Stability-Indicating HPLC Method for the Simultaneous Determination of Paracetamol and Tramadol Hydrochloride in Fixed-Dose Combination Tablets

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Abstract: An accurate, sensitive, precise and isocratic reversed-phase *high-performance liquid chromatography* (RP-HPLC) method, using a UV detector for analysis of paracetamol and tramadol hydrochloride in combination tablets has been developed and validated. The best separation was achieved on Zorbax SB C_{18} 250 mm × 4.6 mm, 5-μm particle, with 30: 70 (v/v) of acetonitrile: 1% trifluoroacetic acid as the mobile phase, at a flow rate of 1.5 ml/min. The detection wavelength was set at 271 nm. The method was validated according to the International Conference on Harmonization (ICH) guidelines. The response was a linear function of concentration over the range of 325 – 2112.50 ppm for paracetamol and 37.5 – 243.8 ppm for tramadol hydrochloride. The correlation coefficient (r^2) was found to be 0.9998 for paracetamol and 0.9961 for tramadol hydrochloride. The limits of detection and quantitation were found to be 35.80 ppm and 108.49 ppm for paracetamol and 4.14 ppm and 12.54 ppm for tramadol hydrochloride. The drug was subjected to hydrolytic and thermal stress and was found to decompose slightly under both acidic and basic hydrolytic stress conditions and also under thermal stress. The degradation products produced as a result of the hydrolytic and thermal stress did not affect the detection of both paracetamol and tramadol hydrochloride, and the assay could thus be regarded as stability-indicating. The developed method was used to assay a sample of fixed-dose combination tablets and was found to be suitable for application in the analysis of fixed-dose combination tablets of paracetamol and tramadol hydrochloride for quality control purposes.

Keywords: RP-HPLC, Paracetamol, Tramadol Hydrochloride, Stability, ICH

1. Introduction

The stability of a drug is defined as the ability of the drug to maintain its physical, chemical, biological and microbial properties during its shelf life [1]. The stability of every pharmaceutical product is affected by certain environmental conditions making stability one of the main important quality requirement for pharmaceutical products. The purpose of stability testing according to the International Conference on Harmonization (ICH) guideline is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, oxygen and light [2].

Different dosage forms of pharmaceutical drugs exist. Notable amongst them is the combination product which according to U. S. FDA includes 'a product comprised of two or more regulated components, that is drug/device, biologic/device, drug/biologic or drug/device/biologic, that's are physically, chemically or otherwise combined or mixed and produced as a single entity' [3]. Fixed-dose combination drugs are known to provide better therapeutic effect, safety, prevent poly-pharmacy and improve compliance [4]. This has led to the increase in combination products used for the management of various medical conditions including painful conditions. One of such combination products is paracetamol/tramadol hydrochloride combination tablets. The development and validation of stability-indicating methods therefore helps to evaluate the drug product in the presence of its degradation products, excipients and other materials that might be present [1].

Paracetamol also known as acetaminophen is chemically known as *N*-(4-Hydroxyphenyl) acetamide (Figure 1). It is a white or almost white crystalline powder that is sparingly soluble in water, freely soluble in alcohol and very slightly soluble in methylene chloride [5]. It is used widely as an analgesic and antipyretic agent but it has no anti-inflammatory properties. Although the exact mechanism of its analgesic action has not been found, it is believed to include the inhibition of prostaglandin synthesis, the inhibition of the nitric oxide pathway and the supraspinal activation of descending serotonergic pathways [6]. Paracetamol is better tolerated than Non-Steroidal Anti-inflammatory Drugs (NSAIDs) because of its lack of gastric mucosae damage and platelet activation inhibition [7].

