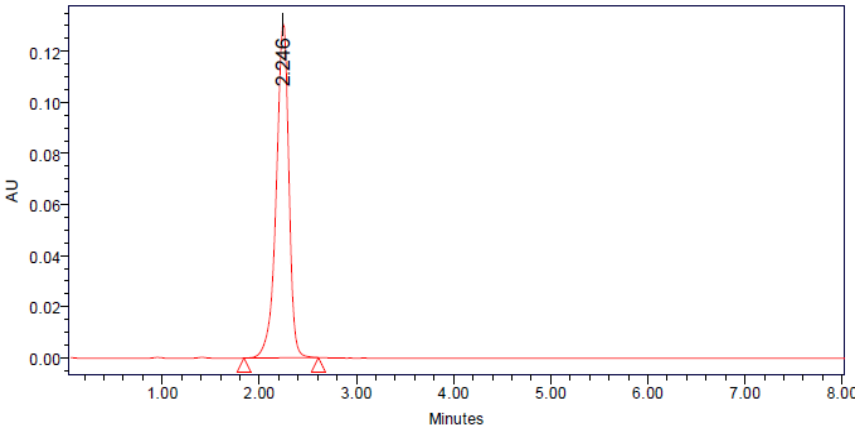


Fig. Chromatogram showing assay of sample injection-2

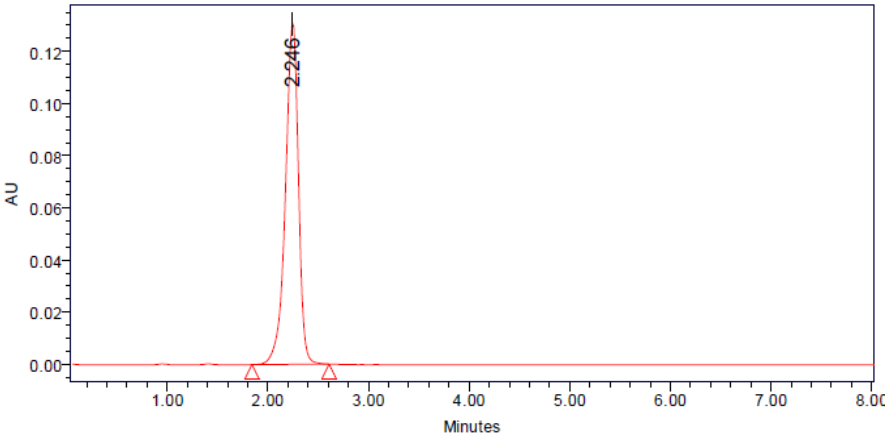


Fig: Chromatogram showing assay of sample injection-3

Table: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Primidone	2.246	1419161	168999	0.85	6352	1
2	Primidone	2.246	1419006	170366	0.86	6521	2
3	Primidone	2.246	1409918	173023	0.85	6921	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

$$=1416028/1419352\times10/30\times30/0.0108\times99.7/100\times0.2737/250\times100$$

The % purity of Primidone in pharmaceutical dosage form was found to be 100.8%.

