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A REVIEW ON THE ESTIMATION OF LENALIDOMIDE IN DIFFERENT MATRICES

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ABSTRACT

Due to the clinical importance of lenalidomide in multiple myeloma, a large number of analytical procedures have been devised to detect the presence of this drug in pharmaceutical samples. In this manuscript, a review on the various techniques described to determine lenalidomide is presented. Special attention has been paid to spectrophotometric and chromatographic techniques, but also to relevant methods using fluorimetry, capillary electrophoresis as well as the detection modes coupled to these techniques. The review also focuses on the different degradation pathways of the drug and methods to identify and quantify it.

KEYWORDS: Lenalidomide, Multiple myeloma, Ultraviolet spectroscopy, Liquid Chromatography, Fluorimetry, Capillary electrophoresis.

INTRODUCTION

Multiple myeloma is a hematologic cancer identified by the accumulation of malignant plasma cells in the bone marrow, which causes bone destruction and marrow failure with subsequent overproduction of monoclonal proteins in most patients.[1-3] The B-cell receptor pathway governs the proliferation, survival, apoptosis, and other phenomena required for normal B-cell functioning.[4] Multiple myeloma is a B cell malignancy often associated with B cell receptor signaling as such in several B cell malignancies like chronic lymphocytic leukemia, Mantle Cell Lymphoma, and Non-Hodgkin's Lymphoma.[5,6] Historically, treatment of B-cell malignancies has involved the use of chemotherapeutic agents like fludarabine, cyclophosphamide, chlorambucil, doxorubicin, vincristine, prednisolone, and rituximab in different permutations and combinations in an attempt to increase patient survival. Considering the fact that chemotherapeutic agents work by killing the dividing cells regardless of their health status and their prolonged usage reduces the quality of life, presently lenalidomide serves as a promising alternative for hematological malignancies.[7]

Lenalidomide is a thalidomide analog with an improved toxicity profile and more potent immunomodulatory activities.[8] It is chemically 3-(4-amino-1-oxo 1, 3-dihydro-2-H-isoindole-2-yl) piperidine-2, 6-dione having the molecular formula $C_{13}H_{13}N_3O_3$ and molar mass of 259.261 g/mol respectively. In 2006, it has been approved by US-FDA for the treatment of patients with multiple myeloma, myelodysplastic syndromes, and transfusion-dependent anaemia in combination with dexamethasone.[9] It works by inhibiting angiogenesis and increases the functional capacity of T cells and NK cells in invitro human systems.[10] It may also act by strengthening the immune response, inhibiting the growth of new blood vessels in the tumor region or decreasing the growth factor and cytokine production.

This novel thalidomide analog has shown remarkable clinical activity in the treatment of multiple myeloma. It has a better side effects profile compared to its parent compound thalidomide and can be managed by careful dose adjustment or combination therapy.[11-17] It has an asymmetric carbon and exists in both *S*(–) and *R*(+) enantiomeric forms, however, it is produced as a racemeic mixture with an optical rotation of zero. Although the *S*(–) enantiomer has been reported to be more potent.[18,19] The progress of single enantioimeric formulations were discontinued due to the fast in vivo racemization.[20,21]

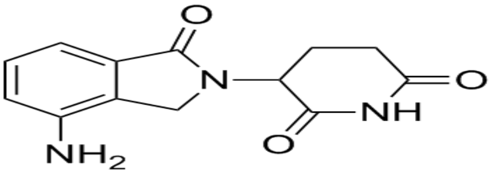

Fig. 1: Structure of lenalidomide.

General methods reported which are used in the analysis of lenalidomide like UV, HPLC, Fluorimetry, Capillary electrophoresis, LC-MS, UPLC-MS are summarized.

ANALYTICAL METHODS

Ultraviolet spectroscopy

Juhi S et al developed a UV method which is simple, rapid, inexpensive, less time-consuming and did not involve any lengthy sample preparation and extraction steps. The RSD of intra and inter day precisions were 0.429% and 0.626 %, which shows that the method is precise. Robustness of the method was performed by checking the effect of variation in solvent characteristics on the absorbance of the method. The absorbance of solutions in plain methanol, methanol with 5% water and methanol with 10% water as solvent was recorded. The developed methods remain robust with a relative standard deviation (RSD) of 1.767%. The ruggedness of the method was confirmed by measuring the absorbance of solutions using two different instruments- Shimadzu UV-1700 double beam spectrophotometer and Shimadzu UV-1800 double beam spectrophotometer. The results showed an acceptable variation of 0.383 % RSD.[22]

Table 1: Estimation of lenalidomide by ultraviolet spectroscopy.

