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Discovery

Stability-indicating RP-HPLC determination of Suberoyl anilide hydroxamic acid (SAHA) in bulk drugs

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ABSTRACT

A Simple, selective, precise and stability-indicating high-performance liquid chromatographic (RP-HPLC) method of analysis Suberoylanilidehydroxamic acid was developed and validated. The chromatographic conditions comprised a reversed-phase C18 column (250x4.6mm), 5 mm with a mobile phase consisting of a mixture of buffer solution (10 mM Potassium dihydrogen phosphate) and acetonitrile in the ratio of (Gradient (Time/%A) 0/90, 2/90, 10/30, 12/10, 15/90 & 20/90). Orthophosphoric acid was added to it and pH of the mobile phase was adjusted to 2.5 at the flow rate of 1mL/min. Detection was carried out at 220nm. The retention time of Suberoylanilidehydroxamic acid was 10.1min. The liner regression analysis data for the calibration plots

showed dood liner relationship with coefficient of regression value,r^2=0.99 in the concentration range 0.02-50 µg/mL. The method was validated for precision, recovery, and robustness. The drug undergoes degradation under acidic, basic and oxidative conditions. All the peaks of degraded product were resolved with significantly different retention time. The sample degraded showed no additional peak. Statistical analysis proves that the method is reproducible and selective for the estimation of drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: HPLC, Gradient method; Forced degradation; Buffer solution; Accuracy; Precision; Recovery; pH; Detector.

Abbreviations: SAHA - Suberoylanilidehydroxamic acid; HDAC - Histone deacetyltransferase; ICH - International Conference on Harmonization; CTCL - Cutaneous T- cell lymphoma; USP - United States Pharmacopoeia; MMP - Matrix Metalloproteinase; HDAC - Histone deacetyltransferase; RNR – Ribonucleotidereductase; PDF - Peptide deformylase; LOX - Lipoxygenase; DMSO -Di methyl sulfoxide; HCl - Hydrochloric acid; NaOH - Sodium Hydroxide; H_2O_2 - Hydrogen Peroxide.

1. INTRODUCTION

Suberoylanilidehydroxamic acid (SAHA, Vorinostat, Zolinza) recently gained US FDA approval for the treatment of advanced cutaneous T- cell lymphoma (CTCL). SAHA inhibits the enzyme Histone deacetylase (HDAC), which arrest the cell growth. Several hydroxamic acid derivatives that exert anti-tumor effect by targeting various enzymes such as Matrix Metalloproteinase (MMP), Histone deacetyltransferase (HDAC), Ribonucleotidereductase (RNR), Peptide deformylase (PDF), Lipoxygenase (LOX) (Rabert Hartman et al., 2004). Mechanism of these inhibitors action is the direct interaction with the active zinc site at the base of the catalytic pocket, which blocks substrate approach active zinc-ion of enzymes (Takayoshi Suzuki et al., 2004). Molecular aspects of HDAC inhibitors effects in tumor cells are complex and not completely elucidated. Therefore, tumor-suppressor genes could be silenced by aberrant histone deacetylation. This epigenetic modification has become an important target for tumor therapy. HDAc inhibitors prevent cell proliferation and survival of tumor cells with very low toxicity towards normal cells (Anton et al., 2007).

C_{18} columns were commonly used for the HPLC analysis. Thus separation was achieved on a 4.6 X 250mm C18 Inertsil ODS 3v 5µm analytical column. This column offered adequate separation of compounds and resulted in well-resolved peaks. The mobile phase or eluent interacts with the solute molecules and the stationary phase itself, with the strength of these interactions determining the resolution obtained and hence the efficacy and efficiency of the separation, resulting in the selection of an appropriate mobile phase being imperative.

Forced degradation studies involve exposing a drug substance to harsher conditions than the product would be expected to experience and determining at what point and how it degrades (Douglas et al., 2005). Generally, a forced degradation study is performed prior to commencing stability testing by exposing the drug to a variety of extreme conditions, such as pH, photolysis, oxidation and temperature, over a very short time period.

2. MATERIALS AND METHODS

Forced degradation study was carried out using different ICH prescribed stress conditions such as acidic, basic and oxidative stresses to assess the specificity of the method. The experimental requirements that were necessary for chemicals, reagents, apparatus and instruments with detailed description of HPLC instruments and other chromatographic conditions are mentioned against each method (Baboota et al., 2007). All the chemicals and solvents used in these experiments were of HPLC and/or analytical grade.

2.1. Chemicals

The reference standard of Suberoylanilidehydroxamic acid was synthesized and purified (99%). HPLC grade acetonitrile was purchased from Alfadan. HPLC grade Ammonium acetate, Acetic acid, Potassium dihydrogen phosphate, Sodium hydroxide, Hydrochloric acid and Hydrogen peroxide were obtained from Merck. The Milli Q water was prepared in our Laboratory.

