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QUALITY CONTROL OF MACA-CONTAINING (*LEPIDIUM MEYENII* WALP.) DIETARY SUPPLEMENTS

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The radish-like hypocotyls of Maca (*Lepidium meyenii* Walp., Brassicaceae) are widely consumed as common vegetable in the Andean highlands. It is considered as healthy food, rich in carbohydrates and protein, a herbal medicine with a general invigorating reputation and fertility and sexual performance enhancer. The latter is the most popular contemporary application of the plant in Europe. The number and variety of industrial products on the market is increasing. Here we report the development of a simple and reliable analytical protocol for the qualitative and quantitative analysis of maca content of preparations and for the detection of synthetic phosphodiesterase inhibitors. Fourteen products were analysed by the method based on TLC and HPLC-DAD analysis developed by us. Our experiments revealed that beside good-quality products, the majority of the multicomponent preparations did not contain the declared herbal component or the quantity of the measured macamide was very low. Furthermore, one preparation is adulterated with a synthetic phosphodiesterase inhibitor. The presented method is suitable for quality control of *L. meyenii* products.

Keywords: *Lepidium meyenii*, maca, potency enhancer, dietary supplement

Maca or Peruvian cress (*Lepidium meyenii* Walp.) belonging to the family of Brassicaceae, is a plant mainly domesticated in the Andes of Peru and Bolivia at elevations of 3500–4000 m above sea level. The radish-like hypocotyls are traditionally consumed by Andean native people as common vegetable for thousands of years (Quirós & Aliaga, 1997). In the Peruvian Central Andes, the average daily dose is about 20 g (Gonzales, 2012). Nowadays a variety of products can be found in the Peruvian markets, such as refreshing or alcoholic drinks, jams, or the hypocotyl powder with the purpose of making cakes or bread (Quirós & Aliaga, 1997; Valentová & Ulrichová, 2003).

The plant has a multiplicity of other uses: it is widely applied as general invigorator, fertility enhancer for people and domesticated animals, and an ingredient of ceremonial concoctions (Quirós & Aliaga, 1997; Wang et al., 2007; Gonzales, 2012).

Maca has been found to contain an array of metabolites, some well-known for the Brassicaceae family (e.g. glucosinolates and its derivatives), others being more characteristic to the genus. Typical markers of the plant are the macamides, which have not been detected in other species. To date, 19 macamides have been described from *L. meyenii* (Wu et al., 2013). Depending on the origin and colour type of the hypocotyl, the composition and quantity of the secondary metabolites of maca may be very different (Gonzales, 2012). According to our actual knowledge on the chemistry of *L. meyenii*, macamides, alkaloids, glucosinolates and their derivatives, polyphenols, and flavonoids may also take part in the pharmacological activity of the species. For the different colour types macaene, macamides

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and glucosinolates differ mainly. Black and lead coloured maca contain the largest quantity of glucosinolates, while yellow maca is the richest in macanenes, macamides, and phenols (Clément et al., 2010).

Outside its native distributional range, maca is usually consumed as food supplement. These products contain the extract or the powdered hypocotyl of the plant. Sexual performance and fertility enhancement are the most popular contemporary applications of the plant in Europe. However, there are several preparations claimed to increase mental and physical performances, too. The number and variety of industrial products (predominantly dietary supplements, mono- and multi-component maca preparations) on the European herbal market – mainly sold via the internet – is increasing. The modes of action responsible for the supposed effects have not been fully elucidated so far. In some pharmacological experiments, the sexual behaviour of male rats and the erectile function of castrated rats have been enhanced (Zheng et al., 2000; Cicero et al., 2001). In a similar experiment maca was inactive, but the cause of this could be the much lower dosage (Lentz et al., 2007). Some clinical trials have been shown that long-term (4–6 weeks) consumption of dry maca hypocotyl with a 1.5–3 g daily dosage leads to the enhancement of sexual activity and fertility of men and women, too (Brooks et al., 2008; Shin et al., 2010; Gonzales, 2012). However, these studies were too heterogeneous and the number of the participants too low to make a firm conclusion.

