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Leveraging elevated temperature and particle size reduction to extract API from various tablet formulations

Beverly Nickerson *, William B. Arikpo, Mark R. Berry, Vincent J. Bobin, Timothy L. Houck, Hany L. Mansour, John Warzeka

Pfizer Global Research & Development, Groton/New London Laboratories, Pfizer Inc., Groton, CT 06340, United States

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Abstract

Several sample preparation techniques were evaluated for extracting active pharmaceutical ingredient (API) from immediate release (IR) and controlled release (CR) tablet formulations. These techniques utilized either elevated temperature [e.g., accelerated solvent extraction (ASE) and microwave assisted extraction (MAE)] or particle size reduction [e.g., ball mill and homogenizer/Tablet Processing Workstation II (TPWII)]. Results were compared for equivalence to those obtained with the existing standard method for each formulation. For the CR formulations, sample preparation times were significantly reduced when using these techniques compared to the standard method. Advantages and limitations associated with each technique are discussed.
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1. Introduction

Sample preparation/extraction of active pharmaceutical ingredients (API) from solid oral dosage forms has been a challenging and time consuming activity for many projects. A typical sample preparation method for an immediate release (IR) tablet formulation often consists of weighing and transferring the tablet(s) into a flask, adding dissolving/extraction solvent and then either shaking or sonicating the sample for a period of time. The sample is then diluted to volume, mixed, filtered and sub-diluted if necessary. These methods rely on the tablet disintegrant and shaking/sonication to disperse the tablet and extract the API. For controlled release (CR) tablet formulations, additional measures are often required to disperse the formulation and extract the API.

A number of different techniques are available to aid sample preparation and extraction of API. This paper describes a study that was performed to evaluate several non-traditional sample preparation techniques to extract API from various IR and CR tablet formulations. The techniques evaluated utilized either elevated temperature or particle size reduction to increase the efficiency of sample extraction and solubilization. The techniques studied that utilize elevated temperature was accelerated solvent extraction (ASE) (also known as pressurized liquid extraction (PLE)) and microwave assisted extraction (MAE). Soxhlet extraction which also utilizes high temperature was not evaluated as this is reported to be time and solvent intensive [1]. Supercritical fluid extraction (SFE) utilizes high temperature and an evaluation of SFE was not performed in this study because the equipment was not available in-house. The techniques evaluated in this work that utilize particle size reduction were milling through the use of a ball mill and homogenization through the use of the tablet processing workstation II (TPWII). These milling techniques were selected based on the availability of the equipment in-house. The techniques used in this study are described in more detail below.

ASE utilizes high temperature and pressure to maximize extraction of the desired component. Increased temperature accelerates the extraction kinetics, and elevated pressure keeps the solvent below its boiling point enabling safe and rapid extractions. ASE has been used for assay and content uniformity determination for pharmaceutical dosage forms, including

* Corresponding author. Tel.: +1 860 441 0668; fax: +1 860 715 8004.
E-mail address: beverly.nickerson@pfizer.com (B. Nickerson).

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extended release tablets [2,3], chewable tablets [4,5], and transdermal patches [3,6]. As an example, Hoang et al. [5] used ASE to extract an LTD4 antagonist from a chewable tablet formulation for content uniformity analysis. Intact tablets were first exposed to water at 40 °C to disperse the tablet, then to methanol at 70 °C to extract the drug. A mean recovery of 98.2% of label claim (1.3% R.S.D.) was obtained and was comparable to results obtained from mechanical extraction (97.6% recovery, 0.9% R.S.D.). In a different application, ASE was used by Blanchard et al. [7] on stressed tablets in order to obtain more concentrated extracts than could be obtained using the manual sample preparation method, resulting in significant time savings to isolate the degradation products in the extracts by semi-preparative HPLC for subsequent structure elucidation studies.

Systems are commercially available and the Dionex ASE system (ASE 200, Dionex, Sunnyvale, CA, USA) was used in this study. The Dionex ASE is an automated system capable of running 24 samples sequentially using various sample cell sizes (1–33 ml). The system has the capability to heat samples up to 200 °C and to perform multiple volume flushes to assure full recovery of extracted sample.

In MAE the sample is placed in a vessel with a microwave-absorbing solvent. Microwaves are then used to heat the sample solution directly. If a non-polar solvent is used, a fluoropolymer Weflon stir bar is used to aid the heating process. As with ASE, increased temperature accelerates the extraction kinetics, and elevated pressure keeps the solvent below its boiling point. Direct heating of the sample solution instead of conductive heating of the vessel results in more rapid heating of the sample and reduced extraction times. Eskilsson et al. [8] used MAE to extract felodipine and one of its degradation products from intact tablets in 10 min using 5% methanol in acetonitrile. The methanol dissolved the outer tablet layer and acetonitrile caused the tablet core to swell and fragment into smaller pieces. Recoveries of 99.0% felodipine (1.5% R.S.D.) and 99.2% of the degradant (5.3% R.S.D.) were obtained when normalized against results obtained with a sample preparation method using grinding and ultrasonication. MAE and ASE have also been evaluated by Lee [9] as a troubleshooting tool to assess suitability of a method to extract API from tablet dosage forms.

Several commercial MAE instruments are available. The instrument used in this study is the Ethos E extraction labstation (Milestone Scientific, Shelton, CT) which is equipped with a microwave diffuser and a magnetic stirrer to ensure a homogeneous field within the microwave cavity and even heating of all samples. Continuous stirring of the solvent/sample within the vessels eliminates sample clumping and achieves uniform temperature inside the vessels for increased extraction efficiency and analyte recoveries. Sample sizes ranging from 1 g to 100 g can be used and up to 12 samples can be prepared simultaneously.

Milling can be used to reduce sample particle size, thereby increasing the surface area of the sample. This in turn can lead to increased extraction efficiency. Kok and Debets [10] used ball milling to prepare immediate release and control release tablets with tablet strengths ranging from 25 μg active (65 mg total tablet weight) to 20 mg active (120 mg total tablet weight). Samples were milled and extracted in 2 min with recoveries of 95–104%.

