# Provisional Biopharmaceutical Classification of Some Common Herbs Used in Western Medicine

**S. Waldmann[1] , M. Almukainzi[1], N.B. Chacra[2], G.L. Amidon[3], Beom-Jin Lee[4], J. Feng[5], I. Kanfer[6], J.Z. Zuo[7], Hai Wei[8], M. B. Bolger[9], R. Löbenberg[(*)1]**

*[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton Canada [2] Faculty of Pharmacy, University of Sao Paulo, Sao Paulo Brazil [3] College of Pharmacy, The University of Michigan, Ann Arbor USA [4] College of Pharmacy, Kangwon National University, Chuncheon, Korea [5] Shanghai University of Traditional Chinese Medicine, Shanghai China [6] Faculty of Pharmacy, Rhodes University, Grahamstown South Africa [7] School of Pharmacy, Chinese University of Hong Kong, Hong Kong China [8]*Center for Chinese Medical Therapy and System Biology, Shanghai University of Traditional Chinese Medicine *[9] Simulations Plus, Inc., Lancaster, California, USA*

[*] Raimar Löbenberg, 3-142-K Health Science and Pharmacy Centre
University of Alberta, Edmonton, AB
Tel: (780) 492-1255
Email: rloebenberg@pharmacy.ualberta.ca

**Abstract.** The aim of this study was to classify some markers of common herbs used in Western medicine according to the Biopharmaceutical Classification System (BCS). The BCS is a scientific approach to classify drug substances based upon their intestinal permeability and their solubility, at the highest single dose used, within the physiologically relevant pH ranges. Known marker components of twelve herbs were chosen from the USP Dietary Supplement Compendium Monographs. Different BCS parameters such as intestinal permeability ($P_{eff}$) and solubility ($C_s$) were predicted using the *ADMET Predictor,* which is a software program to estimate biopharmaceutical relevant molecular descriptors. The dose number ($D_0$) was calculated when information from the literature was available to identify an upper dose for individual markers. In these cases the herbs were classified according to the traditional BCS parameters using $P_{eff}$ and $D_0$. When no upper dose could be determined then the amount of a marker, which is just soluble in 250 mL of water was calculated. This value, $M_x$, defines when a marker is changing from highly soluble to poorly soluble according to BCS criteria. **This biopharmaceutical relevant value can be a useful tool for marker selection.**

The present study showed that a **provisional BCS** Classification of herbs is possible but some special considerations need to be included into the classification strategy. The BCS **Classification can be used to choose appropriate quality control tests for products containing these markers**. A provisional BCS Classification of twelve common herbs and their 35 marker compounds is presented.

**Keywords**: Biopharmaceutical Classification System, markers, herbs, herbal extracts, permeability, solubility, dose number

## Introduction

For any orally administered drug product, the main parameters controlling rate and extent of drug absorption are aqueous solubility and gastrointestinal permeability [1]. The Biopharmaceutical Classification System (BCS) introduced by Amidon *et al*[1]. classifies drugs into four classes according to these two parameters using the highest therapeutic dose within the physiologically relevant pH range of pH 1.2, 7.4:

- Class I – high solubility, high permeability
- Class II – low solubility, high permeability
- Class III – high solubility, low permeability
- Class IV – low solubility, low permeability.

The FDA has adapted the BCS for regulatory and scientific purpose. **The BCS Classification of a drug can make post approval changes of a finished product or generic drug approval possible without the need to undertake *in vivo* studies** [2]. Based on the BCS Classification, waivers for *in vivo* bioequivalence testing of immediate-release oral solid dosage forms of Class I drugs can be granted if dissolution testing can demonstrate that two products are similar *in vitro.* The term "*biowaiver*" is defined by the World Health Organization (WHO) as approving a generic medicine based on strictly defined dissolution criteria relating to the active pharmaceutical ingredient (API) as a surrogate measure for *in vivo* bioequivalence testing [3]. The concept of biowaivers can be traced back to the guidance document issued by the US Food and Drug Administration in 2000. **More recently, consideration has been given to biowaivers for Class III drugs with very rapid dissolution properties and low permeability and is scientifically justified [4] [5], and the procedure has been includedin the recent EMA European Guidance[6].The WHO includesa procedure for weakly acidic compounds in its guidance for a biowaiver if they rapidly dissolve at pH 6.8.**

**Several aspects of the BCS concept should also be valid for herbal medicines. Herbalmaterials usually contain more than one defined substance. Similarly, many phytopharmaceutical / herbal products contain more than one herb.** Therefore, application of the BCS would be more complex compared to conventional/orthodox medicines, which contain one or a few combinations of APIs in a defined matrix of excipients. However, its usefulness might not be as obvious as for orthodox medicines. Herbal medicines are unregulated in many regions of the world or considered as dietary supplements in the United States. Other countries such as Europe and recently Canada have special regulations for traditional medicines, which require regulatory approval [7] [8]. Hence, a BCS Classification of herbals makers in botanical materials will have different implications in different regions of the world. **The concept of phytoequivalence is somewhat theoretical at this time point since there are no established reference products. However, BCS fundamentals can be used for herbal markers to gain critical biopharmaceutical knowledge about them.** From a scientific point of

view the BCS Classification of markers can be used to set *in vitro* quality standards for products. **For example an oral herbal formulation with highly soluble phytochemical components (BCS Class I and III) might only be required to meet disintegration specifications for quality control and release purposes where as a herbal dosage form containing poorly soluble components (BCS Class II and IV) need to pass a dissolution test to demonstrate that its content is appropriately released [(9)] to insure batch to batch consistency**.

