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Excipient compatibility study of *Hypericum perforatum* extract (St. John's Wort) using similarity metrics to track phytochemical profile changes

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Abstract

The formulation of botanical dietary supplements is challenging due to their complex activity–composition relationship, as well as physical and chemical stability issues. As excipient compatibility testing is a major component of sound formulation development, the objectives of this work were: (1) explore excipient compatibility storage paradigms; (2) determine interactions between phytochemicals of interest in Saint John's Wort (SJW) with several excipients; and (3) explore the application of similarity metrics to the data. Modifications to conventional isothermal stress testing paradigms included additional storage conditions of heat and moisture (5, 50 °C, 5 and 0% added water), as well as more rigorous controls. Binary blends of SJW and ten commonly used excipients were prepared and neat SJW was used as control. After 3 weeks, the percentage remaining of each phytochemical was determined by HPLC. Several similarity metrics were applied to the data. Common storage paradigms were suitable for excipient compatibility testing when controls of neat material are stored under similar conditions and the percentage of phytochemicals remaining in excipient:SJW blends and neat SJW are compared. Excipient incompatibilities were determined for SJW phytochemicals of interest. Similarity metrics applied to the phytochemical profiles conveniently summarized the data. This work allows logical decisions to be made regarding the formulation of SJW.

Keywords: *Hypericum perforatum* extract; Excipient compatibility testing; Botanical formulation; Similarity metrics

1. Introduction

Botanical dietary supplements are often used to promote health. One of the most popular botanical dietary supplements is *Hypericum perforatum* extract, commonly known as Saint John's Wort (SJW). It is indicated in the treatment of mild to moderate depression, and is typically dosed at 300

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mg standardized to 0.3% hypericins, taken three times daily. *H. perforatum*, like most botanicals, is quite complex chemically. The complex phytochemical profile of SJW consists of several groups of phytochemicals including the phenolic acids (chlorogenic acid) (1), flavonoids (rutin, hyperoside, isoquercitrin, quercitrin, quercetin) (2–6), napthodianthrones (hypericin, pseudohypericin) (7, 8), and the phloroglucinols (hyperforin, adhyperforin) (9–10). Pharmacologic activity has been attributed to several phytochemicals within SJW. The flavonoids (2–6) may have some anti-depressant activity; (Butterweck et al., 2000; Sparenberg et al., 1993; Calapai et al., 1999, 2001) as well as antioxidant activity (Sloley et al., 2000), which may increase overall extract efficacy by preventing oxidative degradation of other phytochemicals within the SJW matrix. Napthodianthrones, commonly used as marker compounds for SJW standardization, were once thought to impart SJWs anti-depressant activity, but actually demonstrate anti-viral activity (Lavie et al., 1995; Chatterjee et al., 1998b). Current research indicates that the phloroglucinols are the major contributors of anti-depressant activity of SJW, as hyperforin has demonstrated re-uptake inhibition of the neurotransmitters serotonin, norepinephrine, and dopamine, and this response may possibly be dose-related (Chatterjee et al., 1998a; Laakmann et al., 1998; Melzer et al., 1998). Many researchers agree that the overall activity of SJW extract cannot be attributed solely to hyperforin content, and either there are other constituents with anti-depressant activity, or hyperforin's activity is modulated by other phytochemicals. Therefore, it is important to determine the influence of excipients on several phytochemicals of interest, and not merely one or two marker compounds. Excipient compatibility testing of the phytochemical profile is especially necessary given the unstable nature of phytochemicals. Several researchers have reported the instability of many of these compounds to heat, light, oxygen, alkaline pH and elevated humidity (Maisenbacher and Kovar, 1992; Lavie et al., 1995; Fourneron et al., 1999; Orth and Schmidt, 2000; Bilia et al., 2001; Kopelman et al., 2001). Orth investigated the use of ascorbic acid:citric acid (10:1), sodium bisulfite, DL-α-tocopherol, PEG 6000 solid dispersions, and various cyclodextrins on the stability of isolated hyperforin, with modest improvements seen with ascorbic acid:citric acid (10:1) and 1.8-methyl-β-cyclodextrin inclusion complex. Recently, Bilia et al. utilized Orth's ascorbic acid:citric acid chemical stabilizers (except the ratio of acorbic acid:citric acid was altered [200:1]) in SJW preparations for stability studies. This work demonstrated the challenge of simply applying ICH guidelines for long term stability (25 °C/60% RH) and accelerated stability studies (40 °C/75% RH) to the storage of two SJW capsule formulations. Overall, the classes of phytochemicals exhibited variable stability under the different conditions. The complex phytochemical profile of SJW presents a unique challenge to establishing product stability and excipient compatibility. Although not excipient compatibility studies per se, the effect of different excipients on botanicals other than SJW individual phytochemical yields after spray drying have been evaluated with variable results (Soediro-Soetarno et al., 1984; Casadebaig et al., 1989; Moura et al., 1995). As excipient compatibility testing is clearly essential for sound formulation development, especially with unstable compounds, surprisingly there is a dearth of information regarding excipient compatibility testing of botanical extracts.

