Chromatographic Method Development and Validation (HPLC-UV Technique) of p-Formaldehyde Quantification in Duloxetine Hydrochloride Drug Substance by using Chemical Derivatization

Santosh Patil[1], Poonam Koppula[2] and Santhosh Kumar Koppula[3]*

[1]*Ipca Laboratories Ltd., Vadodara -391445, Gujarat, India*

[2]*Sun Pharmaceutical Industries Ltd, Vadodara- 390020, Gujarat, India*

[3]*School of Sciences, GSFC University, Vadodara- 391750, Gujarat, India*

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*Corresponding Author: ksk.india@gmail.com

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Abstract

Trace level determination and quantification of potentially genotoxic impurities (PGIs) in drug substances is a challenging task. p- Formaldehyde is a known PGI and analysis of it is very taxing due to its volatility, low molecular weight, high polarity and absence of any chromophore. The present study demonstrates development and validation of an HPLC-UV method for quantification of p-Formaldehyde in Duloxetine Hydrochloride. The LOD and LOQ achieved are 0.01 and 0.03 % w/w respectively. The calibration curve was found to be linear over a range of 0.03% to 0.15%. The validated method is precise, sensitive, and accurate and has been successfully utilized to ascertain p-Formaldehyde content in scale up batches of bulk drug.

Keywords

Genotoxic impurity, HPLC-UV, derivatization, active pharmaceutical ingredient

Introduction

Duloxetine is a selective serotonin norepinephrine reuptake inhibitor (SNRIs)[1-2], approved by US FDA as safe and effective antidepressant[3]. It is chemically known as y (+) - (S)-N-methyl-γ-(1-naphthyloxy)- 2-thio phenyl propyl amine hydrochloride[4] (**Figure 1**). It is also used for the treatment of generalized anxiety disorder, and relieves the pain of fibromyalgia and peripheral neuropathy[5].

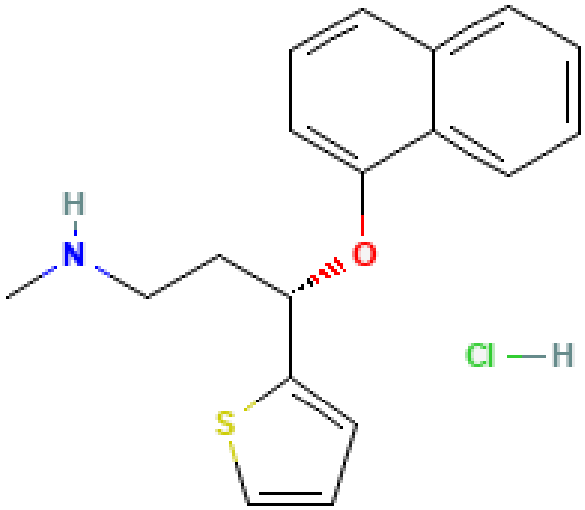

Figure 1: Chemical structure of Duloxetine hydrochloride

p-Formaldehyde when depolymerized results in formaldehyde which is utilized as an important entity in the synthesis of many pharmaceuticals and specialized chemicals[6]. As a formaldehyde releasing agent, p-formaldehyde is a potential carcinogen[7]. Its acute oral median lethal dose in rats is 592 mg/kg[8].

It is highly reactive, volatile, toxic and exposure to it is a significant concern for human body.

Analytical monitoring of potentially genotoxic impurities in drug substances is now a day greatly emphasized by regulatory bodies because these impurities are unwanted chemicals or compounds that harm an organism by inducing its genetic material (DNA) such as genetic mutation, chromosomal breaks or rearrangements and have the potential to cause cancer. The

International Conference on Harmonization (ICH) and The European Medicines Agency has dedicated guidelines for identification and quantification of impurities in new drug substances[9-10]. In 2014, these bodies also have formulated M7 guidelines for identification, categorization, qualification and control of PGIs in pharmaceuticals[11-12].

Due to increased review by regulatory agencies with respect to the potential hazards, there has been an increase in number of analytical methods for determination of PGIs in drug molecules. Based on the maximum daily dose of Duloxetine Hydrochloride, p-formaldehyde needs to be controlled at a limit of 0.1% since it is for oral administration. In the present study, considering the overview facts, a sensitive HPLC - UV method was developed and validated by utilizing derivatization technique. This developed method will help pharmaceutical industries for qualification and quantitation analysis of formaldehyde for various drug molecules by using HPLC.

Materials and Methods

Materials

p-Formaldehyde was purchased from S D Fine Chem Ltd, Mumbai. The solvents acetonitrile, methanol and ortho phosphoric acid were purchased from Merck, India. 2,4-Dinitrophenyl Hydrazine AR was purchased from Molychem, Mumbai, India. Water used during this study was from Milli Q system.

