

First Round Robin test within Bonn-OSINET (RR2006)

Final Report

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Summary

In this RR2006, the comparability of the analytical data between laboratories played the major role. The aim to come to comparable analytical results is well in accordance with the tasks of Bonn-OSINET, which were defined in the Bonn05-workshop in Ostende: co-operation and mutual assistance in difficult cases, promote quality assurance in oil-spill identification (especially through ring-tests, development of common reference materials (CRMs), and sample exchanges) and consider the possibility of a common database of oil sources.

The main purpose of RR2006 was to find out, whether it is possible to identify unknown crude oil pollution found somewhere on the coasts by means of the COSI-database system of the BSH. Participants should produce 17 peak ratios from 4 oil samples for this purpose.

Ideally, all laboratories should measure the same ratios of peaks (representing the relative concentrations of the compounds) in a compound mixture -up to a certain degree of statistical variations due to the analytical error. But it became obvious here that ratios depend also on the analytical parameters. The choice of those parameters might simply be derived from historical developments in the individual labs and/or the fact that the instruments are mainly used for other tasks not connected with oil spill identification. These analytical parameters are thus not necessarily optimized for oil spill identification. Having worked separately so far, laboratories may simply not have noticed this. But when results between laboratories are compared, especially when peaks are measured and ratios are produced, shortcomings in analytical procedures become obvious.

Despite those difficulties, this first intercalibration round within Bonn-OSINET has shown that the COSI-system may serve as a powerful tool for identifying unknown crude oil pollution found somewhere on the coasts. In this sense, RR2006 thus was a first and difficult test. It may be expected that results become even better, when shortcomings in the analytical

procedures are removed and even when the analytical parameters are further adapted between the laboratories.

There is the big chance to come to common analytical parameters in near future. RR2006 thus can be regarded as a big step forward towards cooperation within Bonn-OSINET and a common analytical methodology within the Bonn-Agreement.

1. Introduction

In the first intercalibration round in the framework of the Bonn-Agreement, 13 laboratories from 11 European countries participated. Two “spill samples of a crude oil spill” had to be compared with a suspected source sample. Additionally, a fourth sample (Brent crude oil, used by Germany as a standard oil in the QA-system for producing control charts) was included for clarifying and harmonizing the inter-laboratory comparison.

Participants were asked to compare the three samples by using their own method (intra-laboratory comparison) but also to produce 17 compound ratios from each of the samples, which had to be inserted into excel-spreadsheets.

These spreadsheets should also include the corresponding mass-chromatograms, from which the ratios were produced, and the individual analytical parameters of the laboratories.

The main purpose of RR2006 was to find out, whether it is possible to identify unknown crude oil pollution found somewhere on the coasts by means of the COSI-database system of the BSH, which includes “fingerprints” of more than 300 crude oils (among oils of other types), from all over the world. Variable sets of compound ratios may be used by the COSI system for correlating all oils included in the database with each other, including a part or all of the 17 ratios, which should have been determined here. For including the results of the participants, a special modus for the manual input of compound ratios into the system was developed before.

The mass-chromatograms of the laboratories together with their analytical parameters should help to interpret variations in the calculated ratios.

The general outcome of RR2006 was already discussed at the first Bonn-OSINET meeting in Hamburg, 9.-10. November 2006, together with the discussion about the CEN-methodology. This is reflected in the minutes of this meeting.

RR2006 must thus also be seen in the light of the task of Bonn-OSINET to come to a common method within the Bonn-Agreement.

1.1 RR-2006 Accompanying letter

Dear colleagues,

when we come to a cooperation of laboratories involved in oil spill identification, the focus must be put on the comparability of analytical results.

It actually happened just two weeks ago that we received samples from an oil spill from the German Bight. The suspected ship went to a Spanish harbor, where comparison samples from different tanks of this ship were taken. I asked the water police for these samples and got the reply that these samples will be analyzed by an unknown Spanish laboratory. They were of the opinion that it would be sufficient that we receive the results from this laboratory and compare these with our results of the spill samples.

So, I am waiting for the results –but actually do not expect very much from this case.

The Bonn-OSINET expert group, where the responsible analysts know each other and an exchange of samples and results may be arranged, should improve such a situation.

So, as a first step to achieve comparability of analytical results, let's start with the first intercalibration round within Bonn-OSINET.