**While appropriate clinical efficacy and safety data are usually lacking for herbal preparations and difficult and expensive to acquire, the principles of the BCS applied to herbals materials and their markers may enable scientists to select appropriate markers to ensure batch to batch consistency and related issues of quality for finished products containing herbals.**

The challenge in contrast to chemically defined drug products is that the biopharmaceutical quality of herbal medicines is often not well documented and there is a need for such essential data to be applied to the complex composition of a herbal preparation [(10),(11)].

The European Pharmacopoeia and the International Pharmaceutical Federation (FIP) have developed a classification system for herbals based on the information available about an herbal extract. Accordingly herbal extracts can be classified into 3 categories.

- A – standardized extracts, containing constituents solely responsible for therapeutic activity (Milk Thistle, Senna)
- B – quantified extracts, containing chemically defined constituents possessing active markers (St. John's Wort, Ginkgo)
- C – other extracts, containing no constituents documented as being determinant or relevant for efficacy or as having pharmacological or clinical relevance (Valerian)

**These three categories can be further subdivided into extracts containing negative markers which are substancesthat have to be limited due to their toxicity or phytoequivalence markers which might be used to establish bioequivalence between productsas has been shown for flavonoid glycosides of Ginkgo biloba [(12)].**

In Europe it is recommended that products containing extracts of Type A or B, but not C, should comply with the Note for Guidance on the Investigation of Bioavailability and Bioequivalence [(10)]. For such herbs, the BCS and biowaivers could be used to establish bioequivalence or pharmaceutical equivalence for markers[(12)]. Also the BCS could be used for post approval changes of such herbal products to demonstrate *in vitro* similarity. **However, the BCS Classification with respect to category C extracts would be limited to the demonstration *of in vitro* product similarity since no active is known and therefore bioequivalencebased on markers as for category A extracts and their actives cannot be established at this time point**

[7]. As further knowledge becomes available about category C extracts, they could thus be upgraded to category B or A.[10].

The aim of the present study is to provisionally classify marker components of popular herbs according to the BCS. The classificationhas been applied to the following twelve commonly used herbs, Cascara, Roman Chamomile, Garlic, Ginger, Ginkgo, Ginseng, Licorice, Milk Thistle, Red Clover, Senna, St. John's Wort and Valerian. A provisional BCS Classification strategy for herbalsmarkers according to the available information is presented.

**Materials and Methods**.

To classify herbal extracts according to the BCS, known/published marker compounds were used. These were either bioactive markers as well as chemical markers with no known pharmacological or toxicological effect. The markers were taken from the USP's Dietary Supplements Compendium book [13]. Table 1 lists the extract category assigned by the European Pharmacopoeia and the marker compounds known to be components of each of those herbal extracts.

**Table 1:** Categorization of the Herbal Extracts According to the European Pharmacopoeia and Marker Components According to USP Dietary Supplement Compendium.

| Herb | Category according to European Pharmacopeia | Markers according to USP Dietary Supplement Compendium |
|---|---|---|
| Cascara | A | Cascarosides calculated as Cascaroside A |
| Roman Chamomile | - | Matricin, Chamazulene, Apigenin-7-glucoside, Levomenol |
| Garlic | - | Allicin, Alliin, -glutamyl-(s)-allyl-L-cysteine |
| Ginger | - | Shogaole, Gingerole, Gingerdione and volatile oil |
| Gingko | B | Terpenlactones( Bilobalid, Ginkgolide A, B and C), Flavonoides calculated as Flavonolglycosides with mean molecular mass of 756.7g/mol |
| Ginseng | C | Ginsenosides |
| Licorice | A | Glycyrrhizin Acid |
| Milk Thistle | A | Silymarin calculated as Silibinin(= Silybin A and B) |
| Red Clover | - | Isoflavones |
| Senna | A | Sennosides calculated as Sennoside B |
| St.John's Wort | B | Hypericin, Hyperforin, Pseudohypericin |
| Valeriane | C | Iridoids, Valerenic Acid |

The herbal categories outlined by the European Pharmacopoeia were included for the BCS Classification. For category A extracts the pharmacological active substances were used as

markers. The markers for category B extracts were chosen from the quantifiable phytochemical components in those extracts for e.g. St. John´s Wort; hyperforin, hypericin and pseudohypericin. For category C extracts chemical markers, which might not represent any pharmacological activity but are main phytochemical components in the particular herb were used. For every herb that was not classified by the European Pharmacopoeia, markers that are considered to be the active components or are the common ingredients typical for a particular herb were used. Information such as plant parts used, indications and maximum dose were collected using Health Canada's Licensed Natural Health Products Database Martindale, and the German data base Rote Liste[14].

