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STAYBILITY TESTING OF HERBAL DRUGS – A Review

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ABSTRACT

Stability testing is an important component of herbal drugs and products (HDPs) development process. Drugs regulatory agencies across the globe have recommended guidelines for the conduct of stability studies on HDPs, which require that stability data should be included in the product registration dossier. From the scientific viewpoint, numerous chemical constituents in an herbal drug are liable to varied chemical reactions under the influence of different conditions during its shelf life. These reactions can lead to altered chemical composition of HDP and consequently altered therapeutic profile. Many reports on stability testing of HDPs have appeared in literature since the last 10 years. A review of these reports reveals that there is wide variability in temperature (80 to 100 °C), humidity (0–100%) and duration (a few hours–36 months) for stability assessment of HDPs. Of these, only 1% studies are conducted in compliance with the regulatory guidelines for stability testing. The present review is aimed at compiling all stability testing reports, understanding key challenges in stability testing of HDPs and suggesting possible solutions for these. The key challenges are classified as chemical complexity and biochemical composition variability in raw material, selection of marker(s) and influences of enzymes.

Keywords: enzymes, Stability, biochemical

INTRODUCTION

Herbal drugs constituents are of different kind and have many constituents. The finished products of herbal medicine generally have low concentration of active constituent(s). Stability testing of herbal drugs is a challenging risk, because the entire herb or herbal product is regarded as the active matter, regardless of whether constituents with defined therapeutic activity are known [1]. The most important

aspect in the evaluation of the stability study of a product is its storage condition. The purpose of a stability testing is to provide proof on how the quality of the herbal products varies with the time under the influence of environmental factors such as temperature, light, oxygen, moisture, other ingredient or excipients in the dosage form, particle size of drug, microbial contamination, trace metal contamination, leaching from the container and to establish a recommended storage condition and shelf-life. Based on the climatic conditions only storage conditions can be determined. Stability studies should be performed on at least three production batches of the herbal products for the proposed shelf-life, which is normally denoted as long term stability and is performed under natural atmospheric conditions. With the help of modern analytical techniques like spectrophotometry, HPLC, HPTLC 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.

REGULATORY BASIS OF HERBAL DRUG STABILITY TESTING

Guidelines provided by drugs regulatory agencies such as EMEA (CPMP, 2003a, 2012, 2011a, 2011b; EMEA, 2008a, 2008b, 2010), International Conference on Harmonization (ICH, 2003) and World Health Organization (WHO, 2009) require stability data of any drug product prior to its approval. WHO's Supplementary guidelines for the manufacture of herbal medicines specifically states under its section 17.4 that If the expiry date is given for a herbal material or herbal preparation, some stability data which supports the proposed shelf life under the specified storage conditions should be available. Stability data is always required to support the shelf-life for the finished herbal products (WHO, 2006). EMEA has also issued specific set of guidelines on quality of HMPs, which

Table 1. Recommended conditions for stability testing (WHO, 2009)

Study	Storage condition	Minimum time period covered by data at submission (months)	Testing frequency (months)
Long-term	25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/65 ± 5% RH or 30 ± 2 °C/75 ± 5% RH	12	0, 3, 6, 9, 12, 18, 24 and then annually
Intermediate	30 ± 2 °C/65 ± 5% RH	6	0, 6, 9 and 12
Accelerated	40 ± 2 °C/75 ± 5% RH	6	0, 3 and 6

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clearly states that Since the herbal substance or herbal preparation in its entirety is regarded as the active substance, a mere determination of the stability of the constituents with known therapeutic activity will not suffice. The stability of other substances present in the herbal substance or in the herbal preparation, should, as far as possible, also be demonstrated, e.g., by means of appropriate fingerprint chromatograms. It should also be demonstrated that their proportional content remains comparable to the initial fingerprint (CPMP, 2011b). Current Good Manufacturing Practice USFDA states that Nevertheless, if you use an expiration date on a product, you should have data to support that date. You should have a written testing program designed to assess the stability characteristics of the dietary supplement, and you should use the results of the stability testing to determine appropriate storage conditions and expiration dates (USFDA, 2003). Kruse and Sultan (2010) have discussed the

legal requirement and suggested some specific features for quality control and stability studies on HMPs. From these excerpts, it is evident that drugs regulatory agencies need the shelf life of an herbal product be assessed and recommended on the basis of appropriately generated stability data. Various approaches for assessment of shelf life include assay of markers (active or analytical), biological assays and/or chromatographic chemoprofiling or fingerprinting of control and stability samples of a product under different stability conditions. These conditions are broadly consensual (Table 1) in different international and country-specific guidelines on quality of herbal drugs/products.

STABILITY DETERMINATION OF HERBAL MEDICINAL PRODUCTS

The principle of a stability study is to provide evidence that an active substance or finished product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The importance of stability testing is to; evaluate the efficacy of a drug; provide background information during the development phase of the product or drug discovery; develop suitable packaging information for quality, strength, purity and integrity of a product during its shelf-life [9, 38, 61]. Mechanisms that may indicate a change in stability include; loss of activity; change in concentration of active component; alteration in bioavailability of product; loss of content uniformity; loss of elegance; formation of toxic degradation products and loss of packaging integrity. It is difficult to develop analytical methods for herbal medicines due to the presence of phytochemical constituents which may be susceptible to enzymatic breakdown of these plant metabolites. Predictable chemical changes in herbal products includes; hydrolysis; oxidation (especially in fixed, volatile and essential oils); racemization; geometric isomerization and poly-merization.

Table 1: Acceptance criteria for microbiological tests

Microbiological quality	Acceptance criteria*		
	A	B	C
Total aerobic microbial count	Not more than $5x10^7$ CFU per g or per ml	Not more than $5 x10^4$ CFU per g or per ml	Not more than $5 x 10^5$ CFU per g or per ml
Total yeast and mould count	Not more than $5x10^5$ CFU per g or per Ml	Not more than $5 x10^2$ CFU per g or per ml	Not more than $5 x 10^4$ CFU per g or per ml
Bile-tolerant Gram negative bacteria	Not more than 10^2 CFU per g or per ml	Not more than10^2 CFU per g or per ml	Not more than $5 x 10^4$ CFU per g or per ml
Salmonella spp.	Absent in 10 g or 10 ml	Absent in 25g or 25 ml	Absent in 25 g or 25 ml
Escherichia coli	Absent in 1 g or 1 ml	Absent in 1 g or 1 ml	Absent in 1 g or 1 ml

