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Method Development and its Validation for Simultaneous Estimation of Lornoxicam and Paracetamol as API and in Tablet Dosage form by UV Spectrophotometry using Hydrotropic Agents

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ABSTRACT

A simple, sensitive, economical UV Spectrophotometric method was developed for the simultaneous estimation of Lornoxicam and Paracetamol in bulk and tablet formulation by using Urea solution as Hydrotropic agent. The λ_{max} for Lornoxicam and Paracetamol was found to be 384 nm and 244 nm respectively and both Lornoxicam and Paracetamol obey Beer-Lambert's law in the concentration range of 2-10 µg/ml (r^2=0.999) and 20-60 µg/ml.(r^2=0.999) in 8M Urea (Hydrotropic agent) respectively. The developed method was validated according to ICH guidelines and value of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

Keywords: Method Development, Validation, Simultaneous Estimation, Lornoxicam, Paracetamol, UV Spectrophotometry, Hydrotropic Agents

INTRODUCTION

Hydrotropy is the term originally put forward by to describe the increase in the solubility of a solute by the addition of fairly high concentration of alkali metal salts of various organic acids. However, the term has been used in the literature to designate non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. The chemical structure of the conventional Neuberg's hydrotropic salts (proto-type, sodium benzoate) consists generally of two essential parts, an anionic group and a hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is a prerequisite for a hydrotropic substance. The type of anion or metal ion appeared to have a minor effect on the phenomenon. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. This should imply that hydrotropic agents are molecules having a planar hydrophobic structure brought into solution by a polar group. Hence, it seems rational to propose that molecules with a planar hydrophobic part and a polar group, which is not necessarily anionic, can act as hydrotropic agents. Saleh and El-Khordagui suggested that the phenomenon of hydrotropy is not confined to the metal salts of organic acids, certain cationic salts and neutral molecules may be equally involved. They used procaine HCl, PABA HCl and cinchocaine HCl as cationic salts and resorcinol and pyrogallol as neutral molecules in their studies[1-8].

The review of literature reveals that various methods like UV, HPLC, other spectrophotometric methods have been reported for the estimation of Lornoxicam and Paracetamol individually as API and in combination with other drugs[9-17]. But no method has been reported so far in literature for simultaneous estimation of Lornoxicam and Paracetamol as API and in pharmaceutical tablet dosage form by UV-Visible spectrophotometry using hydrotropic agent. So aim of present research work is to develop a simple, sensitive, accurate, precise, and economical UV-Visible method for simultaneous estimation of Lornoxicam and Paracetamol as API and in tablet dosage form using hydrotropic agents.

MATERIALS AND METHODS

Apparatus

A double beam UV-VIS Spectrophotometer (UV 1800, Shimadzu, Japan) Spectral bandwidth of 1 nm and wavelength accuracy of ± 0.5 nm with a pair of 1 cm matched quartz cells was used to measure the absorbance of all the solutions. Spectra were automatically obtained by UV- Probe system software (UV Probe version 2.31). All weights were taken on Digital electronic balance Sartorius, CP225D.

Reagents and Chemicals

All the chemicals used were of analytical grade.

Marketed Formulation

The commercial fixed dose combination tablet Neucam-P Tablet (contain Lornoxicam 8 mg and Paracetamol 500 mg) was procured from local market.

Methods

Simultaneous estimation of Lornoxicam and Paracetamol by simultaneous equation method[18]

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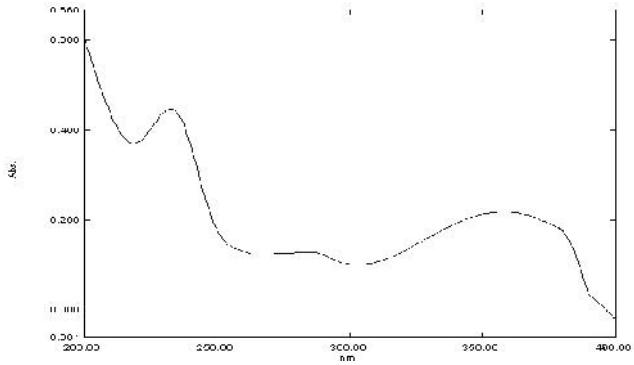

Figure 1: Spectrum showing λ max. of Lornoxicam (384 nm)