As there are only limited experimental data available about the biopharmaceutical properties of herbs, the *ADMET* Predictor (*Simulations plus, Inc.)* was used to predict those properties. Version 5.0 was used for all solubility calculations and version 2.3 for the permeability estimates. ADMET Predictor is computer software used to estimate biopharmaceutical relevant molecular descriptors. The "ADMET" acronym is commonly used in the pharmaceutical industry to indicate phenomena associated with Absorption, Distribution, Metabolism, Elimination, and Toxicity of chemical substances in the human body. The input data were "mol" files of the various chemical structures, created with Symyx Draw 3.2 (Symyx Technologies, Inc.). Using these "mol" files as input to the *ADMET* Predictor, the following parameters were estimated: pKa which is the dissociation constant, $P_{eff}$ which is the effective human jejunal permeability, $C_s$ which is the physiological solubility at pH 1.2, 4.5 and 6.8 and calculated by the software as (S+Sp). (S+Sp) was calculated as a function of the intrinsic water solubility (mg/mL), $pK_a$ values, and solubility factor (the increase in water solubility going from neutral to the cationic or anionic species) using chemical equilibrium theory [15]. $D_0$ is the dose number according to the BCS (see equation 1). The criteria for $P_{eff}$ and $C_s$ are described below.

## BCS Classification Criteria

The two parameters for BCS Classification are aqueous solubility at the highest therapeutic dose within the physiologically relevant pH ranges of pH 1.2, 4.5 and 6.8 and gastrointestinal permeability [1]. Table 2 shows the classification criteria according to these two parameters.

**Table 2.** Classification Criteria for Herbs According to Their Marker Compound's Permeability ($P_{eff}$) and Solubility as a Function of the Dose Number ($D_o$).

| Class | Permeability | Solubility |
|---|---|---|
| I | $P_{eff} \geq 1.78$ | $D_0 < 1$ |
| II | $P_{eff} \geq 1.78$ | $D_0 \geq 1$ |
| III | $P_{eff} < 1.78$ | $D_0 < 1$ |
| IV | $P_{eff} < 1.78$ | $D_0 \geq 1$ |

**Classification criteria according to Permeability properties.**

$P_{eff}$ is one major determinant of the fraction dose absorbed, and quantitatively represents the principal membrane transport coefficient of the intestinal mucosa of a substance [16]. Permeability boundaries were chosen according to the criteria proposed by Amidon et al [1]. Compounds with a higher or equal $P_{eff}$ than metoprolol ($P_{eff}$=1.78) were considered highly permeable and compounds with a $P_{eff}$ below metoprolol were considered as poorly permeable [16].

**Classification Criteria according to Dose number.**

Another important criteria for BCS Classification is the dose number $D_{0,}$ which describes the relationship between solubility and maximum dose strength according to equation 1. Compounds with a $D_o$ lower or equal to one are considered highly soluble [17].

Equation 1 $$Do = \frac{\frac{Mo}{Vo}}{Cs}$$

$M_0$ represents the highest dose strength in mg, $V_0$= 250 ml (volume of water taken with the dose), and $C_s$ is the minimum physiologic solubility at pH 1.2, 4.5 and6.8 at 37˚C in mg/ml [16]. As per definition, the solubility class boundary is based on the highest dose strength of an immediate release dosage form. A drug substance is considered *highly soluble* according to the BCS when the highest dose strength is soluble in 250 ml or less [17]. The volume estimate of 250 ml is derived from typical BE study protocols that require the administration of a drug product with a glass (about 8 ounces) of water [2]. When sufficient information was available, the dose number was used for classification. In the case when only a maximum daily dose was mentioned in literature, that value was used for the calculations.

**Marker Solubility Classification**

When only limited information **about the dose of a marker could be found, its solubility accordingto the BCS was used to classify it:** $M_x$ represents the border value between highly soluble and poorly soluble as defined by the BCS. $M_x$ is calculated according to Eq 1 by solving the equation for $C_s$ with a Dose number value of one. Any dose exceeding $M_x$ cannot dissolve in 250 mL, which would lead to a dose number larger than 1. $M_x$ can be used in method development and quality control to assist in choosing the right marker with sufficient solubility.

**Results**

## Classification of the markers

**The provisional classification, which is presented in this study is based on some necessary assumptions. For example, if markers belong to a similar chemical class (e.g. ginkgo) and the upper dose was only defined for the entire group of markers then each marker was assigned an equal fraction of the dose. It has to be pointed out that a manufacturer of an extract or finished product might arrive at a different BCS Classification because that specific product might have different ratios of the markers within a group. Furthermore, dose differences can cause a marker to change from lower or higher solubility or vise vice versa. However, this has to be established within and between markers groups according to manufacturer specifications set in the product specifications.**

Table 3 shows the classification of different herbs and their relevant associated marker(s) according to the BCS using permeability and dose number at defined pH values. Table 4 shows the solubility approach to classify markers.

