FORCED DEGRADATION STUDIES OF VINCAMINE BY HPTLC

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ABSTRACT

Vincamine is an alkaloid with vasodilator properties. Vinpocetine, a semi-synthetic vincamine derivative alkaloid, is used to treat cerebrovascular diseases like stroke and dementia. Being an important molecule, the current research work examines the forced degradation studies of vincamine using planar chromatography, HPTLC using stability studies parameters such as degradation by acid and base hydrolysis, oxidative stress degradation, hydrolytic induced degradation, photolytic degradation and dry heat degradation. The compound was found to be stable to oxidative stress, but significant degradation occurred under acid hydrolysis, base hydrolysis, water hydrolysis, and to a lesser extent, under thermal stress and photolytic stress. The percentage recovery of vincamine was found to be lower in acid-induced degradation (31.8 %), and base-induced degradation (8.2 %) than in UV-induced degradation (94.9 %) and dry heat-induced degradation (93.1 %). In pharmaceutical research and development, forced degradation experiments are essential for predicting long-term stability.

Keywords: Vincamine, stress testing, stability, high-performance thin-layer chromatography, forced degradation

INTRODUCTION

The safety, purity, and effectiveness of a drug product are impacted by the drug substance stability in different environmental conditions[1]. The ICH Q1A(R2) guideline describes the purpose of stress testing for new medicinal compounds as follows: "Stress testing of the drug substance can help to discover potential degradation products, which can subsequently help to establish degradation routes and, in turn, the intrinsic stability of the molecule. It can also support the effectiveness of the analytical techniques to anticipate stability". The type of stress testing depends on a particular drug molecule and consequently, the drug product concerned[2,3]. Vincamine (Fig. 1), a monoterpenoid indole alkaloid, and white crystalline water-soluble bioactive compound, is present in various *Vinca* species like *Vinca rosea* (*Catharanthus roseus*), *Vinca major*, *Vinca minor*, *Vinca difformis* etc. *Vinca rosea* possesses various therapeutic activities and is active against cancer, diabetes and microbes.

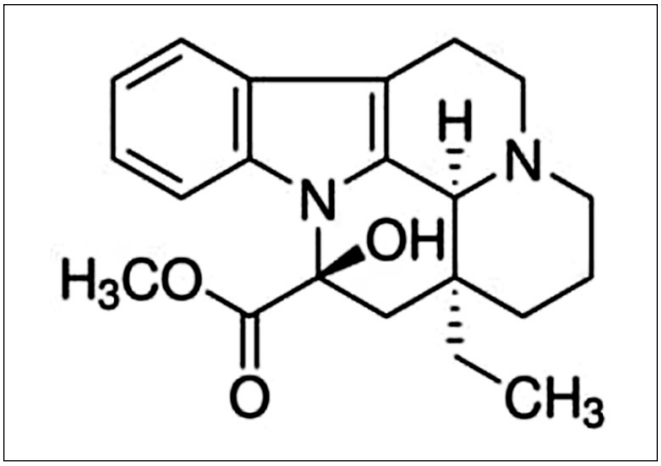

Fig. 1: Vincamine

Vincamine is used to treat hypertension[4,5]. This research work aimed to carry out forced degradation studies of vincamine by the HPTLC method developed by the authors[6,7]. The forced degradation studies of the drug substance were carried out at various conditions such as acid-base degradation, oxidative degradation, hydrolytic degradation, photolytic degradation and dry heat degradation according to stability guidelines by ICH Q1A(R2)[2] and ICH Q1B[8].

