ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Validation of RP-HPLC method for the determination of Ozagrel HCl

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ABSTRACT

Rapid and sensitive high-performance liquid chromatographic method for determination of ozagrel HCl. in bulk using an internal standard, caffiene was developed. chromatographic separation was accomplished by using a C18 analytical column with a mobile phase consisting of Methanol and 0.1% v/v aqueous formic acid (86: 14 v/v). Ozagrel HCl and the internal standard were detected by ultraviolet absorbance at 276 nm. The lower limits of detection and quantification were both 2 and 10 ng/ml, respectively, and the calibration curves were linear over a concentration range of 0.1–1.0 µg/ml of ozagrel HCl.The method provides a sensitive, accurate and reliable analytical procedure suitable for analysis of Ozagrel HCl in bulk.

Keywords: HPLC; Ozagrel HCl; Bulk, Validation.

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ISSN: 0975-8585

INTRODUCTION

Ozagrel HCl, (E)-3-[p-(1H-imidazol-1-ylmethyl) phenyl]-2-propenonate hydrochloride, is a selective inhibitor of thromboxane (TX) A2 synthetase [1]. It is used for the treatment of bronchial asthma and motor disturbance after cerebral thrombosis, since both diseases involve thromboxane A_2 [2]. It also has a highly beneficial effect in the clinical treatment of stroke [3]. It is commercially available as tablets, a solution for injection, and suppositories, and colon-specific delivery and percutaneous penetration studies have also been reported [4-6]. The determination of sodium ozagrel in plasma or serum by HPLC has been reported [7-8]. Ozagrel HCl is poorly soluble in most organic solvents and freely soluble in methanol and water. We have developed a reversed-phase HPLC method to determine ozagrel HCl which is sufficiently specific, sensitive and simple for the measurement of Ozagrel HCl.

MATERIALS AND METHODS

Chemicals

Ozagrel HCl, a gratis sample was supplied by Sun Pharma Advanced Research centre, Baroda, India**.** Caffeine was used as the internal standard (IS) and obtained from the Indo German Alkaloids; Mumbai. All reagents used were of analytical grade except methanol which was of HPLC grade. Water was purified for HPLC with a Millipore Milli-Q water purification system.

Preparation of standard solutions

Stock solution (1000 µg /ml)

25 mg of ozagrel HCl was taken in 25 ml volumetric flask and dissolved and made upto the volume with methanol. Working standards in the range of 0.1 to 10 µg /ml. were prepared by using mobile phase as a diluent.

Preparation of stock and working internal standard solution

Stock solution of caffeine (IS) was prepared by taking 25 mg and by dissolving and making upto 25 ml with methanol.10 µg /ml solution of caffeine (IS) was prepared by further dilution with mobile phase.

Instrumentation and chromatographic conditions

Shimdzu HPLC system consisted of a Spinchrom software (version 2.4.1.93) was used for analysis. Chromatographic separation was achieved using a Phenominex C_{18} analytical column (250 mm × 4.6 mm) which was packed with 5 µm particles and a with a mobile phase consisting of methanol and 0.1 % v/v aqueous formic acid (86:14).The column oven temperature was set at 30°C and the mobile phase was filtered, degassed and pumped at a flow rate of 0.5 ml/min. The detection wavelength was 276 nm.

Method validation

Calibration curves

The Calibration standards ranging from 0.1-10 µg /ml were prepared in mobile phase. To prepare the calibration standards(0.1,0.5,1.0,2.5.5.0,10 µg /ml) ,the aliquots of working standard solutions were transferred into 10 ml volumetric flasks and 1ml of 10 µg /ml (caffeine) internal working standard (IS) solution was spiked in all the flasks and made upto the volume with mobile phase. The calibration line was obtained by linear least squares regression analysis plotting the peak-area ratios (ozagrel HCl / IS) versus the ozagrel HCl concentrations.

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System suitability

For system suitability six replicates of standard drug sample spiked with internal standard were injected. System suitability parameters were evaluated by following USP guidelines by injecting six replicates of 100 µg/ml concentration of working standard stock solution spiked with 10 µg/ml concentration of internal standard (caffeine) solution. Resolution (R),Capacity factor(k^{l}),Theoretical plates(N), Tailing factor(k), HETP, Asymmetry, LOD (µg/mL) and LOQ(µg/mL) were evaluated by following United States Pharmacopoeial guidelines.

