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ORIGINAL ARTICLE

A validated stability-indicating LC method for estimation of etoposide in bulk and optimized self-nano emulsifying formulation: Kinetics and stability effects

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Abstract The present investigation was aimed to establish a validated stability-indicating liquid chromatographic method for the estimation of etoposide (ETP) in bulk drug and self-nano emulsifying formulation. ETP was successfully separated from the degradation products formed under stress conditions on LiChrospher 100 C_{18} reverse-phase column (a 250 mm × 4.6 mm i.d., 5-μm particle size) using 55:45 (v/v) acetonitrile–phosphate buffer saline (pH 4.5) as the mobile phase, at a flow rate of 1.0 mL min^{-1} and detection at 283 nm. The response was a linear function of analyte concentration ($R^2 > 0.9997$) over the concentration range of 0.05–50 μg mL^{-1}. The method was validated for precision, accuracy, robustness, sensitivity and specificity. The % recovery of ETP at three different levels (50%, 100% and 150%) ranged between 93.84% and 100.06% in optimized self-nano emulsifying formulation, Etosid® soft-gelatin capsule and Fytosid® injection. First-order degradation kinetics of ETP were observed under acidic and alkaline conditions. The method was also applied for the stability assessment of self-nano emulsifying formulation under accelerated conditions, the formulation was found to be stable at all storage conditions with the shelf-life of 2.37 years at 25 °C. The method holds promise for routine quality control of ETP in bulk, pharmaceutical formulations as well as in stability-indicating studies.

1. Introduction

Etoposide (ETP), chemically designated as 4′-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene)-β-D-glucopyranoside, is an important antineoplastic agent currently in clinical use for the treatment of small cell lung cancer, testicular cancer and lymphomas (Toffoli et al., 2004; Sissolak et al., 2010). Its mechanism of action involves breakage of DNA strands by reversible interaction with topoisomerase II (Hande, 2008). Low and erratic oral absorption of ETP has been attributed

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to drug precipitation in the gastrointestinal lumen due to poor aqueous solubility, pH-related degradation and efflux by *p*-glycoprotein transporter (Patlolla and Vobalaboina, 2005; Tian et al., 2007; Bansal et al., 2009; Akhtar et al., 2011a). In order to overcome the above mentioned constraints, self-nanoemulsifying (SNE) formulation of ETP has been developed and optimized in our laboratory. SNE formulation comprises of isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants (Date et al., 2010). A selective and sensitive stability-indicating analytical method is required for the evaluation of ETP based novel drug delivery system. Analytical methods such as high-performance liquid chromatography (LC) coupled with UV detection (Shirazi et al., 2001; Kato et al., 2003; Zhang et al., 2010), fluorescence detection (Robieux et al., 1996), electrochemical detection (Eisenberg and Eickhoff, 1993; Duncan et al., 1986; Cai et al., 1999) and solid-phase extraction (Manouilov et al., 1998) have been previously reported for the determination of ETP. However, most of these methods are not ideal for routine measurements, since they necessitate tedious extraction procedures in case of biological fluids and exhibit long retention times. Modern chromatographic method, such as LC-MS/MS, differential pulse voltammetry and UPLC-qTOF-MS/MS have also been developed to determine ETP level in biological matrixes (Chen and Uckuna, 2000; Radi et al., 2007; Sachin et al., 2010). However, these interfaces are extremely complicated and quite expensive to be employed for routine analysis. Moreover, these are not suitable for parent drug stability test guidelines issued by ICH.

