Drug Development and Industrial Pharmacy, 31:907–916, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500272066

Taylor & Francis
Taylor & Francis Group

Content Analysis and Stability Evaluation of Selected Commercial Preparations of St. John's Wort

Amit K. Shah
Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, Mississippi, USA

Bonnie A. Avery and Christy M. Wyandt
Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, Mississippi, USA
and
National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi, USA

ABSTRACT Content analysis and stability studies were performed for the commercial products of St. John's wort. Six marketed formulations were analyzed for their hypericin and pseudohypericin content. These products were standardized to contain 0.3% hypericin. Results revealed total hypericin as 7.72–38.57% of the labeled claim with varying concentrations of pseudohypericin. Stability studies were carried out under three different storage conditions: 1) 25±2°C, 60±5%RH for six months, 2) 40±2°C, 75±5%RH for six months, and 3) 50°C for one month. Tablet formulations were also analyzed for their hardness and friability. Stability studies revealed significant decrease in the content of the marker compounds with time.

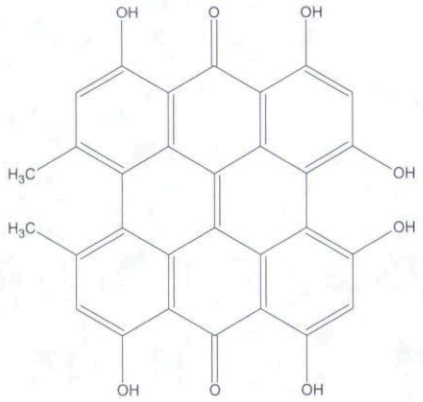
KEYWORDS St. John's wort, Commercial tablets and capsules, Content analysis, Stability, HPLC

INTRODUCTION

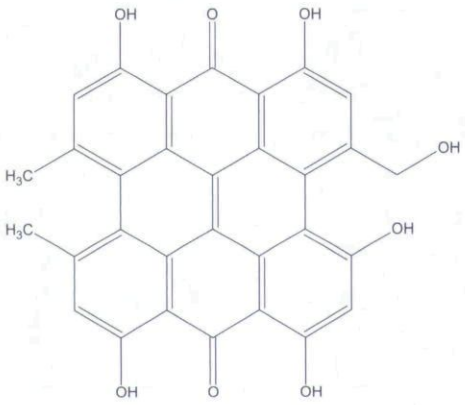
Extract of St. John's wort has been used as an anti-inflammatory and healing agent since ancient times (Brolis et al., 1998; Orth et al., 1999). Over the past few years it has gained tremendous popularity as an anti-viral and anti-depressant agent (Hansgen et al., 1994; Linde et al., 1996; Muller et al., 1997; Ozturk et al., 1996; Schrader et al., 1998; Sommer & Harrer, 1994). Studies also showed the efficacy of extracts of St. John's wort to be comparable to daily dose of imipramine and amitriptyline (Vorbach et al., 1994, 1997; Wheatley, 1997). Whether anti-depressant activity is due to napthodianthrones, i.e., hypericin and pseudohypericin (Baldt & Wagner, 1994; Butterweck et al., 1997) (Scheme 1), phloroglucin derivative (hyperforin) (Chatterjee et al., 1998, 2004; Kaehler et al., 1999; Laakhman et al., 1998), flavonoids (rutin, isoquercitrin, quercetrin) (Butterweck et al., 2000; Calapai et al., 1999, 2001), or other constituent of St. John's wort is still a controversial subject.

In 2000, the Food and Drug Administration (FDA) issued final regulations on the structure/function claims for Dietary Supplements (DS) under the Dietary Supplement Health and Education Act of 1994 (DSHEA) (Israelsen & Blumenthanl, 2000). These regulations require that the safety of dietary supplements be demonstrated, but do not require that their efficacy be established. Due to poor regulations on herbal products in the United States

Address correspondence to Amit K. Shah, Faser Hall 104, Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677, USA; Fax: (662) 915-1177; E-mail: ashah@olemiss.edu



Hypericin



Pseudohypericin

SCHEME 1 **Structures of Hypericin and Pseudohypericin.**

and abroad, most of these products contain significant differences in the amount of actives present. In a recent survey conducted, among 880 herbal products, 43% of the products were consistent in ingredients and recommended daily dose when compared to a benchmark, whereas 20% were consistent only in ingredients (Garrard et al., 2003). A daily dose of 900 mg of St. John's Wort extract is recommended for a desired anti-depressant effect (Fetrow & Avila, 2001). All the extracts are standardized to deliver 0.3% hypericin per serving. Most of the marketed St. John's wort products are standardized in this fashion. The current study was conducted to evaluate the contents and stability of selected commercial preparations of St. John's wort. Few studies have been conducted to determine either the accuracy of the labeled claim or the stability of marketed products. Some surveys have been conducted to evaluate the differences in the amount of extract present in various marketed formulations (Fetrow & Avila, 2001; Garrard et al., 2003). Earlier studies are either based on the stability of solutions (Wirz et al., 2001), stability of extracts/tinctures (Bilia et al., 2001, 2002; Gourneron et al., 1999; Orth & Schmidt, 1998) or compatibility of extracts with excipients (Kopelman & Augsburger, 2002). A study has been carried out in Germany to determine the batch-to-batch reproducibility of various commercial St. John's wort preparations (Wruglics et al., 2001). But no attempt has been made to determine the actual content present in the marketed herbal products versus the amount those products claim to contain. Also, nothing is reported about the stability of the marketed products which clearly is important in determining the quality of these dietary supplements.