*British Pharmacopoeia [65]

CURRENT SCENARIO IN STABILITY TESTING OF HERBAL DRUGS AND PRODUCTS

Many research reports on stability studies on herbal drugs and products have appeared in literature for the last decade. A critical analysis of these reports has revealed that there is a wide variability in conditions employed and duration of stability testing and parameters evaluated for assessment of shelf life. In the present review, we have analysed various such reports on herbal drugs and products in order to (i) compile their stability data; (ii) understand the challenges in their stability testing; (iii) discuss the extent of compliance with stability testing conditions recommended in drugs regulatory guidelines; and (iv) suggest approaches for generating a comprehensive stability data of these drugs and products. Based on the parameters employed to assess stability, these reports are classified into four categories, that is, those assessing only physical characteristics (Physical studies, Table 2), those assessing physical and/or chemical stability (Physico-chemical/Chemical studies, Table 3), those assessing

biological activity (Biological studies, Table 4) and those assessing physical and chemical stabilities as well as biological activity (Physicochemico-biological studies, Table 5). The last type of studies presents an ideal stability testing protocol for herbal products, because evaluation of physical, chemical as well as therapeutic qualities of these products is the key to the scientifically established shelf life.

Table 2. Herbs/herbal products evaluated for physical changes

Herb(s)/herbal product(s)	Stability condition	Findings	Reference(s)
Azadirachta indica cream	25–30 °C, 12 months	Stable	Aremu and Femi-Oyewo, 2009
Clerodendron infortunatum (2.5% and 5%) extract gels	Different temperature and humidity, 3 months	Extract with lower % concentration is found to be more stable	Das *et al.*, 2011
Hippophae rhamnoides emulsion	8, 25, 40 and 40 °C/75% RH, 4 weeks	Stable at temperature ≤25 °C	Akhtar *et al.*, 2010
Sesbania grandiflora	ICH Q1A(R2) recommended temperature and humidity, 3 months	Stable	Dwivedi and Gupta, 2012
Trigonella foenum graecum	8, 25, 40 and 40 °C/75% RH, 4 weeks	Stable except for significant changes in pH	Waqas *et al.*, 2010
Fenugreek seed powder (fast disintegrating tablets)	Accelerated stability, 3 months	Stable	Kulkarni *et al.*, 2011

Table 3. Herbs or herbal products evaluated for chemical or physicochemical stability

Herb(s)/herbal product(s)	Stability condition(s)	Findings	Reference(s)
Actaea racemosa formulations	LT and AS, 6 months; 50 °C, 1 months	Triterpene glycoside (TG) content fall under AS	Budukh *et al.*, 2003
A. racemosa extracts	Varied temperature and humidity, 9 weeks	TG stable and polyphenols unstable at higher temperature	Jiang *et al.*, 2008
Aleurites moluccana (formulations)	AS and LT, 6 months	2–O -rhamnosylswertisin and swertisin remain stableDegradation dependent upon concentration of extract	Cesca *et al.*, 2012
Allium sativum	Fresh blend, −80 °C; tablets, 4 °C and RT, 2–3 years	Stable	Lawson and Gardner, 2005
Andrographis paniculatus extract	LT, IS and 5 °C, 3 months	Insignificant decrease in andrographolide content	Ibrahim and Chong, 2008
Bacopa monnieri extracts	5–80 °C at 75% RH and pH 1.2, 6.8, 9.0 at 40 °C	Unstable at high temperature and low pH	Phrompittayarat *et al.*, 2008
Betula pendula and *Betula pubescens*	LT, 24 months; AS, 80 °C and 100 °C, 6 months	Unstable under accelerated conditions	Hiegl and Franz, 2003
Boswellia serrata extract	LT and AS, 6 months	Extracts have been found physically stable but chemically Unstable	Sahoo *et al.*, 2009
Calendula officinalis tinctures	AS, 3 months; LT, 6 months	Stable	Bilia *et al.*, 2002
C. officinalis	LT, 24 months; AS, 80 °C and 100 °C, 6 months	Flavonoid content decreases under high temperature and humidity	Hiegl and Franz, 2003
C. officinalis cream	8, 25, 40 °C and AS	Stable	Bernatoniene *et al.*, 2011
Calophyllum inophyllum	4 and 37 °C, 3 months	Stable	Mishra *et al.*, 2011
Carum carvi oil	5 °C, LT and AS, 1 months	Carvone content stable	Sachan *et al.*, 2010
Cassia angustifolia	LT, IS and AS		Goppel 2003
Creams and dragees of *Centella asiatica* extract	Different temperature and humidity, 12 months	Marker content decrease depending on severity of conditions	Inamdar *et al.*, 1996
Crataegus oxyacantha fruits and canned drinks	4, 23 and 40 °C, 6 months	Degradation at high temperature	Chang *et al.*, 2006
C. oxyacantha tinctures	LT, 9 months	Complete degradation	Bilia *et al.*, 2007
Curcuma longa PCT-8, PCT-13 and PCT-14	LT, 9 months	Curcumin and oleoresin remain stable	Zachariah and Babu, 1992
Lipid soluble fraction of *C. longa* rhizomes	Different temperature, pH and light conditions	Markers found stable to heat, varied pH and light	Jain *et al.*, 2007
C. longa extracts	LT and 4 °C	Curcuminoids degrade significantly	Green *et al.*, 2008
C. longa tincture	40 °C, 6 months	80% fall in curcuminoid content	Karioti *et al.*, 2010
Echinacea purpurea extract	−20, 25 and 40 °C	Alkamide content remain stable; cichoric acid content decreases at 25 and 40 °C	Livesey *et al.*, 1999
E. purpurea flower extract	Varied temperature, pH, buffer, ionic strength and cosolvent	Alkamide content decreases significantly	Al-Jabari *et al.*, 2008
Ginkgo biloba extracts and formulations	25, 40, 60 and 80 °C, direct sunlight and direct sunlight + humidity, 6 months	Stability decreases at high temperature, humidity and sunlight	Marais, 2001
Guazuma ulmifolia extract	AS, 21 days	Epicatechin found stable; procyanidin degraded significantly	Lopes *et al.*, 2012
Gymnema sylvestre extract	RT, 38 and 45 °C with 65% RH, 1 months	Stable	Killedar *et al.*, 2012
Harpagophytum procumbens tincture	40 °C, 6 months	Iridoids found stable	Karioti *et al.*, 2010
Hieracium pilosella tinctures	LT, 9 months	Caffeoyl quinic acid derivative and flavonoid content remained stable	Bilia *et al.*, 2007
Hippophae rhamnoides juices and berries	−20, 6, 25 and 40 °C, 7 days	Degradation occurred at temperature ≥25 °C	Gutzeit *et al.*, 2008
Hypericum perforatum extract, tablets and capsules	25, 40, 60 and 80 °C, direct sunlight and direct sunlight + humidity, 6 months	Formulations degrade at higher temperature, humidity and in light	Marais, 2001
Dried extract of *H. perforatum*	AS, LT, light, 3 months	Significant chemical degradation	Bilia *et al.*, 2001
Commercial products of *H. perforatum*	AS, LT and thermal conditions, 6 months	Unstable at higher temperature	Shah *et al.*, 2005
Extracted *H. perforatum* oil	Heat and light	Chemical stability of oil affected by extraction method	Isacchi *et al.*, 2007

