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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF DOMPERIDONE AND NAPROXEN IN BULK AND TABLET DOSAGE BY RP HPLC

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Abstract:
A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validation of Domperidone and Naproxen in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Acetonitrile: Water (10:90 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 264nm. The retention time of the Domperidone and Naproxen was 2.121, 3.643 ±0.02 mins, respectively. The method produced linear responses in the concentration range of 5-25µg/ml of Domperidone and 25-125µg/ml of Naproxen. 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. The results have showed that Domperidone and Naproxen and the other degradation products were fully resolved and thus the method is stability-indicating. The developed method can be successfully employed for the routine analysis of Domperidone and Naproxen in API and Pharmaceutical dosage forms.

Key Words: *Domperidone, Naproxen, RP-HPLC, validation*

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INTRODUCTION:

Domperidone is an anti-emetic drug and is chemically **5**-Chloro-1-(1-[3-(2-oxo-2,3-dihydro-1 H-benzo[d]imidazol-1-yl)propyl]piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one. Domperidone is a specific blocker of dopamine receptors. It speeds gastrointestinal peristalsis, causes prolactin release. Naproxen is chemically (*S*)-2-(6-Methoxynaphthalen-2-yl) propionic acid.It works by reversibly inhibiting both the COX-1 and COX-2 enzymes. This results in the inhibition of prostaglandin synthesis. Thus, by inhibiting COX-1/2, Naproxen induces an anti-inflammatory effect. There is no stability indicating analytical method reported for simultaneous estimation of Domperidone and Naproxen. Hence a simple, rapid, sensitive and accurate stability indicating HPLC method was developed for simultaneous estimation of Domperidone and Naproxen from bulk and tablet dosage form.

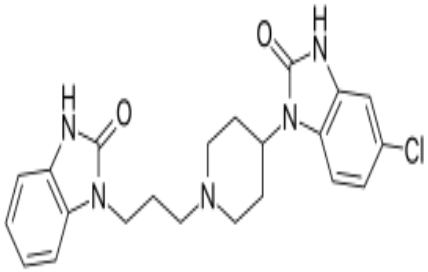

Fig.1: Chemical structure of Domperidone

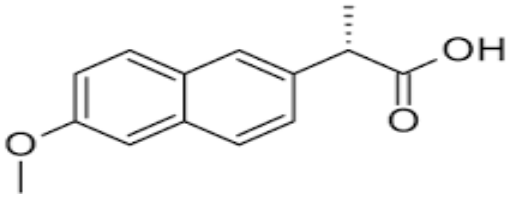

Fig.2: Chemical structure of Naproxen

MATERIALS AND METHODS:

Water, Methanol, Acetonitrile of HPLC grade were procured from Lichrosolv (merck) . Domperidone and Naproxen standards were received as gift samples from SURA LABS.

Equipment:

Chromatographic separation was performed on Waters HPLC with Empower 2 software, Alliance 2695 separation module, 996 PDA detector.

A) Preparation of Domperidone Standard Stock Solution (1000µg/ml)

10mg of Domperidone was weighed and transferred into 10ml of volumetric flask. Mix half diluent and shake it well. Then the volume was made up to the mark using the diluent.

B) Preparation of Domperidone Working Standard Solution (15µg/ml)

Pipette out 0.15ml of standard stock solution of Domperidone in other 10ml volumetric flask. Volume was made upto the mark with the diluent.

A) Preparation of Naproxen Standard Stock Solution (1000 µg/ml)

10mg of Naproxen was weighed and transferred into 10ml of volumetric flask. Mix half diluent and shake it well. Then the volume was made upto the mark using the diluent.

B) Preparation of Naproxen Working Standard Solution (75 µg/ml)

Pipette out 0.75ml of standard stock solution of Naproxen in other 10ml volumetric flask. Volume was made upto the mark with the diluent.

Preparation of Sample solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Domperidone and Naproxen sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.Further pipette 4.5ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Chromatographic conditions:

Phenomenex Gemini C18 (4.6×250mm) was used as the stationary phase. A mixture of ACN and Water (10:90 v/v) was used as a mobile phase. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 264nm. The injection volumes of sample and standard were 10µl. Total run time is 6 mins.