In the present work, the content of the active constituents of six representative St. John's wort products was determined and compared with the labeled claim. The stability of four of these formulations was evaluated for six months and a significant decrease in the concentration of hypericins was observed as a function of time. The hardness and friability of the tablets were evaluated to assess changes in the physical properties of the formulation under different storage conditions.

A novel, simple isocratic high performance liquid chromotography (HPLC) method has been developed to facilitate quantitation of active constituents. Previously reported methods were not selective and either required a complicated gradient system or employed a long run time (Brolis et al., 1998; Li & Fitzloff, 2001).

The specific aims of this study were to determine the inconsistencies in the marketed herbal products and the influence of environmental factors, such as temperature and humidity, on the stability of selected St. John's wort products.

MATERIALS AND METHODS

Reagents and Solvents

Standard hypericin (HPLC purity 96.04%) was purchased from Indofine Chemical Co., (Somerville, NJ) and pseudohypericin (HPLC purity 98.2%) from Calbiochem (La Jolla, CA). HPLC grade methanol, acetonitrile, and ammonium acetate were purchased from Fisher Scientific (Fairlawn, NJ). ACS grade glacial acetic acid was purchased from JT Baker

(Phillipsburg, NJ). Water was purified by a nanopure system from Millipore (Milford, MA).

Test Formulations

Six test formulations (3 tablets and 3 capsules) were purchased from a well-known pharmacy. All the products were randomly selected from over-the-counter medicines. Test formulations from one particular manufacturer belonged to the same lot number and had an expiration date no earlier than 2 years from the date of purchase.

Methods

Sample Preparation for Analysis

For the purpose of evaluating tablets, five tablets from each test formulation were taken and weighed individually. They were then ground using a clean, dry, glass mortar and pestle. When capsule formulations were evaluated, the contents of five capsules were completely emptied. Each powder was carefully mixed, and a 250 mg of aliquot was accurately weighed and transferred to a 5 mL volumetric flask. The extracts were dissolved in methanol. This mixture was homogenized by sonication at room temperature for 10 minutes. The samples were then centrifuged for 5 min to separate the insoluble components from the soluble ones. The supernatant was separated, and 20 µl of the sample was directly injected on a C18 column equipped with a guard column. All the samples were analyzed in triplicate.

HPLC-UV Drug Analyses

A rapid, isocratic HPLC method was developed for the identification and quantification of the marker compounds hypericin and pseudohypericin. The HPLC system consisted of a Waters Millennium Chromatographic manager, equipped with a two pump system. A phenomenex, C18 SYNERGI hydro-RP column having dimensions, 150 × 4.6 mm, with particle size 4 micron, and i.d., 80°A, equipped with a guard column was used. Detection was carried out using a Waters 2487 dual λ absorbance detector. The chromatographic system was monitored using Waters Millennium[32] version 3.20 chromatography software.

Chromatographic separation was achieved using an isocratic solvent system-acetonitrile:methanol:100 mM ammonium acetate:glacial acetic acid (50:30:20:0.15 v/v/v/v). The flow rate was 1.4 mL/min, and the injection volume was 20 µl. Peaks were detected at 590 nm.

Standard Curves

Standard solutions were prepared by dissolving the marker compounds hypericin and pseudohypericin in methanol. Five milligrams of the test compound was dissolved in 10 mL of HPLC grade methanol in a clean, dry, 10 mL volumetric flask. Standards of varying concentrations were prepared for hypericin (10–110 µg/ml) and pseudohypericin (20–130 µg/ml) by serially diluting the appropriate stock solution. All the standards were stored at −20°C. Calibration curves were prepared by plotting the area under the curve as a function of concentration. Regression analysis was performed on the data points using Microsoft Excel software. The equation of the regression line was used to quantitate the unknown samples.

Quantification of Commercial Products

The hypericin and pseudohypericin content of six commercial products (3 tablets and 3 capsules) was quantified. Five products were labeled to contain 300 mg, whereas one was labeled to contain 125 mg of St. John's wort extract. All the products claimed to be standardized to contain not less than 0.3% hypericin by weight of the extract. Based on this, the total labeled amount of hypericin in each tablet or capsule was calculated. The actual amount of hypericin present in each tablet or capsule was determined by using the developed HPLC method and was compared to the label claim. The product labels did not provide information regarding the amount of pseudohypericin. Hence, the amount of pseudohypericin present per tablet or capsule was determined and compared with the total weight of the tablet or capsule.

Stability Studies

Content Analysis

Two tablet and two capsule formulations were randomly selected for stability studies. The test formulations were stored in their original containers as supplied by the manufacturer. The stability study was

