ELSEVIER

International Journal of Pharmaceutics 213 (2001) 199–208

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Evaluation of chemical stability of St. John's wort commercial extract and some preparations

Anna Rita Bilia *, Maria Camilla Bergonzi, Fabrizio Morgenni, Giovanni Mazzi, Franco Francesco Vincieri

Department of Pharmaceutical Science, University of Florence, Via Gino Capponi 9, 50121 Firenze, Italy

Received 31 July 2000; received in revised form 27 October 2000; accepted 3 November 2000

Abstract

Thermal and photostability of a commercial dried extract and capsules of St. John's wort (*Hypericum perforatum* L.) were evaluated under the ICH test conditions. The extract was considered as drug substance and its preparations as drug products. In addition, capsules of different colours corresponding to different opaficient and pigment contents were also evaluated as primary package of drug product and the tests in the secondary pack were performed with amber containers, as well. A selective high-performance liquid chromatography (HPLC) for determination of stability of all the characteristic constituents, namely flavonols, hyperforins and hypericins, was carried out. Photostability testing showed all the constituents to be photosensitive in the tested conditions. However, different opaficients and pigments present in the capsules influenced the stability of the different classes of constituents. Amber containers suggested as secondary packages influenced only in part the photostability of the investigated constituents. Long-term thermal stability testing showed a very low (less than 4 months) hyperforins and hypericins t_{90}, even if ascorbic and citric acids were added to the formulation. From the results we have obtained it is clear that for St. John's wort preparations, a mere translation of the ICH guidelines to the field of herbal products, as suggested by the WPHMP of the EMEA, cannot be accepted. A revision and adaptation of the storage conditions should be elaborated. © 2001 Published by Elsevier Science B.V.

Keywords: St. John's wort (*Hypericum perforatum* L.); Commercial extract and capsules; HPLC; Flavonols; Hyperforins and hypericins; Thermal and photostability

1. Introduction

The general public on a self-selection basis to replace or complement conventional medicines is increasingly using herbal remedies and one of the reasons for their growing popularity is the belief that they are natural and, therefore, 'safe'. Unfortunately, the quality of the majority of them

Abbreviations: DS, drug substance; DP, drug product; EMEA, The European Agency for the Evaluation of Medicinal Products; HD, herbal drugs; HDP, herbal drug preparations; HMP, herbal medicinal products; ICH, International Conference of Harmonisation; MDP, modified preparation; WPHMP, Working Party of the Herbal Medicinal Products.

* Corresponding author. Tel.: +39-055-2757288; fax: +39-055-240776.

E-mail address: ar.bilia@unifi.it (A.R. Bilia).

0378-5173/01/$ - see front matter © 2001 Published by Elsevier Science B.V.

PII: S0378-5173(00)00660-8

remains essentially uncontrolled because they are not generally categorised as medicines (dietary supplements, health foods, and so on).

Stability testing represents a crucial part of the testing program for drug substances because the instability of the product modifies the three essential requisites, i.e. quality, efficacy and safety (Kommanaboyina and Rhodes, 1999). Stability, defined as the time during which a drug retains its integrity in terms of quantity and chemical identity, can be affected by environmental factors such as temperature, pH, light, and air, which can have dramatic effects on some constituents. In the field of herbal drugs (HD), their preparations (HDP) and herbal medicinal products (HMP), if the constituents with therapeutic activity are not known, a limit of $\pm 10\%$ of the initial assay value content has recently been accepted (EMEA, 1999).

In the present work, thermal and photostability of the constituents (Fig. 1) of the commercial dried extract of St. John's wort (*Hypericum perforatum* L.) and two preparations are reported. The International Conference on Harmonisation (ICH) guidelines were applied, as suggested by the Working Party of the Herbal Medicinal Product (WPHMP) of the The European Agency for the Evaluation of Medicinal Products (EMEA) (ICH, 1994, 1996).

St. John's wort has been known since antiquity for many medicinal properties, indicated in gout, hepatic disorders and gastric ulcers. In the last two decades, anti-inflammatory (Brantner et al., 1994), anti-microbial (Shakirova et al., 1970), antiviral (Yip et al., 1996) and antidepressant (Harrer and Sommer, 1994) activities have also been attributed to the total extract or single constituents.

Antidepressant applications of St. John's wort medicinal products (such as Psychotonin®, Neuroplant®, Hyperforat®) have become increasingly popular in Europe, particularly in Germany where physicians routinely prescribe herbal medicines. However, St. John's wort extracts are prescribed, not only as HMP but also as dietary supplements, both standardised using the naphthodianthrones of the hypericin group, calculated as 0.2–1 mg hypericin daily dose.

