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Pharmaceutical prerequisites for a multi-target therapy

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Abstract

The quality of a phytomedicine is defined by the quality of the herbal drug, the manufacturing of the drug preparations and the properties of the finished product, taking into account the special requirements of the individual herbal species in accordance with Good Manufacturing Practice (GMP) standards [2003. Medicinal Products for Human and Veterinary Use. Eudralex, vol. 4 (2003/94/EC)]. The quality control of the complete process is based on pharmacognostic methods, characteristic fingerprint chromatograms, defined amounts of marker substances, physicochemical characteristics and microbiological monitoring. For a herbal multi-component preparation used in multi-target therapy, these pharmaceutical prerequisites have to be ensured for all components and for their combination, as is exemplified by Iberogast® (STW 5) a fixed combination of hydroethanolic extracts of bitter candytuft (*Iberis amara*), angelica root (*Angelicae radix*), milk thistle fruit (*Silybi mariani fructus*), celandine herb (*Chelidonii herba*), caraway fruit (*Carvi fructus*), liquorice root (*Liquiritiae radix*), peppermint herb (*Menthae piperitae folium*), balm leaf (*Melissae folium*) and chamomile flower (*Matricariae flos*) using in the therapy of gastrointestinal compliants (Rösch et al., 2006).

The prerequisites for the quality of each of its components according to actual standards are at first the cultivation of the plant material according to the Guidelines for Good Agricultural Practice (GAP) conditions of Medicinal and Aromatic Plants [1998. Z. Arzn. Gew. Pfl. 3, 166–178] to yield a defined raw material of high quality. Characteristic compounds of the extracts had to be identified and different analytical methods such as HPLC, with low coefficients of variation had to be developed to analyze each of the standardized ethanolic extracts and the finished product.

At the example of the extract of *I. amara* these necessary investigations are described. The variability of the plant material in its natural habitats, the identification of characteristic compounds and exemplary chromatograms for fingerprint evaluation and quantification are shown. These data are required for characterization of the profile of the active substances in the finished product.

Keywords: Herbal multi-component product; *Iberis amara*; Iberogast®; STW 5; Quality control; TLC fingerprint; GLC and HPLC methods; Marker substances; Kaempferol-3,4′-di-*O*-*β*-glucopyranoside-7-*O*-*β*-rhamnopyranoside; Cucurbitacins

Introduction

The pharmaceutical requirements for a herbal product destined for a multi-target therapy are very complex. Generally, there are various possibilities to control the composition of a herbal medicinal product (Gaedcke, 1999, 2004).

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Beginning with the variety of the plant, the seeds, a suitable cultivation, the time of harvest, the preparation by drying and freezing, respectively, the milling and storage which are responsible for the quality of the herbal drug material (Kroth and Steinhoff, 1999; Tobler and Schneider, 2001).

Basic prerequisite for manufacturing of herbal drug preparations and multi-component products is the detailed experience about the constituents and their chemical behavior in the extracts. The different constituents are structured in known therapeutic constituents, co-effectors, matrices creators and undesirable toxic constituents (Franz et al., 2001). Marker substances can be components co-responsible for efficacy (co-effectors) or matrices creators.

Dependent on the extraction procedure, the type of extraction agent and the drug–extract ratio (DER) different constituents in various amounts are extractable. The constituents of an extract or a combination of extracts can react with each other, which can lead to unforseen changes of the composition and instability. With the example of Iberogast® (Table 1), a medicinal product consisting of nine herbal extracts, solutions are shown for receiving a reproducible product of high quality.

Cultivation and quality of the herbal drug

Cultivation is done according to the Guidelines for Good Agricultural Practice (GAP) of Medicinal and Aromatic Plants (1998). This ensures that the plant raw material fulfils high-quality standards. The seeds are identified botanically and are free from pests and diseases, and no genetically modified organisms are used. Standard operating procedures (SOPs) regulate the suitability of the soils and fertilization, the crop maintenance and plant protection. The water used for irrigation is free from contaminants, such as feces, heavy metals, pesticides, herbicides and toxicologically hazardous substances. Harvesting takes place when the plants best comply to the respective SOPs and under the best possible weather conditions. The drying and milling of the drugs are carried out contemporarily. No toxic fumigation or radiation against pest attack and microbiological contamination are permitted.

