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Analytical Method Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Paracetamol and Tolperisone Hydrochloride Drug in Pure and Pharmaceutical Dosage Form

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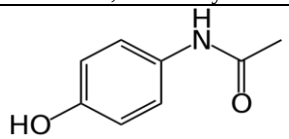
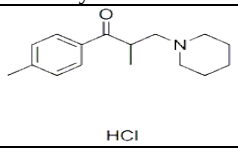
Abstract: ***Two methods for simultaneous estimation of Paracetamol and Tolperisone Hydrochloride in combined tablet dosage form have been developed using Buffer, Acetonitrile and Methanol as a solvent. The first UV spectrophotometric method was a determination using the simultaneous equation method at 246.0 nm and 260.0 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation (isoabsorptive point) at 258.0 nm the maximum absorption of Tolperisone Hydrochloride. The linearity ranges for Paracetamol and Tolperisone Hydrochloride range of 26.96 – 80.88 µg/ml and 12.40 – 37.21 µg/ml respectively. The accuracy of the methods was assessed by recovery studies was found to be 102.03 ± 3.86 and 98.92 ± 0.89 for simultaneous equation method and 100.6 ± 1.80 and 99.41 ± 1.29 for Q analysis (absorption ratio) method for Paracetamol and Tolperisone Hydrochloride respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.***

Keywords: Paracetamol, Tolperisone Hydrochloride, Q–analysis spectrophotometric method ICH

1. Introduction

The Paracetamol is also known as Acetaminophen is a medicine used to treat pain and fever it is typically used for mild to moderate pain relief also used to relieve fever in children, in combination with opiod pain medication, paracetamol is also used for severe pain such as cancer pain and pain after surgery. It is classified as a mild analgesic, Paracetamol is used in reducing fever in all ages of people and children and treatment for people with arthritis pain of the hip bone, hand and knee. Paracetamol adversely effects of more likely to have abnormal liver function tests acute over doses of paracetamol can cause potentially fatal liver damage. Pharmacologically of paracetamol unlike non steroidal anti inflammatory drugs such as aspirin, paracetamol does not appear to inhibit the function of any cyclooxygenase COX enzyme outside the CNS and this appear be the reason why it is not useful as an anti inflammatory. This activity does not appear to direct inhibition by blocking an active site but rather by reducing COX. The Tolperisone Hydrochloride is a Piperdine derivative is a centrally acting muscle relaxant indicated for use in the treatment of pathologically increased tone of the cross striated muscle caused by neurological disease, damage of the multiple sclerosis, myelopathy, pyramidal tract, encephalomyelitis and of spastic paralysis and other uses spondylosis, spondylarthrosis, cervical and lumber syndromes, arthrosis of the large joints. Contraindication of the Tolperisone Hydrochloride should not be used in patients with myasthenia gravis regarding the safety in children, youths during pregnancy and breastfeeding. Side effects fever than one percent of patients and include muscle weakness, headache, nausea, vomiting and dyspepsia. Tolperisone Hydrochloride interactions with other pharmaceutical drug that combination with other centrally acting muscle relaxant, benzodiazepines and non steroidal anti inflammatory drugs. The Pharmacokinetic ally is absorbed nearly completely from the gut and reaches its peak blood plasma concentration after one and half hours it is extensively metabolized in the liver and kidneys excretion half life first with two hours and second is twelve hour and in short description is shown in given table no.1.

Table 1: Introduction of Paracetamol and Tolperisone Hydrochloride:

Overview of Drug	Paracetamol	Tolperisone Hydrochloride
Description	Odorless, Bitter taste, White Crystalline Powder.	White Crystalline Powder.
Structure		
IUPAC Name	N-(4-Hydroxyphenyl) acetamide.	2-Methyl-1-(4-methylphenyl)-3-piperidin-1-one; hydrochloride.
Molecular Formula	$C_8H_9NO_2$	$C_{16}H_{23}NO.HCL$
Molecular Weight	151.163 g/mol	281.82 g/mol
Average Mass	151.163 Da	281.82 Da

Category	Analgesic, Antipyretic action.	Muscle relaxant, Non-steroidal anti-inflammatory drugs (NASAIDs.
Mechanism of Action	It primarily act in the CNS, increasing the pain threshold by inhibiting COX-I, COX-II & COX-III enzymes involved in PG synthesis, no peripheral anti-inflammatory effects.	Acts at the level of spinal cord by blocking Na+ channels and Ca+ channels. It exerts its spinal reflex inhibitory action predominantly via a pre synaptic inhibition of the transmitter release from the primary afferent endings via a combined action on Voltage-gated Na+ and Ca+ channels.

