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# NEW SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF LENALIDOMIDE IN PHARMACEUTICAL FORMULATIONS

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**ABSTRACT:**
Two simple and sensitive spectrophotometric methods (Method A and Method B) were developed for the estimation of Lenalidomide in pharmaceutical formulations. Method A is based on diazo-coupling reaction with N-(1-napthyl) ethylenediamine dihydrochloride (B.M reagent) to form a stable purple coloured chromogen, which can be estimated at 540 nm. Method B is based on the formation of a coloured condensation product with the aromatic aldehyde namely p-dimethylaminocinnamaldehyde (PDAC) which shows absorption maximum at 530 nm. Both the proposed methods (Method A and Method B) obey Beer's law in the concentration range of 1 to 5µg/ml. The methods were validated for use in routine quality control of Lenalidomide in pharmaceutical formulations.
**Keywords:**Lenalidomide,N-(1-napthyl)ethylenediamine dihydrochloride,p-Dimethylaminocinnamaldehyde and spectrophotometry.

## INTRODUCTION :

Lenalidomide (LLD) is an immunomodulatory agent with anti-angiogenic and anti-neoplastic properties[1-6]. LLD is indicated for the treatment of patients with transfusion-dependent anemia due to low or intermediate-1-risk myelodyplastic syndromes associated with a deletion 5q cytogenic abnormality with or without additional cytogenic abnormalities. LLD in combination with dexamethasone is indicated for the treatment of multiple myeloma patients who have received at least one prior therapy. The chemical name of LLD is 3-(4-amino-1-oxo 1, 3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione.

LLD is not official in any pharmacopoeia. For the estimation of LLD few analytical methods such as HPLC[7] and LC-MS[8] were reported. In the present investigation we developed two spectrophotometric methods based on diazotization followed by coupling with B.M reagent (Method A) and Schiff's base formation with PDAC (Method B).

## EXPERIMENTAL DETAILS

**Instrumentation:**

Systronics double beam UV/Visible spectrophotometer 2201 with matched quartz cells were used for the present investigation.

**Reagents preparation:**

1. *Sodium nitrite solution (0.2% w/v):* 200 mg of sodium nitrite was dissolved in distilled water and made up to 100 ml.
2. *Hydrochloric acid (5N):* 425 ml of conc. HCl was taken and diluted to 1000 ml with distilled water.
3. *Ammonium sulphamate solution (0.5 %w/v):* 500 mg of ammonium sulphamate was dissolved in distilled water and made up to 100 ml.
4. *N-(1-napthyl) ethylenediamine dihydrochloride solution (0.1 % w/v):* 100 mg of N-(1-napthyl) ethylenediamine dihydrochloride was dissolved in 100 ml of distilled water.

5. *p-Dimethylaminocinnamaldehyde (PDAC) solution (0.2 %w/v):* 200 mg of PDAC was dissolved in methanol and made up to 100 ml with the same solvent.
6. *Sulphuric acid solution (10 %v/v):* 10 ml of concentrated sulphuric acid was taken and diluted to 100 ml with distilled water.

**Standard preparation:**

About 50 mg of LLD was accurately weighed and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions. This stock solution was further diluted with the same solvent to get working standard solution of 100 µg/ml.

**Sample preparation:**

The content of twenty capsules was taken, thoroughly mixed and ground in a mortar. From this an accurately weighed portion of the capsule content equivalent to 50 mg of the drug was dissolved in 70 ml of methanol and filtered. The filtrate was diluted to 100 ml with methanol. Later this solution was further diluted to get absorbance values within the calibration curve range.