(Continues)

Table 3. (Continued)

Herb(s)/herbal product(s)	Stability condition(s)	Findings	Reference(s)
H. perforatum extract	AS, 6 months		Koyu and Haznedaroglu, 2011
Matricaria chamomilla extract		Greater stability for fresh flower extract	Carle *et al.*, 1989
M. chamomilla	25, 4 and −20 °C, pH, light exposure and LT, 120 days	Stable at −20 °C and to light	Srivastava and Gupta, 2009
Mentha piperita and *Mentha spicata* oils	25 and 40 °C and compatibility with container materials	Stable	Wolf, 2007
Peppermint oil	5 °C, LT and AS	Stable	Sachan *et al.*, 2010
Passiflora alata	Different conditions, 28 days	Spouted bed dried extract found more stable	Bott *et al.*, 2010
Passiflora incarnate tinctures	Long-term and accelerated stability conditions	60% tincture was more stable	Bilia *et al.*, 2002
Piper methysticum	25, 40, 60 and 80 °C, sunlight, sunlight + humidity, 6 months	Tablets more stable than extracts and capsules	Marais, 2001
Piper sarmentosum	LT, AS and 60 °C/85% RH, 6 months	High temperature and humidity increases degradation	Hussain *et al.*, 2011
Plantago lanceolata dried leaves	0%, 40% and 75% RH, 24 weeks	Higher humidity increases degradation. Acteoside less stable than catalpol and aucubin	Gonda *et al.*, 2012
Sambucus nigra	LT, 24 months; AS, 80 °C and 100 °C, 6 months	High temperature and humidity leads to increased degradation	Heigl and Franz, 2003
Silybum marianum tinctures	AS and LT	Stable for not more than 3 months	Bilia *et al.*, 2002
Stevia rebaudiana	4, 22 and 37 °C, 5 months; 60 °C, 137 h; sunlight, 1 week	Marker content lost at 60 °C	Chang and Cook, 1983
Gels containing stevia extract	As per ICH guidelines, 3 months	Higher stability exhibited by lower concentration extract gel	Das *et al.*, 2009
Steviol glycosides	4 and 20 °C, 30 days	Stevioside was found thermolabile, whereas rebaudiside was photosensitive	Gardana *et al.*, 2010
Syzygium cumini extract	0–45 °C at pH 3, 4 weeks, dark and light (2000 Lux)	Anthocyanins found thermo and photolabile	Veigas *et al.*, 2007
Tanacetum parthenium	Different pH, temperature and light conditions	Maximum stability at neutral pH and thermolabile	Fonseca *et al.*, 2007
Parthenolide solution	Varied pH, temperature and humidity	Should be stored at acidic pH ~4.6 under refrigerated conditions	Jin *et al.*, 2007
Withania somnifera	4 and 25 °C	Stable for 72 h	Jirge *et al.*, 2011
Zingiber officinalis	AS and LT	Unstable at temperature ≥25 °C	Phadke *et al.*, 1998
Ziziphus spina-christi	30, 40 and 50 °C/75% RH	Stable for 1 year	Michel *et al.*, 2011

LT, Long term stability; AS, Accelerated stability; IS, Intermediate stability; RT, Room temperature.

Table 4. Herb or herbal product evaluated for biological activity during stability studies

Herb(s)/herbal product(s)	Stability condition	Findings	Reference(s)
Aegle marmelose	Varied pH	Activity lost at higher pH	Reddy and Urooj, 2013; Mansour and Khalil, 2000
Alpinia galanga ethanolic extract	80 °C, 1 h at pH 7	Antioxidant activity retained	Juntachote and Berghofer, 2005
Caesalpinia pulcherrima cream	75 ± 25% RH within six cycles of cooling and heating (5 and 45 °C for 48 h)	Antioxidant activity retained	Soisuwan *et al.*, 2010
Cassia alata leaf extract	Different pH and temperature	Anticryptococcal activity decreases with increase in temperature and pH	Ranganathan and Balajee, 2000
Cinnamomum camphora extract	Heat, 1 h; visible light, 25 °C, 96 h; UV for 4 h; and acid/base, 24 h	Photounstable with respect to (w.r.t.) antimicrobial activity	Chen and Dai, 2012
Echinacea purpurea formulations	4, 30 and 40 °C, 6 months	Total phenolic content and antioxidant activity decreases	Yotsawimonwat *et al.*, 2010
Ocimum sanctum ethanolic extract	80 °C, 1 h	Stable w.r.t. antioxidant activity	Juntachote and Berghofer, 2005
Piper longum	LT, AS and ambient/real-time conditions	Maximally stable under long term stability condition w.r. t. antioxidant activity	Srivastava *et al.*, 2011
Polygonum cuspidatum	Light, heat and pH	Stable w.r.t. antioxidant activity.	Meng and Hang, 2000
Trigonella foenum graecum	4 and 25 °C, 1 months	Unstable at both the temperatures w.r.t. antifungal activity	Haouala *et al.*, 2008

Table 5. Herbs and herbal products evaluated for physicochemical stability as well as biological activity

Herb/herbal product	Stability condition	Findings	References
Allium sativum extracts	pH 6–9, −20, 4 and 23 °C, RT; Time	$T_{1/2}$ (allicin) = 12 days; $T_{1/2}$ (biological) = 17 days antibacterial activity decreases with increase in pH	Fujisawa *et al.*, 2008
Azadirachta indica oil containing formulations	Varied temperature (5–54.5 °C) for 14 days	Azadirachtin A content decreases significantly but antimicrobial activity retained	Kumar and Parmar, 2000
Bacopa monnieri crude plant material	AS and LT	Bacopaside I and Bacoside A, and free radical scavenging activity decreases under AS	Srivastava *et al.*, 2010, 2012
Cassia alata leaves	Sundrying, 85 °C, 1 h	Kaempferol-3-gentiobioside and anti-inflammatory activity decreases in sunlight	Moriyama *et al.*, 2001, 2003
Olea europaea oil	18–28 °C, under light/dark, 24 months	Photosensitive but thermostable	Psomiadou and Tsimidou, 2002
Orthosiphon stamineus	Varied temperature	Marker content and activity decreases at temperature ≥60 °C	Akowuah and Zhari, 2010
Withania somnifera root extract	AS and LT	Unstable after 3 months	Patil *et al.*, 2010

GENERAL METHODS FOR STAYBILITY TESTING

The stability of herbal medicinal products may be determined based on physical and sensory tests, microbial tests and chromatographic/ spectral tests.