2.2. Apparatus

All glassware was washed thoroughly with distilled water and then rinsed with methanol and dried before use. Beaker, Standard flasks, Measuring cylinders, HPLC Vials, Micro pipettes and Membrane Filter.

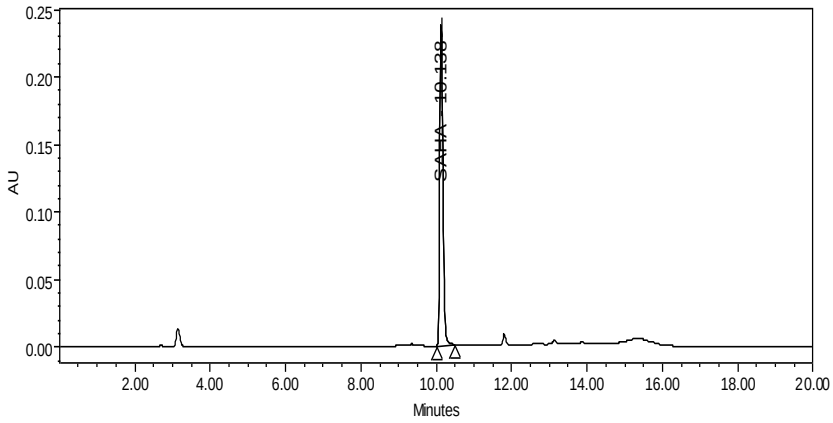

Figure 1

Peak with 10mM Potassium dihydrogen phosphate (pH 2.5) and acetonitrile

2.3. HPLC system and chromatographic conditions

The system used for this work consisted of HPLC and other analytical instruments. Analytical balance (Mettler Toledo AX 205), P^H meter (Elinco Li 610), Vacuum pump (Gastdoa-V130-BW), Sonicator (Sonorex super 10 p digital), Shaking Water Bath (Julabo SW 23), HPLC (Waters 2695 separations Module). The HPLC coupled with a UV variable wavelength detector 240nm. The separations were achieved on a reverse phase C18 column (4.6 X 250mm C18 Inertsil ODS 3v 5µm) preceded by guard column (30X4.6mm) packed with the same material. Mobile phase consisted of 10mM Potassium dihydrogen phosphate (pH 2.5) acetonitrile (Baboota et al., 2007).Gradient (Time/%A) 0/90, 2/90, 10/30, 12/10, 15/90 & 20/90. It was filtered and degassed before use and pumped at 1 ml/min flow rate.

Table 1 Method Development

Column	4.6 X 250mm C18 Inertsil ODS 3v 5µm
Mobile phase	A= 10 mM Potassium Inertsildihydrogen phosphate(pH 2.5) B= Acetonitrile
Flow rate and Detection	1 mL/min and 240 nm
Gradient (Time/%A)	0/90, 2/90, 10/30, 12/10, 15/90 & 20/90.
Retention time	10.1 min;

Table 2 Forced degradation

S. No	Sample	Conc. (µg/mL)		% Remaining	Mean
		0 hr	2 hr		
1	1 M HCl	18.893	18.644	98.68	101.7
2		18.757	18.878	100.65	
3		19.023	20.124	105.79	
4	1 M NaOH	19.308	19.105	98.98	98.68
5		19.844	19.325	97.38	
6		19.407	19.347	99.69	
7	3% H_2O_2	19.497	19.735	101.22	99.78
8		19.986	19.499	97.56	
9		19.559	19.666	100.55	

2.4. Preparation of mobile phase

The mobile phase 'A' was prepared by dissolving 1.36g of Potassium dihydrogen phosphate in 1000 mL of Milli Q Water. And pH was reduced to 2.5 by using ortho-phosporic acid. It was filtered through 0.22 µm membrane filter and degassed by sonication before using in the HPLC system (ArdhaniDwi Lestari et al., 2004).The mobile phase 'B' was acetonitrile and degassed by sonication before using in the HPLC system.

2.5. Preparation of working standard (1mg/mL)

Accurately weigh 5mg of SAHA in 5mL standard flask and add 100µL of DMSO, shake gently until drug completely dissolves (ArdhaniDwi Lestari et al., 2004). Then make up to the volume with acetonitrile.

2.6. For Acidic Degradation

From the stock solution, take 400µL of solution was mixed with 1600µL of 1M HCl was prepared (Acidic Solution).From this acidic solution, take 100µL mixed with 900µL of diluents was added (0 hr) and the same solution incubated at 2 hrs (Atul et al., 2003). The concentration was determined as triplicate.