**Procedure for estimation:**

**Method A:**

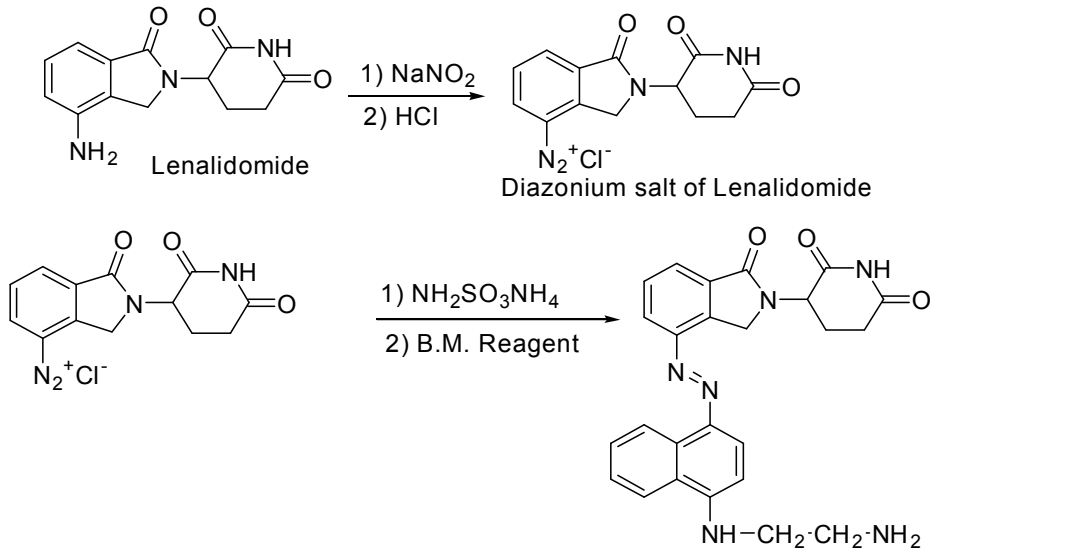
Aliquots of LLD solution (100 µg/ml) ranging from 0.1 to 0.5 ml were transferred into a series of 10 ml volumetric flasks and total volume in all flasks was adjusted to 1.0 ml with methanol. To each flask 1 ml of 5N hydrochloric acid and 1 ml of sodium nitrite solution were added and allowed to stand for five minutes. One ml of ammonium sulphamate solution was then added, mixed and allowed to stand for two minutes. To this solution 1 ml of N-(1-napthyl) ethylenediamine dihydrochloride (B.M reagent) solution was added and mixed well. The final volume was made up to 10 ml with distilled water. The absorbance of pink coloured chromogen was measured at 540 nm against reagent blank. The amount of LLD was computed from calibration curve.

**Method B:**

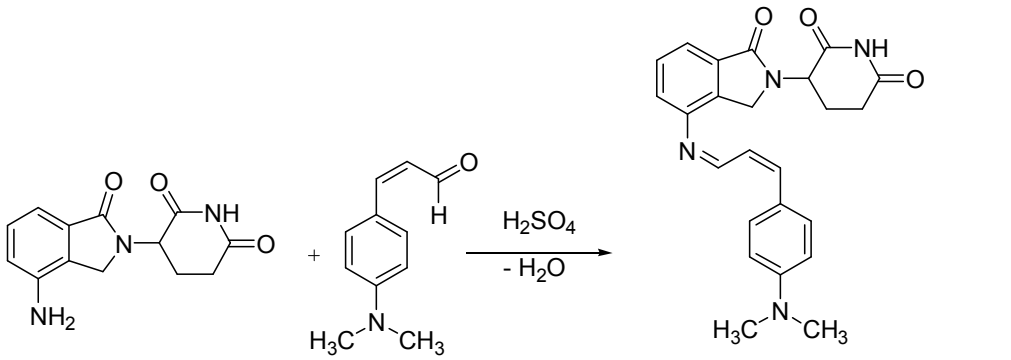
Aliquots of LLD solution (100µg/ml) ranging from 0.1 to 0.5ml were transferred into a series of 10 ml volumetric flasks. To each flask 1.0 ml of p-Dimethylamino- cinnamaldehyde (PDAC) solution and 0.1 ml of sulphuric acid solution were added. After five minutes the volume was brought up to 10 ml with methanol and the absorbance of red coloured species was measured at 530 nm against reagent blank. The amount of LLD was computed from calibration curve.

**RESULTS AND DISCUSSION :**

The primary aromatic amines are specifically and sensitively determinable by diazotization of the amine to corresponding diazonium compound, then coupling of this diazo compound with a phenolic or other amino compound(s). The reaction of aromatic amines with nitrous acid to form diazonium salts is very general and can be carried out regardless of other ring substituents. A vast number of drugs were spectrophotometrically estimated by making use of diazotization and coupling with B.M. reagent [9-16]. LLD contains primary aromatic amine functional group. We developed method A based on diazotization of primary aromatic amine of LLD with nitrous acid (generated in-situ) followed by coupling with B.M. reagent and method B based on Schiff's base formation with PDAC. The mechanism of formation of colored products had been shown in scheme no 1 and 2.




**Scheme no. 1.**




**Scheme no. 2.**

The two developed methods follow Beer's law in the concentration range of 1-5 µg/ml. Interference studies were conducted to see the influence of excipients with the proposed methods. The common excipients usually present in dosage forms do not interfere in the proposed method A and method B. The optical characteristics, regression analysis data and precision of the methods were presented in table no 1. The accuracy of the methods was evaluated by estimating the amount of LLD in previously analyzed samples to which known amounts of LLD was spiked. The accuracy of the methods was also conformed by comparison of the results obtained by proposed and reference methods. The results of accuracy were given in table-2. Some of the commercially available formulations were procured from the local market and analyzed by the developed methods and the results comply with the labeled claim (table-2).

