# Innovative miniaturized approach by MicroNIR and chemometrics for the monitoring of the occupational exposure of workers

# Innovative miniaturized approach by MicroNIR and chemometrics for the monitoring of the occupational exposure of workers

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**Abstract.** The study focuses on the monitoring of the cladribine exposure during production of pharmaceutical ingredients by a novel first level test based on microNIR spectroscopy for the monitoring of the worker's exposure to drugs. The method permits to perform the analyses directly onsite and online as it is portable and miniaturized and record data in wireless mode. The calibration of the response was performed by considering filter membrane fortified with increasing amount of cladribine to reproduce real samples. All the signals were processed by chemometrics and a model of prediction was finally developed to predict unknown samples. Gas chromatography was used to compare the results from a reference technique with the ones from the novel method, obtaining a satisfactory correlation of 0.9989.

## 1. Introduction

The problem of pollution from industrial activities is a very important topic in the various research fields, as leads to the dispersion of many pollutants, which can cause serious consequences for plants, animals, living beings in general and obviously also for the health of man himself. [1-3]. The pollutant elements are varied: plastic dispersion, fine dust, liquids, radioactive waste, radiation, gas, oil waste but also vibrations and loud noises.

In particular, dye and dust pollution [4-7] can affect different environments such as soil, water and air especially from the vehicular emissions [8-10]. Recently, a number of innovative materials have been proposed for the removal of pollutants [11], including nanotubes [12, 13], pellets and zeolites [14-17] from water environment.

Regardless to water treatment strategies, a significant contribution may be related to compound that exhibit sequestering abilities to different ions [18-21]. To this aim, numerous studies in the recent years focused on the evaluation of innovative model systems able to be proposed as novel material for water remediation [22-25].

An other important aspect to take into account when dealing with environmental pollutants is related to the exposition of workers during manufacturing processes, especially in pharmaceutical industries. In this fiel, novel synthetic strategies [29-32] were considered in the art to propose innovative metal free [25-28] and non toxic synthetic procedures by in situ preparations [33-36]

Cladribine is a cytotoxic purine analog resistant to the action of adenosine deaminase, frequently involved in the treatment of indolent lymphoproliferative disorders such as hairy cell leukemia [37-40]. Pharmaceutical processes, transport, and distribution may expose workers to Cladribine during manufacturing [41-44]. As a consequence, the need of rapid and easy to use methods for the

monitoring of the occupational exposure of workers, are more and more required, especially in such situations involving cytotoxic compounds [45-48].

Chromatographic techniques offer fast, automated, and highly accurate method of identifying certain chemicals in a sample, but it can be expensive, complex, and doesn't work for all samples [49]. Medical, forensic, environmental, and manufacturing laboratories use the technique to quantify and separate chemicals in a sample. Chromatographic techniques [50, 51] such as liquid and gas chromatography usually coupled to mass spectrometry [52-54] may be used to detect cladribine in filter membrane. Neverthless, the chromatographic techniques require the pretreatment step [55, 56] in order to extract or concentrate analytes, that may lead to reduce the sensitivity. In addition, liquid chromatography may require a large quantities of expensive organic products. Techniques such as spectroscopy in the InfraRed or Near InfraRed region can be cheaper and even faster, especially for analysis in good manufacturing practice.

Different techniques and instruments are widely used in the physical and chemical characterization of materials both at the level of basic and industrial research and increasingly also in the bio-medical-pharmaceutical fields. Spectroscopic techniques are usually involved for a multiparametric investigation of real matrices because permit to obtain results eliminating the step of the sample pre-treatment [57].

The portable NIR instruments meet these needs, allowing the analysts to carry out the analysis directly in the company and therefore to make changes online if necessary. The Nir is able to "see" only the surface of the samples and therefore it is extremely important that there is an accurate sampling and presentation of the sample to be analyzed [58, 59].

In particular, the coupling of NIR spectroscopy and chemometrics leads to performing results and is able to be used from not specialized personnel after the optimization of the model [60, 61]. Chemometric methods provide solutions for separating useful information from what else is contained in the data, minimizing time and costs [62]. In addition, by the mean of a miniaturized spectrometer, predictions of compounds may be performed directly on site [63, 64] and the optimized methods may be transferred to real situations [65-67]. The advantage of portable device is not only related to the possibility to perform analyses onsite but also permits to obtain results rapidly by not trained personnel.

