The Development of an Inline Raman Spectroscopic Analysis Method as a Quality Control Tool for Hot Melt Extruded Ramipril Fixed-dose Combination Products

Y. Tian[1], Z. Senta-Loys [1], A. Almajaan [1], S. Li[1], D.S. Jones[1], O. Chevallier[3], C. Elliot[3], A.M. Healy [2], J. F. Kelleher., A. M. Madi., G.C. Gilvary., G.P. Andrews [1*]

[1] Pharmaceutical Engineering Group, School of Pharmacy, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom

[2] School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland

[3] Institute for Global Food Security (IGFS), School of Biological Sciences, Queen's University Belfast, Northern Ireland, United Kingdom

** corresponding author: Gavin P. Andrews, Pharmaceutical Engineering Group, School of Pharmacy, 97 Lisburn Road, Belfast, United Kingdom, BT9 7BL.*

Tel: +4428 9097 2646

Email: g.andrews@qub.ac.uk

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Abstract

Currently in the pharmaceutical industry, continuous manufacturing is an area of significant interest. In particular, hot-melt extrusion (HME) offers many advantages and has been shown to significantly reduce the number of processing steps relative to a conventional product manufacturing line. To control product quality during HME without process interruption, integration of inline analytical technology is critical. Vibrational spectroscopy (Raman, NIR and FT-IR) is often employed and used for real-time measurements because of the non-destructive and rapid nature of these analytical techniques. However, the establishment of reliable Process Analytical Technology (PAT) tools for HME of thermolabile drugs is challenging. Indeed, Raman effect is inherently weak and might be subject to interference such as scattering, absorption and fluorescence. Moreover, during HME, heating and photodecomposition can occur and disrupt spectra acquisition. The aim of this research article was to explore the use of inline Raman spectroscopy to characterise a thermolabile drug, ramipril (RMP), during continuous HME processing. Offline measurements by HPLC, LC-MS and Raman spectroscopy were used to characterise RMP and its main degradation product, ramipril-diketopiperazine (RMP-DKP, impurity K). A set of HME experiments together with inline Raman spectroscopic analysis were performed. The feasibility of implementing inline Raman spectroscopic analysis to quantify the level of RMP and RMP-DKP in the extrudate was addressed. Two regions in the Raman spectrum were selected to differentiate RMP and RMP-DKP. When regions were combined, a principle component analysis (PCA) model defined by these two main components (PC 1=50.1% and PC 2=45%) was established. Using HPLC analyses, we were able to confirm that the PC 1 score was attributed to the level of RMP-DKP, and the PC 2 score was related to the RMP drug content. Investigation of the PCA scatterplot indicated that HME processing temperature was not the only factor causing RMP degradation. Additionally, the plasticiser content, feeding speed and screw rotating speed can all contribute to the RMP degradation during HME processing.

1. **Introduction**

The pharmaceutical industry has a well-established quality by testing regulatory framework for safeguarding the manufacture of both small molecule and biopharmaceutical products. This conservative process involves rigorous checks to ensure the quality of the final product and ultimately that it delivers the desired therapeutic efficacy. However, traditional manufacturing platforms and batch-based testing operations present inherent limitations for the commercialisation of modern complex drug delivery systems (Rantanen and Khinast, 2015). The implementation of innovative continuous manufacturing platforms provide process intensification and integration, coupled with real-time quality assurance tools; these developments offer significant potential to reduce the costs associated with traditional pharmaceutical and biopharmaceutical products (Badman and Trout, 2014). Furthermore, certain platforms that enable the use of true continuous manufacturing for "end-to-end" production of complex drug delivery systems may present a much-needed paradigm shift for both research development and commercial scale manufacturing. Additionally, traditional multi-step processes may be naturally integrated with homogeneous processes such as extrusion and spray drying. With a thorough understanding of product processes, tailored complex formulations may be carefully engineered based upon a fundamental understanding of selected manufacturing platforms.