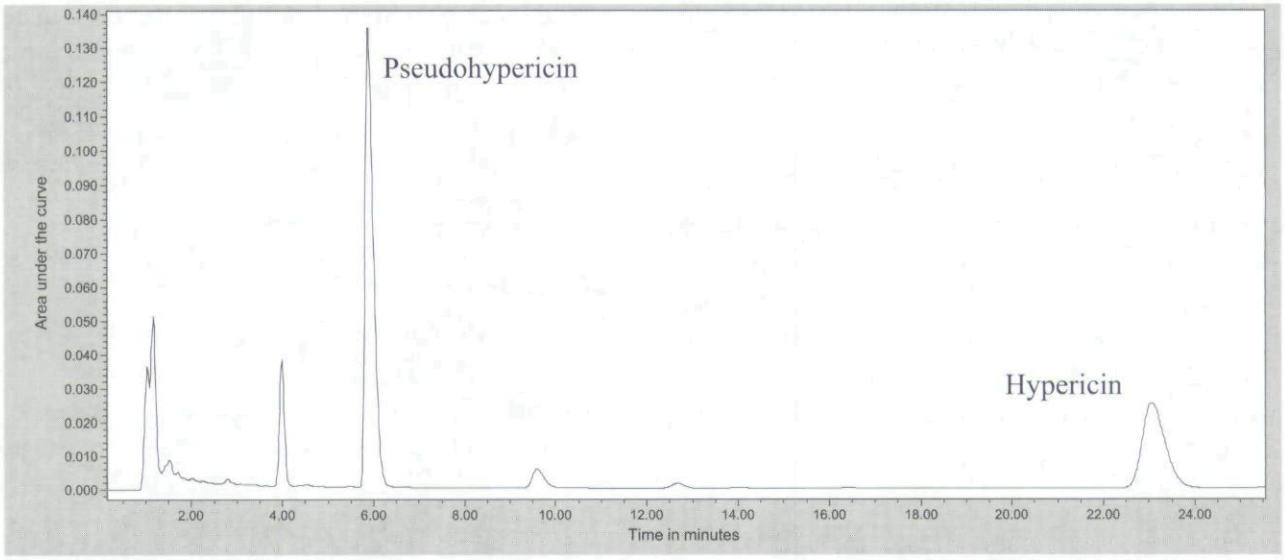


FIGURE 1 **Typical HPLC Chromatogram of Methanolic Extract of St. John's Wort with Detection Wavelength Set at 590 nm. Retention Times of Pseudohypericin and Hypericin were Observed to be 5.9 and 23.2 min, Respectively.**

carried out by exposing the samples to three different storage conditions: long-term stability studies at 25±2°C and 60±5% relative humidity (RH) for six months, accelerated stability studies at 40±2°C and 75±5% RH for six months, and 50°C for one month. Content analysis was carrier out by taking 5 tablets/5 capsules from each manufacturer and by preparing the samples in a manner described earlier. The contents were analyzed to determine the amount of hypericin and pseudohypericin present at the start of the study and after 1, 2, 3, and 6 months for accelerated and long-term studies, whereas 0 time and 1 month for the samples stored at 50°C.

Statistical Analysis

The amounts of marker compounds remaining at each test point were compared with that present at zero time by using a one-way ANOVA followed by Dunnett test, which compares all columns with the control column. An unpaired t-test was used to compare two pairs (storage at 50°C has two data points 0 time and 1 month).

Hardness and Friability

At each sampling period, tablet formulations were assayed for their friability according to USP XXIV. Tablet hardness was also determined at every time point using a Stokes hardness tester.

The physical stability of capsule formulations was not evaluated. All the products were visually observed to see changes in their appearance.

RESULTS AND DISCUSSION

HPLC Method

A novel analytical method that helps in the selective identification and accurate quantification of the most important constituents has been developed.

The marker compounds hypericin and pseudohypericin were best eluted by using the solvent system; acetonitrile:methanol:100 mM ammonium acetate: glacial acetic acid (50:30:20:0.15 v/v/v/v) on a phenomenex, C18 SYNERGI hydro-RP column having dimensions, 150 × 4.6 mm, with particle size 4 micron,

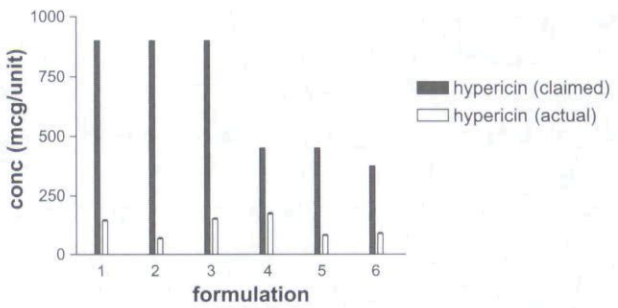


FIGURE 2 **Average Total Hypericin Content in Six Commercial Products in Comparison with its Labeled Claim (1–3 Tablets, 4–6 Capsules).**

TABLE 1 Average Pseudohypericin Content in Six Commercial Products (1–3 Tablets, 4–6 Capsules)

Formulation	Weight of tablet/total capsule content (mgs)	Total hypericum extract (mgs)	Amount of pseudohypericin (mg/formulation)	% of pseudohypericin (per formulation)	% Change (pshyp/formulation)
1	513	300	0.602	0.1173	62.91
2	700	300	0.356	0.0509	275.91
3	479	300	0.505	0.1054	81.33
4	454	300	0.683	0.1504	27.08
5	306	300	0.585	0.1912	0.00
6	456	125	0.287	0.0629	203.75

and i.d., 80°A. Both the components showed appreciable absorbance at a wavelength of 590 nm. Most of the previous analytical methods utilized complicated gradient elution and/or had a long run time. With the current method, elution of the desired components was achieved by using a simple isocratic mode with a run time of less than 25 min. Retention times (t_R) were 5.9 min and 23.2 min for pseudohypericin and hypericin, respectively (Fig. 1). A guard column has been used for two primary purposes. Firstly, it helped in removing almost all the unwanted plant materials from the extract except the components of interest, giving a cleaner and more sensitive chromatogram. Secondly, it increased the column