S. No.	Detection wavelength	Solvent	Linearity range	Accuracy LOD LOQ	References
1	250nm	Methanol	4-24µg/ml	99%-101% 0.2311µg/ml 0.7005µg/ml	Juhi S et al[22]

LOD-Limit of detection; LOQ-Limit of quantification

Fluorimetry

Darwish IA et al successfully developed a fluorimetric method and it was the first study to report the development and quantitative determination of LEN in its bulk form and capsules. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 1.4%. The method was based on the derivatization of LEN with fluorescamine (FLC) in an aqueous medium to produce a highly fluorescent product that was measured fluorimetrically at 494 nm after excitation at 381 nm. Because of the absence of native fluorescence of LEN and the capacity of FLC to form highly fluorescent derivatives with primary amines under relatively mild reaction conditions, it was chosen as a derivatizing reagent. The proposed method is superior to the previously reported spectrophotometric methods in terms of the sensitivity and simplicity of the derivatization procedures, low cost, and wide availability in most quality control laboratories. As well, the proposed procedure used water as a green, inexpensive, and safe solvent, rather than the costive and toxic organic solvents that have been employed in the previously reported HPLC methods.[23]

Table 2: Estimation of lenalidomide by fluorimetry.

S. No	Detection wavelength	Solvent	Linearity range	Recovery LOD LOQ	References
1	381nm(excitation) 494nm(emission)	Methanol	25–300ng/ml	99.46± 1.40% 2.9ng/ml 8.7ng/ml	Darwish IA et al[23]

Capillary electrophoresis

Alshehrt MM et al' s study describes, for the first time, the development and validation of a simple, sensitive and accurate stability-indicating capillary electrophoresis method with a photodiode array detector for the determination of LEN. It 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. No degradation was observed when it is exposed to UV irradiation and dry heat. Degradation was observed in both acid hydrolysis and alkali hydrolysis with a recovery of 99.3% and 85.5% respectively. From the economical point of view, the method involved the native UV-absorbing property of LEN, rather than expensive derivatizing analytical reagents.[24]

Szabo ZI et al developed a capillary electrophoresis method followed by the chiral separation of LEN enantiomers using 30 mM SBE-β-CD, 30 mM phosphate pH=6.5, 12kV applied voltage, 10°C which were then validated for the determination of 0.1% (*R*)-LEN as a chiral impurity, which could be important if a racemic switch is achieved.[25]

Table 3: Estimation of lenalidomide by Capillary electrophoresis.

S. No	Stationary phase, internal standard	Background electrolyte solution	Pressure, method of detection, Migration time	Results	References
1	Deactivated fused silica capillary (52 cm length × 75 µm diameter) at 22˚C Metoclopramide	Phosphate buffer solution (20 mM, pH 7.1): methanol (90:10, v/v).	20 mbar x 25s PDA at 210nm 3.09 ± 0.11min (Metoclopramide) 4.74± 0.24 min (lenalidomide)	Accuracy –98.53 -100.80% w/w r^{2-} 0.9999 LOD- 0.25µg/ml LOQ- 0.80µg/ml	Alshehrt MM et al[24]
2	Untreated bare fused-silica capillary (50 µm id, 48.5 cm total and 40 cm effective length) [sulfobutylether-β-cyclodextrin]	30 mM phosphate buffer pH=6.5	50 mbar x 2s	Accuracy -96.6–103.6%	Szabo ZI et al[25]

PDA-Photo diode array; r^{2-}*correlation coefficient*

High Performance Liquid Chromatography

Juhi S et al also developed a RP-HPLC method with only 15% organic solvent (methanol) consumption. The method passed system suitability parameters including Area ± SD, Theoretical plate ± SD, Tailing factor ± SD were 1106.422 ± 0.729, 6687.83 ± 0.02, 0.865± 1.86 respectively, which has shown acceptable results.[22]

