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Stability studies of berberine using UV spectroscopy

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Abstract:

Nutraceuticals are nutritional supplements that are used to improve health, postpone ageing, fight diseases and support normal bodily functions. Berberine is a nutraceutical drug found in plant species such as berries. It possesses anti-carcinogenic, anti-viral, neuroprotective, cardio-protective, anti-inflammatory and antioxidant properties. The aim of this work is to develop an easy, accurate, precise and economical method for estimating stability of Berberine using UV Spectroscopy.

Our work on stability studies of Berberine got distinct by referring to the ICH guidelines to enlighten the inherent stability characteristics of Berberine. Stability studies involves a series of tests designed to get information on the stability of a drug in order to define its shelf life and usage under specified packaging and storage conditions. The pH, temperature, metal and other compounds may have an impact on the stability of Berberine in various dietary matrices. Forced degradation study is a powerful tool used in pharmaceutical development to develop stability indicating method that leads to quality stability data.

The concentration of the produced degradation products analogous to the intact Berberine was calculated and found to be 96.56%, 66.33% and 75.8 % in case of acid hydrolysis, oxidation, and photolytic degradation respectively. As a result, the developed method for estimation of Berberine in pharmaceutical dosage form and in bulk is simple, definite, reproducible and economical.

Keywords:

Berberine, Stability Studies, Hydrolytic Degradation, Oxidative Degradation, Photolytic Degradation

1. Introduction:

Berberine is a yellow-colored, alkaloid that has been gaining a lot of attention right now due to its potent pharmacological effects. Berberine has a long history of action in, Ayurvedic and Chinese medicine. Its anticancer activity and efficacy in treating neurological, metabolic, and cardiovascular problems have been validated by a recent study. The substance has been used in associated disorders and has undergone numerous clinical assessments in patients with the metabolic syndrome [1].

It functions as a broad range antibiotic against bacteria, fungi, viruses, and parasites it is also having anti-inflammatory and anti-cancer properties in the body. Berberine is one of the rare substances that has shown to activate AMPK-Adenosine Monophosphate Activated Protein Kinase. Berberine strengthens the immune system and the gastrointestinal tract. It can also decrease the impact of excessive alcohol consumption in the body. Berberine increases dopamine, serotonin, and norepinephrine levels in the brain [2].

The molecular formula of berberine is $C_{20}H_{18}NO_4^+$ with the IUPAC name of 9,10-Dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-g] isoquinolino[3,2-a]isoquinolin-7-ium. it has a molecular weight of 336.367g/mole [3].

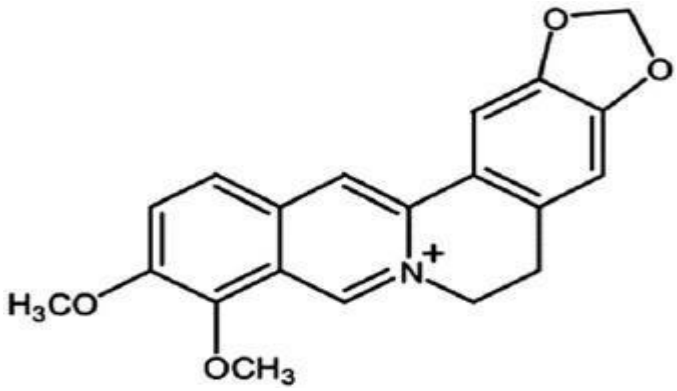

Figure. 1: Chemical Structure of Berberine

2. Degradation studies:

Degradation studies is done by allowing the drug product or the drug substance to various stress conditions [4].These stress conditions can cause degradation of the drug present. This is important to determine the stability of a drug which in turn affects the purity, potency and safety of the drug product [5].

Degradation are of different types. They are:

- Forced degradation
- Hydrolytic degradation
- Oxidative degradation
- Thermal degradation
- Photolytic degradation

2.1. Forced degradation studies:

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus producing degradation products that can be studied to understand the stability of the molecule. The ICH guideline states that 'stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used'.