Tramadol hydrochloride is a centrally acting opioid analgesic used for the management of moderate to severe painful conditions. It is chemically known as (1*RS*, 2*RS*)-2-[(Dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride (Figure 2). It is a white or almost white crystalline powder which is freely soluble in water and in methanol and very slightly soluble in acetone [5]. Tramadol hydrochloride is used for the treatment of painful conditions such as neck pain or waist pain. It acts by activating opioid receptors and inhibiting the neuronal reuptake of noradrenaline and serotonin. Although tramadol affects respiration, it is unlikely to produce clinically relevant respiratory depression at therapeutic dosages [6].
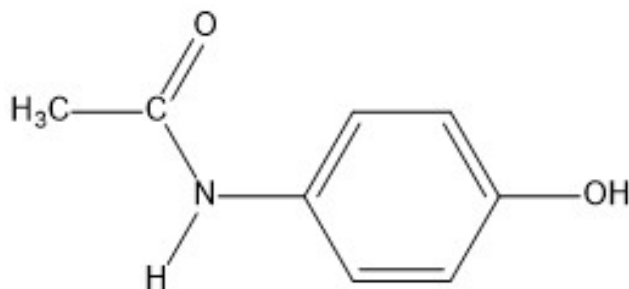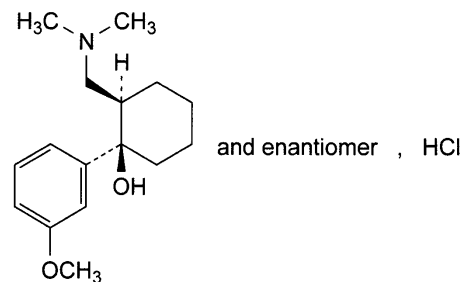
Figure 2. Structure of tramadol hydrochloride.

Tramadol hydrochloride and Paracetamol is an orally administered fixed-dose combination product employed for the symptomatic treatment of moderate to severe pain such as muscoskeletal pain or migraine pain. They provide better analgesia against several types of pain and sources of pain because they have complementary modes of action and target multiple sites [7].

There have been different analytical methods developed and validated for the simultaneous quantification of paracetamol and tramadol hydrochloride with other drug products in fixed-dose combination tablets [8, 9]. Literature also shows that other methods employing different organic modifiers have been used to quantify both drugs in a simultaneous fashion, in the presence of its degradants. M. V. V. N Murali Krishna et al 2015, developed an Ultra Performance Liquid Chromatography (UPLC) method that simultaneously quantifies acetaminophen and tramadol hydrochloride impurities in combination tablets [10]. Kamble and Singh 2012, described a stability- indicating HPLC method for the analysis of paracetamol and tramadol hydrochloride in a pharmaceutical dosage form employing orthophosphoric acid and methanol as mobile phase [11]. Another study also reported on the stability studies of paracetamol and tramadol hydrochloride in combination with domperidone [12]. This suggests that there are just few HPLC methods that simultaneously analyze paracetamol and tramadol hydrochloride in fixed-dose combination tablets in the presence of its degradation products. Therefore, the objective of this research is to study the degradation behavior of paracetamol and tramadol hydrochloride in fixed-dose combination tablets using different organic modifiers and also to develop and validate an accurate, sensitive and rapid RP-HPLC method for quantifying simultaneously paracetamol and tramadol hydrochloride in fixed-dose combination tablets in the presence of its degradation products.

2. Experimental

2.1. Reagents and Materials

Pure paracetamol powder (Tianjin Pharmaceutical Co. Ltd., China), pure tramadol hydrochloride (Emmennar Pharma (P) Ltd., India), HPLC grade acetonitrile (Merck, USA), HPLC grade trifluoroacetic acid (Loba Chemie, India), distilled water, paracetamol/ tramadol hydrochloride combination

Figure 1. Structure of Paracetamol.

tablets (Denk Pharma, Germany).

2.2. HPLC Instrumentation and Column

Agilent 1260 Quarternary pump (Germany)
Agilent 1260 Autosampler
Agilent 1260 Variable Wavelength detector
Agilent Openlab software
Zorbax SB C_{18} (250 mm×4.6 mm) column

2.3. Chromatographic Conditions

Mobile phase: Acetonitrile: 1% trifluoroacetic acid (30: 70 v/v)
Flow rate: 1.5 ml/min
Injection volume: 10 µl
Column temperature: 28°C
Wavelength: 271 nm

2.4. Mobile Phase Preparation

The mobile phase consisted of acetonitrile: 1% trifluoroacetic acid (30: 70 v/v). The volumes of different components of the mobile phase were measured separately into their individual solvent reservoirs. The mobile phase prepared was filtered through a 0.45 µm membrane filter before use.