With the potential to revolutionise manufacturing processing and drug product quality and complexity, it is not surprising that there has been interest in techniques such as hot-melt extrusion (HME) over the last three decades (Repka et al., 2007). This growing interest in HME may be attributed to the wide range of possibilities in the production of pharmaceutical intermediates (e.g. crystalline, amorphous, cocrystalline and nanoparticles) and final dosage forms, (powder, tablet, granule, film, implant and vaginal ring) for different routes of administration, with tailored drug release profiles (e.g. controlled release, solubility enhancement) (Kelly et al., 2012; Li et al., 2016; Paradkar et al., 2010; Patil et al., 2016; Tian et al., 2018). HME also offers a continuous manufacturing platform without the use of solvent,

which is environmental friendly, easy to scale-up and demonstrates good reproducibility. Furthermore, a key aspect of continuous manufacturing is monitoring of process parameters such as screw speed, feed rate, temperature and the potential to implement Process Analytical Technologies (PAT) that permit the management of various Critical Quality Attributes (CQAs) throughout the process (Lang et al., 2014). Introduced by the Food and Drug Administration (FDA) in 2004, the adoption of PAT is part of a scientific risk-based framework intended to support innovation and efficiency in pharmaceutical development, manufacturing, and quality assurance (FDA, 2004). A PAT framework consists of designing and developing continuous monitoring and control strategies to understand and improve the CQAs of pharmaceutical products through inline, online or at-line measurements (Fonteyne et al., 2012; Laske et al., 2017; Wahl et al., 2013). It must be emphasised, however, the concepts and implementation of PAT and QbD framework is not new as process analysis and control have been widely adopted in many other industries (oil refinery, food, plastic and semiconductor). The application of PAT in pharmaceutical science and manufacturing, particularly in conjunction with the development of modern complex formulation and adoption of novel continuous manufacturing platforms is an exciting and challenging area in pharmaceutical manufacturing. Inline vibrational spectroscopic techniques are mainly used as PAT tools to characterise CQAs during continuous manufacturing because of the non-invasive and rapid nature of these methods (De Beer et al., 2011; Netchacovitch et al., 2015). FT-IR and Raman spectroscopy permit materials to be monitored during processing without the sample being removed from the process stream (Laske et al., 2017). Interestingly, when introducing PAT to a HME line, the effects of thermal and mechanical energy input on the integrity of drug may also be investigated. It offers the potential to generate an understanding of the mechanisms associated within extrusion, rather than the traditional 'black-box' treatment of this new pharmaceutical manufacturing technique (Eitzlmayr et al., 2014). Excess thermal and/or mechanical energy input can introduce unwanted changes to the pharmaceutical formulation, particularly when thermally labile drugs and/or excipients are present (Baronsky-Probst et al., 2016; Huang et al., 2017; Karandikar et al., 2015). Therefore, the

ability to capture process induced degradation using PAT within HME can provide significant advantages to the design and understanding of materials and process.

In our previous work, we reported a comparison between spray drying and hot-melt extrusion for the continuous production of fixed-dose combination formulations for the treatment of hypertension (Kelleher et al., 2018). Traditionally, batch manufacturing is utilized to produce FDCs. In such setups, mixtures and intermediate products are going from one container to the next, they are normally tested off-line and stored before being transferred to the next processing step. These multi-batch disconnected processes normally result an extended lead time before the final dosage form can be released. In continuous manufacturing processes, such as spray drying (SD) and hot melt extrusion (HME), there is a continuous feed input and product output in a one-step process. Inline process analytical techniques (PAT) framework ensure desired critical quality attributes of the formulation are assessed and maintained throughout the process, allowing real time manufacture, quality control and product relase (Lee, 2015). With the potential benefts it may offer on product quality, manufacturing agility and flexibility and cost of production, global pharmaceutical continous manufacturing market is gaining moment reaching 1.9 Billion in 2017 with CAGR at 8.4% (ZION market research, 2018).

Ramipril (RMP), a commonly prescribed angiotensin converting enzyme inhibitor was chosen as the model drug. RMP is often prescribed together with other drugs such as hydrochlorothiazide (HCTZ) to form a fixed dose combination (FDC) product with enhanced therapeutic efficacy. However, hot-melt extrusion was found to result in significant degradation of RMP in comparison to solvent-based spray drying. In this work, we report, for the first time, the development of inline Raman spectroscopic analysis in combination with multivariate data analysis to monitor RMP drug content and RMP degradation product during HME processing. In the literature, several studies have shown RMP instability at different pH values, and under

oxidative or thermal stresses (Hanyšová et al., 2005). The main degradation product after the application of heat stress is ramipril-diketopiperazine (RMP-DKP) (De diego et al., 2010). Due to thermal sensitivity, RMP processing by HME represents a real challenge. The aim of this work was to demonstrate the application of inline Raman spectroscopy as a PAT tool for the manufacture of a FDC formulation containing RMP. The presence of RMP-DKP was first highlighted and identified using a series of offline measurements (HPLC and LC-MS). The correlation of these results with the inline Raman spectra collected during HME processing was probed. Multivariate data analysis model (PCA) were then used to characterize RMP and its degradation product during HME processing. Important HME process attributes were identified for RMP based FDC formulations. Using a Quality by Design approach (QbD), the knowledge-based PCA model was further applied to the inline monitoring and analysis of RMP-HCTZ FDC manufactured using HME (European Medicines Agency, 2015).

