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Original Article

Method Development and Validation for the Quantification of Abametapir in Biological Matrices by LC-ESI-MS/MS

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ABSTRACT

Aim: A precise, linear and specific liquid chromatography-tandem mass spectrometry procedure was executed and subjected for validation for the quantification of plasma. **Materials and Methods:** The chromatography elution was carried out using a C_{18}-Discovery column of 15 cm in length and 2.1 mm in internal diameter, packed with stationary phase particles of 5 µm size, at a flow rate of 0.80 mL/min. An isocratic elution process was conducted with a mobile phase solution consisting of methanol and 0.10% V/V HCOOH in a ratio of 90:10. The separation of Abametapir and Metformin was achieved by a liquid-liquid extraction process using ethyl acetate as the solvent. **Results:** A triple quadrupole mass spectrometer was used for the measurement of ions. Electrospray ionization is a technology that ionizes positively, and it was used in Multiple Reaction Monitoring (MRM) with parent/product ionic transitions m/z 185.1→106.06 for Abametapir and 130.1→60.20 for the Metformin internal standard. A calibration graph was constructed with values ranging from 2.05 to 82.00 ng/mL, resulting in the equation y=0.0149x+0.00221, with a r2 value above 0.99. The recovery values of Abametapir exceeded 94.27%, with its accuracy assessed in terms of relative error ranging from -4.04% to 6.52%. **Conclusion:** The accuracy, recovery and sensitivity values of Abametapir in the plasma sample, as shown by the established approach, highlight its significance in pharmacokinetic and bioequivalence research.

Keywords: LC-MS/MS, Abametapir, Metalloproteinase Inhibitor, Validation, Accuracy, Linearity.

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INTRODUCTION

Abametapir chemically designated as 5,5'-dimethyl-2,2'-bipyridine. Its molecular mass and chemical formula (Figure 1) is 184.24 g/mol and $C_{12}H_{12}N_2$ correspondingly. Abametapir is a medicine that is sold under Xeglyze brand name. It is used to treat head lice in people 6 months and older.[1] Some of the most common side effects are red skin, rashes, a burning feeling on the skin, skin inflammation, vomiting, eye irritation, skin itching, and changes in hair colour. Abametapir is a new metalloproteinase inhibitor that kills head lice. It is used to treat head lice infestations. Head lice (*Pediculus capitis*) live for about 30 days, and between 7 and 12 of those days are spent as eggs on hair shaft near scalp. Topical pediculicide, including standard-of-care treatments like permethrin, usually don't have enough ovicidal activity to kill lice eggs, and many of them need to be applied again 7-10 days after the 1st time to kill lice eggs that didn't die from the first treatment.[2]

Metalloproteinases are enzymes that need metal cofactors to work. Metalloproteinases help louse eggs hatch and live. Abametapir is an inhibitor of metalloproteinase that stops louse metalloproteinases from doing their jobs, which are important for their growth and reproduction. In a pharmacokinetic trial with both child and adults, the adult group's AUC_{0-8} h and C_{max} were 121.0 ng. h/mL and 41.0 ng/mL, respectively.[3]

Literature review on Abametapir discloses that no single analytical methodology is designated for the determination of Abametapir in unknown solutions. So, present study was pointed to develop a specific, linear and precise LC-MS/MS procedure for the determination of Abametapir in plasma samples.

MATERIALS AND METHODS

Chemicals and Reagents

The Abametapir (99.18% pure) standard and Metformin (99.79% pure) were acquired from Malladi Drugs and Pharmaceuticals Limited, Alandur, Chennai, Tamil Nadu, India. Water deionization has been carried out using the Milli-Q water system from Millipore, USA. Acetonitrile and methyl alcohol with high liquid chromatography purity were obtained from Merck, Mumbai, India.