Round Robin 2006 consists of 2 parts:

- comparison of oil samples in your laboratory (intra-laboratory comparison, as normal)
- “identifying” unknown oil samples by means of the COSI-system developed by the BSH (and inter-laboratory comparisons).

Several times in the past, results in form of mass-chromatograms have been sent to the BSH by Paul Kienhuis (NL) and Asger Hansen (DK), and the question was asked, whether there are similar oils stored in the BSH-database (COSI). This database contains now results of more than 1200 oil samples (crude oils, products and product mixtures, i.e. sludge- and bilge samples). Meanwhile, Paul is able to include his raw ms-data directly, so that COSI can jointly be used by NL and DE (of course, the analytical parameters have to be adapted as far as possible in order to use the automatic peak detection and ratio calculation algorithms of the COSI evaluation system).

But the COSI-system includes also the possibility for a manual input of compound ratios. It is thus possible to check, whether it is possible to identify an oil sample by using your results, even though your analytical parameters might not be the same as those of the BSH. Of course, only compound ratios, which are easily determinable are chosen in this case (This RR may even be used as a check, whether those ratios are sufficient for this purpose).

You will receive 4 oil samples:

Scenario

Bigger oil pollution of unknown origin was detected at your coast. Two samples were taken: Probe 1 (Probe = sample) and Probe 2. You could easily find out by GC-screening that the oil samples consist of crude oil. Thus, only a tanker or, where feasible, a platform could have been responsible for the discharge.

In order to find out the special type of crude oil involved, you send a request into the Bonn-OSINET expert group, which includes your results (see below). You receive a reply, which leads to a distinct crude oil. Your investigating authorities could arrange that a sample of this oil (Probe 3) was sent to your lab.

Compare the three samples.

The fourth sample (Brent crude oil) is included for clarifying and harmonizing the inter-laboratory comparison:

attached you will find an Excel map containing a spreadsheet (table) for every sample.

Given in table "Brent" are mass-chromatograms of the Brent oil for orientation.

Please

- change A2 and B2 according to your country code and your laboratory.
- Fill in the 4 mass-chromatograms with your figures of the Brent oil (please "try" to use a comparable retention time range and to stay in the frames as given in the "Brent" table of rr2006DE.xls. It is easy to include a graphic in Excel and to resize it correspondingly.). The corresponding mass-chromatograms produced by BSH and RIZA (NL) are already given in table "Brent".
- calculate the ratios (as many as possible by using the peak-heights) and fill in the numbers into column C. This can be done by means of your ms-software but even also by simply measuring the corresponding peaks from a print-out by using a ruler (as I have done it so far with mass-chromatograms from other laboratories). Add 3 ratios from GC-screening.
- fill in the tables for Probe 1, Probe 2 and Probe 3 correspondingly.
- Append the table "Parameters" with the MS-parameters you have used.
- store and rename the Excel map into rr2006(your country code).xls (it is called here rr2006DE.xls – I will also fill in the numbers from our lab (DE) for the inter-comparison).

Brent crude oil is best investigated in the BSH because it is used as the standard oil (within the QM of our lab for determining and fixing the precision of the method by producing Control Charts). In Brent crude, the main compounds of the hopane series, for example, are clearly discernible, and C28 (bisnorhopane) is an outstanding peak. You will need this information, when you start to evaluate the 3 other samples (Probe 1 to Probe 3).

In addition to a “normal” Round Robin test, rr2006 thus offers a big variety of inter laboratory comparisons: all 4 samples from every participant can be used to find out, whether the matching oils can be found in the COSI-database. The peak ratios of the different labs can be compared, and possible deviations/peculiarities can be discussed in connection with the attached mass-chromatograms and the analytical parameters of the participants.

