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Force degradation studies of bioactive berberine and *Tinospora cordifolia* extract

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Abstract
Since ancient times, herbal medicines have been used by many different cultures throughout the world to treat illness. India has seen immense advancement in herbal drug market, which has resulted in development of infinite therapeutic herbal drug formulations by various manufacturers. With the upgrading of ability in the field of phytochemistry it has now been examined that many of drugs may react with each other increasing the serious task about the stability of such formulations. This is the area, which is essential to address in order to regulate the efficacy of the formulation. Stability testing of herbal products is a challenging job, because the herbal product is observed as the active substance, negligent of whether constituents with defined therapeutic activity are known. The objective of the stability testing is to accommodate evidence of how the property of the herbal products changes with the time under the consequence of environmental factors such as temperature and humidity. Different parameters were analyzed at regular intervals. These studies are regulated at 80°C. The present research work deals with the stability studies by using HPTLC through various stability parameters like alkali hydrolysis, acid hydrolysis, oxidative stress degradation, photolytic degradation and dry heat degradation.

Keywords: Berberine, *Tinospora cordifolia*, stability, forced degradation

1. Introduction
Forced degradation (FD) study is a method in which the uniform degradation rate of a pharmaceutical product is raised by the application of an extra stress. Force degradation plays an important role in the evolvement of stability indicating analytical method. Force degradation studies are help to recognize reactions that motive degradation of pharmaceutical product. These studies also detect contaminations which are formed during production, storage and their properties are dissimilar from the desired product. Force degradation studies are accomplished to create product- related variants and promote analytical methods to determine the degradation products formed during accelerated and long term stability studies. Any significant degradation product should be judged for characterization and quantization for its possible hazard [1, 2]. A degradation product is a molecule occuring from a change in the active ingredient as a result of storage (e.g. oxidation, hydrolysis). Compounds that are developed from a reaction of the active ingredient with an excipients or container closure constituent are considered degradation products [3]. Force degradation studies beneficial to conclude the degradation pathway and degradation products of the APIs that could form throughout storage and facilitate formulation development, production and packaging.

1.1 Stability indicating method
Forced degradation is also known as stability indicating methods (SIM). These can be used to bring information about degradation pathways and products that could form during storage and bring formulation development, production, and packaging. It is difficult to get real representative samples in the initial stage of development. Stressing the API produces the sample that contains the products most likely to form under most sensible storage conditions, which is in turn used to develop the SIM [4]. SIM is a validated quantitative analytical procedure that can recognize the changes with time in the properties of the drug substances and drug product.

1.2 Importance and need of force degradation studies
The main reason for stability testing is the responsibility of the well- being of the patient agony from the disease for which the products is outlined. Apart from degradation of the unstable product into toxic decayed products, loss of activity up to a level of 85% of that claimed on the label may lead to loss of the therapy resulting in death. On account of this concern, it has become a legal necessity to provide data for various types of stability tests for the regulatory agencies before authorization of a new product. Other advantages of stability studies of the marketed products are to support a database that may be of value in selection of suitable formulations, excipients and container closure systems to determine shelf life and storage conditions for development of a new product and to verify that no changes have been brought in the manufacturing process that can unfavorably affect the stability of the product [5]. Force degradation studies are important to help develop and exhibit specificity of stability- indicating methods and to decide the degradation pathways and degradation products of the active ingredients. These studies are also useful in the investigation of the chemical and physical stability of crystal forms, the stereochemical stability of the drug substance alone and in the drug product and for differentiating drug substance related degradation products in formulations.

2. Materials and methods

2.1 Chemicals and reagents

Standard berberine was obtained from Sigma chemicals. Plant material *Tinospora cordifolia* was collected from Medicinal garden, Maharshi Dayanand University, Rohtak and was authenticated from Department of Botany, Maharshi Dayanand University, Rohtak. Voucher specimen no. VS/ Phcog/ 213 of the plant was kept in the Department for future reference. For filtration of mobile phase and working solutions membrane filters (0.45µ) were used. Throughout the experimental work distilled and filtered water was used.

2.2 Forced degradation studies

The forced degradation studies were carried out to show whether the analytical method were stability- indicating and could certainly assess the analyte in the presence of impurities and degradation products. Marker berberine and *Tinospora cordifolia* extract were stressed under alkali hydrolysis, acid hydrolysis, oxidative stress conditions, dry heat degradation and photolytic degradation [6-10].

2.2.1 Alkali Hydrolysis

Accurately weighed quantity of berberine and *Tinospora cordifolia* extract were taken and transferred in two different 100 ml volumetric flasks to it 2 ml of 0.1 N sodium hydroxide solution was added. The resulting solutions were heated on water bath for 2 hr at 80° C and allowed to cool at room temperature. After cooling, solutions were neutralized with 0.1 N hydrochloric acid solution and then diluted up to the mark with methanol to obtain concentration of 100 µg/ml.