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Parameters of LC-MS/MS

The LC-MS/MS apparatus comprises an Agilent 3250 liquid chromatographic system equipped with 2 pumping ssystems (dual-SL) and an 6164 Agilent triple quadrupole mass spectrometric detector using electro spray ionization as the ion source (CA, America). An analysis of chromatography data was conducted using the MassHunter program. The chromatography elution was carried out using a Discovery C18 stationary phase with dimensions of 15 cm x 2.1 mm ID and a particles size of 5.0 µm. The elution process was conducted at a flow rate of 0.80 mL/min. An isocratic method of elution was executed, using a mobile phase solution consisting of methanol and 0.10% formic acid at a 90:10 ratio. A triple quadrupoles mass spectrometer was used to measure the concentration of ionic components. Electro-spray-ionization is a process that ionizes positively charged ionic components. This approach was used in multiple reaction monitoring with a parent and product ionic transition of m/z 185.1→106.06 for Abametapir and 130.1→60.20 for the Metformin internal standard. The MS/MS settings were fine-tuned with the following values: voltage of capillary set at 3.5 kV, temperature of the source retained at 450°C, dryer gas (N2) flow rate set at 10 L/min, and nebulization gas pressure adjusted to 40 psi. The autosampling system temperature and infusion volume were maintained at 10°C and 10 µL, correspondingly. The chromatographic elution used 30 volts of collisional energy.

Quality Control Standards

Stock solutions of Abametapir and Metformin at a concentration of 1000 µg/mL were used separately in the mobile phase as a diluent. The Abametapir solutions obtained were subjected to a series of dilutions using the mobile phase in order to prepare working standard controls. The Metformin (IS) working standard at a concentration of 45 ng/mL was treated as required to ensure consistency in all the Abametapir samples. The quality controls that were created were supervised at a temperature of -20°C until the sample analysis was conducted.

Linearity quality controls for Abametapir (2.05, 4.00, 8.00, 18.00, 32.00, 47.00, 64.00, and 82.00 ng/mL) were established using the spiking technique with plasma blanks. Quality control solutions at varying concentrations (5.74, 41.00, and 61.50 ng/mL) were used separately in a consistent way.

Method of Sample Preparation

A 350 µL plasma solution without any substance was relocated into a 10.0 mL tube for further processing. Analyte and 100.0 µL of IS solutions were introduced into tubes to achieve the desired concentration in the final dilution for infusion. The combination was introduced into 5 mL of ethyl acetate for the liquid-liquid extraction technique and used for centrifugation lasting 20 min. The organic solvent phase that was left over was moved to a new glass tube, and steam N_2 was used for the evaporation. The subsequent dried residues were put back together with 100 µL of a movable solvent, and 10.0 µL aliquots were put into LC-MS/MS equipment to be looked at.

Validation

The analytical technique that was created underwent validation following the guidelines set by the USFDA for various validation parameters to meet the necessary criteria.[4-8]

RESULTS AND DISCUSSION

Optimization of Mass Instrument

Throughout the developmental phase, new Abametapir solutions were administered to ensure optimal performance of both

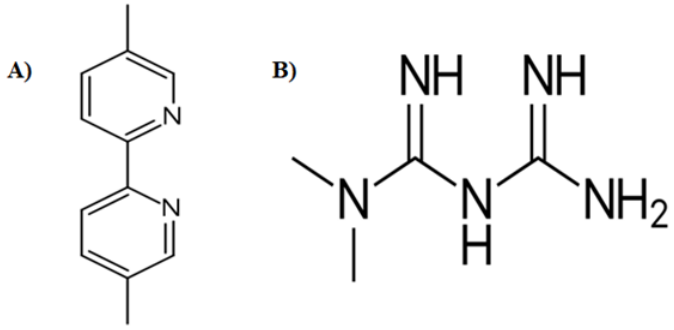

Figure 1: A) Abametapir and B) Metformin chemical structures.

Table 1: Abametapir calibration quality controls.