Table 1. Composition of Iberogast®

Iberogast® contains in 100 ml	
Ethanolic extract (extracting agent: ethanol 50% (v/v)) of fresh plants of	
Bitter candytuft (1: 1.5–2.5)	15.0 ml
Ethanolic extract (extracting agent: ethanol 30% (v/v)) of	
Angelica root (1: 2.5–3.5)	10.0 ml
Chamomile flower (1: 2–4)	20.0 ml
Caraway fruit (1: 2.5–3.5)	10.0 ml
Milk thistle fruit (1: 2.5–3.5)	10.0 ml
Balm leaf (1: 2.5–3.5)	10.0 ml
Peppermint leaf (1: 2.5–3.5)	5.0 ml
Celandine herb (1: 2.5–3.5)	10.0 ml
Liquorice root (1: 2.5–3.5)	10.0 ml

With the example of *I. amara* the advantages of cultivation vs. collected wild plant material are elucidated. *I. amara* L. is an annual, white to violet blooming plant, reaching up to 40 cm of heights with a strong specific smell, and a sharp cress-like taste. The genus *Iberis* grows in Europe, mainly in the mediterranean region (Reichling and Saller, 2003). Analytical parameters include the content of the flavonoids, particularly kaempferol derivates (e.g. kaempferol-3,4′-*O*-rhamnoside and the content of cucurbitacines. The origin of *I. amara* used in Iberogast® is the controlled cultivation in Germany to produce a defined raw material of constant composition with respect to flavonoids and limited amounts of cucurbitacins.

For comparison with this cultivar, specimens of diverse varieties of *I. amara* were gathered on a botanical expedition in the year 2003 or derived from gene banks or cultivators.

Fig. 1 describes the distribution of *I. amara* in Europe (Flora Europaea, 2001) and the places where the specimens were found. Voucher specimens of the plant materials and the accessions are available in the herbarium of Dr. E. Schneider, PhytoConsulting, Freinberg, Germany. Analyses of the contents of

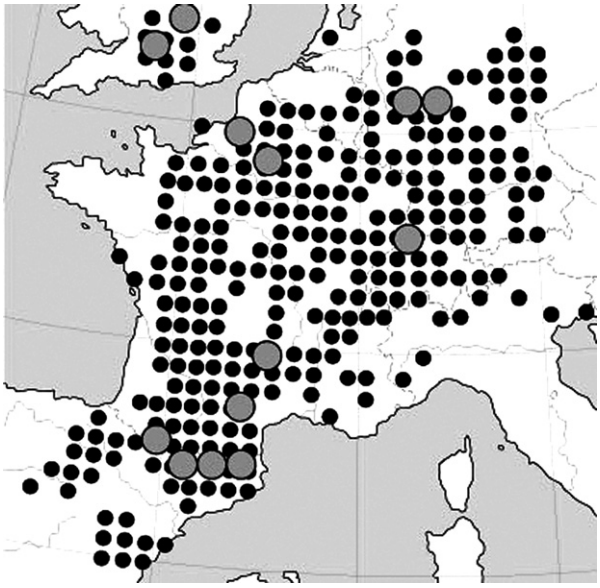

Fig. 1. Distribution of *Iberis amara* in Europe (small points) and the places where the specimens were collected (large points).

Table 2. Contents of kaempferol-3,4′-di-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside (kaempferol[1]), cucurbitacin I and cucurbitacin E in μg/g in specimens from collected plant material, gene banks and seed shops

Species	Specimen no.	Origin	Kaempferol[1] (μg/g)	Cucurbitacin I (μg/g)	Cucurbitacin E (μg/g)
Botanical expedition 2003					
I. amara	10	Ain, F	0	213	308
I. amara	01-120-1	Tarn 01, F	478	525	801
I. amara	02-130	Tarn 02, F	2472	336	591
I. amara	02-150-1	Coll d'Ares 2, S	140	306	715
I. amara	03-150-1	Coll d'Ares 3, S	305	15674	637
I. amara	03-160	Cadi 03, S	3053	706	1102
I. amara	04-160	Cadi 04, S	1667	452	812
I. amara	06-160	Cadi 06, S	2334	354	628
I. amara	07-160	Cadi 07, S	1113	948	1077
I. amara	01-170	Ribes 01, F	2764	372	792
I. amara	02-170	Ribes 02, F	21054	567	1068
I. amara	03-170	Ribes 03, F	2585	568	988
I. amara	01-180	Olot, St. Pivat, S	478	675	1007
I. amara	01-220	Kaiserstuhl, G	2942	655	883
Accessions of gene banks					
I. amara	4	Fritzlar, G	645	182	269
I. amara	5	Fritzlar, G	515	211	293
I. amara	8	Botanical Garden Munich, G	0	213	256
Accessions of seed shops					
I. amara	17	Thompsen and Morgan, UK	557	254	351
I. amara	18	Richters, C	519	204	272
I. amara	19	Borntraeger, G	614	228	253
I. amara	20	Sperling, G	537	180	221
I. amara	21	Sperling, G	588	177	251

