

Development and Validation of an Ultra High-Performance Liquid Chromatographic Method with Ultraviolet Detection for Simultaneous Quantification of Filgrastim and Motixafortide in Human Plasma

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Abstract

Multiple myeloma is a hematologic malignancy characterized by the uncontrolled proliferation of malignant plasma cells in the bone marrow. Filgrastim and motixafortide are used in combination to mobilize hematopoietic stem cells for collection prior to autologous stem cell transplantation, an important component of therapy for eligible patients with multiple myeloma. Accurate quantification of these agents in plasma is essential for therapeutic drug monitoring and bioequivalence studies. The objective of current work is to develop and validate a simple, accurate, and cost-effective ultra-high-performance liquid chromatographic method for the simultaneous estimation of filgrastim and motixafortide in human plasma. Protein precipitation was used for sample preparation. Chromatographic separation was achieved on a CHS C18 column with a mobile phase of 0.01 M ammonium acetate buffer pH 4.8 and acetonitrile (40:60, v/v) at a flow rate of 0.3 mL/min. Metformin was employed as the internal standard, and UV detection was performed at 220

nm. The retention times of filgrastim, motixafortide were 1.638 and 2.031 min., respectively. The method was linear in the range of 5–200 ng/mL for filgrastim and 100–4000 ng/mL for motixafortide, with regression coefficients of 0.9993 and 0.9996, respectively. The lower limit of quantification was 5 ng/mL for filgrastim and 100 ng/mL for motixafortide. Accuracy values were within the range of 98.2% to 101.8%, and precision, evaluated as the percentage coefficient of variation, was below 2.0%. Mean recovery exceeded 95% for both analytes and the internal standard. The developed method is suitable for the simultaneous quantification of filgrastim and motixafortide in human plasma.

Keywords: Bioanalysis; Filgrastim; Human Plasma; Motixafortide; Ultra High-Performance Liquid Chromatography.

Introduction

Multiple myeloma (MM) is a cancer of plasma cells that infiltrates bone marrow, causing pancytopenia and bone lesions with lytic characteristics. MM is the second most com-

Development and validation of an ultra-high-performance liquid chromatographic method with ultraviolet detection for simultaneous quantification of Filgrastim and Motixafortide in human plasma

mon hematologic malignancy worldwide (1) and accounts for approximately 13% of all hematologic malignancies (2). Clinical features include bone pain from fractures due to bone lesions, renal failure, anemia-related fatigue, and recurrent infections (3). Historically, median survival was 24 to 30 months (4), but advances in treatments like autologous stem cell transplantation (ASCT), monoclonal antibodies (mAbs), immunomodulatory drugs (IMiDs), and proteasome inhibitors (PIs) have enhanced overall longevity. ASCT relies on effectively mobilizing hematopoietic stem and progenitor cells (HSPCs), often achieved through colony-stimulating factors (CSFs) or chemotherapy (5).

In September 2023, the FDA authorized the application of filgrastim (Neupogen) and motixafortide (Aphexda) in combination for stem cell mobilization therapy in multiple myeloma residents prior to autologous stem cell transplantation (6). Motixafortide led to the sufficient mobilization of stem cells in approximately 92% of residents across two apheresis processes, when compared with 26% of residents receiving filgrastim alone (7, 8). Filgrastim is another medication used to help with Stem cell mobilization before its collection. A peptide inhibitor named motixafortide, as an orphan drug, has been granted for treating pancreatic cancer and acute myeloid leukemia (9). It inhibits CD184, or chemokine receptor CXCR4, which is utilized in patients suffering from multiple myeloma to mobilize hematopoietic stem cells before they are collected and autologously transplanted. Motixafortide's molecular formula is $C_{97}H_{144}F-$

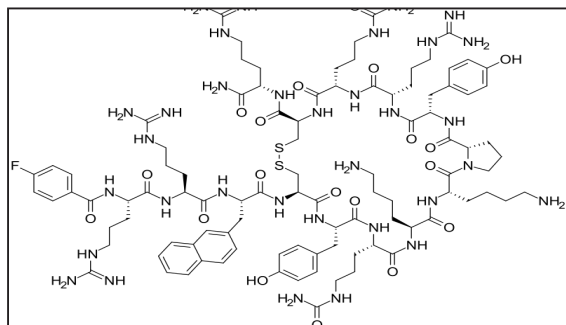


Figure 1. Chemical Structure of Motixafortide

$N_{33}O_{19}S_2$. Its molecular weight is 2159.6 Da. Figure 1 shows its chemical structure.

