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Development and validation of a high performance chromatographic method for determining sumatriptan in niosomes

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ABSTRACT

In this paper, a novel, precise, specific, accurate and rapid reversed-phase high performance liquid chromatographic method was developed, optimized and validated for determining sumatriptan succinate in niosomes with the best chromatographic peak resolution, reduced run time and low cost of analysis. The formulation has been previously optimized in terms of composition and preparation technique to obtain a high drug encapsulation efficiency and adequate vesicle size distribution.

This method showed the best resolution by using Spherisorb® OSD2 C18 column (250 mm × 4.6 mm, 5 μm) using phosphate buffer (0.05 M):acetonitrile (80:20, v/v; pH adjusted to 6.0) as a mobile phase at a flow rate of 1 mL/min and wavelength of 214 nm.

The main objective of this research was to demonstrate the robustness of the reversed-phase HPLC method development by applying the Taguchi robust methodology. The signal-to-noise ratio (S/N) was employed as a quality measurement. This tool permits to establish the influence of some selected factors (acetonitrile:phosphate ratio, pH buffer, oven temperature and flow rate) on two responses (peak areas and retention time). On the basis of the results obtained, we can conclude that this analytical method was robust for all the factors studies, as exception of the flow rate, where the higher quality was obtained for the fewer values (0.8 mL/min). Therefore, this parameter must be carefully controlled when this method was employed, to avoid any modification in the peak areas overall.

1. Introduction

Sumatriptan succinate is a selective serotonin 5-HT agonist at the 5-HT_{1B} and 5-HT_{1D} receptors. It was the first of the so-called "triptan" drugs which have had a significant impact on the treatment of acute migraine episodes and it is available in several dosage forms including products for oral, nasal and parenteral delivery [1].

Despite the oral route could become the definitive step towards the control of migraine episodes, important problems leading to a very low oral bioavailability of sumatriptan have discouraged its clinical application [2].

During the last decade, there has been a continuous interest in the use of colloidal drug delivery systems for the development of sumatriptan delivery systems able to prolong their therapeutic effect. The use of colloidal drug delivery systems, such as liposomes and niosomes, is a suitable strategy to enhance the bioavailability of topically administered drugs, because they offer unique features while preserving the ease of delivery in liquid form [3,4].

Niosomes attract much attention because of their advantages in many aspects, such as chemical stability, high purity, content uniformity, low cost and convenient storage of non-ionic surfactants, and the existence of a large number of surfactants available for the design of these vesicles [5,6]. With this aim, sumatriptan-loaded niosomes were engineered.

However, it is important to develop a specific analytical technique for quantifying the drug from these formulations to have adequate results.

Most of the analytical methods found in the literature, carried out by high-performance liquid chromatography (HPLC) to determine sumatriptan, are aimed at quantifying this substance in plasma and pharmaceutical formulations [7–9], to determine the raw material and its related substances. However, neither of the described methods by HPLC was dedicated to the study of sumatriptan from lipid vesicles as final products.

When an analytical method has been developed, it is important to confirm that it is suitable for its intended purpose. Therefore, the method validation is today an essential concern in the activity of analytical chemistry laboratories.

Taguchi's methodology has been widely applied to the pharmaceutical process design. This methodology constitutes a strategy that involves the use of mathematical and statistical tools to obtain the maximum information through the experimental data and

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to find the optimal conditions for the execution of a particular experimental process. Three specific tools have been used, namely orthogonal arrays, the signal-to-noise ratio (S/N) and analysis of variance (ANOVA) [10].

Successful application of Taguchi method is able to allow operating conditions to be optimized with a minimized sensitivity to noise (a hard-to-control variable). In this approach, the S/N ratio has been introduced as a measurement of the quality characteristic deviating from the desired value. It is also used the S/N ratio to convert the experimental results into a quality parameter to evaluate the optimum analysis [11]. So, the experimental condition having the least variability as the optimum condition can be determined.

Also, Taguchi methods are used for maximizing the robustness of products and processes, thereby achieving high quality at a low cost and time.

Robustness is essential in the production of nearly all products. The variation in the quality of the product may vary from environmental factors and/or manufacturing variables that cannot be easily controlled. Such factors are called 'noise factors'. The Taguchi method uses the S/N ratio, which is directly transformed from the quadratic quality loss function, as a measure to determine the robustness of a process. Thus, optimizing process parameters by means of Taguchi method is an attempt not only to bring the average quality near to the target value, but also to simultaneously to minimize the variation in quality. So, S/N ratio is the best index to measure quality in a robust method and it shows the magnitude of the interaction between 'control factors' and 'noise factors' [12] as showed many authors [13–16].

