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Development of a two-step tier-2 dissolution method for blinded overencapsulated erlotinib tablets using UV fiber optic detection

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ABSTRACT

Measuring dissolution of a comparator drug overencapsulated in a hard gelatin shell is necessary when determining performance of the native and blinded formulations. However, the gelatin in the shell may form cross-links upon storage at stressed conditions, resulting in slow dissolution of the encapsulated drug. The aim of this study was to develop a dissolution approach for a hard-gelatin overencapsulated formulation of a comparator drug, erlotinib, which can overcome cross linking of the capsule shell. In this case, following the USP two-tier dissolution test by simply adding an enzyme did not dissolve the cross-linked capsules because the medium used in the method for erlotinib described in the FDA Dissolution Database contains sodium dodecyl sulfate that inhibits the activity of the enzyme. Changing the method by using different surfactants was not considered acceptable because it is preferable to closely follow the compendial method for the comparator. A two-step tier-2 method was developed as a solution, without significant change to the compendial method conditions. It uses 0.1 N HCl + pepsin as the initial medium to help capsule break-up. SDS is added at 15 min after the testing starts to ensure dissolution of the drug. This may be a useful general approach for dealing with cross-linking in over-encapsulated comparators. A UV fiber optic spectrophotometer was used for in situ, real-time detection of the dissolution profile during method development studies. The fast sampling rate available with this type of detection was important in elucidating the events occurring during dissolution and determining the optimal time of the SDS addition.

1. Introduction

The use of comparator drugs in clinical studies is common practice, in order to evaluate the relative efficacy of a new drug candidate. A comparator product is generally chosen from the most prescribed drugs available on the market in the target therapeutic area. Over-encapsulation of comparator tablets in hard gelatin capsule (HGC) shells with or without filler is a simple and economic way to blind comparators, and typically requires only minimal development work. Overencapsulated tablets (OETs) have been widely used in blinded clinical trials [1]. Even though there is little guidance from regulatory agencies on the development of the comparator formulations, it is expected that the altered or blinded and non-altered products should be bioequivalent. Therefore, analytical methods and testing are needed to ensure that the altered and non-altered products are not only similar in appearance but also in their in vitro behavior [2].

Amongst the analytical characterization required, dissolution is the most critical test to demonstrate "equivalence" between native and overencapsulated tablets. A bioequivalence study can be avoided if similar dissolution profiles are obtained. It is preferable to have a compendial dissolution method with which to test the comparator drug. However, although some of the comparators are described in a Pharmacopeia, and some innovator's products may be recorded in FDA Dissolution Database since January 2004 [3], very little information is available for newer drugs from innovators. The development of dissolution methods for comparators becomes challenging because of often-compressed project timelines, involvement of contract labs, and lack of information as well as experience with the comparator drugs [2].

Another issue in dissolution method development for OET in HGC is to overcome cross-linking of the gelatin shell which may occur under stressed stability conditions. It has long been known that gelatin cross-linking reduces the HGC dissolution rate [4,5]. The dissolution *Q* values of stressed HGC may drop to the point of rejection, although the in vivo bioavailability of the drug from the stressed HGC is not significantly decreased [6,7]. USP has addressed this issue and introduced a two-tier dissolution test to

Abbreviations: HGC, hard gelatin capsule; OET, overencapsulated tablet; RH, relative humidity; UVFO, ultra-violet fiber optic.

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use a medium containing an enzyme for aged capsules that fail to pass the first dissolution test [8]. Two types of enzymes are recommended, depending on the nature of the dissolution medium. Purified pepsin is recommended for water or a medium with a pH of less than 6.8; pancreatin is for media with a pH of 6.8 or greater.

The USP two-tier dissolution test may not work smoothly in some cases. The compendial method used for the first tier may not be suitable in the second tier for direct addition of enzyme because the medium used in the compendial method may contain a surfactant that inhibits the activity of the enzyme [9,10]. Furthermore, surfactants may pose other problems; sodium dodecyl sulfate (SDS) is known to inhibit dissolution of hard gelatin capsules under low-pH conditions [11]. We encountered such a situation when developing a dissolution method for overencapsulated erlotinib tablets. In this case, the OET was made using native erlotinib tablets encapsulated in a HGC shell with filler.

