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Simultaneous estimation and validation of analgesic and antipyretic drugs in combination from solid dosage form by RP-HPLC

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ABSTRACT

To develop the RP-HPLC method for simultaneous estimation of analgesic and antipyretic drugs in combination from solid dosage form by RP-HPLCmethod.To validate the developed RP-HPLC method as per ICH guidelines. Tramadol hydrochloride is a centrally actinganalgesic. HPLC is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. System suitabilitytest in that Capacityfactor, Tailingfactor, Resolution, Selectivity, Separationfactor, Theoreticalplates, Regression co efficient, STD for intercept, LOQ, LOD, Repeatability, Precision studies, Linearity/Calibrationstudies, Robustness, Force degradation/Stability indicatingstudies, Specificity, Drug recovery/accuracy studies. The system suitability test performed for acetaminophen and tramadol has achieved all guideline criteria; including, tailing factor (*T*), separation factors (α), theoretical plates (*N*), capacity factor(*k'*), resolution (*R*) and RSD (%) values as per the obligatory requirements of ICH and US-FDA.The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained no possible degradation products developed for tramadol but as observed acetaminophen was slightly degraded indilute HCl (0.1NHCl) and peroxide (3%H2O2). This developed method by reverse phase liquid chromatography (HPLC) can be used for routine analysis of simultaneous estimation of acetaminophen and tramadol for its high precision, reproducibility, and accuracy for any marketed formulation containing either or both of acetaminophen andtramadol.

INTRODUCTION

Paracetamol or acetaminophen, chemically recognised as N-(4-hydroxyphenyl) acetamide, is most widely used drugs for the treatment of pain and fever.[1] The opiates is almost in effective in intensepain and has no depressant effect on respiration. The major advantage of PR lies in its relative lack of serious side effects.[2] Tramadol is an effective and well-tolerated agent that reduces pain resulting from trauma, renal or biliary colic, and labor, and also for the management of chronic pain of malignantornon-malignantorigin, The analgesic efficacy of tramadol can further be improved by combination with a non-opioid analgesic.[3] various analytical procedures for the assay of PR in bulk powder and dosage forms including cerium sulphatetitrimetry, the assay of the bulk powder whereas HPLC is used for the capsules.[4] Several analytical procedures have been reported for the determination of the two compounds.[5] TR was determined in different matrices using a variety of analytical techniques including HPLC [6], gas chromatography with mass spectrometry (GCMS) [7], thin layer chromatography (TLC) densitometry [8], capillary electrophoresis [9], adsorptive stripping voltammetry[10], square-wave voltammetry and flow injection analysis system with amperometric detection[11], selective PVC membrane electrodes [12], spectro-fluorimetry

[13], and spectrophotometry [14]. The simultaneous determination of the two drugs has been reported in a few publications. They were estimated in human plasma samples using liquid chromatography (LC)MS [15]. In tablets, they were determined using spectrophotometric [16] and reverse-phase HPLC methods using C18 columns [17]. Chromatography is the most powerful analytical technique available to the modern separation sciences. [18,19] Molecules that possess some degree of hydrophobic character, such as proteins, peptides and nucleic acids, can be separated by reversed phase chromatography with excellent recovery and resolution. [20] The proper choice of reversed phase medium is critical for the success of a particular application are, The unique requirements of the application, including scale and mobile phase conditions, The molecular weight, or size of the sample components, The hydrophobicity of the sample components, The class of sample components. [21] The sample should ideally be dissolved in the initial mobile phase. If this is not possible due to stability or solubility problems, formic acid, acetic acid or salt can be added to the sample to increase solubility. It is essential to use reagents and solvents of high purity to ensure minimum detection limits for optimum sensitivity.[22] The four dominant detectors used in LC analysis are the UV, the electrical conductivity, the fluorescence and the refractive index detectors. Importantly, the choice of detector depends on the sample and the purpose of the analysis.[23] Acids such as trifluoroacetic acid, heptafluorobutyric acid and ortho-phosphoric acid in the concentration range of 0.05 - 0.1% or 50 - 100 mM are commonly used. This is due to the fact that the siloxane linkage area cleaved below pH 2.0; while at pH valued above 8.0 silica may dissolve.[24] Most compounds adsorb UV light in the range of 200-350 A°.[25]A large numbers of detectors are used for RP-HPLC analysis. However, among these the five dominant detectors used in LC analysis are the electrical conductivity detector, These detectors are employed in over 95% of all LC analytical applications.[26] The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures.[27] The chromatographic systems used for most pharmaceutical analyses such as assays of the active ingredients, impurity determinations, and dissolution testing (measuring the dissolution rate for a particular form of dosage) must pass a set of predefined acceptance criteria (SST limits) before sample analysis can commence[28] The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used.[29] Which concentration of drug should be used for degradation study has not been specified in regulatory guidance. it is usually possible to get even minor decomposition products in the range of detection.[30] A stability indicating method (SIM) is an analytical procedure used to quantitate the decrease in the amount of the active pharmaceutical ingredient (API) in drug product due to degradation. According to an FDA guidance document, a stability-indicating method is a validated quantitative analytical procedure that can be used to detect how the stability of the drug substances and drug products changes with time.[31]

