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1 **The development and validation of a quality by design based process**
2 **analytical tool for the inline quantification of Ramipril during hot-melt**
3 **extrusion**

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ABSTRACT

Continuous processing is superseding conventional batch processing as a means of manufacturing within the pharmaceutical research/industry. This paradigm shift has led to the implementation of Process Analytical Technology (PAT) as a semi-automatic, predictive tool offering real-time quality control that can be built into the production line. However, PAT tools have been mainly utilised to monitor a single process (e.g. powder blending, synthesis of biopharmaceuticals and small molecules) rather than a full continuous manufacturing process. In addition, there is a paucity of guidance documents that consider the continuous and dynamic conditions of real-time measurements for validation purposes. In this study, the feasibility of developing and validating a predictive and reliable Raman method based on quality by design (QbD) and PAT frameworks for the real-time quantification of Ramipril (RMP) during hot-melt extrusion (HME) were investigated. Through QbD, a design space elucidating the quality attributes of RMP stability was successfully identified based on offline HPLC measurements. Process temperature and powder feeding rate were the main quality attributes to affect the stability of RMP during HME. The optimum combination of process and formulation variables were extracted from the validated design space and used to extrude RMP at a concentration range of 2.5-12.5 %w/w. Three calibration models were established using PLS regression analysis. The developed PLS calibration models showed excellent linearity ($R^2= 0.989, 0.995, 0.992$), accuracy ($RMSE_{cv}= 0.31, 0.26, 0.30\%$) and specificity ($PC1= 81, 85, 89\%$) for models 1, 2 and 3, respectively. Furthermore, the developed QbD-PAT model was able to predict the quantity of RMP at varied process feed rate (10, 35 rpm) operating under long processing time (60 min). The output of this study allows in-process optimisation of formulation and process variables to control the quality and quantity of RMP during HME. Furthermore, it allows the implementation of PAT tools as routine methods of analysis within the laboratory.

1. Introduction

Over the last 20 years, the pharmaceutical industry has observed a paradigm shift in how drug products are manufactured and quality assured. Recent advances and innovation in manufacturing technology has resulted in a drive towards continuous production of pharmaceuticals and biopharmaceuticals (Lee, 2017; Lee et al., 2015; Poechlauer et al., 2012). Continuous manufacturing allows pharmaceutical drug products to be produced in a single-step, eliminating major concerns associated with traditional multi-step, batch processing, which often requires additional guidelines to maintain quality (Badman and Trout, 2015; Kelleher et al., 2018; WHO, 2015). In addition, continuous manufacturing is an environmentally friendly process that offers a more efficient and safe production method of pharmaceuticals with reduced associated human error (Kleinebudde et al., 2017; Lang et al., 2014). Moreover, given the significant and rapid recent advances in machine learning, the transition from the conventional batch production to continuous manufacturing in the near future is inevitable.

Continuous processing has already been implemented as a mean of manufacturing in chemical and petrochemical industries. Despite this, the transition to such an innovative manufacturing platform in the pharmaceutical industry is still in its early stages. This stems from the need to reconsider the infrastructure in the pharmaceutical industry which includes purchasing new equipment and retraining personnel (Lee, 2017). In addition, pharmaceutical companies still perceive regulatory uncertainty as

obstacles to effective use of this technology and ultimately product approval (Woodcock and Woosley, 2008). Despite these challenges, the FDA is helping to facilitate the switch to continuous manufacturing by providing financial incentives to support research, training resources for personnel and guidance documents. For example, the Emerging Technology Program was established by the Centre for Drug Evaluation and Research (CDER) to support modernisation of the pharmaceutical industry and the transition to innovative manufacturing technologies. The sector has responded well to such initiatives and there some have implemented innovative technologies and received FDA approval for drug products prepared using continuous manufacturing (FDA, 2019).

To accommodate the needs of this emerging view of pharmaceutical manufacturing, there was a requirement for parallel innovation in process development and quality control/assurance of drug products. In response, the ICH and FDA released Quality by Design (QbD) and Process Analytical Technology (PAT) regulatory frameworks to facilitate the implementation of innovative manufacturing platforms in the pharmaceutical industry (FDA, 2004; ICH, 2009). QbD allows quality to be built into the process/formulation design (ICH, 2009). Integrating PAT to QbD enables the real-time monitoring and analysis of materials and processes during manufacturing, which offers an in-depth understanding of the critical attributes affecting the quality of the end products (Laske et al., 2017; Moore, 2012). The digital innovation and integration of QbD and PAT in continuous manufacturing has led to a data rich sector for quality

assessment and control. Thus, formulations/processes can be designed and controlled, accordingly, to meet the quality intended. Conversely, conventional batch methods only allow for process performance evaluation through assessment of the quality of the end product (Fonteyne et al., 2015; Gouveia et al., 2017). In essence, the concept of QbD and PAT being that quality cannot be tested into end products, rather it should be built-in to product during the process (Ohno, 1988).

PAT tools collect real-time data of materials during processing by employing non-invasive sensing technologies (Wirges et al., 2013). These technologies, (e.g., vibrational spectroscopy and ultrasound), are commonly used in pharmaceutical manufacturing as tools to monitor the quality of drug product (Netchacovitch et al., 2015). This is due to the non-invasive and rapid nature of these technologies (Müller et al., 2012). Additionally, spectra can be obtained using small amounts of the materials from a wide range of pharmaceutical forms with no sample preparation (Dadou et al., 2020). Recent and significant advances in chemometrics, has enabled the efficient analysis of spectral data. Hence, vibrational spectroscopy has been widely employed in real-time monitoring and quality control of different pharmaceutical processes such as drug synthesis, crystallization, powder blending, granulation, coating, tablet manufacturing and hot melt extrusion (Kelly et al., 2012; Li et al., 2018; Pauli et al., 2019; Šahnić et al., 2016).

