Pharmaceuticals in Environment

Introduction:

A media report of last year pointed out the alarming levels of pollution in Kazipally lake near Hyderabad located in the vicinity of several Pharmaceutical companies. Independent testing revealed not only pollution due to untreated pharmaceutical active ingredients (APIs) but more worryingly with the presence of antibiotic resistant genes.

While the industry may be very well taking steps to ensure that they comply with all relevant norms in treating and discharging effluent but it cannot be denied that there is a definite risk in untreated APIs finding their way into waste water streams.

One of ways in which this risk can be determined is to test the treated waste water for traces of APIs. This is of course no easy task because after passing through a primary, secondary and tertiary treatment process the API may well be no longer in its original form and also due to the sheer amount of background interference and the large dilution factors.

The Swedish Medical Products Agency and Swedish Government have over the past decade surveyed a large number of environmental information on pharmaceuticals and have also adopted a methodology for conducting the risk assessment for Pharmaceuticals in Environment.

This risk assessment is based on several factors like

1. What is the eco-toxic profile of the API?
2. How fast does it degrade?
3. Whether it is bio-accumulative?

GVS Cibatech has done some work in analysing the treated effluent samples for a major pharmaceutical manufacturer to assess the above risk. Most of the APIs are having method of analysis with High performance liquid chromatography (HPLC) for the determination of Assay and related impurities. Hence, HPLC can be used as a useful tool to analyse APIs in treated waste water before discharging it into the water sources.

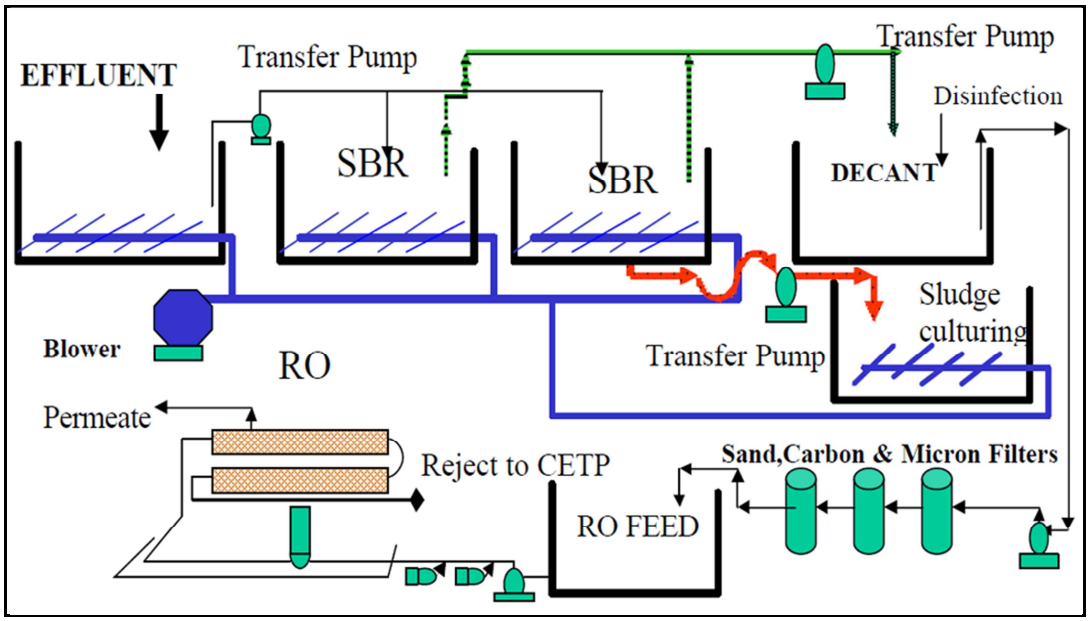
To decide on the acceptable level of these APIs can be decided based on the Predicted no-effect concentration (PNEC). PNEC values are generally used as environmental risk assessment tool in ecotoxicology. FarmaceutiskaSpecialiteteriSverige (FASS) database is generally used for the information related to PNEC values.

Experimental:

Mycophenolate mofetil is used as immunosuppressant drug used to prevent rejection in organ transplantation. PNEC value for Mycophenolate mofetil as per FASS database is 0.068µg/L.