Conventional isothermal stress testing approaches to drug-excipient compatibility evaluation typically involve challenging drug:excipient mixtures (in realistic ratios) with moisture, as the majority of drug degradation reactions involve moisture. These blends may be binary, or for excipient cross comparisons, tertiary or higher. Increasing the moisture content may be accomplished by increasing the relative humidity of the environment, or by adding a fixed amount of water. Serajuddin et al. argue that with exposure of the samples to high humidities, the excipient–drug interaction depends upon the free moisture present and relative hygroscopicities. Drug degradation thus may vary depending on the hygroscopicity of the excipients (Patel et al., 1998; Serajuddin et al., 1999). Therefore, it is suggested that a constant amount of water be added to facilitate interactions between the excipient and

drug, and to surround undissolved particles with an aqueous layer saturated with drug, excipient, and any impurities present, as well as the microenvironmental pH. Serajuddin et al. suggest 20% added water; however, literature values range from 5 to 20% added water (van Dooren and Duphar, 1983; Patel et al., 1998; Serajuddin et al., 1999). Blends of drug and excipient are stored refrigerated without added water as controls. Samples typically are stored at 50 °C with 20% added water, protected from light if necessary, for 3 weeks. Data are reported as percentage drug remaining compared to controls. Since botanicals have complex phytochemical profiles as well as possible instability towards heat, light, moisture, pH etc. this approach must be modified to discern the true effects of the excipients. Optimum sample storage conditions must be rigorous enough to promote an interaction, yet not destroy the samples. A more complex system of controls is necessary to differentiate the effects due simply to the storage conditions from those effects attributable to true phytochemical–excipient interactions.

The complex phytochemical profile makes the reporting of compatability data difficult, and it is preferable to account for the influence of all major phytochemicals within the matrix, rather than one or two marker components. One approach to promote conciseness in the comparison of multi-component profiles is the use of similarity metrics. Similarity metrics have been used to compare dissolution profiles and pH profiles for bioequivalence studies (Moore and Flanner, 1996; Polli and McLean, 2001).

These methods have been referred to as direct curve comparison metrics, since the entire shapes of the two profiles are directly compared. All data points are utilized. The profiles are compared at the same time points, resulting in a single evaluation.

Although these methods have been used for comparison of dissolution profiles and plasma profiles, they are adapted here to compare phytochemical profiles. By substituting the %w/w remaining of each phytochemical (1–9) for the % dissolved or concentration at each time point in excipient: SJW blends or SJW neat compared to controls, the neat SJW phytochemical profile and excipient:SJW phytochemical profile may be compared. For example, in the comparison of lactose:SJW to SJW neat stored under the same conditions, the % remaining of each phytochemical in the lactose:SJW blend is compared to the % remaining of the corresponding phytochemical in the SJW neat. That is, $n = 9$ for nine phytochemicals of interests, with test (T) representing SJW:excipient blends, and reference (R) equivalent to SJW neat. The similarity of the phytochemical profiles of the blend and the neat material may be assessed, necessarily accounting for contributions from each phytochemical.

The f_2 test (Eq. (1)) was introduced by Moore and Flanner, and is used in the SUPAC IR guidance to assess the impact of various formulation and manufacturing changes on drug dissolution. In the context here of comparing phytochemical profiles, f_2 is,

$$f_2 = 50\log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(Rt - Tt)^2\right]^{-0.5} \times 100\right\}, \quad (1)$$

where f_2 is the similarity factor, R_t and T_t are the percentages of the phytochemical of interest remaining (t = phytochemicals 1–9) for reference and test materials, respectively. When $f_2 = 50$–100, the two profiles are considered to be similar, as this range indicates an average point-to-point difference of 10% or less.

Recently, Polli and McLean introduced the use of ratio metrics (ρ) for comparison of two plasma profiles. The metrics compare entire profiles and utilize all plasma profile data, thus reflecting the similarity or dissimilarity of the entire profiles. In the context of comparing phytochemical profiles, the equations are

$$\rho = \frac{\sum_{t=1}^{n}(Rt + Tt) \times \mathrm{RATIO}t}{\sum_{t=1}^{n}(Rt + Tt)}$$

(similar when $\rho < 1.1$), (2)

$$\rho^u = \frac{1}{n}\sum_{t=1}^{n}\mathrm{RATIO}t \quad \text{(similar when } \rho^u < 1.1\text{)}, \quad (3)$$

$$\rho_h^u = \frac{1}{n}\sum_{t=1}^{n} \mathrm{RATIO}t + \%\mathrm{Hyperforin}$$

(similar when $\rho_h^u < 1.1$), (4)

where ρ is the comparison metric, and all n pairs of points are included by using the ratios of concentration of test (T) and reference (R) of phytochemical t, where the larger of T/R or R/T is employed (RATIO_t). Eq. (2) ρ is weighted towards higher concentrations by the sum of the test and reference concentrations and considers RATIO_t ($\rho \geq 1$). Eq. (3) (ρ^u) is the unweighted metric where all time points and pairs of data are given equal importance. Eq. (4) (ρ_h^u) is weighted towards hyperforin, the phytochemical that has shown the most promising anti-depressant activity, by counting contributions to the profile from hyperforin twice. These metrics may aid in the comparison of complex phytochemical profiles.

The objectives of this work were: (1) to explore excipient compatability storage paradigms; (2) assess the extent of interactions between phytochemicals of interest (1–9) in SJW with ten commonly used excipients from various functional categories; and (3) to explore the application of various similarity metrics to the control and excipient:SJW blend phytochemical profiles as an aid in the determination of a feasible formulation.

