ORIGINAL RESEARCH

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Oxyclozanide and Tetramisole Hydrochloride from Formulation

Kanan Gamit[1*], Niraj Vyas[1], Anjalee Shukla[1], Vipul Patel[2], Ankit Anchliya[2]

[1]*Ramanbhai Patel College of Pharmacy, Charotar University of science and Technology CHARUSAT Campus, Changa, Gujarat, India, 388421.*
[2]*Cadila Pharmaceutical Limited, Dholka, Trasad Road, India.*

Received: 01st August, 2020; Revised: 27th August, 2020; Accepted: 05th October, 2020; Available Online: 25th Decemeber, 2020

ABSTRACT

A simple, precise, accurate, and rapid high performance liquid chromatography (HPLC) method has been developed and validated for the determination of oxyclozanide and tetramisole hydrochloride simultaneously, in combined pharmaceutical solid dosage form. The mobile phase used was mixture of solution (2 ml TEA in 1 litre milli-Q water, pH adjusted to 3 with OPA) and acetonitrile (30:70 v/v). Flow rate was set to 1 ml/min. The detection of Oxyclozanide and Tetramisole Hydrochloride was carried out on absorbance detector at 215 nm. Results of the analysis were validated statistically .

Keyword: Oxyclozanide, Tetramisole Hydrochloride, HPLC.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.4.6

How to cite this article: Gamit K, Vyas N, Shukla A, Patel V, Anchliya A. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Oxyclozanide and Tetramisole Hydrochloride from Formulation. International Journal of Pharmaceutical Quality Assurance. 2020;11(4):485-490.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Oxyclozanide (OXY) (3, 3′,5,5′,6 pentachloro-2′-hydroxy salicylanilide) is a salicylanilide anthelmintic drug. The mechanism of action is the uncoupling of oxidative phosphorylation.[1] OXY is a broadspectrum anthelmintic drug widely used in treating infections by *Fasciola gigantica, F. hepatica, Paramphistomum leydeni*, and *Calicophoron daubneyi*. It is also used to treat an infection like intestinal trematodes and gastrointestinal nematodes.[2-5] Various spectroscopic and chromatographic method have been reported for Oxiclozanide structure of OXY is shown in Figure 1.[6-8]

Tetramisole hydrochloride (TET) is (±)-2,3,5,6-Tetrahydro-6-phenyl imidazo [2,1-b] thiazole hydrochloride, used as an anthelmintic drug for the treatment of nematode infection as veterinary medicine.[9] Literature survey reveals that many spectroscopic and chromatographic method have been developed to determine TET structure of TET is shown in Figure 2.[10-21]

The rationale behind development of simultaneous method was, that there have been no method developed for simultaneous determination of oxyclozanide and tetramisole hydrochloride. So, it was thought of interest to develop accurate and precise method for simultaneous determination of both the drugs validation as per International Council for Harmonisation (ICH) guideline (Q2) R1.

MATERIALS AND METHODS

Procurement of API and Formulation

Both API, OXY and TET; and formulation (Bolus tablet) were procured from Cadila Pharmaceuticals.

Reagents and Chemicals

All HPLC grade solvents and chemicals (methanol, acetonitrile, orthophosphoric acid, triethyl amine) were purchased from

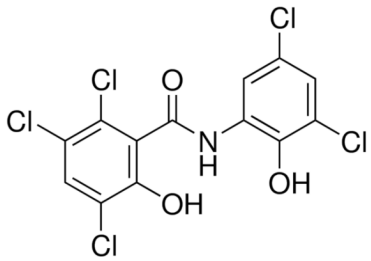

Figure 1: Structure of OXY

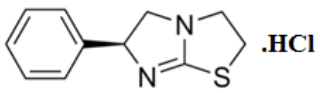

Figure 2: Structure of TET

**Author for Correspondence: kanangamit.ph@charusat.ac.in*

Pectrochem Pvt. Ltd., Mumbai. Water used for HPLC instrument was obtained from Millipore water treatment system. Solvents utilized for HPLC, were filtered through a 0.45 µm filter and degassed at room temperature for 20 minutes using a laboratory sonicator (Remi Instruments, Mumbai, India), prior to use.

