Development and Validation of a Stability-Indicating HPLC Method for the

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Abstract

A stability-indicating High Performance Liquid Chromatography (HPLC) method of analysis of desvenlafaxine suucinate (DSV) in the presence of its acidic induced-degradation product in pure and pharmaceutical preparation had been developed and validated. The chromatographic conditions comprised of an isocratic reversed-phase separation on Discovery C18 column. Elution was carried out using acetonitrile: phosphate buffer pH 3.8 (50:50 v/v) as a mobile phase at a flow rate of 0.7 ml/min and UV detection at 229 nm. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 5-100 µg/ml (r² = 0.9999). The values of slope and intercept were 34.295 and 12.564 respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria. The specificity and stability-indicating capabilities of the method was verified by subjecting DSV to acid hydrolytic stress condition. The acid degradation product was confirmed as 4-(1-cyclohexenyl-2-(dimethylamino)ethyl)phenol. Under the chromatographic condition, the degradation product was well resolved from the active pharmaceutical ingredients with significantly different retention time. Thus the proposed method was found to be stability-indicating and can be used for routine analysis of the drug without interference of acidic degradation product. The proposed method was successfully applied for the analysis of pharmaceutical formulation. The validity of the suggested procedures was further assessed by
applying the standard addition technique which was found to be satisfactory. The results were statistically analyzed and compared with those obtained by the reported method.

A stability-indicating High Performance Liquid Chromatography (HPLC) method of analysis of desvenlafaxine succinate (DSV) in the presence of its acidic induced-degradation
product in pure and pharmaceutical preparation had been developed and validated. The chromatographic conditions comprised of an isocratic reversed-phase separation on Discovery C18 column.

Elution was carried out using acetonitrile: phosphate buffer pH 3.8 (50 : 50 v/v) as a mobile phase at a flow rate of 0.7 ml/min and UV detection at 229 nm. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 5-100 μg/ml (r =0.9999). The values of slope and intercept were 34.295 and 12.564 respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria. The specificity and stability-indicating capabilities of the method was verified by subjecting DSV to acid hydrolytic stress condition. The acid degradation product was confirmed as 4-(1-cyclohexenyl-2-(dimethylamino)ethyl)phenol. Under the chromatographic condition, the degradation product was well resolved from the active pharmaceutical ingredients with significantly different retention time. Thus the proposed method was found to be stability-indicating and can be used for routine analysis of the drug without interference of acidic degradation product. The proposed method was successfully applied for the analysis of
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ABSTRACT
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Keywords: HPLC; desvenlafaxine succinate; acidic degradation product; NMR.
INTRODUCTION

Depression is one among the most rampant form of psychiatric disorders and a leading cause for morbidity and mortality[1].

Desvenlafaxine succinate (DSV) is a newer antidepressant drug which is chemically RS-4-[2-dimethylamino-1-(1-hydroxycyclohexyl) ethyl] phenol succinate monohydrate (Fig. 1). The FDA approved desvenlafaxine in February 2008 for the treatment of patients with major depressive disorder [2]. Moreover, desvenlafaxine is also being investigated as the first non-hormonal treatment for vasomotor symptoms attributed to menopause [3]. Venlafaxine is mainly metabolized in the liver to desvenlafaxine by cytochrome P450 2D6 (CYP2D6).

Desvenlafaxine is pharmacologically active and is not metabolised by CYP2D6 and is excreted unchanged or after conjugation [4].

There is no reference for determination of desvenlafaxine succinate in official compendia.

Several methods have been reported in the literature for simultaneous determination of the DSV with venlafaxine in plasma and biological fluids using HPLC coupled to spectrophotometric [5-8], spectrofluorimetric [9-12] or coulometric detection [13]. Capillary electrophoresis,[14–20], HPLC-ESI/MS [21] and LC-MS/MS [22-27] have been used as well.

To our knowledge, simple UV Spectrophotometric method and difference
spectrophotometric method have been developed for the estimation of desvenlafaxine succinate in tablet dosage

form[28]. Dimal A. Shah et al reported that the DSV is stable to acid hydrolysis at room temperature and at 70°C [29].

N
OH
HO

Figure 1: Chemical structure of Desvenlafaxine

The International Conference on Harmonization (ICH) guidelines [30] require the implementation of stress testing procedures for the identification of degradation products that are potentially occurring in drug substances which can help to understand the possible degradation pathway for the drug. Thus in this work, we described forced degradation of DSV under acidic condition and the product was characterized by IR, MS and 1H NMR spectral data. Today, RP- HPLC is the most popular analytical technique for separating complex mixtures in the chemical, pharmaceutical and biotechnological industry. RP-HPLC is the opposite of normal-phase chromatography, with a nonpolar stationary phase and a polar, largely aqueous mobile phase.

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The most common stationary phases used are octadecyldimethyl (C18) phases with silica as the solid support [31].

The aim of the present study was to develop and validate a simple, isocratic RP-HPLC method for the determination of DSV in tablets in the presence of its acid degradation product.