Motixafortide functions as an inhibitor of chemokine receptor, specifically binding to the chemokine receptor type 4 (CXCR₄). This prevents stromal cell-derived factor 1 from binding to the CXCR₄ receptor and subsequent receptor activation, which may lead to a reduction in tumor cell migration and proliferation. Additionally, suppression of CXCR₄ may cause hematopoietic cells from the bone marrow to migrate into the bloodstream (10). Dosing is 1.25 mg/kg subcutaneously (over about 2 minutes) based on real body weight. 1–14 hours before starting the initial apheresis. If necessary, a subsequent dose can be given 10–14 hours prior to the third apheresis.

Recombinant DNA technology was utilized to synthesize filgrastim, a short-acting granulocyte colony-stimulating factor (G-CSF) analog. It is non-glycosylated, in contrast to endogenous G-CSF, and has an N-terminal methionine inserted into the sequence for E. coli production. Its amino acid sequence is comparable to that of endogenous G-CSF (11). Filgrastim has the molecular formula of $C_{845}H_{1339}N_{223}O_{243}S_9$ and a protein average weight of 18800 Da. Figure 2 illustrates its chemical structure.

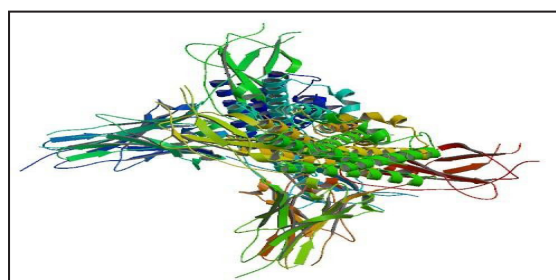


Figure 2. Structure of Filgrastim

The injection of filgrastim is used to treat neutropenia, which is usually brought on by cancer medications. It is a synthetic version of a chemical called colony-stimulating factor that our body naturally produces. Filgrastim aids in the generation of new white blood cells in the marrow.

Filgrastim increases blood neutrophil numbers by imitating the biological effects of granulocyte colony-stimulating factor (G-CSF) (12). It is used therapeutically for a multitude of purposes, including managing infections and preventing febrile neutropenia in patients undergoing myelosuppressive chemotherapy or radiation therapy. One should provide filgrastim at a dosage of 10 µg/Kg subcutaneously or by a brief IV infusion lasting 15 to 30 minutes, four days before the first motixafortide dose and one day before each apheresis.

The FDA's approval of numerous novel medications for various kinds of cancer. It highlights the significance of precisely estimating the drug concentration in patients' bloodstreams. As a result, effective analytical methods are needed to track the medication's content in biological fluids. Ultra-performance liquid chromatography (UPLC) is a frequently preferred analytical technique for the estimation of drugs in biological matrices, and it is a potent instrument.

An extensive literature review reveals that only one LC-MS/MS bioanalytical method (13) has been reported for the simultaneous quantification of motixafortide and filgrastim. Various techniques have been employed for filgrastim quantification, including size exclusion chromatography (SEC) (14-15), ultraviolet (UV) spectroscopy, reverse-phase ultra-fast liquid chromatography (RP-UFLC) (16), cation exchange chromatography (CEX), nuclear magnetic resonance (NMR) studies (17), capillary zone electrophoresis (CZE), and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (18-20). Although liquid chromatography coupled with tandem mass spectrometry offers excellent sensitivity for bioanalysis, its high instrumentation and operational costs may limit routine application in many laboratories. Ultraviolet detection remains widely accessible and economical. Both filgrastim and motixafortide exhibit measurable UV absorbance, making ultraviolet detection a feasible alternative when combined with optimized sample preparation and chromatographic condi-

tions. Therefore, the development of a sensitive ultra-high-performance liquid chromatographic method with ultraviolet detection may provide a practical approach for routine quantitative analysis. The method was developed and validated in accordance with ICH M10 bioanalytical method validation guidelines (21).

