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Intercompartment distribution of monocyclic aromatic hydrocarbons and C_1-C_2 organochlorines in the North Sea environment

Part II
Evaluation of analytical techniques and Experimental section

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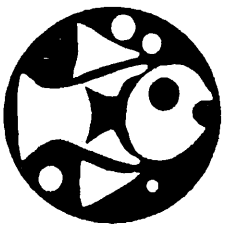

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II.1.1.1. SORBENT SAMPLING TECHNIQUE

II.1.1.1.1. On-line versus off-line analysis desorption

In a first approach the use of the on-line purge and trap (P&T) instrument, equipped with a thermal desorption unit, for the thermal desorption of air samples was evaluated. Desorption of empty tubes at 230°C for 8 minutes in the cartridge desorber gave constant contamination of MAHs: benzene: 4.56±0.63, toluene: 0.89±0.05, m/p-xylene: 0.103±0.016 and ethylbenzene: 0.068±0.012ng (n=3). With the same analysis without heating the cartridge desorber contamination was under control.

Subsequently the original cartridge desorber was replaced by a Chrompack desorber on the on-line P&T system. The blanks were now under control but peak shape was poor in the time window from chloroform till benzene due to water interference and secondly, incomplete desorption of the internal Vocarb 4000 trap was found since a second desorption of the trap gave MAHs up to 42% (benzene) of the first desorption.

On the contrary, when 250µl of air containing 20ng chloroform, 20ng 1,2-dichloroethane, 20ng toluene and 20ng m-xylene was brought on the Vocarb 4000 trap via a Helium stream in an off-line construction, the desorption of the replaced trap was complete. An off-line construction was set up including the Chrompack desorber, a wet trap and the Vocarb trap in order to analyse sampling tubes. Finally, complete desorption of the whole system was obtained by desorption in the desorption unit at 250°C for 14 minutes and purging for 18 minutes at 30ml/min.

In the literature, some solutions are mentioned to minimize water interference. By decreasing the sampling volume, the moisture problem in the analysis is decreased, but the LODs as well (Sweet and Vermette, 1992; Farmer et al., 1994; McClenny et al., 1991). Secondly, cryogenic traps with large internal diameter can be used in order to avoid clogging by ice (Hsu et al.,

1991). Thirdly, the installation of a 5ml water vessel between the cryogenic trap and the sorbent trap kept the water amount entering the desorption unit constant (Hsu et al., 1991). The desorption was preceded by a dry purge (Hsu et al., 1991). As a fourth solution, Farmer et al. (1994) mentioned nickel tubing with glass beads and Chromosorb A. Further on, Greenberg et al. (1994) avoided water entering the analytical instrument in cryogenic sampling by slowly heating up the trap to 80°C in order to transfer only the VOCs. Finally, the technique most often applied is the installation of a water removing trap. Haszpra et al. (1991) installed a granular magnesium perchlorate tube at 60°C before the cryogenic trap. Widely applied are Perma Pure dryers with a tubular hygroscopic ion-exchange membrane (Nafion). They can be used if two assumptions are made. First, the water retaining cartridge is supposed not to trap VOCs. One study reported the loss of methyl chloride (Hsu et al., 1991). Secondly, it may not set free VOCs in order to avoid contamination. McClenny et al. (1984) reported no loss of MAHs and halogenated C_1-C_4-halocarbons at ±100-1000µg.m^{-3} but the dryers had to be cleaned by heated outgassing in order to reach zero air levels. Moreover, Boudries et al. (1994) reported the impossibility of the quantification of butenes, benzene and toluene with Nafion, but later on, quantification was performed by a substraction of 145, 250 and 495ng.m^{-3} i-butene, benzene and toluene respectively.

Using 170mg Carbopack B and 350mg Carbosieve SIII and the breakthrough volume data of Mosesman et al. (1988) 86.4% of water in a 6l air sample is already lost during the sampling step. However, the water retained still disturbed the analysis in the on-line desorption, similar to the analyses of Mc Caffrey et al. (1994). The water trap used in our off-line construction, similar to the trap of Lai et al. (1993), is a simple and efficient method. A glass trap is preferred because stainless steel traps showed unreproducible measurements for C_2-substituted MAHs after a series of analyses. It was demonstrated that the wet trap did not retain VOCs. A thermal desorption system as described by Van Langenhove et al. (1982) was provided with and without the wet trap. Tenax tubes were loaded with 10l humidified gas and with VOCs from a closed two-phase system (CTS) (chloroform, 1,1,1-trichloroethane, tetrachloromethane, toluene, ethylbenzene and p-xylene). When the tubes were analysed in the system equipped with the wet trap, mean recovery values from triple analysis varying from 91.3 to 98.3% were noticed for all VOCs, when compared with analyses executed on the system when it was not provided with the wet trap.

II.1.1.1.2. Limits of detection

The limits of detection (LODs) ($ng.m^{-3}$) are defined as the total amount of VOC at a signal/noise ratio of three (s/n=3) and the blank level. The VOC masses corresponding to s/n=3 were determined by analysing a calibration mixture containing all 13 VOCs at 2.2 - 4.1$\mu g.m^{-3}$ (±670pptv) (6l). The blank levels of chloroform, benzene and toluene had to be considered (0.026, 0.159 and 0.062ng respectively) in order to calculate the LODs. In this way LODs from 2.16$ng.m^{-3}$ (m/p-xylene) to 5.73$ng.m^{-3}$ (o-xylene) were obtained, except for chloroform (41.4$ng.m^{-3}$), benzene (96.0$ng.m^{-3}$) and toluene (48.7$ng.m^{-3}$) (Table II.1.1).
This obtained LODs are similar to LODs of Boudries et al. (1994) for FID-detection. With the same detection (MS in SIM-mode), McClenny et al. (1991) determined LODs of ±105$ng.m^{-3}$ (1,1,1-trichloroethane) to ±1650$ng.m^{-3}$ (toluene) for the same target compounds. Better benzene and toluene LODs (±1.2$ng.m^{-3}$) are reported by Greenberg et al. (1994) with cryogenic sampling and FID-detection. Helmig and Greenberg (1994) suggest their adsorbent sampling with GC-MS analysis is able to detect below pptv levels though no exact determination of LODs is reported. Finally, it has to be mentioned that ECD-detection is capable to detect lower concentration levels of CHCs (Koppmann et al., 1993), but it doesn't cover all target compounds of this work.

II.1.1.1.3. Precision and accuracy

II.1.1.1.3.1. Precision of the analysis

A calibration mixture from a closed two-phase system (CTS) was used to verify the precision of the analysis. The masses analysed were equal to concentrations of 2.2 - 4.1$\mu g.m^{-3}$ (±670pptv) in a 6l sample. The relative standard deviation ranged from 0.9 (chloroform) to 8.3% (1,1-dichloroethane) (Table II.1.2).
Several precision levels are found in literature. With FID- and ECD-detection, deviations of up to 20% were observed for concentrations down to ±20$\mu g.m^{-3}$ (CHCs) and ±200$\mu g.m^{-3}$ (MAHs) (Sweet and Vermette, 1992). Wïedmann et al. (1994) obtained similar reproducibility for tetrachloroethylene at 7 - 70$ng.m^{-3}$.

Table II.1.1. Limits of detection for the analysis of all VOCs in air ($ng.m^{-3}$)

VOC	LOD
chloroform	41.4
tetrachloromethane	3.97
1,1-dichloroethane	3.60
1,2-dichloroethane	3.31
1,1,1-trichloroethane	4.06
trichloroethylene	2.53
tetrachloroethylene	4.40
benzene	96.0
toluene	48.7
ethylbenzene	3.65
o-xylene	5.73
m/p-xylene	2.16

Table II.1.2. Precision of the analysis of all VOCs in air at concentrations of 2.2 - 4.1$\mu g.m^{-3}$ (±670pptv). %SD = relative standard deviation in % (n=4)

VOC	%SD
chloroform	0.9
tetrachloromethane	5.5
1,1-dichloroethane	8.3
1,2-dichloroethane	5.1
1,1,1-trichloroethane	5.0
trichloroethylene	4.0
tetrachloroethylene	6.2
benzene	2.6
toluene	1.1
ethylbenzene	4.0
o-xylene	8.1
m/p-xylene	7.1

Better reproducibility data are reported as well. Hsu et al. (1991) measured 2-5%SD for the same target compounds with GC-MS analysis at 5.6-27.8μg.m^{-3}. At concentrations between ±4 and ±40μg.m^{-3}, %SD below 4.5 are reported by Hisham and Grosjean (1991) with ECD and by Lai et al. (1993) with FID.

In conclusion, no reproducibility data for the measurements of this target compounds at levels below 4μg.m^{-3} (about 1ppbv) are found. Comparable measurements are done by McClenny et al. (1991) with 2.4 (toluene) to 15.1%SD (tetrachloromethane) at average concentrations of ±4.4μg.m^{-3}.

II.1.1.1.3.2. Precision of the sampling procedure

The sampling reproducibility was checked for toluene at a concentration level of 9.25μg.m^{-3} and at a 930ng.m^{-3}. A gas stream was made by application of a dynamic vapour pressure (DVP) system. Gas was sampled on two adsorption tubes in parallel connected, with one common inlet using a Y-shaped teflon tube. Though the standard deviation on one parallel sampling proved to be quite variable (1.9 to 20.7%SD) the mean of the parallel samples was reproducible with 3.2 (n=4) and 3.8%SD (n=4) for 9.25μg.m^{-3} and 930ng.m^{-3} respectively. In this view it is recommended to take air samples in duplicate in a parallel construction in order to improve the precision.

II.1.1.1.3.3. Accuracy of the analysis

A mean accuracy was obtained by analysing ten reference materials. The masses in the reference materials, prepared from a CTS, corresponded to concentrations from 2.62 to 4.90 μg.m^{-3}. The accuracy is presented in Table II.1.3. It can be seen that the recovery was between 91.6 and 122.0% except for chloroform (141.9%) and 1,1-dichloroethane (153.4%). The deviation of these two compounds can be related to the poor reproducibility for these compounds in this test. The relative standard deviations were 58.8 and 62.8% respectively.

Table II.1.3. Mean accuracy of ten analyses of all VOCs in air, where accuracy is defined as measured concentration over expected concentration

VOC	expected concentration ($\mu g.m^{-3}$)	averaged measured concentration ($\mu g.m^{-3}$)	accuracy (%)
chloroform	3.91	5.55	141.9
tetrachloromethane	4.90	4.49	91.6
1,1-dichloroethane	3.37	5.17	153.4
1,2-dichloroethane	3.26	3.40	104.2
1,1,1-trichloroethane	4.00	3.80	94.9
trichloroethylene	4.16	4.36	104.8
tetrachloroethylene	5.44	5.45	100.3
benzene	2.62	2.74	104.4
toluene	3.15	3.10	98.5
ethylbenzene	3.63	3.45	95.1
m/p-xylene	3.63	4.21	115.9
o-xylene	3.48	4.24	122.0

II.1.1.1.4. Analytical quality assurance

The general procedures to ensure data quality, proposed by international organisations dealing with marine sciences as the OSPARCOM (Oslo and Paris Commissions, 1990) and the QUASIMEME working group (Quality Assurance of Information in Marine Environmental Monitoring Programmes in Europe) (Topping et al., 1993) deal with measurements of compounds in matrices like water, sediment and biota. So, no quality control systems for data of concentrations in air are worked out.
However, the principles of a standard addition test and the use of quality control charts can be applied in measurements in air as well in order to assess the analytical performance.

II.1.1.1.4.1. Standard addition test

Two 5 liter samples were taken in parallel on the Belgian Continental Shelf on 31 March 1995 at N 51°21'78" and E 03°08'59". To one of both samples, an amount of each target compound was added from a CTS, corresponding to concentrations from 524 to 1087ng.m^{-3}.

In Table II.1.4 the results of the fortified sample and the non-fortified sample are presented. Next to this, the added amount is given and the recovery of the VOCs from the fortified sample, i.e. the ratio of the concentration found on the fortified sample to the sum of the concentration in the non-fortified sample and the added amount.

From Table II.1.4 it can be seen that for most of the VOCs the recovery is within 71.4 and 130.5%, except for chloroform (52.1%) and trichloroethylene (184.5%). The reason for these deviations is unclear. However, it has to be stated that the sample and the fortified sample are not exactly the same sample. It has already been clear from the evaluation of the precision of the sampling procedure that samples taken in parallel can show different concentrations and that rather the mean of two parallel samples is reproducible than the values from individual samples. This means that the non-fortified sample and the fortified sample before it was fortified, could have had different VOC concentrations.

Table II.1.4. Standard addition test with analysis of a fortified sample (FS) in comparison with the analysis of a non-fortified sample (NFS) taken in parallel (all in $ng.m^{-3}$)

	FS	NFS	added amount	NFS +added amount	recovery(%) (FS/NFS+added amount)
chloroform	560	293	782	1075	52.1
tetrachloromethane	1813	680	980	1659	109.2
1,1-dichloroethane	871	12	674	686	127.0
1,2-dichloroethane	555	125	653	778	71.4
1,1,1-trichloroethane	1896	828	801	1629	116.4
trichloroethylene	1738	111	831	942	184.5
tetrachloroethylene	1660	185	1087	1272	130.5
benzene	2332	1721	524	2245	103.9
toluene	5702	4430	630	5059	112.7
ethylbenzene	2572	2142	726	2867	89.7
m/p-xylene	2954	1883	726	2609	113.3
o-xylene	2354	1257	696	1953	120.6

II.1.1.1.4.2. Analytical quality control charts

The system of quality control and assessment proposed by QUASIMEME is to plot the analytical data for reference materials on an analytical quality control chart (AQCC). Guidelines to assess these charts are given. According to the authors (Topping et al., 1993), this is a better approach than just analyse once a reference material as a check on the quality of analytical data. For the construction of the AQCCs in air analysis, ten reference materials were analysed at random in one batch of samples in a six day period. The obtained results are presented in Figure II.1.1. From 120 measurements, 112 samples were within the warning limits and 8 samples between the warning and control limits. This latter 8 samples were all from the last reference material. Statistically, 95% and 99.7% of the data are expected to be within the warning and control limits respectively, where in this case 93.3 and 100% were found. It can be concluded from the AQCCs that the results indicate an acceptable quality.

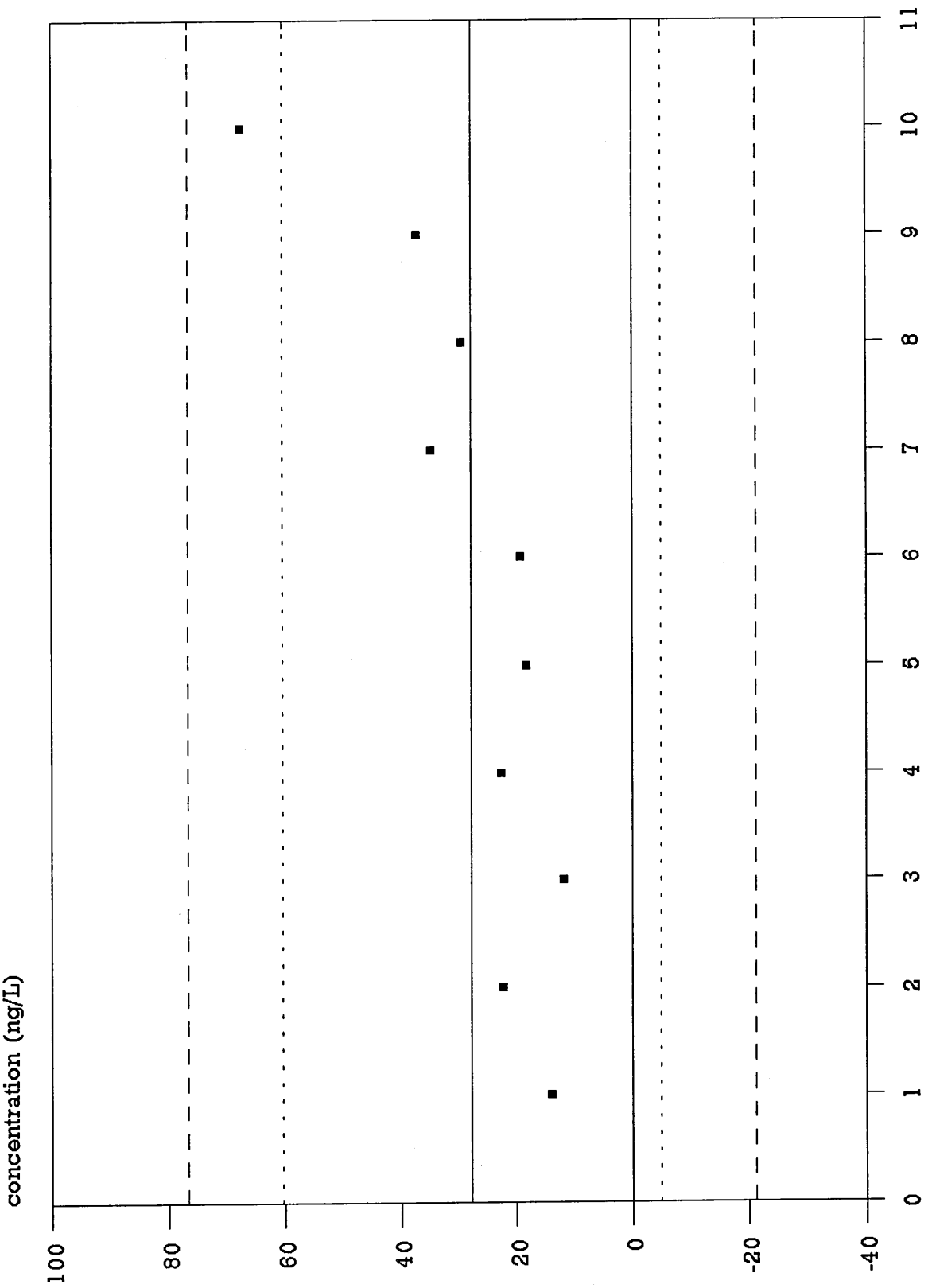

Figure II.1.a. AQQC chloroform. Full line: mean value x, dotted line: X+/-2s, broken line: X+/-3s

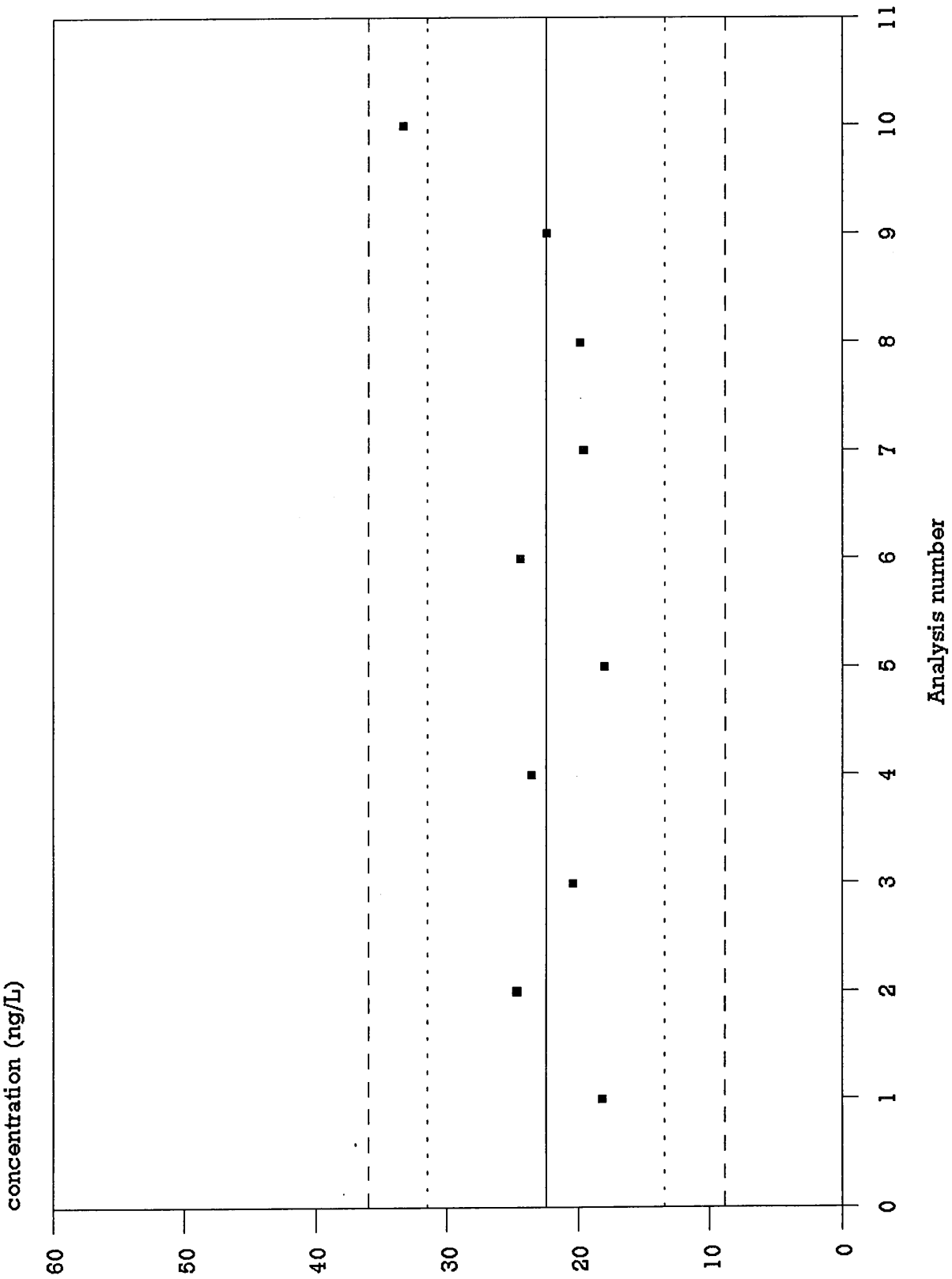

Figure II.1.b. AQQC tetrachloromethane. Full line: mean x, dotted line:x+/-2s, broken line:x+/-3s

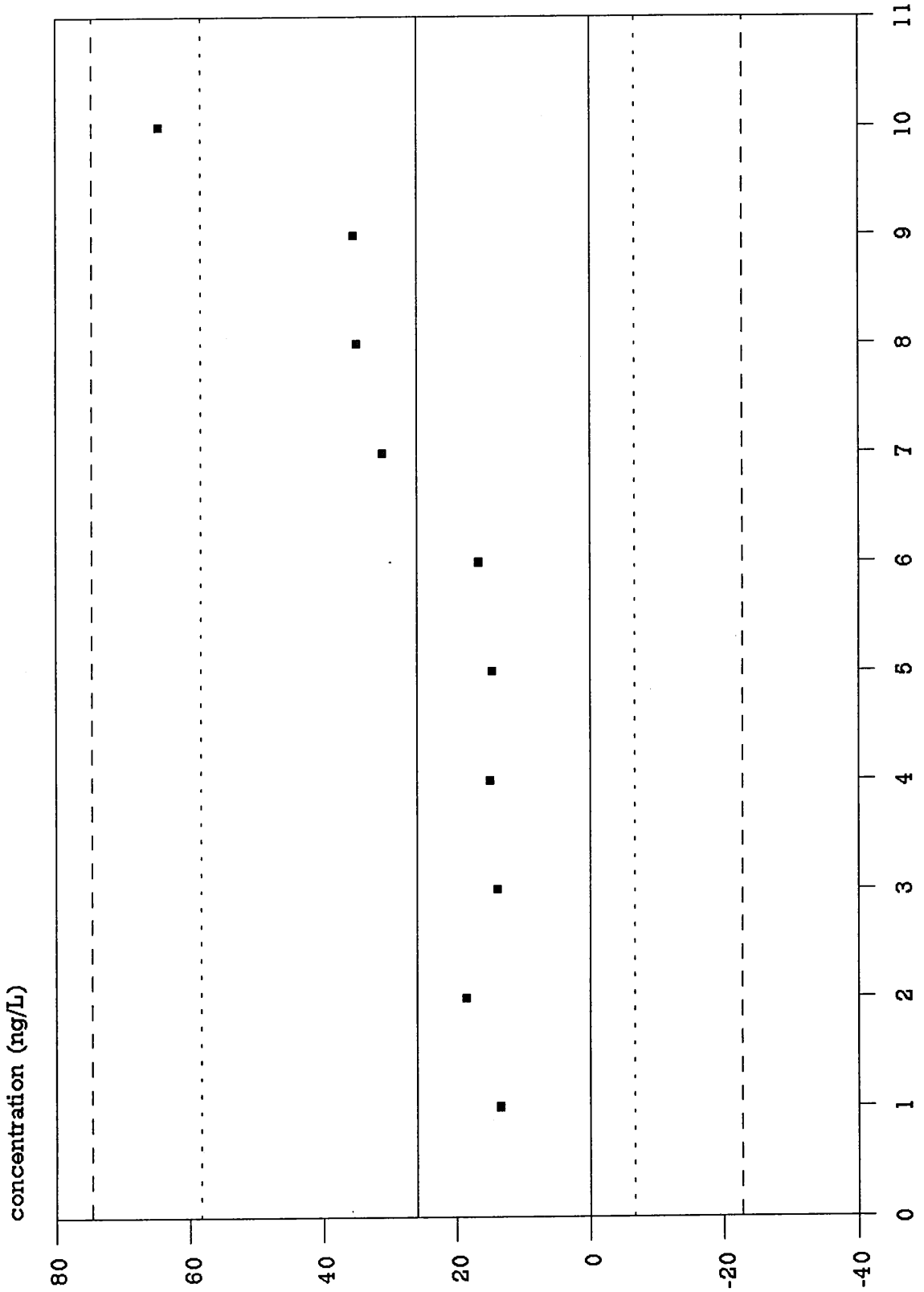

Figure II.1.c. AQQC 1,1-dichloromethane. Full line: mean x, dotted line x+/-2, broken line x+/-3s

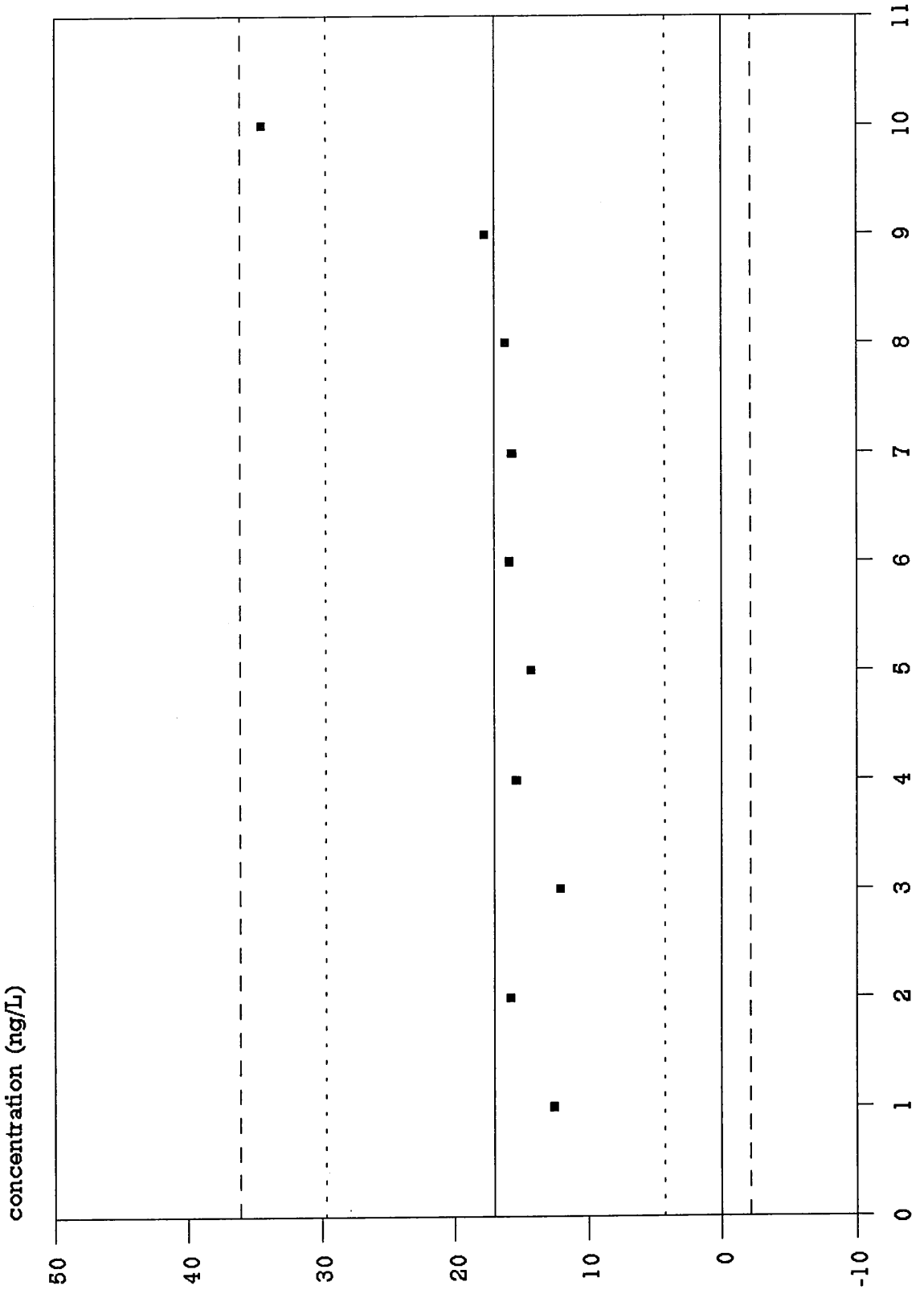

Figure II.1.d. AQQC 1,2-dichloroethane. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

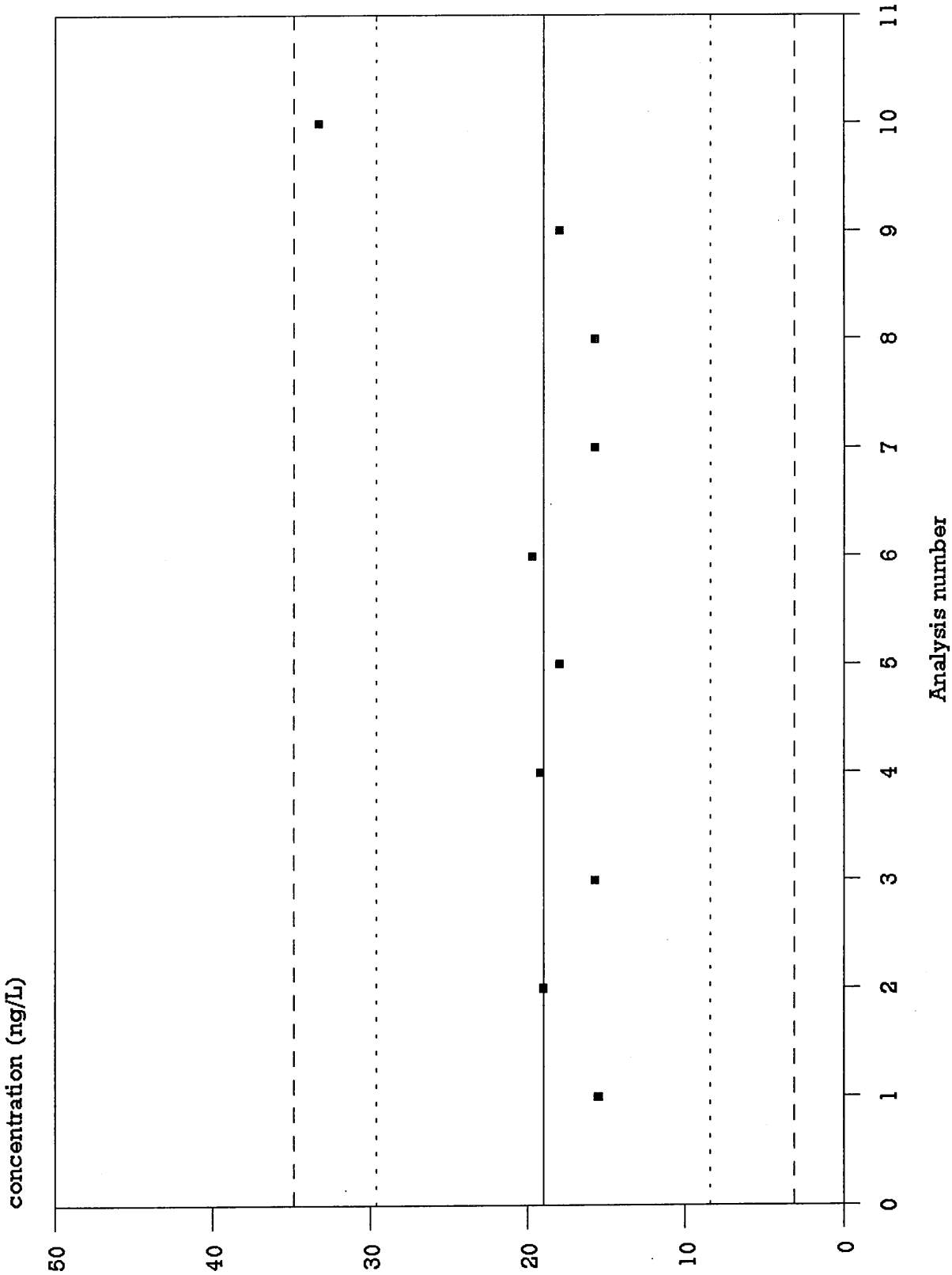

Figure II.1.e. AQCC 1,1,1-trichloroethane. Full line: mean x, dotted line: x+/-2s,broken line x+/-3s

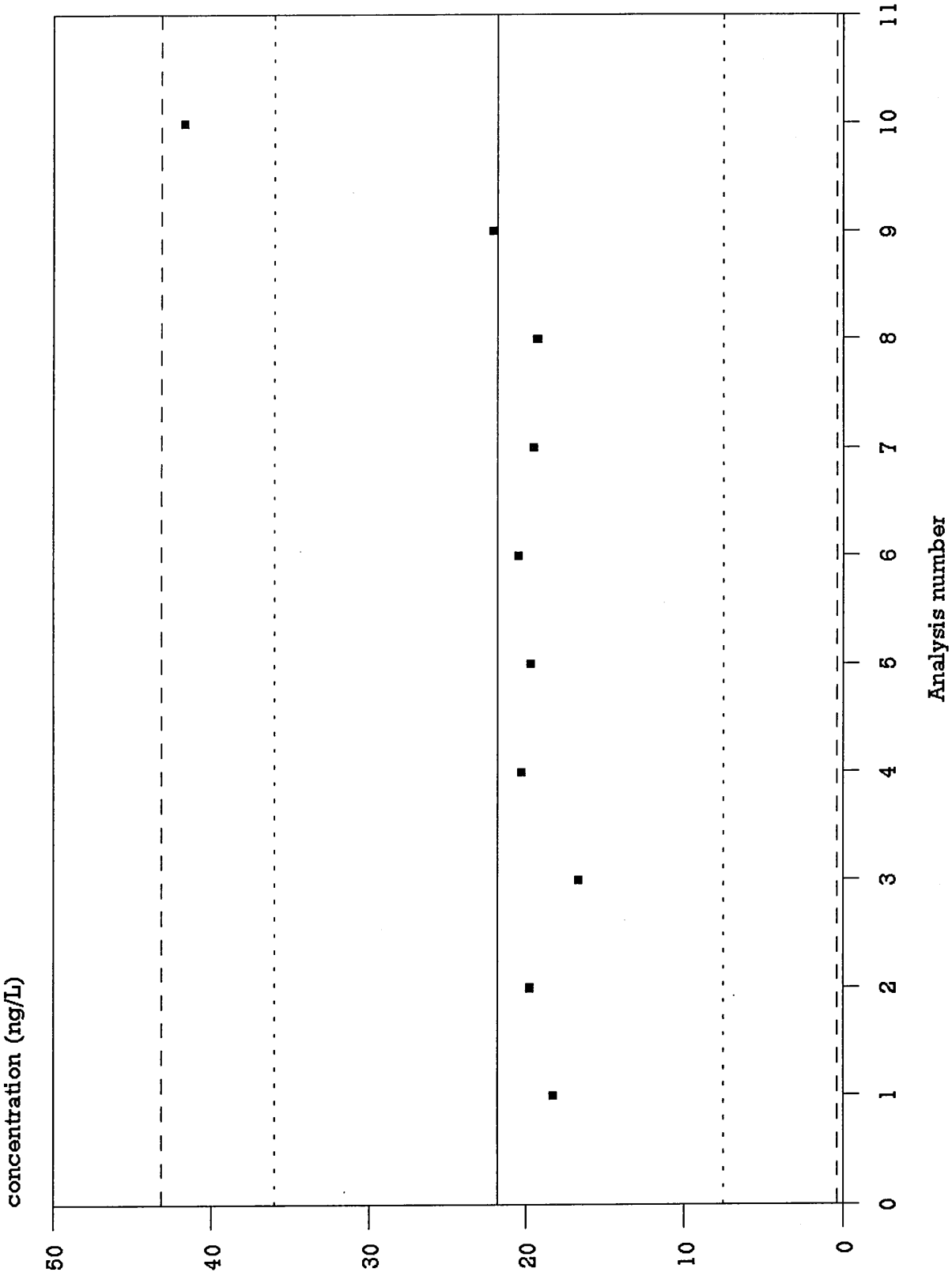

Figure II.1.f. AQQC trichloroethylene. Full line: x, dotted line: x+/-2s, broken line: x+/-3s

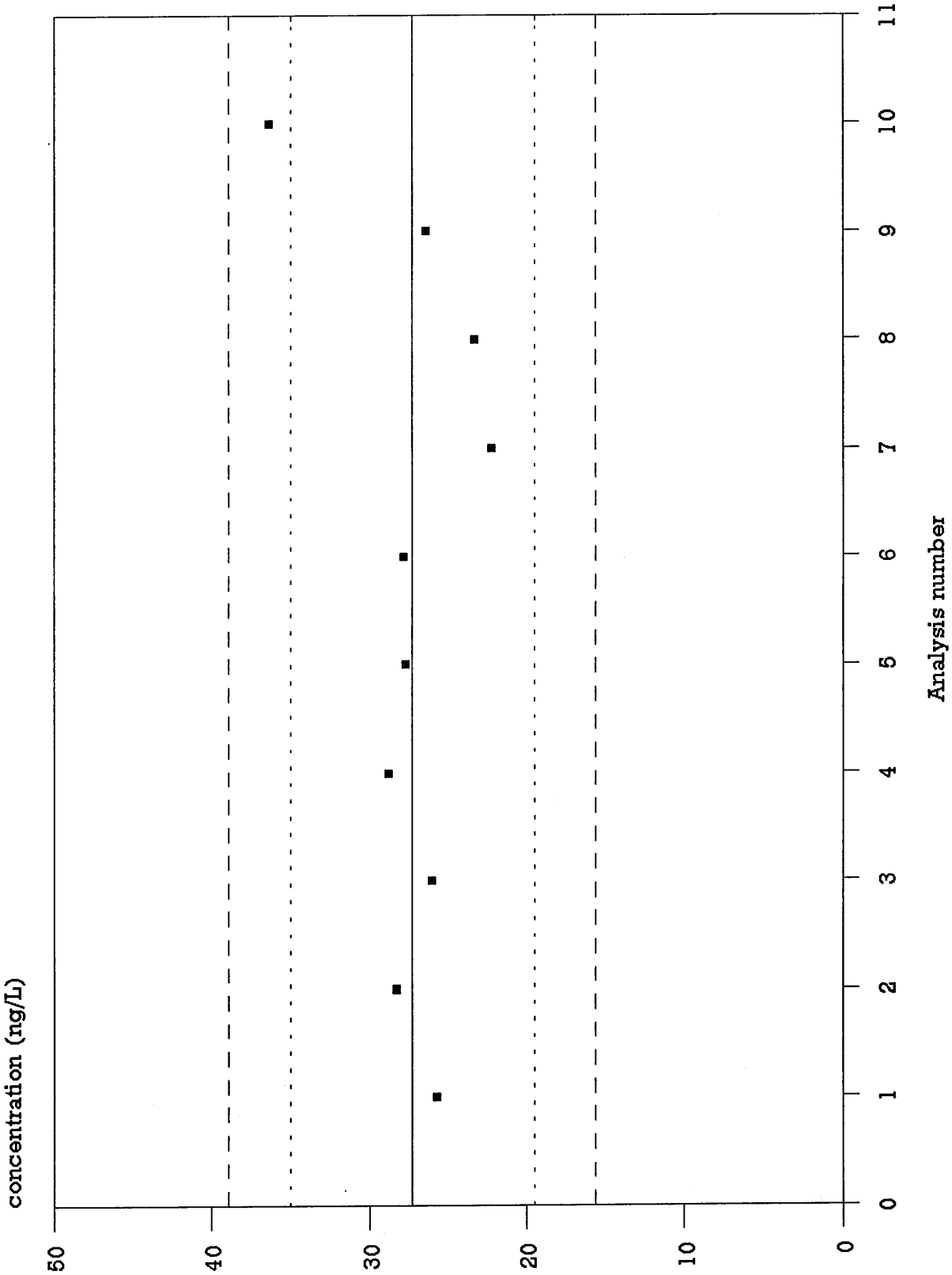

Figure II.1.g. AQQC tetrachloroethylene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-2s