RESULTS AND DISCUSSION:

Method Validation:

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System Suitability

System suitability and chromatographic parameters were validated. Parameters such as resolution, theoretical plates, and tailing factor were calculated. The results are given in table 1.

Table 1: System suitability parameters for Domperidone and Naproxen

NAME	RT	USP TAILING	PLATE COUNT	RESOLUTION
DOMPERIDONE	2.149	1.2	5693.4	
NAPROXEN	3.650	1.1	5428.4	10.1

Linearity:

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Domeridone and Naproxen at different concentration levels. The calibration curve showed (Fig.4 and 5) good linearity in the range of 5-25 µg/ml for Domperidone with correlation coefficient (R^2) of 0.999 and 25-125 µg/ml for Naproxen with correlation coefficient (R^2) of 0.999. Results are given in table 2.

Table 2: Linearity for Domperidone and Naproxen

DOMPERIDONE			NAPROXEN	
S.No.	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**
1	5	135005	25	469094
2	10	277120	50	1049397
3	15	405128	75	1557592
4	20	534643	100	2150412
5	25	672357	125	2648444

Conc*- concentration

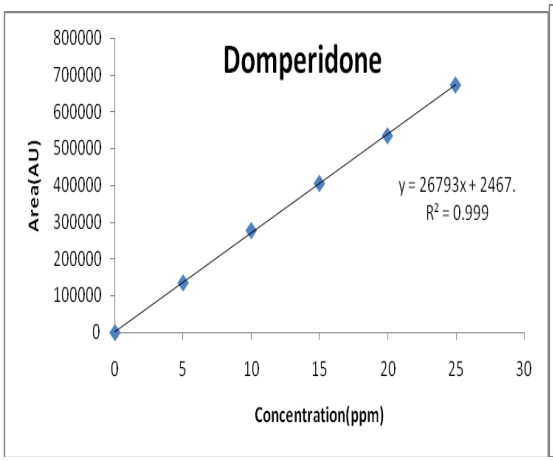

Fig.3: Calibration curve of Domperidone

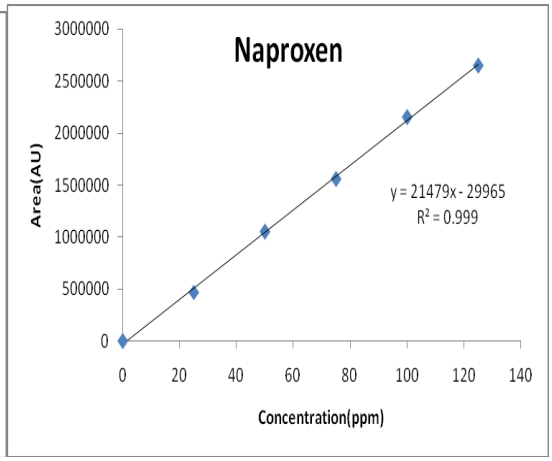

Fig. 4: Calibration curve of Naproxen

Accuracy : Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (50%, 100% and 150%) taking into consideration percentage purity of added bulk drug samples. At each concentration, sample was injected thrice to check repeatability and from the % RSD values it was analyzed that the method was accurate as percentage recovery values were found to be 99.7% for Domperidone and 99% for Naproxen at three different concentrations 50%, 100%, 150%. The results are given in table 3 and 4.

Recovery level	Accuracy of Naproxen					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
50%	37.5	824699	8265277.7	37.49	99.9	99%
	37.5	825468				
	37.5	829416				
100%	75	1613703	1622241	74.9	99	
	75	1625321				
	75	1627698				
150%	112.5	2415799	2422702	112.49	99.9	
	112.5	2423632				
	112.5	2428676				

Table 3: Accuracy data for Naproxen

S.D* - Standard deviation,Conc*- concentration Number of experiments (n) – 3

Table 4: Accuracy data for Domperidone

Recovery Level	Accuracy of Domperidone					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	7.5	200290	2014723	7.5	98.6	99.7%
	7.5	201502				
	7.5	202625				
100%	15	404329	406193	15.5	100.3	
	15	407125				
	15	407125				
150%	22.5	606143	607144	22.5	100.2	
	22.5	606872				
	22.5	608417				

S.D* - Standard deviation Conc*- concentration Number of experiments (n) – 3

Precision: Repeatability- Standard solution containing Domperidone (15 µg/ml) and Naproxen (75 µg/ml) were injected six times and areas of peaks were measured and % R.S.D. was calculated. The results are given in table 5 and 6.

