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Analytical Method Development and Validation for Simultaneous Estimation of Trifluoperazine, Chlordiazepoxide and Trihexiphenidyl in its Pharmaceutical Dosage form by RP-HPLC

Komal V Patel, Mandev B. Patel, Nishith K. Patel

Department of Quality Assurance, A-One college of Pharmacy, Anasan, Ahmedabad, Gujarat, India

ABSTRACT:

A simple, rapid, economical, precise and accurate Reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl in Their Combined Dosage Form has been developed. The RP-HPLC method was developed for the simultaneous estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl in their Combined Dosage Form development method has been achieved. The separation was attained by Column LC- 2010 AT C18 (250mm x 4.6 mm x 5 µm) and Buffer (pH 3.5) : Acetonitrile :TEA (80:20:0.1 v/v/v) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at Wavelength of 228 nm. Retention time of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl were found to be 3.807 min, 6.887 min and 4.667 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Chlordiazepoxide 5-15 µg/ml, for Trifluoperazine HCl 0.5-1.5 µg/ml and for Trihexyphenidyl HCl 1-3 µg/ml. The Percentage recoveries obtained for Chlordiazepoxide, Trifluoperazine HCl and Trihexyphenidyl HCl were found to be in range of 99.27 ± 1.09, 99.57 ± 0.56 and 99.22 ± 0.51 respectively. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl in their combined dosage form.

KEY-WORDS: Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl, RP-HPLC, Mobile phase, Validation.

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For Correspondence:
Komal V Patel

Student of Quality Assurance,
A-One college of Pharmacy, Anasan,
Ahmedabad, Gujarat, India.

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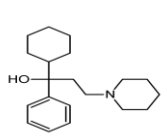
INTRODUCTION:

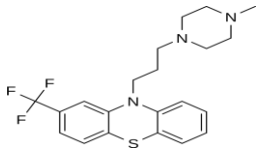
The IUPAC name of the Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl is 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-Oxide, (RS)-1-cyclohexyl-1-phenyl-3-(1-piperidyl)propan-1-ol,10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10H-phenothiazine,1-dioxide respectively, with molecular formula $C_{16}H_{14}ClN_3O$,$C_{20}H_{32}NOCl$ and $C_{21}H_{25}F_3N_3SCl$ respectively and molecular weight 299.75 g/mol,301.466 g/mol and 407.497 g/mol respectively. The molecular structure of the drug is given in Fig.1.

This combinational drug is used in Anxiety and CNS disorder. Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl acts on CNS system. This results in increased binding of the inhibitory neurotransmitter GABA to the GABA (A) receptor. This combination is used in Tension, Behavioural disorders, Insomnia, Emotional disturbances, Pre and post operative apprehensions.

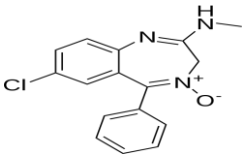
Chlordiazepoxide is Official in British Pharmacopoeia (2010), US Pharmacopoeia 30 (NF 25), Indian Pharmacopoeia (2010). and Trihexyphenidyl is official in British Pharmacopoeia (2010), US Pharmacopoeia 30 (NF 25) and Trifluoperazine British Pharmacopoeia (2010), US Pharmacopoeia 30 (NF 25). However no analytical method has been reported till date for the estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl using the RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl in Pharmaceutical dosage form using RP-HPLC. The proposed method are optimized and validated as per ICH guidelines.



Trihexyphenidyl



Trifluoperazine



Chlordiazepoxide

Figure 1: Chemical structure

MATERIALS AND METHODS

Materials

HPLC Thermo separation Product TSP UV 2000. Chlordiazepoxide and Trifluoperazine HCl was purchased from GITAR LABORATORY. The commercial fixed dose Trihexyphenidyl HCl was purchased from VAIBHAV LABORATORY. All solvents (HPLC grade) were obtained from Merck Chemicals.

Working Standard preparation:

Preparation of standard solution of mixtures of CDZ (10 µg/mL) TFP (1 µg/mL) and THP (2 µg/mL):

Take 1 mL from CDZ stock solution,1 mL from TFP stock solution and 1 ml from THP stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by Potassium Buffer which was used in particular trials.

Procedure for derivatization:

- Potassium buffer (pH ~3.5)

1. Add 25g Ammonium Acetate in 25 ml water and 38ml HCl, make sure all powers dissolve completely.
2. Adjust the pH if necessary with dilute Hydrochloric Acid or dilute Ammonia.
3. Dilute to 100.0 ml with water. pH should be around 3.5.