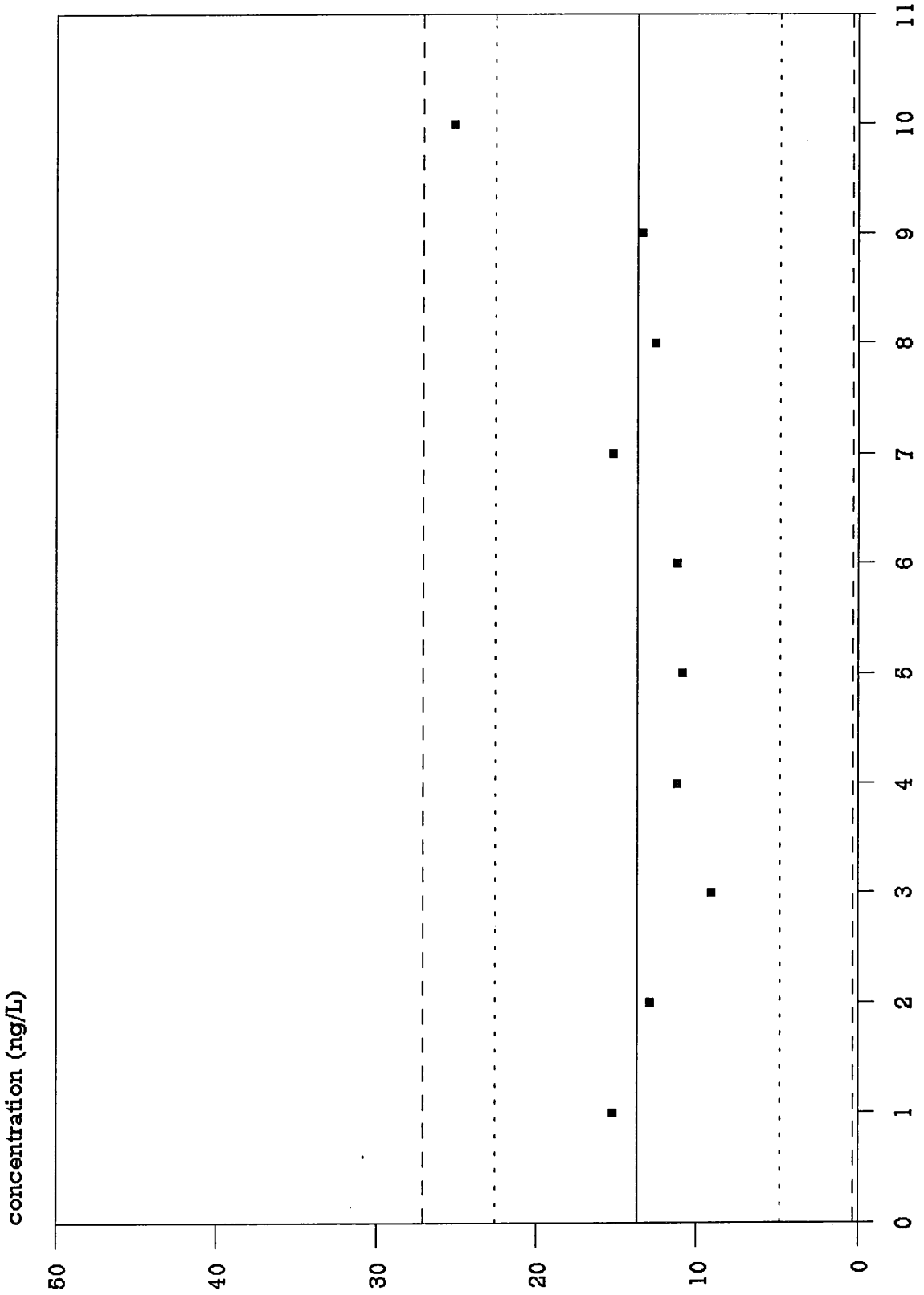

Figure II.1.h. AQQC benzene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

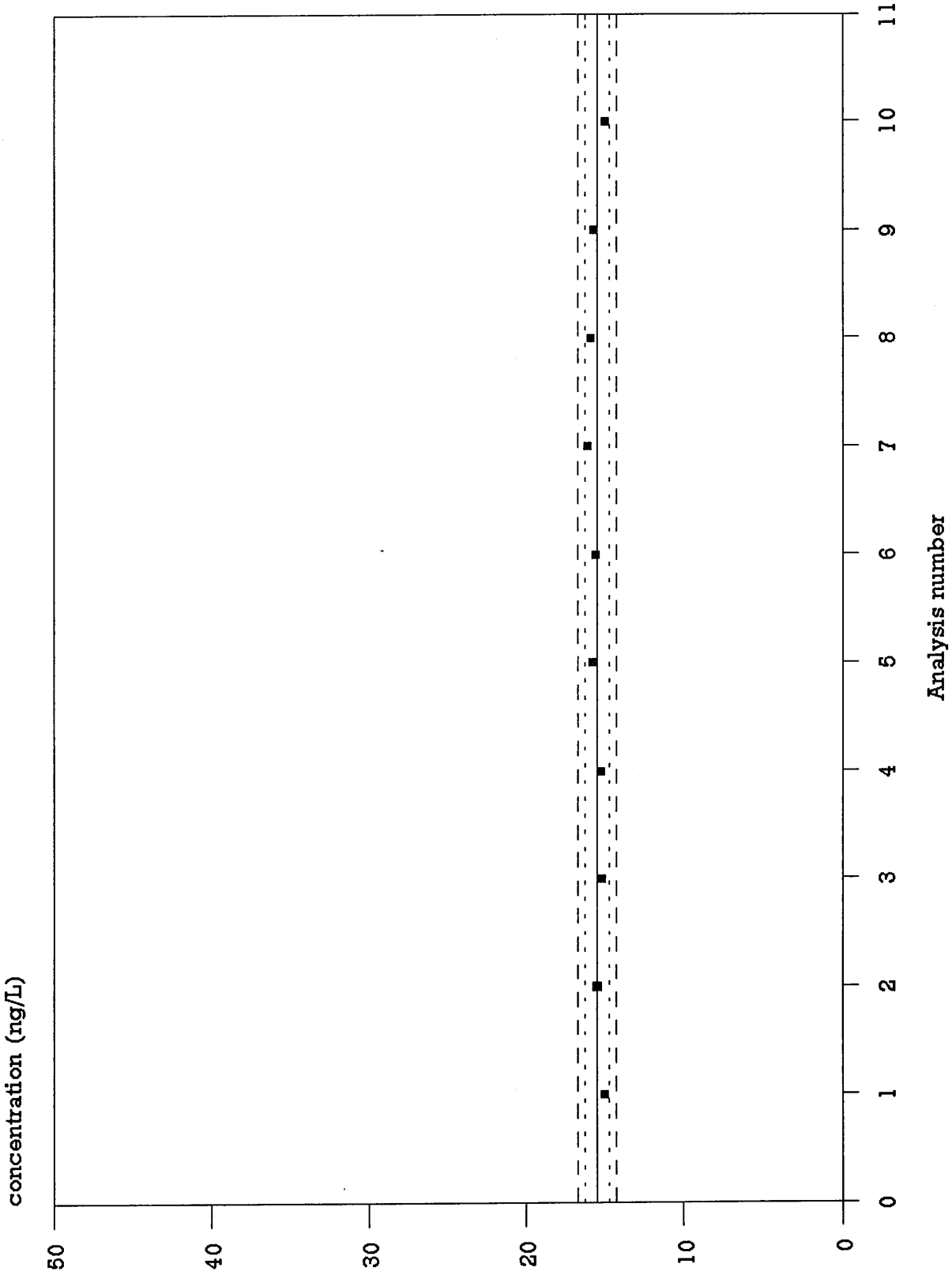

Figure II.1.i. AQQC toluene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

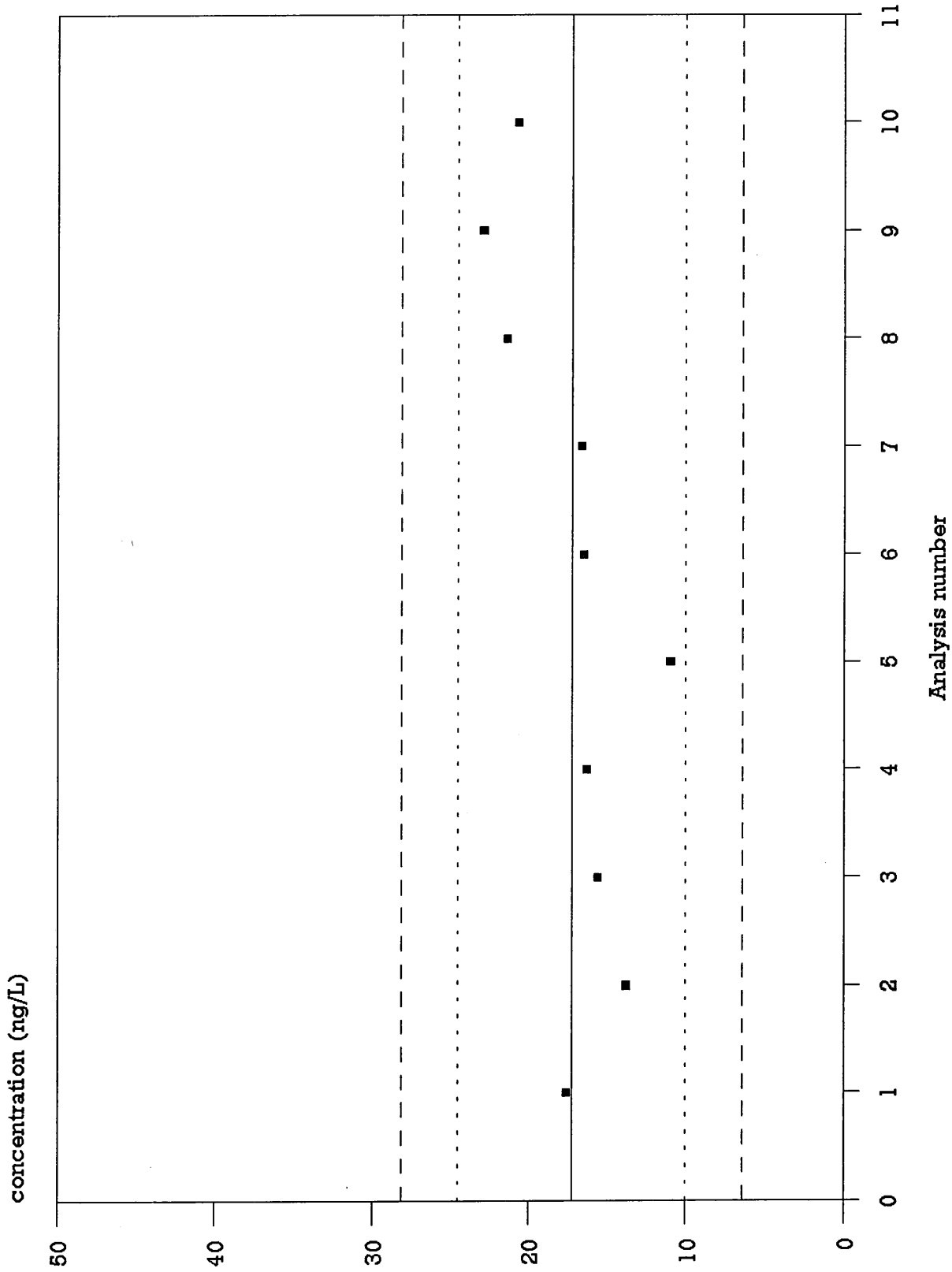

Figure II.1.j. AQQC ethylbenzene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

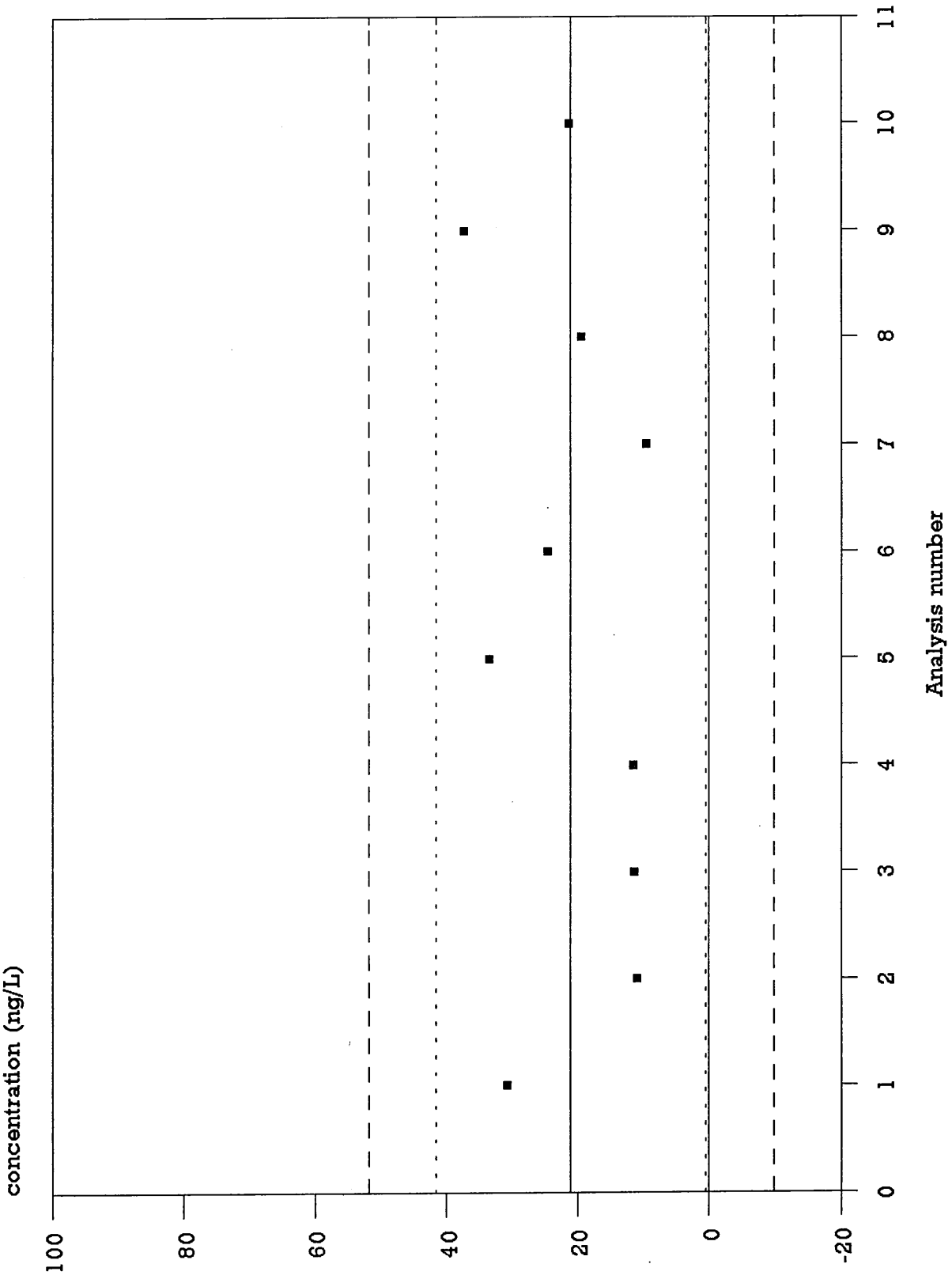

Figure II.1.k. AQQC m/p-xylene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

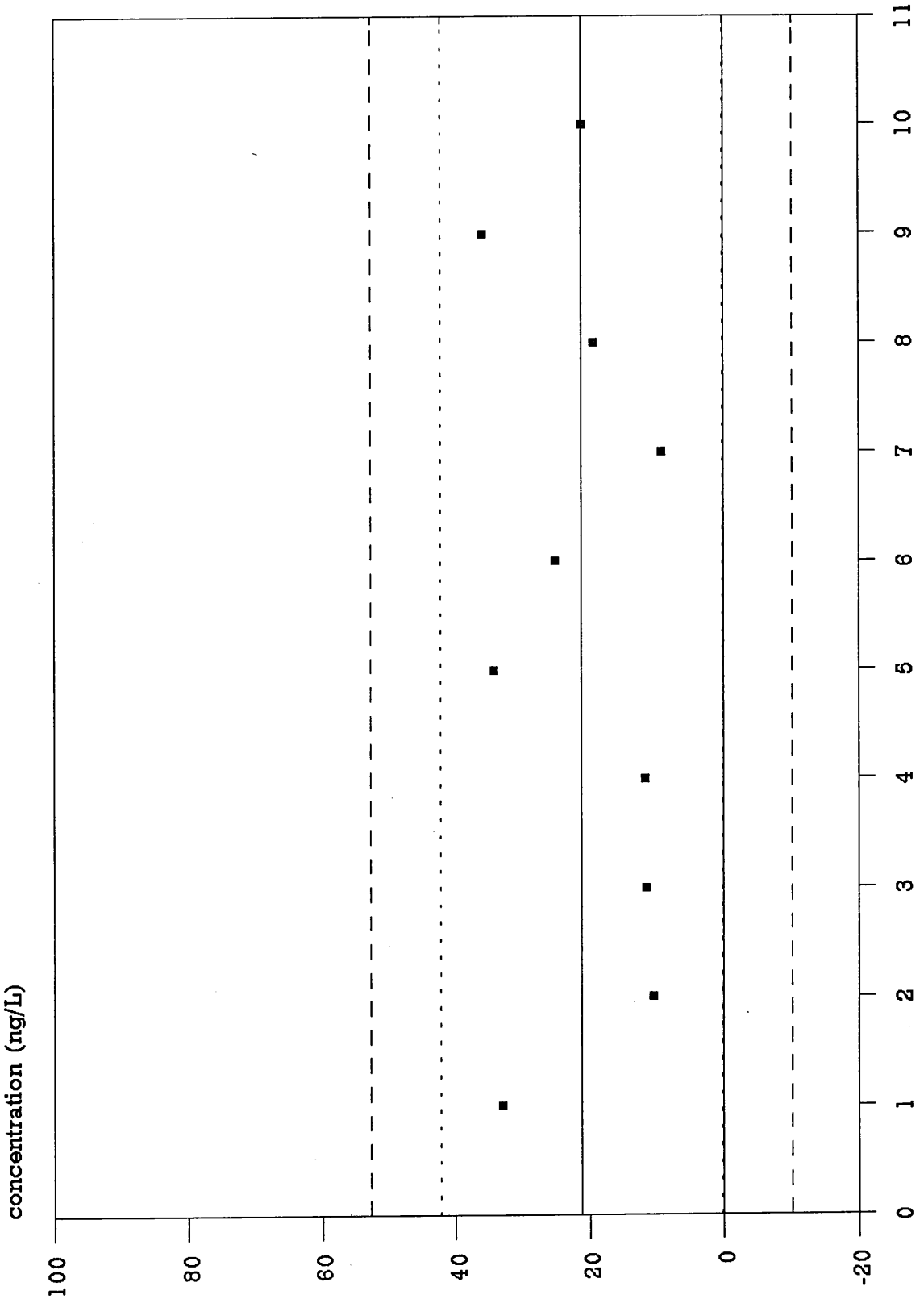

Figure II.1.1. AQQC o-xylene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

II.1.1.2. CALIBRATION METHOD

In order to obtain an accurate and precise calibration system, the dynamic vapour pressure method (DVP) method (Schoene and Steinhanses, 1989) was evaluated for chloroform and toluene and a closed two-phase system (CTS) calibration technique was developed.

II.1.1.2.1. Accuracy of DVP and CTS calibration

The two calibration methods rely on the calculation of the mass brought on the adsorbent. In order to check the validity of the calculation, a GC-FID system equipped with a thermal desorption unit was used. So, the calculated mass from the DVP or from the CTS brought on a sorption tube could be verified versus direct liquid injection.

From a DVP system, 5l air containing 59ng toluene and 448ng chloroform was sampled and analysed. The measured amount significantly exceeded the calculated mass for chloroform (% recovery 138.1±8.6) as well as for toluene (128.8±2.1) (n=6). Possible explanations for this overestimation could be the difference between the real and the stated diameter of capillary tubes of 0.18mm ID.

In Table II.1.5, the analysed masses from the adsorption tubes loaded with chloroform, 1,2-dichloroethane, toluene and m-xylene from a CTS system are presented and compared with the calculated values. As can be seen, the measured masses match very well the calculated masses for chloroform and toluene. It can be concluded that the CTS calibration technique gave satisfactory accurate results with recoveries of 95.5 to 111.2% (n=4) when masses equal to 3.23 till 4.73ppbv in a 6l sample were applied.

Table II.1.5. Calculated masses from a CTS and measured calibration masses from sampling tubes with corresponding standard deviation (ng) (n=4)

VOC	calculated mass	measured mass	recovery
chloroform	149.4	151.3 ± 2.5	101.3 ± 1.7
1,2-dichloroethane	95.5	106.2 ± 1.9	111.2 ± 2.0
toluene	84.4	84.4 ± 1.4	100.0 ± 1.7
m-xylene	88.2	84.2 ± 1.2	95.5 ± 1.4

II.1.1.2.2. Precision of the DVP and the CTS calibration method

The precision of both the DVP and the CTS calibration method were satisfactory. The %SD was 6.2 for chloroform and 1.6 for toluene in the DVP method (n=6). In Table II.1.5 the precision of the calibration mixture from the CTS is presented and ranges from 1.4 (m-xylene) to 1.8%SD (1,2-dichloroethane) (n=4).

When compared to the DVP method and with other methods found in literature, it is clear that the CTS calibration technique is a valuable method since the technique is accurate, reproducible, easy applicable to several compounds and inexpensive. It is clear that the DVP method requires the knowledge of a lot of physical parameters. Permeation tubes are also based on a diffusion process, but knowledge of the parameters governing the mass flux are not required (Berezkin and Drugov, 1991). The diffusion rate can be calculated by measuring the tube weight loss over a long period. In this method regular and very precise weighing of the tube by means of an automatic microbalance is needed. Of all methods, direct liquid injection on the analytical column is the most simple technique (Wiedmann et al., 1994; Helmig and Greenberg, 1994) but it doesn't cover the sampling stage and the thermal desorption. Certified gas cylinders are used directly or after dilution (Greenberg et al., 1994; Gholson et al., 1990; Lai et al., 1993). However, McClenny et al. (1991) reported for 10 compounds of the CHC and MAH target compounds a bias ranging from 4.2 to 21.2% at ±10-20$\mu g.m^{-3}$ concentrations with this methodology. The preparation of a gas calibration mixture by diluting a liquid into a gas cylinder is a third calibration method (Hsu et al., 1991; McClenny et al., 1984; Koppmann et al., 1993; Boudries et al., 1994; Hisham and Grosjean, 1991). For this method, Boudries et al. (1994) reported average deviations of 20% if dilution down to the $ng.m^{-3}$ level is done.

II.1.1.3. CONCLUSIONS

The presented technique consisting of sampling on Carbopack B/Carbosieve SIII sorbents and of a desorption-GC-MS analysis allows the measurement of 13 CHCs and MAHs at $ng.m^{-3}$ levels. This was not achieved by the application of an on-line P&T instrument equipped with a cartridge desorber because of water interference and because of contamination originating from the instrument. On the other hand, the combination of the on-line instrument together with an off-line desorption including a simple water trap, proved to operate reproducibly with

0.9 to 8.3%SD at 2.2 - 4.1$\mu g.m^{-3}$ ($\pm$670pptv) (n=4).

LODs were determined as $\pm$4$ng.m^{-3}$ (2.16 - 5.73) for all compounds except for chloroform, benzene and toluene (41.4 - 96.0$ng.m^{-3}$). This was caused by contamination originating from the P&T instrument. The accuracy of the technique showed averaged data on ten measurements between 91.6 and 122.0%, except for chloroform and 1,1-dichloroethane.

Next to the optimization of the sampling and analysis procedure, a simple calibration technique is presented. It is based on the equilibrium partitioning in a closed air-water system (Henry's law). The accuracy and precision were tested and proved to be good with a recovery of 102.0$\pm$6.6% (n=4) and with a reproducibility for each compound of 1.4 to 1.8%SD. On the contrary the measured concentrations generated by the DVP system (Schoene and Steinhanses, 1989) exceeded the expected concentrations.

II.1.2. EXPERIMENTAL SECTION

II.1.2.1. METHODS FOR THE DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN AIR AT REMOTE SITES

When methods for the determination of VOCs in air at remote sites are considered, attention has to be paid to two critical points. First, a good sampling technique together with an appropiate analysis method is necessary. Secondly, accurate calibration is needed.

II.1.2.1.1. Sampling and analysis techniques

Different sampling techniques are reported in literature. Air sampling methods like canister sampling (Sweet and Vermette, 1992; Farmer et al., 1994; McClenny et al., 1991; Hsu et al., 1991) and Tedlar bag sampling (Tsujino and Kuwata, 1993) are used. Further on, VOCs concentration methods like cryogenic trapping (Greenberg et al., 1994; McClenny et al., 1984; Koppmann et al., 1993; Haszpra et al., 1991) and adsorbent trapping (Ciccioli et al., 1993; Frank and Frank, 1990; Wiedmann et al., 1994; Brown and Purnell, 1979) are applied.

All these sampling methods have some disadvantages with respect to contamination, recovery and water interference. Canisters need a thorough cleaning (Jayanty, 1989; US-EPA, 1988) in order to measure low environmental concentrations. Interactions with the internal sample holder surface have to be considered when air with low relative humidity is sampled (Gholson et al., 1990; Coutant, 1992).

Sampling with cryogenic trapping can be disturbed by the formation of ice in the trap during the concentration step (McClenny et al., 1984). This can be encountered with more complicated cryogenic sampling methods as the double cryogenic trapping method of Greenberg et al. (1994).

Sorbent sampling reduces the water problem if hydrophobic materials are used. However, the disadvantage of this method is a limited safe sampling volume for highly volatile compounds due to breakthrough losses (Brown and Purnell, 1979; Ventura et al., 1993). At low environmental concentration levels, contamination originating from degradation of the sorbent itself, especially of Tenax (Walling et al., 1986) can cause inaccurate results.

Due to the development of new sorbent materials a new impulse to the sorbent sampling technique is given. Carbon based materials such as Carboxen 1000, Carboxen 1001,

Carbotrap, Carbotrap C, Carbosieve SII and Carbosieve SIII are in use (Sturges and Elkins, 1993; Helmig and Greenberg, 1994). The application of different sorbents in series allows sampling and analysis of a wide range of VOCs. They show a good thermal stability and assure low background levels (Helmig and Greenberg, 1994), but some of them such as Carbosieve SIII retain substantial amounts of water (breakthrough volume of 2.3L/g (Mosesman et al., 1988)).

II.1.2.1.2. Calibration techniques

The calibration method is the second important point in the determination of atmospheric VOCs. Several calibration methods for VOCs analysis in air at $\mu g.m^{-3}$ or $ng.m^{-3}$ level are described such as external calibration with direct liquid injection (Wiedmann et al., 1994; Helmig and Greenberg, 1994), calibration with cylinders containing certified gas mixtures or with dilutions of these cylinders (McClenny et al., 1984; Gholson et al., 1990; Lai et al., 1993), the dilution method (Hsu et al., 1991; McClenny et al., 1984; Koppmann et al., 1991; Boudries et al., 1994; Hisham and Grosjean, 1991) and the dynamic vapour pressure (DVP) method (Schoene and Steinhanses, 1989). Briefly, the DVP system consists of a clean air stream passing over the top of a capillary. At the bottom of the tube a vessel with the liquid VOC is installed. The vapour concentration entering the gas stream can be calculated from the vapour pressure and the diffusion coefficient of the VOC, from the pressure and the temperature in the system, and from the length and the diameter of the tube (Schoene and Steinhanses).

II.1.2.2. MATERIALS

II.1.2.2.1. Adsorbents

170mg Carbopack B (graphitized carbon black, $100m^2/g$, 60/80 mesh, Supelco) and 350mg Carbosieve SIII (carbon molecular sieve, $820m^2/g$, 60/80 mesh, Supelco) were used as sorbents in series. They were hold separately with glass wool plugs in open glass tubes (length 16cm, OD 1/4"). The tubes were sealed with 1/4" brass end caps and 1/4" teflon ferrules (Alltech Ass.). Before sampling, the adsorption tubes were conditioned at 270°C with Helium (±50ml/min) during four hours. At the same time, the teflon ferrules placed in a glass

tube (ID 1cm) were conditioned (270°C / 4h / ±50ml/min). This was necessary because it was proven that unconditioned teflon and vespel ferrules contaminated adsorption tubes with VOCs. Teflon showed to be better than vespel in this respect.

II.1.2.2.2. VOCs, internal standard and solvent

The CHCs chloroform, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene (Janssen) and tetrachloromethane (Merck), and the MAHs benzene, toluene (Merck), ethylbenzene and m-, p- and o-xylene (Aldrich) were used as VOCs in the experiments. They were applied without further purification. Toluene-d8 (>99.95 atom%D, Fluka) was used as internal standard. Methanol was obtained from Merck (for Chromatography, 99.8%).

II.1.2.3. APPARATUS

The microprocessor controlled P&T system, CDS Peakmaster (CDS Analytical Instruments, Oxford, USA) was coupled with a gas chromatograph-mass spectrometer (GC-MS) Carlo Erba QMD 1000 (Carlo Erba Instruments, Milan, Italy) by a heated transferline with a cryogenic focuser at the GC injection port. The P&T system was provided with a 60ml vessel, a wet trap and a sorbent trap, and a cartridge desorber. In some preliminary experiments the cartridge desorber was replaced by a Chrompack desorber TCT1 with a control unit (Chrompack, Middelburg, The Netherlands).
As sorbent trap in the CDS instrument a Vocarb 4000 trap (1/8" OD, 8.5cm Carbopack C, 10cm Carbopack B, 6cm Carboxen 1000 and 1cm Carboxen 1001) was used. All parts and also the carrier gas and the transferline were connected to an 8-port switching valve. Separation of the VOCs was done on a RTX-502.2 capillary column (Restek) (length 60m, ID 0.32mm, film thickness 1.8µm, carrier gas helium, inlet pressure 12psi), detection was done using the mass spectrometer in the single ion monitoring (SIM) mode. M- and p-xylene were not separated and were determined together. The selected ions and time windows are presented in Table II.1.6.

Table II.1.6. Selected ion masses, time windows and limits of detection (S/N = 3) for the MS detector in SIM-modus

Compound	Selected ion masses	Time window (min)	Limits of detection (pg)
chloroform	83, 85	14.00 - 15.25	28
tetrachloromethane	117, 119, 121	15.25 - 17.50	35
1,1-dichloroethane	63, 65, 83	12.00 - 14.00	29
1,2-dichloroethane	62, 64	15.25 - 17.50	24
1,1,1-trichloroethane	61, 97, 99	15.25 - 17.50	28
trichloroethylene	95, 130, 132	17.50 - 20.00	28
tetrachloroethylene	129, 164, 166	22.00 - 23.50	18
benzene	77, 78	15.25 - 17.50	12
toluene	65, 91, 92	20.00 - 22.00	5
ethylbenzene	91, 105, 106	23.50 - 27.50	23
m/p-xylene	91, 105, 106	23.50 - 27.50	16
o-xylene	91, 105, 106	23.50 - 27.50	38
α,α,α,-trifluorotoluene	127, 145, 146	17.50 - 20.00	22
chloroform-d	84, 86	14.00 - 15.25	21
toluene-d8	70, 98, 100	20.00 - 22.00	11

Next to the on-line system, an off-line desorption unit was constructed. It consisted of a Chrompack desorber connected to a wet trap. The wet trap in the construction was a 1/4" stainless steel tube (length 1.5 or 3m) or a 2m 1/4" glass column submerged in a temperature controlled ethylene glycol bath (-10 to -15°C). The end of this wet trap was connected to a Vocarb 4000 trap. Helium purge gas was led through a liquid nitrogen trap before entering the off-line system.

II.1.2.4. SAMPLING AND STORAGE OF THE SAMPLES

Samples were taken on board of the Belgian Oceanographic vessel the 'Belgica'. The sampling sites in the Scheldt estuary and on the Belgian Continental Shelf in the North Sea are given in Figure II.1.2. and listed in Table II.1.7. Samples were taken on top of the wheel house. During sampling, the vessel was in an adverse wind position so that exhaust gases were avoided to contaminate the samples.

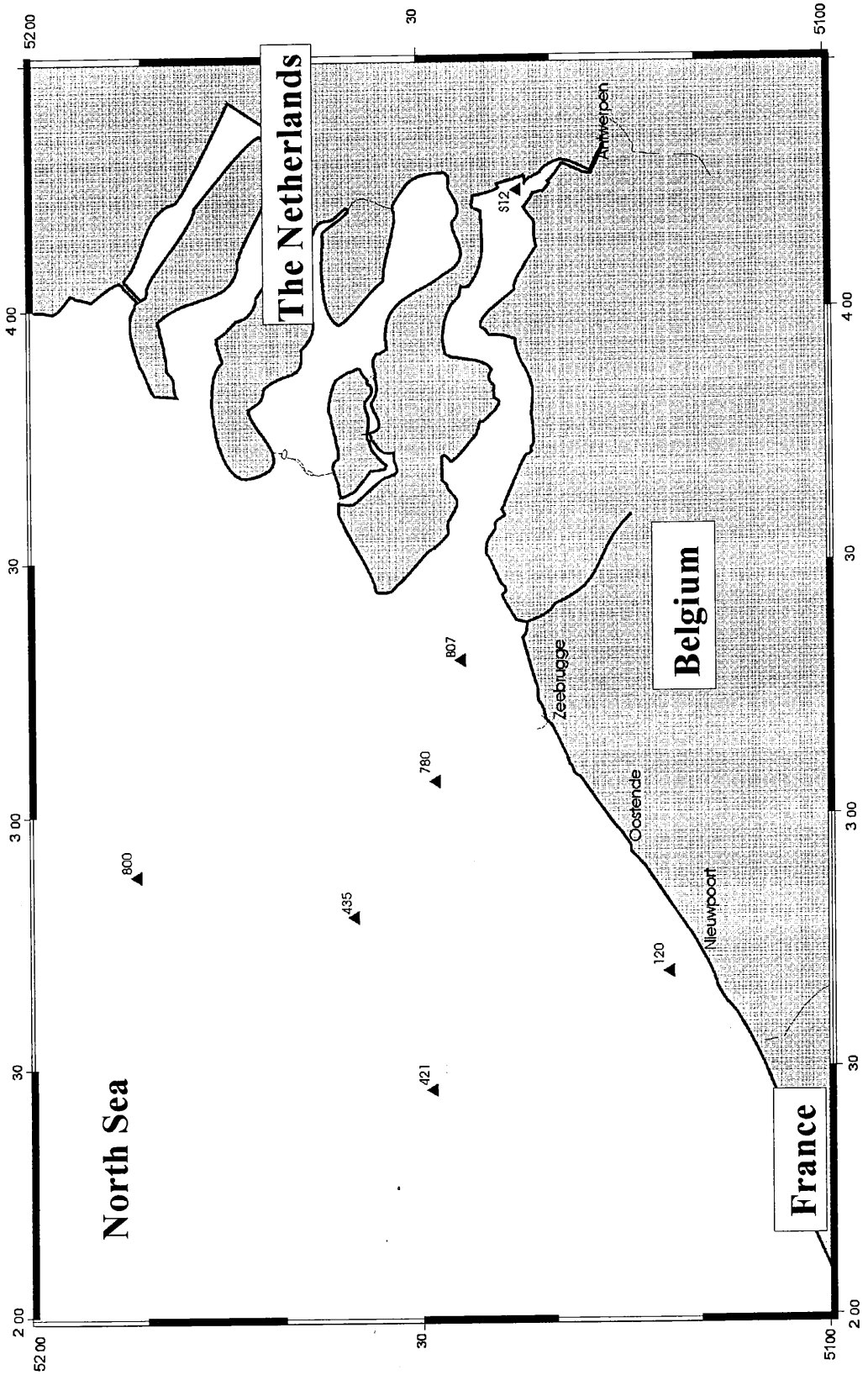

Figure II.1.2. Map of the sampling locations on the Belgian continental shelf and in the Scheldt estuary

Table II.1.7. Coordinates of the sampling locations of the monitoring campaigns.

Location	N	E
B07	51° 25.90	3° 17.80
S12	51° 21.90	4° 13.50
780	51° 28.27	3° 03.48
120	51° 11.10	2° 42.07
421	51° 28.83	2° 27.00
435	51° 34.84	2° 47.42
800	51° 50.83	2° 52.00

Duplicate samples were taken in a parallel position by pumping air through both sorption tubes at a flow rate of ±200ml/min during 25 minutes. After sampling they were sealed and stored in the laboratory till analysis.

II.1.2.5. ANALYTICAL PROCEDURES

II.1.2.5.1. Analysis of the samples

Samples (and also blank tubes and calibration tubes) were placed in the off-line Chrompack desorber. The samples were desorbed at 250°C for 14 minutes and purged for 18 minutes at 30ml.min^{-1}. After desorption of the sampling tubes, the Vocarb 4000 traps with sorbed VOCs were placed in the on-line system for desorption. The Vocarb 4000 trap were desorbed during 6 minutes at 250°C. After desorption the traps were conditioned at 260°C for 4 minutes. The cryofocussing temperature was held at -150°C during the desorption.
After the desorption the cryogenic focuser was heated to 260°C (rate 800°C/min) and held at this temperature during 5 minutes. Temperature programming of the GC and data acquisition were started simultaneously. Temperature of the GC oven was held at 50°C during 10 minutes, then increased to 190°C at a rate of 10°C/min.

II.1.2.5.2. Calibration

II.1.2.5.2.1. Tests on the calibration techniques

Two calibration techniques were tested. A first technique is the dynamic vapour pressure (DVP) calibration technique of Schoene and Steinhanses (1989). Secondly, the closed two phase system (CTS) calibration technique was developed.

A DVP system was set up as described by Schoene and Steinhanses (1989). A fused silica capillary tube with ID=0.18mm was obtained from Alltech Ass.. The length for the toluene and chloroform tubes were 5.0 and 7.5cm respectively. They were held at 24.9±0.1°C and a total pressure of 256kPa was measured. Concentrations of 11.8ng/l toluene and 89.6ng/l chloroform are calculated from the formulas

$$m = D \cdot M \cdot \left(\frac{P_t}{R.T}\right) \cdot \frac{A}{l} \cdot \ln\left(\frac{P_t}{P_t - P_s}\right)$$

and

$$D = \left(\frac{b_o}{P_t}\right) \cdot T^{3/2} \cdot \frac{\left(\frac{1}{M} + \frac{1}{M_a}\right)^{0.5}}{\left(V^{1/3} + V_a^{1/3}\right)^2}$$

with D=diffusion coefficient (cm^2/min), M=molecular mass (g/mol), P_t=total pressure at the top of the capillary tube (kPa), R=gas constant (8314.46ml.kPa/mol.K), T=temperature (K), A=cross section of the capillary tube (cm^2), l=length of the capillary tube (cm), P_s=vapour pressure of the VOC (kPa), b_o=26.133, M_a=average molecular mass of air (29g/mol), V=molar volume of the VOC at boiling point (ml/mol) and V_a=molar volume of air at boiling point (29.9ml/mol).

For the closed two-phase system (CTS) calibration technique, 4.5 to 19µl of the VOCs were added to 25ml methanol. From this solution 40µl was added to 20ml deionized water in a 118ml glass bottle. Immediately after the addition, the bottle was sealed with a mininert valve (Alltech Ass.). This closed two-phase system (CTS) was incubated upside down in a thermostatic water bath at 25.0±0.1°C during at least 3 hours. Knowing the mass M added,

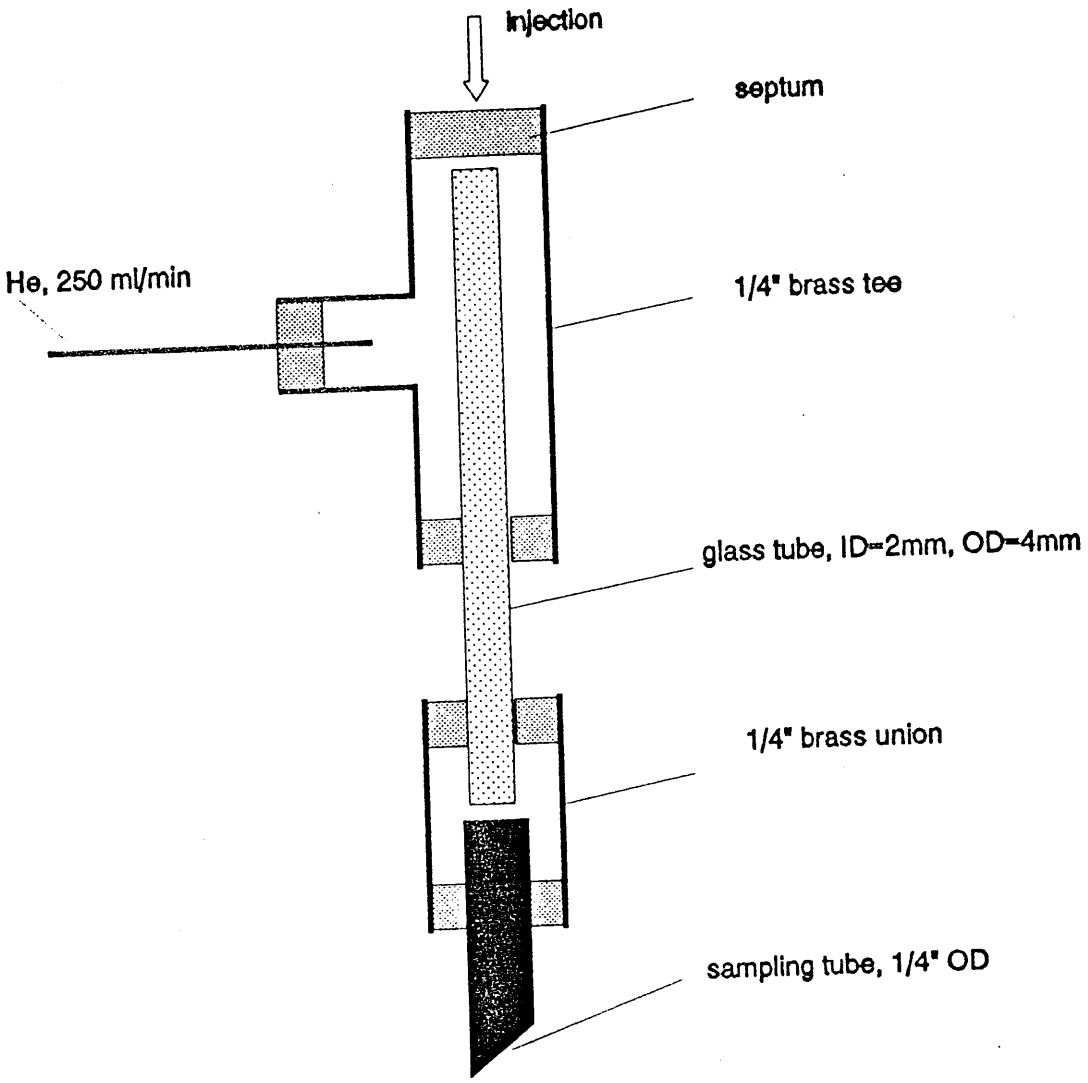

Figure II.1.3. Injection system

the gas and liquid volumes V_g and V_w, and Henry's law constant H (Dewulf et al., 1995), the headspace concentration C_g is calculated from the mass balance:

$$C_g = \frac{M}{V_g + V_w / H}$$

With a gastight syringe (Hamilton Gastight 1750, 500µl) 250µl headspace from the CTS was injected on the adsorption tube via an injection system as illustrated in Figure II.1.3. After injection, the gas stream (250ml/min) was held on for 2.5 minutes.

In order to check the accuracy and the reproducibility of the calibration masses of the CTS and the DVP method, a gas chromatograph (GC) Varian model 3700 equipped with a thermal desorption unit was used (Van Langenhove et al., 1982). The calibration masses could be verified versus liquid injection since this analytical instrument functions as well for thermal desorption of 750mg Tenax TA tubes as for direct liquid injection (Van Langenhove et al., 1982). The GC operated with an injector temperature of 220°C and a detector (FID) temperature of 250°C. Separation of the compounds was done on a RSL 150 fused silica column (30m, film thickness 5µm, ID 0.53mm) and integration by an HP3388A integrator. During analysis the GC oven temperature was kept at 50°C for five minutes, then followed by heating till 200°C at a rate of 7°C/min. He-carrier gas flow rate was 2 mL/min, the FID was fed with air at 400 mL/min and H_2 at 40 mL/min.

II.1.2.5.2.2. Calibration of the samples

For the calibration of the samples a CTS with toluene-d8 was prepared in a same way as described in the previous paragraph, starting with 9µl toluene-d8. In order to know the headspace concentration at equilibrium the dimensionless Henry's law constant for toluene-d8 was determined with the modified EPICS-method (equilibrium partitioning in closed systems) (Dewulf et al., 1995) as 0.183±0.005 (n=9). A second CTS contained all target compounds so that the masses in 250µl headspace corresponded with 2.2 - 4.1µg.m^{-3} (645 till 698pptv) in 6l samples (Table II.1.8.).
On tubes which were used for sampling, 250µl headspace from the first system was injected. For calibration tubes, headspace from both systems was injected on blank tubes.

