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R E S E A E C H A R T I C L E

Stability Indicating RP-HPLC Method for Simultaneous Estimation of Acetaminophen and Tramadol Hydrochloride in Tablet Dosage Form

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A B S T R A C T

A simple, Accurate, rapid and precise method was developed for the simultaneous estimation of the Acetaminophen and Tramadol hydrochloride in Tablet dosage form. The chromatogram was run through. Spurcil, column (4.6 x 150mm, 5µ).Mobile phase containing0.1%OPA: Acetonitrile is taken in the ratio 30:70 was pumped through the column at a flow rate of 1 ml/min. The buffer used in this method was 0.1%OPA buffer. The temperature was maintained at 25°C. Optimized wavelength selected was 280 nm. The retention time of Acetaminophen and Tramadol hydrochloride were found to 1.965 min and 3.826. % RSD of Acetaminophen and Tramadol hydrochloride were found to be 0.7 and 0.2 respectively. % Recovery was obtained at 100.14% and 99.92 for Acetaminophen and Tramadol hydrochloride respectively. LOD, LOQ values obtained from regression equations of Acetaminophen and Tramadol hydrochloride and were 3.03, 10.02 and 3.00, 10.00 respectively. The accuracy and reliability of the method were assessed by evaluation of linearity, precision (intra-day and inter-day % RSD >2 for Acetaminophen and Tramadol hydrochloride, accuracy and specificity, in accordance with ICH guidelines. This method has been successively applied to the pharmaceutical formulation and was validated according to ICH guidelines.

Key words: Acetaminophen and Tramadol hydrochloride, RP-HPLC.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Acetaminophen is generally considered to be a weak inhibitor of the synthesis of prostaglandins (PGs).however the in vivo effects of acetaminophen are similar to those of the selective cyclooxygenase-2(cox-2) inhibitors. Acetaminophen also decreases PG Concentrations In vivo. But and like the selective cox-2 inhibitors. Acetaminophen is aweak inhibitor of PG synthesis of cox-1 and cox-2 in broken cell systems, but, by contrast, therapeutic concentrations of acetaminophen inhibit PG synthesis in intact cells in vitro when the levels of the substrate arachidoinc acid are low (less than about 5 mmol/L).

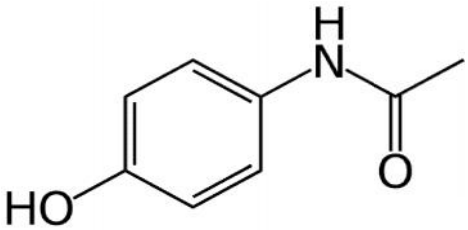

Fig 1: Structure of Acetaminophen

Tramadol and its o-dimethyl metabolite (M1) are selective, weak OP3-receptoragonists.opiate receptors are coupled with G protein receptors and function as both positive and negative regulators of synaptic transmission via G-proteins that activate effector proteins. As the effector system is adenylate cylase and Camp located at the inner surface of the plasma membrane, opioids decreases intracellular cAMP by inhibiting adenylate cyclase. Subsequently, the release of nociceptive neurotransmitters such as substance P, GABA, dopamine, acetylcholine and adrenaline is inhibited. Pharmacologically ,tramadol is similar to tapentadol and methadone in that it not only binds to the MOR,but also inhibits reuptake of serotonin and norepinephrine due to its action on the nor adrenergic and serotonergic systems ,such as its atypical opioid activity.

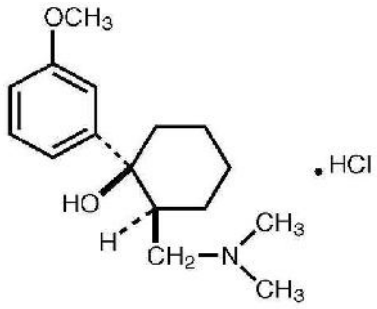

Fig 2: Structure of Tramadol Hydrochloride

The main objective of the study is to develop a method for the estimation of Acetaminophen and Tramadol hydrochloride in tablet dosage forms by RP-HPLC method by optimizing the chromatographic conditions and validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application. To apply the proposed method for the analysis of the drug in its dosages.