Material and Methods

Instrumentation

A Waters UPLC system provided with the column CHS C18 [1.8 µm particle size, L × I.D. 15 cm × 2.1 mm] and tunable UV detector (Empower 2 software) was used for analysis. The mobile phase was composed of acetonitrile and 0.01M ammonium acetate buffer in a 60:40 (v/v) ratio, adjusted to pH 4.8, and was delivered at a flow rate of 0.3 mL/min. Detection was carried out at a wavelength of 220 nm.

Materials and reagents

Filgrastim and motixafortide active pharmaceutical ingredients (APIs) were received as gift samples. An internal standard (IS) was obtained from Akrivis Pharma Pvt. Ltd., Telangana, India. Acetonitrile, methanol, Ammonium acetate, Acetic acid and other required chemicals were purchased from Rankem and Avantor Performance Material India Limited.

Bioanalytical method development

The method development and validation was performed in accordance with ICH M10 bioanalytical method validation guidelines.

Mobile phase and diluent preparation

The mobile phase was composed of ammonium acetate buffer pH 4.8 with diluted acetic acid and acetonitrile in a 40:60 (v/v). The mobile phase was used as diluent.

Standard and stock solution preparation

Primary stock solutions were prepared at concentrations of 200 µg/ml of motixafortide and 10 µg/ml for filgrastim using the diluent, followed by sonication and filtration. Working stocks were then prepared over the concentration ranges of 1-40 µg/mL of motixafortide and 0.05-2.0 µg/mL for filgrastim. The internal stan-

dard (IS), metformin, was prepared at a concentration of 10 µg/mL.

Preparation of calibration curve (CC) standards and quality control (QC) samples

QC samples were prepared by spiking blank plasma with appropriate working stock dilutions of the analytes to produce 0.005 µg/mL Lower Limit of Quantification (LLOQ), 0.01 µg/mL, 0.15 µg/mL Low Quality Control (LQC), 0.04 µg/mL, 0.1 µg/mL Medium Quality Control (MQC), 0.12 µg/mL, 0.16 µg/mL High Quality Control (HQC), and 0.2 µg/mL Upper Limit of Quantification (ULOQ) of filgrastim and 0.1 µg/mL (LLOQ), 0.2 µg/mL, 0.3 µg/mL (LQC), 0.8 µg/mL, 2.0 µg/mL (MQC), 2.4 µg/mL, 3.2 µg/mL (HQC), and 4.0 µg/mL (ULOQ) of motixafortide.

Extraction protocol for bioanalytical sample preparation

Drug extraction from human plasma was carried out using the protein precipitation method. In this procedure, 750 µL of plasma was mixed with 500 µL of internal standard solution and 250 µL of diluent. The mixture was then cyclomixed for 15 seconds to ensure the proper mixing, followed by the addition of 1.5 mL of acetonitrile and vortexing for 2 minutes. The mixture was centrifuged at 3200 rpm for 5 minutes, and the resulting supernatant was filtered using a 0.45 µm polyvinylidene difluoride filter. A 10 µL aliquot of the filtered sample was injected into the UPLC system for analysis.

Results and Discussion

Method Development and Optimization: Several chromatographic trials were performed using different stationary phases and mobile-phase compositions to achieve adequate separation of filgrastim, motixafortide, and the internal standard. Initial experiments using HSS and CHS C18 columns with phosphate and orthophosphoric acid buffer systems resulted in either poor peak shape, insufficient resolution, or longer retention times. Further optimization was carried out by varying the aqueous buffer composition and organic solvent ratio. The final chromatographic conditions employed a CHS

C18 column with ammonium acetate buffer and acetonitrile, which provided well-resolved and symmetrical peaks with acceptable system suitability parameters. Under the optimized conditions, metformin, filgrastim, and motixafortide were eluted at retention times of approximately 1.315, 1.638, and 2.031 min, respectively, with satisfactory resolution and peak shape. The optimized method was therefore selected for subsequent validation and bioanalytical studies.

Optimized chromatographic conditions

Chromatographic separation was performed on a CHS C18 column [1.8 µm particle size, L × I.D. 15 cm × 2.1 mm] using a mobile phase of 0.01 M ammonium acetate buffer: acetonitrile is taken (40:60, V/V) at a flow rate of 0.3 mL/min and injection volume of 10 µL. Metformin is used as an internal standard. Chromatographic analysis was performed at a column temperature of 30°C with UV detection at 220 nm and a total run time of 4 min. The calibration curves were linear ($R^2 > 0.995$) covering a concentration range of 5-200 ng/mL for filgrastim and 100-4000 ng/mL for motixafortide. All analytes were eluted within three minutes, as shown in Figure 3.