This API manufacturing plant is treating waste water in their captive ETP. They are sending the RO reject to external CETP. The ETP set up diagram is given in Figure-1. As you can see the only waste stream which will have impact external environment is RO reject as it goes to external CETP and to river body. So the concentration levels at CETP are to be considered.

Figure-1



Hence method development was carried out with HPLC to evaluate the content of API in treated waste water. Sensitivity limits required are very low hence extraction and concentration techniques were used.

Materials:

HPLC system: Prominence-i, LC-2030C

Column: Shim-pack GWSC185µm x 250 mm, 4.6 mm

Buffers:

Potassium dihydrogen phosphate

Triethyl amine

Mobile phase solvent: Acetonitrile and Water (HPLC grade)

Samples: Treated water samples from different stages of ETP (RO Permeate, ETP inlet, RO reject)

Instrument method:

System:	HPLC System, Prominence-i, LC-2030C
Column:	Shim-pack GWSC_{18}5µm x 250 mm, 4.6 mm
Injection volume:	20 µL
Column Temperature:	40 °C
Auto-sampler Temperature:	10 °C
Eluent A :	0.02 M KH_2PO_4 of pH 5.5 with Triethylamine
Eluent B :	Acetonitrile

Gradient:

Time	Eluent A	Eluent B
0.01 min	70%	30%
3.0 min	55%	45%
12.0 min	50%	50%
15.0 min	50%	50%
20.0 min	25%	75%
25.0 min	50%	50%
30.0 min	70%	30%
35.0 min	70%	30%

Run Time:	35.01min
Flow:	1.5 mL/min
PDA-detector:	190-800 nm

Solution preparation:

Diluent:

Acetonitrile of HPLC grade

Blank preparation:

Use Acetonitrile as blank solution.

Standard solution preparation:

Weigh 10 mg of Mycophenolate mofetil API reference standard in to 100 mL volumetric flask, dissolve the material and dilute up to the mark with diluent (stock solution).

Further dilute 1 mL of the above stock solution to 10 mL with diluent (2nd dilution).

Further dilute 1 mL of the above stock solution to 25 mL with diluent (Standard solution).

Sample solution preparation:

50 mL of sample was filtered through 0.45µm syringe filter and pH of the solution adjusted to 8.5 using 1N sodium hydroxide solution.

Transfer resulting solution in a separating funnel and extracted with 10mL, 5mL, and 5mL volumes of dichloromethane. Collect and combine all the three extract, Add Sodium Sulphate anhydrous to remove the water traces.

Transfer dichloromethane extract in a 25mL volumetric flask, washed sodium sulphate residue with dichloromethane and transfer in same 25mL volumetric flask and make up to the mark with dichloromethane. Evaporate the solution under constant flow of nitrogen till dryness then add 1mL of diluent and vortex for a minute.

Results of relevant method validation:

Method precision: 1.7

Accuracy: 95.0

Linearity: 0.99757

Limit of detection: 0.7

Limit of Quantification: 2.0

Results of sample analysis for Mycophenolate content:

RO feed: 0.45 ppb

RO Permeate: Not detected

ETP inlet: Not detected

RO reject: 2.71 ppb (Equivalent to 0.30 ppt)

- ETP details used for the calculation were as below:

3KL RO reject is sent to CETP per day. CETP capacity is 27 MLD. So effective dilution factor is 9000.

The PNEC value is 68ppt whereas RO reject with 9000 times dilution will have concentration of 0.3ppt. So in this case API concentration in final effluent is below PNEC.

Images:

Image 1: Overlay chromatogram of LOQ precision

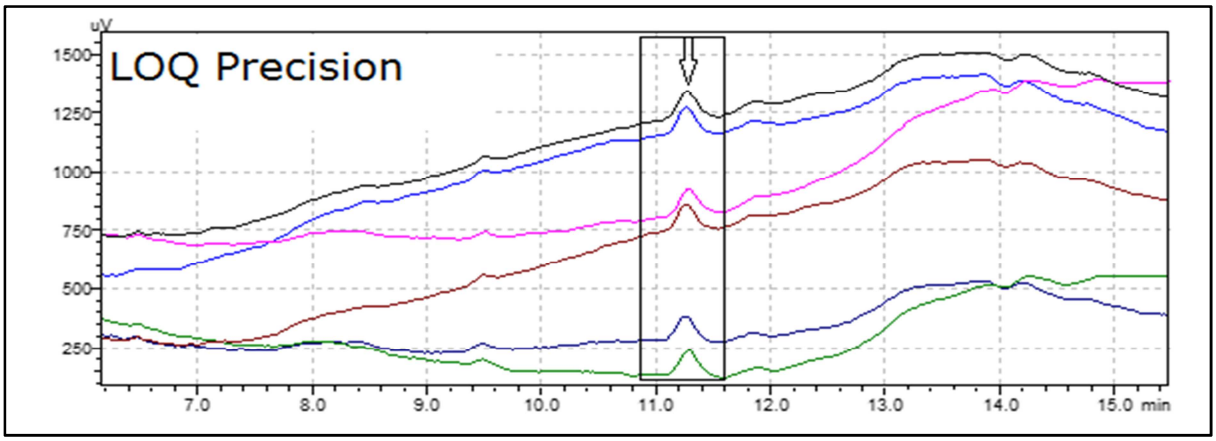


Image 2: Linearity chromatograms (50% to 150% level)

