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Development and Validation of an Extremely Analytical RP-HPLC Technique for the simultaneous estimation of Phentermine and Topiramate in API and mixed Marketed Pharmaceutical Dosage Forms

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ABSTRACT

An accurate, unique and reproducible excessive-commonplace common ordinary performance liquid chromatographic technique for simultaneous estimation of Phentermine and Topiramate in bulk and tablet dosage paperwork. Chromatographic separations of the medicine were completed on a Symmetry ODS C18 (4.6×150mm, five.0 µm) the usage of a cellular segment together with Methanol: TEA Buffer pH-4.8 (35:sixty 5) v/v at a waft price of one.0 ml/min. the medicine elute had been monitored at 276 nm. The retention time received for the Phentermine grow to be 2.090 min and for the Topiramate come to be 5.289 min. The calibration curves had been linear over the form of 20-60µg/ml and 25-seventy-fiveµg/ml for Phentermine and Topiramate respectively. The method is showed as consistent with ICH guiding principle through figuring out its specificity, accuracy, precision, linearity & variety, ruggedness, robustness and tool suitability. The effects of the take a look at understanding knowledge that the proposed technique is easy, speedy, specific and accurate, that is useful for the habitual dedication of Phentermine and Topiramate in bulk and tablet dosage workplace paintings. The approach can be carried out for willpower of in its pill dosage office paintings with none interference from excipients or endogenous substances. The proposed approach is appropriate for habitual manage evaluation.

Keywords: Phentermine and Topiramate, RP-HPLC, Accuracy, ICH guidelines

INTRODUCTION

Method Development

Analytical approach development is considered as an important system in pharmaceuticals. a first-rate analytical approach needs to be clean, used column, mobile phase and buffer should be commonplace. it can be completed without problem little by little. Following are the not unusual HPLC technique development steps.

1. Desire of HPLC Analytical method
2. Choice of Chromatographic conditions
3. Parameter Optimization

PREFERENCE OF HPLC ANALYTICAL APPROACH

initially, are looking for recommendation from the literature this is to be had at the product. it's going to allow you to recognize the character of the product as a manner to help to pick out out the handiest-of-a-kind parameters.

A. Pattern Steerage: pick out outout approach to prepare the pattern in keeping with its solubility, filtration requirements, extraction necessities or special precise necessities to make an easy solution of HPLC evaluation.

B. Chromatography: Reverse segment chromatography is used for maximum of the samples however at the equal time as acidic or number one molecules are gift in the pattern then opposite section ion suppression (for willing acid or base) or contrary phase ion pairing (for strong acid or base) want for use.

C. **Gradient/Isotonic HPLC**: Gradient HPLC is beneficial within the assessment of complex samples having a number of components. it'll help to get higher choice than isotonic HPLC having steady top width while in isotonic HPLC top width increases with the retention time.

D. Column Duration: a hundred-a hundred fifty mm columns are used for max of the samples. It reduces the method improvement and assessment time for the pattern. larger columns are used for complex samples those take greater time in separation.

E. HPLC Detectors: Fluorescence and electrochemical detectors need to be used for trace assessment. Samples having immoderate interest must be analyzed the usage of refractive index detectors.

F. Wavelength: λmax of the sample has the first-rate sensitivity to the UV moderate. It detects the pattern additives that have chromophores. A wavelength above 200 nm offers more sensitivity than the lower wavelengths.

Desire of Chromatographic Conditions

After preference of analytical approach, one-of-a-type chromatographic conditions are decided on. The go together with the float of the analytes thru the column is based upon upon the eye of the solvent within the cell phase.

Parameter Optimization

After taking the same sample runs a few parameters which include column dimensions, particle duration, run time and go with the float charge are optimized. it's far done to get the first-rate choice and minimal run time.

Cause And Goal

Purpose

The primary reason of the triumphing check is improvement of correct, specific, sensitive, selective, reproducible and rapid analytical method for price effective simultaneous estimation of Phentermine and Topiramate in bulk shape and pharmaceutical dosage form.

