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Stability-Indicating LC Method for Analysis of Zaltoprofen in Bulk Drug Formulations

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ABSTRACT

A simple, rapid, and precise LC method has been developed for quantitative analysis of Zaltoprofen (ZLT) in pharmaceutical Bulk dosage forms. Chromatographic separation of ZLT and its degradation products was achieved on a C_{18} analytical column with 0.01 M Potassium dihydrogen phosphate – acetonitrile, 80:20 (v/v), as mobile phase. The flow rate was 1.0 mL min^{-1}, the column temperature 25 °C, and detection was by absorption at 233 nm using a photodiode-array detector and UV detector. The tailing factor for ZLT was 1.07. ZLT was exposed to thermal, photolytic, hydrolytic, oxidative stress conditions as per ICH guidelines and were analysed by use of the proposed method. Peak homogeneity data for ZLT in the chromatograms from the stressed samples, obtained by use of the photodiode-array detector, demonstrated the specificity of the method for analysis of ZLT in the presence of the degradation products. The linearity of the method was excellent over the range 10–140 μg mL^{-1} ZLT. The correlation coefficient was 0.9999. Relative standard deviations of peak areas from six measurements were always less than 2%. Each analysis required no longer than 10 min. The proposed method was found to be suitable, stability indicating and accurate for quantitative analysis of ZLT in bulk drug samples and also study its stability.

Keywords: Column liquid chromatography, Method validation, Bulk Drug determination, Zaltoprofen.

INTRODUCTION

Zaltoprofen (ZLT) is a non-steroidal anti-inflammatory drug, and has excellent effects even on post-surgery or post-trauma chronic inflammation. The chemical name of ZLT is (±)-2-(10,11-dihydro-10-oxodibenzo [b,f] thiepin-2-yl) propionic acid and its structure is shown in Fig. 1[1]. ZLT selectively inhibits cyclooxygenase-2 activity and prostaglandin E2 production[2], It is used

in the treatment of rheumatoid arthritis, osteoarthritis, and other chronic inflammatory Pain conditions [3-8]. ZLT is a unique compound that also has anti-bradykinin activity [9]. It is not only of cyclooxygenases but also of bradykinin-induced 12-lipoxygenase inhibitors [10].

Earlier publications have described a high-performance liquid chromatography (HPLC) methodology useful for the quantification of ZLT in human plasma samples was reported [11]. Liquid– liquid extraction and/or solid-phase extraction techniques were used in the development of this methodology. However, the method has some limitations regarding the recovery yield and extraction protocol. For instance, its recovery yield is poor and the whole extraction process is somewhat laborious. To overcome these problems in this study, we developed a simple column switching HPLC-UV method [12]. These methods are for the analysis of ZLT in biological samples [11-14].However, these methods involve arduous sample preparation and long chromatographic run-times (longer than 12 min) for biological samples.

So far to our present knowledge no stability-indicating analytical method for ZLT is available in the literature for analyzing pharmaceutical bulk drug samples. It is felt necessary to develop a stability-indicating HPLC method for the quantitative determination of ZLT. The current research work deals with the forced degradation of the drug substance under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat and UV. The work also includes the validation of the developed stability-indicating method. The devised method was found to be selective and reliable, and faster and more straightforward than other reported bioanalytical methods.

MATERIALS AND METHODS

Experimental

Chemicals, Reagents, and Solutions

ZLT bulk drug was obtained from Macleods Pharmaceuticals (Mumbai, India), Potassium dihydrogen phosphate (AR Grade) and acetonitrile (HPLC grade) from Merck Fine Chemicals (Mumbai, India), and orthophosphoric acid (AR grade), sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) from Qualigens Fine Chemicals (Glaxo, Mumbai, India). The 0.45µm pump Nylon filter was obtained from Advanced Micro Devices (Ambala Cantt, India). Triple distilled water was obtained from a Milli-Q UF-Plus apparatus (Millipore) and was used to prepare all solutions for the method. Other chemicals used were analytical or HPLC-grade.