2. Materials and methods

2.1. Excipients and extract

Excipients were chosen based on their functionality, widespread use in commercial SJW products, and physico–chemical properties. Fillers (dibasic calcium phosphate, microcrystalline cellulose, pregelatinized starch, anhydrous lactose), lubricants (magnesium stearate, hydrogenated vegetable oil), disintegrants (croscarmellose sodium, crospovidone), and stabilizers (ascorbic acid:citric acid (10:1), malic acid) were examined. All excipients were USP/NF grade.

The excipients and extract were obtained from the following suppliers: magnesium stearate (Mallinkrodt Baker, Paris, KY); hydrogenated vegetable oil, dibasic calcium phosphate, microcrystalline cellulose (Penwest, Patterson, NY); crospovidone (ISP, Wayne, NJ); croscarmellose sodium (FMC, Newark, DE); ascorbic acid, citric acid, malic acid (Spectrum, New Brunswick, NJ); anhydrous lactose (Sheffield, Norwich, NY); pregelatinized starch (Colorcon, West Point, PA); Saint John's Wort Extract (SJW) (Pure World Botanicals, Hackensack, NJ).

2.2. Analytical solvents, standards, and method used were described previously (Kopelman et al., 2001)

2.2.1. Sample preparation

The excipient compatibility protocol was modeled loosely on that proposed by Serajuddin et al. Realistic ratios of excipient to drug were based on a dosage form containing 300 mg SJW, with a target fill weight of 400 mg. Binary blends of SJW (75%—300 mg) and lubricants (0.5%—2 mg), disintegrants (6%—24 mg), fillers (17.5%—70 mg), and stabilizers (1%—4 mg) were prepared by weighing SJW and the appropriate amount of excipient into inert glass vials. The vials were protected from light with a foil covering. A constant percentage of moisture was added to some of the samples to facilitate phytochemical–excipient interactions. Acceptable results with added water (aw) from 5 to 20% have been reported (Carstensen et al., 1964; Gu et al., 1990; van Dooren and Duphar, 1983). Since previous work indicated that many of the phytochemicals in SJW are highly sensitive to moisture, 5% water was added (Kopelman et al., 2001). To further facilitate intimate contact between excipient and SJW particles and promote interactions, the excipient and SJW were blended by briefly (5 s) placing the glass vial on a vortex blender.

2.2.2. Sample storage and analysis

As many phytochemicals are unstable towards heat and moisture, several storage conditions of both the binary blends and neat SJW were necessary to determine the true influence of the excipients on the phytochemical profile. Binary blends of SJW and Excipient and SJW neat were stored at 5 °C/0% aw as controls; 5 °C/5% aw, 50 °C/

0% aw and 50 °C/5% aw. Analysis of the phytochemical profile was performed on days 0 and 21. The entire contents of each vial were analyzed. Samples were visually analyzed weekly for changes in appearance.

2.3. Data analysis

The data are presented as % remaining from controls (samples stored at 5 °C/0% aw). To elucidate the true interactions due to excipients from degradation of phytochemicals within SJW extract itself, several similarity metrics (f_2, ρ, ρ^u, ρ_h^u) were applied to the data to compare the phytochemical profile of SJW neat to SJW:excipient blends.

3. Results

3.1. Storage conditions

Compared to controls (5 °C/0% aw), storage conditions clearly influence the % phytochemical remaining. Storage with increased moisture (5 °C/5% aw) or increased temperature (50 °C/0% aw) resulted in slight decreases in % phytochemical remaining for almost all excipient:SJW binary blends, as exemplified by the lactose:SJW blends in Fig. 1. More dramatic differences are seen with the combination of increased temperature and humidity (50 °C/5% aw). Therefore, these conditions were used to examine excipient compatibility.

3.2. Excipient compatibility

3.2.1. Fillers

At 50 °C/5% aw, binary blends of microcrystalline cellulose:SJW and pre-gelatinized starch:SJW appear to retain a greater percentage of each phytochemical constituent (Fig. 2). Dibasic calcium phosphate and lactose performed similarly, with much less of each phytochemical constituent surviving.