MATERIALAND METHOD

The high-performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP in built with binary pump (LC-10ATVP), UV detector (SPD-10AVP), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Water Symmetry®, 3.5 µm; 150 x 4.6 mm ID., HPLC column purchased from (Newcastle-UK) was used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler-Toledo (USA), ultra-sonicatorLabman® purchased from Ultra Chrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from (Mumbai- India). 50 µ micro-syringe was purchased from Hamilton USA. 0.20µ and 0.45µ nylon membrane filters were purchased from Phenomenex® Mumbai, India.

Reagents and reference samples

HPLC grade acetonitrile and water were purchased from Merck (Mumbai, India). Formic acid (FA) (95%) was purchased from Merck Chemicals (Mumbai, England). 0.20 and 0.45µ nylon membrane filters were used and purchased from Ultra Chrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents were used of analyticalgrade.

Standard stocksolutions

Standard stock solutions of ACP and TRM (1 mg mL-1) were prepared separately by dissolving 10 mg of the drug in methanol-water (2:1 v/v) using a 20 mL volumetric flask and completing the final volume adjusted with either methanol or water based on the solubility of drugs in particular eluents.

Working stock solutions

Working stock solution of TRM (40 µg mL-1) was prepared by serial dilution of 4 ml of its stock solution in a 100 mL volumetric flask by completing to volume with the mobile phase.

Sample preparation

Exactly 20 tablets of Tramacad® P containing 37.5 mg of TRM and 325 mg of MET were weighed separately, powdered and mixed in a mortar. An accurately weighed 10 mg amount of the finely powdered Tramacad® P tablets were transferred into 100 mL volumetric flask and the volume was adjusted with 10 mL of acetonitrile and water and sonicated until completely dissolved. The solutions were filtered with 0.2 µ nylon filters.

Linearity/Calibration studies

Accurately measured aliquots of stock solutions ranging between 32.15-500 µg, of TRM and ACP combination were made transferring 10 mg of each combination of TRM and MET into 25 mL volumetric flasks. It was then mixed with 10 ml of ACN-Water to prepare 1000 ppm. Serial dilutions of the samples were made by adjusting the volume to make 5 dilutions between 32.15-500 µg mL^{-1} with same mobile phase, and then 20 µL were injected into the HPLC instrument.

Precision of the proposed method

Triplicates of similar concentrations of the mixture of TRM and ACP (500 µg.L^{-1}) were analyzed nine times, within the same day, using the procedure mentioned under (5.6). Also the triplicates of similar concentrations of the mixture of TRM and ACP (500 µg.L^{-1}) were analyzed on three successive days using the same procedure mentioned in section 5.6.

Robustness for the chromatographic method

The flow rate of the mobile phase was deliberately changed from 1 ml to ±0.2 mL min^{-1} to make 1.2 mL min^{-1} and 0.8 mL min^{-1} and the results were evaluated to understand the separation

behaviour. Similarly the variation of organic modifier used as acetonitrile was changed by from 83 to ±2% to make 85% and 83% to monitor the peak area and retention time. Finally, the effect of wavelength was monitored by making deliberate variation from 226 by ±2 nm to make 228 nm and 224 nm to understand the peak shape and area.