Antidepressant activity (Chatterjee et al., 1998a) was first attributed to hypericins (**1**, **2**) and the polyphenols flavonols (**3**–**7**), but recent pharmacological and clinical results focus on hyperforins (**8**, **9**) as the main active ingredients of the extract (Chatterjee et al., 1998b; Muller et al., 1998; Laakmann et al., 1998; Chatterjee et al., 1999; Kaehler et al., 1999; Gobbi et al., 1999; Kleber et al., 1999). Thus, the standardisation of the extracts based on hypericin (**1**) can no longer be proposed as a tool to evaluate potential benefits or risks, of St. John's wort preparations. It is well known that hypericins and hyperforins are not stable with regard to heat and light (Adamski and Styp-Rekowoska, 1971; Maisenbacher and Kovar, 1992; American Herbal Phar-

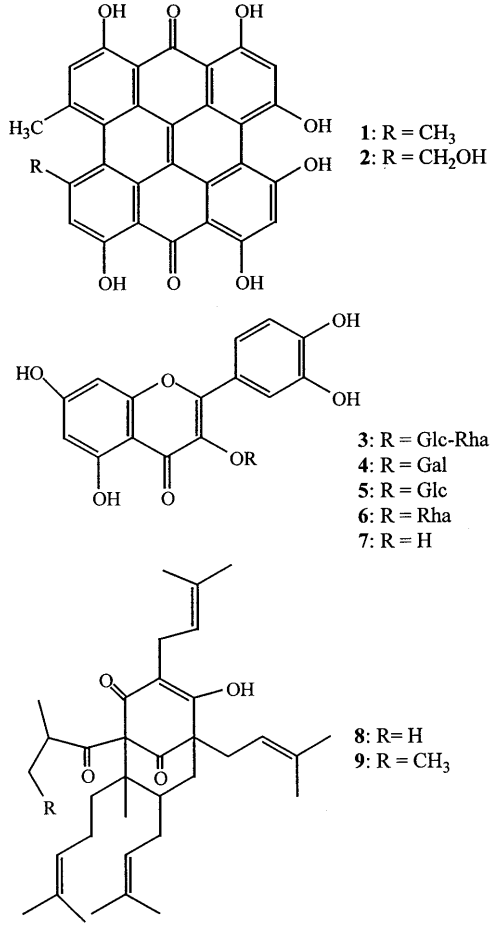

Fig. 1. Structure of compounds **1**–**9**.

macopoeia, 1997; Orth et al., 1999). However, to our knowledge, no studies on the stability of the St. John's wort's commercial preparations have been reported so far. Only the variations in the hypericin content of the wild samples of *H. perfortum* L. and some commercial products were investigated. The aim of these studies were to evaluate both the influence of environmental factors, drying and storage conditions of the spontaneous samples, both the quality of the products available to the consumer with referring to this unique constituent (Constantine and Karchesy, 1998).

Thus, investigations on the thermal and photostability should represent a crucial step in their marketing, even if these products are not sold as herbal medicinal products because they should guarantee, at least, quality and safety.

2. Materials and methods

2.1. Materials

2.1.1. Solvents and reagents

Acetonitrile and MeOH HPLC grade, and 85% phosphoric acid were purchased from Merck (Darmstadt, Germany); 85% formic acid was provided by Carlo Erba (Milan, Italy). Water was purified by a Milli-Q_{plus} system from Millipore (Milford, MA, USA). Ascorbic acid was purchased from Merck (Darmstadt, Germany) and citric acid from Fluka Chemicals (Sigma-Aldrich Division, Milano, Italy).

Indena Research Laboratories (Settala, Milan, Italy) kindly provided the reference rutin trihydrate (**3**) (batch no. K12408717, standard purity 88.17% considering the content of residual solvents, moisture and amount of impurities).

2.1.2. Samples

A commercial sample of St. John's wort's dried extract (lot no. 980600056) was used and it was considered as a drug substance (DS) in the stability testing (ICH, 1994, 1996).

Drug product (DP) was prepared by a mixture (53:46:1) of the dried extract of St. John's wort, lactose (Fluka Chemicals, Sigma-Aldrich Division, Milano, Italy) and magnesium stearate (Fluka Chemicals, Sigma-Aldrich Division, Milano, Italy). This drug product was utilised in the stability testing as such or by adding a mixture of ascorbic and citric acids (200:1), to obtain a modified preparation (MDP) containing 2.85% of antioxidants. DS, DP and MDP were stored at $-20°$ C in filled and well-closed vials, protected from the light by aluminium to avoid the degradation of the constituents as required by the ICH (ICH, 1994, 1996).