Experimental Equipments:
The analysis of the drug was performed on a HPLC system Waters 2695 and Photodiode array detector 2996 was used and RP-HPLC column Hypersil BDS, (C18, 250 mm x 4.6 mm I.D. particle size 5 µm) was used. The output of signal was monitored using waters Empower software.

2. Materials and Methods

Chemical and Reagents:
The pharmaceutically pure standard sample of Paracetamol and Tolperisone Hydrochloride were obtained as gift samples from Emcure Pharmaceutical Ltd. Pune. The percent purity of the drug was evaluated by obtaining its melting point, uv- spectroscopy and Infrared (IR) spectra. HPLC-grade Acetonitrile, Methanol was from Merck. A tablet formulation of Paracetamol and Tolperisone Hydrochloride (Mytop-P 150 mg and 325 mg label claim) were procured from local market.

Buffer And Mobile Phase Preparation
Weight accurately about 1.57 gm of Potassium dihydrogen phosphate dissolve in 1000 ml Milli-Q-Water Mix and adjust pH 3.0 using Ortho phosphoric acid and Sodium hydroxide filter the solution through 0.45µm membrane filter.

Mobile Phase Preparation
Then prepare a filtered and degassed mixture of Buffer, Acetonitrile and Methanol in the ratio of 80: 15: 5 v/v respectively.

Diluent Preparation
The Mobile Phase is used as a Diluent.

Standard Preparation
Weight accurately 54.0 mg of Paracetamol and 25.0 mg of Tolperisone hydrochloride and transferred to 100 ml volumetric flask adds 70 ml of diluent and sonicates up to dissolve and make up the volume up to 100 ml with diluent. Pipette out 5 ml from the above solution and transfer into 100 ml volumetric flask and make up the volume with diluent. Filter through 0.45µm nylon filter.

Chromatographic Condition
A Hypersil BDS, (C18, 250 mm x 4.6 mm I.D. particle size 5 µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at flow rate 1.0ml / min. the sample injection volume was 20µl. the photodiode array detector (i.e. PDA Detector) was set a wavelength of 258nm for the detection and chromatographic run time was 15 minutes.

3. Result and Discussion

Method Development

Determination of Wavelength
Weigh accurately 50.0 mg Paracetamol and Tolperisone Hydrochloride Separately 50 ml of methanol. Take 5 ml of above solution in 25 ml volumetric flask and make up the volume with methanol. Scanned the UV- visible spectra range from 400 – 200 nm and determine the λ max of Paracetamol and Tolperisone Hydrochloride separately using methanol as a blank and determine the Isobestic point. The λ max of Paracetamol was to be 246.0 nm and λ max of Tolperisone Hydrochloride was found to be 260.0 nm. The Isobestic point of Paracetamol and Tolperisone Hydrochloride was found to be 258.0 nm UV Graph shown in Fig. No.1:

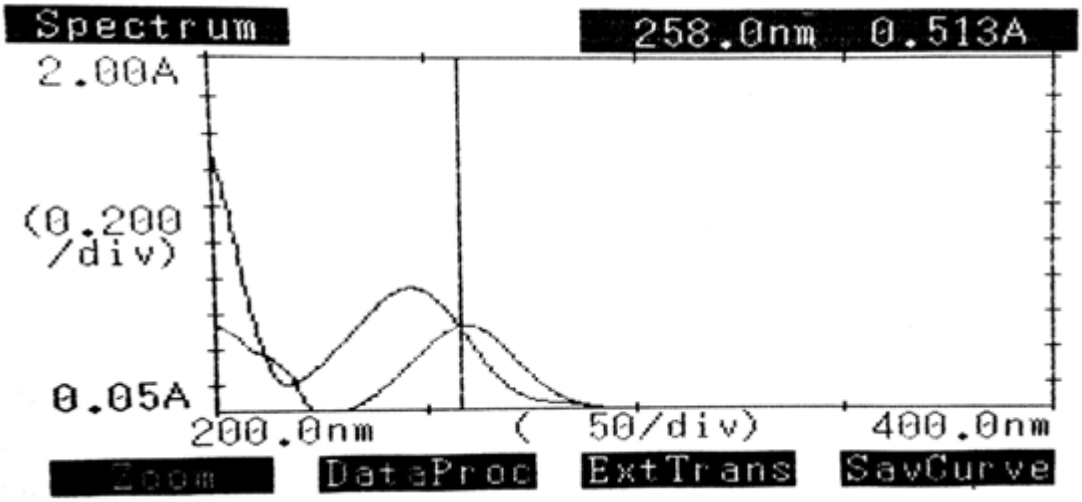


Figure 1

Method Optimized:

The UV- visible spectroscopic of Paracetamol and Tolperisone Hydrochloride Isobestic point at 258.0 nm the chromatographic detection was performed at 258.0 nm using a photo diode array detector as Paracetamol and Tolperisone Hydrochloride is a good result to develop a suitable and robust LC method for the determination of Paracetamol and Tolperisone Hydrochloride were optimized the mobile phase was determined as a mixture of (pH 3.0 by OPA) Buffer, Acetonitrile and Methanol (80:15:5 v/v) at a flow rate 1.0 ml/min under these conditions were eluted at 3.97 and 7.91 minutes respectively with a run time 15 minutes shown in Figure No.2.

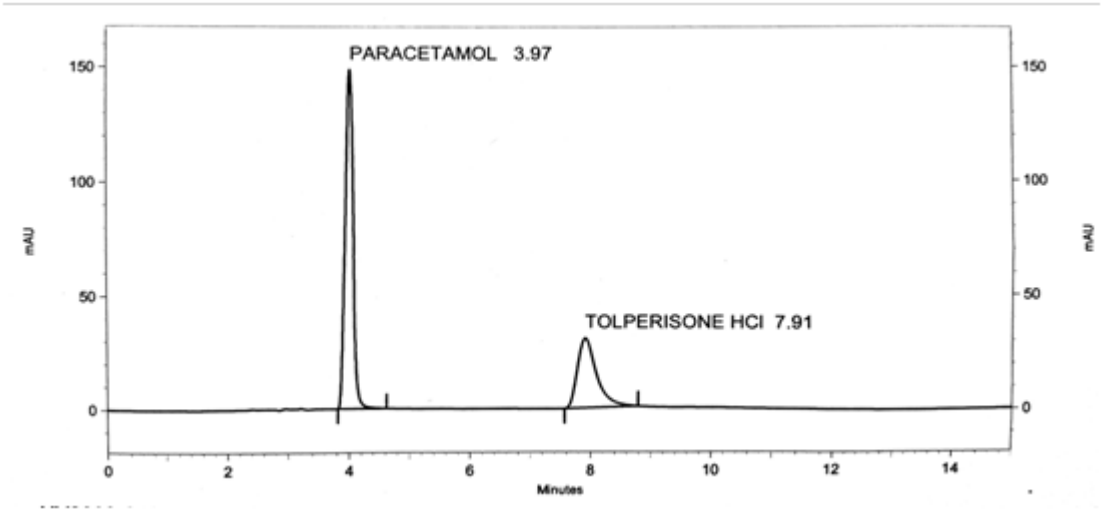


Figure 2

Method Validation

Specificity

Sample Preparation (Tablet Formulation):

Weight of 20 Tablet and crushed finely powdered accurately weighed equivalent to 54.0 mg of Paracetamol and 25.0mg of Tolperisone hydrochloride and transferred into 100 ml volumetric flask added 75 ml of diluent and sonicate for 15.0 minutes with intermediate shaking at controlled temperature and make up the volume with diluent and well mixed. Filtered the Sample solution through whatman filter paper No.41. Take 5 ml of above solution and transfer into 50 ml volumetric flask and make up the volume with diluent shown given table no.2.