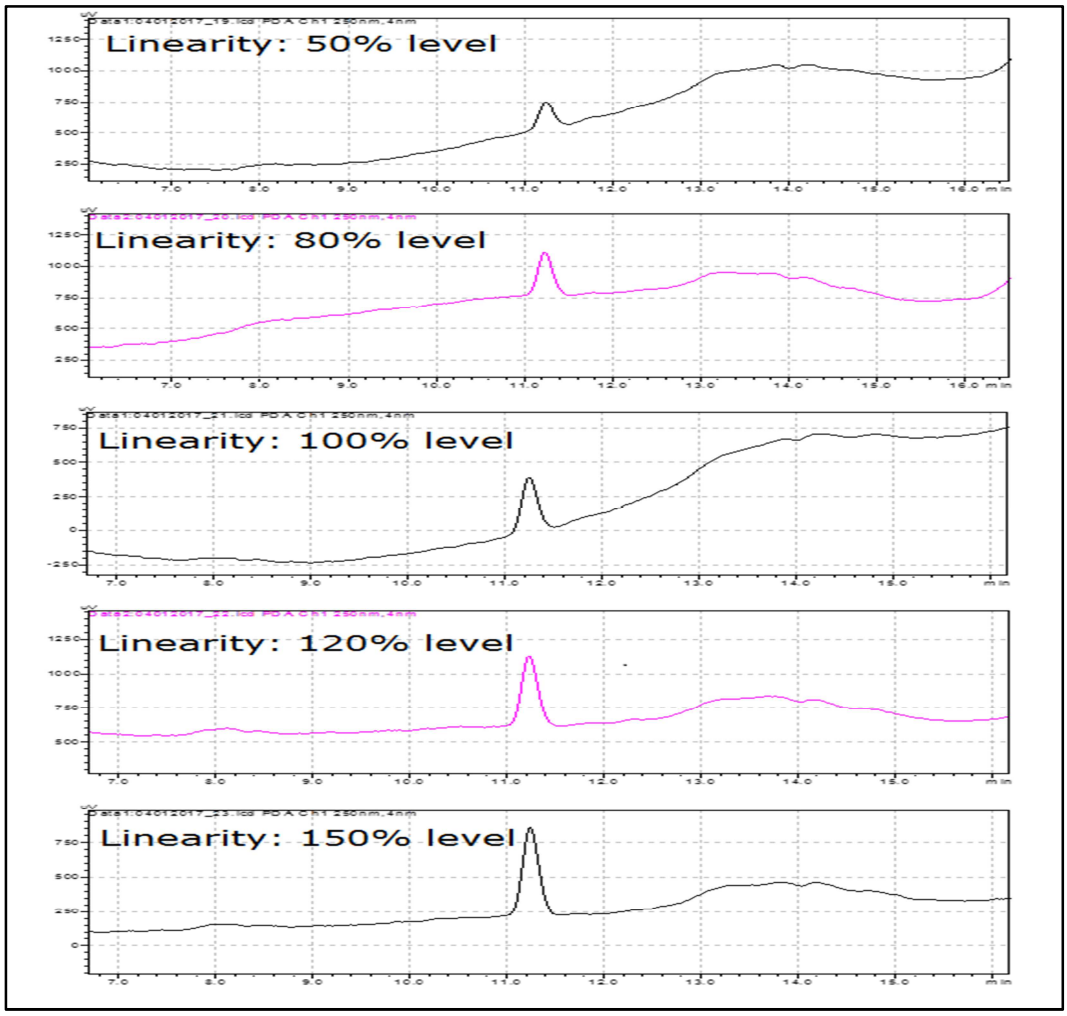
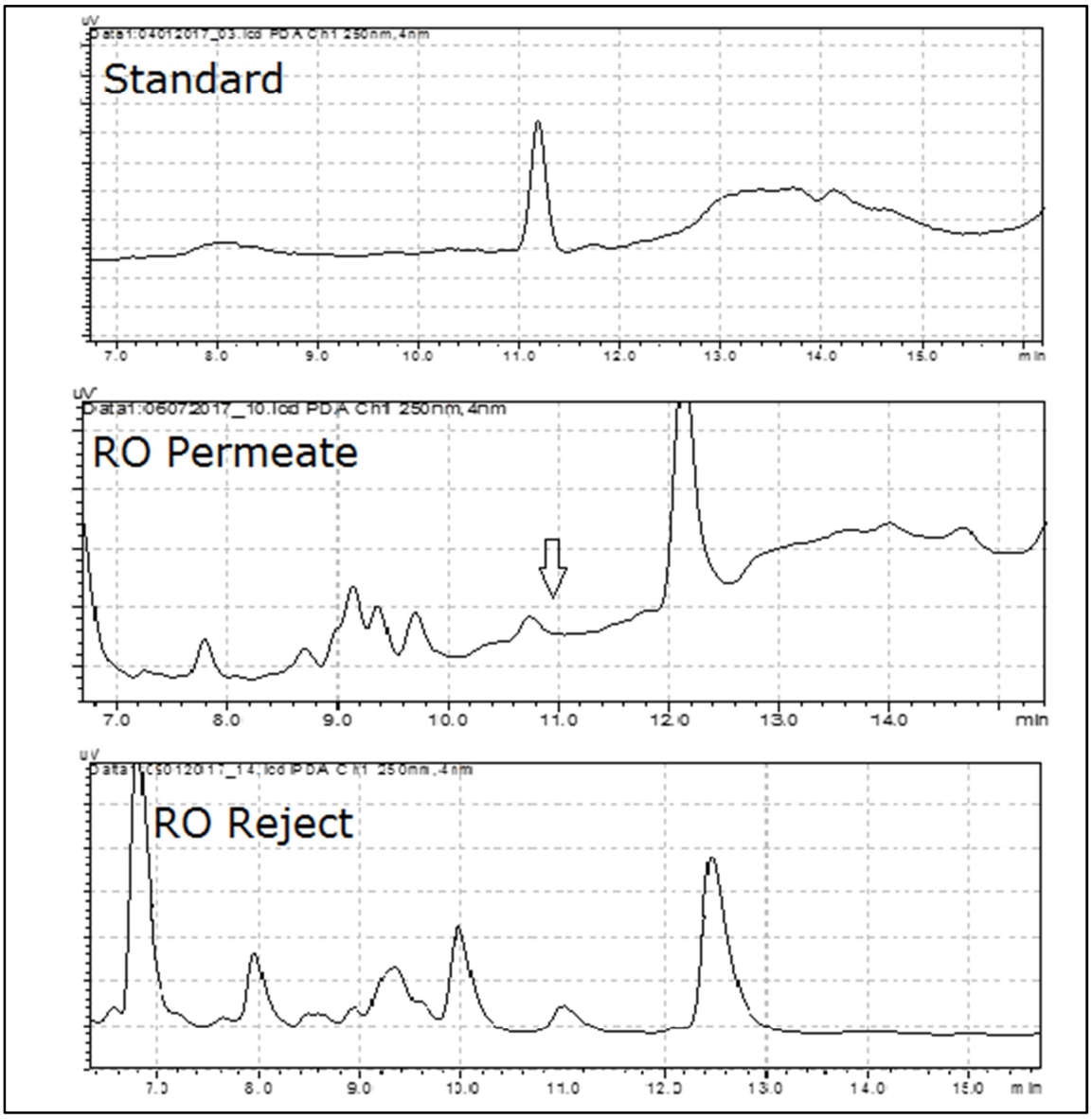


Image 3: chromatograms of Standard and samples (RO Permeate & RO reject)


Standard
RO Permeate
RO Reject
min