



STABILITY STUDY OF MEMORIN CAPSULE BY THIN LAYER CHROMATOGRAPHIC AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD AS A QUALITY CONTROL TOOL FOR AYURVEDIC FORMULATION

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Received for publication: May 17, 2014; Revised: June 21, 2014; Accepted: July 07, 2014

Abstract: Since last one decade India has seen tremendous growth in herbal drug market, which has resulted in development of numerous proprietary herbal drug formulations by various manufacturers, majority of them comprising of polyherbal formulations. With the advancement of knowledge in the field of phytochemistry it has now been observed that many of these constituents present in the drug may react with each other raising the serious concern about the stability of such formulations. This is the area, which needs to address in order to determine the efficacy of the formulation. Stability testing of herbal products is a challenging task, because the herbal product is regarded as the active substance, regardless of whether constituents with defined therapeutic activity are known. The purpose of the stability testing is to provide proof of how the quality of the herbal products varies with the time under the influence of environmental factors such as temperature and humidity. Different parameters were checked at regular intervals. These studies are conducted at 40°C. If any significant change occurs at any time during six months, testing at the accelerated storage condition should be conducted and evaluated against significant change criteria by various analytical methods such as TLC and HPTLC. Therefore evaluation of the parameters based upon chemical, physical, microbiological, therapeutic studies can serve as an important tool in stability studies.

Key Words: Herbal formulation, stability studies, storage condition, TLC, HPTLC

INTRODUCTION

The purpose of the stability testing is to provide proof of how the quality of the herbal products varies with the time under the influence of environmental factors such as temperature and humidity. The evaluation of the stability study of a product is its storage condition based on the climatic conditions only storage conditions can be determined. The products efficacy, safety, and ethical issue have to be confirmed, before launching the product in to market. Stability studies should be performed on at least three production batches of the herbal products for the proposed shelf life, which is called as long term stability and is performed under atmospheric conditions. Stability data can also be generated under accelerated atmospheric conditions of temperature and humidity. This is referred to as short term stability. Stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing. With the help of modern analytical technique likes spectrophotometer, HPLC, GC-MS, IR, DSC, XRD and by employing proper guidelines it is possible to generate a sound stability data of herbal products and predict their shelf life, which will help in improving global acceptability of herbal products. Stability testing of herbal products is a challenging task, because the herbal product is regarded as the active substance, regardless of

whether constituents with defined therapeutic activity are known. Different parameters like TLC HPTLC were checked at regular intervals. These studies are conducted at 40°C. Therefore evaluation of the parameters based upon chemical, physical, microbiological, therapeutic studies can serve as an important tool in stability studies.

Need and scope of Herbal therapy

Today we are more concerned with life style diseases like depression, cancer and heart troubles caused by faulty nutrition and stress. Because these diseases have a mental or emotional component, there is a growing conviction that allopathic is largely unable to cure them, all of it offers is temporary relief from symptoms. There is a need of alternative therapy, to cover a good health for all. Herbal therapy will be one of the best practices to overcome the illness. Traditional Indian practice held that certain drugs should be formulated through the addition of chosen substance that enhances bioavailability of the drug.

Enrichment of memory by using Herbal formulation

The use of herbal products has increased tremendously in the western world as well as in developing countries. Numerous natural products have been evaluated as therapeutics for the treatment of

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variety of purposes, including poor memory. Memory is the most vital aspect for effective survival of human beings. It also differentiates humans from animals. Memory is the ability of an individual to record the information and recall it whenever needed. Traditionally herbal drugs have been used to enhance cognitive functions. A number of medicinal plants and medicines derived from these plants have shown memory enhancing properties by virtue of their medicinal constituents. There has been considerable pharmacological investigation into the memory enhancing activity of some compounds. The herbs acting on the brain are called Nootropic herbs and their isolated constituents are referred to as smart drugs. These herbs enhance the memory as well as increase blood circulation in the brain. So mostly people prefer herbal nootropics over synthetic nootropics because they have less side effects than others. Herbal nootropics mainly act by different ways like by increasing and replenishing neurotransmitter at high concentration in brain, by anti-depression, adaptogenic and mood stabilization, by improved oxygen supply and brain energy, by improved concentration, stamina, and focus, by memory enhancement and learning improvement, by nerve growth stimulation and brain cell protection.