**Table 3**. Permeability ($P_{eff}$) and Dose Number ($D_0$) at pH 1.2 , 4.5 and 6.8 as criteria for the Provisional BCS Classification of Herbs with Related Makers and Known Dose ($M_0$).

| **Herb** | **Marker** | **$M_0$**[mg] | **$D_0$ (pH 1.2)** | **$D_0$ (pH 4.5)** | **$D_0$ (pH 6.8)** | **Peff** | **BCS Class** |
|---|---|---|---|---|---|---|---|
| Cascara | Cascaroside A | 30 | 5.13E-04 | 5.13E-03 | 5.13E-03 | 0.04 | III |
| Garlic | Alliin | 27 | 3.05E-04 | 1.19E-05 | 1.13E-03 | 1.66 | III |
| | Allicin | 12 | 6.66E-03 | 6.66E-03 | 6.66E-03 | 2.72 | I |
| Ginger | 6-Gingerol | 1.88 | 5.33E-02 | 5.33E-02 | 5.33E-02 | 4.47 | I |
| | 8-Gingerol | 1.88 | 2.03E-01 | 2.03E-01 | 2.03E-01 | 5.52 | I |
| | 10-Gingerol | 1.88 | 1.91E+00 | 1.91E+00 | 1.90E+00 | 7.86 | II |
| | 6-Shogaol | 1.88 | 4.53E-01 | 4.53E-01 | 4.53E-01 | 9.99 | I |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 8-Shogaol | 1.88 | 1.54E+00 | 1.54E+00 | 1.54E+00 | 11.6 | II |
| | 10-Shogaol | 1.88 | 4.25E+00 | 4.25E+00 | 4.25E+00 | 12 | II |
| | 6- Gingerdione | 1.88 | 1.79E-01 | 1.79E-01 | 1.65E-01 | 4.21 | I |
| | 8-Gingerdione | 1.88 | 5.83E-01 | 5.83E-01 | 5.26E-01 | 5.24 | I |
| Gingko | Bilobalide | 3.84 | 1.35E-02 | 1.35E-02 | 1.35E-02 | 0.05 | III |
| | Ginkgolide A | 1.36 | 2.37E-02 | 2.37E-02 | 2.37E-02 | 0.04 | III |
| | Ginkgolide B | 1.36 | 9.17E-03 | 9.17E-03 | 9.17E-03 | 0.03 | III |
| | Ginkgolide C | 1.36 | 3.89E-03 | 3.89E-03 | 3.89E-03 | 0.02 | III |
| | Quercetin-3-O-coumaryl-glycosyl-rhamnosid | 32.4 | 2.04E-02 | 2.00E-02 | 4.68E-03 | 0.09 | III |
| Ginseng | Ginsenoside Rb1 | 8.9 | 1.76E-01 | 1.76E-01 | 1.76E-01 | 0.02 | III |
| | Ginsenoside Rb2 | 8.9 | 2.36E-01 | 2.36E-01 | 2.36E-01 | 0.02 | III |
| | GinsenosideRc | 8.9 | 2.36E-01 | 2.36E-01 | 2.36E-01 | 0.02 | III |
| | GinsenosideRd | 8.9 | 4.34E-01 | 4.34E-01 | 4.34E-01 | 0.04 | III |
| | Ginsenoside Re | 8.9 | 3.46E-01 | 3.46E-01 | 3.46E-01 | 0.02 | III |
| | GinsenosideRf | 8.9 | 8.46E-01 | 8.46E-01 | 8.46E-01 | 0.05 | III |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ginsenoside Rg1 | 8.9 | 5.23E-01 | 5.23E-01 | 5.23E-01 | 0.05 | III |
| | Ginsenoside Rg2 | 8.9 | 1.67E+00 | 1.67E+00 | 1.67E+00 | 0.06 | IV |
| Licorice | GlyccyrrhizicAcid | 600 | 5.26E+00 | 1.00E+00 | 2.73E-02 | 0.03 | IV |
| Milk Thistle | Silybin A | 70 | 4.51E-01 | 4.49E-01 | 2.11E-01 | 0.17 | III |
| | Silybin B | 70 | 4.51E-01 | 4.49E-01 | 2.11E-01 | 0.17 | III |
| Red Clover | Biochanin A | 30 | 6.32E+00 | 6.32E+00 | 3.50E+00 | 0.89 | IV |
| | Daidzein | 30 | 2.91E+00 | 2.91E+00 | 2.46E+00 | 1.41 | IV |
| | Formononetin | 30 | 8.76E+00 | 8.76E+00 | 8.16E+00 | 1.91 | II |
| | Genistein | 30 | 2.68E+00 | 2.66E+00 | 1.03E+01 | 0.63 | IV |
| Senna | Sennoside B | 30 | 2.99E-01 | 1.33E-03 | 9.76E-01 | 0.01 | III |
| St.John´s Wort | Hyperforin | 5 | 8.30E+00 | 8.26E+00 | 5.83E+00 | 1.95 | II |
| | Hypericin | 1 | 1.39E+09 | 6.64E+07 | 1.06E+03 | 0.04 | IV |
| | Pseudohypericin | 2 | 1.76E+08 | 8.88E+06 | 1.74E+02 | 0.27 | IV |

**Table 4.**Solubility classification using Permeability and Solubility at pH 1.2, 4.5 and 6.8 and $M_x$ estimates which denotes the solubility value when a marker changes from highly soluble to poorly soluble (mg/mL).