LS-ID	Concentration (ng.mL^{-1})	Analyte Area	IS Area	Drug/IS Area ratio
LS -1	2.05	953	30128	0.031632
LS -2	4	1874	30324	0.061799
LS -3	8	3845	30097	0.127754
LS -4	18	8381	30185	0.277654
LS -5	32	14582	30205	0.482768
LS -6	47	20874	30204	0.691101
LS -7	64	28149	30123	0.934469
LS -8	82	37654	30236	1.245337

LS: Linearity standard.

the product and parent ions. Within the positive ionization technique, a precursor ion measuring 185.1 m/z was detected. Upon fragmentation of the precursor ion, fragments having masses of 158.09, 156.08, 118.06, 106.06, and 51.02 were detected. The daughter ion of Abametapir had the highest intensity at 106.06 m/z. Metformin has comparable physical and chemical characteristics to Abametapir, making it a suitable option as an internal standard for the development of this bioanalytical approach, ensuring consistent recoveries throughout analyte and validation procedures. MRM scans were used to identify the daughter and molecular ionic components of both medication components. The ultimate transitions observed for Abametapir were m/z 185.1→106.06, whereas for the Metformin internal standard, they were m/z 130.1→60.20.

Specificity

Plasma samples containing no analyte and plasma samples spiked at the Lower Limit of Quantification (LLOQ) level of 2.05 ng/mL of Abametapir and Metformin were introduced into an LC-MS/MS apparatus, and the chromatograms obtained are shown in Figure 2. Analysis of the Abametapir and Metformin plasma samples revealed the absence of peaks caused by interference. Abametapir and Metformin were both removed from the system within a 4 min timeframe. Abametapir and Metformin exhibited residence times of 0.83 min/mL and 1.62 min in the system, accordingly.[9]

Sensitivity and Linearity

The signal-to-noise ratio exceeded 10.0 at a concentration level of 2.05 ng/mL. The accuracy and precision were determined to be 3.26% RSD. Consequently, the lower limit of quantification and quantitation for Abametapir was established at 2.05 ng/mL. Analyzed were Abametapir plasma concentrations falling within the range of 2.05-82.00 ng/mL utilizing rectilinear plots 11 (Table 1). Derived from the average results of 6 repeated calibration controls, a linear equation for the graph of Abametapir was established as: y=0.0149x+0.00221 with a r2 value of 0.9993. In this context, 'x' represents plasma concentration and 'y' denotes the peaks ratio of Abametapir/Metformin..

Precision, Recovery and Accuracy

Results regarding the precision and accuracy of both interday and intraday measurements were shown in Figure 3 and Table 2. Accurate results were obtained within a single day, with precision ranging from 3.12% to 4.12% for Abametapir. The accuracy results ranged from a relative error of -4.04% to 6.52%. Across several trial days, precision values for Abametapir ranged from 3.44% to 5.14% (RSD), while accuracy fell between relative errors of -4.45% to 3.89%. The average recoveries of Abametapir ranged from 94.27% to 102.59% across three quality controls, as shown in Table 3. The analysis of the sample solution using the extraction method