In our previous work, the feasibility of utilizing hot melt extrusion (HME) as a continuous processing platform for the thermolabile drug Ramipril (RMP) was

demonstrated (Andrews et al., 2019). Offline HPLC and LC-MS were used as reference methods to quantify RMP and its degradation product ramipril-diketopiperazine (RMP-DKP). Furthermore, a detailed investigation on the selection of the Raman wavenumber region to be used for the analysis of RMP and RMP-DKP using principal component analysis (PCA) scatterplots were investigated. Results revealed that the wavenumber region from 950 to 1250cm⁻¹ showed the best fit for Raman data. The aim of this study was to advance our understanding further and to develop and validate an inline Raman-based PAT tool based on an optimised design space to monitor, control and, thereafter, predict the quality of RMP during HME. A QbD approach was adopted in building the experimental design model, to identify the source of variability for RMP integrity during the HME process and optimise the design space for the development of the PAT method. Additionally, further information needed for the development of the PAT method were extracted from inline Raman spectra using chemometrics. Internal and external validation were carried out to test the suitability of the developed method for the inline quantification of RMP.

2. Materials and Methods

2.1. Materials

Ramipril (RMP) was purchased from Kemprotec (Carnforth, England). Eudragit EPO (EPO) and Eudragit L 100 (L100) were obtained from Evonik Industries (Darmstadt, Germany). Triethyl citrate (TEC) was purchased from Lancaster synthesis Ltd (Morecambe, UK).

2.2. Design of Experiments (DoE)

DoE was employed in this work to optimise the process and formulation by investigating the impact of formulation and process parameters on the integrity of RMP during hot melt extrusion (HME). Four quantitative variables, including process temperature (T), powder feeding rate (FR), screw speed (SS) and plasticiser content (TEC), were investigated (Table 1). RMP concentration was fixed at 10% (w/w) while the polymer to plasticiser ratio was changed depending on the content of TEC (% w/w) used. The ratio of EPO:L100 was kept constant at 1:1 in all formulations. The concentration of RMP and RMP-DKP (% w/w) in the extrudates were assigned as responses in this study and were measured offline using HPLC.

MODDE Pro (version 12.1, Umetrics, Umeå, Sweden) was used to establish the DoE model. A 2-level, full factorial design with centre-point control runs was employed. A total of twenty experiments, containing three repeated runs, having different combinations of variables were designed as shown in Table 2.

2.3. *Hot Melt Extrusion (HME)*

HME was performed using a co-rotating, fully intermeshing twin-screw extruder (Microlab, Rondol Technology Ltd, France). Physical mixtures of the drug, polymers and plasticiser were mixed using a mortar and pestle. The premixed powder was fed into the extruder using a twin-screw powder feeder (Rondol Technology Ltd, France). Extrusion was performed at various conditions as outlined in Table 2. An in-house designed, 2 mm diameter die was fitted to the end of the barrel to accommodate the inline Raman probe (Andrews et al., 2019). The extrudates produced were collected and stored in bags for further analysis.

2.4. *Inline Raman Spectroscopy Measurements*

A high temperature and pressure immersion HME Kaiser Probe (RAMAN RXN™ Probe) was installed into the die. Raman Spectra were collected inline using a Raman Rxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA) with an Invictus NIR diode laser at a laser wavelength of 785 nm (Kaiser Optical Systems). All Raman spectra were recorded using a laser power of 400 mW with a resolution of 2 cm⁻¹ and total exposure time of 10 seconds. Spectra were collected every 20 seconds during extrusion. Data collection was automated using iC Raman software (version 4.1, Mettler Toledo, US). Collected spectra were then transferred to SIMCA software (version 15, Umetrics, Umeå, Sweden) for spectral pre-processing and chemometrics.

2.5. High Performance Liquid Chromatography (HPLC)

HPLC offline analysis was undertaken to quantify RMP and RMP-DKP in the extrudates. The peak areas of RMP and RMP-DKP were used to calculate their concentration. A calibration curve was constructed for RMP with a concentration range from 5 to 50 µg/mL. Linearity was observed with a goodness fit (R^2) of 0.997. For the quantification of RMP-DKP, a sample of RMP was heated on a hot plate for 2 hours at 140°C to induce degradation. Obtained RMP-DKP was then dissolved in a mixture of acetonitrile and HCl (50:50) and concentrations from 0.5 to 50 µg/ml were used to construct the calibration curve. Linearity was observed with a goodness of fit (R^2) of 0.9999. RMP-DKP concentration was then normalised to the concentration of RMP using Equation 1 (Andrews et al., 2019):

$$\text{Normalised DKP (\%)} = \frac{\text{Peak area DKP}}{\text{Peak area RMP}} \times \text{RMP}_{\text{theoretical}} (\%) \quad [1]$$

Following extrusion, pelletised extrudates were dissolved in 0.1M HCl to obtain a final RMP concentration of 25-30 µg/mL. Aliquots were withdrawn from the prepared samples, filtered through hydrophilic PTFE syringe filters (0.45 µm, Fisher Scientific Ireland Ltd., Dublin, Ireland) then analysed using an Agilent 1260 Infinity Series HPLC (Agilent Technologies, Cheshire, UK). A Kinetex® C18 column (150 mm × 4.6 mm, phenomenex, Torrance, USA) was used as the stationary phase. 10 µL of each sample was injected to the column using an auto-sampler. The mobile phase consisting of an aqueous solution of 0.1M sodium perchlorate adjusted to pH 2.5 using phosphoric acid (mobile phase A), and acetonitrile (mobile phase B) was pumped to

the system under gradient mode at varied flow rates between 0.8 and 1.5 mL/min (Andrews et al., 2019). Detection was achieved using a UV detector at a wavelength of 210nm.