Table II.1.8. Volumes V (nl) of VOCs brought in the CTS, density d (g/cm^3), Henry's law constant H at 25°C (dimensionless), mass m in 250µl headspace (ng) and corresponding concentration C in 6l (µg.m^{-3})

VOC	V	d	H*	m	C
chloroform	12.0	1.490	0.153	19.54	3.26
tetrachloromethane	7.2	1.593	1.048	24.49	4.08
1,1-dichloroethane	11.2	1.174	0.206	16.85	2.81
1,2-dichloroethane	30.4	1.253	0.0412	16.32	2.72
1,1,1-trichloroethane	8.0	1.310	0.608	20.02	3.34
trichloroethylene	8.8	1.464	0.351	20.78	3.46
tetrachloroethylene	8.8	1.622	0.601	27.18	4.53
benzene	12.0	0.879	0.194	13.11	2.19
toluene	13.6	0.867	0.224	15.74	2.62
ethylbenzene	14.4	0.867	0.270	18.14	3.02
o-xylene	17.6	0.881	0.173	18.15	3.02
m/p-xylene	14.4	0.863	0.248	17.39	2.90
toluene-d8	14.4	0.944	0.183	16.39	2.73

* Data from Dewulf et al. (1995), except for toluene-d8

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II.2. WATER

II.2.1. EVALUATION OF THE PURGE AND TRAP TECHNIQUE

II.2.1.1. EVALUATION OF THE ON-LINE AND OFF-LINE PURGE AND TRAP TECNIQUE

II.2.1.1.1. On-line Purge & Trap

In a first approach the determination of the target compounds by means of the automated on-line purge and trap (P&T) system was evaluated. Working with the on-line system showed unpredictable amounts of mainly chloroform and benzene (levels up to 800 ng/l) and smaller amounts of 1,1,1-trichloroethane, toluene, ethylbenzene and the xylenes. A series of precautions and measures were taken to eliminate this contamination, or at least, to reduce it to a low constant level. Measures to avoid contamination originating from outside the on-line system, were not effective in controlling the contamination. Further on, several parts of the P&T system were replaced or thoroughly cleaned without improvement of the contamination. In our opinion the 8-port switching valve and the automatic controlled open close valves are the source of contamination at this concentration level. Moreover multiple analysis of a water solution with the internal standard (IS) at a 16.7pl/l concentration, revealed that the reproducibility was low (relative standard deviation on integration areas 37.5% (n=5)).
It was proved that the factor affecting this low reproducibility was the water vapour generated by purging the vessel. A gas mixture of about 5ng IS, tetrachloroethylene and ethylbenzene in 0.5ml air was prepared using a closed gas/water system into which an appropiate amount was brought. With the Henry's law constants (Schwarzenbach et al., 1993), equilibrium concentrations in air and water can be calculated. By means of a gas-tight syringe 500µl of this gas mixture was injected into a gas stream of 50ml He/min before it entered the Tenax TA trap in an off-line construction. The reproducibility for sorbing this VOCs out of a (dry) gas stream combined with a desorption in the on-line apparatus (unchanged conditions) showed 9.3, 9.3 and 10.2%SD on the absolute area for the IS, tetrachloroethylene and ethylbenzene respectively (n=4). The analytical problem was localized in the P&T stage, i.e. purging VOCs out of the water vessel and trapping them out of the water-saturated gas stream. Purging VOCs out of water is not expected to be the cause because it is governed by first order kinetics based on the mass balance $-V.dC_w/dt$

= $Q.C_g$, where V is the purged water volume (l), t time (min) C_w and C_g are the water and gas concentrations (mol/l) and Q is the gas flux rate (l/min) (Mackay et al., 1979; Lin et al., 1993). Assuming the gas-water equilibrium, C_g is $H.C_w$ (H Henry's law constant, dimensionless). So the reproducibility was thought to be dependent on the sorption (trapping), which could be affected by the water vapour.

II.2.1.1.2. Off-line Purge & Trap

The interference of the water vapour with the sorption of the VOCs could be solved by using wet traps which were more effective than the trap in the automatic system, which was only based on adsorption on a metal surface. The existing methods are condensing the water vapour (Plass et al., 1991) and inserting water adsorbing materials between the sparging vessel and the adsorbent trap (Krysell and Nightingale, 1994).

In a first off-line P&T construction a spiral condensor was installed as wet trap. Ethylene glycol of -10±1°C as refrigerant was pumped in the condensor (The contact time for the gas stream was 18s). Using this off-line P&T construction with a Vocarb 4000 sorbent trap, combined with thermal desorption in the automatic P&T system, gave a relative area reproducibility against the internal standard for all 13 VOCs between 2.4 (tetrachloroethylene) and 11.9%SD (ethyl benzene) (n=4).

Although the reproducibility of the quantitation was under control, the peaks at the chromatographic time window of chloroform till benzene were broad and split. When ion 17 and 18 were monitored it was clear that a large amount of water entered the MS-detector at the same time window. The water peak was broad and asymmetric. The fronting peak started at time 14min. and lasted till 18.5min. with a peak maximum at 18min. The peak shapes of the VOCs were getting worse during consecutive analyses on the same day.

It became clear that water accumulated in the CDS Peakmaster system, or at least that the evacuation of water was insufficient. When a desorbed trap was desorbed for a second time, a similar amount of water was detected. The accumulated amount of water could be removed by using the 'auto empty' option of the automated P&T system and by removing the on-line sparging vessel. When using the 'auto empty' option a cleaning gas stream is passing via the 8-port switching valve through the wet trap and finally through the on-line vessel entering at its top to remove the analysed water. This is done while the sorbent trap is thermally desorbed. On the other hand the on-line vessel was never completely dry. Since the gas

stream for conditioning the sorbent trap passes first through the on-line vessel (and the wet trap) via the 8-port switching valve, it was removed.

To conclude, the method developed consisted of an off-line P&T step with a purging time of 18 minutes at a purge rate of 50ml/min and using a wet trap consisting of a 3m empty 1/4" stainless steel tube at -11±1°C and a Vocarb 4000 sorbent trap. Next, the Vocarb 4000 trap was desorbed at 250°C/6 min in the automated P&T system and the VOCs were cryofocussed at -150°C. Finally, they were injected by heating the cryogenic focuser up to 260°C during 5 minutes.

II.2.1.2. LIMITS OF DETECTION

The limits of detection (LODs) (ng/l) of the P&T technique are proportional to the limits of detection of the detector and are inversely proportional with the sample volume V (l) for a given compound. Increasing the volume V lowers the LODs but requires a longer purging time. Using FID-detection, a sample of 870ml and a purge rate of 100ml/min during 30min Plass et al. (1991) analysed C_2-C_4 hydrocarbons with LODs of 0.030 to 0.120ng/l. Even these parameters would be insufficient to purge out MAHs and CHCs because their Henry's constant is considerably lower than the Henry's constant of the C_2-C_4 hydrocarbons ($H \leq 1$ versus H = 20.0, 29.0 and 39.0 for ethane, propane and butane respectively, Schwarzenbach et al., 1993).

Considering the limits of detection of the detector, the H-values of the VOCs, the analysis time and the desired LODs, a sample volume of 60ml was used. In this way LODs for the 13 VOCs, the 2 deuterated surrogates and the IS were calculated by determining the blank results and the signal/noise ration of 3. The blanks were much better under control in the off-line system than in the on-line work. Blanks were measured daily for correction. They were under 0.76 ng/l for all VOCs, except for chloroform (4.46±2.14 (48.0%SD)), for benzene (4.58±1.33 (29.1%SD)) and for toluene (2.59±0.90 (34.7%SD)) (Table II.2.1).

Table II.2.1. Signal/noise 3 ratio's (S/N 3), mean blank values (Blank) (n=9) and limits of detection (LODs) for the analysis of all VOCs, surrogates and the IS in water (ng/l)

Compound	S/N 3	Blank	LOD
chloroform	0.47	4.46	4.93
tetrachloromethane	0.58	0.19	0.77
1,1-dichloroethane	0.49	0.65	1.14
1,2-dichloroethane	0.40	0.70	1.10
1,1,1-trichloroethane	0.47	0.12	0.59
trichloroethylene	0.47	0.04	0.51
tetrachloroethylene	0.30	0.18	0.48
benzene	0.21	4.58	4.79
toluene	0.09	2.59	2.68
ethylbenzene	0.38	0.73	1.11
m/p-xylene	0.27	0.76	1.03
o-xylene	0.63	0.62	1.25
trifluorotoluene	0.36	0.00	0.36
chloroform-d	0.36	0.00	0.36
toluene-d	0.19	0.00	0.19

The amounts of VOCs corresponding to a signal/noise ratio of 3 were determined by analysing a 83.3pl/l solution of all VOCs, surrogates en the IS (Table II.2.1). In this way mean LODs (mean blank + signal/noise 3) from 0.48 (tetrachloroethylene) to 1.25 ng/l (o-xylene) were obtained, except for chloroform (4.93), benzene (4.79) and toluene (2.68) (Table II.2.1).

II.2.1.3. PRECISION AND ACCURACY

The precision of the developed method was checked by analysing 10 times (over a two-week period) a laboratory reference material (LRM). This LRM was made by adding all VOCs and surrogates at a 41.67 pl/l concentration to a matrix being a 30 minutes purged sample. Results shown in Table II.2.2 demonstrate a precision with a standard deviation better than 10% for all VOCs and surrogates except for benzene (11.3%) and 1,2-dichloroethane (15.7%).

Table II.2.2. Measured concentrations (X) and % standard deviation (%SD) of a laboratory reference material and comparison with the expected concentration (X_{exp}) (ng/l)

Compound	X	%SD	X_{exp}	X/X_{exp} (%)
chloroform	61.02	5.7	62.08	98.3
tetrachloromethane	64.75	9.3	66.38	97.6
1,1-dichloroethane	51.65	7.3	48.92	105.6
1,2-dichloroethane	54.74	15.7	52.21	104.9
1,1,1-trichloroethane	54.77	3.9	54.58	100.3
trichloroethylene	64.81	3.5	61.00	106.3
tetrachloroethylene	69.52	2.6	67.58	102.9
benzene	44.87	11.3	36.63	122.5
toluene	45.69	9.3	36.13	126.5
ethylbenzene	43.38	9.1	36.13	120.1
m/p-xylene	38.34	8.4	35.96	106.6
o-xylene	46.69	8.8	36.71	127.2
trifluorotoluene	99.17	0.0	49.58	100.0
chloroform-d	68.47	8.3	62.50	109.6
toluene-d	40.03	6.7	39.33	101.8

The accuracy was assessed by evaluating mean concentrations (X) and the expected concentration X_{exp} (Table II.2.2). The mean X/X_{exp} for all VOCs and surrogates was 109.3±10.4 (9.5%SD, n=14). This slight overestimation is due to the MAHs with X/X_{exp} 117.4±10.7 (9.5%SD, n=6) as compared with 103.2±4.2 (4.1%SD, n=8) for the CHCs.

II.2.1.4. ANALYTICAL QUALITY ASSURANCE

International organizations dealing with marine sciences as the OSPARCOM (Oslo and Paris Commissions, 1990) and the QUASIMEME working group (Quality Assurance of Information in Marine Environmental Monitoring Programmes in Europe) (Topping et al., 1993) propose general procedures to ensure data quality. According to the QUASIMEME guidelines the analytical quality assurance consists of measuring recovery by a standard addition test, a system of quality control and assessment, the use of an appropiate analytical procedure and an evaluation of bias.

II.2.1.4.1. Standard addition test

In order to guarantee the analytical results a standard addition test was done on an environmental sample. A sample from the Scheldt estuary taken in March 1993 was fortified with 16.7pl/l of each VOC and surrogate (laboratory fortified matrix, LFM). For all 13 VOCs and the 2 surrogates a mean recovery of 105.8±11.1% (n=14) for the LFM was found as compared to the concentration expected from the sum of the measured sample results and the added amount (Table II.2.3). So the recovery of stripping VOCs out of a (marine) water matrix is complete. In fact, the recovery is mainly dependent on the possibility of the compounds to be purged out of the matrix. Their high Henry's law constants are even enhanced by the salinity, while the presence of dissolved organic matter is of no importance with respect to the air-water partitioning of these VOCs (Dewulf et al., 1995).

Table II.2.3. Standard addition test with analysis of a laboratory fortified matrix (LFM) in comparison with the analysis of the original matrix (sample) and the added amount (ng/l)

	LFM	Sample	added amount	sample +added amount	recovery(%) (LFM/sample+added)
chloroform	64.12	42.60	24.83	67.43	95.1
tetrachloromethane	32.63	3.04	26.55	29.59	110.3
1,1-dichloroethane	25.82	6.93	19.57	26.50	97.4
1,2-dichloroethane	70.56	47.97	20.88	68.85	102.5
1,1,1-trichloroethane	136.84	101.55	21.83	123.38	110.9
trichloroethylene	83.46	54.65	24.40	79.05	105.6
tetrachloroethylene	86.51	52.87	27.03	79.90	108.3
benzene	39.89	21.02	14.65	35.67	111.8
toluene	67.06	46.36	14.45	60.81	110.3
ethylbenzene	59.13	36.92	14.45	51.37	115.1
m/p-xylene	44.98	22.95	14.38	37.33	120.5
o-xylene	43.67	21.00	14.68	35.68	122.4
trifluorotoluene	99.17	99.17	0.00	99.17	100.0
chloroform-d	130.2	105.21	25.00	130.21	100.0
toluene-d	83.43	59.11	15.73	74.84	111.5

II.2.1.4.2. Analytical quality control charts

The method involving the use of analytical control charts (AQQCs) as an instrument to assess the analytical performance is already discussed previously (*see* I.1.1.1.4.2.). Laboratory reference material (LRM) was analysed 10 times spread at random over a two week period to construct an AQCC with X the mean measured concentration, with X±2.s the upper and lower warning limits (WL), and with X±3.s the upper and lower control limits (CL) for each VOC and surrogate, as illustrated in Figure II.2.1 for all VOCs. Of the 140 obtained data 135 values fell within the warning limits (96.4%) while 95.0% is statistically expected. Four points, one of tetrachloroethylene, benzene, ethylbenzene and chloroform-d, all in different analyses, fell between the warning and control limits so that 99.3% of the data fell within the control limits (99.7% statistically expected). One point fell out of the upper control limit. This was due to the use of a matrix for making the LRM which was insufficiently blank even after 30 minutes purging because the matrix was of a sampling point containing >10000ng/l chloroform. To conclude, these results are of an acceptable quality according to the QUASIMEME guidelines.

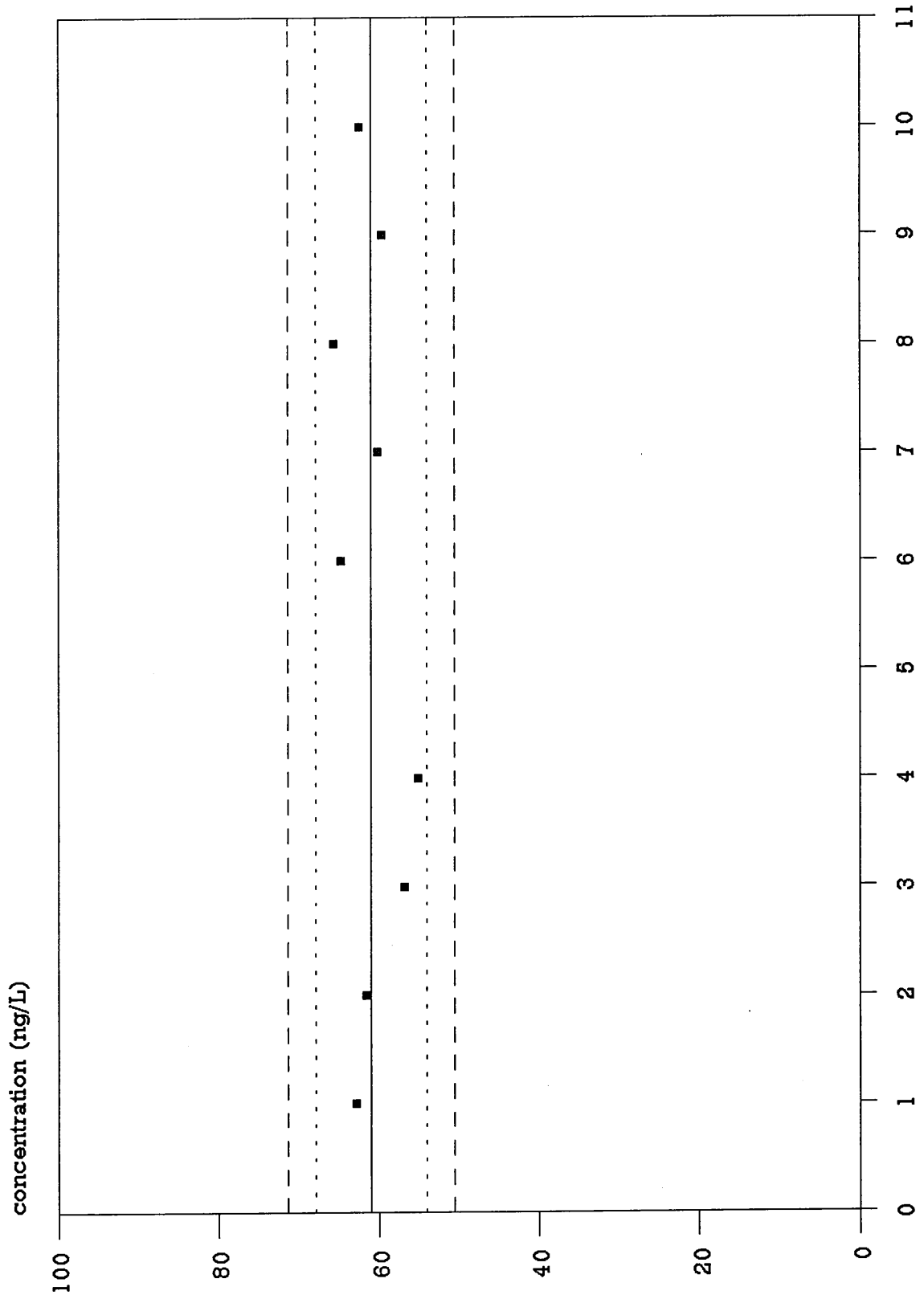

Figure II.2.1.a. AQQC chloroform. Full line: mean x, dotted line x+/-2s, broken line x+/-3s

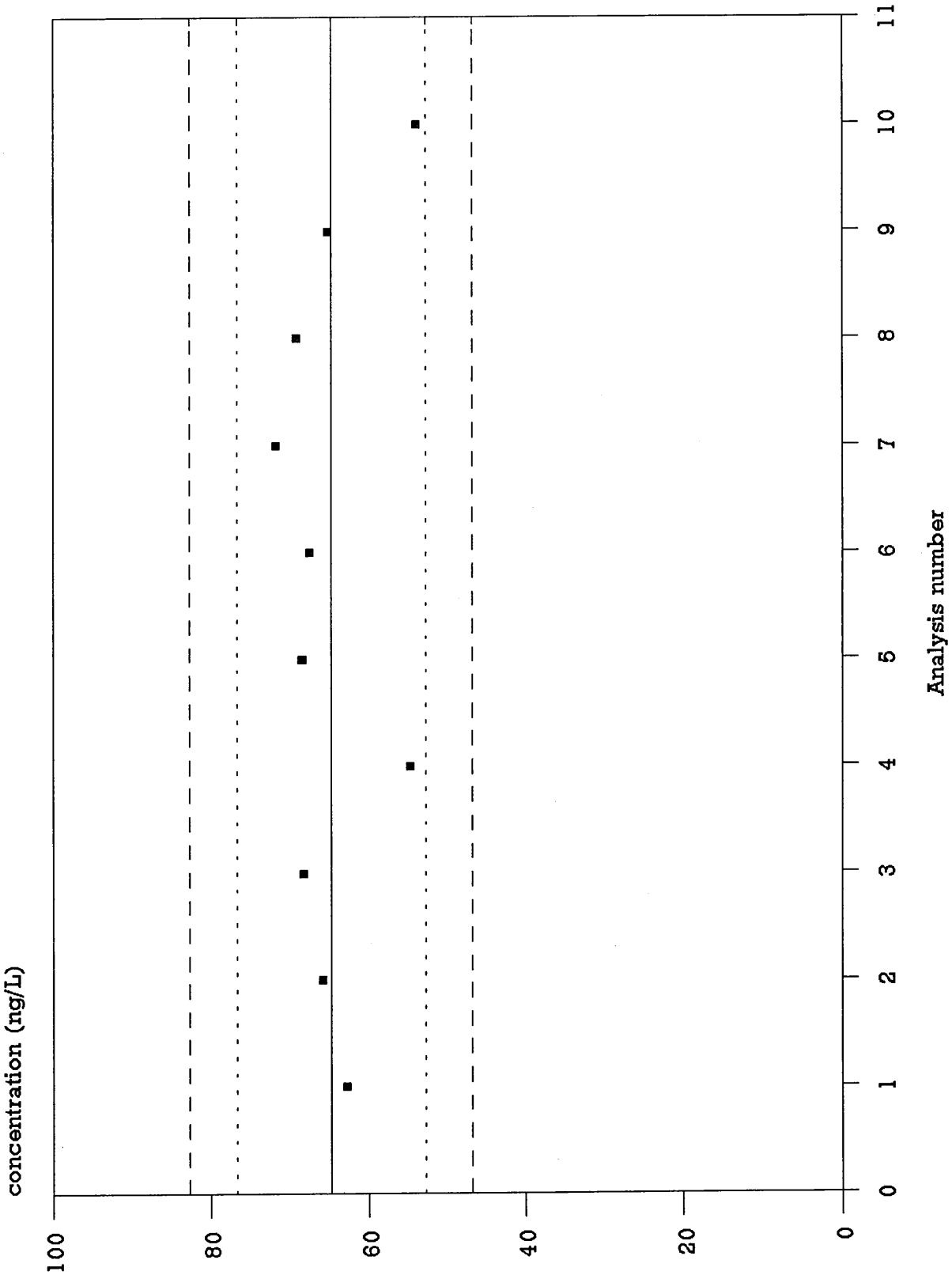

Figure II.2.1.b. AQQC tetrachloromethane.Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

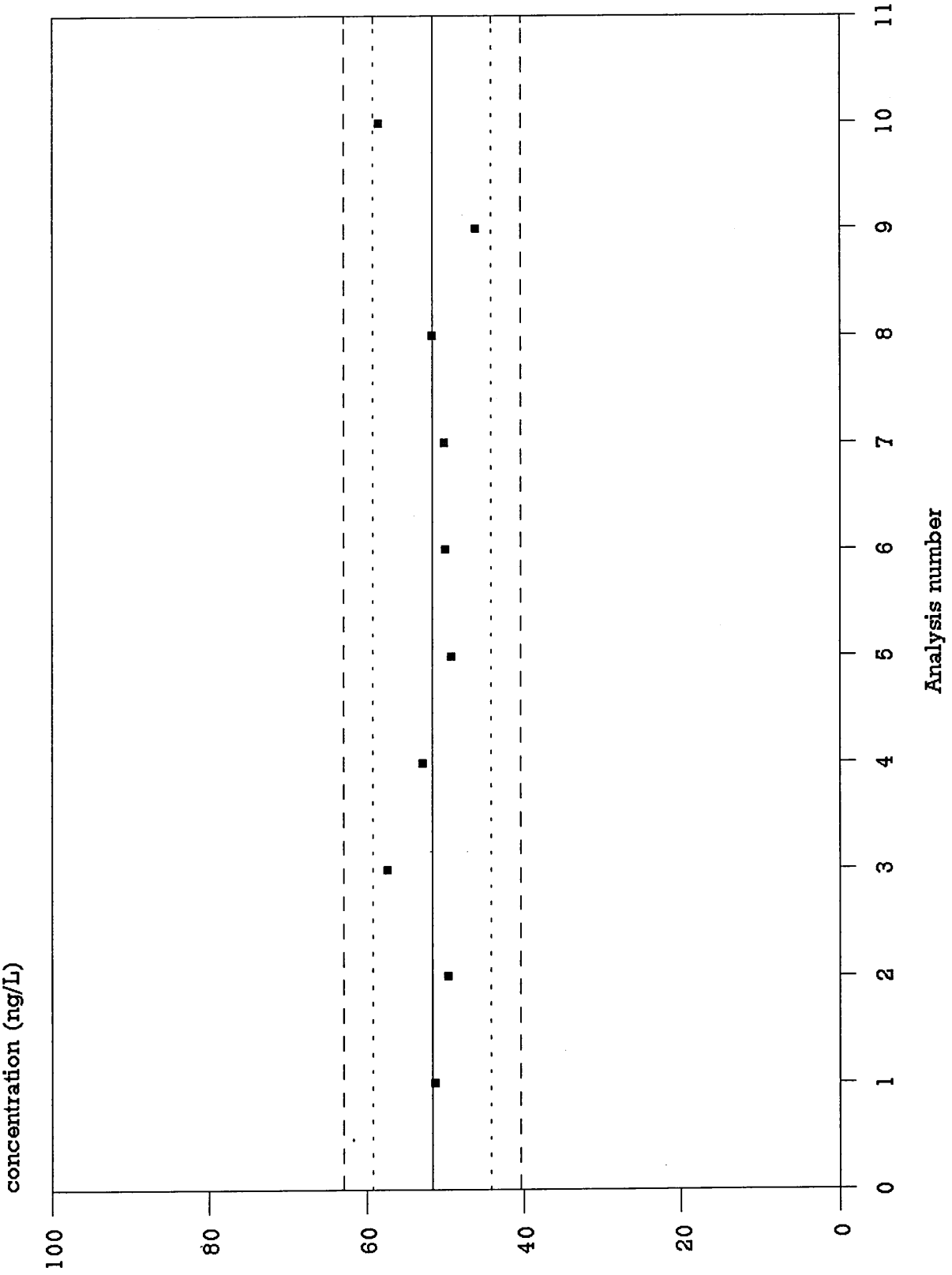

Figure II.2.1.c. AQQC 1,1-dichloromethane. Full line: mean x,dotted line: x+/-2s,broken line: x+/-3s

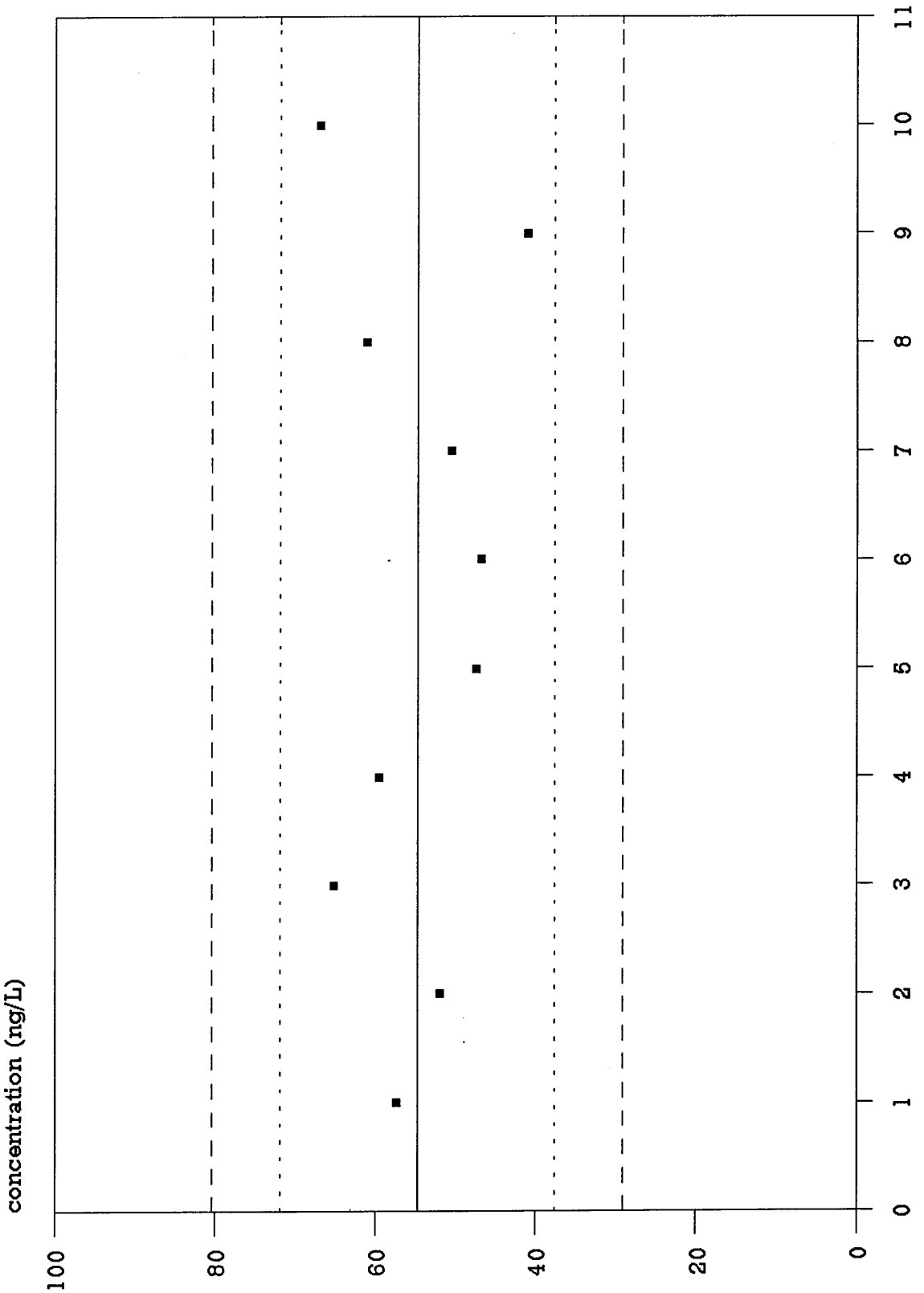

Figure II.2.1.d. AQQC 1,2-dichloromethane: full line: mean x,dotted line: x+/-2s,broken line: x+/-3s

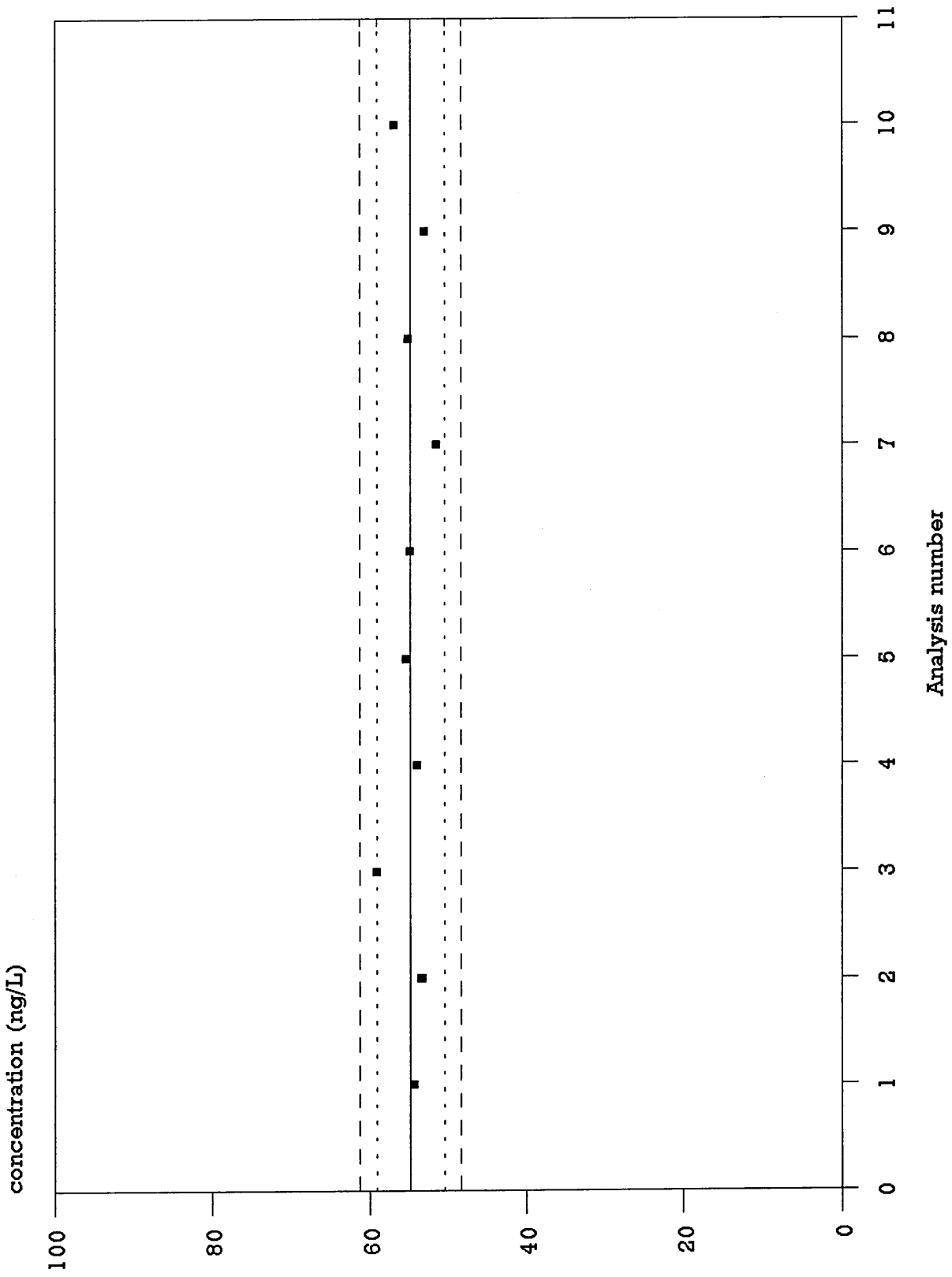

Figure II.2.1.e. AQQC 1,1,1-trichloroethane.Full line:mean x,dotted line: x+/-2s,broken line: x+/-3s

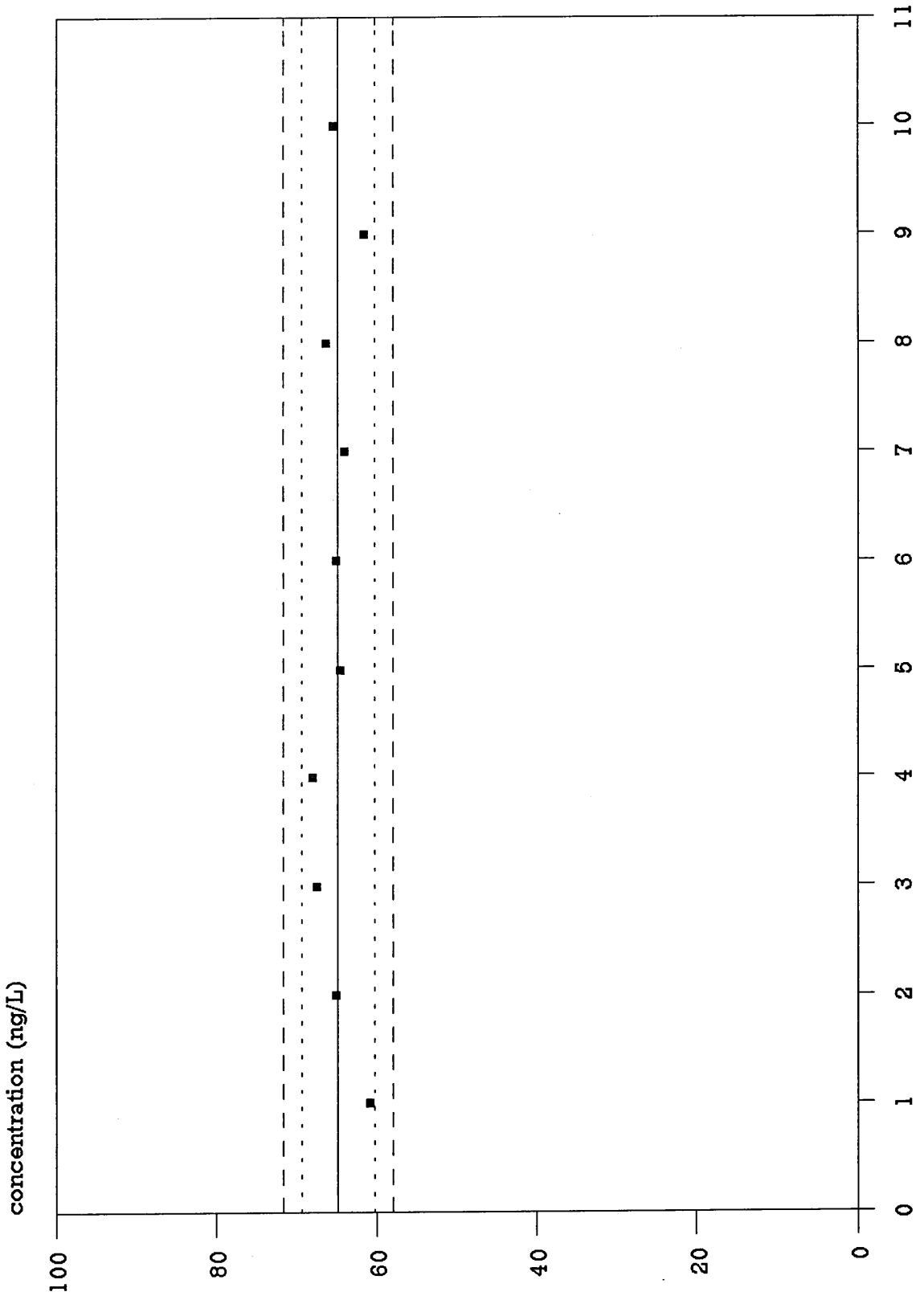

Figure II.2.1.f. AQQC trichloroethylene. Full line: x, dotted line: x+/-2s, broken line: x+/-3s

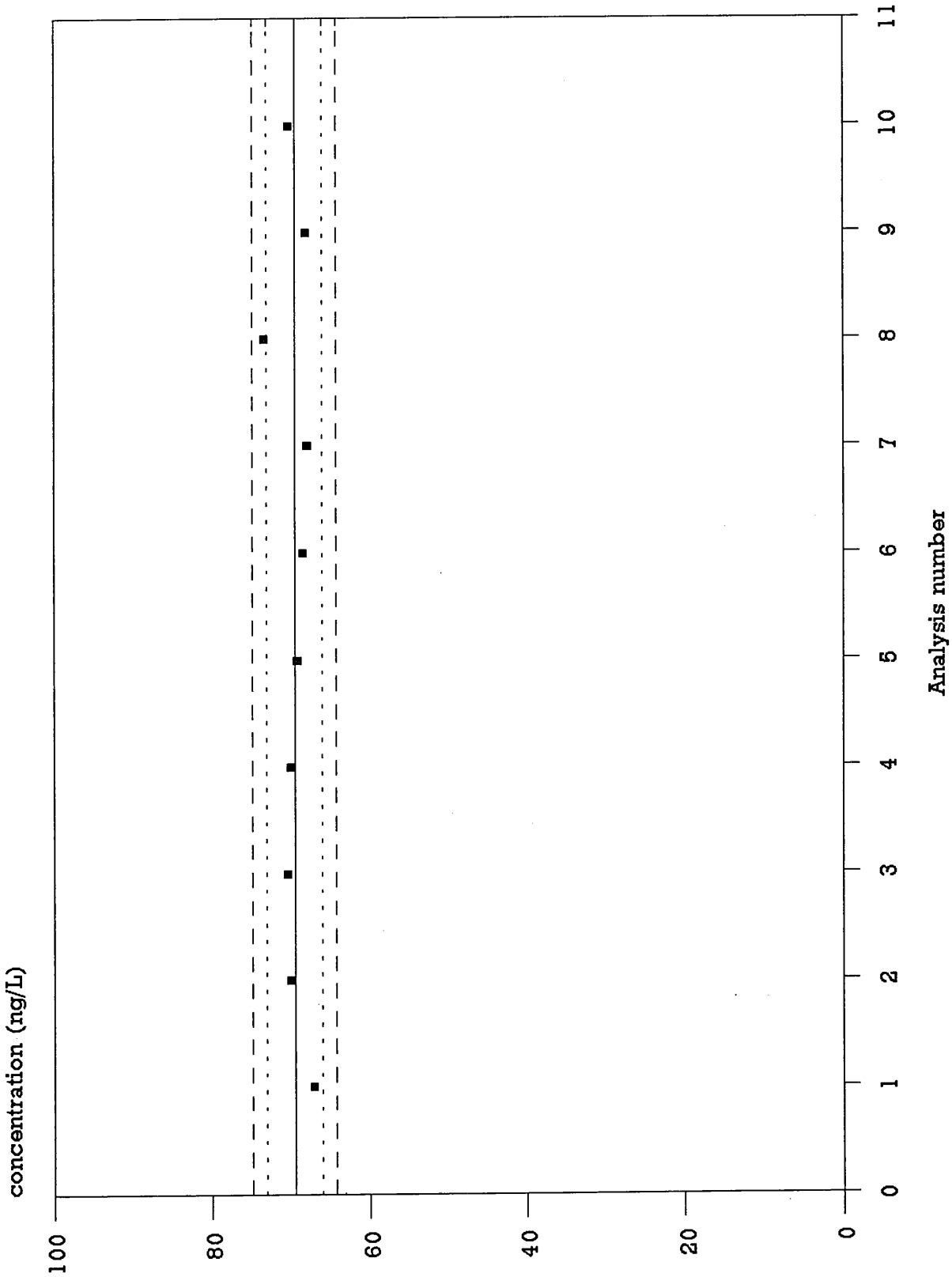

Figure II.2.1. g. AQQC tetrachloroethylene.Full line: mean x,dotted line: x+/-2s,broken line: x+/-3s

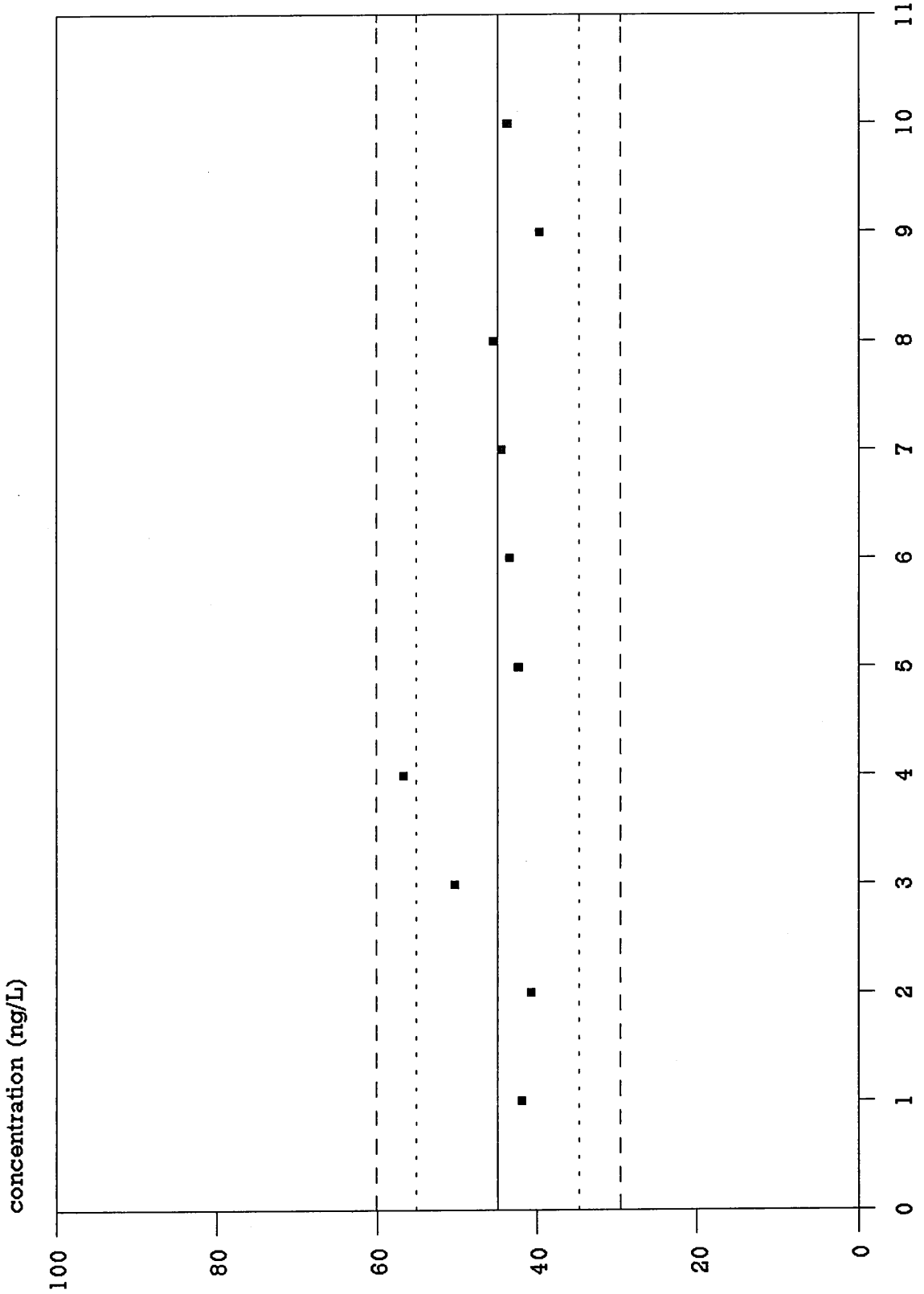

Figure II.2.1.h. AQQC benzene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

