Title of the manuscript: A rapid stability indicating HPLC method for the analysis of Leflunomide and its related impurities in bulk drug and formulations

Makalenin başlığı: Leflunomid ve bununla ilgili kirlilik ve formülasyonlardaki ilgili safsızlıkların analizi için HPLC yöntemini gösteren hızlı bir stabilite

Short title: Quantification of Leflunomide and its related impurities by RP_HPLC

Kısa başlık: Leflunomid ve ilgili safsızlıklarının RP HPLC ile kantifikasyonu

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ABSTRACT:

Objectives: Leflunomide (LFNM) is a drug belongs to isoxazole derivative and having immunosuppressive and anti-inflammatory activities. Literature search confirms that there is no method reported for the simultaneous estimation of LFNM and its related impurities A and B in pharmaceutical dosage forms and in bulk drug. Hence the present work aimed to develop a simple stability indicating RP-HPLC method for the separation and quantification of LFNM and its impurities A and B.

Materials and methods: Systematic trails of method condition like mobile phase ratio, pH, flow rate, stationary phase and detector wavelength was performed for the simultaneous analysis of LFNM and its related impurities A and B. The developed method was validated as per the ICH guidelines including forced degradation studies in different stress conditions.

Results: Optimized separation was achieved on Thermo Scientific Hypersil ODS C18 column (250mm×4.6mm; 5µ id) using mobile phase composition of acetonitrile, methanol and 0.1M sodium perchlorate in the ratio of 40:30:30 (v/v); pH 4.6, at a flow rate of 1.0mL/min in isocratic elution. UV detection was carried at wavelength of 246nm. Well resolved peaks were observed with high number of theoretical plates, less tailing factor and reproducible relative retention time and response factor. The

method was validated and all the validation parameters were found to be within the acceptance limit. Stability tests were done through exposure of the analyte solution to five different stress conditions i.e 1 N HCl, 1 N NaOH, 3% H_2O_2, thermal degradation of powder and exposure to UV radiation. The method can successfully separate the degradation products along with both the impurities studied. The % degradation was also found to be less.

Conclusion: The method developed for LFNM is simple, precise and can applicable for the separation and quantification of LFNM and its related impurities in bulk drug and pharmaceutical formulations.

Keywords: Leflunomide, impurity A, impurity B, stress degradation, HPLC.

ÖZET:

Amaçlar: Leflunomid (LFNM), izoksazol türevine ait ve immünsüpresif ve antiinflamatuar aktiviteye sahip bir ilaçtır. Literatür taraması, farmasötik dozaj formlarında ve toplu ilaçlarda LFNM ve bununla ilişkili impüriteleri A ve B'nin tahmini için rapor edilen bir yöntemin olmadığını doğrulamaktadır. Bu nedenle mevcut çalışma, LFNM'nin ve A ve B safsızlık ayrıştırılması ve nicelenmesi için RP-HPLC yöntemini gösteren basit bir stabilite geliştirmeyi amaçlamıştır.

Malzemeler ve yöntemler: LFNM ve ilgili impüriteleri A ve B'nin eşzamanlı analizi için mobil faz oranı, pH, akış hızı, durağan faz ve detektör dalga boyu gibi sistematik koşulların sistematik yolları gerçekleştirildi. Geliştirilen yöntem ICH yönergelerine göre doğrulandı. Farklı stres koşullarında zorlu bozunma çalışmaları dahil.

Sonuçlar: Optimal ayrıştırma, Thermo Scientific Hypersil ODS C18 kolonu (250mm x 4.6mm; 5µ id) üzerinde, mobil fazın asetonitril, metanol ve 0.1M sodyum perklorat bileşimi kullanılarak 40:30:30 (h / h) oranında elde edildi; izokratik elüsyonda 1.0 ml / dak akış hızında pH 4.6. UV saptaması 246 nm dalga boyunda gerçekleştirildi. İyi çözülmüş zirveler, çok sayıda teorik plaka, daha az kuyruklama faktörü ve tekrarlanabilir bağlanma süresi ve tepki faktörü ile gözlenmiştir. Yöntem onaylandı ve tüm doğrulama parametreleri kabul limitinde bulundu. Stabilite testleri, analit çözeltisinin beş farklı stres koşuluna, yani 1 N HCl, 1 N NaOH,% 3 H_2O_2, tozun termal degradasyonuna ve UV radyasyonuna maruz kalması yoluyla gerçekleştirilmiştir. Yöntem, bozunma ürünlerini hem çalışılan kirliliklerle birlikte başarılı bir şekilde ayırabilir. % Degradasyon da daha az bulunmuştur.

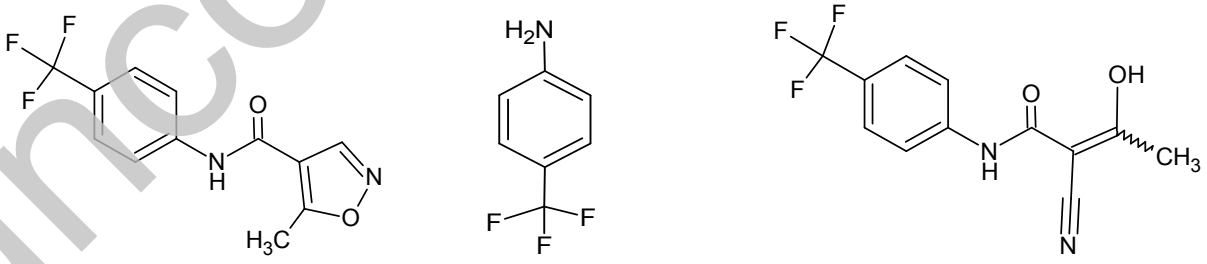
Sonuç: LFNM'nin ayrılması ve miktar tayini için geliştirilen yöntem basit, kesin ve kirliliklerin ayrıştırılması ve analizi için ve toplu ilaç ve farmasötik formülasyonlarda LFNM'nin nicelleştirilmesi için uygulanabilir.