Precision and accuracy

Precision was the level of repeatability as reported between samples analysed on the same day (intra-day) and the samples run on three different days (inter-day).Accuracy is expressed as the closeness of the standard samples to the actual known amounts.

Intra-day variation was measured by analyzing the different calibration standards in six replicate injections in the same day. Inter-day variation based on repeated analysis of the same calibration standards in six analytical runs was determined on different days.

To determine accuracy of proposed method recovery studies were carried out by external addition of known quantities of ozagrel HCl to the calibration standards with in the linearity range and the percentage recovery values were calculated.

LOD and LOQ

The limit of detection (LOD) was defined as the smallest concentration of drug giving a signal-to-noise ratio of 3: 1 in six replicate injections. The lower limit of drug quantification (LOQ) was defined as the lower concentration of ozagrel HCl quantified with a coefficient of variation less than 2.0% in 6 replicte injections. In other words the smallest concentration of drug giving a signal-to-noise ratio of 10: 1 in six replicate injections.

Specificity and selectivity of proposed method

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by comparing the blank chromatogram with the chromatogram obtained for the drug spiked with internal standard to trace out the interfering peaks. The specificity of the method was investigated by the analysis of 12 blank preparations spiked with different concentrations of ozagrel HCl and an internal standard (caffeine).Selectivity of the method was observed based on the separation of ozagrel HCl and an internal standard and by calculating the resolution of the drug peak.

Stability of samples

The concentrations of ozagrel HCl spiked with internal standard in diluent at –80°C for 30 days and followed by three freeze-thaw cycles were analysed to know the stability of sample. Stock solutions of ozagrel HCl spiked with IS were stored at 60 days at 4°C to observe the long term solution stability.

Robustness and ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the Ozagrel hydrochloride (2µg/ml) in two sets as per the proposed method and each dilution injected in triplicate using different column and analyst on different days. The results were shown in table 6(a)

The optimum HPLC conditions set for this method have been slightly modified to evaluate the Robustness of the method. These small modifications include the mobile phase composition, flow rate, the column temperature and the detection wavelength and the results are shown in table 6(b)

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RESULTS AND DISCUSSION

Choice of stationary and mobile phase

A non polar C-18 analytical chromatographic column was chosen as the stationary phase for the separation and determination of Ozagrel HCl. For the mobile phase numbers of eluting systems were examined. The use of methanol-water, acetonitrile–water at any ratio and the solvent systems most frequently used for the separation of neutral compounds ,resulted prolonged retention time and tailing. The choice of optimum composition based on chromatographic response factor, a composition of 86:14(%,v/v) methanol and 0.1%v/v aqueous formic acid found to provide an efficient separation of ozagrel HCl with sufficient retention time .A flow rate of 0.5 ml/min found to be optimum for the studied range of 0.5-2.0 ml/min to get optimum retention time ,baseline stability with minimum noise.Ozagrel HCl and internal standard(caffeine) eluted at retention times of 3.9 min and 5.9 min respectively. The optimum chromatographic conditions represented in **table 3.**