Preparation of Standard Stock Solution

OXY standard stock solution: (500 µg/mL)

50 mg of standard OXY was weighed and transferred to a 100 ml volumetric flask. Then, 30 mL of diluent (mobile phase) was added to volumetric flask. The flask was shaken and sonicated for 10 minutes, and finally volume was made up to the mark with diluent to give a final volume solution containing 500 µg/mL OXY.

TET standard stock solution: (500 µg/mL)

50 mg of standard TET was weighed and transferred to a 100 ml volumetric flask. Then, 30 mL of diluent (mobile phase) was added to volumetric flask. The flask was shaken and sonicated for 10 minutes, volume was made up to the mark with Diluent to give a final solution containing 500 µg/mL TET.

Calibration Curve of OXY and TET

Appropriate volume of aliquots from standard OXY and TET stock solutions were transferred to different volumetric flasks. The volume was adjusted to the mark with diluent to give a solution containing 5, 25, 50, 75 and 100 µg/ml for OXY and TET.

Preparation of Working Standard Solution of OXY and TET

The standard stock solution was prepared by transferring 50 mg of OXY and TET in a 100 mL volumetric flask to obtain 500 µg/mL solution. Then 5 mL solution was diluted in a 50 mL volumetric flask to obtain final concentration of 50 µg/mL solution of OXY and TET.

Preparation of Test Sample Solution of OXY and TET

Twenty tablets were accurately weighed to determine average weight of tablet. Then tablets were finely powdered and powder equivalent to 50 mg OXY and TET was transferred into 100 mL volumetric flask. 30 mL of methanol was added and flask was sonicated for 20 minutes. The flask was shaken and volume was made up to the mark with methanol to get a concentration of 500 µg/mL of OXY and TET. 5 mL of aliquot was taken and transferred to volumetric flask of 50 mL capacity. Volume was made up to the mark with methanol to get a concentration of 50 µg/mL of OXY and TET. This solution was further used for the estimation of OXY and TET both.

Optimization of Chromatographic Parameters

Chromatographic separation was achieved using Agilent, Empower3 HPLC. Mobile phase consisted of mixture of (Add 2 mL TEA in 1000 mL milli-Q-water pH adjusted to 3 with OPA previously filtered through 0.45 µm PVDF filter in a bottle): Acetonitrile (30:70 v/v). The column used was Inertsil C (250* 4.6 mm, 5µm). Flow rate was set at 1 mL/min. Injection volume was 20 µL and chromatogram was recorded at 215 nm. All chromatograms were was processed and integrated using Empower software. Chromatogram of OXY and TET is shown in Figure 3.

System Suitability Parameters

Theoretical plates, tailing factor and resolution r were selected as system suitability parameters (SSP). The value determined for the selected parameters is shown in Table 1.

Validation of Developed Analytical Method

The developed analytical method was subjected to evolve various parameters by adopting methodology proposed in ICH guideline.[22-28]

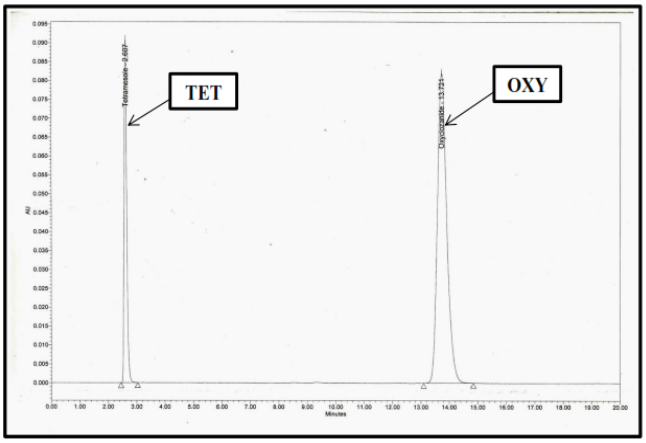

Figure 3: Chromatogram of TET and OXY

Table 1: System suitability data of OXY and TET at 215 nm (n = 3)