Memory is the ability of an organism to store, retain, and subsequently recall information. Memory enhancers are the compounds which improve or enhance the memory. Some physiological conditions such as stress, anxiety affect the memory. Memory loss may be age related and due to some disease condition like Alzheimer's disease, Parkinson's disease etc

MATERIALS AND METHODS

Collection of plant materials

The Memorin capsule was obtained as a gift sample from S. G. Phytopharma, Kolhapur, MH. India. All raw materials like Mandookparni, Shankhpushpi, Yashtimadhu, and Jatamansi were obtained as a gift sample from S. G. Phytopharma, Kolhapur, MH. India.

Stability testing

The newly formulated Ayurvedic health supplement undergone physical, chemical, biological and microbiological characteristics study. The studies are conducted at $40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ RH noted for significant changes occurs during one year at an interval of 0,3 and 6 months. The followed parameters are checked during this interval.

Thin Layer Chromatography

Preparation of the adsorbent: Prepare slurry of the coating material and water and coat the cleaned plates with a layer about 0.25 mm thick. Dry the coated plates in air, heat to activate at 110°C for 30 minutes,

and then allow cooling. Inspect the uniformity of the coating in transmitted light and the texture in reflected light.

Selection of mobile phase: To select suitable mobile phase, initial experiments were carried out on glass TLC plates in saturated chambers. The table no. 6.4 is given below which contains the mobile phase for particular raw materials and Formulation.

Table 1: Mobile phase for Formulation

Formulation	Mobile Phases Used (v/v)
Memorin Capsule	Ethyl acetate : Methanol : Distal water (12:1.7:1.3)
	Toluene : Ethyl acetate (9:1)
	Toluene : Acetone : formic acid (11 : 6 : 1)
	n-Butanol : Water : Glacial acetic acid (7 : 2 : 1)

Table 2: Mobile phase for raw materials

Sr. No	Raw Material	Mobile Phases Used (v/v)
1.	Mandookparni	Methanol : NH_4OH 17 : 3 v/v
2.	Shankhpushpi	Toluene : Ethyl acetate 9:1 v/v
3.	Yashtimadhu	n-Butanol : Water : Glacial acetic acid (7 : 2 : 1)
4.	Jatamansi	Toluene : Acetone : Formic acid (11 : 6 : 1)

Saturation of the chromatographic chamber:

Unless otherwise specified in the test procedure, the chromatography is carried out in a saturated chamber. To achieve saturation, line at least half of the total area of the inside walls of the chamber with filter-paper, pour into the chamber a sufficient quantity of the mobile phase to saturate the filter-paper and form a layer about 5 mm deep. Close the chamber and allow standing for at least 1 hour at room temperature.

Application of the test and reference solutions:

Using a micropipette or a syringe graduated in μl , place spots of the test and reference solutions onto the starting line, which should be parallel to and about 15 mm above the lower edge. The spots should be at least 15 mm from the sides of the plate, and at least 15 mm apart. They should be as small as possible, preferably not more than 4 mm in diameter; if necessary, apply the solution in portions, drying between applications. Mark the distance the mobile phase is intended to ascend as specified in the test procedure, usually 10-15 cm from the starting line.

Development of chromatograms: Allow the spots to dry, place the plate - as nearly vertical as possible - into the chamber, ensuring that the points of application are above the surface of the mobile phase. Close the chamber. Develop the chromatogram at room temperature, unless otherwise specified in the test procedure, allowing the solvent to ascend the specified distance. Remove the plate, mark the position of the solvent front and allow the solvent to evaporate at room temperature or as specified.

Observation and interpretation of the chromatograms: If indicated in the test procedures spray the spots with the specified reagent or place it in iodine chamber and observe and compare the spots with those of a reference material. If required calculate the distance travelled on the adsorbent by a given compound (the R_f value).

