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Biorelevant dissolution testing of St John's wort products

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Abstract

In contrast to chemically defined drugs, most herbal medicinal products (HMPs) are poorly characterized in terms of their pharmaceutical properties. In many cases it is assumed that the plant extract as a whole is the active moiety, since it is often difficult to identify the individual components responsible for the pharmacological activity and even more difficult to assess synergies among the various components. However, where the active components have been identified, it should be possible to compare products with respect not only to content uniformity but also to their biopharmaceutical properties. The aim of this study was to investigate and compare the dissolution characteristics of several St John's wort products under biorelevant conditions. Components of St John's wort known, or suspected, to play a role in its antidepressant activity include phloroglucines, naphthodianthrones and the flavonoids. Since these groups have a broad spectrum of polarity and solubility, dissolution was studied for representative compounds from each group. Although the labelling indicates that several of the products studied should be pharmaceutically equivalent, dissolution under biorelevant conditions revealed that they have quite different release profiles and cannot be considered switchable. It was concluded that biorelevant dissolution testing can be a powerful tool for comparing HMPs as well as synthetically produced drug products.

Introduction

The safety and efficacy of drug products can only be guaranteed when their quality is reliable and reproducible from batch to batch. To ensure the requisite quality, drug manufacturers are required to test their products during and after manufacture and at various intervals during the shelf life of the product. In addition to confirming content uniformity and compliance with the label strength, for oral dosage forms it is also necessary to test whether the drug product has, and maintains, the release properties required to effect absorption from the gastrointestinal tract.

Guidelines for dissolution testing of oral dosage forms have been developed by the FDA and the FIP (FDA Guidance for Industry 1997; Siewert 1997). The application of these guidelines to herbal medicinal products (HMPs) is hindered by the lack of certainty about which components of the extract actually contribute to its activity – in many, if not most, cases it is suspected that several components may play a role.

The regulatory situation in developed countries with respect to quality control of HMPs varies considerably. Although in a few countries (e.g. Germany) the quality requirements for dosage forms containing single active entities are also in general applicable to HMPs, in other countries (e.g. USA) the main drug regulatory agency has little influence over HMPs, since many are introduced to the market as nutritional supplements. With respect to nutritional supplements, there is a chapter in the USP which provides guidelines for dissolution testing (USP 25a). However, these guidelines are intended primarily for products containing trace elements and vitamins. In cases where there are several vitamins and minerals in the preparation, the manufacturer is only required to characterize the dissolution of one representative substance. The USP requirement is that more than 75% of the label strength of this substance must be released within one hour under the test conditions chosen.

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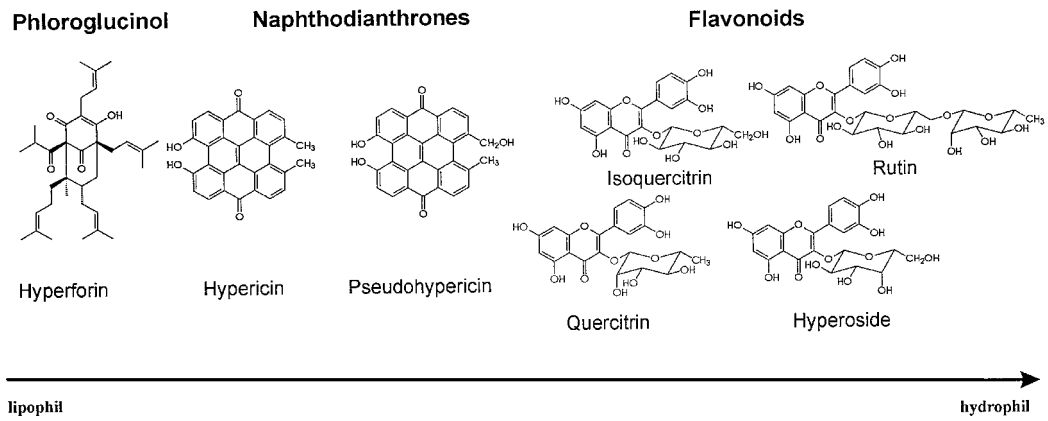

Figure 1 Chemical structure of ingredients from St John's wort.