In this study a Retsch ball mill (model MM301, Retsch Inc., Newtown, PA, USA) was used. This instrument has holders for two equal sized chambers. The chambers are available in different sizes and different materials, such as stainless steel and Teflon. The sample is placed in the chamber along with a ball(s) of the same material as the chamber. The chambers oscillate to mill and pulverize the sample. Viscous materials can be milled in the presence of solvent (i.e., wet milling) and thermally unstable or malleable samples can be frozen prior to milling. The overall process reduces the material particle size to increase the efficiency of sample extraction and solubilization.

Homogenization is a technique that uses a set of rotating blades combined with wet grinding/shredding/shearing to reduce sample particle size and increase surface area for rapid sample disintegration. The homogenizer also provides vigorous mixing, enhancing sample contact with the solvent, thereby facilitating sample extraction [11,12].

Different commercial homogenizers are available. In this study homogenization of samples was performed using the Tablet Processing Workstation II (TPWII, Caliper LifeSceinces, Hopkinton, MA, USA) which is a bench top instrument designed to automate sample preparation and injection onto an HPLC system for solid dosage forms, powders, feeds, capsules and blend samples. Samples are extracted using a wet grinding homogenization technique and the system is able to queue up to 100 samples per run. The use of this system has been reported for a number of pharmaceutical dosage forms, including tablets [13–15] and capsules [16]. These applications include preparation of individual units and composite samples for assay, content uniformity and purity testing and results obtained using the TPWII were comparable to results obtained using manual sample preparation.

2. Experimental

2.1. Equipment

The following instrumentation was used for sample preparation: ASE 200 [Dionex]; Ethos E Extraction Lab Station [Milestone Scientific]; ball mill [model MM301, Retsch Inc.]; Tablet Processing Workstation II [TPWII, Caliper LifeSciences]; and Polytron System PT 3000 [Kinematica, Lucerne, Switzerland]. Prepared sample solutions were analyzed using an Agilent 1100 HPLC system [Agilent Technology, Waldbronn, Germany] and data acquisition and analysis system [Empower Software Service Pack SP-D, Waters Corporation, Milford, MA, USA].

2.2. Samples

Two immediate release tablet formulations, IR-1 and IR-2, and two controlled release tablet formulations, CR-1 and CR-2, were used in this study. Each tablet formulation contains a different API and the composition of these tablets is listed in Table 1. Solubility information for the four APIs is provided in footnotes in Table 1.

Table 1
Formulation composition of tablets used in this study

IR-1		IR-2		CR-1		CR-2	
Component	mg/tablet	Component	mg/tablet	Component	mg/tablet	Component	mg/tablet
Tablet core excipients		Tablet core excipients		Tablet core excipients		Tablet core excipients	
API-1[a]	1.00	API-2[b]	5.48	API-3[c]	10.98	API-4[d]	66.0
Croscarmellose sodium	3.0	Lactose monohydrate	30.25	Hydroxypropyl methylcellulose	30.44	Hydroxypropyl methylcellulose	24.6
Lactose monohydrate	32.0	Magnesium stearate	0.75	Magnesium stearate	0.85	Magnesium stearate	4.8
Magnesium stearate	0.75	Microcrystalline cellulose	60.52	Polyethylene oxide, low MW	164.98	Polyethylene oxide, low MW	244.4
Microcrystalline cellulose	63.25	Sodium starch glycolate	3.0	Polyethylene oxide, high MW	96.25	Polyethylene oxide, high MW	105.8
				Sodium chloride	35.00	Sodium chloride	47.8
				Dye	1.50	Dye	1.6
Coating excipients		Coating excipients		Coating excipients		Coating excipients	
Film coating	4.5	Film coating	4.0	Cellulose acetate	23.75	Cellulose acetate	36.1
				Polyethylene glycol	1.25	Dye	0.5
				Film coating	24.0	Polyethylene glycol	1.9
						Film coating	24.5
Total tablet weight	104.5	Total tablet weight	104.0	Total tablet weight	389.00	Total tablet weight	558.0

[a] API-1 is very slightly soluble in 0.1N HCl and in pH 2 buffer, while practically insoluble in higher pH aqueous solutions. API-1 is also practically insoluble in acetonitrile, very slightly soluble in ethanol and slightly soluble in methanol.
[b] API-2 is slightly soluble in pH 1.5 buffer and in methanol, while practically insoluble in higher pH aqueous solutions.
[c] API-3 is practically insoluble in water and ethanol and soluble in methanol.
[d] API-4 is practically insoluble in water and soluble in ethanol and methanol.

The IR and CR formulations were chosen because they represent the extremes of tablet extraction difficulty: the two IR formulations represent tablets that are relatively easy to extract while the two CR formulations represent extremely challenging tablets to extract. The two CR formulations are osmotically controlled dosage forms. These formulations contain a semipermeable membrane coating that consists of cellulose acetate, a water insoluble polymer, and PEG, a water-soluble polymer. The membrane coating poses a challenge for tablet dispersion. The tablet core consists of hydroxypropyl methylcellulose and polyethylene oxide which poses a challenge for complete extraction of the drug due to potential polymer gelling and inclusion of the drug in the gelled polymer [10,17]. All tablet samples and reference standards were obtained from Pfizer Inc. [Groton, CT].

2.3. Method development

Method development/optimization was performed on each technique focusing on the variables listed in Table 2. Unless otherwise noted, sample preparation method development/optimization for all four techniques was performed using the dissolving solvent in the current approved method for each particular formulation. After method development/optimization was completed, 10 units of each formulation were prepared per each technique. These samples were then analyzed using the HPLC method described in the standard procedure. Results were then pooled and statistically compared to each other.

2.4. Preparation of samples

2.4.1. Standard methods

The standard method for each formulation used in this study is described in Table 3.