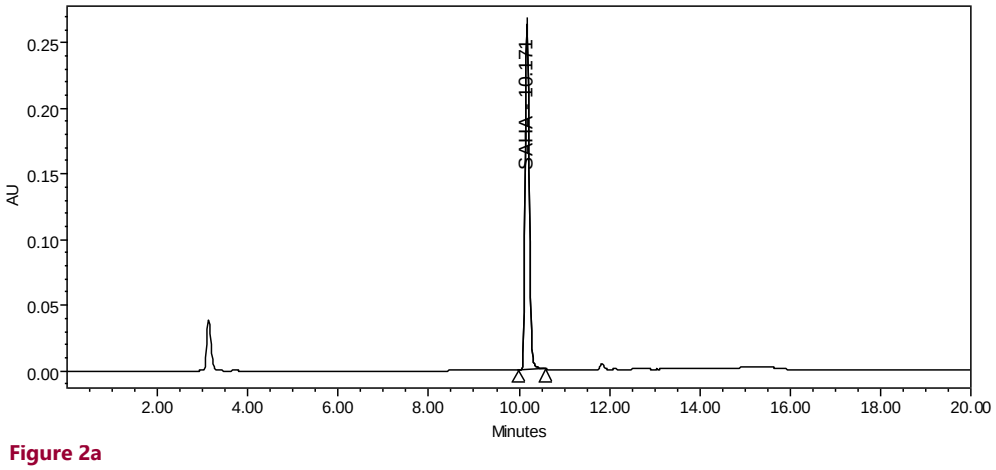

Figure 2a

Peak for SAHA in acidic condition (1 M HCl)

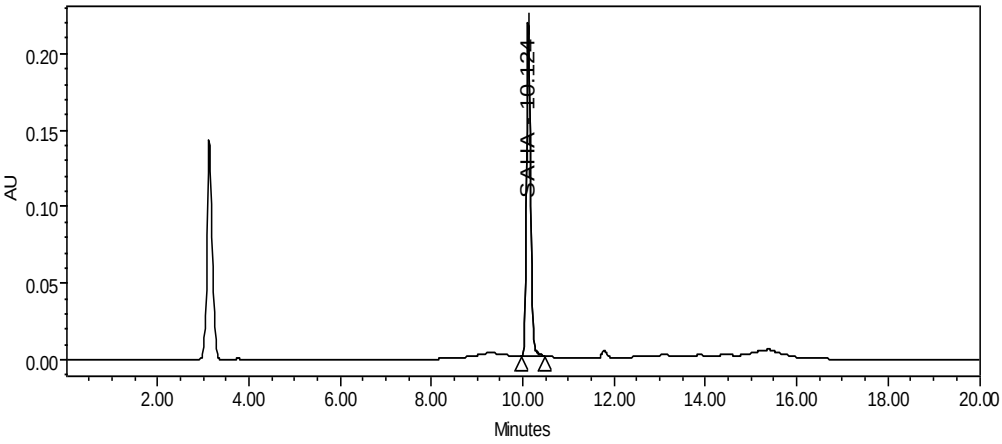

Figure 2b

Peak for SAHA in basic condition (1M NaOH)

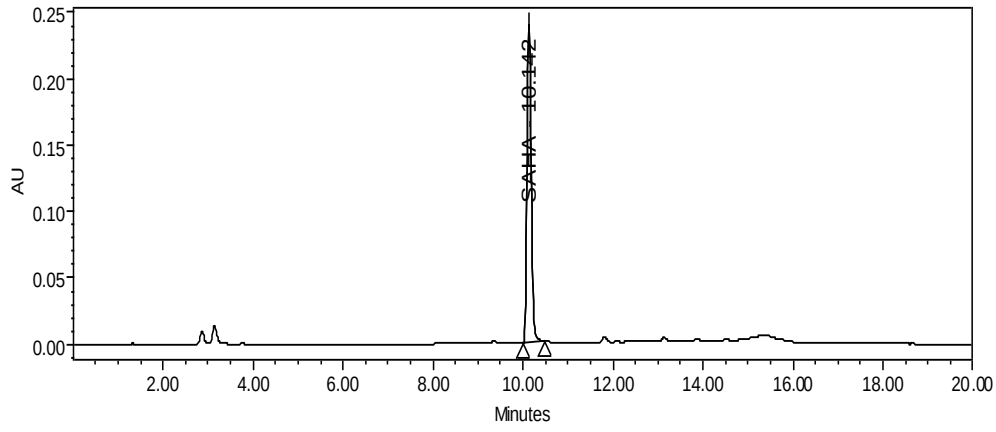

Figure 2c

Peak for SAHA in oxidative condition (3% H_2O_2)

2.7. For Basic Degradation

From the stock solution, take 400µL of solution was mixed with 1600µL of 1M NaOH was prepared (Basic Solution). From this Basic solution, take 100µL mixed with 900µL of diluents was added (0 hr) and the same solution incubated at 2 hrs. The concentration was determined as triplicate.