Table 2: Assay of Tablet Formulation:

No of Injection	Mean Peak Area (Parace-tamol)	% Assay (Paracetamol)	Mean Peak Area (Tolperisone Hydrochloride)	% Assay (Tolperisone Hydrochloride)
1	2793099	99.39	1403348	99.23
2	2804059	99.52	1490473	99.11
3	2808716	99.68	1414008	99.57
4	2811606	100.12	1395371	99.76
5	2801932	99.6	1407160	100.12
6	2819721	100.22	1400119	99.72
Mean		**99.76**		**99.59**
SD		**0.33**		**0.37**
% RSD		**0.34**		**0.38**
Theoretical Plate		**4526**		**3412**

Linearity:

The peak area of Paracetamol and Tolperisone Hydrochloride showed linear calibration curve with respect to concentrations over the range of 26.96 – 80.88 µg/ml and 12.40 – 37.21 µg/ml for Paracetamol and Tolperisone Hydrochloride respectively. The linear regression equations were y = 67785 x + 676092 and correlation coefficient 0.9994 for Paracetamol and y = 36571 x + 310760 and correlation coefficient is 0.9997 for Tolperisone Hydrochloride. Where x is the concentration in µg/ml and y is the peak absorbance in units. The linearity of detector response for Paracetamol and Tolperisone Hydrochloride was established by injecting a series of solutions at the concentration ranging from 25% to 150% level of test concentration with an interval at 50%, 75%, 100%, 125% and 150% of target concentration shown in given Table no. 3 and figure no.3 and figure no 4. Linearity curve for Paracetamol and Tolperisone Hydrochloride respectively.

Table 3: For Linearity Paracetamol and Tolperisone Hydrochloride

Sr. No.	Linearity Solution	Concentration (µg/ml)	Mean Area	STD Deviation	% RSD
Paracetamol					
1	50%	6.25	1362552	2112.012	0.17
2	75%	9.75	2032164	10182.49	0.51
3	100%	13.00	2713816	2918.002	0.12
4	125%	16.25	3343496	12032.98	0.38
5	150%	19.50	4096108	17431.65	0.42
Tolperisone Hydrochloride					
1	50%	3.00	683997	9173.06	1.33
2	75%	4.50	1037716	5794.44	0.56
3	100%	6.00	1406303	2601.59	0.19
4	125%	7.50	1760162	9897.56	0.57
5	150%	9.00	2151345	8614.95	0.39

