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STABILITY-INDICATING CAPILLARY ELECTROPHORESIS METHOD WITH PHOTODIODE ARRAY DETECTOR FOR DETERMINATION OF LENALIDOMIDE IN PHARMACEUTICAL PREPARATION

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This study describes, for the first time, the development and validation of a simple, sensitive and accurate stability-indicating capillary electrophoresis method with photodiode array detector for the determination of lenalidomide (LEN) in its bulk form and pharmaceutical preparation. Metoclopramide was used as the internal standard. Electrophoretic separation was achieved in a deactivated fused silica capillary (52 cm effective length × 75 μm internal diameter) maintained at 22 °C, by a background electrolyte solution consisting of phosphate buffer solution (20 mM, pH 7.1):methanol (90:10, v/v). The samples were injected by pressure at the anodic side at 20 mbar for 25 seconds, and the separation voltage was 30 kV. The detection wavelength was set at 210 nm. LEN was subjected to different accelerated stress conditions. The degradation products, if any, were well resolved from the intact drug with significantly different migration time values. The limit of detection and limit of quantitation were 0.25 and 0.80 μg mL^{-1}, respectively. The intra- and inter-assay precisions were satisfactory; the relative standard deviations did not exceed 0.59%. The accuracy of the method was proved; the mean recovery of LEN was 98.53 – 100.80 (± 0.25 – 0.59%). The proposed method was successfully applied for the determination of LEN in bulk and capsules; the label claim percentage was 99.52 ± 0.43%. The results demonstrated that the method would have a great value when applied in quality control and stability studies for LEN.

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Keywords: Lenalidomide; Capillary electrophoresis, Photodiode array detector; Stability-indicating; Quality control; Pharmaceutical analysis.

1. Introduction

Multiple myeloma (MM) is a B-cell malignancy characterized by proliferation of monotypic plasma cells. It is the second most common hematological malignancy; approximately 20,000 cases of MM have been diagnosed in 2007 [1]. MM is a fatal disease with most patients relapsing after an initial response to the conventional chemotherapy. In the 1990s, thalidomide (Thalomid®, Celgene Corporation) was used empirically in treatment of MM based on its antiangiogenic activity and clinical activity in refractory or relapsed myeloma [2]. However, thalidomide has significant and dose-limiting somnolence, constipation, neuropathy, and teratogenicity [3]. These toxic effects promoted the search for more potent but less toxic thalidomide derivatives [4].

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Lenalidomide (LEN, Fig. 1) is a potent novel thalidomide analog which demonstrated remarkable clinical activity against myeloma cells [5-9] via a multiple-pathways mechanism [4,10-15]. As well, LEN has a more improved side effects profile than its parent compound thalidomide. The strong evidences-based clinical success of LEN in patients has led to its recent approval by US-FDA under the trade name of Revlimid® capsules by Celgene Corporation [16].

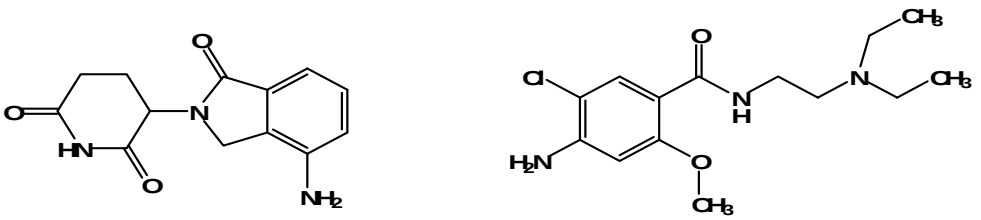

Fig. 1. Chemical structures of lenalidomide (LEN) and the internal standard metoclopramide (MET).

The quality of pharmaceutical product of LEN, in terms of the purity and stability of the active substance and/or finished product is vital for the effective and safe delivery of its therapeutic values to the patient. This is because the presence of impurities and/or potential degradation products may cause changing of chemical, pharmacological, and/or toxicological properties of the active drug entity [17-19]. In general, pharmaceuticals are sensitive to environmental factors such as temperature, humidity, and light. These factors usually vary during manufacturing, transportation, storage, and distribution of the finished product. For these reasons, stability testing of the active substance and the finished product is necessary for providing information about potential degradation products, possible degradation pathways of the drug, compatibility of the drug with the excipients in the finished product, and the long-term effects of the environmental factors on the active drug and its finished products. Results of stability testing are important in developing proper manufacturing process, selecting proper packaging, storage conditions, product's shelf life, and determining the expiration date [20-22]. Furthermore, the climate is widely varying worldwide, and consequently, the quality of pharmaceutical product is greatly affected by the change in the environmental factors. LEN-containing capsules (Revlimid® capsules) are imported from outside of many countries, consequently there is a major concern about its quality after exposure to transportation, storage, and other conditions. Therefore, quality control for LEN-containing capsules in these countries is critically concerned, and the development of stability-indicating methods was very essential, particularly, such methods have become an important aspect of any analytical method validation and a part of International Conference of Harmonization (ICH) guidance for pharmaceutical industry [22].