Transparent gelatine capsules and capsules of different colours (white, orange, yellow, red, blue and light blue) were purchased from Galeno (Comeana, Prato, Italy). The quali-quantitative composition of the capsules of different colours is reported in Table 1. Capsules containing 350 mg ($\pm 2\%$) DP were prepared using a Triplex 100 apparatus, (Galeno-Tecknolab, Comeana, Prato, Italy). Nominal dosage drug substance was according to the marketed products.

Table 1
Constituents of capsules

Capsules	Composition	%
Transparent	Gelatine	100
White	Titanium dioxide	2.00
	Gelatine	till 100
Yellow	Yellow iron oxide	0.50
	Titanium dioxide	1.00
	Gelatine	till 100
Light blue	Indigotin	0.25
	Titanium dioxide	2.00
	Gelatine	till 100
Blue	Erythrosin	0.0257
	Yellow iron oxide	0.12
	Indigotin	0.50
	Titanium dioxide	1.00
	Gelatine	till 100
Red	Erythrosin	1.90
	Indigotin	0.0086
	Titanium dioxide	0.80
	Gelatine	till 100
Orange	Erythrosin	0.0114
	Yellow iron oxide	0.65
	Red iron oxide	0.65
	Indigotin	0.0021
	Titanium dioxide	1.33
	Gelatine	till 100

2.2. Methods

2.2.1. HPLC-DAD and HPLC-MS drug analyses

To evaluate the constituents' content, a HPLC analysis was performed using a method described in the literature (Brolis et al., 1998), modified for our experimental necessities. The identification of the constituents was performed using combined HPLC-diode array detection (DAD) analysis and HPLC-thermospray mass spectrometry. The quantification of the constituents was performed using rutin as an external standard and consideration of each constituent and the relative response factor (RRF) with respect to the rutin, as previously reported (Brolis et al., 1998).

All the samples were analysed in triplicate and a calibration graph with six data points of external standard was used. The content of each constituent of the drug substance is reported in Table 2.

The HPLC system consisted of a HP 1090L instrument with a diode array detector and managed by a HP 9000 workstation (Hewlett & Packard, Palo Alto, CA, USA). The reverse-phase column was a Protein C4 (5 μm, 250 mm, 0.5 mm i.d., 300 Å, Vydac Separation Group Hesperia, CA, USA) maintained at 26°C. The mobile phase was a five-step linear solvent gradient CH_3CN/CH_3OH/H_2O (pH 3.2, H_3PO_4) during a 30-min period at a flow rate of 1 ml/min. Mobil phase: 0 min 85% H_2O–15% CH_3CN; 12 min 80% H_2O–5% CH_3OH–15% CH_3CN; 20 min 10% H_2O–15% CH_3OH–75% CH_3CN; 27 min 5% H_2O–15% CH_3OH–80% CH_3CN; 30 min 85% H_2O–15% CH_3CN, and it was a simple modification of that previously reported (Brolis et al., 1998). Before the HPLC analysis, each sample was filtered through a cartridge-type sample filtration unit with a polytetrafluoroethylene (PTFE) membrane ($d = 13$ mm, porosity 0.45 μm, Lida manufacturing Corp.) and immediately injected. The injected volume of sample was a 10-μl solution. UV–Vis spectra were recorded in the range 200–590 nm, and chromatograms were acquired at 230, 254, 270, 350 and 590 nm.

Table 2
Dried extract (drug substance, DS) composition

Constituent	Content% (mg/100 mg)	Standard deviation (S.D.)
Rutin	4.28	±1.23
Hyperoside	6.35	±1.02
Isoquercitrin	0.61	±0.08
Quercitrin	0.65	±0.08
Quercetin	0.83	±0.13
Total flavonols	12.72	±1.08
Total hyperforins	4.23	±0.01
Total hypericins	0.32	±0.03

The HPLC system was interfaced with a HP 1100 MSD API-electrospray (Hewlett & Packard, Palo Alto, CA, USA). The interface geometry, with an orthogonal position of the nebulizer with respect to the capillary inlet, allowed the use of analytical conditions similar to those of the HPLC-DAD analysis. The same column, mobile phase, time period and flow rate were used. Mass spectrometry operating conditions were optimised in order to achieve maximum sensitivity values; gas temperature 350°C at a flow rate of 10 l/min, nebulizer pressure 30 p.s.i., quadrupole temperature 30°C, and capillary voltage 3500 V. Full scan spectra from m/z 100–800 in the negative and positive ion mode were obtained (scan time 1 s).

The linearity range of responses was determined for five concentration levels with three injections for each level either for the rutin standard or for each component. Calibration graphs for HPLC were recorded with sample amounts ranging from 0.60 to 1.2 μg ($r > 0.998$).