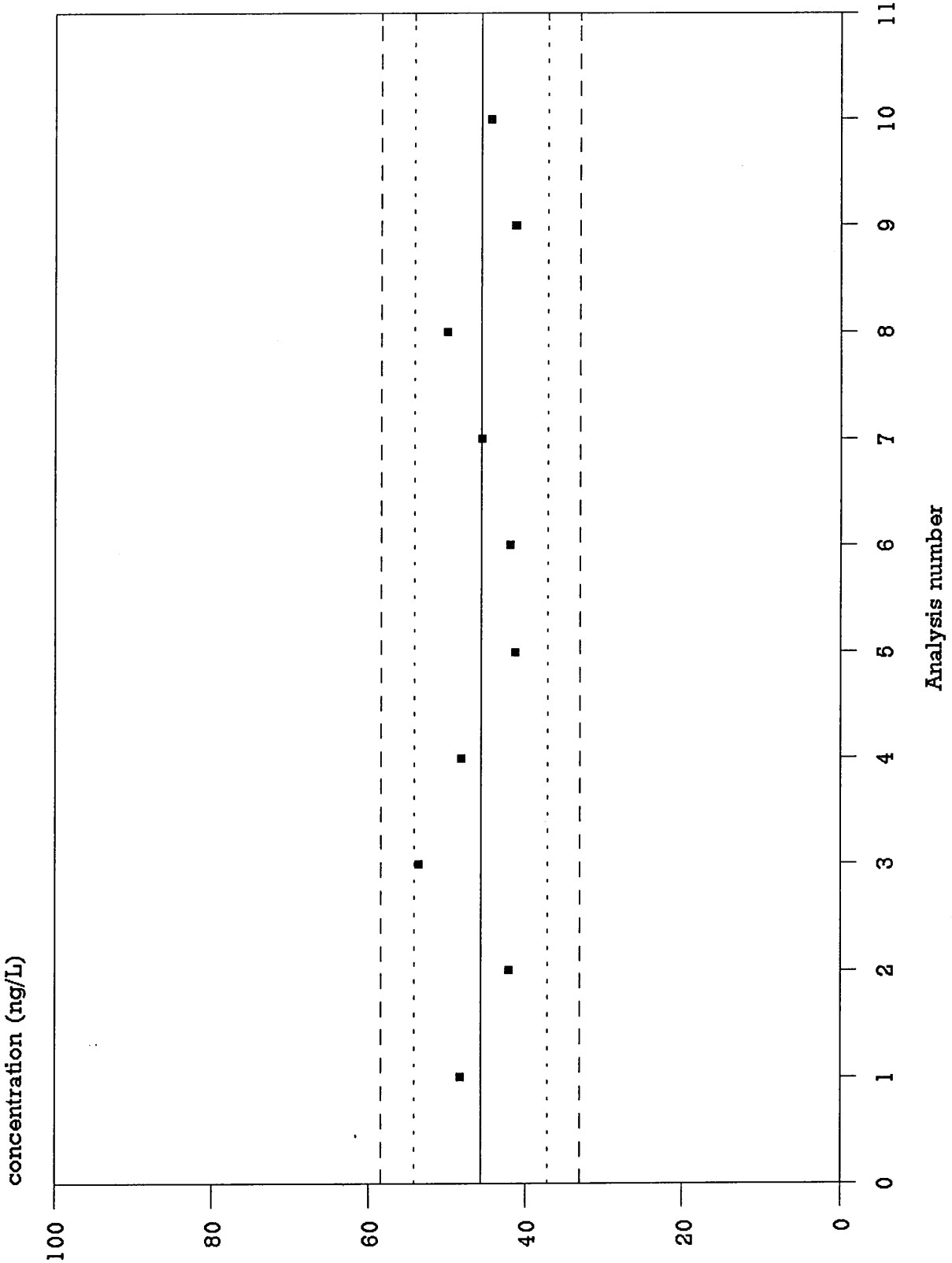

Figure II.2.1.i. AQQC toluene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

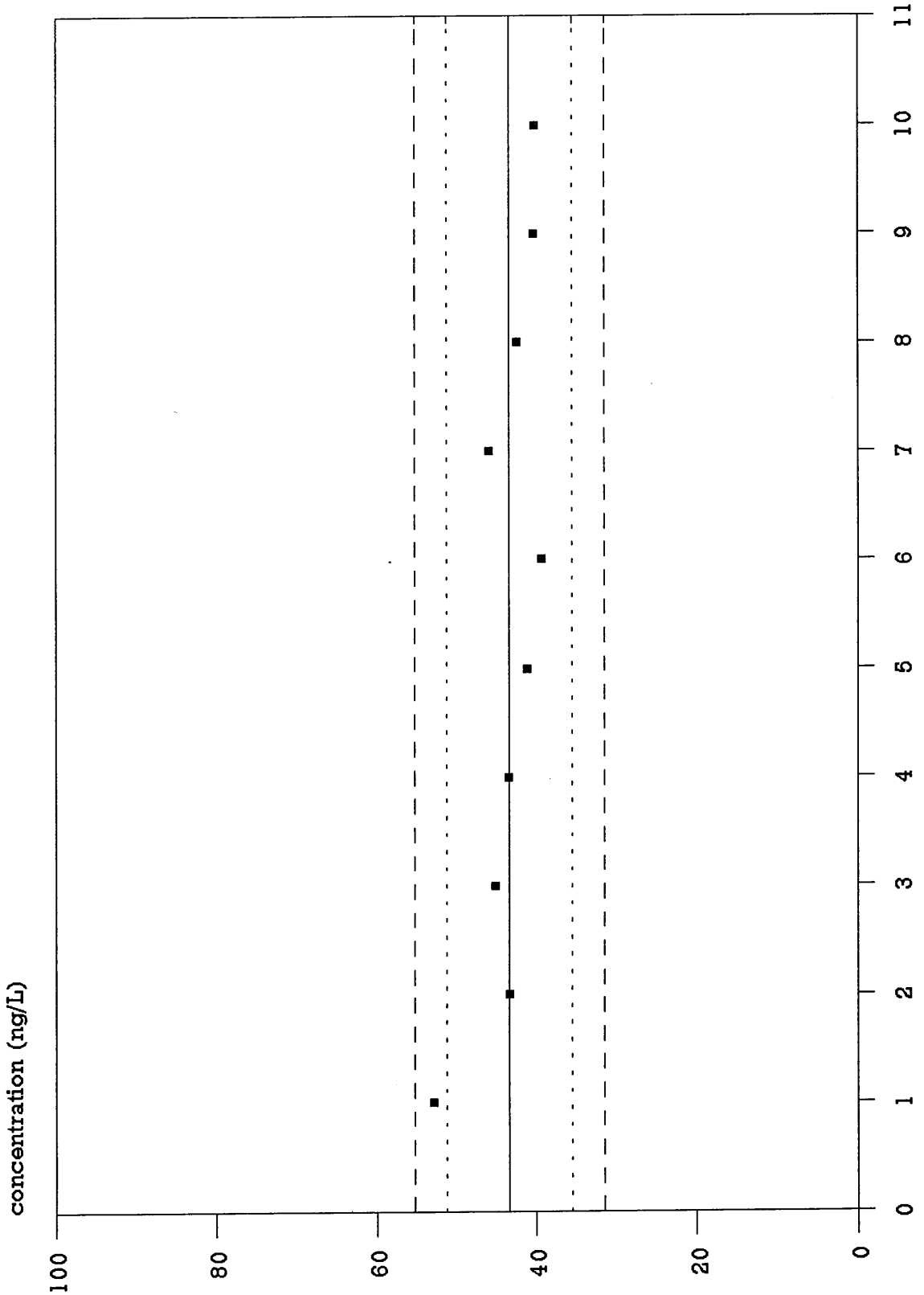

Figure II.2.1.j. AQQC ethylbenzene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

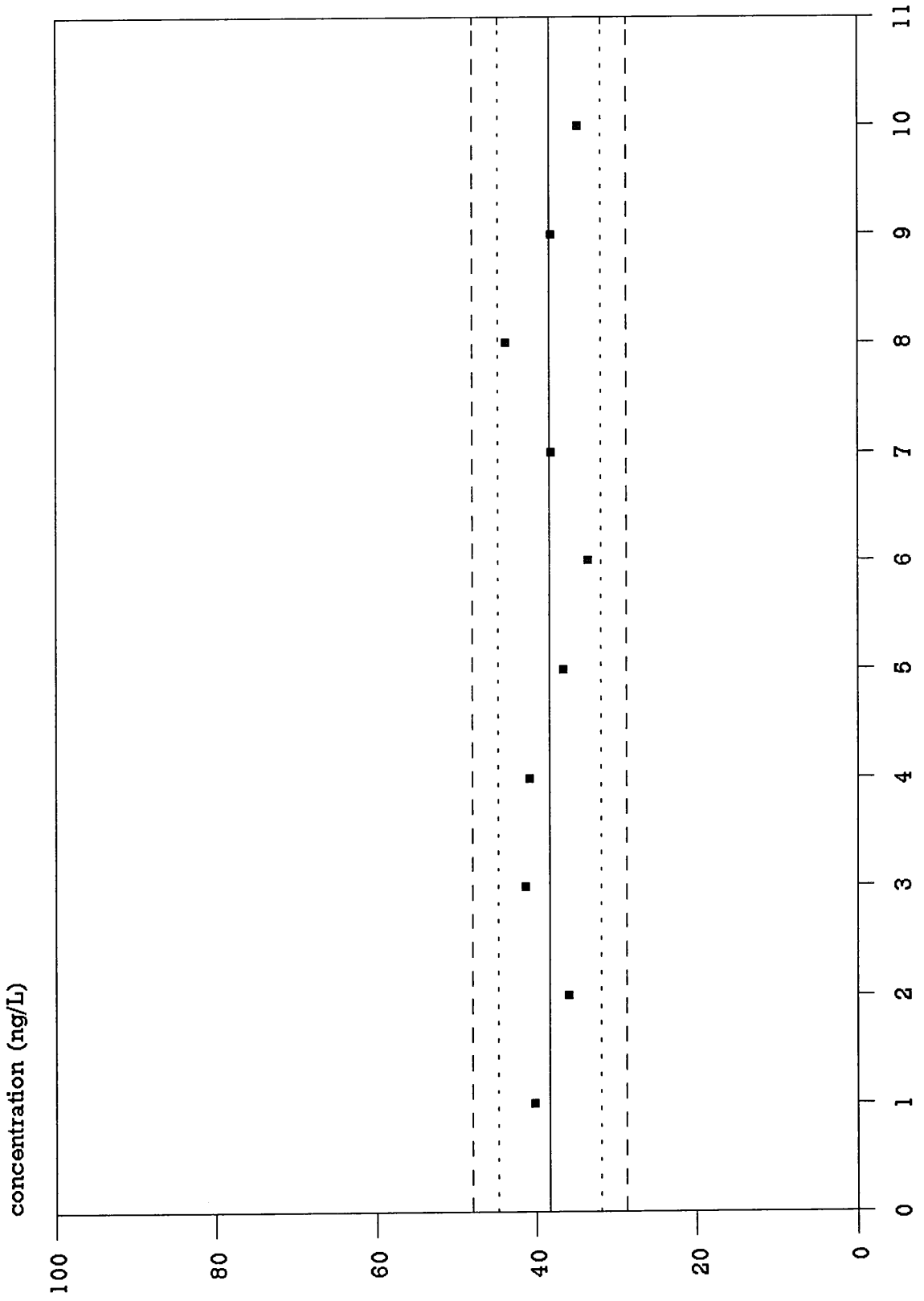

Figure II.2.1.k. AQQC m/p-xylene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

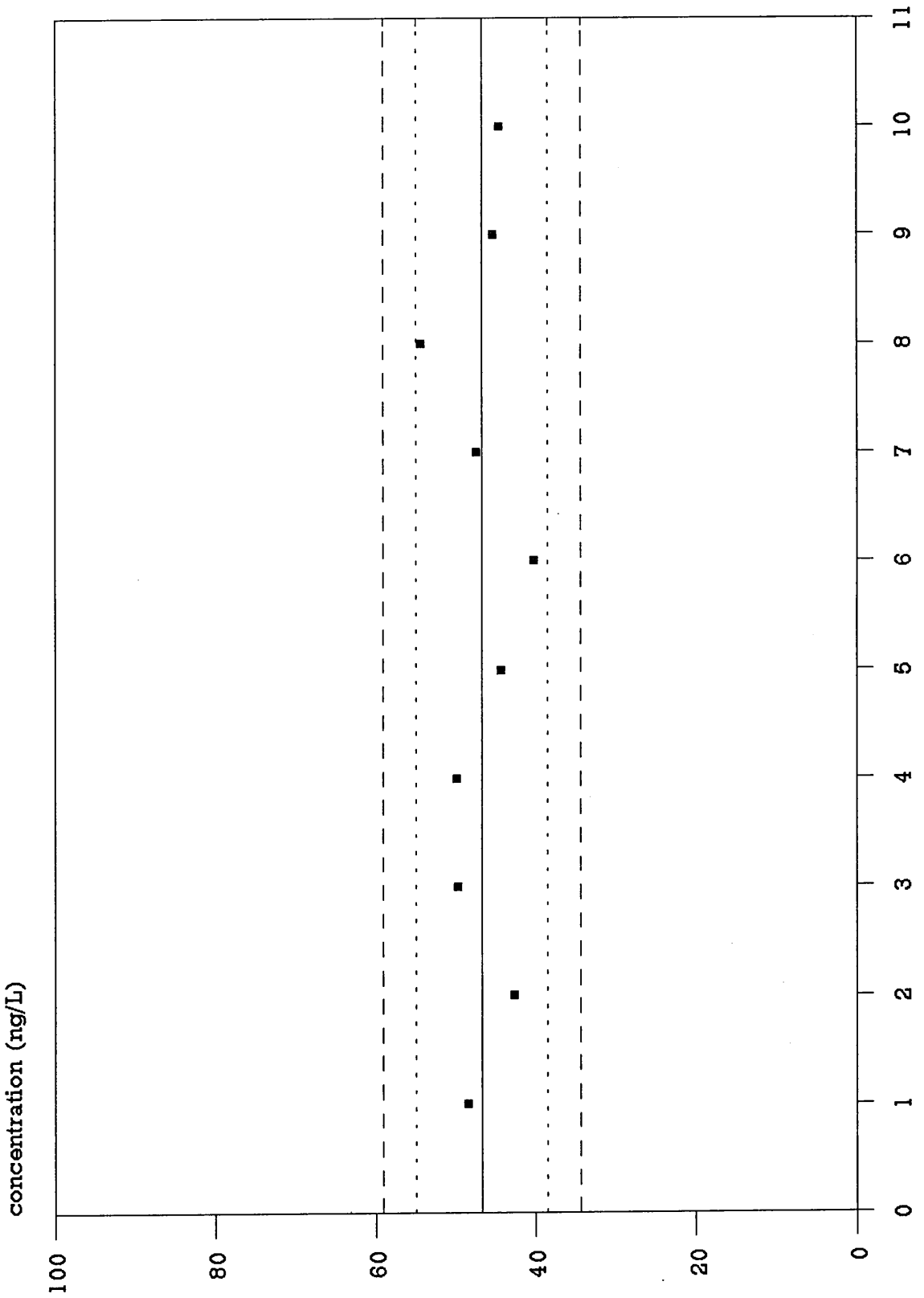

Figure II.2.1.1. AQQC o-xylene. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

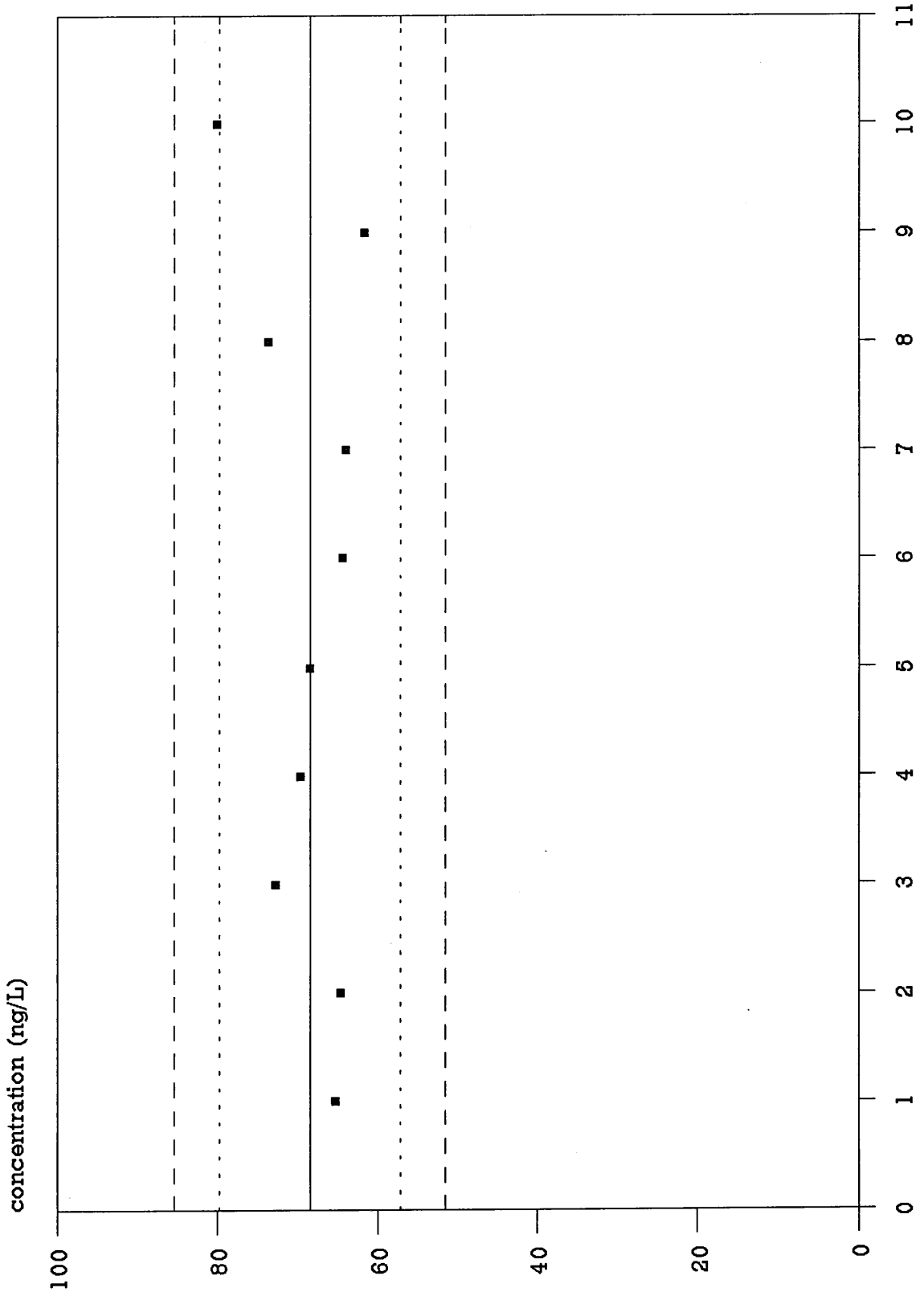

Figure II.2.1.m. AQQC chloroform-d. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

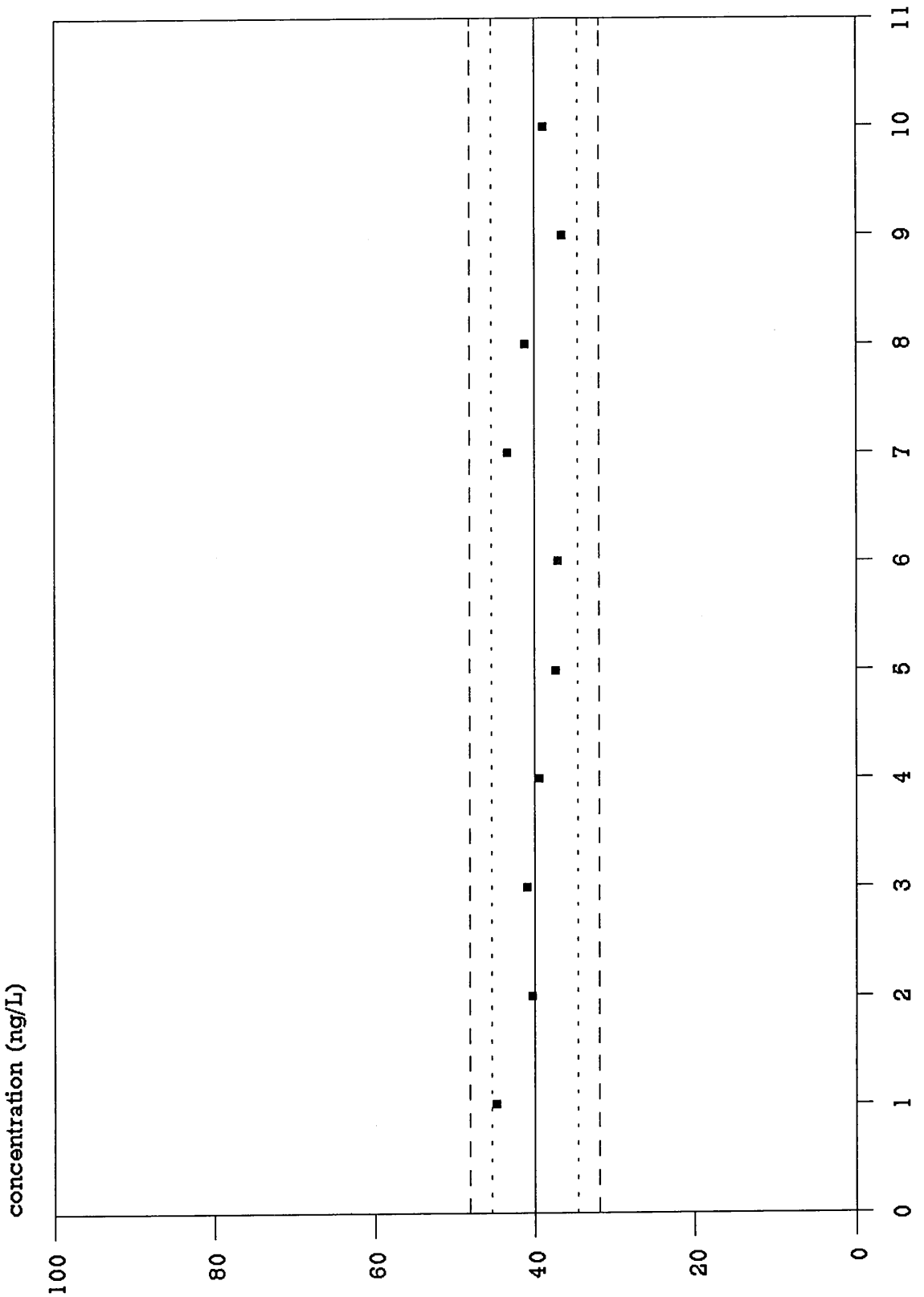

Figure II.2.1.n. AQQC toluene-d8. Full line: mean x, dotted line: x+/-2s, broken line: x+/-3s

II.2.1.5. CONCLUSIONS

This study elaborated the P&T technique to the ng/l level for VOCs with a GC-MS detection system. This means that a wide range of compounds are detectable at this concentration level in the marine environment. The major problems occurring in the application of the on-line automated P&T equipment at this concentration level (reproducability and contamination) were overcome by an off-line P&T stage. In the off-line device the use of a simple constructed wet trap inserted between sparging vessel and adsorbent trap eliminated reproducability problems. The contamination problem in the application of the on-line equipment turned out be originating in the automated P&T apparatus itself during the P&T stage, since it was under control in the off-line P&T work.

The accuracy, precision, limits of detection and the analytical quality on the basis of constructing analytical quality control charts were considered. These checks confirmed the reliability of the method.

The technique was successfully applied in the analysis of VOCs, as well CHCs ás MAHs, in environmental marine samples (*see* Results of the monitoring campaigns). Knowledge of marine concentrations of the latter group is scarce because of shortage of suitable analytical methods applicable in a routine way.

II.2.2. EXPERIMENTAL SECTION

II.2.2.1. METHODS FOR THE DETERMINATION OF VOLATILE COMPOUNDS IN MARINE WATERS

II.2.2.1.1. Liquid-liquid extraction

For C_1- and C_2-halogenated hydrocarbons the liquid-liquid extraction technique is a well developed and accepted analytical method. The procedure consists of a liquid-liquid extraction, gas chromatographic separation and detection by an electron capture detector (ECD). In the seventies the development of the ECD made trace analysis of CHCs possible (Lovelock et al., 1971; Lovelock et al., 1973). The method was further developed (Eklund et al., 1978; Fogelqvist et al., 1982; Fogelqvist, 1985; Klick, 1992; Wallace et al., 1992; Abrahamsson and Ekdahl, 1993) and improved by a mechanized system for extractive sample work-up coupled on-line to an on-column injector (Fogelqvist et al., 1986). This analytical technique is sensitive enough to detect CHCs at marine environmental concentration levels (Fogelqvist et al., 1982; Abrahamsson et al., 1989).

The strength of this liquid-liquid extraction technique is all dependent on the very good sensitivity of the ECD, compared to other commonly used detectors as FID (flame ionization detector) or MSD (mass selective detector). The extraction method is not suitable for the analyses of both CHCs and MAHs in one run at marine environmental concentration levels because of the selectivity of the detector.

II.2.2.1.2. Purge and trap technique

The most widely applied technique for analysing VOCs in water samples is the purge and trap (P&T) technique. A gas stream is stripping the VOCs out of the water sample (Grob and Zürcher, 1976; Grob, 1973; Droszd et al., 1986; Bianchi et al., 1989; Plass et al., 1991; Yamasaki et al., 1992; Eganhouse et al., 1993; Krysell and Nightingale, 1994). The VOCs containing gas stream is subsequently led over a VOCs trap, usually a sorbent trap (in some studies a cryogenic trap is used (Plass et al., 1991; Krysell and Nightingale, 1994)). Between the purge vessel and the VOCs trap a wet trap can be constructed to remove water vapour (Plass et al., 1991; Krysell and Nightingale, 1994). Water vapour can influence the sorption

efficiency or can disturb the detection system. Finally VOCs have to be desorbed of the trap and injected on a GC-detection system. Former analyses (Grob and Zürcher, 1976; Grob, 1973) used solvent desorption of the sorbent trap (activated charcoal). Since this way of desorption limits the detection level and makes determination of compounds eluting in or near the solvent peak impossible, thermal desorption is nowadays commonly used. Out of the several commercially available sorbents, Tenax TA, Tenax GR, silicagel and activated charcoal based sorbents are the most currently used. More recently trapping materials with high retention volumes and low water affinity, like graphitized carbons, activated coal and carbon molecular sieves, are used in series. The P&T method is well accepted and because of this, commercial P&T analytical instruments are available (from Tekmar, CDS, Chrompack, Dynatherm) to automate analyses.

The on-line P&T instrumentation is provided with a microprocessor controlled switching valve in order to integrate on-line the sorbent trap in the purge line as well as in the desorption line to the GC-detection system. The detection limit of the P&T method, coupled with a GC-FID or GC-MS, is of the µg/l level. This is rather high as compared to the liquid-liquid extraction technique combined with ECD. (P&T-GC-ECD combination can be used (Krysell and Nightingale, 1994) but in this case again the range of detectable VOCs is limited.) The lowest reported limits of detection (LODs) with P&T-GC-FID are from 19ng/L (benzene) to 43ng/L (toluene) for benzene, toluene, ethyl benzene and the xylenes (Eganhouse et al., 1993), while Bianchi et al. (1989) reported their developed P&T system to be able to detect VOCs at a 5ng/L level, although no exact LOD values are given.

II.2.2.2. MATERIALS

The CHCs chloroform, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene (Janssen) and tetrachloromethane (Merck), and the MAHs benzene, toluene (Merck), ethylbenzene and m-, p- and o-xylene (Aldrich) were used as VOCs in the experiments. They were applied without further purification.

Deuterated compounds, chloroform-d and toluene-d8 (>99.95 atom % D), used as surrogates were obtained from Fluka. Methanol was obtained from Merck (for Chromatography, 99.8%). As internal calibration standard (IS) α,α,α-trifluorotoluene (Aldrich) was used.

Tenax TA and Vocarb 4000 traps (8.5cm Carbopack C, 10cm Carbopack B, 6cm Carboxen 1000 and 1cm Carboxen 1001) were used as adsorption traps (1/8" OD).

II.2.2.3. APPARATUS

The microprocessor controlled P&T system, CDS Peakmaster (CDS Analytical Instruments, Oxford, USA) was coupled with a gas chromatograph-mass spectrometer (GC-MS) Carlo Erba QMD 1000 (Carlo Erba Instruments, Milan, Italy) by a heated transferline with a cryogenic focuser at the GC injection port. The P&T system was provided with a 60ml vessel, a wet trap and a sorbent trap, and a cartridge desorber. All these parts and also the carrier gas and the transferline were connected to an 8-port switching valve. Separation of the VOCs was done on a RTX-502.2 capillary column (length 60m, ID 0.32mm, film thickness 1.8µm, gas helium, inlet pressure 12psi) (Restek), detection was done using the mass spectrometer in the single ion monitoring (SIM) mode. M- and p-xylene were not separated and were determined together.

The off-line P&T construction consisted of a vessel (internal diameter 3cm, height 12cm) with a glass frit and an injection septum, connected to a stainless steel 1/4" tube (length 1.5 or 3m) submerged in a temperature controlled ethylene glycol bath (-10 to -15°C). The end of this wet trap was connected with the sorbent trap. Helium purge gas was led through a liquid nitrogen trap before entering the purge vessel.

II.2.2.4. SAMPLING AND STORAGE OF THE SAMPLES

Samples were taken on board of the Belgian Oceanographic vessel the 'Belgica'. The sampling sites in the Scheldt estuary and on the Belgian Continental Shelf in the North Sea are already given in Figure II.1.7 and listed in Table II.1.7. In addition to the program, more samples were taken in the Scheldt estuary in 1995. The sample locations are given in Figure II.2.2. and their positions in Table II.2.4.

Samples were taken with a Niskin-Sampling Bottle System (5 or 10l, General Oceanics Inc., Florida) at a sampling depth of 5m. The samples were transferred to 0.75l dark flasks (real volume 780 ml), which were completely filled to avoid headspace. Microbial degradation was prevented by adding 1/1 HCl to obtain a pH level lower than two (Slater and Ho, 1989). Five µl of a methanol solution containing 50pl chloroform-d and toluene-d8 was injected. Flasks were sealed, kept on board at 4.0°C and stored in the laboratory at the same temperature.

Table II.2.4. Coordinates of the sample locations in the Scheldt estuary in the trajectory Vlissingen - Antwerp.

Location	N	E
S01	51 25.00	3 34.20
S04	51 20.70	3 49.50
S07	51 26.20	4 00.00
S09	51 22.20	4 04.70
S12	51 21.90	4 13.50
S15	51 18.80	4 16.40
S15b	51 17.35	4 19.34
S18	51 16.00	4 18.00
S18b	51 15.29	4 19.05
S22	51 13.13	4 23.50

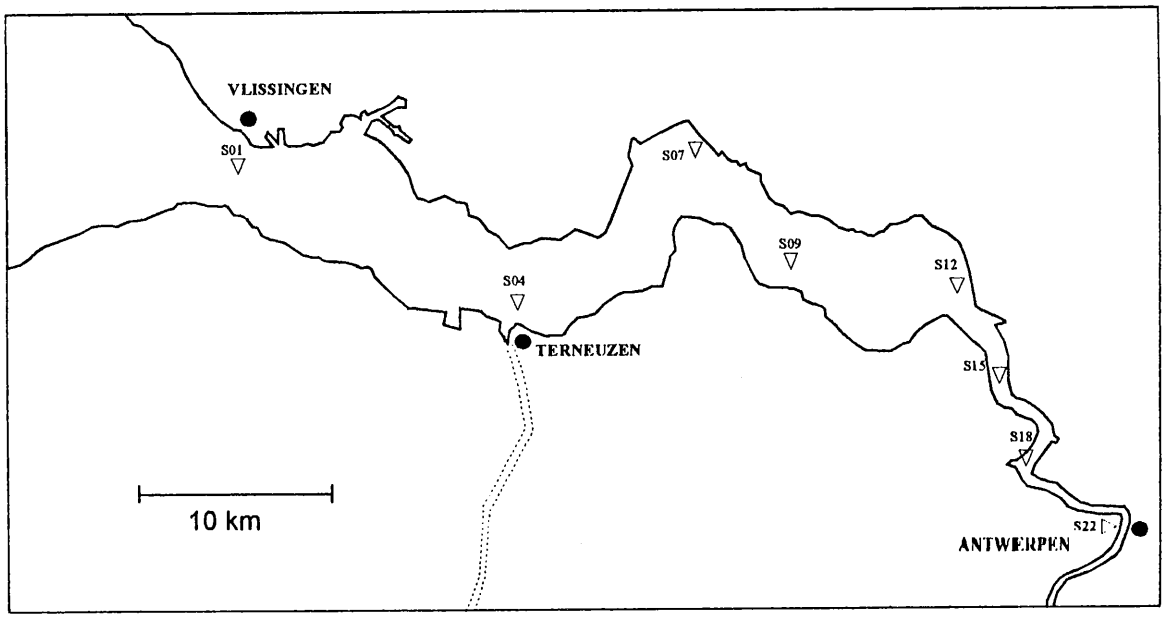

Figure II.2.2. Sampling location in the Scheldt estuary (Antwerp - Vlissingen)

II.2.2.5. ANALYTICAL PROCEDURE

II.2.2.5.1. Preparation of blanks and reference materials

Blanks were prepared by purging sea water for at least 30 minutes at 50ml He/min. Laboratory reference materials (LRMs) and calibration materials were made by adding 5µl methanol stock solution containing the VOCs at the desired concentration.

II.2.2.5.2. Preparation and P&T preconcentration of the sample

60ml water sample was brought into a 100ml syringe. Through the Luer opening of the syringe the IS (5pl) in 5µl methanol was added with a 10µl syringe. In both the on-line and the off-line system, samples were purged during 18min at a rate of 50ml/min. The trapping temperature for the on-line system was 40°C, while for the off-line system the temperature was room temperature.

II.2.2.5.3. Desorption and cryofocussing

In the off-line purge system the trap with sorbed VOCs was replaced in the on-line system for desorption. In some cases the desorption was preceded by a dry purge step in which a cold Helium stream was led over the sorbent trap to flush water from the trap in order to prevent it from entering the GC-MS system. Desorption of VOCs in the off-line as well as in the on-line P&T system, was done in the automated P&T system. Tenax TA traps were desorbed at 180°C during 8 minutes, Vocarb 4000 traps were desorbed during 6 minutes at 250°C. After desorption the trap was conditioned at 200°C for 8 minutes and at 260°C for 4 minutes, for the Tenax and the Vocarb 4000 respectively. The cryofocussing temperature was held at -150°C during the desorption.

II.2.2.5.4. Injection, chromatographic separation and detection

After the desorption the cryogenic focuser was heated to 260°C (rate 800°C/min) and held at this temperature during 5 minutes. Temperature programming of the GC and data acquisition were started simultaneously. Temperature of the GC oven was held at 50°C during

10 minutes, then increased to 190°C at a rate of 10°C/min. The selected ions, the time windows and the limits of detections expressed as signal/noise (S/N) = 3 for the detector are already given in Table II.1.6.

II.2.3. REFERENCES

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II.3. SEDIMENT

II.3.1 GAS-CHROMATOGRAPHY MASS-SPECTROMETRY

II.3.1.1 INTRODUCTION

Gas chromatography (GC) is the method of choice for the analysis of volatile organic compounds. A gas chromatograph coupled to a mass-spectrometer (GC-MS) was therefore selected for the analysis of both CHCs and MAHs. The latter should allow the simultaneous determination of both classes of compounds with sufficient sensitivity (cf. literature overview). Prior to the development of an appropriate method of analysis the GC-MS system was subjected to a number of tests in order to determine the potential and the shortcomings of the system.

II.3.1.2 GAS CHROMATOGRAPHY OF THE TARGET COMPOUNDS

First of all, a GC column was selected that allowed the separation of the target compounds in one analysis. During a first series of tests it became immediately clear that the standard GC-columns (DB-5, 60 m length, 0.25 mm internal diameter (i.d.) and 0.25 µm film thickness (J&W Scientific); DB-1701, 60 m length, 0.25 mm i.d. and 0.25 µm film thickness (J&W Scientific)) used for the determination of other contaminants, such as organochlorine pesticides, provided an insufficient separation of the target compounds. A standard solution was injected on-column into a Carlo Erba Fractovap 4160 gas chromatograph equipped with a flame ionisation detector (FID). Several temperature programs were tested, but the target compounds generally eluted as an unresolved peak at the beginning of the temperature program. The insufficient film thickness was identified as the main cause for this poor resolution. As a result, a Restek speciality column (Rtx-502.2) with a length of 60 m, an internal diameter of 0.32 mm and a film thickness of 1.8 µm was tested under the following conditions:

Injector: cold on column

Detector: FID, 250°C

Make up: O_2, inlet pressure 0.9 kg/cm^2 and H_2, inlet pressure 0.5 kg/cm^2

Carrier: helium, inlet pressure 1.6 kg/cm^2

Temperature program: 40°C for 16 min, from 40 ° C to 200 °C at 4° C/min, hold 3 min.

A second temperature program was tested in order to enhance the separation and the speed of analysis:

Injector: cold on column

Detector: FID, 250°C

Make up: O_2, inlet pressure 0.9 kg/cm^2 and H_2, inlet pressure 0.5 kg/cm^2

Carrier: helium, inlet pressure 1.6 kg/cm^2

Temperature program: 50°C for 10 min, from 50 ° C to 150 °C at 5° C/min, hold 3 min.

This was later changed to the following program:

Injector: cold on column

Detector: FID, 250°C

Make up: O_2, inlet pressure 0.9 kg/cm^2 and H_2, inlet pressure 0.5 kg/cm^2

Carrier: helium, inlet pressure 1.6 kg/cm^2

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The latter program gave the best compromise between speed and separation. Under these conditions, the column allowed the separation of most target compounds with the exception of 1,2-dichloroethane that coelutes with benzene and m-xylene that coelutes with p-xylene (figure II.3.1.). Benzene and 1,2-dichloroethane eluted as a broad distorted peak (figure II.3.2.), while m- and p-xylene eluted as a sharp undistorted but relatively high peak (figure II.3.1.). α,α,α,-Trifluoromethylbenzene was chosen as internal standard and didn't coelute with any of the target compounds (figure II.3.1.). This column was subsequently installed into the GC-MS system in order to test its performance in conditions similar to those described above. An identical standard solution was injected with the SPI (Septum Programmable Injector) under the following conditions:

Injector: SPI, initial temperature: 40 °C for 0.3 min, from 40 ° C to 200 ° C at 180° C /min.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

By using a mass spectrometer as detector, benzene and 1,2-dichloroethane could be individually determined on the basis of selected ion masses (figure II.3.2.) but this was unfortunately not the case for m-xylene and p-xylene. Further analysis of the column types provided by the different manufacturers, at the time, revealed no solution for this problem. The retention times of the target compounds and relative retention times to the internal standard are given in table II.3.1.

Table II.3.1: Retention windows and relative retention times (to the internal standard trifluorotoluene) of the target compounds.

Compound	Retention window (min)	Relative retention time
1,1-Dichloroethane	4:30-4:50	0.56
Chloroform	6:10-6:30	0.76
Trichloroethane	6:40-6:60	0.82
Tetrachloromethane	7:00-7:20	0.86
1,2-Dichloroethane	7:10-7:30	0.87
Benzene	7:10-7:30	0.87
Trichloroethylene	8:00-8:20	0.97
Trifluorotoluene	8:15-8:35	1.00
Toluene	9:45-9:65	1.18
Tetrachloroethylene	10:40-10:60	1.29
Ethylbenzene	12:00-12:20	1.44
m-Xylene	12:05-12:25	1.46
p-Xylene	12:05-12:25	1.46
o-Xylene	12:45-12:65	1.54

II.3.1.3 MASS-SPECTROMETRY OF THE TARGET COMPOUNDS.

Each of the target compounds was individually injected in order to obtain a representative mass spectrum. The mass spectra for the different target compounds are given in figures II.3.3. to II.3.16. The mass spectra were collected with a Magnum Ion trap and the conditions were as follows:

Injector: SPI, initial temperature: 40 °C for 0.3 min, from 40 ° C to 200 ° C at 180° C /min.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 4° C/min, hold 0 min.

The spectra were used to identify selected masses that were to be used for quantification (table II.3.2).

Table II.3.2: Selected ion masses, detection (LOD) and quantification limits (LOQ) of the target compounds.

Compound	Selected ion masses	LOD (pg)	LOQ (pg)
1,1-Dichloroethane	63,64	3	11
Chloroform	83,85	26	85
Trichloroethane	61,97,99	19	62
Tetrachloromethane	117,119	8	27
1,2-Dichloroethane	62	3	9
Benzene	78	3	9
Trichloroethylene	60,130	6	21
Toluene	91	3	11
Tetrachloroethylene	94,129,166	8	27
Ethylbenzene	91,105	3	8
m-Xylene	91,106	3	11
p-Xylene	91,106	3	11
o-Xylene	91,106	3	11

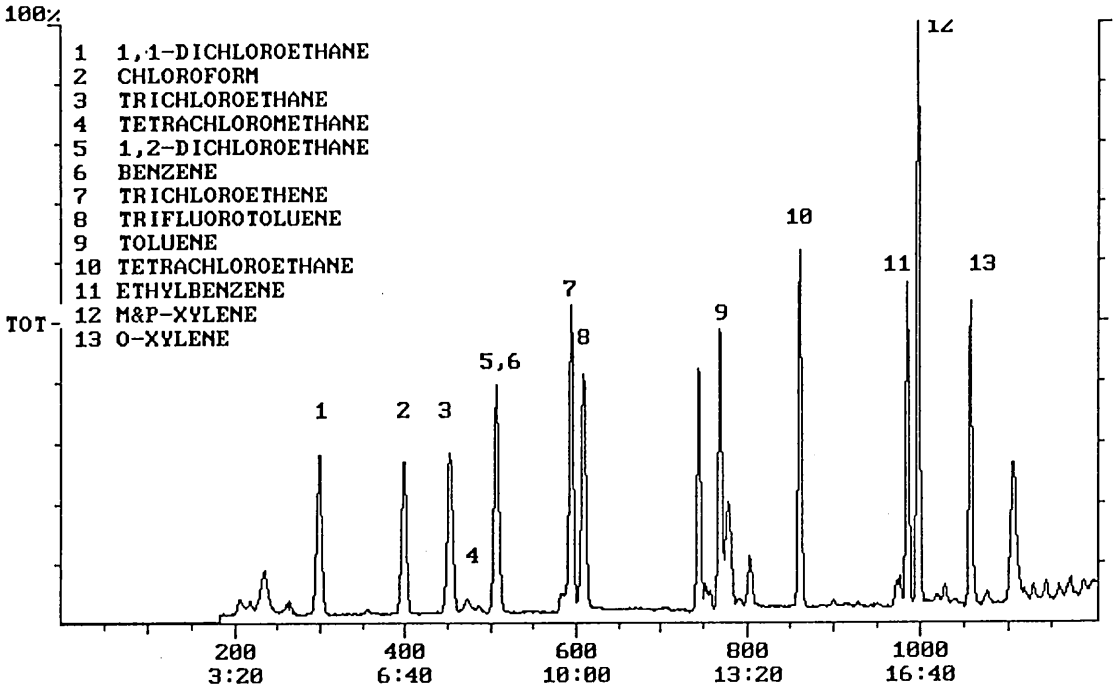


Figure II.3.1: Chromatogram of the target compounds and the internal standard on a Restek Rtx-502.2 with a length of 60 m, an internal diameter of 0.32 mm and a film thickness of 1.8 µm, x-axis: retention time and scan number, y-axis relative height to total ion count.