2.2. Hydrolytic degradation studies:

Hydrolysis usually means the cleavage of chemical bond by the addition of water. The main source of impurities in the formulation is for hydrolytic degradation. Water either as a solvent or a moisture in the air comes in contact with the pharmaceutical product is responsible for degradation of most of the drugs. In hydrolytic degradation study under acid and basic condition, 0.1M NaOH and O.1M HCI are employed for generating acidic and basic stress samples respectively. If sufficient degradation is not observed, higher strength is employed to induce degradation (1M NaOH/ 1M HCI).

2.3. Oxidative degradation studies:

The increase in oxidation state of an atom through a chemical reaction is known as an oxidation. Most of the drug undergoes auto oxidation because of oxygen in air. Therefore, it is an important degradation pathway of many drugs. Hydrogen peroxide, metal ions in a drug product act as initiator for auto oxidation. Hydrogen peroxide is a common oxidant that produce degradation to products during long term stability studies. Hydrogen peroxide is much more popular for the oxidative degradation studies than any other oxidizing agent.

2.4. Thermal degradation studies:

Temperature also plays a role in degradation of drugs. High temperature leads to increase in degradation of drugs. Most of the drugs are temperature sensitive. Thermal degradation can lead to other reactions like pyrolysis, hydrolysis, decarboxylation, isomerization, rearrangement and polymerization.

2.5. Photolytic degradation studies:

Exposure of sun light to drug is called photolytic degradation. The rate of degradation is directly proportional to the intensity; quantity of sun light absorbed by the drug. It is carried out by exposing the drug substance and drug product to a sun light. (ICH Guidelines). In degradation studies UV plays one of the important roles in detection of impurities and the assay values of drug products can be calculated using spectroscopy. In ultra violet region every molecule will give its own absorption at particular wavelength this is called λmax. Every compound has unique λmax and absorption. Absorption values differ only with the change in concentration of the compound. Degraded samples spectrum are compared with standard spectrum to identify the degradation. After degradation, the absorption and the λmax of the samples can vary from the standard that shows the degradation of samples [6, 7, 8, 9].

3. Materials and methods:

3.1. Drug:

- The reference standard of Berberine was purchased from Sami Labs, Bangalore.
- The commercial product of Berberine capsule was purchased from the market (Vokin Biotech Private Limited).

3.2. Reagents used:

- 0.1M Sodium hydroxide
- 0.1M Hydrochloric acid
- 5% Hydrogen Peroxide
- Methanol
- Distilled water

3.3. Preparation of reagents:

3.3.1. Preparation of 0.1M Sodium hydroxide:

4 gm of Sodium hydroxide pellets were weighed and dissolved in small amount of distilled water then made up the volume to 1000ml

3.3.2. Preparation of 0.1M Hydrochloric acid:

8.33ml of concentrated hydrochloric acid was measured and diluted with distilled water to 1000ml

3.3.3. Preparation of 5% Hydrogen peroxide:

50ml of Hydrogen peroxide was diluted with distilled water and the volume made up to1000ml

4. Instrumentation:

All absorption spectrum was measured using Shimadzu UV-1650 Spectrophotometer with 1 cm matched quartz cells.

Table. 1: Instrumentation

Sl.no.	**Equipment**	**Model/ company**
1.	Digital weighing balance	Infra digi IN 600
2.	UV Visible Spectrophotometer	UV 1800 240V Shimadzu
3.	UV Software	UV Probe 2.34

5. Solubility:

Table. 2: solubility

Solvent	**Berberine**
Water	Insoluble
Ethanol	Soluble
Acetonitrile	Sparingly soluble
0.1N HCl	Sparingly soluble
0.1N NaOH	Sparingly soluble
Methanol	Soluble

6. Experimental methodology:

6.1. Selection and optimization of solvent:

It is well known that the peaks, quality, and shape are significantly influenced by the solvents. Water, ethanol, acetonitrile, 1.0M HCl, 0.1M NaOH, and methanol were among the solvents that were employed.

Solvent methanol was optimized as it met all the requirements in terms of the stated time, peak quality, and non-interference as required.