By contrast, the European monograph (still under discussion) proposes three categories for herbal medicinal products according to how well their active components have been characterized (Lang & Stumpf 1999). Category A (standardized extracts) includes those HMPs for which the active moiety has been definitively identified. Examples include silymarin, aescin and sennoside. Category B (quantified extracts) includes those products for which the active ingredient(s) has not been clearly identified, but for which substances have been identified which are known to contribute to the activity. Examples include ginsenosides, procyanidines, flavonoides and hyperforin. Last but not least is category C. This category includes herbal medicinal products for which no individual active ingredients have been identified, but which have a traditional place in the therapy of certain diseases. Examples here include Extr. Valerianae and Extr. Echinaceae.

In Europe, the EMEA (European Agency for the Evaluation of Medicinal Products) does not require dissolution tests for HMPs falling under category B or C if the extract is formulated as an immediate-release product. Further, if the active ingredient is known to be highly soluble in aqueous solutions at pH values typical of the gastrointestinal tract, a disintegration test may be substituted for the dissolution test (EMEA 1999). In Germany, the situation varies a little from the EMEA guidelines, requiring that all herbal medicinal products belonging to Category A be subjected to dissolution tests. Similar to the EMEA guideline, those extracts belonging to categories B and C need not undergo dissolution tests so long as they are formulated as immediate-release products (Bundesanzeiger 1997).

Clearly, one could argue that whenever the components that contribute to the activity have been identified, that dissolution testing can and should contribute to an evaluation of the pharmaceutical and biopharmaceutical quality of the product. This would mean that dissolution testing is appropriate for HMPs containing either category A or B extracts. St John's wort extract is a typical example of a category B extract. It has been well documented in the pharmacological literature that the lipophilic phloroglucine derivative, hyperforin (2–5% of the extract; Figure 1), contributes to the antidepressive activity of the extract (Wonnemann et al 2000; Greeson et al 2001). Still under discussion is the role of the naphthodianthrones (Figure 1), which are present in the extract in a concentration of 0.15–0.40% (Nahrstedt & Butterweck 1997). These compounds tend to exhibit medium polarity. It is also thought that the more hydrophilic flavonoids (Figure 1), present in a concentration of about 2–4%, may contribute to the pharmacological activity (Butterweck et al 2000). To determine how useful dissolution testing might be in characterizing the biopharmaceutical properties of St John's wort products, five products were selected from those currently available on the German market and the dissolution of typical phloroglucines (hyperforin), naphthodianthrones (hypericins) and flavonoids (isoquercitrin, quercitrin, rutin and hyperoside) was characterized to cover the entire spectrum of polarities present in the extract. In addition to standard compendial media, recently developed biorelevant media (Galia et al 1998) were used in an attempt to better simulate in-vivo release and therefore to better predict the influence of the formulation on the bioavailability of the extract.

Materials and Methods

Drugs

The drug products selected for study are listed in Table 1. All were purchased from pharmacies in the Frankfurt/Main area.

Sodium taurocholate (purity >98%) was obtained from Tiefenbacher GmbH (Hamburg, Germany). Egg-lecithin (Lipid E PC 99.1% pure) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Potassium chloride, sodium chloride, sodium hydroxide, hydrochloric acid, acetic acid and potassium hydrogen phosphate, all analytical grade, were purchased from E. Merck (Darmstadt, Germany). Acetonitrile, ethanol, methanol (Rotisolv quality) were obtained from Carl Roth GmbH (Darmstadt, Germany). For the preparation of mobile phase for the HPLC, Milli-Q water was used.

Table 1 Characteristics of the products studied.

