Environmental Quality:
Monitoring and Analysis
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Lecture No. 26
Analysis Methods – Gas Chromatography
(Mass Spectrometry)

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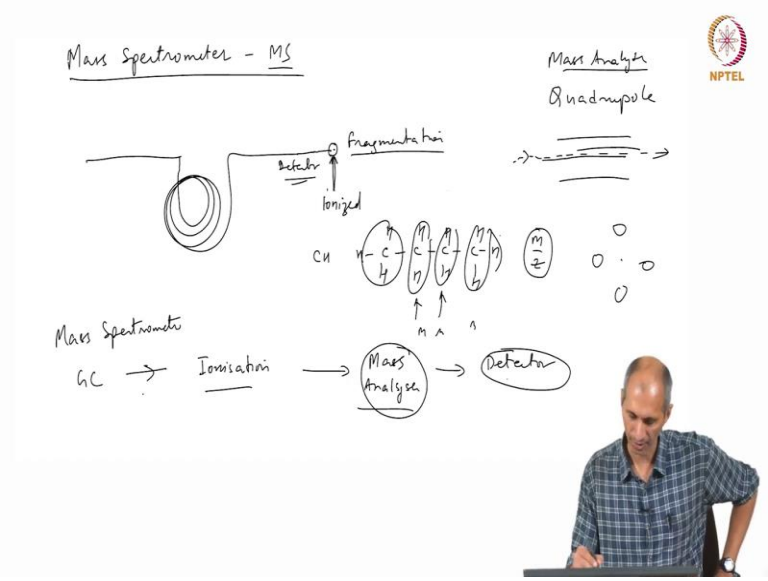

Okay, so we are continuing our discussion for chromatography. So, discuss little bit about mass spectrometer for organic analysis. So, the mass spectrometer has the identification of in a mass spectrometer detector, what happens is this so in the regular GCV we see that GC column and goes out and you have the FID or something that comes here so the trip detectors is here in the mass spectrometer is a big detector.

It is not a small device like the FID or GCV what happens in the mass detector is the each peak everything that comes anything that comes to that into the detector is ionized were essentially it is ionized and fragmented broken into small segments. And this trivializing the theory of it, mass spectrometry is a very complex field and involves the interaction with energy, different forms. In this case, energy is in the form of electrons, high energy electrons and not that high energy also, it is fairly moderate.

There are other devices where you can ionize it to a much larger extent and do further things. But what essentially happens here is that the there is a fragmentation that happens of the molecules organic molecule is coming. And each when it fragments, you have the capacity the instrument has the capacity to measure the intensity of each fragment. So for example, if you have a compound that looks like the CC-H what this can do is it will fragment in terms of CH2, CH2, CH2, CH3 and so on.

So, it knows and each of these has a mass there is a certain mass for each of these fragments and this mass and there is also a charge because it is already ionized the mass and charge and based on this mass and mass pay charge you can separate each fragment, you can analyze for each fragment history we do for photography, we analyze each fragment and then measure the amount that is contained in each fragment. So to give you an example so, I will show you an example in a minute.

So, what this does this the mass spectrometer has an instrument has a device called as a Quadrupole mass analyzer. So, the first thing the mass spectrometer the first thing is an ionization is a source is ionization sample is coming from the GC flowing and it comes in ionization then it is a mass analyzer. And then there is a detector, this is actual detector what we are doing is if you put the detector here we are analyzing everything and mass you put the detector before the GC or analyzing.

Without even separating component we are putting after this GC separate incompetent analyzing it, but you are do not have any information about the qualitative part the detector is here at the end of it, before this you are separating it so, this mass analyzer what it does is this the mass analyzer itself is a is different types of mass analyzer exists but 1 of the common mass analyzer is called as a quadrupole. Very simply it is a channel which is about 4 magnets. If you look at it from the way is a 4 magnets that are and there is a path this is the pathway of the analyzer are going here.

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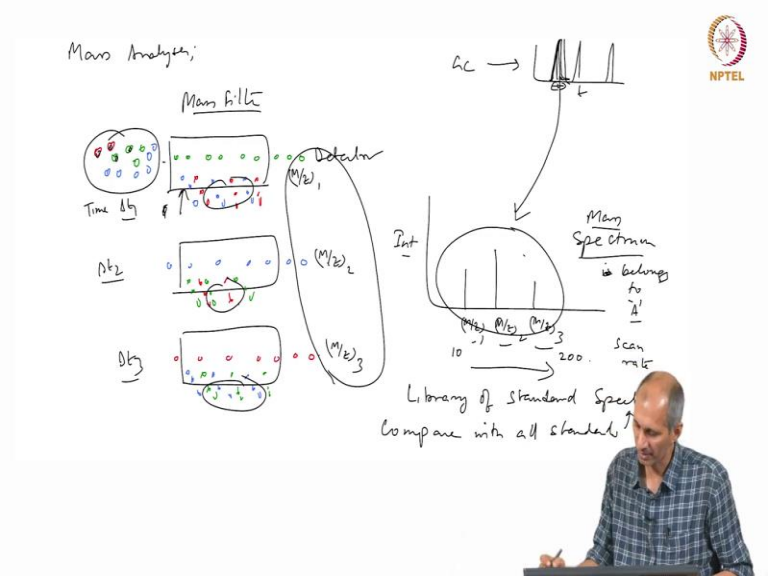

