Original scientific paper
UDC 615.322.074:543.422.3-74

APPLICATION OF NEAR INFRARED SPECTROSCOPY FOR DETECTION OF GROUND MEDICINAL HERBS

Antonela Režan[1], Maja Benković[1], Tamara Jurina[1*], Ana Jurinjak Tušek[1], Jasenka Gajdoš Kljusurić[1], Davor Valinger[1]

[1]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

[*]e-mail: tlekic@pbf.hr

Abstract

Medicinal plants represent a rich source of biologically active compounds that could have a beneficial effect on human health and a wide range of medicinal plants can be found on the market today. Regular or in-line particle size measurement can be one of the ways to control the quality of certain products where medicinal plants are used as raw materials for their production. The aim of this study was to examine the possibility of differentiating different particle sizes of the same plant and different particle sizes of different plants.

The non-invasive spectroscopy in the near infrared range of 904 - 1699 nm (NIR) was used to record spectra of five different plants: sage (*Salvia officinalis* L.), thyme (*Thymus serpyllum* L.), lavender (*Lavandula x hybrida* L.), lemon balm (*Melissa officinalis* L.), and mint (*Mentha piperita* L.) for seven different particle sizes (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm and 1000 µm). NIR spectra were used for principal component analysis.

The results showed a uniform particle size distribution for smaller fractions (< 100 µm, and 100 µm) for each medicinal plant individually, while for larger fractions the presence of smaller particles influenced the uniformity. This was confirmed by microscopic images of all the fractions. The presence of smaller particles was observed especially for 800 µm particle sizes of lavender and sage. By comparing the same particle size for all medicinal plants NIR showed very good differentiation between medicinal plants of the same particle size.

Results confirm that NIR spectroscopy can be successfully used in qualitative and quantitative differentiation of different particle sizes of different plants and its very high sensitivity to different particle sizes of the same plants.

***Key words**: NIR spectroscopy, Medicinal plants, Principal component analysis, Grounding.*

1. Introduction

Accelerated rhythm of life and everyday responsibilities leave very little time to care about proper nutrition and health, consequently increasing number of chronic diseases such as cancer, vascular diseases and neurodegenerative diseases. Lack of natural antioxidants in the diet can also be one of the causes of many chronic, degenerative diseases and various cancers [1]. Since ancient times and in various cultures around the world, people have used herbs as a healing asset. It is considered that medicinal plants are effective and safe for use with more than a thousand years of experience in use [2]. Most medicinal herbs are the richest natural source of bioactive compounds in the manufacture of drugs for traditional medicine, functional food products, pharmaceutical intermediates and dietary supplements [3]. The *Lamiaceae* family is rich in phytochemical properties and is widely used as medicinal and spice herbs. Some of the plants from this family that are used in folk medicine are sage, lavender, lemon balm, mint and thyme [4]. A large number of studies have been carried out to demonstrate the effectiveness of modern plant medicine, but one of the main challenges is still the use of well-defined and standardized plants and herbal ingredients. The excretion of bioactive components from medicinal herbs has led to the development of numerous methods for their extraction but also for the method of monitoring the physical and chemical properties of bioactive components.

Today, scientists, despite numerous analytical methods, find it difficult to determine the standards for a given product derived from a medicinal plant. The reason for this may be numerous differences in the chemical composition, functional and structural diversity of chemical substances present in the plant, and inconsistent methods for extraction of active substances [5]. Increasing market and consumer desires for quality food with positive health benefits created the need for efficient and accurate analytical methods for estimating raw materials and finished products. Regular or line measurements of the particle size of plant parts can be one of the ways to control the quality of certain plant products. One of the methods for monitoring the physical and chemical properties of food products is NIR spectroscopy (NIRs). The NIR technique is specific and sensitive to changes in the chemical and physical properties of the sample and thus can be used to monitor the authenticity and origin of different products and as a fingerprint in the agricultural and food industry [6].