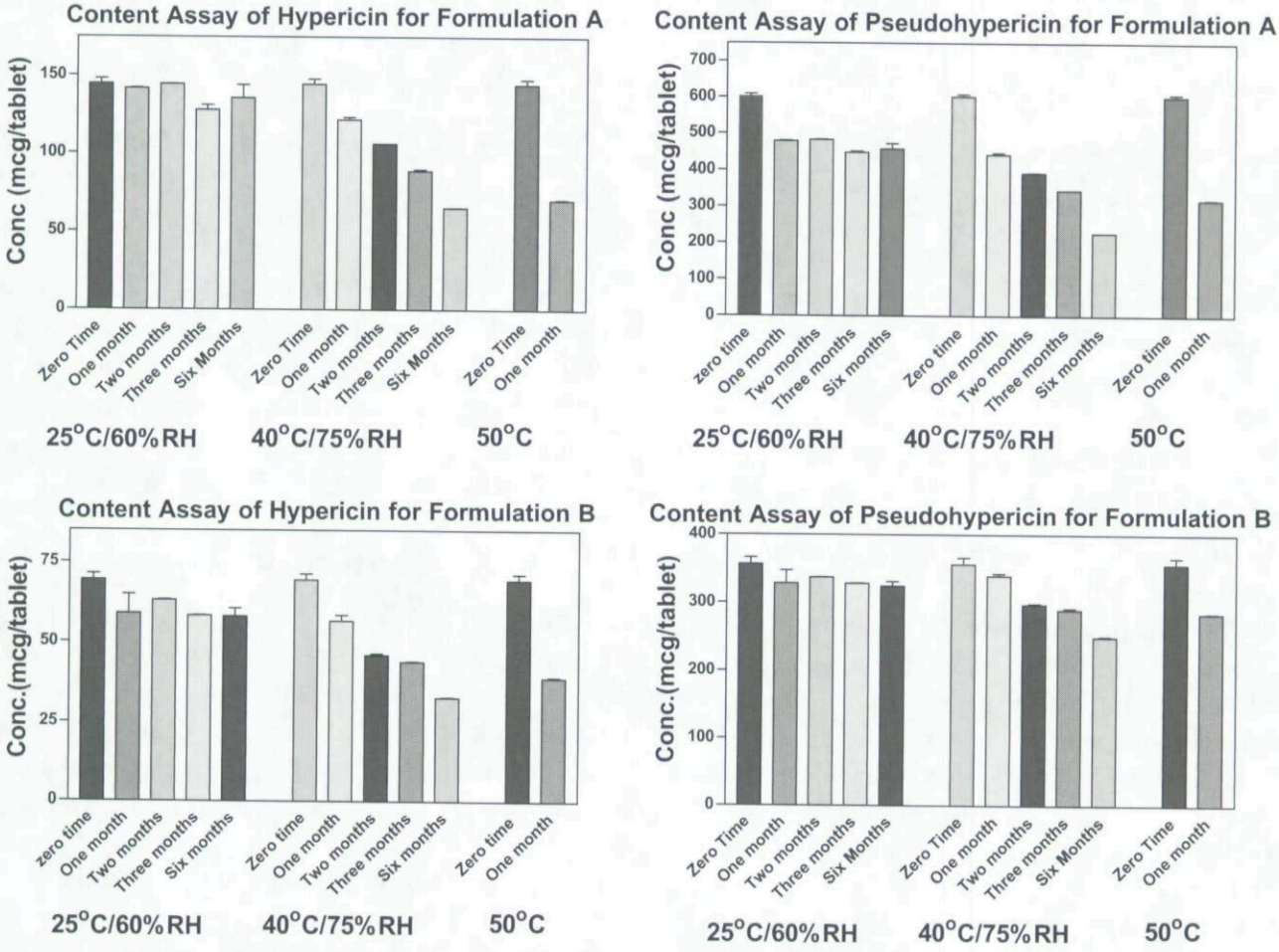


FIGURE 3 Content Assay of Four Formulations A, B, C, and D under Stability Studies. Formulations were Subjected to Three Storage Conditions, 25±2°C/60±5% for 6 Months, 40±2°C/75±5%RH for 6 Months, and 50°C for 1 Month. The Decrease in Concentration of Hypericin and Pseudohypericin is Determined as a Function of Time (Formulation A and B Tablets, C and D Capsules).

