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Seasonal distribution of Type-2 anti-diabetic drug Metformin HCl in River Yamuna of Delhi NCR "During Covid-19 Havoc"

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ABSTRACT

Metformin HCl is a highly prescribed drug of the anti-diabetic class around the Globe. In India, it is also highly prescribed and India is the second-largest consumer of Metformin HCl in the world. More than 90% of the drug excreted to the environment (Mainly in soil and Water) as waste of total consumption. Which is untraced due to the non-availability of a suitable & convenient analytical method for the quantification of this drug in an aqueous medium. because as per the WHO reports the quantity above 1ppm of Metformin HCl adversely affects the Flora and fauna in water. In this paper, developed an analytical methodology for the quantification of Metformin HCl in an aqueous medium. Extraction of the compound is through Solid-phase and quantification is through HPLC coupled with UV-Visible spectrophotometer. The suitability of the method for intended use has been validated as per the ICH guideline. The method has been applied for the quantification of Metformin HCl in the Yamuna river of Delhi NCR. The average recovery of Metformin HCl in river water has been found 65.4%. The highest concentration of Metformin HCl has been observed 282 µg/L in the winter season and lowest in the rainy season below LOQ. It may be due to migration of population due to lockdown, limited human and industrial activity of COVID -19, caused to increase the flow of the river which resulted in reduction of Metformin HCl in Yamuna river.

Keywords- Environment, Metformin, analytical methodology, Yamuna River, New Delhi

INTRODUCTION

These days, several typesof organic pollutants are being delivered in the environment, especially in surface water. To assess this phenomenon and keep away from conceivable unfavorable consequences for humans and wildlife, it is basic to increase the greatest information about events and destiny of natural micro-pollutants in the Aquatic Environment. Pharmaceuticals are a significant classification of water contaminants that have pulled in expanding worry in the most recent decade. A high amount of administered pharmaceuticals passes the human body unchanged by excretion and enters wastewater. The excreted and unchanged pharmaceuticals pass the sewage treatment plant (STP) and the incomplete removal contributes to environmental presence[1]. Even very low concentrations present in the water bodies or environment, drugs causes adverse effects on marine life. Mostly the problems are visible after certain time rather than spontaneous effects. Which is directly proportional to bio availability, susceptibility and degradability of the compound. [2]

Diabetes is the most common non communicable disease globally and have increased morbidity and mortality rates. It had affected 88 million in 2019 and expected 115 million in 2030 of the Indian population. [3][4] India has become the second-largest number of diabetic patients. The changing lifestyle with less physical activity, increased fast food trends, sedentary lifestyle, obesity are the etiological factors.[5][6]
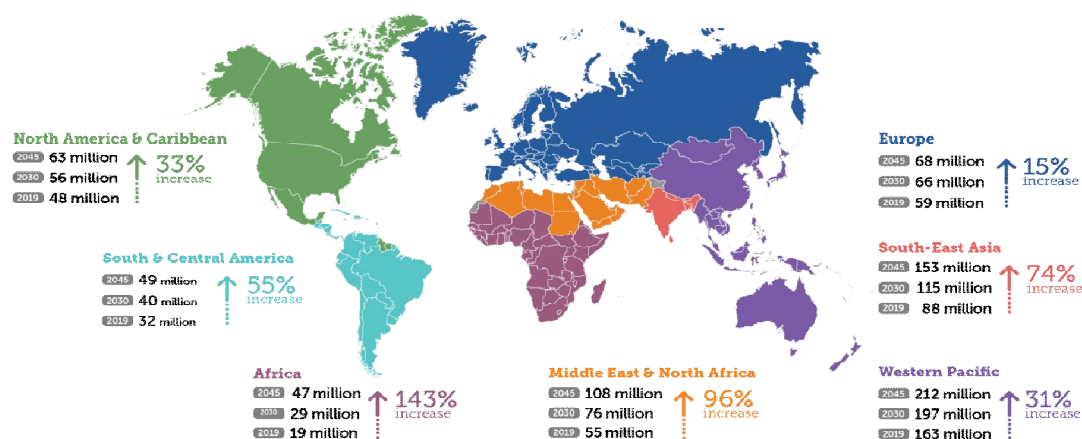
Fig. 1-Number of people (20-79 years) with diabetes globally and by IDF Region

As the number of patients increased globally the consumption of anti-diabetic drugs increased globally. Metformin HCl is the highest prescribed drug for diabetic type-2 patients.[7,8] It decreased down the blood sugar level by inhibiting hepatic glucose production and gluconeogenesis.[9][10]