Erlotinib was approved by the U.S. Food and Drug Administration in November 2004 for the treatment of non-small cell lung cancer [12], and an erlotinib tablet formulation has been available on the market for some years under the trade name of Tarceva. There is little published literature on the analysis of erlotinib formulations. A recent report described a validated stability-indicating method for erlotinib and its degradants in bulk powder and tablets [13]; the long runtime of the method was not optimized for use in dissolution testing. Dissolution of different crystalline forms of pure erlotinib has been described in the literature [14]. Dissolution information on the formulated product is available via the FDA Dissolution Database [3], and this method was used as a basis for our studies, with 0.1 N HCl and 1% SDS as medium. Comparable profiles were initially generated using this method for the native and the OET during formulation development and a 2-week lab stability study. The dissolution specification was established based on these profiles. During a formal stability study, the dissolution result from initial time point met the specification however, after four weeks at 40 °C/75% relative humidity (RH) the overencapsulated tablet showed a significant dissolution slowdown, which was attributed to cross-linking of the gelatin shell under the stress conditions. It was not possible to conduct a tier-2 test according to USP, since the activity of the enzyme would be affected by the SDS.

In this paper, we describe development of a two-step tier-2 method to allow characterization of the dissolution of the overencapsulated erlotinib tablets for use as a comparator. The first stage of dissolution was started in 0.1 N HCl with pepsin. In the second stage, SDS was added to enhance erlotinib solubility after the cross-linked HGC shell disintegrated.

UV fiber optic (UVFO) spectrophotometry was used in this study to determine the dissolution profiles during the method development. In comparison with traditional dissolution finishes, the UVFO methodology has many advantages, such as in situ measurement, no sampling and filtering, less operational errors, real-time calculation of results, measurement of more time points, and more accurate determination of the dissolution profile [15–18]. In this study, the high temporal resolution of the in situ UVFO measurement was particularly important for observing the different dissolution events and determining the optimal starting time of step 2 for the tier-2 method. The validation of the proposed method was also carried out.

2. Materials and methods

2.1. Chemicals

The following materials were used: hydrochloric acid (J. T. Baker, Phillipsburg, NJ, USA, Baker Analyzed Reagent), sodium dodecyl sulfate (SDS, J. T. Baker, Ultrapure-Bioreagent), pepsin (Sigma–Aldrich, St. Louis, MO, USA, Premium, purity 92%, enzyme activity 3260 units/mg), acetonitrile (EMD Chemical Inc. Gibbstown, NJ, USA, HPLC grade), and trifluoroacetic acid (TFA, J. T. Baker Phillipsburg, NJ, USA, HPLC grade). Water was obtained from in-house Milli-Q water purification system (Millipore, Danvers, MA, USA, Ultra-Pure).

2.2. Samples

Erlotinib hydrochloride tablets, 25 mg/tablet (Tarceva®, OSI Pharmaceuticals, Inc., Melville, NY, USA), were purchased on the open market. An overencapsulated formulation, two tablets/capsule, was prepared in-house using the native tablets with 286 mg backfill (microcrystalline cellulose 99.75% and magnesium stearate 0.25%), in hard gelatin capsule shells (#0, gray opaque, Capsugel, Peapack, NJ, USA). Stability samples of both the native and overencapsulated tablets were stored at 40 °C/75%RH in closed bottles for four weeks. Stressed overencapsulated tablets were stored at 40 °C in an open dish under very high humidity (in a covered 2-L beaker, containing a 100-mL beaker filled with ~70 mL of water to increase the humidity) for one week. The stressed samples were used for method development.

2.3. Reference standard

Reference standard solution was made by dissolving one native erlotinib tablet (25 mg) in 500 mL of 0.1 N HCl containing 1% (w/v) SDS, to make a working concentration of 0.05 mg/mL.

2.4. Dissolution method conditions

The conditions used for dissolution of the native tablet were USP Apparatus II, at 75 rpm, with 1000 mL of 0.1 N HCl media containing 1% (w/v) SDS, at 37 °C [3]. Sampling time points were 15, 30, 45, and 60 min. The same method was applied to the overencapsulated tablet samples.

2.5. Dissolution set-up

An Evolution 6100 bathless dissolution system (Distek, North Brunswick, NJ, USA) was used. The media was degassed by purging with helium for 15 min before adding SDS. Three-prong magnetic sinkers (Vankel #12-3055, Cary, NC, USA) were employed for both native and overencapsulated tablet samples. PVDF 0.45 μm filters were used for sample filtration. The method was validated before use.

2.6. HPLC finish

An HPLC method was developed and validated for the determination of the concentration of the active drug in the dissolution samples from formal release and stability testing. This method used a Waters X-Bridge C18 column, 150 mm × 4.6 mm, 3.5 μm, at 35 °C. The mobile phase was 55% A (0.1% TFA in water) and 45% B (0.1% TFA in acetonitrile) for an isocratic run with a flow rate of 1.8 mL/min. The injection volume was 10 μL. UV wavelength for detection was 345 nm. The run time was 2.5 min. The retention time of erlotinib was 1.6 min.