C = Canada, F = France, G = Germany, S = Spain, UK = United Kingdom.

flavonoids and cucurbitacins were conducted with validated HPLC methods.

Table 2 shows the content of kaempferol-3,4′-di-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside and cucurbitacin E and I of diverse specimens. The data confirm the variability of the available varieties. The content of all these compounds shows a great genetic variation, so demonstrating the superiority of plants from defined cultivars and cultivation conditions to obtain a standardized composition of active substances with so low variations as possible.

I. amara whole plants have to be harvested by hand at the optimal crop time and frozen within a few hours after harvesting to obtain best possible quality and constant quantities of the main important compounds such as flavonoids and cucurbitacins.

All herbal drugs and *I. amara* fresh plant are analyzed according to the Pharmacopoeia Europaea (Europäisches Arzneibuch (Ph. Eur.), 2004), German Pharmacopoeia (Deutsches Arzneibuch (DAB), 2004) or Steigerwald monographs. Individual tests of identity (macroscopic, microscopic and chromatographic methods), assays of characteristic constituents and tests of purity are conducted. In addition, all drugs are tested on microbiological contaminations, the content of heavy metals, pesticides and aflatoxins or other mycotoxins, as for example ochratoxin in liquorice root. These measures ensure that only drugs of a high quality level are used, which is a precondition for the production of standardized drug preparations complying with the quality requirements (Fig. 2).

Manufacturing and quality of the drug preparation

The extraction of drug material is determined under standardized manufacturing conditions and in process controls. Relevant quality parameters are the DER, the quality of the herbal drug (grade of comminution, content of water, content of extractable substances), the extraction solvent (type, concentration, amount, flow speed), the procedure (type of extraction, time, temperature, pressure, batch size, filtration) and the equipment (type, level of filling, static pressure) (Gaedcke, 1997). All these parameters are defined for each drug by SOPs, and all steps of the manufacturing process are validated.

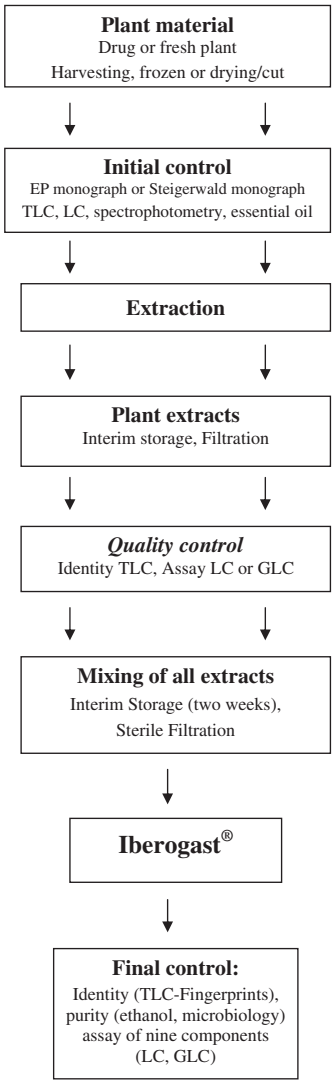

Fig. 2. From herbal drugs to a multi-component herbal medicinal product: manufacturing and quality control scheme of Iberogast®.