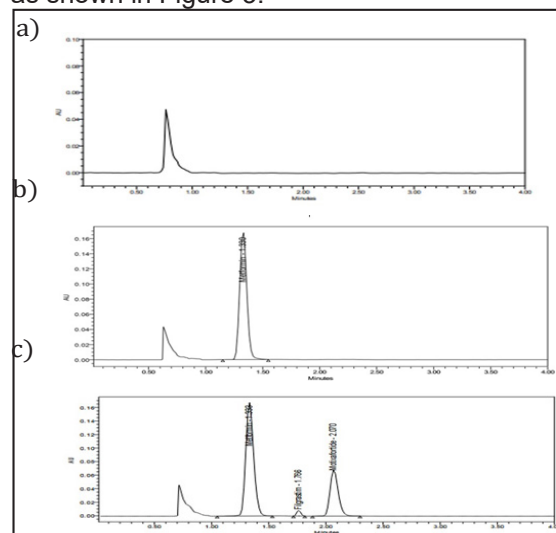


Figure 3. Representative Chromatogram of A) Blank B) Matrix Spiked IS C) Matrix spiked with Motixafortide, Filgrastim, and internal standard (IS)

Bioanalytical method validation

System suitability

It was evaluated using six replicate injections of an MQC sample. Plate count, tailing factor, and resolution of filgrastim and motixafortide were according to ICH guidelines. The plate count (> 2000), the tailing factor (< 2), and Table 1. System suitability result

S. No.	Analyte	R_t	Peak Area	Peak Resolution	Peak Tailing	Plate Count	% Coefficient of Variation of R_t
1.	Filgrastim	1.63	19265	3.1	1.3	3897.6	0.31
2.	Motixafortide	2.03	1925476	2.9	1.1	4462.7	0.26

Analyte identification and selectivity assessment

The identities of filgrastim and motixafortide were initially established by comparing the retention times of individual reference standards with those obtained from plasma samples spiked with the respective analytes. Standard-spiking experiments were performed by adding known quantities of filgrastim and motixafortide to blank human plasma, followed by sample preparation and chromatographic analysis. The spiked samples exhibited proportional increases in the corresponding peak responses without the appearance of additional co-eluting peaks or changes in retention times. Furthermore, chromatograms of blank plasma demonstrated the absence of endogenous interferences at the retention times of filgrastim and motixafortide, confirming the selectivity of the method. These observations support the chromatographic assignment of the analyte peaks under the developed UHPLC-UV conditions.

Specificity, carryover, and matrix interferences

No interfering peaks were observed at the retention times of filgrastim and motixafortide in blank plasma, demonstrating high method specificity. Carryover assessment revealed no notable peaks in pre- and post-injections of LLOQ and ULOQ calibration standards, indicating the absence of carryover. The area obtained is less than 20% of the extracted LLOQ stan-

dard area to the unextracted area by injection in a replicate manner. All of these findings confirm the method's reliability in complex biological matrices. The carryover response observed in blank samples injected after the upper limit of quantification sample was less than 20% of the mean response at the lower limit of quantification.

Sensitivity

The LLOQ values for filgrastim and motixafortide are assessed by injecting six replicates. Both analytes showed acceptable accuracy (100.90 and 100.40%, respectively) and precision (%CV < 2), meeting the specifications of bioanalytical validation represented in figure 4. This sensitivity is suitable for clinical pharmacokinetic applications, particularly where low plasma concentrations are expected post-dosing.

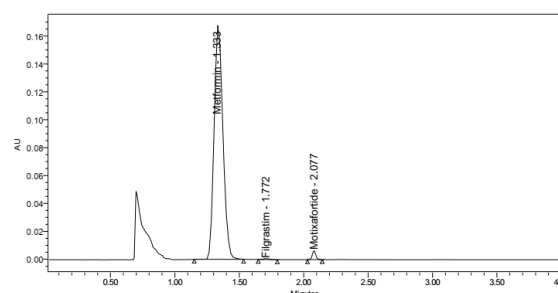


Figure 4. Sensitivity (LLOQ) Chromatogram of Motixafortide, Filgrastim, and internal standard (IS)

Development and validation of an ultra-high-performance liquid chromatographic method with ultraviolet detection for simultaneous quantification of Filgrastim and Motixafortide in human plasma

Matrix effect

Matrix effect was evaluated using six independent plasma lots at LQC and HQC levels, each analyzed in triplicate. The matrix effect was evaluated using at least three replicates each of LQC and HQC samples, prepared from matrices obtained from a minimum of six differ-

ent sources. Minimal ion suppression/enhancement was observed, with mean recoveries ranging from 100.81 to 101.66% and % CV values below 3. These findings demonstrate that the method is robust across diverse biological matrices, an essential requirement for clinical studies involving multiple donors.