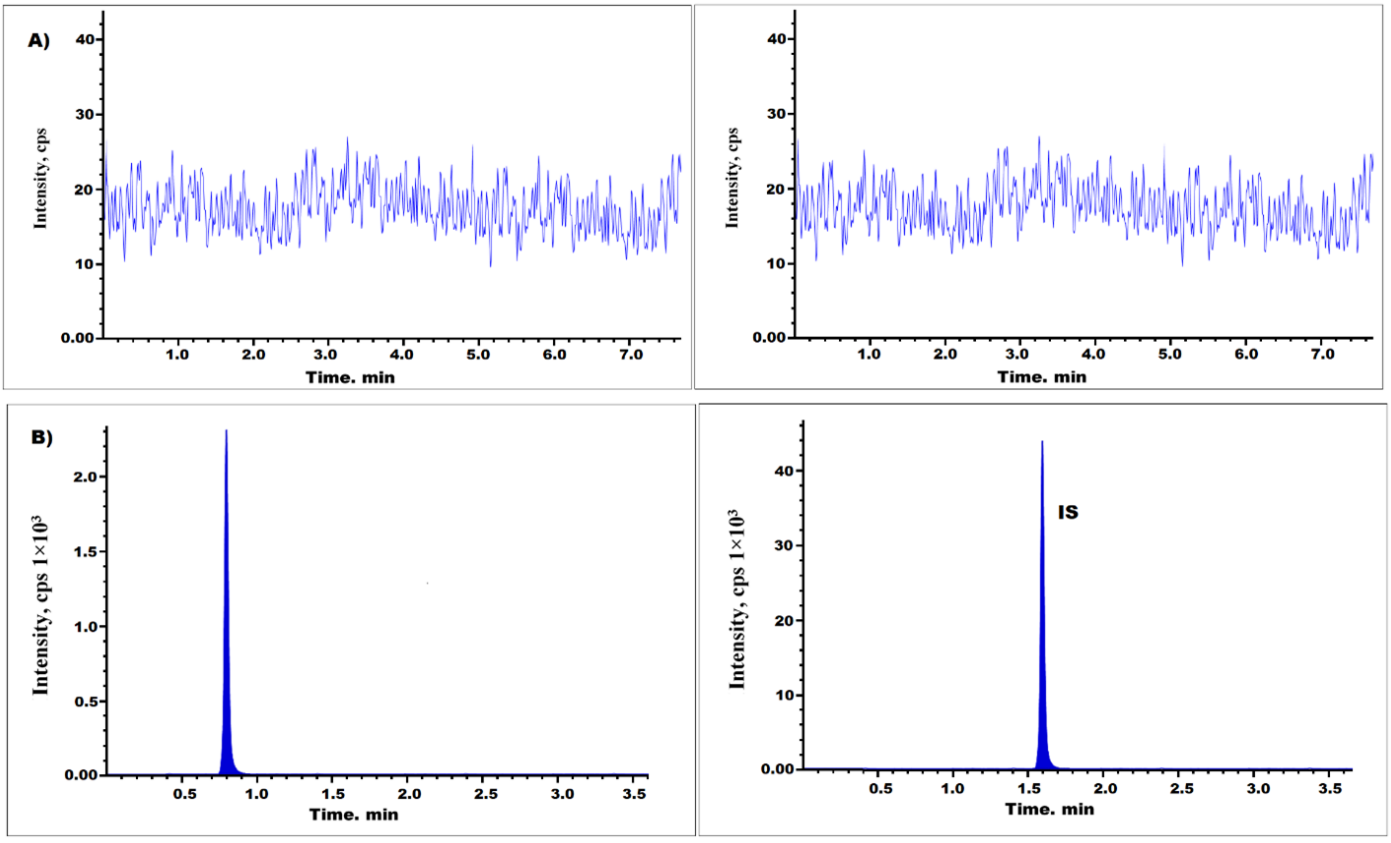

Figure 2: Abametapir A) Blank plasma and B) LLOQQC chromatograms.