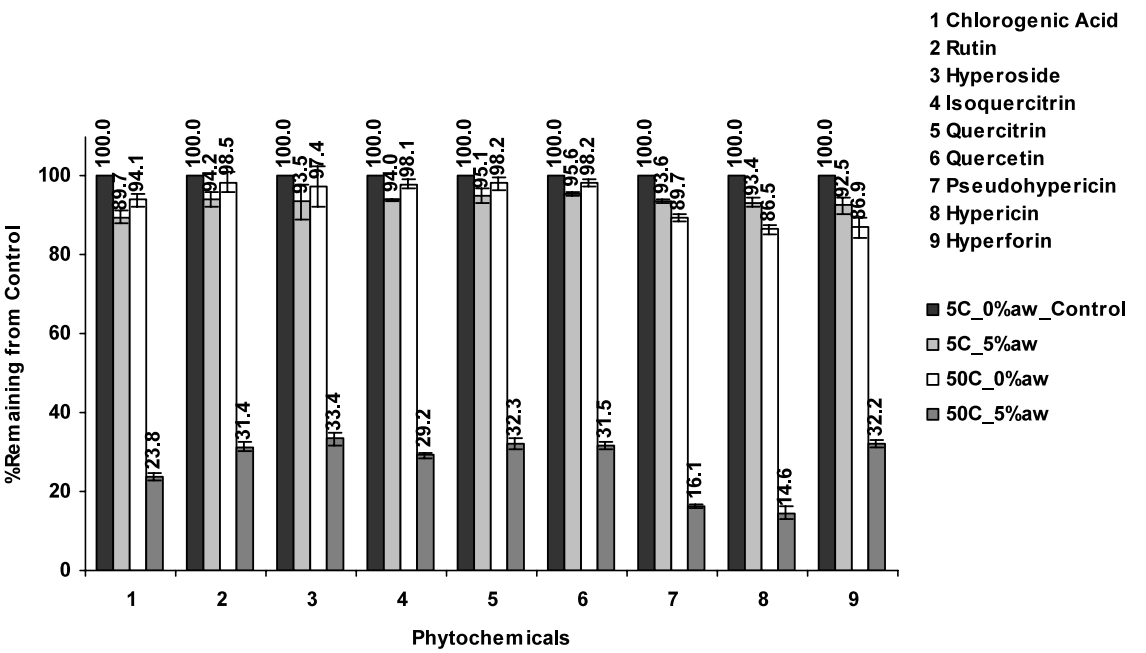

Fig. 1. Influence of storage conditions of lactose:SJW binary blends on SJW phytochemical profile (data are presented as the mean of three replicates ± standard deviation).

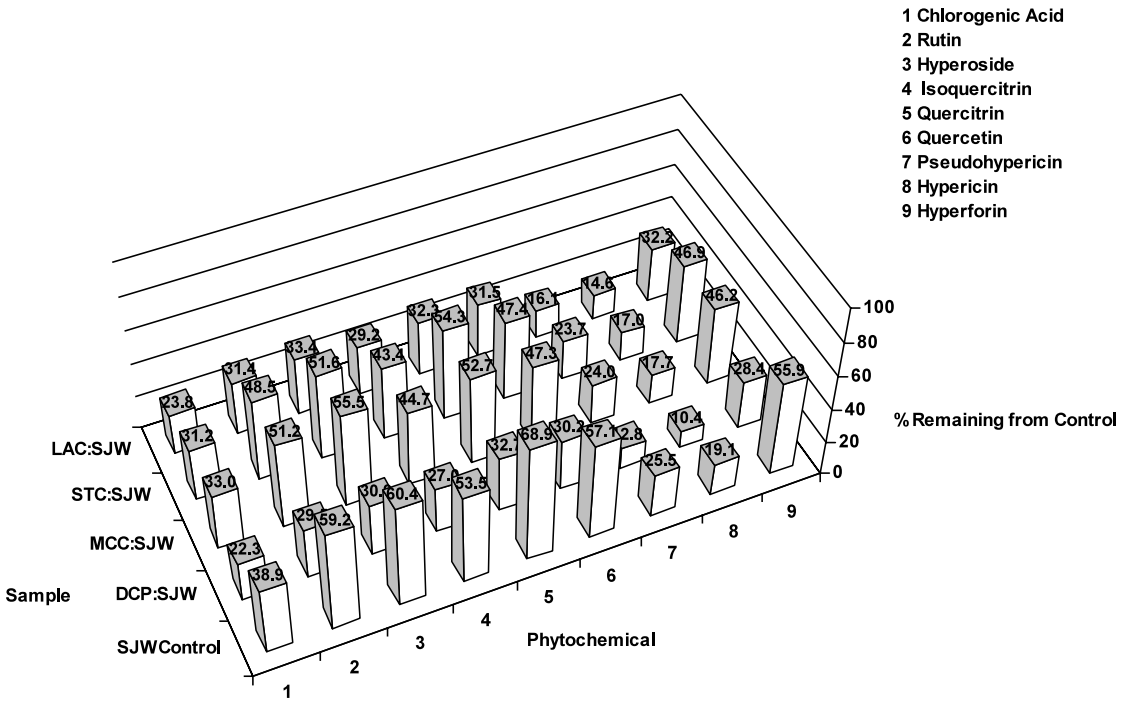

Fig. 2. Excipient compatibility-filler:SJW blends (a,b) stored at 50 °C/5% aw: (a) LAC:SJW = lactose:SJW; STC:SJW = pre-gelatinized starch:SJW; MCC:SJW = microcrystalline cellulose:SJW; DCP:SJW = dibasic calcium phosphate:SJW; (b) standard deviations ranged from 0.04–2.1 for all Filler:SJW combinations tested.

3.2.2. Disintegrants

The crospovidone blend clearly retains a greater percentage of each phytochemical than the croscarmellose sodium blends, except the napthodianthrones (7, 8) (Fig. 3). In fact, crospovidone:SJW blends retain a greater percentage of each constituent than SJW neat.

3.2.3. Lubricants

Binary blends of SJW with hydrogenated vegetable oil or magnesium stearate retained similar percentages of the phytochemicals (Fig. 4). Overall, magnesium stearate showed a small advantage over hydrogenated vegetable oil, and a very slight advantage for most phytochemicals (1–4, 6–9) over SJW alone.