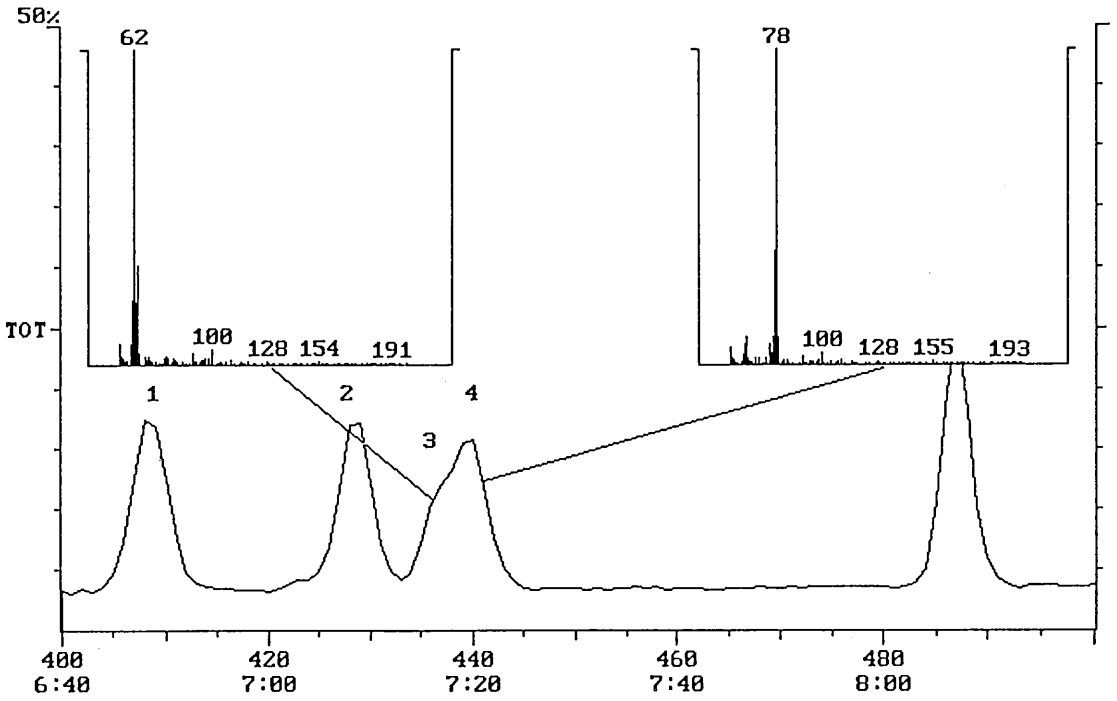


Figure II.3.2: Peak distortion as result of the incomplete separation of benzene and 1,2-dichloroethane on the Restek Rtx-502.2 (60 m, i.d. 0.32 mm and film thickness 1.8 µm), 1 = 1,1,1-trichloroethane, 2 = tetrachloromethane, 3 = 1,2-dichloroethane and 4 = benzene, left insert = mass spectrum of 1,2-dichloroethane, right insert = mass spectrum of benzene, x-axis: retention time and scan number, y-axis relative height to total ion count.

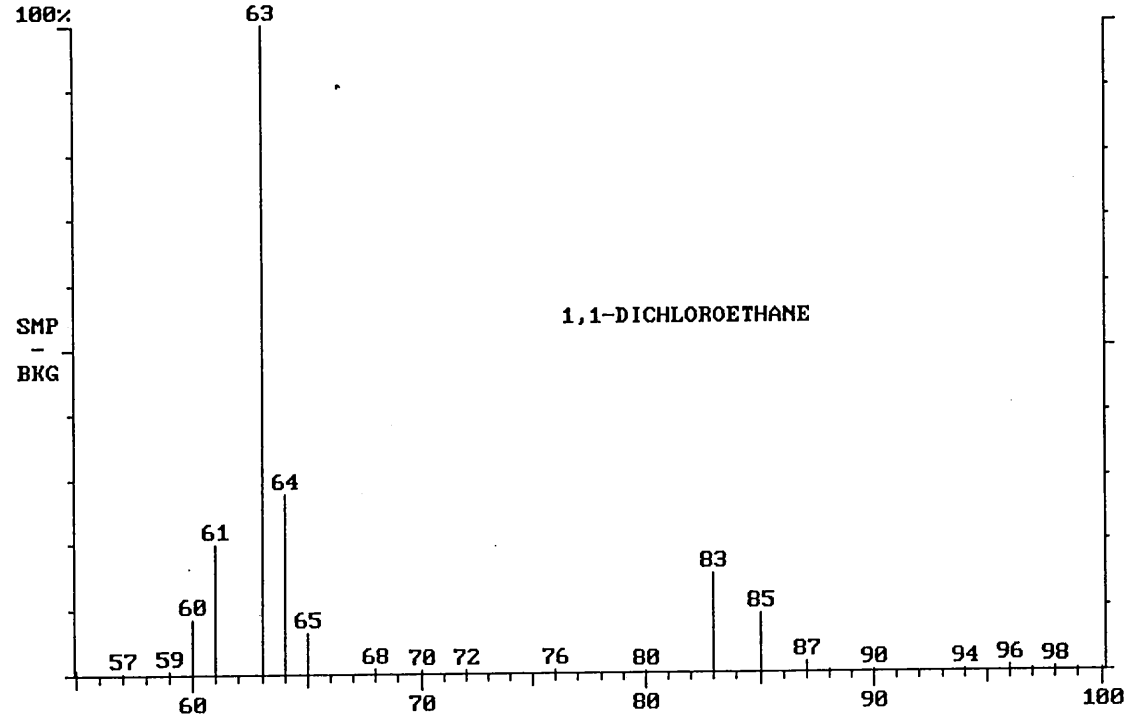


Figure II.3.3: Mass spectrum of 1,1-dichloroethane, x-axis: mass number, y-axis relative height to total ion count.

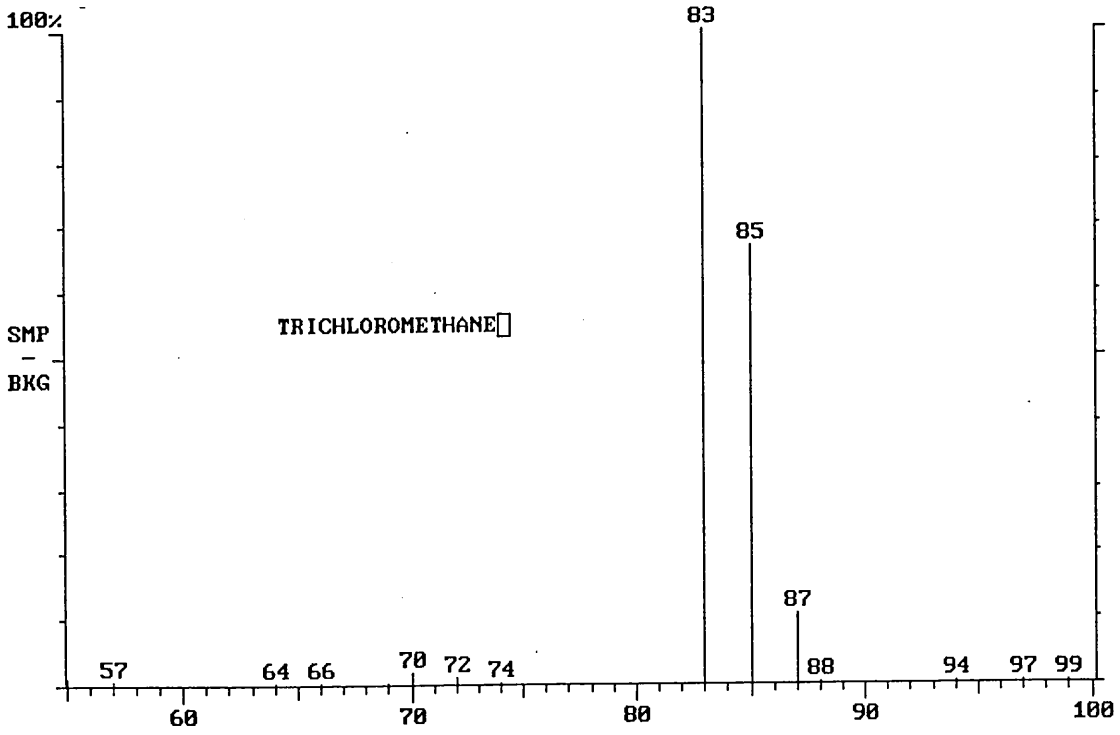


Figure II.3.4: Mass spectrum of chloroform (trichloromethane), x-axis: mass number, y-axis relative height to total ion count.

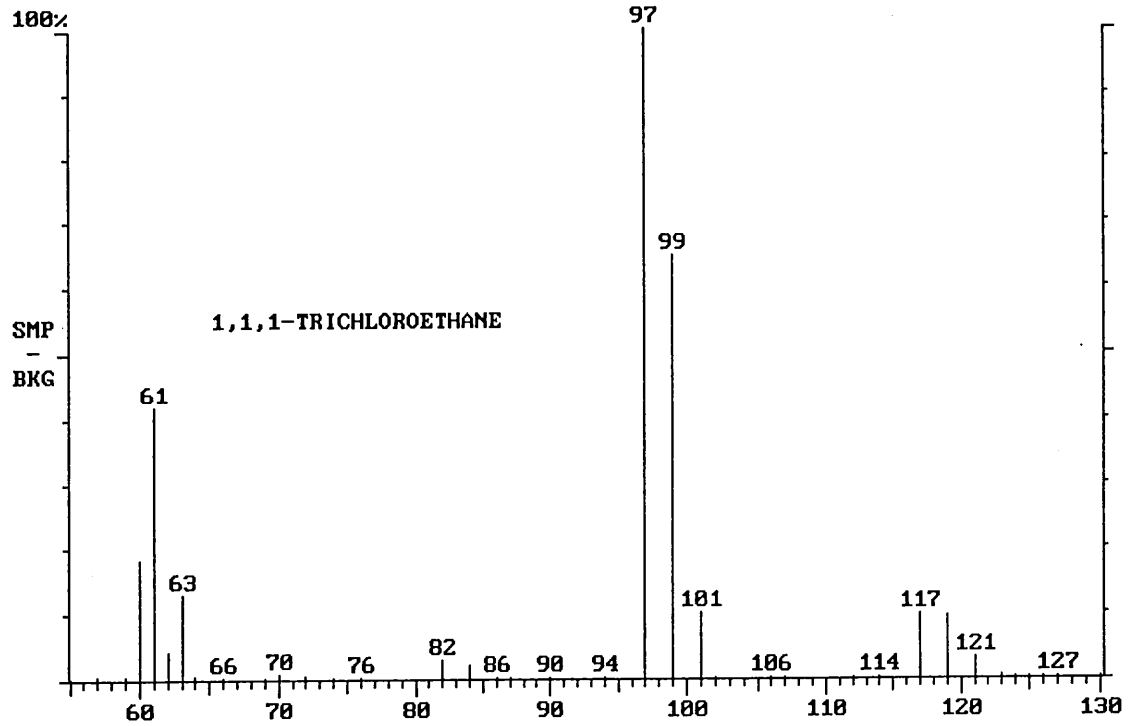


Figure II.3.5: Mass spectrum of 1,1,1-trichloroethane, x-axis: mass number, y-axis relative height to total ion count.

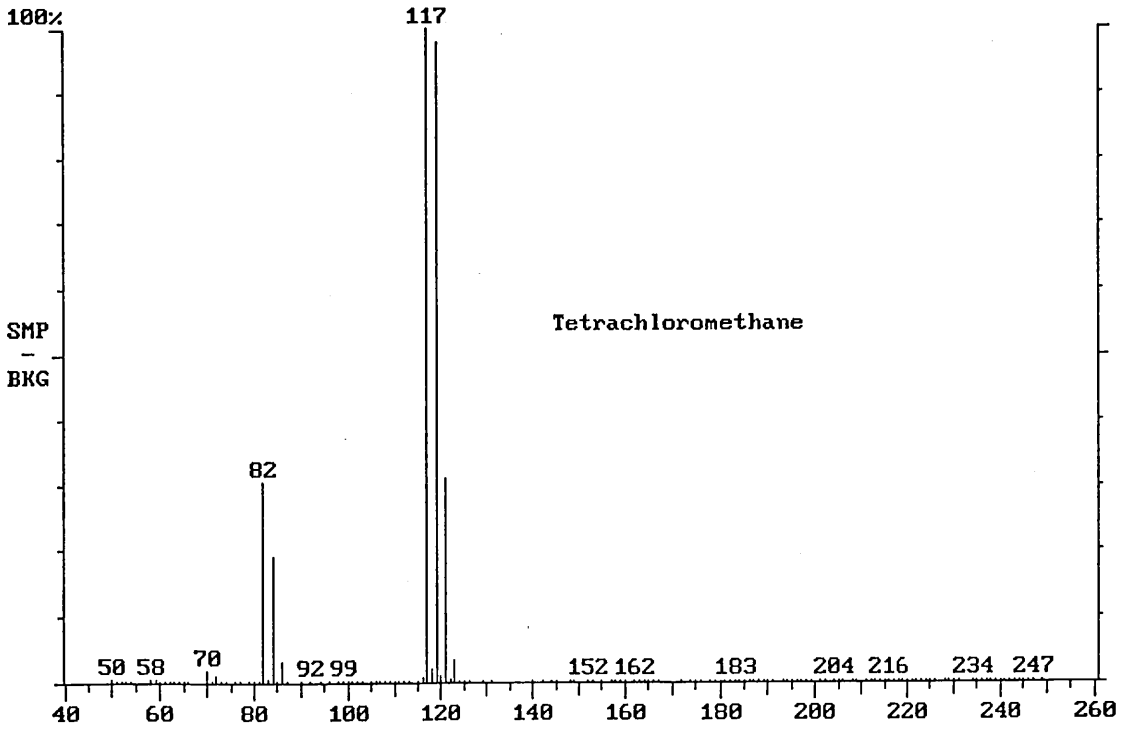


Figure II.3.6. Mass spectrum of tetrachloromethane, x-axis: mass number, y-axis relative height to total ion count.

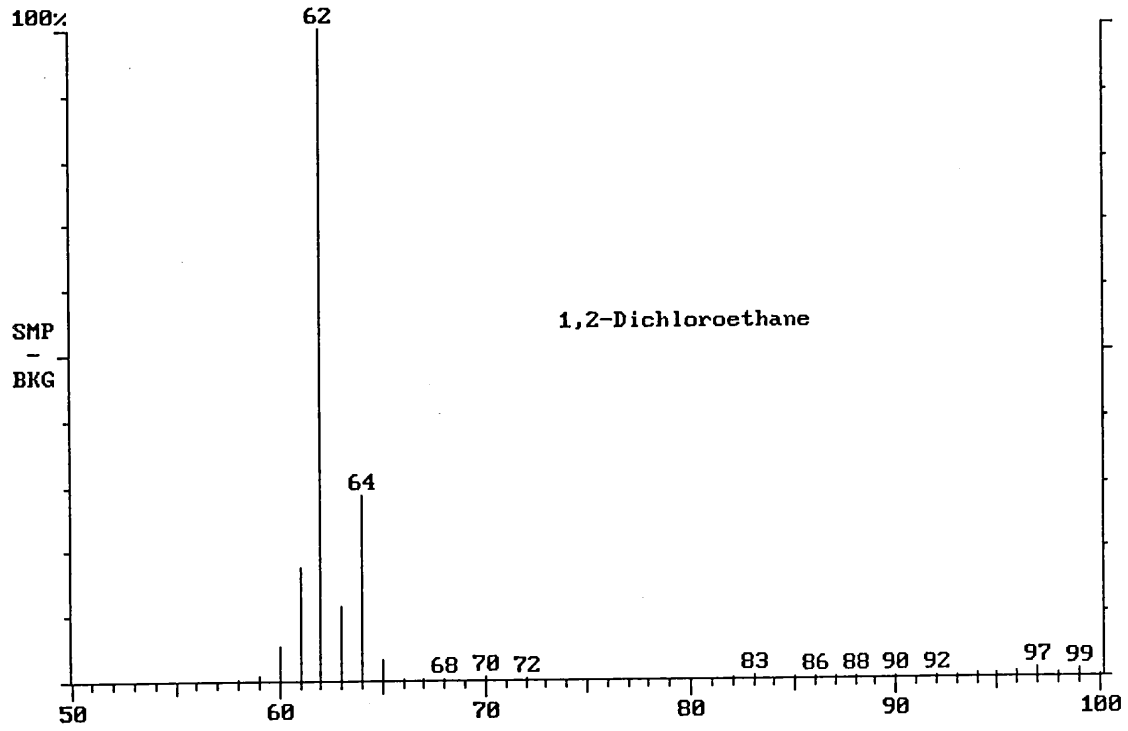


Figure II.3.7: Mass spectrum of 1,2, -dichloroethane, x-axis: mass number, y-axis relative height to total ion count.

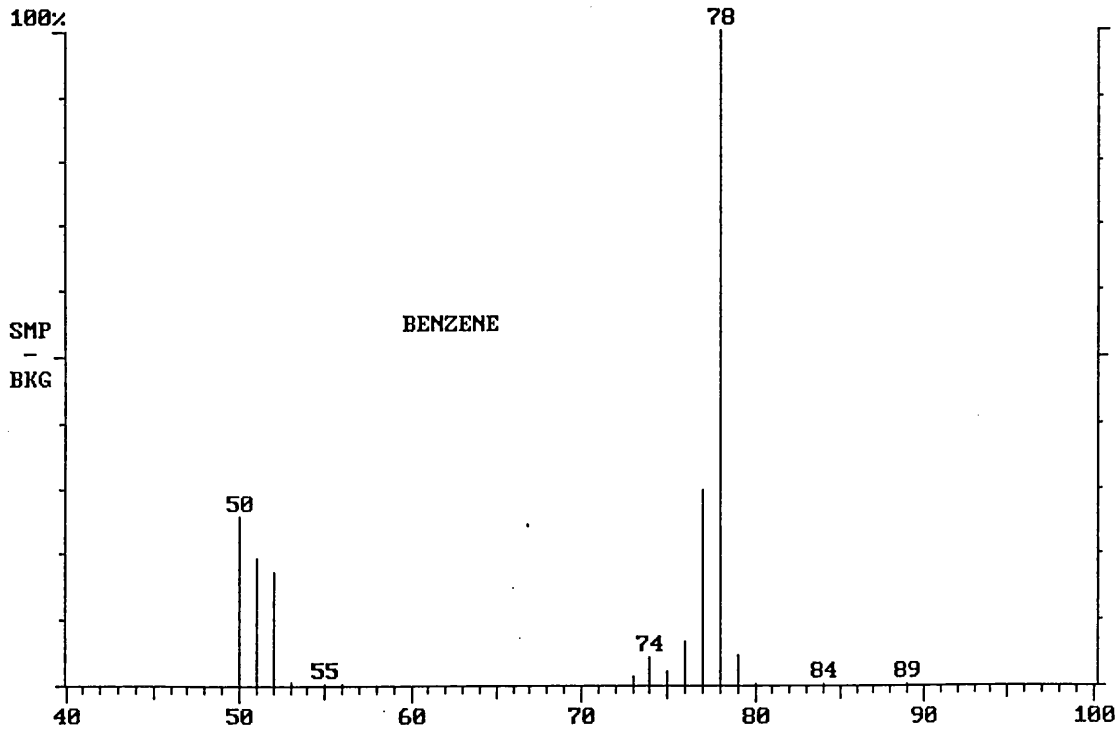


Figure II.3.8: Mass spectrum of benzene, x-axis: mass number, y-axis relative height to total ion count.

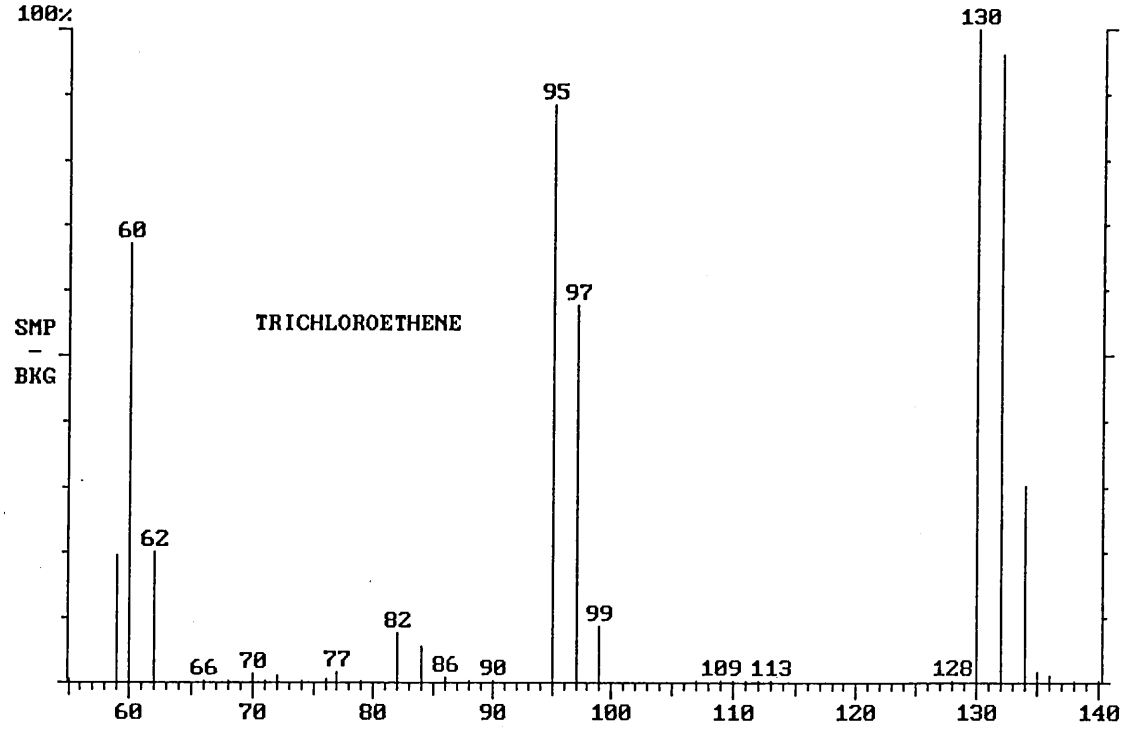


Figure II.3.9: Mass spectrum of trichloroethylene (trichloroethene), x-axis: mass number, y-axis relative height to total ion count.

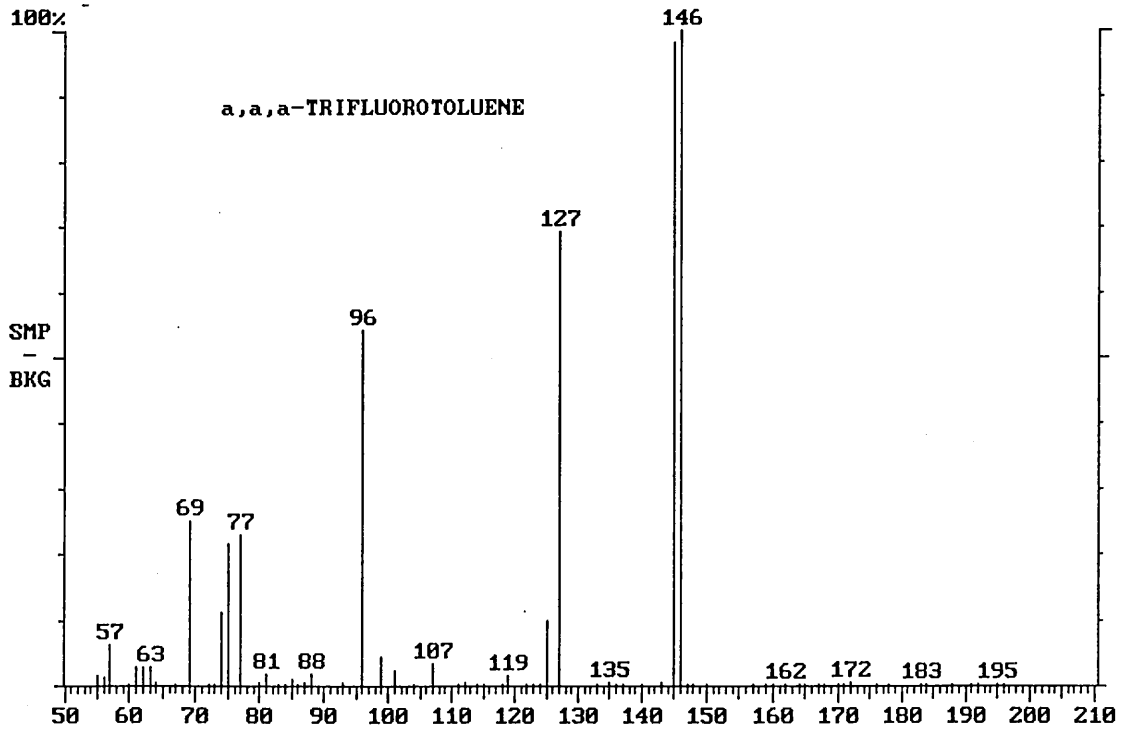


Figure II.3.10: Mass spectrum of α,α,α,-trifluorotoluene, x-axis: mass number, y-axis relative height to total ion count.

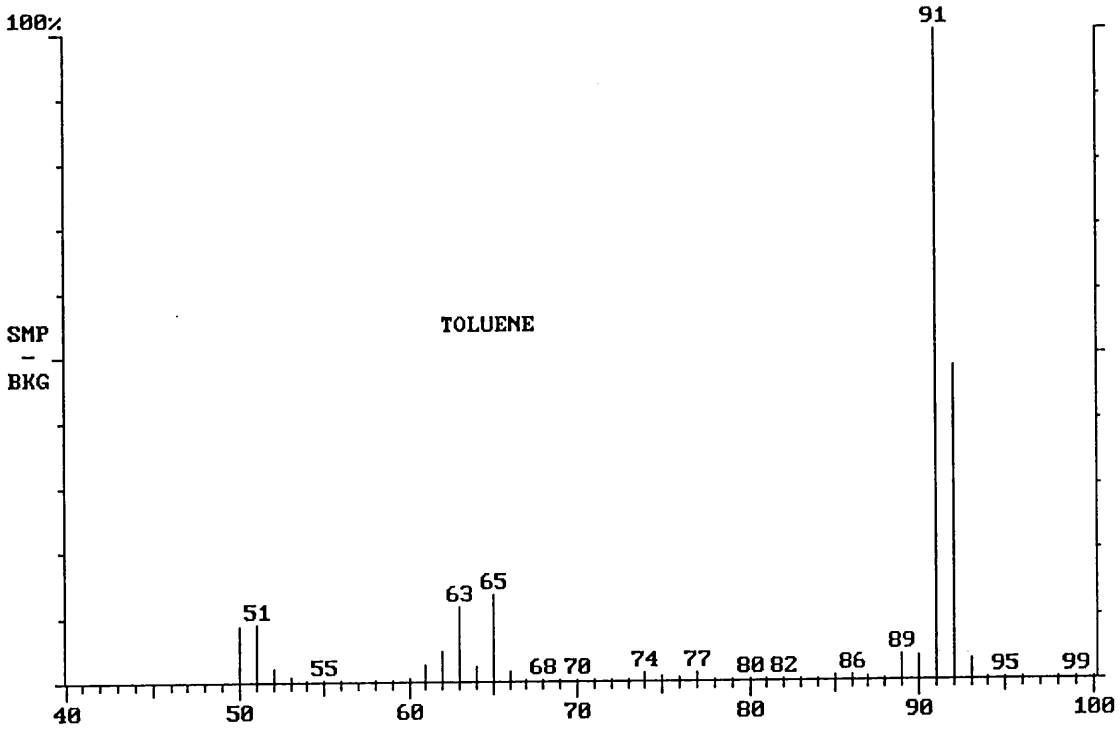


Figure II.3.11: Mass spectrum of toluene, x-axis: mass number, y-axis relative height to total ion count.

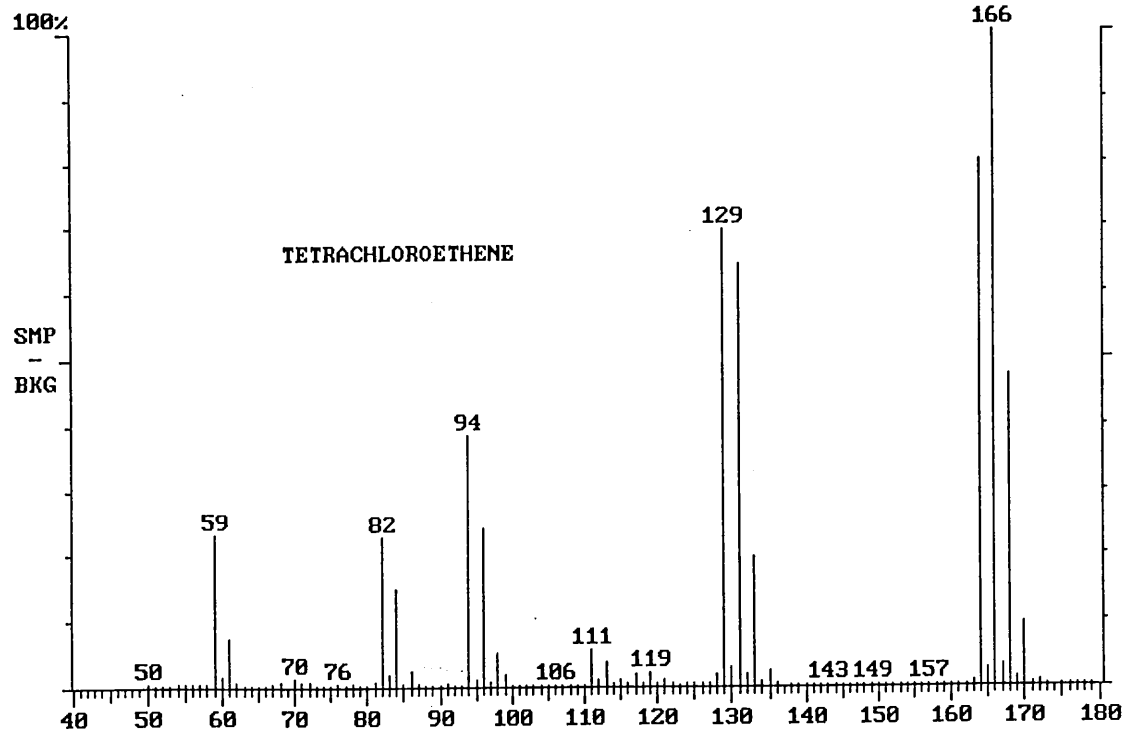


Figure II.3.12: Mass spectrum of tetrachloroethylene (tetrachloroethene), x-axis: mass number, y-axis relative height to total ion count.

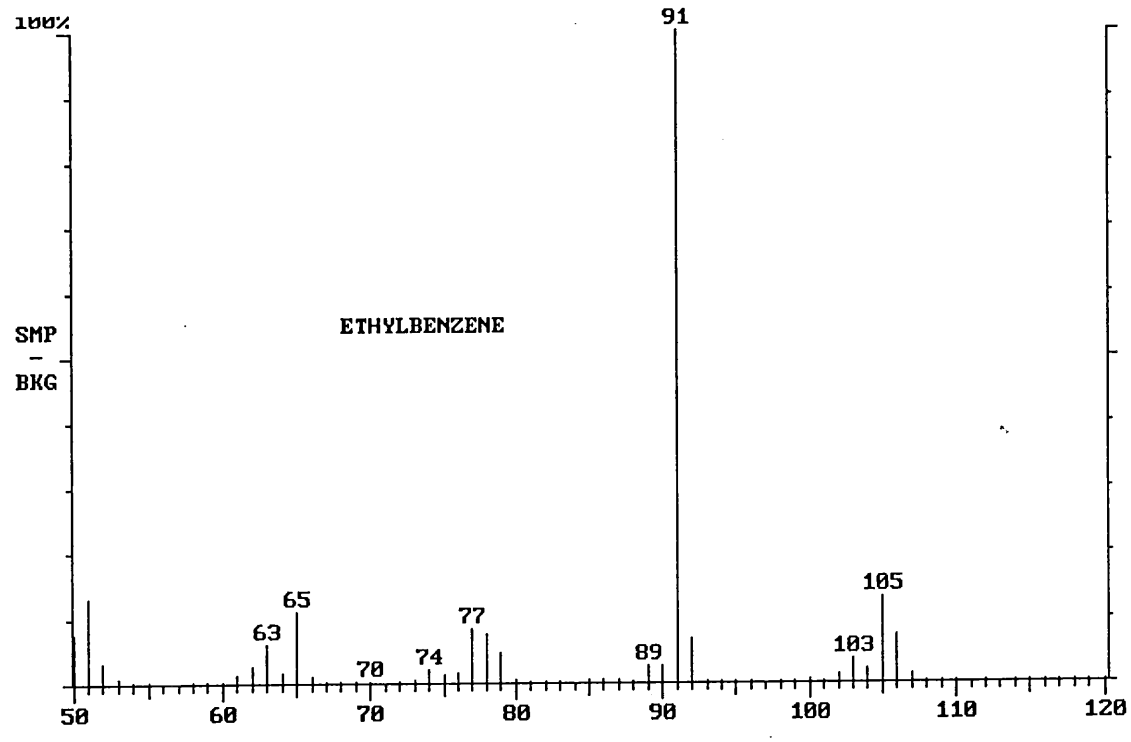


Figure II.3.13: Mass spectrum of ethylbenzene, x-axis: mass number, y-axis relative height to total ion count.

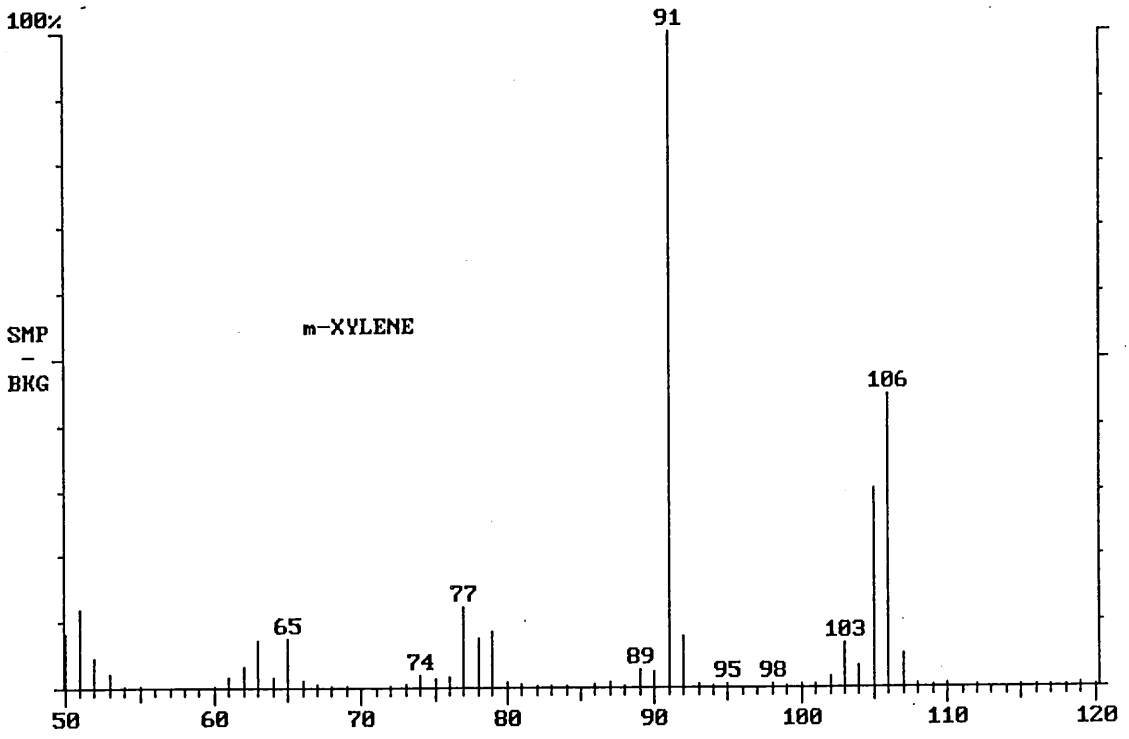


Figure II.3.14: Mass spectrum of m-xylene, x-axis: mass number, y-axis relative height to total ion count.

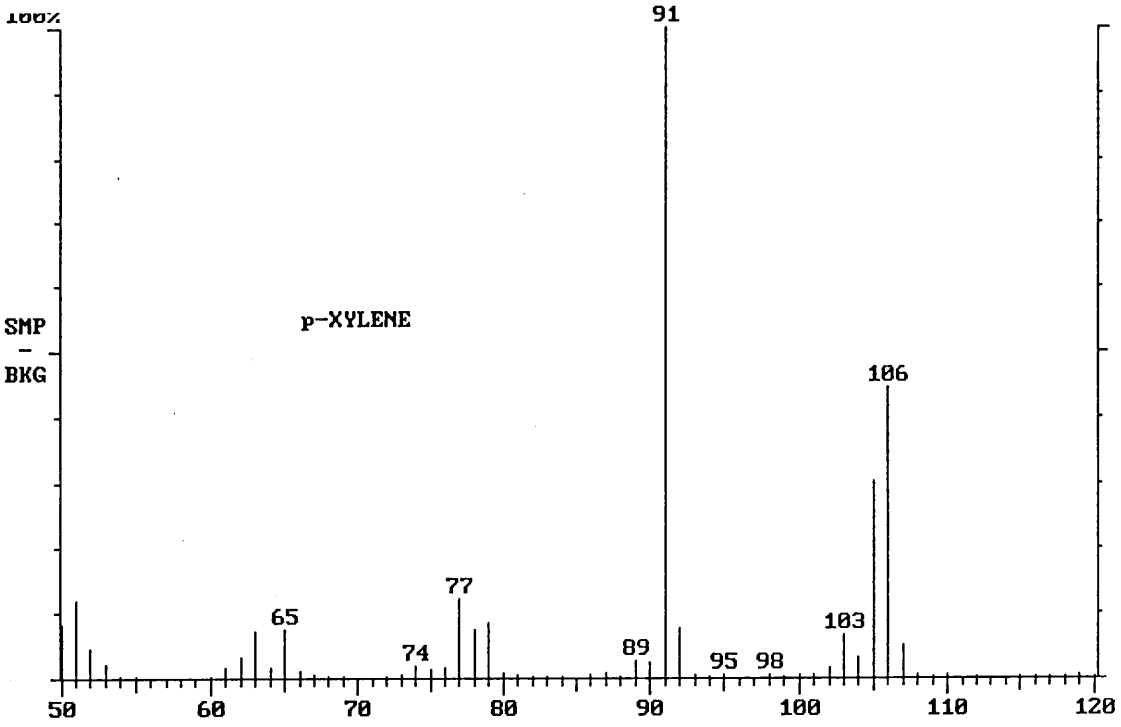


Figure II.3.15: Mass spectrum of p-xylene, x-axis: mass number, y-axis relative height to total ion count.

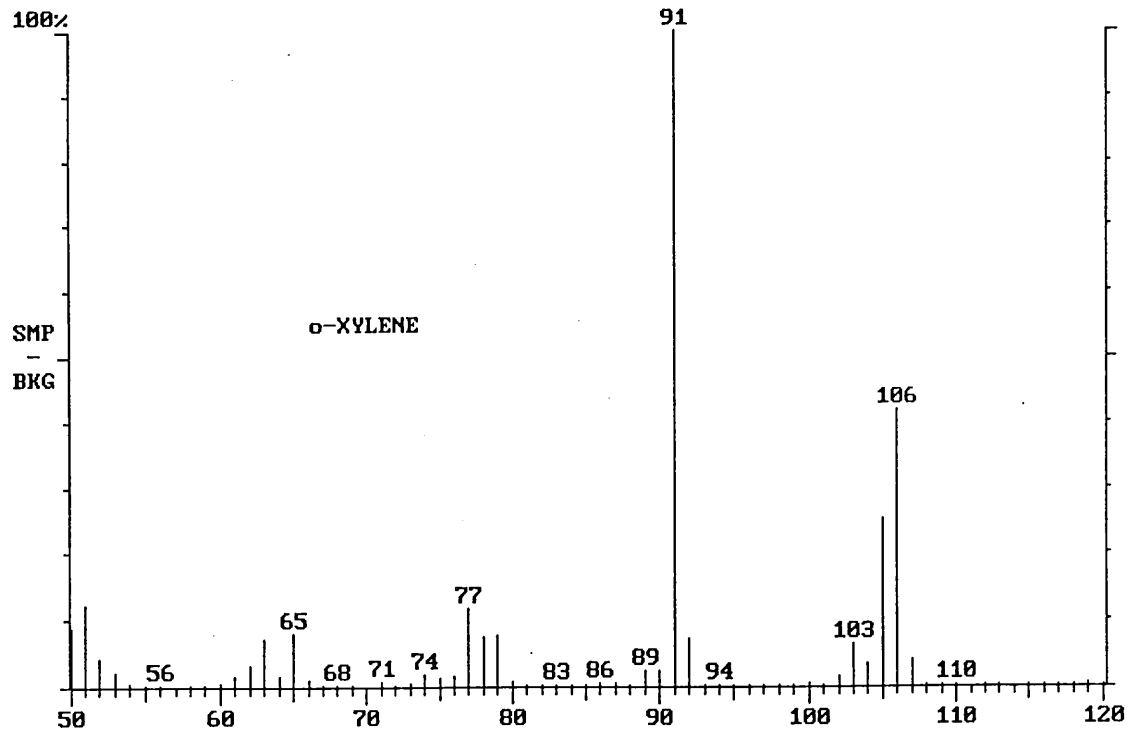


Figure II.3.16: Mass spectrum of o-xylene, x-axis: mass number, y-axis relative height to total ion count.

II.3.1.4 DETECTION LIMITS

Detection limits of the system were determined by injecting a number of standard solutions of known concentrations and determining the signal to noise ratio (S/N). The experimental conditions were:

Injector: SPI, initial temperature: 40 °C for 0.3 min, from 40 ° C to 200 ° C at 180° C /min.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The detection limit (LOD) is defined as the amount that can be positively identified by the system and is calculated as the amount that corresponds with a S/N of three. The limit of quantification or determination (LOQ) is defined as the amount that can be positively quantified with the system and is calculated as the amount that corresponds with a S/N of ten. LODs and LOQs are given in table II.3.2. for the target compounds. The LOD varied between 26 pg for chloroform and 3 pg for the MAHs. The LOQs varied between 85 pg for chloroform and 8 pg for ethylbenzene.

The greater sensitivity of the system for MAHs is apparent, but the overall sensitivity was considered to be sufficient for the envisaged determinations.

II.3.1.5 LINEARITY OF THE GC-MS SYSTEM

The linearity of the system was tested by injecting a series of standard solutions with analyte masses ranging from 40 pg to 17 ng. Each standard solution was individually prepared and injected into the GC-MS under the following operational parameters:

Injector: SPI, initial temperature: 40 °C for 0.3 min, from 40 ° C to 200 ° C at 180° C /min.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The resulting areas were corrected with the area of the internal standard and analysed with the least squares method for linearity. The results of the analyses are given in table II.3.3 and illustrated in figure II.3.17.

Table II.3.3: Correlation coefficients (r) for the different target compounds.

Compound	r
Chloroform	0.9999
Trichloroethane	0.9999
Tetrachloromethane	0.9997
Trichloroethylene	0.9999
Benzene	0.9999
Toluene	1.0000
Tetrachloroethylene	0.9993
Ethylbenzene	0.9981
m&p-Xylene	0.9976
o-Xylene	1.000

The correlation coefficients prove that the instrument is linear over the mass range mentioned above. The linearity of the system was therefore considered to be satisfactory for the envisaged analysis.