RESULTS

It is the first simultaneous estimation of acetaminophen (ACP) and tramadol (TRM) attempted on C18 column with good resolution (R), sensitive to UV detector and capacity factor (k) was achieved. [32] the acetaminophen was eluted with void volume/solvent front (t0) which is strictly not acceptable by ICH guidelines. In addition, the sensitivity of tramadol was found quite low in UV detection. [33,34,35,36,37] UV detection was specifically carried out (Figure 1,2,3,4) at 226 nm for both selected ACP and TRM as both compound sex hibitoptimum absorption. The flow rate was adjusted to 1ml. min^{-1} to achieve better resolution, and peak symmetry.

System suitability tests for ACP and TRM

System suitability test reveals the factors such as, theoretical plate (N), capacity factor (k'), resolution (R), separation factor (α), tailing factor (*T*), Mean±SD and RSD% which should in acceptable range for at least 6 successive injections of same analytes (Figure 5).

Methodvalidation:

Repeat ability:

Implementing the procedure mentioned under section (5.6), the homologous mixture of both ACP and TRM of same concentrations (500 $\mu g.mL^{-1}$), were tested for six injections with in the sameday. The % RSD was calculated and found it is less than 2 % ; shown in Table1.

Intradayprecision:

Implementing the procedure mentioned under section (5.6), the homologous mixture of both ACP and TRM of three replicates of three similar concentrations 250 ppm were tested and evaluated within the same day (intra-day precision). The %RSD was calculated and found less than 2%; shown in (Table2).

Linearity:

Under linearityor calibration studies, a linear relationship between area under peak values and selected drug concentration ($\mu g.\ mL.\ min^{-1}$) was plotted for five-six chosen concentrations of

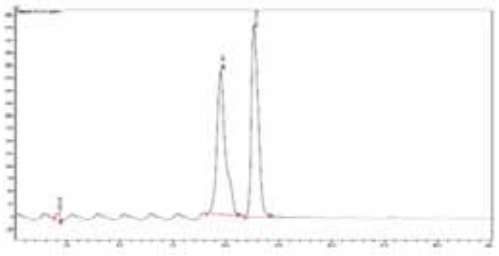

Figure 1. Method development reports of ACP

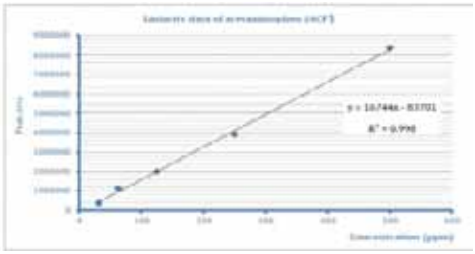

Figure 2. Calibration curve of(9.74 min.) and TRM (11.32) BY RP-HPLCacetaminophen

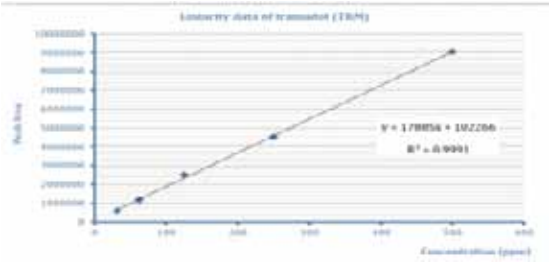

Figure 3. Calibration curve of tramadol

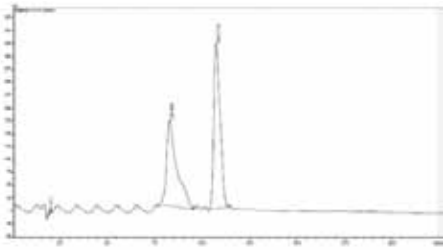

Figure 4. Chromatograph of ACP &TRM

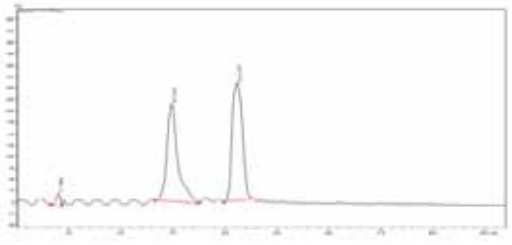

Figure 5. Chromatograph of ACP (7.43 min)