Parameter	*OXY*	*TET*
Tailing factor (< 2)	1.35	1.35
Theoritical plates (> 2000)	8274	3865
Resolution (>2)	8.9	

Table 2: Calibration Data of OXY and TET at 215 nm

Conc. (µg/ml)	*TET*		*Conc. (µg/mL)*	*OXY*	
	Mean ± S.D	*%RSD*		*Mean ± S.D*	*%RSD*
5	66390 ± 75.27	0.11	5	211485 ± 851.75	0.27
25	294991 ± 1157.42	0.39	25	946040 ± 1308.79	0.13
50	581753 ± 477.77	0.08	50	1890857 ± 694.97	0.03
75	863357 ± 1853.96	0.21	75	2809793 ± 1566.29	0.35
100	1134774± 1516.04	0.13	100	3718206 ± 6726.41	0.18

Linearity

Linearity was established by injecting five different dilutions of OXY and TET solutions covering the concentration range of 5–100 µg/mL (5, 25,50,75,100 75 µg/mL) individually, each 20 µL. The experiment was repeated six times. Area and corresponded concentration of OXY and TET were recorded. Correlation coefficient (r^2) was also determined. Result of the studies are shown in Table 2.

Precision

Precision of the developed analytical method was determined by performing interday and intraday variation in peak area of OXY and TET for three selected concentration (25µg/mL, 50µg/mL, 75 µg/mL) from linearity experiment. Intraday variation was determined by recording peak area of OXY and TET in standard solutions of OXY and TET six times in a day while those were determined for six days, once in a day, to determine interday precision. The data set obtained for each concentration was subjected to statistical treatment to determine mean and %RSD. The results of the studies are shown in Tables 3 and 4.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, 20 Tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of 50 % (12. µg/mL), 100 % (25 µg/mL) and 150 % (37.5 µg/ml) of OXY and TET to the synthetic mixture taking into consideration percentage purity of added bulk drug samples. The Amount of OXY and TET recovered was calculated and recorded as % recovery in Tables 5 and 6.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

LoD and LoQ of the developed method were determined initially by visual observation by noting signal to noise ratio (S/N), while selecting concentrations for linearity experiments. S/N for LoD was considered as 3, while that for LoQ was considered as 10. Later, LoD and LoQ were determined from the line equation, by adopting formula give below:

$$\text{Limit of Detection (LoD)}: 3.3 \times \sigma/S$$

$$\text{Limit of Quantification (LoQ)}: 10 \times \sigma/S$$

Where, σ = Standard deviation of the y- intercept, S = Slope of calibration curve (m).

Repeatability

Repeatability was established by determining the assay of three standard preparations and sample preparation under same conditions. The repeatability was expressed in terms of relative standard deviation (RSD).Results for repeatability is shown in Table 7.

Robustness

A robustness study was carried out by changing flow rate by 10%. (i.e. 1.1 mL/min and 0.9 mL/min) , an organic component in the mobile phase by ± 2 % and flow rate by 1%. The effects of changes in chromatographic response were recorded. The result of robustness study is shown in Table 8.