Anahtar kelimeler: Leflunomide, kirlilik A, kirlilik B, stres bozulması, HPLC.

1. INTRODUCTION:

Leflunomide is an isoxazole derivative having both immunosuppressive and anti-inflammatory activities.[1] LFNM acts as dihydroorotate dehydrogenase inhibitor used for the treatment of rheumatoid arthritis.[2,3] It is also used to treat psoriasis, psoriatic arthropathy[4] and other inflammatory conditions like bullous pemphigoid, felty syndrome, sjogren syndrome, wegener granulomatosis, and vasculitis.[5]The most common side effects associated with LFNM are nausea, vomiting, diarrhea, abdominal pain, alopecia, and hypertension.[6]

Literature reveals that few methods are reported for the estimation of LFNM in pharmaceutical formulations using HPLC[7-15], UPLC[16] and Spectrophotometer. [17, 18] Only one HPLC method was reported for the simultaneous estimation of LFNM with other NSAIDs.[19] Few bio analytical methods reported for estimation of LFNM in biological samples using HPLC,[20] HPTLC,[21] and liquid chromatography.[22] The other methods reported were found to be determination of active metabolite of LFNM in biological samples using HPLC. [23-25] No methods reported for the estimation of LFLM and its USP related impurities in pharmaceutical formulations. Hence in this paper we attempt to develop a simple method for the estimation of LFLM (5-methyl-N-[4-(trifluoromethyl) phenyl]-isoxazole-4-carboxamide) and its related impurities A (α,α,α-Trifluoro-p-toluidine, 4-(Trifluoromethyl)aniline, 4-Aminobenzotrifluoride) and B (2-Cyano-3-hydroxy-N-(4-trifluoromethylphenyl) crotonamide) in pharmaceutical formulations. Molecular structure of LFNM and its related compounds in the study are given in Figure 1.



LFNM Impurity A Impurity B

Figure 1: Molecular structure of LFNM and its related compounds in the study

2. MATERIALS AND METHODS:

2.1 Instrumentation:

The separation and estimation of LFNM with impurities A and B was done on PEAK HPLC (India) system. Mobile phase was pumped in to column using LC-P7000 isocratic pump. 20 µL fixed volume sample was injected for the analysis using rheodyne injector (model 7725) with fixed 20µl loop. Variable wavelength programmable (Waters – 486) UV-Visible detector was used for detecting the compounds. The detector response signals were monitored and integrated using Young Lin Autochro -3000 software (Korea). Samples were injected using Hamilton (USA) manual HPLC syringe. Double beam UV-Visible spectrophotometer (Teccomp UV-2301 – India) was used for spectral analysis. Denver electronic analytical balance (SI-234) was used for weighing the standards and samples. pH of the mobile phase was adjusted by using Systronics (India) digital pH meter (Sr No S 1326).

2.2 Chemicals and Reagents:

The active pharmaceutical ingredient LFNM with 99.20% purity and its two impurities A and B were obtained as gift sample from Torrent Pharmaceuticals Limited, Secundrabad, Telangana, India. The marketed formulation of LFNM (Lefno© – 10mg) was purchased in local pharmacy. Laboratory reagent grade sodium perchlorate monohydrate ($NaClO_4.H_2O$) and perchloric acid ($HClO_4$) are purchased from SD fine chem. Limited, Mumbai. HPLC grade methanol, acetonitrile and water were purchased from Merck chemicals, Mumbai. 0.2 µ nylon membrane filter papers were used for filtration of samples and mobile phase and were purchased from Millipore (India).

2.3 Preparation of solutions:

2.3.1 *Sodium perchlorate solution (0.1M)*:

14.046g of $NaClO_4.H_2O$weighed accurately and was dissolved in 500 mL water (HPLC grade). Then it was sonicated for 2-5 min to dissolve the compound completely in water. The final volume was made the mark in a 1000 mL volumetric flask using water. The solution was filtered through 0.45 micron nylon membrane filter paper.

2.3.2 *Perchloric acid solution (0.1M)*:

70% perchloric acid having molarity 11.6 M was used for the preparation of 0.1 M solution. 8.6 mL $HClO_4$ was pipetted and was further made up to 1000 mL using water. The solution sonicated and filtered through 0.45 micron nylon membrane filter paper.

2.3.3 *Standard drug and impurity solutions*:

100 mg of standard drug LFNM was weighed accurately and was taken in a 100 mL volumetric flask. The drug was dissolved in approximate 75 mL of methanol. Then the final volume was made up to 100 mL with methanol. LFNM standard stock solution of 1000 µg/mL was obtained. 1ml from 1000 µg/mL was accurately pipetted in to a 100 mL volumetric flask and the final volume was made up to the mark to get LFNM working standard solution of 10 µg/mL.

The procedure explained for the preparation of LFNM standard solution was followed for the preparation of 10 µg/mL Imp A and Imp B separately. 10 mL of LFNM, Imp A and Imp B were mixed separately and the mixture solution was used for method development.