EXPERIMENTAL SECTION

Materials and Reagents:

All chemicals and reagents used were of analytical or pharmaceutical grade. Solvents were of HPLC grade. Analytically pure DSV was purchased from AFINE CHEMICALS LIMITED, Hangzhou, China its purity found to be 99.33%. D-VENIZ 50 tablets (SUN pharmaceutical ind. Ltd.; each tablet was labeled to contain desvenlafaxine succinate equivalent to 50 mg desvenlafaxine. Acetonitrile, HPLC grade (LAB SCAN), Water, HPLC grade (LAB SCAN), Potassium dihydrogen phosphate (Adwic) and Ortho phosphoric acid were used.

Diluent: A mixture of acetonitrile: Water in the ratio 50:50 v/v.

Instrument used

The HPLC system consisted of Younglin instrument equipped with gradient HPLC pump 9000, mixer and degasser, and 9000 UV-detector, manual 20 µL loop, autochrome3000 software.
system controller. The separation is made on Discovery® C-18 column, 5 μm particle size (250 × 4.6 mm). The samples are injected with a 100 μl Agilent analytical syringe. Teflon membrane filter, pore size 0.45 μm and 47mm diameter for solvents, Teflon disposable membrane filter pore size 0.45 μm for samples. An ultrasonic, Soniclean 120T, Australia, A digital pH meter and HANNA HI 9321, Portugal were used.

Methodology

Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column.

The mobile phase consisted of acetonitrile : 5 mM potassium dihydrogen phosphate solution (50:50 v/v) at a flow rate of 0.7 ml/min. Potassium dihydrogen phosphate solution was prepared by dissolving 680mg KH₂PO₄ in 1000ml HPLC grade water. Final pH of the mobile phase was adjusted to 3.8 by orthophosphoric acid. The mobile phase so prepared was filtered through 0.22 μm nylon membrane filter and degassed by sonication. Flow rate of 0.7ml / min was maintained.

Detection was carried out at 229 nm. The injection volume was 20 μl for assay and degradation level.

Preparation of stock solutions

preparation of intact DSV standard solution

An accurate weight of DSV (25 mg) was introduced into a 25 ml volumetric flask,
dissolved in and completed to volume with water to prepare a stock solution of concentration (1000µg/ml).

Aliquots (5, 6 and 7 ml) of stock solution were diluted with diluent to prepare working standard solutions of DSV of concentration 100, 120 and 140µg/ml.

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Preparation of Acid Induced Degradation Product.

Phosphoric acid (1ml) was added dropwise to a solution of DSV (500mg) in diethyl ether (10ml) in ice bath during addition and keeping the stirring for 10 hours at room temperature. The pH of the reaction mixtures was adjusted to 7.5 with 10% sodium carbonate solution where the product was precipitated, the diethyl ether was allowed to evaporate and then the formed product was filtered, dried and recrystallized from acetonitrile.

The Deg. product was accurately weighed (25 mg), transferred to a 100 ml volumetric flask, dissolved and diluted up to the mark with the diluent (250µg/ml).

Preparation of D-VENIZ 50 tablets solution:
Twenty tablets were accurately weighed and finely powdered. An amount of powder equivalent to 25 mg DSV was transferred to 100 ml volumetric flask containing 70 ml water, sonicated for 15 minutes, diluted up to the mark with water and filtered discarding the first 15 ml. An aliquot (25 ml) was transferred to 50 ml volumetric flask and complete to volume with acetonitrile to prepare tablet solution of concentration (125 μg/ml).

Method Validation

System suitability

According to the USP 28, the system suitability tests are an integral part of chromatographic analysis and should be used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed.

To ascertain the effectiveness of the method developed in this study, system suitability tests were performed on standard stock solutions of DSV and its acidic degradation product.

Linearity and Limits of Detection and Quantitation

Accurately measured aliquots of working standard solution DSV (100 μg/ml) equivalent to (50-1000 μg) were transferred separately into a series of 10 ml volumetric flasks and diluted up to the mark with diluent. Twenty micro liters for each solution was injected in duplicate injection in to Discovery C18 column and the chromatogram was recorded at a flow rate of 0.7 ml/min.; the eluent was monitored at 229 nm. The area under peaks (AUPs) were recorded and used for the
construction of calibration curve. A regression equation was computed.

LOD and LOQ were calculated using following equation as per ICH guidelines.

\[ \text{LOD} = 3.3 \times \sigma / \text{S} \]

\[ \text{LOQ} = 10 \times \sigma / \text{S} \]

where \( \sigma \) is the standard deviation of y-intercept of regression line and \( S \) is the slope of the calibration curve.

Precision

Pure samples of DSV (24, 66, & 90 \( \mu \)g/ml) were analyzed over different days to obtain inter-day (intermediate precision, \( n = 3 \)) and within the same day to obtain intra-day precision (repeatability, \( n = 3 \)) , then the RSDs % values were calculated.

Accuracy

Aliquot portions of DSV working standard solution (120 \( \mu \)g /ml) equivalent to (120-840 \( \mu \)g) were transferred into a series of 10 ml volumetric flasks and complete to volume with diluent.
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