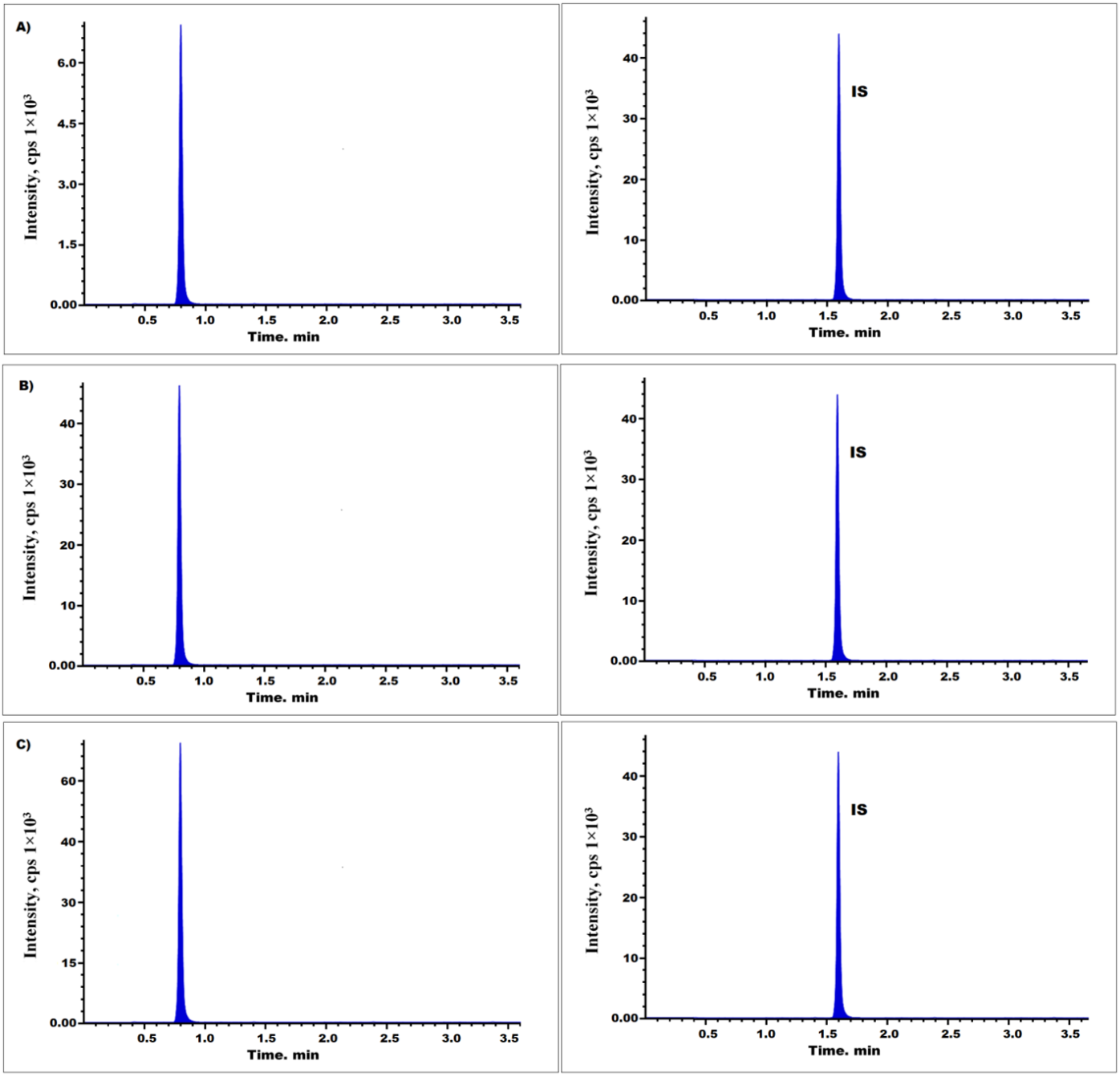

Figure 3: Abametapir outcomes at A) Low-QC B) Median-QC and C) High-QC level.

revealed that Abametapir and Metformin showed significant improvement, with a high percentage of 97.9% obtained from blank plasma.

Matrix Effects

The peak response ratios of Abametapir/Metformin in blank plasma extract compared to those with diluent ranged from 94.67% to 102.94% for Abametapir at the Low-QC level and from 95.22% to 103.05% at the High QC level, as shown in Table 4.

Stability Study

The stability of Abametapir was shown by the analysis of control samples subjected to different storage conditions.[12] The exposed environments consist of long-term stability during sample storage at -20°C for 30 days, short-term stability at room temperature for up to 8 hr, and undergoing 3 complete freeze and thaw cycles (frozen at -20°C for 12.0 hr), as well as stability of treated (extracted) samples after 24 hr at 4.0°C.The number 8 As per regulatory standards, the assessed accuracy levels of the

Table 2: Intra-day and interday precision and accuracy of Abametapir.

Spiked conc. ng/mL)	Intraday(*n*=6)			Interday(*n*=6×3)		
	Measured conc. (mean±SD; ng/mL)	Precision (RSD %)	Accuracy (RE %)	Measured conc. (mean±SD; ng/mL)	Precision (RSD %)	Accuracy (RE %)
2.05	1.98±0.06	3.26	-3.59	2.13±0.10	4.88	3.84
5.74	5.94±0.25	4.12	3.52	5.48±0.19	3.45	-4.45
41	43.67±1.79	4.09	6.52	42.70±1.65	3.87	4.15
61.5	59.01±1.84	3.12	-4.04	59.26±3.05	5.14	-3.64

RE: Relative error; RSD: Relative standards deviation.