2.3.4 *Formulation solution*:

Ten tablets of LFNM (Lefno© – 10 mg) were powdered using sterile mortar and pestle to get a fine powder. From the tablet powder an amount of drug equivalent to 10 mg of LFNM was weighed accurately and was dissolved in 10 mL methanol. Keep the solution in an orbital shaker for 15 min to dissolve the drug completely in solvent. Then it was filtered through 0.45 micron nylon membrane filter paper. Sample solution containing 1000 µg/mL LFNM was obtained. The sample stock solution was further diluted to get working sample solution having LFNM concentration of 250 µg/mL. This solution was used for the identification and estimation of LFNM and its impurities A and B in pharmaceutical formulations.

2.4 Method development:

The standard drug solution containing 10 µg/mL concentrations of both impurities and LFNM was initially used for method development studies. The wavelength of detector was maintain based on the iso-absorption wavelength obtained in UV spectrophotometer for LFNM, impurity A and B. System suitability, resolution, responses factor, peak symmetry are the key factors that are keep in consideration for optimization of mobile phase. Mobile phase was confirmed by change in different solvent ratios, strength of organic modifiers and pH. Separation was performed on different column configurations and manufactures. Flow rate of the mobile phase also changed in order to get better resolution. The conditions that give best resolution, response and peak symmetry and considered as suitable conditions and these conditions were further validated for the applicability of the method for the estimation of LFNM and its related impurities A and B in pharmaceutical formulations.

2.5 Method Validation:

The method was validated as per ICH guidelines [13].

2.5.1 System suitability:

System suitability tests were carried out on a freshly prepared standard solution at three concentrations (10, 20 and 30 µg/mL) of the LFNM, Imp A and Imp B to scrutinize the various optimized parameters such as retention time, relative response factor, resolution, tailing factor and USP plate count.

2.5.2 Linearity and range:

Standard calibration curves were prepared with six calibrators over a concentration range of 0.5–3.0 µg/mL for LFNM, Imp A and B. the solutions were analyzed in triplicates in the optimized conditions. The data of peak area vs drug concentration were treated by linear least square regression analysis.

2.5.3 Precision:

Precision was carried by six standard solutions containing 2 µg/mL of LFNM, Imp A and B were prepared and were analyzing in the optimized method conditions. For intraday precision, the solutions were prepared and analyzed six times in the same day at different time intervals and for interday precision, the solutions were analyzed

in three different days. Peak area responses of six replicate analyses were calculated in terms of relative standard deviation (RSD).

2.5.4 Ruggedness:

Ruggedness of the method was studied by different analysts analyzing standard solutions containing 8 µg/mL of LFNM, Imp A and B in the optimized conditions in the same laboratory conditions. %RSD of Peak area responses of six replicate analyses were calculated.

2.5.5 Robustness:

Robustness of the proposed method was carried out by the slight variation in optimized method conditions. Change in ±5 nm of detector wavelength, ± 5mL variation in mobile phase organic and pH modifier, ±0.1 mL mobile phase flow rate and ±0.1 factor of pH was studied. In each of the changed condition, standard solution containing 8 µg/mL of LFNM, Imp A and B were analyzed in triplicate. The % of change was calculated.

2.5.6 Recovery:

Standard addition method was carried for determining the accuracy of the method. 50%, 100% and 150% level concentrations were spiked to a known concentration of 1 µg/mL. Accuracy was determined by comparing the difference between the spiked value and actual found value.

2.5.7 Force degradation studies:

Acid Hydrolysis:

50 mg of drug was mixed with 50 mL of 0.1 N HCl solutions. After the incubation of 12hours (AH 1) and 24 hours (AH 2), the sample solution was neutralized and diluted up to standard concentration of 250 µg/mL and was analyzed in the developed method conditions.

Base Hydrolysis:

50 mg of drug was mixed with 50 mL of 0.1 N NaOH solutions. After the incubation of 12hours (BH 1) and 24 hours (BH 2), the sample solution was neutralized and

diluted up to standard concentration of 250 µg/mL and was analyzed in the developed method conditions.

Oxidative Degradation:

50 mg of drug was mixed with 50ml of 3% H_2O_2 solution. After the incubation of 12hours (OD1) and 24 hours (OD2), the sample solution was neutralized and diluted up to standard concentration of 250 µg/mL and was analyzed in the developed method conditions.

Photolytic Degradation:

50 mg of drug sample was kept in UV light (254 nm). After the incubation of 12hours (PD 1) and 24 hours (PD2), the sample solution was neutralized and diluted up to standard concentration of 250 µg/mL and was analyzed in the developed method conditions.

Thermal Degradation:

50 mg of drug sample was kept in oven at 60^0C. After the incubation of 12hours (TD 1) and 24 hours (TD 2), the sample solution was neutralized and diluted up to standard concentration of 250 µg/mL and was analyzed in the developed method conditions.

2.5.8 Formulation analysis:

250 µg/mL formulation sample solution that prepared from marketed formulation tablets of LFNM (Lefno© – 10 mg) was analyzed triplicates in the optimized conditions. The peak area response obtained in the formulation analysis was used for the determination of applicability of the developed method for the estimation of LFNM in pharmaceutical formulations.

3. RESULTS AND DISCUSSIONS:

The present work is aimed to develop a simple, accurate RP-HPLC-UV method for the quantification of LFNM and its related impurities A and B in pharmaceutical formulations. Literature survey reveals that there is no method was reported previously for the separation, qualitative and quantitative analysis of LFNM and its related impurities A and B. Hence the attempt made here is found to be novel and having significant importance in simultaneous detection and quantification of LFNM and its related impurities A and B.

