Journal of Pharmaceutical and Biomedical Analysis 96 (2014) 135–143

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Implementation of design of experiments for optimization of forced degradation conditions and development of a stability-indicating method for furosemide

Moolchand Kurmi [a], Sanjay Kumar [a], Bhupinder Singh [b], Saranjit Singh [a,*]

[a] Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, 160062 Punjab, India
[b] Division of Pharmaceutics, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India

ARTICLE INFO

Article history:
Received 21 December 2013
Received in revised form 18 March 2014
Accepted 23 March 2014
Available online 31 March 2014

Keywords:
Furosemide
Stability-indicating method
Design of experiment
Degradation products

ABSTRACT

The study involved optimization of forced degradation conditions and development of a stability-indicating method (SIM) for furosemide employing the design of experiment (DoE) concept. The optimization of forced degradation conditions, especially hydrolytic and oxidative, was done by application of 2^n full factorial designs, which helped to obtain the targeted 20–30% drug degradation and also enriched levels of degradation products (DPs). For the selective separation of the drug and its DPs for the development of SIM, DoE was applied in three different stages, *i.e.*, primary parameter selection, secondary parameter screening and method optimization. For these three, IV-optimal, Taguchi orthogonal array and face-centred central composite designs were employed, respectively. The organic modifier, buffer pH, gradient time and initial hold time were selected as primary parameters. Initial and final organic modifier percentage, and flow rate came out as critical parameters during secondary parameter screening, which were further evaluated during method optimization. Based on DoE results, an optimized method was obtained wherein a total of twelve DPs were separated successfully. The study also exposed the degradation behaviour of the drug in different forced degradation conditions.

1. Introduction

Forced degradation study is a complementary part of stability testing wherein influence of environmental stress factors like pH, temperature, humidity, oxygen and light are evaluated on drug substances and products [1]. Various approaches are available in the literature to conduct forced degradation studies [2–4], and most of them recommend the use of specified and limiting forced degradation conditions, like stressor strength, temperature, time of exposure, *etc.* The usual target is 10–30% drug degradation, however, chances exist that critical degradation products (DPs) may be formed in relatively low concentration (<10%) in the forced degradation conditions employed. To enrich the DPs, the normal practice is to change the forced degradation conditions, which is usually done by hit and trial. A very new approach in the field of forced drug degradation is to evaluate inter-dependency of forced degradation parameters by applying the concept of design of experiment (DoE) [5–7]. In turn, this can help to arrive at a combination of forced degradation conditions where maximum enrichment of DPs is obtained. DoE is also an established tool for the development of analytical methods [8–10]. The same has been extended lately to the development of stability-indicating [11–13] and impurity-profiling methods [14,15] involving separation of multiple components.

The twin objectives of the present study were: (i) optimization of forced degradation conditions for furosemide (4-chloro-2-[(furan-2-ylmethyl) amino]-5-sulfamoylbenzoic acid) by using DoE strategy, and (ii) application of DoE concepts to the development of stability-indicating method (SIM) for the drug, which was also suitable for LC–MS studies. Many studies are reported in the literature on stability behaviour of furosemide, including development of HPLC methods for evaluation of the same [16–20], but yet there is no study on its forced degradation behaviour according to ICH guidelines. Moreover, no reports exist on the application of DoE to the optimization of forced degradation conditions and the development of a SIM for this drug. In general, the development of HPLC analytical methods by DoE approach involves an arbitrary selection of parameters influencing the method. This study was done in three stages to systematically evaluate all the parameters involved in HPLC analysis.

* Corresponding author. Tel.: +91 172 2292031; fax: +91 172 2214692.
E-mail address: ssingh@niper.ac.in (S. Singh).

http://dx.doi.org/10.1016/j.jpba.2014.03.035
0731-7085/

Table 1
Levels of forced degradation parameters used for optimization of hydrolytic and oxidative conditions.

Conditions	Stressor	Levels of forced degradation parameters		
		Concentration	Temperature (°C)	Time (h)
Hydrolytic	HCl (N)	0.01 and 0.1	50 and 80	6 and 12
	NaOH (N)	0.01 and 0.1	50 and 80	6 and 12
	ACN:H_2O (50:50, v/v)		50 and 80	12 and 24
Oxidative	H_2O_2 (%)	5 and 15	30 and 60	24 and 48
	AIBN (mmol%)	5 and 20	30 and 60	24 and 48

Table 2
Design matrices for optimization of forced degradation conditions along with responses in terms of percent drug degradation and percent of targeted DPs formed.