2.7. UV fiber optic method

A UV fiber optic spectrometer (Opt-Diss, Leap Technologies, Inc., Cary, NC, USA) was used for in situ determination of the concentration of erlotinib during development of the two-tier method. Six channels of the spectrometer were employed and connected to six Arch probes (Leap Technologies, Inc.). The pathlength of the probes

was 10 mm. The detection wavelength was 350 nm with a two-point baseline correction at 305 and 390 nm. The exposure time was 50 ms. The measurement was made at a rate of 1/min, and the number of scans was three for each data point.

2.8. Method validation procedure

The two-tier 2-step dissolution method was validated with respect to the parameters of specificity, accuracy, precision, linearity and range.

2.8.1. Specificity

The specificity was assessed by measuring the spectra of the following media and samples using the UVFO methodology: (1) compendial dissolution medium (1% SDS in 0.1 N HCl), (2) two-tier step 1 medium (pepsin in 0.1 N HCl), (3) reference standard solution (0.05 mg/mL erlotinib in 1% SDS/0.1 N HCl), and (4) OET sample solution (0.05 mg/mL erlotinib in 1% SDS/0.1 N HCl) containing the backfill and HGC shell. The UV detection wavelength was determined based on the wavelength of maximum absorption of erlotinib without any interference.

2.8.2. Accuracy and precision

Two procedures were performed to determine the method accuracy and precision: (1) perform a dissolution run on six individual OET samples and calculate RSD of the % drug release at three time points: (i) the end of the step 1 (at 15 min, before the SDS was added); (ii) the end of step 2 (at 60 min); and (iii) the infinity point (at 90 min, after increasing the paddle speed to 200 rpm for 30 min). (2) Measure the erlotinib content six times from the same vessel after completing the dissolution test, and calculate the RSD.

2.8.3. Linearity and range

A linearity evaluation was conducted over seven concentration levels. Standards were prepared by diluting a reference standard solution to give solutions over the range of 10–150% of the target concentration. These diluted solutions were measured by UVFO in triplicate.

3. Results and discussion

3.1. Effect of accelerated conditions on dissolution of OET stability samples

The OET formulation was developed based on its similar dissolution profile in comparison with the innovator's native tablet, as determined using the dissolution method described above. The dissolution specification was set up based on the profiles from the development samples: the absolute difference between the average percent dissolved of the OET and the native tablet at 30, 45, and 60 min should be ≤10%.

A stability study was conducted to further assess the comparability of the in vitro behavior of the OET and native tablet. The samples were stored for various times under two conditions: 25 °C/60%RH (intended storage conditions; 4 weeks, 3 months, 6 months, and 12 months) and 40 °C/75%RH (4 weeks, and 3 months). Samples were tested initially and at each time point for appearance, assay/degradants, and dissolution. The dissolution results tested manually using the compendial dissolution method with the HPLC finish (see Section 2.6) showed that the OET and the native tablet samples at the initial time point and those stored under 25 °C/60%RH up to 4 weeks gave comparable dissolution profiles. However, after storage under 40 °C/75%RH conditions for 4 weeks, the absolute differences of the % dissolved at 30 min between the OET and the native tablet exceeded 10%. While there was no significant change in the dissolution profile of the native tablet, the OET displayed a significant slow-down in dissolution (Table 1). It was observed during the dissolution of the OET 40 °C/75%RH samples that two out of six units from the 4-week time point were not fully disintegrated at 15 min and some of them were still in lump form and covered by a gel-like film at 45 min. The slowdown of dissolution for the stressed OET samples was thus attributed to the poor dissolution of the gelatin shell, presumably due to cross-linking driven by the high temperature and humidity storage conditions [5].

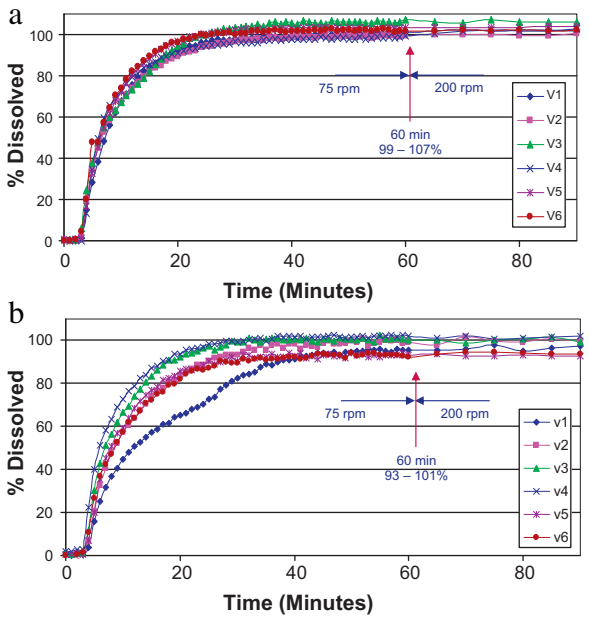

Fig. 1. Dissolution results for erlotinib over-encapsulated samples, using the compendial dissolution conditions and a UV-FO finish. (a) Fresh OET samples; (b) stressed OET samples. Note the initial opening of all the capsules occurs at approximately 4 min. However, the rate of the stressed OET dissolution is quite variable thereafter, due to inconsistent complete breakup of the capsule shells.