Product	Trade name	Dosage form	DEV[a]	Extraction solvent
300 mg extract per dosage form				
P1	Jarsin 300	Sugar-coated tablet	4–7:1	Methanol 80%
P2	Neuroplant 300	Film tablet	2.5–5:1	Ethanol 60%
P3	Texx 300	Film tablet	4–7:1	Methanol 80%
425 mg extract per dosage form				
P4	Felis 425	Capsule	3.5–6:1	Ethanol 60%
612 mg extract per dosage form				
P5	Laif 600	Film tablet	5–8:1	Ethanol 50%

[a]Raw material:extract ratio (on a dry basis).

Drug products were assayed as follows: 10 dosage-form units of each product were weighed (in the case of capsules, after emptying the capsule shell) and mixed together in an analytical mill until homogenous. A mass equivalent to that of one dosage form unit was then transferred into a 50-mL standard flask and brought to volume with 80% ethanol. After sonicating for 10 min at room temperature, the samples were filtered (0.45 μm polypropylene filters), and transferred directly into brown glass vials for assay of hypericines and hyperforin. For analysis of flavonoids, 200 μL of the filtered sample was diluted before HPLC analysis using 300 μL 80% ethanol and 500 μL of mobile phase.

Dissolution testing

Dissolution testing was conducted in Type 2 (Paddle) apparatus of the USP at a temperature of 37 ± 0.5°C. Since hyperforin is known to be light sensitive, all tests were conducted under reduced light. Hydrodynamic conditions were standardized at 100 rev min^{-1} and 500 mL medium for each test. For each product under each dissolution condition, three tests were run in parallel. Dissolution of hyperforin and flavonoids was performed with one dosage form in each vessel. Due to problems with the minimum quantifiable concentration for investigation of hypericin release, five dosage forms were used in each vessel in each test. Samples (5 mL) were withdrawn at appropriate intervals and replaced immediately with pre-warmed medium. Samples were filtered through 0.45-μm poly(tetrafluoroethylene) (PTFE) filters, discarding the first 2 mL and transferring about 1.2 mL into brown glass HPLC vials and about 2.5 mL into brown glass polarography vials. No further work-up was required before analysis.

Dissolution media

Simulated Gastric Fluid (SGF) was prepared according to the USP (USP 25b), without pepsin. FaSSIF (Galia et al 1998), used to simulate conditions in the fasted state in the proximal small intestine, contains 3 mmol sodium taurocholate and 0.75 mmol lecithin in a pH 6.5 phosphate buffer adjusted to isoosmolarity. FeSSIF (Galia et al 1998) is used to simulate fed-state conditions in the proximal small intestine and contains 15 mmol sodium taurocholate and 3.75 mmol lecithin in a pH 5 acetate buffer adjusted to an osmolarity of 670 mOsm.

Analyses

HPLC analysis was used to quantify dissolution of hyperforin and the flavonoids. Hyperforin analyses were carried out on a 5-μm, 125 × 4 mm Merck LiChrospher 100 RP-8 column with acetonitrile–phosphate buffer pH 2.1 (83:17; isocratic) as the eluent system and a 1 mL min^{-1} flow rate. Injection volume was 20 μL and detection wavelength 254 nm. Hyperforin concentrations were determined relative to an external standard, kindly donated by Schwabe GmbH (Karlsruhe, Germany). The method was validated over the range 0.73–400 μg mL^{-1}. Flavonoids were separated on a 4-μm, 250 × 4 mm Merck Supershper RP-18e column. A gradient elution was performed with acetic acid–mixture of acetonitrile with methanol (3:1) as the mobile phases. The flow rate was 1 mL min^{-1} and the injection volume was 20 μL. Peaks were detected at 354 nm. Both methods were validated according to ICH guidelines (ICH Topic Q2A 1994; ICH Topic Q2B 1996). Flavonoid concentrations were also determined via an external standard method using rutin, quercitrin and hyperoside as the standard substances (Roth, Karlsruhe, Germany). The validated range for rutin was 0.29–100 μg mL^{-1}, for the combined concentration of hyperoside and isoquercitrin 0.56–50 μg mL^{-1} and for quercitrin 0.32–20 μg mL^{-1}.