LINEARITY

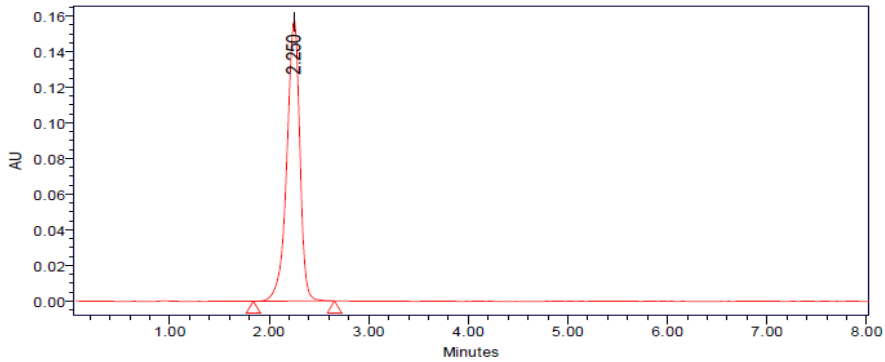


Fig. Chromatogram showing linearity level-1

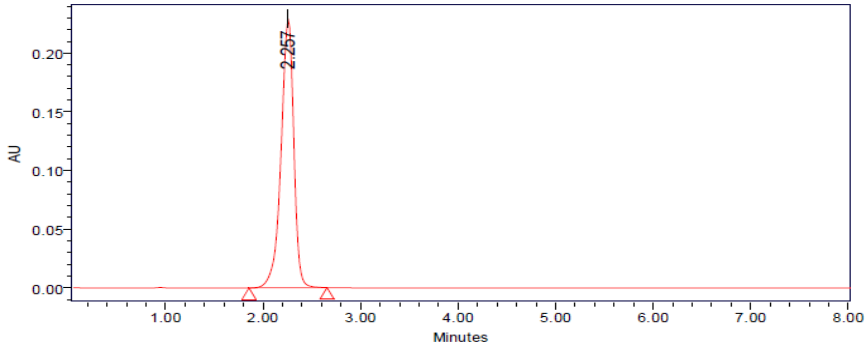


Fig. Chromatogram showing linearity level-2

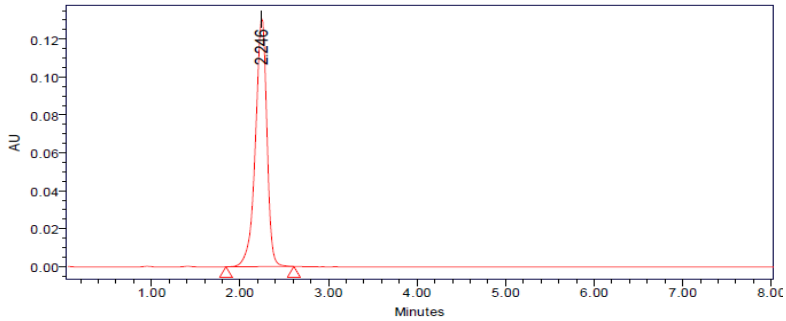


Fig. Chromatogram showing linearity level-3

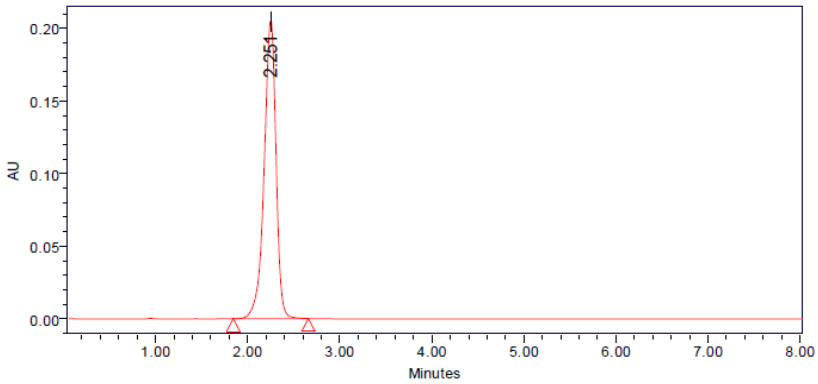


Fig. Chromatogram showing linearity level-4

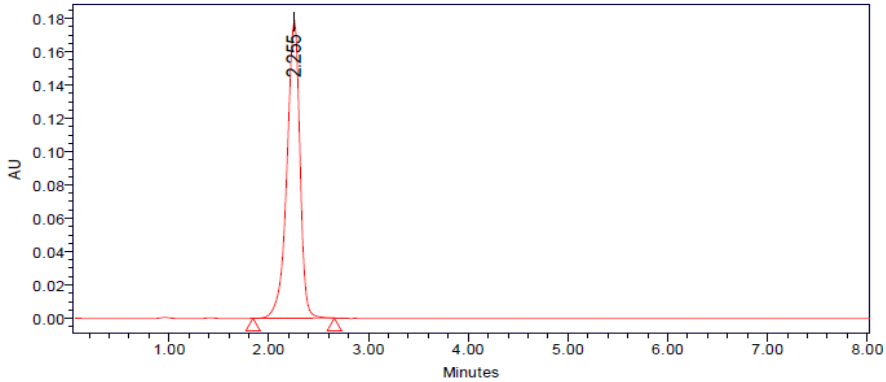
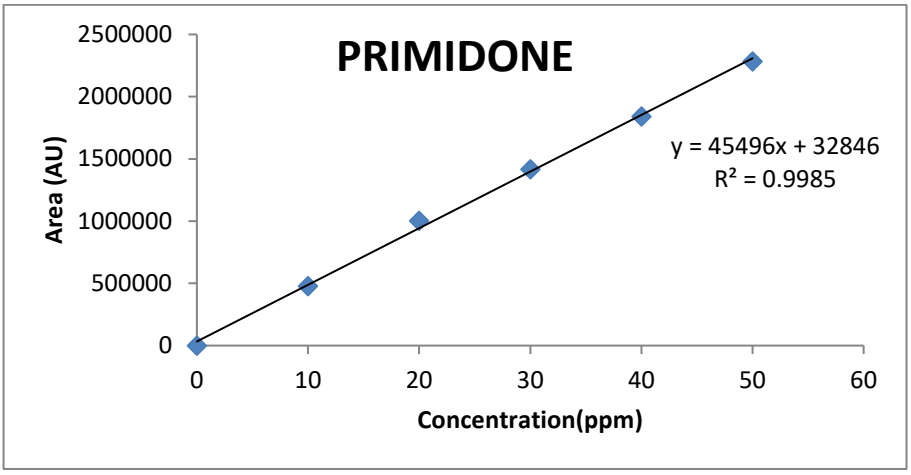


Fig. Chromatogram showing linearity level-5

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	478475
66	20	1001129
100	30	1416507
133	40	1841573
166	50	2283778



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of DRUG is a straight line.

Y = mx + c

Slope (m) = 45496

Intercept (c) = 32846

Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is **0.99**. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