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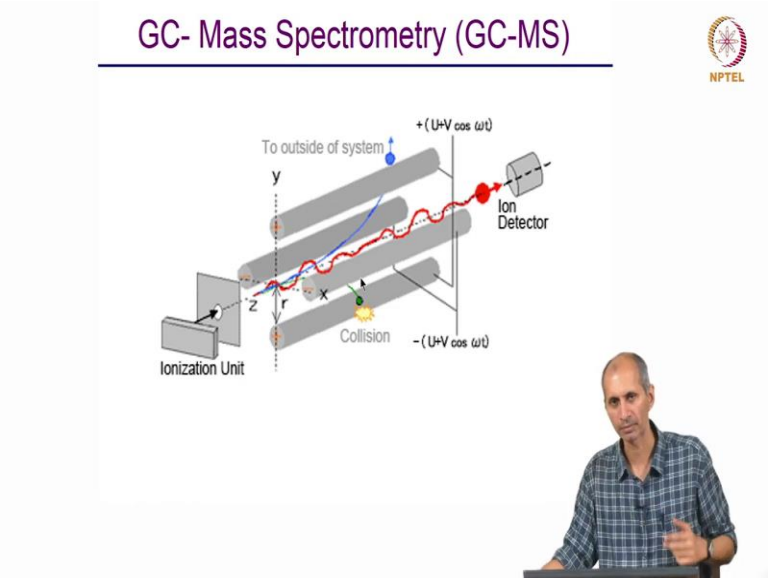

This is a mass analyzer so, this is coming from the GC. And here, this is the place where we this thing happens to the ionization happens, energy is given large amount of energy is given and the compound that is coming in it bombards, and it goes fragments are going through this. These 4 rods you see are the analyzers rods essentially have some, it is like a magnet. Very simple terminology is like a magnet really, it is some sort of an electromagnetic device. What it does is it pulls there is a trajectory.

The some of the things that are have a certain mass what it what is programmed to do is it is programmed to allow 1 mass to go through the rest of it is pulled out. It is also called the mass filter let us assume that it is like a mass filter they do not worry about the details of it. What it allows is, there are several things that are let us give different colors to it this is some reds, some green fragments and some blue fragments are all going in what it does is for a particular setting of the mask filter.

It pulls out if I can pull out all the blues come out all the reds come out and only the greens go through to the detectors. This happens, which means that it is separating the fragments of a compound itself 1 compound is breaking it up into fragments and 1 of these fragments is being analyzed. And then it switches to the next one. So this is from, say from time t1 to t2 we will call it us some delta t1 and the next delta t2 to the same thing happens but now allowing it will allow all the green and the red ones are pulled out it only allows the blue to go through and so on.

And the third time segment in allows the red ones to go through this is done very rapidly very quickly. So, from time delta t1 times time intervals delta t2, delta t3 it is trying to analyze the doing and M by Zed 1, M by Zed 2, M by Zed 3 corresponding to 3 different fractions of the thing. So, the detector is really allowing 1 of these fragments to go through and then this all his information is constructed.

So, the signal now look like this at one particular time as a compound is going to the chromatograph GC system and then you will get M by Zed 1, M by Zed 2, M by Zed 3, all these 3 intensity this is the intensity of the signal all these 3 intensities together represent the compound that is getting out. So, in other words in the GC system as a GC system we are getting peaks that are coming out each peak is coming out sometime as the peak is coming out.

This is a flow of flow system it is coming out as a function of time. So, each 1 of these things is going through the detector. there is mass analyzer and at every point if you are able to quickly scan all the mass by charges I want to get mass by charges from say 10 to about 200 how I will know this must be charged the maximum must be charged the molecule rate of a compound beyond that you cannot have anything.

So, if the molecule does not fragment it will come in as it is so, then the molecular rate will be the mass of that fragment. So, it does not have anything to fragment it was all full and request higher energy to fragment and we are not providing that much energy in the GC some things which are fragmentable in the GC energy, if you need to fragment more, you need to go to really high energies and you do not have that in the instrument here.

So, in the range that you are looking at, while the compound this is a time that it has the beginning of a peak to the end of the peak is the speed at which the compound is going. In this within this time, it has to know go and scan everything, all the fragments, it has to scan from 10 to 200 as many times as possible it keeps going 110 to 200 as many times as well as it stays for some time. So, what it will do is it has a certain order scholars can wait.

So, it will do that so, you can imagine that if something is flowing at a certain speed, so, everything is going right, you have a mixture of these things which is flowing through this mass analyzer while it goes through this mass and literally all of it is going through this mass analyzer you have to scan through everything, you have to scan all the mass that is of interest to you. And therefore, you will get intensities assuming that this is all well mixed, what we mean will mixes at a time at 1 second and say 1.2 seconds or 1 second and 1.2 seconds.

The composition is the same, or 1 of this fragment does not get out of the others which means they will be a problem. So we assume that they are all well mixed and then you pull out all of them except one and then measured that this is intensity? This is recreated when the compound comes out like this in the exhibit, this is the spectrum, this is the mass spectrum of whatever has come out how do you identify.

Now is this is compound X here to check if this is compound, say all peaks so, everything corresponding to this peak has now come out. So the intensity of all these 3 will add up together the intensity of this peak that is the general idea. There may be there may be losses, of course, obviously, there are losses because it is not the entire thing, you are lost some of them you are only collecting the signal for a fraction of it because you are losing all of it here.

You are, not analyzing it really. This is another important point to remember. But nevertheless you get signal corresponding to the fraction fragments of this and this is how do you use this to identify the compound. You have a mass spectrum, which is like a signature of this particular compound. What this says is, there is a certain fraction of MZ 1 there is a certain fraction of MZ 2 it was a certain fraction ends at MZ 3.

