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

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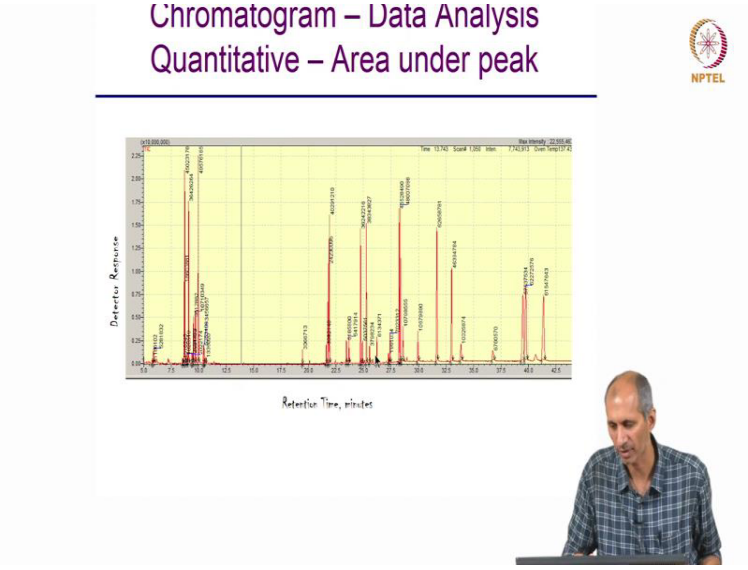

So, you can integrate the entire chromatogram you will get all of this.

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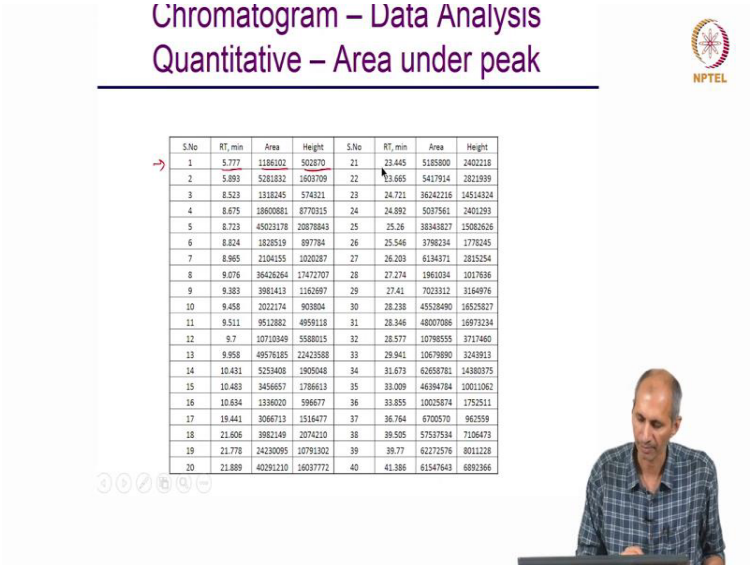

S.No	RT, min	Area	Height	S.No	RT, min	Area	Height
1	5.777	1186102	502870	21	23.445	5185800	2402218
2	5.893	5281832	1603709	22	23.665	5417914	2821939
3	8.523	1318245	574321	23	24.721	36242216	14514324
4	8.675	18600881	8770315	24	24.892	5037561	2401293
5	8.723	45023178	20878843	25	25.26	38343827	15082626
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 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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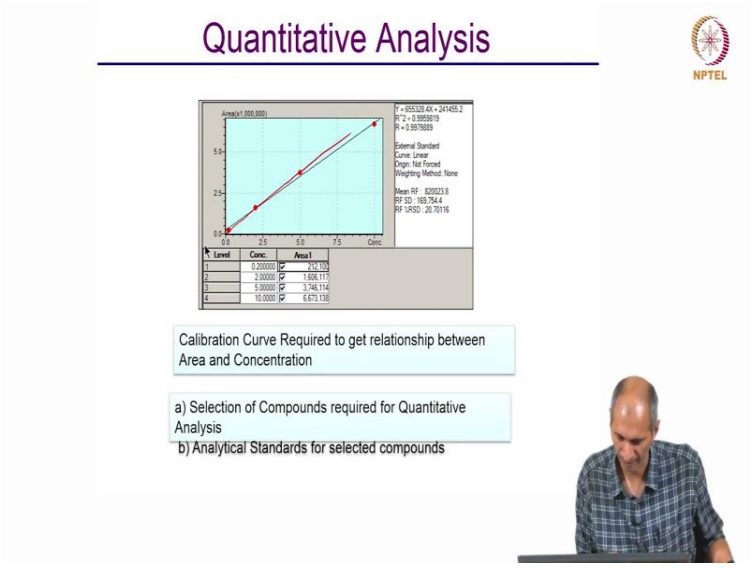