For preparation of 0.01 M buffer, 1.36 g of Potassium dihydrogen phosphate as dissolved in 1000 mL of distilled water. The pH of the resulting solution was observed to 4.8 using SevenMulti pH meter (Make Mettler Toledo).

Diluent used was 20:80; 0.01 M Potassium dihydrogen phosphate-acetonitrile. Standard stock solutions were prepared by dissolving the drug in the acetonitrile and diluting them to the desired concentration using diluent.

Chromatographic conditions

The HPLC apparatus was a Waters chromatographic system equipped with an injection valve (Rheodyne 033381); Waters 2487 UV dual *k* absorbance detector was used. Peak area integration was performed using Empower 2 software. Also a gradient high-performance liquid chromatograph of an Agilent 1100 series instrument comprising degasser, quaternary pump, auto injector, column compartment, and photodiode-array detector was used. Chromatography was performed on Peerless C_{18} (250 X 4.6 mm, 5µm particle) column (Supplier- HYM Brothers, Mumbai, India) at 25 ^{0}C temperature. The isocratic mobile phase was a 20:80 v/v mixture of 0.01 M potassium dihydrogen phosphate buffer (pH 4.8) and acetonitrile at a flow rate of 1 mL min^{-1}. the variable wavelength programmable UV–Vis detector was set at 233 nm. Before use, the mobile phase was filtered through a 0.45 µm membrane filter and degassed in an ultrasonic bath. The injection volume was 10 µL and detection was by ultraviolet (UV) absorption at 233 nm. For analysis of samples obtained by forced degradation, the photodiode-array detector was used in scan mode in the range 200– 400 nm. Peak homogeneity was expressed as peak purity and was obtained directly from the spectral analysis report by use of the above-mentioned software

Preparation of the Standard Solution

An accurately weighed sample (50 mg) of ZLT reference standard was transferred to a 100 mL volumetric flask and dissolved in acetonitrile to make a stock solution of 0.5 mg mL^{-1}. Aliquots from the stock solution were diluted with the diluent to give the solutions in the concentration range 10-140 µg $mL^{-1.}$ The solutions were injected in HPLC and area was measured for each solution. The calibration curve was obtained by plotting peak area on ordinate against drug concentration on abscissa.

Preparation of Sample Solution

A 50mg of API from bulk powder was transferred to a 100 mL volumetric flask and 50 mL acetonitrile were added. The mixture was sonicate for 5 min with intermittent shaking then diluted to volume with acetonitrile. The 10 mL of above solution was transferred to a 50 mL volumetric flask and diluted to volume with diluents.

Procedure for Forced Degradation Study

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, light. It provides recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies. The objective of this work was to develop an analytical LC procedure which would serve as a stability-indicating method for assay of ZLT in bulk drug product. Forced degradation of the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. For photolytic stress, drug product in the solid state was irradiated with UV radiation with peak intensities at 254 and 366 nm. The UV dose from the lamp of peak intensity at 366 nm was measured by use of a quinine monohydrochloride (2% solution in water) chemical actinometer as mentioned in the ICH guidelines [15]. The ICH guidelines state that the minimum desired exposure should be 200 Wh m^2 which corresponds to a

change in absorbance of 0.5 AU of the quinine actinometer at 400 nm. This change was observed after irradiation for 24 h. A second photolytic stress test experiment with greater irradiation time, 48 h, was performed to establish the specificity of the method. Forced degradation of the drug products under acidic, basic, and oxidizing conditions was performed on filtered solution (as described in the section ''Preparation of Sample Solution'') containing 100 µg mL^{-1} ZLT. For thermolytic degradation 200mg of powder was store in a flint glass vial with rubber septum and aluminum flange collar at 80 ^{0}C for two weeks. For photolytic degradation, 200mg powder was exposed. The solutions were then prepared as described in the section ''Preparation of Sample Solution''.