The quality of each extract is tested according to the individual specification. Characteristic parameters are the general properties of the extract (color, odor), the content of ethanol and residual solvents, the density, the identity of the used drug (TLC fingerprint of the chromatographic profile) and the content of the analytical marker substances within a defined range. For analyzing the characteristic marker substances Steigerwald has developed HPLC or GLC methods which comply with the relevant guidelines with respect to precision and robustness when used in the individual extracts as well as in the complex herbal matrix of the final product (Figs. 3–5). Regularly not all of the detectable peaks can be assigned to chemically defined substances. But it is of prime importance that an active substance responsible for the pharmacological action is known or, otherwise, a marker substance that analyzes well and is quantitatively predominant, be used for the quantification of the single extracts and the finished product. Characteristic marker substances are, for example, carvone in caraway fruit, menthol in peppermint leaf, rosmarinic acid in balm leaf and the flavonoid kaempferol-3,4′-di-*O*-*β*-glucopyranoside-7-*O*-*α*-rhamnopyranoside in bitter candytuft (Table 3). The defined quality of the drug and the standardized manufacturing process ensure a high batch-to-batch reproducibility of the extracts.

Manufacturing and quality of the finished product

The quality of a herbal multi-component product cannot only be defined by its release specification. The complete production process is a precondition for a defined quality of the medicinal product, including the use of herbal drugs of defined quality, the manufacturing of the extracts and the finished product in concordance with the GMP standards (Good Manufacturing Practice (GMP), 2003), and the analytical control of all intermediate products and the finished product. Besides a well-defined process, still wide experience on the manufacturing of the extracts and the finished product is required to receive a reproducible product of high quality.

For the better quality of the finished product, the right sequence of mixture, the mixing time (30 min), the interim storage (2 weeks) and the filtration (sterile filter) are essential for avoiding unwanted reactions between the different extracts (for example major precipitations) and for ensuring stability.

The identity and quality is secured by tests covering all components of the combination. Tests parameters include TLC fingerprint chromatograms covering all of the polarity range, the appearance (color, clearness), the density, the content of ethanol, the microbiological purity and the content of all nine components by the assay of characteristic marker substances. The proportion of each component in the final product has to be within a range of 95–105% of the specified content. The measurement is carried out by validated HPLC or GLC methods. In Fig. 2 the complete manufacturing and quality control scheme is shown.

In all complex herbal matrices, a great number of herbal compounds can be suggested. In chromatographic methods, a lot of peaks appear and a complete separation to the baseline is very difficult. In comparison to a single extract, the analysis of Iberogast® as a combination product of nine extracts is considerably more demanding. Not only the number of peaks increases, but in particular the number of chemically similar constituents with the same behavior in