2. Material and methods

2.1. Material

Ramipril (RMP) was purchased from Kemprotec (Carnforth, England). Eudragit EPO was generously gifted from Evonik Industries (Essen, Germany). Triethyl citrate (TEC) was purchased from Lancaster synthesis Ltd (County, Country).

2.2. Experimental Design

An initial scoping design of extrusion process parameters and formulation factors (Table 1) was conducted, where high and low levels of each process parameter were chosen in order to gain maximum responses for the degradation of RMP. To highlight the degradation of RMP, several parameters were considered: RMP and TEC (plasticiser) concentrations, feed rate, screw speed and temperature. A total of fifteen experiments were conducted in order to probe the influence of these factors on the content of RMP and RMP-DKP.

Table 1: Overview of the composition and process conditions employed in HME studies

	Formulation variables			Process variables		
Ext	**RMP_{theo} (%)**	**TEC (%)**	**EPO (%)**	**Feed speed (rpm)**	**Screw speed (rpm)**	**Temp. (°C)**
S1	15	0	85	manual	60	140
S2	15	0	85	manual	60	110
S3	5	10	85	manual	60	110
S4	10	10	80	manual	60	110
S5	15	10	75	manual	60	110
S6	5	5	90	7	60	110
S7	10	5	85	7	60	110
S8	15	5	80	9	60	110
S9	5	5	90	2	5	110
S10	10	7.5	82.5	15	50	110
S11	10	7.5	82.5	15	50	110
S12	15	10	75	25	90	110
S13	15	10	75	20	70	100
S14	15	10	75	manual	100	110
S15	15	10	75	manual	100	140

RMP_{theo} (%) is the drug loading in the physical mixture that was introduced into the extruder

2.3. Hot-melt Extrusion

Polymer/plasticizer and drug at defined ratios were premixed using a mortar and pestle then fed into a 10 mm twin-screw co-rotating extruder (Rondol Technology Ltd, France) with a twin-screw powder feeder (Rondol Technology Ltd, France) at various feeding speed or manually. The extruder die was 2 mm in diameter and an in-house custom designed inline Raman probe was used (Figure 1). The extrudates were pelletised using a VARICUT pelletiser (ThermoScientific, Germany)

2.4. Inline Raman Measurements and Principle Component Analysis

Inline Raman Spectra were collected with a Raman Rxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA). For inline measurements, a high temperature and pressure immersion probe (RAMAN RXN™ Probe specifically designed for HME) was inserted into the die head with a custom-made fitting (Figure 1). An Invictus NIR diode laser was employed, with a wavelength of 785 nm (Kaiser Optical Systems). All inline Raman spectra were recorded with a resolution of 2 cm^{-1} and an exposure time of 0.5 seconds using a laser power of 400 mW. Spectra were collected every 20 seconds during experimentation. The run-time for a typical experiment was approximately 30 minutes, with a 10-minute stabilisation period. Data collection was automated using iC Raman software (version 4.1, METTLER TOLEDO) after stabilisation. Raman spectra were automatically transferred and analysed using SIMCA software (version 14.1, Umetrics, Umeå, Sweden). Principal Component Analysis (PCA) was used to highlight principle components of all the spectra collected during HME processing. To reduce random noise and undesired perturbations in the signal particularly at high temperature, Standard Normalised Variate (SNV) and Multiplicative Scatter Correction (MSC) pre-processing and Savitzky-Golay (SG) smoothing were applied before PCA.

2.5. HPLC Analysis

HPLC offline analysis was performed in order to quantify both RMP and RMP-DKP. Pelletised extrudates (25-30 mg) were dissolved in 0.1M HCL (representing gastric media) to obtain a

concentration of RMP of 25 µg/mL. After filtration through hydrophilic PTFE filters (0.45 µm, Fisher Scientific Ireland Ltd., Dublin, Ireland), samples were analysed with an Agilent 1260 Infinity Series HPLC (Agilent Technologies, Cheadle, UK). A Kinetex® C18 column (150 mm length, diameter 4.6 mm, particle size 5 µm) was used as the stationary phase. The mobile phase consisted of an aqueous solution containing 0.1M sodium perchlorate adjusted with phosphoric acid to pH 2.5 (mobile phase A), and acetonitrile (mobile phase B). The samples were injected automatically with an auto-sampler (10 µL), and the flow rate varied between 0.8 and 1.5 ml/min as described in **Table 2**. Detection was performed using a UV detector at 210nm.