The aim of this paper was to use the non-invasive spectroscopy in the near infrared range of λ = 904 - 1699 nm to record spectra of five different plants: sage (*Salvia officinalis* L.), thyme (*Thymus serpyllum* L.), lavender (*Lavandula x hybrida* L.), lemon balm (*Melissa officinalis* L.), and mint (*Mentha piperita* L.) for seven different particle sizes (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm and 1000 µm). The recorded spectra were analysed using Principal Component Analysis (PCA) in order to examine the possibility of differentiation between different particle sizes of the same medicinal herbs and the same particle sizes of different medicinal herbs, as well as to confirm the applicability of a fast and non-destructive NIRs for medical plant characterization.

2. Materials and Methods

2.1 Plant materials

Sage (*Salvia officinalis* L.), thyme (*Thymus serpyllum* L.), lavender (*Lavandula x hybrida* L.), lemon balm (*Melissa officinalis* L.) and mint (*Mentha piperita* L.) were purchased from a local herbal store (Suban d.o.o., Zagreb, Croatia). Plants were harvested during the season of 2015. Harvested plants were sun dried and packed in paper bags which were stored at ambient conditions prior to use.

2.2 Milling

Dried plants were milled using IKA Tube mill control (IKA-Werke, Staufen, Germany). Milling conditions were as follows: rpm = 20 000 min^{-1} for t = 40 s. Since the plant material had different organic structures and thus different hardness, for some samples milling time was reduced to t = 20 s. After milling, samples were kept in a desiccator until further used.

2.3 Separation of particle size fractions

Milled plant material was subjected to sieving in order to separate the particle size fractions. The following sieves (Fritsch, Idar-Oberstein, Germany) were used: 1000, 800, 500, 355, 250 and 100 µm pore diameter. Seven different particle size fractions were obtained marked as < 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm, and 1000 µm, which were then subjected to NIR analysis.

2.4 NIR spectroscopy

The basic principle in NIRs is that the examined sample is irradiated with NIR radiation and the reflected or transmitted radiation is recorded in the form of a spectrum [7]. In this study, the NIR spectra (in the range from λ = 904 nm to λ = 1699 nm) of ground medicinal herb fractions were collected with the setup for NIRS studies previously described by Valinger *et al.*, [8], that included NIR-128-1.7-USB/6.25/50 µm scanning monochromator (Control Development, Inc., South Bend, USA), provided with Spec32 software. For every ground fraction of medicinal herbs 6 measurements were performed without any prior mechanical or chemical treatment.

2.5 Microscopic imaging

In order to identify size and structure of particles present in the ground medicinal herb samples a BTC Type LCD-35 (Bresser, Rhede, Germany) light microscope imaging was performed at 4x magnification and photographed by an integrated Moticam 3 microscope camera (Motic, Kowloon, Hong Kong).

2.6 Data analysis and chemometrics

NIR spectroscopy is based on electromagnetic radiation in the near infrared spectrum and in order to analyse the recorded spectra one of different chemometric techniques such as multivariate linear regression analysis (MLRA), principal component analysis (PCA) or canonical variate analysis (CVA) has to be applied [9, 10]. In this case, PCA was used for identifying patterns in data and expressing the data to highlight similarities and differences. Its goal is to extract the important information from the data table and to express this information as a set of new orthogonal variables called principal components or factors [11]. Pre-processed spectra were used to perform PCA by means of statistical software Statistica v. 10 (StatSoft Inc., USA) and were also plotted in 3D using Wolfram Mathematica v.10 (Wolfram Research, USA).

3. Results and Discussion

For each of seven particle size fractions (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm, and 1000 µm), 6 recordings were performed for each of medicinal herb

(sage, thyme, lavender, lemon balm, and mint) using NIR instrument and the example of 42 sage raw spectra is presented in Figure 1.

As mentioned before, it is not possible to see the difference between the samples from the spectral data unless at least one of the chemometric methods is applied. Although a shift in the spectra is visible when looking at the absorbance units, principal component analysis analyses the data in a way that it searches similarities and differences for every single wavelength of all the samples. PCA normalizes these shifts so they have no influence on analysis. Only the heights of certain peaks that are either rising or descending are the base for clustering. In order to test the differences between all seven particle size fractions PCA analysis was performed for all medicinal herbs which is shown in Figure 2 a-e.