2.5. Preparation of Standard Solution

325 mg of paracetamol and 37.5 mg of tramadol hydrochloride were accurately weighed and dissolved in 100 ml of the mobile phase to give a stock solution of 3250 ppm of paracetamol and 375 ppm of tramadol hydrochloride. Furthermore, standard solutions were made by diluting the stock solution with the mobile phase to give solutions in the concentration range of 325 -2112.50 ppm for paracetamol and 37.5 – 243.8 ppm for tramadol hydrochloride.

2.6. Preparation of Sample Solution

For sample preparation of the combination tablets, twenty (20) tablets were weighed and finely powdered. A quantity (0.5000 g) of the powder was weighed and dissolved with 30ml of the mobile phase. The solution was quantitatively transferred into a 100 ml volumetric flask and sonicated for 5 minutes. It was then made up to the mark and filtered to remove any insoluble excipients. A volume of 5 ml of the solution was pipetted into a 10 ml volumetric flask. The solution was then made to volume using the mobile phase. The solution was injected onto the column three times and the peak areas were recorded.

2.7. Stress Degradation Studies

2.7.1. Acid Hydrolysis

Acid-induced, forced degradation was performed by adding an aliquot of stock solution of paracetamol and tramadol hydrochloride mixture and 5 M HCl and refluxing the mixture for approximately six hours. The solution was then left to reach room temperature, neutralized by the addition of the same strength of HCl, and diluted to 100 ml with the mobile phase so as to obtain a final concentration of 162.5 ppm for paracetamol and 18.75 ppm of tramadol hydrochloride.

2.7.2. Base Hydrolysis

Base-induced, forced degradation was performed by adding an aliquot of stock solution of paracetamol and tramadol hydrochloride mixture and 5 M NaOH and refluxing the mixture for approximately six hours. The solution was then left to reach room temperature, neutralized by the addition of the same strength of NaOH, and diluted to 100 ml with the mobile phase so as to obtain a final concentration of 162.5 ppm for paracetamol and 18.75 ppm of tramadol hydrochloride.

2.7.3. Thermal Degradation

To study the effect of temperature, approximately 162.5 mg and 18.75 mg of paracetamol and tramadol hydrochloride mixture was stored at 120°C in a controlled temperature oven for 6 hours. It was then dissolved in 100 ml of mobile phase to obtain a solution of final concentration of 1625 ppm and 187.50 ppm of tramadol hydrochloride.

2.7.4. Stability of Solution

The solution stability was carried out by storing standard solutions of paracetamol and tramadol hydrochloride mixture in tightly capped volumetric flasks at room temperature for three days. These solutions were assayed after three days against fresh samples.
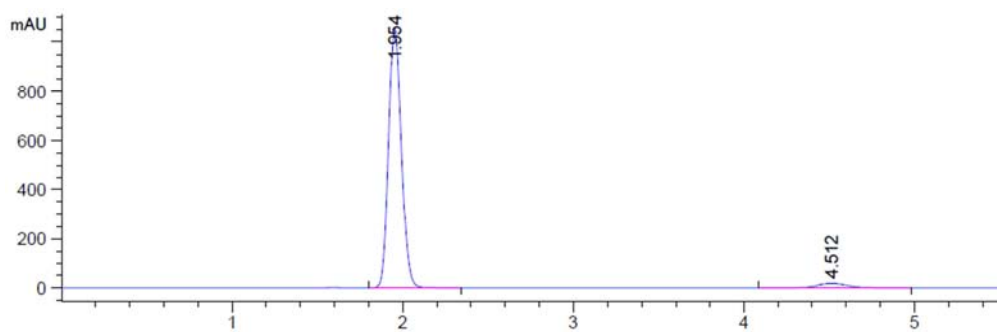
2.8. Validation of the Developed Method