2.2.2 Acid Hydrolysis

Accurately weighed quantity of berberine and *Tinospora cordifolia* extract were taken and transferred in two different 100 ml volumetric flasks to it 2 ml of 0.1 N hydrochloric acid solution was added. The resulting solutions were heated on water bath for 2 hr at 80° C and allowed to cool at room temperature. After cooling, solutions were neutralized with 0.1 N sodium hydroxide solution and then diluted up to the mark with methanol to obtain concentration of 100 µg/ml.

2.2.3 Oxidative Stress Degradation

Accurately weighed quantity of berberine and *Tinospora cordifolia* extract were taken and transferred in two different 100 ml volumetric flasks to it 2 ml of 3% hydrogen peroxide solution was added. The resulting solutions were heated on water bath for 2 hr at 80° C and allowed to cool at room temperature and then diluted up to the mark with methanol to obtain concentration of 100 µg/ml.

2.2.4 Dry Heat Degradation

To execute dry heat degradation, pure samples of berberine and *Tinospora cordifolia* extract were kept in oven at 80°C for 2 hr, allowed it to cool at room temperature. From the above, 10 mg each of berberine and *Tinospora cordifolia* extract were weighed. Transferred to two separate volumetric flasks (100 ml) and dissolved in few ml of methanol. Volumes were made up to the mark with methanol to obtain working standards of 100 µg/ml of both the drug.

2.2.5 Photo Degradation

Pure samples of berberine and *Tinospora cordifolia* extract were exposed to UV light for 6 hr. From the above, 10 mg each of berberine and *Tinospora cordifolia* extract were weighed. Transferred to two separate volumetric flasks (100 ml) and dissolved in few ml of methanol. Volumes were made up to the mark with methanol to obtain working standards of 100 µg/ml of both the drug.

3. Results and discussion

Various methods available for determination of berberine and *Tinospora cordifolia* extract are not stability indicating. Force degradation studies are beneficial to determine the degradation pathway and degradation products of the APIs that could form during storage and promote formulation development, production and packaging. Acid, base, hydrogen peroxide and light degraded samples showed well separated spots of pure berberine. Details of degradation products with their R_f values is summarized in Table 1 and 2. Chromatogram of acid hydrolysis, dry heat degradation and photolytic degradation shows degradation of standard berberine and the degradation product peak at R_f value 0.65. Degradation peak shows that there is a major difference in peak for degraded *Tinospora cordifolia* extract. Alkali hydrolysis and oxidative degraded samples shows degradation peaks at R_f 0.68 and 0.66 respectively for standard berberine and no peak was found for extract of *Tinospora cordifolia.* Figure 2 shows comparisons of chromatogram of standard berberine before degradation and chromatogram of standard berberine after degradation parameters. Figure 3 shows the comparisons of chromatogram of extract before and after degradation parameters.

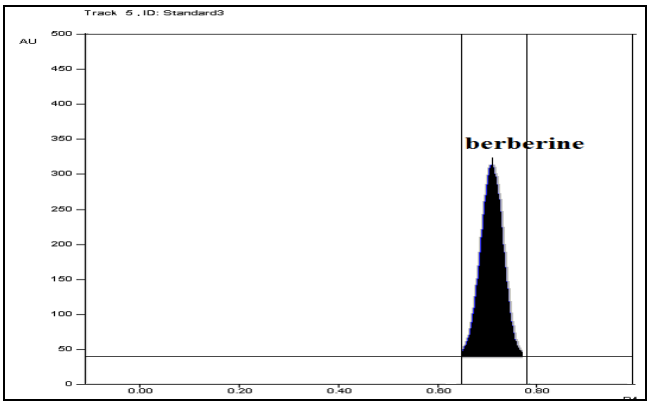
Table 1: Degradation studies of standard berberine at different parameters

S. No	Sample	Parameters	Condition	Time	R_f value of standard berberine before degradation	R_f value of standard berberine after degradation
1	Berberine standard	Alkali hydrolysis	1 N NaOH at 80° C in water bath	2 hrs	0.61	0.68
2	Berberine standard	Acid hydrolysis	1 N HCL at 80° C in water bath	2 hrs	0.61	0.65