1. Physical and sensory methods

Herbal products, like pharmaceutical products, usually undergo physical changes during storage. These changes though not usually quantitative in nature may be used as a guide to check if the products are deteriorating. These include evaluation of changes in parameters such as colour, taste, odour, clarity, specific gravity, total solid residue, viscosity, the moisture content of powders, dissolution and disintegration tests for capsules and tablets. It must be noted that some of these methods of assessment such as taste and odour should be carried out only if they do not affect the safety of the personnel involved [8, 9, 10, 38].

2. Microbial tests

Microbial contamination or load tests and preservative efficacy or challenge tests (where preservatives are used) of finished herbal products are essential in the determination of stability and shelf life of the product [62]. Key factors affecting the efficacy of the antimicrobial preservative added are the active ingredient, excipients, storage conditions, the container and its closure. The British Pharmacopoeia states that for a product "it shall be demonstrated that the antimicrobial activity of the preparation as such or if necessary, with the addition of a suitable preservative or preservatives provides adequate protection from adverse effects that may arise from microbial contamination or proliferation during

storage and use of the preparation" [63]. Analyses of such parameters with time allows the tracing of stability of the product and subsequent prediction or estimation of shelf-life. These tests should be done according to Pharmacopoeia methods (British Pharmacopoeia, United States Pharmacopoeia, European Pharmacopeia.), WHO methods, or any other internationally recognized methods [64]. The microbial tests should involve: Total viable aerobic plate count; contaminating fungus (yeast and mould); Salmonella spp.; Escherichia coli and Staphylococcus aureus. Table 1 shows the British Pharmacopoeia acceptance criteria for microbiological testing of herbal products. Criteria 'A' represents herbal medicinal products containing herbal drugs, with or without excipients, intended for the preparation of infusions and decoctions using boiling water (for example herbal teas, with or without added flavourings). 'B' represents herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where the method of processing (for example, extraction) or, where appropriate, in the case of herbal drugs, of pre-treatment reduces the levels of organisms to below those stated for this category). 'C' represents herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where it can be demonstrated that the method of processing (for example, extraction with low strength ethanol or water that is not boiling or low-temperature concentration) or, in the case of herbal drugs, of pre-treatment, would not reduce the level of organisms sufficiently to reach the criteria required under B.

3. Chromatography and spectral methods

Chromatographic methods used to assess the chemical stability of herbal products include thin layer chromatography (TLC), high (ultra) performance liquid chromatography (HPLC, UPLC), high performancethin layer chromatography (HP-TLC), gas liquid chromatography (GLC), etc., while spectral methods used include ultraviolet-visible (UV-VIS) spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) and mass spectroscopy (MS). These techniques allow tracing of changes which may occur during storage of a complex mixture of biologically active substances contained in herbal materials. Comparisons of appropriate characteristic/fingerprint chromatograms allow the determination of the stability of identified active ingredients (if any) and other substances present in the finished herbal product (which may appear as markers) [10, 37, 66].

ASSESSMENT OF SAFETY PARAMETERS

The acute toxicity of the herbal medicinal product may be assessed at the beginning and end of the stability study for products used in the treatment of acute conditions. Subchronic and chronic toxicity studies may also be done for products meant for the treatment of chronic conditions [27, 67]. However, due to the time is taken in doing such studies, they should be determined on a case by case basis depending on the nature of the herbal medicinal product involved with consideration to other already determined parameters. These tests if not possible for inclusion in a stability study should be used as quality control measures or tests [64, 68].

CONDITIONS FOR STAYBILITY TESTING OF PRODUCTS

The shelf-life of a product depends on its storage temperature and also on humidity. These conditions may vary from country to country. Four climatic zones have been defined in order to enhance determination of stability testing conditions for products (table 2). The definition is based on observed temperatures and relative humidity, both inside and outside rooms, from which mean temperatures and

average humidity values are calculated [8]. The general conditions for testing products in various containers and storage temperatures are shown in tables 3-6. The stability study should be conducted in the container closure system in which it will be marketed [8, 10, 64, 69]. In general, "significant change" for a finished herbal product may be defined as a 10 % change in assay from its initial value with respect to the marker being used; or failure to meet the acceptance criteria for potency when using biological or immunological procedures; any degradation product exceeding its acceptance criterion; failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e. g., colour, phase separation, re-suspendibility, caking, hardness, dose delivery per actuation, pH). It should be noted that accelerated stability tests may cause some dosage forms such as ointments and creams to change form due to the relatively high temperatures to be employed. This should not be mistaken for the failure of the product to meet acceptance criteria since such changes should be expected [8, 10, 38, 70].

Table 2: Temperature and humidity distribution in climatic zones

Climatic zone	Climate	Mean temperature	Average humidity
I	Temperate	21	60
II	Subtropical	26	65
III	Tropical (dry)	31	60
IV	Tropical (wet)	31	70

Aulton [8], I-Temperate climate includes Canada, New Zealand, Northern Europe, United Kingdom and Russia, II-Mediterranean and subtropical climate includes Japan, Southern Europe and the USA, III-Hot and dry climate includes Argentina, Australia, Botswana and the Middle East, IV-Hot and Humid Brazil, Ghana, Indonesia, Nicaragua, Nigeria and the Philippines

Table 3: General conditions for testing of products (impermeable containers)

Study	Storage conditions	Time points for testing	Minimum data covered at submission
Long term	25 °C±2 °C/60% RH± 5% RH or 30 °C±2 °C/65% RH±5% RH	0, 3, 6, 12, 18, 24, 36, 48, 60	12 mo
Intermediate	30 °C±2 °C/65% RH±5% RH	0, 1, 3, 6	6 mo
Accelerated	40 °C±2 °C/65% RH±5% RH	0, 1, 3, 6	6 mo