Mobile phase was confirmed by change in different solvent ratios, expected peak shape, resolution was achieved using mobile phase composition of acetonitrile, methanol and 0.1 M sodium perchlorate in the ratio of 40:30:30 (v/v). The pH of the mobile phase was adjusted to 4.6 using 0.1 M perchloric acid. Mobile phase was pumped at a flow rate of 1.0 mL/min in isocratic elution. UV detection was carried at wavelength of 246 nm and separation was achieved on Thermo Scientific Hypersil ODS C18 column (250 mm×4.6 mm; 5 µ id). In the optimized conditions, well retained, resolved and symmetric peaks are observed in the standard chromatogram contains 10 µg/mL of LFNM, imp A and B. Standard chromatogram obtained in the optimized conditions was given in Figure 2. The blank analysis was performed by analyzing the mobile phase and confirms that there is no detection was observed in the blank chromatogram (Figure 3). This proved that the method developed was found to be specific and no mobile phase interference was observed in the chromatogram.

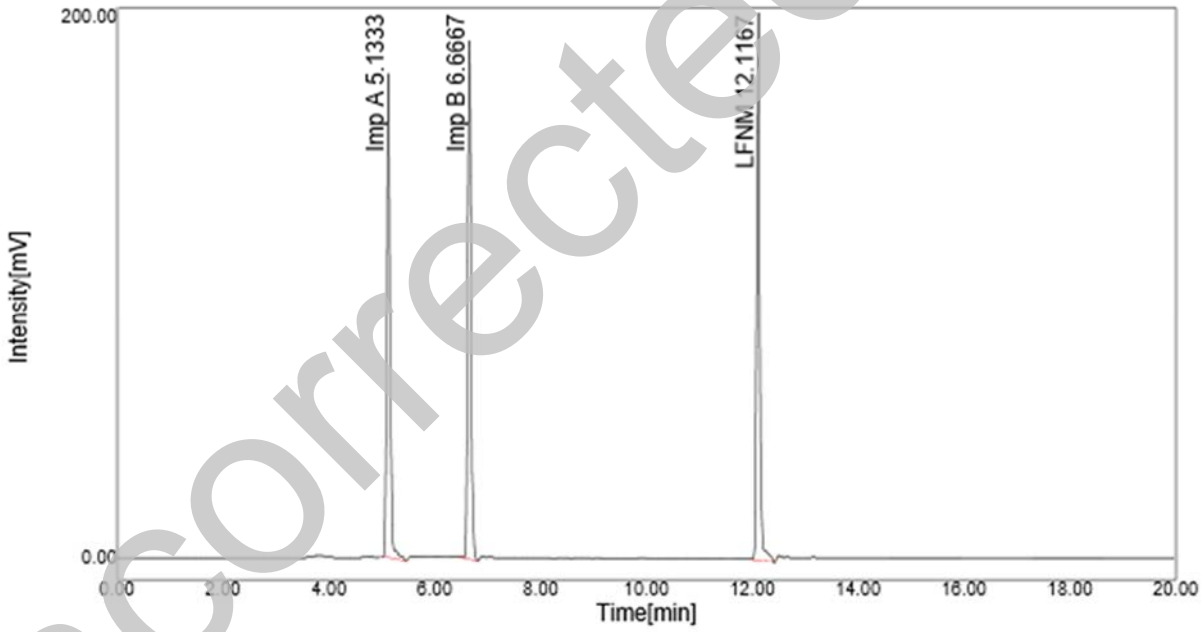


Figure 2: Standard chromatogram of LFNM, impurity A and B at a concentration of 10 µg/mL

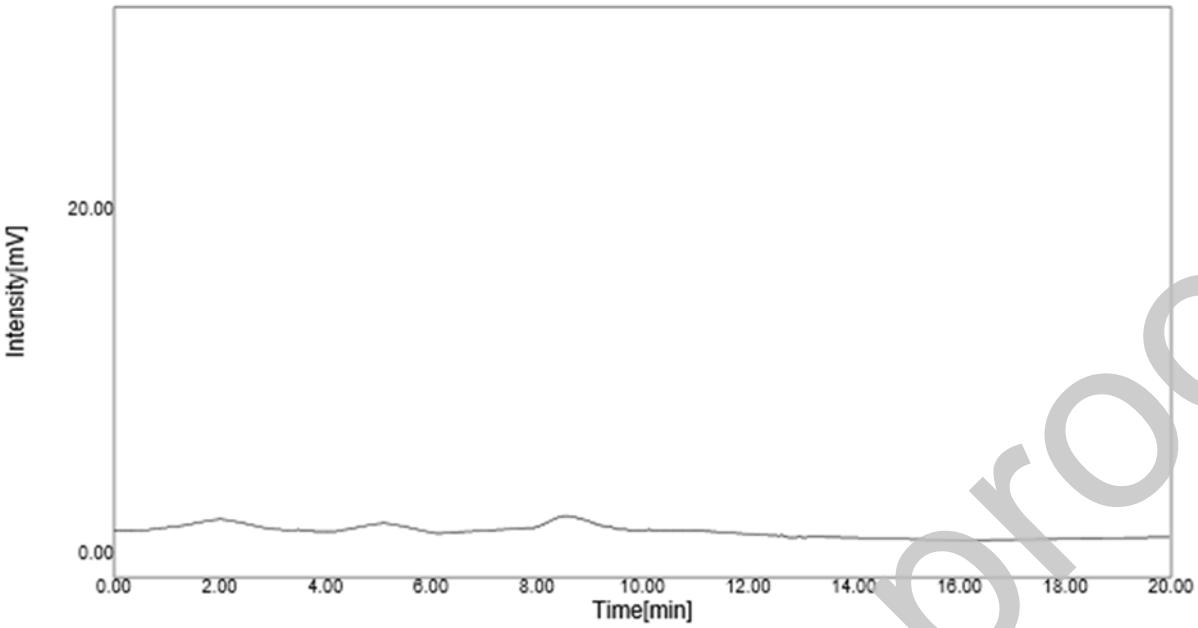


Figure 3: Blank (Mobile phase) chromatogram of LFNM, impurity A and B

Prior to validation of the developed method, repeatability and system suitability was determined at standard solution concentrations of 10, 15 and 20 µg/mL. The standard solutions were prepared and were analyzed in the developed method conditions in triplicates. The system suitability conditions like plate count, asymmetric factor, resolution was determined and was found to be within the acceptance limit. The response factor (RF), relative response factor (RRF) and relative retention time (RRT) were also calculated and found to be reproducible. Hence the developed method was found to be reproducible and all the system suitable parameters are in the acceptable limits (Table 1).