Instrumentation

The analysis was carried out using Waters Alliance HPLC system (e2695 separating module) (Waters Co., Milford, MA, USA) with an Ultraviolet - Visible detector (Waters 2489) with an auto sampler and column heater. Data were collected and processed using Empower™ software (Version 3) from Waters.

Chromatographic Conditions

The method for p-Formaldehyde was developed, validated and applied to study the estimation of p-Formaldehyde in Duloxetine Hydrochloride. The mobile phase was filtered through 0.45µ filter (Millipore) and degassed using sonicator. p-Formaldehyde was analyzed

using Inertsil ODS 3V (250mm × 4.6mm, 5.0µm) column set at 35°C with mixture of Solution-A, Solution-B and orthophosphoric acid (50:50:0.1 v/v) as the mobile phase in isocratic mode. Solution-A is water and Solution-B is pre-mixed solution of acetonitrile and methanol in 1:1 ratio. A flow rate of 1.0 mL/min with an injection volume of 10µL and an absorption wavelength of 355 nm were used. The run time was 30 minutes and the retention time was 18.1 minute for p-Formaldehyde.

Preparation of derivatizing solution, standards stock, working standard and sample solutions

1 mg/mL derivatizing solution of 2,4-Dinitrophenyl Hydrazine (DNPH) was prepared by using acetonitrile. Diluent was prepared by using mixture of derivatizing solution of 2,4-Dinitrophenyl Hydrazine (DNPH) and water in ratio 5:95 v/v. p-Formaldehyde standard stock solution of 0.0025 mg/mL concentration was prepared by dissolving in 0.1N NaOH solution, further 1 mL of this solution was diluted to 100 mL of water. Working standard solution was prepared by diluting 10.0 mL of p-Formaldehyde standard stock solution to 100 mL volumetric flask containing 10 mL of 1% solution of ortho phosphoric acid solution, made to the volume by using diluent as derivatizing solution. The solution was allowed to stand for 4 hours for derivatization at room temperature before injection.

Sample solution was prepared by dissolving accurately 25 mg of Duloxetine Hydrochloride in 10 mL of 1% solution of ortho phosphoric acid and made up to the volume of 100 mL by using diluent as derivatizing solution. The solution was allowed to stand for 4 hours for derivatization at room temperature before injection. Blank solution was prepared by transferring 10 mL of 1% solution of ortho phosphoric acid into 100 mL volumetric flask and made up with volume by using diluent as derivatizing solution.

Results and Discussion

Method development and optimization

Based on maximum daily dose of selected drug substance, p-Formaldehyde is required to be controlled to a limit of 0.1 % since the drug substance is administered orally. p-Formaldehyde is volatile in nature and does not have any chromophore and therefore chromatographic method development was initiated by using gas chromatograph (GC) with

FID detector. During development, it was found that GC method is quite sensitive to detect the p-Formaldehyde peak after derivatization, however the analysis becomes difficult in Duloxetine Hydrochloride drug substance due to its non-solubility in major organic solvents. Eventually, High Performance Liquid Chromatography (HPLC) technique was tried for the determination and estimation of p-Formaldehyde content in drug substance. But due to the absence of chromophores in formaldehyde, it could not be detected by using UV detector in HPLC. Hence, a derivatizing agent, namely 2,4-Dinitrophenyl hydrazine, was selected to introduce UV chromophores into a carbonyl group of p-Formaldehyde. p-Formaldehyde and 2,4-Dinitrophenyl hydrazine form an instant reaction at 1:1 mole ratio and immediately form formaldehyde-2,4-dinitrophenyl hydrazone derivative at room temperature **(Figure 2)**, which is UV active and shows UV maximum at a wavelength of 355 nm**.** Based on peak response obtained for formaldehyde-2,4-Dinitrophenyl hydrazone complex, content of p-Formaldehyde was estimated. The retention time for the p-Formaldehyde derivative was observed at 18.1 minute. The objective of this work was to develop an analytical method, which could not only separate p-Formaldehyde complex peak from drug substances and its related components but also could quantify at least less than 30% to the specification limit of formaldehyde which is 0.1%. Several development trials were carried out by using water and acetonitrile as mobile phase and using different columns but poor peak shape was observed. In some cases, there were interference from sample matrix observed at the peak of p-Formaldehyde. Desired resolution and sensitivity were achieved by using 5 µm particle size, Inertsil ‘‘ODS-3V’’ HPLC column of 250 mm length, 4.6 mm internal diameter having 17% of carbon load. Elution mode was selected as isocratic with Solution-A, Solution-B and orthophosphoric acid (50:50:0.1 v/v) as the mobile phase in isocratic mode. Solution-A is water and Solution-B is pre-mixed solution of acetonitrile and methanol in 1:1 ratio. Impurity was monitored by using UV detector at wavelength 355 nm as shown in **Figure 3** for chromatograms of standard solution, sample as such and sample spiked with p-Formaldehyde. Batch of Duloxetine Hydrochloride was analysed for p-Formaldehyde content and it was not detected in batch sample.