Table. 3: selection and optimization of solvent

Solvent	**Berberine**
Water	Insoluble
Ethanol	Soluble
Acetonitrile	Sparingly soluble
0.1N HCl	Sparingly soluble
0.1N NaOH	Sparingly soluble
Methanol	Soluble

6.2. Selection of wavelength:

The wavelength at which maximum absorption takes place in UV analysis was at a range of 200 to400 nm and the λ max was at 344 nm

6.3. Preparation of standard stock solution:

The standard stock solution of Berberine was prepared by dissolving 10 mg of the drug in methanol and the final volume was made upto 10 ml with the same solvent in a volumetric flask to get a solution containing 1000µg/ml berberine (Bs).

6.4. Preparation of working standard:

In a 10 ml standard flask pipette out 1 ml of Bs and dilute it up to the mark with Methanol to a concentration of 100µg/ml (Bw) the solution was scanned between 200-400nm and 344 nm was found to be the maximum wavelength of absorption was shown in the Fig. 12. This wavelength was selected for the development of UV method for estimating Berberine.

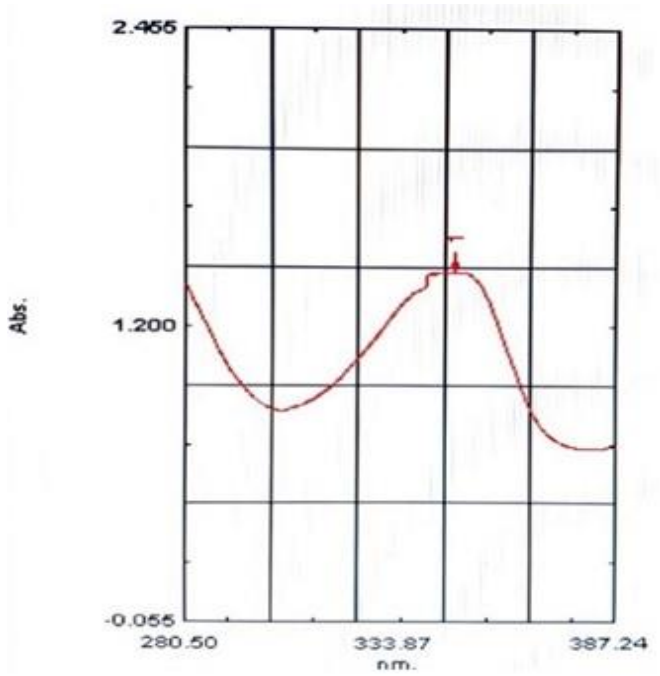

Figure. 2: Uv Spectra Of Berberine

7. Force degradation studies:

Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule

7.1. Hydrolytic degradation using 0.1M HCL:

7.1.1. Standard preparation:

Aliquot of 1 ml of Berberine (containing concentration of 30µg/ml) was transferred to a small round bottom flask. The solution was mixed with 9 ml of 0.1 N hydrochloric acid. The prepared solutions were subjected to reflux for 2 h in a boiling water bath.

The samples were cooled to room temperature (25ºC), neutralized with an amount of acid equivalent to that of the previously added. From the resulting neutral solution, 2 ml was taken in cuvette and absorbance was recorded.

7.1.2. Sample preparation:

100mg equivalent of Berberine Capsule were crushed weighed and transferred to volumetric flask, dissolved 0.1M Hydrochloric acid to achieve a concentration of 1mg/ml. The solution was kept at room temperature. Then the next day (1st day), an aliquot solution was diluted with methanol to get final concentration of 3µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded.

7.1.3. Blank preparation:

100ml of 0.1M HCl solutions was taken in a 100ml volumetric flask. The solution was kept at room temperature the next day; an aliquot solution was diluted with ethanol to get final concentration.