Table 1: System suitability results

Compound	Concentration in µg/mL	Retention Time (min)	RRT	RRF	Theo plate*	Tail Factor*	Resolution*
LFNM	10	12.109±0.008	---	---	9933	0.745	11.524
	15	12.155±0.019	---	---	9862	0.740	11.607
	20	12.144±0.010	---	---	9863	0.757	11.850
Impurity A	10	5.132± 0.001	0.423±0.004	0.649±0.005	4270	1.157	---
	15	5.133± 0.028	0.422±0.002	0.647±0.001	4277	1.160	---
	20	5.137±0.010	0.423±0.003	0.651±0.002	4333	1.163	---
Impurity B	10	6.654±0.018	0.050±0.002	0.843±0.001	6055	1.540	5.980
	15	6.672±0.009	0.549±0.001	0.834±0.005	6157	1.485	5.877
	20	6.611±0.009	0.544±0.003	0.846±0.002	6086	1.540	5.893

*Values given in table are the average values of three replicate experiments

Six points linear calibration curve was obtained in the concentration range of 0.5-3.0 µg/mL. Linear regression equation was fund to be y = 50344x + 2396.2 (R = 0.999), y = 33020x - 169.16 (R = 0.999), y = 42853x + 606.76 (R = 0.999) and for LFNM, imp A and B respectively. Very high correlation coefficient value (more than 0.999) was observed for LFNM and both impurities confirm that the method follows linear relation accurately within the concentration range studied. Linearity results were given in Table 2 and calibration curve was shown in Figure 4.

Table 2: Linearity results

S No	Concentration in µg/mL	Peak Area observed		
		Imp A	Imp B	LFNM
1	0.5	16243±198.596	22220±314.079	26647±185.176
2	1	32607±533.094	42538±227.027	52314±254.161
3	1.5	50051±109.768	65490±173.463	80610±109.587
4	2	65467±360.266	86523±253.997	102530±1945.648
5	2.5	82575±117.091	108259±1057.730	128587±2427.108
6	3	98748±152.533	128567±761.748	152506±1489.122

*Values given in table are the average ± standard deviation of three replicate experiments

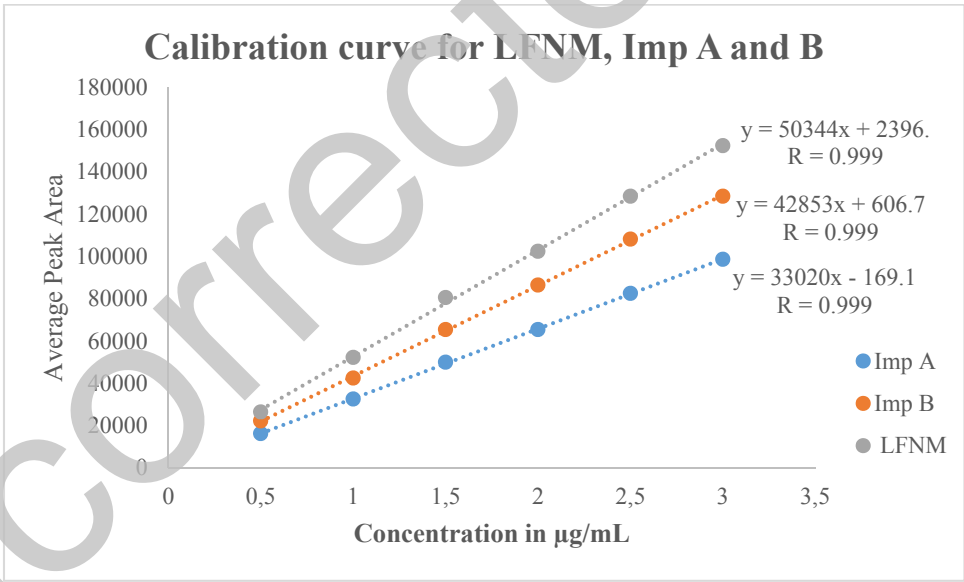


Figure 4: Linear calibration curve for LFNM, Imp A and B in the developed method

Precision of the developed method was carried by analyzing the standard solution at a concentration of 2.0 µg/mL. The solution was analyzing six times in the same day for intraday precision, three different days for interday precision. Preparing and analyzing the same concentration solution three days by three different analysts for

ruggedness. The %RSD was calculated and was found to be 0.383, 0.394, 0.915 in intraday precision, 0.258, 0.236, 0.281 in interday precision and 0.578, 0.458, 0.491 in ruggedness study for impurity A, B and LFNM respectively. Results confirm that the developed method was found to be rugged and precise.

Robustness was carried by analyzing the standard solution in the optimized conditions that are changed deliberately. The % change in each changed conditions was calculated and was found to be less than 2 (Table 3). This confirms that small change in the analytical conditions did not influence the chromatographic separation and detection of LFNM and its related impurities A and B. Hence the method was found to be robust.