Goals

- To amplify analytical technique
- choosing the HPLC separation mode.
- choosing/ optimizing the cellular section.
- selecting column for evaluation.
- choosing the proper detector machine.
- choosing suitable gradient/ isocratic medium.
- selecting appropriate drift fee, temperature and pH.
- To validate extraordinary parameters.
- Specificity
- Linearity
- Precision
- Accuracy
- restriction of detection
- limit of quantitation
- Robustness

MATERIALS AND STRATEGIES

Selection of Chromatographic Strategies

The right choice is based upon upon the individual of the sample, (ionic or ion robust or impartial molecule) its molecular weight and balance. the medicine determined on are polar, ionic and because of this reversed segment chromatography have grow to be determined on.

Optimization of Column

The technique changed into finished with numerous columns like HypersilC18 column, X-bridge column and X-terra (four.6 ×150mm, fiveμm particle length), Symmetry ODS C18 (4.6 x 150mm, five□m) modified into decided to be quality as it

gave top height shape and backbone at 1ml/min go with the drift.

Mobile phase Optimization

to start with the cellular section tried emerge as Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with numerous proportions. ultimately, the cell section come to be optimized to Methanol: TEA Buffer pH-four.eight (35:sixty five) v/v respectively.

Estimation of Phentermine and Topiramate in pharmaceutical dosage form:
Procedure
Preparation of Mobile Phase

as it have to be measured 350 ml (350%) of HPLC Methanol and 650 ml of TEA (sixty five%) were blended and degassed in a digital quite sonicater for 10 mins and then filtered through 0.45 µ clear out underneath vacuum smooth out.

Diluent

cell phase can be used as diluent.

Assay Steerage of Stylish Solution: (Phentermine &Topiramate)

Because it should be weigh and switch 10 mg of Topiramate going for walks well-known proper right proper into a 10ml of smooth dry volumetric flasks upload about 7ml of diluent and sonicate to dissolve and removal of air definitely and make amount up to speed with the diluent. in addition pipette zero. Four ml of Phentermine, zero.5ml of Topiramate from inventory solutions in to a 10ml volumetric flask and dilute on pinnacle of factors with diluent.

Technique

Inject the samples thru manner of changing the chromatographic situations and record the chromatograms, word the conditions of right height elution for appearing validation parameters as regular with ICH suggestions.

Coaching of Pattern Solution

Take common weight of 10 pills and overwhelm in a mortar with the beneficial aid of the use of way of the use of pestle and weight 10 mg identical weight of Phentermine, Topiramate pattern right rightrightright into a 10ml clean dry volumetric flask and upload about 7ml of Diluent and sonicate to dissolve it in reality and make amount on pinnacle of factors with the equal solvent.

Technique

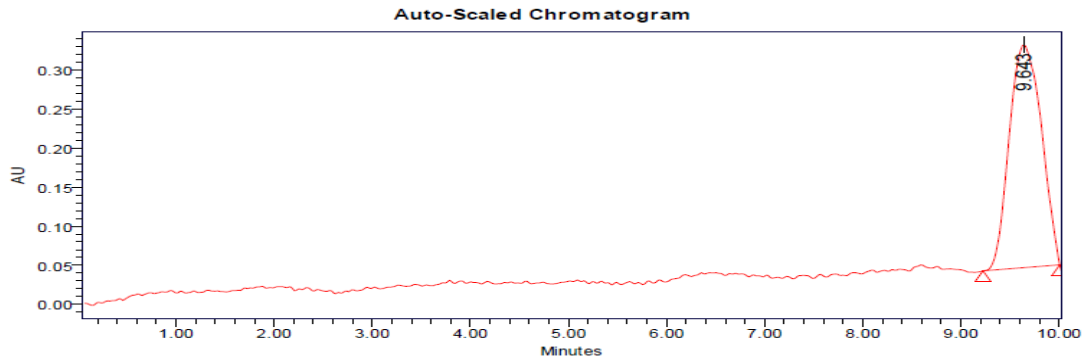
Further pipette zero.4ml of Phentermine and 0.5ml of Topiramate from above inventory solution right proper right right into a 10ml volumetric flask and dilute up to the mark with diluent. Inject the three replicate injections of desired and sample solutions and calculate the assay through the use of using tool:

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

RESULTS AND DISCUSSION

Trails for Method Development
Trail 1

Mobile phase : Methanol: Water (60:40%v/v)
Column : Zodiac C18 (4.6×250mm) 5µm particle size
Flow rate : 0.8ml/min
Wavelength : 276 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 10 minutes



S.No	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Phentermine	9.643	46589	2542		7.3	189

Observation

In a separation of Phentermine and Topiramate peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Method Validation

Blank

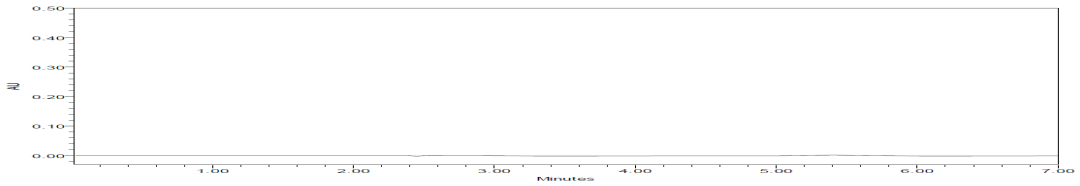


Fig 1 -: Chromatogram showing blank (mobile phase preparation)

System Suitability

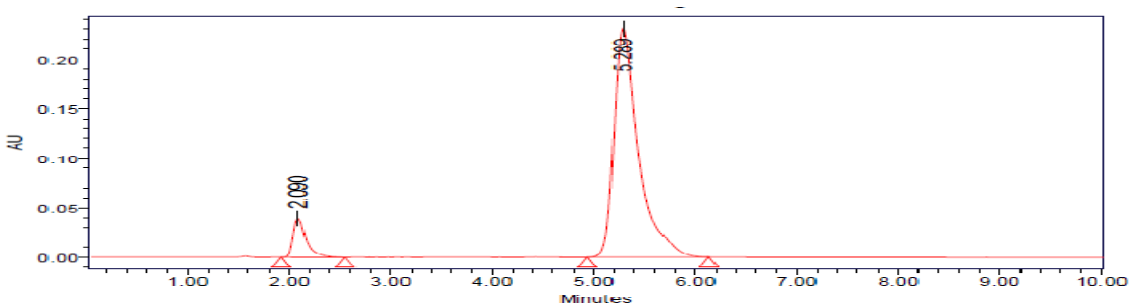


Fig 2 : Chromatogram showing injection -1

Table 1: Results of system suitability for Phentermine

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Phentermine	2.090	325896	39689	5653	1.42
2	Phentermine	2.090	326989	39689	5695	1.42
3	Phentermine	2.089	327985	39698	5598	1.44
4	Phentermine	2.089	329477	40198	5569	1.43
5	Phentermine	2.085	325858	40259	5612	1.47
Mean			**327241**			
Std. Dev			**1527.944**			
% RSD			**0.466917**			

Table 2 : Results of system suitability for Topiramate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Topiramate	5.289	3576859	232352	5785	1.46	9.80
2	Topiramate	5.289	3585695	232365	5915	1.47	9.81
3	Topiramate	5.338	3596885	232451	5895	1.48	9.81
4	Topiramate	5.327	3565874	231653	5987	1.40	9.83
5	Topiramate	5.262	3598654	233658	5861	1.43	9.82
Mean			3588946				
Std. Dev			3585486				
% RSD			11360.78				

Specificity

Assay (Standard)