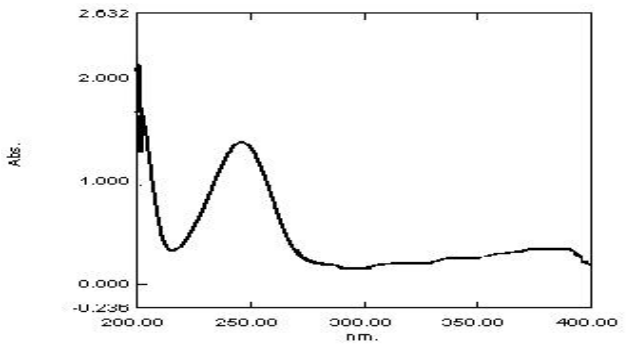

Figure 2: Spectrum showing λ max. of Paracetamol (244 nm)

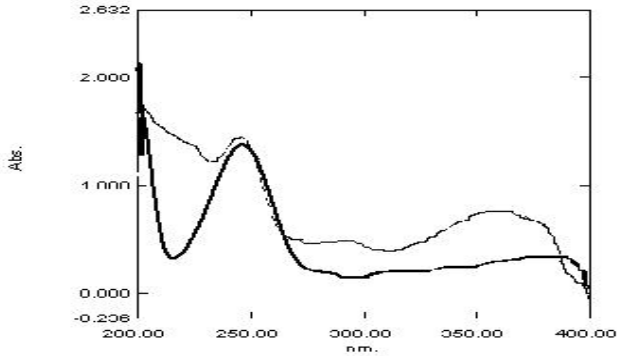

Figure 3: Overlain spectra showing λ_{max} of Lornoxicam (384nm) and Paracetamol (244nm)

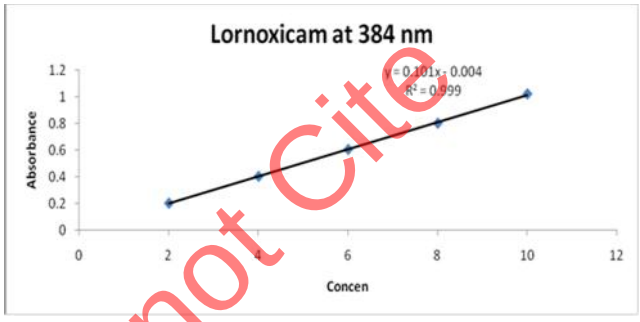

Figure 4: Calibration curve for Lornoxicam at 384 nm.

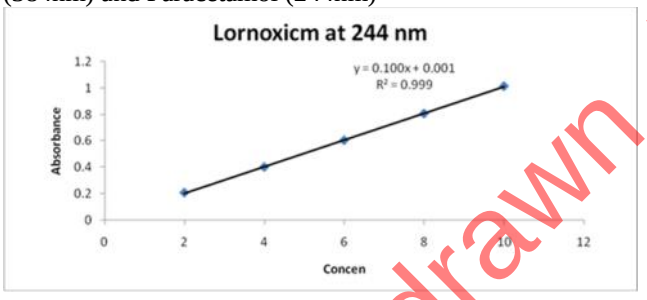

Figure 5: Calibration curve for Lornoxicam at 244 nm.

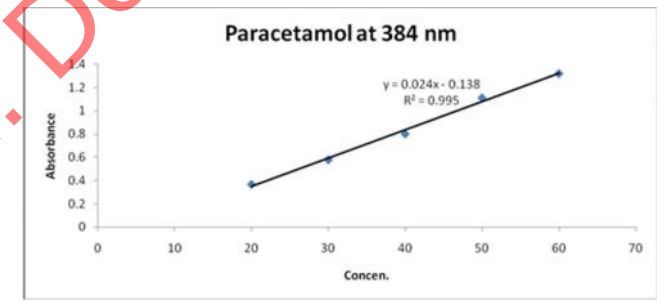

Figure 6: Calibration curve for Paracetamol at 384 nm.