2.8. For Oxidative Degradation

From the stock solution, take 400µL of solution was mixed with 1600µL of 3% H_2O_2 was prepared (Oxidative Solution). From this oxidative solution, take 100µL mixed with 900µL of diluents was added (0 hr) and the same solution incubated at 2 hrs (Baboota et al., 2007). The concentration was determined as triplicate. The Stressed sample after completion of stress condition was analysed by the proposed method and the percentage degradation of each analyte was calculated under each condition.

3. RESULTS AND DISCUSSION

3.1. Method Development

Initially various mobile phases and stationery phases were tested to obtain the best separation and resolution of SAHA in 10 mM potassium dihydrogen phosphate and acetonitrile in 4.6 X 250mm. C18

Inertsil ODS 3v 5µm analytical column were found to be the most appropriate for the separation of the components at a the flow rate of 1.0 mL/min (Rolando Gouzalez-Hernandez et al., 2001). Using the mentioned chromatographic conditions, well resolved sharp peaks can be obtained at retention time of 10.1 minute for SAHA.

The mobile phase of 10mM Potassium dihydrogen phosphate and Acetonitrile was formed tailing in the peak. After the pH was adjusted to 2.5 (to reduce the pH using Ortho-phosphoric acid) the sharp peak was obtained. At the flow rate of 1 mL/min., using the Gradient condition at the different time point at different ratio of mobile phase combinations, well resolved sharp peak can be obtained at retention time of 10.1 minutes for SAHA (Table 1).

Method development was started with more polar mobile phase (90 % of 10mM Potassium dihydrogen phosphate) however; no peak could be obtained (Ardhani Dwi Lestari et al., 2004). The polarity of the mobile phase was then decreased by the addition of acetonitrile. A 10mM Potassium dihydrogen phosphate (pH 2.5) and acetonitrile resulted in good separation and sharp peaks (Figure 1).

3.2. Forced Degradation study

To evaluate the specificity of the proposed method different stress conditions were applied to SAHA (ArdhaniDwi Lestari et al., 2004). The stress conditions applied were acidic, bases and oxidation.

3.2.1. For Acidic Degradation

After 2 hours of SAHA in acidic condition (1 M HCl) the peak area was no changes to the initial amount (0 hr). The peak was detected at the retention time 10.1 (Rabert Hartman et al., 2004). So SAHA was stable in acidic condition (Fig. 2a).

3.2.2. For Basic Degradation

After 2 hours of SAHA in basic condition (1M NaOH) the peak area was no change to the initial amount (0 hr). The peak was detected at the retention time 10.1. So SAHA was stable in basic condition (Fig. 2b).

3.2.3. For Oxidative Degradation

After 2 hours of SAHA in oxidative condition (3% H_2O_2) the peak area was no change to the initial amount (0 hr). The peak was detected at the retention time 10.1 (Baboota et al., 2007). So SAHA was also stable in oxidative condition (Fig. 2c), (Table 2).

4. CONCLUSION

SAHA was stable in various conditions such as acidic, basicand oxidative, which can be concluded by the absence of the degradation products as evident from the forced degradation studies.To conclude, the developed method can be utilized for the sample analysis of SAHA. Moreover, the experimental results showed the compound was stable under various adverse conditions hasprove the compound has excellent “DRUGGABLE” properties.

SUMMARY OF RESEARCH

1. Suberoylanilidehydroxamic acid (SAHA) recently US FDA approval for the treatment of advanced cutaneous T- cell lymphoma (CTCL).
2. SAHA inhibits the enzyme Histone deacetylase (HDAC), which arrest the cell growth. A simple, sensitive HPLC method was developed and determines the force degradation of the drug substance. After many attempted, that mobile phase 10mM potassium dihydrogen phosphate (pH 2.5) and acetonitrile was chosen.
3. It got sharp peaks and less retention time of SAHA. Under the various condition acidic, basic and oxidative conditions, SAHA was no degradation occurred.

FUTURE ISSUES

1. The method was developed and determines the forced degradation of the drug in accordance with ICH guidelines.
2. Statistical analysis proved the method got sharp peaks and less retention time of SAHA.
3. Under the various condition acidic, basic and oxidative conditions, SAHA was no degradation occurred.
4. The same method developed was used for the validation, stability studies, stress test, physicochemical parameters of the drugs, etc.

DISCLOSURE STATEMENT

The raw material of the drug substance, solvents, instruments etc., are provided and supported for doing this research work helped as Orchid Research Laboratories Ltd, Chennai-119.

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