Linearity Curve for Paracetamol

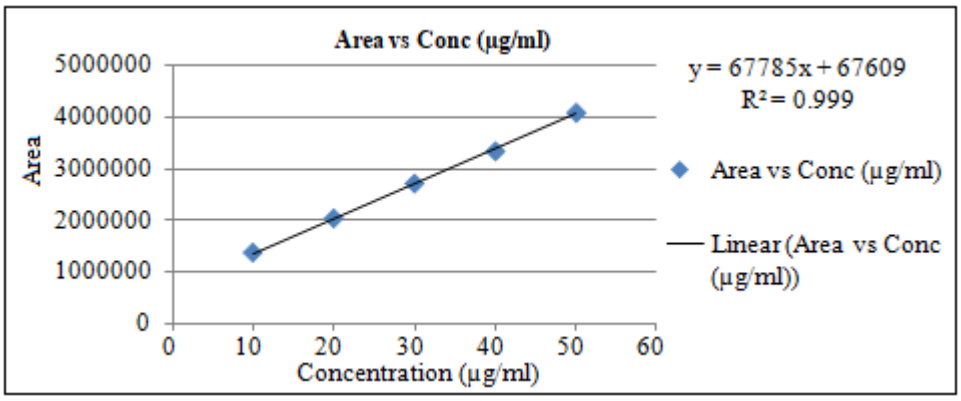


Figure 3

Linearity Curve for Tolperisone Hydrochloride

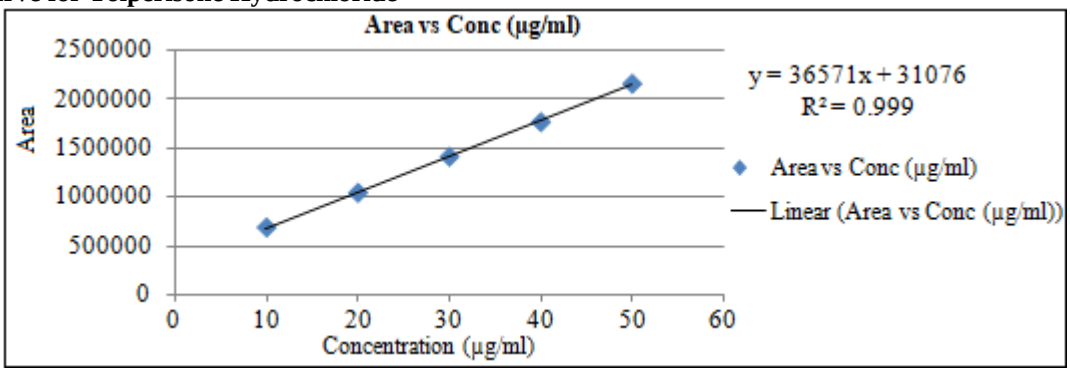


Figure 4

Accuracy:

The accuracy of the method recovery studies were carried out by addition of standard drug solution to sample solution was determined on fifth concentration levels to 50%, 75%, 100%, 125% and 150% of formulation the reference standard of paracetamol and Tolperisone Hydrochloride analyzed as per proposed method, percentage recovery and percentage mean recovery were calculated. From the data obtained given in Table no. 4. The method was found to be accurate.

Table 4: Accuracy study for Paracetamol and Tolperisone Hydrochloride:

% Accuracy Level	Prepared Concentration (µg/ml)	Observed Concentration (µg/ml)	Mean Recovery (%)	% RSD
Paracetamol				
At 50 %	54.7	53.65	99.35	0.61
At 75 %	81.87	81.63	99.44	0.37
At 100 %	106.67	106.05	98.99	0.28
At 125 %	135.5	134.78	99.12	0.46
At 150 %	162.4	161.34	99.18	0.43
Tolperisone Hydrochloride				
At 50 %	23.63	23.28	99.21	1.28
At 75 %	36.34	36.16	99.41	1.47
At 100 %	47.64	47.05	99.11	0.36
At 125 %	59.94	59.08	98.89	1.26
At 150 %	72.15	71.91	98.86	1.25

Precision:

The agreement between true value and the value founded opinions (degree of scatter) between a series of measurements repeat ability different time (same test) was studied method repeatability was achieved from % RSD values obtained by repeating the assay six sample replicate on the same day for inter day precision. The intermediate intraday precision of the method was check by performing same procedure on the different days under the same experimental procedure and prepared individually of Paracetamol and Tolperisone Hydrochloride as per test method and inject each solution into HPLC. The calculation and result shown in given Table no. 5.

Table 5: Method precision (Interday and Intraday) studies for Paracetamol and Tolperisone Hydrochloride by Proposed Method

Summary Showing Method Precision by Proposed Method				
	For Paracetamol		For Tolperisone Hydrochloride	
	Method Precision of Inter day & Intra day		Method Precision of Inter day & Intra day	
	99.21	99.21	98.91	99.31
	99.25	99.79	99.38	99.42
	99.31	99.65	99.04	99.56
	99.12	99.28	99.32	99.35
	99.13	99.22	99.64	99.21
	99.28	99.78	98.86	99.13
Mean	**99.22**	**99.49**	**99.19**	**99.33**
SD	**0.78**	**0.28**	**0.31**	**0.15**
% RSD	**0.8**	**0.29**	**0.31**	**0.16**

Robustness:

In the robustness study influences of different chromatographic conditions were evaluated by assaying test solution after small but deliberate changes in the analytical conditions. The method was determine by carrying out the

analysis under conditions during which flow rate (± 0.1 ml/min), mobile phase pH (±), wavelength (± 0.2 nm) and changes in mobile phase composition (± 5%) were determined for each variable and % RSD was calculated results of robustness study are shown in Table no.6.