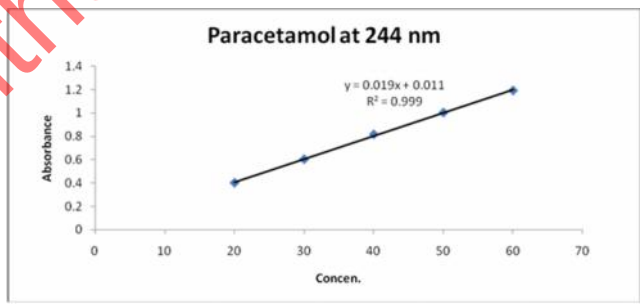

Figure 7: Calibration curve for Paracetamol at 244 nm.

Accurately weighed lornoxicam (10 mg) was transferred to 100 ml volumetric flask, dissolved in 8M urea and made-up the volume to 100 ml with same solvent system. The final solution contained 100 µg per ml of lornoxicam solution.

Preparation of standard stock solution of Paracetamol

Accurately weighed paracetamol (10 mg) was transferred to 100 ml volumetric flask, dissolved in 8M urea and made-up the volume to 100 ml with same solvent system. The final solution contained 100 µg per ml of paracetamol solution.

Determination of wavelength of maximum absorbance for Lornoxicam

Standard lornoxicam solution (1ml) was transferred to separate 10 ml volumetric flask. The volume was adjusted to 10 ml with same solvent. The absorbance of

Table 1: Calibration curve data and Absorptivities of Lornoxicam

Conc. (µg/ml)	Absorbance		Absorptivity Coefficient	
	At 384 nm	At 244 nm	a_{x1} At 384 nm	a_{x2} At 244 nm
2	0.202	0.207	101.00	103.50
4	0.403	0.401	100.75	100.25
6	0.605	0.603	100.83	100.50
8	0.802	0.805	101.00	100.65
10	1.021	1.012	102.10	101.20
MEAN			100.75	101.22
S.D			0.549	1.32

Table 2: Calibration curve data and Absorptivities of Paracetamol

Conc. (µg/ml)	Absorbance		Absorptivity Coefficient	
	At 384 nm	At 244 nm	a_{y1} At 384 nm	a_{y2} At 244 nm
20	0.401	0.368	20.050	18.400
30	0.603	0.580	20.100	19.300
40	0.816	0.802	20.400	20.050
50	1.002	1.112	20.040	22.240
60	1.290	1.320	21.500	22.000
MEAN			20.418	20.398
S.D			0.622	1.67

Table 3: Optical parameters & regression characteristic for Lornoxicam and Paracetamol

Parameters	Lornoxicam		Paracetamol	
	384 nm	244 nm	384 nm	244 nm
Beers's law limit (µg/ml)	2-10	2-10	20-60	20-60
Molar absorptivity (l $mole^{-1}cm^{-1}$)	3.7×10^4	3.8×10^4	0.3×10^4	0.3×10^4
Sandell's sensitivity (mg/cm^2/.001absorbance unit)	9.9×10^{-3}	9.6×10^{-3}	5.0×10^{-2}	5.4×10^{-2}
Regression equation (y= a + bc)				
slope (b)	0.1019	0.1007	0.0244	0.0198
intercept (a)	0.00045	0.00014	-0.138	0.011
Correlation coefficient (r^2)	0.9997	0.9999	0.9954	0.9993

Table 4: Value of absorptivities

a_{x1}	a_{x2}	a_{y1}	a_{y2}
100.75	101.22	20.418	20.398

Table 5: Specificity study for the synthetic mixture in 8:500 (LOR: PARA) ratio

Mix	Conc. (µg/ml)	λ_{max} (nm)	Before addition of excipients		After addition of excipients		% Interference
			Abs.	Conc. (µg/ml)	Abs.	Conc. (µg/ml)	
1	0.5+31.25	384	0.0505	0.49	0.055	0.54	10.20
		244	0.575	31.24	0.578	31.41	0.544
2	1.0+62.50	384	0.100	0.99	0.102	1.01	2.02
		244	1.250	64.65	1.245	64.39	0.402
3	2.0+125	384	0.201	1.99	0.207	2.04	2.51
		244	2.55	127.18	2.50	124.68	1.96
Mean	LOR						4.91
	PARA						0.96

the final solution was scanned in the range 400 to 200 nm against solvent as blank (Figure 1).

Determination of wavelength of maximum absorbance for Paracetamol

Standard paracetamol solution (1 ml) was transferred to separate 10 ml volumetric flask. The volume was adjusted to 10 ml with same solvent. The absorbances of the final solution were scanned in the range 400 to 200 nm against solvent as blank (Figure 2).

