Journal of Pharmaceutical and Biomedical Analysis 100 (2014) 279–283

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# Detection of sibutramine in adulterated dietary supplements using attenuated total reflectance-infrared spectroscopy

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ARTICLE INFO

*Article history:*
Received 2 June 2014
Received in revised form 24 July 2014
Accepted 6 August 2014
Available online 15 August 2014

*Keywords:*
ATR-IR
Sibutramine
Adulterated dietary supplements
Chemometrics
Mobile detection approaches

ABSTRACT

Sibutramine is one of the most occurring adulterants encountered in dietary supplements with slimming as indication. These adulterated dietary supplements often contain a herbal matrix.

When customs intercept these kind of supplements it is almost impossible to discriminate between the legal products and the adulterated ones, due to misleading packaging. Therefore in most cases these products are confiscated and send to laboratories for analysis. This results inherently in the confiscation of legal, non-adulterated products. Therefore there is a need for easy to use equipment and techniques to perform an initial screening of samples.

Attenuated total reflectance-infrared (ATR-IR) spectroscopy was evaluated for the detection of sibutramine in adulterated dietary supplements. Data interpretation was performed using different basic chemometric techniques. It was found that the use of ATR-IR combined with the k-Nearest Neighbours (k-NN) was able to detect all adulterated dietary supplements in an external test set and this with a minimum of false positive results. This means that a small amount of legal products will still be confiscated and analyzed in a laboratory to be found negative, but no adulterated samples will pass the initial ATR-IR screening.



## 1. Introduction

Sibutramine is a neurotransmitter reuptake inhibitor, structurally related to amphetamines, that reduces the reuptake of serotonine, norepinephrine and noradrenaline, resulting in higher concentrations of these compounds at the synaptic clefts and a reduction of appetite. Sibutramine was marketed in Europe under the brand name Reductil® (Abott Laboratories, Illinois, USA) and was used for the treatment of obesity. In 2010 the European Medicines Agency ordered the withdrawal of sibutramine from the market, based on several studies indicating serious cardiovascular risks. The agency considered that the beneficial effects of sibutramine could not justify the potential occurrence of cardiovascular incidents in treated patients.

Despite the withdrawal of sibutramine of the market, the product can still be bought through illegal channels like the Internet and the black market. This can be in the form of counterfeit medicines or as adulterated dietary supplements. Especially this last group of products is often encountered in laboratories analysing suspicious pharmaceutical products. The risks associated with these adulterations cannot be underestimated. The product is sold as 100% natural, composed of different herbal extracts or as dietary supplement containing a mixtures of plants, vitamins and minerals. The consumer is therefore not aware that he is taking a synthetic drug [1–4].

In literature several methods are described to detect and quantify sibutramine as adulterant in dietary supplements. Most of these methods are using liquid chromatography, either with UV-detection or hyphenated with mass spectrometry [4–9]. Other techniques as UV–vis and IR spectroscopy and voltammetry were also reported [3,9]. The disadvantage of all these methods is that the sample in which adulterants are expected have to be send to a laboratory for analysis. This means that the products have to be confiscated, analyzed and if no adulterants were found released.

Inspectors and customs are often confronted with products, especially dietary supplements, with indication weight loss. All these products can be considered as potentially containing adulterants. Off course it is impossible to confiscate all of them. The

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http://dx.doi.org/10.1016/j.jpba.2014.08.009
0731-7085/

more it should be mentioned that a wide variety of dietary supplements composed of 100% natural products are available on the legal market, and so are not considered as a risk. This differentiation is very difficult for inspectors and custom personnel. Therefore easy to use screening methods that can be used on the inspection site itself would be very useful.

Infrared spectroscopy (IR) is a technique that was already regularly used for the detection of adulterations [9–12]. Attenuated total reflectance (ATR) is a sampling technique that is often used in combination with IR, which enables samples to be examined directly in their solid or liquid state without any further sample preparation steps. ATR-IR is therefore very interesting, since it is simple, no sample preparation is necessary and the instruments are rather compact.

In this study ATR-IR was evaluated for the detection of sibutramine, which is by far the most encountered adulterant in dietary supplements for weight loss. Therefore the ATR-IR spectra were measured for a set of 125 suspected dietary supplements, which were previously analyzed in our laboratory for the presence of adulterants. The samples were divided in two groups, based on the presence of sibutramine. In a next step chemometric tools were applied in order to perform a data exploration and to develop classification models based on the ATR-IR data. The performance of the models were evaluated and compared based on both cross validation as on the prediction error of an external test set.