Since the male sexual performance enhancer effect of the plant and its metabolites has not been confirmed convincingly preclinically or clinically, maca products marketed for this purpose are candidates for quality control focusing on activity-potentiating synthetic compounds to the products. Although there are data published on the maca content of several dietary supplements (Ganzera et al., 2002), there is a lack of studies aiming both the analysis of macamide content and the potential synthetic adulterants of the products. The aim of our work was to develop an analytical protocol for the qualitative and quantitative analysis of maca content of food supplements and to screen selected products for the presence of synthetic phosphodiesterase inhibitors.

1. Materials and methods

1.1. Chemicals and reagents

The macamide *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (Fig. 1) previously reported only from *L. meyenii* (McCollom et al., 2005) was isolated in our laboratory (=MACA–3) from dry *L. meyenii* hypocotyl powder (Raw Organic Maca Powder, EverTrust Ltd, UK, originating from Peru) using chromatographic methods. The structure of this compound was elucidated by ^{1}H and ^{13}C NMR experiments. Peak purity analysis carried out with the Empower Pro software confirmed that the chromatographic peak of the compound is spectrally pure. Acetonitrile (LiChrosolv® HPLC grade) was obtained from Merck (Darmstadt, Germany). Millipore Direct-Q UV3 clarifier (Millipore Corporation, Billerica, MA, USA) was used to produce purified water for HPLC measurements. Synthetic phosphodiesterase inhibitors were isolated from medicaments (sildenafil (Viagra), tadalafil (Cialis), vardenafil (Levitra)); their derivatives (pseudovardenafil, nor-acetildenafil, thiosildenafil, dimethyl-thiosildenafil, and aminotadalafil) were isolated previously from adulterated dietary supplements. Ten maca-containing dietary supplements were purchased in Hungarian shops, 3 maca powders were provided by Ashaninka Pharma Ltd. (Hungary), 1 maca powder was purchased in a webshop (UK).

1.2. Sample preparation for validation and product analysis

Dry maca hypocotyl powder (Raw Organic Maca Powder, EverTrust Ltd, UK, originating from Peru) was used for validation of the analytical method. Five hundred milligrams of the product was extracted with 10 ml *n*-hexane (10 min extraction in ultrasonic bath at room temperature), then diluted with *n*-hexane to 25.0 ml in a volumetric flask, and centrifuged (10 min, 2500 r.p.m.). Then 20.0 ml of the supernatant were pipetted to a round-bottom flask and evaporated under vacuum. Extracts were redissolved in 2 ml *n*-hexane, filtered through a filter membrane (Acrodisc® GHP 13 mm, 0.45 μm, Waters, USA). The first 0.5 ml was dismissed; the other 1.5 ml was analysed by HPLC-DAD. Three extracts were prepared and injected in triplicate.

In case of product quality analysis, 500 mg of the powder type products and 380–1000 mg of the multicomponent preparations (depending on the tablet or capsule sizes) were processed as described above.

1.3. Calibration and linearity

As calibration standard *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (MACA–3), a major alkamide of *L. meyenii*, was applied. In order to obtain the linear range of quantification, stock standard solution (1.2 mg ml^{-1}) was prepared with *n*-hexane. Of the stock standard solution, 1.0 ml was transferred to 10.0 ml volumetric flask and diluted with *n*-hexane. Different amounts of this solution were diluted with *n*-hexane to obtain six different concentrations between 0.0048–0.06 mg ml^{-1}. The calibration range of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide was 0.048–12.0 μg/injection. Ten microlitres of the standards were injected in triplicate. The slope, intercept, and the correlation coefficient were determined. For calculation of the calibration curve, peak areas were plotted against the injected mass of the macamide standard.

1.4. HPLC apparatus and measurement conditions

HPLC analysis was carried out on a Waters 600 system (Waters Corporation, Milford, USA), equipped with a 2998 photodiode array detector, on-line degasser, column thermostat, and autosampler using a reversed phase Kinetex XBC18 (2.6 μm, 100 Å, 100×4.6 mm column (Phenomenex, Torrance, USA) at 25 °C). Chromatographic elution of the samples was accomplished by an isocratic solvent system consisting of acetonitrile – H_2O 85:15 with a flow rate of 0.7 ml min^{-1}. For analysis, 10.0 μl extract was injected. The samples were monitored at 210 nm. Data acquisition and evaluation were performed using Empower Pro software.

