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Detection and identification of multiple adulterants in plant food supplements using attenuated total reflectance—Infrared spectroscopy

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ABSTRACT

Due to the rising popularity of dietary supplements, especially plant food supplements, and alternative herbal medicines, a whole market developed and these products became freely available through internet. Though several searches revealed that at least a part of these products, especially the ones obtained from websites disclosing their physical identity, are aldulterated with pharmaceutical compounds. This causes a threat for public health, since these compounds are not declared and therefore adverse effects will not immediately be related to the product. The more the adulterants can interfere with other medicinal treatments.

Since the present active pharmaceutical ingredients are not declared on the package and the products are sold as 100% natural or herbal in nature, it is very difficult for custom personnel to discriminate between products to be confiscated or not. Therefore easy to apply analytical approaches to discriminate between adulterated and non-adulterated products are necessary.

This paper presents an approach based on infrared spectroscopy combined with attenuated total reflectance (ATR) and partial least squares-discriminant analysis (PLS-DA) to easily differentiate between adulterated and non-adulterated plant food supplements and to get a first idea of the nature of the adulterant present.

The performance of PLS-DA models based on Mid-IR and NIR data were compared as well as models based on the combined data. Further three preprocessing strategies were compared. The best performance was obtained for a PLS-DA model using Mid-IR data with the second derivative as preprocessing method. This model showed a correct classification rate of 98.3% for an external test set. Also eight real samples were screened using the model and for seven of these samples a correct classification was obtained.

Generally it could be concluded that the obtained model and the presented approach could be used at customs to discriminate between adulterated and non-adulterated herbal food supplements and even get a first idea of the nature of the adulterant present. The more the presented approach hardly needs sample preparation.

1. Introduction

The past years a significant increase in the consumption of dietary supplements and especially plant food supplements is observed. This is mainly due to a renewed interest in natural and herbal products to prevent or even cure diseases. A trend that is also promoted in marketing campaigns, which seriously influence the public opinion about the choice of products they use, especially when it concerns over the counter available products that can be used in the context of self-medication or the prevention of sickness. The more the concerns about adverse effects of chemical drugs and the questioning of allopathic medicines has boosted the popularity of plant food supplements and herbal based medicines [1,2].

Due to this increased popularity a whole market developed and the trade in dietary supplements became very lucrative. Nowadays plant as well as other food supplements can be bought in grocery stores, super markets, etc., but are also freely available on the internet and some speciality shops. On the internet a whole scale of

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websites sell dietary supplement promoted as being 100% effective and composed of 100% herbal and/or natural compounds. Despite this, several investigations revealed the presence of active pharmaceutical ingredients as adulterants in these products [3–8]. This is another trend, which increased rapidly during the past decades and represents a serious risk for public health. The adulterants present are not labelled on the package and are often present in the wrong dosages. This means that the consumer/patient is not aware of the fact that he is taking a medicines and so adverse or toxic effects are not immediately related to the dietary supplement, neither by the patient nor by the medical staff. The more the adulterants can interfere with other, sometimes lifesaving, medical treatments [2]. Therefore caution is advised when using dietary supplements purchased from internet or other irregular sources.

Essentially four main groups of adulterated dietary supplements can be distinguished: weight loss products, potency enhancers, muscle building and sport performance enhancers and products for the treatment of pain [2].

In literature a lot of papers can be found dealing with the detection and characterisation of adulterants in food supplements. The most classical approaches are based on chromatography hyphenated with mass spectrometry for identification, followed by an assay with liquid chromatography with UV detection [5–7,9]. The disadvantage of such an approach is the fact that the samples need to be confiscated and send to a competent laboratory. In case no adulterants are found the products have to be released resulting in a loss of resources for both the customs and the laboratory but also for the distributors of the products.

The problem with dietary supplements, encountered at customs is that they can all be considered as potentially adulterated, since the adulterants are not labelled. Off course it is impossible to confiscate and analyse them all. Therefore there is a need for easy to use screening methods, needing no or minimal sample preparation that can be used on the inspection site itself.