The newly developed method was validated using the various parameters suggested by the International Conference on Harmonization guidelines on validation of analytical procedures: text and methodology. These parameters are linearity, accuracy, specificity, precision and robustness [13].
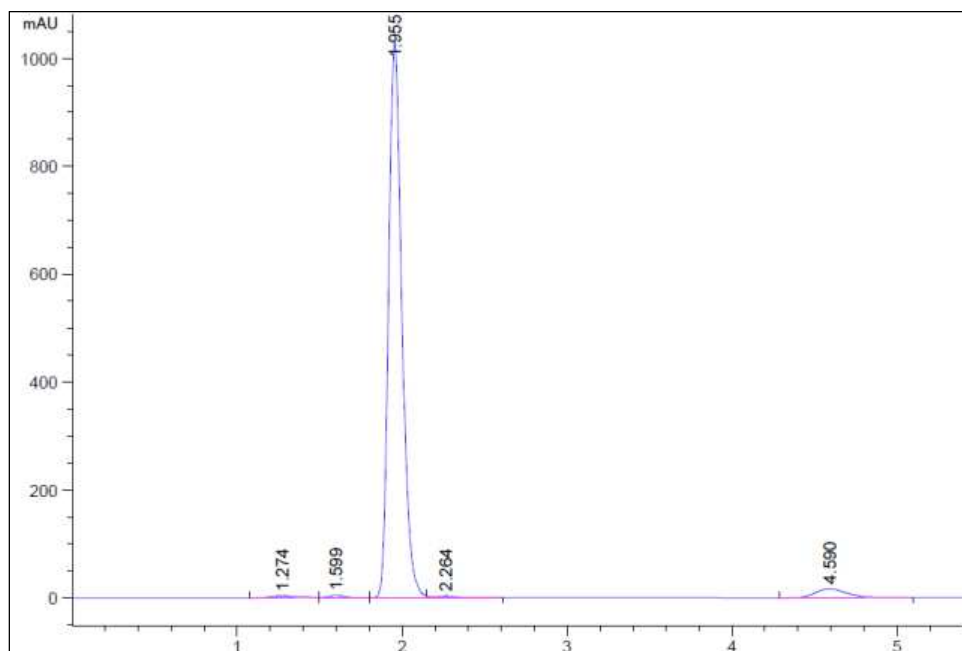
3. Results and Discussion

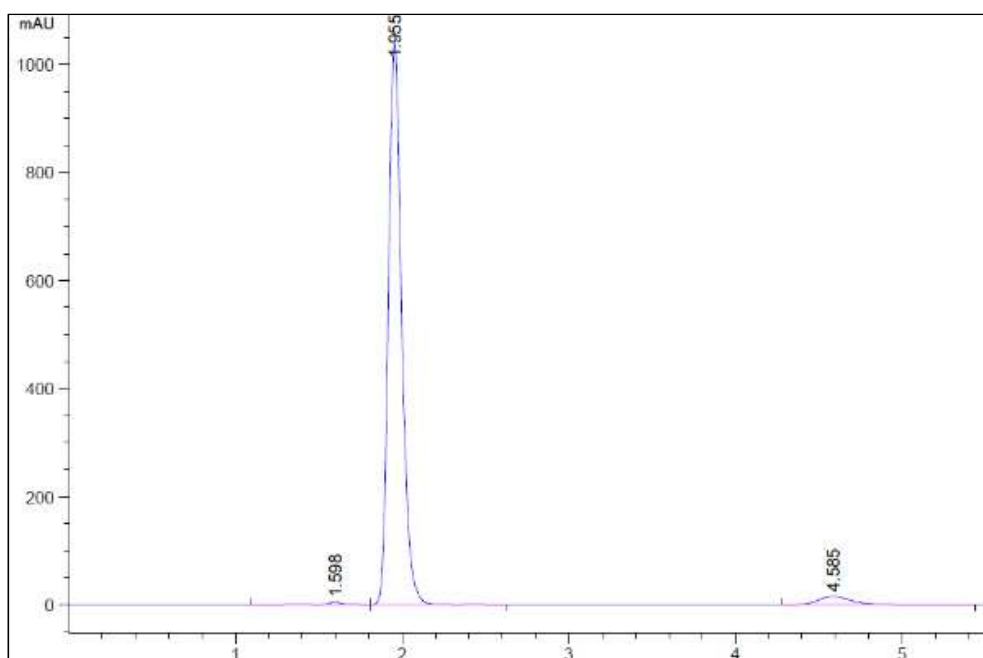## 3.1. Degradation Behavior of Paracetamol and Tramadol Hydrochloride