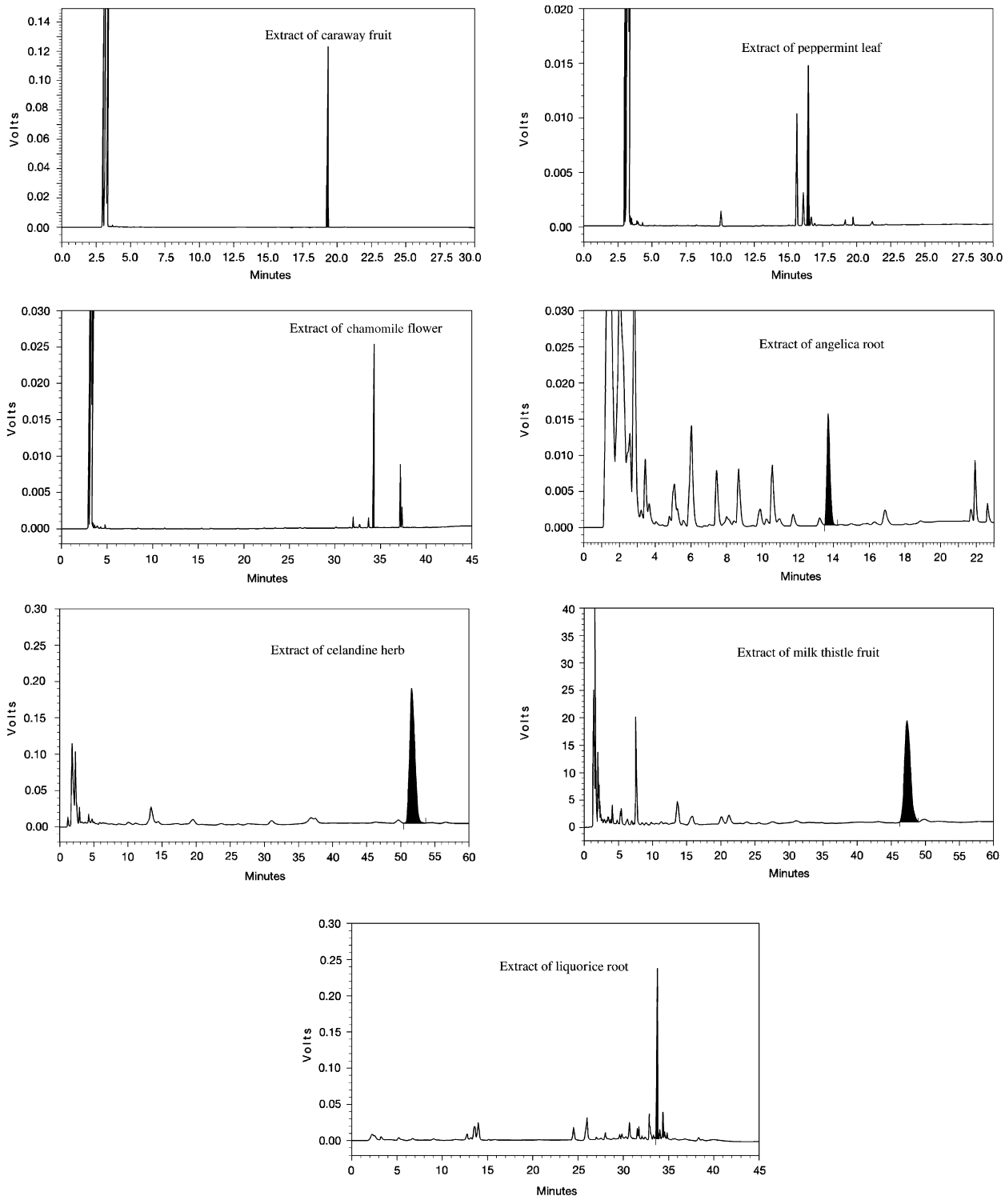

Fig. 3. HPLC and GLC chromatograms for quantitative determination of characteristic compound in the ethanolic extracts of caraway fruit (carvone), peppermint leaf (menthol), chamomile flower (bisabolol oxide A), angelica root (osthole), celandine herb (chelidonic acid), milk thistle fruit (*N*-malonyltryptophan) and liquorice root (glycyrrhizic acid).

chromatographic methods also increases. In Fig. 4, the comparison of the HPLC chromatogram of the extract of balm leaf and that of the combination product Iberogast® shows that the marker substance rosmarinic acid can be analyzed even in the finished product precisely and is reproducible. In a herbal multi-component product, the requirements for analyzing such a complex combination are considerable