Model chromatogram for Ozagrel HCl

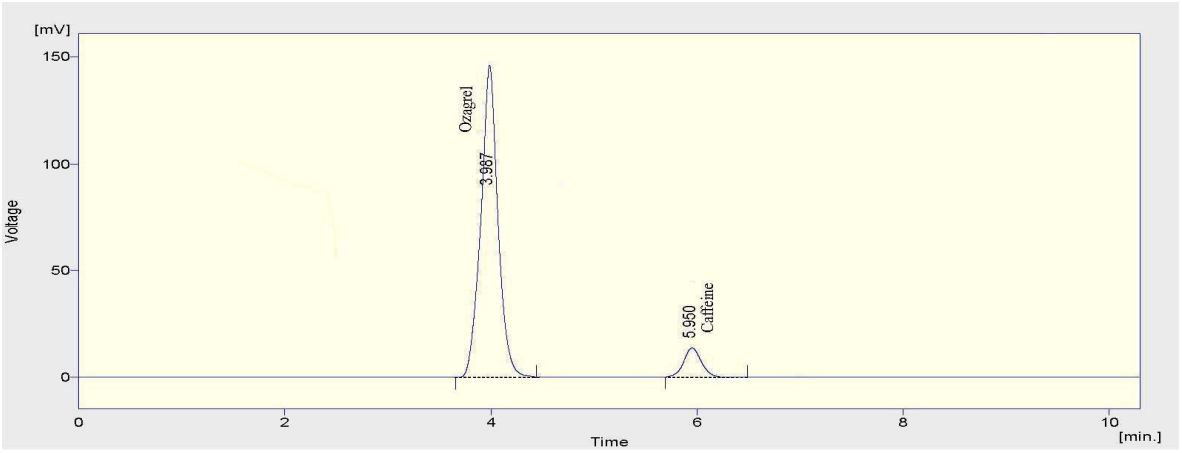

Fig: 1 Chromatogram of ozagrel (t_R: 3.9 min.) spiked with caffeine (t_R: 5.9 min.)

System Suitability

Table: 1. System suitability for ozagrel HCl

S. No	Parameter	Result
1	Resolution	2.527
2	Capacity factor	0.423
3	Theoretical plates	5487
4	Tailing factor	1.02
5	HETP	4.5×10^{-5}
6	Asymmetry	1.09
7	LOD (ng/ml)	2
8	LOQ (ng/ml)	10

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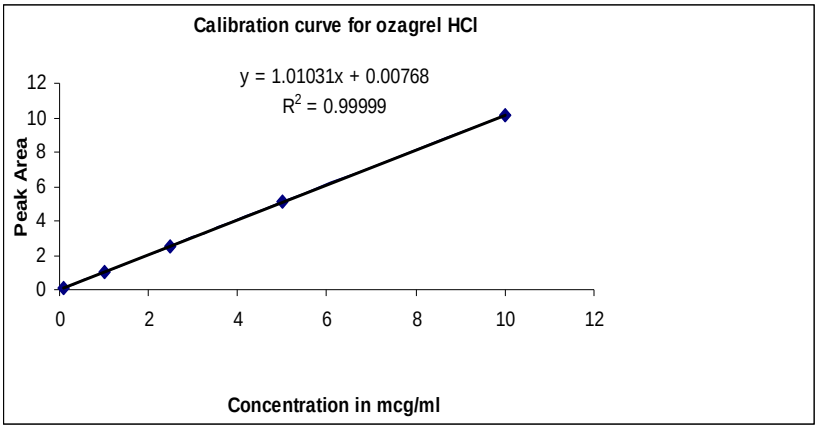
Linearity

Table: 2 Linearity for ozagrel HCl

Concentration of Ozagrel (μg/ml)	Peak area ratio's of Ozagrel / Internal Standard	Statistical data		
		Slope	Intercept	Correlation coeff.
0.1	0.101	1.01031	0.000768	0.9999
1	1.010			
2.5	2.540			
5	5.0795			
10	10.1000			

Calibration curve for ozagrel HCl



Precision and accuracy

Precision was the level of repeatability as reported between samples analysed on the same day (intra-day) and the samples run on three different days (inter-day).Accuracy is expressed as the closeness of the standard samples to the actual known amounts. The precision of the proposed method either the inta-and inter-day variations in the peak area ratios of the drug and internal standard were calculated in terms of % RSD (%C.V) and the results are presented in the **table 4.** The coefficient of variation of both intra and inter-day analysis of calibration standards in six replicate injections found to be less than 2.0%, while the accuracy of the method was 99.2% –100.07%.

To determine accuracy of proposed method recovery studies were carried out by external addition of known quantities of ozagrel HCl to the calibration standards with in the linearity range and the percentage recovery values were tabulated in the **table 5**. The percentage recovery found to be good agreement with the amount added, which conforms the suitability of method for quantitative analysis.

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Chromatographic conditions for Ozagrel HCl.

Table: 3 Chromatographic conditions for Ozagrel HCl.

S. No	Parameter	For Ozagrel HCl.
1	Mobile Phase	Methanol and 0.1 % v/v formic acid (86:14).
2	Stationary phase	Phenominex C_{18} analytical column(250 mm × 4.6 mm) which was packed with 5 µm particles
3	Flow rate(ml/min)	0.5
4	Run time(min)	10
5	Column temperature(^{o}C)	Ambient
6	Volume of injection(µL)	10
7	Detection wavelength(nm)	276
8	Internal standard	Caffeine
9	Retention time of the drug(min)	3.9
10	Retention time of internal standard(min)	5.9

Table: 4 Precision of proposed HPLC method Ozagrel HCl.