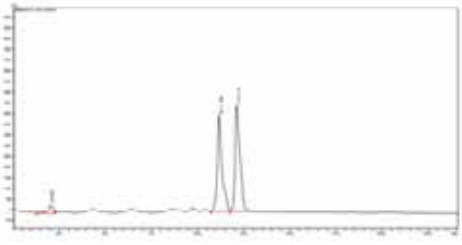

Figure 6. Robustness studies for ACP (11.15 min)and TRM (10.57min) depicts and TRM (12.11 min) at acetonitrile 9% effects of flow

Table 1 : Repeatability data of ACP and TRM

S. No.	Acetaminophen	Tramadol
	PeakArea; Conc. 250ppm	PeakArea; Conc. 250ppm
1	8215706	8554961
2	8369033	8109306
3	8384149	8384650
4	8369033	8109306
5	8381901	8394260
6	8443727	8456561
Mean	8360591	8334840
STD. DEV.	76229.76	184939.01
RSD (%)	0.91	2.21

each drug. The regression equations, correlation coefficient values (r), standard error of intercept (Se), standard deviation of intercept (Sa), limit of detection (LOD) and limit of quantification (LOQ) have been calculated. The linearity of the calibration curves was validated by the high value of correlation coefficient, acceptable values of regression coefficient, standard deviation of the slope and standard deviation of the intercept; shown in (Table 3).

Robustness:

From all above studies, after making deliberated changes in flow rate (± 0.2mL.min^{-1}), organic modifier concentration; acetonitrile (±2%) and wave length (±2nm) have not made any significant changes in resolution, capacity factor and tailing factor. Nonetheless, it seems minute changes in robustness studies makes significant changes in the oretical platecounts. Robustness studies for ACP and TRM displayed in (Table4), (Figure6).

Accuracy:

Accuracy of the results was calculated by % recovery of 5 different concentrations of each drug. The results including the mean of the recovery and standard deviation are shown in (Table 5), (Figure7)

Force Degradation studies:

Which are observed in (Table 6), their effect observed in (Figure 8)

DISCUSSION

The developed analytical method for the simultaneous estimation of acetaminophen (ACP) and tramadol (TRM) in both bulk and tablet formulation has accomplished main ICH

Table 2 : Intraday Precision data of ACP&TRM

S. No.	Concentration (ppm)	Area		Mean ± SD		%RSD	
		ACP	TRM	ACP	TRM		
1	250 ppm	8369033	8554961	8374071± 8727.22	8449639± 92040.87	0.10	1.08
	250 ppm	8384149	8409306				
	250 ppm	8369033	8384650				
2	250 ppm	7915706	8109306	7836008± 72913.79	8295598± 161428.41	0.93	1.94
	250 ppm	7819669	8394260				
	250 ppm	7772651	8383230				
3	250 ppm	8443727	9161683	8504332± 161500.5	9116709± 64067.54	1.89	0.70
	250 ppm	8687369	9145091				
	250 ppm	8381901	9043353				

Table 3 : Linearity data of acetaminophen

Name of Drug; **Acetaminophen**					
S. No.	Concentration	Area		Average (Mean)	
	($\mu g.mL^{-1}$)	ACP	TRM	ACP	TRM
1	500 PPM	8369033	9043353	8369033	9043353
	500 PPM	----	------		
2	250 PPM	3903412	4506799	3903412	4506799
	250 PPM	------	------		
3	125 PPM	2010372	2513192	2010372	2513192
	125 PPM	----	-----		
4	62.5 PPM	1140369	1184214	1140369	1184214
	62.5 PPM	-----	------		
5	31.25 PPM	379017	590049	379017	590049
	31.25 PPM	-----	------		
6	Regression Equation			Y=16744x-83701	Y= 17885x + 102266
7	Correlation coefficient (R^2)			0.998	0.9991
8	Std. Error of intercept			111428.4996	79653.06
9	Std. Dev. of intercept			249161.6997	178109.67
10	LOQ			49.10 $\mu g.ml^{-1}$	32.86 $\mu g.ml^{-1}$
11	LOD			148.80 $\mu g.ml^{-1}$	99.58 $\mu g.ml^{-1}$

