International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
April - May, 2016, Vol. 5, No.3, pp 2092-2098
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

HPLC - VALIDATION OF MOXIFLOXACIN

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(Received: January 24, 2016; **Accepted:** March 28, 2016**)**

ABSTRACT

The structure formula of moxifloxacin is described as follows: 8 methoxy flouroquinolone: and it is broad spectrum antibiotic [1, 2].we study moxifloxacin by IV drug delivery system. And for its study following apparatus is used; 1-a reversed-phase Lichrospher RP-18 column, 2-a mobile phase which is composed of different solvents.

It is detected at a wavelength of 290 nm. By this method the retention time of moxifloxacin is very short. When we detect it in plasma and brain tissue linear curves are obtained. .Advantage of this method is that we can detect the bacteria in plasma and brain tissues by this method.

Keywords: Lichrospher RP-18 column, moxifloxacin, MIC.

INTRODUCTION

Most commonly founded bacteria in environment are Listeria monocytogenes it is actually a gram-positive bacteria [3]. This bacteria is charectrized as a facultative intracellular pathogen and produce in the food also responsible for various diseases, like

1- Septicemia

2- Abortions

3- CNS infections [3].

This Listeriosis attacks the people of high risk and also involves the immune compromised patients. [4].so we can say that it is very effective against the functions of extracellular and intracellular bacteria [5]

However it is noted that to detect the MIC of L. monocytogenes strains we did not give much importance to its origin that from where it is produced.[5] The function and efficacy of moxifloxacin is analyzed by a model which is named as "murine model of central listeriosis" [7], however its drawback is that for it we needed the comprehensible study (pharmacokinetic) of the drug in the entire body of experimental animal. However, this is not very appropriate way because of following reasons;

1- We obtain the false results by it like: fosfomycin is very efficient inside the body of animal against L. monocytogenes but it failed to do work outside the body [8].
2- No method 0r assay has been developed to find out the moxifloxacin concentration in the brain and spinal cord (CNS) and we cannot asses its toxic and effective conc. in CNS by this method.

So there was need to search a method to find its concentration in CNS thus, we developed a HPLC method which is helpful in determining moxifloxacin amount in both the; 1- Plasma, 2- CNS

So we assess the pharmacology of this drug by model that is "murine model of CNS listeriosis"

PARAMETERS USED IN HPLC METHOD VALIDATION

Precision, determination range, quantization range, straight line, accuracy, repeated reproduction, Reestablishment, stability of sample solution and Specificity.

The accuracy is the proximity of the test results. Accuracy should be recognized across its range. Accuracy is assessed by using a minimum of 9 determinations. The value which is recommended for accuracy and precision is ±15%, excluding for the ±20% which is lower limit of quantification average and standard differences were calculated. Precision is calculated by using coefficient of variation.[27]

Q.C samples were separately prepared from the calibration standards. The sample solutions have three dissimilar moxifloxacin quantities: close to the lower, mid and upper LOQ straight line was obtained by scheming measurement of curves in plasma and in copied three part different runs. Calculation was made by different quantities of moxifloxacin in brain tissues and plasma with sample solutions: involving 5ug/L and 250µg/L and involving 0.1 and 2.5µg/g for brain. The curved lines were fixed by a straight line regression system for the calibration of maximum area value of this antibiotic in the in vivo standard solution. Drug Moxifloxacin stability seen in analyzing replicates at three different samples. These method are use in regards to receipt criteria of industrial principle to the bio investigative validation .(26)The value which is suggested for accuracy and precision is ±15%, except for the lower limit of quantification for which lower limit of quantification ±20% is up to standard. Average and standard difference were calibrated. Precision is calculated by using coefficient of variation (CV)[27]. Stability is tested at the initial and at the end of the sample injection time limit of 15 h, at temp 9°C but beta-lactam stability was founded in patient's plasma at 4°C at room temperature for 12 hours.

Moxifloxacin has a broad spectrum antibacterial activity fluoroquinolone Gram+ve bacteria Listeria monocytogenes which is common in the atmosphere. It causes severe infections and facultative intracellular bacteria that is food-borne Listeriosis[3] mostly occurs in persons with insensitive basic diseases or having less strong immune system[4]. Moxifloxacin has fast antibacterial action not in support of in vitro and in vivo L.monocytogenes[5]. As a result moxifloxacin could be well thought-out much competent as compared to amoxicillin, against L. monocytogenes[6]. To evaluate the specificity of moxifloxacin in this murine[7], pharmacokinetic study of this fluoroquinolone was essential in plasma. Definitely, some results can even be deceptive[8]. Though, no bio analytical testing has been done to find out moxifloxacin quantity in brain tissues. Therefore, we promote a high-performance liquid chromatographic technique for the purpose to find out moxifloxacin concentration in plasma as well as in brain tissue. Our methodology was then utilized to validate the pharmacokinetic aspects of moxifloxacin in brain and plasma in evaluated murine model of central nervous system[9-14].