The paper proposes a new system, the MicroNIR, operating in the Near Infrared region of the spectrum and uses chemometrics to interprete results and correlate for the monitoring of the occupational exposure 2-chlorodeoxyadenosine (cladribine, 2-CdA).

## 2. Experimental

A Chronos sampling device was used to collect air on glass fiber filters during Cladribine manufacturing. Spectra in the NIR region were collected by a miniaturized instrument (45 x 42 mm), the MicroNIR weighting 60 g that transfers spectra in a wireless mode directly to the computer for processing by chemometric algorithms.

Prior to the acquisition of the sample, the instrument requires calibration though a special collar that permits the registration of a signal related to the total absorbance (dark reference) and a total reflectance (Spectralon). The instrumental settings included: an integration time of 10 ms and a nominal spectral resolution at 6.25 nm. The total time of analysis was 2.5 s per sample. Multivariate statistical analysis was performed by V-JDSU Unscrambler Lite (Camo software AS, Oslo, Norway). In addition, GC-MS technique was used to compare results and validate the model.

## 3. Results

The first step for the development of reproducible methods is related to calibration of the spectral response using simulated samples that reproduce the real samples. In this case, fortified glass filter membranes were used to collect air and standard solutions of cladribine were used to add increasing amount of cladribine on the filter (Figure 1). Spectra of Cladribine spiked onto fiber glass membrane were acquired and compared to those recorded for the filters without the molecule (the blank). A

significant matrix effect may be observed in Figure 2, as the two average spectra of blank (blue) and spiked blank (green) seemed to be not distinguishable.