2.6. Principal component analysis (PCA)

The analysis of the Raman spectra collected during extrusion was achieved using SIMCA software (version 14.1, Umetrics, Umeå, Sweden). To reduce random noise and baseline drift in the signal, particularly at high temperature, Standard Normalised Variate (SNV), Multiplicative Scatter Correction (MSC) and 15-point quadratic Savitzky-Golay (SG) pre-processing spectral filters were considered. Analysis was performed on the spectral region of 950-1250 cm^{-1} which allowed for the characterization of pure RMP and its degraded product (RMP-DKP) (Andrews et al., 2019). Principal Component Analysis (PCA) was performed to identify the principle components of the spectra collected during HME.

2.7. The Development and Validation of the inline Raman based Calibration Models

Partial Least Square (PLS) calibration models for RMP (%) was established by correlating the Raman spectra obtained during HME processing with the concentration measured using offline HPLC. Parameters used to evaluate the PLS model performance were the coefficient of determination of predictions (R^2X), coefficient of determination of observations (R^2Y), model validity (Q^2), root mean

200 squared error of estimation (RMSEE) and root mean squared error of cross validation
201 (RMSE_{cv}).

202 The optimum combination of formulation and process conditions, obtained from the
203 DoE design space, was used to develop and validate the PAT method. A calibration
204 set composed of formulations containing 2.5, 5, 7.5, 10 and 12.5 % w/w of RMP was
205 prepared and extruded. Inline Raman spectra were collected and predicted levels of
206 RMP in the extrudates were plotted against the values measured using the reference
207 method (HPLC).

208 For the validation, a validation set (which includes runs that were not used in the
209 calibration set) composed of formulations containing 4, 6, 7 and 9 (% w/w) RMP was
210 formulated and extruded. Validation parameters including selectivity, range,
211 linearity, accuracy, precision and robustness were assessed to meet the criteria
212 established in the International Council for Harmonization on the validation of
213 analytical procedure (ICH-Q2), the European Medicines Agency (EMA) guideline on
214 the use of near infrared spectroscopy and the US-FDA draft guidance on the
215 Development and Submission of Near Infrared Analytical Procedures as follows
216 (EMA, 2009; FDA, 2015; ICH, 2005):

217 *Linearity.* Three calibration models were established, on three consecutive days, using
218 Raman spectra collected for the calibration set. The linearity was evaluated using PLS
219 regression to correlate the values of RMP (%) predicted from Raman with the values

220 measured from the HPLC reference method. The coefficient of determination (R^2),
221 slope, and intercept of the regression line were reported.

222 *Selectivity.* To evaluate the selectivity/specificity of the method, inline Raman spectra
223 obtained from a formulation containing RMP was compared with (i) the spectra
224 collected from a formulation containing the carrier only (polymers mixture and TEC)
225 to assess the capability of the method to distinguish and quantify RMP unequivocally
226 in presence of other ingredients, and (ii) the loading plot obtained for the principal
227 component of the PLS calibration model to assess its capability to describe the main
228 features of the collected inline Raman spectra.

229 *Accuracy.* Accuracy measures the closeness of the results obtained by the PAT method
230 to the true values measured using the reference method. Three parameters were
231 considered to assess accuracy. Root mean square error of estimation (RMSEE) and
232 cross validation (RMSEcv) were estimated from the calibration set while root mean
233 square error of prediction (RMSEP) was obtained from the validation set. The
234 RMSEE/cv and RMSEP can be calculated using Equations 2 and 3 (Li et al., 2018):

235
$$RMSE(E/cv) = \sqrt{\frac{\sum(\hat{y}_i - y_i)^2}{n-N}} \quad [2]$$

236
$$RMSEP = \sqrt{\frac{\sum(\hat{y}_i - y_i)^2}{n}} \quad [3]$$

237 where $\hat{y}_i$ is the predicted RMP concentrations by Raman, y_i is the measured RMP
238 concentrations by HPLC, n represents the number of samples in the data set, and N is
239 the number of variables considered in the model.

Precision. Precision measures the closeness of multiple measurements of the same sample under same condition. Relative standard deviation (RSD, %) and RMSEP of the validation set are used as validation parameters to evaluate precision. The definition of precision in existing guidelines and the recommended methods for assessment are more suitable for offline measurements and not applicable for inline measurements. In this work, repeatability (intraday precision) was evaluated by acquiring Raman spectra at predetermined time intervals of the extrusion process and collecting samples from the produced extrudates that match the time at which the spectra were obtained. Intermediate/Interday precision (within-lab) and reproducibility (between-lab precision) are not achievable in agreement with the ICH, EMA and US-FDA guidelines since the same sample cannot be analysed inline more than once. Therefore, in this work the experiments, for the validation set, were repeated at three consecutive days, RSD and RMSEP were calculated, and the values obtained were reported as parameters for the interday precision considering that the same formulations, methods, conditions and Raman probe were used.

Robustness. Robustness is a measure of the capability of the method to remain unaffected by small variations in process/formulation variables. Raman spectra employed in the DoE investigation were used to address the robustness of the method since the spectra were collected at different formulation and process conditions. Parameters including R^2 , slope, intercept, RMSEE, RMSEcv and RMSEP were used to assess the robustness of the PAT method.