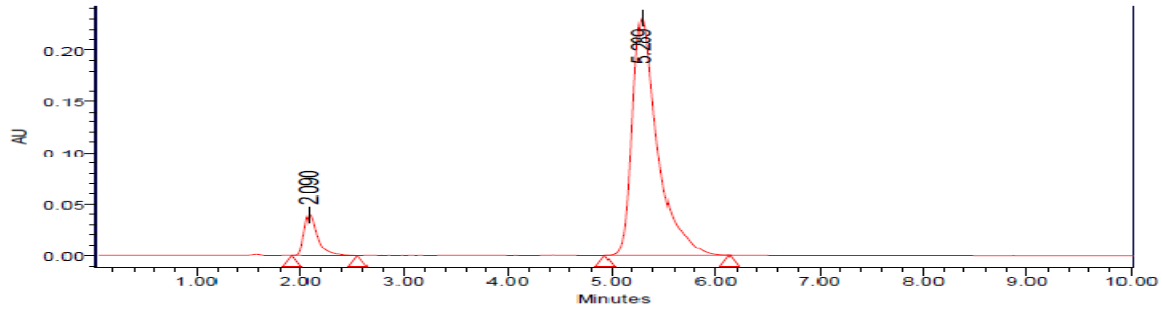


Fig 3-: Chromatogram showing assay of standard injection -1

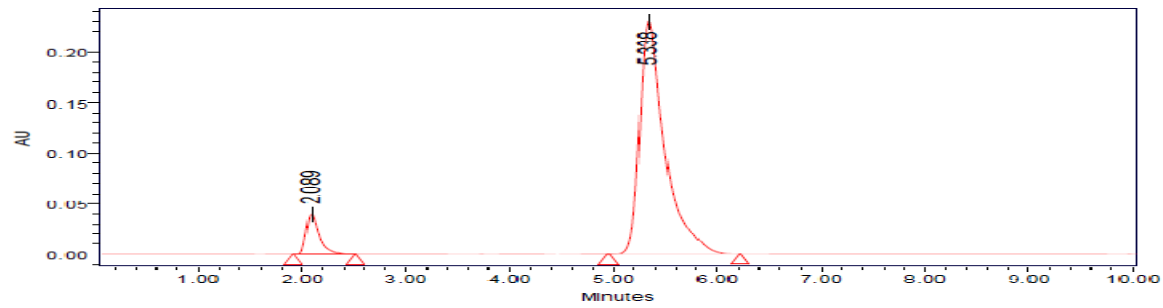


Fig 4-: Chromatogram showing assay of standard injection -2

Table3 : Peak results for assay standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Phentermine	2.090	328966	39586		1.70	5563	1
2	Topiramate	5.289	3574898	232356	9.80	1.77	5665	1
3	Phentermine	2.089	327898	39568		1.66	5584	2
4	Topiramate	5.338	3569854	232548	9.93	1.83	5646	2
5	Phentermine	2.089	328657	40526		1.68	5584	3
6	Topiramate	5.327	3565874	232547	9.91	1.86	5783	3

Assay (Sample):