Total hypericin (hypericin and pseudohypericin) concentrations were measured using an electrochemical method (DPP; differential pulse polarography). Samples were analysed after exposure to a daylight-lamp for 30 min using a standard addition process according to the method of Michelitsch et al (2000).

Statistical methods

All results are presented as the mean (n = 3). The percentage dissolved at defined time points related to the specified dissolution curves were statistically compared

using the Kruskal–Wallis test for non-parametric multiple comparisons.

Results

The content of the various components in the five St John's wort products studied is shown in Table 2. These values were then used as the 100% values for calculating dissolution test results.

Dissolution of hyperforin

Dissolution was first conducted in Simulated Gastric Fluid USP 25 (without pepsin) (SGF_{sp}), since the stomach is the first part of the gastrointestinal tract to come in contact with the dosage form. In this medium, no hyperforin dissolution could be detected (Figure 2). To determine whether this lack of release might be due to wettability problems, Triton X 100 (0.1%) was added to the medium. However, no release was observed, even after addition of the surfactant.

In conditions simulating the fasted state in the proximal small intestine, dissolution of hyperforin was also poor (about 5%). Studies comparing dissolution in FaSSIF in the presence and absence of the bile components (sodium taurocholate and lecithin) revealed that most of the (modest) increase in the percentage release compared with SGF_{sp} could be attributed to the bile components. When the concentration of bile components was further increased to approximate postprandial conditions in the proximal small intestine, the dissolution of hyperforin was substantially improved, with 90% release from Texx 300 within 2 h (Figure 2). The results for the other products are compared with those of Texx 300 in Figure 3. The results indicate that, even in the medium with the most advantageous composition for the release of hyperforin, some of the products performed poorly. Neither Jarsin 300 nor Felis 425 released more than 50% of their hyperforin content within 2 h of testing in FeSSIF.

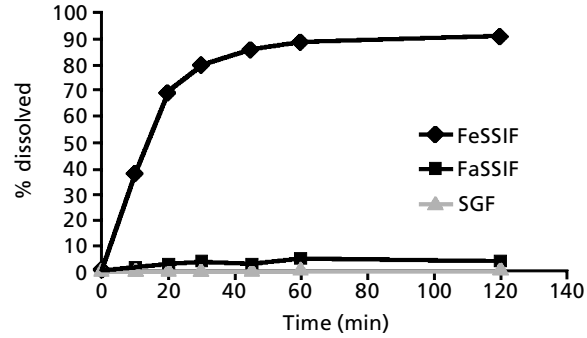

Figure 2 Dissolution of hyperforin from Texx 300 in different media (*P*-values at 10, 20, 30, 45, 60 and 120 min: 0.0143, 0.0036, 0.0036, 0.0036, 0.0036 and 0.0036, respectively).

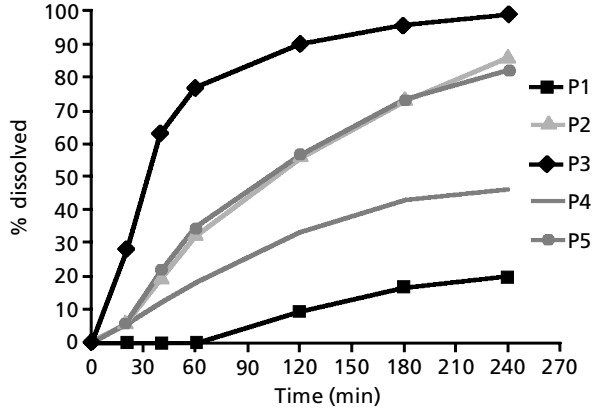

Figure 3 Dissolution of hyperforin in FeSSIF (P1, Jarsin 300; P2, Neuroplant 300; P3, Texx 300; P4, Felis 425; P5, Laif 600 (*P*-values at 20, 40, 60, 120, 180, and 240 min: 0.0311, 0.0060, 0.0033, 0.00017, 0.00017 and 0.00017, respectively).

