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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE AND PARACETAMOL IN BULK DOSAGE FORMS

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ABSTRACT

To guarantee the precision and dependability of analytical techniques used to ascertain the concentration of active pharmaceutical ingredients (APIs) in compounded dosage forms, method development is a methodical and crucial procedure. These techniques' reproducibility and suitability for producing data for submission are established through validation, which also guarantees adherence to legal criteria. The analytical assessment of two commonly used pharmaceutical substances, tolperisone and paracetamol, is the main emphasis of this work. A common analgesic and antipyretic, paracetamol is mostly used to treat mild to moderate fever and pain. Conversely, tolperisone, a derivative of piperidine, functions as a centrally acting muscle relaxant and is widely used to treat neurological disorders that cause elevated muscle tone, such as multiple sclerosis, myelopathy, encephalomyelitis, spastic paralysis, and pyramidal tract damage. These medications work in concert to provide a synergistic therapeutic impact in the treatment of muscle spasms and pain. This review looks at different analytical techniques for identifying tolperisone and paracetamol in pharmaceutical formulations and biological samples. There is discussion of methods like liquid chromatography-mass spectrometry (LC-MS), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and spectrophotometry. The study highlights the advantages and disadvantages of different techniques by assessing them according to their sensitivity, accuracy, and suitability for pharmaceutical investigation.

KEYWORDS: Paracetamol, Tolperisone, analytical methods, muscle relaxant, HPLC, pharmaceutical analysis.

INTRODUCTION

Tolperisone is a muscle relaxant with a distinctive ability to alleviate muscle tension without causing sedation, incoordination, weakness, mental confusion, or withdrawal symptoms, unlike other drugs in its class. It acts as a cholinergic muscarinic antagonist and is also classified under gastrointestinal anticholinergic or antispasmodic agents based on its pharmacological properties.[1] Tolperisone hydrochloride is highly soluble in water, with increased solubility in acidic environments (pH < 4.5). However, it is prone to degradation in aqueous solutions, particularly at higher pH levels (2–4).[2]

The advantages of Tolperisone include a low adverse event profile, lack of sedation and no interaction with alcohol as well as no potential for tolerance and addiction. The structure of Tolperisone is given below.[3]

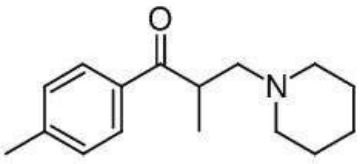


Figure 1: Structure of Tolperisone.

Table no. 1: Details of Tolperisone.

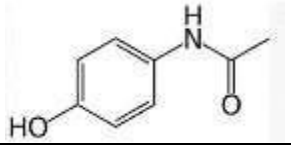
Property	Details
Molecular Formula	$C_{16}H_{23}NO \cdot HCl$
Molecular Mass	281.83 g/mol
IUPAC Name	2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride
Appearance	White crystalline powder
Nature	Basic
pKa	9.4
Solubility	- Soluble in water, methanol, chloroform, and ethanol
	- Slightly soluble in acetone
	- Insoluble in benzene and ether

MECHANISM OF ACTION

Tolperisone is a centrally acting muscle relaxant widely used for its therapeutic efficacy in managing muscle spasticity. Its mechanism of action primarily involves targeting the spinal cord, where it inhibits neural transmission by blocking sodium and calcium channels.[4] By

acting at this level, Tolperisone suppresses spinal reflexes through presynaptic inhibition, reducing the release of neurotransmitters from primary afferent nerve endings. This dual action on voltage-gated sodium and calcium channels contributes to its effectiveness in controlling excessive muscle activity.[5]

Table no. 2: Details of Paracetamol.

Parameter	Paracetamol
Description	Odorless, Bitter taste, White Crystalline Powder
Structure	
IUPAC Name	N-(4-Hydroxyphenyl) acetamide
Molecular Formula	C8H9NO2
Molecular Weight	151.163 g/mol
Average Mass	151.163 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.[6]

EXPERIMENTAL EQUIPMENTS

The analysis of the drug was conducted using a high-performance liquid chromatography (HPLC) system. The **Waters 2695 HPLC system** was equipped with a **Photodiode Array (PDA) Detector 2996**, providing high sensitivity and precise detection capabilities. The chromatographic separation was achieved on an **RP-HPLC Hypersil BDS C18 column** (250 mm × 4.6 mm I.D., particle size 5 µm), ensuring optimal performance for analyte resolution. The signal output was monitored and processed using **Waters Empower software** for data acquisition and analysis.[7]