Following the assay conditions moxifloxacin was taken from the in vivo standard retention time was 8 minutes. The chromatographic papers taken from the examination of two empty matrixes reveal no invasive peaks with the same times. Straight line curve was experiential in moxifloxacin analysis from brain and plasma (r^2 = 0.999) on the validated quantity limits. Good results were taken for accuracy (99.2–111.5%; 94.8–106.2%) and precision (CV < 10%; CV < 5%) for brain tissue and plasma.

CHEMICALS:

Chemicals used here are, potassium dihydrogen phosphate, orthophosphoric acid (85%), tetrabutylammonium bromide (TBABr) ciprofloxacin, moxifloxacin, dipotassium hydrogen phosphate, acetonitrile and methanol, potassium salt, piperacillin sodium salt, Ceftazidime, ticarcillin, and meropenem Amoxicillin, ceftriaxone, cloxacillin sodium salt, oxacillin sodium salt, Cefotaxime sodium salt, penicillin G. The working standard solutions, internal solutions and stock solutions of moxifloxacin HCl and ciprofloxacin are in methanol.

Phosphoric acid (85%) was used throughout the procedure. Stationary phase is Atlantis T3 used for separation. Citric buffer consisted of 25mM citric acid, 10mM sodium dodecyl sulfate and 10mM TBABr in 500mL water and the pH was set at 3.5 by 0.1 M NaOH.[25]. The mobile phase is firstly filtered and then gases are removed by degasser, consist of 10Mm phosphoric acid solution and pH is adjusted at 2 with hydrochloric acid and acetonitrile. A linear gradient from 7% to 19% acetonitrile in 6 min and 19% to 49% from 6 to 16 min was used by means of a flow rate of 2 ml/min. Run time

was delayed to 22 min to go back early point. It was monitored at 210 nm for cloxacillin, at 230 nm for amoxicillin, at 298nm for imipenem and meropenem then the data is recorded. The Quantification was based on the peak heights. The baseline is inspected and adjusted visually and also manually.

INSTRUMENTATION

Chromatographic analysis was executed on a HPLC system that consists a fluorescence detector, a pump, degasser and a thermostat (at 25C) 717 plus auto sampler waters. Separation was performed by the use of a reversed-phase Lichrospher C18 column (250 × 4 mm, 5µm). The mobile phase consisted of mixture of acetonitrile-methanol-buffer (pH 3.5) (40 : 3 : 57, v/v/v) at a flow rate of 1.0mL/min All the data was evaluated by using Chrome eon.. Fluorescence findings were performed at an emission wavelength that is 550 nm and an excitation wavelength that is 290 nm.

SAMPLE PREPARATION

In case of moxifloxacin 100µL of plasma is taken and then add working standard internal solution 20µL for brain (in 0.5 milliliters of water 0.5g crumpled).Then tasters were deproteinized with the help of 100µL acetonitrile. Then for 10 minutes at 3000rpm centrifuge the samples. 400µL of sterile water was adding in 100µL supernatant. Then 20µL of this solution is injected into system of HPLC. We mixed the ciprofloxacin soln.20µL to 100µL for brain. Then, repeat the above injecting technique for plasma samples.

For beta-lactam antibiotics prepare stock solution and stockpiled at -80 °C. To prepare standard solution of 10µg/ml, mixed with water to acquire a 1-mg/ml. Three operational solution of beta-lactam were prepared from stock solution and diluted 10-folds in complete plasma.

Table: Reproduction of moxifloxacin in plasma (a) and drug quantity in brain tissue (b) calculation: test performance data (n=6)

(a)

Concentration Nominal (µg/L)	Concentration Measured (µg/L)	Variation Coefficient %	Accuracy %	concentration Measured (µg/L)	Variation Coefficient (%)	Accuracy%
		Within-performance			B/w-performance	
20	22.3 ± 0.3	1.5	111.5	19.9 ± 1.9	9.5	99.0
125	125.5 ± 1.3	1.0	100.4	125.6 ± 2.8	2.2	100.5
225	240.4 ± 3.6	1.5	106.8	230.9 ± 8.1	3.5	102.6

(b)

