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Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 599–604

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Fast sample preparation for analysis of tablets and capsules: the ball-mill extraction method

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Received 12 December 2000; received in revised form 7 March 2001; accepted 11 May 2001

Abstract

A new ball-mill extraction method for solid dosage forms was developed. It was used for tablets, and compared with a conventional (powdering and sonication) method applied in pharmaceutical analysis of solid dosage forms. The ball-mill sample preparation procedure is both quantitative and fast. No powdering, weighing and sonication steps are needed in the sample preparation. The complete procedure takes 2 min (milling and extraction) and 5 min (centrifugation), respectively, much less than the conventional method in which sample preparation takes approximately 45–90 min. The samples are centrifuged in the mill vial, which saves time and avoids evaporation of solvent. Stainless steel extraction vials with different diameters were fabricated to enable the use of various extraction volumes. The extraction recovery was tested using various types of tablets (small, large and extended release tablets) with active compounds at low and higher concentrations, recoveries were comparable with the conventional method. The relative small investment and simplicity of the method makes it excellently suited for use in various pharmaceutical (development and quality assurance) laboratories.

Keywords: Pharmaceutical analysis; Solid dosage forms; Content uniformity; Automation; Grinding; Powdering

1. Introduction

For the analysis of active compound and related substances in tablet or capsule formulations, usually chromatographic methods are applied. The speed-up of these methods has been the subject of many studies [1,2]. In general, this type of analysis requires quantitative extraction of the analytes. Conventional sample preparations for tablet and capsules usually start with a laborious and time-consuming grinding (powdering) step. To enable the determination of the contents per tablet both the intact tablets and the powder must be accurately weighed. Next, the analytes are extracted by sonication and/or mixing with solvent on a vortex mixer for a certain period of time. In all recent articles concerning the analysis of tablets or capsules found in this journal, such steps were incorporated, see, e.g. [3–7]. Most of these steps cannot easily be done in parallel, so that the sample preparation for larger series of tablets is a major part of the total analysis time.

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0731-7085/01/$ - see front matter
PII: S0731-7085(01)00488-5

Automation of tablet extraction by applying robotized systems can in some cases be cost-effective since it can be operated unattended [8]. However, significant effort is required for method development and validation of such an automated method [9]. Highly skilled operators are needed for the development of the methods and maintenance of these systems [10]. Therefore, the additional effort can only be compensated for when large series of tablets are to be analyzed, e.g. in a production quality assurance lab. These automated systems are not suited for all strengths and sizes of tablets, depending on the size and shape of the homogenizer probe. Furthermore, the cleaning steps in these systems, which are needed to avoid sample carry-over, require up to hundreds of milliliters of solvent per analysis and, therefore, also produce substantial waste [9].

During the oral drug product development for a new active entity (AE), several tablet formulations are tested for stability, content uniformity and release characteristics. This implies series of 10–100 analyses per formulation, so that sample preparation times are considerable, but the extra effort needed for transfer of the method to an automated system cannot be justified.

Therefore, in the present study, the feasibility is determined of a new, faster and more general applicable method that uses a ball-mill for both grinding (powdering) and extraction of the tablets in a single step. Such ball-mills can be found in many pharmaceutical industries, since they are routinely used for milling (micronization) of active compounds and granulates [11,12]. Since series of samples have to be handled and no time should be wasted for cleaning procedures in between the sample preparations, a larger number of grinding vials is needed. These vials were somewhat modified and constructed in-house, to ensure more flexibility in volume of the vials.

2. Experimental

A Retsch ball-mill model MM200 (Retsch, Haan, Germany) that can hold 2 vials, and operates at frequencies between 3 and 30 Hz was used. Retsch as well as in-house (Technical Services Department, Diosynth, Oss, The Netherlands) constructed stainless steel vials and balls were tested. The total investment for the mill and 50 vials was approximately Euro 7.000. After extraction, the vials were centrifuged at 3500 rpm for 5 min in a Megafuse 1.0 (Heraeus, Hanau, Germany) in 50 or 100 ml tube holders, so that 8 or 4 vials, respectively, can be centrifuged in parallel. For extraction, mixtures of analytical grade water, acetonitrile and methanol (J.T. Baker, Deventer, The Netherlands) were used, depending on the specific application. Organon production batch tablets of Marvelon® were tested, as well as Organon development batches containing different steroids and a central nervous system (CNS) compound at various amounts (for a description of the tablet constituents and dose levels, see Table 2). The contents of the tablets were determined with fully validated reversed phase HPLC methods. This implies that the deviation from linearity within 70–130% of the declared value is $< 1\%$, and that the repeatability and reproducibility of the methods are within 2 and 3%, respectively. The selectivity of the method for tablet excipients and degradation products is checked by comparing the peak purities (with diode array detection) of standards with light- and temperature stressed samples.