Dissolution of total hypericin

As for hyperforin, no dissolution of hypericines could be detected in SGF. In FaSSIF the dissolution was considerably better, with up to 55% of the total hypericin content

Table 2 Hyperforin, total hypericin and flavonoid content of the five St John's wort products studied.

Analyte	P1	P2	P3	P4	P5
Hyperforin (mg/dosage form)	9.82 (0.71)	12.39 (0.92)	7.67 (0.34)	12.77 (1.56)	15.19 (0.46)
Hyperforin (% in extract)	3.27	4.13	2.56	3.00	2.48
Rutin (mg/dosage form)	8.48 (3.4)	9.85 (4.38)	8.81 (2.52)	14.60 (2.91)	10.82 (4.53)
Rutin (% in extract)	2.83	3.28	2.94	3.44	1.77
Hyperoside/isoquercitrin (mg/dosage form)	15.33 (3.34)	9.31 (4.43)	12.18 (2.51)	18.27 (3.04)	19.45 (4.82)
Hyperoside/isoquercitrin (% in extract)	5.11	3.10	4.06	4.29	3.18
Quercitrin (mg/dosage form)	1.07 (2.64)	0.71 (4.70)	1.23 (2.86)	1.51 (2.92)	2.67 (4.87)
Quercitrin (% in extract)	0.36	0.24	0.41	0.36	0.44
Total hypericin (mg/dosage form)	0.83 (3.46)	0.61 (7.14)	0.75 (3.44)	1.14 (7.27)	1.92 (5.98)
Total hypericin (% in extract)	0.28	0.20	0.25	0.27	0.31

Relative s.d. (%) is given in parentheses.

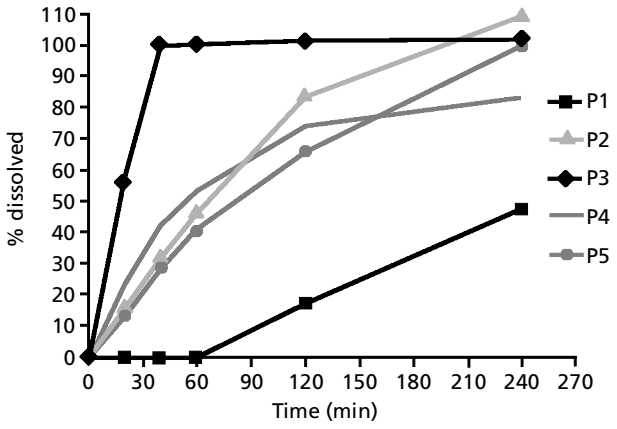

Figure 4 Dissolution of rutin in FeSSIF (P1, Jarsin 300; P2, Neuroplant 300; P3, Texx 300; P4, Felis 425; P5, Laif 600 (*P*-values at 20, 40, 60, 120, and 240 min: 0.0011, 0.00080, 0.00080, 0.00047 and 0.0012, respectively).

released within 2 h. This percentage may represent a falsely low value, due to an artefact of the experiment. Because five dosage-form units per vessel had to be used to obtain quantifiable concentrations, some coning occurred at the bottom of the vessel. This phenomenon is known to inhibit dissolution and lead to poorer than expected results (Beckert & Quach 1996). Due to analytical problems (interference of the medium), no results could be obtained in FeSSIF.

Dissolution of flavonoids

The dissolution of four flavonoids, rutin, hyperoside, isoquercitrin and quercitrin, was followed. Because the structures of hyperoside and isoquercitrin are closely related (see Figure 1), complete separation by HPLC was not possible. Isoquercitrin concentrations were therefore calculated together with those for hyperoside and the results presented as the sum concentration.

The relatively hydrophilic flavonoids dissolved well into all media tested.

To compare results among products, dissolution in FeSSIF was used, since this enabled us to simultaneously characterize the dissolution of both hyperforin and the flavonoids from the various products. Results for rutin are shown in Figure 4.