You can reconstruct the structure of the compound using this, but that is a longer method, but is there an easier way of checking if this is a spectrum, the spectrum corresponds to a particular compound. So, is the signature of this compound is there a way in which you can verify cross check if this mass spectrum is belongs to a particular compound. How will you verify this is a spectrum corresponding to 1 particular compound easy method?

Time normal this is a signature spectrum, how will you come what will you how will you verify this is this belongs to a particular compound your identifier but lot of data now, this data is a spectrum. So, spectral information is like a signature, how do you know that this signature belongs to a compound. There is no wavelength here, you are talking about different spectrum come to that we have been doing this also this discussion.

If you do not know what it is, what is 1 option whenever we are stuck like this we make use of a standard. So, what standard will you check it against? You did not know so, there is a you will compare this is a library of standard spectra for all known chemicals that are being manufactured and you think it is there in this compound library of standard mass spectrum. Every time somebody manufactures a chemical, you they will measure properties and one of the properties is mass spectrum.

There are other spectra information that you also get this infrared, UV visible absorption to other things we will talk about briefly fluorescence spectrum knowledge is that information that pertains to the signature of that particular compound. So, you compare with the reference with all standard spectrum a very painful process which means compare means I will give you an example.

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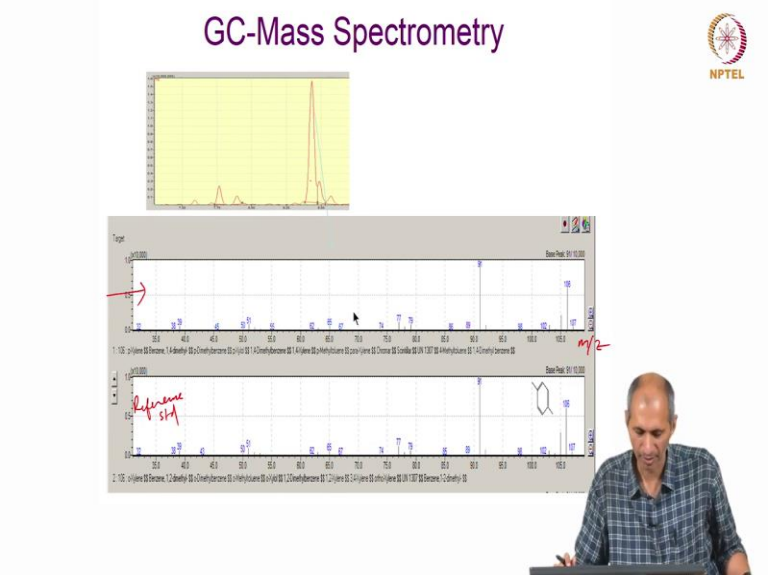

So, suppose I take this speak, this is a chromatogram from a GC and then I take this particular peak and we have said that now, this is a 2 dimensional thing. So, here this is the time axis and this is the intensity, this is a signal intensity this is time and you can see these numbers here are 7.5, 10 minutes, this time in minutes. Now, if I take this 1 particular peak expanded it in the software, the mass spectrum of this peak corresponds to this.

So, the first one here is the mass spectrum corresponding to this peak. Why I am doing is I am taking the mass spectrum for the entire thing. So first the entire thing. This peak the mass spectrum of this corresponds to this. So you can see that on the X axis here is M by Zed and the Y axis is some intensity. Again, you can see small lines and you can see some numbers there are some mass spectra and there is one big number big peak is 91 and another one of the big peak is 106.

Now I have a library of such spectra for if I do not have a library, I can guess I can say, this is benzene. Can I go and see this? This is benzene really, I know benzene maybe in a sample and they supposed to come around this time from the retention time analysis, I want to confirm this is really benzene. So I inject benzene and I compare the spectra of reference spectra in it. So this 1 is a reference standard of benzene second peak.

So what it does really is you see that some of the peaks which are there in the first one or not, they are in second and some peaks are there here probably are not there in the first one. So, we do a match.

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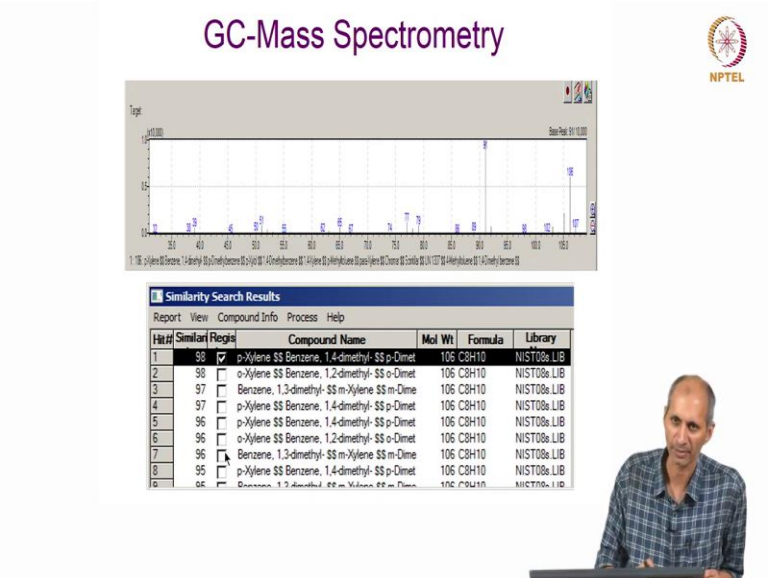

So, when we do a match the instrument will allow you this when you do the match then this is you can you get what is called a similarity search in the olden time it used to be done manually, but now software very quickly. So, it will give you a match says 98% match to benzene to several things, which are all isomers you know compounds have same molecular formula it will look the same, you have to figure out based on some other thing whether which one of the SMS uses.

