Talanta 171 (2017) 45–52

Contents lists available at ScienceDirect

Talanta

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

Global approach for the validation of an in-line Raman spectroscopic method to determine the API content in real-time during a hot-melt extrusion process

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ARTICLE INFO

Keywords:
In-line validation
Real-time analysis
Raman spectroscopy
Hot-melt extrusion
API content
Accuracy profile

ABSTRACT

Since the Food and Drug Administration (FDA) published a guidance based on the Process Analytical Technology (PAT) approach, real-time analyses during manufacturing processes are in real expansion.

In this study, in-line Raman spectroscopic analyses were performed during a Hot-Melt Extrusion (HME) process to determine the Active Pharmaceutical Ingredient (API) content in real-time. The method was validated based on a univariate and a multivariate approach and the analytical performances of the obtained models were compared. Moreover, on one hand, in-line data were correlated with the real API concentration present in the sample quantified by a previously validated off-line confocal Raman microspectroscopic method. On the other hand, in-line data were also treated in function of the concentration based on the weighing of the components in the prepared mixture. The importance of developing quantitative methods based on the use of a reference method was thus highlighted. The method was validated according to the total error approach fixing the acceptance limits at ± 15% and the α risk at ± 5%. This method reaches the requirements of the European Pharmacopeia norms for the uniformity of content of single-dose preparations. The validation proves that future results will be in the acceptance limits with a previously defined probability.

Finally, the in-line validated method was compared with the off-line one to demonstrate its ability to be used in routine analyses.

1. Introduction

In the pharmaceutical field, more and more applications used the Process Analytical Technology (PAT) approach to monitor their manufacturing process in real-time time, as shown in Fig. 1 [1]. Indeed, since the Food and Drug Administration (FDA) published in 2004 a guidance for industry entitled [2]: "PAT – A framework for innovative pharmaceutical development, manufacturing and quality assurance", the number of studies focused on the development of real-time analyses during a manufacturing process increased.

The objective of the PAT approach is to analyze and control the process and the product during the manufacturing by implementing specific tools leading to the development of on- or in-line methods. During the last decade, applications in the pharmaceutical domain applying this approach spread out as can be read in the review Netchacovitch et al. [1]. It is possible to notice that vibrational spectroscopy (IR, NIR and Raman) was often used as PAT tool [1–5]. Raman spectroscopy is a non-destructive and non-invasive technique needing no or few sample preparation. The sample is irradiated by a monochromatic radiation and the scattered light is analyzed by the spectrometer. The Raman spectrum represents thus the intensity of the scattering in function of the wavelength. According to the acquired Raman spectrum, it is possible to perform qualitative and quantitative analyses determining the chemical composition of a sample, the polymorphism and solid-state, the content of the Active Pharmaceutical Ingredient by measuring the intensity of a Raman specific band, and so on [6,7]. During this work, Raman spectroscopy was implemented as PAT tool during a Hot-Melt Extrusion (HME) process. The hot-melt extrusion process is a continuous one leading to an increase of the API bioavailability. The pharmaceutical mixture goes

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http://dx.doi.org/10.1016/j.talanta.2017.04.060
Received 2 February 2017; Received in revised form 20 April 2017; Accepted 25 April 2017
Available online 26 April 2017
0039-9140/

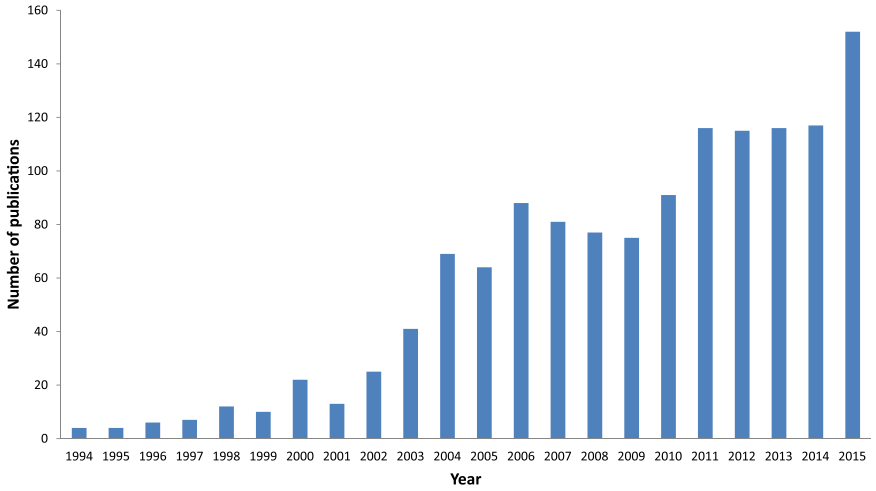

Fig. 1. Representation of the number of publications in the 1994–2015 period, illustrating the evolution of the application of Process Analytical Technology (PAT) tools. Data were obtained with the database Scopus® and using the analyze TITLE (Process Analytical Technology) AND (LIMIT TO (SUBJAREA, "PHAR")). These data were assessed on January 2017.