As for hyperforin, the products varied widely with respect to the release of rutin in FeSSIF. Whereas product Texx 300 released 100% of its rutin content within 30 min, Jarsin 300 released less than 50% within 4 h. Table 3 summarizes the results for all of the products with respect to hyperforin and flavonoid release.

Discussion

The need for quality assurance, including confirmation of label strength, content uniformity and release properties, has long been recognized for drug products containing chemically defined, synthetically produced drugs.

Table 3 Dissolution of investigated analytes from the St John's wort preparations studied.

Product	Analyte	Percentage dissolved after				
		20 min	40 min	60 min	120 min	240 min
P1	Rutin	< LOQ	< LOQ	0.86 (173)	17.03 (63.4)	47.35 (5.6)
	Σ Hyperoside, isoquercitrin	< LOQ	< LOQ	0.78 (173)	17.32 (62.8)	47.19 (4.8)
	Quercitrin	< LOQ	< LOQ	< LOQ	19.31 (87.8)	50.66 (3.3)
	Hyperforin	< LOQ	< LOQ	< LOQ	9.16 (0.4)	20.11 (0.3)
P2	Rutin	15.70 (83.2)	32.17 (61.9)	45.99 (30.0)	83.51 (8.8)	109.22 (7.5)
	Σ Hyperoside, isoquercitrin	14.64 (86.9)	30.62 (63.4)	44.06 (30.5)	81.24 (9.9)	106.61 (7.6)
	Quercitrin	15.02 (91.1)	30.67 (63.8)	47.41 (30.0)	81.21 (9.6)	107.38 (5.6)
	Hyperforin	5.97 (104.3)	19.97 (65.3)	32.44 (40.9)	55.74 (12.8)	86.03 (9.5)
P3	Rutin	58.19 (19.6)	99.41 (0.4)	100.14 (1.4)	101.40 (1.0)	101.59 (1.2)
	Σ Hyperoside, isoquercitrin	55.80 (19.9)	96.02 (0.7)	96.51 (1.2)	97.91 (1.2)	97.99 (0.8)
	Quercitrin	56.64 (19.5)	96.50 (0.7)	96.11 (2.6)	99.24 (2.3)	97.52 (1.1)
	Hyperforin	28.29 (12.8)	63.17 (2.7)	76.71 (2.2)	90.16 (3.9)	99.02 (0.6)
P4	Rutin	23.26 (28.5)	42.26 (24.3)	53.14 (13.0)	74.09 (15.6)	83.00 (13.8)
	Σ Hyperoside, isoquercitrin	22.46 (28.8)	40.67 (24.5)	51.33 (13.4)	71.48 (15.8)	80.00 (14.1)
	Quercitrin	24.28 (30.0)	40.24 (27.4)	50.10 (16.5)	69.58 (22.0)	81.09 (14.8)
	Hyperforin	6.08 (29.2)	12.46 (37.3)	18.59 (34.4)	32.63 (14.9)	45.97 (32.1)
P5	Rutin	13.31 (13.7)	28.78 (1.0)	40.67 (0.9)	65.75 (1.7)	99.78 (1.2)
	Σ Hyperoside, isoquercitrin	12.52 (13.6)	27.37 (1.4)	38.93 (0.9)	63.31 (2.0)	96.49 (1.5)
	Quercitrin	12.36 (12.9)	27.84 (0.9)	38.42 (0.7)	62.77 (3.8)	96.17 (0.5)
	Hyperforin	5.96 (33.6)	21.65 (2.2)	34.25 (0.2)	56.77 (2.0)	82.07 (4.2)

LOQ = lower limit of quantification. Relative s.d. (%) is given in parentheses; n = 3 vessels.