Table 5: Method precision data for Domperidone

S.No.	Injection	PeakName	RT	Area
1	Injection-1	Domperidone	2.198	405262
2	Injection-2	Domperidone	2.196	405637
3	Injection-3	Domperidone	2.160	405628
4	Injection-4	Domperidone	2.160	405647
5	Injection-5	Domperidone	2.160	405948
6	Injection-6	Domperidone	2.186	408732
Mean			2.17	406142.3
Std. Dev.			0.0187	1287.197
% RSD			0.8	0.316933

S.D* - Standard deviation R.S.D* - Relative standard deviation Conc*- concentration Number of experiments (n) – 5

Table 6: Method precision data for Naproxen

S.No.	Injection	PeakName	Rt	Area
1	Injection-1	Naproxen	3.623	1608292
2	Injection-2	Naproxen	3.611	1609283
3	Injection-3	Naproxen	3.696	1617836
4	Injection-4	Naproxen	3.696	1619743
5	Injection-5	Naproxen	3.696	1614262
6	Injection-6	Naproxen	3.642	1608471
Mean			3.660	1611315
Std. Dev.			0.039	6077.093
% RSD			1.06	0.377151

S.D* - Standard deviation R.S.D* - Relative standard deviation Conc*- concentration Number of experiments (n) – 5

ROBUSTNESS:

To demonstrate the robustness of the method, prepared solution as per test method are injected at variable conditions like using different conditions like flow rate and nature of organic phase. System suitability parameters were compared with that of method precision.

Table 7: Robustness data for Domperidone and Naproxen

Parameter	DOMPERIDONE		NAPROXEN	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.9 ml/min	2.210	0.9	4.498	0.9
1.0 ml/min	2.121	1.2	3.643	1.1
1.1 ml/min	2.184	1.1	3.505	0.8
Organic phase				
Less organic phase	2.200	0.9	4.504	0.9
More organic phase	2.127	0.7	3.512	0.9

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were found to be0.5 µg/ml and 1.5µg/ml for Domperidone and 4.0µg/ml and 12.3µg/ml for Naproxen estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantified with very low concentration. The result is given in Table 8.

Table 8: LOD and LOQ of Domperidone and Naproxen

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg/ml)	LOQ (µg/ml)
Domperidone	4269.8	26793	0.5	1.5
Naproxen	26594	21479	4.0	12.3

FORCED DEGRADATION:

ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses were carried out.

Acid degradation:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Domperidone and Naproxen sample into a 10mL clean dry volumetric flask and add about 3mL of 0.5N Hcl and kept a side for 3hours and add 3mL of 0.5N NaOH solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely.

Further pipette 0.15ml of Domperidone and 4.5ml of Naproxen from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Chromatogram of acid degradation on sample solution is shown below in figure 5.