HPTLC Method Development and Method Validation

Instrumentation: The following HPTLC system was used to develop method, which consist of,

- LINOMAT-V automatic TLC sample applicator
- CAMAG TLC Scanner 3 equipped with Wincats software (version 1.4.2)
- Merck HPTLC plates coated with silica gel 60 F₂₅₄ (0.2 mm thickness) on aluminum sheets,
- ILS micro syringe (100µl)

Selection of stationary phase: Development of standard and isolated CPT was performed on (10 cm x 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) aluminum backed silica gel 60 F₂₅₄ TLC plates.

Selection of mobile phase for Memorin capsule:

To select suitable mobile phase, initial experiments were carried out on glass TLC plates in saturated chambers. Initially, solvent system containing n-Butanol: Water: Glacial acetic acid (7: 2: 1v/v) was used. This system had given satisfactory result.

Sample preparation

Preparation of sample solution of Memorin capsule:

For sample preparation, 500 mg of drug taken in 5 ml of methanol and sonicate for 1hr. This resulting solution was further used for application on pre coated TLC plate. Same procedure was used for preparation of 3 month and 6 month for all temperature condition.

Chromatographic Conditions

The experiment was performed on a silica gel G₆₀ F₂₅₄ (0.2 mm thickness) HPTLC plates (20x10cm) without prewashing. Samples were applied to the plates as 4 mm bands, 4 mm apart and 10 mm from the edges of the plate, with an LINOMAT-V automatic sample applicator. The plates were developed by the ascending technique, to a distance of 80 mm, at 25 ± 5°, relative humidity 50-60%, in a Camag twin-trough glass chamber with a stainless steel lid, using a mobile phase, composed of n-Butanol: Water: Glacial acetic acid (7 : 2 : 1 v/v) for CPT. The chamber saturation time was kept as 20 min. After development, plates were dried with a hot-hair dryer, viewed in a UV cabinet, and then scanned with a Camag TLC Scanner, using win CATS software (version 1.4.2), in absorbance mode, with slit dimensions 6.00 x 0.45 mm. The detection wavelength 254 nm was selected. And then dipped in Ninhydrin reagent and viewed in visualizing chamber.

RESULTS

Thin layer chromatography

TLC was carried out by taking most effective extracts of row material and Memorin capsule. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution. The number of spot and R_f value of row material was shown in table 3 and the number of spot and R_f value of Memorin capsule was shown in table 4

Table 3: TLC for raw materials

S. No.	Raw materials	Solvent system	No of spot	RF values
1	Mandukparni	Methanol : NH ₄ OH (17 : 3 v/v)	2	0.76 0.66
2	Shankhpushpi	Toluene : Ethyl acetate (9:1 v/v)	2	0.85 0.62
3	Yashtimadhu	n-Butanol : Water : Glacial acetic acid (7 : 2 : 1 v/v)	2	0.25 0.21
4	Jatamansi	Toluene : Acetone : formic acid (11 : 6 : 1 v/v)	2	0.77 0.66

Table 4: TLC for Memorin capsule

Finished product	Solvent system	No of spot	RF values
Memorin capsule	n-Butanol : Water : Glacial acetic acid (7 : 2 : 1 v/v)	4	0.77 0.66 0.49 0.25

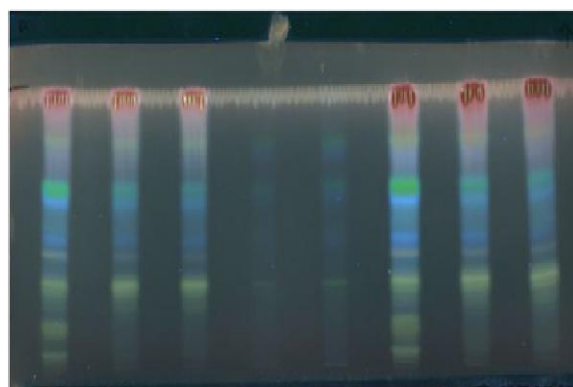


Figure 1: HPTLC chromatography interpretation of Memorin capsule for stability study at 40°C