guidelines [30], the system suitability test performed for acetaminophen and tramadol has achieved all guideline criteria[; including, tailing factor (*T*), separation factors (α), theoretical plates (*N*), capacity factor(*k'*), resolution (*R*) and RSD (%) values asper the obligatory requirements of ICH [33,34,35], should in acceptable range for at least 6 successive injections of same analytes. In repeatability the % RSD was calculated and found it is less than 2%; shown in Table1, Precision found less than 2%; shown in Table2, The linearity of the calibration curves was validated by the high value of standard deviation of the slope and standard deviation of the intercept. Minute changes in robustness studies makes significant changes in theoretical platecounts Figure 6. The validated stress degradation studies under thermal, oxidative, alkaliand acid ascertained no possible degradation

Table 4 : Robustness data of ACP&TRM

S. No.		F. (-0.2 ml.mL-1)	F (+0.2 ml.mL-1)	A(-2ml)	A(+2ml)	WL(-2nm)	WL (+2 nm)
Resolution	ACP	----	----	----	----	----	----
	TRM	2.38	3.006	3.86	2.24	3.005	2.73
Tailing factor	ACP	1.41	1.82	1.43	1.47	1.28	1.31
	TRM	1.58	1.46	1.08	1.51	1.24	1.53
Capacity factor	ACP	3.775	3.32	2.76	4.56	3.83	2.78
	TRM	4.38	4.64	4.33	5.037	3.005	3.44
Theoretical plates	ACP	5913	1147	1423	12757	4399	3264
	TRM	6531	4306	2547	11282	5353	5697

Table 5 : Accuracy data of ACP&TRM

Conc. (%)	S. No.	S. amt. (µg.mL-1)	D. added (µg.mL-1)	Amt. rec. (µg.mL-1)	% recovery	Mean±SD	% RSD
80%	1	325	260	572.22	97.81	98.98±1.30	1.31
	2	325	260	587.31	100.39		
	3	325	260	577.71	98.75		
100%	1	325	325	641.90	98.75	100.44±2.42	2.41
	2	325	325	671.02	103.23		
	3	325	325	645.90	99.36		
120%	1	325	390	712.09	99.59	100.15±1.12	1.18
	2	325	390	710.88	99.42		
	3	325	390	725.34	101.44		

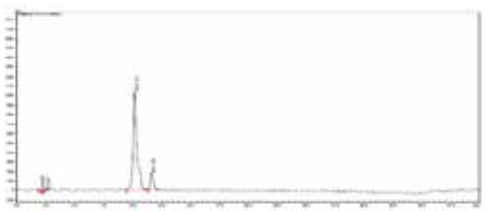

Figure 7. Accuracy dada of ACP and TRM at 80%TRM; thermal degradation

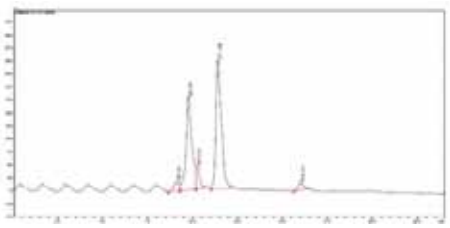

Figure 8. Force degradation data of ACP

Table 6 : Degradation data of ACP and TRM; thermal degradation at 60˚C

Peak#	Ret. Time	Area	Height	Area%
1	9.12	345038	17866	3.2389
2	9.753	4067314	179330	38.1797
3	10.233	629986	45264	5.9137
4	11.398	5331117	248147	50.043
5	16.015	279613	15456	2.6247

products developed Figure 8. so its reliable method as compared to others.

So far, no any development of methods are available for simultaneous quantification of these two drugs, we make it adolescent method in high throughput analysis, these method is simple, easy and efficient having not any incompatibility, In future, with the help of these drug we formulate new dosage form.

CONCLUSION

This developed method by reverse phase liquid chromatography (HPLC) can be used for routine analysis of simultaneous estimation of acetaminophen and tramadol for its high precision, reproducibility, and accuracy for any marketed formulation containing either or both of acetaminophen and tramadol.

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