Acidic Degradation

10 mL sample stock solution was transferred to a 50 mL volumetric flask and 10 mL 1 M HCl was added. The mixture was left at 60 ^{0}C for 8 h in a water bath then left to equilibrate to ambient temperature. The solution was then neutralised with 1 M sodium hydroxide and then diluted to 50 mL with diluent.

Alkaline Degradation

10 mL sample stock solution was transferred to a 50 mL volumetric flask and 10 mL 0.01 M sodium hydroxide was added. The mixture was left for 2 h at ambient temperature. The solution was then neutralised with 0.01 M HCl, then diluted to 50 mL with diluent.

Oxidative Degradation

10 mL sample stock solution was transferred to a 50 mL volumetric flask and 10 mL 30% hydrogen peroxide was added. The mixture was left for 2 h at ambient temperature then diluted to 50 mL with diluent.

Thermal Degradation

Approximately 200 mg drug product powder was left at 80 ^{0}C for two weeks. The sample was then treated to obtain solution containing 100 µg mL^{-1} ZLT.

Short-Wavelength (254 nm) UV Degradation

Approximately 250 mg drug product powder was exposed to short-wavelength UV light for 24 h. The sample was then treated to obtain solution containing 100 µg mL^{-1} ZLT.

Long-Wavelength (366 nm) UV Degradation

Approximately 250 mg drug product powder was exposed to long-wavelength UV light for 48 h. The sample was then treated to obtain solution containing 100 µg mL^{-1} ZLT.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

To develop a stability-indicating LC method, we have investigated different stationary phases like C18 (Agilent, Waters, YMC, and ACE) and CN (YMC and Alltima), and different mobile phase compositions containing organic modifier (acetonitrile) and different buffers. The buffers used include phosphate, ammonium acetate, and trifluoroacetic acid of different pH (3–5). Our objectives in chromatographic method development was to achieve a peak tailing factor <1.5 and

retention times from 3 to 10 min. Chromatographic separation of ZLT and its degradation products was achieved on a 250 mm X 4.6 mm, 5µm Peerless C_{18} column. Development studies revealed that 0.01 M Potassium dihydrogen phosphate - acetonitrile 20:80 (v/v) at a flow rate of 1.0 mL min^{-1} and a column temperature of 25 ^{0}C were suitable conditions for a stability-indicating method for study of the degradation of ZLT. ZLT peak shape was excellent, with little tailing, and ZLT was well resolved from its degradation products. The retention time of ZLT was, typically, approximately 4.6 min and chromatographic analysis time was less than 10 min. Under the optimized conditions ZLT and its degradation products were well separated.

Although the conditions used for forced degradation were attenuated to achieve degradation upto 10 % [15], this could not be achieved for thermal degradation even after exposure of API for 80 ^{0}C for two weeks and photolytic degradation even after prolonged exposure. During the initial forced degradation experiments, it was observed that alkaline hydrolysis of ZLT was a slow reaction. The drug was slightly degraded by acid hydrolysis, alkaline hydrolysis, and oxidative condition. Table 1 indicates the extent of degradation, peak purity, and assay of ZLT under the various stress conditions. Chromatographic peak purity data were obtained from the spectral analysis report. A peak-purity value greater than 990 is indicative of a homogeneous peak; peak purity values for the analyte peak in the range 999–1,000 for the tablets were indicative of homogeneous peaks and thus established the specificity of the assay. Chromatograms obtained from ZLT bulk sample solution, and solutions after acidic and alkaline hydrolysis and oxidative degradation of the drug product are shown in Fig. 1b–e, respectively.

Method validation

The developed method was validated as per ICH guidelines [16], and accordingly the parameters were evaluated for Linearity, Specificity, Accuracy, Precision, Robustness and Solution Stability.