3.2. Stressed overencapsulated tablets as a model system

In order to develop a method to overcome the cross linking problem, appropriate samples were needed. Since samples from the formal stability study were limited, the possibility of using a model system with rapidly stressed overencapsulated tablets was investigated. 20 OET 50 mg/capsules were prepared and stressed at 40 °C in an open dish at 80% humidity for one week. Dissolution testing was performed for the stressed OET samples and freshly prepared OET samples using UVFO in situ measurements. Results demonstrated that the dissolution of six fresh OET samples is fast and the profiles are smooth and very alike (Fig. 1(a)). However, the stressed samples behaved very similarly to the 4W 40 °C/75%RH stability samples in exhibiting a slowdown of dissolution. Fig. 1(b) shows the dissolution profile of six stressed OET samples, in which the dissolution profiles are variable, and in particular the dissolution of the sample in vessel 1 slowed down significantly. It was observed during the testing that the capsule in this vessel started to break at the jointed end of the sinker at around 3 min. The shell then formed a tube held by the sinker with one open end at which the filler and tablet were exposed. UVFO showed that the drug was only ~4% dissolved at 4 min. A part of the shell started to deform at 5 min and turned into stringy gel-like material spinning around sinker. The solution became cloudy, and the dissolved drug rose to ~15%. The remaining capsule dissolved very slowly thereafter. Pieces of the shell still covered some of the drug and filler on the sinker even at ~35 min when much smaller pieces of

Table 1
Tier-1 dissolution results from the stability study generated by using the compendial dissolution method with HPLC finish.

Sample (6/set)	Time (min)	15	30	45	60
Native tablets, initial	AVG	97	98	99	99
	SD	1.2	0.8	1.0	1.0
Over-encapsulated tablets, initial	AVG	80.0	94	96	99
	SD	10.2	7.2	4.2	2.2
Native tablets, 40 °C/75%RH, 4W	AVG	95	97	98	98
	SD	0.9	0.6	0.5	0.7
Over-encapsulated tablets, 40 °C/75%RH, 4W	AVG	71	85*	91	94
	SD	22.7	19.5	11.4	5.5

* A significant dissolution slowdown was observed for the OET samples stored at 40 °C/75%RH for 4W. The absolute differences of the % dissolved at 30 min between the OET and the native tablet exceeded 10%.

remaining shell and higher drug release was observed in the other vessels.

3.3. Development of a two-step USP tier-2 method

Dissolution slowdown due to gelatin capsule cross-linking is a recognized phenomenon [4,5], and is typically addressed using a tier-2 method [8] where an enzyme such as pancreatin or pepsin is added to dissolve the cross-linked shell. However in the current case, the compendial method is not well suited to enzyme addition due to the presence of SDS which is an effective denaturing agent [10].

3.3.1. Effect of SDS in the dissolution of erlotinib

In order to develop an approach to perform measurement without interference from cross-linking, the effect of SDS in the compendial method was first studied; if SDS could be eliminated, a traditional tier-2 method could be employed. Native erlotinib tablets were tested for dissolution in 0.1 N HCl without and with SDS. During the first run, two 25-mg native tablets were placed in 1000 mL of 0.1 N HCl with a three-prong magnetic sinker. As displayed in Fig. 2(a), the native tablet dissolution was very slow without surfactant, with only 54.9% dissolved after 60 min. Thus, removal of SDS from the medium was not a suitable approach in this case.

After the first run, 100 mL of the dissolution solution was removed from the top level in the vessel. Another run was started with the samples still in the vessels, using the 900 mL remaining solution. 100 mL of 10% SDS in 0.1 N HCl was added 5 min after the run was restarted. Thus, from 5 min onwards, the media composition was that specified in the compendial method. The result was that the dissolution reached a plateau within the next 15 min. This plateau remained at the same level through 60 min under normal conditions and after additional 30 min infinity test with 200 rpm paddle speed (Fig. 2(b)), which indicated that the dissolution of the native tablets was complete. These experiments indicated that SDS plays an important role in solubilizing erlotinib, and that delayed addition of SDS solution could still result in complete dissolution.