3. Results and discussion

The ball-mill is frequently used at the development departments of Organon for micronization of both active compounds and tablet excipients. For this, agate vials and balls are used. Obviously, for extraction purposes the vials should be solvent-tight, therefore, two stainless steel vials with screw caps were purchased (Retsch, see Fig. 1). The Teflon® ring in the cap of the vials ensures proper solvent-tight closure. Since the Retsch vials did not fit in the centrifuge-tube holders used in the laboratory, other vials were manufactured in-house. By making vials with different internal diameters, the extraction volumes that can be used can more easily be varied. The features of the ball-mill, vials, balls and centrifuge tube holders are summarized in Table 1.

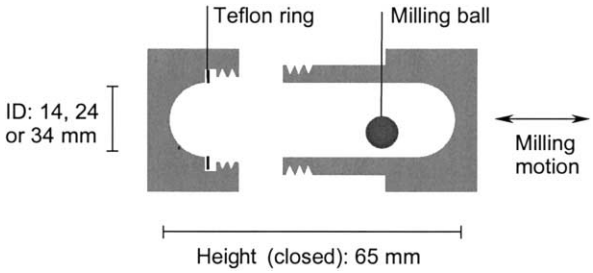

Fig. 1. Schematic view of the solvent-tight stainless steel vial.

For starting an extraction procedure, intact tablets, one or more milling balls and finally the required amount of extraction solvent are put together in the vials, which were closed by hand and clamped in the ball-mill.

First, the milling-potential of the set-up was studied using 1–10 lactose-based placebo tablets (65 mg, 5 mm) of different hardnesses (crushing strengths, 20–80 N). The break-down (grinding to powder) of the tablets was followed in time while using different numbers and sizes of mill-balls of both agate and stainless steel, and applying different solvent compositions and volumes. Here, it was found that a fast (<3 min) grinding of the tablets could not be accomplished, even at maximum mill-speed when using the agate balls, regardless of their size and number, presumably because they are too light. When using the stainless steel balls (7–12 mm) at maximum mill-speed, all tablets where completely crushed within 1 min, at all solvent compositions and -volumes. However, under these circumstances, an increase in temperature of the samples was noted after prolonged milling (>3 min). Furthermore, the sample was colored grayish obviously by steel-particles from the vial and/or balls. By applying the mill at 15 Hz, these problems were circumvented, and the tablets where completely powdered (regardless of their hardness and the tested solvents) within 1.5 min. The particles in the slurry obtained after the mill extraction step were much smaller than 100 μm (by visual comparison with 75 μm particles). These observations led to a standard mill extraction time of 2 min, using a single 12 mm ball in the 18 and 35 ml vials and a 9 mm ball in the 6 ml vials.

To determine the extraction efficiency of the procedure, assay results for manual and ball-mill method of a number of tablets originating from Organon production (Marvelon®) as well as pharmaceutical development batches were compared. The results of these studies are presented in Table 2. It should be noted that the dosage of AE in the

Table 1
Characteristics of the ball-mill, vials, balls and centrifuge tube holders

Type	Number of vial holders	Mill speed	Size	Material	i.d. (mm)	o.d. (mm)	Height (closed) (mm)	Volume (ml)
Ball-Mill								
MM200	2	3–30 Hz	$30\times50\times17$ cm					
Vials								
Retsch				Stainless steel	24	35	65	18
In-house				Stainless steel	14	24	65	6
In-house				Stainless steel	24	24	65	18
In-house				Stainless steel	34	44	65	35
Balls								
				Stainless steel		5, 7, 9, 12		
				Agate		5, 7, 9		
Centrifuge tube holders								
				Plastic, rubber base	34.5, 44.5			

i.d., internal diameter; o.d., outer diameter.