Table 2. Matrix effect result

S. No.	Plasma Lot No.	Filgrastim		Motixafortide	
		High Quality Control (HQC)	Low Quality Control (LQC)	High Quality Control (HQC)	Low Quality Control (LQC)
		Nominal Concentration (ng/mL)		Nominal Concentration (ng/mL)	
		160.00	15.00	4000.00	300.00
		Calculated Concentration (ng/mL)		Calculated Concentration (ng/mL)	
1	LOT1	161.15	15.54	4000.300	298.360
		160.22	15.14	3975.589	310.235
		160.65	14.92	3579.484	299.106
2	LOT2	163.20	15.04	4089.478	306.741
		164.01	14.99	4103.589	300.897
		159.30	15.14	4198.669	311.148
3	LOT3	160.05	15.54	4127.103	300.500
		164.12	14.91	4018.401	303.361
		165.50	14.54	4122.748	310.000
4	LOT4	167.20	15.04	4103.334	297.589
		164.30	15.06	4009.975	309.482
		159.03	15.13	4015.600	312.103
5	LOT5	168.20	15.68	4173.000	301.367
		161.10	16.10	4176.357	308.321
		162.00	15.04	4096.470	301.562
6	LOT6	163.10	16.06	4065.474	304.896
		164.02	15.08	4199.879	303.397
		159.10	15.01	4110.700	309.785
n		18	18	18	18
Mean		162.5697	15.2200	4064.7861	304.9361
SD		2.73629	0.40622	139.22000	4.84312
% CV		1.68	2.67	3.43	1.59
% Mean Accuracy		101.61	101.47	101.62	101.65
No. of QC Failed		0	0	0	0

Linearity

Calibration curves exhibited excellent linearity over the concentration ranges of 5-200 ng/mL for filgrastim and 100-4000 ng/mL for motixafortide with correlation coefficients (r^2) exceeding 0.999 (Tables 2A & 2B), indicating a

strong and excellent relationship between the analyte concentrations and detector response. Confirming the method's ability to accurately quantify analytes across the intended concentration range.

Table 3.A Result of Linearity

S.NO	Motixafortide		Filgrastim	
	Con. (ng/ml)	Area response ratio	Con. (ng/ml)	Area response ratio
1.	100	0.021	5	0.0002
2.	200	0.042	10	0.0005
3.	300	0.064	15	0.0007
4.	800	0.170	40	0.0018
5.	2000	0.426	100	0.0043
6.	2400	0.514	120	0.0052
7.	3200	0.662	160	0.0067
8.	4000	0.851	200	0.0082

Table 3.B. Calibration Curve Parameters, LOD, and LOQ for Filgrastim and Motixafortide

Analyte	Linearity range	Calibration equation	R ²	Slope (±SD)	Intercept (±SD)	LOD (ng/mL)	LOQ (ng/mL)
Filgrastim	5-200	$y = 0.00004x + 0.0001$	0.999	0.00004 ± 0.000002	0.0001 ± 0.00001	0.0008	0.003
Motixafortide	100-4000	$y = 0.0002x + 0.0887$	0.999	0.0002 ± 0.00001	0.0887 ± 0.0021	0.0347	0.105

Precision and accuracy

Within-run and between-run at six replicates were injected at each QC concentration level in each analyte, with results given as high accuracy (within ±15% of nominal values) and

precision (%CV < 15), with slightly relaxed criteria for LLOQ (%CV < 20) (Table 3). This highlights the method's reliability in both single- and multi-batch analyses, essential for clinical sample handling over extended periods.