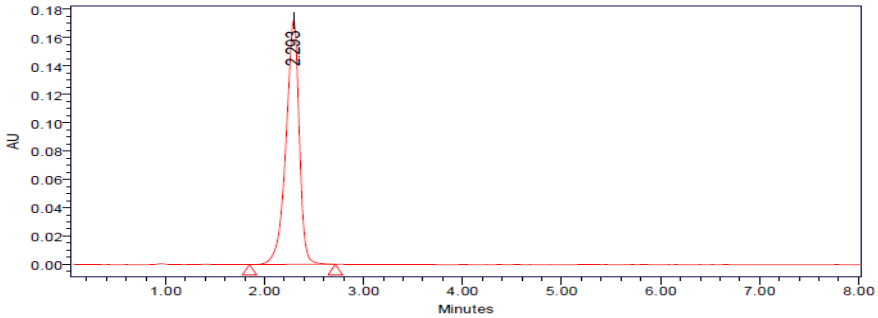


Fig. Chromatogram showing precision injection -1

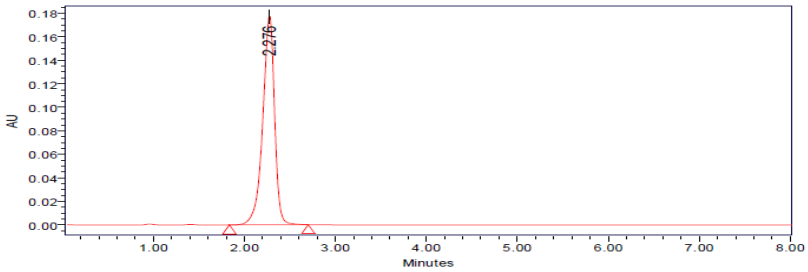


Fig. Chromatogram showing precision injection -2

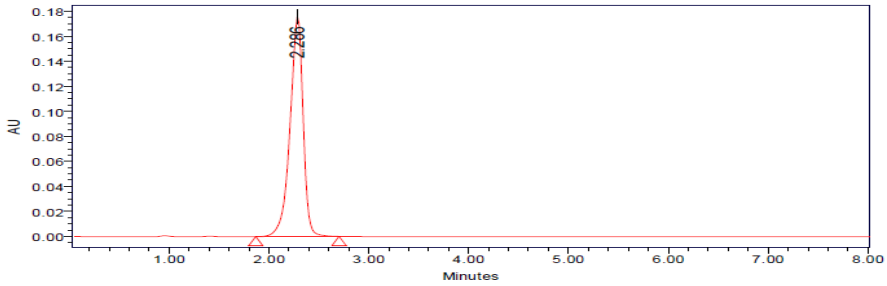


Fig. Chromatogram showing precision injection -3

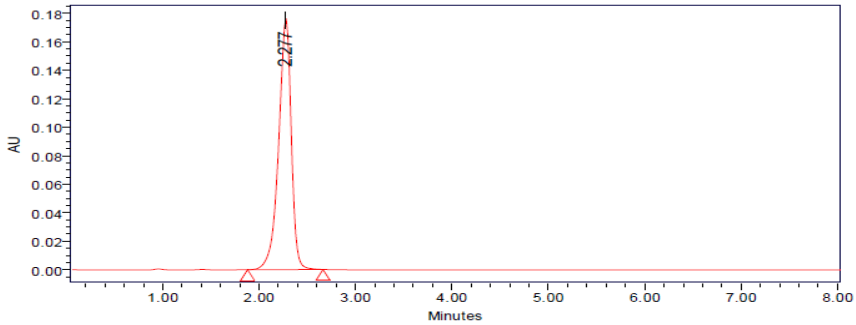


Fig. Chromatogram showing precision injection -4

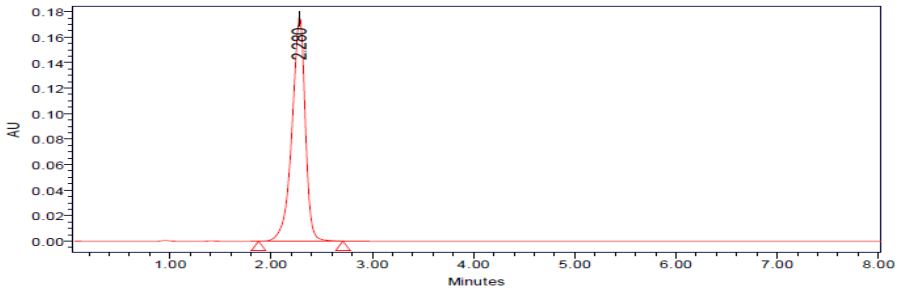


Fig. Chromatogram showing precision injection -5

Table: Results of repeatability for Primidone:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Primidone	2.293	1413527	173368	6413	0.85
2	Primidone	2.276	1401699	175290	6214	0.85
3	Primidone	2.286	1411309	175314	6528	0.85
4	Primidone	2.277	1425886	176294	6624	0.85
5	Primidone	2.280	1412465	178661	6982	0.85
Mean			1412977			
Std.dev			8619.49			
%RSD			0.61			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