Infrared spectroscopy combined with attenuated total reflectance as sampling technique (ATR-IR) and chemometrics was already regularly proposed as an approach to detect adulterants in dietary supplements and other products [4,10–13]. ATR-IR is interesting in this context since it allows samples to be analysed directly in their solid or liquid state without further sample preparation. The more the instruments are rather compact and are suited to be used on site. Several chemometric techniques were described in literature to treat infrared spectra and classify samples or identify some compounds in the samples. In the context of adulterated products principal component analysis (PCA), soft independent modelling of class analogy (SIMCA) and partial least squares analysis-discriminant analysis (PLS-DA) are most often used, although also other techniques, like projection pursuit, classification and regression trees, random forests and support vector machines, were mentioned. PCA is an unsupervised projection technique that is often used for data exploration before modelling, while SIMCA and PLS-DA are supervised techniques used for modelling and classification of samples [4,10–13]. The problem with the studies already reported in literature is that they all focus on the detection of a certain molecule, e.g. sibutramine in weight loss products [11] or on a particular group of dietary supplements, e.g. potency enhancers [10,13].

In this study it was investigated if adulterated and non-adulterated products could be distinguished based on ATR-IR and if the adulterants in the positive samples could be identified based on the same data. Therefore a series of plant food supplements, previously analysed in our laboratory and found negative for adulterants, were intentionally adulterated with six different active pharmaceutical ingredients. ATR-IR spectra were recorded for these mixtures and the non-adulterated products and this both in Mid-IR as in near infrared (NIR). PLS-DA was then used to model the obtained spectra in order to differentiate between adulterated and non-adulterated products and classify adulterated ones in function of the adulterant present. The obtained model was validated using both cross validation as an external test set. As a proof of concept the proposed approach was applied to screen a number of real samples, suspected of containing adulterants.

2. Methods and materials

2.1. Standards and samples

Active pharmaceutical ingredients (API) for paracetamol, acetyl salicylic acid, caffeine, codeine phosphate hemihydrate and ibuprofen were purchased from Fagron (Waregem, Belgium). All products were delivered with a certificate of analysis conform to the European Pharmacopoeia [14]. Phenolphtaleine was purchased from Sigma Aldrich (St. Louis, USA).

The selection of the six API's was a compromise between adulterants, regularly encountered in our laboratory and the price of the API's. The most encountered adulterants are by far the PDE-5 inhibitors and slimming agents like sibutramine. Though the purchase of these types of molecules is expensive and seen the number of triturations to be prepared these could not be taken into account for this project. Caffeine was also taken as API since it is present in a lot of plant food supplements encountered in the laboratory and this in products with a whole scale of indications.

58 herbal dietary supplements, previously send to our laboratory for screening on illegal API's and found negative, were used as matrices to prepare triturations of the selected API's to serve as "self-made" adulterated food supplements.

As a proof of concept eight real samples, send to us by the competent authorities and screened with mass spectrometry, were analysed with the proposed approach. All samples were sold as plant food supplements with a 100% herbal composition. No indication were mentioned on the packaging.

2.2. Sample preparation

Mid-IR and NIR spectra were recorded for the 58 blank herbal matrices (negative samples) considered in this paper. The measurements were done directly using the ATR sampling tool on a part of the herbal mixture present in the capsules.

For each of the selected API's triturations were prepared of 1.6, 3.3 and 10% API in herbal matrix. For each of the API's and at each concentration level ten different triturations were prepared with ten different herbal matrices. To reflect the real life situation the selection of the herbal matrices was done randomly. Table 1 shows the estimated dosage per dosage unit (300 mg) of the API's in the different triturations and the lowest therapeutic dose on the market. As can be seen from the table the selected concentrations of the triturations are equal or significantly lower than the lowest therapeutic dose for all API's

For the selected API's also combined preparations are available on the market [15]. For paracetamol and acetyl salicylic acid combinations exist with caffeine and codeine respectively, as well as a combined preparation paracetamol, acetyl salicylic acid and caffeine. These combinations can also be encountered in adulterated products, therefore triturations were prepared with repectively 1.6% caffeine and 10% paracetamol, 1.6% caffeine and 10% acetyl salicylic acid, 1.6% codeine phosphate hemihydrate and 10% paracetamol, 1.6% codeine phosphate hemihydrate and 10% acetyl salicylic acid, and 1.6% caffeine, 10% paracetamol and 10% acetyl salicylic acid. Again ten triturations were prepared using 10 at random selected herbal matrices from the set of 58. The selected