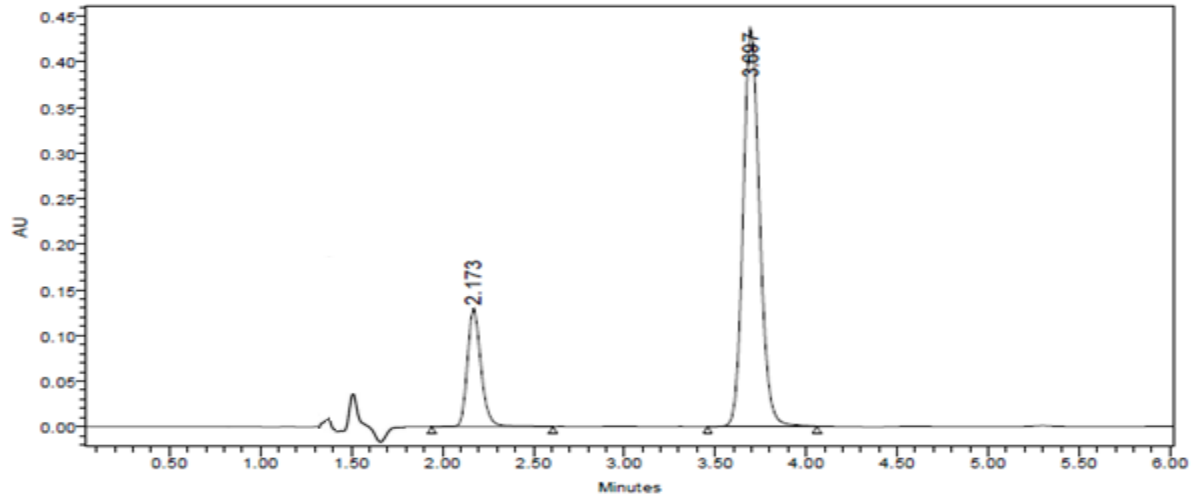

Fig. 5: Chromatogram of Acid Degradation

Alkaline degradation:
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Domperidone and Naproxen sample into a 10mL clean dry volumetric flask and add about 3mL of 0.5N Na OH and kept a side for 3hours and add 3mL of 0.5N Hcl solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely.

Further pipette 0.15ml of Domperidone and 4.5ml of Naproxen from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. Chromatogram of alkaline degradation on sample solution is shown below in figure 6

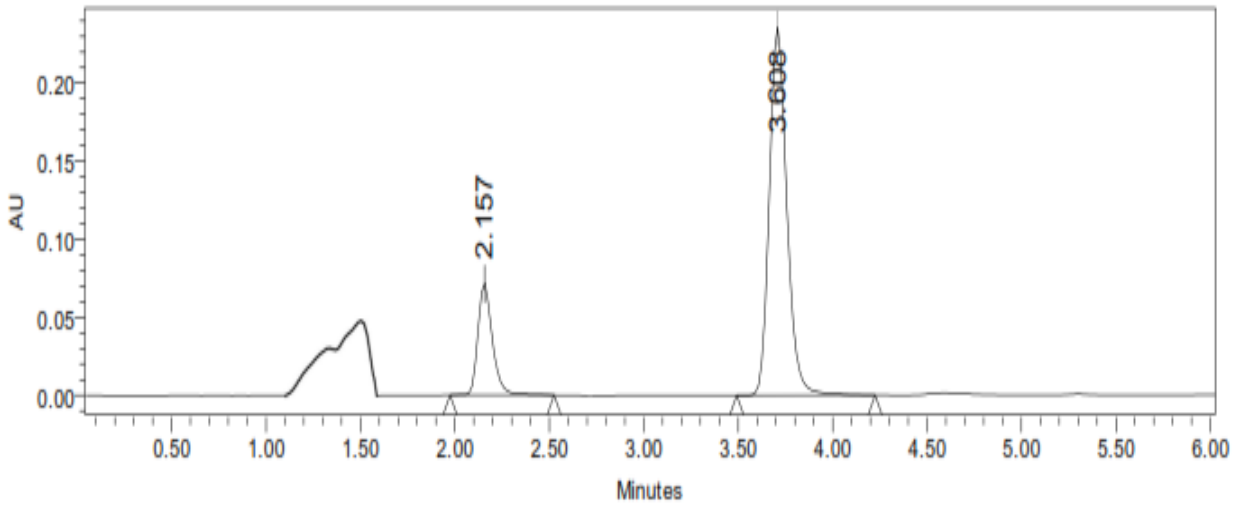

Fig. 6: Chromatogram of Alkaline Degradation

Oxidative degradation:
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Domperidone and Naproxen sample into a 10mL clean dry volumetric flask and add about 3mL of Hydrogen peroxide solution and kept a side for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely.

Further pipette 0.15ml of Domperidone and 4.5ml of Naproxen from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. Chromatogram of Oxidative degradation on sample solution is shown below in figure 7.