Szabo ZI et al aims to develop a novel chiral methods for enantioseparation of LEN. To get access to the individual enantiomers at first, a chiral HPLC method development was carried out. The absolute configuration of the isolated pure enantiomers (i.e. >99%) was elucidated by combined electronic circular dichroism spectroscopy and quantum chemical time-dependent density functional theory calculation method and the individual enantiomers can also be used for CE method development. The enantio discriminating performances of eight chiral stationary phases (CSPs) including four CD (Quest-BC, Quest-C2, Quest-CM and Quest-SB) and four polysaccharide-type columns (Chiralpak AD, Chiralcel OD, Chiralcel OJ and Chiralpak AS) were evaluated. The most promising results displaying this particular order of elution were obtained using Chiralcel OJ column and 100% ethanol as mobile phase.[25]

Saravanan. G et al developed and validated a RP-HPLC method for the separation of LEN and its impurities. Degradation studies were performed on bulk samples of LEN subjected to 0.5N hydrochloric acid, 0.5N sodium hydroxide, 10% v/v hydrogen peroxide, heating to 60˚C and UV light at 254 nm. Degradation was observed only under base hydrolysis conditions. The developed LC method gave a mass balance close to 99.5%, proving it to be unaffected by the presence of Imp-A and other degradation products confirming the stability indicating power of the method. The organic solvent consumption of this method is about 20% with a run time of 24 min.[26]

Nourah Z. Alzoman et al proposed a stability-indicating chiral HPLC method was designed for the enantiomeric separation of LEN in the presence of its degradation products. LEN was exposed to different accelerated stress factors. The degradation products were well resolved from the pure drug enantiomers. LEN was found to be more stable under thermolytic and photolytic stress conditions in its solid form than in acidic or basic stress conditions in solution. The mobile phase is completely organic with a short run time of 12 min and most sensitive with a quantification limit of 2ng/ml compared to other methods.[27]

Meghdad P et al developed a RP-HPLC method for the determination of LEN and its related substances by using a usual C_8 coloum and it was suitable for the high-resolution separation of the drug. The purity of the LEN is 99.91%. Due to the high purity of the active pharmaceutical ingredient, it was stressed to produce new impurities. The results show that the resolution of the peaks for fresh, acid stress, and thermal stress were considerably high. Gradient elution has been opted with a run time of 40 minutes.[28]

Maheshwara LR et al established a RP-HPLC method for the determination of LEN and related substances using a C_{18} coloumn under gradient conditions. The degradation studies were performed using 0.1N HCl, 0.1 N NaOH, 1% v/v hydrogen peroxide, humidity, UV at 254 nm, Sunlight, and heat at 60˚C. No significant degradation of LEN was observed. However, a slight degradation was observed in presence of NaOH. The developed HPLC method gave the peaks purity angle was less their threshold angle, proving it to be suitable for stability studies with a run time of 85 min.[29]

Punna V et al developed a stability indicating RP-HPLC method for the estimation of LEN with a wide linearity range from 25 to 150µg/ml. The organic solvent consumption is 45%. The retention timeless compared to established methods proving it to be rapid, precise and accurate.[30]

Prasadh SS et al designed a RP-HPLC method for the determination of LEN related substance with the inclusion of another LEN Impurity-III for the quantitative analysis of LEN in pharmaceutical formulations using analytical quality by design approach to overcome this risk-sensitive to pH of buffer solution. The design of experiments was performed using fractional design by in view of the pH of the buffer in mobile phase-A, flow rate, the percentage of methanol in mobile phases- A and B and resolution between the close eluting impurities (Impurity-I&II) as responses. Samples are subjected to different forced degradation studies and found that impurity degradant peaks in chromatograms of all stressed samples and LEN are resolved. The purity angle was found fewer than the purity threshold for the all forced degradation samples. This shows that there is no interference and co-elution from degradants in the quantification of impurity in the drug product.[31]

Krishna P et al developed an isocratic HPLC method for the quantitative and enantioselective determination of R- and S- LEN. The drug was subjected to hydrolytic, oxidative, photolytic, and thermal stress and substantial degradation occurred in alkaline and acidic media and under oxidative and hydrolytic stress conditions. The major product obtained as a result of aqueous hydrolysis was different from that produced by acid hydrolysis and basic hydrolysis. The stress samples were assayed against a reference standard and the mass balance was found to be close to 99.7%.[32]

Table 4: Estimation of lenalidomide by high performance liquid chromatography.