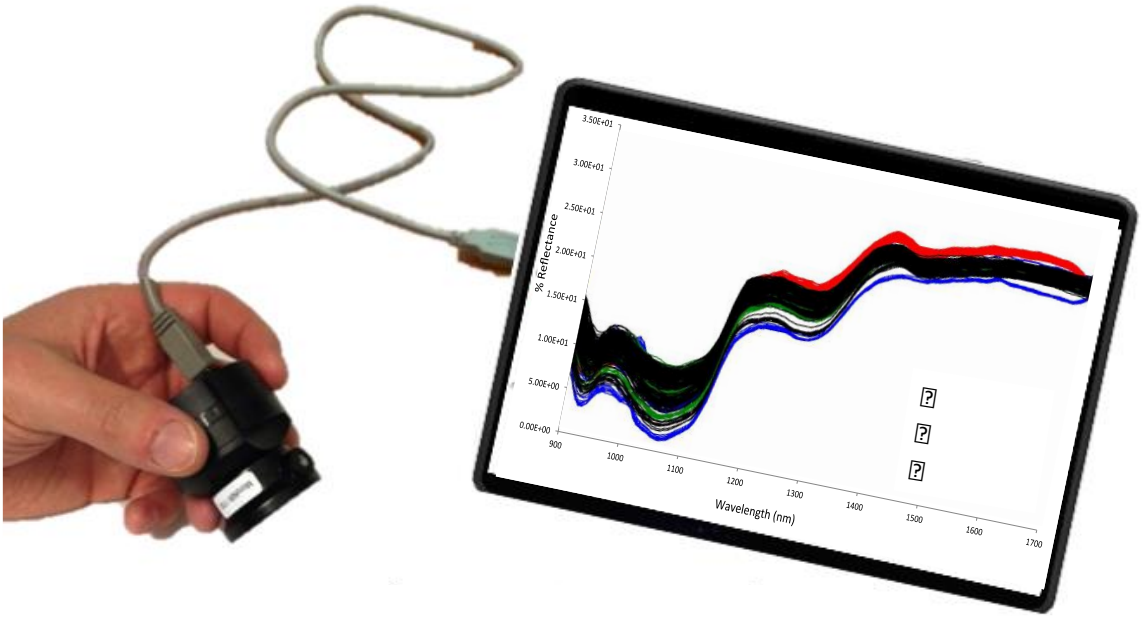


**Figure 1.** MicroNIR platform for real-time acquisition and prediction of cladribine on filter membrane.

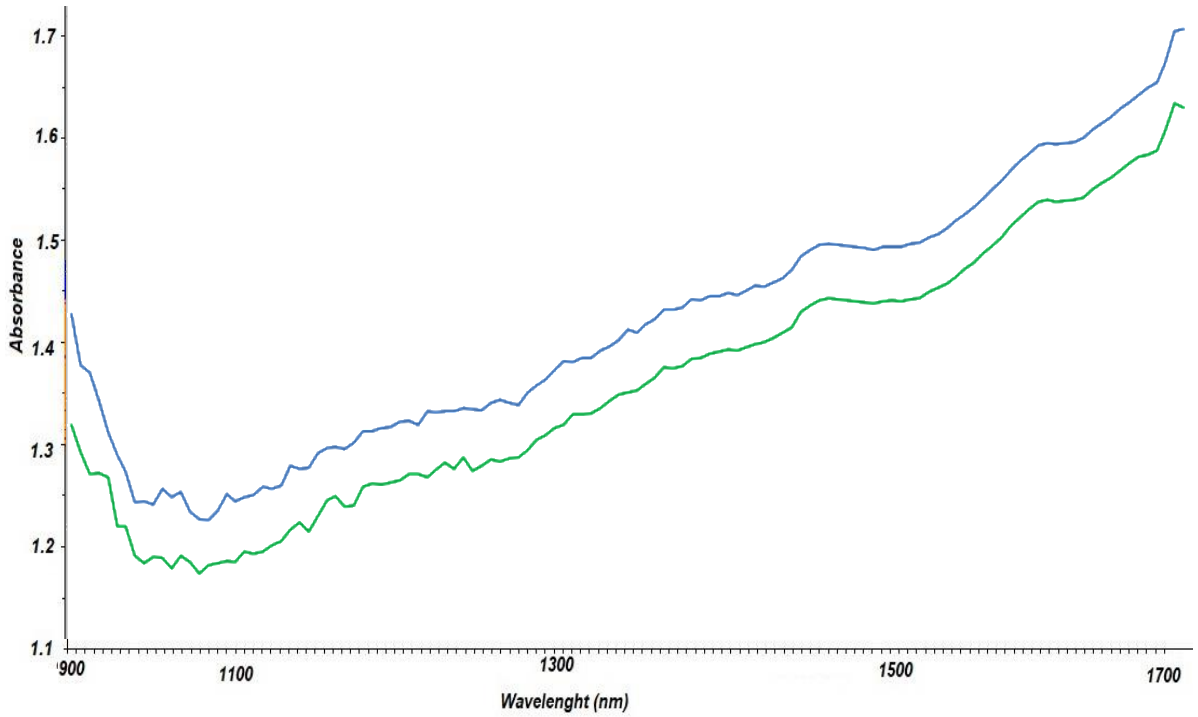


**Figure 2.** Spectra collected by MicroNIR of blank (blue) and spiked blank with Cladribine (green).

Chemometric methods require that there is maximum comparability between the variables. This easily does not happen to natural data, which for example can have dramatically different orders of

magnitude. The basic pre-treatments are: transformation of variables, data scaling, replacement of missing data.

The most common problems of non-comparability are solved by applying scaling, also called standardization. The most frequently used are Centering (centering, CS), Scaling with respect to the maximum (maximum scaling, MS), Interval scaling (range scaling, RS), Autoscaling (autoscaling, AS).

Principal component analysis (PCA) is the most important among the data exploration techniques and consists in transforming the original variables into new variables, called principal components (or latent variables), obtained by linear combination of the original variables and such as to be orthogonal to each other.

The PCA allows you to reduce the dimensionality of the data, representing them in an orthogonal space; to eliminate spurious information (eg: instrumental noise); to assess the relative relevance of the variables; to view objects and search for outliers, clusters, classes. Consequently, PCA is the starting point for many multivariate techniques

In this work, signal pretreatments [68] of the data matrix were investigated in order to improve sample separation were evaluated: in particular, Standard Normal Variate transform (SNV), Multiplicative Scatter Correction (MSC), Mean Centering (MC) and normalization [69,70] as scatter-correction methods and spectral derivation including Savitzky-Golay (SG) polynomial derivative filters. The most performing mathematical pre-treatment uses a combination of the baseline correction, first derivative transform and Multiplicative Scatter Correction (MSC). By this way, samples resulted well separated according to the amount of cladribine on the filter membrane.

PCA was used to evaluate results and the corresponding scores plot is reported in Figure 3, where each point represents the average of 10 spectra and colors are used to differentiate the Cladribine amount.