Please send in return

- a short report of your results (intra-laboratory comparison).
- rr2006(your country code).xls

1.2 Origin of the samples

In fact, the given scenario was invented: the two spill samples consisted of crude oil from the first production platform in the Baltic Sea, which was installed in the Kaliningrad-area (D6), and which started its main production in 2005. Samples of this oil had been send by the operating company to different laboratories (SINTEF, No, ETC-Canada, BSH), in order to fulfill an obligation made by HELCOM: the oil should be analyzed in detail before the main production started, and its characteristics (“fingerprints”) should be known, so that it can be identified, if involved in a pollution case. The D6 oil production in the Baltic Sea is highly criticized by environmental organizations, which fear increased oil pollution by the platform itself but also by the increased tanker transports. So, part of the “environmental strategy” of the operating company was especially to include this oil into the COSI-database system, which will definitely detect this oil (because spill samples are compared with all oils in the database in every case), and which may be used by different countries (RR2006 served as a first test here). In addition, it was intended that this oil is available now in the laboratories participating in the Bonn-OSINET expert group. But it was also pointed out that care must be taken not to blame the operating company without justification.

Sample 3 was a crude oil sample taken in 1992 from Kaliningrad crude oil, i.e. many years before the D6 exploitation in the Baltic Sea started. It was found by the COSI-system as the best matching sample, when the D6 crude oil samples were included into the database. Thus sample 3 points to Kaliningrad crude oil and can indeed be regarded as a suspected source sample.

2 Results of the intra-laboratory comparison

	Probe1/Probe2	Probe1, Probe2/ Probe3	remark
Be	match	non-match	
De	match	non-match	but same area
Dk	match	non-match	but closely related
No	match	non-match	
NI RIZA	match	non-match	
NI NFI	match	non-match	
Lv	match	non-match	possible that all 3 are of same source
Es CEDEX	match	probable match	
Es CSIC	match	non-match	
Fr	match	non-match	
Se	match	non-match	
Uk	match	possible match	ultimately non-match
Fi	match	possible match	

All participants found a correct “match” between the two “spill samples”. But nearly one half of the participants also found that sample 3 was “closely related” up to a “probable” or “possible” match. Generally, this example shows that the “meaning” of differences between oil samples may be quite different: because of the differences between the spill samples and the suspected source sample a rigid “non-match” was actually a right conclusion in this case. But such a finding is not of any help, if crude oil pollution by a distinct platform is found on a beach and only a crude oil sample from the general production area is available.

Generally, oil spill identification must support investigating authorities in finding the source of oil pollution. The mere comparison of analytical data is not sufficient. Experience is needed here.

3 Results of the inter-laboratory comparison

All ratios are given in “Tableall” (upper tables) of overall.xls (attached). Given here are also the mean values, the standard deviations of the ratios and their ranges: min-values marked red, max-values marked blue. The corresponding ratios of the participants (or ratios near the minimum and maximum values) are marked with the corresponding color –if significant (see below).

The ratios found by the BSH are achieved in the following manner: for controlling the samples sent for this RR, subsamples were taken before sending and analyzed from every third set. The De values in “Tableall” thus each represent the mean values of four samples.

The lower three lines of the Brent-table contain the mean values of the BSH control charts, measured weekly over nearly two years (and the +/-10% range of these values).

For the Brent-crude, all ratios are also shown in diagrams, which also contain those values from the BSH control charts (2 = mean; 1 = mean+10%; 3 =mean-10%).

In addition, in “Brentall” a diagram is given, which contains a built-in Excel routine for the better visualization of outliers. The region of the lower values of this diagram is enhanced in “Brentallm”.

In the lower 4 tables of “Tableall”, outliers are excluded (and obvious “failures” are corrected). A value is regarded as an outlier, if a significant reduction of the coefficient of variation (CV, i.e. the relative standard deviation) is achieved for this value.

3.1 Isoprenoid-ratios (GC-FID)

The extremely low c17/pr- and C18/ph-ratios of Es (CEDEX) could not be explained (unfortunately it was not asked for the GC-FID-chromatograms in this RR2006). These are definitely outliers, which might be caused by “human error”. Such low values (found in all 4 samples) would indicate very high bacterial degradation.

Remark: some obvious “human errors” caused by e.g. unintended “shifting” of values in excel-tables or incorrect integration by the acquisition-software in five cases were already noticed directly after the arrival of the results in the BSH, and those participants had made already corresponding corrections. Generally it must be noted here that “human errors” should not happen in forensic investigations, as the results may lead to severe consequences here.

Excluding those 2 ratios of Es (CEDEX) would highly improve the CV of the C17/pr- and C18/phy-ratios to up to one half for all 4 samples (lower tables).