Table 2: Details of HPLC gradient elution method including time (min), mobile phase composition and flow rate (mL/min) (%)

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow (mL/min)
0	70	30	0.8
5	40	60	1.5
6	70	30	1
9	70	30	1
10	70	30	0.8

The specificities required by for RAM formulation are i) an assay level of 90-105% of the label claim for RAM; ii) Levels of the major metabolite RAM-DKP of ≤ 5.0%.(Angeli and Trezza, 2009) Therefore, we have constructed a calibration curve with RMP concentration from 5 to 50 µg/mL. Linearity was observed with a goodness fit (R^2) of 0.999. We also normallised the RMP degradation product in respective to the stated RMP content. It was defined as the area ratio between the RMP-DKP peak and the RMP reference peak expressed as equation 1. Pure RMP peak area at a defined concentration of 25 µg/mL was used as internal standard.

$$Normalised\ \%RMP\text{-}DKP = \frac{Area\ (RAM-DKP)}{Area(RAM_{ref})} \times \%RAM_{theo} \qquad \text{equation 1}$$

The percentage of RMP-DKP was classified at three levels:

- Low ➔ from 0 to 4.9%; - High ➔ > 5%

2.6. LC-MS Analysis

LC-MS analysis was performed in order to identify RMP impurities after HME. To test for RMP degradation product (RMP-DKP), pure RMP was heated at two temperatures, 100°C and 140°C, for two hours on a hotplate. Samples were then dissolved in simulated gastric media (0.1M HCL), filtered and analysed. Analyses were carried out on a Waters Acquity UPLC I-Class system (Milford, MA, USA) coupled to a Waters Xevo G2-XS QTof mass spectrometer (Manchester, UK) with an electrospray ionisation source operating in positive or negative mode with lock-spray interface for real time accurate mass correction. Instrument settings were as follows: source temperature was set at 120 °C, cone gas flow at 50 L/h, desolvation temperature at 450 °C, and desolvation gas flow at 850 L/h. The capillary voltage was set at 1.0 kV in positive mode and 0.5 kV in negative mode, respectively. Source offset was 80 (arbitrary unit). Mass spectra data were acquired in continuum mode using the MSE function (low energy: 4 eV; high energy: ramp from 15 to 30 eV) over the range m/z 50-1200 with a scan time of 0.1 s. A lock-mass solution of Leucine Enkephalin (1 ng/µL) in methanol/water containing 0.1% formic acid (1:1, v/v) was continuously infused into the MS via the lock-spray at a flow rate of 10 µL/min. The chromatographic separation was conducted on a Waters Acquity UPLC HSS T3 column (100 mm x 2.1 mm, 1.8 µm) equipped with an Acquity UPLC HSS T3 VanGuard pre-column (100Å, 1.8 µm, 2.1 mm X 5 mm). The column oven temperature was set at 45°C, injection volume at 5 µL and flow rate at 0.4 mL/min. The mobile phase consisted of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. The gradient was set as follows: 2.00 min of 99% (A) followed by a linear increase from 1 to 99% (B) over 16.00 min, isocratic cleaning step at 99% (B) for 0.50 min, then returned to initial conditions 99% (A) over 0.25 min and column equilibration step at 99% (A) for 1.75 min.

3. Results and discussion

3.1. HPLC and LC-MS analysis of RMP and its degradation product

Inline Raman spectroscopic analysis and robustness of offline analytical techniques are of paramount importance. Therefore, in order to establish the PCA model from inline Raman spectra, offline analyses were first performed using HPLC and LC-MS. To gain an understanding of the impact of HME processing on the integrity of RMP within the extruded formulations, the RMP content within the extrudates was analysed by HPLC. A characteristic peak of pure RMP was observed at a retention time of 6.1 min. However, HPLC analyses of some extrudates (depending on the HME process conditions employed) also showed an additional peak, which appeared at a retention time of 7.1 min (Figure 2). As observed in figure 2, the intensity of the second peak was clearly higher for sample S15 (Temp.=140°C) than for S14 (Temp.=110°C), and the RMP peak decreased in intensity for S15 compared to S14, suggesting that various levels of RMP degradation were generated through HME processing. To further investigate the pathway of potential RMP degradation relating to the appearance of the UV absorption peak at a retention time of 7.1 min in the HPLC, LC-MS analysis was performed. The exact molecular weight may be obtained using this method for RMP before and after exposure to the heating stress induced by HME. As an example, as shown in Figure 3a, the mass spectrum of pure RMP was tested after thermal treatment at 100°C for two hours on a hotplate, considered to be similar to the HME temperature environment. It was observed that with heat treatment at 100°C, molecular ion at m/z 417.2381 (elemental composition $C_{23}H_{33}N_2O_5$ calculated with +/-0.8 mDa accuracy) in positive mode with m/z 415.229 (elemental composition $C_{23}H_{31}N_2O_5$ calculated with +/- 0.4 mDa accuracy) representing the elements of unchanged RMP (C23H32N2O). Whilst, when RMP was treated at 140°C for two hours on a hotplate, LC-MS analyses showed different results both in positive and negative ionisation modes. The negative ionisation mode did not provide any signal which is indicative of removal of the carboxylic acid functional group after heat treatment. The trace in the positive mode ionisation showed the presence of a peak with a molecular ion at m/z 399.2291 (elemental composition $C_{23}H_{31}N_2O_4$ calculated with +/- 0.7mDa accuracy, Figure