The absorption of the resulting solution showed maximum against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

*Table. 4: **Hydrolytic Degradation of Berberine Using 0.1m HCL***

Sl. No		Concentration(µg/ml)	Actual absorbance at 344 nm	Acid Hydrolysis absorbance at 344 nm
1.	Blank	3	0.412	0.410
2	Standard		0.813	0.689
3	Sample		0.731	0.706

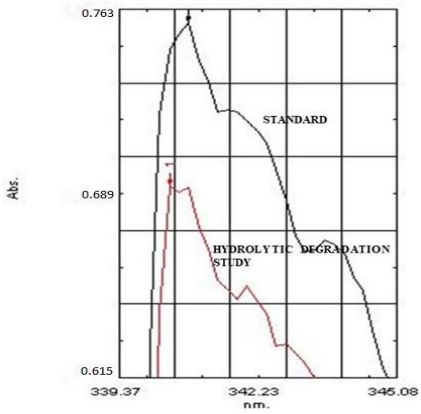

Figure. 3: Overlay Spectra of Hydrolytic Degradation of Berberine Using 0.1m Hcl

8. Oxidation degradation using 5% H_2o_2:

8.1. Standard preparation:

100mg of Berberine was weighed and transferred to volumetric flask, dissolved to 5% H_2O_2 achieves a concentration of 1mg/ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with methanol to get final concentration of 3µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded.

8.2. Sample preparation:

100mg equivalent of Berberine Capsules were crushed weighed and transferred to volumetric flask, dissolved 5% H_2O_2 to achieve a concentration of 1mg /ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with methanol to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded.

8.3. Blank preparation:

100ml of 5% H_2O_2 solution was taken in a 100ml volumetric flask. The solution was kept at room temperature. After 30mins an aliquot solution was diluted with distilled water to get final concentration. This is used as a blank.

Table. 5: oxidative degradation of berberine using 5% H_2O_2

Sl. No		Concentration (µg/ml)	Actual absorbance at 344 nm	Oxidative Degradation absorbance at 344 nm
1.	Blank	3	0.410	0.410
2	Standard		0.756	0.739
3	Sample		0.635	0.594

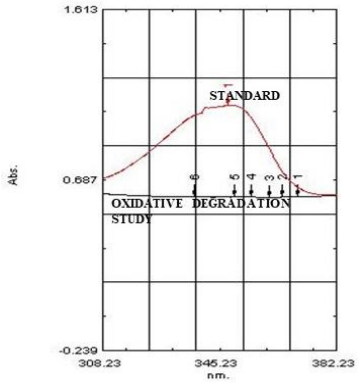

Figure. 4: overlay spectra of oxidative degradation of berberine using 5%H_2O_2

9. Irradiation with ultraviolet light:

A sample powder of Berberine (10 mg) was exposed to UV light (254 nm) for 48 h. The material was dissolved in 10 ml methanol. The solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted to obtain a solution containing concentration of 3µg/ml and a volume of 2 ml was taken in cuvette and absorbance was recorded. As well, an aqueous solution of Berberine (1mg/ml) was exposed to UV light (254 nm) for 48 h, and after diluting, a volume of 2 ml was taken in cuvette and absorbance was recorded.

Table. 6: irradiation with uv light

Sl. No		**Concentration (µg/ml)**	**Actual absorbance at 344 nm**	**Oxidative Degradation absorbance at 344 nm**
1.	Blank	3	0.409	0.407
2	Standard		1.080	0.897
3	Sample		0.811	0.615