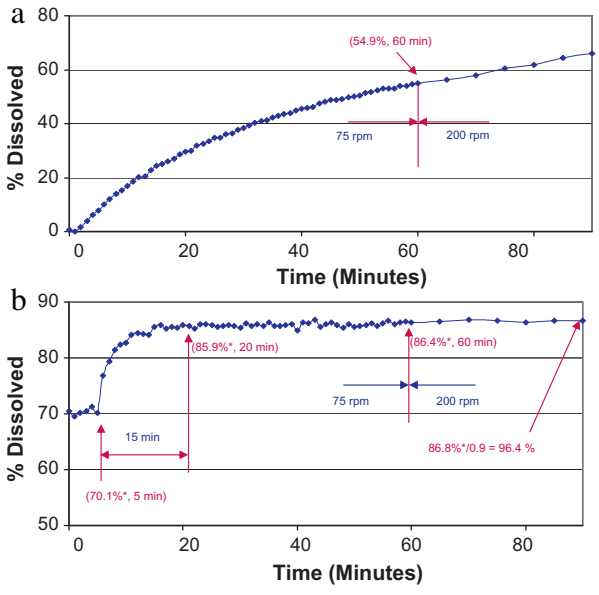

Fig. 2. Dissolution of native erlotinib tablets: (a) in 0.1 N HCl, 1000 mL; (b) in 900 mL of bottom solution from (a) with addition of 100 mL 10% SDS in water at 5 min.

3.3.2. Effect of SDS on the dissolution of HGC shells

It has been reported in the literature that SDS significantly slows down the dissolution of gelatin shells at pH < 5 because of the formation of a less-soluble product [11]. To confirm whether the slow dissolution of the erlotinib OET could be partially attributed to effects on the shell by SDS in the medium, another test was conducted with the HGC shell only without the native tablets and filler; 0.1 N HCl was used as the medium with and without 1% SDS for comparison. Observations from this testing are displayed in Table 2. The HGC shell dissolved within 13 min in 0.1 N HCl without SDS. However, some capsule residue was still visible after 60 min in 0.1 N HCl with 1% SDS. The observations in this study are in agreement with the literature [11].

Considering the SDS effect on both the HGC shell dissolution and the enzyme activity and thus its ability to disrupt gelatin cross linking, the question may be raised as to why the surfactant was not changed. According to the literature, compared to SDS, Tween 80 showed far better disintegration and solubility results for HGC in dissolution media with neutral or low pH [10]. Although chang-

Table 2
Observations during HGC shell dissolution with or without SDS.

Time (min)	Shell in 0.1 N HCl	Shell in 0.1 N HCl + 1% SDS
0:40	Capsule cracked on one end	Capsule cracked on one end
2:00	Two ends of the shell dissolved	Two ends open, shell is sticky, gel-like, dissolves very little
3:00	Shell body area dissolving fast	Shell is sticky, gel-like, dissolves very little
4:00	~20% of the shell visible	~90% shell is sticky, gel-like, dissolves very slowly
10:00	Trace left on bottom	~More than 50% shell left, shell is sticky, gel-like, dissolves very slowly, fine silk-like material floating around
13:00	Dissolved	~<50% shell left, shell is sticky, gel-like, dissolves very slowly, fine silk-like material floating around
45:00	–	>95% dissolved, some left on the vessel bottom and some on the sinker
60:00	–	Very little shell residue visible

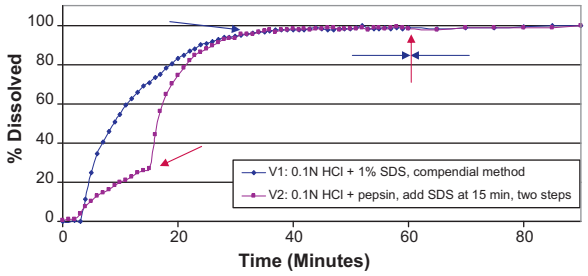

Fig. 3. Comparison of dissolution profiles of OETs with: vessel 1, single-step tier-1 testing: vessel 2, two-step tier-2 testing. Note that in this case, the OET in single-step tier-1 testing dissolved relatively rapidly, however, as shown in Fig. 2 dissolution under these conditions is quite variable.

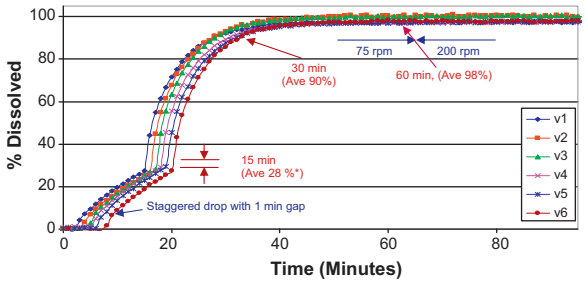

Fig. 4. Dissolution profiles of stressed OET samples using the finalized two-step tier-2 dissolution method.

ing the surfactant is a reasonable choice if a method is developed by an innovator for their original HGC formulations, this not such a useful approach for development of a dissolution method for a comparator OET formulation because of two reasons: (1) it is preferable to follow the available compendial method closely to avoid concerns as to whether the developed method adequately probes dissolution differences between the native and over-encapsulated formulations. (2) On a practical note, project timelines usually do not allow for extensive development work for comparators. For instance, this tier-2 method development was initiated when cross linking occurred with the 4W stability sample, and the method has to be in place when the 3M stability sample arrived. Therefore, quick development of a tier-2 method that could overcome the SDS effect and gelatin cross linking was the focus, and the two-step approach was considered an acceptable solution.