It could be noticed that the best separation of different particle sizes was observed in Figure 2 c for lavender, but the main problem in all the figures was that some of the different particle sizes were overlapping. While smaller particle size fractions were better clustered for all the medicinal herbs especially in the case of < 100 µm particle diameter (Figure 2 a-d) larger particle sizes had higher dispersion. This is very interesting since in all the cases eigenvalue of the first two factors is extremely high (over 99.6%) and despite that the first factor that accounts for a maximal amount of total variance in the observed variables was in all the cases in range of 98.94 – 99.70%. The reason for overlapping of the larger particle size samples could be seen on microscopic images (Figure 3) where 800 µm particle size samples of lavender and sage are shown.

It is visible from the microscopic images (Figure 3) that different particle sizes are present in both samples. The fractions that were best separated (< 100 µm) did not have any larger particles and as it is previously shown in the work of Valinger *et al.*, [12], stating that the NIR instrument is very good at separating very small particle sizes. For example, the particle size fraction of 800 µm still has some particles in the size range from 800 - 1000 µm, but also smaller particles which tend to bind

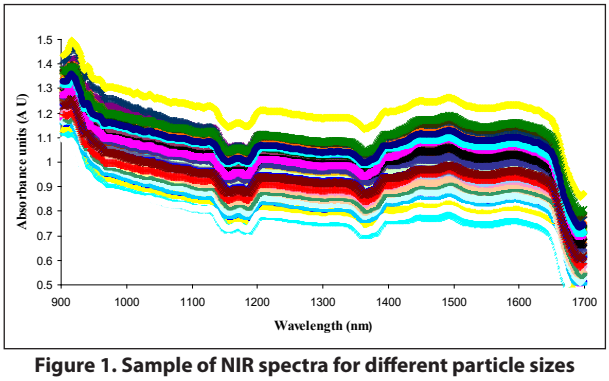


Figure 1. Sample of NIR spectra for different particle sizes (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm, and 1000 µm) of sage

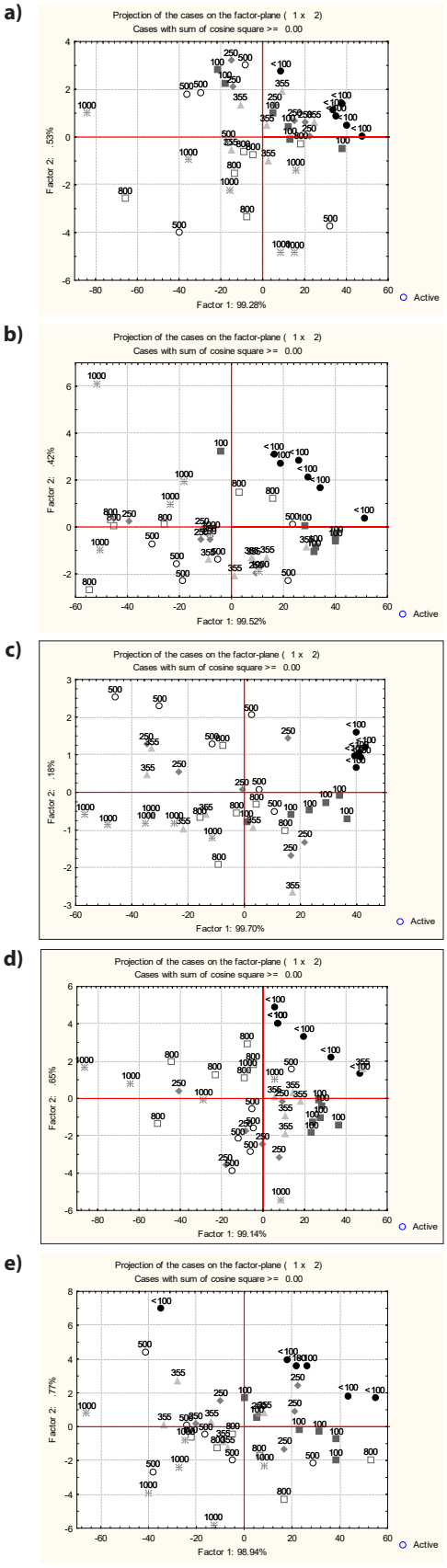
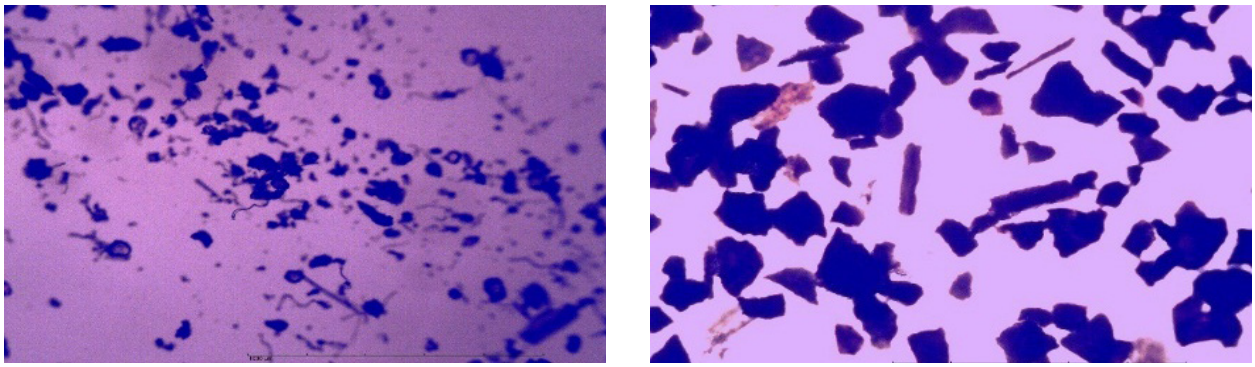


