Journal of Saudi Chemical Society (2017) **21**, S101–S111

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

An experimental design approach for optimization of spectrophotometric method for estimation of cefixime trihydrate using ninhydrin as derivatizing reagent in bulk and pharmaceutical formulation

Yogita B. Wani [a], **Dipak D. Patil** [b,*]

[a] *Department of Quality Assurance, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dhule, Maharashtra, India*
[b] *Department of Pharmaceutical Chemistry, H. R. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dhule, Maharashtra, India*

Received 6 May 2013; revised 29 October 2013; accepted 2 November 2013
Available online 11 November 2013

KEYWORDS

Cefixime trihydrate;
Ninhydrin;
Experimental design;
Full factorial;
Box-Behnken design

Abstract The aim of the present work is to use experimental design to screen and optimize experimental variables for developing a spectrophotometric method for determining cefixime trihydrate content using ninhydrin as a derivatizing reagent. The method is based on the reaction of the amino group of cefixime with ninhydrin in an alkaline medium to form a yellow-colored derivative (λ_{max} 436 nm). A two-level full factorial design was utilized to screen the effect of ninhydrin reagent concentration (X_1), volume of ninhydrin reagent (X_2), heating temperature (X_3) and heating time (X_4) on the formation of the cefixime–ninhydrin complex Y (absorbance). One way ANOVA and Pareto ranking analyses have shown that the ninhydrin reagent concentration (X_1), volume of ninhydrin reagent (X_2) and heating temperature (X_3) were statistically significant factors ($P < 0.05$) affecting the formation of the cefixime–ninhydrin complex Y (absorbance). A Box-Behnken experimental design with response surface methodology was then utilized to evaluate the main, interaction and quadratic effects of these three factors on the selected response. With the help of a response surface plot and contour plot the optimum values of the selected factors were determined and used for further experiments. These values were a ninhydrin reagent concentration (X_1) of 0.2% w/v, volume of ninhydrin reagent (X_2) of 1 mL and heating temperature (X_3) of 80 °C. The proposed method was validated according to the ICH Q2 (R1) method validation guidelines. The results of the present

* Corresponding author. Tel.: +91 9960878673.
E-mail address: dipakpatil888@rediffmail.com (D.D. Patil).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2013.11.001

study have clearly shown that an experimental design concept may be effectively applied to the optimization of a spectrophotometric method for estimating the cefixime trihydrate content in bulk and pharmaceutical formulation with the least number of experimental runs possible.

1. Introduction

Cefixime trihydrate (CEF) is an oral third generation cephalosporin antibiotic used in the treatment of gonorrhea, tonsillitis and pharyngitis [1]. Chemically, CEF is (6*R*, 7R)-7-{(2-(2-amino-1, 3-thiazol-4-yl)-2-(carboxymethoxyimino) acetyl) amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo (4.2.0) oct-2-ene-2-carboxylic acid (Fig. 1) [2].

Two-level full and fractional factorial designs as well as Plackett–Burman designs are used to screen the important factors that influence process output measures or product quality [3]. A two-level full factorial design consists of two levels of each experimental factor and has a design matrix made up of all combinations of these factor levels. It can provide the direction for further experimentation [3].

Response surface methodology (RSM) is a statistical technique used for the development and optimization of complex processes [4–6]. RSM is used after preliminary screening of experimental factors that significantly affect the response using factorial designs [4]. The technique has several advantages over conventional optimization method in which one variable is used at a time (OVAT). RSM provides a large amount of information and is a relatively economical approach because a small number of experiments are performed for monitoring the interaction of the independent variables and the response. In conventional optimization, the increase in the number of experiments necessary to carry out the research, leads to an increase in time and expenses as well as an increase in the utilization of reagents and materials for experiments [7].

Many types of response surface designs are used for optimization, such as central composite, Doehlert and Box-Behnken designs. The Box-Behnken design is preferable to central composite and Doehlert designs because it requires fewer test runs and is rotatable. A design is rotatable only when the experiments are roughly situated on a (hyper) sphere. By selection of an adequate number of center points, it is possible to modify the precision of the response of a predicted design to be similar over the whole domain. Such a design is said to have uniform precision [3].

The Box-Behnken design is advantageous because it does not contain any points at the extremes of the cubic region created by the two-level factorial combinations [4,5,8]. The Box-Behnken design was selected in the present investigation and used to optimize, validate and analyze CEF spectrophotometrically, because the design provides three levels for each factor and requires fewer runs in the three-factor case compared with the central composite and Doehlert design.

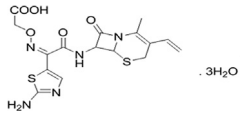

Figure 1 Structure of CEF.

Numerous studies have been carried out on CEF. Indian Pharmacopoeia [2], British Pharmacopoeia [9], United States Pharmacopoeia [10] and European Pharmacopoeia [11] described a liquid chromatographic method for estimating the CEF content in bulk form and performing assays of oral suspensions and tablet formulations of CEF. The use of spectrophotometry [12–18], spectrofluorimetry [17], high performance liquid chromatography (HPLC) [12,19] and voltammetry [20] to estimate the content of CEF in single-component formulations was reported in the literature. Estimation of the content of CEF in multicomponent formulations by spectrophotometry [21–23], HPLC [24–27] and high performance thin layer chromatography (HPTLC) [28,29] was described in the literature.

The visible spectrophotometric method described by [12] was based on the oxidative coupling reaction of CEF with 3-methyl-2-benzothiazolinon hydrazone hydrochloride in the presence of ferric chloride. [16] developed a stable, product with a concentration dependent cherry-red color after the reaction of CEF with sodium nitrite in an acidic solution. A spectrophotometric method based on the acidic oxidation of CEF with cerium at elevated heating temperature was studied by [17] and [18] described a spectrophotometric method in which cephalosporins are converted into hydroxamic acid, which forms a colored complex with iron(III). A simple, accurate and precise spectrophotometric method based on the derivatization of CEF with sodium 1,2-naphthoquinone-4-sulfonate was developed by [30]. An accurate, precise and eco-friendly spectrophotometric method for estimating the content of CEF using ninhydrin was developed by [31]. The conventional experimental method was utilized to optimize the reaction variables in the above spectrophotometric methods. The one variable at a time method is inefficient and gives misleading results [7]. So it must be avoided. Therefore, there is a need to use a systematic and statistical way of optimizing the reaction variables so as to obtain significant and precise results.

The aim of the present work was to utilize the experimental design approach for screening and optimizing the experimental variables for developing a spectrophotometric method for determining the content of CEF in bulk and pharmaceutical formulations using ninhydrin as a derivatizing reagent.

2. Experimental

2.1. Instrument

A Shimadzu UV–visible spectrophotometer 1700 (UV probe software) with 1-cm matched quartz cells was used for measuring the absorbance.

2.2. Materials

CEF was obtained as a gift sample from Centurion Laboratories, Vadodara (Gujarat). HIFEN DT 100® tablets (100 mg)