Using HPLC to study the effect of different stress conditions on paracetamol and tramadol hydrochloride, the chromatograms obtained suggest the degradation behavior of both drugs (Table 3). The representative degradation chromatograms are shown in Figures 3a-d. There was major decomposition of the drugs under alkaline hydrolysis, followed by decomposition under acid hydrolysis and thermal degradation (Figures 3b, 3c and 3d respectively). All these were recorded in a time frame of 5 minutes.
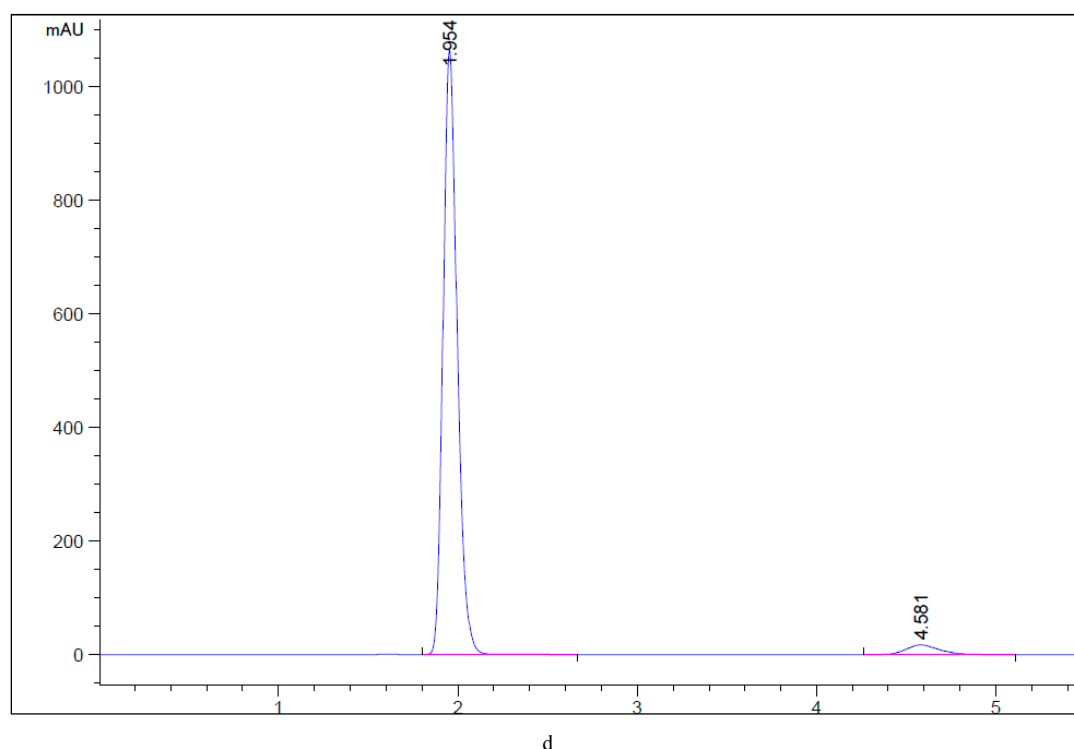
a

b

c

Figure 3. *Chromatograms (x-axis – time in minutes and y-axis – Absorbance in mAU) representative of the stability profile of paracetamol and tramadol hydrochloride using this method. (a) Untreated solution (b) Alkaline hydrolysis (c) Acid hydrolysis (d) Thermal hydrolysis.*

3.2. Development of Stability-Indicating Method

The stability-indicating ability was established under stress conditions. Maximum degradation of the combination product was observed under alkaline conditions, followed by acid hydrolysis and thermal degradation. The percentage degradation of paracetamol and tramadol hydrochloride under stress conditions and retention times of the degradants are shown in Table 1. The separation of paracetamol and tramadol hydrochloride from the degradation products was performed on a Zorbax SB C_{18} column. Using different proportions of acetonitrile and 1% trifluoroacetic acid, the drugs were separated to give individual peaks. However, good resolution of the peaks were obtained at a ratio of 30% of acetonitrile and 70% of 1% trifluoroacetic acid (v/v). Using a variable wavelength UV detector, the eluents were monitored at a wavelength of 271 nm, where maximum sensitivity was observed.