Relatively recently, a discussion about the extension of these concepts to HMPs has been initiated, with the result that it is now the norm (at least in Germany) for information such as the composition of the extraction fluid, the ratio of raw material to extraction fluid and the amount of extract in the product to appear on the label. Moreover, the better manufacturers strive to maintain batch-to-batch conformity of their herbal medicinal products by appropriate blending of extracts. Due partly to the variability in the raw material used to obtain the extract, however, it is not always possible to achieve the kind of content uniformity usually expected of products containing chemically defined actives (Wurglics et al 2001). But even when the batch-to-batch content conformity is good, this doesn't necessarily guarantee that the product will be bioequivalent, since absorption from the gastrointestinal tract will depend, at least partly, on the release profile of the actives from the dosage form. Dissolution testing is the traditional yardstick of release from the dosage form, and in recent years, the focus of dissolution testing has moved increasingly to the prediction of bioavailability and bioequivalence (Shah 2001). For these purposes, it is advantageous to simulate conditions in the gastrointestinal tract in the in-vitro test. Up till now, biorelevant dissolution testing has been used primarily for oral products containing single, chemically defined active ingredients, but the time is ripe to consider applying these tests to the biopharmaceutical evaluation of HMPs belonging to categories A and B as well.

The advantages of the biorelevant media in comparison with typical compendial media for dissolution testing of herbal products are clearly demonstrated in this study. Although the dissolution of hydrophilic components such as the flavonoids can be adequately studied in simple aqueous media, the more lipophilic components (in the case of St John's wort, hypericines and hyperforin) are not sufficiently soluble in simple aqueous media to be able to compare release among products. The biorelevant media, which contain bile components at concentrations typically found in the gastrointestinal tract, offer the possibility of comparing release among formulations in a way that should reflect the in-vivo release. The dissolution results in the biorelevant media clearly demonstrate that there are glaring differences in the release properties of the various products studied and that these products could not be considered interchangeable. Since the dissolutions in FeSSIF were all conducted under sink conditions and the results were all normalized to the measured content of the various components in the products studied, the differences in the profile shapes must be attributed to differences in formulation among the products and not to differences in the product content.

According to the film model for dissolution (Dressman et al 1998), differences in the dissolution profiles could result from solubility, surface area, hydrodynamic or diffusivity differences among experiments. Of these factors, only the hydrodynamics were held constant by standardizing the test design in terms of volume of medium and stirring rate. Excipients in the formulation could influence solubility and wettability of the actives and perhaps exert a minor effect on their diffusivity. In addition, the particle size of the dry extracts used to manufacture the product could play a role in the release rate. Although the influence of formulation factors such as these on the biopharmaceutical properties of the product has been repeatedly documented for products containing chemically defined active ingredients (especially those which are lipophilic and tend to exhibit poor dissolution), it is just beginning to be appreciated that these factors could be equally important for the in-vivo performance of HMPs.

In the case of St John's wort, the evidence for the contribution of the lipophilic component, hyperforin, to the antidepressant activity is convincing. A comparison of the dissolution results in the various media indicates that the release of this component is far better under fed-state (FeSSIF) conditions than under fasted-state (SGF, FaSSIF) conditions. These results suggest that ingesting the dosage form with or after a meal may likely favour absorption of the hyperforin fraction of the extract.

Combined with our previous data concerning the batch-to-batch conformity of various St John's wort products (Wurglics et al 2001), the dissolution data suggest that even when the labelling suggests that two products are pharmaceutically equivalent (e.g. Jarsin 300 and Texx 300), neither the hyperforin content nor its release from the dosage form under biorelevant conditions could be considered essentially similar for the two products. It is therefore recommended that patients taking St John's wort products should not be switched from one product to another without considering the possibility that the dose may have to be adjusted to maintain the same therapeutic effect.

Conclusion

In this study, the advantages of the biorelevant media in comparison with typical compendial media for dissolution testing of herbal medicinal products are clearly demonstrated. The results of dissolution in biorelevant media show that there are glaring differences in the release properties of various products containing St John's wort extract. Therefore, it was concluded that these products could not be considered interchangeable. As a result, it is recommended that patients taking St John's wort products should not be switched from one product to another without considering the possibility that the dose may have to be adjusted to maintain the same therapeutic effect.

Additionally, the results suggest that ingesting the dosage form with or after a meal may likely favour absorption of the hyperforin fraction of the extract.

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