Table 4. Result of inter- and intraday precision

S.No.	Analyte	QC Level	Intra-day					Inter-day			
			n	Mean	SD	% CV	% Recovery	Mean	SD	% CV	% Recovery
1.	Motixafortide	HQC*	6	4044.31	86.4	2.1	101.11	4059.70	61.5	1.5	101.49
		MQC*	6	2010.17	6.7	0.3	100.51	2015.00	26.1	1.3	100.75
		LQC*	6	303.89	3.5	1.2	101.30	302.84	3.6	1.2	100.95
		LLOQ*	6	100.87	1.9	1.9	100.87	100.69	2.2	2.1	100.69
2.	Filgrastim	HQC	6	163.03	3.7	2.3	101.90	160.97	3.7	2.3	100.60
		MQC	6	102.38	2.6	2.5	102.38	101.34	3.1	3.1	101.34
		LQC	6	15.10	0.5	3.4	100.68	15.11	0.5	3.4	100.72
		LLOQ	6	5.04	0.7	13.9	100.84	5.04	0.5	9.9	100.79

*Lower Limit of Quantification (LLOQ), Low Quality Control (LQC), Medium Quality Control (MQC), High Quality Control (HQC).

Development and validation of an ultra-high-performance liquid chromatographic method with ultraviolet detection for simultaneous quantification of Filgrastim and Motixafortide in human plasma

Recovery

Extraction recovery was consistently high at LQC, MQC, and HQC levels for both analytes, confirming the method's efficiency in sample preparation (Table 4). The %CV values

were within the acceptance limit of 15%, indicating reproducibility. The use of protein precipitation ensured simplicity while maintaining high recovery, aligning with strategies used in similar LC-MS/MS-based bioanalytical studies.

Table 5. Recovery Results

S.No	Drug Name	QC Level	n	Mean	SD	% Coefficient of Variation	% Recovery
1.	Motixa fortide	Low Quality Control (LQC) (300 ng/mL)	6	19483	131.15	0.67	82.23
		Medium Quality Control (MQC) (100 ng/mL)	6	252938	4208.01	1.66	84.98
		High Quality Control (HQC) (4000 ng/mL)	6	661228	3205.59	0.48	88.03
2.	Filgras-tim	Low Quality Control (LQC) (15 ng/mL)	6	115578	1723.62	1.49	85.76
		Medium Quality Control (MQC) (100 ng/mL)	6	1458941	14026.46	0.96	83.90
		High Quality Control (HQC) (160 ng/mL)	6	3058626	32152.97	1.05	85.98

Stability studies

Stability studies confirmed that both analytes remained stable under various storage and processing conditions, including long-term storage at $-20 \pm 5^\circ\text{C}$ and $-80 \pm 5^\circ\text{C}$ for 30 days (Tables 5–7). QC levels of HQC and LQC retained concentrations within $\pm 15\%$ of nominal values. Long-term stability samples were stored at -20°C , corresponding to the operating temperature of the laboratory deep freezer used for sample storage. The temperature was routinely monitored throughout the study to ensure

consistent storage conditions. These results validate the method's suitability for extended pharmacokinetic studies, especially where sample storage over time is necessary. Long-term stability was evaluated for 30 days under frozen storage conditions. This duration was selected to exceed the anticipated storage period between sample collection and final analysis in future pharmacokinetic studies, thereby ensuring the integrity of filgrastim and motixafortide during routine bioanalytical sample handling and storage.

Table 6. Stability assessment of motixafortide and filgrastim (zero days)

S No.	Motixafortide		Filgrastim	
	High Quality Control (HQC)	Low Quality Control (LQC)	High Quality Control (HQC)	Low Quality Control (LQC)
1	3261.46	308.06	160.96	15.55
2	3172.64	282.06	159.89	15.13
3	3220.63	296.06	159.23	14.98
4	3250.71	299.06	158.25	14.75
5	3192.80	296.07	162.10	15.00

6	3189.61	301.06	161.46	15.19
n	6	6	6	6
Mean	3214.64	297.06	160.31	15.10
SD	35.78	8.58	1.451	0.27
% CV	1.11	2.89	0.90	1.78
%Mean Accuracy	100.46	99.02	100.20	100.68

Table 7. Stability assessment of the Matrix sample stored at $-20 \pm 5^{\circ}\text{C}$ over a 30-days period of motixafortide & filgrastim.