Extensive literature survey showed that there was only one report describing two spectrophotometric methods for the quantitation of LEN in its pharmaceutical formulations [23], however, these methods were not stability-indicating assays. Saravanan *et al.* [24] have developed a reversed phase liquid chromatographic (RP-LC) method with UV detection at a single-wavelength (245 nm) for determination of LEN in presence of process-related impurities. Therefore, this method was not adequately informative when applied in the stability testing of LEN. For these reasons, an alternative stability-indicating technique is essential for determination of LEN in its bulk drug and Revlimid® capsules. Capillary electrophoresis (CE), since it is based on different separation principles and consequently results in a unique selectivity compared to RP-LC, it is more advantageous when applied as a stability-indicating technique. Nevertheless, RP-LC is still a dominant technique in pharmaceutical analysis, the extensive use of CE will generate alternative and complementary methods. On the other hand, the main active component and structurally related impurities and degradants in pharmaceutical formulations have similar

chemical properties and thus their separation becomes difficult. However, the greater separation efficiency of CE makes this separation possible [25]. Furthermore, CE equipped with photodiode array detector (PDA) provides even more information.

The present study describes, for the first time, the development and validation of a stability-indicating CE-PDA method for stability evaluation and quantitative determination of LEN in the presence of its potential degradation products.

2. Experimental

Materials

Lenalidomide (LEN) was purchased from LC Laboratories (Woburn, MA, USA). Revlimid® capsules (Celgene Corporation, New Jersy, USA) labeled to contain 5 mg LEN per capsule was obtained from the local market. Metoclopramide (MET, Fig. 1), as hydrochloride salt was obtained from Sigma Chemical Co. (St. Louis, CA, USA). A deactivated fused silica capillary was obtained from Agilent Technologies (Böblingen, Germany). HPLC-grade methanol, and reagent-grade sodium hydroxide, hydrochloric acid, and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Deionized water and Millipore membrane filter (0.2 μm) from Nihon, Millipore was used throughout the experiments.

Electrophoretic instrumentation and conditions

The employed CE system consisted of an Agilent capillary electrophoresis instrument (Agilent Technologies, Germany) equipped with PDA detector and a data handling system comprised of an HP computer and Agilent Chem station software provided with the instrument. Detection was performed at 210 nm. A deactivated fused silica capillary was obtained from Agilent Technologies and had the following dimensions: 61 cm total length, 52 cm effective length, and 75 μm internal diameter. The temperature of the capillary and the samples was maintained at 22 °C. The background electrolyte solution (BGE) consisted of phosphate buffer (20 mM, pH 7.1):methanol (90:10, v/v). Samples were injected into the capillary by pressure at the anodic side at 20 mbar for 25 seconds. The electrophoresis was carried out by applying a voltage of 30 kV to the capillary, with the cathode being at the detector end. The capillary was washed between run with deionized water for 2 min, then equilibrated with the running buffer for another 2 min; this was to ensure reproducibility of the analysis. The solutions were filtered through a 0.2 μm Millipore membrane filter (Millipore, Nihon, Japan) before injection.

Preparation of standard solutions and constructing the calibration curve

A stock solution (1 mg mL^{-1}) of LEN was prepared by dissolving an accurately weighed quantity (25 mg) of LEN reference standard material in 25 mL methanol. A stock solution (1 mg mL^{-1}) of the internal standard (IS) metoclopramide (MET) was prepared in water by dissolving an accurately weighed quantity (25 mg) of MET in 25 mL water. This stock solution was further diluted with water to obtain a working solution of 0.3 mg mL^{-1}. Aliquots of the standard stock LEN solution were transferred into 10-mL volumetric flasks, 1 mL of IS working solution was added to each flask, and the solutions were complete to the mark with the BGE to yield final LEN concentrations of 1, 5, 10, 20, 30, and 50 μg mL^{-1}. Triplicate injections of each concentration were performed. The peak-area ratio of each LEN concentration to that of the IS was plotted as a function of the corresponding standard LEN concentration to construct the calibration curve, and the corresponding regression equation was derived.

Preparation of capsules solution

The contents of 10 Revlimid® capsules (Celgene Corporation, NJ, USA), labeled to contain 5 mg of LEN per capsule were evacuated and weighed. An accurately weighed portion equivalent to 5 mg LEN was transferred into 10-mL volumetric flasks containing 5 mL methanol. The solutions were stirred and sonicated for 20 min, then made up to volume with methanol. The completed solutions were mixed well, and centrifuged at 3000 rpm for 10 min. Aliquot of the capsule solution was transferred to 10-mL volumetric flasks, 1 mL of the IS working solution was

added, and the volume was completed with the BGE. This solution containing 25 μg $mL^{-1}$for LEN was subjected to the analysis by the proposed CE-PDA method.

Forced degradation studies

Forced degradation studies of LEN in its bulk drug and capsules including appropriate solid and solution states were carried accordance to the ICH regulatory guidance [26,27] of stress testing.