- Std stock soln of CDZ : 10mg-100ml with methanol (100 µg /ml)
- Std stock soln of TFP: 1mg-1000ml with methanol (10 µg /ml)
- Std stock soln of THP: 2mg1000ml with methanol (20 µg /ml)

Take 0.1ml of working STD (CDZ+TFP+THP) to a 10ml volumetric flask. Inject above Solution 20 µl.

Sample preparation:

Working Sample Preparation (CDZ 100 µg/mL, TFP 10 µg/mL and THP 20 µg/mL):

- Take 1 mL from this and transferred to 10 ml volumetric flask and made up volume up to the mark with potassium Buffer. Use This Solution for Derivatization as Mention Below.
- Take 0.1ml of working STD (CDZ+TFP+THP) to a 10ml volumetric flask. Incubate this solution at 50C for 15 minutes in a water bath.

Method validation:

Chromatographic conditions and System Suitability Parameters:

Pumps:

Mode of chromatography: Reversed Phase Chromatography

Mode of Elution: Isocratic

Flow Rate: 1.0 ml/min

Oven:

Oven Temperature: 30° ± 2°C

Detector:

Type: DAD detector

Lamp: D2 lamp

Wavelength: 228 nm

Auto sampler Configuration:

Rinsing Volume: 1000 µl

Sampling speed: 20 µl/sec

Other parameters:

Column: C_{18} (25cm x 0.46 cm) Hypersil BDS Sample Volume: 20 µl

Mobile Phase: Buffer (potassium pH3.5): acetonitrile: TEA (80:20:0.1 v/v/v)

Diluent: Methanol

System Suitability Parameters:

Retention time: CDZ (3.807), TFP (4.667), THP (6.887)

Asymmetry: CDZ (1.360), TFP (1.379), THP (1.350)

Theoretical plates CDZ (7056), TFP (7520), THP (9459)

Linearity and Range (n=3):

The linearity for CDZ, TFP and THP were assessed by analysis of combined standard solution in range of 5-15 µg/ml, 0.5-1.5 µg/ml and 1-3 µg/ml respectively, solutions were pipette out from the Stock solution of CDZ(10 µg/ml), TFP (1 µg/ml) and THP (2 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml, 0.5,0.75,1,1.25 and 1.5 µg/ml and 1,1.5,2,2.5 and 3 µg/ml for CDZ, TFP and THP respectively. The plot of peak area against concentration was plotted. Correlation coefficient and regression line equations were calculated.

Linearity range was established through consideration of required practical range and according to each drug concentration present in the pharmaceutical product, to give accurate, precise and linear results**.**

Precision

Repeatability

The data for repeatability of peak area measurement for CDZ (10 µg/ml), TFP (1 µg/ml) and THP (2 µg/ml), based on six measurements of same solution of CDZ (10 µg/ml), TFP (1 µg/ml) and THP (2 µg/ml),.The % RSD for CDZ,TFP and THP was found to be 0.271,0.249 and 1.153 respectively.

Intraday Precision

Standard solution containing (0.359,0.148,0.554 µg/ml) of CDZ and (0.198,0.200,0.312/ml) of TFP and (0.914,0.568,1.062 µg/ml) of THP were analyzed three times on the same day and % R.S.D was calculated**.**

Interday Precision:

The inter-day precision of the proposed method was determined by measuring the corresponding responses on 3 different days over a period of 1 week for 3 different concentration of Standard solution containing (0.236,0.979,0.252 µg/ml) of CDZ and (0.199,0.757,0.806 µg/ml) of TFP and (0.385,0.540,1.290 µg/ml) of THP were analyzed three times on the same day and % R.S.D was calculated.

Accuracy (% Recovery)

- ✓ The accuracy of the method was determined by calculating recovery of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl by the Standard addition method.
- ✓ **For CDZ**

  10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 228 nm. The amount of CDZ was calculated at each level and % recoveries were computed.
- ✓ **For TFP**

  1 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 228 nm. The amount of TFP was calculated at each level and % recoveries were computed.
- ✓ **For THP**

2 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 228 nm. The amount of THP was calculated at each level and % recoveries were computed.

Table 1: Linearity data for CDZ.

Sr.No	Concentration (µg/ml)	Area
1	5	776.733
2	7.5	1162.735
3	10	1570.086
4	12.5	1949.034
5	15	2348.984

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of y-intercept of calibration curve (σ) and average of slope (S) of the calibration curve.

LOD = 3.3 × σ /s,

LOQ = 10 × σ /s

Robustness

The robustness was studied by analyzing the sample of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl by deliberate variation in the method parameters. The change in the response was noted. Robustness of the method was studied by changing different experimental conditions like temperature of column by ±2°C, Flow rate by ±0.2 ml/min, Mobile phase by ±2%.