3b) matching the formula of the suggested RMP degradation product (RMP-DKP) (Angeli and Trezza, 2009; De diego et al., 2010; Hogan et al., 2000).

Previously, our study showed that RMP is thermolabile upon melting in the DSC with a first degradation beginning at 123°C associated with a weight loss of ~5% (by TGA) (Kelleher et al., 2018). This percentage of RMP degradation corresponds to the loss of one H_2O molecule (molar mass 18) representing with the difference in molecular weights between RMP and RMP-DKP. To further access the thermal degradation products post-HME processing, the extrudates were analysed by both HPLC and LC-MS. Results obtained for each experiment in terms of RMP content and level of RMP degradation are summarised in **Table 3**.

Table 3: Overview of the composition and process conditions performed during this study and resultant HPLC data.

	Formulation variables			Process variables			CQAs (Responses)		
Ext	**RMP_{theo} (%)**	**TEC (%)**	**EPO (%)**	**Feed speed (rpm)**	**Screw speed (rpm)**	**Temp. (°C)**	**Drug load (%)**	**Normalised RMP-DKP (%)***	**Level of RMP degradation (%)**
S1	15	0	85	manual	60	140	4.2±0.2	7.9±0.5	High
S2	15	0	85	manual	60	110	9.5±1.2	4.0±0.06	High
S3	5	10	85	manual	60	110	4.6±0.4	0.5±0.03	Low
S4	10	10	80	manual	60	110	8.9±0.3	1.0±0.07	Low
S5	15	10	75	manual	60	110	13±0.5	1.7±0.08	Low
S6	5	5	90	7	60	110	3.2±0.5	1.2±0.03	Low
S7	10	5	85	7	60	110	7.2±0.8	2.1±0.3	Low
S8	15	5	80	9	60	110	10.5±0.35	4.8±0.3	High
S9	5	5	90	2	5	110	5.1±0.2	0.7±0.1	Low
S10	10	7.5	82.5	15	50	110	10.45±1.3	1.2±0.3	Low
S11	10	7.5	82.5	15	50	110	10.75±0.5	1.0±0.1	Low
S12	15	10	75	25	90	110	15.2±0.35	0.8±0.03	Low
S13	15	10	75	20	70	100	14.9±0.1	1.0±0.3	Low
S14	15	10	75	manual	100	110	14.7±0.3	0.2±0.2	Low
S15	15	10	75	manual	100	140	10.6±0.3	2.0±1.1	Low

* RMP-DKP level was normalised according to equation 1

As previously described in the methods section, the level of RMP degradation was ranked into Low, Medium and High, presented in **Table 3**. It is evident through the use of offline analyses,

the percentage of RMP-DKP (degradation) and differences in RMP content before and after HME processing can be quantified. These quantitative responses can be further used to probe the design space for RMP processing using HME. For example, with regard to samples S14 and S15, temperature change (110-140°C) resulted in a significant decrease in RMP drug loading and a subsequent increase in the concentration of RMP-DKP.