2. Materials and Methods

Instrument used

Water HPLC with proving software of empower and 2965 separation module with PDA detector. UV-Visible spectrophotometer for the model of Lab india3000+, pH meter and weighing balance.

Chemicals: Acetaminophen, Tramadol, KH2PO4, HPLC grade methanol, Acetonitrile, Water and Ortho phosphoric acid.

Chromatographic Conditions:

Table No 1: Chromatographic Conditions

Parameter	Description
Instrument used	Waters HPLC with auto sampler and UV detector
Temperature	Ambient (25^0c)
Mode of separation	Isocratic mode
Column	Spurcil, column (4.6 x 150mm, 5µ)
Buffer	0.1% OPA
Mobile phase	0.1% OPA: Acetonitrile (30: 70)
Flow rate	1 ml/min
Wavelength	280 nm
Injection volume	20 µ l
Run time	10 min

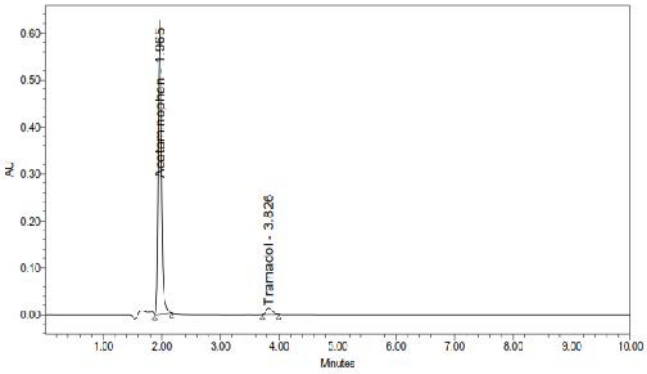

Fig 3: Optimized Chromatogram of Acetaminophen and Tramadol

Observation: The separation was good, peak shape was good, so we conclude that there is no required for increase the retention times of peak, so it is taken as final method.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of 0.1% OPA Buffer, 700 ml (70%) of Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation:

Accurately weigh and transfer 162.5 mg of Acetaminophen and 18.75 mg of Tramadol working standard into a 100ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 1.2 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 162.5 mg of Acetaminophen and 18.75 mg of Tramadol sample into a 100ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 30 µL of the standard, sample into the chromatographic system and measure the areas for Acetaminophen and Tramadol peaks and calculate the %Assay by using the formulae.

System Suitability Results:

Tailing factor for the peaks due to Acetaminophen and Tramadol in Standard solution should not be more than 2.0Theoretical plates for the Acetaminophen and Tramadol peaks in Standard solution should not be less than 2000. Resolution for the Acetaminophen and Tramadol peaks in standard solution should not be less than 2.

Method Validation:

Specificity:

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

Precision:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Acetaminophen & Tramadoland calculate the individual recovery and mean recovery values.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 162.5 mg of Acetaminophen and 18.75 mg of Tramadol working standard into a 100ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I:

0.4 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II:

0.8 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – III:

1.2 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – IV: 1.6 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Preparation of Level – V: 2.0 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Procedure: Inject each level into the chromatographic system and measure the peak area.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