RESULT AND DISCUSSION:

Validation parameters:

Linearity:

The linearity for CDZ, TFP and THP were assessed by analysis of combined standard solution in range of 5-15 µg/ml, 0.5-1.5 µg/ml and 1-3 µg/ml respectively, solutions were pipette out from the Stock solution of CDZ(10 µg/ml), TFP (1 µg/ml) and THP (2 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml, 0.5,0.75,1,1.25 and 1.5 µg/ml and 1,1.5,2,2.5 and 3 µg/ml for CDZ, TFP and THP respectively.

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted. Correlation co-efficient for calibration curve CDZ, TFP and THP was found to be 0.999, 0.999 and 0.999 respectively.

The regression line equation for CDZ, TFP and THP are as following:

For CDZ: **y = 156.7x - 7.929**

For TFP: **y = 387.8x - 4.668**

For THP **y = 851.7x - 5.533**

Table 2: Linearity data for TFP.

Sr.No	Concentration (µg/ml)	Area
1	0.5	384.037
2	0.75	574.785
3	1.0	776.051
4	1.25	963.331
5	1.5	1162.027

Table 3 : Linearity data for THP

Sr.No	Concentration (µg/ml)	Area
1	1	420.91
2	1.5	630.213
3	2	851.093
4	2.5	1056.653
5	3	1272.432

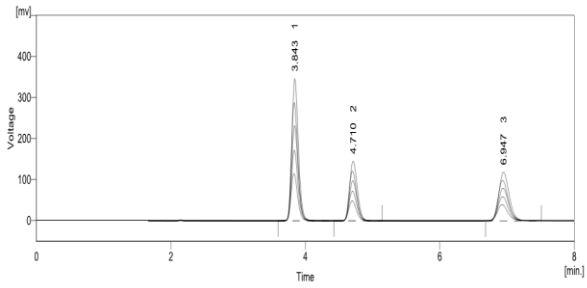


Figure 1:Overlay chromatogram of different concentrations of mixtures of CDZ ,TFP and THP

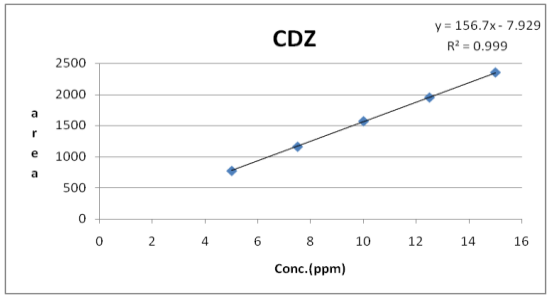


Figure 2: Calibration Curve of CDZ (5-15 µg/ml).

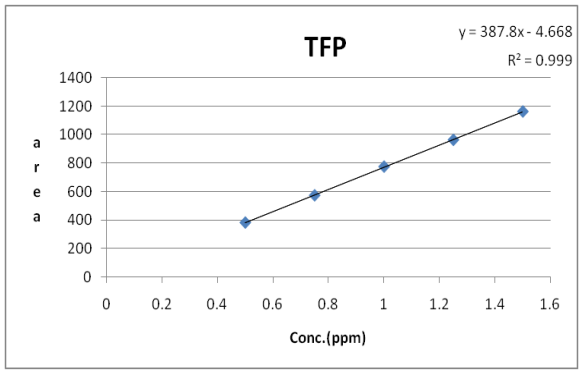


Figure 3: Calibration Curve of TFP (0.5-1.5 µg/ml).

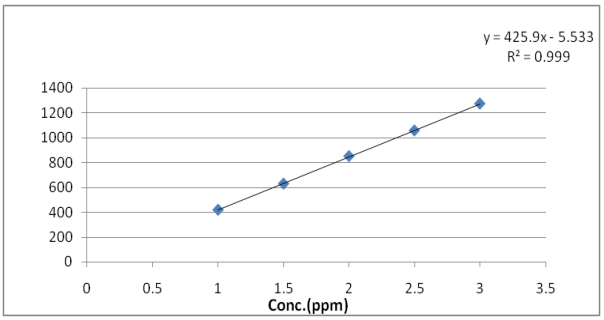


Figure 4: Calibration Curve of THP (1-3 µg/ml).

Precision

Repeatability

The data for repeatability of peak area measurement for CDZ (10 µg/ml), TFP (1 µg/ml) and THP (2 µg/ml), based on six measurements of same solution of CDZ (10 µg/ml), TFP (1 µg/ml) and THP (2 µg/ml).The % RSD for CDZ,TFP and THP was found to be 0.271,0.249 and 1.153 respectively.