To evaluate the repeatability of the method, six samples from methanol solution (2.5 mg/ml) of the extract were analysed by RP-HPLC. Each constituent of the extract was evaluated to calculate the relative standard deviation. The following data were obtained; rutin (**3**) 3.0%, hyperoside (**4**) 3.7%, isoquercitrin (**5**) 1.5%, quercitrin (**6**) 1.5%, quercetin (**7**) 1.4%, hypericin (**1**) 1.8%, hyperforin (**9**) 1.3%.

To evaluate the reproducibility of the injection integration, 10 μl of a standard solution of rutin (0.1 μg/1 μl) and of methanol solution of the extract were injected six times and the relative standard deviation (R.S.D.) values were calcu-

lated. The following data were obtained; rutin (**3**) 1.0%, hyperoside (**4**) 4.0%, isoquercitrin (**5**) 1.3%, quercitrin (**6**) 2.0%, quercetin (**7**) 1.9%, hypericin (**1**) 1.2%, hyperforin (**9**) 1.8%.

2.2.2. Stability study

The thermal stability testing was carried out in triplicate using transparent capsules, introduced in inert and transparent containers. All samples were wrapped in aluminium foil and they were exposed during a 3-month period, under two different temperature conditions $+25 \pm 2$°C with 60 ± 5% relative humidity (RH) for the long-term testing and at $+40 \pm 2$°C with 75 ± 5% RH for accelerated testing. The constituent content was evaluated fortnightly. The apparatus utilised for these tests are the climatic chambers (Angelantoni Industry S.p.A. Massa Martana, Perugia, Italy) of the Analytical Research Division of the A. Menarini s.r.l. Florence, Italy.

In the accelerated photodegradation testing, the analysis was carried out in triplicate; DS, DP and MDP were introduced into chemically inert and transparent glass containers and were spread in the containers to give a thickness typically not more than 3 mm (at least not more than 300 mg) according to the ICH guidelines. In addition, the experiment was carried out in triplicate using capsules. Two transparent capsules and two capsules of each colour were introduced into inert and transparent or amber glass containers. Characteristics of amber and transparent glass containers were according to European Pharmacopoeia (3rd edn., 1997, p. 171).

Three vials of each sample were stored for 7 h, as suggested by the ICH guidelines, in a Solarbox 1500 instrument equipped by a xenon lamp (1500 W) that emitted radiation in the range of 310–700 nm (Angelantoni Industry S.p.A., Massa Martana, Perugia, Italy). The samples were exposed providing an overall illumination of 176 Klux and an integrate energy of 75.8 W/m^2. After exposition, the samples were covered with aluminium and immediately analysed by HPLC.

After the stability testing both DS (125.0 mg), DP (237.5 mg) and MDP (244.5 mg) were treated with 50 ml of methanol HPLC grade and placed in a sonicator for 20 min to obtain a standard solution of 2.50 mg/ml of dried extract. Two capsules from each vial were opened, mixed and 237.5 mg (DP) or 244.5 mg (MPD) were treated as described above.

Some samples were wrapped in aluminium foil as dark controls to evaluate the contribution of thermally induced change of the total observed change.

2.2.3. Dissolution study

A Sotax ATX dissolution apparatus (USA) with paddles was employed to carry out all of the tests. The volume of the dissolution medium, experimental temperature, and paddle speed were 1000 ml, 37 ± 0.5°C and 50 rpm, respectively. All of the analyses were assayed in demineralised and unbuffered (pH 6) water. The duration of test was 1 h and the dissolved drug was assayed at a wavelength of 270 nm in an UV 100 Perkin Elmer spectrophotometer at the measured time intervals (7.5 min). The calibration curve was obtained from a dissolution test of three samples (350, 500 and 700 mg) of the drug product. No interference of the opaficients and the pigments of capsules at the wavelength of 270 nm was detected.

The dissolution test was carried out in triplicate on capsules of DP and MDP as such or submitted to the stability testing.

3. Results and discussion

The WPHMP of the EMEA has recently suggested that HD, HDP and HMP in their entirety are regarded as active substances, thus, it is necessary to control the fingerprint chromatograms for the evidence that all the metabolites present are likewise stable and that their proportional content remains constant. If the active constituents are unknown as in St. John's wort's preparations, a limit of ± 10% of the initial assay value can be accepted.