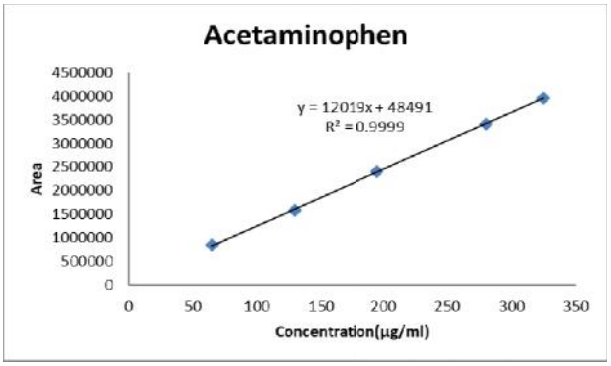

Fig 4: Calibration graph for Acetaminophen

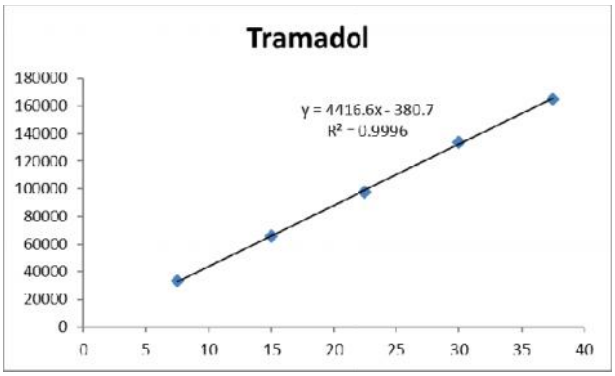

Fig 5: Calibration graph for Tramadol

Limit of Detection and Limit of Quantification:

The LOD solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.The LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

Flow rate was varied at 0.9 ml/min to 1.1ml/min.Standard solution 195 ppm of Acetaminophen& 22.5 ppm of Tramadol was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

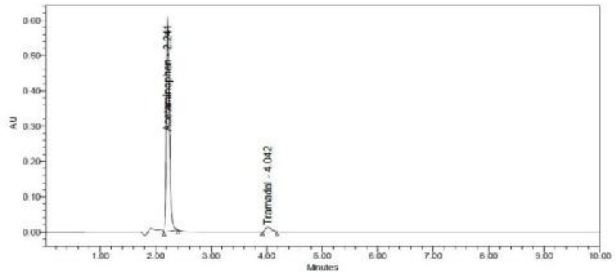

Fig 6: Chromatogram showing less flow

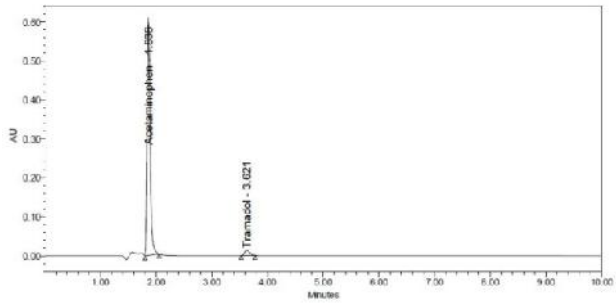

Fig 7: Chromatogram showing more flow

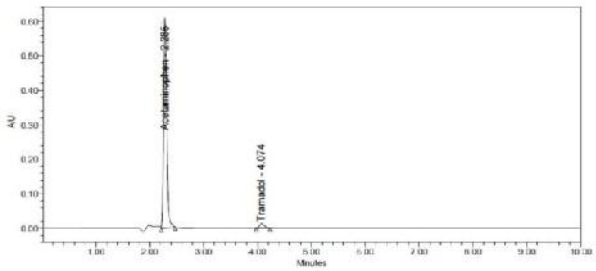

Fig 8: Chromatogram showing less organic composition
Degradation Studies

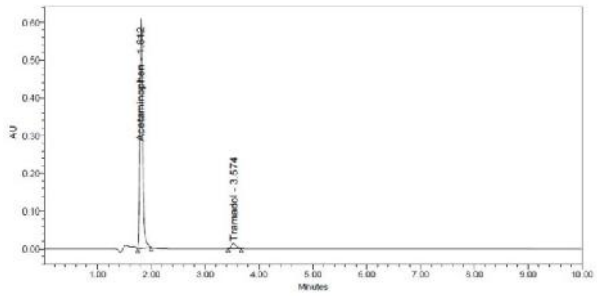

Fig 9: Chromatogram showing more organic composition

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Acetaminophen and Tramadol using the proposed method.

