Journal of Pharmaceutical and Biomedical Analysis 95 (2014) 229–237

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Validation of a multipoint near-infrared spectroscopy method for in-line moisture content analysis during freeze-drying

Ari Kauppinen[a,*], Maunu Toiviainen[b], Marko Lehtonen[a], Kristiina Järvinen[a], Janne Paaso[c], Mikko Juuti[b], Jarkko Ketolainen[a]

[a] School of Pharmacy, Promis Centre, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, Kuopio FI-70211, Finland
[b] Optical Measurement Technologies, VTT Technical Research Centre of Finland, P.O. Box 1199, Kuopio FI-70211, Finland
[c] Optical Measurement Technologies, VTT Technical Research Centre of Finland, P.O. Box 1100, Oulu FI-90571, Finland

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 6 March 2014
Accepted 8 March 2014
Available online 15 March 2014

Keywords:
Freeze-drying
Moisture
Multipoint near-infrared spectroscopy
Validation
Accuracy profile

ABSTRACT

This study assessed the validity of a multipoint near-infrared (NIR) spectroscopy method for in-line moisture content analysis during a freeze-drying process. It is known that the moisture content affects the stability of a freeze-dried product and hence it is a major critical quality attribute. Therefore assessment of the validity of an analytical method for moisture content determination is vital to ensure the quality of the final product. An aqueous sucrose solution was used as the model formulation of the study. The NIR spectra were calibrated to the moisture content using partial least squares (PLS) regression with coulometric Karl Fischer (KF) titration as the reference method. Different spectral preprocessing methods were compared for the PLS models. A calibration model transfer protocol was established to enable the use of the method in the multipoint mode. The accuracy profile was used as a decision tool to determine the validity of the method. The final PLS model, in which NIR spectra were preprocessed with standard normal variate transformation (SNV), resulted in low root mean square error of prediction value of 0.04%-m/v, i.e. evidence of sufficient overall accuracy of the model. The validation results revealed that the accuracy of the model was acceptable within the moisture content range 0.16–0.70%-m/v that is specific for the latter stages of the freeze-drying process. In addition, the results demonstrated the method's reliable in-process performance and robustness. Thus, the multipoint NIR spectroscopy method was proved capable of providing in-line evaluation of moisture content and it is readily available for use in laboratory scale freeze-drying research and development.

1. Introduction

Near-infrared (NIR) spectroscopy has attracted considerable attention in the pharmaceutical industry since the launch of United States Food and Drug Administration (FDA) process analytical technology (PAT) initiative [1]. NIR spectroscopy fulfills many of the criteria of an ideal PAT tool for pharmaceutical applications; it is fast, noncontact, nondestructive, requires minimum or no sample preparation and it provides both physical and chemical information about the sample [2,3]. In addition, the use of remote probes and optic fibers makes it possible to implement NIR spectroscopy for in-line analysis [3–6]. In most cases, the NIR spectroscopy techniques require calibration with the reference method and a suitable multivariate data analysis (MVDA) tool such as partial least squares (PLS) regression or multiple linear regression (MLR) [2,3]. In any case, the validity of NIR spectroscopy method needs to be assessed.

The validation of an analytical method is an integral part of the method development and implementation prior to routine operation. The goal of validation is to confirm that the future results obtained by the method will fall close enough to the true value of the analyte of interest during a routine analysis. During validation, parameters such as specificity, linearity, trueness, precision, accuracy and robustness need to be determined. In short, the validation of the method is the collection of documented evidence that an analytical procedure is suitable for its intended purpose [7]. Several

Abbreviations: CQA, critical quality attribute; KF, Karl Fischer; LV, latent variable; MLR, multiple linear regression; MSC, multiplicative scatter correction; MVDA, multivariate data analysis; PAT, process analytical technology; QbD, quality-by-design; RMSECV, root mean square error of cross-validation; RMSEP, root mean square error of prediction; RSS, residual sum of squares; SNV, standard normal variate.

* Corresponding author. Tel.: +358 40 3553 870.
E-mail address: ari.kauppinen@uef.fi (A. Kauppinen).

http://dx.doi.org/10.1016/j.jpba.2014.03.008
0731-7085/

independent agencies and regulatory bodies have published guidelines to aid in the development and validation of analytical methods [8–12].

Despite, or rather due to, the plethora of guidelines, the terminology and methodology related to the validations of analytical methods are inconsistent. Moreover, it has been noted that the terminology can be contradictory even within the same document [13]. Therefore, as a part of a proposal to harmonize validation protocols of quantitative analytical methods, the commission of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) has introduced the use of accuracy profiles as a decision tool in method validation [14–17]. The accuracy profiles are based on the concept of β-expectation tolerance limits as a measure of the total measurement error. The accuracy profile provides a single visual representation of the validation statistics and it can be used to evaluate the future performance of an analytical method. With the predefined acceptance limits, these accuracy profiles utilizing β-expectation tolerance limits have been shown to be highly informative when assessing the validity of an analytical method [18–25]. A recent review of validations of the pharmaceutical NIR spectroscopic methods [26] demonstrated that the use of accuracy profiles complies with the requirements of Q2(R1) guideline of International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use [9]. In the same review, it was also noted that a method validation using only traditional chemometric parameters (e.g. coefficient of determination R^2 and root mean square error of prediction RMSEP) is inadequate for the regulatory requirements of the pharmaceutical industry.