Acid and alkali hydrolysis. Aliquot of 0.5 mL of LEN solution (1 mg mL^{-1}) was transferred into a 10-mL volumetric flask. The solution was mixed with 1 mL of 0.5 N hydrochloric acid, or 0.5 N sodium hydroxide. The prepared solutions were left at room temperature for 48 hours and 10 min in case of acid and alkali hydrolysis, respectively. Blank samples were prepared and treated similarly with hydrolytic reagent, however the samples were left the same periods in the dark in order to exclude the possible effects of light on degradation. Samples were withdrawn at an appropriate time, neutralized, and diluted with the BGE to obtain the predicted concentration of non-stressed LEN. The samples were subjected to the analysis by the proposed CE-PDA method. Several control samples were prepared and analyzed by the same procedures, and their results were compared with those samples subjected to the stress-hydrolytic conditions.

Irradiation with ultraviolet light. A sample powder of LEN (25 mg, 1 mm thick layer in a Petri plate) was exposed to UV light (245 nm) for 10 days. The material was dissolved in 5 mL methanol. The solution was filtered with syringe filtration disk and diluted to 25 mL with methanol to obtain a claimed concentration of 1 mg mL^{-1}. This solution was diluted with BGE to give a final concentration of 50 μg mL^{-1} then subjected to the analysis by the proposed CE-PDA method.

Exposing to dry heat. A sample powder of LEN (25 mg, 1 mm thick layer in a Petri plate) was exposed to dry heat at 60 °C for 8 days. A control parallel set of samples was kept in dark at refrigerator temperature. The material was dissolved in 5 mL methanol. The solution was filtered with syringe filtration disk and diluted to 25 mL with methanol to obtain a claimed concentration of 1 mg mL^{-1}. This solution was diluted with BGE to give a final concentration of 50 μg mL^{-1} then subjected to the analysis by the proposed CE-PDA method.

3. Results and discussion

Method development

The initial method development was conducted on pure drug using working standard solutions, protected from light, of LEN and the internal standard (IS) metoclopramide (MET, Fig. 1). The electrophoretic parameters were preliminarily optimized to develop a stability-indicating CE-PDA method for the determination of LEN in its bulk and pharmaceutical capsules.

Optimization of electrophoretic conditions

The background electrolyte (BGE) is still one of the key parameters in the successful development of a CE method for the purpose of pharmaceutical analysis since its role is very complex. The main purposes of a BGE are providing the transport of the electric current and the electrophoretic separation of the analytes. However, when an electric current passes through the BGE, some additional phenomena occur, such as the electroosmotic flow (EOF), which plays an important role in the over whole electrophoretic processes. The BGE should primarily provide an appropriate migration of the analytes in a reasonable time with no peak broadening and migration interferences [28]. Therefore, the most important conditions that affect the electrophoretic efficiency of BGE were investigated in order to achieve the most appropriate BGE system for the

optimum separation of LEN and MET (IS); these investigations are discussed in the following sections:

Effect of buffer type and strength. Two different buffer systems (acetate and phosphate) were used with and without an organic modifier, and the results were compared in terms of the achieved selectivity, reproducibility, baseline separation, and the analysis time. Under keeping the other electrophoretic parameters constant, phosphate buffer gave a better resolution, shorter migration times, and more sharp peaks. Phosphate buffer of varying ionic strengths (20, 30, and 40 mM) were investigated. The best resolution and shortest migration times were achieved when the buffer strength was 20 mM (Table 1).

Table 1. Effect of various parameters on the electrophoretic resolution of lenalidomide (LEN) and the internal standard metoclopramide (MET)

Parameter	Resolution (Rs)	Migration time (min)	
		LEN	MET
pH of buffer system			
4.5	5.6	6.4	3.6
5	5	6.0	3.5
6	4.6	5.3	3.2
6.4	3.75	4.5	3.0
7.1	2.4	2.6	2.0
7.6	3	3.2	2.3
Buffer concentration (mM)			
20	4	3.7	2.5
30	4	3.8	2.6
40	3.5	4.4	3.0
Methanol organic modifier (%, v/v)			
10	4.5	5.1	3.5
20	3.1	6	4.3
Voltage (kV)			
30	5.2	5	3.7
25	3.6	3.7	2.8
10	ND [a]	> 20	19
Injection time (seconds)			
15	1.7	2.6	2.0
25	1.8	2.7	2.0
30	1.3	2.7	2.1
50	1	2.7	2.1

[a] ND = not determined

Effect of pH. The electrophoretic migration of weak electrolytes such as LEN and MET (weak bases) is greatly affected by the pH value of the BGE. Therefore, the pH of the BGE should be controlled to keep the migration velocity of weak electrolytes and the velocity of the EOF constant. This usually results in a stable and reproducible migration behaviour of the analytes. As well, the effective mobility (the electrophoretic migration) of weak electrolytic species is strongly dependent on their pK values, which are related to the pH of the BGE. However, it should be emphasized, that even substances with zero effective mobility may move in the capillary due to the EOF, and this EOF is also strongly dependent on the pH of the BGE [28].