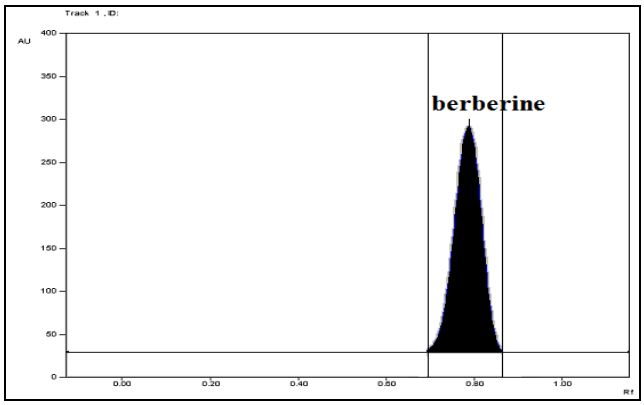
3	Berberine standard	Oxidative stress degradation	3% H2O2 at 80˚C in water bath	2 hrs	0.61	0.66
4	Berberine standard	Dry heat degradation	Dry heat hot air oven at 80˚C	2 hrs	0.61	0.65
5	Berberine standard	Photolytic degradaon	Photolytic expose to UV	6 hrs	0.61	0.65

Table 2: Degradation study of *Tinospora cordofolia* extract at different parameters

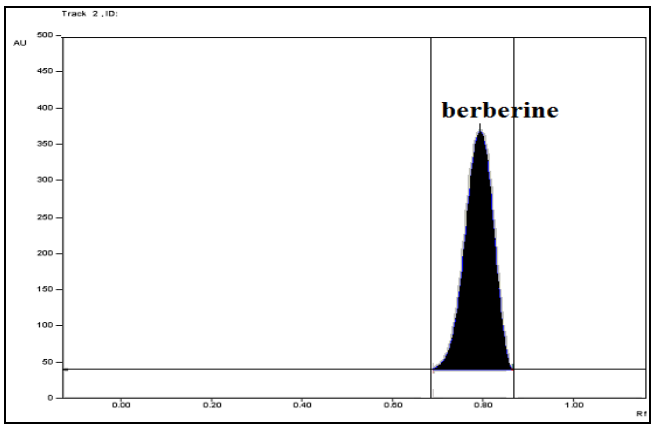
S. No	Sample	Parameters	Condition	Time	R_f value of berberine present in extract of *Tinospora cordifolia* after degradation parameters	Rf value of berberine present in extract of *Tinospora cordifolia* after degradation Parameters
1	Extract of *Tinospora cordifolia*	Alkali hydrolysis	1 N NaOH at 80˚ C in water bath	2 hrs	0.65	No peak found
2	Extract of *Tinospora cordifolia*	Acid hydrolysis	1 N HCL at 80˚C in water bath	2 hrs	0.65	No peak found
3	Extract of *Tinospora cordifolia*	Oxidative stress degradation	3% H2O2 at 80˚C in water bath	2 hrs	0.65	No peak found
4	Extract of *Tinospora cordifolia*	Dry heat degradation	Dry heat, hot air oven at 80˚C	2 hrs	0.65	0.75
5	Extract of *Tinospora cordifolia*	Photolytic degradation	Photolytic, expose to UV	6 hrs	0.65	No peak found



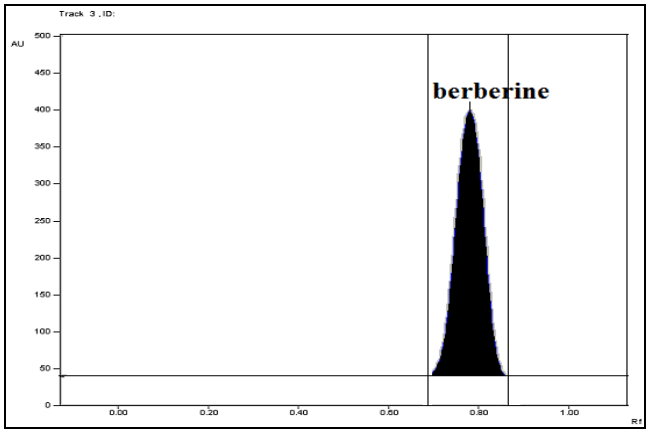
Standard berberine peak before degradation



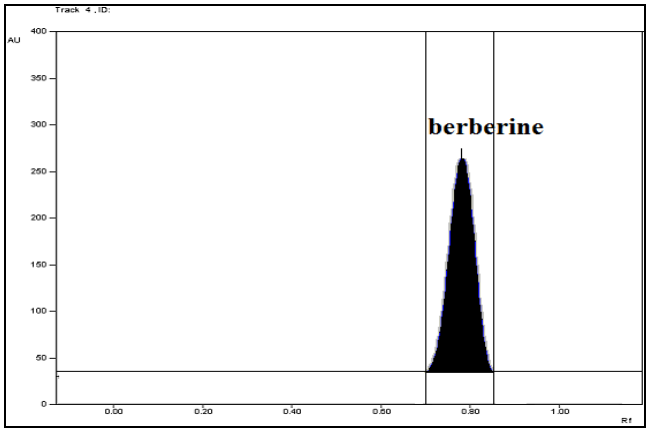
Standard berberine after alkali hydrolysis