| **Herb** | **Marker** | **$M_x$pH 1.2** | **$M_x$pH 4.5** | **$M_x$pH 6.8** | **$P_{eff}$** |
|---|---|---|---|---|---|
| Chamomile | Chamazulene | 0.2775 | 0.2775 | 0.2775 | 12 |

| | | | | | |
|---|---|---|---|---|---|
| | Matricin | 252.5 | 252.5 | 252.5 | 0.09 |
| | Apigenin-7-glucoside | 510 | 510 | 550 | 0.14 |
| | Levomenol | 9.075 | 9.075 | 9.075 | 3.96 |
| Garlic | -glutamyl-(S)-allyl-L-cysteine | 8050 | 3250 | 21000 | 2.44 |
| Valerian | Didrovaltrate | 25 | 25 | 25 | 0.06 |
| | Isovaltrate | 19.925 | 19.925 | 19.925 | 0.05 |
| | Valtrate | 22.225 | 22.225 | 22.225 | 0.05 |
| | Valerenic Acid | 23.175 | 54.75 | 5175 | 2.46 |

**Classification of herbal markers according to the BCS.**

The provisional BCS Classification of the herbs is described below. If markers of one herb were classified as different BCS Classes, then the whole herb was assigned the higher BCS Class.

**Class I**

No herb was classified as entirely BCS ClassI for all its marker components.

**Class II**

Rhizomes of Ginger (Zingiber officinale, Roscoe) are often used to relieve or prevent the symptoms of motion sickness [17]. There is no categorization according to the European Pharmacopoeia and a single dose of 500 mg was found in the German Database. The volatile oil content is described as 2-3 % [14][18][19] .Therefore, each marker in Table 3 was calculated using 1.88 mg, all markers are highly permeable and thus belong to BCS Class II as some of the mentioned markers e.g. 8-Shogaol and 10- Shogaol in table 3 are poorly soluble.

**Class III**

Ginkgo biloba L. leaves help to enhance cognitive function in an aging population and also help to support peripheral circulation. It is a category B plant according to the European Pharmacopoeia and its extracts are commonly standardized to 24% flavone glycosides and 6% terpene lactones with a maximum single dose of 120 mg [20]. Tebonin™ a German ginkgo medicine contains: 26.4-32.4 mg flavonoids and 6.0-8.4 mg terpenlactones, The terpene lactones are further differentiated into 3,36-4,08 mg Ginkgolide A, B, C (1.36 each) and 3.12-3.84 mg bilobalide [14]. With a permeability of 0.04, 0.03, 0.02, and 0.05 respectively and a dose number smaller 1 for all markers.The evaluated ginkgo markers belong to BCS Class III.

The bulb of Garlic or Allium sativum L. is traditionally used to help relieve the symptoms associated with upper respiratory tract infections and catarrhal conditions, to reduce elevated blood lipid levels and to help maintain cardiovascular health in adults [21]. The European Pharmacopoeia does not categorize garlic extract; however many garlic products are standardized on the markers Allicin and Alliin. Allicin has a permeability of 2.72 and a recommended maximum daily dose of 12 mg, leading to a dose number smaller than 1 and is therefore classified as a BCS Class I substance. Alliin with its carboxylic acid group has also a dose number smaller than 1 (maximum daily dose of 27mg), but a lower permeability of 1.66, thus making it a BCS Class III substance. For the third marker, -glutamyl-(S)-allyl-L-cysteine, given in the USP Supplement Compendium, no maximum dose strength is known. $M_x$ is 700 mg, meaning the solubility behavior changes at a very high dose, and a $P_{eff} = 2.44$ so no BCS Class was assign for this marker. However, the other two markers are well known, and BCS Class III, can be assigned to them.

The dried and aged bark of Cascara or Rhamnuspurshiana DC is traditionally used as a laxative. The maximum dose is 30 mg per day, which is also the maximum single dose, and standardization is based on the content of hydroxyanthracene derivatives calculated as cascaroside A [22]. The European Pharmacopoeia categorizes cascara as a category A herb extract. With a calculated $P_{eff}$ of 0.04 and a dose number smaller than 1, cascaroside A is classified as a class III substance.

Senna or Cassia senna L. leaf dry extract is used as a laxative. It is a category A plant extract according to the European Pharmacopeia and is standardized on sennosides calculated as sennoside B with the highest dose strength of 30 mg per day, which is also the maximum single dose [23]. Sennoside B with a $P_{eff}$ of 0.01 and a dose number smaller than 1 is considered a BCS Class III herb.

Milk thistle or Silybummarianum L. is a category A plant extract and is standardized on silymarin calculated as silibinin (silybin A and sylibin B). The fruits are extracted and used for hepatic protection [24]. The maximum single dose of the commercial product Legalon® is 140 mg, therefore 70 mg was used for each active [14]. With a permeability of 0.17 and a dose number less than 1, Milk Thistle's markers belong to BCS Class III.

## Class IV

Ginseng root or Panax Ginseng C. A. Mayer is used in herbal medicine as a stimulant and as supportive therapy for the promotion of healthy glucose levels. It is a Category C plant extract but is often standardized on ginsennosides. One commercial product, Roter Imperial Ginseng von Gintec®, recommends a single dose of 475mg (ginsennosides content of 15%) [14][25]. Therefore, 8.9 mg was used for each marker for all ginsennoides. Most ginsennoides belong to BCS Class III, except, Ginsenoside Rg2, which is BCS Class IV marker.