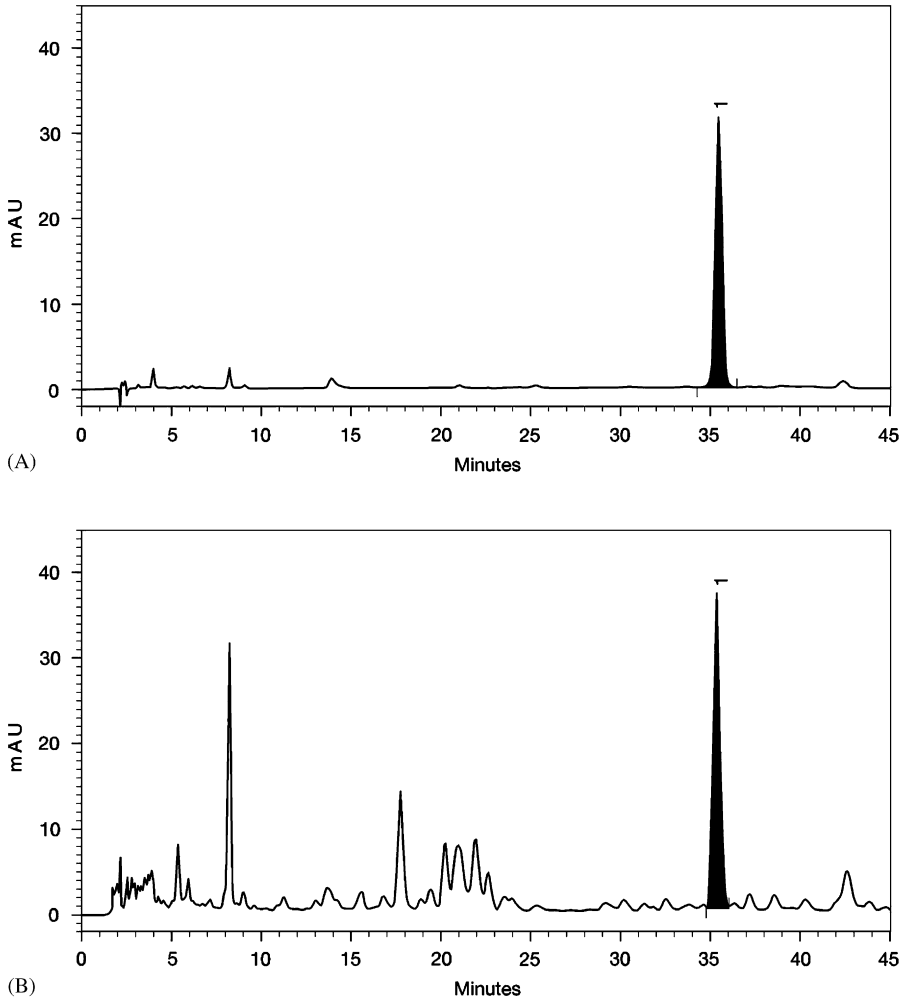

Fig. 4. HPLC chromatogram of the determination of rosmarinic acid (1) in the ethanolic extract of balm leaf (a) and in Iberogast (b).

and amount to a multiple of the mono-preparation product.

The development of the methods for quality control of a complex herbal matrix as Iberogast® is a continuous process that ensures that the methods applied comply with the actual state of scientific progress. This is demonstrated in the following by the example of the quantitative determination of *I. amara*.

Establishing the prerequisites for the quality of a component of a herbal fixed combination according to actual standards: the example of *Iberis amara*

The fresh whole plant of *I. amara* contains, as the main group of phytochemical components, a wealth of flavonol glycosides, besides of which only glucosinolates and low amounts of cucurbitacines have to be mentioned (Dalgaard et al. 1977; Nielsen et al. 1977; Bauer 1984; Bauer and Wagner, 1983), The known flavonol glycosides of the kaempferol- and quercetin-type were kaempferol-3-*O*-arabinoside-7-*O*-rhamnoside, kaempferol-3-*O*-glucoside-7-*O*-rhamnoside, kaempferol-7-*O*-rhamnoside and quercetin-3-*O*-glucoside-7-*O*-rhamnoside (Kowalewski and Wierzbicka, 1971) but were not suitable as marker substances for a specific quantification of the *I. amara* extract in the final combination product.

The chromatogram of the flavonoid pattern shows a major compound at 35 min (Fig. 5). As a result of the search for a suitable marker substance, this peak was isolated by preparative HPLC methods from the ethanolic extract of *I. amara* and the structure was established as a new flavonol glycoside named kaempferol-3,4′-di-*O*-*β*-glucopyranoside-7-*O*-α-L-rhamnopyranoside by a combination of 1D and 2D NMR techniques (COSY, HSQC, HMBC, NOESY) as well as UV, IR and mass spectral data. This structure had not yet been described in literature.

This substance, a characteristic marker for the flavonoids as a major compound of the phytochemical