So, this is why it does not give you 100% again, it only gives you a very high probability of the compound. So, this is xylene in this case, the 1, 4 dimethyl benzene, is 1, 2 dimethyl benzene is para xylene, ortho xylene all these are possible. So, that information is not available here. You have to go to some other way of finding out but that at least you know it xylene. So this is the way mass spectral GCMS is used. So, you need if you have a new compound, you need the spectra for that and you will compare most of the times.

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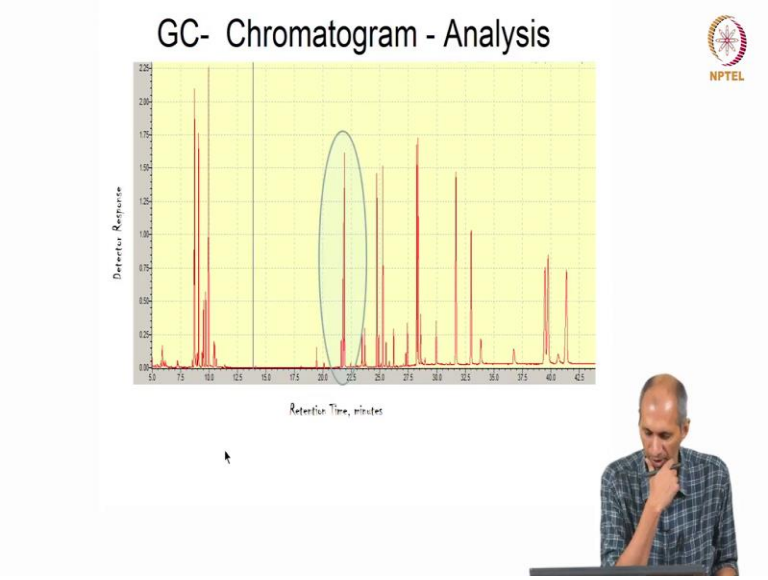

So, yesterday's lecture, we are talking about quantification, and we will look at that a little bit. So this is how the chromatogram looks at detention time at detector response. Then I can current modern software, you can do all these things, you can expand and go and look into a peak and all that.

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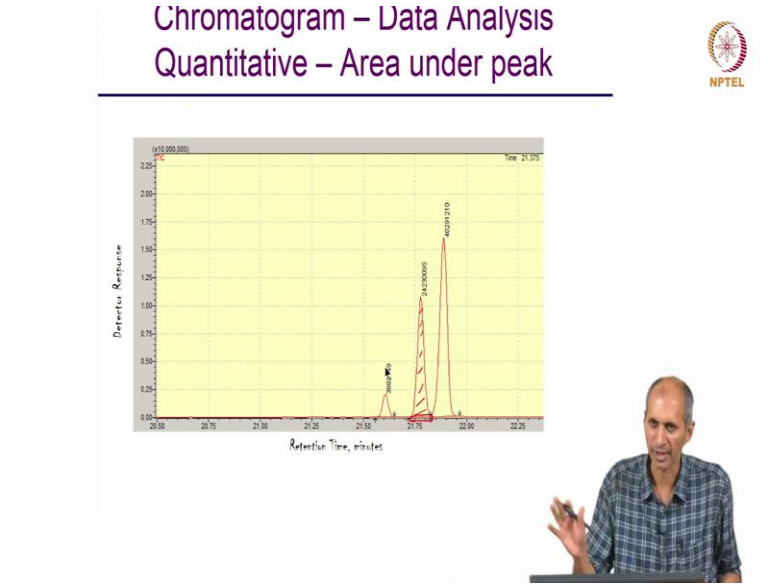

So I am expanding that particular section of the chromatogram. It look like this. You can CP capes. They look very nice scene. But you can also see that sometimes if you notice this point, there is a particular it is not going back to baseline. It is stopping short, which means that it is separated and not exactly nice. But do you see there are 2 peaks so it is your judgment, whether

you can live with it or not. So there are some statistical tools this area calculation is done like this and they will adjust the numbers there.

But if the peak is really overlapping and they will go back to separation again you run the chromatography with different set of conditions. So, the area under the peak the number now you are seeing is the peak has been integrated, you see how it has been integrated cut it there and they have integrated area. So, it is integrated from this point to this point and throughout and this is some units, arbitrary area units.

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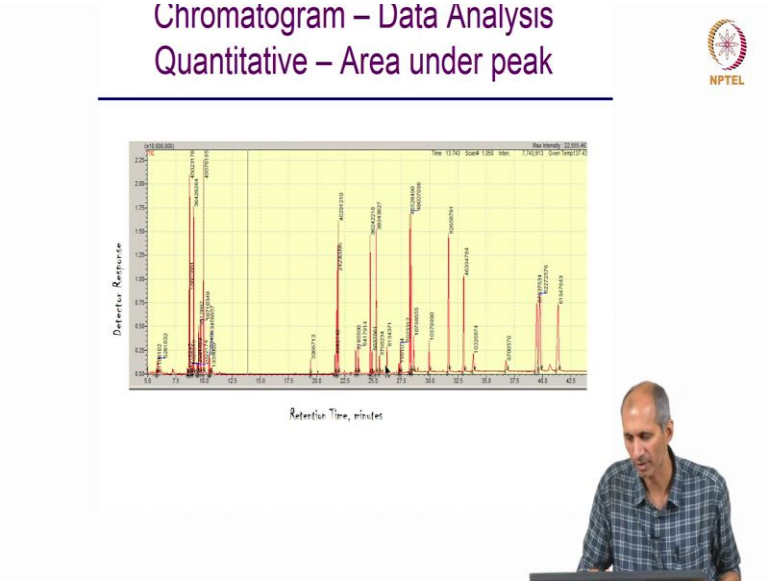

So, you can integrate the entire chromatogram you will get all of this.