Concentration Nominal (µg/g)	Concentration Measured (µg/g)	Variation coefficient (%)	Accuracy (%)	Concentration Measured (µg/g)	Variation Coefficient (%)	Accuracy (%)
		Within-performance			B/w-performance	
0.2	0.19 ± 0.01	3.6	94.8	0.21 ± 0.01	5.0	105.5
1.25	1.33 ± 0.03	2.5	106.2	1.30 ± 0.04	3.0	104.3
2.25	2.17 ± 0.03	1.4	96.6	2.26 ± 0.11	5.0	100.4

Then take plasma in heparin zed tubes and transported to ice and centrifuge for 10 mints at4°C.700µl and 20µl of water added to 100µl patient plasma sample. Each sample was assorted and centrifuged. 900µl of water is used to dilute 100 µL of the supernatant. 20µl were inoculated into the system. These dilutions provide the same percentage of acetonitrile as early condition of linear gradient.

EXPERIMENTAL MODELS

7-8 weeks old female mice were used. The bacteriologist in laboratory used to stimulate listeriosis in 3 mice.The mice had weighed 23-27gm and afterward injected intravenously by means of the tangential in the vein of tail 105L× 1. An intraperitoneal injection of drug (50mg/kg) was injected in the mice, at 36 hours after infection.

PHARMACOKINETIC ANALYSIS:

In the mouse plasma and brain the pharmacokinetic profiles were examine after administering a single dose of moxifloxacin. They were deliberated from specimens collected at zero, five, fifteen, thirty, sixty, 120, 240, 360, and 480 minutes after 1st dose. Six mice from every group were slater on every sampling time. Concentration time data were examined using a non-compartmental model with first-order elimination and zero-order absorption through a nonlinear least squares method.

Pharmacokinetic limitations were anticipated via consistent techniques. After directly review of investigational concentrations time curves highest brain and plasma concentrations (Cmax) were determined. We determined the AUC via Trapezoidal rule until determine the variation of last pharmacokinetic parameter between control and infected mice. Results are stated as means ± S.D. The procedures defined above were employed in the determination of mean moxi concentrations. In plasma, for diseased mice, the moxi Cmax for controlled and infected group 6.7±1.2 mg/L, 17.3±6.6 mg/L respectively.

After intraperitonial administration of moxifloxacin concentration in brain tissues was quickly perceived within 5 min and peaked point at 1.8- 2.2µg/g as compared with 0.5-1.1µg/g in the control group within 60 minutes.

We used HPLC technique to measure moxi diffusion in blood and cerebral tissues. Ba et al. was performed optimization of the method by the modification of numerous pre-analytical and investigative stages [9]. Mice blood and cerebral tissues testers were treated by deproteinization due to its low protein binding. We decided, confirm the method using ciprofloxacin as an internal standard owing to the extraction efficiency [1].

Parting was accomplished by ion-pairing reversed-phase chromatography with 10 milimole strength of SDS [12]. Liang exposed that over this strength, resolution of fluoroquinolone was not upgraded and column equilibrium time was ideal. TBABr was mixed at the strength of 10mM to rise resolution between moxi and cipro, and to increase the peak nature [9,12]. The pH of the mobile phase was fixed at 3.5 with citric acid buffer solution at strength of 25mM. Lastly, the adjusted mobile phase comprised of 10mM TBABr, 10mM SDS, 3% methanol, 25mM citric acid, 40 percent acetonitrileat 3.5pH.Moxi and cipro have more sensitivity and specificity for flouresence detection than ultra voilet detection that's why it is used.

For determining moxifloxacin dual matrix the HPLC method was used. The lower LOQ was 0.1 g/L & 5g/L in brain and plasma respectively [11;15]. The HPLC procedure is very helpful and necessary for determining the cerebral penetration as well as pharmacokinetics of the moxifloxacin. For moxifloxacin quantification in body fluids, many methods were illustrated [12; 13; 17]. After administration of moxifloxacin in peritoneal cavity its cerebral penetration was determined. Rodriguez-Cerrato induced E. coli meningitis in rabbits and then determined penetration of moxifloxacin in CSF [13]. Pharmacokinetic study of moxifloxacin on non-inflamed meninges was done by Kanellakopoulou [10; 13]. Moxifloxacin was administered by Wise et al. in control and infected mice and was detected in brain after 6 and 8 hours respectively [7; 18]. Twelve beta lactam antibiotics were separated at wavelength 210, 230, and 298 nm. Mobile phase absorbed UV which results in variation from baseline. Another method was used in which polar drugs were retained and non-polar drugs were eluted. Mobile phase pH also effect the retention time. Drugs were well separated at acidic pH. Increase in pH decreases the retention time. These compounds were separated in 22 min. Imipenem shows the phenomenon of tautomerism due to which 2 peaks were obtained which were not resolved even on making dilutions with buffer of different pH (31; 28). 1st peak is considered as plasma concentration

Table: 1 Moxifloxacin concentration (50mg/kg) in brain tissues after intraperitonial administration in brain tissues.