3.2. Offline Raman spectroscopic analysis on RAM and its degradation product

To assess the feasibility of using inline Raman spectroscopic analysis to simultaneously detect and quantify RMP and RMP-DKP during HME processing, it is important to check if such differences between RMP and RMP-DKP (involving the loss of one H_2O) can be captured using offline Raman signals. Offline Raman spectra of RMP and RMP-DKP are shown in Figure 4a. Signals representing characteristic groups of the RMP molecule were observed at 1004 cm^{-1} (aromatic ring), 1654 cm^{-1} (carboxylic acid) and 3278 cm^{-1} (secondary amine). With respect to RMP-DKP, a strong peak corresponding to the aromatic group was still observed, while the characteristic peaks of carboxylic acid and secondary amine were absent. The wavelength of the aromatic peak was chosen to characterise drug content for this study due to it being present in the RMP spectrum. Knowing that aromatic peak was not specific to RMP, a second signal region need was selected in order to characterise the part of RMP-DKP generated during HME processing. Due to moderate intensity of carboxylic acid and amine groups in Raman spectroscopy, wavelength regions associated with these changes were further investigated and appropriate region(s) were chosen as suitable for identification of the RMP degradation product through multivariate analysis study. To clarify the interferences may be contributed from other excipients in the extrudates as well as the process temperature (100-140°C), the inline Raman spectra at the regions of interests were presented in Figure 4b. It is clear that, the regions around 1004 cm^{-1} indicating of aromatic ring in RMP were not influenced. A detailed comparison between RMP, triethyl citrate and Eudragit EPO at suggested regions using Raman spectroscopic analysis was provided in support document.

3.3. Wavelength region selections on inline Raman spectra

Our main objective of this article is to identify the most important Raman spectra regions where the main quality attributes (RMP content and RMP-DKP content) can be described using a multivariate data analysis model. Further enrichment of this model and subsequently development of Raman-based partial least square model will be conducted in our following work. As previously discussed, from offline Raman spectra, Region 1 (950 to 1050 cm^{-1} in Figure 4) was selected to represent the variation of RMP content in the formulations due to the intense peak corresponding to the aromatic group in RMP. Inline Raman spectra were also collected for all the fifteen extrusion samples during HME processing. Similar features were observed from the inline Raman spectra where Region 1 data collected for all HME experiments are presented in Figure 5. To decrease the additional noise during inline Raman acquisition, pre-processing filters (SNV+MSC+SG) were applied to the spectra. As observed in Figure 5a, the intensity of the peak at 1004 cm^{-1} is linked to RMP concentration. The loading plot (Figure 5b) confirmed the significance of this wavelength region in relation to the drug loading. In this case, the aromatic peak has the largest absolute value in the loading plot (p1) justifying the importance of this variable in building the PCA scatter plot. Thus, a PC 1 versus PC 2 scatter plot for the region 1 in Raman spectra was obtained (Figure 6). The correct selection of the wavelength region on the Raman spectra resulted in the main scores being oriented along PC 1 (R^2 = 94%). The spectra having the highest PC 1 score correspond to the extrudates containing 15% w/w of RMP (orange) while a low value of PC 1 was associated with RMP concentration of 5% w/w (blue). However, some samples containing 15% w/w RMP (theoretical content) were present with medium scores on PC 1, indicating the complexity of developing a PCA model using single region data. The discrepancy between high and low RMP drug loading when using Region 1 alone may be attributed to the corresponding Raman spectra region characteristic of benzene ring group. The benzene ring structure is present in both RMP and RMP-DKP, and thus various levels of RMP-DKP present in the extrudates under different process conditions will affect the accuracy of the PCA model when only Region 1 was used. Thus, the PCA model based on wavelength range

Region 1 alone was not sufficient to describe the mixture of RMP and RMP-DKP within the extrudates.

After further investigation, Region 2 (1150 to 1250 cm^{-1}) in the Raman spectra collected from inline Raman signals was selected and filters were applied to highlight the level of RMP degradation, i.e. %RMP-DKP in the extrudates as quantified by HPLC (Figure 7). Spectral regions associated with the carboxylic acid and secondary amines were not significant in the PCA models and hence were neglected from consideration. Spectra from Region 2 have also shown significant variations in responding to RMP degradation, a high amplitude in Raman signal was observed for RMP degradation at low level, whilst a significant decrease in the amplitude was observed with increased level of RMP degradation (%RMP-DKP). The loading plot (Figure 7b) confirms that peaks selected between 1150 cm^{-1} and 1250 cm^{-1}, are indeed responsive variables. This can also be correlated with offline measurement of pure RMP-DKP samples showing broadened peaks with lower amplitude (Figure 4). Across the different degradation levels, a correlation of 72.3% was obtained along the PC 1 (Region 2) (Figure 8). A high level of degradation (> 4%) was observed on the negative side of PC 1 while a low-level degradation was observed on the positive side. When PC 2 (R^2=23%) was considered, RMP drug content (RMP_{theo}) can also be identified (labelled at 5%, 10% and 15%). Furthermore, clusters corresponding to 5% RMP content were observed in the negative values of PC 2, clusters representing 10% of drug content were localised close to zero and groups corresponding to 15% of RMP were found in the positive values of PC 2. These indicate that Region 2 can also be used to describe the level of RMP degradation as well as RMP drug content.