The dried root of licorice or Glycyrrhiza Glabra L., is used in herbal medicine as an expectorant

to help relieve chest complaints, such as catarrhs, coughs and bronchitis. The maximum dose strength is 600 mg per day of glycyrrhizic acid [26] which is the marker for this standardized category A plant extract. The low permeability and dose number higher than I, classifies licorice as BCS Class IV.

Traditionally, red clover or Trifolium pratense L. ointments have been applied to the skin to treat psoriasis, eczema, and other rashes. Red clover also has a history of use as a cough remedy in children [27]. Sometimes red clover is standardized to a specific isoflavone content with a maximum dose of 120 mg of isoflavones per day; 30 mg was used for the calculations for each marker. Red clover contains BCS Class IV markers.

The aerial parts of St. John's Wort or Hypericum perforatum L. are used for mild depression and as a sedative for relief of restlessness and nervousness [28]. It is a category B plant extract according to the European Pharmacopeia. Active ingredients are thought to be hypericin, pseudohypericin and hyperforin. Extracts are often standardized on hypericin or hyperforin with a maximum dose of 1mg and 5 mg per day, respectively [28]. The classification of these different markers vary. In newer studies on St. John's Wort, hyperforin is considered the main active ingredient. According to the classification, hyperforin is a BCS Class II compound, with a permeability of 1.95 and a dose number higher than 1, and hypericin and pseudohypericin with a permeability of 0.4 and 0.27 and a dose number higher than 1, are BCS Class IV makers, **thereby placing St. John´s Wort in a mixed BCS Classification for its main markers**.

**Marker Solubility Estimates**

The Roman Chamomile or Chamaemelum Nobile L. is traditionally used to relieve mild digestive disturbances, such as nausea or dyspepsia. The flower heads are extracted [29], but Roman Chamomile extract is not categorized by the European Pharmacopoeia. As there is no information about the maximum dose of specific makers available, no BCS Class was assign for this herb's markers. Chamazulen and Levomenol with permeabilities of 12 and 3.96 and $M_x$ values of 0.278 mg, 9.075 mg . Matricin and Apigenin-7-glucoside have a low permeability of 0.19 and 0.14 respectivly and a $M_x$ of 252.5 mg.

Valerian or Valeriana officinalis L. root is used as a sleep aid and is sometimes standardized on valerenic acid with a maximum dose of 81 mg (9 g of dried root daily standardized to 0.9% valerenic acid) [30]. The European Pharmacopoeia categorizes valerian as plant C extract. Since valerenic acid is not an active ingredient, it was therefore not included in the classification, but is given as "marker" in the USP Supplement Compendium, as it is a toxic substance and therefore harmful to health. A commercial product, Baldurat®, contains 650 mg valeriana extract, but no information about any other marker content could be found. Didrovaltrate, valtrate and isovaltrate have low permeability $P_{eff}$0.06, 0.05, and 0.05 respectively and $M_x$ values around only 20mg.

**The classification of aglycons.**

Some markers of the reported herbs have sugars attached to their molecular structure. Often it is not scientifically conclusive if the entire molecule or only the aglycon gets absorbed *in vivo* and if the entire molecule or only the aglycon represents the active molecule. This may also depend on the individual plant. In the case of quercetin, one of the markers of Gingko biloba, studies showed that this marker has poor bioavailability and no quercetin could be detected in human plasma after oral administration [(31), (32)]. Quercetin circulates in plasma only in its conjugated form. However, the absorption process is still poorly understood [(33)]. It has been suggested that the intestinal sodium-glucose co- transporter might be involved in the absorption of quercetin glycosides and Graefe et al. [(34), (35)] reported that the entire molecule was absorbed by an active transport mechanism where the sugar component seems to be involved. Since these mechanisms are not conclusively known, Table 5 lists the classification of the aglycon only. As shown, the solubility behavior as well as the permeability behavior of some markers changed when only the aglycon was classified according to the BCS. Active transport was not considered for the permeability estimation.

**Table 5.** Different BCS Classification of the Aglycons Using Permeability and Dose Number/*Solubility* at pH 1.2, 4.5 and 6.8. In bold the BCS Class if the aglycon was classified differently compared to the entire molecule.

| **Herbs** | **Marker** | **$D_0$ pH1.2/*Mx*** | **$D_0$ pH4.5/*Mx*** | **$D_0$/*Mx* pH6.8** | **$P_{eff}$** | **BCS** |
|---|---|---|---|---|---|---|
| | | **[mg/ml]/*[mg]*** | **[mg/ml]/*[mg]*** | **[mg/ml]/*[mg]*** | **[cm/s x$10^{-4}$]** | **Class** |
| Cascara | Cascaroside A Aglycon | 3.95E-01 | 3.95E-01 | 3.90E-01 | 0.98 | III |
| Chamomile | Apigenin | *1.56E+01* | *1.57E+01* | *2.75E+01* | 0.57 | - |
| | Quercetin | 1.10E+00 | 1.05E+00 | 8.85E-02 | 0.4 | **IV** |
| Gensing | Protopanaxadiol | 4.10E+02 | 4.10E+02 | 4.10E+02 | 1.38 | **IV** |
| | Propanaxatriol | 1.60E+02 | 1.60E+02 | 1.60E+02 | 0.72 | **IV** |
| Senna | Sennidin B | 2.69E+04 | 4.33E+00 | 1.63E+00 | 0.19 | **IV** |

The hydroxyanthracene marker of senna changed from class III to class IV as the solubility of the aglycon is less as shown in Table 5. Apigenin has no class assign since the highest dose is unknown. Apigenin-7glycoside is considered a potential class III compound. Also the aglycon of quercetin shows a different solubility behavior and is therefore classified differently.