Metformin HCl is not metabolized in the body. hence, It excreted as such in urine more than 90%.[8,11,12] due to which in so many country sewage water and surface water found contaminated with it. The concentration of Metformin HCl had been observed upto 50μg/L in freshwater potable water.[13,14]which is below the defined limit of WHO but it increase in environment gradually which is the concern of current society and aquatic ecosystem. Its impact on the environment is not available but it may be seen in the future.[15] So it'snecessary to be monitor in highly populated areas.
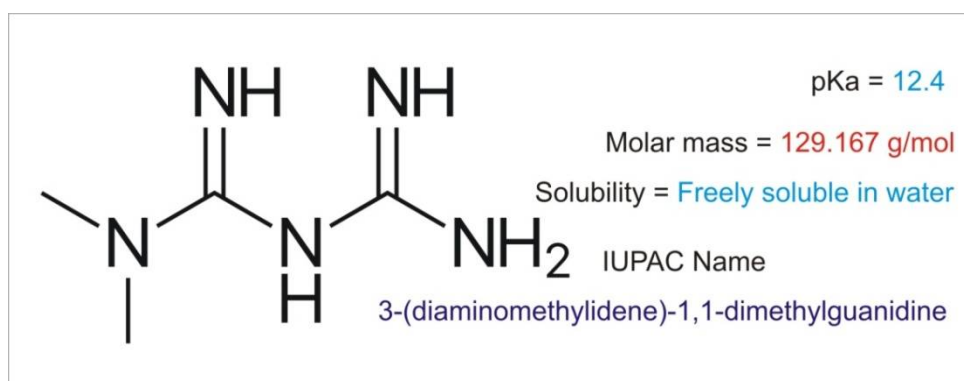

Figure.2- Structure and chemical detail of Metformin

Here we have determined the concentration of Metformin HCl in the Yamuna river of 22 KM stretch of the New Delhi region. Before this, So many analytical had been developed to determine Metformin in sewage water and surface water through HPLC coupled with LC-MS/MS. But this technique not easily available due to the high cost in developing countries. So here, we have used HPLC with a UV spectrophotometer. This is easily available in all pharmaceutical and environment research departments.

MATERIAL AND METHODS

Chemical and material

Metformin API with potency of 100.% was gifted for research purpose by Alkem laboratories Ltd, Navi-Mumbai (India), Ortho-phosphoric acid (AR-Grade from Merck), Sodium Dodecyl Sulphate (AR-grade from Sigma Aldrich), Acetonitrile (HPLC grade from RANKEM), and 1-heptane sulphonic acid(AR-grade from Merck), Oasis HLB SPE cartridge (Waters, India),Milli-Q-Water is from Laboratory Millipore Purifier. 0.45 μ Whatman filter paper, 0.22μ Nylon Millipore syringe filter.

Solid-phase extraction

Extraction methodology has been performed through oasis HLB cartridge with SDS ion pair. first of all, condition the cartridge with 10 ml of methanol followed by 5ml of water and final conditioning with 2mM SDS then sample is percolated at neutral pH. Elution of metformin was performed through 4 ml methanol.[16]To demonstrate the efficiency of Cartridge recovery had been performed at milli- Q water with spiking at a concentration of LOQ label and 500μg/L.

Sampling site description

Sewage and discharge from the industries are brought to The Yamuna river by 18 channels in Delhi NCR. Contingent upon the area at which these channels releasing the wastewater into the stream, six testing destinations were chosen (Figure-3). Sampling destinationswere1. Sur Jamuna ghat, Wazirabad This site doesn't have any domestic or industrial release in the nearby region and subsequently, the contamination load was fundamentally from the agrarian overflow. The water quality now is significant, as it is utilized as crude water (feed-water) for the water treatment plants providing consumable water in Delhi;2. Khazoori Pontoon bridge Nazafgarh Downstream- it's having a total load of 2064 MLD which is 61% load of total drainage from NCR,[17]3. Qudsia Ghat, 4. ITO bridge 5. Nizamuddin bridge, 6. Okhla barrage collecting from Shahdara drain which transferring a load of 638 MLD from eastern Delhi.

Sampling procedure

Water Sample is collected in 500ml amber colored glass bottle in triplicate from each sampling location and stored in an ice-cooled box. Time points of each sample were the first week of February, May, and August month, Which cover all seasons of Winter, Summer, and Monsoon. Sample processed through solid-phase extraction and analyzed on the same day in the laboratory.

Chromatographic parameter

Separation of analyte was performed on a Waters HPLC system equipped with UV-Vis Spectrophotometer, a quaternary pump, andan auto sample. Inertsil ODS 3V (150 mm × 4 mm) 5µ particle size Column used for separation. The injection volume was 1000 µL. 20% Acetonitrile with buffer of 0.1 % Heptane Sulphonic Acid pH 3.5 wth Ortho-phosphoric acid is used as mobile phase. Isocratic elution with 1 ml/minute flow rate.