3.2.4. Stabilizers

For most phytochemicals, greater degradation occurred in the malic acid:SJW binary blends than occurred in the ascorbic acid/citric acid (10:1):SJW blends. However, a significantly greater percentage of hyperforin (9) remains in the malic acid blend than remains in the ascorbic acid:citric acid (10:1) blend (36.6 vs. 26.9%, respectively). SJW alone retained a greater percentage of each phytochemical than either of the stabilizer systems (Fig. 5).

3.3. Similarity metrics

Similarity metrics were applied to excipient:SJW binary blends and SJW neat stored at 50 °C/5% aw. All metrics (f_2, ρ, ρ^u, ρ_h^u) indicate that hydrogenated vegetable oil, magnesium stearate, and croscarmellose sodium binary blends have percentage remaining of components 1–9 (phytochemical profiles) similar to SJW neat stored under the same conditions (Table 1). The f_2 test also indicates microcrystalline cellulose:SJW and pregelatinized starch:SJW blends have similar profiles to SJW neat.

3.4. Visual analysis

Samples stored at 50 °C/5% aw appeared resinous. As SJW extract is a brown powder, no color change was discerned for any of samples under all storage conditions. Visual observations, therefore, did not lend any insight to degradation that may have occurred.

4. Discussion

4.1. Storage conditions

To increase the likelihood of interactions between the excipient and drug substance in a timely manner, conventional excipient compatibility studies specify the use of high temperatures and added moisture. Clearly, for drugs that are heat and moisture sensitive, this paradigm must be approached with caution. The difficulties are further compounded with botanicals, which contain several phytochemicals of interest. The true effects of heat, moisture, and each excipient were determined by storing excipient:SJW blends, as well as SJW neat, under several conditions (Fig. 6). In Fig. 6, only one phytochemical (hyperforin-9) is shown and one excipient class (fillers), highlighting the complexity of the process. For example, the percentage remaining of hyperforin in SJW neat appears to be almost equivalent to blends at 5 °C/0% aw. Increasing moisture from 0 to 5% aw facilitates the degradation of hyperforin in all cases, whether a filler is added or not. The influence of increasing temperature from 5 to 50 °C results in even greater degradation, with filler:SJW blends (except microcrystalline cellulose) retaining a greater percentage of hyperforin than SJW neat. In this example, the degradation is obviously due to the heat, and not the fillers. Increasing both heat and moisture results in a clearer picture of excipient compatibility. The interactions between excipient and SJW and subsequent influence on the percentage hyperforin remaining, regardless of temperature and humidity, can be seen. For example, dibasic calcium phosphate blends result in a 49.1% decrease in hyperforin compared to SJW alone, whereas pre-

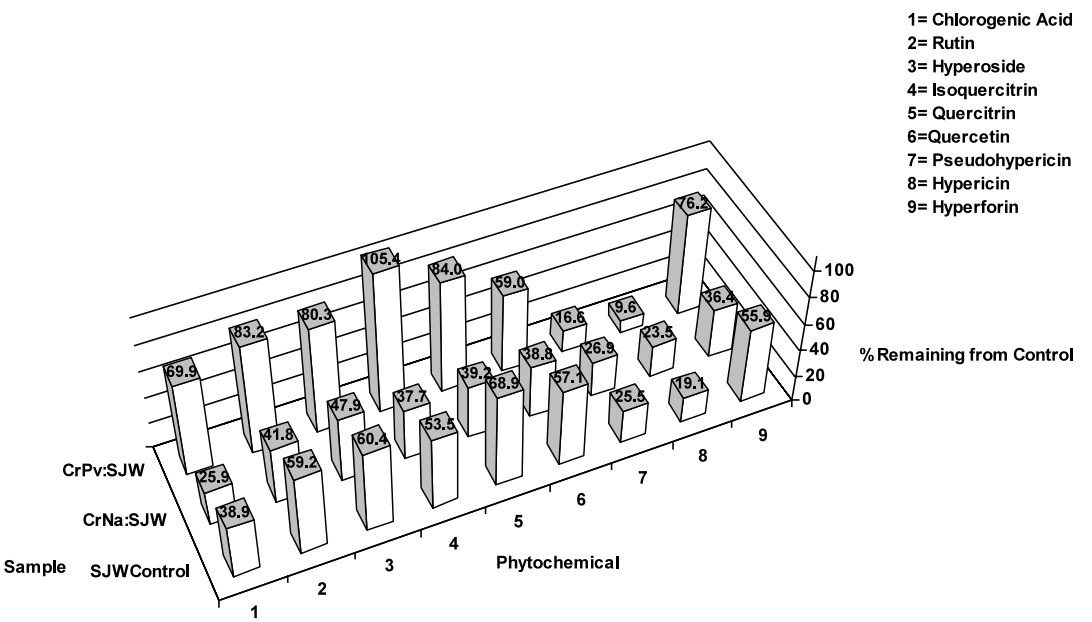

Fig. 3. Excipient compatibility-disintegrant:SJW blends (a,b) stored at 50 °C/5% aw: (a) CrPv:SJW = crospovidone:SJW; CrNa:SJW = croscarmellose sodium:SJW; (b) standard deviations ranged from 0.1 to 0.9 for all Disintegrant:SJW combinations tested.