Table 2
Experimental conditions and extraction recoveries of the various tablets tested

AE	AE weight	Tablet weight (mg)	Number of tablets	Tablet excipients	Solvent component	Solvent volume (ml)	Vial volume (ml)	Recovery for manual method	Recovery for ball-mill method
Ethynyl estradiol[a]	30 μg	65	5	Lac (85%), PotSt (l0%), PVP (3%), Aerosil (1%), SteaAc (1%)	ACN/H_2O, 80/20	10	18	97.7% ($\pm$0.5, $n=3$)[b]	99.3% ($\pm$0.8, $n=3$)
Ethynyl estradiol[a]	30 μg	65	1	Lac (85%), PotSt (l0%), PVP (3%), Aerosil (1%), SteaAc (1%)	ACN/H_2O, 80/20	2	6	98.3% ($\pm$2.2, $n=3$)	98.3% ($\pm$1.3, $n=3$)
Desogestrel[a]	150 μg	65	5	Lac (85%), PotSt (10%), PVP (3%), Aerosil (1%), SteaAc (1%)	ACN/H_2O, 80/20	10	18	99.1% ($\pm$0.6, $n=3$)	100.2 ($\pm$0.8, $n=3$)
Desogestrel[a]	150 μg	65	1	Lac (85%), PotSt (10%), PVP (3%), Aerosil (1%), SteaAc (1%)	ACN/H_20, 80/20	2	6	98.1% ($\pm$1.9, $n=3$)	97.5% ($\pm$1.5, $n=3$)
Steroid III	500 μg	65	5	Lac (80%), CrnSt (15%), HPC (3%), MgStea (0.5%)	MeOH	12.5	18	102.6% ($\pm$0.9, $n=12$)	103.9% ($\pm$1.2, $n=12$)
Steroid IV	150 μg	65	5	Lac (80%), CrnSt (15%), HPC (3%), MgStea (0.5%)	MeOH	12.5	18	102.1% ($\pm$0.9, $n=12$)	103.7% ($\pm$1.7, $n=12$)
Steroid V	25 μg	65	10	Lac (95%), Primojel (4%), MgStea (0.5%)	MeOH	10	18	97.5% ($\pm$0.2, $n=3$)	97.5% ($\pm$1.1, $n=3$)
CNS compound	20 mg	120	10	HPMC (40–77%), $CaHPO_4$ (0–40%), Aerosil (1.5%), MgStea (1%)	MeOH/H_2O, 80/20	20	35	–[c]	95.4% ($\pm$1.0, $n=12$)

Abbreviations, Lac, lactose; PotSt, potato starch; PVP, polyvinylpyrrolidone; SteaAc, stearic acid; CrnSt, corn starch; HPC, hydroxypropyl cellulose; MgStea, magnesium stearate; HPMC, hydroxypropylmethyl cellulose; ACN, acetonitril; MeOH, methanol.

[a] Marvelon®.

[b] Standard deviation (S.D.); *n*, number of analyses.

[c] Grinding could not be accomplished manually.

tablets varies from very low (25 μg/65 mg tablet) to relatively high (20 mg/120 mg tablet). Analysis was performed using single tablets, as well as on a total of five or 10 tablets. The former is commonly done to obtain information on the content uniformity of a batch of tablets, the latter to determine its mean content [13,14]. In standard manual methods, usually the amount of solvent used per tablet is the same for both content-uniformity and mean-content analyses, which simplifies the validation of the procedure. This implies that for the mean-content analysis, usually 5–10-fold more solvent is used as for the content-uniformity analysis. Here, because of the limited amount of solvent that can be used in the vials, also the influence of the extraction volumes on the results of the content analysis was studied to some extend.

The tablets containing the CNS compound contained large amounts of hydroxypropylmethyl cellulose (HPMC), which influences the break-down of the tablet in water and thereby extends the in-vitro release times to over 8 h. The large amounts of HPMC, however, also influence the physical properties of the tablets. These tablets are quite ductile and tend to smear, and are, therefore, very difficult to grind manually in a mortar. The recoveries of the extraction using either acetonitrile or methanol were 69.6 and 94.1%, respectively, (when using water in the extraction solvent a viscous extract was obtained). With the ball-mill, however, and by applying methanol as solvent an extraction recovery for the AE of 99.4% was obtained. Therefore, for these tablets, all content analyses including tablets for clinical trials and potential market products will be performed with the ball-mill method.