3.4. Establishment of PCA model from inline Raman spectra

Finally, when RMP drug content (950-1050 cm^{-1}, Region 1) and RMP degradation (1150-1250 cm^{-1}, Region 2) regions were combined, a PCA model was established based on chosen 420 inline Raman spectra (Figure 9). PC 1 with R^2 of 50.1% and PC 2 with R^2 of 45% were obtained, indicating a good identification for both RMP drug content and its degradation within

extrudates. The level of RMP-DKP was oriented along PC 1 scores, while pure RMP content was oriented along PC 2 scores. With this PCA model obtained through inline Raman spectra, we were able to characterise CQAs (RMP drug content and %RMP-DKP level) for our systems during HME processing. The RMP drug content and its degradation product were separated along two main principal components in this PCA model. Combining observations of RMP and its degradation product with results obtained from offline HPLC analysis (Table 1), maps were established to summarise the CQAs based on the chosen responses (Figure 10 a & b). Regarding degradation, RMP-DKP (PC 1 scores alone), three levels of degradation were identified, from low, medium and high (Figure 10a).

More importantly, we were also able to simultaneously identify the level of RMP drug content within the extrudates during HME processing.

3.5. Probing the effects of process parameters on the CQAs

In the past decade, the pharmaceutical and biotechnological industry have developed integrated "one input and one output" powder to tablet production lines. The possibility of inline quality assessment of CQAs and real-time quality control will provide the necessary framework for the future of pharmaceutical manufacturing (Fonteyne et al., 2015). In this work, RMP content and the level of %RMP-DKP were considered as two of the most important quality attributes of the prepared extrudates. The development of a PCA model was based on highlighting these CQAs from an inline Raman-based multivariate data analysis technique. As discussed, CQAs might be influenced by material attributes (CMAs) and process parameters (CPPs). In this scoping design, plasticizer TEC concentration, barrel temperature, feed speed and screw rotation speed were all changed. The influence of these investigated parameters on the CQAs were assessed *in situ* using inline Raman spectroscopy, based upon our established PCA map.

Although this scoping design was not a full experimental design normally used to probe the critical factors on the CQAs, the inline Raman-based PCA mapped using the existing dataset revealed important aspects for the current study. For example, the effects of TEC on the level of

RMP degradation has been highlighted in Figure 10b. Along PC 1, extrudates with a high level of TEC were observed with low level of RMP degradation (positive scores in PC 1) and samples without TEC were observed with high level of RMP degradation (negatively scored in PC 1). As previously discussed, PC 1 is associated with the level of RMP degradation, this observation allows showing positive effect of TEC on RMP integrity with a degradation decreases when TEC increases. Additionally, when samples prepared with a TEC concentration of 10% (blue clusters in Figure 10b) were considered, variations can also be observed throughout PC 1, suggesting the influence of other parameters on the level of RMP degradation. For example, formulation trials S1 and S15 were both prepared at 140°C. S1 was negatively scored along PC 1, while S15 was localised at zero, implying high and medium RMP degradation (RMP-DKP), respectively. The addition of TEC and increase in screw speed in S15 results in a decrease in degradation, even though the process temperature was set at 140°C.

Results therefore suggest that the level of TEC, feed speed and screw rotating speed are all contributed to the level of RMP degradation produced via HME. Recently, the contributions of HME process parameters to the specific mechanical energy inputs and residence time of the process have also been highlighted in the literature (Repka et al., 2018). Plasticizer lowers the glass transition temperature (T_g) as well as the melt viscosity of the polymer, resulting in a reduction in the shear-induced temperature changes (Chokshi and Zia, 2004). Therefore, inclusion of plasticizer decreases the heat stress experienced during HME processing, hence reducing the generation of RMP-DKP. The factors that influence the discrepancy between the extruder temperature and local material temperature within the extruder are pertinent to understanding and controlling this platform for the production of thermolabile drugs (Huang et al., 2017).

Furthermore, the effect of heat stress on the degradation of RMP is well known in the literature (Figure 3). The PCA scatterplot was also used to identify the influence of processing temperature, as shown in Figure 11. As predicted using offline measurements, when a

temperature of 100°C was used during HME processing, the level of RMP degradation was not observed/measurable, whilst when a high temperature (140°C), was used for HME processing, medium to high levels of RMP degradation were observed. In order to highlight the potential temperature effect on both CQAs during processing, extrudates were prepared at different temperatures (100°C for S14 and 140°C for S15) may be compared. These extrudates were obtained under the same conditions, except for processing temperature (S14 at 110°C and S15 at 140°C). S14 resulted in positive scoring of PC 1, while S15 was localised in the centre of the scatterplot, implying a lower %RMP-DKP level for S14 compared to S15. This result was also confirmed by HPLC analysis with 0.2% and 2% of RMP-DKP obtained for S14 and S15 respectively (Table 1).