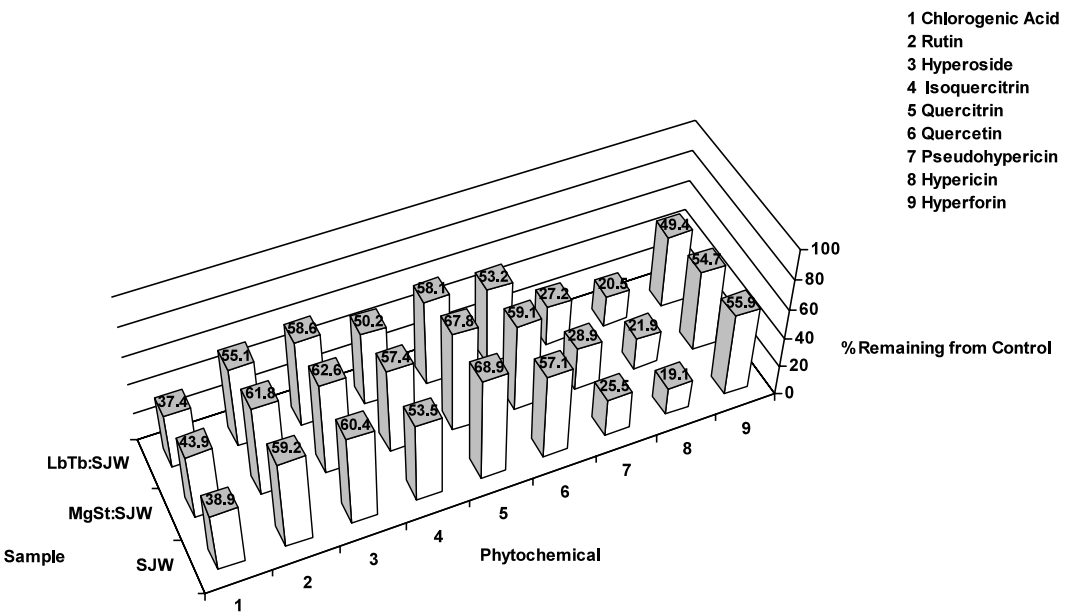

Fig. 4. Excipient compatibility-lubricant:SJW blends (a,b) stored at 50 °C/5% aw: (a) MgSt:SJW = magnesium stearate:SJW; LbTb:SJW = hydrogenated vegetable oil:SJW; (b) standard deviations ranged from 0.1 to 0.9 for all disintegrant:SJW combinations tested.

gelatinized starch decreases hyperforin by only 16.1%. The conventional method of excipient compatibility testing appears to work well when appropriate controls are employed. Although many botanicals exhibit both heat and moisture sensitivity, by increasing both heat and moisture and comparing results to neat botanical extract stored under the same conditions, interactions between excipient and SJW are facilitated and appropriate excipient choices can be made based truly on excipient compatibility.

4.2. Excipient compatibility

4.2.1. Fillers

The overall greater percentage of phytochemicals remaining upon storage with microcrystalline cellulose and pre-gelatinized starch versus lactose and dibasic calcium phosphate may be due primarily to pH differences and possibly hygroscopic tendencies (Fig. 2). The slightly alkaline nature of dibasic calcium phosphate (Wade and Weller, 1994) may contribute to severe degradation of many of the phytochemicals (Orth and Schmidt, 2000). Although dibasic calcium phosphate is non-hygroscopic, a desirable property for physical and chemical stability of the formulated product, it is not a good choice for formulation with SJW. Lactose incompatabilities often occur by base catalyzed degradation, and the well documented Maillard reaction that occurs with primary amines (Wade and Weller, 1994). However, there are no primary amines in phytochemicals 1–9. Microcrystalline cellulose and pre-gelatinized (corn) starch are slightly acidic, with pH ranging from 5 to 7 and 4.5 to 7, respectively (Wade and Weller, 1994). The acidic character may contribute to the survival of the phytochemical profile. In addition, microcrystalline cellulose and pre-gelatinized starch are hygroscopic, which may have promoted stabilization. For example, Durig and Fassihi (1993) report the stabilizing effects of various cellulose derivatives on pyridoxal hydrochloride. They theorize that the excess water becomes strongly bound to free hydroxy groups in the amorphous regions of the cellulose, reducing water activity, and decreasing the likelihood of degradation reactions. Therefore, microcrystalline

cellulose and pre-gelatinized starch are possible filler choices.

4.2.2. Disintegrants

Crospovidone appeared to exert a *protective* effect on the phytochemical profile, with much higher percentages remaining of each phytochemical versus SJW alone (Fig. 3). The pH is only slightly acidic (5–8) and crospovidone is generally regarded as inert and insoluble. Similarly, Desai et al. report the stabilizing effect of crospovidone on the dissolution stability of hydrochlorothiazide capsules. The large, positive effect of crospovidone is attributed to its moisture scavenging ability, which prevented deleterious interactions from occurring (Desai et al., 1994). Although croscarmellose sodium is slightly acidic (pH 5–7), severe degradation of all phytochemicals except the napthodianthrones occurred in disintegrant blends. In addition, the efficacy of croscarmellose sodium decreases when formulated with hygroscopic materials (Wade and Weller, 1994). Further, crospovidone has greater moisture sorption properties than croscarmellose sodium (Kornblum and Stoopak, 1973). Therefore, crospovidone is the clear choice of disintegrant.