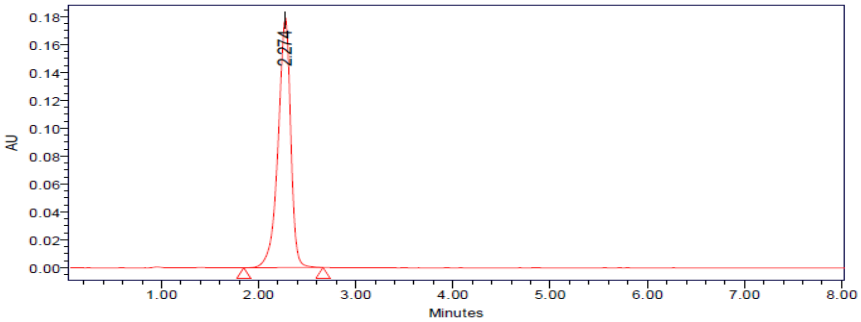


Fig: Chromatogram showing injection -1

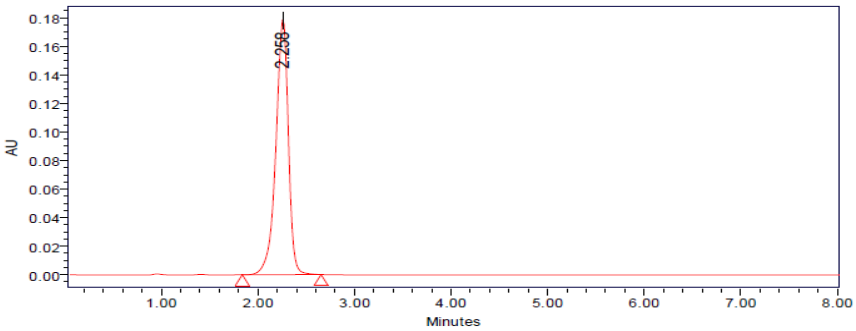


Fig: Chromatogram showing injection -2

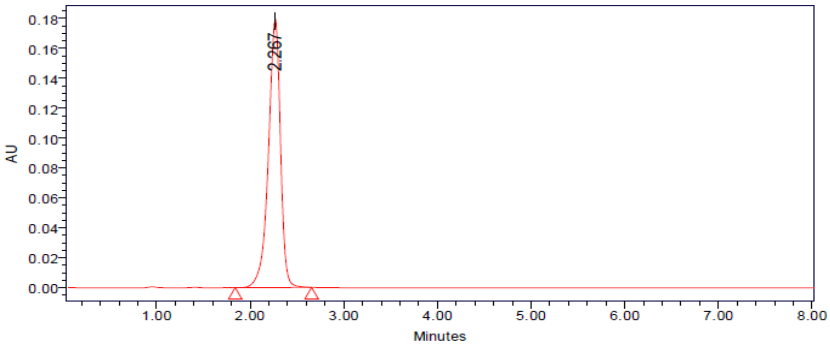


Fig: Chromatogram showing injection -3

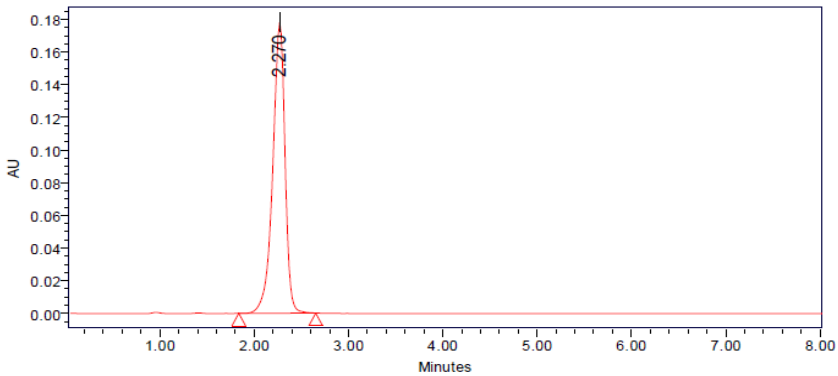


Fig: Chromatogram showing injection -4

Table: Results of Intermediate precision for Primidone

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USPTailing
1	Primidone	2.274	1414528	179491	6587	0.85
2	Primidone	2.258	1407782	177765	6321	0.85
3	Primidone	2.267	1404009	179939	6385	0.85
4	Primidone	2.270	1406800	178900	6580	0.85
Mean			1409550			
Std. Dev.			3991.819			
% RSD			0.2			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2

6.3.4: ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Accuracy 50%:

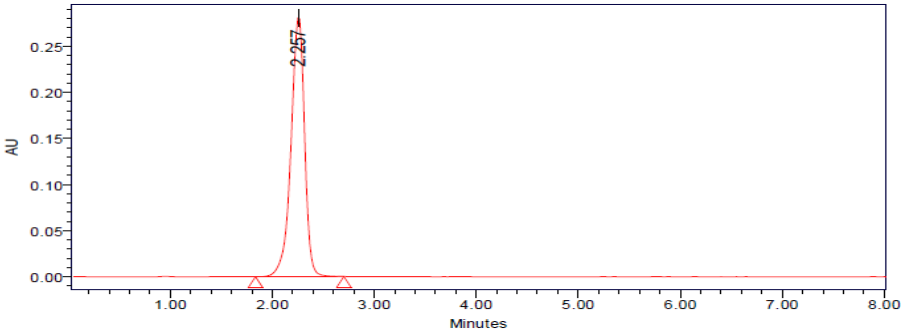


Fig. Chromatogram showing accuracy-50% injection-1

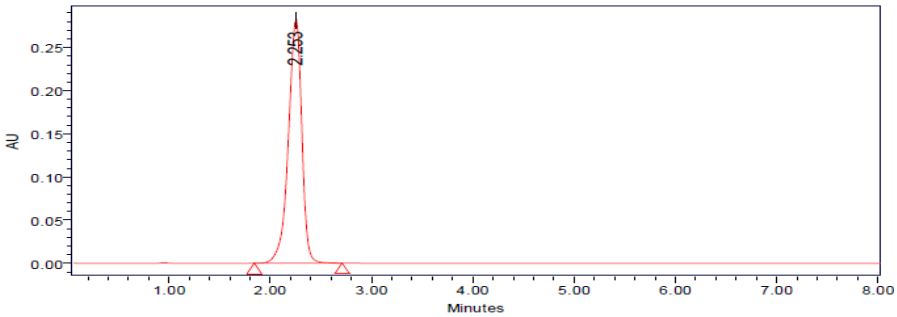


Fig. Chromatogram showing accuracy-50% injection-2

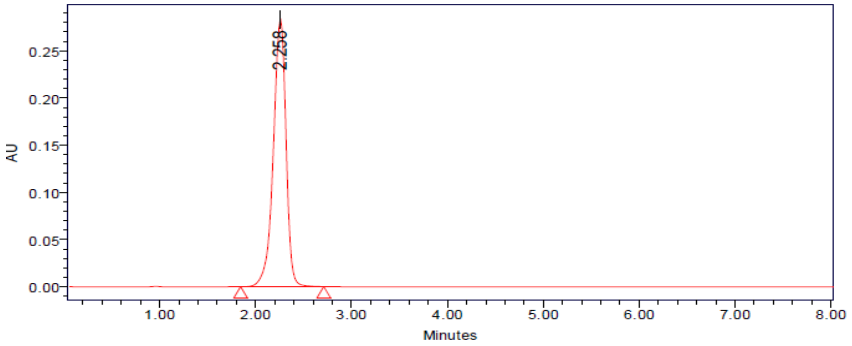


Fig. Chromatogram showing accuracy-50% injection-3

Table: Results of Accuracy for concentration-50%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Primidone	2.257	702161	284053	0.85	6951	1
2	Primidone	2.253	718725	281676	0.85	6582	2
3	Primidone	2.258	705329	284024	0.85	6345	333

LIMIT OF DETECTION FOR PRIMIDONE

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: =3.3×33428.61/45496

= 2.42µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: =10×33428.61/45496

= 7.34µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Primidone. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Primidone were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