However, when a medium temperature of 110°C was applied during HME processing, various levels of RMP degradation were obtained (highlighted as blue, Figure 11). The effect of TEC content, for example, at the same processing temperature can be demonstrated by a comparison between S2 and S14 formulations, which were both produced at a temperature of 110°C. A 4% difference in RMP degradation was observed between the two samples, simply due to the TEC loading (0% w/w TEC in S2 extrudates versus 10% w/w TEC in S14 extrudates). Additionally, the effect of initial drug loading may also play an important role on the rate of degradation; in comparing formulations with different initial drug loads (S8 and S9 formulations). For the S8 formulation (15% RMP, processed at 110°C with 5% TEC and 60 rpm screw speed), 4.8% RMP-DKP was detected in the final product, whilst for the S9 formulation (5% RMP that was processed at similar conditions), only 0.7% RMP-DKP was observed. This suggests that, with increasing RMP drug loading, there is an increased chance of RMP exposure to heating and shearing stresses, which may encourage the generation of the RMP-DKP degradation product. Therefore, the process conditions are highly sensitive to the initial RMP drug loading. Cares have to be taken in consideration of the design space for HME platform at different drug loadings to ensure for efficient production of thermally liable drugs.

In general, the PAT tools equipped with multivariate data analysis reduces process errors and provide a quality product (Challa and Potumarthi, 2013). Through the investigation of these critical factors using an established PCA scatterplot and offline RMP/RMP-DKP results, we could directly identify, in principle, the main route causes for RMP degradation within extruded formulations, during HME processing. Furthermore, results also indicate that the TEC content and process temperature are not the only parameters influencing the generation of RMP-DKP during HME (Figure 10 and 11). Further investigations of the formulation design in relation to the HME process parameters will be conducted using a full design of experiments and PLS model. The design space for RMP-HTCZ based fixed-dose formulations will be assessed in more detail through the inline Raman-PLS tool.

3.6. Inline Raman based partial least square model

Although, the main objectives of this article was to test the feasibility of inline Raman based PCA model for the identification of RMP and RMP-DKP within the hot-melt extrusion process, with the offline HPLC quantification on the RMP content, a quantitative model based on partial least square method (PLS) may also be constructed. The establishment of inline Raman-based PLS model can help us to further test the suitability of the selected Raman regions (950 – 1250 cm^{-1}) in the PCA model. Figure 12 shows the constructed PLS model based on the inline Raman spectra collected from extrusion trials and the RMP drug loadings quantified by HPLC (Table 3). A goodness fitting (R^2) of 0.9427 has been obtained with a root mean square error of estimation (RMSEE) value of 1.05 and root mean square error of internal cross validation (RMSEcV) value of 1.12. Clearly, external validations are required to further develop the quantitative model, nevertheless, the values of RMSEE and RMSEcV indicate a good level of predictively for RMP content and validate the selection of the regions from inline Raman spectra.

4. Conclusions

The application of HME as a means of continuously manufacturing thermally liable drugs has been limited due to the degradation caused during processing. In this article, we have demonstrated the use of a Raman-based process analytical tool to probe the effects of formulation design and process parameters on the degradation of thermally liable drug RMP. Inline monitoring and characterisation were performed using Raman spectroscopy and meaningful conclusions were drawn via the establishment of a multivariate data analysis model. With this model, it was possible to convert the complex Raman data into a principle component map, where the levels of RMP drug content and RMP-DKP degradation were successfully mapped into clusters. Two specific Raman regions were firstly selected to characterise RMP content (Region 1) and its degradation product (Region 2). Through the combination of both regions (950 cm^{-1} to 1250 cm^{-1}), the PCA scatterplot obtained correlated to the level of RMP-DKP along principle component one (R^2=50.1%) and RMP content along principle component two (R^2=45%). This map could differentiate between the level of RMP-DKP and RMP drug content, which can also be related to the critical formulation design and process parameters for the HME processing. Data analysis of the PCA scatterplot highlighted the impact of TEC concentration and process temperature on RMP degradation within the extruded formulations. The establishment of Raman-based PCA map will be further utilised for the optimisation of HME processing to produce RMP-HCTZ fixed-dose combination formulations.

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