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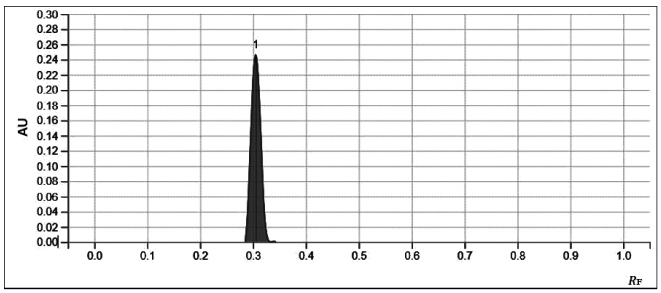
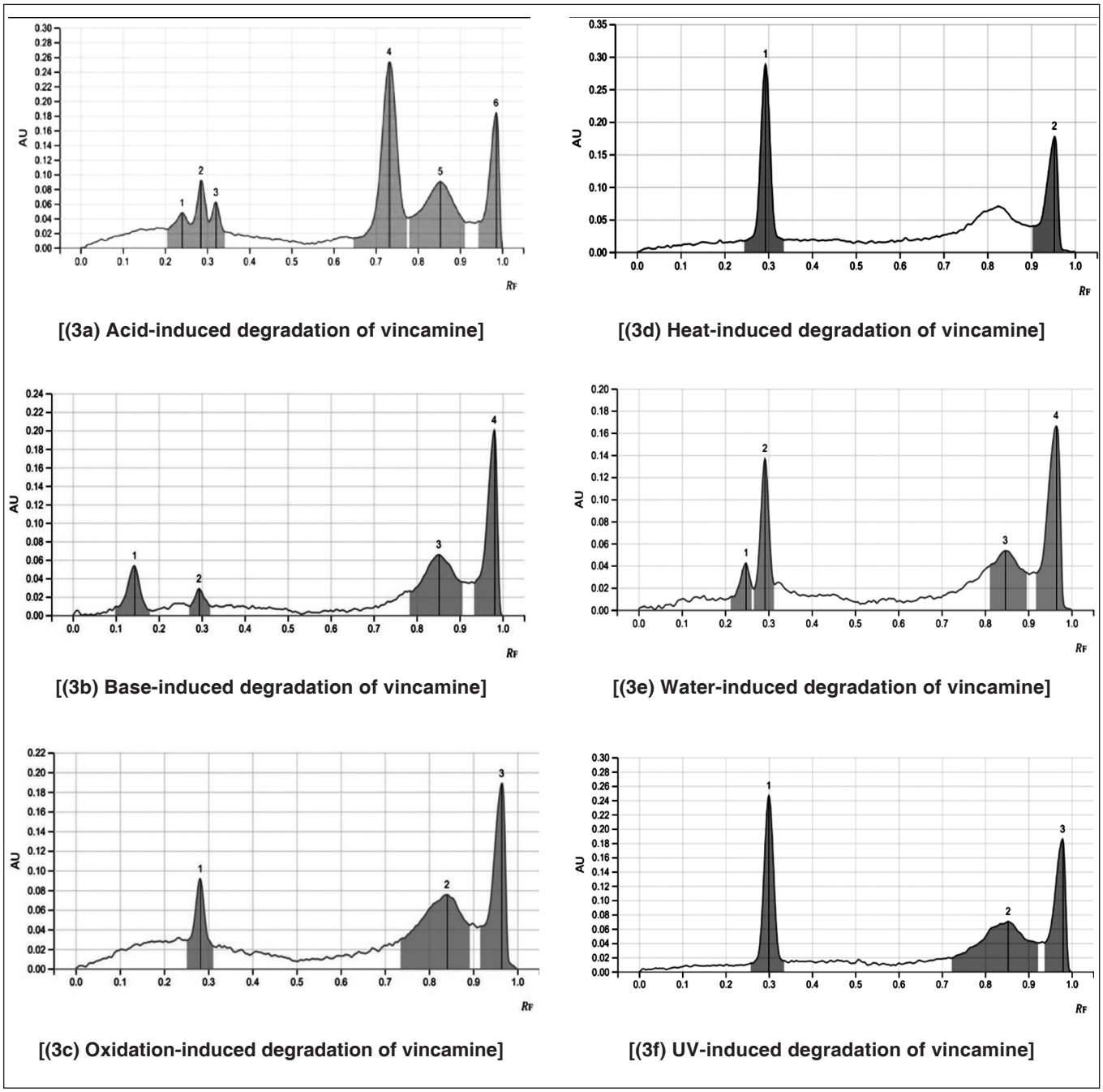


Fig. 2: HPTLC densitogram of vincamine

MATERIALS AND METHODS

Chemicals and reagents

The marker, vincamine, was obtained from Yucca Enterprises, Wadala, Mumbai, Maharashtra. Solvents, 2-propanone or dimethyl ketone (acetone), methanoic acid (formic acid), trichloromethane (TCM, or chloroform), and methyl alcohol (methanol) were procured from S.D. Fine Chem. Ltd. Merck TLC plates silica gel 60 F_{254}, 1.05554.007, were used for analysis.