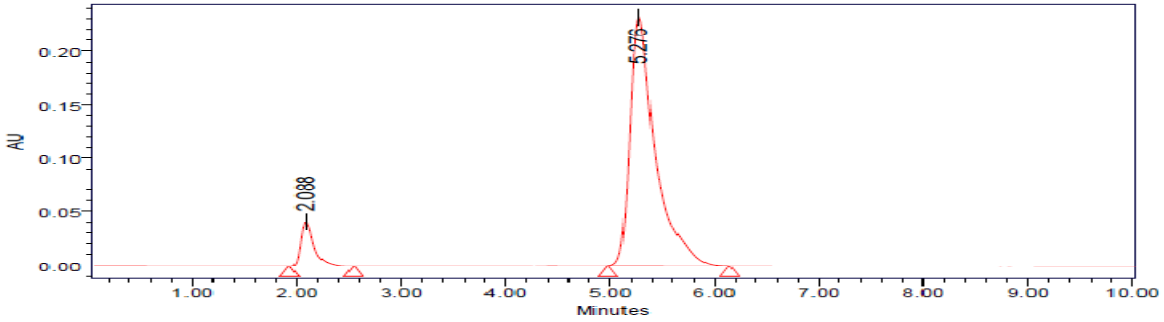


Fig 5: Chromatogram showing assay of sample injection-1

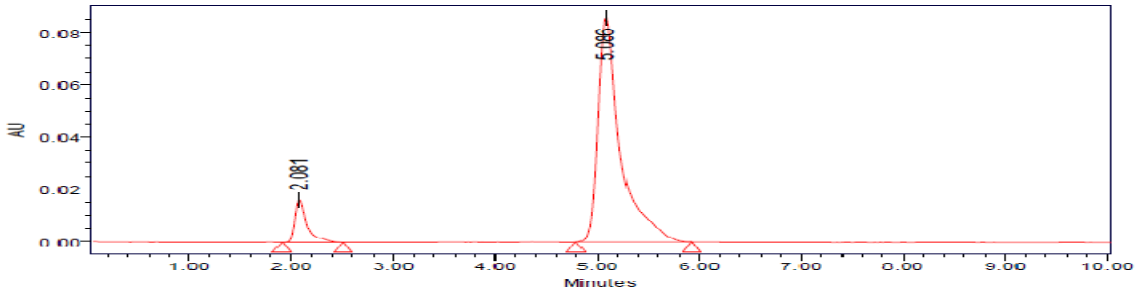
Table 4: Peak Results for Assay Sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Phentermine	2.088	336589	40365		1.69	5569	1
2	Topiramate	5.276	3586985	232565	9.75	1.89	5658	1
3	Phentermine	2.087	335684	41245		1.72	5548	2
4	Topiramate	5.268	3587896	235685	9.82	1.91	5864	2
5	Phentermine	2.085	335876	40898		1.75	5496	3
6	Topiramate	5.262	3586848	234588	9.78	1.95	5754	3

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

The % purity of Phentermine and Topiramate in pharmaceutical dosage form was found to be99.494%.

Linearity



Chromatographic Data For Linearity Study

Phentermine:

Concentration µg/ml	Average Peak Area
20	164436
30	255571
40	348687
50	439024
60	534830

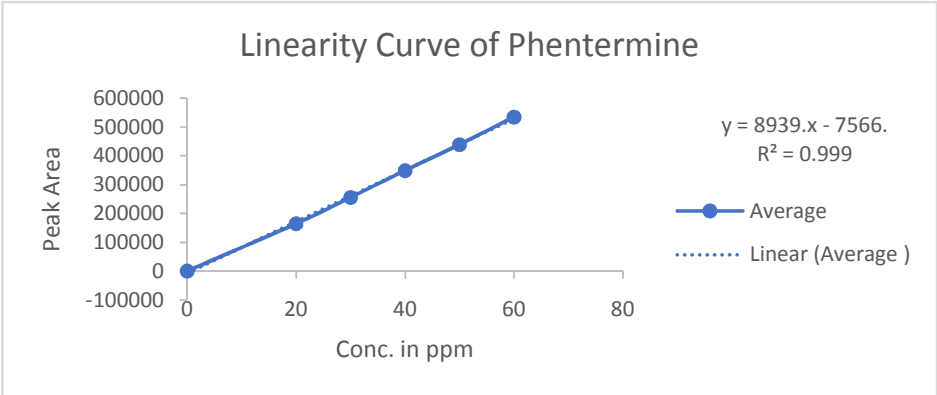


Fig 6: Calibration graph for Phentermine

Topiramate

Concentration µg/ml	Average Peak Area
25	1782454
37.5	2728974
50	3688678
62.5	4658022
75	5592695