2.4.2. ASE/PLE

The ASE methods used for the five formulations are summarized in Table 4. Additional ASE parameters used were pressure (2000 psi), preheat time (0 min), heat time (5 min) and purge time (60 s). For tablet preparation method 1, tablets were quartered and crushed on filter paper and then the tablets and paper were transferred to an 11 ml ASE cell. For tablet preparation method 2, tablets were crushed, mixed with 5 ml of sand and placed into a 22 ml ASE cell. All sample extracts were filtered or centrifuged and diluted as specified in the standard method or as needed to arrive at the desired nominal concentration.

2.4.3. MAE

Intact IR tablets or crushed (with hammer) CR tablets were placed either in 100 ml or 270 ml sample cells. The appropriate sample diluent was quantitatively added to the sample and capped. The capped cells were loaded on the rotor with the temperature sensor inserted into the reference cell and then placed in the microwave cavity. The microwave energy entered in the run program ranged from 75 W to 800 W: the actual energy applied was dependent on the extraction temperature and the rate of

Table 2
Method development/optimization parameters used for each technique

Extraction technique	ASE/PLE	MAE	Ball mill	TPWII
Initial parameter(s) optimized for complete extraction of API	Temperature varied to determine optimum extraction without degradation	Temperature varied to determine optimum extraction without degradation	Milling time investigated to assure full pulverization of tablet to a powder	Sample volume and diluent addition strategy, dispersion time
Secondary parameter(s) evaluated if API extraction issues	Solvent, number of extractions and sample preparation/loading varied depending on dose form/extraction issue	Varied sample preparation/loading depending on dose and formulation. Use of intact or crushed tablet. Rate of temperature ramp and hold time	Wet milling instead of dry milling	Dispersion speed
Any "default" parameters used	Pressure, preheat time, heat time and purge time	Temperature ramp, static time, stir bar size and speed, and cool down time	Oscillation speed maxed out through the entire experiment	Ten 10 s pulses at 8000 rpm
Any additional factors to consider or comments	Sample generally introduced in the sample cell as a powder		Ball size and type	Probe height, volume and soak time

Table 3
Standard methods used to prepare the tablet formulations

Operation	IR-1	IR-2	CR-1	CR-2
Tablet treatment	Transfer intact tablet into 25-ml volumetric flask. Protect sample solutions from light	Transfer intact tablet into 25-ml volumetric flask	Cut tablet into quarters with a razor then transfer into 100-ml volumetric flask	Cut tablet into quarters with a razor then transfer into 100-ml screw cap test tube. Protect sample solutions from light
Sample diluent	0.1N HCl/ACN, 80/20 (v/v)	20 mM phosphate buffer, pH 3/MeOH, 45/55 (v/v)	0.1N phosphate buffer, pH 6/MeOH, 55/45 (v/v)	ACN/MeOH, 50/50 (v/v)
Agitation	Add 12 ml diluent, then shake on reciprocating shaker for 30 min at 200 oscillations/min	Add 8 ml diluent, then shake on a mechanical shaker for 60 min at 200 oscillations/min	Add 50 ml diluent, then stir overnight with a magnetic stirrer	Add 30 ml diluent, allow to sit for 24 h in the dark. Then blend with a polytron for 20 s at a speed setting of six
Sample dilution	Dilute to volume with diluent and mix well	Dilute to volume with diluent and mix well	Remove magnetic stirrer, dilute to volume with diluent and mix well	Quantitatively transfer into a 250-ml volumetric flask. Rinse polytron twice into the flask with diluent. Then dilute to volume with water
Filtration	Filter using an Acrodisc CR PTFE 0.45 μm filter	Filter using an Acrodisc CR PTFE 0.45 μm filter	Centrifuge an aliquot at 2500 rpm for 20 min	Centrifuge an aliquot at 2000 rpm for 30 min
Additional dilution	None	None	A 1:2 dilution is made with diluent	A 1:2 dilution is made with diluent
Final sample concentration	0.04 mg/ml	0.2 mg/ml	0.12 mg/ml	0.05 mg/ml
Sample Analysis by HPLC (column/column temperature, mobile phase, detection)	Symmetry C18 (5 μm, 4.6 mm × 50 mm, Waters Corp., Milford, MA)/25 °C; 20 mM $NH_4H_2PO_4$, pH 3.0/ACN, 65/35 (v/v); UV at 260 nm	Symmetry C18 (3.5 μm, 4.6 mm × 75 mm, Waters Corp., Milford, MA)/35 °C; MeOH/20 mM KH_2PO_4, pH 3.0, 55/45 (v/v); UV at 285 nm	Nova-Pak C18 (4 μm, 3.9 mm × 150 mm, Waters Corp., Milford, MA)/ambient; 0.1N phosphate buffer, pH 6.0/MeOH, 55/45 (v/v); UV at 225 nm	Ultrasphere-ODS (5 μm, 4.6 mm × 250 mm, Beckman Coulter Inc., Fullerton, CA)/ambient; ACN/MeOH/water, 20/30/50 (v/v/v); UV at 265 nm

ACN: acetonitrile. MeOH: methanol.

Table 4
ASE method parameters used in this study

Parameter	IR-1	IR-2	CR-1	CR-2
Temperature (°C)	80	80	60	80
Static time (min)	5	5	5	5
Flush volume (%)	60	60	100	100
Number of cycles	3	3	1	1
Solvent	80/20, 0.1N HCl/ACN	80/20, 0.1N HCl/ACN	MeOH	50/50, ACN/MeOH
Number of extractions	1	1	10	8
Tablet preparation method	2	2	1	1

ACN: acetonitrile. MeOH: methanol.

temperature ramp. The stir bar size and sample stir rate were predetermined based on sample cell volume. Very small star bars (e.g., Teflon coated, egg or octagon shaped, 5/8 in. × 1/4 in. or 5/8 in. × 5/16 in., VWR International, West Chester, PA) and fast stir rate were used for the 100 ml cell while small to medium size stir bars (e.g., Teflon coated, assorted shapes, 5/8 in. × 1/4 in. to 1–1/2 in. × 3/8 in., VWR International) were used for the 270 ml cell. The extraction was performed using the following program: temperature ramp to 45 °C in 1–3 min (IR tablets) or to 65 °C in 3–5 min (CR tablets); 10 min hold (IR tablets) or 15 min hold (CR tablets); and 10 min cool down for all formulations. In some experiments, the cool down time to ambient temperature was reduced by placing the sample rotor in a cold water bath or sink. After temperature equilibration to ambient, the samples were either filtered or an aliquot was diluted to the specified concentration and filtered.