After excluding those 2 values for all samples (lower tables), there still seems to be the general tendency that the highest ratios for C17/pr and C18/phy are found by Fi and Dk over the 4 samples, whereas the lowest values are found by Ni(NFI) and Es(CESIC). Absolute differences of more than 1 in the C18/phy-ratios, for example, may only be explained by different resolutions and/or the different ways how the analysts prefer to measure the peaks (especially where they set the baselines).

Asger Hansen has included in his results a comparison between the isoprenoid ratios calculated by the software integrator and calculated manually. His results show that –by chance- bigger differences may appear (especially in his sample1 -whereas those differences were noticeable but distinctly smaller for the other 3 samples):

		sample1		
		a	b	Avg.
ratios	(Height measured by integrator)			
	C17/Pr	1,24	1,32	1,28
	C18/Ph	2,64	2,85	2,74
	Pr/Ph	2,41	2,39	2,40
	(height measured by ruler)			
	C17/Pr	1,82	1,47	1,65
	C18/Ph	3,17	2,99	3,08
	Pr/Ph	1,95	2,27	2,11
	Diff			
	C17/Pr	-0,58	-0,15	-0,37
	C18/Ph	-0,53	-0,14	-0,33
	Pr/Ph	0,46	0,12	0,29

When the pr/phy ratios of all participants are compared, a remarkable low CV of only 6% is found for the Brent-crude and values of only up to 10% for the other 3 samples. One reason for these very similar pr/phy-ratios could be that differences in the resolution of C17/pr and C18/phy between the different labs and the different measurement techniques are leveled out, when pristane is related to phytane.

Remark: The uncertainty in measuring peaks in the GC-chromatograms may have led to the decision not to use or recommend those isoprenoid-ratios in corresponding publications about oil spill identification although it is well known that those ratios belong to the most important “biomarkers” for the discrimination between oils. Because there is no full separation of peaks in the GCs, differences are easily calculated, where

there are no –especially when the concentrations of the oil samples to be compared are different. Calculating and comparing those ratios from a mass-fragment of the alkanes (e.g. 85) would circumvent this problem. An extreme example is shown in prphy.doc (attached).

3.2 GC/MS-ratios

a) Some bigger deviations from the mean value of all participants in single ratios could be explained by the fact that in these cases the analytical parameters were not optimized for oil spill identification.

Remark: In this RR, no directive was given with regard to the analytical parameters, and participants may well come to right conclusions by using their own sets of parameters. The following comments are made in the light of the comparability of the data and should thus not be regarded as “bad criticism” nor should advice given be regarded as the only possible solution.

Mainly three analytical/technical problems could be identified:

- A rapid “rough” chromatography (too high temperature gradient used by the Uk) leads to the fact that the biomarker-clusters are found on the descending shoulder of an unresolved hump (Figure 1 in figures.ppt, attached). This leads to too high values of the first compounds in a homologues series, e.g. Ts and Tm in the hopan-series, and even reduces the “discrimination power” of these ratios between oils.
- A similar phenomenon is present, when the temperature gradient ends too early (i.e. before the peaks are measured and the corresponding ratios are produced). The corresponding homologues series of the hopanes, steranes and aromatic steranes are then measured under constant temperature, which leads to the fact that peaks become broader and broader (Figures 2 and 3). This effect was especially observed, when 60m-columns were used, as by Fr and No (and the same or a similar gradient was used as with the 30m-columns used by most of the participants). For better visualization, the temperature gradient is included in some mass-chromatograms of the participants (Figures 4 to 8). Generally, all biomarker values of Fr and No are effected. Of course this effect is bigger, when ratios of peaks are calculated, which are far apart from each other (such as the TA21 and the values of the tricyclics, recommended in the CEN-methodology and presented by No and NI in their result reports, Figure 9 and 10).
- Insufficient sensitivity of the instrument leads to a too high influence of scatter and noise on the ratios. This might explain some outliers calculated by Es(CEDEX) throughout all 4 samples.

Remark: Es(CEDEX) participated the first time in such an intercalibration and mentions that there were time constraints because their method is still under development.

b) Differences also appeared because obviously the peak area was taken for producing the ratios in some cases, although it was requested to take the peak height (Es(CSIC), Figure 11, Fi, Figure 12, -especially obvious in connection with the second point mentioned above, i.e. end-temperature reached too early).

c) Fi decided not to measure gammacerane in the Brent crude (too small peak) , and No decided not to measure MF (overlapping peaks), Figure 13.