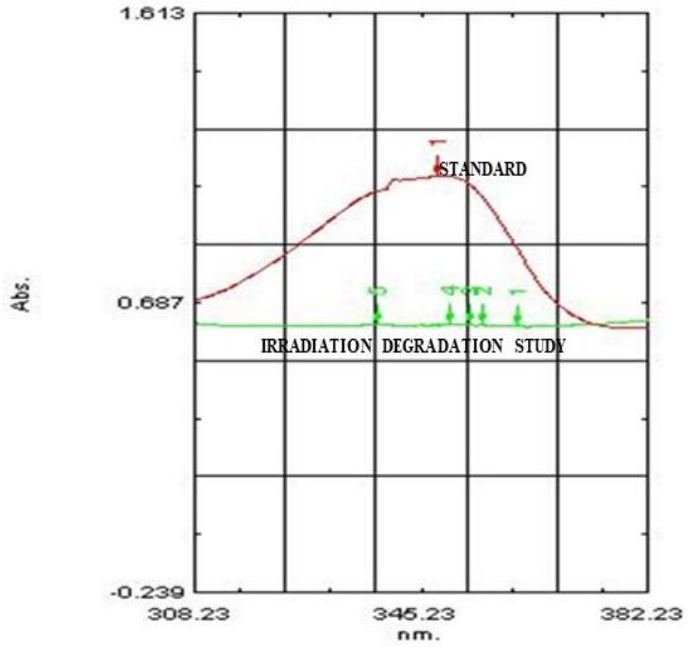

Figure. 5: overlay spectra of irradiation of berberine with uv light

10. Results and discussion:

From the forced degradation, it was observed that in case of hydrolytic degradation stability Berberine was most stable under the employed stress conditions as shown in Fig. 15. Maximum degradation was seen oxidative degradation using hydrogen peroxide (Fig. 16). Nonetheless, the method was able to isolate completely the degradation products from the intact Berberine. This confirmed stability indicating property of the proposed method.

The concentration of the produced degradation products analogous to the intact Berberine was calculated and found to be 96.56%, 66.33% and 75.8% in case of acid hydrolysis, oxidation, and photolytic degradation respectively

Table. 7: stability studies

Sl.No	Stability Studies of standard Berberine	Concentration Used(µg/ml)	Concentration Left After Degradation (µg/ml)	%Recovery
1	Acid hydrolysis	3	2.897	96.56
2	Oxidation degradation	3	1.990	66.33
3	Photolytic degradation	3	2.274	75.8

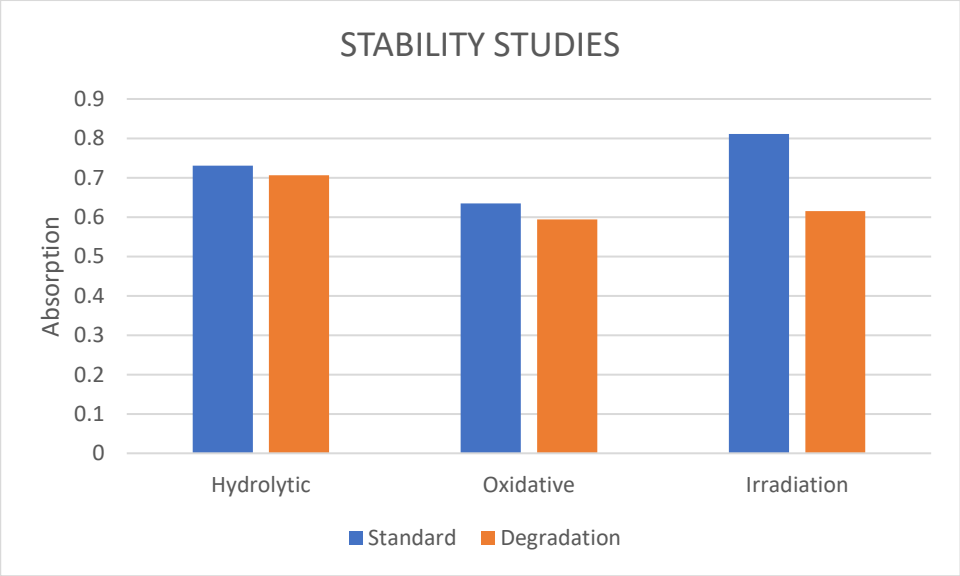

Figure. 6: stability studies

11. Conclusion:

The present study involves the stress induced stability studies such as acid hydrolytic degradation, oxidative degradation and photolytic degradation. Degraded samples were

quantified by UV method and the results of bulk and samples are compared with that of standard.

Berberine is very sensitive so it is unstable in oxidative and photolytic but is stable hydrolytically. Statistical analysis for the results clearly demonstrates that the method is suitable for the determination of Berberine in bulk and capsules without any interference from the degradation products, and it is endorsed for routine use in quality control industry laboratories.

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