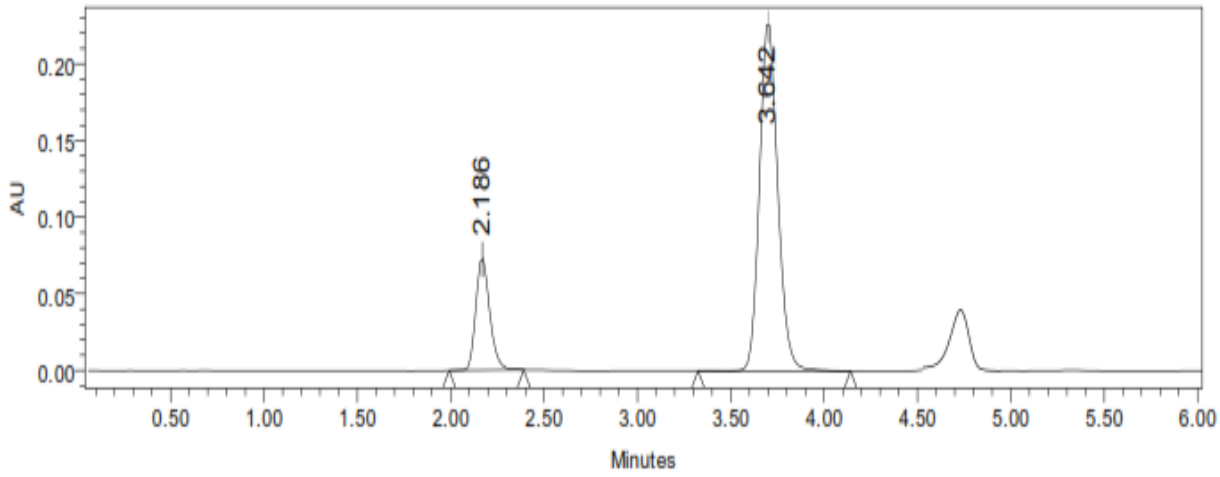

Fig 7: Chromatogram of Oxidative Degradation

Photolytic degradation:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Domperidone and Naproxen sample into a 10ml clean dry volumetric flask and expose to sunlight for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely.

Further pipette 0.15ml of Domperidone and 4.5ml of Naproxen from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Chromatogram of Photolytic degradation on sample solution is shown below in figure 8.

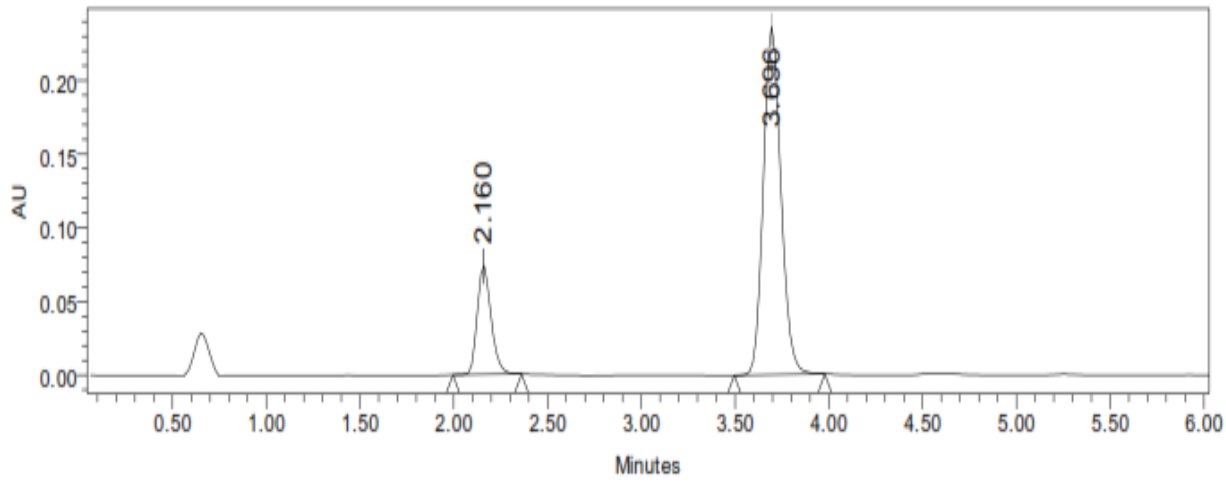

Fig. 8 : Chromatogram of Photolytic Degradation

Thermal degradation:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Domperidone and Naproxen sample into a 10mL clean dry volumetric flask and expose to heat at 80-90ºc for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 0.15ml of Domperidone and 4.5ml of Naproxen from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. Chromatogram of Thermal degradation on sample solution is shown below in figure 9.