In order to select the optimum pH, phosphate buffer (20 mM) of varying pH values in the range of 4.5 – 7.6 were investigated under keeping the other conditions constant. It was observed

that the resolution of LEN from IS and their migration times decrease as the pH value increases up to pH value of 7.1. Beyond pH value of 7.1, a better resolution was achieved; however the migration times were longer (Table 1). Since an adequate resolution with short migration times was achieved at pH of 7.1, this pH was selected for the subsequent investigations.

Effect of organic modifier. It has been demonstrated that the organic solvents in the run buffer offer some potential advantages: (1) extends the range of analytes that can be investigated, due to an enhancement in the solubility of various substances; (2) increase the selectivity compared to the entirely aqueous buffers through changes in the physicochemical properties, such as changes in acid–base properties of the analytes, viscosity and dielectric constant of the separation medium, as well as interactions between analytes and solvent; (3) reduce the analysis time by employing relatively high applied voltages, since most organic solvents have lower dielectric constants compared to water and, thus, relatively high voltages can be applied without causing any significant band broadening due to Joule heating [29]. For these reasons, the use of methanol, as an organic modifier, was attempted in the present study. Methanol was added to the phosphate buffer ranges at two different concentrations (10 and 20%, v/v). The use of 10% gave better results than 20%, in terms of better resolution and shorter migration time (Table 1), thus this concentration was used in the subsequent experiments.

Effect of applied voltage. McLaughlin *et al.* [30] have demonstrated the direct proportion of the electrophoretic resolution of analytes to the applied voltage. In the present study, three varying voltages (10, 25, and 30 kV) were applied. At 10 kV, long migration time and less sharp peaks were observed. The use of 30 kV gave better resolution and shorter migration time than 25 kV, thus all the subsequent investigations were carried out by applying 30 kV (Table 1).

Sample injection time. In order to select the most appropriate sample injection time, varying times (15 – 50 seconds) were tested. It was found that the injection time had no significant effect on the migration time of both LEN and MET (IS), however it has an obvious effect on the resolution. There was no significant difference in the resolution when the injection times were 15 and 25 seconds; Rs values 1.7 and 1.8, respectively (Table 1). When the injection time was increased, the resolution was significantly decreased (Table 1). Thus an injection time of 25 seconds was chosen for the analysis.

Applying the above-mentioned optimum electrophoretic conditions, the migration times of MET (IS) and LEN were 3.09 ± 0.11 and 4.74 ± 0.24 min (n = 3), respectively (Fig. 2B).