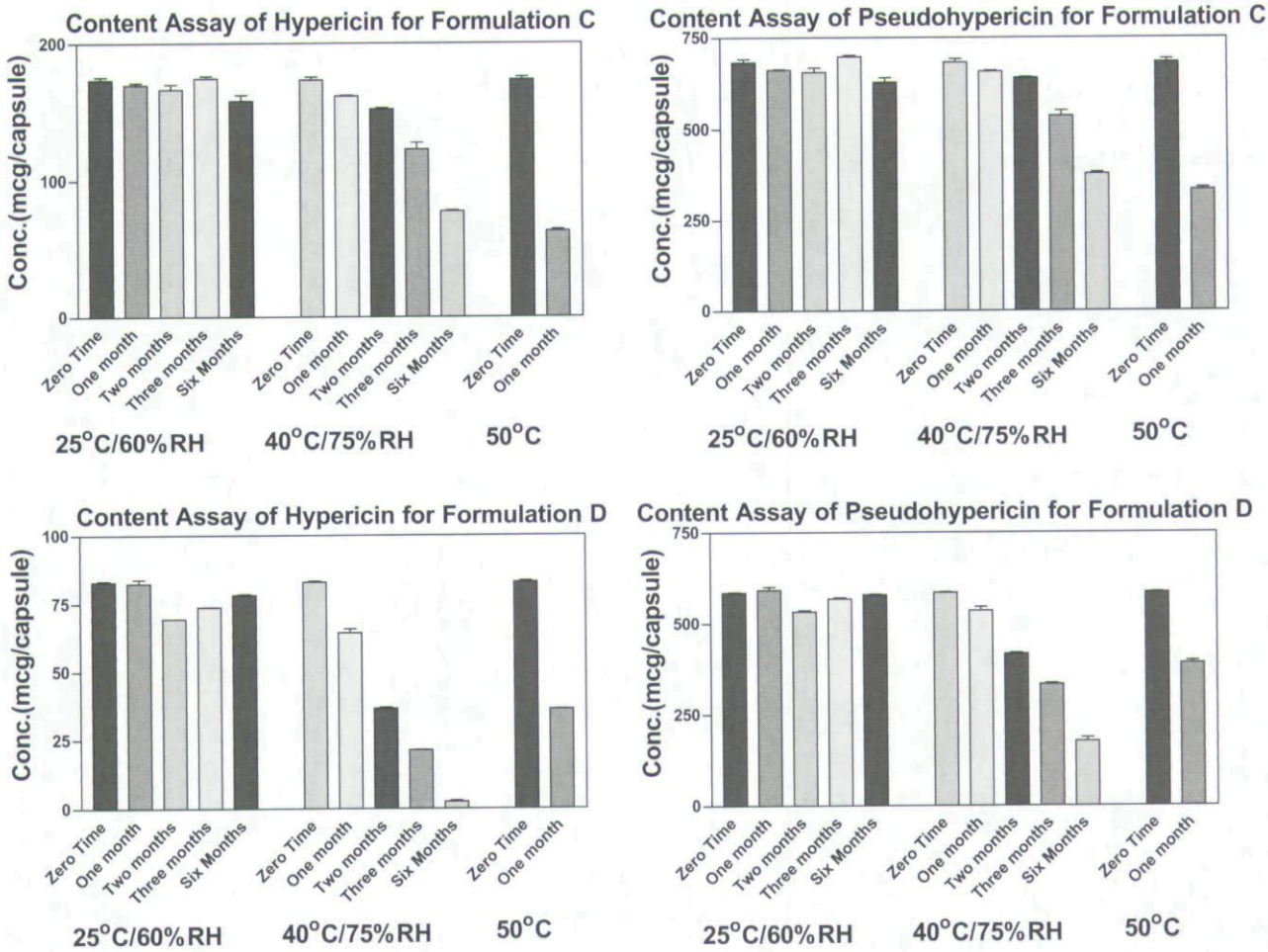


FIGURE 3 Continued.

shelf life by trapping irreversibly retained compounds which would otherwise permanently bind to and foul stationery phase of the column.

Identification, Standard Curves, and Linearity

The marker compounds hypericin and pseudohypericin were identified in the extract by comparing their t_R values obtained from the chromatogram to those of the commercially obtained pure compounds. The results were further confirmed by spiking the extract solutions with a known amount of each of the pure sample and by observing a corresponding increase in the AUC's of the compound peak and, hence, the concentration as calculated from the calibration curve.

Analyses of standards indicated that the calibration curve of hypericin was linear (r=0.9998) in the concentration range of 10–110 μg/ml and that of pseudohypericin was linear (r=0.9996) in the concentration range of 20–130 μg/ml. Each calibration was followed by a blank injection of methanol in order to determine whether trace quantities of standards were retained on the column, but no significant peaks were observed at the t_R of either of the standards, indicating complete elution of both the components.

Content Analysis

Out of these six formulations five were labeled to contain 300 mg of St. John's wort extract, whereas one was labeled to contain 125 mg of extract per unit. All the extracts were standardized to deliver 0.3% hypericin per serving (in the case of formulations 4 and 5, 2 units per serving). Based on this, the amount of hypericin claimed to be present in each tablet/capsule was calculated to be 900 μg, 900 μg, 900 μg, 450 μg, 450 μg, and 375 μg for formulations 1 through 6, respectively. As evident from Fig. 2, all the 6 formulations showed tremendous variation in the actual amount of hypericin present. The formulations

TABLE 2 Residual % of Hypericin and Pseudohypericin of Four Formulations A, B, C, and D at the End of Study Period (Formulation A and B Tablets, C and D Capsules)

Formulation	Storage conditions	Residual % hypericin	Residual % pseudohypericin
A	zero time	100.00	100.00
	25°C/60% RH	93.87	75.98
	40°C/75% RH	45.08	37.59
	50°C	48.73	53.00
B	zero time	100.00	100.00
	25°C/60% RH	83.69	91.04
	40°C/75% RH	47.09	69.94
	50°C	56.16	79.78
C	zero time	100.00	100.00
	25°C/60% RH	91.37	91.97
	40°C/75% RH	44.81	55.35
	50°C	36.41	48.95
D	zero time	100.00	100.00
	25°C/60% RH	94.34	98.94
	40°C/75% RH	3.11	29.99
	50°C	43.72	65.79

contained hypericin in the concentration range of 69.46 µg to 173.56 µg contributing to a lowest percentage of 7.72% and a highest of 38.57% of the labeled claim. A daily dose of 900 mg of hypericin is required for anti-depressant effect (Fetrow & Avila, 2001), but the results from this investigation reveal that these products do not deliver the minimum required dose.

Table 1 shows the amount of pseudohypericin present in the six test formulations. The total amount of pseudohypericin present in the six formulations ranged from 0.287 mg to 0.683 mg per tablet/capsule. The percentage of pseudohypericin per unit ranged from 0.05%–0.19%, with the maximum percent variation of 275%. Clearly, the content of the active ingredients in the six formulations varied significantly.

Stability Studies

Earlier, most of the herbal products were made as tinctures and the literature suggest that 40% and 60% alcoholic solution of St. John's Wort exhibit very low stability at room temperature. Now, since tablet and capsule formulations of herbal products are widely available, it is very important to test their stability because of very few regulations.

The stability results for four formulations in terms of decreasing concentrations of the markers, hypericin and pseudohypericin are summarized in Fig. 3. Each graph represents the content assay of one marker compound at the above mentioned three storage conditions along with the error bars.