Figure 2. Principal component analysis for the first two factors for particle size fractions (< 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm, and 1000 µm) of a) sage; b) thyme; c) lavender; d) lemon balm; and e) mint

onto the larger particles. This presence of different particle sizes certainly has an influence in clustering of same particle size fractions, and the only way this could be resolved is to use certain fractions that are of different size and then do a calibration with mixes of different ratios. In order to get good calibration with NIR, some other techniques based on laser diffraction are employed especially in pharmaceutical industry [13].

In order to see if NIR can distinguish different medicinal herbs of the same particle size, another PCA analysis was performed. Figures 4 a-e give the PCA results of the same particle sizes of different medicinal herbs ranging from < 100 µm, 100 µm, 250 µm, 355 µm, 500 µm, 800 µm, and 1000 µm respectively. As can be seen in all the figures, lavender samples are completely separated from the rest of the medicinal herbs and clustered in the opposite quadrants of other medicinal herbs and, in all cases, quite isolated from other plants, which could be attributed to certain physical and chemical properties that should be further explored in future research.



a) b)

Figure 3. Microscopic images of 800 µm particle size samples of a) lavender and b) sage

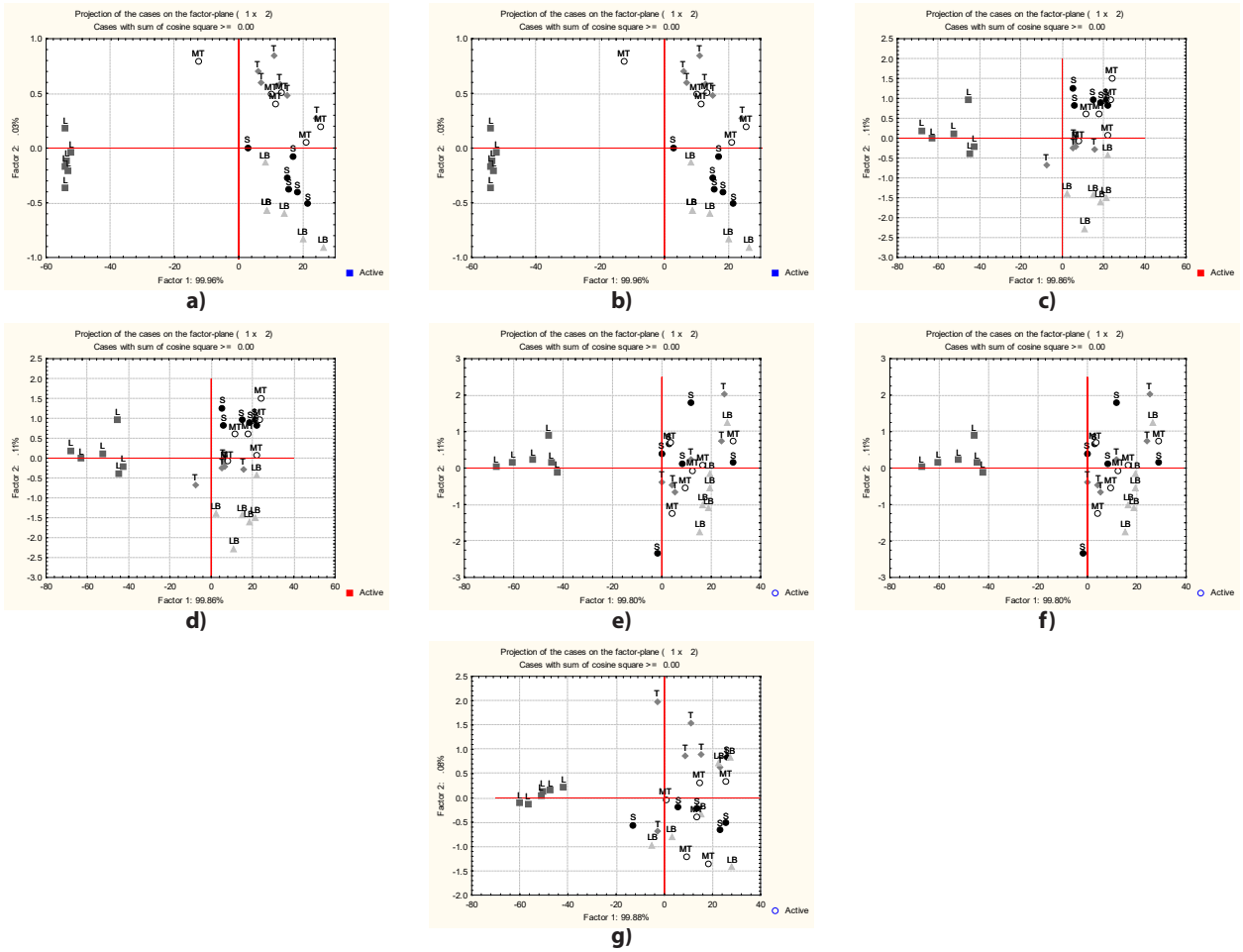


Figure 4. PCA images of samples for particle sizes of a) < 100 µm; b) 100 µm; c) 250 µm; d) 355 µm; e) 500 µm; f) 800 µm; and g) 1000 µm, for five different medicinal plants (S - sage, L - lavander, LB - lemon balm, T - thyme, MT - mint)

Very good separation was achieved for particle sizes ranging from < 100 to 500 µm for all the medicinal herbs while at larger particle size fractions of 500 µm to 1000 µm (Figure 4 e-g) sage was the most dispersed. Sometimes it is hard to see the distinction between the samples on two dimensional figures and in such cases 3D PCA is used. Furthermore, sometimes the third factor can be very influential in separating the samples that are otherwise overlapping in 2D. This is more beneficial if the first two factors have lower eigenvalues and high variance in the observed variables. Example of 3D PCA for particle sizes of < 100 µm samples of medicinal herbs is shown in Figure 5.