The pharmaceutically pure standard samples of Paracetamol and Tolperisone Hydrochloride, were evaluated for purity using melting point determination, UV spectroscopy, and infrared (IR) spectroscopy.[8] HPLC-grade acetonitrile and methanol were obtained from Merck, and a commercial tablet formulation (Mytop-P, containing 150 mg Tolperisone Hydrochloride and 325 mg Paracetamol) was sourced from the local market. The buffer was prepared by

dissolving 1.57 g of potassium dihydrogen phosphate in 1000 mL of Milli-Q water, adjusting the pH to 3.0 with ortho-phosphoric acid and sodium hydroxide, and filtering through a 0.45 µm membrane.[9] The mobile phase, serving as the diluent, was a filtered and degassed mixture of buffer, acetonitrile, and methanol in an 80:15:5 (v/v) ratio. A standard solution was prepared by dissolving 54.0 mg of Paracetamol and 25.0 mg of Tolperisone Hydrochloride in 70 mL of diluent, sonicating to dissolve, and making up to 100 mL.[10] A working solution was then prepared by diluting 5 mL of this stock solution to 100 mL with the diluent and filtering through a 0.45 µm nylon filter.[11]

CONDITIONS FOR CHROMATOGRAPHY

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.[12]

RESULTS AND DISCUSSIONS

The wavelength determination for Paracetamol and Tolperisone Hydrochloride was conducted using UV-visible spectrophotometry. Accurately weighed 50.0 mg of each drug was dissolved separately in 50 mL of methanol. From these stock solutions, 5 mL was further diluted to 25 mL with methanol to prepare the working solutions.[13] The UV-visible spectra of these solutions were scanned over a wavelength range of 200–400 nm, with methanol as the blank. The maximum absorbance wavelengths (λmax) for Paracetamol and Tolperisone Hydrochloride were determined to be 246.0 nm and 260.0 nm, respectively. Additionally, the isosbestic point, indicating a common absorption wavelength for both drugs, was identified at 258.0 nm.[14]

The UV-visible spectroscopic analysis of Paracetamol and Tolperisone Hydrochloride revealed an isosbestic point at 258.0 nm, which was selected as the detection wavelength for chromatographic analysis using a photodiode array detector.[15] This wavelength provided optimal results for the development of a robust and reliable LC method for the simultaneous determination of both compounds. The mobile phase was optimized as a mixture of buffer (pH 3.0, adjusted using ortho-phosphoric acid), acetonitrile, and methanol in an 80:15:5 (v/v) ratio.[16] The method employed a flow rate of 1.0 mL/min, and under these conditions, Paracetamol and Tolperisone Hydrochloride were eluted at retention times of 3.97 minutes

and 7.91 minutes, respectively, with a total run time of 15 minutes.[17]

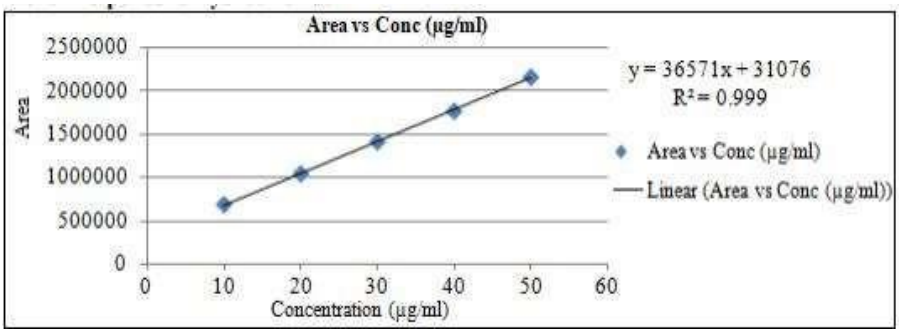
LINEARITY

The peak area responses for Paracetamol and Tolperisone Hydrochloride demonstrated linearity across the concentration ranges of 26.96–80.88 µg/mL and 12.40–37.21 µg/mL, respectively.[18] The linear regression equations were determined to be y=67785x+676092y = 67785x + 676092y=67785x+676092 with a correlation coefficient (r2r^2r2) of 0.9994 for Paracetamol, and y=36571x+310760y = 36571x + 310760y=36571x+310760 with an r2r^2r2 of 0.9997 for Tolperisone Hydrochloride, where xxx represents the concentration in µg/mL and yyy the peak absorbance.[19] Linearity was further validated by injecting solutions at concentrations ranging from 25% to 150% of the target concentration, with intervals at 50%, 75%, 100%, 125%, and 150% levels. The results confirmed the linear detector response for both drugs. The corresponding calibration curves and linearity data are depicted in Table 3.