II.3.1.6 REPEATABILITY AND REPRODUCIBILITY OF THE GC-MS SYSTEM

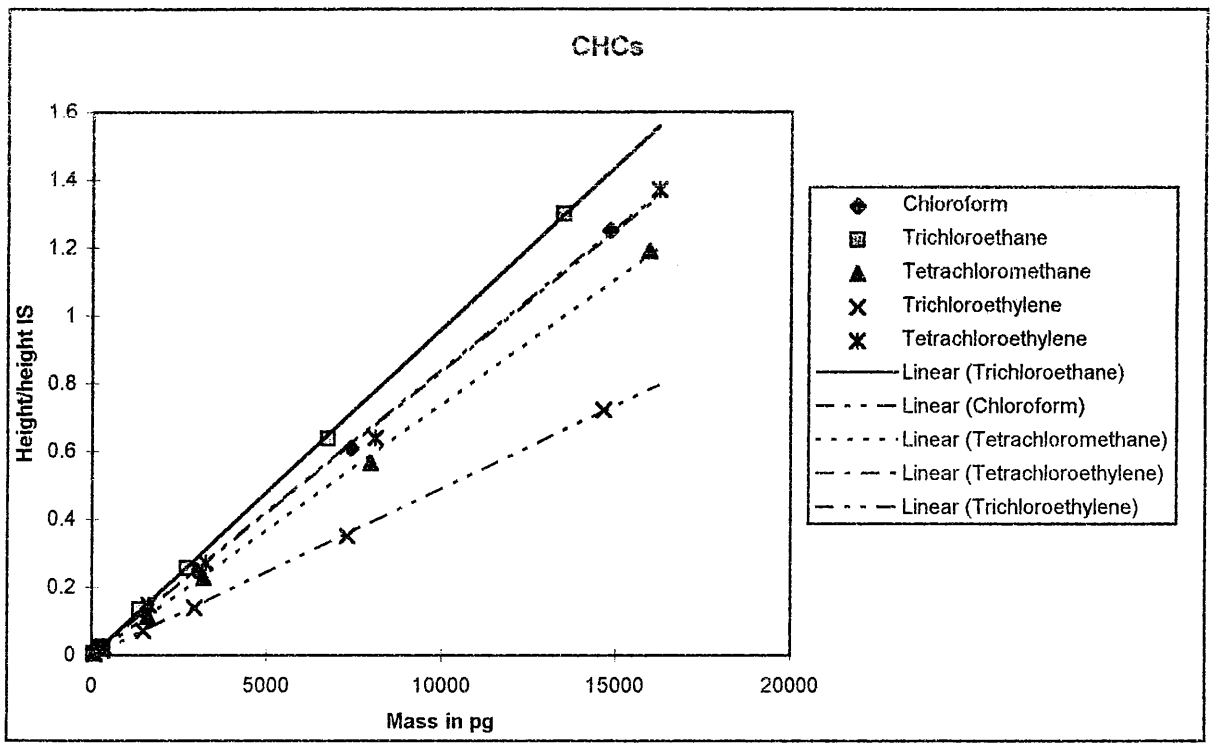
The repeatability of the GC-MS was tested by injecting the same standard concentration repeatedly during one day. The reproducibility of the GC-MS was tested by injecting the same standard concentration over a period of one week. The analytical conditions were as above. The resulting relative standard deviations are given in table II.3.4.

Table II.3.4: Repeatability and reproducibility of the GC-MS.

Compound	Repeatability (%) n = 5	Repeatability (%)* n = 5	Reproducibility (%) n = 5
Chloroform	2.2	8.48	5.6
Trichloroethane	2.3	8.15	4.8
Tetrachloromethane	3.3	8.90	7.4
Trichloroethylene	3.3	8.11	10.6
Benzene	1.8	8.87	15.8
Toluene	2.8	8.93	6.3
Tetrachloroethylene	4.8	9.47	20.0
Ethylbenzene	5.4	10.93	19.3
m&p-Xylene	8.1	12.60	26.3
o-Xylene	2.2	8.51	5.4

*Repeatability without internal standard, n = number of injections.

The results suggest that the system remains stable for at least one week. The latter would imply that one calibration at the beginning of the week is sufficient for an entire week of analysis. Current practice at the lab, for other types of analysis, is to calibrate the equipment before each new batch of samples. It seems in any case advisable to keep this practice for the GC-MS system. The results further illustrate the effect of compensating for differences in injection with the internal standard. Not using an internal standard definitely leads to a higher variability.



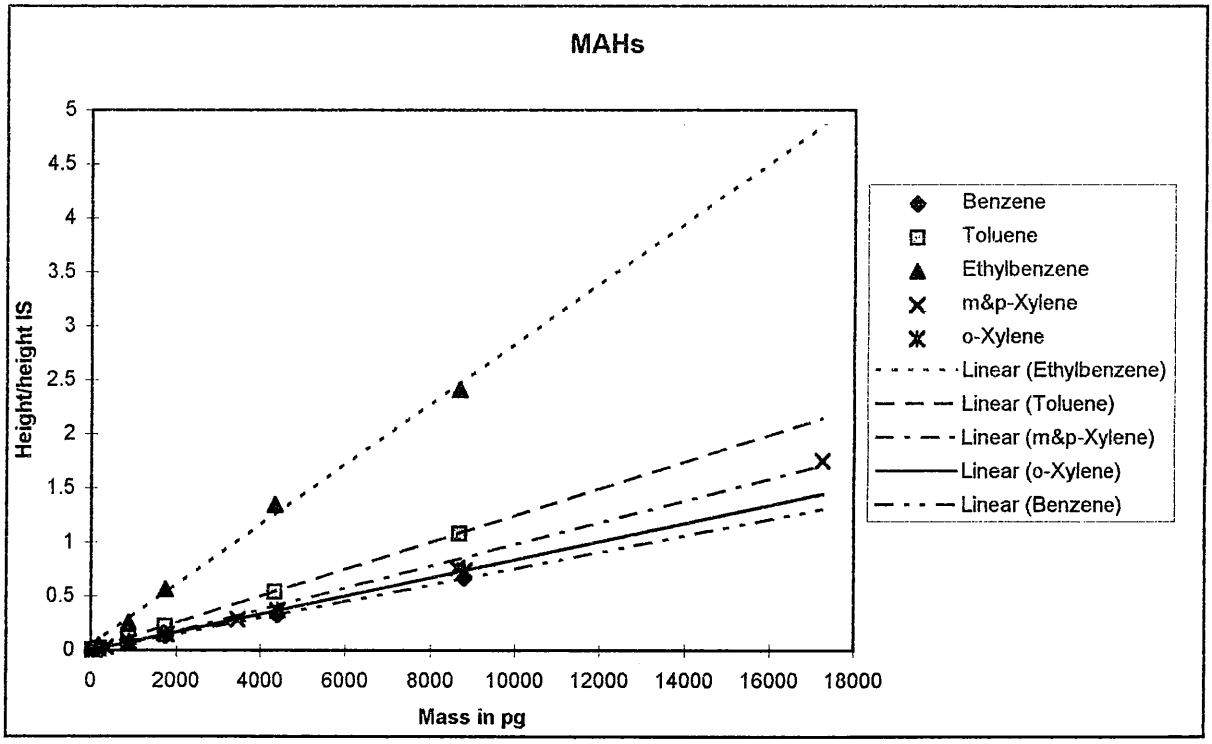


Figure II.3.17: Linearity of the GC-MS for CHCs (upper graph) and MAHs (lower graph).

II.3.2 PURGE AND TRAP TECHNIQUE.

II.3.2.1 INTRODUCTION.

The selection of an appropriate technique for the analysis of VOCs in marine sediments was based on a number of criteria set beforehand. First of all, the basic analytical criteria such as a sufficient recovery, reproducibility and detection limits should be met. It seemed further essential that the method is easy to use, rapid and involves as little sample handling as possible.

Reviewing the different methods documented in the literature (see earlier), several analysis strategies for the determination of VOCs in marine sediments were identified. The basic strategies that can be identified are the purge and trap technique or P&T (Charles and Simmons, 1987; Bianchi and Varney, 1989b; Bianchi *et al.*, 1991; Al-Rekabi *et al.*, 1995), the vacuum extraction technique (Hiatt, 1981; Hiatt *et al.*, 1994), the static headspace technique (Kawata *et al.*, 1988; Bianchi and Varney, 1989a), closed loop stripping in combination with steam distillation (Amin and Narang, 1985), steam distillation (Kawata *et al.*, 1988), a methanol extraction combined with purge and trap analysis of the extract (Marchand *et al.*, 1994) and supercritical fluid extraction or SFE (Levy and Roselli, 1989). Of these techniques the purge and trap technique was selected. Static headspace techniques require a thorough knowledge of the sediment sample and are, in principle, only applicable for a given sediment type. As different sediment types were expected during the monitoring campaign, the method was rejected. The methanol extraction in combination with P&T was feasible but only for higher concentrations. Steam distillation and vacuum distillation were equally feasible, but were rejected for practical reasons. And finally, SFE requires specialised equipment that was not available at the institute. The P&T further offers the possibility of on-line pre-concentration and analysis of the samples. As sorbent trap Tenax (Charles and Simmons, 1987; Bianchi and Varney, 1989b; Bianchi *et al.*, 1991), Chromosorb-106 (Bianchi and Varney, 1989b; Bianchi *et al.*, 1991) or Sphericarb (Bianchi *et al.*, 1991) are reported in literature.

As a first step, a Tekmar LSC-2000 P&T apparatus was coupled to the GC-MS system and a number of tests was run to determine the applicability of the apparatus for the planned analyses. A sorbent trap filled with Tenax was selected for the initial tests.

II.3.2.2 COUPLING TO THE GC-MS.

II.3.2.2.1 Introduction.

The Tekmar LSC-2000 was coupled to the GC-MS with a heated transfer line consisting of a 1 m deactivated fused silica column enclosed in heated isolating mantle (figure II.3.18) A cryofocussing module connects the transfer line with the analytical column in the GC (figure II.3.18.).

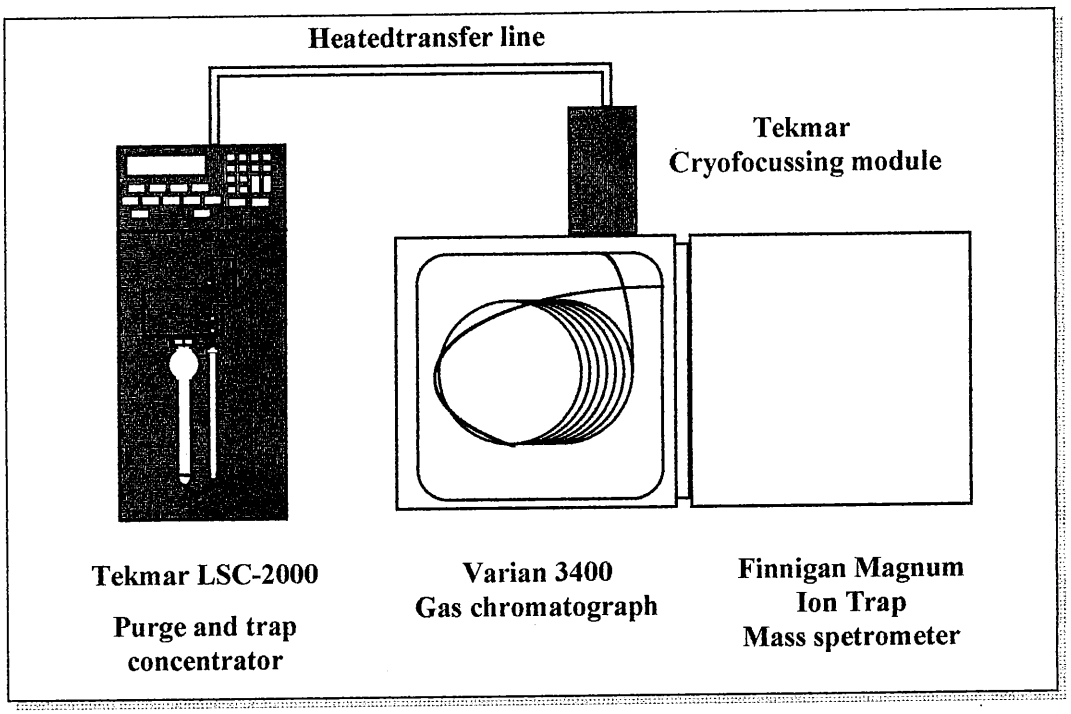


Figure II.3.18: Schematic overview of P&T coupled to GC-MS.

The principle of the apparatus is as follows: a glass vessel (purging vessel) , an adsorbent trap and the transfer line are connected to each other through a six-way valve placed in a heated compartment. Sorbent trap, purging vessel and transfer line are connected with the valve through glass-lined stainless steel tubing (figure II.3.19.). In the first step, a liquid or solid sample is introduced into the purging vessel and

purged with a constant flow of an inert gas (helium), the position of the valve allows at that stage the gas to pass over the adsorbent trap (figure II.3.20.). An optional dry purge step allows the purge gas to pass over the sorbent trap, bypassing the purging vessel (figure II.3.21.). This step aids the elimination of water accumulated on the trap during the purging phase. In the next phase (desorption phase), the trap is rapidly heated and back-flushed with the gas. The valve connects at that stage the sorbent trap with the transfer line (figure II.3.22.). An optional "Moisture Control Module" (MCM) is used to trap water that is introduced into the system during purging (figure II.3.22.). The principle is adsorbing and condensing the water onto the wall of a glass lined stainless steel tube cooled between 8 an 4 °C with the aid of a Peltier element. During, the bake phase the MCM tubing is heated to 120 °C to eliminate the water collected during desorbing (figure II.3.21.). The analytes are carried with the gas to the cryofocussing module (figure II.3.18.). The module is cooled to allow trapping of the compounds prior to introduction into the GC. The analytes are then injected onto the column by rapidly heating the module. The analytes are thus introduced into the column as a narrow liquid plug which results in better chromatographic conditions. The column is temperature programmed similar to programs used for splitless and on-column injection (see earlier). During a first series of tests a blank water sample and a water sample spiked with a standard solution were analysed to determine the characteristics of the equipment.

II.3.2.2.2 Preparation of standard solutions and blank water.

Preparation of standard solution

A test was made to select a proper solvent for the preparation of standard solutions. To that purpose a weighed quantity of the candidate solvents methanol and dichloromethane was stored in identical vessels in the refrigerator at 4 °C. The weight loss recorded for dichloromethane far exceeded that of methanol. Methanol was therefore chosen as solvent of choice for the preparation of standard solutions.

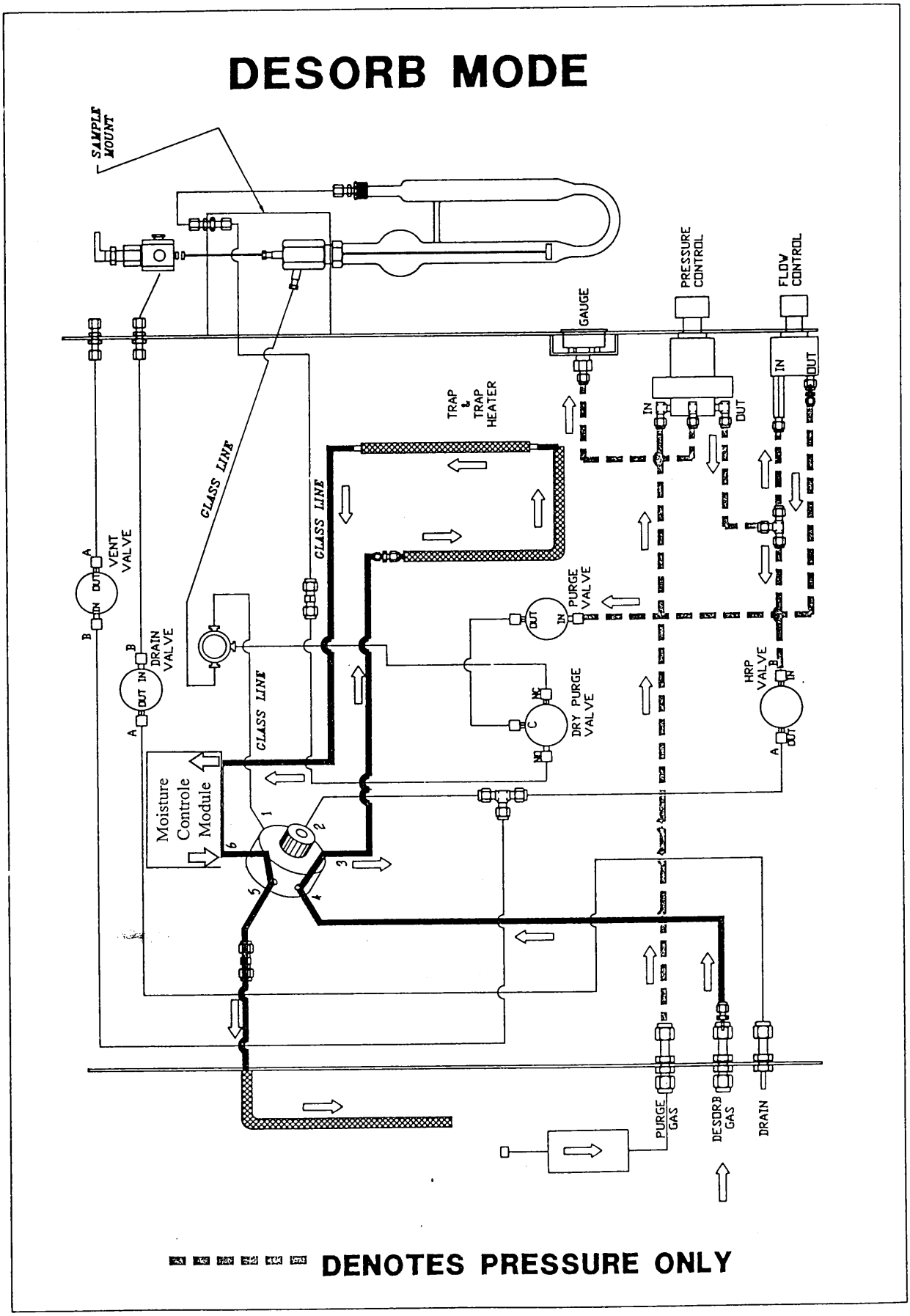


Figure II.3.19: Schematic overview of the gas flows during the standby mode of the Tekmar P&T.

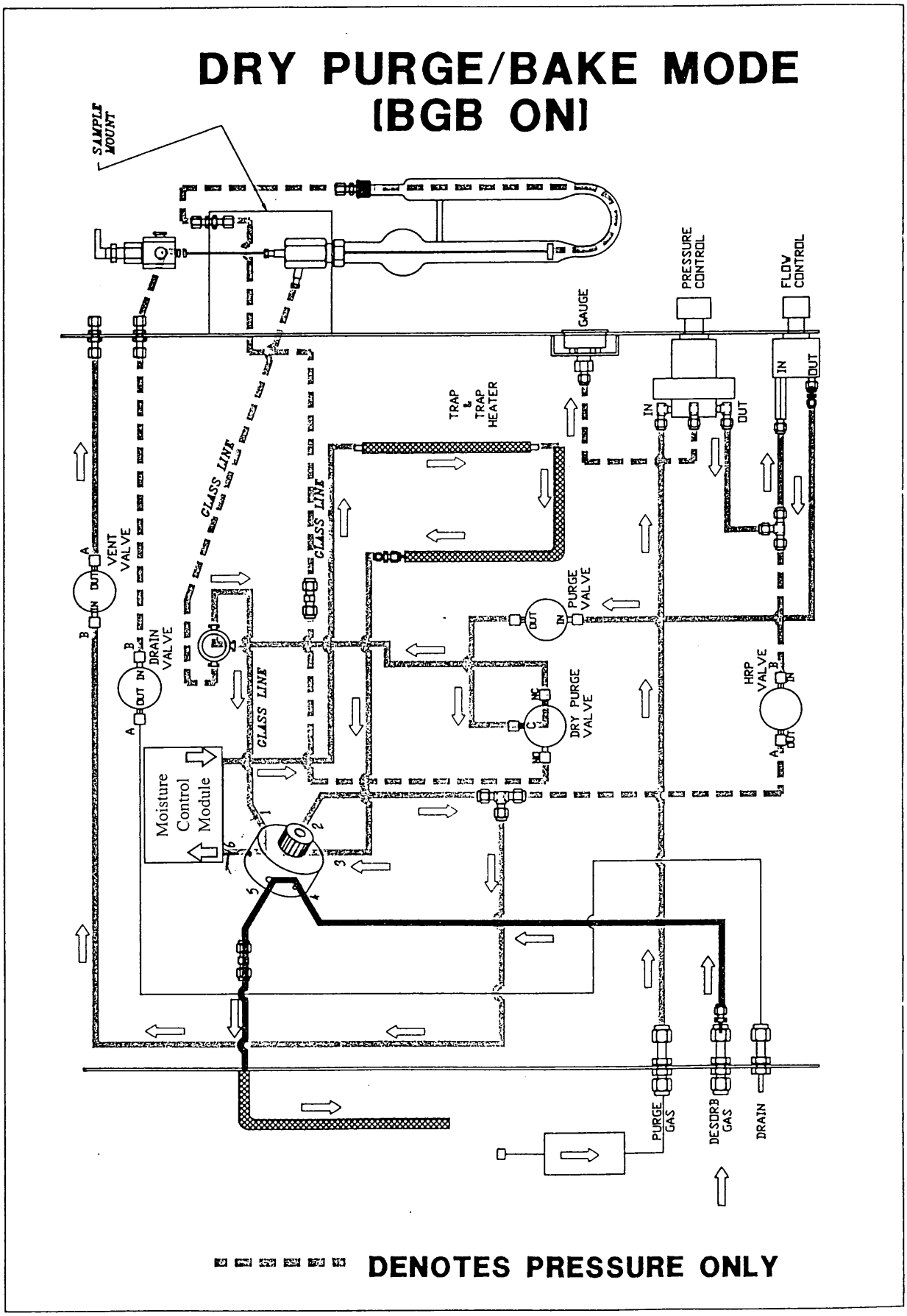


Figure II.3.20: Schematic overview of the gas flows during the purge/bake mode of the Tekmar P&T.

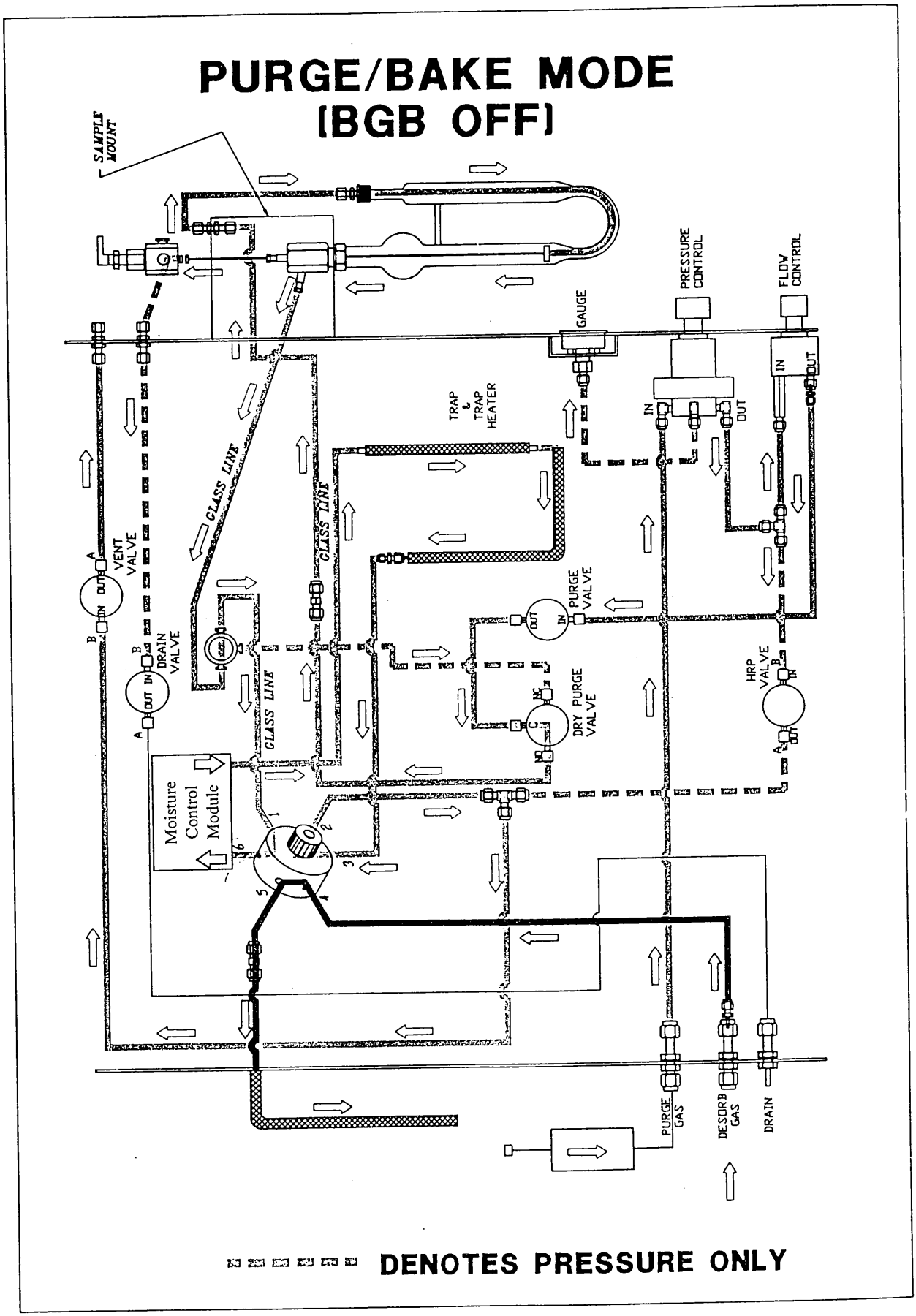


Figure II.3.21: Schematic overview of the gas flows during the dry purge/bake mode of the Tekmar P&T.

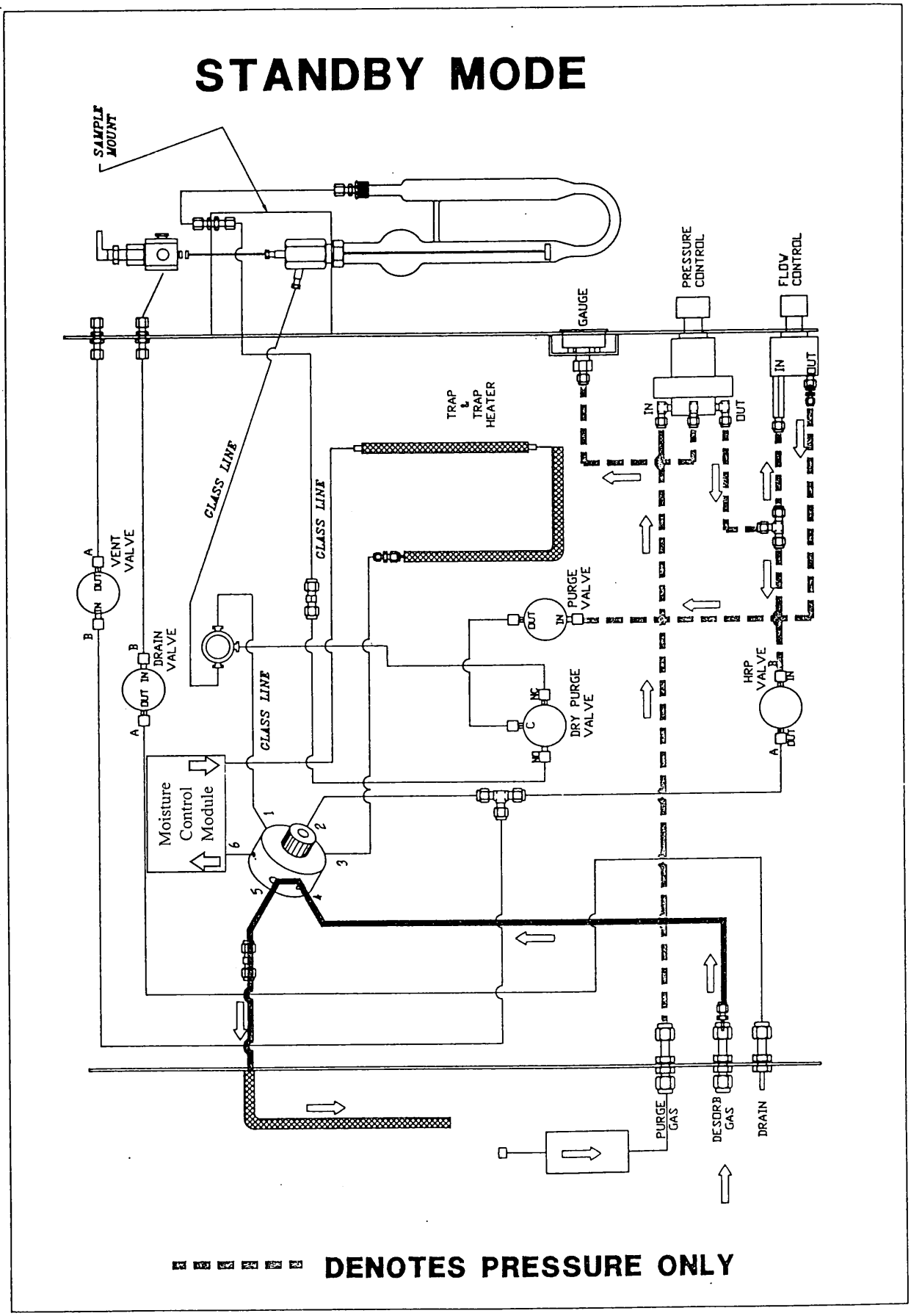


Figure II.3.22: Schematic overview of the gas flows during the desorb mode of the Tekmar P&T.

An initial standard solution (stock solution) was made by diluting 1 ml of the different target compounds in 100 ml of methanol as follows: a small quantity (approx. 20 ml) of solvent was introduced in a volumetric flask and the weight was recorded. One ml of each of the target compounds was added to the methanol and at each occasion the weight is recorded. Finally, the volume was brought to 100 ml and the weight was again recorded. This allows correction for eventual weight losses. This method allows calculation of the concentration on both a volume and a weight basis. Reporting and using standard solutions on a weight basis is recommended for analytical purposes (Wells *et al.*, 1992) but by using accurate volumes at the same time, a rapid calculation of concentrations and of dilution series is possible. In any case, standards were used on a weight basis for analytical purposes. From the stock solution, dilution series are made by dissolving known quantities in methanol, again on a weight basis. Frequent renewal of standard solutions is recommended due to the high volatility of the analytes. The dilutions were therefore continuously monitored for concentration differences. As a rule, no concentration differences should be allowed that exceed the analytical variability.

Preparation of blank water.

Three types of water were used for the preparation of blanks namely: mineral water (Spa Reine, Spa), double distilled water prepared in the laboratory and water specially prepared for the analysis of VOCs (Baker). For the first two types the water was treated by heating it to a temperature of 90°C and simultaneous purging with helium (N 7.0, l'Air Liquide) or N_2 (N 6.0, l'Air Liquide). In all the cases and as a routine, water, used for preparations, was continuously purged during storage with the gasses mentioned above.

II.3.2.2.3 Recovery, system blank, linearity and repeatability.

Recovery of the system

To determine the recovery of the system, a know quantity of the internal standard was added to a series of blank water samples (5 ml) and was analysed under the following conditions:

Purging apparatus: Tekmar LSC-2000 with 5 ml sparger, oven temperature: 250° C, mount temperature: 250° C, purge time: 12 min, dry purge time: 6 min, purge flow: 40ml/min.

Trap: Tenax, at room temperature during purging, desorbed at 180°C for 4 min, baked at 180° C for 7 min.

Transfer line: 250 °C

Injector: cryofocussing module, cooled to -120° C during desorbtion, heated from -120° C to 200 °C in 0.75 min during injection, standby temperature 250°C.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The relative surface of the internal standard (surface divided by mass) was then compared with the average relative surface the IS obtained from a number of injections with the SPI and by using the following operational conditions:

Injector: SPI, initial temperature: 40 °C for 0.3 min, from 40 ° C to 200 ° C at 180° C /min.

Detector: as above

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The results are given in table II.3.5. The concentration-height ratio was not significantly different for the analysis with the Tekmar compared to a manual injection with the SPI.

This was confirmed with a Kruskal-Wallis ANOVA test and further specified with Dunn's post test.

Table II.3.5: Comparison of height/concentration ratios obtained with manual injection and concentration with the Tekmar system. RSD % = relative standard deviation, n = number of measurements, p = p - value obtained with the Dunn's post test when comparing the SPI analysis with the Tekmar analysis.

Method	Injected mass in pg	Height/Concentration	RSD %	n	p
SPI	299	31.49	11.29	4	
Tekmar 1	619	47.75	25.12	3	> 0.05
Tekmar 2	31	31.81	17.96	4	> 0.05
Tekmar 3	29	43.5	20.57	4	> 0.05
Tekmar 4	196	33.15	19.11	4	> 0.05

System blank.

A 5 ml blank water sample (Spa Reine, Spa), prepared as described above, was introduced into the purging vessel of the P&T apparatus and analysed using the following analytical conditions:

Purging apparatus: Tekmar LSC-2000, oven temperature: 250° C, mount temperature: 250° C, purge time: 12 min, dry purge time: 6 min, purge flow: 40ml/min.

Trap: Tenax, at room temperature during purging, desorbed at 180°C for 4 min, baked at 180° C for 7 min.

Transfer line: 250 °C

Injector: cryofocussing module, cooled to -120° C during desorbtion, heated from -120° C to 200 °C in 0.75 min during injection, standby temperature 250°C.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

After the first series of analyses, it became evident that traces of chloroform, trichloroethene, benzene, toluene, ethylbenzene and the xylenes were present in the water or in the system. As a first remedy, a different kind of water was used for the preparation of the blank (double distilled water prepared in the lab by percolating tap water over an ion exchange apparatus (Gelman Sciences) and treated as described above). This proved to be unsuccessful as identical traces of VOCs were still detectable. Even the use of specially prepared water for the analysis of VOCs (Baker) resulted in the detection of the VOCs mentioned earlier. As contamination levels were, as a rule, lower when an analysis was performed with an empty purging vessel, additional steps were taken to reduce the possibilities of contamination originating from water. In a first attempt, water (Spa Reine, Spa) was eluted over a bed of activated carbon (35-50 mesh, Merck , Darmstadt -Germany) and subsequently boiled while being purged with nitrogen (N 6.0, l'Air Liquide). Secondly, water was extracted several times with hexane (Promochem nanograde, Wesel-Germany) and purged after extraction with nitrogen as above. None of the remedies described above resulted in a significant improvement of the contamination levels. Desorbing the trap without a preliminary purging step resulted in nearly perfect blank values, with the exception of a small quantity of benzene that was thought to originate from the trap itself. Tenax is known to release small quantities of benzene when the material becomes deteriorated. Reconditioning of the trap by baking it overnight at 180 °C resulted indeed in a decrease of benzene. In any case, water was not the primary cause of the contamination problem. The contamination was therefore thought to originate from the equipment. After consulting representatives of the manufacturer a series of tests were performed in order to determine the cause of this contamination. Firstly, the glassware was thoroughly cleaned and treated with chromosulfuric acid (Merck, Darmstadt-Germany). Secondly, a series of analyses was performed with boiling water in the purging vessel in order to clean the system (steam-cleaning). These measures did not produce immaculate blanks and during this step it became clear that the MCM did not succeed in retaining all the water vapour released during purging. The ion trap became saturated with excess water that could only originate from the P&T device. Further cleaning of the system consisted of the following actions: all the glassware was again treated with chromic acid as above. A Shimadzu

LC-9 HPLC pump was coupled to the inlet of the trap and the system was flushed by pumping boiling water through it for 4 hours at a flow of 1 ml/min. Afterwards, the trap was replaced with a length of copper tubing of the same dimensions and the system was purged with helium overnight according to the scheme in figure II.3.3.. After a number of analyses of blank water samples the system was further cleaned as above, but by replacing the boiling water with a 50/50 mixture of water and methanol (Promochem nanograde, Wesel-Germany). Using this mixture the system was backflushed for 1 hour at a flow rate of 0.5 ml/min. To eliminate the methanol, the previous procedure was applied. Finally, after another batch of experiments, the entire previous procedure was again applied, this time by using 100 % methanol (Promochem nanograde, Wesel-Germany) in the first step. Afterwards the system was operated for an entire week with boiling water in the purging vessel (steam-cleaning). Following that, a series of analyses was carried out with an empty purging vessel. As a last resort, the entire tubing in the system and the six-way valve were replaced. Subsequent analysis of a number of blank water samples revealed acceptable blank levels, that were at or below the limit of detection of the system. However, during the next series of experiments described in the following paragraph, blank levels gradually increased. In our opinion the switching valve and the automatically controlled opening valves serve as sources of contamination that can never be entirely eliminated with the present equipment.

Variability of the blank

As series of test were run to determine the short and the long-term variability of the blank in water and in air.

For the blank in water, internal standard was injected into a luer lock syringe filled with 5 ml blank water and analysed with the experimental conditions described above. This procedure was repeated every 2 hours. The same procedure was then repeated 10 times at equal intervals during one week. The results are given in table II.3.6 .

The short-term (1 day) variability of the blank ranged from 16 % for ethylbenzene to 70 % for tetrachloroethene. No background concentrations could be determined for

tetrachloromethane, trichloroethane and the dichloroethanes. The long-term variability (1 week) varied between 59 % for chloroform and 232 % for benzene (table II.3.6) and is illustrated in Fig. II.3.23.

Table II.3.6: Average (pg), median (pg) and relative standard deviation (pg) (RSD) of blank values measured during 1 day in water. N = number of measurements.

Compound	1 Day Average	1 Day Median	1 Day RSD % n=4	1 Week Average	1 Week Median	1 Week RSD % n=10
1,1-Dichloroethane						
Chloroform	17	16	53	85	81	59
Trichloroethane	-	-	-	218	110	143
Tetrachloromethane	-	-	-	-	-	-
1,2-Dichloroethane	-	-	-	-	-	-
Benzene	123	125	19	3407	325	232
Trichloroethylene	222	194	32	542	132	156
Toluene	128	128	30	4312	4312	88
Tetrachloroethylene	354	354	70	947	219	196
Ethylbenzene	65	64	16	239	60	134
m&p-Xylene	57	65	60	-	-	-
o-Xylene	26	27	60	230	192	67

Although the blank values did not change too much during one day, there was a considerable variability both in time and in concentrations over the period of one week.

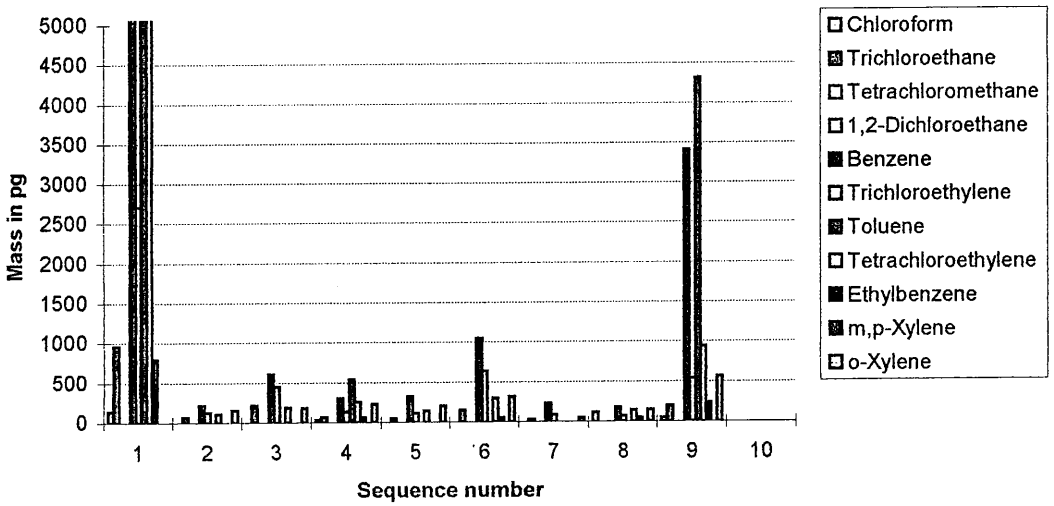


Figure II.3.23: Variability of the blank in water over a period of 1 week.

Moreover, no distinctive patterns could be observed with the exception that when blanks were high they were generally high for all the analytes. Although, some blank samples were characterised by extreme values, the values generally did not exceed a mass of 500 pg which amounts to a concentration of 100 pg/ml or 0.1 ppb. The high concentrations of chloroform could be partially attributed to the use of chloroform in another part of the building. However, the high concentrations of other volatiles could not be attributed to practices in the institute. Remarkably, no background concentrations could be demonstrated for tetrachloromethane and dichloroethane.

The variability of the blank value in air was determined by analysing the air in an empty sparging vessel of the Tekmar under identical conditions as above. This was done immediately after extensive cleaning of the instrument (see earlier). The short-term variability was again determined during one day and the long-term variability was determined during one week. The short-term (1 day) variability of the blank ranged from 119 % for benzene to 224 % for chloroform. No background concentrations could again be determined for tetrachloromethane, trichloroethane and the dichloroethanes. The variability is at a first glance much higher for the empty sparger. However, the tests were done after an extensive cleaning and the blank was nearly perfect for most compounds. Afterwards there was a steady increase of background concentrations. The long-term variability (1 week) varied between 142 % for chloroform and 428 % for toluene (table II.3.7) and is illustrated in Fig. II.3.24. As above, the background levels generally do not exceed a mass of 500 pg which amounts to a concentration in the air of 100 $\mu g/m^3$.

Table II.3.7: Average, median and relative standard deviation (RSD) of blank values measured during 1 day in air (empty sparging vessel). N = number of measurements.

Compound	1 Day			1 Week		
	Average	Median	RSD % n=4	Average	Median	RSD %n=10
1,1-Dichloroethane	-	-	-	-	-	-
Chloroform	36997	-	224	33443	1209	142
Trichloroethane	214415	8704	135	113706	26904	253
Tetrachloromethane	-	-	-	-	-	-
1,2-Dichloroethane	-	-	-	-	-	-
Benzene	68465	9972	129	47723	17001	152
Trichloroethylene	47162	-	144	25407	2712	211
Toluene	43635	-	186	43356	0	428
Tetrachloroethylene	19706	-	196	85250	1812	193
Ethylbenzene	27990	-	148	18396	0	182
m&p-Xylene	65127	-	163	46144	4248	186
o-Xylene	-	-	-	1961	-	210

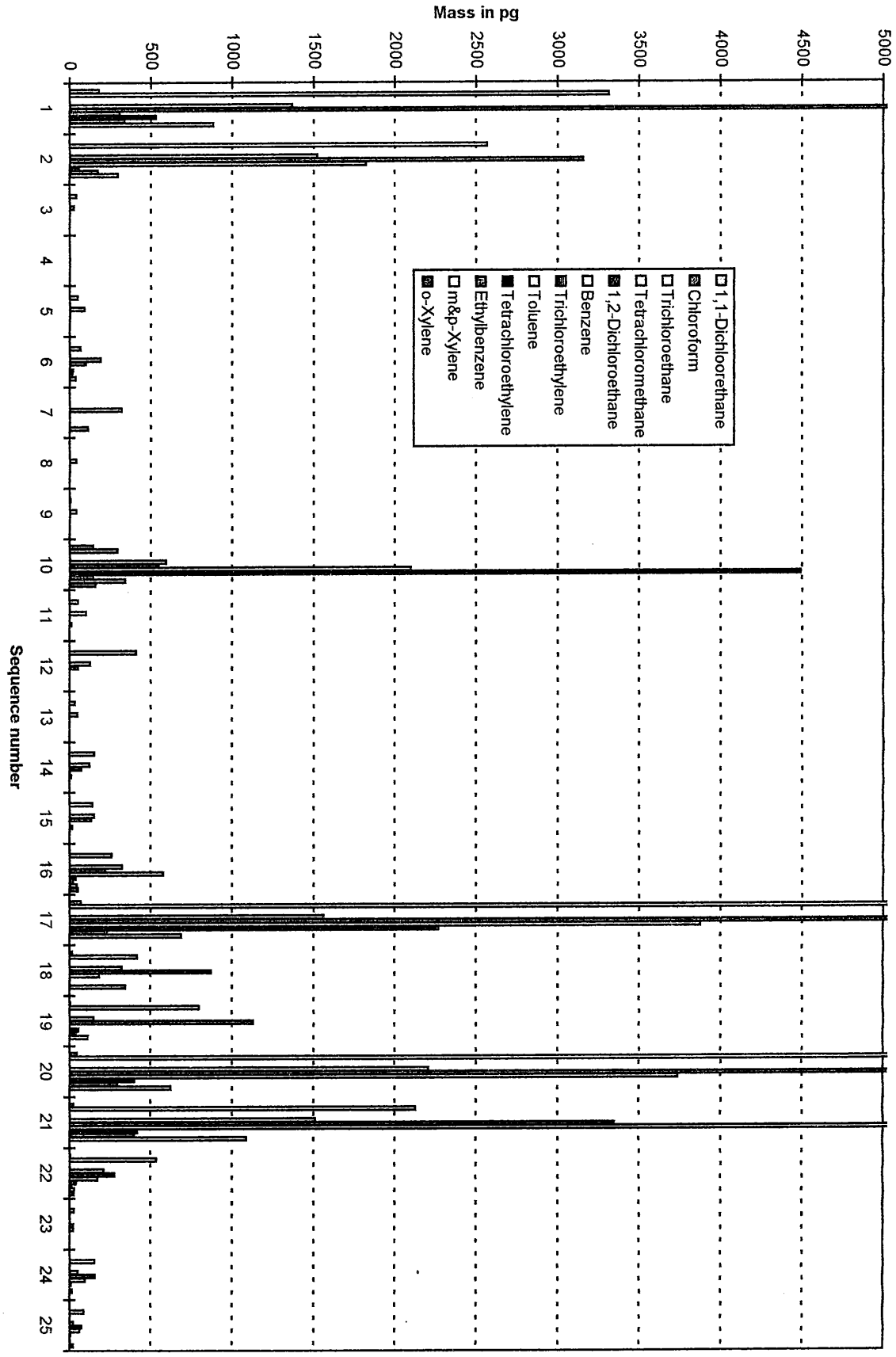


Figure II.3.24: Variability of the blank in air over a period of 1 week.