2.8. *In line Monitoring of RMP using a Raman-PLS Model*

The capability of the developed Raman-PLS model to capture the changes in the quality of RMP when altering the process conditions was evaluated. The PLS calibration model obtained from the inline Raman spectra collected during the DoE experiments was used. The experiment was performed using a sample with 10% RMP, w/w. The potential impact of feed rate on the quality of RMP during HME processing was demonstrated by varying the FR between its upper and lower limits of the optimised/validated design space (10 and 35 rpm) over 60 min extrusion time. The extrudates were then analysed using offline HPLC. RMP content obtained from HPLC and the developed Raman-PLS model were then directly compared. In addition, the RMP values predicted using the optimised design space were reported.

3. Results and Discussion

3.1. *Screening of Variables Affecting RMP Integrity using DoE*

Having a good understanding of the formulation and the manufacturing process is a critical part of drug development (Moore, 2012). A DoE approach was adopted in this work to deliver a further understanding of the formulation and process parameters that affect the stability of RMP during HME. A total of 20 experiments were performed (Table 2). It is worth noting that experiments N3 and N7 were not extrudable due to the high back pressure at the die exit which exceeded the upper limit of the extruder, 100 bar.

Given the fact that all the trial runs were conducted at a fixed initial RMP loading (10% w/w), results shown in Figure 1 clearly indicate that temperature (T) is the main variable affecting the RMP and RMP-DKP (% w/w) content in the extrudates. An increased temperature results in a negative contribution on RMP stability and a subsequent positive contribution to RMP-DKP content. No direct effect on RMP integrity was associated with SS and TEC within the studied ranges, therefore, their roles were not highlighted in this work. Feed rate (FR), on the other hand, showed a positive impact on RMP stability and a negative impact on RMP-DKP content. This can be explained by the fact that increasing the FR will reduce the residence time of materials within the extrusion barrel and, therefore, the formulation will be exposed to the high T in the barrel for shorter time periods (Reitz et al., 2013). In contrast, the square factor of feed rate (FR^2) showed a negative impact on RMP content. This can

be explained by the specific mechanical energy in the extruder barrel. Specific mechanical energy is defined as the amount of energy input during extrusion (Repka et al., 2013). It is affected by the shear stress, pressure and torque in the extruder and, hence, can be optimized by varying the FR and SS. And since SS showed no significant effect on RMP stability within the studied range, we postulate that at a certain level of FR, the pressure and torque, and hence the specific mechanical energy, reach values sufficiently high to promote RMP degradation during HME processing. This observation is supported by the positive effect that $FR*FR$ exerted on RMP-DKP content (Figure 1b), suggesting a non-linear relationship between FR and RMP concentration in the extrudate. On the other hand, it is evident from the coefficient plot (Figure 1) that the interaction term between FR and T ($FR*T$) promotes RMP integrity. This observation implies that increasing the FR can compensate the degradation caused by high T.

Figure 2 shows the design space predicting RMP concentration in the extrudates as a function of each set of variables. The contour lines represent the probability of failure of the variables to result in the optimum RMP concentration (9-11 %) which was based on a 95% confidence interval. Therefore, the green area represents the combination of variables that results in RMP concentration in the specified range with a probability of failure of $\leq 5\%$, whereas the red area represents the set of variables that will fail to meet the target concentration of RMP with a probability of failure $> 5\%$.

3.2. Validation of the Design Space Model

The validity of the design space that was obtained from the DoE model in predicting RMP concentration within the hot melt extrudates was investigated. Six validation runs (Table 3) having different combinations of the independent variables were selected from the design space (Figure 2) and extruded. RMP and RMP-DKP (% w/w) contents in the extrudates were measured using HPLC to verify the predicted values from the DoE. Three runs were selected from the green region (V3, 4 and 6), whereas the other three runs (V1, 2 and 5) were selected from the red region. Obtained results from the HPLC were close to the prediction established by the DoE model (Table 3). RMP-DKP content in extrudates was in general aligned to values obtained from HPLC.

3.3. Chemometrics

To shed more light on the correlation between studied variables and responses and try to find patterns and/or categories that can better describe the interaction between the variables, chemometrics was employed (Gabrielsson et al., 2002). In a previous study, we conducted a thorough investigation of the Raman shift region that best represents RMP and RMP-DKP in the inline Raman spectra, collected from the die, using principal component analysis (PCA) (Andrews et al., 2019). Based on the findings, a Raman spectral region of 950 - 1250 cm^{-1} was adopted in this work (Figure 3a). Partial least square (PLS) regression was used to fit the collected spectra. A comparison between the most common filters was conducted to examine the pre-

processing filter(s) which generate the best PLS fit. A Summary of models fit (Table 4) shows better linearity and lower values of residual errors (RMSEE, RMSEcv, RMSEP) for the PLS model established from the spectra that were pre-processed using SNV, MSC and SG spectral filters. Thus, Raman spectra were pre-processed using the three filters throughout this study.

The PC1 versus PC2 scatterplots are presented in Figure 3 (b, c). PCA scatterplots have been obtained with PC1 explaining 88% of results. Sample clusters were orientated along PC1 with weakly degraded samples observed on the negative side and highly degraded samples on the positive side of x-axis. The concentration of RMP and RMP-DKP were clearly linked since RMP is transformed into RMP-DKP by cyclization and/or condensation (Shafiq and Shakeel, 2008). Thus, when RMP content was high (8-10%), a low percentage of RMP-DKP (0.2- 3%) was recovered. In contrast, when RMP content was low (2- 7%), high concentrations of RMP-DKP (4- 8%) were observed.

