Evaluation of praziquantel properties upon milling and comilling at room and cryogenic temperatures

Zanolla D. [1]; Perissutti B.[1]; Bertoni S.[2]; Invernizzi S.[3]
Voinovich D.[1]; Moneghini M.[1]; Passerini N. [2]*;Albertini B.[2]

[1]Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.le Europa 1, 34127 Trieste, Italy; bperissutti@units.it

[2]Department of Pharmacy and BioTechnology, University of Bologna, Via S. Donato 19/2, 40127 Bologna, Italy; nadia.passerini@unibo.it

[3]Società dei Naturalisti e Matematici di Modena, Via Università 4, 41121 Modena, Italy; invernizzi2@gmail.com

INTRODUCTION

This study aimed to a comprehensive evaluation of Praziquantel (PZQ, a BCS II class drug) behavior upon the milling process in vibrational mill considering the influence of milling temperature (cryogenic vs room temperature), frequency, time and presence of different polymers (milled raw PZQ vs comilled PZQ-crospovidone and PZQ-povidone at 1:1 wt ratio) on two experimental responses (residual crystallinity and PZQ recovery), as depicted in Fig. 1.

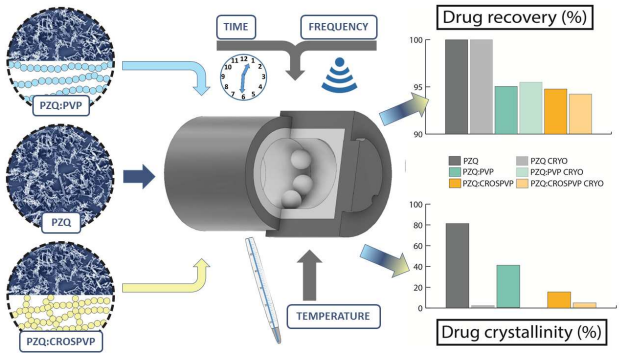

Figure 1: Graphical abstract

MATERIALS AND METHODS

Materials

Praziquantel (Ph. Eur. grade donated by Fatro S.p.A.); PZQ impurity A and impurity B Ph. Eur. grade from Endotherm Gmbh; Povidone (Fluka Chemie-PVP) and micronized crospovidone (BASF-CROS). Methanol (Ph. Eur. for HPLC Gradient Grade-VWR Chemicals BHD PROLABO®).

Milling experiments

Before grinding, PZQ and each polymer (PVP, CROS) were manually mixed in an agate mortar in a 1:1 wt ratio for 3 min. The grinding process was performed in a vibrational mill-Retsch MM400 (Retsch GmbH, Haan, Germany) 35 ml zirconium oxide jars, using 1.072 g of powder per jar and three 15 mm spheres, as previously [1]. PZQ was ground by itself and in combination with the polymers (PVP or CROS) one by one. No cooling was provided to the grinding jar during room temperature (RT) milling. For cryogrinding (CC) experiments, prior to milling, the jars containing the samples were immersed in liquid nitrogen for 1 minute every 15 minutes of milling. Vibrational frequency and milling duration were varied according to the experimental plan below reported (Table 1).

Sample temperature measurement

Temperatures of the samples were recorded using a 35XP-A Amprobe K-type thermocouple (Amprobe, Test Tools Europe). Averaged measurements were from three replicates.

Experimental Design

For planning the milling trials, a factorial design was employed by means of JMP software (SAS Institute Inc). The variables were considered at different levels as reported in table 1. A number of 2^3 x 3 duplicated trials (total trial number=48) were conducted in random order to reduce systematic error.

Experimental Variables	**Levels**		
Time (min)	30	90	
Frequency (Hz)	15	25	
Temperature	room (RT)	cryogenic (CC)	
Product Formulation	PZQ	PZQ: PVP (1:1)	PZQ: CROS (1:1)
Experimental Responses	**Y1 Drug Recovery %**	**Y2 Residual Crystallinity %**	

Table 1: The process (milling) and formulation factors, test levels, and experimental responses

Determination of Experimental responses

The quantification of PZQ was assessed using a HPLC method adapted from literature [2] and suitably modified according to our analytical system [1].
Drug residual crystallinity was calculated by the measurements (by Differential Scanning Calorimetry) of the enthalpy of fusion (ΔH) of the PZQ using the equation: residual cristallinity (%) = (ΔH_{sample} x 100) / ΔH_{PZQ}.

Statistical analysis

Statistical analysis was performed by using JMP software, considering the physical variables (time and frequency) as continuous-numerical variables. The screening of the effects was performed by a multiple comparison of each level against a control level (Dunnett's test).