Table 3: Abametapir and Metformin recovery study.

Concentration	Y	Z	% Recoveries	% Mean recoveries	%RSD
LQC	2668	2583.691	96.84	97.90	3.56
MQC	19060	17967.86	94.27		
HQC	28590	29330.48	102.59		
Metformin	30267	29973.41	99.03		

Y, un-extracted sample mean recoveries; Z, extract sample mean recoveries.

Table 4: Abametapir matrix effects at Low-QC and High-QC level.

	Low-QC			High-QC		
Sl. No.	Area without matrix	Area with matrix	Matrix effect	Area without matrix	Area with matrix	Matrix effect
1	2651	2528	95.37	28485	27177	95.41
2	2674	2531	94.67	28549	27352	95.81
3	2681	2604	97.14	28607	29479	103.05
4	2672	2580	96.58	28492	29158	102.34
5	2634	2696	102.36	28542	27177	95.22
6	2619	2695	102.94	28581	27514	96.27
Mean			98.18			98.02
±SD			3.58			3.65
% RSD			3.64			3.72

SD: Standard deviations; RSD: Relative standards deviation.

Abametapir medication, falling within the range of 93.67% to 104.08%, were deemed satisfactory.

CONCLUSION

An analytical approach utilizing liquid chromatography-tandem mass spectrometry was establilshed and validated for accurately measuring the levels of Abametapir in plasma samples. An elution chromatography process was carried out using a Discovery C18 column of 15 cm in length and 2.1 mm in ID, packed with a 5.0 µm stationary phase. The elution was executed at a flowing rate of 0.80 mL/min. Electrospray ionization is a process that ionizes positively charged ions. This approach was used in MRM with a parent/daughter ionic transition of *m/z* 185.1→106.06 for Abametapir and 130.1→60.20 for the Metformin internal standard. A calibration graph was constructed with values ranging from 2.05 to 82.00 ng/mL, yielding the equation y=0.0149x+0.00221 with a R^2 value over 0.99. The recovery values of Abametapir exceeded 94.27%, with accuracy assessed using relative error ranging from -4.04% to 6.52%. Ultimately, the developed technique adhered to the standards for validating bioanalytical methods and is suitable for quantifying the concentration of Abametapir in various biological specimens.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LC-ESI-MS/MS: Liquid chromatographic-electrospray-ionisation tandem mass spectrometry; **LLE:** Liquid-liquid extraction; **FDA:** Food and Drug Administration; **QC:** Quality control; **SD:** Standard deviation; **LQC:** Low quality control; **MRM:** Multilple reaction monitoring; **LLOQ:** Lower limit of quantification; **HQC:** High quality control; **MQC:** Median quality control; **CV:** Coefficient of variation.

SUMMARY

Abametapir is a medicine that is sold under Xeglyze brand name. It is used to treat head lice in people 6 months and older. Present study was pointed to develop a specific, linear and precise LC-MS/MS procedure for the determination of Abametapir in plasma samples. Discovery C^{18} column of 15 cm x 2.1 mm ID with a 5.0 µm stationary phase, chromatography elution was executed at a flowing rate of 0.80 mL/min. An isocratic method of elution was processed with a mobile phase solution consisting of methanol and 0.10% formic acid in a 90:10 ratio. Electrospray ionization is a method that ionizes positively, as seen in the MRM technique using the parent/product ionic transitions of m/z 185.1→106.06 for Abametapir and 130.1→60.20 for the Metformin internal standard. A calibration graph was constructed with values ranging from 2.05 to 82.00 ng/mL, yielding the equation y=0.0149x+0.00221 with a r2 value over 0.99. The recovery values of Abametapir exceeded 94.27%, with accuracy assessed using relative error ranging from -4.04% to 6.52%.

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