Brent crude

Excluding of outstanding ratios caused by extreme –and not optimized- analytical parameters (differences caused by a) mentioned above), and correcting other ratios by re-measuring the corresponding peak heights directly from the chromatograms (differences caused by b) mentioned above) leads to CV-values of all hopanes (except the ratio of gammacerane) and aromatic steranes below 10% for the Brent crude oil (“Tableall”, lower table). Excluding the values of zero mentioned in c) (just to show the effect) highly improves the CV of these ratios from 38% to 23%, and from 36% to 19%, respectively.

Generally, the ratios of the non-polar compounds were thus much better comparable than the ratios of the more polar aromatic and aromatic sulfur compounds (except the low ratio for gammacerane).

Further, control charts produced by the BSH, which include regular measurements of those ratios in the Brent crude oil over nearly two years, show that the ratios of the non-polar biomarkers could be measured much more precisely than those aromatic compounds (Figures 14 to 17). The biomarker ratios are much less dependent on the instrumental conditions and remained stable even after severe instrumental changes (e.g. change of the column), whereas the aromatics reach a similar precision only in shorter time intervals (Figure 18 shows that the chromatographic conditions change continuously and retention times have to be settled from time to time, due to column-bleeding. In November 2004 bigger problems appeared and the column was changed).

It can also be shown that smaller peaks, e.g. the gammacerane in the Brent oil, can only be measured less precisely (Figure 19). This corresponds also to a bigger deviation of this ratio between the participants.

Samples 1 to 3

The “basic” problems described above are pronounced, when the results of samples 1 to 3 are compared: although the same amount of oil was weighted, the concentration of the compounds, which had to be measured, was only about 1/3 of the concentration of these compounds in the Brent-crude. This led to a steeper decline of the shoulder of the unresolved hump, on which the homologous series of the hopanes, steranes and aromatic steranes were found, in case of a too rapid chromatography, and in case, the temperature gradient ended before the peaks were measured (e.g. when 60m-columns were used). Of course, also a too low sensitivity led to larger uncertainties and bigger differences here.

But, in addition, the oil of these 3 samples was more complex than the Brent-crude. Bigger deviations were observed because wrong peaks were measured. Further differences appeared, when smaller peaks in highly overlapping clusters were included.

Because the analyses were conducted under different analytical conditions, some kind of a “multi-dimensional chromatography” was achieved. By this, it became obvious that not always the right peaks were measured. Especially Se (using in contrast to all others a pure non-polar column) demonstrated that C28-bisnorhopane was not present in the 3 samples (Figure 1 of proben.ppt). Peaks more or less in the vicinity of this peak in these samples were falsely taken as bisnorhopane by nearly all other participants (except Be, including De, Figures 2 to 14). The same problem was found with gammacerane, 2-MF and retene, which were either not present or appeared as very small peaks in highly overlapping clusters. In addition, an additional peak appeared in the region of Tm in the 3 samples, which was partly not resolved from Tm (or appeared together with Tm as one peak). This led to a high range of the Tm/hop-ratios between the participants from about 0,2 to about 0,4 and 0,5 in these samples. This effect is shown for sample 1 by N1 (RIZA), which used two different sets of analytical parameters (Figure 15), whereas no differences are found with the Brent-oil (Figure 16), when these two parameter sets are used.

3.3 Conclusions

Ideally, all laboratories should measure the same ratios of peaks (representing the relative concentrations of the compounds) in a compound mixture -up to a certain degree of statistical variations due to the analytical error.

But it became obvious here that ratios depend also on the analytical parameters used. The choice of those parameters might simply be derived from historical developments in the individual labs and/or the fact that the instruments are mainly used for other tasks not connected with oil spill identification. They may thus not necessarily be optimized for oil spill identification. Having worked separately so far, laboratories may simply not have noticed this. But when results between laboratories are compared, especially when peaks are measured and ratios are produced, shortcomings in analytical procedures become obvious.

For comparing ratios between laboratories, the adaptation of the analytical parameters might additionally be necessary. Since those parameters, such as column-type, temperature gradient etc., are more or less arbitrarily chosen so far, the latter point does not seem to be a bigger barrier.