Forced degradation conditions	Runs	Variables			Responses[a]		
		A	*B*	*C*	R_1	Targeted DPs[b]	R_2
Acid (N HCl)	1	0.01	80	6	12.0	DP III	11.7
	2	0.1	50	6	15.0		14.4
	3	0.01	50	12	1.9		1.8
	4	0.1	80	12	98.5		87.6
	5	0.1	50	12	23.0		21.4
	6	0.01	50	6	1.3		1.2
	7	0.1	80	6	85.0		74.3
	8	0.01	80	12	45.0		38.3
Neutral (ACN:H_2O (50:50, v/v))	1		50	24	0.3	DP VIII	0.1
	2		50	12	0.17		0.0
	3		80	24	82.7		26.0
	4		80	12	6.9		1.7
H_2O_2 (%)	1	15	30	48	3.7	DP I and II[c]	1.2
	2	5	60	24	22.4		3.4
	3	5	30	48	3.6		0.6
	4	5	60	48	59.7		6.0
	5	15	60	48	92.2		27
	6	5	30	24	2.0		0.7
	7	15	60	24	46.5		5.6
	8	15	30	24	2.2		0.7
AIBN (mmol%)	1	20	60	48	46.5		
	2	20	30	48	11.1		
	3	5	30	48	2.8		
	4	5	30	24	0.8		
	5	20	60	24	32.4		
	6	20	30	24	1.5		
	7	5	60	24	9.6		
	8	5	60	48	15.9		

DPs, degradation products; *A*, concentration; *B*, temperature (°C); *C*, time (h); R_1, observed total drug degradation; R_2, percent of targeted DPs.
[a] On relative area percent basis.
[b] DPs other than I, II, III and VIII were not targeted because they were not affected significantly by changing of forced degradation conditions.
[c] A single peak was observed for DPs I and II at this stage. However successful separation was achieved during subsequent step of secondary parameter screening at the time of method development.

2. Experimental

2.1. Chemicals and reagents

Pure furosemide was supplied by Sigma–Aldrich Inc. (St. Louis, USA). Hydrochloric acid (HCl) was procured from LOBA Chemie Pvt. Ltd. (Mumbai, India) and sodium hydroxide (NaOH) was purchased from Ranbaxy Laboratories (S.A.S. Nagar, India). Hydrogen peroxide (H_2O_2) was procured from Merck Specialities Pvt. Ltd. (Mumbai, India). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck KGaA (Darmstadt, Germany). Buffer salts and all other chemicals were of analytical reagent grade. Ultra pure water was obtained from ELGA water purification unit (Bucks, England).

2.2. Apparatus and equipment

Precision water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for the forced degradation studies in the solution state. A Dri-Bath (Thermolyne, Iowa, USA) was used for solid state thermal forced degradation studies. Accelerated

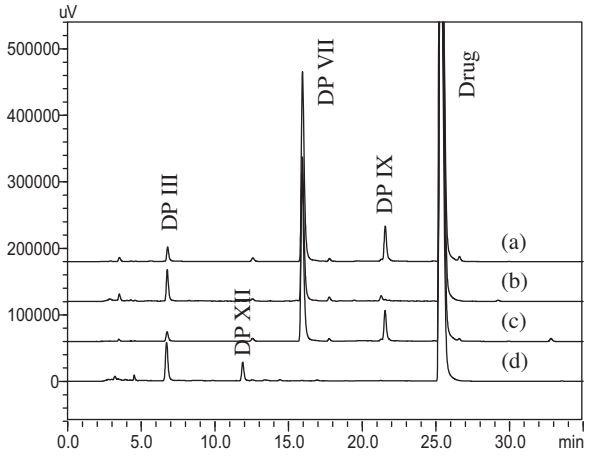

Fig. 1. Chromatograms obtained under optimized photolytic conditions at 273 nm. (a) Photoacid, (b) photobase, (c) photoneutral, and (d) photosolid.