Table 3: Intraday precision of OXY and TET

Conc. (µg/ml)	*TET*		*Conc. (µg/ml)*	*OXY*	
	Abs ± S.D	*%RSD*		*Abs ± S.D*	*%RSD*
25	294991 ± 1157.42	0.39	25	946040 ± 1308.79	0.13
50	581753 ± 477.77	0.08	50	1890857 ± 694.97	0.03
75	863357 ± 1853.96	0.21	75	2809793 ± 1566.29	0.35

Table 4: Interday precision of OXY and TET

Conc. (µg/ml)	*TET*		*Conc. (µg/ml)*	*OXY*	
	Abs ± S.D	*%RSD*		*Abs ± S.D*	*%RSD*
25	295517 ± 1206.55	0.40	25	948988 ± 3003.59	0.31
50	585138 ± 1526.13	0.26	50	1899225± 3043.04	0.16
75	865613 ± 4369.95	0.50	75	2856132± 21810.04	0.76

Table 5: Accuracy of TET (n = 3)

%Level	*Sample conc. (µg/mL)*	*Std. added (µg/mL)*	*Total conc. of TET (µg/mL)*	*Amt. recovered (µg/ml)*	*Mean % Recovery ± S.D*	*%RSD*
50%	25	12.5	7.5	37.3	99.66±0.524934	0.52
100%	25	25	50	49.7	99.56±0.471405	0.48
150%	25	37.5	62.5	62.3	99.73±0.899383	0.90

Table 6: Accuracy of OXY (n = 3)

%Level	*Sample conc. (µg/mL)*	*Std. added (µg/mL)*	*Total conc. of TET (µg/mL*	*Amt. recovered (µg/ml)*	*Mean % Recovery ± S.D*	*%RSD*
50%	25	12.5	7.5	37.2	99.35 ± 0.10403	0.10
100%	25	25	50	50.06	100.2±0.216025	0.21
150%	25	37.5	62.5	62.4	99.8 ± 0.852447	0.85

Assay

From the calibration curve, the line equation was constructed using the least square regression analysis. Amount of OXY and TET present in table was calculated from line equation using area of peak corresponded to OXY and TET. Results are shown in Table 9.

RESULTS AND DISCUSSION

TET and OXY was determined by RP-HPLC method using column and optimized mobile phase

Figure 3 showed the chromatogram of TET and OXY in selected mobile phase. The Retention times of OXY and TET are 13.72 min and 2.62 min respectively

System suitability parameter, which is tailing factor, theoretical and plates and resolution, were determined from the peak of TET and OXY, shown in Table 1. All selected parameters were within the permitted value that indicates that the system is suitable for the operation of method.

Linearity range for OXY and TET were 5-100µg/mL, (r^2 = 0.99) and (r^2 = 0.99), for TET and OXY respectively. The linear regression equations are Y = 11268 x +13561 for TET and Y36986x+ 28975 for OXY.

The correlation coefficient value for plot of concentration of OXY and TET was found approaching 1.0, which ensured the linearity of area for the concentration range selected for the studies. Calibration curves for TET and OXY are shown in Figures 4 and 5 respectively. Linearity graph of OXY and TET is shown in Figures 6.

Precision of the developed method was established for OXY and TET by performing while performing intraday and interday analysis. The results, in Tables 3 and 4, showed that percentage RSD value for all three selected concentrations was within the recommended limit. Intraday precision were found to be 0.08 – 0.39 and 0.03 -0.35% RSD and interday precision was found to 0.26 – 0.40% RSD and 0.16 – 0.31%RSD for OXY and TET respectively. As the value for %RSD was very low, it may confirmed that method was precise.

Accuracy studies were carried out at three different level, i.e. 50,100, and 150%. Concentration of the sample solution selected was 25 µg/mL for both TET and OXY. Results of accuracy studies are shown in Tables 5 and 6

LoD and LoQ values were determined to establish the sensitivity of the developed analytical method for OXY and TET. LoD was found to 0.26 µg/mL and 0.19 µg/mL for TET and OXY respectively. LoQ was found to 0.79 µg/mL and 0.62 µg/mL for TET and OXY, respectively

Repeatability was performed by injecting OXY and TET for selected concentration, 50 µg/mL. The results are shown in Table 7. Robustness was determined initially, by incorporating