through a die under controlled conditions (pressure, temperature, feed rate and screw speed) in order to form a solid dispersion. The API state conversion is a critical step during a HME process and can be studied by Raman spectroscopy [8]. As recent works, Monteyne et al. studied the link between rheology and continuous twin-screw melt granulation on molecular level. They used in-line Raman spectroscopy and in situ Fourier Transform Infrared during twin screw hot-melt granulation to study the polymorphic conversion of anhydrous caffeine in order to enhance the process understanding and development [9]. The research of Van Renterghem et al. was focused on a mini hot-melt extrusion process using in-line Raman spectroscopy [10] and compared this research with the results of Saerens et al. [11,12] performed on a larger scale extruder. The trend is also to develop in-line quantitative methods based on a validation protocol. Saerens et al. [13] validated an in-line Raman spectroscopic method determining the API content during a hot-melt extrusion process. The validation protocol was based on the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) approach, the accuracy profile [14–17]. This approach is also used in this study and is exposed in the following paragraph.

In this study, the validity of the method was also demonstrated according to a risk-based protocol using the accuracy profile as decision tool. Indeed, the SFSTP commission developed a simple and visual decision tool for the validation of quantitative analytical procedures. It was based on the total error (bias and standard deviation) of the method. First, based on the calibration samples, a response function was selected. It represented the relationship between the response and the analyte quantity. Then, the bias and precision of the method were estimated. The bias represented the systematic error while the precision represented the random error. Using these estimations, it was possible to determine the accuracy of the method including limitations such as the β-expectation tolerance interval representing an area where the future results will fall and the acceptance limits fixed by the analyst taking into account the intended use of the method [14–17].

This study was focused on the development and validation of a quantitative in-line Raman spectroscopic method implemented on a hot-melt extrusion process in order to quantify the API content in real-time. The validation protocol was based on the ICH Q2 (R1) guidelines [18] and the total error approach was applied. Moreover, the importance of using a reference method to determine the accuracy of the validated method was demonstrated. This is an important step when developing HME processes. Finally, the ability to use this in-line method in routine was demonstrated by comparing the concentration of test samples estimated with the in-line method and with the off-line reference one.

2. Materials and methods

2.1. Chemicals

Itraconazole (ITZ) was purchased from Lee Pharma Ltd. (Andhra, Pradesh, India), Soluplus® and Croscarmellose sodium (AcDiSol®) were provided from BASF (Ludwigshaven, Germany) and FMC Biopolymer (Brussels, Belgium), respectively.

2.2. Hot-melt extrusion process

Mixtures containing 70%, 85%, 100% and 110% of itraconazole (ITZ) targeted concentration were prepared as shown in Table 1. They were composed of ITZ, AcDiSol® and Soluplus®. 150 g of these mixtures were extruded.

The mixtures were premixed in a *TURBULA*® Shaker-Mixer for 20 min and then put inside the volumetric feeder to be extruded. An 18 mm twin screw hot-melt extruder was used (L/D=32, Scamex®, Crosne, France) with a common screw configuration containing two kneading zones (45° forward). The temperature gradient (70–135–145–155 °C) was applied to the four heating zones of the barrel. A volumetric feeder was used and the feeding rate was set at 4 rpm corresponding with the formulation mixture to 6g min^{-1}. The rotational screw speed was set at 100 rpm. The melt was analyzed during the extrusion process by an in-line Raman spectroscopic probe and extrudates were collected after cooling to ambient temperature for off-line analysis.

2.3. Validation protocol

The in-line Raman spectroscopic method was validated following

Table 1
Mixtures preparation for the validation of a quantitative in-line spectroscopic method for the determination of ITZ content during a hot-melt extrusion process.

Calibration standards	%ITZ	%ITZ inside the mixture	ITZ (g)	AcDiSol® (g)	Soluplus® (g)
	70	17.5	26.25	3.75	120.00
	100	25	37.50	3.75	108.75
	110	27.5	41.25	3.75	105.00
Validation standards	%ITZ	%ITZ inside the mixture	ITZ (g)	AcDiSol® (g)	Soluplus® (g)
	70	17.5	26.25	3.75	120.00
	85	21.25	31.88	3.75	114.37
	100	25	37.50	3.75	108.75
	110	27.5	41.25	3.75	105.00