Table 4: Conditions for testing of products in semipermeable containers*

Study	Storage conditions	Time points for testing	Minimum data covered at submission
Long term	25 °C±2 °C/40% RH±5% RH or 30 °C±2 °C/35% RH±5% RH	0, 3, 6, 12, 18, 24, 36, 48, 60	12 mo
Intermediate	30 °C±2 °C/35% RH±5% RH	0, 1, 3, 6	6 mo
Accelerated	40 °C±2 °C/not more than 25% RH±5% RH	0, 1, 3, 6	6 mo
Accelerated	40 °C±2 °C/75% RH±5% RH	0, 1, 3, 6	6 mo

*Alternatively for products in semipermeable containers, samples may be stored under general conditions like that of impermeable containers and water loss calculated by determining permeation coefficient or using calculated ratio of water loss

Table 5: Conditions for testing of products intended for storage in a refrigerator

Study	Storage conditions	Time points for testing	Minimum data covered at submission
Long term	5 °C±3 °C	0, 3, 6, 12, 18, 24, 36, 48, 60	12 mo
Accelerated	25 °C±2 °C/60% RH±5% RH	0, 1, 3, 6	6 mo

If significant change occurs between 3 and 6 mo at accelerated storage conditions then shelf life based on real time data should be conducted in the long term

Table 6: Conditions for testing of products intended for storage in a freezer

Study	Storage conditions	Time points for testing	Minimum data covered at submission
Long term	-20 °C±5 °C	0, 3, 6, 12, 18, 24, 36, 48, 60	12 mo

CHALLENGES IN STABILITY TESTING OF HERBAL DRUGS/PRODUCTS

Stability testing helps in establishing the storage conditions to ensure sustained QSE of a drug product throughout its shelf life. But ensuring QSE under the influence of various storage conditions is much more complicated for an herbal product than that for a synthetic drug product. The major challenges that make stability testing of an herbal drug/product a herculean task include its chemical complexity, variability in biochemical composition of raw material, selection of marker(s) for its stability testing and influences of enzymes present in it.

1. Chemical complexity

An herbal drug is an extremely complex and heterogeneous mixture of chemicals. These range from

being highly polar to non-polar, hydrophillic to lipophillic, acidic to basic and having very low to very high molecular mass. This diversity in chemical composition is the major deterrent in development of a comprehensive chromatographic fingerprint, which is recommended by regulatory guidelines for establishing shelf life of an herbal product. Moreover, the contents of these diverse constituents vary from traces to few milligrammes per unit mass of an herbal drug. As a result, all or most of the constituents are not isolated or available with sufficient purity for their use as active/analytical makers in qualitative or quantitative analysis of the drug. Further, different constituents, present in trace or appreciable amounts, in an herbal drug elicit multiple pharmacological responses by acting independently, synergistically or antagonistically, which accounts for the diverse biological activities of the drug. Exposure of an herbal drug to varied environmental conditions (light, humidity, heat and/or air) during its shelf life may trigger chemical reactions between or among its different chemical constituents that may alter the levels of constituent(s) responsible for an activity or different activities. Hence, there is a need to identify chemical markers, whose levels may be correlated with a specific or multiple biological activities. This objective can be achieved by closely monitoring the fingerprint profiles as well as biological activity(ies) of stability samples with respect to those of control sample during stability studies of an herbal drug.

2. Variability in biochemical composition

Content and nature of phytoconstituents in an herbal raw material are governed, directly or indirectly, by seed selection, growth conditions, fertilizers, pesticides, heavy metal content, microbial contamination and methods of harvesting, drying and post-harvest processing (38). It implies that a particular herbal drug procured from different sources is most likely to have variable biochemical compositions. As a result, the products of an herbal drug procured from different sources developed by different manufacturers tend to have different biochemical compositions. Shah and co workers (Shah et al., 2005) have tested different marketed formulations of Hypericum perforatum, which are claimed to have hypericin 0.3% of the product. But, that study has revealed that hypericin content varies from a mere 5% to just 50% of the label claim (0.3%). Similarly, content of Azadirachtin A is found to vary significantly (617.3 to 1149.65 ppm) in different batches of neem oil formulations (39). These studies suggest that similar products from different manufacturers may have widely variable content of marker(s). Such products (i) are required to be administered in different doses to elicit a specific biological response and (ii) will have varied shelf lives depending upon the initial content of the markers. Microbial contamination of an herbal product is another quality attribute that may not only pose a potential health hazard but also lead to degradation of certain constituents by actions of microbial enzymes. Therefore, monitoring of microbial load in an herbal product should also be an integral part of stability testing protocol. Pesticide residues and heavy metal contamination are the other unwanted components of an herbal product, which not only attract toxic manifestations but also can react with markers that may result in altered chemical composition of the product during its shelf life and complication of the stability testing programme.

3. Selection of marker(s)

The active pharmaceutical ingredients in a synthetic medicinal drug product are well defined, and these serve as markers for stability testing of the product. Any change in content of that marker during its