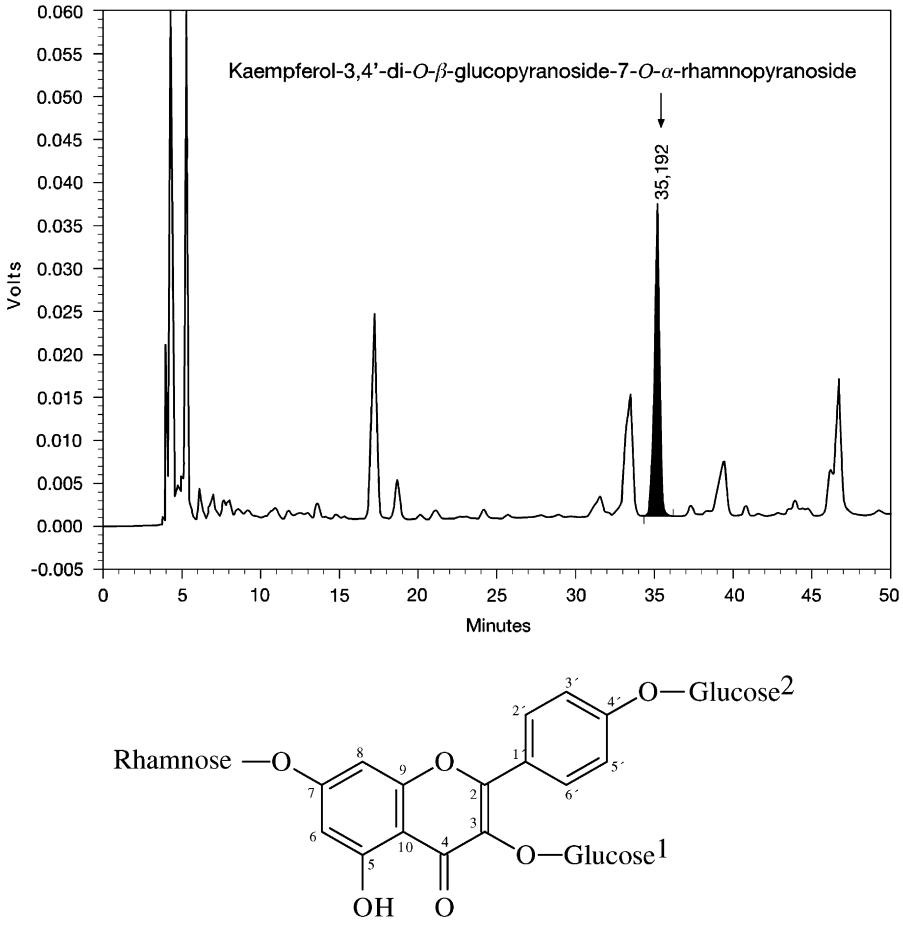

Fig. 5. HPLC chromatogram for quantitative determination of kaempferol-3,4′-di-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside in the ethanolic extract of *Iberis amara*.

Table 3. Characteristic compounds of the individual ethanolic extracts of Iberogast®

Ethanolic extract	Characteristic compounds
Bitter candytuft (*Iberis amara*)	Flavonoids (e.g. kaempferol-3,4′-di-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside), cucurbitacins
Chamomile flower (*Matricaria recutica*)	Essential oil (e.g. bisabolol oxide A)
Caraway fruit (*Carum carvi*)	Essential oil (e.g. carvone)
Peppermint leaf (*Mentha piperita*)	Essential oil (e.g. menthol)
Balm leaf (*Melissa officinalis*)	Hydroxycinnamic acid derivates (e.g. rosmarinic acid)
Angelica root (*Angelica archangelica*)	Coumarins (e.g. osthole)
Celandine herb (*Chelidonium majus*)	Dicarboxylic acids (e.g. chelidonic acid), alkaloids
Milk thistle fruit (*Silybum marianum*)	Silymarins, amino acid derivatives (e.g. *N*-malonyltryptophan)
Liquorice root (*Glycyrrhiza glabra*)	Glycyrrhizic acid, flavonoids

constituents of *I. amara* extract, can be detected with sufficient specificity and precision in the extract as well as in the finished product, allowing its use for in process control. In general, concentrations of 0.05–0.2 mg/ml were determined by HPLC analysis with RP18 material using acetonitrile–water with 2% acetic acid gradient in *I. amara* extracts.

Conclusion

The preconditions for a multi-target therapy by a herbal multi-component preparation are partly identical with those relevant in herbal drugs in general, as is demonstrated by the data on the development of a marker substance for *I. amara* and the advantages of its

controlled cultivation. But in addition, special preconditions are necessary, which by far exceed those in herbal mono-preparations. They include a specified production process for each extract and for the combination, the characterization of marker substances characteristic for each component and the development of analytical methods for their quantitation in the extract as well as in the final product. Such methods allow high precision in process controls of each component by qualitative and quantitative methods. Taking together the specified cultivation and processing of the plant parent material, the optimized production process, and the development and use of highly sensitive methods of analysis have led to an unprecedentedly high degree of standardization for the multi-drug product Iberogast® allowing a modern multi-target therapy.

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