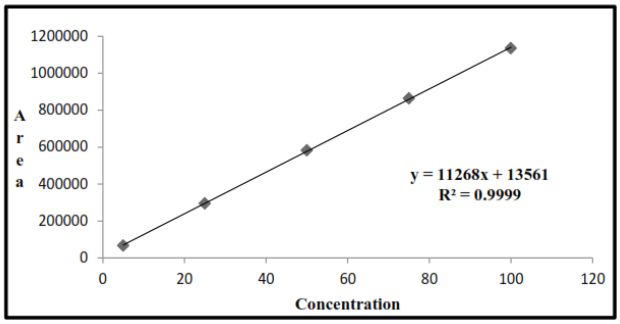

Figure 4: Calibration Curve of TET at 215 nm

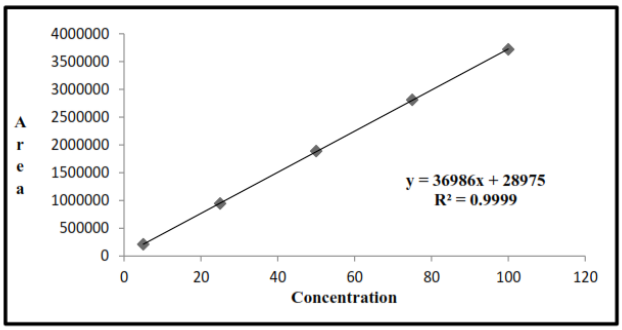

Figure 5: Calibration Curve of OXY at 215 nm

Table 7: Repeatability of OXY and TET (n = 6)

Drug Name	*Conc. (µg/ml)*	*Mean ± S.D*	*% RSD*
TET	50	581753 ± 477.77	0.08
OXY	50	1890857 ± 694.97	0.03

Table 8: Robustness data of OXY and TET (n=3)

Parameter		*TET*		*OXY*	
Area ± S.D		*%RSD*	*Area ± S.D*	*%RSD*	
pH	2.8	582928 ± 1231.71	0.21	1895966 ± 2081.34	0.10
	3.2	586384 ± 2492.18	0.42	1903976 ± 1461.54	0.07
Organic Solvent	+2%	599162 ± 506.63	0.08	1898301 ± 1353.59	0.07
	-2%	597651 ± 641.28	0.10	1893209 ± 524.53	0.02
Flow Rate (mL/min)	0.9	629633 ± 4603.257	0.73	2114087 ± 34269.43	1.62
	1.1	556699 ± 3704.052	0.66	1667623 ± 24305.42	1.45

Table 9: Assay of OXY and TET (n=3)

API	*Label Claim (mg)*	*Amount Obtained (mg)*	*(%)Mean Conc.± S.D*	*% RSD*
OXY	450 mg	455 mg	101 ± 0.20	0.20
TET	450 mg	448 mg	99.6 ± 0.28	0.28

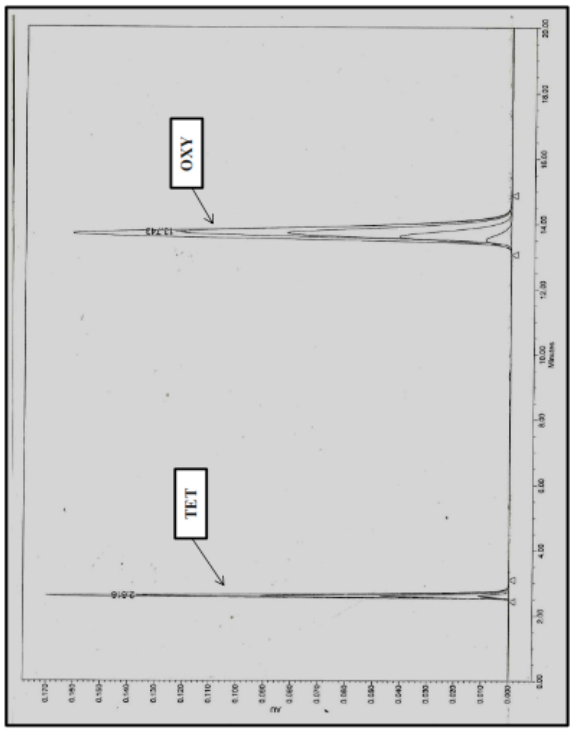

Figure 6: Linearity Graph of OXY and TET

deliberate small alteration in selected chromatographic parameters. The results showed that minor changes in selected chromatographic parameters did not affect the peak area.