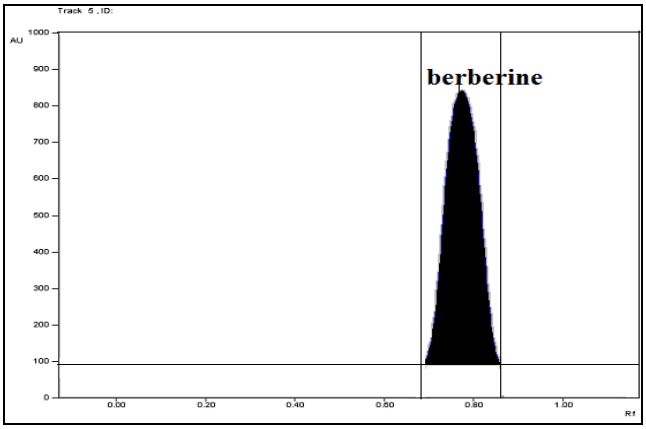
Standard berberine after acid hydrolysis



Standard berberine after oxidative hydrolysis

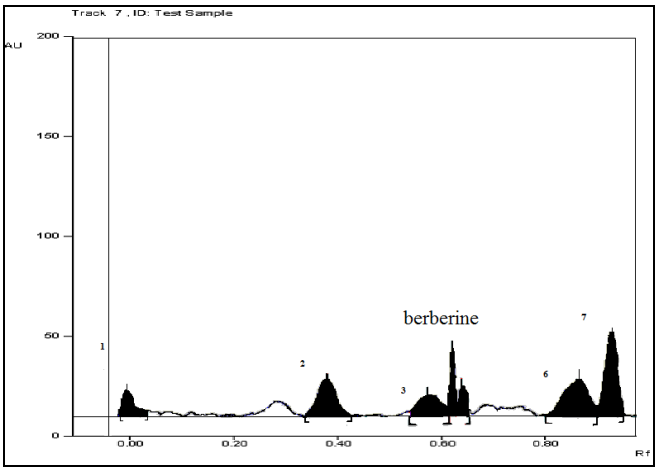


Standard berberine after dry heat degradation



Standard berberine after photolytic degradation

Fig 1: Chromatogram comparison of standard berberine before degradation and after degradation parameters.



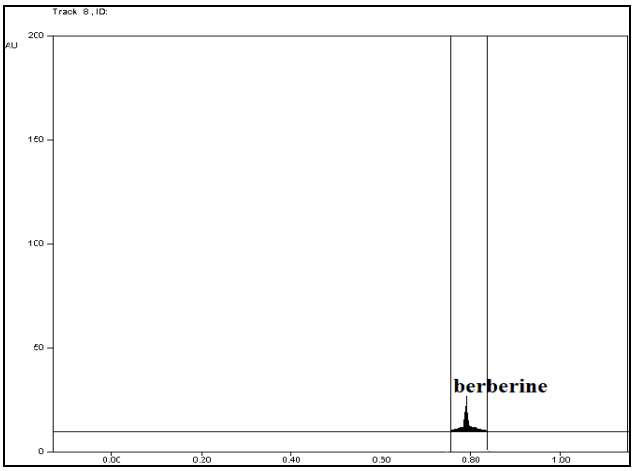


Fig 2: Chromatogram comparison of berberine present in extract of *Tinospora cordifolia* before degradation and after degradation parameters

4. Acknowledgements

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5. References

1. FDA. Guidance for Industry. Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation", Draft guidance. 2000.
2. ICH. Guidance for Industry, Q5C. Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. ICH- Q5C. 1996.
3. Singh S, Bakshi M. Development of stability-indicating assay methods- A critical review, Journal of pharmaceutical and biomedical analysis. 2002; 28:1011-1040.
4. Swartz M, Krull I. Developing and validating stability-indicating methods, *LCGC* North America. 2005; 23(6):586-593.
5. Carstensen JT. Drug stability, Principles and practices, Marcel Dekker, New York. 2000.
6. Kumarn BSP, Annapurna MM, Pavani S. Development and validation of a stability indicating RP- HPLC method for the determination of Rufinamide. Journal of pharmaceutical analysis. 2013; 3(1):66-70.
7. Ngwa G. Forced degradation as an integral part of HPLC stability- indicating method development. Drug delivery technology. 2010; 10(5):20.
8. Shah DA, Bhatt KK, Mehta RS, Baldania SL, Gandhi TR. Stability indicating RP-HPLC estimation of nebivolol hydrochloride in pharmaceutical formulations. Indian journal of pharmaceutical sciences. 2008; 70:591-595.
9. Damle DM, Chaudhari D, Sinker M. Stability indicating HPTLC method for estimation of nebivolol hydrochloride and amlodipine besylate in combination. Eurasian journal of analytical chemistry. 2010; 5(2):161-169.