Then I can go to each one of these compounds if I know which one they are, I will do a calibration and the calibration is done again in terms of say concentration that you are injecting into the GC. Calibration can be done in mass or concentration. But here we are doing concentration because you do not 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 for 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 that can happen in the injection system. When the sample's getting vaporized and gets pushed into the column.

It may not come out of the column sometimes, and it may get adsorbed onto 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 if it is happening systematically. And it is happening not as an 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, I do not care if I am getting the same. How much is getting lost in the system. This is happening, that is for sure. The calibration is done.
Student: If mass is getting lost then concentration will also change no sir.
Professor: No, but that's what your calibration is based on that as long as it is consistent. How do you know what is happening in the system? You are not getting mass units, you are not even measuring mass you are measuring voltage or something. So that is why we sometimes do concentration calibration because it does not matter.

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

So you can demonstrate that you have a good quantitative analysis that you can do, as you can see here is fitting a linear curve with 0 intercept. Sometimes you can choose without an intercept and all that is depending on how your calibration looks. You can do that but you have to be very careful. So intercept is very large in this case, this slope is 655238 and intercept is 241455. It means if you run a blank and you have no compound it should give you that area. But if it is not giving, which means that it is an artifact of the fitting, it is really a nonlinear fits at that level. So

then is not reliable really. So generally what people do is they do not take it all the way, they will ignore this intercept because you are not going back there.

So they will use this curve in this region that is all intercept is used. It is not ignored, but there is also a process suppose 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 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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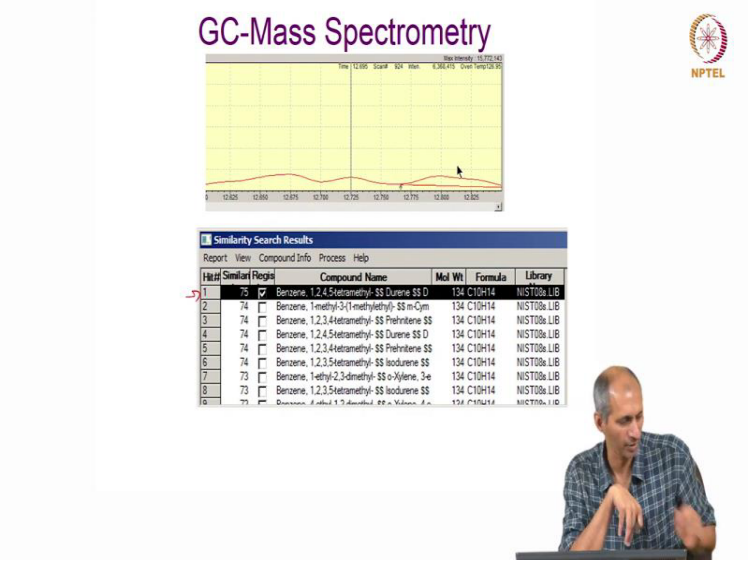

Now, sometimes if you have peaks like this very small peaks, you may get a similarity search that looks, let us say 75% its your call whether you are ok with75 or not, you will get a similarity match of 30% 40% 80% 95% 98% so, typically, from this chromatogram I am not really sure, if it is noise or signal or whether it is enough information for me to judge if it is benzene or not. So, I will discard that and say I am unable to determine.