Table 3: Robustness results

S No	Condition	Robustness at 2 µg/mL					
		Imp A		Imp B		LFNM	
		Peak Area	% Change	Peak Area	% Change	Peak Area	% Change
1	Optimized	65467	---	86523	---	102530	---
2	MP 1	65633	0.253	85901	0.719	102981	0.439
3	MP 2	65782	0.481	85257	1.464	103102	0.557
4	WL 1	64990	0.728	85637	1.024	103392	0.840
5	WL 2	64628	1.281	86030	0.569	103782	1.221
6	pH 1	65125	0.522	86132	0.451	103075	0.531
7	pH 2	64528	1.434	85998	0.606	102903	0.363
8	FR 1	64593	1.335	85813	0.820	102745	0.209
9	FR 2	64520	1.446	86121	0.464	102134	0.386

MP (Mobile Phase) 1: acetonitrile, methanol and 0.1 M sodium perchlorate in the ratio of 34:35:30 (v/v), MP 2: acetonitrile, methanol and 0.1 M sodium perchlorate in the ratio of 45:25:30 (v/v); WL (Wavelength) 1: 241 nm, WL 2: 251 nm; pH 1: 4.5, pH 2: 4.7; FR (Flow rate) 1: 0.9 mL/min, FR 2: 1.1 mL/min

Accuracy of the method was determined spiked recovery studies. 50%, 100% and 150% concentrations were spiked and analyzed in triplicates to a known concentration of 1 µg/mL. The % recovery was found to be within the acceptance limit of 98-102% (Table 4). The %RSD in each spiked level was calculated and was found to be within the acceptance limit of <2. Hence the proposed method was found to be accurate.

Table 4: Recovery results

S No	Compound	Recovery level	Concentration in µg/mL				% Recovery*
			Target	Spiked	Final	Amount Recovered*	
1	Imp A	50%	1	0.5	1.5	1.491±0.003	99.359±0.165
2		100%	1	1	2	1.980±0.010	99.011±0.522
3		150%	1	1.5	2.5	2.464±0.009	98.548±0.373
4	Imp B	50%	1	0.5	1.5	1.489±0.004	99.254±0.243
5		100%	1	1	2	1.979±0.002	98.977±0.116
6		150%	1	1.5	2.5	2.487±0.004	99.504±0.183
7	LFNM	50%	1	0.5	1.5	1.497±0.006	99.803±0.409
8		100%	1	1	2	1.974±0.003	98.699±0.141
9		150%	1	1.5	2.5	2.495±0.017	99.838±0.685

*Values given in table are the average ± standard deviation for three replicate experiments

Standard drug was exposed in different stress conditions and was analyzed in the optimized conditions and the results were compared with un-stressed standard (Figure 5). The % degradation was found to be very high in acid degradation study. In this condition the drug was found to be degraded up to 2.518% in 12 h and 10.808% in 24 h of stress expose. The number of degradation products was also found to be high in this condition. Three additional peaks along with both impurities and LFNM were detected in acid condition (Figure 6). In base degradation study, the drug was found to be degraded up to 2.19% and 8.619% in 12 and 24 h of stress expose respectively. One additional peak in 12H and two additional peaks in 24H (Figure 7) stress expose along with LFNM and both impurities were detected in base conditions. In oxidative degradation, very less degradation of 1.14% was observed in 12 H with no additional degradation compounds whereas after 24 H one additional peak was detected and the % degradation was found to be 5.289 (Figure 8). In photolytic degradation, 3.13% degradation with one additional detection was observed at 12H and two additional degradation products with % degradation of 8.887 (Figure 9) was observed. In thermal degradation, 5.581% degradation was observed and the chromatogram show one additional detection along with LFNM and both impurities (Figure 10).In all degradation conditions, the % degradation was found to be increase with increase in time. The % degradation and the formation of number of degradation products increased with increase in the stress degradation time from 12 H to 24 h in all the conditions studied. The additional detection observed in the stress degradation study along with LFNM and both impurities

studied may be unknown impurities formed or the compound degraded due to the stress study. These additional compounds were not observed in standard unstressed chromatogram. Both the impurities and the degradation products were successfully separated in the optimized conditions and hence the method can separate and quantify the potential impurities in LFNM. Forced degradation results were given in Table 5.

Table 5: Forced degradation results

S No	Condition	No of additional peaks observed	Peak Area	% Amount Remained	% Amount Degraded
1	AH 1	1	12371397	97.481	2.518
2	AH 2	3	11319358	89.191	10.808
3	BH 1	1	12412803	97.807	2.192
4	BH 2	2	11597145	91.380	8.619
5	OD 1	0	12546917	98.864	1.136
6	OD 2	1	12019823	94.710	5.289
7	PD 1	1	12293071	96.864	3.135
8	PD 2	2	11563280	91.113	8.887
9	TD 1	0	12481397	98.350	1.652
10	TD 2	1	11982822	94.419	5.581