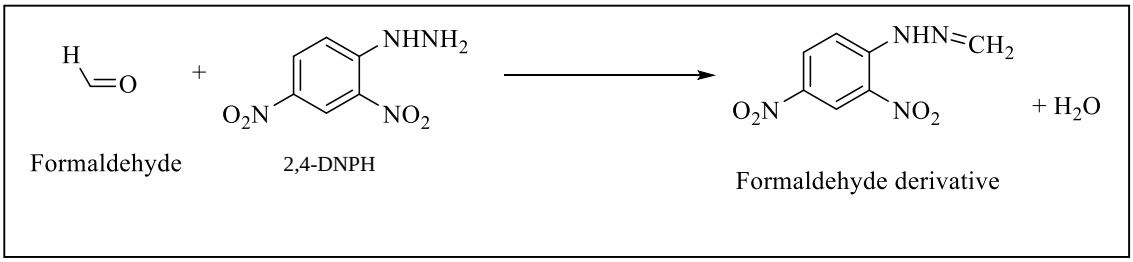

Figure 2: Reaction scheme of Formaldehyde derivative formation

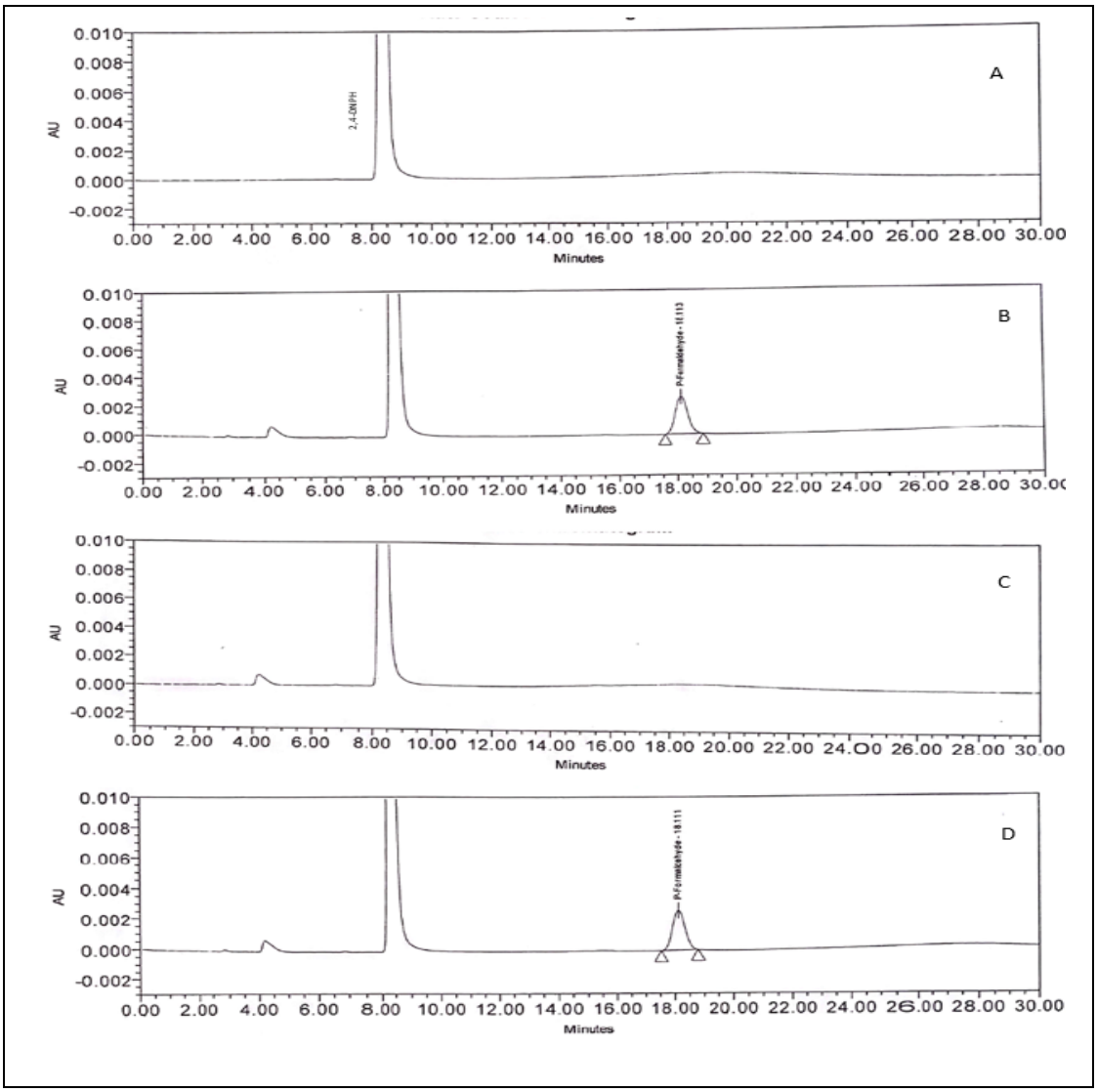

Figure 3: Representative chromatogram (A) blank, (B) formaldehyde-2,4-dinitrophenyl hydrazone (2,4-DNPH derivative), (C) sample chromatogram as such and (D) sample spiked with formaldehyde.

Method validation

The method was validated according to validation of analytical procedures provided in the ICH guidelines[13] and the results are presented in **Table 1**.

Specificity

Specificity was used to test the ability of the method to eliminate the effects of all interfering substances on Formaldehyde derivative peak, specifically by comparing the chromatograms to the blank samples. The validated method showed that there is no interference observed at the peak of interest.

Linearity and range

A linear relationship was observed between the area response for Formaldehyde derivative and corresponding concentrations. The calibration curves exhibit a linearity over a range of 0.03% to 0.15% with respect to sample concentration for formaldehyde with regression coefficient value greater than 0.9800. The method provided a good correlation between the area response and component concentration.

Sensitivity

The LOD (Limit of Detection) was evaluated by determining the minimum levels of concentration of Formaldehyde derivative that could be detected using this analytical method. The LOQ (Limit of Quantitation) was studied by estimating the minimum concentration that could be quantified with acceptable accuracy and precision. The precision study was also carried out at LOQ level by injecting six preparations of formaldehyde derivative. LOD, LOQ and Percentage RSD (Relative Standard Deviation) for the area response of impurity at LOQ level for formaldehyde derivative is shown in **Table 1**.

Precision

The precision for the said Formaldehyde derivative was evaluated by injecting six individual Duloxetine Hydrochloride samples spiked with 0.1% of p-Formaldehyde. Percentage relative standard deviation for actual recovered amount was calculated and were within 15% confirming the good precision of the developed analytical method.

Accuracy