shelf life or stability testing is directly related to change in its therapeutic efficacy. In contrast to it, an herbal drug/product is a very complex mixture of chemicals, and its therapeutic actions are usually a function of additive or synergistic actions of chemically diverse phytoconstituents. It implies that any change in content of a specific marker or a set of specific markers during stability testing of an herbal drug/product is not likely to transcend to similar change in its therapeutic effectiveness. Therefore, selection of constituents as markers is the most challenging task in rational stability testing protocol for assessment of shelf life of an herbal drug/product. The major proportion (78%) of stability studies reported on herbal drugs/products involve evaluation of only physical characteristics (Table 2) and physico-chemical stability (Table 3). Assessment of physical stability provides no quantitative information about chemical stability and therapeutic effectiveness of a product during its shelf life. Hence, it can be argued here that only physical stability evaluation does not provide any concrete and reliable information about shelf life of a product. On the other hand, studies on physico-chemical stability involve monitoring of content of some selected markers (Table 3). A critical look into these available physicochemical stability reports revealed that different research groups have used different markers for stability testing of an herbal drug/product. For stability studies on Actaea racemosa, (40) have used cimiracemoside-A, actein and 27- deoxyactein (triterpenes) as markers, whereas (41) have used cimiracemoside F, 3-epi-26- deoxyactein, actein (triterpenes) and polyphenols as markers. Comparative analysis of these two studies reveals that polyphenols are unstable in comparison with triterpenes at higher temperatures. Therefore, shelf life of A.racemosa with respect to polyphenolic content is shorter than that with respect to triterpene content. Similarly, stability studies on Echinacea purpurea are conducted using alkamide and cichoric acid (42) as well as using alkamides (43). Cichoric acid is found very unstable in comparison with alkamides, which again implies two different shelf lives for the same herb. Another case is of stability studies on Calendula officinalis, wherein one research group has used total carotenoid content as marker (44), whereas the others have used flavonoid content for stability assessment (45,46). Both the markers belong to different chemical categories, which may be responsible for different shelf lives of C. officinalis with respect to different markers. These analyses of reports suggest that there is wide variability in (i) susceptibility of different classes of makers to chemical change during shelf life and (ii) selection of markers for monitoring of stability of an herbal drug/product. It implies that shelf lives assigned to a particular herbal drug/product are liable to be different with respect to different markers. Therefore, the major question, which needs to be answered in the very first place, in assessment of shelf life of an herbal drug/product is that 'which phytoconstituent is to be selected for monitoring during stability testing so that a reliable shelf life of the drug/product is established?' As the sole purpose of consuming any herbal drug/product is to get some therapeutic and/or nutritional benefits, and these benefits are functions of individual, additive or synergistic actions of its different phytoconstituents. An ideal stability studies protocol for such a drug/product should involve quantitative analysis of marker(s), whose levels can be extrapolated to its intended benefits. For instance, withanolides in Withania somnifera are related to immunomodulatory activity (47); rebaudiside and stevioside are related to sweetness of Stevia rebaudiana (33); curcumin is responsible for antiinflammatory activity of Curcuma longa (49); and hyperforin is chiefly responsible for antidepressant activity of H. perforatum (50). But still, knowing

the biologically active constituent in an herbal drug may not be sufficient, because most herbal products are composed of a cocktail of herbal drugs. In such cases, it is usually not possible to ascribe a particular biological activity to a set of active markers. In this regard, the WHO's Supplementary guidelines on good manufacturing practices for the manufacture of herbal medicines says that it is often not feasible to determine the stability of each active ingredient. Moreover, because the herbal material, in its entirety, is regarded as the active ingredient, a mere determination of the stability of the constituents with known therapeutic activity will not usually be sufficient. Chromatography allows tracing of changes which may occur during storage of a complex mixture of biologically active substances contained in herbal materials. It should be shown, as far as possible, e.g. by comparisons of appropriate characteristic/fingerprint chromatograms, that the identified active ingredient (if any) and other substances present in the herbal material or finished herbal product are likewise stable and that their content as a proportion of the whole remains within the defined limits. (WHO, 2006). The USFDA has also included biological assay as one of the quality control parameter (USFDA, 2004). Therefore, in light of these regulatory recommendations, it becomes imperative to assess shelf life of an herbal drug/product in terms of chemical stability as well as biological activity. But out of pool of stability studies on such drugs/products, only a few studies are conducted with respect to both marker compound as well as biological activity. For instance, immunomodulatory activity of W.somnifera is found to vary proportionally to the concentration of withanolide (47); decrease in free radical scavenging activity of Bacopa monnieri corresponds well with decrease in concentration of Bacopaside I (52); free radical scavenging activity of Orthosiphon stamineus extract is proportional to the content of polyphenols (53); and antiangiogenic activity of Matricaria chamomilla extract is directly related to flavonoids and apigenin-7-O-glucoside content (54). Nevertheless, contrary to these correlating reports, some studies have defied a correlation between stability of a selected marker and a particular biological activity tested during the stability studies. Exposure of Olea europaea to high temperature causes decrease in pheophytin (marker), but the antioxidant activity is increased (55). Change in azadirachtin A content in Azadirachta indica formulations does not conform to similar changes in antibacterial and anti-diabetic activities of the formulation (56). Determination of biological half life (in terms of antibacterial activity) and chemical half life (in terms of allicin) of garlic extracts indicates that allicin alone is not responsible for antibacterial activity of the extract (57) Therefore, shelf life assignment to an herbal product to ensure consistent therapeutic efficacy and safety should be based on systematic stability testing that include evaluation of physical and chemical stabilities as well as the intended biological activity of the stability samples by appropriate in vitro and/or in vivo methods. Table 5 discloses such comprehensive stability studies, and this number constitutes only a very small fraction (8%) of the total number of stability testing reports on different herbal drugs and products.

4. Enzymatic activities during shelf life

Chemical constituents in an herbal drug are plant secondary metabolites. Their levels in plants are regulated by synchronized activities of various enzymes belonging to the categories of oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. These enzymes catalyse the metabolism of constituents by interconversion reactions, conjugation reactions, oxidative