The above figure 3 reveals that both the drugs absorbs at the λ_{max} of each other so, it may be possible to determine both the drugs by Simultaneous equation method/Vierodt's method.

Preparation of calibration curve for Lornoxicam and Paracetamol

Table 6: Data showing Repeatability of absorbances

S. No.	Conc.	Wavelength (nm)	ABS.	Mean ± S.D.	% C.V.
1	(LOR 0.5 + PARA 31.25) µg/ml	384	0.0505	LOR	LOR
		244	0.575	0.0517±0.0009	1.80
2		384	0.0530		
		244	0.570		
3		384	0.0525		
		244	0.574		
4		384	0.0515	PARA	PARA
		244	0.570	0.573±0.003	0.536
5		384	0.0510		
		244	0.573		
6		384	0.0520		
		244	0.578		

Table 7: Data showing intra-day study

Conc. µg/ml	Time	WL (nm)	Absorbance (nm)			Conc. Found (µg/ml)			Mean ± S.D	% C.V.
			I	II	III	I	II	III		
0.5:31.25	9 AM	384	0.0550	0.0520	0.0545	0.54	0.53	0.54	0.54±	1.30
		244	0.578	0.579	0.576	31.41	31.39	31.42	0.007	
	12PM	384	0.0545	0.0560	0.0530	0.55	0.54	0.53		
		244	0.577	0.580	0.579	31.44	31.47	31.41	31.42±0.	0.081
	3 PM	384	0.0560	0.0545	0.0530	0.54	0.55	0.54	025	
		244	0.578	0.580	0.575	31.46	31.42	31.44		
1.0:62.5	9 AM	384	0.102	0.105	0.103	1.01	1.07	1.05		1.89
		244	1.240	1.235	1.245	64.39	64.35	64.37	1.03±	
	12PM	384	0.103	0.107	0.105	1.03	1.05	1.04	0.019	
		244	1.245	1.240	1.250	64.38	64.37	64.35	64.37±0.	0.025
	3 PM	384	0.104	0.102	0.105	1.01	1.04	1.05	016	
		244	1.240	1.250	1.245	64.39	64.35	64.38		
2.0:125	9 AM	384	0.207	0.209	0.208	2.04	2.05	2.03	2.05±0.0	0.73
		244	2.50	2.53	2.54	124.68	124.65	124.62	15	
	12PM	384	0.205	0.204	0.206	2.06	2.08	2.06		
		244	2.52	2.50	2.54	124.65	124.62	124.63	124.64±	0.017
	3 PM	384	0.206	0.205	0.204	2.04	2.05	2.04	0.021	
		244	2.54	2.55	2.52	124.67	124.63	124.65		
Mean	LOR									1.30
%C.V.	PARA									0.041

Standard solutions of Lornoxicam in the concentration range of 2 µg/ml to 10 µg/ml obtained by transferring (0.2, 0.4, 0.6, 0.8, 1.0 ml) of lornoxicam stock solution (100 ppm) to the series of 10 ml volumetric flasks and standard solutions of paracetamol in the concentration range of 20 µg/ml to 60 µg/ml were obtained by transferring (2.0,3.0,4.0,5.0,6.0 ml) of paracetamol stock solution (100 ppm) to the series of 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 384 nm and 244 nm against the solvent system as blank and calibration curves were plotted. The Lambert-Beer's Law is linear in concentration range of 2 to 10 µg/ml at 384 nm and 2 to 10 µg/ml at 244 nm for lornoxicam. The Lambert-Beer's Law is linear in concentration range of 20 to 60 µg/ml at 384 nm and 20 to 60 µg/ml at 244 nm for paracetamol.

Calibration Curve for Lornoxicam

It can be concluded that the method was linear and the Lambert – Beer's law was obeyed in concentration range of 2 to 10 µg/ml at 384 nm.

It can be concluded that the relationship between concentration and absorbance was linear in concentration range of 2 to 10 µg/ml at 244 nm.

Calibration Curve for Paracetamol

It can be concluded that the method was linear and the Lambert – Beer's law was obeyed in concentration range of 20 to 60 µg/ml at 384 nm

It can be concluded that the method was linear and the Lambert –Beer's law was obeyed in concentration range of 20 to 60 µg/ml at 244 nm.