S. No	Stationary phase	Mobile phase	Flow rate, method of detection, Run time	Results	References
1	Waters C-18 (250 mm x 4.6 mm, 5µm)	Acetate buffer at pH 5 : methanol (85:15)	1 mL/min, UV at 250nm	R_{t} – 7.9 min r^2-0.998 Accuracy-100.36 LOD- 40ng/mL LOQ-122ng/mL	Juhi S et al[22]
2	Chiralcel OJcolumn	Pure ethanol	0.6ml/min 210nm	97.5–98.2%	Szabo ZI et al[25]
3	Inertsil ODS-3 V (150 · 4.6 mm ID, 5 lm)	Buffer : acetonitrile : methanol, 80:8:12v/v	1ml/ min, PDA at 210nm 24 min	R_{t} – 6.1 r^2>0.999 Accuracy – 99-101%w/w	Saravanan. G et al[26]
4	LUX 5U Cellulose-2 chiral column (250 × 4.6 mm i.d.).	Methanol : glacial acetic acid : triethylamine , (100 : 0.01 : 0.01, v/v/v),	1.2ml/min, UV at 220nm 12 min	R_{t} – 3.153 ± 0.013 r2- 0.9999 Accuracy *S*(–)-100.09 ± 0.80% w/w *R*(+)99.97 ± 0.93% w/w LOD- 1ng/ml LOQ- 2ng/ml	Nourah Z. Alzoman et al[27]
5	C-8 (250×4.6 mm ID, 5 µm)	Phosphate buffer at pH=3.30 (1000ml) : (methanol:acetonitrile) (100:500 V/V)(gradient)	0.8ml/min UV at 220nm 40 min	R_{t} – 12 min (Impurity 1)15 min (Impurity 2) 17 min (lenalidomide)	Meghdad P et al[28]
6	Sun fire C-18(250×4.6 mm ID, 5 µm)	A:B (85:15 v/v) Phosphate buffer at pH 2.0: (methanol & acetonitrile, 55: 45 v/v)	1ml/min UV at 254nm 85 min	R_{t} – 12.4 r^2- 0.9984 Accuracy – 93.6% to 99.4%w/w	Maheshwara LR et al[29]
7	C-18 (250 X 4.6 X mm X 5µm)	Phosphate Buffer: Acetonitrile (55:45) v/v	1ml/min UV at 242nm	R_{t} – 2.5 min LOD- 58 ng/ml. LOQ-174ng/ml	Punna V et al[30]
8	X-bridge-C-18 column (150 mm × 4.6 mm × 3.5 µ)	Buffer (90:10 v/v): methanol [35:65 v/v] (gradient)	0.8ml/min, PDA at 210nm 65 min	R_{t} – 11.9 Accuracy - 92.6-106.6 %.	Prasadh SS et al[31]
9	Inertsil ODS -3V (250 x 4.6 mm, 5 µm)	Orthophosphoric acid buffer, pH 3.0: acetonitrile. 50:50 (v/v)	1ml/min PDA at 240nm 30 min	R_{t} – 8.7 min LOD &LOQ 15ng/ml r^2- 0.999 accuracy- 100.07-100.17%	Krishna P et al[32]

UV-ultraviolet; R_{t}- retention time; ODS-octa decyl silane.

Liquid Chromatography-Mass Spectroscopy (LC-MS)

Raghu NS et al was the first to propose a LC-MS method for the identification and quantitation of degradants, impurities of LEN in capsules with its drug product excipients. The drug excipient blend was subjected to various stress conditions of thermal stress, pH hydrolysis, oxidation and photolysis. The stress studies and subsequent LC-MS analyses showed the intrinsic stability of LEN and the possible degradation pathways under hydrolytic and oxidative conditions. An added finding was that the LEN drug excipient blend was practically stable to dry heat and photolysis. The same analytical method can be adopted for the multiple strengths of LEN, which is the added advantage of this method.[33]

Table 5: Estimation of lenalidomide by liquid chromatography mass spectroscopy.