Figure.3- Sampling destinations point from The Yamuna River

Method Validation

Verification of the intended method had been performed based on the regulatory guideline. Calibration range kept between 10 µg/L and 625µg/L with 6 calibration points.

System precision was performed by injecting 500 µg/L concentration spiked std in six replicates. To check the specificity of method blank, individual compound std spiked in milli-Q water sample was injected. Limit of detection (LOD) and limit of quantification (LOQ) is determined using the slope method of calibration curve,[18]

$$LOD = \frac{3.3\,\sigma}{S} \text{ and } LOQ = \frac{10\sigma}{S} \qquad\qquad ....Eq.1\&2$$

Where,

σ = Standard deviation error for y to x-axis linear regression of least square,

S = Slope of calibration curve

Accuracy of the method, Studied by spiking both compounds surface water at LOQ and 1000ppb concentration level (In triplicate).

RESULT AND DISCUSSION
Method development and validation
Metformin salt found in the form of HCl So it is commonly known as Metformin HCl but whenever go for quantitative and qualitative determination it was analyzed in the form of Metformin. As see about its chemical properties It's a highly polar compound with low molecular weight, basic with pKa 12.4 and -1.43octanol-water coefficient.[12]

Extraction of Metformin was carried out through Oasis HLB cartridge withSDS, The recovery without ion pair found to be below 20%hence to enhance the recovery SDS is used as ion pair. After conditioning with 2mM SDS,the recovery average was found to be 65.4%.

λ-max for Metformin was found to be 233 so the response of peak was captured at 233 nm. The concentration of the compound is very low hence the injection volume of the sample is kept1000μL, Sample is stable at room temperature so sample tray temperature is kept ambient. The column selection for separation was based onthe polarity of a molecule as the compound is highly polar and having a pKa of 12.4, but the range of column for pH is 2-11, Due to its high polarity it would not retain in the column and flush out in void volume.Hence, To retain the compound used 1-heptane sulphonic acid as an ion pair reagent and kept buffer acidic at pH 3.5 with Orthophosphoric acid, the ratio of buffer and Acetonitrile kept 80% and 20% respectively. Flow kept Isocratic,The column compartment temperature kept at 30°C,Retention time of Metformin peak is obtained at about 4 minutes.

The above chromatographic condition had been optimized after several trials, the suitability of the developed method had been verified by validation of method as per ICH guideline.[18] The results of the validation parameter have been summarized in Table-1.

Table .1- Summary of validation parameter

S.No.	Parameter	Result
1	Linearity	0.999 (Figure.4)
2	Limit of Detection	19.32 μg/L
3	Limit of Quantification	58.56 μg/L
4	Recovery at LOQ level (n=3)	68.1 % (%RSD 4.9)
5	Recovery at 500μg/L	62.4 % (%RSD 6.3)
6	Repeatability	
	(a) Intraday (%RSD)	8.5
	(b) Inter day (%RSD)	9.2
7	Solution stability	72 hours at room temperature

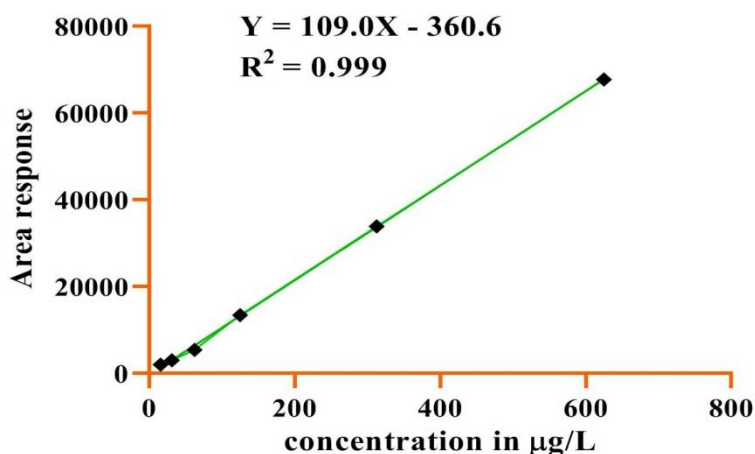$$Y = 109.0X - 360.6$$
$$R^2 = 0.999$$

Figure.4- Linearity graph of Metformin HCl

Applicability of method in real sample analysis
The developed method had been used for the quantification of Metformin HCl in the Yamuna river. Variation in concentration was observed with season and with distance. Wazirabad Barrage is the entry point of the Yamuna River in New Delhi before this point river stream not received water from domestic and industrial discharge hence the concentration of Metformin was found below LOQ level not quantify. After that, it collects drain from the Najafgarh drain which having the highest load of industrial discharge and domestic waste of2064 MLD which is the 61% of the total load fromDelhi NCR. Similarly, all points of sampling in the Yamuna River only received discharge of domestic and STP. Which subsequently increases the concentration of Metformin HCl.