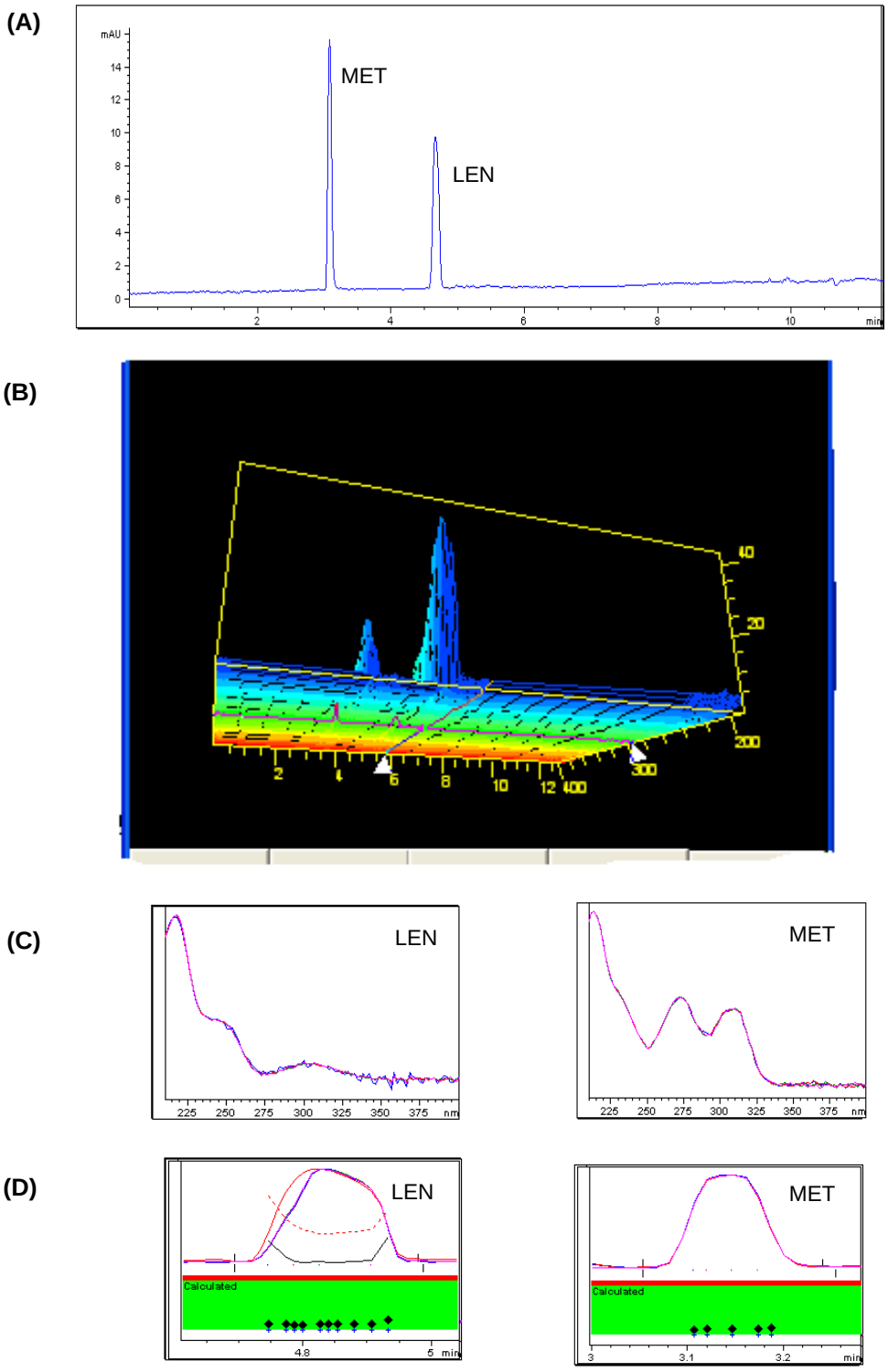

Fig. 2. A typical electropherogram of a standard mixture of 10 µg mL^{-1} of LEN and 30 µg mL^{-1} of MET (A), the corresponding 3D plot (B), their absorption spectra (C), and their corresponding peak purity graphs (D).

Method validation

The proposed CE-PDA method was validated according to the ICH guidelines [31], in terms of linearity, range, sensitivity, accuracy, precision, robustness, ruggedness, and specificity.

Linearity, range and sensitivity. Using the above-mentioned optimum electrophoretic conditions, a calibration curve was constructed by plotting the peak area ratios of LEN to that of the IS versus the corresponding concentrations of LEN, and a linear least-square regression analysis was conducted to determine the intercept, slope, and correlation coefficient (r) of the line to demonstrate its linearity, and establish the linear range of the proposed method. The results revealed a good linear calibration fit in the range of 1 – 50 μg mL^{-1}. The calibration equation was: Y = –0.0018 + 0.0920 C (r = 0.9999), where Y is the peak area ratio, C is the concentration of LEN, and r is the correlation coefficient. The high value of correlation coefficient indicated the good linearity, and the low values of standard deviations of the intercept and the slope (Table 2) indicated the significant validity of the calibration points used for constructing the calibration curve.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the signal-to-noise ratio [31]. Their values were 0.25 and 0.80 μg mL^{-1}, respectively (Table 2).

Table 2.Validation parameters for the quantitative determination of LEN by the developed CE-PDA method

Parameter	Value
Linear range (μg mL^{-1})	1–50
Intercept (a)	–0.0018
Slope (b)	0.0920
Correlation coefficient (r)	0.9999
LOD (μg mL^{-1})	0.25
LOQ (μg mL^{-1})	0.80

Precision . Precision was determined in accordance with ICH recommendations [31]. Within-day precision was assessed by injecting three consecutive injections of LEN solution at three varying concentrations (10, 30, and 50 μg mL^{-1}), as a single batch. The results showed excellent within-day precision as the relative standard deviation (RSD) values of the measured concentrations did not exceed 0.33% (Table 3). Between-day precision was determined by analyzing the same concentrations of LEN on three consecutive days; triplicate injections were done for each concentration. The low RSD values (≤ 0.59%) indicated the high precision of the proposed method [31].

Table 3. Precision and accuracy of the proposed CE-PDA method for the determination of LEN

Nominal LEN (μg mL^{-1})	Measured LEN (μg mL^{-1} ± SD)	Recovery (%)	RSD (%)	Error (%)
Within-day [a]				
10	10.05 ± 0.03	100.50	0.29	0.50
30	29.56 ± 0.10	98.53	0.33	-1.46
50	50.21 ± 0.13	100.42	0.25	0.42
Between-day [b]				
10	10.08 ± 0.06	100.80	0.59	0.80
30	29.59 ± 0.08	98.63	0.27	-1.36
50	50.21 ± 0.21	100.42	0.41	0.42

[a] It was assessed by three replicate injections as a single batch (n = 3).
[b] It was determined by three replicate injections on three consecutive days (n = 9).

Accuracy . Accuracy was determined by the recovery study of known concentrations (10, 30, and 50 μg mL^{-1}) of LEN standard solution. The concentrations were calculated from the calibration curve. The recovery was presented as percentages, (calculated concentration / nominal concentration × 100). The recovery values ranged from 98.53 to 100.80 (± 0.25─ 0.59%), Table 3. As well, the errors, presented as percentages, ranged between 1.46 and 0.80% (Table 3). These results indicated the acceptable accuracy of the method [31].