The long-term levels in the empty sparger were further compared with the blank concentrations in water. To that purpose a non-parametric Krukal-Wallis ANOVA test was run. The concentrations in water and the empty sparger of the different analytes were further compared with Dunn's post test.

Table II.3.8: Dunn's Multiple Comparison Test for the concentrations in water versus air. ns = not significant, ** significant.

Compound	Comparison	P value	Conclusion
1,1-Dichloroethane	water vs air	P > 0.05	ns
Chloroform	water vs air	P > 0.05	ns
Trichloroethane	water vs air	P > 0.05	ns
Tetrachloromethane	water vs air	P > 0.05	ns
1,2-Dichloroethane	water vs air	P > 0.05	ns
Benzene	water vs air	P > 0.05	ns
Trichloroethylene	water vs air	P > 0.05	ns
Toluene	water vs air	P > 0.05	ns
Tetrachloroethylene	water vs air	P > 0.05	ns
Ethylbenzene	water vs air	P > 0.05	ns
m&p-Xylene	water vs air	P > 0.05	ns
o-Xylene	water vs air	P < 0.01	**

The medians of the concentrations varied significantly for the different compounds ($p<0.001$). However, the concentrations in air and water of the different target compounds were not significantly different (table II.3.8), with the exception of o-xylene where the concentrations in water were significantly higher. Blank values were therefore more than likely originating from the same source. It was already argumented that the instrument itself serves as a trap and therefore as a source of contamination. The other possibility is the presence of these volatile compounds in the laboratory air. This was clearly demonstrated by the increased concentrations of chloroform in the blanks, when the product was used in another lab. Successive analyses of the empty sparger without exposing the system to the laboratory air resulted in a decrease of the blank values, although not an elimination. It is therefore concluded that both the instrument and the laboratory air are responsible for the presence of background concentrations of the analytes. In the light of further quantitative analysis of environmental samples, the blank values need to be accounted for both in the calculation of the actual concentrations and in the calculation of the limits of detection. As the daily blank values were not too variable it was thought safe to base the limit of detection on the standard deviation (SD) of the daily blank. The

limit of detection was therefore defined as the amount corresponding to the blank plus three SDs of the blank. In practice the variability of the blank lies around 30 %. Three times the standard deviation would amount to 90 %. The limit of detection was therefore in the first instance set at two times the daily blank. Bianchi *et al.* (1991) defined their blank values on the as the amount of analyte that gives a peak area response three times as great as the standard of the response obtained from the blank. The limit set for these experiments is more severe but was maintained none the less.

Repeatability and linearity of the system.

A series of tests was further run to determine the repeatability and the linearity of the system for this type of analysis.

The linearity of the system is readily demonstrated by plotting the average concentrations of the masses reported in table II.3.1 to their respective average heights (Fig. II.3.8). Considering the r^2 value of 0.9905 it was concluded that a linear relationship exists between the amount of analyte in the sample and the amount that is purged out.

To determine the repeatability a standard solution (see earlier) was injected into a luer lock syringe filled with 5 ml of water under identical analytical conditions as above. The results of the repeatability are given in table II.3.9.

The high variability of chloroform could be attributed to background problems, since high concentrations of chloroform were used in an adjacent room. Analysis were from that point forward performed in a room were no other solvents were present and the use of other solvents was carefully monitored. For the other compounds the variability ranges from 1.41 to 12.27 %. The overall variability was considered to be sufficient.

Table II.3.9: Short-term (1 day) variability of the Tekmar purge and trap system for the analysis of a water sample containing a standard solution of the target compounds. RSD = relative standard deviation, n = number of measurements.

Compound	Concentration in ng/g	RSD % n = 5
Chloroform	1.483	29.14
1,1,1,-Trichlororethane	1.349	3.77
Tetrachloromethane	1.594	6.03
Benzene	0.879	6.78
Trichloroethylene	1.464	4.03
Toluene	0.867	3.85
Tetrachloroethylene	1.623	12.27
Ethylbenzene	0.867	1.41
m,p-Xylene	1.623	3.78
o-Xylene	0.880	3.58

II.3.3 EVALUATION OF THE PURGE AND TRAP TECHNIQUE FOR SEDIMENT ANALYSIS

II.3.3.1 INTRODUCTION

The most straightforward method of analysis was to use the fritless sparger supplied with the Tekmar P&T apparatus. It was already demonstrated that the equipment could be used to quantitatively purge VOCs out of water with a sufficient repeatability. However, testing this set-up with a spiked sediment sample immediately revealed that additional heating was required to obtain an acceptable repeatability. The latter was subsequently tested by submerging the sparger in a water bath at 40 °C. Purging at elevated temperatures, unfortunately resulted in excess water in the system, which could not be handled by the moisture control module (MCM). This water seriously hampered subsequent analysis with the ITD-MS. The problems mentioned earlier with the background concentrations gave an additional impulse to the search for an alternative.

Based on P&T devices reported in literature several systems were tested. Initial tests used the set-up of Bianchi *et al.* (1991). This was soon abandoned for practical reasons. The method uses a large 1l bottle, also used for sampling, and contains ca

300-400 g of sediment. The authors report detection limits in the range 10-300 pg/g which is precisely the range aimed for. The bottles are, however, difficult to handle and it was soon discovered that similar LODs could be obtained using different purge vials and smaller sample sizes. Several other purge devices were then tested with varying results over a period of several months, but repeatability was generally below the standard that was set at minimum 30 %. Describing all tests would take too long, but the most successful method was finally based on Yan *et al.* (1992). The authors used a 8 ml vial with septum, that is punctured by two needles that serve respectively as the purge gas inlet and the purge gas outlet. They reported detection limits in the range of 3-50 ng/g. A similar approach was therefore tested using a 100 ml screw cap glass vial, as the bigger volume would allow a larger sample intake and therefore a lower detection limit. Finally, the method used for the determination of biota samples was adapted and tested for marine sediments. The method proved superior to the previous approach and was used form that point onward.

II.3.3.2 EVALUATION OF THE OFF-LINE P&T ANALYSIS OF SEDIMENT SAMPLES

II.3.3.2.1 Introduction

The off-line method for the determination of marine sediments is using a modified 100 ml screw cap vial as purging vessel. The cap was perforated and two lengths of 1/8" tubing were inserted (figure II.3.9). The system was rendered gas tight by pushing the tubing through a septum that was placed between the screw cap and the glass vessel. The purge inlet was connected directly to a gas cylinder and the purge outlet to a coil of 1/4" tubing inserted in a glycol bath cooled at -10 °C as a water trap. The P&T set-up was placed in an oven heated at 40 °C during the analysis. The analytes were finally trapped onto a Tenax trap coupled to the water trap. The latter is subsequently inserted in the Tekmar P&T, desorbed and analysed as described above. Up to 50 g of sediment could be analysed with this method. with a repeatability ranging from to and a recovery ranging from to. Optimum purge flow was set at 40ml/min and optimum purge time was set at 60 min. Further tests included the use of a 2 µm HPLC filter for better dispersion of the purge gas. The latter proved impractical as it greatly hindered introduction of the tubing into the sediment. The

major drawbacks of this method are the frequent occurrence of leaks at the level of the septum seal and frequent blocking of the water trap due to ice formation.

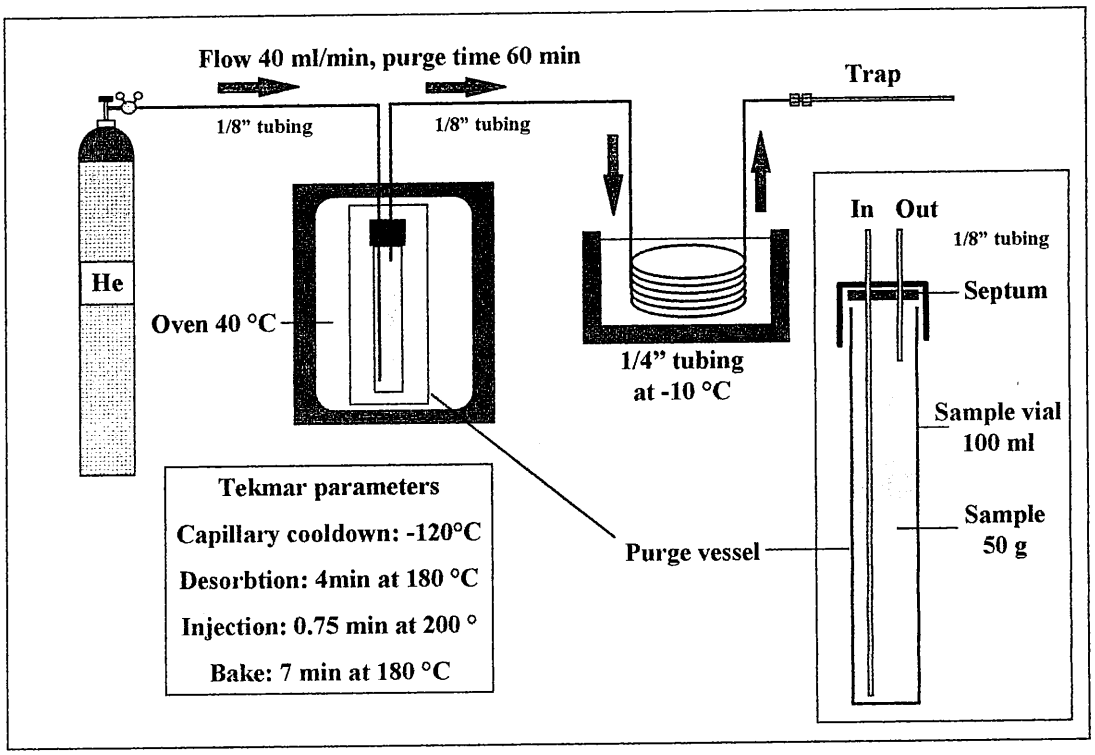


Figure II.3.9: Schematic overview of the off-line purge and trap method for the analysis of marine sediments.

II.3.3.2.2 Repeatability and recovery

In order to determine the repeatability and recovery a spiked sediment sample of about 70 g with VOC concentrations between 9 and 16 pg/g was prepared and analysed over a period of one week. The spiked sample was prepared by adding 5 ml of organic free water containing the VOCs to a pre-treated sediment sample and letting it stand for 24 hours. The sediment was treated prior to the test by heating at 150 °C in order to evaporate the VOCs. The blank sediment was stored in a sparging vessel and continuously purged with He. Charles and Simmons (1987) stated that the performance of sediment P&T techniques is primarily dependent on the physico-chemical properties of the analytes, rather than the intrinsic properties of the sediment. The effect of the treatment of the sample was therefore considered negligible and the recoveries and repeatability obtained with the test should reflect the

performance for actual samples. The sediment samples were analysed using the following operational parameters:

Tekmar: oven temperature: 250° C, mount temperature: 250° C, purge time: 12 min, dry purge time: 6 min, purge flow: 40ml/min.

Trap: Tenax, at room temperature during purging, desorbed at 180°C for 4 min, baked at 180° C for 7 min.

Transfer line: 250 °C

Injector: cryofocussing module, cooled to -120° C during desorbtion, heated from -120° C to 200 °C in 0.75 min during injection, standby temperature 250°C.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The results of the test are given in table II.3.10 and illustrated in figure II.10.

The recoveries thus obtained varied between 148 and 33 %. The high value of chloroform could be related to the use of the solvent in another laboratory in the same building, resulting in high background concentrations. This was, however, not the case for ethylbenzene, so a different reason lay behind the observed high recovery. The recoveries for the xylenes were particularly poor. Generally, the recoveries reported here are situated between those reported by Charles and Simmons (1987), who reported recoveries ranging from 38 % to 67 %, and those reported by Bianchi *et al.* (1991), who reported recoveries generally above 60%.

Table II.3.10: Recovery and repeatability of the off-line P&T analysis.

Compound	Recovery in %	RSD % n = 5
Chloroform	125	11
1,1,1,-Trichlororethane	49	18
Tetrachloromethane	na	na
Benzene	79	20
Trichloroethylene	55	17
Toluene	99	13
Tetrachloroethylene	62	9
Ethylbenzene	148	19
m,p-Xylene	37	20
o-Xylene	33	31

n = number of samples, RSD = relative standard deviation, na = not available.

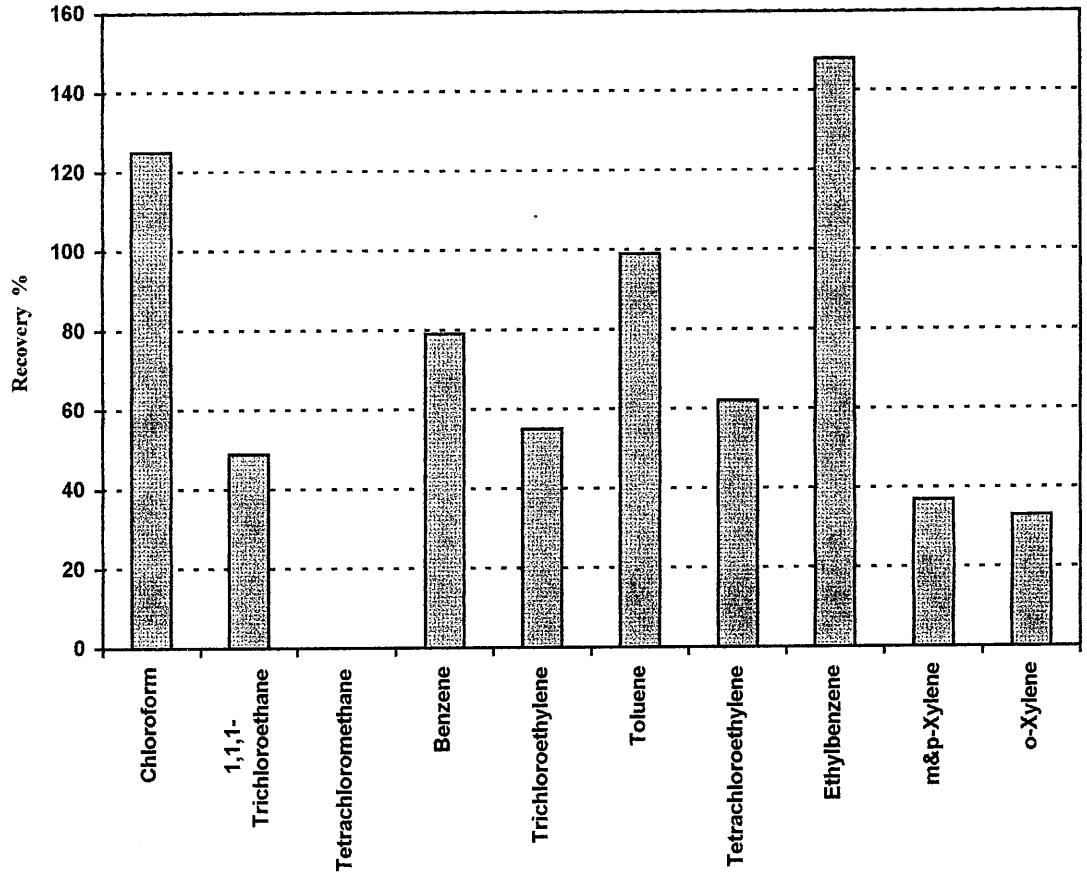


Figure II.3.10: Recovery of the different VOCs for the off-line method.

Bianchi *et al.* (1991) reported RSDs between 1 and 3 % on average. The RSDs observed for this method were a factor 10 higher but were , on the other hand,

considered to be quite acceptable. It can further be noted that the RSDs for the different VOCs are very comparable.

II.3.3.2.3 Limits of detection

The limit of detection was based on the blank value or on the S/N, in the absence of background concentrations, as described above. The variability of the daily blank was determined by analysing the blank sediment described in the previous section. As for the recovery test, 5 ml of water was added that contained in this case only the internal standard. The samples were analysed with the same operational parameters as given in the previous section.

The results of the calculation are given in table II.3.11. As the LOD is dependent on the sample intake the LODs were calculated for a standard sediment sample of 50 g.

For all VOCs the detection limit is in the lower pg/g range. Detection limits reported in literature range from 5 to 50 pg/g for the P&T techniques (Bianchi and Varney, 1989b; Bianchi *et al.*, 1991; Al-Rekabi *et al.*, 1995). The detection limits obtained with the method were therefore considered as sufficient.

Table II.3.11: LOD for the different VOCs with the off-line P&T analysis.

Compound	LOD in pg/g	RSD % n = 5
Chloroform	23	17
1,1,1,-Trichlororethane	9	17
Tetrachloromethane	9	7
Benzene	10	13
Trichloroethylene	7	18
Toluene	11	11
Tetrachloroethylene	16	20
Ethylbenzene	5	7
m,p-Xylene	6	14
o-Xylene	5	13

n = number of samples, RSD = relative standard deviation, LOD = limit of detection

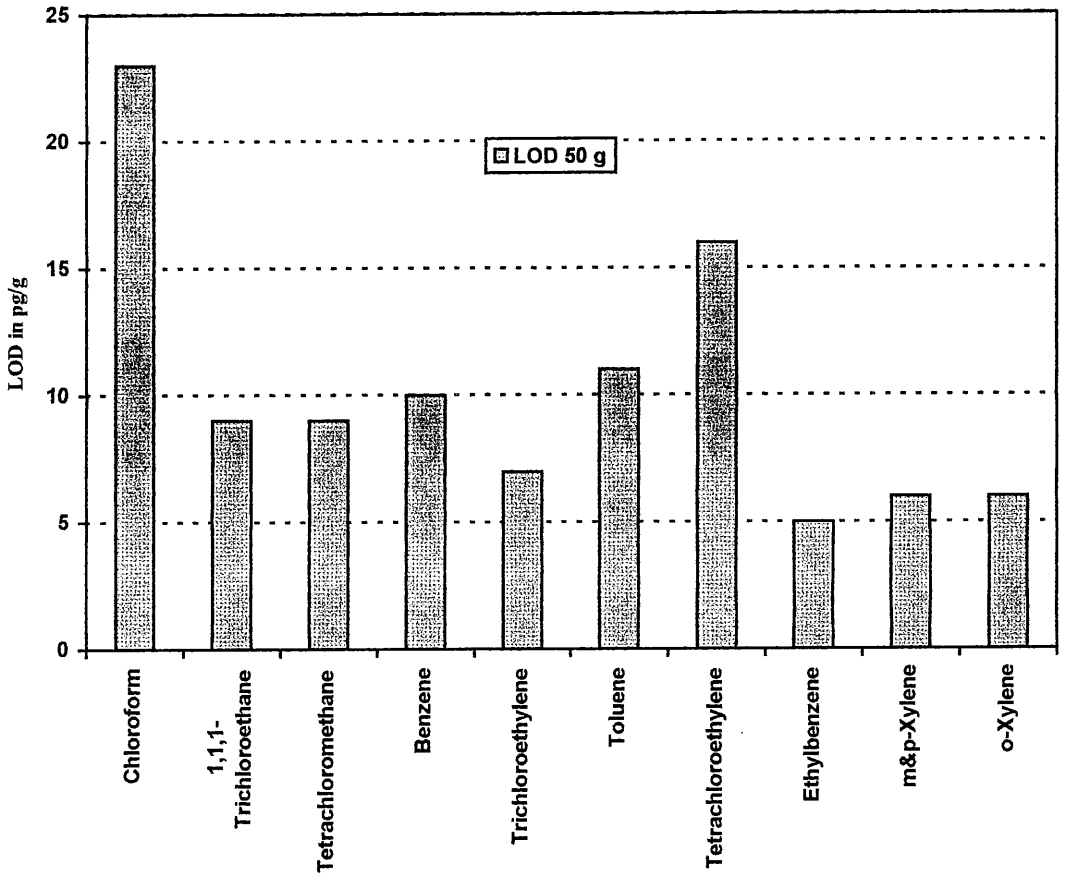


Figure II.3.11: LOD for the different VOCs with the off-line method.

II.3.3.3 EVALUATION OF THE ON-LINE P&T ANALYSIS OF SEDIMENT SAMPLES

II.3.3.3.1 Introduction

After a number of North Sea sediment samples was analysed using the previous technique it became obvious that the concentrations were generally below the detection limit. As a result, the research then focused on the development of an appropriate technique for biota samples. This lead to development of the method described in section II.4. The latter could, however, equally well be used for the analysis of VOCs in sediments and this was again tested. The method was finally adopted both for the analysis of sediments and biota samples and the validation of the methodology for sediments is given below. A detailed description of the determination of optimal purge time and flow and other operational parameters is given in II.4.

II.3.3.3.2 Recovery, repeatability and LOD

Recovery and repeatability were determined as above using a blank sediment sample spiked with a known concentration of the different VOCs. LODs were again determined on the basis of the variability of the blank or the SN. The practical sample intake for the 24 ml vial is limited to about 30g. For these experiments, a sample intake of about 10 g was used. The operational parameters are given in section II.3.4:

Table II.3.12: Recoveries, repeatability and LOD for the different VOCs with the on-line method.

Compound	LOD in pg/g			Recovery in %	RSD%
	10g	20g	30g		
Chloroform	25	12	8	89	8
Trichloroethane	5	3	2	109	7
Tetrachloromethane	6	3	2	na	na
Benzene	91	45	30	95	6
Trichloroethylene	7	3	2	111	10
Toluene	55	28	18	91	1
Tetrachloroethylene	32	16	11	96	7
Ethylbenzene	16	8	5	91	4
m&p-Xylene	46	23	15	93	6
o-Xylene	18	9	6	88	3

LOD = limit of detection, RSD = relative standard deviation, na = not available

Compared to the off-line method, The recovery and repeatibility of the on-line method are superior and much closer to those reported by Bianchi *et al.* (1991). Moreover, the LOD is as good or better than for the on-line method and again in the same order of magnitude as those reported in literature for the P&T techniques (Bianchi and Varney, 1989b; Bianchi *et al.*, 1991; Al-Rekabi *et al.*, 1995). The on-line method was therefore preferred to the off-line method and used for the determination of VOCs in marine sediments during the monitoring campaign.

II.3.3.4 ANALYTICAL QUALITY ASSURANCE

II.3.3.4.1 Introduction

Current QA practice for the determination of organic contaminants in sediments consists of the routine analysis of internal reference materials (IRMs), certified standard materials (CRMs), procedural blanks and recovery analysis (QUASIMEME).

Participation in intercalibration exercises is also recommended. The analysis of IRMs and CRMs allows the construction of a control chart that is a monitor for the analytical quality of the analysis.

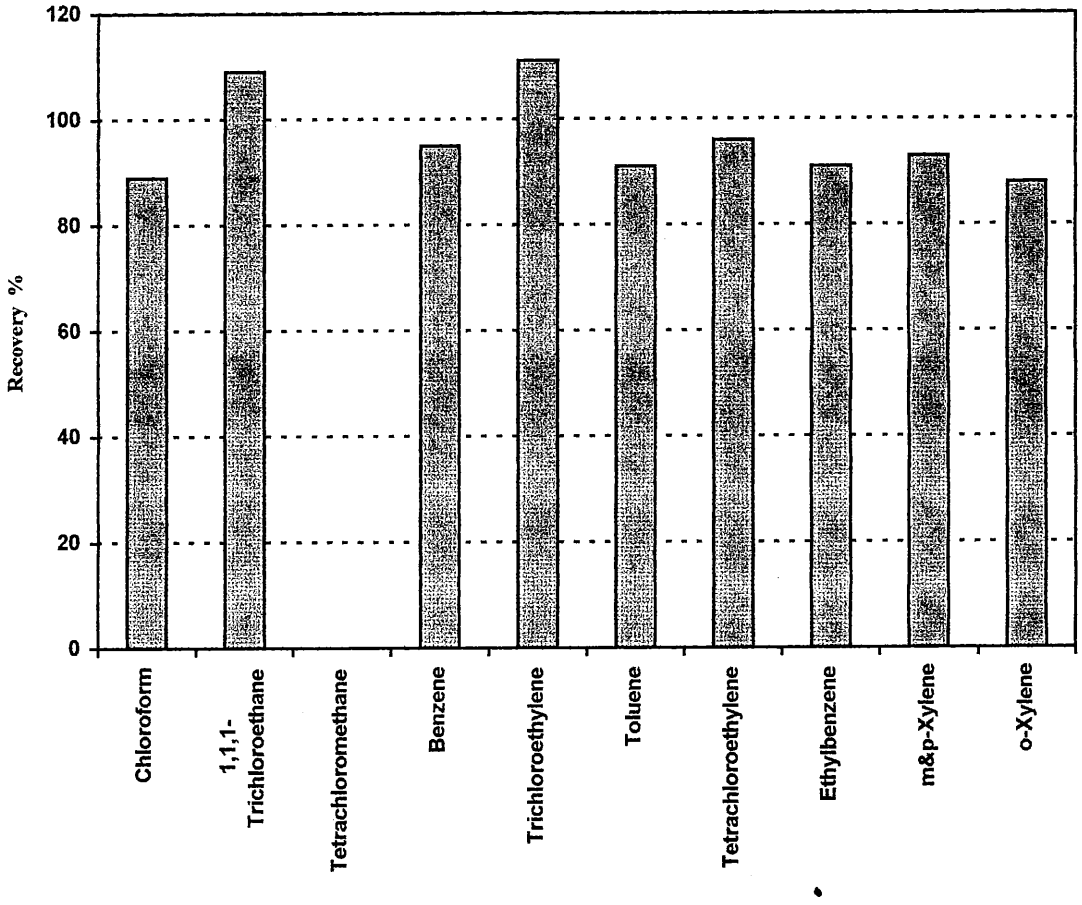


Figure II.3.12: Recoveries of the different VOCs for the on-line method.

Unfortunately, there is at present no CRM available for the analysis of VOCs in marine sediments. It is therefore impossible to verify the accuracy of the developed analytical technique other than testing recovery of the method. To monitor the reproducibility of the method over a long period of time an IRM could be developed. This would in turn allow the construction of a control chart and thus control of the analytical quality.

The development of an IRM would require to study the long term behaviour of VOCs in a homogenous sediment sample of sufficient quantity. This work was not

undertaken in the framework of this project, but it would probably become necessary in the prospect of continued monitoring. A number of QA measures was, none the less, used to assure the quality of the data produced. Most methods with the exception of the analysis of spiked samples are discussed more extensively in section II.4.

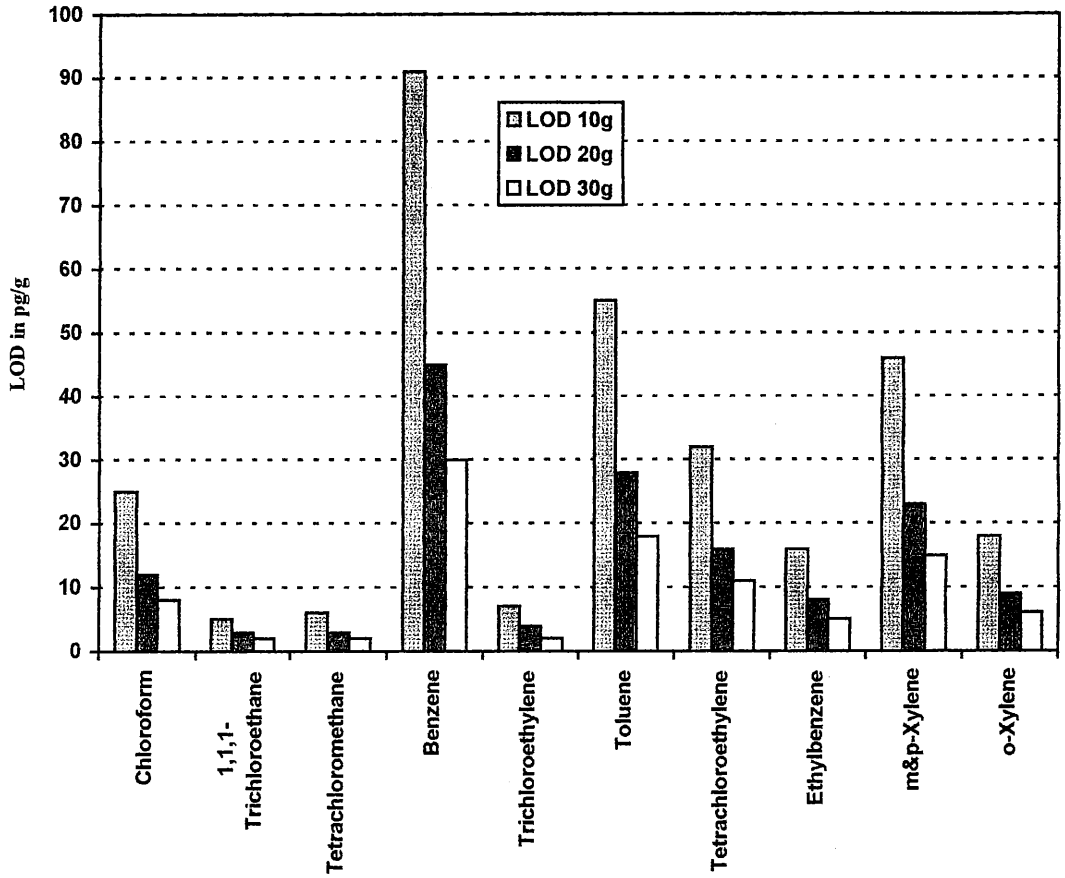


Figure II.3.13: LODs for the different VOCs, for different sample intakes using the on-line method.

II.3.3.4.2 QA measures

Blank analyses

First of all, a daily blank analysis accompanied each batch of samples. The latter is extremely important in view of the omnipresence of these contaminants. This is the first warning signal for the proper functioning of the analytical system. Very high blank values prohibit the analyses of real samples in which low concentrations are expected. As a rule blank values should be at least ten times below the those of the calibration solution.

Variability of the standard and standard analysis

A second QA measure is to monitor the relative height of the different VOCs during the analysis of the standard solution used for calibration. If the system is working properly no deviations from the median relative height are expected higher than 30 %. In the case that the warnings limits were exceeded, the analysis was halted and the system was evaluated for proper functioning. Very low values could often be attributed to a leak in the system and on some occasions to a degradation in the performance of the MS. Very high values were mostly due to high system contamination and a change in the response of the MS.

Analysis of spiked sediment samples

A third QA measure consists of analysing a spiked sediment sample as an unknown and to compare the calculated concentrations with the expected ones. Spiked sediment samples were prepared in the same way as described for the determination of the recovery. The analytical method is under control when the determined values falls within three standard deviations of the expected value. The standard deviation is determined by analysing a series of samples. As the sediments were analysed immediately after validation of the method, and that, due to the fact that concentrations of most VOCs were below detection limits, the analysis sequence was halted at an early stage, not enough data points are available to construct a quality control chart. The use of this QA measure, although imperative in the advent of future monitoring, is therefore not further discussed.

Identification of the target compounds

Positive identification of the target compounds was performed using both the retention time and the MS spectrum of the analyte. The high sensitivity of the ion-trap MS allows full scan spectra even at low concentrations. The practice for the identification and quantification of the target compounds was as follows: first the presence of the compound is verified by determining the presence of a peak in the retention window were the compound is expected, using the selected ions given in table II.3.2. If a peak is detected, the full scan mass spectrum is compared with the

one stored in the library. Only when a positive identification is made, the peak is quantified using the selected masses mentioned earlier.

II.3.4 EXPERIMENTAL SECTION

II.3.4.1 METHODS FOR THE DETERMINATION OF VOLATILE COMPOUNDS IN MARINE SEDIMENTS

Two methods, off-line and an on-line, were developed that allow the simultaneous determination of the volatile organochlorines chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene and the volatile aromatics benzene, toluene, ethylbenzene and the xylenes (BTEX) in marine sediments. As only the second method was retained for quantification of the sediment samples, this section only deals with the operational parameters for that method.

The method consists of transferring the sediment from the sampling container to a 24 ml sample vial. After addition of 15 ml of water with internal standard the vial is connected to a Tekmar LSC 2000 purge and trap apparatus coupled to a gas Finnigan-Magnum GC-MS. The volatiles are forced out of the sediment by purging with a stream of helium gas while heating at 70°C and trapped onto a Vocarb 4000 sorbent trap. After purging the trap is backflushed while being rapidly heated and the analytes are desorbed into a cryofocusing module connected to the analytical. The analytes are injected into the column by rapidly heating the module.

II.3.4.2 MATERIALS

All materials used for the various experiments and analyses were of research grade quality. The CHCs chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene were obtained (Merck) and the MAHs benzene, toluene, ethylbenzene and the xylenes (Merck) were used, without further purification, as standards in the experiments. Methanol (Baker, VOC) was used as solvent for the preparation of standard solutions. As internal standard (IS) α,α,α-trifluorotoluene (Aldrich) was used. Vocarb 4000 traps (8.5 cm Carbopack C, 10 cm Carbopack B, 6 cm Carboxen 1000 and 1 cm

Carboxen 1001) were obtained from Supelco and used as adsorption traps (1/8" OD). Water used for the preparation of blanks and standards was obtained from Baker. Purified sea sand (Merck) was used for the preparation of blank sediment samples and spiked sediment samples.

II.3.4.3 APPARATUS

A microprocessor controlled P&T system, Tekmar LSC-2000 (Tekmar, Cincinatti, USA) was coupled with a gas chromatograph - mass spectrometer system (Finnigan Magnum Ion trap, Finnigan, San Jose, USA), by a heated transfer line ending in a cryogenic focuser at the GC. The P&T system was provided with a 25 ml fritted sparger and moisture control module (MCM) as wet trap. The internal lines of the P&T are glass-lined stainless steel. The transfer line and internal lines are connected with a heated 6-port switch valve. The samples were purged using an impinger (Alltech) connected to purge gas outlet and the 25 ml frit sparger of the Tekmar (Tekmar, Cincinatti, USA). Samples were stored prior to analysis in 100 ml (Hach) or 24 ml sample vials (Alltech). The latter were for analysis coupled to the impinger with a Wheaton connector (Wheaton).

II.3.4.4 SAMPLING AND STORAGE OF THE SAMPLES

II.3.4.4.1 Sampling

Samples were collected using a Van Veen grab sampler in a similar way as Bianchi *et al.* (1991) and immediately transferred to sample containers that were filled until zero headspace was reached. The two major concerns during sampling are volatilisation of the analytes and contamination of the sample. Both are minimised by keeping the exposure time to the surrounding air as small as possible. No additional measures were taken.

II.3.4.4.2 Sample storage

As for sampling the two major concerns are contamination and losses due to volatilisation. Marchand *et al.* (1994) stored there samples frozen until analysis. This seemed the most practical approach for the current program as well. Sample

containers were therefore stored immediately after sampling at -28 °C in closed containers with zero headspace. As there are no direct sources of the VOCs in the storage room contamination would have to originate from the surrounding air. Yet the freezers are air tight. It therefore seems unlikely that this form of contamination would significantly attribute to the observed concentrations. The latter has not been investigated during this project, although it would become imperative in the prospect of prolonged monitoring of these compounds.

II.3.4.5 ANALYTICAL PROCEDURE

II.3.4.5.1 Preparation of blanks and standard solutions

Preparation of blanks

Water specially prepared for the analysis of VOCs (Baker) was used to prepare blanks and standard solutions (see further). The water was treated by heating it to a temperature of 90°C and simultaneous purging with helium (N 7.0, l'Air Liquide) or N_2 (N 6.0, l'Air Liquide) in a glass sparger. As a routine, water used for preparations, was continuously purged during storage with the gasses mentioned above.

For the preparation of blank samples 15 ml of the treated water was inserted in a 100 ml syringe and 4 µl of a known concentration of internal standard (α,α,α-trifluorotoluene, Aldrich) was added to the water in the syringe. The latter was done by inserting a 10 µl HPLC syringe in the opening of the 100 ml syringe. The water sample was then added to a blank sediment sample (see further) and the mixture is run through the entire analytical procedure.

Preparation of a blank sediment sample

A blank sediment sample is obtained by heating purified sea sand (Merck) in an oven at 150 °C and further purging it with He in a sparger. Purging is done continuously until the sediment is used for analysis.

Preparation of standard solutions

Methanol was chosen as solvent of choice for the preparation of standard solutions (see earlier). An initial standard solution (stock solution) was made by diluting 1 ml

of the different target compounds in 100 ml of methanol as follows: a small quantity (approx. 20 ml) of solvent was introduced in a volumetric flask and the weight was recorded. One ml of each of the target compounds was added to the methanol and at each occasion the weight is recorded. Finally, the volume was brought to 100 ml and the weight was again recorded. From the stock solution, dilution series are made by dissolving known quantities in methanol, again on a weight basis. Standard solutions are frequently renewed due to the high volatility of the analytes. The dilutions were continuously monitored for concentration differences. As rule, no concentration differences should be allowed that exceed the analytical variability.

For calibration of the system 4 µl methanolic solution with a known concentration (generally between 0.4 and 0.8 ng/µl) of the various target compounds was injected with a 10 µl syringe in an 100 ml syringe containing 15 ml of blank water (see above). Afterwards, another 4 µ of a methanolic solution containing the internal standard (about 0.4 ng/µl) was introduced into the 100 ml syringe with different 10 µl syringe. The water was then injected into a 24 ml sample vial (Alltech) that contained blank sediment (see above). The sample vial was subsequently connected to the on-line P&T set-up, pre-concentrated and analysed with the GC-MS.

II.3.4.5.2 P&T concentration of the sample

The glass vial containing the sample and VOC free water is coupled to an impinger connected to the P&T. The volatiles are forced out of the tissue by purging the sample for 30 min with a stream of helium gas at 10 ml min while heating at 70°C in a water bath. The analytes are trapped onto a Vocarb 4000 sorbent trap (Supelco) mounted in the P&T apparatus. The operational parameters of the P&T apparatus are given below:

Tekmar: Trap standby temperature: 45 °C, purge temperature: 35 °C, mount temperature: 40 °C, oven temperature: 250 °C, line temperature: 250 °C, cryo union standby temperature: 250 °C.

II.3.4.5.3 Desorption and cryofocussing

After purging the trap is backflushed while being rapidly heated at 250 °C and the analytes are desorbed into a cryofocusing module cooled to -120°C and connected to the analytical column.

II.3.4.5.4 Injection, chromatographic separation and detection

The analytes are injected into the column by rapidly heating the cryofocussing module from -120°C to 200 °C in 0.75 min. Separation is done on a Restek, RTx-502.2, 60 m, 0.32 mm i.d., 1.8 µm film. With the following GC conditions:

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The target compounds are detected on a Finnigan Magnum Ion trap detector on the basis of retention times and mass spectra and quantified using the total mass of selected ions (table II.3.2). The operational parameters of the mass spectrometer are given below:

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

II.3.5 CONCLUSIONS

A method was developed and validated that allowed the quantification of the target compounds in marine sediments with sufficient sensitivity, repeatability and recovery. The method can however be further improved with an emphasis on the reduction of background concentrations of the analytes, further reduction of the analysis time and easier handling.

The purge and trap technique has proven to be a valid approach, but some drawbacks are, nevertheless, encountered. The major problem is associated with the inability to obtain perfect blanks with the current P&T apparatus. On frequent occasions the instrument has proven to be either a source or a trap of the analytes in question. Problems are also associated with the sorbent trap that were used. Usually a Vocarb 4000 trap (Supelco) is preferred. However, after a period of extensive used the trap will contribute seriously to the blank problems by releasing benzene and presumably also toluene.

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II.4. BIOTA

II.4.1 INTRODUCTION

Prior to selecting an appropriate technique for the analysis of VOCs in marine biota, a number of criteria were set. The method should, first of all, meet the basic analytical criteria such as a sufficient recovery, good reproducibility and low detection limits. Apart from that, it seemed essential that the method should be easy to use, rapid and involve as little sample handling as possible.

Reviewing the different methods documented in the literature (see earlier), several analysis strategies for the determination of VOCs in marine biota were identified. Of these a number were selected for further evaluation in view of the criteria set above. In a first strategy volatiles are forced out of the tissue by using vacuum distillation (VD) (Hiatt, 1981; Hiatt, 1983; Newman and Gshwend; 1987; Hiatt *et al.*, 1994) and trapping (VD&T). A second strategy uses an inert gas to purge the volatiles out of the system (Easley *et al.*, 1981; Ferrario *et al.*, 1985), followed by trapping (P&T). Finally steam distillation can be used (Page and Lacroix, 1995) again followed by trapping (SD&T). Either glass vessels cooled to -78 °C or -196 °C (Hiatt, 1981; Hiatt, 1983) or adsorbent traps with materials such as Tenax (Easley *et al.*, 1981; Ferrario *et al.*, 1985) or Vocarb (Page and Lacroix, 1995) are used as trapping devices. On the onset of the project the various techniques were evaluated and a number of tests were performed using steam-distillation, static headspace and various P&T devices. As a result, the P&T technique in combination with adsorbent traps (Tenax, Vocarb) was selected as the most appropriate, especially in view of the possibility of the on-line determination of VOCs by combining a commercial P&T apparatus with a GC-MS system (see earlier).

II.4.2 EVALUATION OF THE PURGE AND TRAP TECHNIQUE

II.4.2.1 INTRODUCTION

The method of Easley *et al.* (1981) served as a basis for development. The authors used a sparging vessel similar the fritless sparger of the Tekmar LSC-2000 P&T and Tenax/Silicagel/Activated carbon (1/1/1) as sorbent trap. The P&T apparatus described in section II.4.1. was used both as an on-line P&T system and as a desorbtion unit for off-line P&T. An identical sparger was purchased and coupled to the Tekmar (on-line analyisis) or used as a stand-alone purging vessel (off-line analysis). The use of other spargers was further compared with the design of Easley *et al.* (1981). This set-up was tested on-line and off-line and the optimal conditions were determined.

Using the sparger of the Tekmar directly was unfeasible, as the sample is purged at elevated temperatures which results in excess water. Previous experiments already demonstrated the inability of the MCM (moisture control module) to deal with excess water so an additional water removing step is required.

II.4.2.2 PRELIMINARY TESTS

In order to obtain an idea of the presence of VOCs in fish tissue and to test the applicability of the P&T technique a series of preliminary tests were performed.

In a first series of tests 5 g tissue was extracted in 5 ml methanol. The mixture was subsequently centrifuged at 5000 rpm. 50 µl supernatant was injected into a luer-lock syringe filled with 5 ml of water. After addition of 100 µl of a methanolic IS solution, the mixture was transferred into the sparging vessel of the Tekmar P&T. The sample was then analysed using the following operational parameters:

Purging apparatus: Tekmar LSC-2000 with 5 ml sparger, oven temperature: 250° C, mount temperature: 250° C, purge time: 12 min, dry purge time: 6 min, purge flow: 40ml/min.