Linearity

Linearity was studied in the concentration range of 10-140 µg mL^{-1}. All measurements were repeated three times for each concentration. Correlation coefficient (r) of the line constructed by plotting mean of peak areas against corresponding concentration and was found to be 0.9999 with slope 14.755 X 10^3.

Specificity

The optimized solvent system yielded a symmetric peak for the drug with Rt 4.6 min (**Figure 1a**). The peak for the bulk drug was identified by comparing the Rt and also comparing its absorbance spectrum with that obtained with the standard drug. Peak purity values were > 999 for the drug product, which shows that the analyte peaks were pure and there were no interferences in the analyte peak.

Accuracy

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level to pre-analyzed samples and subsequent solutions were re-analyzed. At each level three determinations were performed. Recovery of ZLT was in the range of 98.5 – 101.3%. The average recovery for the three levels (nine determinations) was 100.25%, RSD 0.56%.

Precision

Precision of the method was determined in terms of intra-day (repeatability) and inter-day precisions (intermediate), analyzing the drug at three different concentrations. The solutions were analyzed using the method on same day for intra-day an for three consecutive days for inter-day precision. The results for intra-day & inter-day were 99.63% & 99.52% respectively.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method the experimental conditions were deliberately altered and assay, peak tailing, and peak area were evaluated. The mobile phase flow rate was 1.0 mL min^{-1}. This was changed by 0.1 units to 0.9 and 1.1 mL min^{-1} and the effect was studied. Similarly, the effect of column temperature was studied at 23 and 27 ^{0}C instead of 25 ^{0}C. The effect of mobile phase composition was studied by use of aqueous 0.01 M Potassium dihydrogen phosphate - acetonitrile 76.5:23.5 and 83.5:16.5 (v/v). The effect of detection wavelength was studied at 228 and 238 nm. For all changes of conditions the sample was assayed in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay of ZLT for all deliberate changes of conditions was within 98.5– 101.5%. The complete are results shown in Table 2.

Table 1. Results from the forced degradation study

Stress Condition	Degradation of ZLT (%)	Peak Purity [a]	Assay (%)
Acidic	8.33	999.567	91.67
Alkaline	6.37	999.687	93.63
Oxidation	8.11	999.678	91.89
Thermal	No Degradation	999.778	98.78
Short Wavelength UV	No Degradation	999.819	99.67
Long Wavelength UV	No Degradation	999.865	99.35

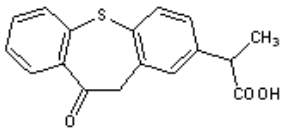
[a] *- Peak Purity in the range of 990 – 1000 indicate homogeneous peak and also Purity angle is smaller than purity threshold in chromatogram indicate pure peak.*

Table 2. Robustness study

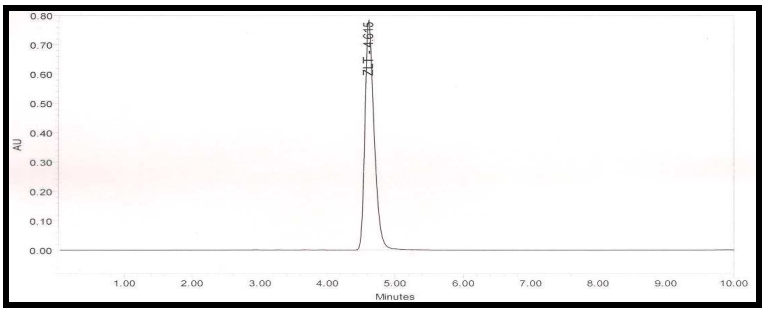
Condition	Peak area	USP peak tailing	Assay % (n = 3)	SD
Flow rate (±10% of the optimum flow)				
0.9 mL min^{-1}	1458177	1.08	99.23	0.24
1.1 mL min^{-1}	1473754	1.11	100.29	0.53
Mobile phase composition (± 5% of optimum organic modifier concentration)				
76.5 mL	1454356	1.22	98.97	0.21
83.5 mL	1454944	1.12	99.01	0.13
Temperature (± 2 ^{0} C of optimum temperature)				
23	1445098	1.07	98.34	0.49
27	1464643	1.08	99.67	0.24
Wavelength (± 5 nm of the optimum wavelength)				
228	1463026	1.11	99.56	0.67
238	1470227	1.19	100.05	0.41