RESULTS AND DISCUSSION

In the linearity study at respective wavelengths, the linear regression equation for Lornoxicam, calibration curve at 384 nm was calculated by y = 0.1019x - 0.0045, (r^2 = 0.999), where y is absorbance and x is the value of various concentrations of standard solutions and the

Table 8: Data showing inter-day study

Conc. µg/ml	Days	WL. (nm)	Absorbance (nm)			Conc. Found (µg/ml)			Mean ± S.D	% C.V.
			I	II	III	I	II	III		
0.5:31.25	DAY I	384	0.0550	0.0545	0.0540	0.54	0.54	0.55	0.54±0.010	1.85
		244	0.578	0.570	0.576	31.42	31.45	31.42		
	DAY II	384	0.0560	0.0550	0.0545	0.53	0.56	0.54	31.43±0.012	0.038
		244	0.575	0.574	0.578	31.44	31.45	31.43		
	DAY III	384	0.0545	0.0550	0.0560	0.55	0.56	0.54		
		244	0.578	0.575	0.577	31.45	31.43	31.44		
1.0:62.5	DAY I	384	0.102	0.105	0.104	1.01	1.05	1.06	1.04±0.018	1.73
		244	1.240	1.245	1.240	64.39	64.37	64.33		
	DAY II	384	0.105	0.103	0.107	1.02	1.05	1.03	64.37±0.020	0.032
		244	1.240	1.244	1.247	64.38	64.35	64.39		
	DAY III	384	0.102	0.104	0.105	1.03	1.06	1.05		
		244	1.250	1.245	1.240	64.39	64.38	64.36		
2.0:125	DAY I	384	0.207	0.208	0.210	2.04	2.06	2.05	2.05±0.016	0.821
		244	2.50	2.45	2.48	124.68	124.63	124.65		
	DAY II	384	0.209	0.208	0.207	2.06	2.04	2.05	124.64± 0.026	0.021
		244	2.54	2.48	2.49	124.65	124.68	124.63		
	DAY III	384	0.205	0.204	0.207	2.06	2.09	2.08		
		244	2.54	2.55	2.50	124.60	124.62	124.65		
Mean	LOR									1.467
% C.V.	PARA									0.030

Table 9: Limit of detection and quantification

S. No.	(x) Slope LOR	(x) Slope PARA	(y) Intercept LOR	(y) Intercept PARA	L.O.D	L.O.Q
1	0.101	0.019	0.0045	0.011	LOR	LOR
2	0.103	0.018	0.0046	0.012	0.119	0.362
3	0.101	0.019	0.0045	0.011		
4	0.101	0.017	0.0045	0.013	PARA	PARA
5	0.102	0.018	0.0046	0.012	0.0693	0.210
6	0.101	0.018	0.0045	0.011		
Mean	0.1015	0.018				
S.D.			0.001414	0.001211		

linear regression equation for lornoxicam calibration curve at 244 nm was calculated by y = 0.1007x + 0.0014 (r^2 = 0.999).

Moreover, in the linearity study at consecutive wavelengths, the linear regression equation for Paracetamol, calibration curve at 384 nm was calculated by y = 0.0244x - 0.138 (r^2 = 0.995) and the linear regression equation for Paracetamol calibration curve at 244 nm was calculated by y = 0.0198x + 0.011 (r^2 = 0.999).

Determination of optical parameters

The molecular absorptivity and Sandell's sensitivity were calculated as

Molecular absorptivity (Є) = AM/ct Sandell's Sensitivity = M/Є

Here A = Absorbance, M = molecular weight, M = Molecular weight, Є = Molecular absorptivity, C = Concentration of sample, t = path length

Other optical parameters i.e. Beer's limit, slope, intercept and correlation coefficient were calculated from calibration curve.

The results are shown in Table 3.