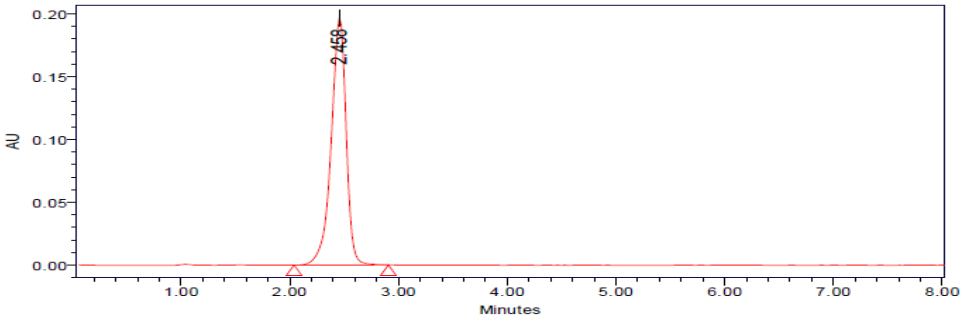


Figure: chromatogram showing less flow of 0.9ml/min

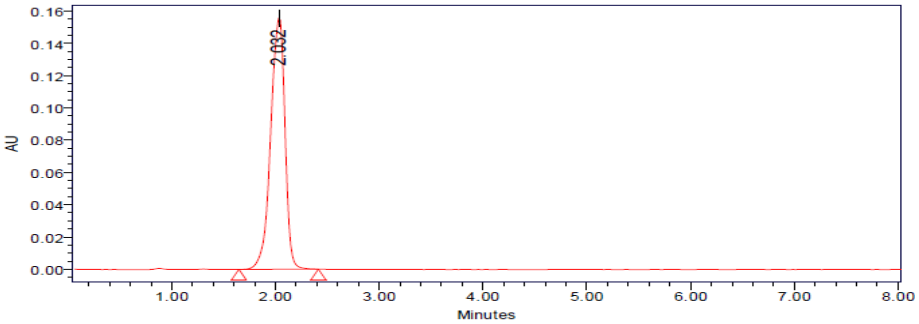


Figure: chromatogram showing more flow of 1.1 ml/min

Variation of mobile phase organic composition

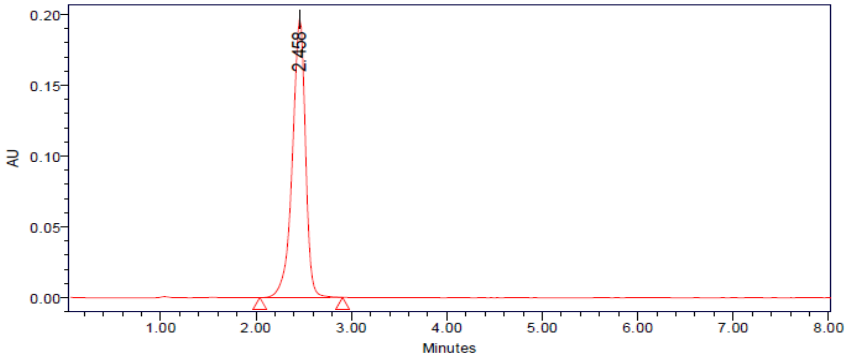


Figure: chromatogram showing less organic composition

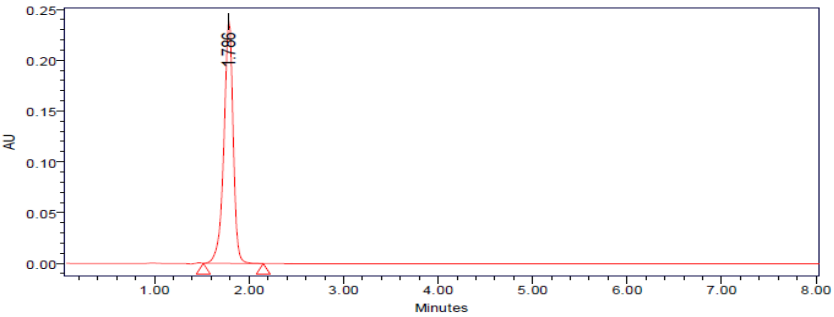


Figure: chromatogram showing more organic composition

Table: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1417834	2.312	6291	0.86
Less Flow rate of 0.9 mL/min	1680410	2.458	6584	0.85
More Flow rate of 1.1	1290965	2.032	6254	0.86
More Organic phase	1528843	1.786	6987	0.88
Less organic phase	1779784	2.458	6184	0.87

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Primidone in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Primidone was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol and ammonium acetate buffer p^H 3.5 (65:35) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Primidone in bulk drug and in Pharmaceutical dosage forms

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