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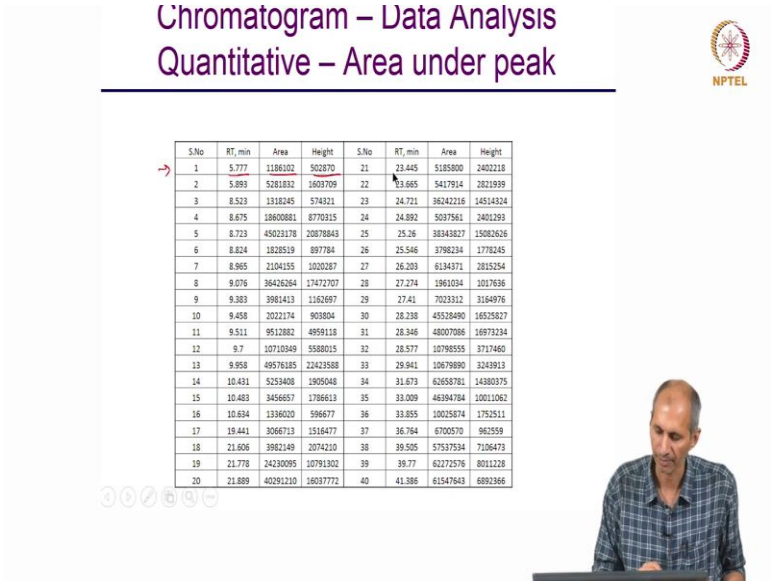

S.No	RT, min	Area	Height	S.No	RT, min	Area	Height
1	5.777	1186102	502870	21	23.445	5185800	2402218
2	5.893	5281832	1603709	22	23.665	5417914	2821939
3	8.523	1318245	574321	23	24.721	36242216	14514324
4	8.675	18600881	8770315	24	24.892	5037561	2401293
5	8.723	45023178	20878843	25	25.26	38343827	15082626
6	8.824	1828519	897784	26	25.546	3798234	1778245
7	8.965	2104155	1020287	27	26.203	6134371	2815254
8	9.076	36426264	17472707	28	27.274	1961034	1017636
9	9.383	3981413	1162697	29	27.41	7023312	3164976
10	9.458	2022174	903804	30	28.238	45528490	16525827
11	9.511	9512882	4959118	31	28.346	48007086	16973234
12	9.7	10710349	5588015	32	28.577	10798555	3717460
13	9.958	49576185	22423588	33	29.941	10679890	3243913
14	10.431	5253408	1905048	34	31.673	62658781	14380375
15	10.483	3456657	1786613	35	33.009	46394784	10011062
16	10.634	1336020	596677	36	33.855	10025874	1752511
17	19.441	3066713	1516477	37	36.764	6700570	962559
18	21.606	3982149	2074210	38	39.505	57537534	7106473
19	21.778	24230095	10791302	39	39.77	62272576	8011228
20	21.889	40291210	16037772	40	41.386	61547643	6892366

So, I will get I can get a report like this. So, it also reports for a particular retention time it will give me the area and the height.

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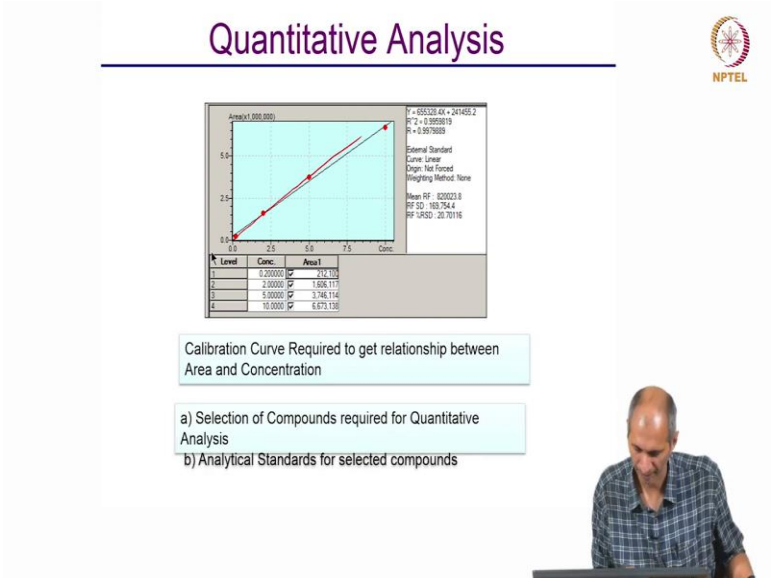

Then I can go to each one of these compounds if I know which one they are, I will do a calibration and the calibration is done in terms of the concentration that injecting into the GC versus now we have we have studied calibration. Calibration can be done in mass or concentration. But here we are doing concentration because you do not know what is happening to the mass in the system.