1.5. Method validation

Repeatability was evaluated based on experiments with Raw Organic Maca Powder extracts. Intraday precisions were calculated from data acquired during a 3-day validation. Precision was expressed as relative standard deviation (RSD%). Signal to noise ratio was used to express the limit of detection (three times the noise) and quantitation (ten times the noise). Recovery analysis was carried out by adding known amounts of macamide (64, 128, and 256% of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide) to the maca extract (n=3 at each concentration). To assess the stability of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide in the *n*-hexane extract, the extracts were stored at room temperature and analysed after 3 and 4 days.

1.6. Detection of phosphodiesterase inhibitors

For the presence of phosphodiesterase inhibitors, multicomponent preparations were analysed by TLC and HPLC-DAD according to a protocol developed in our laboratory (Csupor et al., 2011). Briefly, one capsule or tablet was extracted with 10 ml methanol for 10 minutes by ultrasonic bath at room temperature, and then filtered through a membrane filter.

Ten microlitres of the extracts were applied to silica gel stationary phase (Merck 105553 60 20×20) and developed in toluene – acetone – ethanol – 25% NH_3 70:50:10:3 in a developing chamber saturated with solvent vapour. The development distance was 20 cm. The detection was realized under UV (254 nm) and by spraying the plate with Dragendorff's reagent or vanillin-sulphuric acid and heating at 120 °C. This method allows the identification of phosphodiesterase inhibitors by comparing their retention factors and the colours of the spots to those of analytical standards. Selective detection carried out with Dragendorff's reagent makes it possible to detect so far unidentified synthetic derivatives.

For HPLC-DAD analysis a Gemini-NX 5u C18 100A, 100×4.6 mm column was used. The solvent system was NH_4HCO_3 (10 mM pH=10.00±0.2) – acetonitrile 7:3 with a flow rate of 1 ml min^{-1}. Twenty millilitres of extract was injected. The samples were monitored in the whole UV range (200–400 nm) and at 220, 254, 282, and 292 nm. Data acquisition and evaluation were performed using Empower Pro software. Synthetic phosphodiesterase inhibitors can be identified based on their retention times and characteristic UV spectra.

2. Results and discussion

2.1. Method validation

Linear calibration curve (y=1871×710.20x+3015.43) was established with a correlation coefficient (R^2) of 1.00, which allows reliable quantification. *N*-Benzyl-(9*Z*,12*Z*)-octadecadienamide content of 6 preparations was below the limit of detection and above the limit of quantification in case of 8 products. The intraday precision, obtained during the validation for *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide, ranged between 0.80 and 1.11%. These results underline the good repeatability of our method. The recovery was 97% (RSD% 0.80) for the first concentration, 99.6% (RSD% 2.90) and 98.8% (RSD%) for the second and third concentrations, respectively. These results are in accordance with recovery values of previously published methods (Ganzera et al., 2002; McCollom et al., 2005) and confirm the good accuracy of our method. According to the stability measurements, the *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide content of *n*-hexane extracts stored for 3 and 4 days at room temperature were 96.53% and 103.40%, respectively, of the original samples. These results indicate that the decomposition of this macamide is not significant and therefore analysis can be carried out reliable from fresh samples.

2.2. Product analysis

Fourteen maca products containing mono- (5) and multicomponent (9) preparations (distributed by 11 companies) were randomly selected and purchased (Table 1). The origin of the monocomponent products was Peru, the multicomponent preparations derived from China (5), UK (2), and other EU countries (2). Their pharmaceutical form was powder (4), capsule (6), tablet (2), ampoule (1), and spray (1). In case of monocomponent preparations (1–5 products) the recommended daily posologies (1500–5000 mg dry hypocotyl powder)

overlap with the effective dosages used in human studies (Quirós & Aliaga, 1997; Wang et al., 2007; Gonzales, 2012), but for multicomponent products (6–14.) are much lower, between 80–125 mg for 4 products, 500 and 750 mg in two cases, and not declared in one case. However, 4 products (6, 8, 12, and 13) were claimed to contain "maca extract" (unspecified), in 1 case dry hypocotyl powder (11), and in 4 cases (7, 9, 10, and 14) no further information was given. The most preferred recommendations for the products were "potency enhancer", "for men", "for the reproductive system", and "mental and physical capacity enhancer". Among thirty other herbal (28), animal (2), or fungal (1) ingredients the most preferred ones were *Serenoa repens* (labelled in 7 products), *Panax ginseng* (5), *Ginkgo biloba* (4), *Rhodiola rosea* (3), and *Urtica dioica* (3). The majority of these are widely used either for symptoms of the genitourinary system or as physical or sexual performance enhancer.