2.4.4. Ball mill

Sample preparation of all the studied formulations used the following common parameters: 3 min milling time at 30 oscillations/s, sample diluent from the standard method used for wet milling, and stainless steel chambers and two 11 mm stainless steel balls used for all formulations except IR-1 which used Teflon vessels and balls due to drug/solvent incompatibility with stainless steel. After milling, samples were quantitatively transferred to a volumetric flask and diluted to volume. An aliquot was then filtered or centrifuged as described in the standard method.

2.4.5. TPWII

Sample preparation of the IR formulations was accomplished by automatically adding 50 ml of diluent to the extraction vessel and homogenizing. Dispersion parameters for the homogenization step varied slightly between the two formulations. Four 12 s pulses at 8000 rpm were used for IR-1 and ten 10 s pulses at 5000 rpm for IR-2. The resulting solutions were automatically filtered.

Sample preparation of the CR formulations required multiple homogenization steps. An initial aliquot of diluent (e.g., 100 ml for CR-1 and 125 ml for CR-2) was automatically added to the extraction vessel followed by several dispersion cycles (e.g., ten 20 s pulses at 12,000 rpm for CR-1 and seven 20 s pulses at 12,000 rpm for CR-2). A second addition of diluent (e.g., 100 ml for CR-1 and 375 ml for CR-2) was then performed followed by additional dispersion cycles (e.g., ten 20 s pulses at 12,000 rpm for CR-1 and six 20 s pulses at 12,000 rpm). This ensured a rapid and complete extraction of the API from the tablet matrix.

2.5. Statistical analysis

Analysis of variance (ANOVA), multiple comparison, and equivalence testing techniques combined with graphical illustrations were used to compare the methods under investigation. SAS v8.2 was used for all analysis and graphical enhancements.

3. Results and discussion

3.1. Method development

Method development approaches used for each of the techniques is described below. Challenges related to each technique and the various formulation types are also highlighted.

3.1.1. ASE

Method development for CR-1 tablets will be discussed as it was a difficult formulation to extract and the work done for this formulation is representative of most of the parameters that can effectively be varied in developing an ASE method. Temperature is the most effective way to increase extraction efficiency with the ASE and a temperature must be found that allows for rapid extraction but which does not degrade the sample. For CR-1 API recovery increased from 40 °C to 80 °C, however at 100 °C the amount recovered decreased relative to the lower temperatures, most likely due to degradation. Other parameters studied were: extraction solvent volumes (modified by changing ASE cell sizes), extraction solvent, number of static cycles, time of static hold, number of extractions done on a sample and sample preparation.

Extraction solvent volume and different extraction solvents were explored to increase solubility of the API. The number of static cycles and the time of the static hold explore the solubilization and diffusion of the API. For CR-1 a large number of extractions was necessary for complete extraction. It was found that more extractions over a given time yielded better results than one extraction in that same time. CR-1 required 10 extractions of a single sample which took ∼2.5 h to complete. The ASE does not agitate the sample and that is especially problematic with a formulation like CR-1 which contains polymers that swell or gel and can trap the API.

Preparation of the sample prior to loading it into the ASE cell by mixing the crushed sample with sand was explored. Mixing with sand increases the effective surface area of the powdered sample and could help to minimize gelling and clumping of the polymer, which is a problem for the CR-1 and CR-2 formulations. Sand also aids in the immersion of particles in the diluent.

The large number of parameters that can be varied makes ASE method development complicated and time consuming. For example, a set of parameters can be optimized for a specific extraction solvent but they may not be optimized if the solvents are changed. The same is true for altering the preparation of the sample. For CR-1, eight different experiments were run each varying different parameters and combinations of parameters.

The two parameters that were most effective in the extraction of API from CR-1 tablets were the solvent and the number of extractions. Extraction was greatly enhanced by switching from the diluent used in the standard method (55/45, v/v, 0.1N phosphate buffer, pH 6.0/methanol) to 100% methanol. Increasing the number of extractions also increased the amount of API recovered most likely due to the agitation the sample received during loading and unloading of solvent from the cell and the increased interaction time of the solvent and API. While these two parameters were important in the analysis of CR-1, that may not be true for other formulations. For example, CR-2 showed little to no change in recoveries when different solvents were used.

Based on the work done in this study, it is recommended that tablet samples be crushed or ground to a fine powder. Initial experiments should evaluate different static hold times (e.g., 5 min and 30 min), temperatures (e.g., 40 °C and 80 °C) and solvents. Information on the API such as thermal stability and solubility in different solvents can assist in selecting the appropriate temperatures and solvents. Each sample should be extracted three times and each extract analyzed individually. Multiple extractions and individual analysis of the extractions allows one to see if complete extraction has occurred and when, or if there is still API left in the sample cell.

3.1.2. MAE

In MAE the rate and efficiency of extraction is directly related to temperature, sample type and length of extraction time. Therefore, the primary parameters that were considered during method development were tablet formulation type, sample diluent and extraction temperature.

All experiments were performed using the sample diluent in the standard method and the initial extraction temperature used was that in the standard dissolution test method (e.g., 37 °C, since sample solutions typically stable under these conditions) plus 8 °C (e.g., 45 °C). Higher extraction temperatures were determined by adding 20 °C (e.g., 65 °C) increments where needed. For example, if an efficient extraction was achieved (e.g., ≥98%), no further temperature increases were evaluated, since higher temperatures would decrease throughput. However, if sample degradation was observed, experiments were performed using lower temperatures (e.g., 5–10 °C decrease).