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

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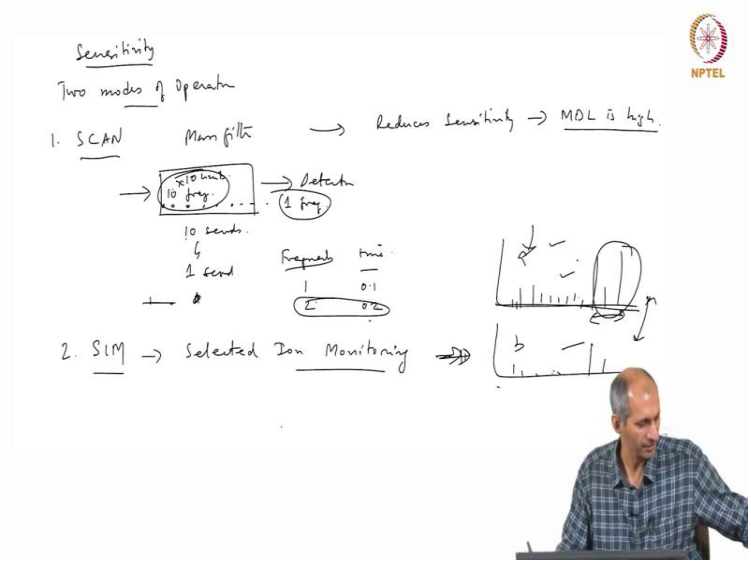

So, now one of the things that we have seen is that the goals in terms of sensitivity. So, we know that the GCMS can be run in two modes of operation, one is called as a SCAN. SCAN is what we just explained. If I want to scan the entire range of mass by charge by looking at whatever is there in the system.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

So, potentially it has the power to find out what else organic compounds are there in the system. I have 100 peaks there it can tell you what each peak is, problem is to scan it. We also saw that a group of ions fragments are going in, we are only analyzing it 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, 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 if, I have to divide the time that compound goes from here to there, it takes 10 seconds for I am dividing into 10. And I am only giving 1 second per 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 the detector. The other 9 fragments are getting out

except 1. For the first 0.1s fragment one is going through, 0. 2 seconds, fragment 2 is going through and so on. So there is only 1 fraction of the time each fragment is going through 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 that's the time it is allowing it to go through. So, in order to prevent this, what this does is it reduces sensitivities i.e., SCAN which means that to have my minimum detection limit high, I need higher concentration detected. So, if there are really large number of units of the detection, the detector has an MDL.

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 must be able to have enough concentration here that goes to detector so that for the same principle that we are using for MDL we give the maximum opportunity for 1 particular fragment to go through a detector. hence scan is sometimes not possible.

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

If I want to focus I want to spend time looking at all of them. I am losing valuable time in analyzing these 3 which are significant fractions of it. So, in 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 of 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 and 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. This is a mass spectra of 1 compound. So, your difference between a and b in terms of information what can you do with this.

Calibration is different but right here we are using mass spectra for something. We are using mass spectra for one but here what you cannot do in this spectra with a I can do something that I

cannot do with b. So you cannot use a signature as it is unreliable because it is a fraction of the entire thing. It can give you a large signal but 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 it 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 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 quantification, if you want to find out very trace levels you have to do SIM. So, in 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 may look like this.

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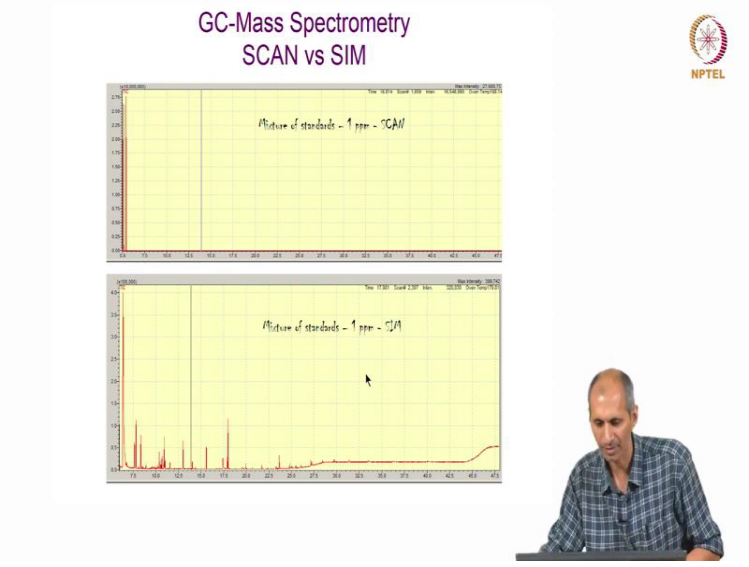

So, I will show you so this is what it looks like if I take a scan on top of chromatogram and has 1 ppm of some mixture. I have a standard mixture 1 ppm you cannot see anything does not mean there is nothing if you cannot see anything because it is all below detection. But if I change it to

sim 1 ppm I can see no peak. So, what I am doing is I am not looking for everything in the mass spectrum from mass of 2 to mass of 300 and all and that I am selecting specific compound and I am looking for them alone.