ProtopanaxadiolistheparentcompoundofGinsenoside Rb1, Rb2, Rc, and Rd. Whereas, PropanaxatriolistheparentcompoundofGinsenoside Re,Rg1, and Rg2. Both are changed from class III to class IV.

**Discussion**.

The BCS is a framework to classify active pharmaceutical ingredients according to their solubility and permeability properties **to assess their ability to become orally bioavailable.** For the 35 components considered in the 12 herbs, the BCS Class breakdown (in terms of numbers) was 6, 5,17,7 for BCS Class I-IV respectively with **some herbs where their markers were in mixed BCS Classes.** Thus nearly 50 % of the components were BCS Class III, which is **somewhat higher than the percentageobserved by Takagi et al. for pharmaceuticals [(36)] .In their study, the share of BCS Class I + III (High Solubility Drugs) was 66% of all evaluated drugs.**

**The provisional BCS Classification described in this study provides a possible decision tree of some relevant criteria, which need to be considered when herbs and their products are developed or are compared to other products. In the first case the BCS Classification can provide valuable information about markers and thus assist to choose the best biopharmaceutically suitable marker(s). In the second case the knowledge of the BCS Classification can be used to decide if an *in vitro* comparison between two products may be meaningful to assess possible therapeutic differences.**

**As mentioned earlier, the concept of phytoequivalence and biowaivers for herbal products is currently only in development and not an acceptable regulatoryrequirement even though some studies have been performed to show bioequivalencefor selected markers. The present study showed that herbs might contain markers, which belong to differentBCS Classes. Herbal products, which contain only BCS Class I and III markers could be compared by dissolution tests and consequently the biowaivers principles used for drugs could possibly be applied for such herbs and their products.If an herbal materialalso contains BCS Class II or IV markers, then biowaiver criteria cannot be applied to such products containing them. However, for such products dissolution testscan still discriminate product differences, but therelevance of such differences will need to be established via *in vivo* testing.**

Although herbal extracts show a complex composition of either known active compounds or chemical markers, each ingredient – active or inactive – can be classified according to the BCS system. However, the implications as outlined above for a BCS Classification of herbs are different. For a category A extract with known markers, a BCS Classification might be used to establish **bioequivalence (phytoequivalance)** for a marker because the well researched components responsible for an *in vivo* effect are used for the classification. For such an herb with

BCS Class I markers, dissolution testing could be used as a surrogate for bioequivalence as outlined in the biowaiver guidance [3] and as required by the EMA to establish bioequivalence between therapeutic products. [6] However, if such an herb is marketed in other regions of the world where no regulatory requirements exist, scientific approaches should be used to set quality standards. If the BCS Class I marker shows very rapid dissolution (>85% in 15 minutes) then disintegration testing rather than a dissolution testing may be scientifically justified to ensure the product performance[17]and batch to batch consistency. The foregoing illustrates the different utility of the BCS approach for either regulatory or scientific purposes to ensure product performance and quality to the patient.

For category B herbs where only some of the ingredients may be used as active markers it will be challenging to establish bioequivalence other than by complex clinical studies comparing different products. However, dissolution tests may be used in such cases to establish pharmaceutical equivalence between products and ensure consistency *in vitro* performance within and between products.

For category C herbs where only chemical markers are known, disintegration and dissolution tests can be used to compare products. *In vitro* similarity **between herbal markers** can be used to improve product quality and consistency, which can be seen as a major step forward in the quality control of botanical medicines.

The present study shows that a BCS Classification of herbs is possible but some special considerations need to be included in the classification strategy such as category A, B or C characteristics of the markers. If available, $D_0$ and $P_{eff}$ as main parameters need to be used. Marker classification according to the BCS, which is based on the solubility of individual markers, seems to be suitable for herbs where the dose is not known. The application of the solubility-based classification may be used in product development to choose a suitable marker for dissolution studies. Similarly, clinical researchers can use the classification to choose markers, which have suitable solubility and permeability properties and can be detected *in vivo*.

**Conclusion:** The present provisional BCS Classification of herbs and their markers has shown that some special considerations need to be **included in the classification strategy such as the pharmacological knowledge about markers to categorize herbal extracts. For category A extractswith known active markers the principles of the BCS can be applied in a similar way that has been applied to drugs and their products. However, for category B and C extracts a BCS Classification will be limited to assist in marker selection and to select appropriate performance tests.** When an upper dose limit is not knownfor a marker or when the actives are not known, a solubility based classification of markers provides information when a marker changes from highly soluble to poorly soluble which can help to chose the right marker for quality control purposes. **Applying the principles of the BCS to herbals and their markers can help to improve thequality of herbal medicines.**

**Acknowledgments:**

The authors thank Simulations Plus for their support. The High Ministry of Education of the Kingdom of Saudi Arabia is acknowledged for their support.