Table 6: For Summary Data of Robustness Study of Paracetamol and Tolperisone Hydrochloride

Robustness Conditions	RT (min)	System Suitability Parameters		% Assay	% RSD
		Theoretical Plates	USP Tailing Factor		
Summary of Paracetamol					
Flow rate 0.9ml/min	4.38	4151	1.62	99.24	0.99
Flow rate 1.1ml/min	3.56	4543	1.81	99.11	0.19
Detection in Wavelength 256 nm	3.93	4485	1.87	99.28	1.28
Detection in Wavelength 260 nm	3.92	3889	1.96	99.21	1.29
Detection of pH 2.8	4.25	4042	1.42	99.24	0.71
Detection of pH 3.2	4.28	4455	1.36	99.22	0.54
Summary of Tolperisone Hydrochloride					
Flow rate 0.9ml/min	8.75	3375	1.62	99.98	1.3
Flow rate 1.1ml/min	7.09	3465	1.61	99.29	1.07
Detection in Wavelength 256 nm	7.77	3468	1.96	99.63	0.23
Detection in Wavelength 260 nm	7.75	3589	1.92	99.62	0.24
Detection of pH 2.8	8.58	3247	1.66	99.11	1.03
Detection of pH 3.2	8.49	3593	1.28	99.24	1.71

Ruggedness:

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the assay by two different analyst different column and different day. The ruggedness can be describe as the ability to reproduce the analytical method in different laboratories or under different analyst without the occurrence of unexpected differences in the obtained result in given Table no.7.

Table 7: The Ruggedness study for Paracetamol and Tolperisone Hydrochloride

Paracetamol	% Purity	Tolperisone Hydrochloride	% Purity
Analyst 01	99.82	Analyst 01	99.67
Analyst 02	99.64	Analyst 02	99.89
% RSD	0.13	% RSD	0.16
Column ID	3V, AD 470	3V, AD 470	
Day	05-04-2018	06-04-2018	

Limit of Detection and Limit of Quantification

The measurement of LOD value and LOQ value were performed by preparing the dilution of stock solution until the signal to noise ratio were LOD value of Paracetamol is 0.587µg/ml and Tolperisone Hydrochloride is 0.404µg/ml. and LOQ value of Paracetamol is 1.78µg/ml and Tolperisone Hydrochloride is 1.23µg/ml respectively shown in given table no. 8.

Table 8: For Value of LOD and LOQ:

Sr. No.	Name of Drug	LOD µg/ml	LOQ µg/ml
1	Paracetamol	0.587µg/ml	1.78µg/ml
2	Tolperisone Hydrochloride	0.404µg/ml	1.23µg/ml

System Suitability

The preparation of standard solution of 270 µg per ml of Paracetamol solution and 250 µg per ml of Tolperisone Hydrochloride solution was prepared and injected before all parameter of validation. The system suitability parameters like Theoretical plates, Resolution, Asymmetry were calculated and compared with the standard solution is given table no 9.

Table 9: System Suitability for Paracetamol and Tolperisone Hydrochloride

Injection No.	RT (min)	Tailing Factor	Theoretical Plate	Peak Area	RT (min)	Tailing Factor	Theoretical Plate	Peak Area
For Paracetamol					For Tolperisone Hydrochloride			
1	3.96	1.23	4514	2697736	7.98	1.69	3126	1432329
2	3.96	1.24	4536	2685767	7.91	1.21	4534	1427907
3	3.96	1.22	4588	2694098	7.90	1.65	3182	1428284
4	3.97	1.21	4598	2694097	7.90	1.68	3167	1419283
5	3.97	1.23	4476	2703242	7.98	1.67	3045	1436046
Mean				2694988	Mean			1428769
SD				6369.710	SD			6256.812
% RSD				0.24	% RSD			0.44

4. Conclusion

The RP- HPLC method was developed and validated for simultaneous estimation of Paracetamol and Tolperisone Hydrochloride in combined dosage form. The present analytical method was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, robust and having stability indicating characteristics. The present analytical method can be used for its intended purpose. The results of the validation tests were found to be satisfactory and therefore this method can be applied successfully to analyze drug formulations.

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