Both the markers were present in the lowest amounts in formulation D, with hypericin degrading more than 95% and pseudohypericin degrading more than 70% of the theoretical value after six months storage. Formulations A, B, and C also exhibited more than 50% degradation of hypericin compared to its theoretical value and more than 30% degradation of pseudohypericin. The residual percentages (mentioned in Table 2) of hypericin for formulations A, B, C, and D under accelerated stability testing were 45.1%, 47.1%, 44.8%, and 3.1% (*$p<0.001$ for all †except one time period, $p>0.05$) respectively at the end of the 6 month period. The residual percentages of pseudohypericin for formulations A, B, C, and D were 37.6%, 69.9%, 55.4%, and 30.0% ($p<0.001$ except for two time periods, $p>0.05$), respectively.

All the formulations responded similarly when exposed to long-term stability testing, except formulation A, which showed 24% degradation of pseudohypericin at the end of the study period. The residual

*For one particular stability storage condition, p-values are based on the comparison of amount of hypericin/pseudohypericin remaining at 1, 2, 3 or 6 months to that of zero time.

†Except values—for example, for formulation A at 40±2°C/75±5% RH, residual concentration of hypericin remaining at 1 month (compared to 0 time) is not in the range of the mentioned p-values. Amongst different formulations, except values are for different time points.

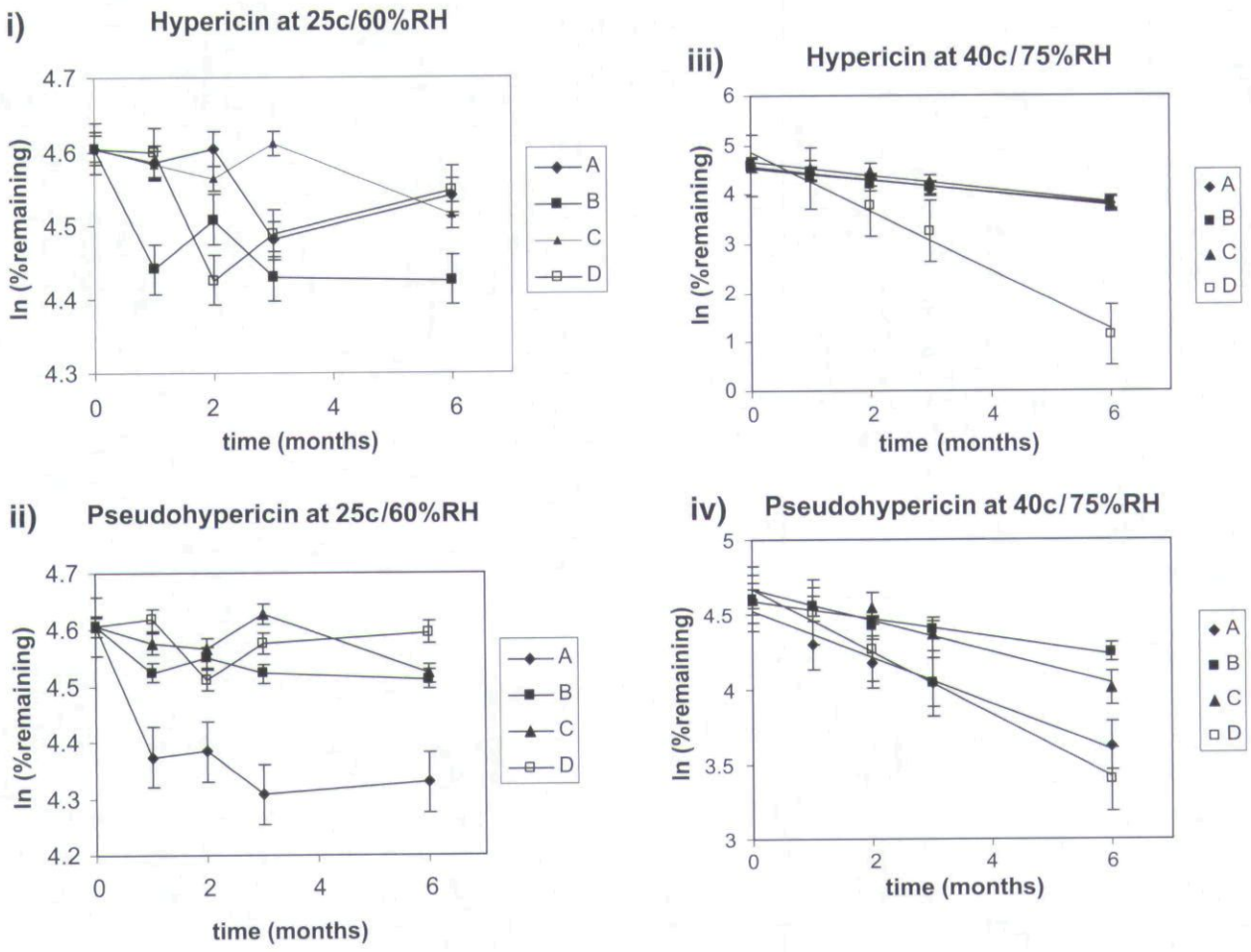


FIGURE 4 i and ii: Semi-log Plot of Percentage of Hypericin and Pseudohypericin Remaining as a Function of Time in Months, Stored at 25±2°C/60±5% RH. iii and iv: Semi-log Plot of Percentage of Hypericin and Pseudohypericin Remaining as a Function of Time in Months, Stored at 40±2°C/75±5% RH. (Formulations A and B Tablets, C and D Capsules).

percentages of hypericin for formulations A, B, C, and D under long-term stability testing were 93.9%, 83.7%, 91.4%, and 94.3% ($p>0.05$—not significant, except for four time points, $p<0.05$), respectively, at the end of the 6 months period. The residual percentage of pseudohypericin for formulations A, B, C, and D were 76%, 91%, 92%, and 98.9% ($p>0.05$—not significant, except seven, $p<0.05$), respectively, at the end of 6 months.