So your relationship is mainly with the concentration, you are eventually going to calculate concentration anyway in what you are injecting into the GC. So you might as well do it with that. So you forget about what is happening inside the system. So, one of the things that happens in the system as you are seeing that there is a lot of loss can happen in the injection system. When you are when the compound is getting vaporized, samplers getting vaporized and pushed into the column.

It may not come out of the column sometimes, and it may get absorbed on the injection system, it may get lost in the detector, it may be for various reasons compound may not come out fully. So you do not want to worry about all that is happening systematically. And it is happening not as is this an error, but it is a regular predictable error? You do not worry about what is happening here. If I inject concentration, say 1 milligram per liter, I will get some area response that is all.

Every time I do that, if I am getting the same, I do not care. How much of one is getting lost in the system. This is happening, that is for sure. This calibration is done. No, but that is what you calibration is based on as long as it is consistent. How do you know what is happening in the system? You are not getting masters, you are not even measuring voltage or something. So that is why we sometimes do concentration calibration it does not matter.

We do not know how much mass concentration. People do not worry about that. Also, concentration is used directly because volume injected is most of the times is fixed. If you inject different volumes and your problem concentration was saying there was no change. The other thing that people do sometimes is so the calibration is paramount. So if your calibration is reliable, which means that every time you do a calibration, you are getting the linear response, then you are, with it.

So that is the demonstrable you can demonstrate that you can you have a good quantitative analysis that you can do, as you can see here is fitting a linear curve with 0 with an intercept. Sometimes you can choose without an interceptor and all that that is depending on how your calibration looks, you can do that or to be careful. So intercept is very large in this case, this slope is 655238 and interceptors 241455 which means that.

When you have what it means this physically if you run a blank what this means is that when you have no compound it should give you that area. But if it is not giving, which means that it is an artifact of fitting, which means it is really a nonlinear fits at that level. So then is not reliable really. So what generally what people do is they do not take it all the way to they will ignore this intercept because you are not going back there.

So they will use this curve in this region that is all interest every use. It is not it is not ignored, but there is also a process where I can also fit it to 0 it will look like this if I fit it 0 this curve will slightly shift a little bit. These issues are there and you have to take a call based on how do you think how much error is happening and where you are which part of the calibration curve are you in? If you are on the lower end, then you have a problem. So you have to fix that we talked about this.

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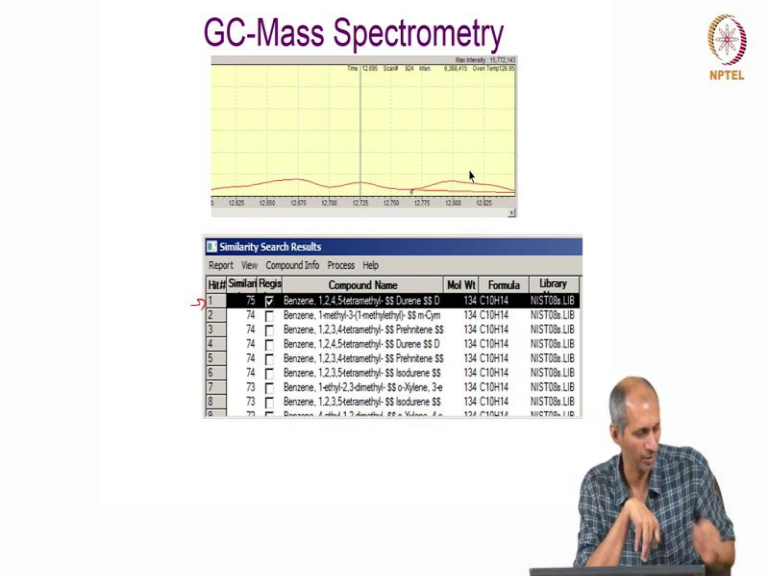

Now, sometimes if you have peaks like this very small peaks, you may get a similarity search that looks, let us say 75% your call whether you are 75 or not, you will get a similarity match of 30% 40% 80% 95% 98% so, typically, from this chromatogram I am not really sure if it is noise or signal whether it is enough information for me to judge whether it is benzene or not. So, I will discard that I say and I am unable to determine.

So, we will say below detection limit some such thing. So, if you cannot identify come on, you cannot even calibrate for it to this. It is equivalent to saying it is below detection limit, you cannot detect the compound.

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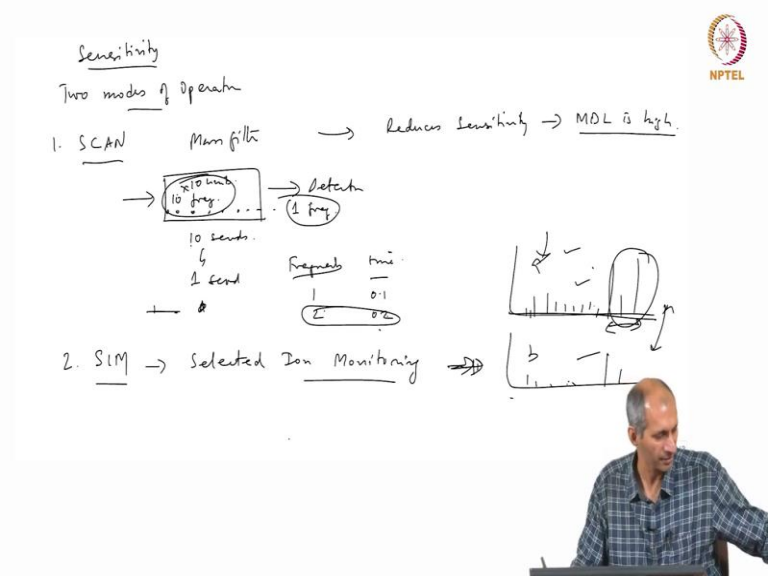

So, now one of the things that we have seen is that the goals of the in terms of sensitivity. So, we know that the GCMS can be run into 2 modes of operation, 1 is called as a SCAN is what we just explained just that I want to scan the entire range of mass by charge and looking at whatever is there in the system. So, this is what we do normally GCMS is a very useful tool so, what people get very analysts are very optimistic and say I want I want to know what is there in the compound everything.