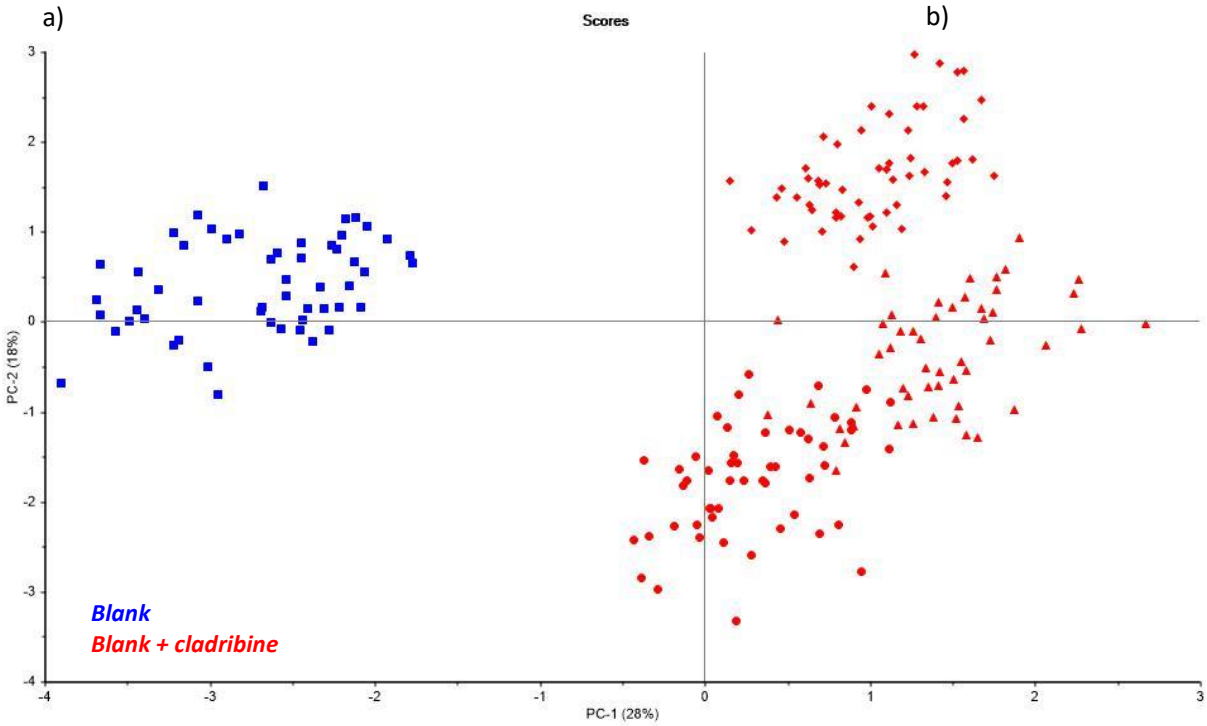


**Figure 3.** Scores plot from PCA of: a) blank (blue) and b) spiked blank with cladribine (red).

Figure 3 demonstrate that samples are located in the plot according to PC 1, thus demonstrating that samples may differentiated as a function of the presence of Cladribine on the filter membrane.

## 4. Model of prediction

The models summarize the state of knowledge of a problem and allow to predict the evolution of the system studied. The construction of a mathematical model is always preparatory to chemometric procedures such as classification and regression. The classification is based on qualitative models, the regression on quantitative models. The procedure on which the search for a model is based is divided into 4 phases: 1) Identification. It consists in establishing whether the model is of a deterministic type, that is, based on a priori knowledge of functional connections between the variables, or stochastic, that is, based on statistics; 2) Construction. It consists in giving a numerical form to the model, through a fitting procedure; the latter involves the estimation of parameters that define the model and the evaluation of uncertainty; 3) Validation. It consists in checking the model (testing), quantifying its descriptive capacity and predictive capacity; 4) Application. The model is used to predict unknown events.

In this work, a model of prediction for Cladribine on a filter membrane was developed and validated by the regression algorithm PLS in the range 1-200 ng/filter.

Partial least squares regression is probably the least restrictive of the various multivariate extensions of the multiple linear regression model. This flexibility allows to use this method in situations where the use of traditional multivariate methods is very limited. Partial least squares regression has become a standard tool for the calibration of chemical-analytical methodologies that provide multivariate measurements. In general, in the face of a more complicated operation, the PLS still provides simpler models and is able to give exhaustive answers even in the presence of inaccurate data [62,62,69].

Validation of a model is the evaluation of its predictive capabilities. It can be done in two ways. The first is CROSS VALIDATION, that consists in excluding some objects and building the model with the rest, and then using the excluded objects as a reference standard. Two different ways are available: a leave-one-out (LOO), where given n objects, n models are calculated by excluding a object at a time: each model is calculated on n-1 objects and verified on the object excluded from the model calculation; leave-more-out (LMO). This is similar to LOO, with the difference that k is excluded objects at a time. A set of k objects excluded from the calculation and used to test the model is called a deletion group.