3.3.3. *Investigation of method parameters in a two-step tier-2 approach*

To overcome the SDS effect on the activity of the enzyme, the tier-2 dissolution method can be designed in two steps: (1) the dissolution starts in the medium with enzyme but no surfactant; (2) SDS is added after the cross-linked HGC shell is disintegrated. Although we know of no papers in the primary literature describing the development of such a method, three records were found in the FDA Dissolution Database describing two-step tier-2 procedures [3]. These are for the dissolution of capsule formulations of Celecoxib, Dutasteride, and Ziprasidone HCl. Each of these methods used different volumes of medium for steps 1 and 2. The time to switch to step 2 was also different for each case. During method development for the OET formulation in this study, the two steps were designed as follows: step 1 starts with 900 mL of pepsin in 0.1 N HCl for 15 min. The amount of pepsin is ≤750,000 units/1000 mL, according to USP. Pepsin is added to the 0.1 N HCl 30 min before testing starts to ensure adequate enzyme activity. Step 2 starts at 15 min by adding 100 mL of 10% SDS in 0.1 N HCl to the step 1 solution.

The dissolution profile of an OET sample (tested in vessel 2) using the two-step procedure is displayed in Fig. 3. In comparison with a profile from the single-step tier-1 method (tested in vessel 1), the two-step tier-2 profile detected by using UVFO in situ measurement displayed a unique shape with two lobes. The first lobe represents the first step wherein the use of pepsin made the capsule shell open and dissolve quickly, but the drug was released very slowly, only 21.7% dissolution at 15 min prior to the addition of SDS. The second lobe represents the second step wherein the drug release was quickly increased after SDS addition and reached to 94.5% at 30 min. Considering that the drug release of the unstressed native erlotinib tablet at 30 min was 98% (Table 1), the vessel 2 results from the two-step tier-2 approach could easily pass the specification which requires that the absolute difference between % dissolved of the OET and the native tablet is ≤10%. Note that in Fig. 3, the OET sample in vessel 1 did not display a significant slowdown (as seen in Fig. 1(b), the capsule-to-capsule variability is quite high). Fig. 3 also illustrates the particular utility of the UVFO measurement approach in these studies, since frequent data reporting (one point per min) results in the dissolution curves being very clearly defined.

Visual observations of the OET dissolution in the two-step tier-2 and single-step tier-1 testing confirmed the differences between the dissolution processes. Pictures were taken during the dissolution of the OET samples in vessel 1 and vessel 2 above. These are provided as supplemental material, Figs. S1 and S2. The visual observations confirm the relatively slow opening of the shell in the single-step tier-1 testing.

3.4. *Optimization of the two-step tier-2 method*

A number of operational procedures needed to be carefully defined because they may have a significant impact on the two-step tier-2 dissolution testing. The first is pre-heating of the solution of 10% SDS in 0.1 N HCl. It was observed during the first two-step tier-2 testing, when the 10% SDS solution prepared at room temperature (~22 °C) was added into the dissolution vessel, the medium temperature dropped from 37.1 °C to ~32 °C. The temperature only reached to 36.2 °C at 60 min, which was not in compliance with USP requirements. Therefore, it is necessary to pre-heat the concentrated SDS solution to 37 °C before addition of the surfactant-containing medium. Another issue is timing of the drop of the HGC samples. Because transferring exactly 100 mL of the pre-heated SDS solution into 6 vessels is not simple, and takes time, a staggered drop was chosen. A staggered drop of the capsule to each vessel at the beginning of the tier-2 testing gives time for the solution addition and allows operational consistency over the six vessels.

The dissolution profiles of six stressed HGC samples obtained by using the finalized two-step tier-2 method detected by the in situ UVFO instrumentation are shown in Fig. 4. Capsules were dropped with a 1 min staggering time into 900 mL of medium containing 0.1 N HCl and 207 mg pepsin in each vessel. At 15 min, the average drug release of the six vessels was 28%, when 100 mL of pre-heated 10% SDS in 0.1 N HCl was added into each vessel. At 30 min, the average drug release of the six vessels was 90%. These profiles reached a plateau of 98% on average at 45 min, which was maintained up to 90 min including 30 min infinity testing. These profiles from 30 min onwards are very comparable with those of the native tablets.