Robustness. In order to measure the extent of the method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged, and in parallel the electrophoretic profile was observed. The electrophoretic parameters were interchanged within the range of 1-10% of the optimum recommended conditions. The results indicated that the small change in the conditions did not significantly affect the determination of LEN.

Sample solution Stability. The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution (10 μg mL^{-1}) for 24, 48 and 72 hours under laboratory bench conditions (25 ± 1°C). There was no significant change in analysis over a period of 72 hours. The mean RSD between peak areas was found to be 0.47%, suggesting that the drug solution can be stored without any degradation over the time interval studied.

Specificity. Specificity of the method is defined as its ability to assess unequivocally the analyte in the presence of components which may be expected to be present. These might include impurities, degradation product, and excipients. The electropherogram demonstrated the specificity of the proposed CE-PDA method, as there were no peaks at the migration times of either LEN or the internal standard (MET) from excipients that is commonly co-formulated with LEN in its capsules; the electropherogram of capsules solution spiked with MET (IS) was typically the same as that given in Fig. 2. As well, peak purity tests may be useful to show that the analyte peak is not attributable to more than one component. Peak purities of LEN and MET were confirmed by the use of the PDA detector.

Stability-indicating study

The ICH guideline entitled stability testing of drug substances and products [22] requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance, and provide a rapid identification of differences that might result from changes in the manufacturing processes or source sample. Susceptibility to acid-base hydrolytic, and photolytic stability are the required tests. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products. As described in the experimental section, different stress conditions were applied: acid-base hydrolysis, exposure to dry heat, and irradiation with UV light.

The electropherograms of the blank solutions, consisting of stress agents without the drug were inspected in order to mark the peaks corresponding to stress agents and to distinguish them from the potential LEN degradation products. The stressed samples were detected under different wavelengths using the PDA detector in order to ensure that no additional degradation products were formed with different extinction values than the parent drug. Peak purity test performed by PDA detector was useful to prove that the analyte electrophoretic peak did not contain more than one substance. After recording UV spectrum (200-400) of the LEN and the representative samples from each stress condition, the detection wavelength of 210 nm was finally selected.

From this investigation, it was clear that LEN was stable against UV irradiation, and exposure to dry heat, as no significant degradation products were detected in their electropherograms, which were identical to the electropherogram of LEN sample that has not been subjected to any stress conditions (Fig. 3 a-c). As well, the recovery values of the intact LEN in these stressed samples were 100.1 and 99.8% in case of UV irradiation, and exposure to dry heat, respectively (Table 4). In case of acid hydrolysis, a degradation product was detected at a migration time of 8.3 min (Fig. 3d). The total concentration of this degradation product was

calculated and found to be 0.7% of the intact LEN, which was 99.3% (Table 4). In case of alkali hydrolysis, degradation product was observed at a migration time of 9.2 min (Fig. 3e). The concentration of this degradation product was calculated and found to be 14.5% of the intact LEN (Table 4). In both acid and alkali hydrolysis, the proposed CE-PDA method was able to separate completely the degradation products from the intact LEN. This confirmed the selectivity and stability-indicating property of the proposed method.