Table 4: repeatability data for TFP.

		CDZ		
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	10	1565.283 1563.976 1566.882 1570.032 1568.622 1575.875	1568.45±4.25	0.271

Table: Repeatability data for CDZ.

		TFP		
Sr No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	1	773.736 775.27 774.495 776.049 776.831 779.169	775.93 ±1.93	0.249

Table 5: repeatability data for THP.

		THP		
Sr No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	2	848.569 850.25 849.382 851.102 851.945 851.473	850.45 ±1.30	1.153

II. Intraday precision

Standard solution containing (5-15 µg/ml) of CDZ and (0.5-1.5 µg/ml) of TFP and (1-3 µg/ml) of THP were analyzed three times on the same day and % R.S.D was calculated**.**

Table 6: Intraday precision data for estimation of CDZ

		CDZ	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	769.78 ± 2.76	0.359
2	10	1558.92± 2.31	0.148
3	15	3498.33± 19.38	0.554

Table 7: Intraday precision data for estimation of TFP

		TFP	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	0.5	381.73 ± 0.76	0.198
2	1	771.40± 1.53	0.200

3	1.5	1734.56 ± 5.42	0.312

Table 8: Intraday precision data for estimation of THP

		THP	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	416.24 ± 3.80	0.914
2	2	844.11± 4.80	0.568
3	3	1886.32 ± 20.03	1.062

III. Interday precision

Standard solution containing (5-15µg/ml) of CDZ and (0.5-1.5 µg/ml) of TFP and (1-3 µg/ml) of THP were analyzed three times on the different day and % R.S.D was calculated.

Table 9: Interday precision data for estimation of CDZ

		CDZ	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	769.55 ± 1.82	0.236
2	10	1544.75± 15.12	0.979
3	15	3502.97± 8.83	0.252

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Table 10: Interday precision data for estimation of TFP.

		TFP	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	0.5	381.36 ± 0.76	0.199
2	1	768.07± 5.82	0.757
3	1.5	1723.19 ± 13.88	0.806

Table 11: Interday precision data for estimation of THP.

		THP	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	416.99 ± 1.60	0.385
2	2	843.80± 4.56	0.540
3	3	1886.90 ± 19.43	1.29

Accuracy:

- ✓ **For CDZ**

  10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 228 nm. The amount of CDZ was calculated at each level and % recoveries were computed.

- ✓ **For TFP**

  1 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 228 nm. The amount of TFP was calculated at each level and % recoveries were computed.

- ✓ **For THP**

  2 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 228 nm. The amount of THP was calculated at each level and % recoveries were computed.

Table 12: Recovery data for CDZ.

SR. NO.	**Conc. Level (%)**	**Sample amount (µg/ml)**	**Amount Added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	80 %	5	4	3.93	98.17	99.81 ± 1.48
2		5	4	4.04	101.02	
3		5	4	4.01	100.24	
4	100 %	5	5	4.90	98.02	99.27 ± 1.09
5		5	5	5.00	100.09	
6		5	5	4.98	99.70	
7	120 %	5	6	5.99	99.83	99.57 ± 0.38
8		5	6	5.95	99.13	

9		5	6	5.99	99.75	

Table 13: Recovery data for TFP

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	0.5	0.4	0.40	98.77	99.99 ± 1.12
2		0.5	0.4	0.40	100.99	
3		0.5	0.4	0.40	100.22	
4	100 %	0.5	0.5	0.49	98.97	99.57 ± 0.56
5		0.5	0.5	0.50	100.07	
6		0.5	0.5	0.50	99.67	
7	120 %	0.5	0.6	0.60	100.28	99.70 ± 0.59
8		0.5	0.6	0.59	99.10	
9		0.5	0.6	0.60	99.72	

Table 14: Recovery data for THP

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1	0.8	0.79	98.88	99.41 ± 0.80
2		1	0.8	0.79	99.03	
3		1	0.8	0.80	100.33	
4	100 %	1	1	0.99	99.08	99.22 ± 0.51
5		1	1	0.99	98.79	
6		1	1	1.00	99.79	
7	120 %	1	1.2	1.20	100.41	99.44 ± 1.22
8		1	1.2	1.18	98.08	
9		1	1.2	1.20	99.84	

LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Limit of Detection:

Table 15: Limit of Detection data for CDZ and TFP and THP.