Figure 4a shows PCA scatterplot grouped based on T and FR values due to the significant effect they have on the integrity of RMP. It can be observed that samples extruded at 140°C are on the positive side corresponding to samples with high RMP-DKP content. Conversely, samples extruded at 100 and 120 °C were observed on the negative side associated with RMP drug content $\geq 8\%$. This implies, again, the direct relationship between T and RMP degradation and the subsequent production of RMP-DKP. This effect can be reduced by increasing the FR. Samples extruded at 140°C show

higher RMP content when mixtures were fed at a rate of 40 rpm (blue) compared to 10 rpm (pink). This is in agreement with the results obtained from the DoE study.

PCA was used to describe samples depending on their colour and TEC content (w/w). Figure 4b shows that samples were well orientated along PC 2. Extrudates obtained without TEC were observed on the negative side, while extrudates with 5% and 10% of TEC were observed on the origin and the positive side of the y-axis, respectively. Additionally, different clusters were identified based on the colour of the extrudates. According to Figure 4b, the most degraded samples were brown regardless of TEC concentration. Slightly yellow and transparent extrudates were localized on the positive side of PC 2, corresponding to formulations prepared at TEC concentrations of 5% and 10% w/w, respectively. Samples localised on the negative side of PC 2, at 0% TEC, were white and opaque. This indicates, again, that TEC content does not affect the integrity of RMP when incorporated in the formulation at ratios up to 10% w/w.

3.4. The Development and Validation of the Raman based PAT Method

Following the DoE and chemometrics studies, optimisation of the process and formulation variables that result in the desired concentration of RMP within the extrudates was carried out. The plot in Figure 5a displays the predicted RMP (% w/w) at each variable set combination. The green and blue regions represent the values of variables that result in the target concentration of RMP (9-11 %). The dynamic plot (Figure 5b) presents a summary of the effect of each factor on RMP content. The

377 concentration of RMP during extrusion was not changed as a function of TEC and SS.
378 On the other hand, an inverse linear correlation was observed between RMP and T.
379 FR exhibited a complex relationship with RMP. The integrity of RMP was enhanced
380 with FR up to a certain limit where a curvature in the plot is observed. This, again, can
381 be explained by the specific mechanical energy inside the extruder barrel previously
382 discussed and supports our claim of the presence of a non-linear relationship between
383 FR and RMP content. The optimum set point suggested was adopted for the
384 development and validation of the Raman based PLS calibration model as follows: T=
385 106 °C, FR= 32 rpm, SS= 80 rpm and TEC= 7.3 % w/w.

386 The objective of validation is to ensure that each measurement performed using the
387 method developed was similar to the true value of the unknown sample (ICH, 2005).
388 Existing guidelines do not accommodate the needs of such innovative platforms. The
389 European Medicines Agency (EMA) and United States Food and Drug Administration
390 (US-FDA) have published practical guidance on the development and validation of
391 near infrared (NIR) analytical procedures (EMA, 2009; FDA, 2015). The guidelines
392 outline the tests and statistics required to ensure the applicability and reliability of the
393 developed offline spectroscopy based chemometrics method. The stress conditions
394 and dynamic nature of samples during continuous processing were not considered in
395 the guidance. EMA guidelines state: "Because PAT NIRS procedures are specific to
396 the nature of the manufacturing process, it is not appropriate to prescribe exact
397 requirements for such procedures in this guideline". On the other hand, US-FDA

guidelines propose the applicability of the guidance for inline methods. Nevertheless, intermediate precision which involves measuring the same sample on different days or by different analysts “should also be determined” (FDA, 2015). Such requirement is not attainable for inline methods. It is worth noting that both guidelines were proposed for NIR spectroscopy, however, they are applicable to Raman spectroscopy as well.

Therefore, whilst several investigations have developed inline method for drug quantification purposes, very few have considered the feasibility of validating the established methods (De Leersnyder et al., 2018; Hubert et al., 2007; Saerens et al., 2014b; Zhang et al., 2019). A NIR based PAT method was developed and validated for the production of a drug product (Zhang et al., 2019). All validation parameters were calculated with the exception of precision which was considered unachievable. When developing a PAT method for blend monitoring, the intraday precision (repeatability) parameters can be obtained by shutting the feeding and the turret off (De Leersnyder et al., 2018). Thus, spectra can be collected from the same rotating powder. This is not achievable when employing hot melt extrusion (HME). In another study, a validation strategy which was based on the total error of the method, was adopted (Hubert et al., 2007) wherein acceptable drug levels and tolerance levels were identified and each measurement represented the true value and the bias (De Leersnyder et al., 2018; Saerens et al., 2014b). Again, not all validation parameters were obtainable.

418 Therefore, in this work, several guidance documents that are implemented for offline
419 analysis were considered. These include, the ICH Harmonised Guideline on the
420 Validation of Analytical Procedure, the EMA Guideline on the use of near infrared
421 spectroscopy and the US FDA draft guidance on the Development and Submission of
422 Near Infrared Analytical Procedures (EMA, 2009; FDA, 2015; ICH, 2005). For the
423 development of chemometrics based models, the following criteria should be fulfilled
424 (EMA, 2009; FDA, 2015):

425 (i) The selection of the wavenumber range of the Raman spectra does not need to
426 include the full range. However, considering a narrow region around the
427 characteristic Raman shift can restrict the model performance (FDA, 2015). Therefore,
428 a thorough study on the model performance using different regions should be
429 delivered. In our previous work, a detailed investigation was delivered in order to
430 find and justify the optimum spectral region to avoid under and/or overfitting of the
431 data (Andrews et al., 2019). Using PCA analysis, the wavenumber region of 950 to
432 1250 cm^{-1} showed high capability in identifying the variables that affect RMP integrity
433 during HME qualitatively and in distinguishing between RMP and its degradation
434 product (RMP-DKP). In addition, the PLS calibration model developed was able to
435 predict RMP concentration with good linearity and accuracy (R^2 0.94, RMSEC 1.05,
436 RMSEcv 1.12). Therefore, the same region was adopted in this work. Table 4 shows
437 the suitability of the selected spectral pre-processing filter for the inline quantification
438 of RMP during HME.