Assay of OXY and TET was performed from tablet. Results showed that tablet contained 455 mg of OXY and 448 mg of TET. Result for assay of OXY and TET is shown in Table 9.

CONCLUSION

The proposed HPLC method was successfully developed and validated as per ICH guideline (Q2) R1 . The method is very simple and selective as OXY and TET were well separated, which makes it especially suitable for routine quality control analysis work. Thus, the proposed method was applied for simultaneous estimation of OXY and TET in formulation.

REFERENCES

1. Walley JK. Oxyclozanide (3,3′,5,5′,6-pentachloro-2,2′-dihydroxybenzanilide-- 6Zanil″) in the treatment of the liver fluke Fasciola hepatica in sheep and cattle. Vettrinary Research. 1966;78:267–276.
2. Paraud C, Fournier E, Robergeot V, Kulo A, Pors I, Baudry C, Chartier C. Calicophoron daubneyi infection in grazing goats: Results from a cross-sectional coprological survey in France. Small Ruminant Research. 2009 Mar 1;82(1):66-68. https://doi.org/10.1016/j.smallrumres.2009.01.009.
3. Paraud C, Gaudin C, Pors I, Chartier C. Efficacy of oxyclozanide against the rumen fluke Calicophoron daubneyi in experimentally infected goats. The Veterinary Journal. 2009 May 1;180(2):265-267. https://doi.org/10.1016/j.tvjl.2008.01.002
4. Rolfe PF, Boray JC. Chemotherapy of paramphistomosis in cattle. Australian Veterinary Journal. 1987 Nov;64(11):328-332.
5. Shokier KM, Aboelhadid SM, Waleed MA. Efficacy of five anthelmintics against a natural Fasciola species infection in cattle. Beni-Suef University Journal of Basic and Applied Sciences. 2013 Mar 1;2(1):41-45. https://doi.org/10.1016/j.bjbas.2013.09.006
6. Kumar AR, Shetty AS. Development and Validation of RP-HPLC Method for the Estimation of Oxyclozanide in Bulk Drug and Pharmaceutical Formulations. Development. 2012 Apr;4(2): 700-704.
7. Mohamed AO, Ramadan NK, Shawky SE, Salem MY. Simultaneous determination of oxyclozanide and levamisole by spectrophotometric and chromatographic methods. Journal of Applied Pharmaceutical Science. 2014 Sep 1;4(9):36-45.
8. Anusha G, Gupta R, Manohar Babu S. Development and Validation
9. of RP-HPLC Method for estimation of Oxyclozanide in pure and Pharmaceutical formulation. International Research. 2014 June;3(2):185–191.
10. Sweetman SC. Martindale, the complete drug reference 36th Ed., Pharmaceutical Press, London, UK, 2009.
11. Sane RT, Sapre DS, Nayak VG. An extractive spectrophotometric method for the determination of tetramisole hydrochloride in pharmaceutical preparations. Talanta. 1985;32:148–149. https://doi.org/10.1016/0039-9140(85)80045-X
12. Amin AS, Dessouki HA. Facile colorimetric methods for the quantitative determination of tetramisole hydrochloride; Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2002;58:2541–2546. https://doi.org/10.1016/S1386-1425(02)00005-7
13. Sastry CS, Aruna M, Reddy MN, Sankar DG. Extractive spectrophotometric determination of some anthelmintics using fast green FCF or orange-II. Indian Journal of Pharmaceutical Sciences. 1988;50(2). 140–142
14. Amin AS. Quantitative determination of some pharmaceutical veterinary formulations using bromocresol purple and bromocresol green. Analytical Letters. 2006;30:2503–2513. https://doi.org/10.1080/00032719708001761
15. Patil H, Wani M, Kuchekar BS. Development and validation of UV-spectrophotometric method for the estimation of tetramisole hydrochloride in bulk and pharmaceutical dosage form. Indo American Journal of Pharmaceutical Research. 2014;4:1903-1909.
16. Nassar MWI, Attia KAM, Mohamad AA, Said RA, Abdel-monem AH. Development and validation of spectrophotometric methods manipulating ratio spectra for determination of tetramisole hydrochloride in the presence of its alkali-induced degradation product. Journal of analytical & pharmaceutical research. 2018;7(1):67-72.
17. Gupta VK, Singh AK, Gupta B. Potentiometric sensor for the high throughput determination of tetramisole hydrochloride. Combinatorial Chemistry & High Throughput Screening. 2007;10:583–594. https://doi.org/10.2174/138620707782152371
18. Issa YM, Rizk MS, Shoukry AF, Abdel-Aziz R, Abdel-Fattah HM, Atia EM. Construction and performance characteristics of new tetramisole selective plastic membrane electrodes. Talanta. 1994;41:135–141. https://doi.org/10.1016/0039-9140(94)80179-7
19. Holbrook A, Scales B. Polarographic determination of tetramisole hydrochloride in extracts of animal tissue. Analytical Biochemistry. 1967;18:46-53. https://doi.org/10.1016/0003-2697(67)90054-1
20. Wagh JS, Mokashi AA, Datta A. High-performance liquid chromatographic method for the monitoring of the synthesis of the precursor for tetramisole; Journal of Chromatography.