Peaks in highly overlapping clusters should not be measured because there is the high risk that differences are “produced” where there actually are no differences (It may be possible that corresponding ratios were nevertheless determined in this RR because it was asked to determine those ratios here).

Again, the adaptation of the analytical parameters as far as possible -together with a common reference oil- would help to ensure that the same peaks are measured by all laboratories – although this RR has shown that sometimes a “multi-dimensional chromatography” (or a structure determination) may be needed in order to be sure that peaks are identified correctly.

4 Finding the oils in the COSI-database

Because of the problems mentioned above, one could expect that the oils could hardly be identified by means of the COSI-system. But when a multivariate comparison is made (i.e. ratios are compared jointly as a data set) the error (or difference) of the single values can be much bigger than in case, each ratio is compared individually.

For every sample, the ratios calculated by the participants were included into the COSI system, and a unique name was given to these sample (e.g. BrentBe, Brent ratios calculated by Belgium). The results of the correlation of the Brent-values and the values of sample 1 and sample 3 are shown in form of screen dumps in the corresponding directories of the

participants. As the raw data of the participants were not included, the chromatograms in these figures don't have any meaning. Important information is given in the second tables above (best matching samples) and on the right side of the figures, where the ratios produced by the participants are given in blue, the values of the best matching sample are given in red, and where the % difference of these values is given (marked green, if below 10% of the mean of the two values, marked red, if above).

For Brent-crude, generally all 17 ratios were used for correlation, whereas for the other samples 28-norhopane, gammacerane and MF/Mphy were excluded –according to the comments given in 3.

4.1 Results

With rare exceptions, the oils could be identified or at least the general areas were found, where they were produced (Brent-Statford-Oseberg area in case of the Brent oil or Kaliningrad-area in case of the other 3 samples).

4.2 Conclusion

The COSI-system may serve as a powerful tool for identifying unknown crude oil pollution found somewhere on the coasts by comparing analytical results achieved by different laboratories with those included in the database.

RR2006 was a first and difficult test. It may be expected that results become even better, when shortcomings in the analytical procedures are removed or even when the analytical parameters are further adapted between the laboratories.

5 Adaptation of analytical parameters

Instruments and columns used by the different laboratories:

	instrument	type	column type	L in m	ID in mm	Film in um
DE (BSH)	HP 6890N/5973 MS	quadrupole	Varian CP-Sil 8 CB	30	0,25	0,25
BE (MUMM)	trace ms	quadrupole	DB-5	30	0,25	0,25
DK (NERI)	VG 70-250S	double-sector	HP5-MS	30	0,25	0,25
ES (CSIC)	Thermo-Finnigan Trace GC-MS	quadrupole	Zebtron ZB-5MS	30	0,25	0,25
ES (CEDEX)	HP 5890 SII/5972 MS	quadrupole	HP 5MS	30	0,25	0,25
F (CEDRE)	HP 6890N/5973 MS	quadrupole	Varian FactorFour VF-1 ms	60	0,25	0,25
FIN (NBI)	HP 6890N/5973 MS	quadrupole	Agilent HP-5MS	30	0,25	0,25
LV (VGMA)	HP 6890N/5973 MS	quadrupole	HP5MS	30	0,25	0,25
NL (NFI)	HP 6890N/5973 MS	quadrupole	HP-5ms	30	0,25	0,25
NL (RIZA)	HP 6890N/5973 MS	quadrupole	J&W DB 5	30	0,25	0,25
NO (SINTEF)	HP 6890N/5973 MS	quadrupole	HP5-MS	60	0,25	0,25
SE (SKL)	HP 6890N/5973 MS	quadrupole	Ultra 1 Methyl Siloxane	25	0,2	0,33
UK (ERT)	ThermoFinnigan Trace DSQ	quadrupole	Phenomenex ZB-5	30	0,25	0,25

Obviously, there is the big chance to come to general, common parameters within Bonn-OSINET:

After the Bonn-OSINET workshop in Hamburg, Se and Fr have promised already to adapt their columns to the majority of the laboratories.

Because then the polarity of the columns used is comparable, all laboratories will soon use comparable columns with the same length, inner diameter and film thickness (except No).

As the columns are more or less arbitrarily chosen, it should be discussed, whether even a further adaptation should be achieved by using the same columns from the same manufacturer.

Dk has indicated, that they will soon change from their very old sector-field instrument to a quadrupole MS.