(iii) Potential outliers in the calibration model should be investigated. A potential outlier is a sample that shows an atypical spectrum and/or has high residuals (EMA, 2009). Such sample should be considered an outlier and be excluded from the PLS model. It is recommended to use built-in diagnostic tools within the software to analyse outliers (FDA, 2004). Hotelling's T-squared Distribution (Hotelling's T2) test and distance to the model (DModX) analysis were used. The Hotelling's T2 test is an extension of the student's t-test and is adopted in multivariate analysis. Calculated values represent the distance for each sample from the origin/centre of the model (Nagy et al., 2017). Thus, a large T2 range for a given sample is an indication that it is far from other samples in the score space of the model. Hence, it is likely to be an outlying observation that may detrimentally distort the calibration model and, subsequently, give false results for unknown samples (Brouckaert et al., 2016). Values larger than the 95% confidence limit are suspect, while values larger than the 99% confidence limit can be considered as serious outliers and should be excluded from the calibration model. DModX is the distance of a sample/observation in the calibration model from the X plane (predicted values plane) (Saerens et al., 2014a). Potential outliers can be identified from the critical value (Dcrit) of the DModX which is computed by depending on the selected significance level ($\alpha=0.5$) (Müller et al., 2012). Samples that show DModX values twice as large as Dcrit are considered as moderate outliers. To investigate if these samples are outliers, we investigated their residuals. Samples that showed extreme absolute residuals were considered as outliers. Three PLS calibration models were developed for the quantification of RMP

(Figure 6). Twenty, two and seven outliers were identified and excluded from the developed model 1, 2 and 3, respectively. Hotelling's T² and DModX plots for the three models after excluding the outliers are shown in Figure 7.

After addressing the guidance requirements for the development of a chemometrics based method of analysis, validation was carried out. Two levels of validation were considered, internal and external validation (EMA, 2009; FDA, 2015). Cross validation method was considered for internal validation. This method comprises the removal of one or more spectra from the calibration set and applying the calibration model on the removed spectra to predict its value. Subsequently, the difference between the value predicted by the model and the true value of the removed sample was calculated. The root mean square error of cross validation (RMSE_{cv}) was used as the validation parameter for this method. The smaller the number; the optimum the model was for the analysis. Cross validation was carried out for the three developed PLS calibration models. Calculated RMSE_{cv} values are reported in Table 5. It is observed that calculated values are very similar to the RMSEE values which indicates the validity of the three models to accurately predict excluded samples. However, results may indicate a possibility of overfitting (Zhang et al., 2019).

Following the development and internal validation of the inline Raman based PLS calibration models, external validation was performed which included validating the method for linearity, specificity, accuracy and precision.

481 Linearity was estimated from the developed calibration curves. For PLS regression,
482 the predicted RMP values from Raman spectra were plotted as the independent
483 variables (x-axis); whereas, the measured values using the reference HPLC method
484 were the dependent variables (y-axis). Coefficient of determination (R^2), slope and
485 intercept were the metrics used to assess the linearity. For good linearity, it's expected
486 that the R^2 is close to unity with slope and intercept values that are not statistically
487 different from 1 and 0, respectively. Results obtained (Figure 6 and Table 5) clearly
488 show good linearity between the predicted and the measured RMP values with the
489 ability of the PLS models to describe more than 98.9 % of the variation in the results
490 (model 2 and 3 can describe $\geq 99\%$ of the variance). The slope value was 1 in the three
491 models whereas the values of the intercept were very close to zero. Thus, linearity
492 requirements were met in the developed model.

493 To confirm the specificity/selectivity of the method to quantify RMP in the presence
494 of other ingredients, collected Raman spectra obtained for the formulations containing
495 Ramipril were compared to the spectra obtained for a blank formulation (EPO, L100
496 and TEC). As shown in Figure 8a, the characteristic peak of Ramipril (1004 cm^{-1}) was
497 not observed in the spectra collected for the blank polymer mixture. No interference
498 was observed between the Raman shift of RMP (1004 cm^{-1}) and other ingredients
499 present in the formulation when RMP was included in the formulation which proves
500 the specificity of this method for RMP. Furthermore, the loading plot for the principal
501 component of the PLS calibration model (Figure 8b) was able to describe the main

features of the original spectra that was used for the development of the PLS calibration model, i.e. the characteristic peak of RMP. R^2X values obtained from the principal component of models 1, 2 and 3 were 81, 85 and 89%, respectively. This, again, confirms the specificity of the established PLS calibration models for the inline quantification of RMP.

Accuracy was estimated from the RMSEE, RMSEcv and RMSEP values. RMSEE and RMSEcv were calculated from the calibration set whereas the validation set was used to calculate RMSEP. Large differences between the three parameters indicate a poor calibration model that needed further optimisation/investigation. The values calculated for RMSEE, RMSEcv and RMSEP from the three calibration models (Table 5) were lower than 1%. This indicates the capacity of the developed model to predict unknowns accurately.

Precision was evaluated from the calculated RSD and RMSEP values (Table 6). Despite measurements being carried out in stress and dynamic conditions, the method showed high precision in predicting the concentration of RMP in the validation set with RMSEP values < 1% and RSD < 7% for both the intra and (the developed) interday precision tests.