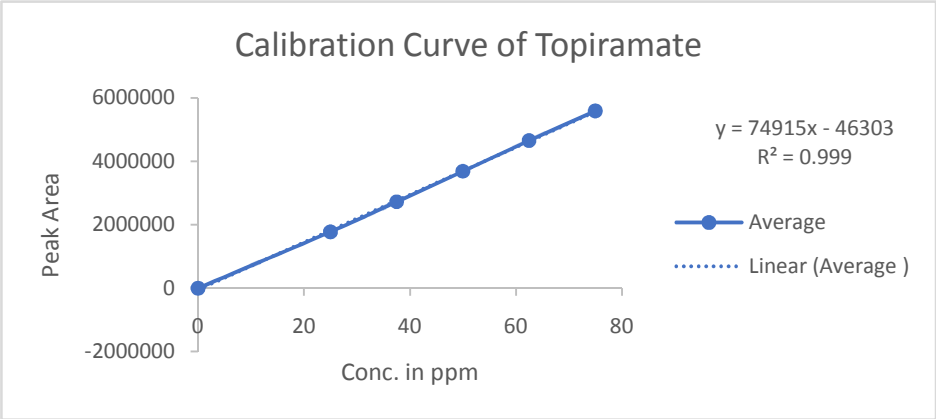


Fig 7: Calibration graph for Topiramate

Precision: Repeatability

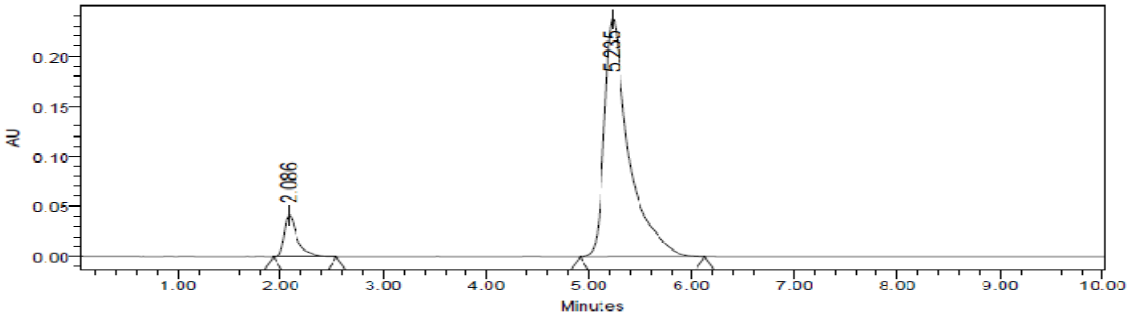


Fig 8: Chromatogram showing precision injection -1

Table 5: Results of Repeatability for Phentermine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Phentermine	2.086	327689	41697	5081.3	1.8
2	Phentermine	2.083	327978	41402	5144.1	1.8
3	Phentermine	2.083	327879	41540	5118.1	1.8
4	Phentermine	2.081	327868	42256	5147.3	1.8
5	Phentermine	2.081	327859	42143	5101.8	1.8
Mean			**327854.6**			
Std. Dev			**104.2176**			
% RSD			**0.031788**			

Table 6: Results of method precision for Topiramate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Topiramate	5.178	3576985	241253	5969.5	2.0	9.8
2	Topiramate	5.199	3578989	2365824	5865.1	2.0	9.7
3	Topiramate	5.235	3576859	239568	5936.4	2.0	9.9
4	Topiramate	5.202	3578458	2386547	5964.4	2.0	9.8
5	Topiramate	5.206	3579864	241425	5045.6	2.0	9.5
Mean			**3578231**				
Std. Dev			**1296.889**				

% RSD	0.036244

Accuracy

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Phentermine	2.060	545657	78854		1.87	5658	1
2	Topiramate	4.991	5696874	582543	8.99	1.09	5654	1
3	Phentermine	2.063	546231	78142		1.86	5628	2
4	Topiramate	5.001	5692579	586895	9.05	1.09	5797	2
5	Phentermine	2.058	545878	78245		1.89	5666	3
6	Topiramate	5.017	5697485	586582	9.13	1.09	5674	3

Table 7: The Accuracy Results for Phentermine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	186584.7	20	20.026	100.13	
100%	367968.7	40	40.32	100.80	100.435%
150%	545922	60	60.225	100.375	

Table 8: The Accuracy Results for Topiramate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1925532	25	25.084	100.336	
100%	3790965	50	49.985	99.970	100.284%
150%	5695646	75	75.410	100.546	

CONCLUSION

In the gift research, a smooth, touchy, particular and accurate RP-HPLC approach have grown to be advanced for the quantitative estimation of Phentermine and Topiramate in bulk drug and pharmaceutical dosage office work. This approach grows to be clean, because of the fact that diluted samples are without delay used without any preliminary chemical derivatisation or purification steps. Phentermine end up decided to be soluble in water and decrease alcohols, slightly soluble in chloroform and insoluble in ether, very barely soluble in benzene & acetone, soluble in methanol, ethanol. Topiramate have come to be decided to be soluble in water and acetone. it's miles freely soluble in acetone, chloroform, dimethyl sulfoxide, and ethanol, DMSO and Dimethyl Formamide, Acetonitrile. Methanol: TEA Buffer pH-four. Eight (35: sixty-five) have become selected due to the fact the cellular phase. The solvent device used in this method become reasonably priced. The %RSD values were below 2 and the approach changed into decided to be unique. The outcomes expressed in Tables for RP-HPLC technique changed into promising. The RP-HPLC approach is more touchy, correct and unique in evaluation to the Spectrophotometric techniques.

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