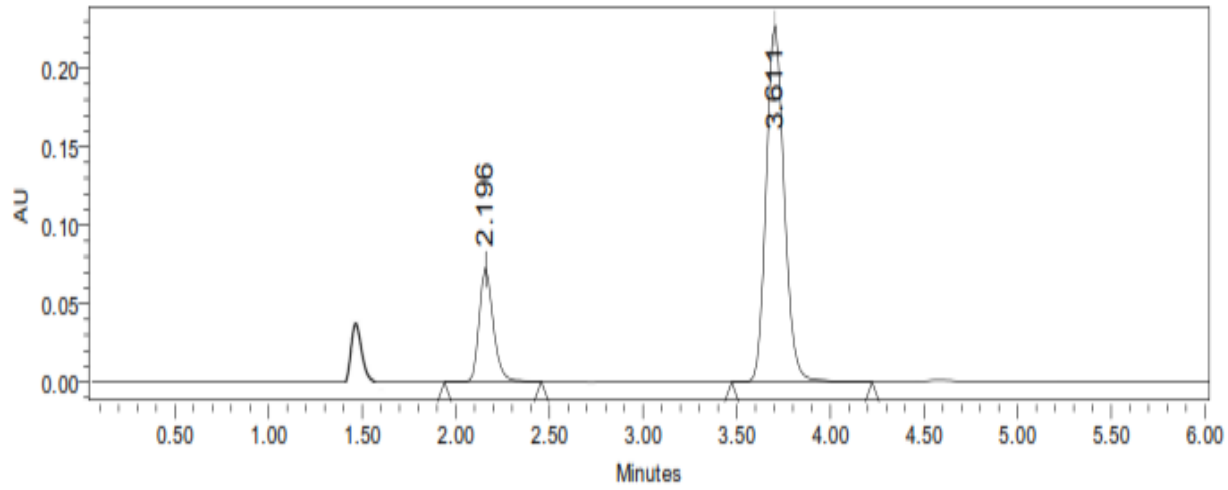

Fig. 19: Chromatogram of Thermal Degradation.

Table 10: Stability data for Domperidone and Naproxen

S. No	Type of degradation	Weight of sample (µg/ml)	Area of sample		Assay content (% w/w)	
			Domperidone	Naproxen	Domperidone	Naproxen
1	Acid (0.5N HCl)	50µg/ml of Domperidone and 75µg/ml of Naproxen	21074471	2971811	90% (10%)	92% (8%)
2	Base (0.5N NaOH)	50µg/ml of Domperidone and 75µg/ml of Naproxen	21074674	3078484	92% (8%)	91% (9%)
3	Peroxide (3% H_20_2)	50µg/ml of Domperidone and 75µg/ml of Naproxen	20011633	3071191	95% (5%)	95% (5%)
4	Thermal (at 60^0 c)	50µg/ml of Domperidone and 75µg/ml of Naproxen	21001918	3071919	93% (7%)	93% (7%)
5	Photolytic (Sunlight)	50µg/ml of Domperidone and 75µg/ml of Naproxen	21016363	3072992	91% (9%)	92% (8%)

CONCLUSION:

Stability indicating RP-HPLC method have been developed and validated for the determination of Domperidone and Naproxen in tablet dosage form. The method was found to be specific as there was no interference of any co-eluting impurities after stress degradation study. The degraded products are well resolved, indicating the method can also be useful for determination of degraded products. The proposed method is found to be simple, accurate, precise and robust. Hence, it can be used successfully for the routine analysis of Domperidone and Naproxen in pharmaceutical dosage forms and for analysis of stable samples obtained during accelerated stability study.

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REFERENCES:

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition,Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/columncare.pdf
9. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online) ,URL:http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
13. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf
14. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
15. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
16. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1-8.
17. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
18. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
19. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
20. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.