4.2.3. Lubricants

Compared to hydrogenated vegetable oil, magnesium stearate appears to exert a protective effect on the phytochemicals (Fig. 4). Similar results with drug substances have been reported (Durig and Fassihi, 1993). This is particularly interesting since hydrogenated vegetable oils tend to be inert, and magnesium stearate is alkaline in nature and is considered more reactive. Since many phytochemicals are sensitive to moisture, the hydrophobic nature of magnesium stearate may protect SJW particles from exposure to the added moisture. Although hydrogenated vegetable oil is also hydrophobic, the laminar nature of magnesium stearate may provide a better barrier to moisture of each particle than hydrogenated vegetable oil. Magnesium stearate particles are laminar in structure and tend to delaminate during mixing, thereby enhancing their ability to coat host particle surfaces (Shah and Mlodozeniec, 1977).

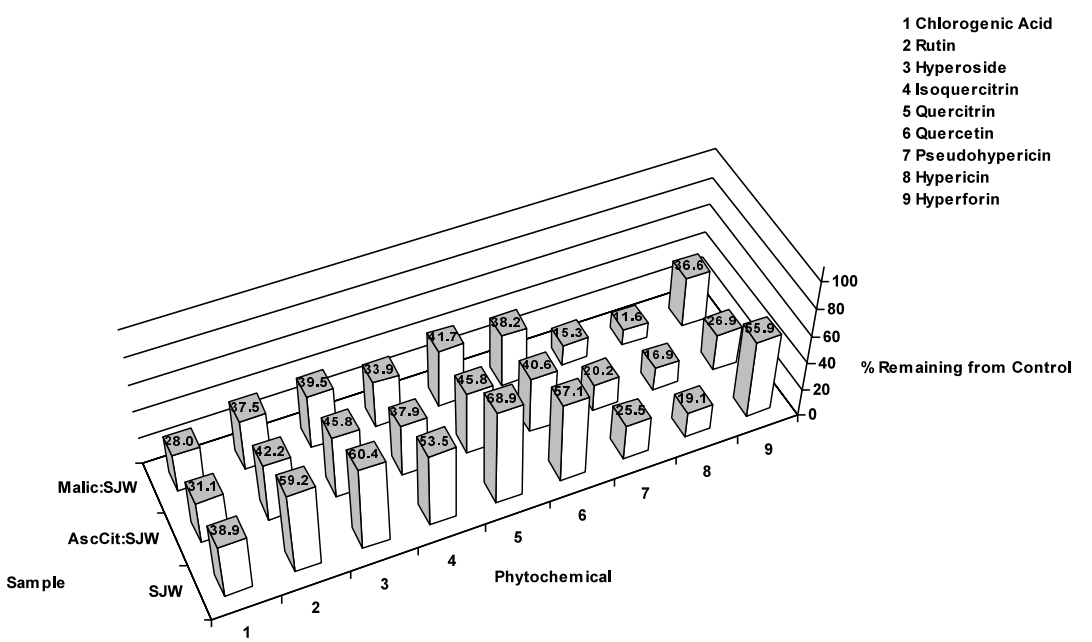

Fig. 5. Excipient compatibility-stabilizer:SJW blends (a,b) stored at 50 °C/5% aw: (a) AscCit:SJW = ascorbic acid/citric acid blend:SJW; Malic:SJW = malic acid:SJW; (b) standard deviations ranged from 0.1 to 2.9 for all stabilizer:SJW combinations tested.

Table 1
Application of similarity metrics to compare phytochemical profiles of SJW controls to binary blends of excipient:SJW stored under the same conditions (50 °C/5% aw)

	f_2	ρ	ρ_u	ρ_h^u
MCC:SJW	53.2	1.18	1.17	1.17
DCP:SJW	29.9	1.96	1.94	1.94
LAC:SJW	31.4	1.81	1.75	1.75
STC:SJW	51.9	1.21	1.19	1.19
AscCit:SJW	41.6	1.38	1.35	1.36
Malic:SJW	36.7	1.56	1.56	1.56
LbTb:SJW	65.2	1.09	1.08	1.09
MgSt:SJW	75.4	1.06	1.07	1.07
CrNa:SJW	65.2	1.09	1.08	1.09
CrPv:SJW	30.5	1.47	1.52	1.50

(a) Similar when $f_2 = 50$–100; (b) Similar when ρ, ρ_u, $\rho_h^u < 1.1$; (c) LAC:SJW = lactose:SJW; STC:SJW = pre-gelatinized starch:SJW; MCC:SJW = microcrystalline cellulose:SJW; DCP:SJW = dibasic calcium phosphate:SJW; AscCit:SJW = ascorbic acid/citric acid blend:SJW; Malic:SJW = malic acid:SJW; MgSt:SJW = magnesium stearate:SJW; LbTb:SJW = hydrogenated vegetable oil:SJW; CrPv:SJW = crospovidone:SJW; CrNa:SJW = croscarmellose sodium:SJW.

4.2.4. Stabilizers

Although, the combination of ascorbic acid:citric acid (10:1) and malic acid have both acidifying and antioxidant properties, neither appeared to stabilize the phytochemicals whatsoever (Fig. 5). Bilia et al. similarly reported that ascorbic acid:citric acid in combination with formulated SJW capsules provided no significant stabilization of the phytochemicals within the matrix (Bilia et al., 2001). It is possible that the level (1%) was not high enough to promote stabilization. If the formulation requires the use of an alkaline excipient (e.g. dibasic calcium phosphate) in a powder blend, or exposure to conditions where oxidation is likely to occur (e.g. sub-optimal storage conditions), perhaps the true value of the stabilizers would be revealed. Further research is warranted on the use of chemical stabilizers (acidifiers and antioxidants) for botanical formulation development.