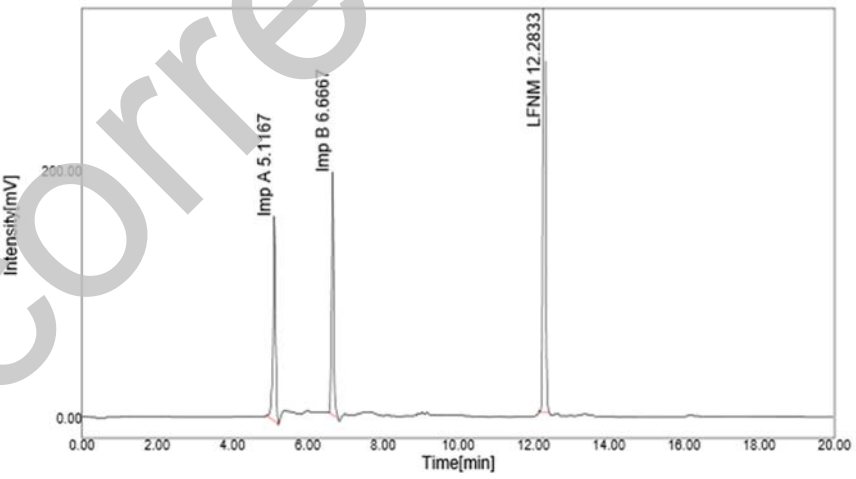


Figure 5: Unstressed (Standard) chromatogram of LFNM

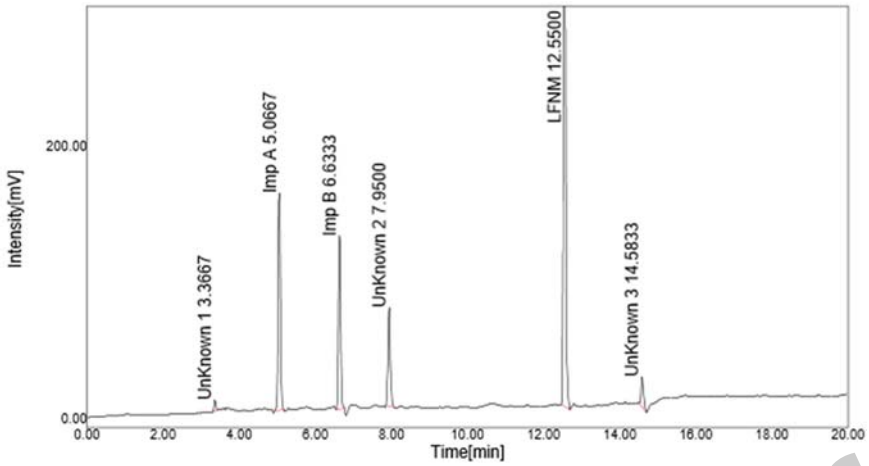


Figure 6: Acid degradation chromatogram of LFNM

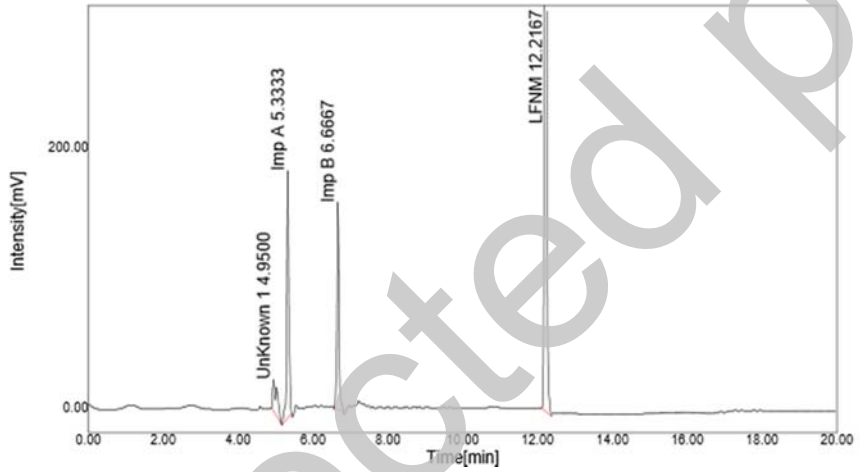


Figure 7: Base degradation chromatogram of LFNM

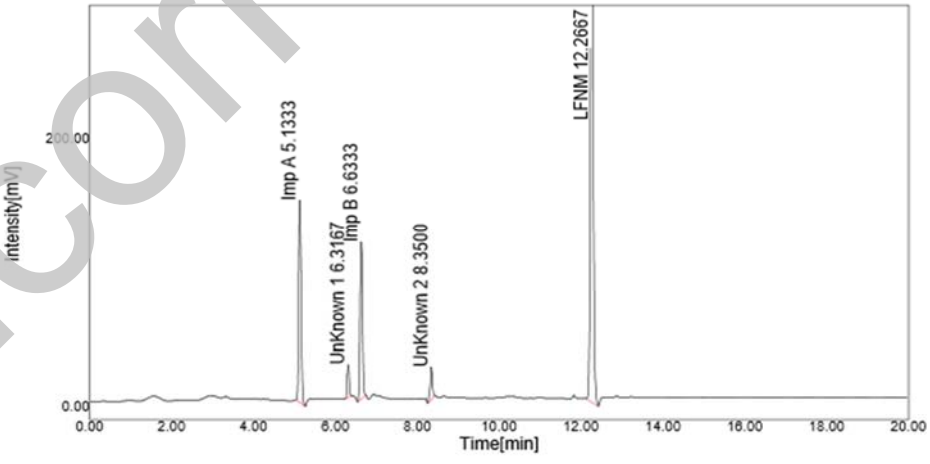


Figure 8: Oxidative degradation chromatogram of LFNM

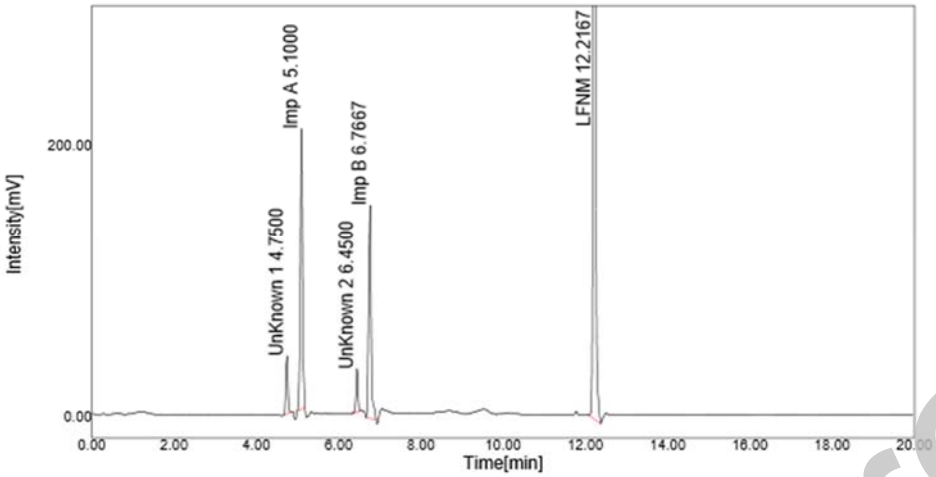


Figure 9: Photolytic degradation chromatogram of LFNM

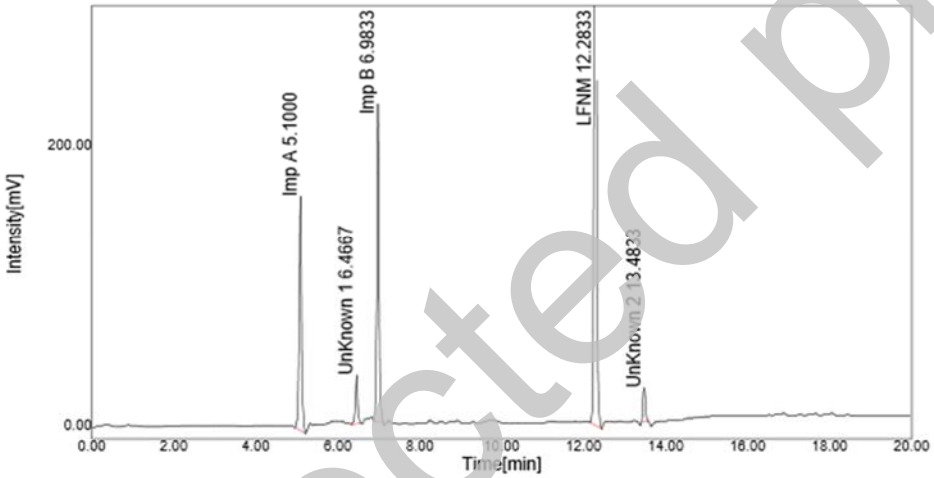


Figure 10: Thermal degradation chromatogram of LFNM

The formulation sample solution of LFNM was analyzed in the developed method conditions in triplicates. The peak area response of LFNM was used for determination of the applicability of the developed method for the analysis of LFNM in pharmaceutical formulations. Standard regression equation was used for the determination of formulation assay and the % assay was found to be 98.735%. In the formulation chromatogram both the impurities were detected (Figure 11) and other chromatographic impurities and formulation excipients did not interfere the results. Hence the developed method was found to be suitable for the quantification of LFNM and can separate and analyze impurities A and B