The residual percentages of hypericin for formulations A, B, C, and D stored at 50°C were 48.7%, 56.2%, 36.4%, and 43.7% ($p \leq 0.0005$ except one, $p=0.001$), respectively, whereas the residual percentages of pseudohypericin were 53.0%, 79.8%, 48.9, and 65.8% ($p<0.05$ except one, $p=0.019$), respectively, at the end of the 1 month period.

The kinetics of degradation revealed that both hypericin and pseudohypericin were significantly stable and did not follow any specific rate order reaction at 25°C, whereas they followed a first order kinetics at 40°C (Fig. 4). The natural logarithmic plots of % remaining v/s time yield straight lines with correlation coefficients between 0.95 and 1.00.

TABLE 3 Hardness of Two Tablet Formulations A and B as Determined at Each Time Interval of the Stability Studies

	25°C/60% RH	40°C/75% RH	50°C
Formulation A			
Zero time	12.35	12.35	12.35
One month	12.5	13	12.9
Two months	12.55	14.85	—
Three months	13.7	15.05	—
Six months	15.4	15.45	—
Formulation B			
Zero time	11.65	11.65	11.65
One month	9.35	12.05	12.45
Two months	10.7	12.85	—
Three months	11.1	12.7	—
Six months	12.1	12.65	—

Hardness and Friability

The results from the hardness study are shown in Table 3. The hardness of the two tablet formulations at the end of each sampling period increased at all three storage conditions. The hardness of formulation A was 12.35 kg at 0 time and reached a maximum of 15.45 kg when stored at 40±2°C/75±5%RH, whereas that of formulation B reached a maximum of 12.65 kg from 11.65 kg when stored at 25±2°C/60±5%RH, at the end of the 6 month period. The increase in hardness is attributed to high humidity conditions in the stability chambers. The tablets appeared moist on removal from the stability chambers, but became hard due to the loss of extra moisture, hence attributing to tablets of greater hardness when stored under accelerated stability storage conditions. The fact that a change in tablet hardness causes a significant change in tablet bioavailability is a point for further investigation.

The friability of the tablet formulations determined at each assay time point revealed a maximum percentage weight loss of 0.25% and minimum of 0.006%. The low friability on storage under stability conditions might be attributed to the increase in hardness and the formation of unbreakable mass of the tablet. This may significantly alter the dissolution of these tablets.

Appearance

At the beginning of the study period, the capsule powder was free flowing, but upon exposure to stability storage conditions, all the products appeared dark, and opening the capsule shell revealed a solid plug difficult to break.

CONCLUSION

A novel, rapid HPLC method was developed for the determination of hypericins in commercial products. The quantification of the commercial products revealed that all of them (3 tablets and 3 capsules) contained significantly less hypericin than the label claimed. These products also exhibited a large variation in the quantity of pseudohypericin. The tablet hardness was observed to increase under certain storage conditions, which might result in unacceptable changes in the release rate. Content analysis of the test formulations revealed very low amounts of the actives, which when subjected to stability storage condition resulted in significant degradation and, hence, further decrease in the concentration of actives. This can cause a great decrease in potency of these herbal products.

REFERENCES

Baldt, S., & Wagner, H. (1994). Inhibition of MAO by fractions and constituents of hypericum extract. *Journal of Geriatric Psychiatry and Neurology, 7*, 273–275.

Bilia, A. R., Bergonzi, M. C., Morgenni, F., Mazzi, G., & Vincieri, F. F. (2001). Evaluation of chemical stability of St. John's wort commercial extract and some preparations. *International Journal of Pharmaceutics, 213*, 199–208.

Bilia, A. R., Bergonzi, M. C., Mazzi, G., & Vincieri, F. F. (2002). Analysis and stability of the constituents of artichoke and St. John's wort tinctures by HPLC-DAD and HPLC-MS. *Drug Development and Industrial Pharmacy, 28*, 609–619.

Brolis, M., Gabetta, B., Fuzzati, N., Pace, R., Panzeri, F., & Peterlongo, F. (1998). Identification by high-performance liquid chromatography-diode array detection-mass spectrometry and quantification by high-performance liquid chromatography-UV absorbance detection of active constituents of *Hypericum perforatum*. *Journal of Chromatography A, 825*, 9–16.

Butterweck, V., Wall, A., Lieflander-Wulf, U., Winterhoff, H., & Nahrstedt, A. (1997). Effects of the total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity. *Pharmacopsychiatry, 30*, 117–124.

Butterweck, V., Jurgenliemk, G., Nahrstedt, A., & Winterhoff, H. (2000). Flavonoids from *Hypericum perforatum* show antidepressant activity in the forced swimming test. *Planta Medica, 66*, 3–6.

Calapai, G., Crupi, A., Firenzuoli, F., Costantino, G., Inferrea, G., Campa, G. M., & Caputi, A. P. (1999). Effects of *Hypericum perforatum* on levels of 5-hydroxytryptamine, noradrenaline and dopamine in the cortex, diencephalons and brainstem of the rat. *Journal of Pharmacy and Pharmacology, 51*, 723–728.