S. No	Selected drug	Conc. Taken (µg / mL)	Intra – day		Inter – day	
			Measured Conc. Mean ± S.D	% C.V	Measured Conc. Mean ± S.D	% C.V
1	Ozagrel HCl	1	0.99 ± 0.1	0.45	0.97 ± 0.2	0.23
		5	5.05 ± 0.15	0.52	5.01 ± 0.18	0.57
		10	9.98 ± 0.18	0.34	9.80 ± 0.15	0.38

Table: 5 Accuracy of proposed method

S. No	Selected drug	Conc. Taken (µg / mL)	Measured Conc. Mean ± S.D	% measured(µg / ml)
1	Ozagrel HCl	2.5	2.48 ± 0.32	99.2
		7.5	7.49 ± 0.25	99.8
		10	10.07 ± 0.14	100.07

LOD and LOQ

The LOD of bulk drug found to be 2 ng/ml and the LOQ was 10 ng/ml.

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Specificity and selectivity of proposed methods

A satisfactory chromatographic separation was obtained using the system described above. Representative chromatogram of blank spiked with IS and ozagrel HCl are shown in Fig. 1. Under the chromatographic conditions described, ozagrel HCl and IS were eluted with retention times of 3.9 and 5.9 min respectively. No extra peaks were found to interfere with the elution of the drug and IS.

Stability of samples

Stock solutions of ozagrel HCl and IS were stable for at least 60 days when stored at 4°C. The concentrations of ozagrel HCl in diluent at –80°C for 30 days and following three freeze-thaw cycles were found to be 98.5% ± 2.6% and 99.0% ± 3.2% of the initial values.

Ruggedness and Robustness

These small modifications in the mobile phase composition, HPLC system, the column, the column temperature, the detection wavelength and the analyst did not affected the quantification of ozagrel HCl by the proposed method. Hence the method found to be robust and rugged.

Table 6(a): Ruggedness of the method

S. No.	Theoretical amount(µg/ml)	*Amount estimated(µg/ml	Mean ± SD	% RSD
Set 1	2	2.03	2.03 ± 0.03	1.52
Set 2	2	1.97	1.97 ± 0.02	1.04

*Mean of 6 samples in 3 replicate injections

Table 6(b): Robustness of the method

Parameter	Variation	System suitability		
		Theoretical plates	Tailing factor	%RSD
Standard	----------	5487	1.02	0.98
Flow rate	0.4	5348	1.05	1.08
	0.6	5578	1.04	1.50
Wave length	+ 2nm	5643	0.99	1.06
	- 2nm	5341	0.99	1.09
Mobile Phase	85:15	5346	1.03	1.24
	87:13	5379	0.98	1.35
Temperature	-5°C	5447	1.0	1.40
	+5°C	5367	1.03	1.58

ISSN: 0975-8585

CONCLUSION

The proposed chromatographic conditions ascertains resolution and reproducibility and the system suitability tests were conducted on freshly prepared standard stock solutions of Ozagrel HCl and the parameters obtained such as retention times, limit of detection (LOD),limit of quantification(LOQ) were tabulated.

In conclusion, the proposed method has given good resolution between Ozagrel and internal standard within short analysis time and low values of RSD indicate that the proposed method is very simple, rapid and highly precise. The proposed method is found to be very selective which was indicated by good relative retention time between ozagrel and internal standard and no interference was observed between two peaks. Therefore the method can be applied for routine quality control analysis of Ozagrel HCl.

REFERENCES

[1] K Iizuka, K Akahane, D Momose et al. J Med Chem 1981;24:1139-1148.

[2] JM Dogne, X Leval, P Benoit et al. Am J Respir Med 2002; 1: 11-17.

[3] T Imamura, S Kiguchi, K Kobayashi et al. Arzneimittelforschung 2003;53: 688-694.

[4] NX Zhang, SH Sato, F Kobayashi et al. Biol Pharm Bull 1997;20: 282- 284.

[5] T Yamaoka, Y Makita, H Sasatani et al. J Control Release 2000; 66: 187-197.

[6] T Ogiso, K Koike, M Iwaki et al. Biol Pharm Bull 2000; 23: 844-849.

[7] NX Zheng, I Adachi, I Horikoshi et al. Biol Pharm Bull 1994;17: 302-305.

[8] O Taro, I Masahiro, H Yoko et al. J Pharm Sci 1997;86: 1111-1114.