S. No.	Internal Standard	Sample preparation, Stationery phase Mobile phase	Flow rate, Detection (m/z) LOQ, linearity, accuracy, Rt, run time	References
1	-	ACE1 C-18, (150X4.6mm i.d, 3 mm) A, orthophosphoric acid buffer, pH 2.0 : Water and acetonitrile 200:800 (v/v) (gradient)	1ml/min UV at 220nm m/z value of 260.0(lenalidomide) LOD- 0.05 µg/Ml LOQ- 0.11 µg/Ml r^2-0.999 accuracy – 99.56 Rt-10min Run time : 65 min	Raghu NS et al[33]

m/z-mass to charge

BIOANALYTICAL METHODS

High Performance Liquid Chromatography

Khalil NY et al proposed a non extractive and simple pre-column derivatization procedures for the trace determination of LEN in human plasma by HPLC with fluorescence detection. Plasma samples were treated with acetonitrile for protein precipitation and then with copper acetate to form stable complexes with the biogenic amines and mask their interference with the derivatization reaction of LEN. It has no native fluorescence therefore a pre-coloum derivatization procedure with fluorescamine is carried out which reacts widely with a variety of nucleophiles including primary amines, even at very low concentrations, forming a fluorescent pyrrolinone moieties whereas the reagent and its degradation products are not reactive and non fluorescent. The proposed method is best suitable for the accurate quantification of LEN in human plasma with good sensitivity compared to other methods at concentrations as low as 2.3ng/mL, with a wide linear range and a relatively shorter run time of 15 min. The simple procedure is valuable for the combined pharmacokinetic and bioavailability studies of LEN in human subjects.[34]

Guglieri-López B et al developed a wide linearity range RP-HPLC method for the determination of LEN in plasma samples. Plasma samples were ultrasonicated for protein precipitation followed by solid-phase extraction. In view of the fact that none of the published methods has a linearity range that allows therapeutic drug monitoring of LEN for pharmacokinetic studies. Thus this method allows estimation on a wide linear range, from 100-950ng/mL, thereby making it a valuable method for pharmacokinetic studies of LEN in human subjects.[35]

Hara S et al developed a HPLC method with fluorescence detection in the treatment of anemia associating with low- or intermediate-risk myelodysplastic syndromes patients and recurrent multiple myeloma patients. It has 30% organic solvent consumption and is highly sensitive with a lower limit of quantifying of 5ng/ml.[36]

Table 6: Estimation of lenalidomide by high performance liquid chromatography in biological samples.

S. No.	Stationary phase	Mobile phase	Flow rate, method of detection, Run time	Results	References
1	Hypersil BDS C-18 250 × 4.6 mm, 5 µm	Phosphate buffer (pH 4):methanol: tetrahydrofuran (70:10:20, v/v)	1ml/min 495nm (emission) 382 (excitation) 15 min	R_t -11.6 min r^2-0.9995 Accuracy- 100.91 ±1.87% LOD-0.8ng/ml LOQ-2.30ng/ml	Khalil NY et al[34]
2	Xterra RP C-18 (250 mm length × 4.6 mm i.d., 5 µm)	Phosphate buffer: acetonitrile (85:15, v/v, pH 3.2)	0.5ml/min PDA at 311nm 15 min	R_t -10.8 min r^2-0.997 85.98% ± 5.33%. LOD-28ng/ml LOQ-100ng/ml	Guglieri-López B et al[35]
3	Capcell Pak C-18 UG120 (150 × 4.6mm, 5 µm) Internal standartd -3-aminobenzyl alcohol	50mM phosphate buffer (pH4.0) : methanol : tetrahydrofurane (70:10:20, v/v);	0.7ml/min 382nm(excitation) 495nm(emission)	LOQ – 5ng/ml	Hara S et al[36]

BDS-base deactivated silane; RP-reverse phase

Liquid Chromatography-Mass Spectroscopy (LC-MS)

Krishna P et al developed a simple, sensitive, rugged, high throughput, and effective method for determination of lenalidomide in rat plasma by LC-MS/MS. The greater advantages of the current method are, short run time of 1.2 min because the mobile phase adopted is completely organic. This method was successfully adopted for the analysis of the samples received from a pharmacokinetic study, which were conducted in rats. The present study again demonstrated that lenalidomide is eliminated predominantly via urinary excretion of the unchanged drug.[32]