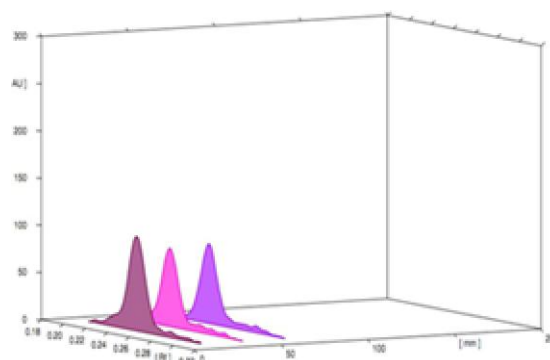


Figure 2: Graphical representation of Memorin capsule during stability study at 40°C

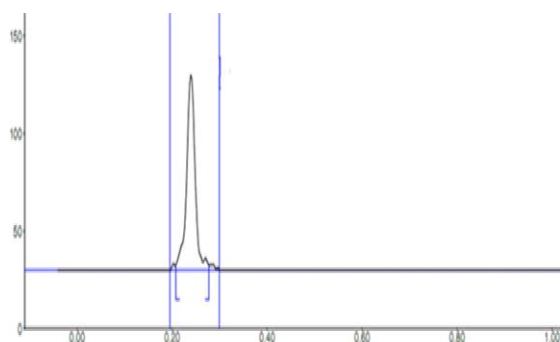


Figure 3: Peak area and Rf value during initial study at 40°C

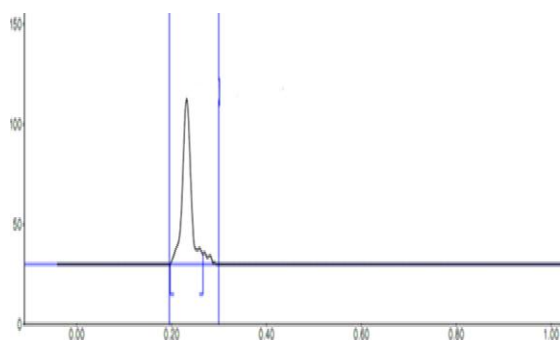


Figure 4: Peak area and Rf value during 3 month study at 40°C

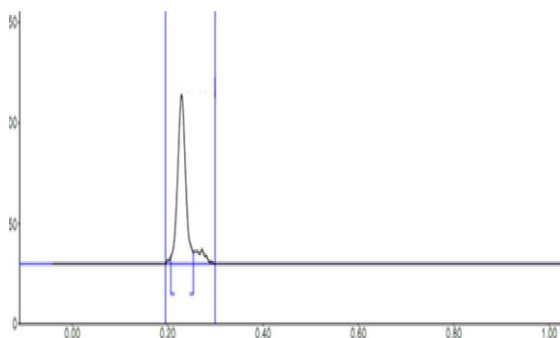


Figure 5: Peak area and Rf value during 6 month study at 40°C

Table 5 HPTLC evaluations for peak area and Rf value during stability study at 40°C

Stability periods	Area	Rf value
Before study	1525.1	0.24
After 3 months	1263.9	0.23
After 6 months	1183.3	0.23

CONCLUSION

- Stability testing is now the key procedural component in the pharmaceutical development program for a new drug as well as new formulation.
- The outcome of this study indicates that the formulation could be preserved at low temperature as well as moderate temperature.

- When compared to the marketed formulation, the herbal formulation shows a very good response in stability studies.
- The outcome of the TLC and HPTLC analysis study indicates that the Memorin capsule was slightly degraded at 40°C after 3 and 6 months study within limits.

ACKNOWLEDGEMENT

Authors are thankful to Principal and Management Tatyasaheb Kore College of Pharmacy, Warananagar for providing necessary facilities to complete this work.

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Source of support: Nil

Conflict of interest: None Declared