Preparation of synthetic mixture of Lornoxicam and Paracetamol

The synthetic mixture of Lornoxicam and Paracetamol was prepared in ratio of 0.5 :31.25 Accurately weighed 16 mg of lornoxicam and 1000 mg of paracetamol were transferred to 1000 ml volumetric flask, 700 ml of solvent system was added, dissolved and made the volume up to mark, then 10 ml of above solution was transferred into 100 ml volumetric flask. Common excipients, 8 % starch, 2 % magnesium stearate, 2 % talc and 84 % lactose (for 1000 µg/ml) which were used in tablet formulation, were added in this mixture and sonicated for 20 minutes. This solution was filtered through the Whatmann filter paper No. 41 and residues were washed with solvent system. The filtrate and washings were combined and volume was made-up to the 100 ml with solvent system. Then 1 ml of the solution was diluted to 100 ml with the solvent system. The decision of this ratio of drugs in the synthetic mixture was based upon the dosage strength of combination, which is available in the market.

Estimation of Lornoxicam and Paracetamol in synthetic mixture

The synthetic mixture (0.2,0.3,0.4,0.5,0.6,0.7,0.8)ml was transferred to a series of seven 10 ml volumetric flasks separately and volume was made up to the mark with

Table 10: Data showing Recovery study

S.No.	WL. (nm)	Conc.of tablet sol^{n} (ppm)	Std. Added (ppm)	Abs.	Amt. Found (mg)	% Recovery	Mean Recovery ± S.D.	% C.V.
1	384	6.23	4.80	0.525	5.198	94.25204	LOR	LOR
	244	40.15	32.00	0.729	36.359	100.7872	97.848 ±	0.003
	384	6.23	4.80	0.528	5.228	94.79601	0.321	
	244	40.15	32.00	0.726	36.209	100.3714		
	384	6.23	4.80	0.526	5.208	94.43336	PARA	PARA
	244	40.15	32.00	0.7236	36.09	100.0416	100.228	0.023
2	384	6.23	6.00	0.617	6.109	99.82026	±2.323	
	244	40.15	40.00	0.804	40.1	100.0624		
	384	6.23	6.00	0.621	6.149	100.4739		
	244	40.15	40.00	0.8072	40.259	100.4591		
	384	6.23	6.00	0.613	6.069	99.16667		
	244	40.15	40.00	0.8022	40.01	99.8378		
3	384	6.23	7.20	0.675	6.683	99.52345		
	244	40.15	48.00	0.885	44.14	99.97735		
	384	6.23	7.20	0.671	6.644	98.94267		
	244	40.15	48.00	0.890	44.389	100.5413		
	384	6.23	7.20	0.673	6.663	99.22561		
	244	40.15	48.00	0.885	44.14	99.97735		

Table 11: Statistical analysis for Neucam-P Tablet

S. No.	Absorbance Data		Conc. Found in µg/ml		Labelled amount in tablet (mg/tablet)		Amount found in (mg/tablet)	
	384 nm	244 nm	LOR	PARA	LOR	PARA	LOR	PARA
1	0.0505	0.6265	0.500	31.247	8.00	500.00	8.00	499.95
2	0.0509	0.6273	0.504	31.287	8.00	500.00	8.06	500.59
3	0.0512	0.6259	0.507	31.217	8.00	500.00	8.11	499.47
4	0.0506	0.6264	0.501	31.242	8.00	500.00	8.02	499.87
5	0.0513	0.6270	0.508	31.272	8.00	500.00	8.13	500.35
Mean			0.504	31.253			8.06	500.05

(For LOR 0.5 µg/ml; PARA 31.25 µg/ml respectively)

Table 12: Summary of Optical Parameters and Regression Characteristics of Lornoxicam and Paracetamol by UV Method

Parameters	Lornoxicam		Paracetamol	
	384 nm	244 nm	384 nm	244 nm
Beers's law limit (µg/ml)	2-10	2-10	20-60	20-60
Molar absorptivity (l mole^{-1}cm^{-1})	3.7 x 10^{4}	3.8 x 10^{4}	0.3 x 10^{4}	0.3 x 10^{4}
Sandell's sensitivity (mg/cm^{2}/.001absorbance unit)	9.9 x 10^{-3}	9.6 x 10^{-3}	5.0 x 10^{-2}	5.4 x 10^{-2}
Regression equation (y= a + bc)	0.1019	0.1007	0.0244	0.0198
slope (b)	0.00045	0.00014	-0.138	0.011
intercept (a)				
Correlation coefficient (r$^{2)}$	0.9997	0.9999	0.9954	0.9993

solvent system. The absorbances of these solutions were measured at 384 nm and 244 nm.