Hasnain MS et al designed a LC-MS/MS method for the quantitation of LEN in human plasma using Box–Behnken experimental design. The method was validated and a 32 factorial was employed using Box–Behnken experimental design for the validation of robustness. These designs have three factors such as mobile phase composition (X1), flow rate (X2) and, pH (X3) while peak area (Y1) and retention time (Y2) were taken as response. This showed that little changes in the mobile phase and flow rate affect the response while pH has no affect. LEN and carbamazepine were stable in human plasma after five freeze thaw cycles, at room temperature for 23.7 h, bench top stability for 6.4 h thus proving to be effective for the pharmacokinetic and bioequivalence study of LEN.[37]

Ranganathan P et al developed a LC–MS/MS method for the quantification of LEN in human plasma. The sample is prepared by liquid-liquid extraction using ethyl acetate followed by isocratic elution on a C_{18} coloum with 90% organic solvent consumptioin. The method was found to be sensitive, stability indicating under various storage conditions and ultrafast with a run time of 2 min together with less sample volume can facilitate the bio study of LEN with additional time point’s inclusion.[38]

Gopinath R et al proposed a LC-MS/MS method for the quantification of LEN in human plasma. Ethyl acetate and 0.1% formic acid were used for extaction of the sample from the biological matrices followed by isocratic elution with 85% organic solvent consumption. This simple and sensitive assay is suitable for bioequivalence and bioavailability studies of LEN in human subjects.[39]

Veeraraghavan S et al was the first to developed a LC-MS/MS method was developed for the simultaneous determination of lenalidomide, ibrutinib, and its active metabolite PCI45227 in rat plasma. liquid-liquid extraction was opted with ethyl acetate and dichloromethane in the ratio of 90:10. Previously published methods indicate longer run times with a high plasma volume requirement besides issues with low sensitivity. This method involves 50 µl plasma followed by a single step liquid-liquid extraction procedure. Furthermore, this extraction procedure decreases both the cost and duration of the assay. Chromatographic run time of 7.5 min per sample with good sensitivity and a gradient reverse-phase renders the method useful in high-throughput bioanalysis.[40]

Qing Liu MS et al was the first to develop a LC-MS/MS method to simultaneously quantify lenalidomide and flavopiridol in human and mouse plasma to facilitate their combined clinical development. Samples were prepared by liquid-liquid extraction with acetonitrile. This assay is the most sensitive reported to date for both compounds with lower quantification limits of 1nM for lenalidomide and 0.3 nM for flavopiridol. This superior sensitivity will enable accurate estimation of pharmacokinetic parameters in current and future trails.[41]

Shu C et al was the first to demonstrate a sensitive, reliable and high-throughput developed a LC-MS/MS method for the rapid and simultaneous measurement of lenalidomide, cyclophosphamide, bortezomib, dexamethasone and adriamycin opted for the treatment of multiple myeloma to monitor the drug pharmacokinetic profile for ensuring safety and efficacy during chemotherapy. This method shows excellent specificity, linearity, intra- and inter-day precision and accuracy, extraction recovery, matrix effect and stability within a run time of 5 min and is the most sensitive with 2-4ng/ml of quantification limits.[42]

Veeraraghavan S et al was the first to develop a LC-MS/MS method for the simultaneous quantification of idelalisib, fludarabine and lenalidomide used in the treatment of B-cell Chronic Lymphocytic Leukemia a progressive disease of the blood and bone-marrow. The method is shows utmost sensitivity that even concentrations as low as 1.15, 0.95, and 0.97ng/mL of idelalisib, fludarabine, and lenalidomide were detectable by using 50 µL of rat plasma proving it to be most suitable for regular analysis.[43]

Gopinath S et al developed a LC-MS/MS method to simultaneously quantify LEN and dexamethasone in human plasma which has been used as combination therapy in the treatment of multiple myeloma and to facilitate their combined clinical development. The extraction was carried out with ethyl acetate followed by isocratic elution on a C_{18} coloumn. This method has 90% organic solvent consumption with a shorter run time of 2 min.[44]

Table 7: Estimation of lenalidomide by liquid chromatography mass spectroscopy in biological samples.