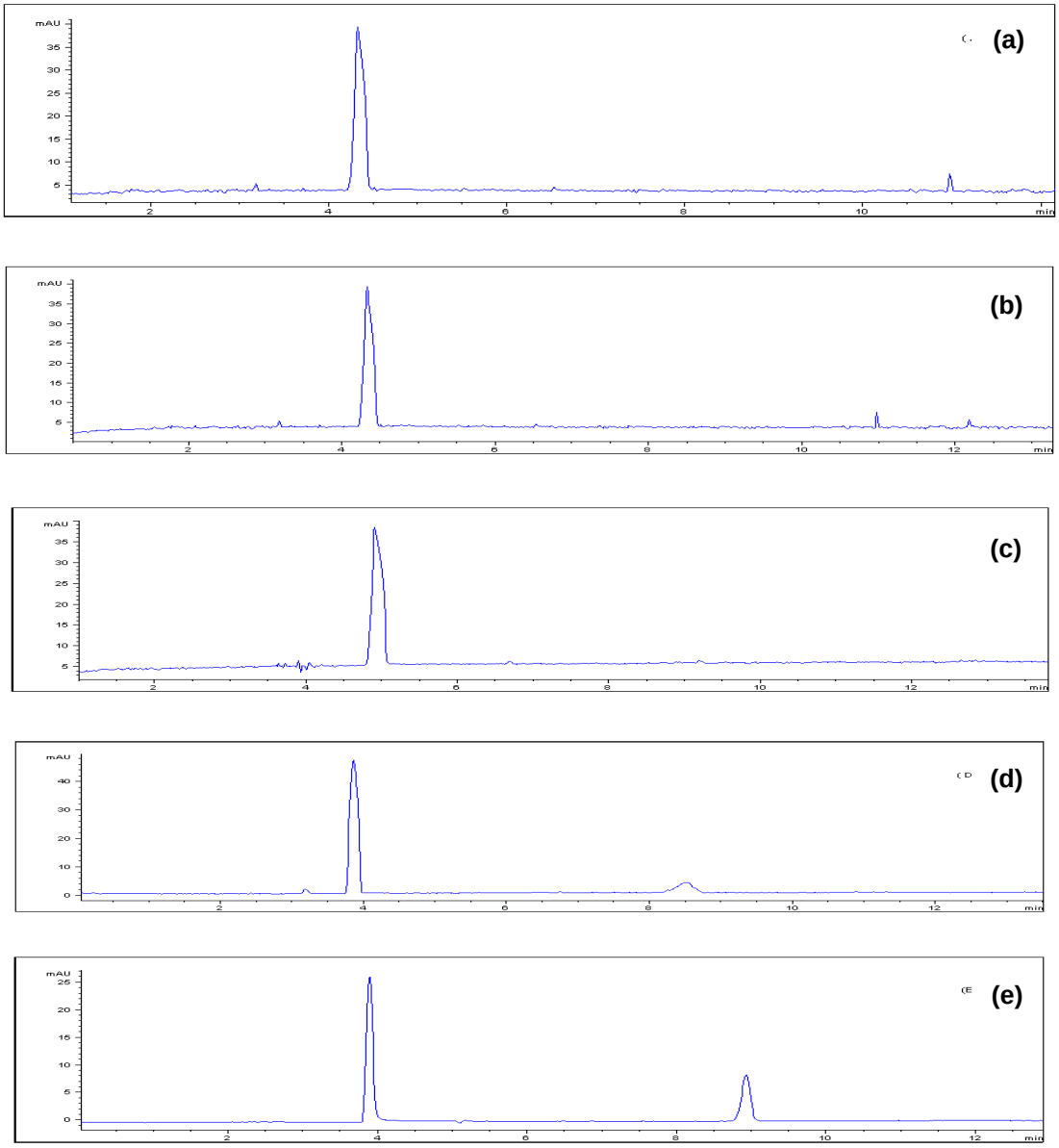

Fig. 3. Typical electropherograms that have been obtained from stress testing studies of LEN. Panel A is the mixture of standard solution containing LEN (50 µg mL^{-1}) and MET (30 µg/mL) that has not been subjected to any stress condition. Panels from B to D are samples that have been subjected to UV irradiation, dry heat, acid hydrolysis, and alkaline hydrolysis, respectively.

Table 4. Summary of accelerated degradation of LEN under different stress conditions

Stress condition	Recovery of intact LEN (%)	Remarks
UV irradiation	100.1	No observed degradation
Exposure to dry heat	99.8	No observed degradation
Acid hydrolysis	99.3	Minor degradation
Alkaline hydrolysis	85.5	Observed degradation

Applicability of the Method

It is evident from the results obtained previously that the proposed CE-PDA method gave satisfactory results with the analysis of LEN in bulk. Thus, LEN-containing capsules were subjected to the analysis by the proposed method. The label claim percentage was 99.52 ± 0.43%. This acceptable value indicated the applicability of the method for the routine quality control of LEN capsules without interference from the excipients. This was evidenced from the good label claim percentage as well as the absence of any peaks in the electropherogram of the capsule extract solution, which was identical to the standard solution (Fig. 2A). Since there was no official method for the quantitative determination of LEN in its capsules, a reported method [24] was used as a reference method. The analytical result obtained by the proposed CE-PDA method was compared with those obtained from the reference method by statistical analysis with respect to the accuracy (by *t*-test) and precision (by *F*-test). No significant differences were found between the calculated and theoretical values of *t*- and *F*-tests at 95% confidence level proving similar accuracy and precision in the determination of LEN by both methods (Table 5).

Table 5. Determination of LEN in capsules by the proposed CE-PDA and reference methods

Capsules [a]	Recovery (% ± SD)		*t*-value [d]	F-value [d]
	Proposed method [b]	Reference method [c] [24]		
Revlimid®	99.52 ± 0.43	99.69 ± 0.21	0.88	4.50

[a] Capsules are labeled to contain 5 mg of LEN per capsule.
[b] Values are mean of five determinations ± SD.
[c] Values are mean of three determinations ± SD.
[d] The tabulated t- and F-values at 95% confidence limit are 2.77 and 19.2, respectively.

4. Conclusions

The present study represents the first report that deals with the development of a stability-indicating CE-PDA method for determination of LEN in its bulk and pharmaceutical capsules. This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH/FDA guidelines. The proposed method showed acceptable accuracy, precision, selectivity, and wide linear concentration range. From the economical point of view, the method involved the native UV-absorbing property of LEN, rather than expensive derivatizing analytical reagents. Statistical analysis for the results proved that the method is suitable for the determination of LEN in bulk and capsules forms without any interference from the degradation products, and it is recommended for routine use in quality control industry laboratories.