polymerization and degradative reactions (58). Barz and Koster (1981) have provided a comprehensive compilation of degradation of many phytoconstituents under the influence of different enzymes. In pre-harvest stage, activities of enzymes are controlled by feedback mechanisms. After harvesting, the enzyme activities depend upon the conditions employed for processing and/or storage of the plant material/produce. A large volume of research has been performed on studying post-harvest behaviour and stability of enzymes in edible plant produce like fruits and vegetables. These post-harvest active enzymes, which mainly include catalase, peroxidase, lipoxygenase, chlorophyllase, pectin, esterase, ascorbate oxidase, polygalactouronase, galactolipase and phospholipase, continue to act during storage of the plant produce and cause changes in chemical composition of the produce. Such changes are reflected as changes in colour, texture, consistency and taste. An edible plant produce can be used for nutritional as well as therapeutic purposes. For example, garlic, zinger, blackberries, olive, Indian blackberry (black plum, jamun), Indian gooseberry (Amla) and many others are used both as herbal drugs and edible produce. However, their post-harvest treatments vary with their intended use. The product intended to be used as edible products are stored at low temperature and high humidity and have shelf life of a few days to a few months (Aked, 2000). Marked changes in colour, texture/consistency and taste are noted in these products in a few days, which are attributed to catalytic actions of phenylperoxidase (PPO), polygalactouronase and lipoxygenase, respectively (Toivonen and Brunnell, 2008). In contrast, the product intended to be used as herbal drugs are usually dried under controlled environment that hampers/decreases the degradation associated with microbes, enzymes, and hydrolytic reactions. While the microbial and hydrolytic degradation can be controlled to large extent by reducing moisture content, the enzymatic degradation may not be completely avoided during the shelf life. It is so, because some of the enzymes are not completely inactivated/denatured by heat treatment, and thus remain active during shelf life. Peroxidase and catalase (CAT) can remain active after treatment with temperature as high as 60 °C, whereas the other enzymes are inactivated (49). This complete or partial inactivation of enzymes leads to a sequence of events, which is subjective to individual enzyme. For instance, heat treatment of red ginseng results in inactivation of catabolic enzymes, which are responsible for metabolism of antioxidant constituents, hydrolysis of saponins to ginsenosides Rh2, Rh4, Rs3, Rs4 and Rg5 that possess anticancer properties and production of 20(S)-ginsenoside Rg3 responsible for antimetastatic, vasorelaxant and antiplatlet aggregation properties. On the other hand, when it is subjected to steam processing, yield of two ginsenosides Rg3 and Rg5 is increased, and panaxytriol is formed, which ultimately leads to increase in anticancer activity of the herb (64). Another such case is of sinigrin, which is an important constituent (glucosinolate) in many cruciferous vegetables. It is hydrolysed by myrosinase, an enzyme present in plant or in gut microflora, to allyl isothiocyanate (an anticancer compound). But upon heating, myrosinase gets inactivated, and hence, anticancer property of allyl isothiocyanate cannot be availed off (65) Post-harvest browning is the most commonly observed visible change in herbal products. The culprits for this change are PPO, peroxidase (POD) and/or Phenylalanine ammonia lyase (PAL) that oxidize a phenolic compound to a quinone, which imparts brown appearance to the product (Fig. 2). Quinone itself is highly reactive species that can further react with other constituents to form complex products. In an herbal product, phenolic compounds can be present as such or generated in situ from terpenoidal/flavonoidal

glycosides by the action of glycosidases. For instance, browning in apple is initiated by glycosidase catalysed hydrolysis of quercetin glycoside to form quercetin, which subsequently is converted into proanthocyanidin via flavan-3,4-diol. Finally, PPO catalysed oxidation of proanthocyanidine produces quinones. Amine oxidases, oxalate oxidases and superoxide dismutases present in mitochondria, chloroplast and peroxisomes are the other enzymes responsible for browning (Adams, 2010). Based on these different reports on post-harvest activities of enzymes, it can be inferred that enzymes can play an important role in determining shelf life of an herbal product. Nevertheless, there are some arguments that discourage the post-harvest degradative roles of enzymes in plant produce intended for use as drugs. These are as follows: 1 Almost all herbal drugs, except those required for isolation/extraction of essential or volatile oils, are subjected to different treatments to conserve their physical, chemical, organoleptic and pharmacological characteristics. These treatments mainly aim at removal of water by drying under sunlight or with artificial methods such as freeze drying, heat drying, microwave drying, far infrared drying, vacuum drying and spray drying. During these treatments, the moisture content in plant material falls from 60– 80% to 5–12%, and weight is reduced by 15–80% depending upon the types of plant organ (67). Removal of water as well as exposure to heat as such inactivates most of the enzymes. 2 Many fresh or dried plant products are extracted with organic solvents to formulate HMPs. In these solvents, the enzymes are either denatured or not extracted or their activity is significantly reduced. Hence, the herbal extracts are either free from enzymes or contain inactive enzymes. These arguments do not give the liberty to assume for sure that no enzyme may be active during shelf life. Therefore, in order to assess the impact of enzyme activity on shelf life of an herbal product, it is recommended that the enzymes, which have high probability of remaining active post-harvesting, should be identified by appropriate enzyme assays. Further, their biochemical roles and their levels during shelf life should be monitored through specific enzyme assay(s) and/or the levels of specific substrate(s) or product(s) during the shelf life.

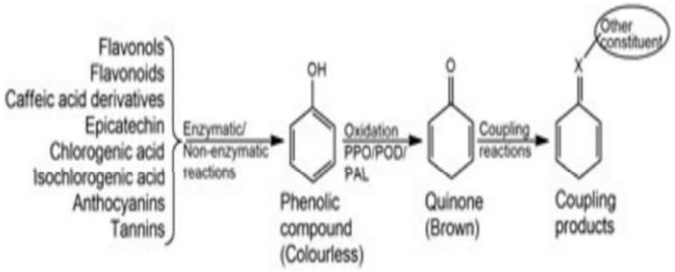

Figure 2. Degradation of constituents leading to browning and other products.

EVALUATION OF STAYBILITY DATA

A systematic approach should be adopted in the presentation and evaluation of the stability information. This should include results from the physical, chemical, biological, and microbiological tests, including particular attributes of the dosage form (for example, dissolution rate for solid oral dosage forms), where appropriate [69]. The stability study should help establish the shelf life of future batches of an herbal medicine based on testing a minimum of two or three batches. The degree of variability of individual batches affects the confidence that a future production batch will remain

within specification throughout its shelf life. Where the data show so little degradation and so little variability that it is apparent from looking at the data that the requested shelf life will be granted, it is normally unnecessary to go through the formal statistical analysis. The overall shelf life should be based on the minimum time a batch can be expected to remain within acceptance criteria. The evaluation should consider not only the assay but also the degradation products and other appropriate attributes [9, 10, 69, 70]. The level of selected markers and possible degradation products should also be ascertained with time in order to help determine the shelf-life of the products. Depending on the availability of equipment, selected tests such as TLC, HPLC, HPTLC, UV-Visible spectrophotometry may be used to quantify selected markers as well as determine the levels of degradation products. It is not expected that every listed test be performed at each time point. The list of tests presented for each dosage form is not intended to be exhaustive, nor is it expected that every listed test be included in the design of a stability protocol for a particular finished herbal product [8, 9, 10]. Table 7 presents some recommended stability tests for various herbal dosage forms.