3.5. *Validation of the two-step tier-2 method*

According to USP, the validation elements addressed for a dissolution method depend on the intended use of the data [19]. The ICH Harmonized Tripartite Guideline requires a number of parameters to be evaluated in the validation of a dissolution method, including

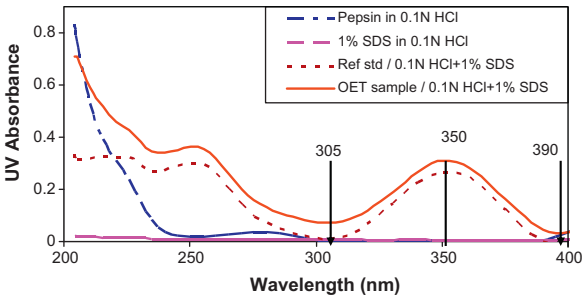

Fig. 5. UV spectra of the media, reference standard, and OET sample. Measurement at 350 nm provides a good signal due to the drug with minimal interference from the medium.

accuracy, precision, specificity, linearity and range [20]. In the case of the validation for a two-step tier-2 dissolution method of a comparator OET using UVFO in situ detection, it was decided to focus on the following.

3.5.1. Specificity

Method specificity was evaluated by measuring the spectra of the dissolution media: 1% SDS in 0.1 N HCl and pepsin in 0.1 N HCl, reference standard solution, and the OET sample solution containing the backfill and HGC shell. The spectra showed that erlotinib has a UV maximum at 350 nm. In addition, none of the other components interfere with the erlotinib measurement (Fig. 5). Therefore, 350 nm was chosen as the measurement wavelength. It was also noted that the baseline of the UV spectrum of the OET sample solution shifted upwards. This was caused by the turbidity of the solution due to undissolved particulates from the backfill and the other excipients in the native tablets. A two-point baseline correction methodology using 305 and 390 nm was selected based on the characteristics of the UV spectra. This baseline correction was compared with a number of other correction methodologies, including single-point, 2nd derivative, and two-point using the other wavelengths. The 305/390 nm methodology was determined to be most effective and robust in overcoming the spectral baseline shift for this method.

3.5.2. Accuracy and precision

Because of the lack of a true reference standard in development and validation of this comparator test method, a spiking test for assessment of the method accuracy was performed by using the OET samples. The accuracy was demonstrated in this case by the % drug release of six OET samples. At the end of step 1, the average % released compared to label claim was 27.95, RSD = 3.93%. At the end of step 2, the average % released compared to label claim is 98.24, RSD = 1.26%. At the infinity point, the average % released compared to label claim is 98.80, RSD = 1.30%.

The UVFO instrument employs six channels and probes for the dissolution measurement in six vessels. Each channel should be treated as an individual spectrophotometer [12]. Therefore, the precision of the method was demonstrated by determining the variability of six measurements from each channel/vessel at the end of a dissolution test. The RSDs for the six channels/vessels ranged from 0.24% to 0.55%, an acceptably tight precision for the purpose of this method.

3.5.3. Linearity and range

The linear regression for the UV absorbance of seven levels of reference standard solution at 10, 25, 50, 75, 100, 125, and 150% of the target concentration showed an excellent correlation coefficient (R^2 = 0.9999). The *y*-intercept was 0.37% of the target concentration. The range of the UV absorbance was from 0.0230 AU to 0.3307 AU for the assessed reference standard solutions which ranged in concentration from 0.005 to 0.075 mg/mL, adequate for quantitation of the % drug release through the entire dissolution.

The above validation results demonstrated that this method is suitable for its intended use. Some parameters related to ruggedness of the method were not evaluated in this study because they had been validated for the original manual method and are not unique or not required for UVFO measurement, including medium degassing, compatibility of filter, and stability of the reference standard solution and dissolution sample solution.

4. Conclusions

Dissolution of comparator drugs over-encapsulated in hard gelatin shells can be challenging because of HGC cross-linking. The use of SDS in the dissolution medium of the compendial method further complicates the situation. Simply adding pepsin or pancreatin to the dissolution medium may not resolve the cross-linking problem due to the interference of SDS with enzyme activity. Changing the method by using different surfactants may not be acceptable because it is preferable to follow the compendial method for comparators closely. A two-step tier-2 method as developed in this study is the first described in the literature for the dissolution of a comparator drug with an OET formulation. It serves as a practical solution, by separately adding the surfactant and enzyme without significant change to the compendial method conditions. This method effectively resolved the problems encountered in use of the compendial method for the dissolution of erlotinib OET formulation. In this study, the testing results from the 30, 45, and 60 min time points in this two-step method could be used for evaluation of the overencapsulated comparator against the acceptance criteria.