[(3a) Acid-induced degradation of vincamine]

[(3d) Heat-induced degradation of vincamine]

[(3b) Base-induced degradation of vincamine]

[(3e) Water-induced degradation of vincamine]

[(3c) Oxidation-induced degradation of vincamine]

[(3f) UV-induced degradation of vincamine]

Fig. 3: Results of forced degradation studies of vincamine

Table I: Forced degradation data of vincamine

Exposure condition	Time (h)	Percent recovery	R_f of degradation product
Acid (0.1 N HCl) reflux	2	31.8	0.23, 0.27, 0.71, 0.84,0.97
Base (0.1 N NaOH) reflux	2	8.2	0.14, 0.85, 0.97
H_2O_2 (6 % V/V)	4	64.1	0.84, 0.96
Dry heat (105 °C)	8	93.1	0.95
Water, reflux	8	68.4	0.24, 0.84, 0.96
Photostability-UV	8	94.9	0.84, 0.96

Each result is an average of three measurements

Chromatographic conditions

Utilizing a CAMAG Automatic TLC Sampler 4 (ATS 4) with a 100 µL applicator syringe, 5 µL of samples were applied on the TLC plate in duplicate as 0.8 cm wide bands which were 1 cm apart. CAMAG glass chamber of 20 cm x 10 cm size was preconditioned for 20 min., with the eluent selected for the development of the plates. The eluent consisting of TCM: dimethyl ketone: methanoic acid (5:1:0.5 V/V/V), was used in the linear ascending mode to the migration distance of 7 cm. The slit dimension was kept at 0.6 cm × 0.045 cm with the scan speed of about 2 cm per second. CAMAG TLC scanner 4 was used at 222 nm, λmax of vincamine, for densitometric evaluation. CAMAG visionCATS software was used for data evaluation.

Preparation of stock solution

To compare the R_f of the degradant with the R_f of the marker, a standard stock solution was prepared by dissolving 0.01 g of vincamine in 10 mL of methyl alcohol in a 10 mL graduated flask.

Stress degradation studies

10 mg of vincamine was subjected to various stress agents followed by its analysis using the HPTLC method. Table I shows stressors and time of treatment in hours. After acid, base, oxidative, and water-induced degradation, samples (10 mg of vincamine in 10 mL stressors) were diluted to 100 mL using methyl alcohol for chromatographic analysis. For heat-induced degradation, vincamine was directly placed in an oven, while for photolytic degradation, vincamine was directly placed in the UV cabinet at 254 nm. After 8 h, both degraded samples of vincamine were dissolved in 100 mL of methyl alcohol and subjected to chromatographic analysis.

RESULTS AND DISCUSSION

An accurate, precise, robust, planar, chromatographic HPTLC method was developed using TCM: dimethyl ketone: methanoic acid (5:1:0.5, V/V/V) as eluent. The vincamine peak was detected at R_f 0.33±0.03 (Fig. 2). Linearity was obtained in the range of 200-1000 ng spot^{1} ($y = 7.9521x + 653.65$ with $r^2 = 0.9957$). The % RSD for repeatability, intermediate precision, and robustness studies, was found to be less than 2 %, indicating a precise and robust method.

Vincamine was found to be more prone to acid degradation (Fig. 3a). In base-induced degradation, the area under the curve of vincamine decreased, showing that vincamine undergoes degradation under basic conditions (Fig. 3b). Vincamine undergoes moderate degradation in the presence of H_2O_2, and, in water, (neutral) hydrolysis (Fig. 3c and 3e). Vincamine shows less degradation when exposed to dry heat and UV light (Fig. 3d and 3f). The concentration of unaffected vincamine was calculated based on the area under the curve. All the degradant peaks' R_f values are provided in Table I.

CONCLUSION

Forced degradation studies on vincamine were carried out. These degradation studies provide information on the intrinsic stability of vincamine and accordingly, vincamine should be handled carefully while working in such conditions. In future, the studies can be carried out to find out the structures of these degradants. This study may also be helpful in the derivatization of vincamine.

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