CDZ	TFP	THP
LOD = 3.3 x (SD / Slope) = 3.3 x (11.709/156.7) = 0.246 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (5.784/387.8) = 0.049 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (6.415/851.7) = 0.002 µg/ml

Limit of Quantitation:

Table 16: Limit of Quantitation data for CDZ and TFP and THP.

CDZ	TFP	THP
LOQ = 10 x (SD / Slope) = 10 x (11.709/156.7) = 0.747 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (5.784/387.8) = 0.149 µg/ml	LOQ = 10 x (SD / Slope) = 3.3 x (6.415/851.7) = 0.007 µg/ml

Robustness:

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 3.3 and 3.7.
3. Ratio of Mobile phase was changed (±2) Buffer : Acetonitrile (82:18) and Buffer : Acetonitrile (78:22)

Table 17: Robustness data for CDZ.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	1611.009	1521.656	1597.439	1488.475	1595.1	1520.022
2	1629.813	1533.886	1612.474	1502.462	1614.094	1533.886
3	1639.308	1538.695	1623.572	1508.516	1618.7	1544.878

% R.S.D	0.885	0.574	0.814	0.685	0.777	0.812

Table 18: Robustness data for TFP

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	800.912	753.54	791.556	738.01	793.148	753.54
2	805.609	758.188	801.985	742.655	797.837	758.188
3	810.316	760.536	802.535	747.293	799.419	763.61
% R.S.D	0.584	0.470	0.774	0.625	0.409	0.664

Table 19: Robustness data for THP.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	878.345	826.448	868.092	809.448	869.84	826.448
2	871.187	819.804	871.991	805.584	872.866	821.574
3	888.635	834.112	880.104	819.59	877.463	837.464
% R.S.D	0.997	0.866	0.702	0.891	0.439	0.982

Analysis of marketed formulation by developed method

Sample Stock Solution (CDZ 100 µg/mL, TFP 10 µg/mL and THP 20 µg/mL):

Take Crushed Tablet powder equivalent to 10 mg of CDZ, 1 mg TFP and 2 mg of THP was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (CDZ 10 µg/mL, TFP 1 µg/mL and THP 2 µg/mL):

Take 1 mL from this and transferred to 10 ml volumetric flask and made up volume up to the mark with Borate Buffer. Use This Solution for Derivatization as Mention Below.

Take 0.1ml of **working sample (CDZ+TFP+THP)** to a 10ml volumetric flask. Incubate this solution at 50C for 15 minutes in a waterbath.Inject above Solution 20 µl for Assay Analysis.

Table 20: Analysis on marketed formulation

Tablet	Chlozep Plus		
mg/Tablet powder	**CDZ (10 mg)**	**TFP (1 mg)**	**THP (2 mg)**
Assay (% of label claim*) Mean ± S. D.	100.05±0.544	101.45±0.313	100.92±0.277

The assay results were comparable to labeled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

In Estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl in their combined tablet dosage form, separation was achieved on C_{18} (25cm x 0.46 cm) Hypersil BDS at 30°C temperature by using a mobile phase Buffer (Potassium pH 3.5): Acetonitrile :TEA (80:20:0.1 v/v/v) at a flow rate of 1.0 ml/min and UV detection for Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl was carried out at 228 nm. Data suggests that the results obtained were found within the acceptance criteria.

Results of the validation for Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl of the above method were linear in the range of 5-15 µg/ml,1-3 µg/ml and 0.5-1.5 µg/ml respectively. The % recovery was found to be **99.27 ± 1.09 %, 99.57 ± 0.56 %** and **99.22 ± 0.51 %** respectively. The results of the precision study indicate that the proposed method shown good repeatability with a % RSD of 0.271, 0.249 and 1.153 respectively. Similarly %RSD from the intraday precision data was found to be 0.148-0.553 %, 0.198% - 0.312%, 0.568% - 1.062% respectively and %RSD from the Interday precision data were found to be 0.236-0.978 %, 0.198-0.805 %, and 0.384-1.029% respectively. Absolute difference between mean assay values of method precision and intermediate precision was found to be less than 2.0 %. Robustness is

performed by making changes in flow rate, Mobile phase composition and temperature. The assay obtained after proposed changes compared with the assay obtained in normal conditions. According to the acceptance criteria difference in the assay should not be more than 2%. The results obtained are well within the acceptance criteria.

Hence, the method can be termed as robust. Since the results are well within the limit of acceptance criteria for all validation parameters, therefore the method can be considered as validated and suitable for intended use. So, the proposed RP-HPLC assay method can be successfully applied for the estimation of Chlordiazepoxide, Trihexyphenidyl HCl and Trifluoperazine HCl in their combined tablet dosage form.

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