The ICH guideline entitled "Stability testing of new drug substances and products" requires stress testing to be carried out in order to elucidate the inherent stability characteristics of the active substance. These include various stress tests like hydrolytic stability, oxidative stability and photolytic stability testing (ICH, Q1A (R2), 2005). An ideal stability-indicating analytical method should be able to quantify the active constituents and at the same time resolve the drug from its degradation products. This would also enable the detection and measurement of the drug and its degradation products in the presence of excipients employed in the formulation. The literature is silent on the development of a validated stability-indicating assay method in routine analysis of ETP in the presence of its degradation products. Therefore, it was thought necessary to study the stability of ETP under different stress conditions. The main objective of the present manuscript was to develop and validate stability-indicating high-performance liquid chromatographic method for the determination of ETP in the presence of its degradation products in accordance to ICH guidelines. The proposed stability-indicating method is simple and allows rapid for stability studies and quality control analysis of drug in bulk, pharmaceutical formulations as well as in stability-indicating studies. Moreover, the proposed LC method was utilized to investigate the kinetics of this antineoplastic agent under acidic and alkaline conditions at different temperatures and their respective degradation kinetic parameters were calculated with the help of Arrhenius plot. The proposed LC method was also utilized for stability assessment of the optimized self-nano emulsifying formulation under accelerated conditions.

2. Materials and methods

2.1. Materials

Etoposide was (assigned purity: 99.5%) originated as gift sample from the Dabur Research Foundation (Ghaziabad, India). Marketed products (Etosid® soft-gelatin capsule, claimed to contain 50 mg and Fytosid® injection, claimed to contain 20 mg mL^{-1} of ETP) were commercially purchased. Sefsol 218 was gift sample from Nikko Chemicals (Japan). Transcutol P was kindly provided by Gattefosse, France (Mumbai, India). Cremophor RH 40 was achieved from BASF (Mumbai, India). Triacetin was purchased from E-Merck (Mumbai, India). HPLC-grade methanol and acetonitrile were obtained from Spectrochem Pvt. Ltd. Mumbai, India. Buffers and other chemicals were of analytical-reagent grade. Ultra-pure water was obtained from a Milli-Q system (Millipore, USA).

2.2. Methodology

2.2.1. Standard solution

The stock solution of ETP was prepared by dissolving 10 mg in 20 mL methanol in a 100-mL volumetric flask following dilution to 100 mL with mobile phase. This solution was then diluted as needed to prepare different standard solutions from 0.05 to 50 μg mL^{-1}. The quality control (QC) samples of ETP at three different levels were independently prepared at concentrations of low QC (LQC, 2 μg mL^{-1}), medium QC (MQC, 10 μg mL^{-1}) and high QC (HQC, 40 μg mL^{-1}).

2.2.2. Optimized SNE, capsule and injection preparation

The self-nano emulsifying formulation of ETP was prepared by aqueous titration (low energy emulsification technique) method (Shafiq et al., 2007; Azeem et al., 2009a). The composition of optimized SNE formulation of ETP (ETP_{SNE}) was 28.57% (w/w) of sefsol 218 and Triacetin (1:1) (oil), 47.62% (w/w) of Cremophor RH 40 (surfactant) and 23.81% (w/w) of Transcutol P (co-surfactant). The optimized ETP_{SNE} formulation (label claim 50 mg), Etosid® soft-gelatin capsule (label claim 50 mg) and Fytosid® injection (label claim 20 mg mL^{-1}) with an appropriate volume of 300, 200 and 500 μL were suitably diluted with methanol, respectively, to yield a stock concentration of 100 μg mL^{-1}. These solutions were sonicated for 10 min and further diluted with the mobile phase to yield a concentration of 10 μg mL^{-1}. The above solutions were then filtered through a 0.22-μm nylon membrane filter and analyzed in triplicate by proposed LC method.

2.2.3. Instrumentation and chromatographic conditions

The analysis was carried out on a Waters Alliance e 2695 separating module (Waters Co., MA, USA) using photo diode array detector (waters 2998) with autosampler and column oven. The instrument was controlled by the use of Empower software installed with equipment for data collection and acquisition. Compounds were separated at an ambient temperature (25 ± 2 °C), on a 250 mm × 4.6 mm i.d., 5-μm particle size, LiChrospher 100 C_{18} reverse-phase column with 55:45 (v/v) acetonitrile–phosphate buffer saline (pH 4.5) as the mobile phase at a flow rate of 1.0 mL min^{-1}. Before use, the mobile phase was filtered through a 0.22-μm Nylon filter.