4.3. Metrics

Similarity metrics provided a convenient means to summarize large amounts of data into a single evaluation. Each metric compares two phytochemical profiles, consisting of the mean percentage remaining of each of nine phytochemicals. A notable disadvantage is the inability of the metrics to reveal whether the percentage remaining of the phytochemical profiles for the binary blends was greater than or less than that of SJW neat stored under similar conditions. For example, crospovidone:SJW blend has an f_2 value of 30.5, indicating the phytochemical profiles of SJW neat percentage remaining and crospovidone:SJW blend percentage remaining are different. All other metrics (ρ, ρ^u, ρ_h^u) also indicated that the profiles differed from one another (Table 1). However, a difference in profiles does not necessarily mean that the excipient should be rejected. In this case, the profiles were different because crospovidone *stabilized* the SJW phytochemical profile! It is thus important to understand why the profiles are different. To that end, the graphical data in Fig. 3 provide an important perspective. Taking the metric data together with the graphical data it is clear that crospovidone is the disintegrant of choice. These similarity metrics will be used in further studies to compare the influence of different pharmaceutical processes on the phytochemical profiles of botanical extracts.

5. Conclusions

Common excipient compatibility storage paradigms (5 °C/0% aw control and 50 °C/5% aw sample) are suitable for excipient compatibility testing of botanical extracts. Controls of neat material must be stored under similar conditions and the percentage of phytochemicals remaining in excipient:botanical blends and neat material must be compared. For SJW, the excipients tested which have the most favorable percentage phytochemicals remaining are: Fillers-microcrystalline cellulose, pre-gelatinized starch; Disintegrants-crospovidone; and Lubricants-magnesium stearate. The stabilizers did not appear to protect the phytochemicals from oxidation or base catalyzed degradation; however, the levels may not have been high enough (1%) and the compatibility testing only investigated binary blends.

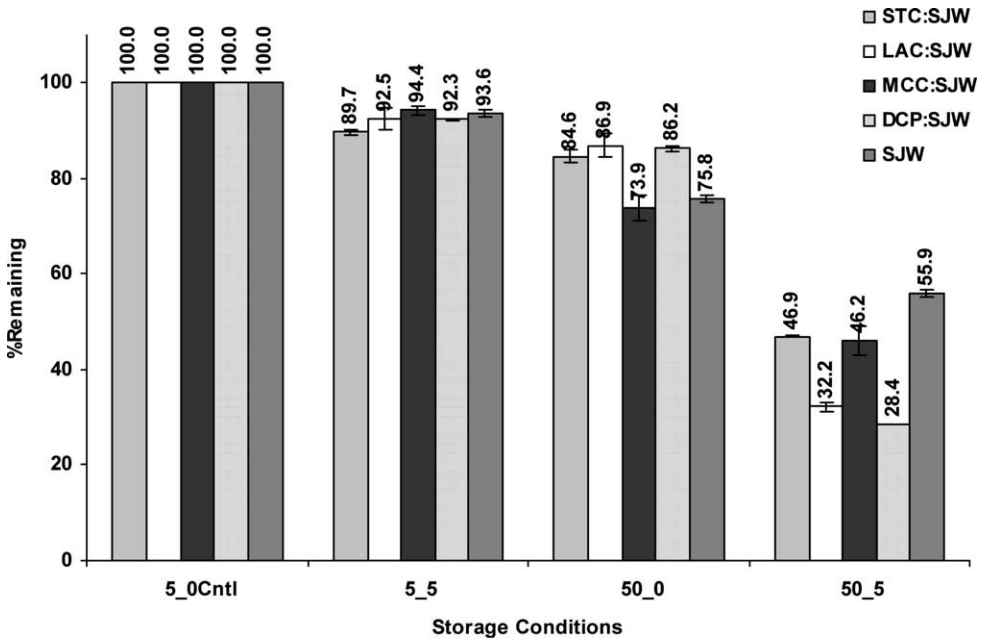

Fig. 6. Hyperforin in filler:SJW blends stored at 5 °C/0% aw, 5 °C/5% aw, 50 °C/0% aw, 50 °C/5% aw (a,b): (a) Data are presented as the mean of three replicates ± standard deviation; (b) LAC:SJW = lactose:SJW; STC:SJW = pre-gelatinized starch:SJW; MCC:SJW = microcrystalline cellulose:SJW; DCP:SJW = dibasic calcium phosphate:SJW.

Similarity metrics (f_2, ρ, ρ^u, ρ_h^u) applied to the phytochemical profiles provided a convenient means to summarize large amounts of data. Nonetheless, meaningful conclusions regarding excipient choices could only be obtained in combination with raw data. Since botanical extracts typically have several active components (direct or indirect), this approach is especially relevant. These results also invite consideration for possible application of similarity metrics to assess changes in the phytochemical profiles of botanical extracts in general as they age. For the purposes of this preliminary work, the criteria applied in SUPAC IR to indicate similarity of dissolution profiles, i.e. $f_2 = 50–100$, was applied. Other criteria may be more appropriate to specific cases and situations. Overall, this research allows logical decisions to be made regarding the formulation of SJW.

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