No further runs were considered to evaluate the robustness of the method since 20 runs were executed previously under different conditions of T, FR, SS and TEC concentration to establish the DoE model (Table 2). The PLS calibration model developed from the DoE model (Figure 9a) was used to assess Robustness. The model

showed good linearity ($R^2 = 0.955$, slope=1, intercept ~ 0) and accuracy (RMSEE= 0.63, RMSEECv= 0.66). External validation was carried out using inline Raman spectra collected from the validation runs employed in the DoE study (Table 3). The model showed good prediction capability when compared with the true HPLC values (RMSEP= 0.88). This indicates the capacity and robustness of the Raman based PAT model to identify and quantify RMP under variable conditions.

3.5. *Inline Monitoring of RMP Content During HME Processing*

To demonstrate the capability of the established QbD-PAT model in predicting the quantity of RMP during HME processing, feed rate was altered between the upper and lower limits in accordance to the design space obtained from DoE (Figure 5). The RMP content within extrudates were predicted using the QbD design space and inline Raman-PAT tool during the 60-min trial run. The predicted RMP contents were then compared to the values obtained through the offline HPLC method. It was observed from the obtained RMP (%) profile (Figure 10) that the predicted values for RMP using the developed Raman-PAT tool and the QbD optimised design space were within the 10% relative error window from true values (HPLC). This provides future evidence of the potential impact of process parameters on the integrity of RMP within extrudates which was successfully captured using the inline QbD-PAT digital tool during the HME process.

4. Conclusion

This work demonstrated through the adoption of a QbD-PAT framework, hot-melt extrusion can be used as a continuous processing platform for the manufacture of solid dosage forms containing thermolabile drugs. This was achieved through a careful design of the HME process and in-process monitoring. A design space indicating the probability of maintaining RMP integrity during the HME process was first established based on a DoE, full-factorial model and offline HPLC measurements. Following the identification and optimisation of the quality attributes that affect the in-process stability of RMP and the validation of the obtained design space, an inline Raman-based PLS model was developed and validated for the quantification of RMP during HME. The established Raman-based PLS model enabled the correlation between the design space for quality attributes and the quality of the response (RMP) during the HME process. Thus, the quality of the dosage form can be designed and built-in into HME and monitored inline throughout the process. It is envisaged that the implementation of QbD-PAT will undoubtedly improve the quality and efficiency of pharmaceutical manufacturing.

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Table 1. Overview of variables and responses that were used to build the HME process design space

Variables				
Name	Abbreviation	Unit	Lower limit	Upper limit
TEC concentration	TEC	%	0	10
Feed rate	FR	rpm	10	40
Screw Speed	SS	rpm	50	100
Temperature	T	°C	100	140
Responses				
Name	Abbreviation	Unit	Lower limit	Upper limit
Ramipril	RMP	%	9	11
Ramipril-Diketopiperazine	RMP-DKP	%	-	-

Table 2. Overview of the combinations of the formulation and process variables adopted for the HME processing and the results obtained for the responses.

	<u>Variable</u>				<u>Response</u>	
	TEC (%)	FR (rpm)	SS (rpm)	T (°C)	RMP (%)*	RMP-DKP (%)*
N1	0	10	50	100	9.2 ± 0.2	1.2 ± 0.6
N2	10	10	50	100	8.6 ± 0.2	2.8 ± 0.2
N3	0	40	50	100	-	-
N4	10	40	50	100	10.0 ± 0.1	0.5 ± 0.1
N5	0	10	100	100	8.4 ± 0.3	1.6 ± 0.1
N6	10	10	100	100	8.7 ± 0.1	2.1 ± 0.1
N7	0	40	100	100	-	-
N8	10	40	100	100	10.2 ± 0.4	2.3 ± 0.2
N9	0	10	50	140	2.0 ± 0.2	7.9 ± 0.5
N10	10	10	50	140	1.6 ± 0.1	8.6 ± 1.3
N11	0	40	50	140	7.7 ± 0.4	2.7 ± 0.6
N12	10	40	50	140	6.5 ± 0.5	4.1 ± 0.5
N13	0	10	100	140	2.7 ± 0.5	8.7 ± 0.7
N14	10	10	100	140	1.8 ± 0.2	7.9 ± 0.8
N15	0	40	100	140	6.3 ± 0.2	3.6 ± 0.3
N16	10	40	100	140	6.6 ± 0.2	4.6 ± 0.3
N17	5	25	75	120	9.1 ± 0.7	1.8 ± 0.2
N18	5	25	75	120	9.0 ± 0.1	1.6 ± 0.0
N19	5	25	75	120	8.7 ± 0.1	1.4 ± 0.0
N20	0	25	75	120	8.8 ± 0.1	1.2 ± 0.1

**Values were obtained offline using HPLC and represent the average ± standard deviation (n=3)*

Table 3. Process predictions for the validation of the DoE model; HPLC was used as the reference method of quantification^a

	<u>Variable</u>				<u>Response</u>							
	TEC (%w/w)	FR (rpm)	SS (rpm)	T (°C)	RMP (% w/w)				RMP-DKP (% w/w)			
					P	L	U	M ^b	P	L	U	M ^b
V1	10	25	75	120	8.7	8.3	9.1	8.3(0.5)	1.9	1.6	2.4	0.9(0.3)
V2	5	15	75	140	4.0	3.5	4.5	4.3(0.6)	5.1	3.9	6.6	4.9(0.5)
V3	10	20	50	100	10.4	10.0	10.8	10.0(0.3)	0.9	0.7	1.2	0.7(0.1)
V4	6	30	100	110	10.0	9.6	10.4	9.7(0.5)	1.0	0.7	1.3	0.8(0.1)
V5	10	25	100	140	6.3	5.8	6.8	6.1(0.8)	5.1	3.9	6.6	3.2(0.6)
V6	8	34	80	103	10.6	10.2	10.9	9.9(0.5)	0.9	0.6	1.1	0.6(0.2)

^a P, values predicted from the DoE model; L, lower limit; U, upper limit; M, values measured using HPLC.