In the present study, the dried extract of St. John's wort was compared with drug substance (DS), and the mixture of extract and excipients as drug product (DP). Capsules of DP were also prepared and tested. Hard gelatine capsules of different colours and different opaficient and pig-

Table 3
Content (mg/100 mg DS), S.D. and content residual percentage of the capsules using DP and MDP in the long-term testing (+25°C, 60% RH)[a]

Sample days	Flavonols		Hypericins		Hyperforins	
	mg/100 m ± S.D.	Residual%	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual %
DP, 15 days	12.67 ± 1.12	99.6	0.29 ± 0.08	90.6	4.14 ± 0.16	97.8
DP, 30 days	12.64 ± 1.19	99.4	0.27 ± 0.04	84.4	4.03 ± 0.08	95.3
DP, 45 days	12.60 ± 1.21	99.1	0.23 ± 0.02	71.8	3.99 ± 0.12	94.3
DP, 60 days	12.57 ± 1.21	98.8	0.18 ± 0.03	56.3	3.94 ± 0.09	93.1
DP, 75 days	12.49 ± 1.23	98.2	0.11 ± 0.02	34.3	3.89 ± 0.10	92.0
DP, 90 days	12.46 ± 1.13	98.0	0.09 ± 0.04	28.1	3.81 ± 0.08	90.0
MDP, 15 days	12.70 ± 1.10	99.9	0.29 ± 0.02	90.6	4.13 ± 0.13	97.6
MDP, 30 days	12.69 ± 1.18	99.8	0.27 ± 0.04	84.4	4.06 ± 0.08	96.0
MDP, 45 days	12.64 ± 1.23	99.4	0.23 ± 0.05	72.0	4.05 ± 0.10	95.7
MDP, 60 days	12.63 ± 1.15	99.3	0.19 ± 0.03	59.4	3.98 ± 0.08	94.1
MDP, 75 days	12.59 ± 1.23	99.0	0.14 ± 0.02	43.8	3.93 ± 0.10	92.9
MDP, 90 days	12.54 ± 1.13	98.6	0.07 ± 0.04	36.8	3.90 ± 0.18	92.2

[a] DP, drug product; MDP, modified drug product containing 2.85% antioxidants (ascorbic acid/citric acid 200:1).

ment contents (see Table 1), were used and considered as the immediate pack and investigated as possible light-absorber substances. Furthermore, amber containers were considered as secondary pack to evaluate the protection from light penetrates.

Since dried extracts are standardised only in hypericin content, as the first step in this investigation, the quali–quantitative content of all the constituents was achieved by HPLC analysis, using a previously published method modified for our needs. The results are reported in Table 2. From these data, flavonols represented the main constituents of the DS (12.72%) and among them hyperoside (**4**) (quercetin-3-galactoside) and rutin (**3**) (quercetin-3-glucosylrhamnoside) represent the principal compounds (4.28 and 6.35%, respectively). Hyperforins (including hyperforin **8**, adhyperforin **9**, and their metabolites) were 4.23% of content, and the typical naphtodianthrone hypericins (including hypericin **1** and pseudohypericin **2**) were 0.32%.

After the analytical investigation of DS, DP was prepared by mixing magnesium stearate and lactose as reported above in the experimental section. In addition, a modified drug product (MDP) was obtained by adding a mixture of antioxidants (ascorbic/citric acids 200:1) to obtain a nominal content of 2.85%. DP and MDP were then placed into transparent and coloured capsules to obtain doses of 350 mg (± 2%).

HPLC analysis of the samples was carried out fortnightly. The results of thermal stability testing, during the 3 months' storage are reported in Tables 3 and 4.

The residual percentage of hyperforins of the samples submitted to accelerated thermal stability testing was 85.1% after 15 days storage and became 60% after 45 days. Hypericins degraded very quickly and after 15 days only 43.8% of the theoretical value content was still present; after 45 days its content was no longer detectable. Flavonols were unexpectedly very unstable and after 15 days, only 85.5% of the theoretical value content was still present. This fact was probably due to the presence of two main glycosides hyperoside (6.35%) and rutin (4.28%) that can easily hydrolyse to the aglycone quercetin (for both the derivatives) or to isoquercitrin (for rutin alone).

In the long-term testing, after 3 months' storage of DP, hypericins degraded more than 70% of the theoretical value, having a t_{90} of only about a couple of weeks. Hyperforins showed a t_{90} of about 3 months, while flavonol content was 98% of the initial value after the same period.

Thermal stability testing on MDP, as reported in Tables 3 and 4, showed only a small increase of the stability of hypericins. Thus, in the thermal accelerated stability testing, after 15 days, the flavonol content was 86.1% as compared with 85.5% found in the same product without stabilisers. After the same storage period, hyperforins' content was 88.9% of the theoretical value content, while hypericins were 68.7% as compared with 43.8% of the DP.