n = 3 determinations

Fig.1. Chemical structure of ZLT (a) Chromatograms obtained from drug solution (b) and solutions degraded by acid hydrolysis (c) alkaline hydrolysis (d) and oxidation (e)



(a)



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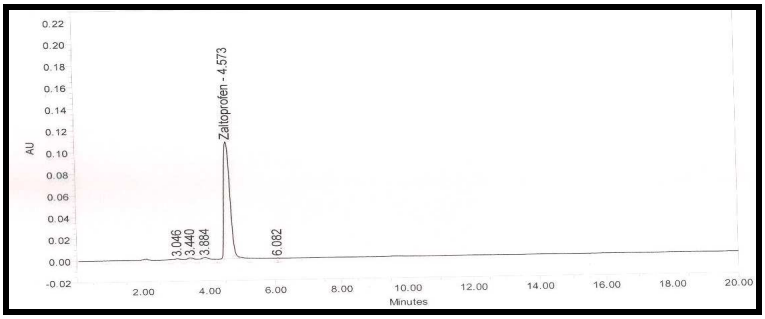

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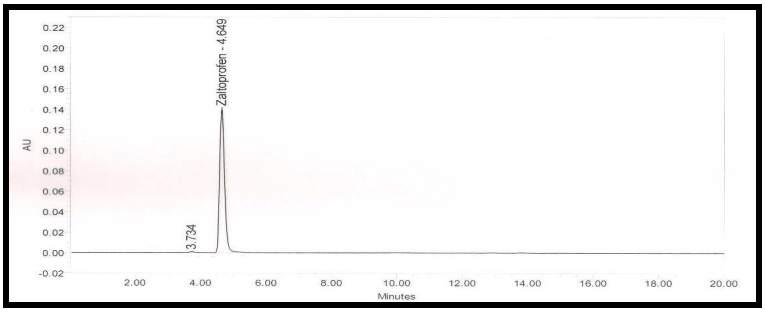
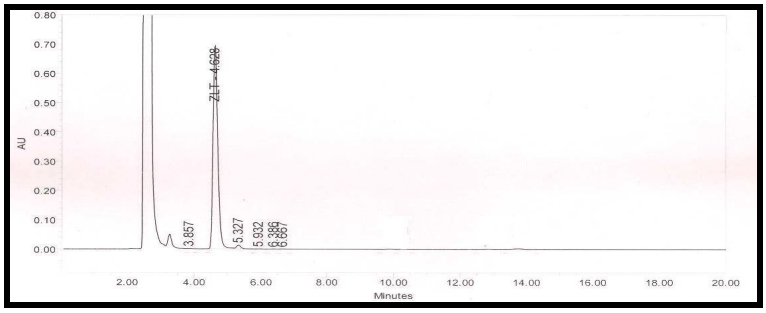


(d)



(e)

Solution Stability

The stability of the standard solution was tested at intervals of 4, 8, 12, 30 and 48 h. The stability of solutions was determined by comparing area% and peak purity results for ZLT. The area% values were within 0.5% after 30 h. These results indicate the solution was stable for 30 h at ambient temperature, because there was no formation of any unknown peak. The assay values of three determinations are 99.25 % & 99.19% at 12 and 48 h respectively.

CONCLUSION

A simple and quick, new HPLC analytical method has been developed which may be applied in routine quality control to determine ZLT in bulk sample. The LC method is stability indicating and as by validation data, enables specific, accurate, precise & robust for determination of ZLT in pharmaceutical bulk products.

Acknowledgements

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