So, it is potentially it has the power to find out what else what is there in all other compounds in the system 100 peaks there it can tell you what each peak is, problem is suppose you have a in order to scan it, this is the mass filter. We also saw that a group of ions mass fracked fragments are going in, we are only analyzing in the detector, we are only analyzing a fraction of what is going in so if we are measuring 1 fragment you are measuring it for a certain period of time.

So let us say there are 10 fragments, each fragments and your total time is say 10 seconds. This detector can only detect 1 fragment. So let us say there are 10 into 10, 10 fragments and 10 units of each fragment. So 1 of the things that happens is if I want to, I have to divide the time that

compound goes from here to here, it takes 10 seconds for I am dividing the time, I am dividing into 10. And I am only giving 1 second for each fragment.

So what happens is I am only able to spend 1, 10th of the time for each fragment. The rest of the time this particular fragment is not going to record it is getting out. The other 9 fragments are getting out except 1. So for the first second fragment, fragment number time. For the first point, 1, second fragment 1 is going through point 2 seconds, fragment 2 is going through and so on. So there is only 1 fraction of the time.

That each fragment is going to which means that even though there are 10 units of a particular fragment I am only able to measure one small fraction of it because at the time it is allowing it to go through. So, in order to prevent this, this is what this does is it reduces to sensitivities scan which means that my minimum detection limit is high I need higher concentration detected. So, if there are there are really large number of units of the detection the detector has an MDL is high.

The detection the detector that we are using has an instrument detection limit below which it cannot measure. So, if I want to get above the detection limit of the instrument I need to be able to have enough concentration here that goes to detector the same principle that we are using for MDL terms of so, we give the maximum opportunity for 1 particular fragment to go through a detector for when we are doing scan is sometimes not possible to do it.

Even though it is very attractive to do the scan. So instead if you want to increase sensitivity what we do is something called as SIM just call a selected ion monitoring we do not we do not look for everything, we only look for specific main fragments. But if you do that, for example, in this case of let us say that a mass spectrum consists of small things and then 3 big ones, now these 3 big ones are in this this mass range here and there are 10 other small peaks here.

If I want to focus if I want to if I want to spend time on looking at all of them, I am losing valuable time in analyzing these 3 which are significant fractions of it you will get a very high. So, insane sim what I am doing is I am only analyzing these 3, I will not worry about the others.

So, it gives me more time to analyze the larger mass fractions mass this thing, what is the payoff what is the compensation? So, it increases sensitivity which means I am able to detect lower concentrations.

But what is the side effect what is the consequence of this? Now, time is the same what is the difference between this and this? What is the difference between a and b? This is 1 compound is a mass spectra of 1 compound. So, your difference between a and b in terms of information what can you do with this that you cannot do with this what can you not do with be this if I give you a mass spectrum like this the original mass spectrum scan mass spectrum is a is cannot be what is the consequence of that.

Calibration is different but here we are using mass spectra for something What are we using mass spectra for one but here are what can I what can you not do this this spectra with a I can do something I cannot do that will be I just discuss that, so you cannot use a signature you is unreliable a signature because it is a fraction of the entire thing. It can give you a large signal but it is it does not contain all these peaks.

So when you are doing a match, it will give you a 70% 60% match, you are really not sure if it is that compound or something else, which means that you have to use SIM. SIM is not used for identification sim is used for increasing sensitivity, which means is used for quantification only, which means by this time you should be absolutely clear that the retention time and the compounds that are coming at that particular in your sample are is what you think of it is so, you have to do a scan to ensure that this is this compound.

And then when you want to go to compound which have so when you are doing calibration and all that you are establishing retention time and the identity of a particular compound. So when you want to do when you want to do quantification, you want to find out to raise levels you have to do SIM. So, practically what this does is the following now you have 2 problems here. I have unknown sample which may contain a lot of compound at very small concentrations. If I do a scan my chromatogram a look like this.

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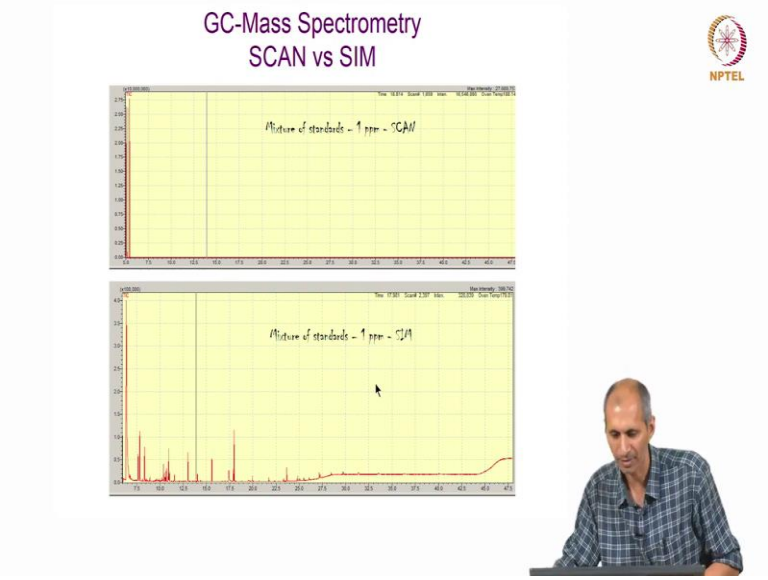