The IR tablets were placed in the MAE vessels as intact tablets since they typically disintegrate rapidly. The use of intact and broken/crushed tablets were evaluated for the CR formulations to determine if the broken/crushed tablets would facilitate a more rapid and efficient extraction.

The secondary parameters evaluated to improve efficiency and reduce extraction time were rate of temperature ramp, extraction hold time and stir bar size and stir speed. Faster temperature ramps were applied to aqueous solutions and diluents that were aqueous/polar organic mixtures rather than polar organics due to better microwave energy absorption in the aqueous diluents.

The 270 ml or the 100 ml sample cells were used based on the nominal standard and sample concentration specified in the current test methods and to improve throughput. The larger sample cells allow the use of larger stir bars which facilitate more rapid tablet disintegration and dissolution of the API. In addition, for high dosage strengths the use of a larger volume can eliminate the need for subsequent sample dilution steps. However, due to the fact that the MAE carousel only holds six 270 ml cells, additional runs were required for tests on samples greater than 6. For samples which required 60 ml or less, the 100-ml cells were preferred since 12 cells can be prepared at one time, and higher pressure is typically achieved in the smaller cells. Since the Ethos E labstation used in this study is not equipped with a pressure sensor, the effect of higher pressure in the 100-ml cells cannot be evaluated.

As described in the ASE method development, the CR formulations evaluated in this study tended to gel at elevated temperature and this resulted in the formation of clumps that trapped the API and prevented homogeneous dispersion and dissolution. The very small stir bars would get trapped in the clumps if a suitable stir rate was not used, especially in the 100 ml cells. This also led to low API extraction and higher sample-to-sample extraction variability.

The stir bar tended to just rock from wall to wall instead of rotating smoothly or semi-smoothly in the cell if a suitable stir rate was not applied. Since the cells are not transparent, it was necessary to pre-determine the stir rate to be applied. The stir rate pre-determination was performed in the sample cell or in a beaker. A suitable stir bar was placed in the cell or a beaker of an approximately equivalent volume. The uncovered cell or beaker was then placed in the oven cavity and the appropriate stir rate (e.g., as fast a rotation as possible without causing "rocking" of the stir bar) and rotation was set by adjusting the stir bar dial to the desired position. The stir rate pre-determination helps to prevent the stir bar from getting trapped and to achieve a homogeneous mixing of the samples.

The sample rotor is rotated one half circle in both direction on order to assure even distribution and exposure of all samples to microwave energy. CR-2 was the first formulation of the four formulations to be evaluated using MAE. Low API recovery in some CR-2 samples was observed when the oven cavity was fully populated during testing. This was not the case during method development and optimization when using a maximum of two cells in the oven cavity per experiment. The low recovery in some cells when the oven cavity is fully populated is

probably due to non-uniform exposure of all sample cells to the microwave energy. As a result, an additional 3 min for IR formulations and 5 min for CR formulations was added to the hold time when six or more samples were being tested. The additional hold time allowed longer exposure of all samples which resulted in equivalent recoveries in all cells, improved %R.S.D., and no degradation was observed.

Based on the work done in this study it is suitable to use intact tablets for IR formulations since these tablets typically disintegrate rapidly. Although intact, crushed or broken CR tablets may be used, crushed or broken tablets are recommended to reduce extraction time and improve API recovery. If sample diluent is aqueous or a mixture of aqueous and polar organic, then a 2 min temperature ramp to 45 °C followed by a 10 min hold time and subsequent cool down is recommended as a starting point for MAE method development for IR tablets. For CR tablets, a 5 min temperature ramp to 65 °C followed by a 15 min hold time and subsequent cool down is recommended. A longer ramp time may be needed if the diluent is not very polar. Higher temperatures may be evaluated if needed to improve recovery provided that no sample degradation is observed at the initial run temperature. Longer hold time may also be explored to improve extraction efficiency.

3.1.3. Ball mill

The variables investigated during method development were ball size, vessel and ball material, and wet vs. dry milling. Milling speed and time were kept constant to reduce development time and were maintained relatively high to assure effectiveness. It was determined that wet milling/extraction of all the formulations was more effective than dry milling following by sample extraction. The CR-1 and CR-2 formulations required an extra extraction step to recover most of the drug.

Stainless steel vessels and balls were used for all the formulations except for IR-1 where Teflon vessels and balls were used to eliminate low recovery caused by the interaction of the drug with the acidic media (0.1N HCl/acetonitrile, 80/20, v/v) and stainless steel. In this case the drug degraded and the stainless steel balls became pitted and corroded. This issue was not observed with IR-2 and the use of acidic media (20 mM phosphate buffer, pH 3/methanol, 45/55, v/v) and stainless steel balls. A wide range of ball sizes is available and only two sizes were investigated (e.g., 11 mm and 20 mm). It was observed that the smaller ball size yielded higher recovery and that using two 11 mm balls gave higher recovery than one 11 mm ball.

The greatest challenge encountered during the study was transfer of the sample solution from the vessel to the final volumetric flask. A minimum of 100 ml final volume is essential to include three rinses of the vessel and its cover. Only two samples at a time could be milled and extracted, however this is not considered a major limitation due to the extremely fast milling and extraction times. It was a challenge to fully recover both CR-1 and CR-2, therefore additional wash cycles of the vessel were added. Quantitative transfer of the vessel contents to the final volumetric flask was quite difficult and required extreme care and was time consuming to perform. In addition, cleaning the vessels between individual extractions requires additional time that cannot be avoided. As a final consideration, the ball mill generates extreme noise during operation, which can be a distraction to colleagues.

Based on the work done in this study, a recommended starting point for developing a ball mill method for tablets is to place the tablet, two balls (e.g., 11-mm) and diluent in the ball mill vessel, then wet mill and extract the API from the tablets at 30 oscillations/s for 3 min.