Calapai, G., Crupi, A., Firenzuoli, F., Costantino, G., Inferrea, G., Squadrito, F., Parisi, A., Sarro, G. D., & Caputi, A. (2001). Serotonin, norepeinephrine, and dopamine involvement in the antidepressant action of *Hypericum perforatum*. *Pharmacopsychiatry, 34*, 45–49.

Chatterjee, S. S., Bhattacharya, S. K., Wonnemann, M., Singer, A., & Muller, W. E. (1998). Hyperforin as a possible antidepressant component of hypericium extracts. *Life Science, 63*, 499–510.

Chatterjee, S. S., Filippo, V., Lishko, P., Maximyuk, O., Noldner, M., & Krishtal, O. (2004). Hyperforin attenuates various ionic conductance mechanism in the isolated hippocampal neurons of rat. *Life Science, 65*, 2395–2405.

Fetrow, C. W., & Avila, J. R. (2001). *Professional's Handbook of Complementary and Alternative Medicines*. Springhouse Corp.

Garrard, J., Harms, S., Eberly, L. E., & Matiak, A. (2003). Variations in product choices of frequently purchased herbs. *Archives of Internal Medicine, 163*, 2290–2295.

Gourneron, J. D., Herbette, G., & Caloprisco, E. (1999). Pseudohypericin and hypericin in St. John's wort extracts. Breakdown of pseudohypericin. *Analytical and Environmental Chemistry, 2*, 127–131.

Hansgen, K. D., Vesper, J., & Ploch, M. (1994). Multicenter double-blind study examining the antidepressant effectiveness of the hypericum extract LI 160. *Journal of Geriatric Psychiatry and Neurology, 7*, S15–S19.

Israelsen, L. D., & Blumenthanl, M. (2000). FDA issues final rules for

structure/function claims for dietary supplements. *HerbalGram, 48*, 32–38.

Kaehler, S. T., Sinner, C., Chatterjee, S. S., & Philippu, A. (1999). Hyperforin enhances the extracellular concentrations of catecholamines, serotonin and glutamate in the rat locus coeruleus. *Neuroscience Letters, 262*, 199–202.

Kopelman, S. H., & Augsburger, L. L. (2002). Excipient compatibility study of *Hypericum performatum* extract (St. John's wort) using similarity metrics to track phytochemical profile changes. *International Journal of Pharmaceutics, 237*, 35–46.

Laakhman, G., Schule, C., Baghai, T., & Keiser, M. (1998). St. John's wort in mild to moderate depression: the relevance of hyperforin for the clinical efficacy. *Pharmacopsychiatry, 31*, 54–59.

Li, W., & Fitzloff, J. F. (2001). High performance liquid chromatographic analysis of St. John's wort with photodiode array detection. *Journal of Chromatography B, 765*, 99–105.

Linde, K., Ramirez, G., Mulrow, C. D., Pauls, A., Weidenhammer, W., & Melchart, D. (1996). St. John's wort for depression-an overview and meta-analysis of randomized clinical trials. *British Medical Journal, 313*, 253–257.

Muller, W. E., Rolli, M., Schafer, C., & Hafner, U. (1997). Effect of hypericum extract (LI160) in biochemical models of antidepressant activity. *Pharmacopsychiatry, 30*, 102–107.

Orth, H. C. J., & Schmidt, P. C. (1998). Isolation, purity analysis, and stability of hyperforin as a standard material from *Hypericum perforatum L. Journal of Pharmacy and Pharmacology, 51*, 193–200.

Orth, H. C. J., Rentel, C., & Schmidt, P. C. (1999). Analysis and stability of hyperforin as a standard material from *Hypericum perforatum L. Journal of Pharmacy and Pharmacology, 51*, 193–200.

Ozturk, Y., Aydin, S., Beis, R., Baser, K. H. C., & Berberoglu, H. (1996). Effect of *Hypericum perforatum L.* and *Hypericum calycinum L.* extracts on the central nervous system in mice. *Phytomedicine, III*, 139–146.

Schrader, E., Meier, B., & Brattstrom, A. (1998). Hypericum treatment of mild-moderate depression in a placebo-controlled study. *Human Psychopharmacology, 13*, 163–169.

Sommer, H., & Harrer, G. (1994). Placebo-controlled double-blind study examining the effectiveness of an hypericum preparation in 105 mildly depressed patients. *Journal of Geriatric Psychiatry and Neurology, 7*, S9–S11.

Vorbach, E. U., Hubner, W. D., & Brattstrom, A. (1994). Effectiveness and tolerance of the hypericum extract LI160 in comparison with imipramine, randomized double-blind study with 135 outpatients. *Journal of Geriatric Psychiatry and Neurology, 7*, S19–S23.

Vorbach, E. U., Arnoldt, K. H., & Christl, D. (1997). Efficacy and tolerability of St. John's wort extract LI 160 versus imipramine in patients with severe depressive episodes according to ICD-10. *Pharmacopsychiatry*, 30, S81–S85.

Wheatley, D. (1997). LI 160, an extract of St. John's wort, versus amitriptyline in mildly to moderately depressed outpatients—a controlled 6-week clinical trial. *Pharmacopsychiatry, 30*, S77–S80.

Wirz, A., Meier, B., & Sticher, O. (2001). Stability of hypericin and pseudohypericin in extract solutions of *Hypericum perforatum* and in standard solutions. *Pharmaceutical Industry, 63*, 410–415.

Wruglics, M., Westerhoff, K., Kounzinger, A., Wilke, A., Baumeister, A., Dressman, J., & Schubert-Zsilavecz, M. (2001). Batch-to-batch reproducibility of St. John's wort preparations. *Pharmacopsychiatry, 34*, S152–S156.