Table 1. Analysis of 14 maca containing dietary supplements

Product (origin)	Pharmaceutical form	Recommended daily dosage	MACA-3 (μg 1^{-1} g product)	MACA-3 in the recommended daily dosage (μg)
1. (Peru) Raw Organic Maca Powder (yellow coloured)	powder[1]	3–5 g	193.1	579.3–955.5
2. (Peru) Red Maca Powder	powder[1]	no data	225.8	
3. (Peru) Black Maca Powder	powder[1]	no data	63.0	
4. (Peru) Gelatinazed Maca Powder	powder[1]	no data	28.0	
5. (Peru)	tablet[1]	1500 mg	45.2	67.8
6. (EU)	capsule[2]	one capsule	30.9	15.45
7. (UK)	capsule[2]	one capsule per day for 5 days, then 2 days break	2.6	1.3
8. (China)	tablet[2]	one–two tablets per day, for 4–6 weeks	13.9	13.9–27.8
9. (China)	capsule[2]	maximum one capsule in every three days	n.d. Presence of thiosildenafil	
10. (China)	capsule[2]	one–two capsules 30 minutes before the sexual activity	n.d.	
11. (EU)	capsule[2]	two capsules before sexual activity	n.d.	
12. (China)	capsule[2]	one–two capsules one hour before sexual activity	n.d.	
13. (China)	ampule[2]	one ampule	n.d.	
14. (UK)	spray[2]	3–5 puffs locally	n.d.	

[1]: monocomponent preparations; [2]: multicomponent preparations, n.d.: not detected

The HPLC method developed by us allowed the reliable analysis of one of the major macamides of *L. meyenii*. The good separation of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide from other components of the extracts provided robustness for the method. HPLC-DAD analysis revealed that significant peaks with higher retention times than that of this compound do not belong to the class of macamides. From the 14 analysed preparations, the presence of maca was confirmed in 8 products. The HPLC-UV chromatograms (210 nm) of maca containing products were similar to that of Raw Organic Maca Powder (Fig. 1).

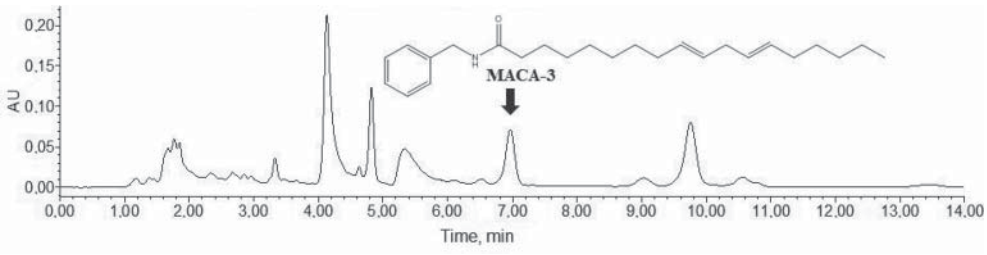

Fig. 1. HPLC-UV chromatogram of a maca extract (210 nm) and the structure of *N*-benzyl-(9Z,12Z)-octadecadienamide (MACA-3)

In the maca powders 28.0–225.8 μg *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide/g was present. Marked differences in macamide content could be detected in case of the different colour types. In 6 preparations the concentration of the marker macamide was below the detection limit and one preparation was adulterated with a synthetic phosphodiesterase inhibitor. Thiosildenafil, a derivative of sildenafil has been detected by TLC and HPLC-DAD (retention time 7.2 min, maximal UV absorption at 282 nm) in this product.

3. Conclusions

Increased use of dietary supplements is a worldwide phenomenon. The public opinion that these products are natural and hence safe plays important role in their increased commerce. The way and goals of application of maca in Europe is largely different from the traditional use. Due to the lack of proper quality control in some cases the declared and the real compositions of the products are different and certain products contain non-labelled substances, including synthetic compounds, to enhance the effect.

In half of the 14 randomly selected maca products the maca content could be detected by our method, however, in 6 food supplements the concentration of the marker macamide was below the detection limit and one of the products was adulterated with a synthetic compound. The calculated maca content in the recommended daily doses of the majority of products were below the widely accepted posology of the plant. Our results draw attention to the need for proper quality control of maca-containing dietary supplements.

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