Table 1. *Percentage degradation and the retention times of paracetamol and tramadol hydrochloride and their degradants.*

Conditions	Retention time of drug/degradation products (min)	Percentage degradation of drug	
		Paracetamol	**Tramadol hydrochloride**
Untreated solution	1.96	-	-
Acid hydrolysis	1.60, 1.96, 4.59	0.60	2.93
Alkaline hydrolysis	1.27, 1.60, 1.96, 2.26, 4.59	0.50	3.26
Thermal stability	1.95, 4.58	5.45	0.94

3.3. Validation of the Developed Method

The stability-indicating method was validated per the ICH parameters such as specificity, accuracy, linearity, range, precision and robustness [13].

The Limit of Detection (LOD) and Limit of Quantification (LOQ) concentrations for paracetamol and tramadol hydrochloride were found to be 35.80 ppm and 4.14 ppm and 108.49 ppm and 12.54 ppm respectively.

A summary of the method validation results is shown in Table 2.

Table 2. *Summary of method validation parameters for paracetamol and tramadol hydrochloride.*

Parameter	Paracetamol	Tramadol hydrochloride
Specificity	Specific	Specific
Limit of Detection (ppm)	35.80	4.14
Limit of Quantification (ppm)	108.49	12.54
Linearity (r^2)	0.9998	0.9961
Robustness	Robust	Robust

The system suitability results are shown in Table 3.

Table 3. *System suitability results.*

Description	Paracetamol	Tramadol hydrochloride
Peak symmetry	0.79	0.79
Width	0.0798	0.1744
Theoretical plates	3232	3643
Resolution	2.12	11.30
Selectivity	1.20	2.25

The precision and recovery data are listed in Table 4.

Table 4. *Precision and recovery data.*

Samples	Actual concentration (ppm)	Precision (% RSD)		Accuracy
		Intra-day	Inter-day	
Paracetamol	1300	0.10	0.41	100.70
	1625	0.05	0.44	100.01
	1950	0.27	0.84	99.64
Tramadol hydrochloride	150	0.06	0.88	97.93
	187.5	0.28	1.09	98.67
	225	0.93	0.43	99.47

Comparing this method to already existing methods, this method is relatively faster since it requires an analysis time of about 5 minutes as evidenced in the chromatograms (Figure 3).

The influence of small changes in chromatographic conditions on the method was studied. Change in flow rate (1.5 ml/min to 2.0 ml/min), column temperature (28°C to 30°C) and column (Zorbax C_{18} to Agilent Prep C_{18}) was studied to determine the robustness of the developed method. The %RSD was found to be < 2%. This suggests that the method is robust and small changes in chromatographic conditions would not affect the assay. The stability of the drug solution was determined by storing it for three days. There were no significant changes over the observation period. This data confirms the stability of the solution for at least three days.

The system suitability testing evaluates the effectiveness of the entire chromatographic system prior to use and during the time of analysis. Parameters such as theoretical plates, resolution and selectivity were all within the limits stipulated by CDER guidelines [14]. The chromatographic system used, can therefore be said to be suitable.

4. Conclusion

A simple, accurate and sensitive reversed-phase HPLC method for the simultaneous quantification of paracetamol and tramadol hydrochloride in fixed-dose combination tablets have been developed and validated per the International Conference on Harmonization guidelines. The method was also used to study the stability profile of paracetamol and tramadol hydrochloride under stress conditions. The newly developed method is easy to operate, requires a relatively shorter analysis time and is stability-indicating (it can be used to study the stability profile of paracetamol and tramadol hydrochloride). This new method can therefore be used for the routine analysis of fixed-dose combination tablets of paracetamol and tramadol hydrochloride.

Acknowledgements

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