Table no. 3: Linearity data.[20]

Sr. No.	Linearity Solution	Concentration (µg/mL)	Mean Area	STD Deviation	% RSD
Paracetamol					
1	50%	6.25	1,362,552	2,112.012	0.17
2	75%	9.75	2,032,164	10,182.49	0.51
3	100%	13.00	2,713,816	2,918.002	0.12
4	125%	16.25	3,343,496	12,032.98	0.38
5	150%	19.50	4,096,108	17,431.65	0.42
Tolperisone Hydrochloride					
1	50%	3.00	683,997	9,173.06	1.33
2	75%	4.50	1,037,716	5,794.44	0.56
3	100%	6.00	1,406,303	2,601.59	0.19
4	125%	7.50	1,760,162	9,897.56	0.57
5	150%	9.00	2,151,345	8,614.95	0.39

LINEARITY CURVE FOR TOLPERISONE HYDROCHLORIDE



ACCURACY

The accuracy of the developed method was evaluated through recovery studies by adding a standard drug solution to the sample solution at five concentration levels: 50%, 75%, 100%, 125%, and 150% of the formulation. The analysis was performed using the proposed method for both Paracetamol and Tolperisone Hydrochloride, and the percentage recovery and mean recovery values were calculated. Method provided accurate recovery for both drugs across the tested concentration levels, confirming its reliability and robustness for quantitative analysis.[21]

Table no. 4: Accuracy data.

% 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

The robustness of the method was assessed by evaluating the impact of deliberate, small variations in chromatographic conditions on the analysis of the test solution. The parameters studied included changes in flow rate (± 0.1 mL/min), mobile phase pH (± 0.1 units), detection wavelength (± 0.2 nm), and mobile phase composition (± 5%). The method's performance was analyzed under these varied conditions, and the % RSD values were calculated to evaluate consistency. The results demonstrated the method's robustness, as the % RSD values remained within acceptable limits, confirming the reliability of the method under minor variations in analytical conditions.[22]

The ruggedness of the method was evaluated by analyzing the assay under varying conditions, including different analysts, columns, and days. This study assessed the method's reproducibility across different laboratories or analysts without significant variation in the results, demonstrating its robustness.[23]

The sensitivity of the method was determined by evaluating the limits of detection (LOD) and quantification (LOQ) using serial dilutions of the stock solution until the signal-to-noise ratio was within acceptable limits. The LOD values were found to be 0.587 µg/mL for Paracetamol and 0.404 µg/mL for Tolperisone Hydrochloride, while the LOQ values were 1.78 µg/mL and 1.23 µg/mL, respectively. These results are presented in Table 8, confirming the method's ability to detect and quantify low concentrations of the analytes with precision.[24]

Table no. 5: Summary of Robustness Conditions for Paracetamol.

Condition	RT (min)	Theoretical Plates	USP Tailing Factor	% Assay	% RSD
Flow Rate 0.9 mL/min	4.38	4151	1.62	99.24	0.99
Flow Rate 1.1 mL/min	3.56	4543	1.81	99.11	0.19
Wavelength 256 nm	3.93	4485	1.87	99.28	1.28
Wavelength 260 nm	3.92	3889	1.96	99.21	1.29
pH 2.8	4.25	4042	1.42	99.24	0.71
pH 3.2	4.28	4455	1.36	99.22	0.54

Table no. 6: Summary of Robustness Conditions for Tolperisone Hydrochloride.

Condition	RT (min)	Theoretical Plates	USP Tailing Factor	% Assay	% RSD
Flow Rate 0.9 mL/min	8.75	3375	1.62	99.98	1.30
Flow Rate 1.1 mL/min	7.09	3465	1.61	99.29	1.07
Wavelength 256 nm	7.77	3468	1.96	99.63	0.23
Wavelength 260 nm	7.75	3589	1.92	99.62	0.24
pH 2.8	8.58	3247	1.66	99.11	1.03
pH 3.2	8.49	3593	1.28	99.24	1.71

Table no. 7: The Ruggedness study for Paracetamol and Tolperisone Hydrochloride.[25,26]

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

CONCLUSION

A robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Paracetamol and Tolperisone Hydrochloride in combined dosage forms. The method validation was performed in accordance with ICH Q2 (R1) guidelines, meeting all specified acceptance criteria. The results demonstrated that the method is specific, precise, linear, accurate, robust, and stability-indicating. Based on the satisfactory outcomes of the validation tests, this method is suitable for its intended purpose and can be reliably employed

for the analysis of drug formulations.

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