^b Numbers between parentheses represent standard deviation (n=3).

Table 4. Calibration parameters calculated from the PLS models developed using Raman spectra that were collected and preprocessed using different spectral filters*

Spectral filter	R²X	R²Y	Q²	RMSEE	RMSEcv
Raw spectra	1	0.909	0.909	0.886	0.885
SNV	0.976	0.961	0.954	0.587	0.627
MSC	0.973	0.955	0.950	0.624	0.660
1st D	0.916	0.922	0.986	0.823	0.953
2nd D	0.792	0.803	0.734	1.288	1.500
MSC:SNV:SG	0.983	0.955	0.950	0.628	0.660
1st D:MSC:SNV:SG	0.754	0.89	0.859	0.987	1.162
2nd D:MSC:SNV:SG	0.55	0.844	0.772	1.179	1.417

* R²X, R² predicted; R²Y, R² observed; Q², model validity; RMSEE, root mean square error of estimation; RMSEcv, root mean square error of cross validation; SNV, standard normalised variate; MSC, multiplicative scatter correction; SG, Savitzky-Golay; 1st D, first derivative; 2nd D, second derivative.

Table 5. Linearity and accuracy validation parameter obtained for each Raman based PLS calibration model.

Parameter	Model 1	Model 2	Model 3
<u>Linearity</u>			
R ²	0.989	0.995	0.992
Slope	1	1	1
Intercept	-1.24e-07	2.01e-07	1.31e-07
<u>Accuracy</u>			
RMSEE (%)	0.308	0.235	0.304
RMSE _{cv} (%)	0.306	0.258	0.304
RMSEP (%)	0.487	0.635	0.625

Table 6. Precision parameters calculated for the validation set using the three established PLS calibration models*

RMP (%)	<u>Model 1</u>				<u>Model 2</u>				<u>Model 3</u>			
	<u>Intraday</u>		<u>Interday</u>		<u>Intraday</u>		<u>Interday</u>		<u>Intraday</u>		<u>Interday</u>	
	RSD	RMSEP	RSD	RMSEP	RSD	RMSEP	RSD	RMSEP	RSD	RMSEP	RSD	RMSEP
4	2.76	0.15	3.91	0.34	2.20	0.45	5.54	0.42	6.90	0.67	5.87	0.61
6	1.24	0.55	3.95	0.66	2.09	0.56	4.07	0.49	1.81	0.11	3.49	0.47
7	4.12	0.83	3.85	0.85	3.37	0.80	2.64	0.74	2.69	0.26	3.71	0.41
9	4.91	0.41	3.24	0.56	2.18	0.20	2.06	0.36	2.20	0.21	2.33	0.42

* RSD, relative standard deviation; RMSEP, root mean square error of prediction. Values reported are in (%).

Figure 1. Coefficients plots representing variables affecting (a) the stability of RMP and (b) the formation of RMP-DKP during HME process as determined by the DoE study.

Figure 2. Design space obtained from the DoE model. Colour scale bar on the right-hand side represents the probability of failure for variables to generate the response, RMP (%), within the targeted range. RMP was quantified offline using HPLC.

Figure 3. (a) Raman spectra obtained from the wavenumber region 950 - 1250 cm^{-1} during HME. PC 1 versus PC 2 scores scatterplots generated for (b) RMP and (c) RMP-DKP from the collected inline Raman spectra. Colour code, which represents the concentration (% w/w) of RMP (a, b) and RMP-DKP (c) measured using offline HPLC, is on the right side of the plots.

Figure 4. PCA scatterplots showing: (a) the effects of FR (at 10 rpm, pink; 25 rpm, purple; and 40 rpm, blue) and T.(b) TEC concentration (0, 5 and 10%) along PC 2; while the appearance of the produced extrudates are presented in a colour code as follows: White-Opac, Red; light yellow, blue; yellow, yellow; and brown, green.

Figure 5. (a) Response surface and (b) dynamic plots showing the predicted RMP concentration at different combinations/limits of the studied variables, and the set point that result in the optimum content of RMP in the extrudates ($T=106$, $FR=32$, $SS=80$, $TEC=7.3$). Colour code on the right side of the figure (a) represents the concentration of RMP in the extrudates (% w/w) measured using HPLC.

Figure 6. The developed PLS calibration models. (a) Model 1, (b) Model 2 and (c) Model 3.

Figure 7. Distance (DMoDX) and Hotelling's T2 Plots obtained from the three developed PLS calibration models. Model 1, green; Model 2, blue and Model 3, red.

Figure 8. (a) Raman spectra obtained from the extrudates with (red) and without (blue) Ramipril. (b) Loading plot of the first principal component obtained from the Raman based PLS calibration (model 3).

Figure 9. PLS calibration model developed using the collected inline Raman spectra during the 20 DoE extrusion runs vs the concentration of RMP in the extrudates measured using offline HPLC.

Figure 10. Ramipril content profile monitored for 60 min during the HME process at varied FR (0-19 min = 35 rpm, 20-44 min = 10 rpm and 45-60 min = 35 rpm). The process was allowed to stabilise for 10 min at FR 35 rpm before collecting the inline Raman spectra. Values of RMP (%) were recorded as predicted from the design space (QbD) and the PLS based PAT method (PAT), and as the true values (HPLC). Dashed lines represent the relative error from the true HPLC values ($\pm 10\%$).