Accuracy was studied by analyzing three replicates at four different concentration levels: at LOQ, 80%, 100% and 150% **(Table 1).** The observed accuracy values were within a range of 100.7% to 102.5% for Formaldehyde derivative. This indicates high accuracy of new method developed.

Sr. No.	**Parameters**	**Formaldehyde derivative**
1	System Suitability, Retention Time (RT) min	18.1
2	Linearity, Correlation coefficient (r)	0.9998
3	Detection limit, LOD (%)	0.01
4	Quantitation limit, LOQ (%)	0.03
5	Precision %RSD. LOQ, (n = 6)	0.38
6	Method Precision %RSD. (n = 6)	0.5
7	% Average Accuracy	
	Accuracy at LOQ level (n = 3)	102.5
	Accuracy at 80% level (n = 3)	101.5
	Accuracy at 100% level (n = 3)	101.2
	Accuracy at 150% level (n = 3)	100.7
8	Sample	Not detected

Table 1: Method validation summary

Conclusion

An isocratic reversed phase - HPLC method was successfully developed for the estimation of p-Formaldehyde in Duloxetine Hydrochloride drug substance. The method developed is simple for the purpose of quantification for the current specified limit 0.1%. The method validation results proved that the method is specific, linear, precise and accurate and can be applied for estimation of p-Formaldehyde in Duloxetine Hydrochloride for monitoring drug safety. Also, this method can be easily adopted for the determination at trace level i.e., less than 1 ppm level considering p-Formaldehyde as known genotoxic by using appropriate sample preparation technique based on solubility of individual molecule.

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Glossary

LOD- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOQ- The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

RSD- Relative standard deviation is also called percentage relative standard deviation formula, is the deviation measurement that tells us how the different numbers in a particular data set are scattered around the mean.

HPLC-UV- High-performance liquid chromatography (HPLC) is a technique used to separate molecules based on size and surface charge, among other properties. The incorporation of ultra-violet (UV) spectroscopy with HPLC allows the concentration of molecules to be determined following separation.

Derivatization- Derivatization, or chemical structure modification, performed by high performance liquid chromatography technique in order to enhance detectability or to improve the chromatographic performance for the target analytes.