## 2. Theory

### 2.1. Exploratory methods

#### 2.1.1. Principal component analysis (PCA)

PCA is a chemometric technique in which high dimensional data is projected into a low dimensional space of new latent variables, called principal components. These principal components are linear combinations of the manifest variables, in which the weights or loadings of the different variables are chosen in such way that the first principal component represents the highest variance in the data set and the higher principal components the highest remaining variance. In this way the number of variables is reduced, allowing a graphical representation of the data. By definition the different principal components are orthogonal. The loadings of the variables show their respective contribution to a given PC and the correlation between the different explanatory variables. The projections of the objects on the PCs are called the scores and are a measure for the similarities among objects [13].

#### 2.1.2. Projection pursuit (PP)

PP is similar to PCA, though the latent variables, called projection pursuit features (PPFs) are obtained by maximizing a projection index describing inhomogeneity of data. In such way PP can be complementary to PCA in revealing the data structure and groups of similar samples. The algorithm as described by Croux and Ruiz-Gazen was used [14] in this study. Two projection indices were tested: entropy and yenyukov.

### 2.2. Modelling methods

#### 2.2.1. k-Nearest Neighbours (k-NN)

k-Nearest Neighbours (k-NN) [13], is a classification technique where classification rules are defined based on neighbourhoods of training set objects. The neighbourhoods are defined by calculating the Euclidian distances between the unknown object and each of the objects of the training set. For a training set with n samples, n distances are calculated. The unknown object is classified into the group to which the majority of the k neighbouring objects (the k objects with the smallest Euclidean distances) of the training samples belong.

#### 2.2.2. Partial least squares-discriminant analysis (PLS-DA)

Partial least squares is a projection technique, similar to PCA. The latent variables, called PLS-factors, are also linear combinations of the original manifest variables, but are defined in such way that they maximize the co-variance with the response variables. This means that contrary to PCA PLS is a supervised technique, taking in account the response variables. PLS-DA is a variant, used when the response variables are categorical [13].

#### 2.2.3. Classification and regression trees (CART)

CART is a non-parametric statistical technique that is able to solve both classification (categorical dependent variables) as regression problems (continuous dependent variables) [15]. In both cases the method builds a decision tree, describing a response variable as a function of different explanatory variables. Classification trees are obtained when the response variable is categorical. Regression trees are calculated in the case of continuous dependent variables.

A CART analysis consists of three steps. In a first the maximum tree is build, using a binary split-procedure. To choose the most appropriate variable (wavelength in our study) and split value, CART uses an algorithm in which all descriptors and all possible split values are considered. The split resulting in the highest decrease in impurity between the mother group and the daughter groups is selected. For classification trees the impurity can be defined by different split criteria [15]. The three commonly used split criteria are the Gini index, the Twoing index and the Information index.

The maximum tree usually shows overfitting and therefore it is pruned in a second step of the analysis. This procedure results in a series of less complex subtrees of the maximum tree. In the third and final step the optimal tree is selected using a cross validation procedure. More details about CART can be found in Refs. [12,15,16].

#### 2.2.4. Random Forests

Random Forests are based on a combination of tree based classifiers, similar to the ones obtained with CART [17]. The set of classification trees is trained using random bootstrap samples from the training set (generally two third from the samples in the set). The feature selection for the respective trees is based on bagging and random feature selection. The number of trees to be included in the model is based on the minimization of misclassification. During cross validation and external validation the prediction from the different trees are combined in one final prediction, based on the principle of voting [17].

## 3. Methods and materials

### 3.1. Samples

All samples used in this research were donated by the Federal Agency for Medicines and Health Care Products (FAMHP) in Belgium. They all come from postal packs ordered by individuals via Internet sites. The sample set contains a variety of pharmaceutical forms namely capsules, gel capsules, powders, powders for fruit beverages and instant coffee. All samples, once received were stored at ambient temperature and protected from light. All samples were screened previously in our laboratory using LC-Ion trapMS. The sibutramine containing samples contained concentrations between 3 and 20 mg per dosage unit. 5 mg (±1 mg) per dosage unit was the most frequent occurring concentration.