Freeze-drying is one of the main downstream processes used in formulating biopharmaceuticals (e.g. proteins, peptides, viral vectors) as a way to improve storage stability via conversion from a liquid phase into a solid [27]. The moisture content is a one of the major critical quality attributes (CQA) of a freeze-dried product and it is known to influence the stability of the product. The optimum residual moisture level is a formulation specific characteristic that needs to be assessed by empirical studies [27]. The zero level moisture is not often preferred as over-drying can compromise the stability of the product [28,29]. The residual moisture is usually determined by the time-consuming, off-line and destructive KF titration analysis. In contrast, NIR spectroscopy offers a rapid and non-invasive alternative for moisture content determination during freeze-drying and it can also be incorporated into in-line applications. However, due to its single-vial feature, in-line applications of NIR spectroscopy have been generally considered restricted [30,31]. We recently reported the feasibility of using a multipoint NIR spectroscopic method for in-line moisture content determination during a freeze-drying process [6]. It was demonstrated that the multipoint NIR method, when combined with the appropriate MVDA tool, could be used for the estimation of moisture content during the latter stages of a freeze-drying process.

This paper describes the validation of this multipoint NIR spectroscopy method for in-line moisture content analysis during a freeze-drying process. More specific, the focus was on assessing the validity of low moisture content predictions obtained at the end of the process. A secondary objective was to establish a protocol for calibration model transfer in order to ensure validity of NIR system performance in the multipoint mode. The validation was implemented using accuracy profiles that are in accordance with the regulatory guidelines [9,10]. The validation results demonstrated an acceptable specificity, trueness, precision and accuracy over the relevant moisture content range. The method was also found to be robust to a deliberate change in process temperature which represents a critical process parameter during freeze-drying. These results proved the validity of the multipoint NIR spectroscopy method for in-line moisture content analysis during freeze-drying.

2. Materials and methods

2.1. Materials

An aqueous sucrose solution (50 mg ml^{-1}) was used as a model formulation in this study. Sucrose was selected as it is one of the most commonly used stabilizers in the freeze-dried pharmaceutical formulations [32]. Analytical grade sucrose (≥99.5%, Sigma–Aldrich, USA) was dissolved in the filtered (0.22 μm filter) and deionized Milli-Q water (Millipore, USA). Washed and autoclaved 2 ml clear glass tubing injection vials (Schott AG, Germany) with an inner diameter of 13 mm were used for freeze-drying. The fill volume of the vials was 1 ml, resulting in a sample thickness of 7.2 mm. Bromobutyl freeze-drying stoppers and aluminum seals (West Pharmaceutical Services Inc., USA) were used to seal the sample vials that were removed from the freeze-drying process.

2.2. Multipoint near-infrared spectroscopy

The detailed information of the instrumentation has been described in our previous publication [6]. Briefly, the NIR system consisted of a hyperspectral camera (Specim Oy, Finland), a multichannel fiber-optic light source (VTT, Finland), and three fiber-optic noncontact diffusion reflectance probes (VTT, Finland). The instrumentation of the NIR system into a freeze-dryer was executed using tailor-made optic fibers (Oplatek Group Oy, Finland) and a vacuum-proof fiber-optic feedthrough (Vacom GmbH, Germany). NIR spectra were measured noninvasively from the bottom side of the vials. The NIR spectra of the calibration set **A**, independent validation set **B** and calibration transfer set **C** were the averages of 300 frames measured using a frame rate of 60 Hz and an integration time of 15 ms. During robustness tests, the NIR spectra were collected continuously at a rate of 0.9 Hz with an integration time of 15 ms. In the analysis, the NIR data were averaged in blocks of 113 consecutive spectra representing two-minute periods of the process data. Before and after the processes, black and white reference spectra were measured using the built-in shutter operation of the camera and a 98% ODM98 diffuse reflectance standard (Gigahertz-Optik GmbH, Germany), respectively.

2.3. Freeze-drying

Freeze-drying was performed using a LyoStar II laboratory scale freeze-dryer (SP Scientific Inc., USA) equipped with the sample extractor door.

2.3.1. Samples for calibration and validation

Samples for calibration set **A** and independent validation set **B** for external validation were prepared in three series as independent freeze-drying processes. These processes were conducted at a primary drying temperature −36 °C and a secondary drying temperature +40 °C under pressure of 55 mTorr. The freezing step was followed by a 2-h isothermal annealing step at −10 °C. The NIR probes were placed on the shelf of the freeze-dryer with the sample vials. The sample extractor door was used to remove samples from the process at fixed intervals during the end of the primary and secondary drying stages. The end point of primary drying was confirmed with a comparative pressure measurement using a capacitance manometer and a Pirani gauge. At each sampling point, 25–30 sample vials were measured with primary NIR probe P1, removed from the process, stoppered under vacuum, sealed and measured with KF reference method. The number of