So, show you so this is what it looks like if I take a scan on the top chromatogram and top 1 ppm of some mixture I have a standard mixture 1 ppm you cannot see anything does not mean there is nothing you cannot see anything we got is all below detection. But if I change it to sim 1 ppm I can see no peak. So, what I what I am doing is I am not looking for everything from in the mass spectrum from mass of 2 to mass of 3 and all that I am selecting specific compound and I am looking for them alone.

Then I get peaks then I know that I before I get to this point of sim I need what I am looking for, which means that I am looking for a specific set of compounds. So, which is why analysis of all these compounds, you have to have an objective very clear in the beginning, the objective will be I am looking for PAHs, I am looking for pesticides I am looking for polychlorinated by finance and looking for a specific class of compounds alkanes.

And then you have to set up this program the system to look for only those ions those must be charged and why is it? So, which means that you have to go to the original spectra and pick the mass by charge.

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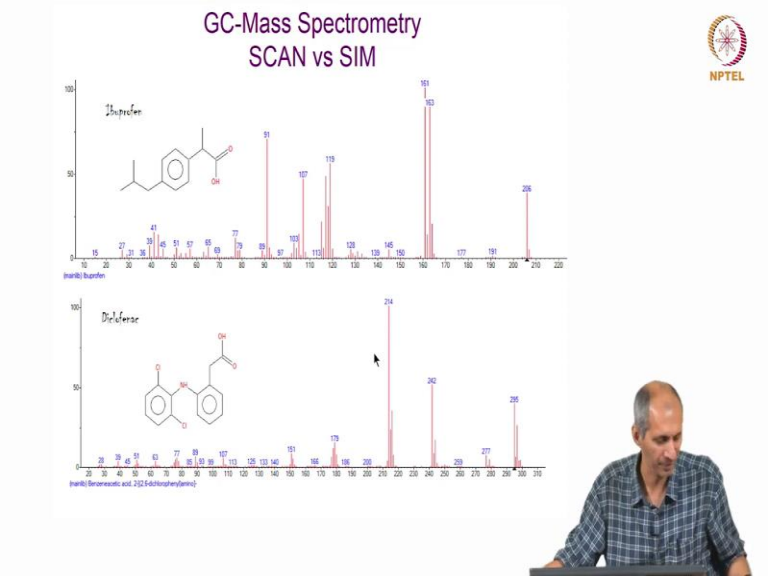

So, in this case, I can I have a choice in this case, Phase Ibuprofen or diclofenac is a compound that we want to look in water. So there are some very prominent, large peaks. But I can find out certain which is the largest I can take 161, 163 maybe 91 signatures, representatives of ibuprofen and in diclofenac to 14 to 42, and 296. And I run under a lot of other peaks, which it will not detect, it will not. Which means I am I have to make sure using a scan and using my calibration and all that that procedure to make sure that the peak that is coming at that time is diclofenac.

And the fragmented peak that I am getting a sample is part of that peak that is coming. So you can also use this spiking method which I described yesterday, in the GCMS, you can take the original compound and do a sim for specific peaks then you can spike it with the compound of interest, you have a guess now that it could be this, you already know the compound you are looking for then you spike that compound standard.

Then you go to the scan and see which is not showing up because the scan can detect higher concentrations, your sample does not have that high concentration so you increase concern and verify whether you are getting the same thing. So anyways, this is a very large, this thing.

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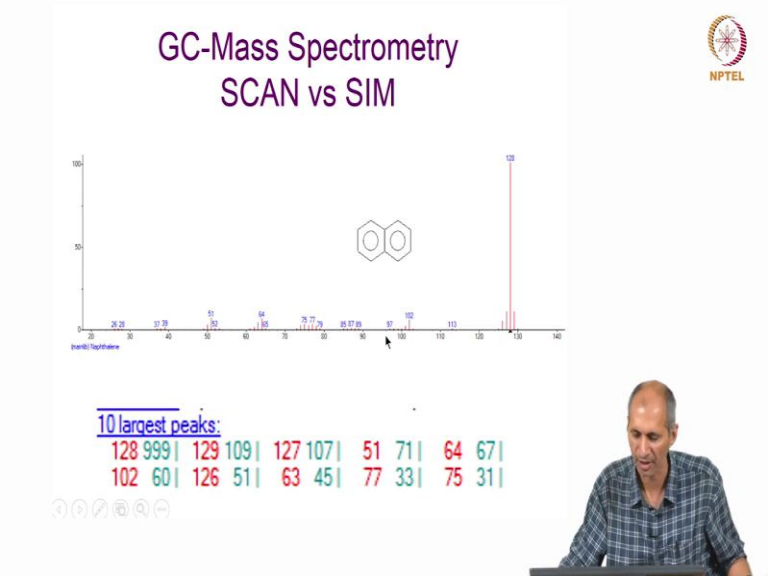

And there is other complications doing organic analysis, especially if you are doing things like organic acids and other things, which you need more further processing. In order to do this. There is something called dramatization, which will, which will increase the stability of the compound during the analysis. We would not talk about that here. But the summary of the thing is that full quantitative and qualitative analysis of the system.