In the long-term thermal stability testing of MDP, flavonol content was not affected by the presence of antioxidants, hyperforin content was affected by only about 2%, while hypericin residual content showed an increased stability of about 8%.

The approach to photostability testing was systematic and involved (a) tests on DS; (b) tests on the DP outside its immediate pack; (c) tests on the DP in the immediate pack (hard gelatine capsules of different colours) and (d) tests on the finished product in the marketing pack (amber containers).

DS, DP and MDP were submitted to photoexposition in thin layers (Table 5) according to the ICH guidelines. As expected, DS was more stable than DP; the total flavonols represented 84.6 and 69.5% of the initial value, respectively. Similar residual content value for hypericins (28.1%) and hyperforins (27.4%) were found in DP. DS, as expected, showed high residual content of hyperforins (47.7%), but the same percentage of hypericins (28.1%).

For the photostability testing of the product in the immediate pack, capsules of different colours were evaluated (Table 6). The lowest residual content of flavonoids was found in the transparent and white capsules 80.5 and 86.7% of the initial content values, respectively. All the other capsules showed a flavonol residual content of more than 93%. However, white capsules contained the highest content of hyperforins (about 75.7%), while dark blue capsules the highest content of hypericins (62.5% of the initial value content).

Amber containers, considered as secondary pack, did not show high positive variations with respect to the residual content of total flavonols and hypericins in the tested capsules. However, residual content of hyperforins exhibited an increase of 2–6%.

Due to unacceptable changes of residual content in the photostability testing, MDP was investigated as well (see Table 5). The total flavonol and hyperforin residual content in MDP exposed to the light was 72.6 and 47.7%, respectively, as compared with 69.5 and 27.4% found in the same product without stabilisers. Hypericin content was not affected by the presence of the stabilisers. However, if the test using the immediate and secondary packs is considered, small variations in the residual content percentage of hypericins and flavonols occurred. On the contrary, hyperforin content reached at least 85.1% of the initial value content in white capsules in amber containers (Table 6).

Table 4
Content (mg/100 mg e. s.), S.D. and content residual percentage of the capsules using DP and MDP in the long-term testing (+40°C, 75% RH)[a]

Sample, days	Flavonols		Hypericins		Hyperforins	
	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual %
DP, 15 days	10.88 ± 1.23	85.5	0.14 ± 0.02	43.8	3.60 ± 0.18	85.1
DP, 30 days	10.75 ± 1.34	84.5	0.09 ± 0.04	28.1	3.13 ± 0.08	74.0
DP, 45 days	10.60 ± 1.19	83.3	—		2.54 ± 0.13	60.0
MDP, 15 days	10.95 ± 1.42	86.1	0.22 ± 0.05	68.7	3.76 ± 0.09	88.9
MDP, 30 days	10.81 ± 1.20	85.0	0.11 ± 0.06	34.4	3.40 ± 0.14	80.3
MDP, 45 days	10.90 ± 1.30	85.7	0.07 ± 0.08	21.9	2.94 ± 0.38	69.5

[a] DP, drug product, MDP, modified drug product containing 2.85% antioxidants (ascorbic acid/citric acid 200:1).

Table 5
Content (mg/100 mg e. s.), S.D. and content residual percentage of DS, DP, and modified drug product (MDP, containing 2.58% antioxidants) after photo exposition in thin layer

Sample	Flavonols		Hypericins		Hyperforins	
	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual%
DS	10.76 ± 1.08	84.6	0.11 ± 0.00	34.3	2.73 ± 0.09	64.5
DP	8.84 ± 1.21	69.5	0.09 ± 0.01	28.1	1.16 ± 0.13	27.4
MDP	9.24 ± 0.02	72.6	0.09 ± 0.02	28.1	2.02 ± 0.10	47.7

Finally, the dissolution performance of the capsules containing DP and MDP submitted to the accelerated photodegradation and submitted to the long-term thermal stability testing did not change according to the variations of the constituents' contents.

4. Conclusions

The stability data requirements for human pharmaceutical products in the European Union (EU) are based on a series of directive and regulation requirements and a series of advisory guidelines have been developed and adopted through the International Conference of Harmonisation (ICH) procedures. A translation of these guidelines has recently been reported by the WPHMP of the EMEA in the evaluation of HD, HDP and HMP, and 90% of the labelled potency is generally recognised as the minimum acceptable potency level if active principles are not known.