## References


(1) Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm.Res. 1995;12(3):413-420.

(2) Löbenberg R, Amidon GL. Modern Bioavailability, Bioequivalence and Biopharmaceutical Classification System. New scientific approaches to international regulated standards. European Journal of Pharmaceutics and Biopharmaceutics 2000;50:3-12.

(3) World Health Organization, Geneva. Fortieth report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations.*WHO Technical Report Series*.**2006,** No. 937, Annex 7

(4) Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. Pharm.Res. 2002;19(7):921-925.

(5) Blume HH, Schug BS. The biopharmaceutics classification system (BCS): Class III drugs – Better candidates for BA/BE waiver? European Journal of Pharmaceutical Sciences 1999;9(2):117-121.

(6) EMA-CHMP *Guideline on the Investigation of Bioequivalence*. 2010.

(7) European Medicines Agency, Herbal medicinal products http://www.ema.europa.eu/ema/index.jsp?curl=/pages/home/Home_Page.jsp&jsenabled=true Accessed 1/6, 2011.

(8) Health Canada, Natural Health Products Directorate.Http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/hpfb-dgpsa/nhpd-dpsn/index-eng.php .Accessed 1/6, 2011

(9) Tong C, Lozano R, Mao Y, Mirza T, Ldbenberg R, Nickerson B. The value of in Vitro dissolution in drug development: A position paper from the AAPS in Vitro release and dissolution focus group. Pharm Technol 2009;33(4):52-64.

(10) Lang F, Keller K, Ihrig M, Van Oudtshoorn-Eckard J, Möller H, Srinivasan S. Biopharmaceutical characterization of herbal medicinal products. Pharmacop.Forum 2003;29(4):1337-1345.

(11) Lang F, Arzneimittel WS, Keller K, Ihrig M, Van Oudtshoorn-Eckard J, Möller H. Biopharmaceutical characterisation of herbal medicinal products: FIP-discussion paper. Part 1. PharmazeutischeIndustrie 2003;65(6):547-550.

(12) *Wójcicki J,Mater Med Pol. 1995 Oct-Dec;27(4):141-6.*

(13)United State Pharmacopeia Compendium, USP Compendium. ; 2009-2010.

(14) Drug information for Germany. Www.rote-liste.de

(15) Fraczkiewicz, R. In silico prediction of ionization. In *Comprehensive Medicinal Chemistry II*, Vol. 5; Testa B., van de Waterbeemd H., Eds.; Elsevier: Oxford, UK, 2006; pp. 603-26

(16) Mannhold R, Kubinyi H, Folkers G. *Drug Bioavailability, Estimation of Solubility, Permeability, Absorption and Bioavailability*. Wiley VCH Verlag GmbH & Co, KgaA: Weinheim 2009;40:538.

(17) FDA *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*. 2000.

(18) M.Wichtel. Teedrogen. Second edition ed.: WVG, Stuttgrat; 1989

(19) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_ginger-gingembre-eng.php.

(20) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/archive_mono_ginko_biloba-eng.php.

(21) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_garlic-ail-eng.php.

(22) Canada, 18. Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_cascara-eng.php.

(23) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_senna_sene-eng.php.

(24) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_thistle-chardon-eng.php.

(25) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_ginseng-eng.php.

(26) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono licorice-reglisse-eng.php.

(27) Herbal Medicines. Available at: http://www.medicinescomplete.com/mc/herbals/2008/1000736746.htm?q=%22red%20clover%22&t=search&ss=text&p=1#hrb1000736758. Accessed 1/6, 2011

(28)Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_rom_chamom-eng.php.

(29) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_stjohnswort-millepertuis-eng.php

(30) Canada, Health. Natural Health Products Database.Http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_valerian-eng.php.

(31) Hollman PCH, Bijsman MNCP, Van Gameren Y, Cnossen EPJ, De Vries JHM, Katan MB. The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in

man. Free Radic.Res. 1999;31(6):569-573.

(32) Gee JM, Dupont MS, Rhodes MJC, Johnson IT. Quercetinglucosides interact with the intestinal glucose transport pathway. Free Radical Biology and Medicine 1998;25(1):19-25.

(33) Gugler R, Leschik M, Dengler HJ. Disposition of quercetin in man after single oral and intravenous doses.Eur.J.Clin.Pharmacol. 1975;9(2-3):229-234. .

(34) Graefe EU, Wittig J, Mueller S, Riethling A-, Uehleke B, Drewelow B. Pharmacokinetics and bioavailability of quercetin glycosides in humans. J.Clin.Pharmacol. 2001;41(5):492-499.

(35) Graefe, E. U.; Wittig, J.; Drewelow, B.; Riethling, A. K.; Mueller, S.; Wukasch, I.; Derendorf, H.; Veit, M. Relative systemic availability of the flavonoids quercetin and rutin in humans. *Pharm MedChem*. **1999**, 332:20

(36)Takagi T, Ramachandran C, Bermejo M, Yamashita S, Yu LX, Amidon GL. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. Molecular Pharmaceutics 2006;3(6):631-643.