3.1.4. TPWII

For the TPWII, variables that influenced the efficiency of API extraction from the formulation include the solvent volume and composition, dispersion speed, dispersion time, and the number of dispersion pulses (a pulse is one homogenization cycle) per extraction, and probe height for most CR and large tablet. Each of these parameters was optimized in order to achieve complete and rapid extraction of the API.

Although sample degradation is unlikely for most methods, dispersion parameters need to be chosen carefully in order to avoid thermal degradation since heat is generated during the homogenization process, especially if excessive (too many and lengthy) pulses are used. Different extraction solvents may be added in between dispersion cycles in order to promote dissolution of tablets. For IR tablets, a soak time prior to homogenization was generally included in the method in order to induce tablet disintegration.

Starting with smaller volumes facilitates a more rapid extraction because of higher probability of the homogenizer hitting the sample particles more times in a shorter time. As a result, particle size reduction occurs faster. A serial dilution step or additional volumes and subsequent pulses may then be added to achieve the desired concentration. This helps to avoid excessive pulsing in one large volume which may cause thermal degradation.

Other factors that are critical to method development involve sample handling after homogenization. The type of filter and the filtration rate had to be optimized in order to prevent clogging of the filter. In addition, the clean up procedure had to be designed to prevent carry-over and cross-contamination between samples.

Based on the work done in this study, recommended starting parameters for developing a TPWII method for tablets is proposed as shown in Table 5. For film coated IR and CR tablets, an initial soak of the tablet in an appropriate solvent may be required to facilitate tablet disintegration before dispersing. In addition, if the tablet diameter is greater than or equal to 7 mm, ensure that the “allow clearance for large tablet” option is selected in order to elevate the probe height.

3.2. Method comparison

Data obtained by the four techniques and the standard method for each tablet formulation are provided in this section. Tables 6–9 show the results obtained from the different extraction techniques for the four formulations tested. Minimum (Min), maximum (Max) and average (Avg) recovery results as %label claim (%LC) are presented. Results from each technique are compared for equivalence (within ±5%) with the standard method by the Two One-Sided Test (TOST [18]) procedure and

Table 5
Recommended starting parameters for developing a TPWII method

IR tablet method (steps 1 and 2 may be reversed)	
Step 1	Add tablet to dispersion vessel
Step 2	Add 50 ml of diluent to the vessel (may use a different or additional volume to achieve desired nominal concentration)
Step 3	Disperse tablet using six 10 s pulses at 8000 rpm
Step 4	Let dispersion settle for 30 s
Step 5	Filter dispersion at 0.10 ml/s and include filter pre-wash with 3 ml to waste
Step 6	Wash vessel 2 times with 100 ml of water Wash vessel 1 time with 100 ml of diluent Wash filter path 2 times with 3 ml of diluent
CR Tablet method (steps 1 and 2 may be reversed)	
Step 1	Add tablet to dispersion vessel
Step 2	Add 50 ml of diluent to the vessel (if tablet is large, more diluent and probe height adjustment may be required)
Step 3	Disperse tablet using ten 15 s pulses at 12,000 rpm
Step 4	Add 100 ml of diluent to the vessel (diluent may differ from that used in step 2)
Step 5	Disperse tablet using ten 15 s pulses at 12,000 rpm
Step 6	Let dispersion settle for 30 s
Step 7	Filter dispersion at 0.10 ml/s include a 3 ml filter pre-wash to waste
Step 8	Wash vessel 2 times with 100 ml of water Wash vessel 1 time with 100 ml of diluent Wash filter path 2 times with 3 ml of diluent

Table 6
Summary of IR-1 tablet results

	Lot 1[a]		Lot 2			
	Standard method	ASE	Standard method	Ball mill	MAE	TPWII
Min recovery (%LC)	97.7	95.3	98.4	98.1	100.2	96.5
Max recovery (%LC)	103.0	100.1	102.0	110.5	104.2	104.2
Avg recovery (%LC)	100.9	97.5	100.2	102.6	102.4	100.7
%R.S.D.	1.8	1.7	1.1	3.1	1.5	2.7

[a] Lot 1 was used for the ASE because of low sample availability of lot 2.

are shown in Fig. 1. In these plots, 90% confidence intervals for differences between methods are shown. Methods are considered equivalent by the TOST procedure at 95% confidence if the 90% confidence interval for the difference lies inclusively within the ±5% equivalency bounds. For each of the two IR formulations, the average recoveries and %R.S.D. values obtained using the various techniques compare well to the results obtained by the standard method as shown in Tables 6 and 7. In addi-

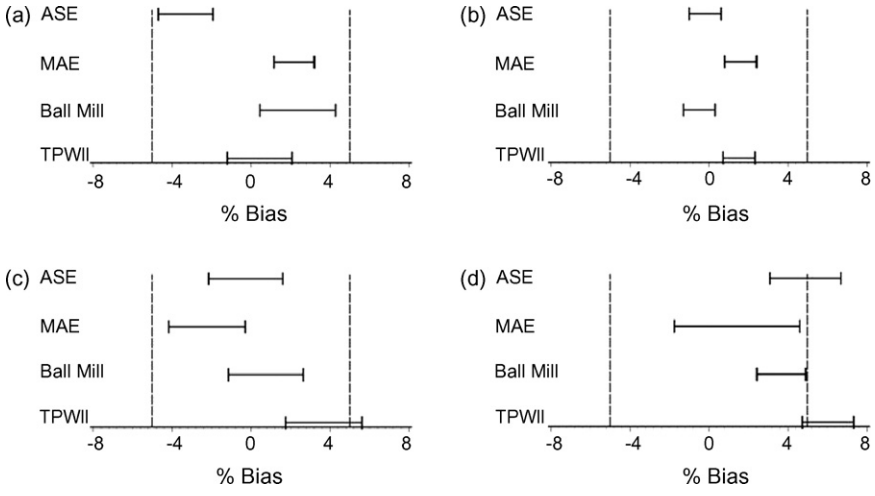

Fig. 1. Method equivalence analysis for (a) IR-1 tablets, (b) IR-2 tablets, (c) CR-1 tablets and (d) CR-2 tablets.