1993;644:428–433. https://doi.org/10.1016/0021-9673(93)83357-X
21. Mourot D, Delepine B, Boisseau J, Gayot G. High-pressure liquid chromatographic analysis of veterinary anthelmintics I: quantitative determination of tetramisole. Journal of Pharmaceutical Sciences. 1979;68:796–797. https://doi.org/10.1002/jps.2600680636
22. Bertuccia C, Tedescoa D, Fabinia E, Di Pietraa AM, Rossib F, Garagnanib M, Borrellob ED, Andrisano V. Determination of levamisole and tetramisole in seized cocaine samples by enantioselective highperformance liquid chromatography and circular dichroism detection. Journal of Chromatography. 2014;1363:150–154. https://doi.org/10.1016/j.chroma.2014.07.069
23. International Conference on Harmonization (ICH) Guidelines Q2 (R1): Validation of analytical procedures: Text and methodology, 2005
24. Raval MA, Gamit KG, Vyas NY, Patel ND. Estimation of Withaferin–A from Ashwagandhadi lehya using HPLC. International Journal of Pharmaceutical Sciences and Research. 2020; 11(1): 334-342.
25. Gamit KG, Vyas NY, Patel ND, Raval MA. Estimation of Guggulsterone-Z in Gokshuradi Guggulu using Reversed-Phase High-Performance Liquid Chromatography. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(11): 204-208.
26. Vyas NY, Patel SH. Simultaneous estimation of Curcuminoids, Piperine and Gallic Acid in an ayurvedic formulation by validated HPTLC method. Asian Journal of Pharmaceutical and Clinical Research. 2016;9:117-122
27. Vyas NY, Patel SH: Validated spectrofluorimetric method for estimation of Piperine in an Ayurvedic formulation. Asian Journal of Pharmaceutical and Clinical Research.2012;5, 231-233.
28. Vyas NY, Panchal S. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Nebivolol and Indapamide in Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Analysis. 2014;4(3):98-102
29. Patel M, Vyas NY. Development and validation of stability indicating HPTLC method for estimation of Ursolic acid. Asian Journal of Research in Chemistry. 2013;6(6):546-551.