Preparation of stock:

Accurately weigh and transfer 162.5 mg of Acetaminophen and 18.75 mg of Tramadol working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Hydrolytic degradation under acidic condition:

Pipette 1.2 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition:

Pipette 1.2 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation

Acetaminophen and Tramadolsample was taken in petridish and kept in Hot air oven at 110^0 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Oxidative degradation

Pipette 1.2 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 1.2 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials

3. Results and Discussions

Table No 2: System suitability results for acetaminophen and tramadol

S.No	Peak name	Retention time	Area	USP resolution	USP tailing	USP Plate count
1	Acetaminophen	1.98	2380851		1.23	5143.77
2	Tramadol	3.85	96441.3	12.61	1.27	6900.18

Table No 3: Accuracy (recovery) data for Acetaminophen

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1194061.3	81.25	81.61	100.44	99.74
100%	2365150.7	162.5	161.65	99.48	

150%	3540917.7	243.75	242.01	99.29	

Table No 4: Accuracy (recovery) data for Tramadol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	48659.3	9.375	9.45	100.82	100.53
100%	96624.3	18.75	18.77	100.11	
150%	145727.7	28.125	28.31	100.65	

Table No 5: Results of precision for Acetaminophen and tramadol

Injection	Area for Acetaminophen	Area for Tramadol
Injection-1	2373684	96855
Injection-2	2345262	96785
Injection-3	2364533	96564
Injection-4	2398744	96432
Injection-5	2376766	96243
Injection-6	2364758	96443
Average	2370624.5	96553.7
Standard Deviation	17621.4	231.5
%RSD	0.7	0.2

Table No 6: Results of Intermediate precision

Injection	Area for Acetaminophen	Area for Tramadol
Injection-1	2364544	96753
Injection-2	2375755	96554
Injection-3	2364746	96656
Injection-4	2387744	96242
Injection-5	2365757	96533
Injection-6	2365757	96454
Average	2370717.2	96532.0
Standard Deviation	9362.3	176.0
%RSD	0.4	0.2

Table No 7: Linearity results for Acetaminophen

S. No	Linearity Level	Concentration	Area
1	I	65	843096
2	II	130	1586386
3	III	195	2401704
4	IV	280	3415267
5	V	325	3954729
Correlation Coefficient			0.999

Table No 8: Linearity results for Tramadol

S. No	Linearity Level	Concentration	Area
1	I	7.5	33224
2	II	15	65703
3	III	22.5	97583
4	IV	30	133512
5	V	37.5	164942
Correlation Coefficient			0.999

Table No 9: Analytical performance parameters of Acetaminophen and Tramadol

Parameters	Acetaminophen	Tramadol
Slope (m)	12019	4416.6
Intercept (c)	48491	380.7
Correlation coefficient (R^2)	0.9999	0.9996

Table No 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Acetaminophen	62	188	3.03
Tramadol	62	186	3.00

Table No 11: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Acetaminophen	62	621	10.02
Tramadol	62	620	10

Table No 12: Robustness system suitability results of acetaminophen for variation in flow

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.26	5372.86
2	1.0	1.21	5114.07
3	1.1	1.26	5624.39

Table No 13: Robustness system suitability results of tramadol for variation in flow

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	13.95	1.29	6618.78
2	1.0	12.38	1.30	6445.83
3	1.1	10.67	1.29	6454.23

* Results for actual flow (1ml/min) have been considered from Assay standard.

4. Conclusion

An attempt was made to develop and validate a stability indicating RP-HPLC method for the simultaneous estimation of the acetaminophen and tramadol hydrochloride in tablet dosage form. The separation was eluted on spuril (4.6×150mm,5µ)column. mobile phase containing orthophaspohoric acid 0.1% (PH: 3.5) and acetonitrile in the ratio of 30: 70 was pumped through column at a flow rate of 1ml /min. optimized wavelength for acetaminophen and tramadol hydrochloride was 280nm. The developed method is validated based on ICH guidelines.The RP-HPLC method developed for the quantification of acetaminophen and tramadol hydrochloride.The advantages present in the simplicity of sample preparation, requires less run time for recording chromatograms and the low costs of reagents used.The proposed methods ensure sufficient resolution and the precise quantification of the compounds. Hence, the proposed method is rapid, accurate, precise, specific, robust,and economical. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, the proposed method can be used for routine drug analysis of acetaminophen and tramadol hydrochloride pharmaceutical dosage form.

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