Table 7
Summary of IR-2 tablet results

	Standard method	ASE	Ball mill	MAE	TPWII
Min recovery (%LC)	97.8	97.8	96.8	99.7	99.8
Max recovery (%LC)	100.9	101.7	101.1	102.8	102.2
Avg recovery (%LC)	99.6	99.4	99.1	101.2	101.2
%R.S.D.	1.1	1.1	1.3	1.0	0.8

tion, as shown in Fig. 1a and b, the ASE, MAE, ball mill, and TPWII are equivalent to the standard method at 95% confidence for both IR formulations. Statistically significant bias can be identified in Fig. 1a and b as well. Confidence intervals that do not cover 0% bias indicate statistically significant bias between methods at the 90% confidence level. For example, Fig. 1b shows a confidence interval for the difference between TPWII and the standard method that does not include zero. This statistically significant bias, however, is of marginal practical concern since the confidence interval falls within the ±5% equivalency bounds.

As shown in Table 8 the results obtained for CR-1 by the ASE, ball mill, and MAE compared well to the standard method. The average recoveries for these techniques ranged from 108.8% to 111.8% compared with 111.1% obtained for the standard method. The average recovery obtained by the TPWII was the highest at 114.8%. Both CR-1 and CR-2 are manufactured with a 10% drug overage so the expected recovery is 110%. The R.S.D. results ranged from 1.8% to 3.3% with an R.S.D. of 1.5% for the standard method. As shown in Fig. 1c, ASE, ball mill and MAE results are equivalent to those obtained by the standard method. The results obtained by the TPWII, however, are not equivalent to the standard method as these results are higher.

As shown in Table 9 the average recoveries obtained for CR-2 by the four techniques ranged from 107.0% to 113.0% compared with 107.0% obtained for the standard method. The R.S.D. results ranged from 1.2% to 4.9% with an R.S.D. of 1.7% for the standard method. The MAE technique showed the greatest variability with an R.S.D. of 4.9%. This high %R.S.D. is most likely due to uneven exposure of all samples to the microwave energy as discussed previously under the method development section. CR-2 was the first formulation of the four formulations that was evaluated by MAE. Analysis of subsequent formulations used longer hold times (additional 3 min for IR formulations and additional 5 min for CR formulations) when six or more sample cells were placed in the oven to compensate for this effect. Therefore,

Table 8
Summary of CR-1 tablet results

	Standard method	ASE	Ball mill	MAE	TPWII
Min recovery (%LC)	108.3	109.3	108.5	104.5	110.6
Max recovery (%LC)	112.9	115.9	115.5	116.8	118.4
Avg recovery (%LC)	111.1	110.8	111.8	108.8	114.8
%R.S.D.	1.5	1.8	2.1	3.3	1.8

Tablets manufactured with a 10% API overage, therefore recovery should equal 110% LC.

Table 9
Summary of CR-2 tablet results

	Standard method	ASE	Ball mill	MAE	TPWII
Min recovery (%LC)	102.7	107.6	108.0	100.1	110.6
Max recovery (%LC)	108.9	116.0	113.1	114.9	115.3
Avg recovery (%LC)	107.0	111.9	110.7	108.4	113.0
%R.S.D.	1.7	2.3	1.2	4.9	1.4

Tablets manufactured with a 10% API overage, therefore recovery should equal 110% LC.

the other three formulations tested have better %R.S.D. values for the MAE analysis.

As shown in Fig. 1d, ball mill and MAE results are equivalent to those obtained by the standard method, although the ball mill results are biased high. The results obtained by the ASE and TPWII, however, are not equivalent to the standard method as these results are higher. These results suggest that the standard method is not extracting the entire drug from the samples.

Differences in method variability are indicated by the lengths of the confidence intervals shown in Fig. 1. For example, in Fig. 1b and c for IR-2 and CR-1, respectively, the lengths of the confidence intervals are similar for the four methods for each formulation, indicating that variability within a method is similar for each method. This is not the case for IR-1 and CR-2 as shown in Fig. 1a and d, respectively, where there are differences in method variability as indicated by the varying lengths of the confidence intervals (i.e., longer confidence intervals indicate larger variability). From the way the data were collected, the sources of variation (method, sample, analyst, standard, etc.) cannot be partitioned out, and so statistically identifying the possible sources of variation is not possible.

A summary of the method equivalency to the standard method is shown in Table 10. Results obtained by the ball mill and the MAE for all four formulations evaluated were equivalent to the results obtained by the standard method. Results obtained by the ASE were equivalent for three formulations and higher for one of the CR formulations compared to the results obtained by the standard method. Results obtained by the TPWII were equivalent for the two IR formulations and higher for the two CR formulations compared to the results obtained by the standard method, suggesting that these two standard methods may not be extracting all the drug. Overall, these results show that the ASE, ball mill, MAE and TPWII are viable sample extraction and preparation techniques for tablets.

During the analysis of the four formulations the advantages and disadvantages of each technique became apparent.

Table 10
Summary of method equivalency for each formulation

	ASE	Ball mill	MAE	TPWII
IR-1	√	√	√	√
IR-2	√	√	√	√
CR-1	√	√	√	× (+)
CR-2	× (+)	√	√	× (+)

√: method equivalent to standard method. × (+): method not equivalent to standard method due to higher results.