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References

[1] A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu, M.J. Thun, Cancer J. Clin. **57**, 43 (2007).
[2] S. Singhal, J. Mehta, R. Desikan, D. Ayers, P. Roberson, P. Eddlemon, P., N. Munshi, E. Anaissie, C. Wilson, M. Dhodapkar, J. Zeldis, D. Siegel, J. Crowley, B. Barlogie, N. Engl. J. Med. **341**, 1565 (1999).
[3] J.D. Tariman, Clin J. Oncol. Nursing **7**, 143 (2003).
[4] P.G. Richardson, R.L. Schlossman, E. Weller, T. Hideshima, C. Mitsiades, F. Davies, R. LeBlanc, L. P. Catley, D. Doss, K. Kelly, M. McKenney, J. Mechlowicz, A. Freeman, R. Deocampo, R. Rich, J.J. Ryoo, D. Chauhan, K. Balinski, J. Zeldis, K.C. Anderson, Blood **100**, 3063 (2002).
[5] J.D. Tariman, Clin, J. Oncol. Nursing **11**, 569 (2007).
[6] P. Sonneveld, A. Palumbo, EJHPP **14**, 58 (2008).
[7] S.R. Shah, T.M. Tran, Drugs **67**, 1869 (2007).
[8] P. Falco, F. Cavallo, A. Larocca, A.M. Liberati, P. Musto, M. Boccadoro, A. Palumbo, Expert Rev. Anticancer Ther. **8**, 865 (2008).
[9] T. Hideshima, P.G. Richardson, K.C. Anderson, Expert Opinion on Invest. Drugs **15**, 171 (2006).
[10] L.G. Corral, P.A. Haslett, G.W. Muller, R. Chen, L.M. Wong, C.J. Ocampo, R.T. Patterson, D.I. Stirling, G. Kaplan, J. Immunol. **163**, 380 (1999).
[11] K.C. Anderson, Seminars Hematol. **42**, S3 (2005).
[12] P. Richardson, K. Anderson, Anderson. J. Clin Oncol. **22**, 3212 (2004).
[13] J.D. Tariman, Clin J. Oncol. Nursing **7**, 143 (2003).
[14] N. Raje, T. Hideshima, K.C. Anderson, Expert Rev. Anticancer Ther. **6**, 1239 (2006).
[15] D. Verhelle, L.G. Corral, K. Wong, J.H. Mueller, L.M. Parseval, K.J. Pergakes, P.H. Schafer, R. Chen, E. Glezer, G.D. Ferguson, A. Lopez-Girona, G.W. Muller, H.A. Brady, K.W.H. Chan, Cancer Res. **67**, 746 (2007).
[16] Celgene Corporation, http://ir.celgene.com/phoenix.zhtml?c=111960&p=irol-newsArticle&ID=877894&highlight.
[17] S. Ahuja, K.M. Alsante, Handbook of Isolation and Characterization of Impurities in Pharmaceuticals. Academic Press, San Diego, 2003.
[18] S. Ahuja (Ed.), Impurities Evaluation of Pharmaceuticals, Marcel Dekker, Inc., New York, 1998.
[19] FDA: Guidance for Industry: Impurities in Drug Product, Draft guidance, Center for Drug Evaluation and Research (CDER), 1998.
[20] W. Grimm, in: J.T. Carstensen, Rhodes CT (Eds.): Drug Stability, Principles and Practices. Marcel Dekker, Inc., New York, 2002, p. 520.
[21] H. Khan, M. Ali, A. Ahuja, J. Ali, Stability Testing of Pharmaceutical Products - Comparison of Stability Testing Guidelines. Current Pharm. Anal. **6**, 142 (2010).
[22] ICH Guidance for Industry Q1A (R2): Stability Testing of New Drug Substances and Products, IFPMA, Geneva, 2000.
[23] B.S. Sastry, S. Ganadhamu, S.V. Prasad, K.V. Rraju, Intl. J. PharmTech Res. **1**, 416 (2009).
[24] G. Sravanan, B.M. Rao, M. Ravikumar, M.V. Suryanarayana, N. Someswararao, P.V.R. Acharyulu, Chromatographia **66**, 287 (2007).
[25] K.D. Altria, M.M. Rogan, Introduction to Quantitative Applications of Capillary Electrophoresis in Pharmaceutical Analysis, Beckman Primer, Vol. VI, Fullerton, CA, USA, 2006.
[26] ICH, Q2B Validation of Analytical Procedure: Methodology in: Proceeding of the International Conference on Harmonization, Geneva, March 1996.
[27] ICH, Guidance on Analytical Method Validation, in: Proceedings of International Convention

on Quality for the Pharmaceutical industry, Toronto, Canada, September 2002.
[28] J.L. Beckers, P. Bocek, Electrophoresis **24**, 518 (2003).
[29] C.W. Huie, Electrophoresis **24**, 1508 (2003).
[30] G.M. Mc Laughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison, T.J. Bronzert, J. Liq. Chromatogr. **15**, 961 (1992).
[31] ICH Guideline Q2 (R1): Validation of Analytical Procedure: Text and Methodology, ICH, London, 2005.