As a model for the investigation of stability of HD, HDP and HMP, using ICH guidelines, a St. John's wort dried commercial extract and its preparations were evaluated. St. John's wort preparations are sold as herbal medicinal products only in part (mainly in Germany), thus the majority of them are marketed as dietary supplements (rest of Europe and USA). These latter products are subjected to a different legislation, where they cannot declare therapeutic qualities they boast of, i.e. pseudo-medicinal and health properties. All these products are standardised in total hypericins, corresponding to the 0.27–0.31% and they are marketed in US and Europe for their antidepressant activity (which was initially attributed to the flavonoids, then to the content of total hypericins and only recently to the hyperforins, even if pharmacological or clinical studies are still controversial).

Thus, in this work the evaluation of the quality and stability of St. John's wort following the preparations has been carried out through the content of all the constituents because the active principles of this HD have not been yet established. So only the stability of all the constituents present in the extract can assess if the product remains at an acceptable level throughout the period during which it is in the marketplace available for supply to the patient/consumer.

Storage at 25°C in an immediate pack which is impenetrable to light, such as aluminium tubes or cans, leads to a different t_{90} shelf life of the herbal product: several months if we considered total flavonol content, 3 months if we consider the hyperforin content and finally, only a couple of weeks if we consider the hypericins. Even if ascorbic and citric acids were added to the preparation as stabilisers, the shelf-life of the constituents increases only in part.

As regards the forced photodegradation testing, both hypericins and hyperforins resulted very unstable to light. If capsules of different colours were investigated as protective immediate pack, they affected the stability of the different classes of constituents. Thus, opaficients and inorganic and organic pigments selectively shielded the wavelengths, which cause the degradation of the different substances of the extract. From the data obtained from our studies it seems that the white and light blue capsules containing 2% of titanium dioxide, are more protective with regard to the hyperforin content. Dark blue capsules, contain-

Table 6
Content (mg/100 mg DS), standard deviation (S.D.) and content residual percentage in capsules of different colours after photo exposition

Sample	Flavonols		Hypericins		Hyperforins	
	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual%	mg/100 mg ± S.D.	Residual%
Cp B DP	12.2 ± 0.95	95.3	0.20 ± 0.07	62.5	2.86 ± 0.13	67.6
Cp T DP	10.3 ± 1.00	80.5	0.09 ± 0.06	28.1	2.53 ± 0.26	59.8
Cp LB DP	12.6 ± 1.03	98.4	0.16 ± 0.01	50.0	3.18 ± 0.06	75.2
Cp R DP	12.4 ± 1.09	96.9	0.11 ± 0.04	34.4	3.08 ± 0.08	72.8
Cp O DP	12.8 ± 1.12	100.0	0.13 ± 0.05	40.6	3.04 ± 0.13	71.9
Cp W DP	11.1 ± 1.10	86.7	0.12 ± 0.04	37.5	3.20 ± 0.15	75.7
Cp Y DP	12.0 ± 1.08	93.8	0.12 ± 0.06	37.5	2.99 ± 0.04	70.7
Cp T DP/AM	10.7 ± 1.03	83.6	0.10 ± 0.09	31.2	2.58 ± 0.31	61.0
Cp W DP/AM	11.2 ± 1.13	87.5	0.12 ± 0.12	37.5	3.45 ± 0.15	81.6
Cp T MDP	10.9 ± 1.09	85.2	0.10 ± 0.06	31.2	2.66 ± 0.19	62.8
Cp W MDP	11.3 ± 1.13	88.3	0.13 ± 0.03	40.6	3.49 ± 0.13	82.5
Cp T MDP/AM	11.1 ± 1.25	86.7	0.11 ± 0.06	34.4	2.68 ± 0.19	63.4
Cp W MDP/AM	11.7 ± 1.05	91.4	0.13 ± 0.03	40.6	3.60 ± 0.13	85.1

ing the highest percentage of the organic pigment indigotin, are more protective for the hypericins. Finally, orange capsules, containing the highest value of yellow and red iron oxides, are the most protective for the flavonols.

As a conclusion, we can assess that ICH guidelines cannot translate as such to all the herbal products. Furthermore, an alternative storage conditions should be accepted if justified, in accordance with the structure of the constituents present in the extract (i.e. lower relative humidity conditions, different temperature of storage, 20 instead of 25°C for the long-term testing, and so on), such as in the case of St. John's wort.

Standardisation based on a characteristic constituents or class of metabolites (as hypericins in St. John's wort extracts) might not offer a guarantee of pharmacological equivalence.

It is important to point out that the field of HD, HDP and HMP is confused and pharmaceutical scientists and regulators must consider not only the totality of the product-drug, excipients, and pack, as happens for pharmaceutical products, but also the presence of an extract.