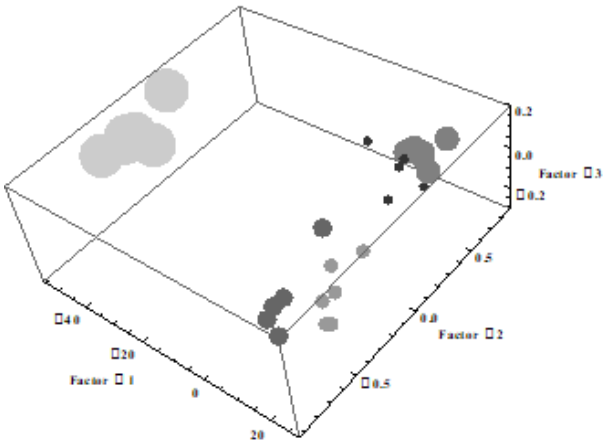


Figure 5. Principal Component Analysis of five medicinal plants ground samples for particle sizes of < 100 µm by the first three factors (● sage, ● lavander, ● lemon balm, ● thyme, ● mint)

In comparison with the Figure 4 a where the samples of thyme and mint are overlapping Figure 5 shows better separation of those two medicinal herbs.

4. Conclusions

- NIR spectroscopy combined with PCA analysis as a chemometric method shows good potential as an analytical technique for particle size monitoring. Although data processing requires knowledge of chemometry, the calibrated system could be used as continuous, without the need for additional data processing.

- Regular or on-line measurement of particle size of medicinal herbs with NIR spectroscopy can be one of the ways to control the quality of certain products where medicinal herbs are used as raw materials for their production and also when developing new food products.

5. References

[1] Gupta R. K., Patel A .K., Shah N., Choudhary A. K., Jha U. K., Yadav U. C., Gupta P. K., and Pakuwal U. (2014). *Oxidative stress and antioxidants in disease and cancer: A review.* Asian Pacific Journal of Cancer Prevention, 15, pp. 4405-4409.

[2] Yang C. L., Or T. C., Ho M. H., and Lau A. S. (2013). *Scientific basis of botanical medicine as alternative remedies for rheumatoid arthritis.* Clinical Reviews in Allergy & Immunology, 44, pp. 284-300.

[3] Handa S. S., Khanuja S. P. S., Longo G., and Rakesh D. D. (2008). *Extraction technologies for medicinal and aromatic plants*. International centre for science and high technology, Trieste, Italy.

[4] Nikolić T. (2013). *Systematic botanics* (in Croatian). Alfa, Zagreb, Croatia.

[5] Cravotto G., Boffa L., Genzini L., and Garella D. (2010). *Phytotherapeutics: An evaluation of the potential of 1000 plants.* Journal of Clinical Pharmacy and Therapeutics, 35, pp. 11-48.

[6] Cozzolino D., and Roberts J. (2016). *Applications and developments on the use of vibrational spectroscopy imaging for the analysis, monitoring and characterisation of crops and plants. Review*. Molecules, 21, pp. 755.

[7] Nicolai B. M., Beullens K., Bobelyn E., Peirs A., Saeys W., and Theron L. I. (2007) *Non-destructive measurement of fruit and vegetable quality by means of NIR spectros¬copy: A review*. Postharvest Biology and Technology, 46, pp. 99-118.

[8] Valinger D., Gajdoš Kljusurić J., Kovačević D., and Kurtanjek Ž. (2011). *Application of NIR spectroscopy for monitoring quality of surimi.* Proceedings of 13th Ružička days, Today Science - Tomorrow industry, Vukovar, Croatia, pp. 431-438.

[9] Ding H. B., and Xu R. J. (1999). *Differentiation of beef and kangaroo meat by visible/near-infrared reflectance spectroscopy.* Journal of Food Science, 64, (5), pp. 814-817.

[10] Alishahia A., Farahmanda H., Prietob N., and Cozzolino D. (2010). *Identification of transgenic foods using NIR spectroscopy: A review.* Spectrochimica Acta A., 75, pp. 1-7.

[11] Abdi H., and Williams L. J. (2010). *Principal component analysis.* WIREs Computational Statistics, 2, pp. 433-459.

[12] Valinger D., Benković M., Gajdoš Kljusurić J., Bauman I., and Kurtanjek Ž. (2011). *Application of NIR spectroscopy for monitoring different particle sizes of sucrose.* Journal on processing and energy in agriculture, 15, (3), pp. 188-190.

[13] Carrilho Figueiredo J. L. (2008). *FT-NIR spectroscopy and Laser Diffraction particle sizing of APIs in Pharmaceutical formulations.* Superior Technical Institute, Technical University of Lisbon, Lisbon, Portugal.