Then I get peaks then I know that 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 biphenyls or I am 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 mass by charge m by z. Which means that you have to go to the original spectra and pick the mass by charge.

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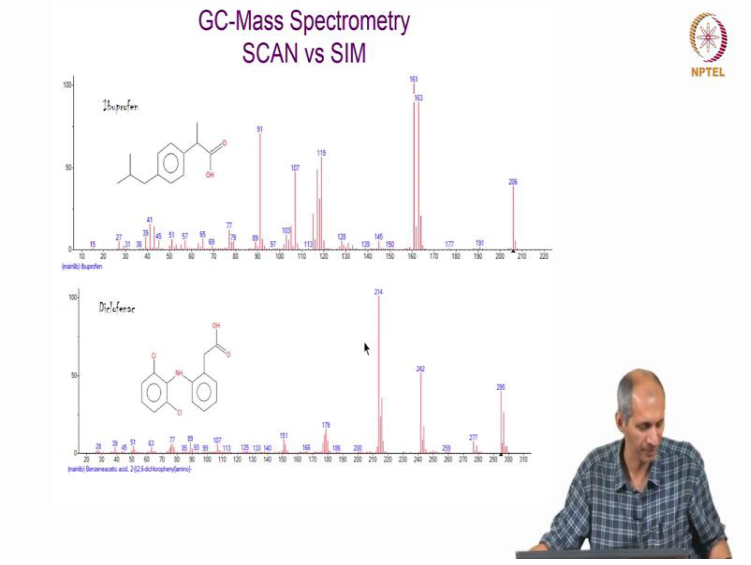

So, in this case, I have a choice in this case, Phase Ibuprofen or diclofenac these are the compounds that we want to look in water. So there are some very prominent, large peaks. But I can find out which is the largest I can take 161, 163 maybe 91 as signatures, representatives of ibuprofen and in diclofenac 214, 242, and 296. I run and there are a lot of other peaks, which it will not detect, it will not. Which means 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 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.

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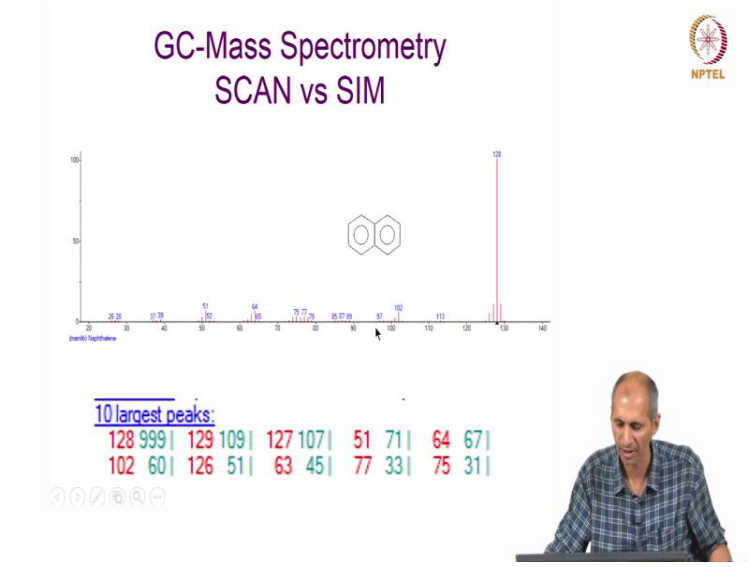

And there are other complications in doing organic analysis, especially if you are doing things like organic acids and other things, which you need further processing. In order to do this. There is something called derivitization, which will, increase the stability of the compound during the analysis.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