S. No.	Internal Standard	Sample preparation, Stationery phase Mobile phase	Flow rate, Detection (m/z) LLOQ, linearity, accuracy, Rt, run time	References
1	Thalidomide	Liquid–liquid extraction X-terra RP C-18 (50´4.6 mm, 5 mm) 10mM Ammonium formate: acetonitrile : glacial acetic acid in ratios of 20:80:0.1 (v/v/v) (pH 3.5)	260.2 → 149.1(lenalidomide) 259.3 → 149.1(thalidomide) LLOQ- 0.78 µM r2 > 0.9989 - Rt-0.69 (lenalidomide) 0.60 (thalidomide) Run time – 1.2 min	Krishna P et al[32]
2	Carbamazepine	Solid-phase extraction, Lichrocart 125-4.Lichrospher 60, RP-Select B, 5 µm Ammonium formate : Acetonitrile (15:85)	1ml/min MRM- 260.08/149.20 (lenalidomide), 237.02/194.40 (carbamazepine) 2ng/ml r^2>0.998 - 1.04 min(lenalidomide) 1.2 min(carbamazepine) 3 min	Hasnain MS et al[37]
3	Fluconazole	Liquid Extraction XTerra RP-18, (4.6 X 50mM, 5µm) 0.1 % Formic acid: Methanol (10:90% v/v)	0.400 mL/min.- 9.999 ng/mL r^{2-}0.9953 94.67% 1.054(lenalidomide) 1.073(fluconazole) 2 min	Ranganathan P et al[38]
4	Fluconazole	Liquid-liquid Extraction Waters C-18 (50 × 4.6 mm) 5 µm 0.1% formic acid & water-methanol (15:85, v/v)	0.5 mL/min. MRM-260.1/148.8(lenalidomide), 307.1/238.0(Fluconazole) 10ng/ml r^2-0.99 98.06 ±1.62% 1.1 min(lenalidomide) 1.12 min(Fluconazole) 2 min	Gopinath R et al[39]
5	Tolbutamide	Liquid-liquid extraction YMC pack ODS AM (150 mm ×4.6 mm, 5 µ m)	1 ml/min SRM-441.18/138.08(ibrutinib) 260.08/149.09(lenalidomide)	Veeraraghavan S et al[40]

		A- acetonitrile: B- 0.1% formic acid buffer, Gradient elutioon (0, 25), (2.0, 90), (5.0, 90), (5.2, 25), (7.5, 25).	475.20/304.07(PCI-45227) 271.10/91.12(Tolbutamide) 0.72 ng/ml(ibrutinib) 1.87 ng/ml(lenalidomide) 0.76 ng/ml(PCI-45227) r^2-0.9981 77.5%(ibrutinib) 81.0 - 87.5% (PCI-45227) 95.6 - 98.8%(lenalidomide) 92.3% (Tolbutamide) All compounds are eluted within 5 min 7.5 min	
6	Genistein	Liquid-liquid extraction Zorbax (Agilent) C-18 column (3.5 µm, 2.1 × 50 mm) ionized water :ACN,each with 0.1% formic acid.	0.4ml/min APCI- 260.06 > 149.10 (lenalidomide), 402.09 > 341.02 (flavopiridol) 1nM (lenalidomide), 0.3nM (flavopiridol) r^2-0.998 99% to 116% 4.38 (lenalidomide), 4.45(flavopiridol) 10 min	Qing Liu MS et al[41]
7	Vindoline	Solid phase extraction Waters XBridge® BEH C-18 column (2.5 µm, 2.1 mm×50 mm) A, 0.1% Formic acid aqueous solution: phase B, CAN (gradient)	0.3ml/min - 4 ng/mL (thalidomide) 2 ng/mL (lenalidomide, Cyclophosphamide, Bortezomib, Dexamethasone, adriamycin) r^2- 0.99 - 1.75 min(thalidomide) 0.76 min(lenalidomide) 2.33min(Cyclophosphamide) 4.39 min(Bortezomib) 4.80 min (Dexamethasone) 3.65 min(adriamycin) 5 min	Shu C et al[42]
8	Tolbutamide	Liquid–liquid extraction C-18column (150 mm × 4.6 mm i.d., 5µm) (YMC®-PACK, JAPAN). A, methanol: B, 0.1% formic acid buffer (70:30v/v).	1ml/min SRM- 416.25/176.48(idelalisib) 286.11/154.10 (fludarabine) 260.15/149.15(lenalidomide) 1.15 ng/mL (idelalisib) 0.95 ng/mL (fludarabine) 0.97 ng/mL (lenalidomide) (r^2)- 0.9996 (idelalisib) 0.9985 (fludarabine) 0.9988 (lenalidomide) 84 to 87% (idelalisib) 53 to 65% (fludarabine) 50 to 53% (lenalidomide) - 4 min	Veeraraghavan S et al[43]
9	Fluconazole	Liquid–liquid extraction XTerra RP-18 column (50 mmx4.6 mm, 5µm) Methanol: water containing 0.1% formic acid (90:10, v/v)0.5 mL/min.	0.5 mL/min MRM- 260.1→148.8(lenalidomide) 393.3→147.0 (dexamethasone) Run time – 2 min	Gopinath S et al[44]