Table 11
Advantages and limitations of each extraction technique

	Advantages	Limitations
Manual	• Minimal analyst training needed • Specialized or expensive equipment not needed	• May require long extraction times • Can be labor intensive
ASE	• Semi-automated • Multiple samples (e.g., up to 24) can be extracted sequentially • Multiple extractions can be performed on a given sample to "prove" total extraction	• No sample agitation • High temperatures may cause sample degradation • Sample pretreatment necessary (e.g., crushed tablet) • Extensive method development usually required
MAE	• Extract multiple samples (e.g., 12) in parallel • Fast extraction	• High temperatures may cause sample degradation • Cooling time required (adds to overall sample preparation time) • Labor intensive to prepare sample cells (e.g., torque needed to close cells)
Ball mill	• Minimal analyst training needed • Ease of method development • Wet or dry milling can be performed	• Lack of automation • Only two samples can be prepared at a time • Quantitative transfer of sample is labor intensive • Minimum of 100 ml flask required to collect sample and rinses for quantitative transfer
TPWII	• Fully automated • Multiple samples (e.g., up to 100) can be prepared sequentially	• Minimum of 50 ml sample volume • Large reagent volume required for clean up between samples

These advantages and limitations are summarized in Table 11. Although each of the techniques gave equivalent or higher results than the standard method, some techniques are preferred based on the application. The TPWII proved to be the most desirable technique when compared to the others in terms of speed, efficiency and hands-on analyst time required. The automation of the TPWII allows a large number of samples to be analyzed with minimal analyst hands-on time and would be excellent for high volume routine use. A potential drawback to the TPWII is the higher cost of the equipment relative to the other techniques. For low product volumes a stand-alone polytron homogenizer would be a cost effective alternative.

The ball mill and MAE were ranked second to the TPWII. Advantages for the ball mill include minimal analyst training, ease of method development and fast turn around for small sample numbers. The drawback to the ball mill is the extensive analyst time required to quantitatively transfer the milled sample and to clean the milling chambers and balls between samples. The MAE can extract multiple samples in parallel, which allows for a rapid turnaround time. The MAE uses elevated temperatures which could lead to sample degradation and more analyst hands-on time is required to prepare samples compared to the TPWII. The ASE was the last choice based on potential degradation due to elevated temperatures, extensive method development time and long extraction times required for the CR formulations.

Despite their limitations, the ball mill and ASE are useful alternative methods that can be used in troubleshooting or investigating low assay results. The ball mill requires little training to use, has shown to be very effective in completely extracting API and requires little method development. The ASE can be useful in troubleshooting as it has the ability to extract the sample multiple times until there is no more drug left in the sample. The disadvantage of this technique is the extraction time required and the uncertainty of how many extractions are enough (before no more API is detected). While a stand-alone homogenizer (i.e., polytron) was not evaluated in this study, it would be a good choice for troubleshooting or to investigate low assay results for the same reasons as those listed for the ball mill.

4. Conclusion

A study was conducted using four extraction techniques for extracting various solid dosage formulations. Two of the techniques, ASE and MAE, utilized elevated temperature to increase extraction efficiency. The other two techniques, milling with a ball mill and homogenization with a TPWII, used particle size reduction to increase sample surface area and hence increase extraction efficiency. All four techniques were shown to be viable extraction and sample preparation techniques for tablets. As discussed in the previous section, however, the advantages and limitations of each of these techniques make certain techniques more amenable to specific applications. The TPWII, due to its automation, is well suited for routine preparation of large numbers of samples with minimal analyst hands-on time. The MAE can extract multiple samples in parallel to increase turnaround time. The ball mill is simple to learn and samples are milled in a short period of time. The ASE can perform multiple extractions on a sample until all the drug is extracted, and therefore would be useful for investigations where verification of low potency assays are needed.

References

[1] D. Kou, S. Mitra, in: S. Mitra (Ed.), Sample Preparation Techniques in Analytical Chemistry, John Wiley & Sons Inc., Hoboken, 2003, pp. 139–182.
[2] E. Björklund, M. Järemo, L. Mathiasson, L. Karlsson, J.T. Strode III, J. Eriksson, A. Torstensson, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 535–549.

[3] F. Höfler, R. Neufang, B. Raffelsberger, O. Stähler, LaborPraxis 23 (1999) 52–54.
[4] A.M. Abend, L. Chung, D.G. McCollum, W.P. Wuelfing, J. Pharm. Biomed. Anal. 31 (2003) 1177–1183.
[5] T.H. Hoang, R. Farkas, C. Wells, S. McClintock, M. Di Maso, J. Chromatogr. A 968 (2002) 257–261.
[6] Dionex Application Note 327, Extraction of Nitroglycerin from Transdermal Patches by Accelerated Solvent Extraction, 1997.
[7] A. Blanchard, C. Lee, B. Nickerson, L.L. Lohr, A.J. Jensen, K.M. Alsante, T.R. Sharp, D.P. Santafianos, R. Morris, K.D. Snyder, J. Pharm. Biomed. Anal. 36 (2004) 265–275.
[8] C.S. Eskilsson, E. Björklund, L. Mathiasson, L. Karlsson, A. Torstensson, J. Chromatogr. A 840 (1999) 59–70.
[9] C. Lee, Am. Pharm. Rev. 10 (2007) 142–147.
[10] S.J. Kok, A.J.J. Debets, J. Pharm. Biomed. Anal. 26 (2001) 599–604.
[11] R.E. Majors, LC–GC 16 (1998) 436–441.
[12] C.K. Choi, M.W. Wong, in: S. Ahuja, M.W. Dong (Eds.), Handbook of Pharmaceutical Analysis by HPLC, vol. 6, Academic Press, San Diego, 2005, pp. 123–144.
[13] I. Toro, J.F. Dulsat, J.L. Fabregas, J. Claramunt, J. Pharm. Biomed. Anal. 36 (2004) 57–63.
[14] U. Holler, C. Brodhad, P. Hofmann, V. Spitzer, J. Pharm. Biomed. Anal. 31 (2003) 151–158.
[15] W.F. Shamrock, K. Reilly, D.K. Lloyd, J. Pharm. Biomed. Anal. 21 (2000) 1225–1232.
[16] S.M. Han, A. Munro, J. Pharm. Biomed. Anal. 20 (1999) 785–790.
[17] R.O. Williams III, M.A. Sykora, V. Mahaguna, AAPS PharmSciTech. 2 (2001) (article 8).
[18] R.L. Berger, J.C. Hsu, Stat. Sci. 11 (1996) 283–319.