RESULTS AND DISCUSSION

Powder temperature was found to increase with increased milling frequency and time, up to maximum recorded value of 46.9°C, when milling at 25 Hz (for 90 min in the ambient conditions). Interestingly, by comparing the temperatures detected in identical operating conditions, the values are almost superimposable in the three powder systems (PZQ, PZQ:PVP, PZQ:CROS, Figure 2).

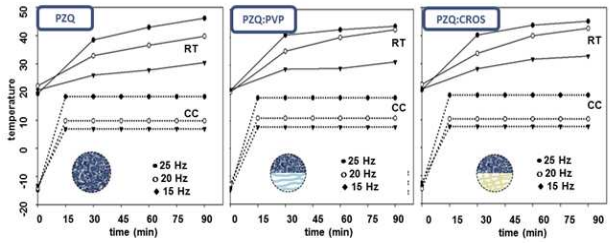

Figure 2. Average powder temperature *vs* milling time at different frequencies at RT (solid lines) and in CC (dashed lines).

The effects of the variation of factor levels on both experimental responses are reported in following Tables 2 and 3.

	Y1 Drug recovery		
	PZQ	**PZQ-PVP (1:1)**	**PZQ-CROS (1:1)**
Milling parameters	**p-value**	**p-value**	**p-value**
Time (30)	n.s.	*** p<.001	*** p<.001
Frequency (15)	n.s.	*** p<.001	*** p<.001
Temp. cond (RT)	n.s.	n.s.	n.s.
	Y2 Drug residual crystallinity		
	PZQ	**PZQ-PVP**	**PZQ-CROS**
Milling parameters	**p-value**	**p-value**	**p-value**
Time (30)	n.s.	n.s.	* p=.015
Frequency (15)	*** p=.006	n.s.	** p=.007
Temp.cond. (RT)	*** p<.001	n.s.	n.s.

Table 2. Effect of variations of parameters on experimental responses. Comparison of each level against a control level (in brackets).

Praziquantel recovery after neat grinding by itself remained unchanged, highlighting its resistance and optimal propensity to mechanochemical activation, since even increasing frequency or time of grinding no tendency to drug degradation was noticed. On the contrary the presence of the polymers in the systems favored a certain drug degradation at ambient temperature, that could be minimized using cryogenic conditions.

When considering drug crystallinity degree upon grinding at RT conditions, the presence of the polymers promoted a high de-structuration of the drug, while in the system only based on PZQ a similar amorphisation could be achieved exclusively using cryogenic conditions.

CONCLUSION

The results reported in this work highlight the role of both polymers as catalyst toward physical de-structuration and chemical degradation of the drug upon milling. The use of a cryogenic environment appeared as a necessity for a pronounced physical de-structuration, also preserving the chemical integrity of the drug.

	Y1 Drug recovery		Y2 Drug residual Crystallinity	
	RT	**CC**	**RT**	**CC**
Parameters	**p-value**	**p-value**	**p-value**	**p-value**
Time (30)	n.s.	n.s.	n.s.	*** p<.001
Frequency (15)	* p=.027	n.s.	n.s.	*** p<.001
Presence of PVP (PZQ)	*** p<.001	*** p<.001	*** p<.001	*** p<.001
Time (30)	n.s.	n.s.	n.s.	** p=.003
Frequency (15)	* p=.038	n.s.	n.s.	*** p<.001
Presence of CROS (PZQ)	*** p<.001	*** p<.001	*** p<.001	** p=.007
Time (30)	*** p<.001	n.s.	n.s.	** p=.006
Frequency (15)	*** p<.001	n.s.	n.s.	*** p<.001
Polymer choice (PVP)	n.s.	n.s.	n.s.	n.s.

Table 3. Effect of variations of parameters on experimental responses. Comparison of each level against a control level (in brackets).

AKNOWLEDGMENTS

The authors thank "Università degli Studi di Trieste - Finanziamento di Ateneo per progetti di Ricerca Scientifica - FRA 2015.

REFERENCES

1. Perissutti, B.; Passerini, N.; Trastullo, R.; Keiser, J.; Zanolla, D.; Zingone, G.; Voinovich, D.; Albertini B.. An explorative analysis of process and formulation variables affecting comilling in a vibrational mill: The case of praziquantel. Int. J. Pharm. 533 (2), 402-412 (2017).
2. Sun, Y.; Bu., S.J. Simple, cheap and effective high-performance liquid chromatographic method for determination of praziquantel in bovine muscle. J. Chromatogr. B. 899, 16 (2012).