On seasonal variation it's found that due to COVID-19 lockdown around the country the pollution level of River had been decreased and population load of NCR region had been decreased due to which flow of freshwater also increased, So increase in water flow and decrease in population (due to migration of pupil towards native place) result in decrease in organic pollutants in water. Than we go for Monsoon season the flow of water increased due to rain hence the concentration of organic pollutant decreased. That's why winter season showing higher concentration of Metformin in river water. Availability of Metformin HCl concentration is summarized in Table-2.

Table.2- Occurrence of Metformin in the Yamuna river (New Delhi)

S.NO.	Sampling destinations	Month		
		February (Winter)	May (Summer)	August (Monsoon)
		Mean (%RSD)	Mean(%RSD)	Mean (%RSD)
1	Sur Jamuna Ghat	52 µg/L (27.4%)	35 µg/L (29.5%)	25 µg/L (35.6%)
2	Khazoori Pontoon bridge	156 µg/L (10.7%)	110 µg/L (15.4%)	48 µg/L (28.9%)
3	Qudsia Ghat	188 µg/L (12.6%)	145 µg/L (14.5%)	58 µg/L (31.4%)
4	ITO bridge	240 µg/L (15.8%)	130 µg/L (11.8%)	55 µg/L (35.2%)
5	Nizamuddin Bridge	218 µg/L (12.6%)	166 µg/L (16.2%)	47 µg/L (33.6%)
6	Okhla barragge	282 µg/L (10.4%)	182 µg/L (19.1%)	59 µg/L (29.8%)

The result obtained is comparable to data obtained in other countries, Highest load of Metformin in wastewater observed in US 248µg/L,[19] Germany 129 µg/L,[14] Canada 99µg/L[20] and Netherland 98 µg/L.[21]based on literature available occurrence of Metformin HCl summarized in Figure-5. But the concentration of Metformin obtain in the river water is slightly higher than data obtained in the US stream. That can be predictedbased on population load in that particular area. New Delhi is among the highly populated city in the world and the water treatment facility in New Delhi not sufficient only 2083 MLD of sewage water is treated out of 3268 MLD per day and remaining discarded as such in River water. So Metformin HCl observed in water was higher in comparison to global appearance.
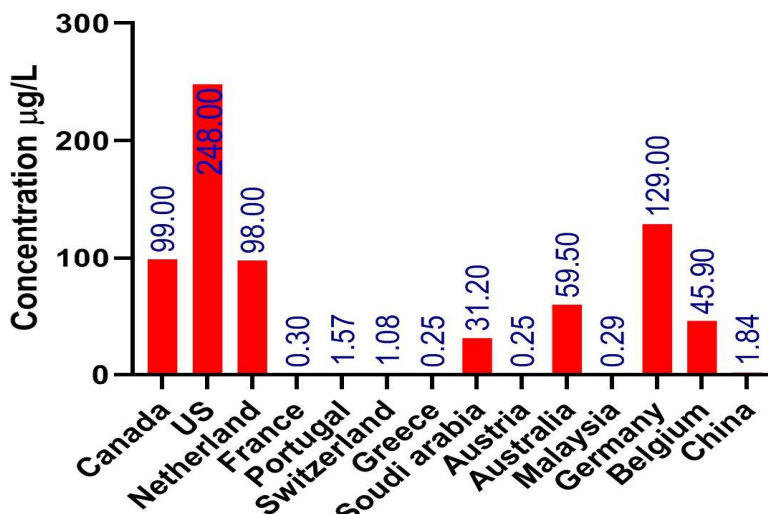

Figure.5- Bar diagram for occurrence of Highest concentration of Metformin observed Globally.

CONCLUSION

In this paper developed an analytical methodology for the quantification of Metformin HCl in wastewater. Method verified for intended use as per ICH guidelines. Method is capable to detect compound of interest for a range of58.56µg/L to 625 µg/L. Method was used to determine the concentration of Metformin HCl in the River Yamuna stretch of New Delhi. At the entry point of the Yamuna river in New Delhi concentration of Metformin was found below LOQ but as it moves ahead the quantity of wastewater increased the concentration of Metformin HCl availability increased. So this information concludes out that regular monitoring of water is compulsory and Delhi Jal Board needs to increase the sewage treatment capacity to prevent untreated wastewater into the river.

As every method have its advantage and disadvantage so that described below

Advantage – This method is convenient, the cost of analysis is not too high, can be use for STP effluent, river water, and Hospital / domestic wastewater analysis.

Disadvantage – It cannot be used to quantify Metformin HCl below the LOQ limit of 58.56µg/L.

CONFLICTS OF INTEREST
There are no conflicts of interest to declare.

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