Blood	T_{max} (hours)	C_{max} (milligram per litre)	$T_{1/2}$ (hours)	Cl (litre per hour)	Vd (litre per kilogram)	AUC $_{24hours}$ (milligram hour per litre)
Infec.	.75	11.7-23.9	1.9	.02	3.5	27.1
Cont.	one	5.5 -7.9	1.1	.08	6.5	9.5
Cerebral tissue		(microgram per gram)				(microgram hour per gram)
Infec.	.75	1.8- 2.2	21.7			3.3
Cont.	One	0.5 – 1.1	10.7			1

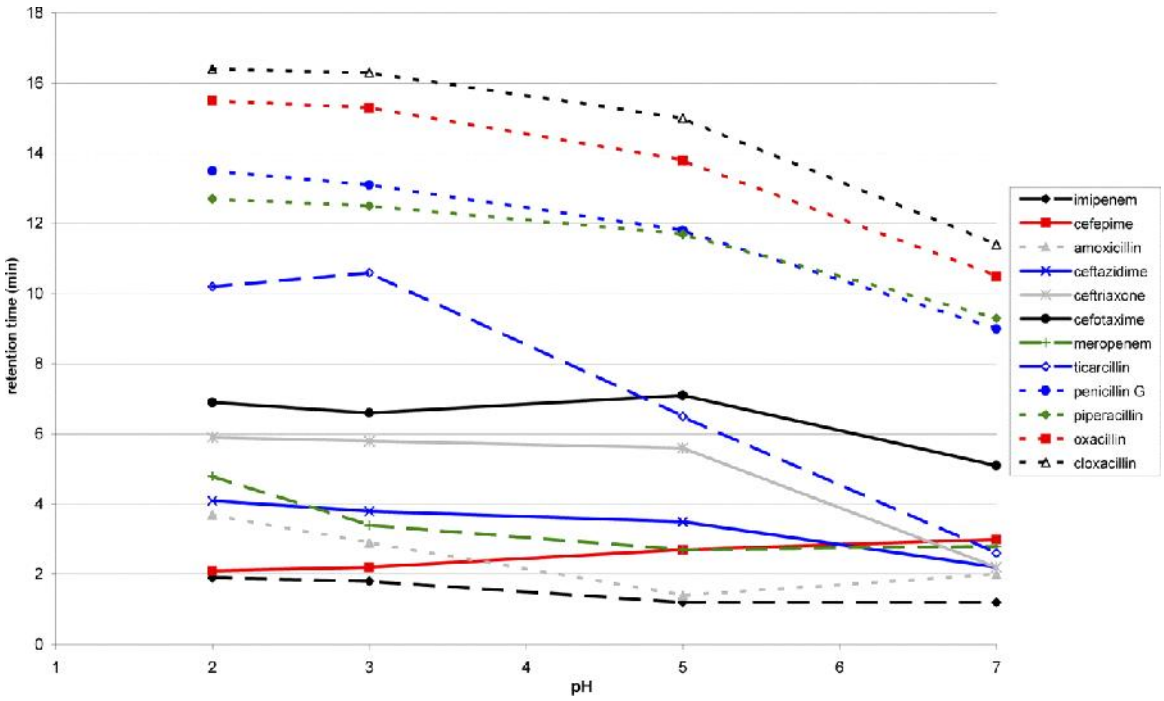

Figure: Compartive retention time of different antibiotics

peak. When a graph is plotted between retention time and mobile phase pH, linear line of cefepime, ceftazidime, amoxicillin, cefotaxime, meropenem and imipenem is obtained at concentration of 2-250 g/ml and of penicillin G, aoxacillin, piperacillin, cloxacillin and ceftriaxone at concentration of 5-250 g/ml.

CONCLUSION:

We authorized and developed HPLC method for moxifloxacin in brain and plasma and its entrance in CNS was verified. We recommended that any infection may alter the pharmacokinetic data of the moxifloxacin as expected. Penetration of drug in the infectious tissue is much more than

healthy tissue when the concentration of drug is increased. This method can also be used for quantification of drug in other body organs and diseases. It is a quick method and used for quantification of antibiotics in plasma in daily life (21; 32). e.g use for TDM. (29; 33).

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