As can be seen in Table 2, the values found with the ball-mill show no significant difference with the manual methods. The values found for analytical standard deviation (S.D.) are slightly higher with the ball-mill method, but are still acceptable. The time that is saved per analysis is, as expected, 15–30 min, mainly since the manual grinding and weighing of the tablet powder is circumvented. Clean up of the vials was performed in the standard washing apparatus used for other lab glassware, and no sample carry-over was observed. For all types of samples included in this study, the stability of the solutions in the mill-vials after grinding was tested, this way, the situation was mimicked where an analyst postpones the actual analysis to, for example, the next day. All values found were satisfactory ($>98\%$ after 24 h).

Two drawbacks of the method should be mentioned, first the limited amount of extraction solvent (35 ml) that can be used could be a problem for high-dosed or poorly soluble compounds. This can usually be dealt with by the choice of a better extraction solvent. Due to the instant grinding, no water is needed for the decomposition of the tablets prior to extraction, so that e.g. pure methanol can easily be used. As a comparison, in robotized systems, a minimum amount of solvent is needed, for a proper preconditioning of the tubing and filters used [9]. This implies that for low-dosed tablets, a too low extract concentration can be obtained.

The complexity of the full automation of the ball-mill method is a second drawback; the vials should remain solvent tight under shaking with the ball. Now, the vials are closed and clamped in the mill by hand, which requires some force, this will be difficult for a 'standard' robot-arm. Therefore, this method is most suited for applications that now include a manual grinding step, which will not or cannot be easily fully automated. The relative small investment (approximately Euro 7.000 in total for mill and vials) and the simplicity of the method makes it excellently suited for use in various (development and quality assurance) laboratories.

4. Conclusions

The new ball-mill method is simple, reliable and fast. Savings in total analysis time are up to 30 min per batch analysis compared with the conventional method using a powder step in a mortar. The use of the same closed vial for grinding, extraction and centrifugation ensures no loss of compounds or solvents. No extra solvents are needed for washing steps, as is frequently the case in robotized systems. The main drawback of the

method is the limited amount of extraction solvent that can be used, 1–35 ml. Furthermore, the sample preparation cannot easily be done in parallel, so that for large amounts of samples, full automation can sometimes save more time. The method is suited for tablets of various size and hardness. Due to the relative small investments required, it can simply be introduced in pharmaceutical laboratories involved in analysis of tablets, capsules or granulates.

References

[1] M. Wieser, P. Malz, T. Arendt, S. Kuppers, Chromatographia 51 (2000) 478–482.
[2] D.J. Phillips, M. Capparella, U.D. Neue, Z. el Fallah, J. Pharm. Biomed. Anal. 15 (1997) 1389–1395.
[3] J. Lambropoulos, A.B. Bergholdt, J. Pharm. Biomed. Anal. 24 (2000) 251–258.
[4] D. Ivanovic, M. Medenica, S. Kojic Marinkovic, D. Radulovic, J. Pharm. Biomed. Anal. 23 (2000) 965–971.
[5] S. Carda-Broch, J. Esteve-Romero, M.C. García-Alvarez-Coquec, J. Pharm. Biomed. Anal. 23 (2000) 803–817.
[6] B. Paw, G. Misztal, J. Pharm. Biomed. Anal. 23 (2000) 819–823.
[7] B.L. Hogan, M. Williams, A. Idiculla, T. Veysoglu, E. Parentec, J. Pharm. Biomed. Anal. 23 (2000) 637–651.
[8] M.D. Luque de Castro, A. Velasco-Arjona, Anal. Chim. Acta 384 (1999) 117–125.
[9] W.F. Shamrock, K. Reilly, D.K. Lloyd, J. Pharm. Biomed. Anal. 21 (2000) 1225–1232.
[10] C. John, S. Jonathan, J.A. Short, J. Autom. Chem. 16 (1994) 131–133.
[11] J.N. Staniforth, in: M.E. Aulton (Ed.), Pharmaceutics, The Science of Dosage Form Design, Churchill Livingstone, Edinburgh, 2000, pp. 581–590.
[12] J.C. Chaumeil, Methods Find. Exp. Clin. Pharmacol. 20 (1998) 211–215.
[13] The United States Pharmacopoeia, XXIV Revision, National Formulary XIX, American Pharmaceutical Association, Washington, DC, 2000
[14] European Pharmacopoeia III, Council of Europe, Strasbourg, France, 1997