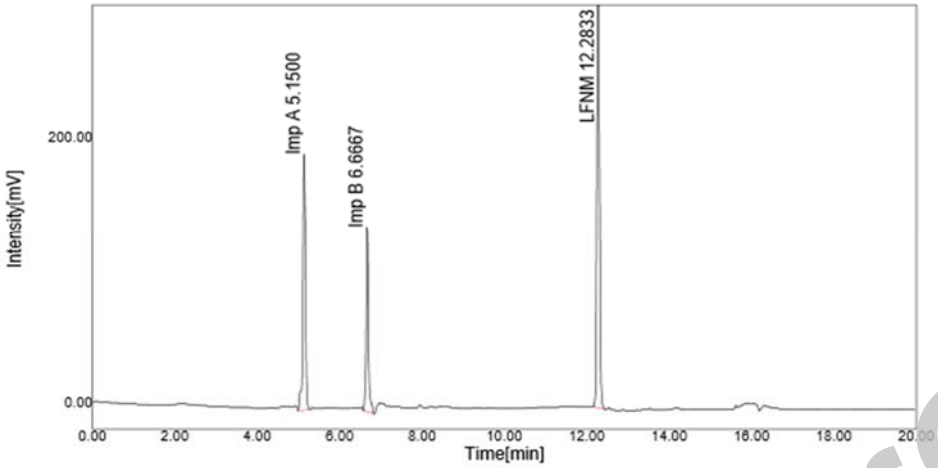


Figure 11: Formulation chromatogram of LFNM (Lefno© – 10 mg)

4. CONCLUSION:

A simple, validated and fast stability indicating HPLC method is established for quantification of LFNM and its potential USP impurities A and B. In literature no method was found to be established for the simultaneous quantification of LFNM and its potential impurities A and B. Hence the method represents the first report about a stability indicating method for the determination of LFNM in the presence of impurities. The proposed method achieves satisfactory separation of LFNM from impurities and the degradation products, extended linear range and rapid analysis time. A high recovery of LFNM in formulation was obtained. The proposed method ensured a precise and accurate determination of LFNM in pharmaceutical formulations. The excipients present in the formulation were not interfering in the method. Hence the method is simple, convenient and suitable for analyzing LFNM in bulk and in pharmaceutical formulations in presence of its potential impurities A and B.

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