At 384 nm and 244 nm two simultaneous equations were formed using absorptivity coefficient values.

Two simultaneous equations were formed using the obtained absorptivity coefficient values. 100.75 and 20.627418 are absorptivities at 384 nm for Lornoxicam and Paracetamol respectively, while 101.22 and 20.398 are absorptivities at 244 nm for Lornoxicam and Paracetamol respectively.

The synthetic mixture of the combination of both the drugs was prepared in the ratio of 0.5:31.25 (lornoxicam: paracetamol). The decision of this ratio of drugs in the synthetic mixture was based upon the dosage strength of formulation in combination, which is available in the market. Now the absorbance of the synthetic mixtures

Table 13: Summary of Validation Parameters of UV Method

Parameter	Observation	
	Lornoxicam	Paracetamol
Specificity	No interference was found w.r.t. excipients	
Linearity (Correlation coefficient r)	0.999	0.999
Range	0.362 – 10	0.210 – 60
Accuracy* (% Recovery)	99.361 %	99.498 %
Precision RSD**		
Repeatability (n= 6)	1.80	0.536
Intra-day (n=3)	1.30	0.041
Inter-day (days=3)	1.46	0.030
LOD (Limit of Detection)	0.119	0.069
LOQ (Limit of Quantitation)	0.362	0.210

* Acceptance Criteria 90-107 %. ; **Acceptance Criteria: RSD ≤ 2 %

were measured at two wavelengths and the concentration of lornoxicam and paracetamol were calculated using following two equations.

Validation of the Developed Method According to ICH Guidelines[19]

Following parameters were taken into consideration for validation of proposed methods

Specificity

Method: The synthetic mixture of Lornoxicam and Paracetamol was prepared in ratio of 0.5:31.25. Accurately weighed 16 mg of Lornoxicam and 1000 mg of Paracetamol were transferred to 1000 ml volumetric flask, and 700 ml of solvent system was added. Common excipients, such as 8% of starch, 2% of magnesium stearate and 84% of lactose and 2% talc (for 1000 µg/ml) which were used in tablet formulation, were added in this mixture and sonicated for 10 minutes. This solution was filtered through the Whatmann filter paper and residues were washed with solvent system. The filtrate and washings were combined and volume was made-up to the 100 ml with solvent system.

Then 10 ml of the solution was diluted to 100 ml with the solvent system. From this stock solution, synthetic mixture (0.8, 1, 1.2, 1.4 ml) were transferred to a series of four 10 ml volumetric flasks separately and volume was made upto the mark with solvent system. The absorbances of these solutions were measured at 384 nm and 244 nm which are shown in table 5. The decision of this ratio of drugs in the synthetic mixture was based upon the dosage strength of combination, which is available in the market.

The results are shown in Table 5.

The results obtained for the specificity study from five samples studies (n = 3) after addition of excipients had a very small change in concentration from the concentration before addition of excipients. It can be concluded from the results that developed method is specific as percent interference was found to be 4.91 and 0.96 for Lornoxicam and Paracetamol respectively.

Precision

Repeatability was assessed using: Six time repetition of target concentration 100 % that is (0.5+31.25 µg/ml).

Intermediate precision can be assessed by intra-day and inter day analysis.

Repeatability

Method: It was conducted on the solution which has the concentration value of 100 % of the target concentration (n = 6).

The results are shown in Table 6.

Repeatability study showed a R.S.D of 1.80 % of lornoxicam and 0.536 % of paracetamol. It is concluded that the analytical technique showed a good repeatability precision.

Intra-Day Precision

Method: In the study of the intra-day which was conducted at three different time such as 9 am, 12 pm, 3 pm on the solution having the concentration value 80%, 100 %& 120% of the target concentration (n = 3). The results are shown in Table 7.

Intraday study showed a R.S.D of 1.30% for lornoxicam and a R.S.D of 0.041% for paracetamol thus showing that the analytical technique had a good intraday precision.

Inter-Day Precision

Method: In the study of the inter-day which was conducted on the solution having concentration value 80%, 100 %& 120% of the target concentration (n = 3), at three different days. The results are shown in Table 8.

The R.S.D of 1.467 % for lornoxicam and a R.S.D of 0.030 % for paracetamol has been found thus showing that the analytical technique had a good inter-day precision.