LLOQ-lower limit of quantification;MRM-multiple reaction monitoring; SRM-Selected reaction monitoring; APCI-atmospheric pressure chemical ionization.

Ultra Performance Liquid Chromatography-Mass Spectroscopy (UPLC-MS)

Iqbal M et al's study was the first to describe the determination of LEN in both animal and human plasma by UPLC-MS/MS method. It is based on simple one step protein precipitation for sample preparation and isocratic flow with a short run time of 2.5 min. The proposed method could be practical and reliable and most sensitive having a quantitation limit of 0.23ng/ml thus it will be most useful in the pharmacokinetic and toxicokinetic study for LEN in both animal and humans.[45]

Hadir M. Maher et al's proposed method utilized solid phase extraction along with the super selectivity potential of LC-MS/MS detection for the the simultaneous determination of lenalidomide and dexamethasone in rat plasma and within very short runtime of 1.5 min. This method is the most sensitive reported to date with a limit of quantitation of 0.01ng/mL for both compounds. The proposed method enabled the determination of the pharmacokinetics of both dexamethasone and LEN following their oral administration to rats, whether alone or in combination. This issue is extremely important in therapeutic drug monitoring of patients with multiple myeloma and those suffering from renal impairment.[46]

Table 8: Estimation of lenalidomide by ultra performance liquid chromatography mass spectroscopy in biological samples.

S. No.	Internal Standard	Sample preparation, Stationery phase Mobile phase	Flow rate, Detection (m/z) LLOQ, linearity, accuracy, Rt, run time	References
1	Carbamazepine	Protein precipitation using methanol` Acquity UPLC BEH™ C-18 (50 × 2.1 mm, i.d., 1.7 µm, Waters, USA) acetonitrile: water:formic acid (65:35:0.1%, v/v/v)	0.2 mL/min. MRM- 260.1 > 149.0(lenalidomide) 237.0 > 179.0 (carbamazepine) 0.23ng/mL. r^2>0.98 93.5–113% Rt-0.71 min 2.5 min	Iqbal M et al[45]
2	Domperidone	Solid-phase extraction Acquity UPLC BEH™C-18 column (100 _ 1.0 mm, i.d., 1.7 µm A, 0.1% formic acid in water: 0.1% formic acid in acetonitrile (20 : 80, v/v)	0.2ml/min MRM- 393 > 147(Dexamethasone) 260 > 149(lenalidomide) 0.01ng/ml Linearity(r^2)-0.999(dexamethasone) 0.998(lenalidomide) 87.23–101.73% (dexamethasone) 86.89-106.44% (lenalidomide) 0.38 min (dexamethasone and lenalidomide) 1.5 min	Hadir M. Maher et al[46]

BEH- ethylene bridged hybrid

CONCLUSION

The use of lenalidomide for certain myelodysplastic syndromes and also its combination with dexamethasone in treatment of multiple myeloma makes it clinically significant. Consequently, the development of procedures to carry out pharmacokinetic or bioequivalence studies, as well as control of the drug in pharmacological or biological samples is of great interest in the clinical field. Spectrophotometry and liquid chromatography are the techniques most widely chosen to develop lenalidomide analysis. LC offers simplicity, and the sensitivity may be conveniently adjusted by coupling different detectors. Spectrophotometric methods are attractive due to their low cost and rapidity.

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