UVFO has been demonstrated to be a particularly effective tool in dissolution method development and investigations. In this case, the excellent time resolution was critical in determining the optimal time of the surfactant addition. In formal studies of overencapsulated erlotinib, an HPLC finish was used to accommodate the capabilities of a contract lab. However, UVFO is a preferred approach for in-house dissolution testing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.04.026.

References

[1] R.G. Myers, C.M. Cratty, Blinding clinical supplies utilizing overencapsulation, Pharm. Eng. 23 (2003) 1–5.
[2] K.C. Huynh-Ba, A.-F. Aubry, Analytical development strategies for comparator products, Am. Pharm. Rev. 6 (2003) 92–97.
[3] US FDA Dissolution Database, www.fda.gov/Drugs/InformationOnDrugs/ucm-135742.htm, records since 2004.
[4] G.A. Digenis, T.B. Gold, V.P. Shah, Cross-linking of gelatin capsules and its relevance to their in vitro—in vivo performance, J. Pharm. Sci. 83 (1994) 915–921.
[5] S. Singh, K.V.R. Rao, K. Venugopal, R. Manikandan, Alteration in dissolution characteristics of gelatin-containing formulations, Pharm. Technol. 26 (2002) 36–58.
[6] J. Brown, N. Madit, E.T. Cole, I.R. Wilding, D. Cade, The effect of cross-linking on the in vivo disintegration of hard gelatin capsules, Pharm. Technol. 15 (1998) 1026–1030.
[7] G.A. Digenis, E.P. Sandefer, R.C. Page, W.J. Doll, T.B. Gold, N.B. Darwazeh, Bioequivalence study of stressed and nonstressed hard gelatin capsules using amoxicillin as a drug marker and gamma scintigraphy to confirm time and GI location of in vivo capsule rupture, Pharm. Technol. 17 (2000) 572–582.

[8] The United States Pharmacopeia, Dissolution, General Chapter<711>, USP 32-NF 27, USP, Rockville, MD, USA, 2009, p. 263.
[9] H. Marchais, G. Cayzeele, J.-Y. Legendre, M. Skiba, P. Arnaud, Cross-linking of hard gelatin carbamazepine capsules: effect of dissolution conditions on in vitro drug release, Eur. J. Pharm. Sci. 19 (2003) 129–132.
[10] F.H. Pennings, B.L.S. Kwee, H. Vromans, Influence of enzymes and surfactants on the disintegration behavior of cross-linked hard gelatin capsules during dissolution, Drug Dev. Ind. Pharm. 32 (2006) 33–37.
[11] F. Zhao, V. Malayev, V. Rao, M. Hussain, Effect of sodium lauryl sulfate in dissolution media on dissolution of hard gelatin capsule shells, Pharm. Res. 21 (2004) 144–148.
[12] M.H. Cohen, J.R. Jonson, Y.F. Chen, R. Sridhar, R. Pazdur, FDA drug approval summary: erlotinib (Tarceva®) tablets, Oncologist 10 (2005) 461–466.
[13] S.S. Pujeri, A.M.A. Khader, J. Seetharamappa, Validated stability-indicating chromatographic method for the assay of erlotinib active pharmaceutical ingredient, Anal. Lett. 42 (2009) 1855–1867.
[14] Y.-C. Tien, C.-S. Su, L.-H. Lien, Y.-P. Chen, Recrystallization of erlotinib hydrochloride and fulvestrant using supercritical antisolvent process, J. Supercrit. Fluid 55 (2010) 292–299.
[15] X. Lu, R. Lozano, P. Shah, In situ dissolution testing using different UV fiber optic probes and instruments, Dis. Technol. 10 (2003) 6–15.
[16] K.H. Wiberg, U.-K. Hultin, Multivariate chemometric approach to fiber-optic dissolution testing, Anal. Chem. 78 (2006) 5076–5085.
[17] V. Bijlani, D. Younayel, S. Katpally, B.N. Chukwumezie, M.C. Adeyeye, Monitoring ibuprofen release from multiparticulates: in situ fiber-optic technique versus the HPLC method: a technical note, AAPS PharmSciTech 8 (2007) E9–E12, doi:10.1208/pt0803052.
[18] T. Mirza, Q. Liu, R. Vivilecchia, Y. Joshi, Comprehensive validation scheme for in situ fiber optic dissolution method for pharmaceutical drug product testing, J. Pharm. Sci. 98 (2009) 1086–1094.
[19] The United States Pharmacopeia, The Dissolution Procedure: Development and Validation, General Chapter<1092>, USP 32-NF 27, USP, Rockville, MD, USA, 2009, p. 599.
[20] International Conference on Harmonization, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Harmonized Tripartite Guideline Q2A, 27, October, 1994, p. 3.