The second way is VALIDATION ON TEST SET. The original data is divided into two groups, called training sets and evaluation sets or test sets. The model is calculated on the training set. The predictive capacity is quantified on the evaluation set. The optimal situation is that in which training sets and test sets are defined following experimental design [69,70].

The entire data set of measurements were splitted into calibration and validation set in order to instruct the platform to predict cladribine. To evaluate model's ability, 15 independent samples were analyzed by the microNIR and results were compared to the parallel GC-MS analysis. This step ensures reproducibility and effectiveness and confirm that results are not bath-dependent. 3D scores plot is reported in Figure 4.

MicroNIR results were found to be significantly different concluding that the chemometric investigation of data provided for an accurate and sensitive method to quantify cladribine on a filter membrane without requiring extraction procedures. The correlation of the results from microNIR and GC-MS showed a correlation coefficient of 0.9989. In addition, the novel method permits to investigate even amount of cladribine on the filter that are 100 times lower than the reference chromatographic method.

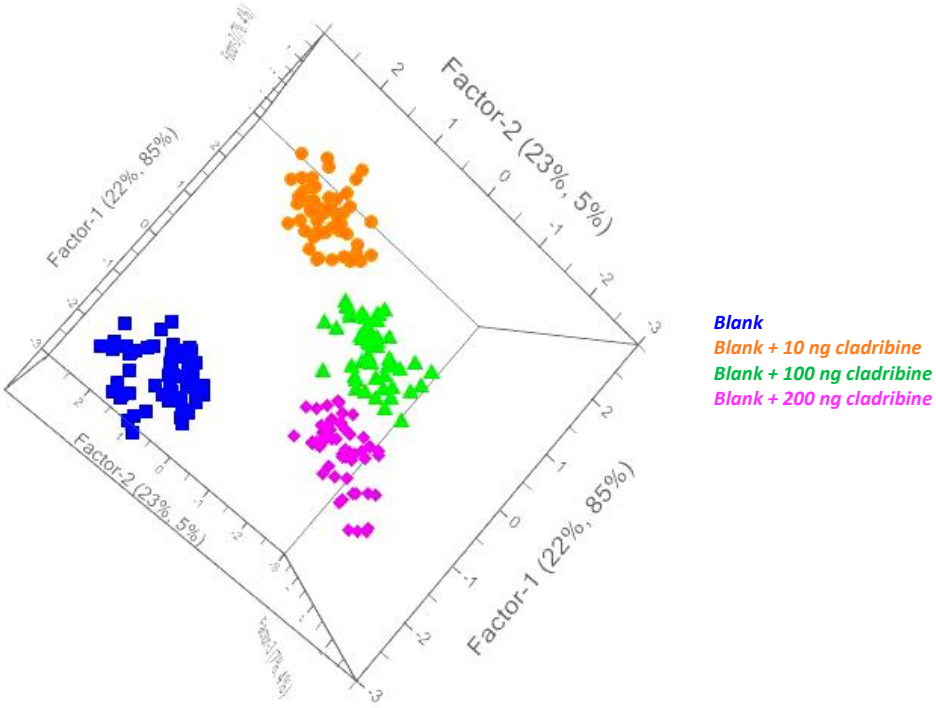


**Figure 4.** 3D Scores plot from PLS-DA of blank (blue) and spiked blank with 10 ng (orange), 100 ng (green) and 200 ng (pink) of Cladribine.

## 5. Conclusions

This work presents a novel promising research focusing on the monitoring of cladribine on glass filter membrane by a MicroNIR/Chemometric approach. This new method demonstrated to be useful to accurately detect cladribine at low amount as it directly measures the sample without pretreatment. In addition, the errors of estimation are those required for confirmatory test, suggesting the high potential of the platform. The preliminary results demonstrated the feasibility of the miniaturized approach to provide an innovative and timely monitoring of workers exposed to Cladribine during manufacturing in pharmaceutical industries.

The coupling of NIR spectroscopy and chemometrics, through a portable instrument, allows for quick and easy analyzes that can be used for monitoring air pollution from different materials. In particular, the work demonstrates to be able to analyze sampled air on filter membranes with high sensitivity and can be proposed for the investigation of other molecules.

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