S No.	MOTIXAFORTIDE				FILGRASTIM			
	High Quality Control (HQC) (320 ng/mL)		Low Quality Control (LQC) (300 ng/mL)		High Quality Control (HQC) (160 ng/mL)		Low Quality Control (LQC) (15 ng/mL)	
	Comparison Samples	Stability Samples	Comparison Samples	Stability Sample	Comparison Samples	Stability Samples	Comparison Sample	Stability Samples
1	3197.99	3164.39	319.54	319.05	161.85	159.42	15.59	15.55
2	3220.95	3172.65	305.59	302.20	158.86	159.81	14.63	14.92
3	3186.98	3164.04	291.64	292.05	163.12	163.99	16.63	16.58
4	3280.91	3172.21	294.00	296.06	156.99	156.45	14.66	15.26
5	3230.91	3199.61	298.59	297.60	161.35	161.21	15.53	14.94
6	3260.99	3200.59	301.06	300.60	159.45	160.84	14.61	14.65
n	6	6	6	6	6	6	6	6
Mean	3229.79	3178.91	301.74	301.26	160.27	160.286	15.28	15.31
SD	36.08	16.82	10.03	9.41	2.24	2.47	0.80	0.69
%CV	1.12	0.53	3.33	3.12	1.40	1.54	5.26	4.52
% Mean Accuracy	100.93	99.34	100.58	100.42	100.17	100.18	101.87	102.13
% Mean Stability	98.42		99.84		100.01		100.26	

Table 8. Stability assessment of the Matrix sample stored at $-80 \pm 5^{\circ}\text{C}$ over a 30-days period of motixafortide and filgrastim.

S No.	MOTIXAFORTIDE				FILGRASTIM			
	High Quality Control (HQC) (320 ng/mL)		Low Quality Control (LQC) (300 ng/mL)		High Quality Control (HQC) (160 ng/mL)		Low Quality Control (LQC) (15 ng/mL)	
	Comparison Samples	Stability Samples	Comparison Samples	Stability Sample	Comparison Samples	Stability Samples	Comparison Sample	Stability Samples
1	3199.38	3189.46	299.15	298.53	161.21	161.65	15.02	15.02

Development and validation of an ultra-high-performance liquid chromatographic method with ultraviolet detection for simultaneous quantification of Filgrastim and Motixafortide in human plasma

2	3212.62	3192.60	292.58	296.60	158.92	158.98	16.04	16.04
3	3224.63	3194.57	290.26	290.20	154.98	159.58	14.90	14.95
4	3264.63	3198.56	296.05	292.05	161.98	161.85	15.17	15.16
5	3297.69	3272.80	309.06	307.61	159.32	156.21	14.96	14.32
6	3214.43	3196.59	301.06	300.45	160.52	160.98	15.01	15.65
n	6	6	6	6	6	6	6	6
Mean	3235.56	3207.43	298.03	297.57	159.48	159.87	15.18	15.19
SD	37.70	32.17	6.72	6.25	2.48	2.12	0.42	0.59
%CV	1.17	1.00	2.26	2.10	1.56	1.33	2.82	3.93
% Mean Accuracy	101.11	100.23	99.34	99.19	99.68	99.92	101.25	101.29
% Mean Stability	99.13		99.85		100.24		100.04	

Conclusion

The method shows high sensitivity (LLOQ: 5 ng/mL for filgrastim and 100 ng/mL for motixafortide), reproducibility (% CV ≤ 13.9), specificity (no interference observed), and stability (98.42-100.26%), making it suitable for future pharmacokinetic and therapeutic drug monitoring studies involving filgrastim and motixafortide. Its accessibility, affordability, and ease of use, this technique may serve as an alternative analytical method in laboratories lacking sophisticated instrumentation. The simultaneous quantification of these two drugs, which are generally used combination, represents a novel contribution to the analytical field. This method may facilitate improved drug monitoring and therapeutic management in stem cell mobilization therapy. Future studies should investigate the applicability of the method in clinical pharmacokinetic investigations and validate its performance through cross-comparison with established LC-MS/MS methods.

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Conflict of interest

All authors have approved the manuscript, and there are no conflicts of interest to declare.

Data availability statement

The corresponding author can provide the data supporting the study's conclusions upon request. Due to ethical and privacy concerns, the data are not publicly accessible.

Author contributions

Lurdhu Mary. k: Investigation; Methodology; Validation: Writing-original draft. Naresh Podila: Conceptualization, validation. Pedamallu Niharika: Resources. Venkata Ramana Maddula: Writing review and editing.

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