Table 7: Recommended stability tests for different herbal dosage forms

Type of dosage form	Recommended stability tests
Decoctions (oral), Glycerites, Aceterites, oxymels	Change in colour, odour, taste, formation of a precipitate, clarity, specific gravity, total solid residue, pH, viscosity, extractable, phytochemical constituents, microbial contamination, preservative efficacy/challenge tests, and toxicity/safety [14].
Tablets	Change in colour, pH, total water or solvent extractive, phytochemical constituent, dissolution (or disintegration, if justified), water content, hardness, friability, swelling, crakes and clumping (coated tablets), microbial contamination, preservative efficacy/challenge tests, toxicity/safety [14].
Capsules	Change in colour, pH, total extractive, phytochemical constituent, brittleness, hardening or softening of shell, dissolution (or disintegration, if justified), water content, microbial contamination, preservative efficacy/challenge tests, toxicity.
Alcoholic beverages, Tinctures	Clarity, pH, specific gravity, alcohol content, extractable, change in colour, odour, taste, the formation of a precipitate, total solid residue, extractable, phytochemical constituents, microbial contamination, toxicity.
Teas and powders	Change in odour, moisture content, pH, total water extractive, formation of hard mass, caking, Phytochemical constituents, microbial contamination, and toxicity.
Ointment, balms	Change in colour, odour, homogeneity, pH, consistency, grittiness, excessive bleeding, phytochemical constituents, microbial contamination, and toxicity.
Oils	Rancidity, change in colour, odour, pH, phytochemical constituents, microbial contamination, toxicity
Pastes and creams	Change in colour, odour, homogeneity, pH, consistency, grittiness, cracking, shrinking due to evaporation of water, phytochemical constituents, microbial contamination, preservative challenge tests (where preservative are used), toxicity
Soaps	Change in colour, odour, homogeneity, pH, phytochemical constituents, microbial contamination, preservative challenge tests (where preservative are used), toxicity/skin sensitivity tests
Suppositories and Pessaries	Softening, hardening or drying, dissolution

CONFORMITY AND CONTRAVENTION WITH RECOMMENDED STABILITY CONDITIONS

Drugs regulatory bodies recommend that a drug product should be subjected to accelerated stability testing (at 40 °C/75% RH) for 6 months, intermediate stability testing (at 30 °C/65% RH) for 6–12 months and longterm stability testing (at 25–30 °C/60–75% RH) for a period equal to the proposed shelf life of the product. Intermediate stability testing is, however, not required when the conditions for long-term testing are same as those for intermediate ones (ICH, 2003). Meta-analysis of literature

reports on stability studies on different types of herbal drugs/products (Tables 2–5) reveals that there is a wide variation in conditions employed (temperature of 80 to 100 °C, relative humidity of 0– 100% and duration of a few hours to 3 years) for the studies. Out of these, only 23% studies comply with storage conditions and sampling schedule recommended for accelerated stability studies, and only a negligible proportion (<1%) of the studies comply with conditions recommended for long-term and accelerated stability studies. Therefore, there is almost total noncompliance with the recommended conditions in generation of stability data of different herbal drugs/products. Polyherbal products are far behind the monoherbal ones in terms of their systematic stability studies because chemical analysis of such products is much more tedious than that of the latter. Most polyherbal products are analysed only for their physical stability, and only a few products are evaluated through chemical stability studies (Table 6). Among these, the studies by (69,70) have actually followed the regulatory guidelines for storage condition and duration.

Table 6. Studies conducted on polyherbal products

Polyherbal product (therapeutic use)	Stability condition	Testing evaluation criteria	References
Physical studies			
Ointment of *Eucalyptus globulus, A. indica, Elsholtzia fructicosa, Ocimum sanctum* and *Rhododendron setosum* (Antimicrobial)	2, 25 and 37 °C, 4 weeks	Physical attributes such as spreadability, diffusion and irritant effect	Chhetri *et al.*, 2010
Tablets of *Myristica fragrans, Codiaeum variegatum, Cinnamomum zeylanicum* and *Aloe vera* extracts (Antiinflammatory)	5, 25 and 37 °C, 1 months	Physical attributes of tablets	Rajiah and Mathew, 2010
Oral formulation of *Piper nigrum* and *Nyctanthes arbortristis* (Antipyretic)	4, 25 and 47 °C	Physicochemical characteristics of formulation	Ghiware *et al.*, 2010
Formulation of *C. rotundus, C. sativus* and almond oil.	4 °C, 20 days	Physical stability of formulation	Rajvanshi *et al.*, 2011
Capsules containing *Sereona repens, A. racemosa, Crataeva nurvala, A. catechu* and *Orchis muscular* (for benign prostatic hyperplasia)	40 °C/75% RH, 3 months	Physicochemical properties of capsules, disintegration time, pH (1% *w/v* formulation), total ash, water and alcohol soluble extractives, total saponin content and microbial load	Sakthivel *et al.*, 2012
Safoof Burs (Unani antidiabetic formulation)	40 °C/75% RH, 6 months	Organoleptic characteristic microbial content	Hussain *et al.*, 2011
Physicochemical/chemical studies			
Eazmov capsules composed of *Picrorrhiza kurroa, Glycyrrhizha glabra, Cyperus rotundus* (anti-inflammatory, analgesic and antiarthritic)	45 °C/75% RH, 6 months	Specific markers, that is, kutkin, glycyrrhizin and β-sitosterol	Chauhan *et al.*, 1999
Formulation of extracts of *Trichilia catigua* and *Ptychopetalum olacoides* (stimulant and aphrodisiac)	5, 24 and 40 °C, 90 days	Total flavonoids content expressed as rutin	Baby *et al.*, 2007
Mixture of peppermint oil and caraway oil	Different temperature and humidity for 1 months	Menthol and carvone content	Sachan *et al.*, 2010
Ashwagandha (*Withania somnifera*) capsules, Shilajeet (*Asparagus racemosus*) capsules and Ashwashila (*W. somnifera* and *A. racemosus*) capsules	40 °C/75% RH and 37 °C, 6 months	Physical characters of capsules, contents of fulvic acid, withanolides and alkaloids	Bankoti *et al.*, 2012
Beverages of *Carica papaya* and *Aloe barbadensis*	4 °C, 3 months	Physico-chemical properties chemical profile	Boghani *et al.*, 2012
Blended juices of *Cucumis sativus* and *Cucumis melo* (antidiabetic, hypolipidemic and antioxidant)	Ambient condition, 4 months	Total solid content, pH, content of reducing and non-reducing sugars	Kausar *et al.*, 2012
Biological studies			
Mixture of *Crescentia alata* and *O. sanctum* (anticryptococcal)	Different pH and temperature	Anticryptococcal activity	Ranganathan and Balajee, 2000

CONCLUSION

Herbal products have gained wide acceptance in both developing and advanced countries and are being produced in commercial quantities. The stability of these herbal products is of paramount importance to assure product quality, safety and efficacy. It is expected that herbal product manufacturers will apply the necessary protocols and techniques to achieve and maintain the stability of their products during manufacture, storage, transportation and usage. This will contribute to patient safety, product efficacy and enhance patient confidence in herbal products and improve compliance.

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