**CONCLUSION:**

The proposed methods are economic, simple, sensitive, reproducible and accurate and can be used for the routine analysis of LLD in bulk as well as in its pharmaceutical preparations.

**Table-1: Optical characteristics and regression analysis parameters**

| Parameter | Method-A | Method-B |
|---|---|---|
| $\lambda_{max}$ (nm) | 540 | 530 |
| Beer's law limits (µg/ml) | 1 - 5 | 1 - 5 |
| Molar absoptivity (L. mole$^{-1}$ cm$^{-1}$) | 3.721x $10^3$ | 3.656x $10^3$ |
| Sandell's sensitivity (µg/cm$^2$/0.001 absorbance unit) | 0.0070 | 0.0071 |
| Regression equation* (Y = a + bx): Slope(b) | 0.1429 | 0.1409 |
| Intercept(a) | 0.000476 | 0.000143 |
| Correlation coefficient | 0.9998 | 0.9995 |
| % RSD** | 0.3906 | 0.615 |
| % Range of error** (Confidence limits): 0.05 level | 0.4099 | 0.645 |
| 0.01 level | 0.6429 | 1.012 |
| % Error in bulk samples*** | 0.10 | -0.82 |

*Y = a + bx, Where 'x' is the concentration of LLD in µg/ml and 'Y' is the absorbance value.

**Average of six determinations

*** Average of three determinations

**Table-2: Estimation of LLD in pharmaceutical formulations**

| Sample | Labeled amount (mg) | Amount found by proposed methods* (mg) ± S.D | | Amount found by reference method [7] (mg ± S.D ) | % recovery by proposed methods**± S.D | |
|---|---|---|---|---|---|---|
| | | Method A | Method B | | Method A | Method B |
| 1 | 5 | 4.99 ± 0.011 | 5.02 ± 0.014 | 4.98 ± 0.021 | 99.69 ± 0.21 | 101.11 ± 0.18 |
| 2 | 10 | 9.98 ± 0.012 | 10.03 ± 0.014 | 10.04 ± 0.008 | 99.89 ± 0.18 | 100.03 ± 0.11 |

* Average of six determinations

**** AVERAGE OF THREE DETERMINATIONS**

**REFERENCES:**


1. Naing A. Sokol L, List A.F., J Natl Compr Canc Netw., 2006, 1, 78-82.
2. Giagounidis A.A,Germing U, Aul C., Clin Cancer Res., 2006, 1, 5-10.
3. Anderson KC., Lenalidomide and thalidomide: mechanisms of action similarities and differences, Semin Hematol., 2005, 42, 53-8.
4. Giagounidis A.A, Germing U, Strupp C, Hildebrandt B, Heinsch M, Aul C., Ann Hematol., 2005, 9, 569-571.
5. Dredge K, Horsfall R, Robinson S.P, Zhang L.H, Lu L, Tang Y, Shirley MA, Muller G, Schafer P, Stirling D, Dalglesih A.G, Bartlet J.B., Microvasc Res., 2005, 69, 56-63.
6. List A, Kurtin S, Roe D.J, Buresh A, Mahadevan D, Fuchs D, Rimsza L, Heaton R, knight R, Zeldis J.B N Engl J Med., 2005, 6, 549-557.
7. Sravanan G, Rao B.M, Ravikumar M, Suryanarayana M.V, Someswararao N, Acharyulu P.V.R., Chromatographia., 2007, 66, 287-290.
8. Liu Q, Farley K, Johnson A, Muthusamy N et al, The Drug Monit. 2008, 30, 620-627.
9. National formulary XIV, American pharmaceutical association, Washington, D.C., 1975, 271.
10. The united states Pharmacopoeia XXIX, The United States Pharmacopeial Convention, Rockville, Md., 1974, pp 527-528.
11. Koch-wester, J., Klein, S. W., Foo Canto, L.L., Kastor, J. A., and Desanctis, R.W., N.Eng. J. Med., 1969, 281, 1253.
12. Mark, L. C., Kayden, H.J., Steele, J.M., Cooper, J. R., Berlin, I., Rovenstine, E. A. and Brodie, B.B., J. Pharmacol. Exp. Ther., 1951,102, 5.
13. Brunner, C. A., J. Assoc. Off. Anal. Chem., 1972, 55,194.
14. Brunner, C. A., J. Assoc. Off. Anal. Chem., 1973, 56,689.
15. Philips, W.F. and Trafton J.E., J. Assoc. Off. Anal. Chem., 1975, 58, 44.
16. Flinn, P.E., J.Chromatogr. Sci., 1975, 13, 580.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