In this work, two main purposes have been planned. First, the validation process of the analytical method by HPLC for the determination of sumatriptan in niosomes, describing validation parameters stated both by USP 29 and by the ICH guidelines to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility, ensuring that the findings achieved, when this method was applied, were correct. On the other hand, the Taguchi optimization methodology has been proposed to optimize robustness parameter in this validation process.

2. Materials and methods

2.1. Chemical and reagents

Sumatriptan succinate (SS) was received as a gift sample from Glaxo Smith Kline (Brentford, UK). Chloroform, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), potassium di-hydrogen phosphate, acetonitrile HPLC quality, HCl 37%, sodium hydroxide and hydrogen peroxide 33% (v/v) were received from Panreac Chemistry (Barcelone, Spain). Stearylamine (SA) was purchased from Fluka-Biochemika (Switzerland). Cholesterol (CH) was obtained from Sigma–Aldrich (Barcelone, Spain). Eumulgin® B2 was obtained from Acofarma (Barcelone, Spain). Deionized and purified water using a Milli-Q system (Millipore) was selected as solvent for the standard solutions preparation. All other reagents used in this study were of analytical grade.

2.2. Chromatographic system

The chromatographic apparatus consisted of a Hitachi Elite LaChrom, equipped with a quaternary pump L-2130, a diode array detector L-2455, an automatic injector L-2200 and oven L-2350. For data collection and calculation, EZChromElite Data System Manager Software was used.

The chromatographic conditions were used as previously described [17]. This analytical method was optimized by using a column C18 (Waters, Spherisorb® OSD2 250 mm × 4.6 mm, 5 μm). The mobile phase consisted of phosphate buffer (0.05 M):acetonitrile (80:20, v/v) adjusted to pH 6 with sodium hydroxide (0.05 M). The mobile phase was filtered through a 0.22 μm nitrocellulose-membrane filter (Millipore, Barcelone, Spain) and degassed under vacuum prior to use. Absorbance was measured at 227 nm and the flow rate was 1 mL/min. The injection volume was 10 μL. Peak areas were measured and high-performance liquid chromatography analysis was conducted at room temperature.

2.3. Preparation of sumatriptan niosomes and determination of drug content into vesicles

Multilamellar vesicles (MLV) were prepared by a modification of the thin film-hydration method. Briefly, Eumulgin® B2 as non-ionic surfactant was completely dissolved in about 8 mL of chloroform. This solution was deposited as a thin film in a round-bottom flask by rotaevaporating the chloroform under vacuum. Then, 5 mg of SA and a constant amount of CH (3:1 surfactant/CH molar ratio) were added. The mixture was again dissolved in about 8 mL of chloroform and again rotaevaporated to remove the organic solvent and to form a lipid thin film. The vacuum was applied for 1 h to ensure the total removal of trace solvents. The film was then hydrated by adding 6 mL of the hydrophilic phase, containing 15 mg of SS dissolved in HEPES 10 mM. Samples were submitted to five vortexing cycles (each cycle consisting in stirring for 2 min and heating at 58 °C for 5 min) until vesicle formation. The temperature was maintained at 58 °C until the end of the process, above the gel–liquid transition temperature (T_c) of the amphiphilic and lipid substances. All formulations were quickly sealed in glass containers and stored in the dark at 4 °C.

The composition of this formulation has been selected on the basis of previously reported works (data not shown).

2.4. Standard and sample solutions

Standard solutions of SS at a concentration of about 2.5 mg/mL have been prepared by dissolving the appropriate amount of SS (2.5 mg) in 1 mL of deionized water.

This standard solution will be used to compare with the drug amount in the final product. These solutions have been stored in the dark under refrigeration at 4 °C and have been found to be stable for several weeks. The stability of the standard solutions has been checked over this period by preparing and injecting daily a solution of the analyte.

To carry out the sample solution preparation (assay of pharmaceutical preparation), an appropriate amount of niosomes equivalent to 2.5 mg of SS was placed in a 1 mL volumetric flask with 500 μL of methanol. This sample was vortexed for 5 min and diluted to volume with water, and was finally filtered through a 0.22 μm nylon-membrane filter (Millipore, Barcelone). The resulting filtered solution was placed in a HPLC vial and injected.

2.5. Validation study

The method was validated in agreement with the International Conference on Harmonization Guidelines (ICH Q2(R1)), using the following parameters: linearity, precision, accuracy, specificity, detection and quantitation limits and robustness.

2.5.1. System suitability

A system suitability test has been performed by injecting the SS solution six times into the HPLC system. From these injections, the RSD was calculated.