Linearity

Linearity range was found to be 2-10 µg/ml for lornoxicam at 384 nm and 244 nm. The correlation coefficient was found to be 0.999 & 0.999 which reveals good linearity between above range. The slope was found to be 0.101 & 0.100 and intercept was found to be 0.0045 & 0.0014 which were close to zero intercept.

For paracetamol at 384 nm and 244 nm linearity range was found to be 20-60 µg/ml .The correlation coefficient was found to be 0.999 & 0.999 which adhere good linearity between above range. The slope was found to be 0.024 & 0.019 and intercept was found to be 0.138 & 0.011 which were close to zero intercept.

Range

Range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. It includes working range, linearity range and target range and 100% concentration or test concentration.

Working range: It begins from limit of quantification to the maximum concentration used for the development of the analytical method. In this case it is found to be 0.362

to 10 µg/ml & 0.210 to 60 µg/ml for lornoxicam and paracetamol respectively.

Linearity range: It is the interval in which the response is directly proportional to the concentration between the upper and lower levels including the level (which is generally ± 5% of the intercept having slope equal to zero). In this case it is equal to 2-10 µg/ml & 20-60 µg/ml for lornoxicam and paracetamol.

Target range: It is that concentration which is 80%, 100% and 120% of the target concentration. In this case these are equal to 4.8 µg/ml, 6 µg/ml and 7.2 µg/ml for Lornoxicam and 32 µg/ml, 40 µg/ml and 48 µg/ml for paracetamol.

Target concentration: It is defined as the concentration, which is equal to the midpoint of linearity range. It is equal [(10 +2)/2] =6 µg/ml for Lornoxicam and [(60 +20)/2] =40 µg/ml for paracetamol respectively.

Limit of detection and limit of quantification

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The results are shown in Table 9.

The LOD was found to be 0.119 µg/ml and 0.069 µg/ml and LOQ was found to be 0.362 µg/ml and 0.210 µg/ml for lornoxicam and paracetamol respectively which represents that sensitivity of the method is high.

Accuracy: The results of analysis, obtained in three group containing three replicate experiments with API and different tablet dosage forms, had good agreement with the labeled amount of the drug.

Method: In nine different 10 ml volumetric flasks, 2.5 ml of the pre-analyzed tablet solution (100 µg/ml) was taken and added 1, 2, 3 ml of standard solution of bulk (API) mixture (1000µg/ml) and the volume was made up to 10 ml with 8M urea solution. The results are shown in Table no.10.

The results obtained for the accuracy study (recovery method) from three sample studies (n = 3) for each level indicated that the mean of the % recovery was 97.848% and 100.228% and R.S.D was 0.003 % and 0.023 % for lornoxicam and paracetamol respectively in synthetic mixture (LOR 8 µg/ml: PARA 500 µg/ml)

Estimation of Lornoxicam and Paracetamol in Tablet dosage form

Twenty tablets were taken and the I.P. method was followed to determine the average weight.

Method: Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 14 mg of drug was transferred to 100 ml volumetric flask, and mixed with 70 ml of urea solution and solution was sonicated for 10 minutes there after volume was made up to 100 ml with same solvent. The solution was filtered through Whatmann filter paper No. 40. From the filtrate, 10 ml in each was transferred to five different 10 ml volumetric flasks. The absorbances of these solutions were measured at 384 nm and 244 nm using 8M urea as blank. The Percentage of label claim for lornoxicam and paracetamol tablets was determined by Vierodt's method. The results are shown in Table 11.

The developed method was evaluated in the assay of commercially available tablets containing 8 mg of lornoxicam and 500 mg of paracetamol. The Tablet content amount was found to be 8.06 mg/tab for lornoxicam and 500.05 mg/tab for paracetamol by Vierodt's method.

CONCLUSION

A new, sensitive, accurate, precise UV-spectroscopic method was developed for the simultaneous estimation of Lornoxicam and Paracetamol as API and in tablet dosage form. The summary of results is shown in Table 12 and 13.

Industrial Application

The proposed method is new, simple, economically, accurate, safe and precise. It can be successfully employed in routine analysis of Lornoxicam and Paracetamol as API and in tablet dosage form. The main advantage of this method is that we use water as a solvent which decrease the cost of routine analysis.

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