Some important matters should be reconsidered during the revision of the ICH guidelines in view of their application in the field of herbal products and some important questions should be solved; what degree of change is acceptable in the field of HD, HDP and HMP? If the level of confidence about quality is the same of drug product (90% of the labelled potency), how can we establish the exact quantity of constituents we need to explain the biological activity if the quali-quantitative content of the different classes of constituents have an extreme variability? Are we sure that the clinical studies are carried out on all the extracts and preparations in which all the substances are quali-quantitatively determined?

These and many others are the matters linked to the chemical composition and biological and pharmacological activities of HD, HDP and HMP to be solved before considering them as medicines.

Acknowledgements

This work was supported by M.U.R.S.T. (Ministero dell'Università e della Ricerca Scientifica e Tecnologica), Rome.

References

Adamski, R., Styp-Rekowoska, E., 1971. Trwalosc, Hiperycyny w soku, suchym wyciagu i tabletkach z ziela dziurawka pospolitego (*Hypericum perforatum* L.). Farmacja Polska 3, 237–241.

Brantner, A., Della Loggia, R., Sosa, S., Kartnig, T., 1994. Untersuchungen zur antiphlogistichen Wirkung von *Hypericum perforatum* L. Sci. Pharm. 62, 7–8.

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*. J. Chromatogr. A 825, 9–16.

Chatterjee, S.S., Noldner, M., Koch, E., Erdelmeier, C., 1998a. Antidepressant activity of *Hypericum perforatum* L. and hyperforin, the neglected possibility. Pharmacopsychiatry 31 (1), 7–15.

Chatterjee, S.S., Bhattacharya, S.K., Wonnemann, M., Singer, A., Muller, W.E., 1998b. Hyperforin as a possible antidepressant component of hypericum extracts. Life Sci. 63 (6), 499–510.

Chatterjee, S.S., Filippo, V., Lishko, P., Maximyuk, O., Noldner, M., Krishtal, O., 1999. Hyperforin attenuates various ionic conductance mechanism in the isolated hippocampal neurons of rat. Life Sci. 65 (22), 2395–2405.

Constantine, G.H., Karchesy, J., 1998. Variations in hypericin concentrations in *Hypericum perforatum* and commercial products. Pharmac. Biol. 36, 365–367.

EMEA., 1999. Working Part on Herbal Medicinal Products (HMPWP), Stability testing of HD, HDP, and HMP, guidelines 25, p. 48.

Gobbi, M., Valle, F.D., Ciapparelli, C., Diomede, L., Morazzoni, P., Verotta, L., Caccia, S., Cervo, L., Mennini, T., 1999. *Hypericum perforatum* L. extract does not inhibit 5-HT transporter in rat brain cortex. Naunyn Schmiedebergs Arch. Pharmacol. 360 (3), 262–269.

Harrer, G., Sommer, H., 1994. Treatment of mild/moderate depressions with Hypericum Phytomedicine 1, 3–8

Working Group, Stability Testing of New Drug Substances and Products, guideline Q1A, 22 September 1994.

ICH Working Group, Photostability Testing of New Drug Substances and Medicinal Products, guideline Q1B, 6 November 1996.

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. Neurosci. Lett. 262 (3), 199–202.

Kleber, E., Obry, T., Hippeli, S., Schneider, W., Elstner, E.F., 1999. Biochemical activities of extracts from *Hypericum perforatum* L. 1st Comunication: inhibition of dopamine-β-hydroxylase Arzneimittelforschung 49(2), 106–109.

Kommanaboyina, B., Rhodes, C.T., 1999. Trends in stability testing, with emphasis on stability during distribution and storage. Drug Dev. Ind. Pharm. 25 (7), 857–868.

Laakmann, 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 (Suppl. 1), 54–59.

Maisenbacher, P., Kovar, K.A., 1992. Analysis and stability of *Hyperici oleum*. Planta Medica 58, 351–354.

Muller, W.E., Singer, A., Wonnemann, M., Hafner, U., Rolli, M., Schafer, C., 1998. Hyperforin represents the neurotransmitter reuptake inhibiting constituents of hypericum extract, Pharmacopsyachiatry Suppl. 1, 16–21.

Orth, H.C.J., Rentel, C., Schmidt, P.C., 1999. Isolation, purity analysis and stability of hyperforin as a standard material from *Hypericum perforatum* L. J. Pharm. Pharmacol. 51, 193–200.

Shakirova, K.K., Garagulya, A.D., Khazanovich, R.L., 1970. Antimicrobial properties of some species of St. John's wort cultivated in Uzbekistan. Mikrobiol. Zh. 32, 494–497.

Yip, L., Hudson, J.B., Gruszecka-Kowalik, E., Zalkow, L.H., Neil towers, G.H., 1996. Antiviral activity of a derivative of the photosensitive compound hypericin. Phytomedicine III (2), 185–190.