Trap: Tenax, at room temperature during purging, desorbed at 180°C for 4 min, baked at 180° C for 7 min.

Transfer line: 250 °C

Injector: cryofocussing module, cooled to -120° C during desorbtion, heated from -120° C to 200 °C in 0.75 min during injection, standby temperature 250°C.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The relative surface to the IS was compared with the values obtained from the analysis of blank and a standard solution. The results for various organisms are given in table II.4.1.

Table II.4.1: Estimated concentrations (A) in ng/g and ratio between the height of the sample and the height of the blank (B) of VOCs in marine biota.

	Flounder 1 *Platichthys flesus*		Flounder 2 *Platichthys flesus*		Plaice *Pleuronectes platessa*		Brown Shrimp *Crangon crangon*	
	A	B	A	B	A	B	A	B
Chloroform	14.29	5.5	nd	0.8	27.99	3.6	121.28	4.9
Trichloroethane	nd	na	nd	na	nd	na	35.65	27.6
Benzene	nd	1.3	nd	0.3	22.60	1.7	nd	1.2
Trichloroethylene	nd	na	nd	0.6	nd	0.6	nd	1.0
Toluene	13.44	1.7	nd	0.3	25.17	2.7	34.44	2.3
Ethylbenzene	nd	0.1	nd	1.5	18.48	1.8	22.92	2.1
m&p-Xylene	nd	1.5	nd	0.3	38.13	19.7	nd	0.9
o-Xylene	nd	1.4	nd	0.9	10.27	4.3	nd	0.4
Tetrachloroethylene	nd	na	nd	1.5	36.80	8.5	nd	na

nd = not detectable, na = not applicable

A peak with a height of approximately two times that of the blank (see earlier) was considered detectable and a concentration was estimated. The most important conclusion of this experiment was the presence of significant amounts of VOCs in marine biota. Unfortunately, the procedure involves a 100 fold dilution of the original

extract and therefore allowed only a detection limit of approximately 20 ng/g for most VOCs.

The effect of the homogenisation technique was investigated in the next phase. About 5 g fish tissue, originating from one fish (*Limanda limanda*) was homogenised in 5 ml of methanol both with an ultra-turrax homogeniser (Janke & Kunkel) and a motor driven Potter-Elvejhem homogeniser (B. Braun). The extract was further treated and analysed as above. The results are given in table II.4.2. In each instance, the relative height of the sample (to the IS and the sample amount) is compared with the relative height of the blank obtained under the same conditions. These experiments clearly illustrated the fact that homogenisation with the Ultra-Turrax results in higher concentrations of most VOCs. The reason for this is assumed to be the intense tissue disruption caused by this type of homogeniser.

Table II.4.2: Ratios between the relative height of the samples (to IS and sample amount) and the blanks obtained for the analysis a 100 µl methanolic homogenate (ultra-turrax mixer and Plotter- Elvejhem homogeniser) in 5 ml of water and direct analysis in the fritless sparger of the Tekmar filled with 5 ml of water.

Compound	Ultra-turrax	Potter-Elvejhem	Sparger
Chloroform	10.96	nd	10.55
Trichloroethane	2.78	nd	2.53
Tetrachloromethane	na	na	nd
Dichloroethane	na	na	na
Benzene	2.71	nd	7.38
Trichloroethylene	3.26	nd	nd
Toluene	5.97	nd	18.75
Ethylbenzene	9.09	nd	17.91
m&p-Xylene	8.03	nd	12.71
o-Xylene	7.96	nd	15.56
Tetrachloroethylene	15.87	2.61	159.54

nd = not detectable, not applicable.

In another experiment, biota tissue was purged directly in the Tekmar sparger under identical analytical conditions as described above. Again, significant amounts of VOCs could be purged out of the tissue (table II.4.2). The results further suggest that the concentrations obtained with this method would be higher. The detection limits were in any case lower since the dilution factor was eliminated.

II.4.2.3 DETERMINATION OF ANALYTICAL CONDITIONS

II.4.2.3.1 Sample treatment

Sample treatment involves dissection of the organism to obtain the edible tissue and processing of the tissue. The different ways of sample treatment reported in literature are homogenisation (Hiatt (1983), Ferrario *et al.* (1985), Pearson and Mc Connel (1975))and ultrasonication (Easley *et al.* (1981)) (see earlier). Samples are generally treated under cooled conditions. Hiatt (1983) added liquid nitrogen during chopping while Easley *et al.* (1981) cooled the sample in an ice bath during ultrasonification. Finally, Ferrario *et al.* (1985) performed the entire procedure at 4 ° C. As the method of Easley *et al.* (1981) was selected as a basis for development, the ultrasonication step was used. Although the latter results in a severe tissue disruption it remained to be determined whether additional homogenisation was necessary.

The preliminary experiments demonstrated the higher performance of the Ultra-Turrax homogeniser (Janke & Kunkel). This type of homogeniser was therefore used in this experiment. In order to compare the effect of homogenisation the left fillet of whiting (*Merlangius merlangus*) was entirely homogenised in an ice bath at 0 °C and divided into four portions. The right fillet was cut into four pieces from head to tail. The samples were transferred to a 24 ml sample flask and 10 ml water containing 1 ng α,α,α,-trifluoromethylbenzene (trifluorotoluene or TFT) as internal standard was added. The samples were analysed with a similar set-up as Easley *et al.* (1981). The experimental set-up is illustrated in figure II.4.1.

After sonication for 2 min, the samples were connected to an impinger type purge vial (Easley *et al.* (1981)). The entire vessel was thans placed in a water bath heated at 70 °C and conditioned for 2 min. The sample was then purged with a flow of 10 ml/min for 5 min. An additional water trap consisting of a glass spiral cooler (-10 °C) is placed between the purging vessel and a Vocarb 4000 trap (Supelco). After purging the trap is transferred to the Tekmar LSC-2000 to desorb the analytes.

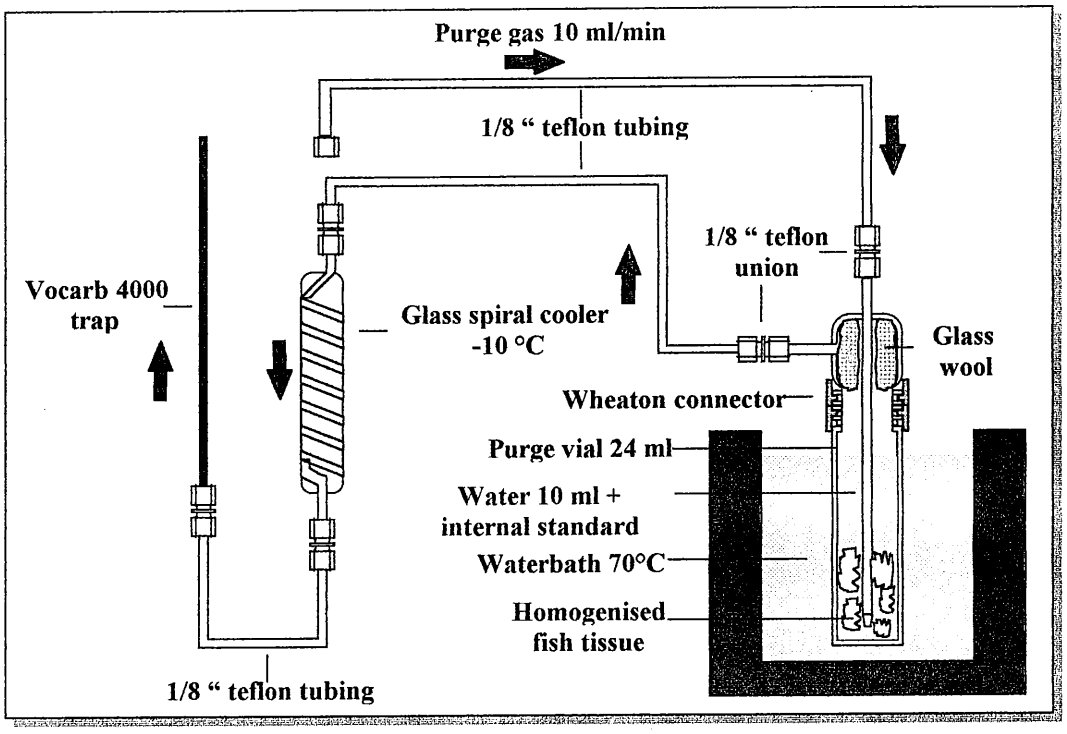


Figure II.4.1: Off-line P&T setup.

The analytical conditions were as follows:

Trap: Vocarb 4000, at room temperature during purging, desorbed at 250°C for 10 min, baked at 260° C for 4 min.

Injector: cryofocussing module, cooled to -120° C during desorbtion, heated from -120° C to 200 °C in 0.75 min during injection, standby temperature 250°C.

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

Transfer line GC-MS: 250 ° C.

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The results of the experiment are given in table II.4.3 and illustrated in fig. II.4.2.

Table II.4.3: effect of homogenisation on the analysis of fish tissue samples with the method of Easley *et al.* (1981).

	Not homogenised		Homogenised	
	Average (ng/g)	RSD %	Average (ng/g)	RSD %
Chloroform	3.20	43	13.62	21
Trichloroethane	0.19	47	0.16	18
Tetrachloromethane	nd	nd	nd	nd
Benzene	1.94	13	2.04	51
Trichloroethylene	nd	nd	5.53	10
Toluene	2.52	57	1.20	28
Tetrachloroethylene	5.28	45	2.07	19
Ethylbenzene	2.44	50	1.70	9
m&p-Xylene	20.10	133	2.96	15
o-Xylene	1.40	53	1.44	13

nd = not detectable, RSD = relative standard deviation.

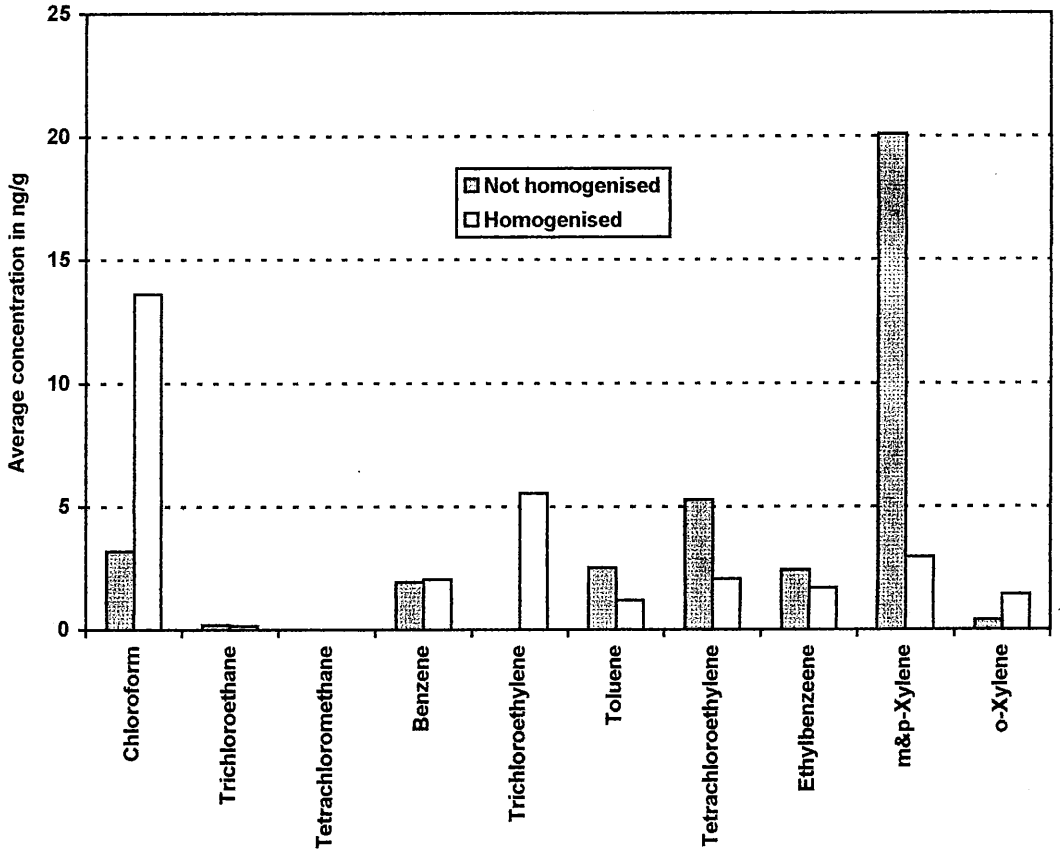


Figure II.4.2: Comparison of concentrations obtained with homogenised and unhomogenised tissue.

The average concentrations in homogenised and non-homogenised tissues were similar, with the exception of chloroform, trichloroethylene and m&p-xylene, but the relative standard deviations were a lot higher when the tissue was not homogenised. Only for benzene the RSD was worse for the homogenised samples. The results thus indicated a non-homogeneous distribution of the contaminants in the tissue and homogenising is therefore recommended. The results further indicated that homogenising under controlled conditions will not lead to significant losses of analytes. The high average values for tetrachloroethylene and m&p-xylene in the first column were explained by a single high value. The reason for this could either be related to the analytical technique or to the tissue itself, but this was not further investigated.

II.4.2.3.2 Purge flow and purge time

Increasing the purge flow in the experimental set-up described in the previous section resulted in a severe foaming. The high temperature of the sample (70 °C) causes denaturation of tissue proteins, which on their turn cause foaming at elevated flows. The lean muscle tissue analysed thus far exhibited little or no foaming at a flow rate of 10 ml/min. Searching an optimal purge time seemed therefore more advantageous than searching an optimal purge flow. However, in certain circumstances and for certain tissues, sample foaming did occur. This is discussed in the next section.

In order to determine the optimal purge time a homogenised fish muscle tissue sample (*Merlangius merlangus*) was prepared according to the procedure described above. After sonication for 2 min the sample was analysed as above (purged at 10 ml/min for 5 min). The analysis was subsequently repeated three times with the same sample in order to determine whether additional VOCs could be purged out of the tissue. The results are illustrated in fig. 5.II.3.

The figure indicates that significant amounts of at least tetrachloromethane were still purged out of the tissue after 20 min. A purge time of at least 20 is therefore required.

To further evaluate the purge time another sample was prepared in the same manner and immediately purged for 25 min, again followed by 5 min increments of the purge time. The results of this experiment are given in fig. II.4.4.

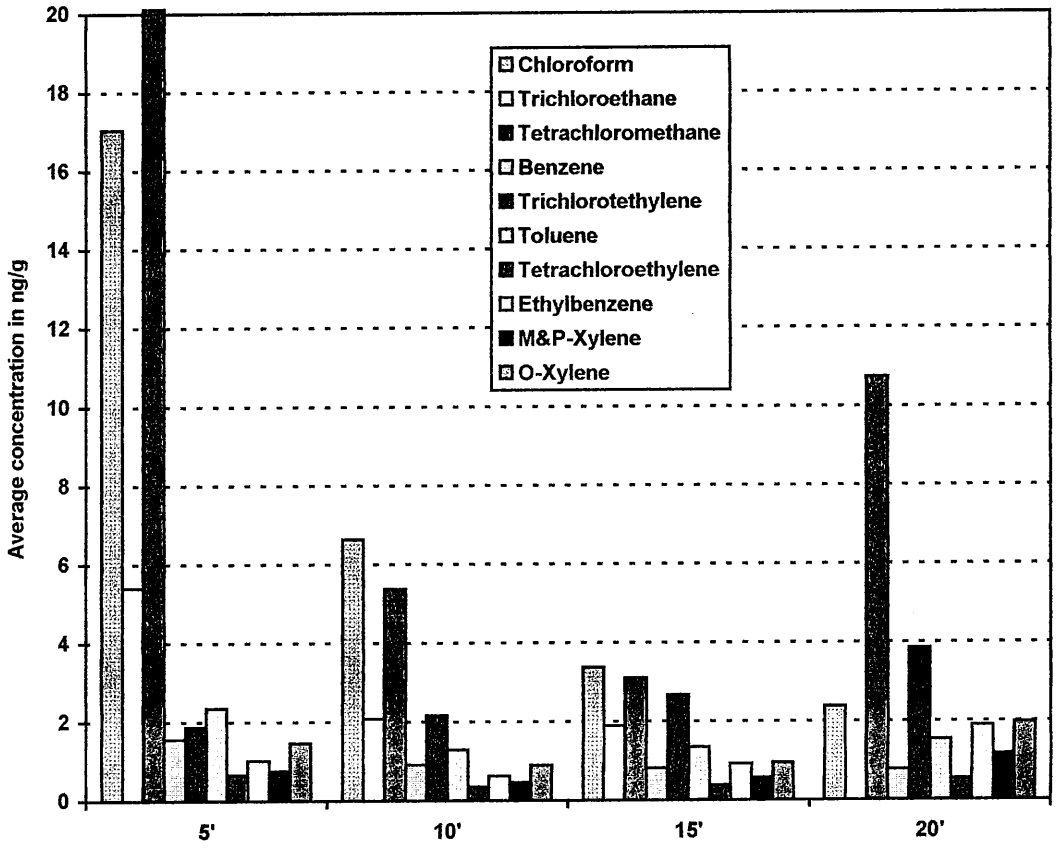


Figure II.4.3: Height relative to the blank of VOCs after successive purging for 5 min.

The figure indeed suggests that a purge time of 25 min is sufficient to purge all the VOCs out of the tissue.

In a final experiment homogenised fish muscle tissue was prepared and divided into 5 portions. The portions were individually analysed as described above with a purge time of respectively 20, 30, 40 and 60 min. The results are illustrated in fig. II.4.5.

The results indicate that the optimum purge for the CHCs is around 30 min and for the MAHs around 40 min. A purge time of 40 min is, however, not recommended as

the amount of tetrachloromethane purged out of the tissue drastically decreases after 30 min.

In view of the experiments in this section a purge time of 30 min was considered optimal for the simultaneous determination of CHCs and MAHs in fish tissue. In a final step the breakthrough of the trap was tested using a second trap coupled to the first. The results revealed that the presence of VOCs in this trap was not different from that of a blank. It was therefore concluded that no breakthrough occurs under the above conditions.

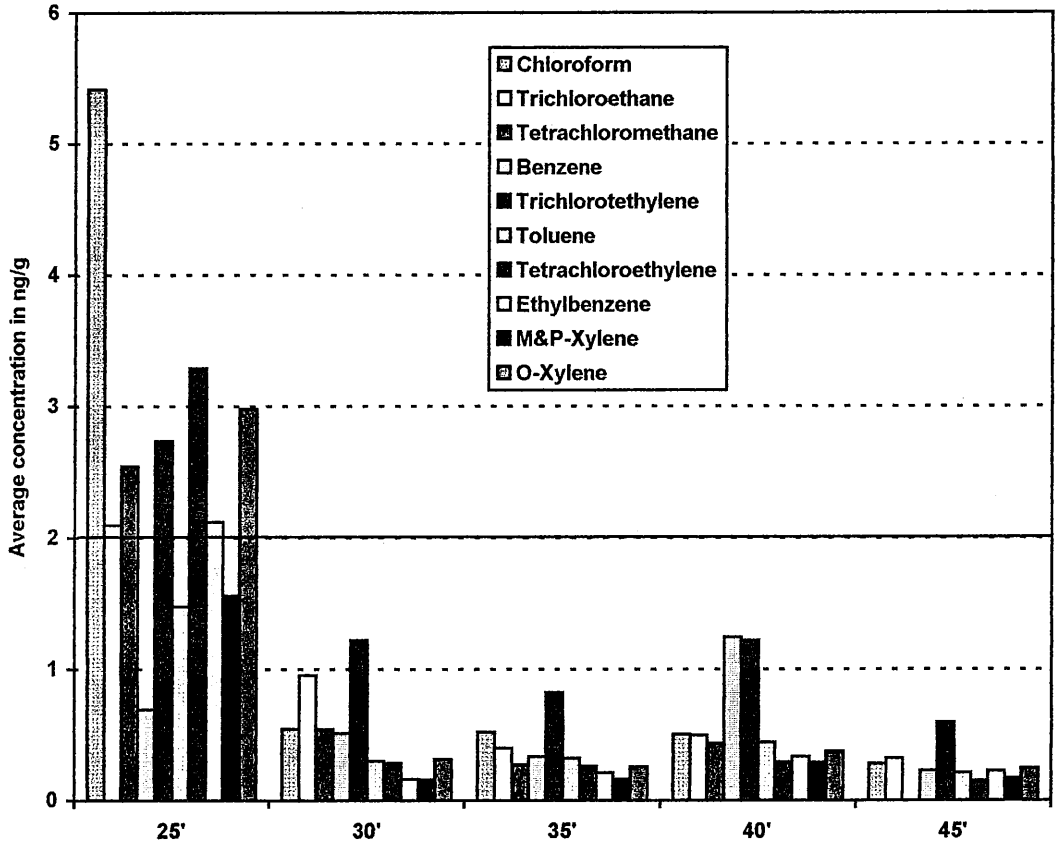


Figure II.4.4: Height relative to the blank of VOCs after successive purging for 5 min from 25 min onwards.

II.4.2.3.3 Sample foaming

Sample foaming is the result of denaturation of proteins at the elevated temperatures during purging and can cause a serious contamination of the experimental set-up. If foam reaches the trap it can lead to deactivation of the trap and introduction of thermal decomposition products from labile, non volatile materials (Rose and Colby,

1979). The most straightforward way to reduce sample foaming is reducing the purge flow or by inserting a mechanical barrier. Easley *et al.* (1981) observed no foaming at the flow rates and with the equipment they used. Using a similar set-up, sample foaming did occur for certain tissues. Inserting glass wool in the purge vial as a mechanical barrier generally prevents the foam from reaching the trap, but on certain occasions this proved not sufficient. Michael *et al.* (1980) did also not succeed in breaking the foam bubbles by using glass wool as a "foam trap". A method was therefore searched that eliminated sample foaming.

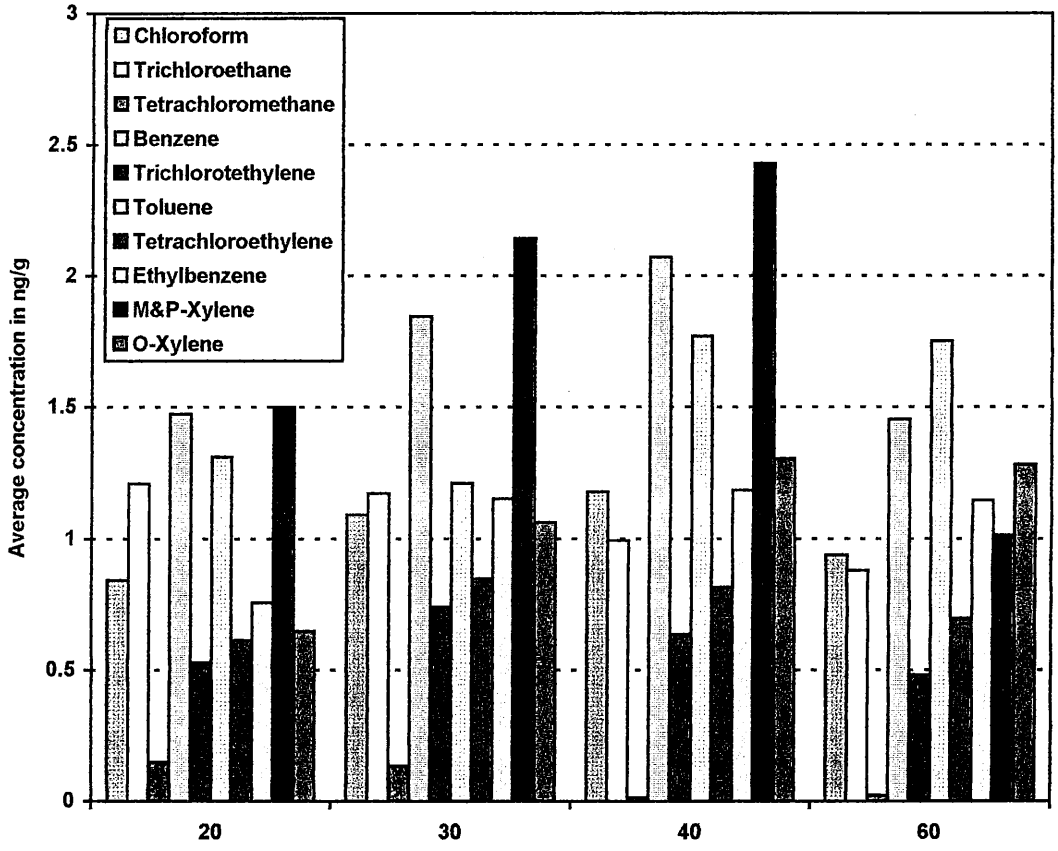


Figure II.4.5: Relative height of VOCs with purge times of 20, 30, 40 and 60 minutes.

Rose and Colby (1979) discussed the use of both a silicone antifoaming reagent and heat dispersion. The use of the antifoaming agent resulted in spurious peaks in the chromatogram. The severity of these peaks increased with each analysis and it eventually became necessary to replace the trap and bake the transfer lines. Using a similar antifoaming agent (antifoam, Vel), the occurrence of a number of unknown interfering peaks was indeed observed. Michael *et al.* (1980) made similar

observations when using Dow corning antifoam. The presence of high amounts of chloroform could be attributed to the fact that the antifoaming agent was used in a lab were chloroform is frequently used for lipid extractions. To remedy this, antifoaming agent was added to water and this solution was purged overnight with He. However, using this water for analysis did not reduce the presence of interfering peaks. Using a heatgun as did Colby and Rose (1979) was not entirely efficient and proved very impractical, as it requires the presence of the analyst for a period of 30 min.

As an alternative, 1 and 10 % phosphor wolframic acid (Merck) in the water were used as antifoaming agents. However, no sufficient reduction of sample foaming could be reached. Coagulation of the proteins with NaCl (Merck) in combination with defrosting in the microwave was equally inefficient in reducing sample foaming.

During the first period of analysis a combination of a reduced purge flow and glass wool as a mechanical barrier were used to prevent or reduce sample foaming. This method is generally successful, but nevertheless requires careful monitoring of the set-up. Sample foaming still occurs under these conditions with possible disastrous effects.

The use of n-octanol (50 µl) as an anti-foaming agent was recently investigated. The first results indicate an efficient reduction of the sample foaming and did not reveal any adverse effects on the analysis. Strikingly, Michael *et al.* (1980) reported only moderate success when using n-octanol. This method will be further investigated.

II.4.2.3.4 Comparison between off-line and on-line determination

All the analyses performed thus far were performed with an off-line P&T device. In the next step the feasibility of on-line P&T was investigated and compared with off-line P&T.

To investigated the feasibility of on-line P&T, the vessel used for the off-line determination was coupled to the sparger of the Tekmar (figure II.4.6). As for the off-

line set-up, a glass cooler (-10 °C) serving as a water trap was initially placed between the purge vial and the Tekmar sparger. As there is no substantial change in the set-up, apart from the coupling to the Tekmar, the experimental conditions defined above should apply to both methods. The main focus was therefore set on the values and the variability of system background concentrations. To this purpose, a blank water sample was analysed several times using both experimental set-ups. The analytical conditions were as above with some additional parameters for the on-line determination given below:

Tekmar: Trap standby temperature: 45 °C, purge temperature: 35 °C, mount temperature: 40 °C.

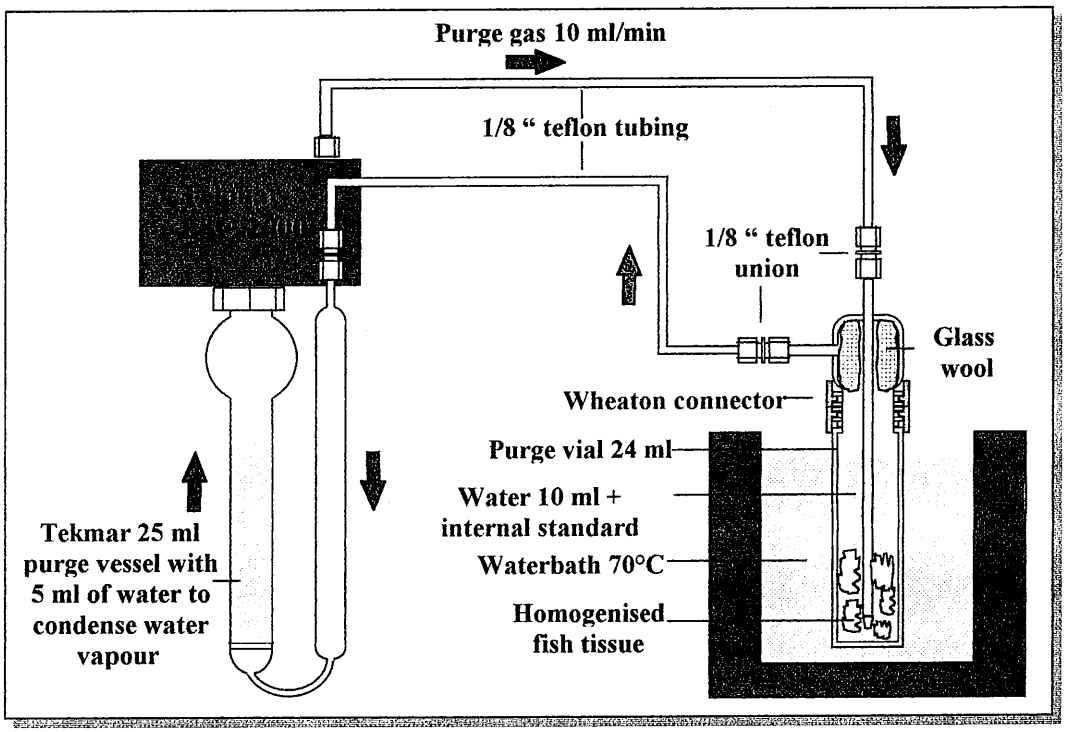


Figure II.4.6: On-line P&T set-up.

The results of both series of analysis are given in table II.4.4.

The average values of the peak surface of the internal standard were 685577 ± 19% and 680696 ± 13% for the off-line and on-line set-up, respectively. This proved that the experimental conditions apply for both methods. However, the blank values were in general significantly lower when using the on-line method, with the exception of benzene (originating from the trap) and trichloroethylene. The surface of the peak of

the latter was however very small and the compound was only detected in two of the blank analyses, which explains the high standard deviation. The variability of the blanks was, in general, lower for the analyses performed with the on-line set-up. As a result, further test to validate the method were carried out using the on-line set-up.

Table II.4.4: Average, relative standard deviation (RSD) and ratio between the averages of analyses blank water with the off-line and on-line set-up.

Compound	Off- line		On-line		Ratio
	Average (n=6)	RSD %	Average (n=5)	RSD %	
Chloroform	213544	37	1880	36	114
Trichloroethane	2919	14	0	nd	na
Tetrachloromethane	nd	na	nd	na	na
Benzene	264922	41	349242	16	1
Trichloroethylene	1631	26	1044	127	2
Toluene	183325	37	20603	12	9
Tetrachloroethylene	48726	36	nd	na	na
Ethylbenzene	195927	30	4993	12	39
m&p-Xylene	284111	26	11037	17	26
o-Xylene	66034	25	1413	20	47

n = number of analyses, ratio = average off-line/average on-line

II.4.2.3.5 Elimination of excess water

It was already discussed that eliminating the water vapour formed during purging at elevated temperatures is a prerequisite to a proper analysis. The glass cooler used so far had the disadvantage of frequent clogging of the line due to the formation of ice. As an alternative, the sparger of the Tekmar was filled with water kept at room temperature, in order to condense the water vapour present in the purge gas. Water is then further eliminated in the MCM of the Tekmar (see section II.3). This method has proven valid in most cases, but under certain circumstances the system does not entirely eliminates the water. This was especially the case after extended periods of operation. A more recent method consists of cooling a purge vessel, identical to the one used for the samples, to -5 °C. This is done by enveloping the vial with a plastic bag through which glycol (- 5 °C) is circulated. This method combines the successful elimination of water at low temperatures and the ease of using the sparger and is routinely used at present.

II.4.2.4 REPEATABILITY

In order to determine the repeatability of the method a homogenised fish muscle tissue sample was prepared and analysed on five separate occasions with the on-line method described above. To determine the long term reproducibility a homogenous sample of sufficient size would be needed that allows a series of analyses over a period of at least one week, but preferably one month. As the behaviour of the VOCs in homogenised tissue is unclear, this would need to be investigated. The use of a sufficiently large fish is not recommended, as it was proven that the VOCs are not homogeneously distributed in the tissues. The development of a reference material was not undertaken in the framework of this project due to the considerable practical difficulties that still accompany the current methodology. However, it would be inevitable in the prospect of further monitoring. This test was therefore limited to the repeatability or the short term variation of independent analyses of the same sample. The number of samples, namely five, is representative for the average number of samples that can be analysed during per day. It was already discussed that the variability of the blank was only more or less within limits during the timespan of one day and that background values tended to vary considerably over extended periods of time. The long term reproducibility will therefore be largely influenced by the background levels and a day to day approach is preferred. Samples are therefore processed batchwise in such way that each batch can be analysed the same day, with an accompanying blank and standard analysis. The homogenised sample used for the determination of the repeatability therefore represented the actual analysis of a batch.

The results are given in table II.4.5. and illustrated in fig. II.4.7. The repeatability varies between 8 % for toluene and 36 % for chloroform. RSDs reported in literature for the various methodologies vary between 2 and 30 % (Easley *et al.*, 1981; Hiatt, 1981; Reinert *et al.*, 1983; Hiatt *et al.*, 1994). Specifically for purge and trap techniques RSDs between 5 and 20 % are reported (Easley *et al.*, 1981; Reinert *et al.*, 1983). In view of these facts, the repeatability obtained with this methodology was deemed sufficient.

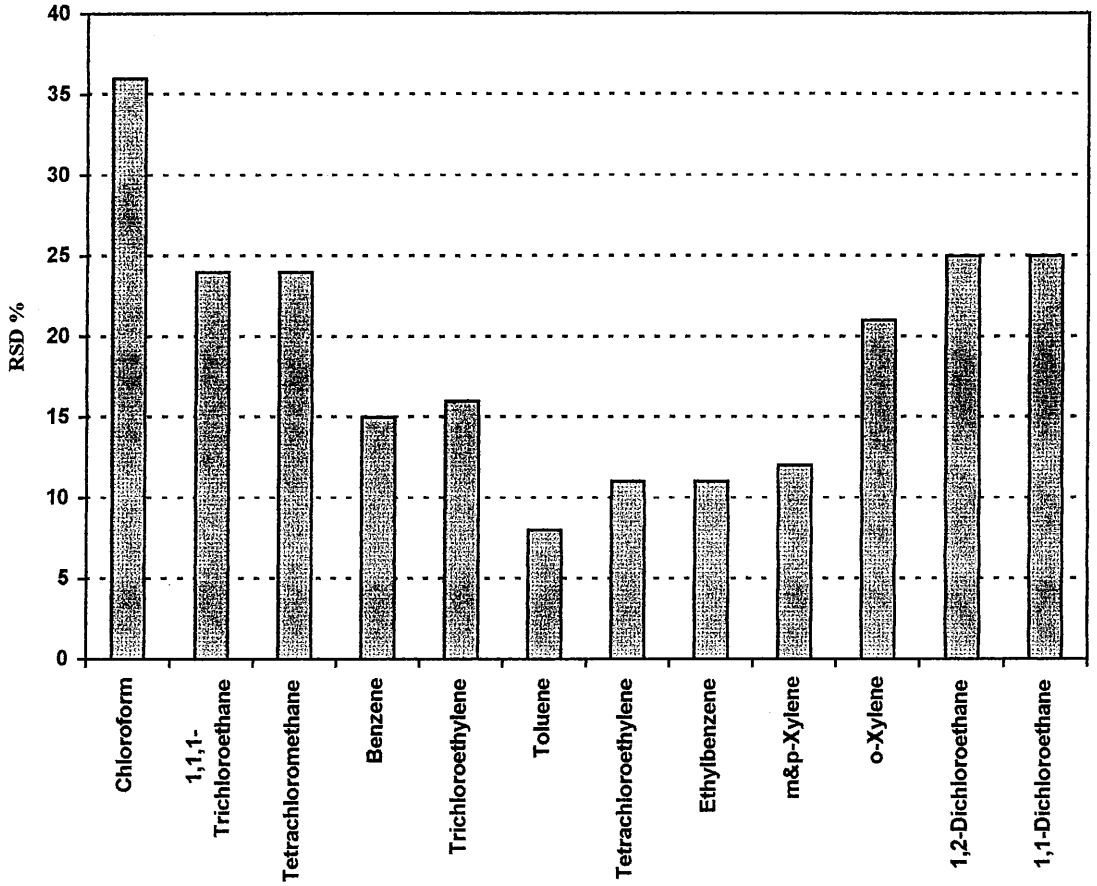


Figure II.4.7: Repeatability of the analysis of VOCs in marine biota.

Table II.4.5: LODs, repeatability and recovery for the analysis of VOCs in marine biota.

	LOD 5g (ng/g)	LOD 10g (ng/g)	Repeatability (%)	Recoveries (%)
Chloroform	0.4	0.2	36	95 ±36
1,1,1-Trichloroethane	0.01	0.006	24	66 ±24
Tetrachloromethane	0.01	0.005	24	70 ± 24
Benzene	0.2	0.08	16	80 ± 18
Trichloroethylene	0.04	0.02	16	63 ± 17
Toluene	0.2	0.08	8	115 ± 11
Tetrachloroethylene	0.1	0.06	11	74 ± 11
Ethylbenzene	0.04	0.02	11	72 ± 15
m&p-Xylene	0.2	0.08	12	69 ± 15
o-Xylene	0.04	0.02	21	77 ± 25
1,2-Dichloroethane	0.01	0.005	25	115 ± 25
1,1-Dichloroethane	0.01	0.005	25	115 ± 25

II.4.2.5 RECOVERY

To determine the recovery, a homogenised fish muscle tissue sample was prepared and divided in 5 parts. The homogenised samples were immediately transferred to sample vials and water was added which contained both the internal standard and a known concentration of the target compounds. The samples were stored for 24 hours at room temperature and in the dark prior to analysis. Afterwards the samples were analysed with the methodology given above. The results of the analysis are given in table II.4.5 and illustrated in fig. II.4.8.

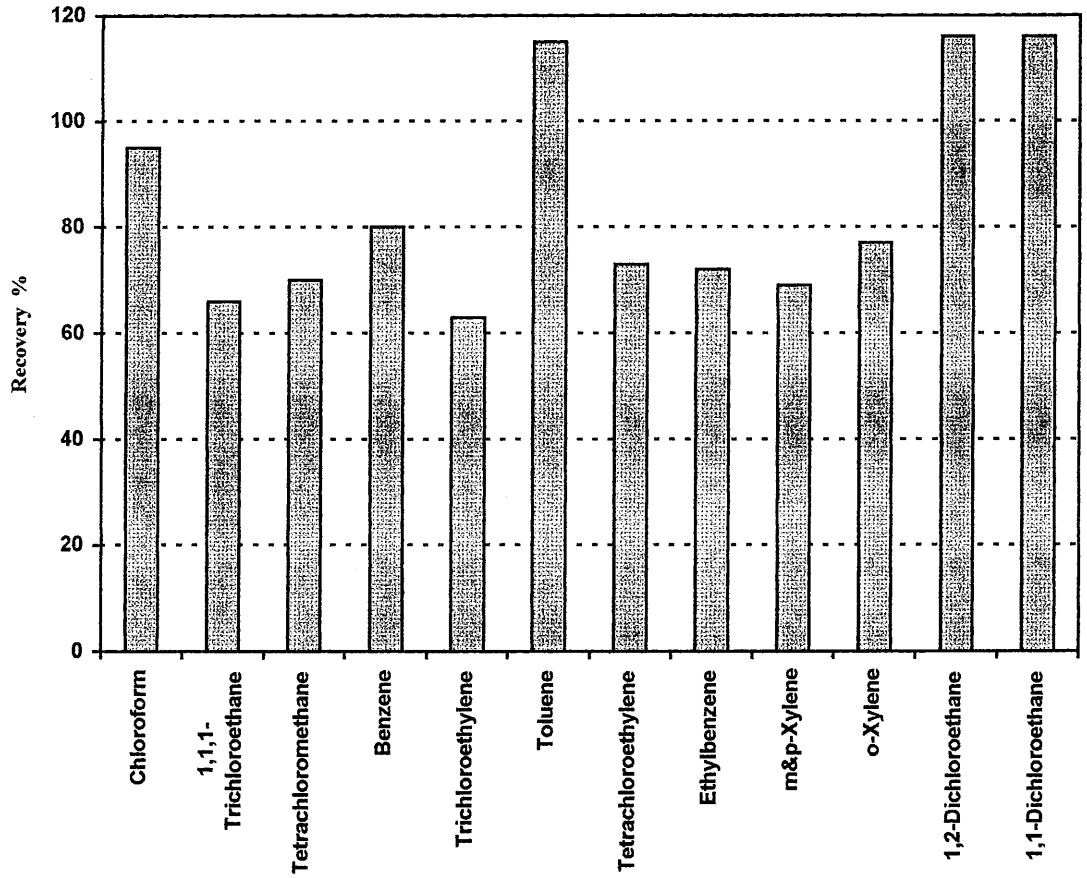


Figure II.4.8: Recovery for the different VOCs of the analysis of VOCs in marine biota.

The recovery varies between 63 % for trichloroethylene and 115 % for toluene and the dichloroethanes. Recoveries reported in literature range from 46 to 129 % for the various techniques and from 60 to 90 % for the purge and trap techniques (Murray and Riley, 1973; Easley *et al.*, 1981; Hiatt, 1981; Reinert *et al.*, 1983; Gotoh *et al.*, 1992; Hiatt *et al.*, 1994). The recovery for the various VOCs corresponds well with

reported values in literature and was therefore considered to be sufficient for the analyses at hand.

II.4.2.6 LIMITS OF DETECTION

The limit of detection (LOD) was calculated using two methods. As for most target compounds a significant background concentration is unavoidable, this blank value will have to be incorporated into the calculation of the LOD. The approach used, was to determine the variability of the blank over a period of one day. A peak was considered detectable the moment its area was higher than the area of the blank plus three standard deviations of the blank. The daily variability of the blank in the Tekmar was already discussed in section II.4.2.2.3. and averages around 30%. Applying the rule set above would render a peak detectable when its area is higher than the area of the blank augmented with 90% of that value. For all practical purposes the LOD was, as a consequence, set at two times the blank value (blank+100%). For the compounds for which no significant blank levels could be observed the LOD was calculated on the basis of the signal to noise ratio (S/N) of the peak. In that case a peak is detectable when its S/N is higher than three. The LOD is further dependent on the amount of analysed tissue. LODs were thus calculated for sample sizes of 5 and 10 g using the methods described above. The results are given in table II.4.5 and illustrated in figure II.4.9.

The present methodology allows LODs from 0.4 to 0.01 ng/g for a sample of 5 g and 0.2 to 0.005 ng/g for a sample of 10 g. The lowest reported LOD for the analysis of VOCs in marine samples is 0.01 ng/g (Gotoh *et al.*, 1992) using an ECD. Other authors reported detection limits between 1 and 10 ng/g (Easley *et al.*, 1981; Hiatt, 1983; Hiatt *et al.*, 1994). It can therefore be concluded that the LODs obtained with the current methodology are generally superior to those reported in literature for the analysis of marine samples. It should be noted, however, that Page and Lacroix (1995) reported slightly superior LODs for the analysis of VOCs in food. Further reduction of blank values or an increased sample intake may further improve the LODs. However, increasing the sample intake could result in additional problems such as increased sample foaming and system contamination due to high loads of the target compounds

and other volatiles. The worst cases for the present methodology are definitely those for which the background contamination is worst. An attempt to reduce the background levels should therefore be the first approach.

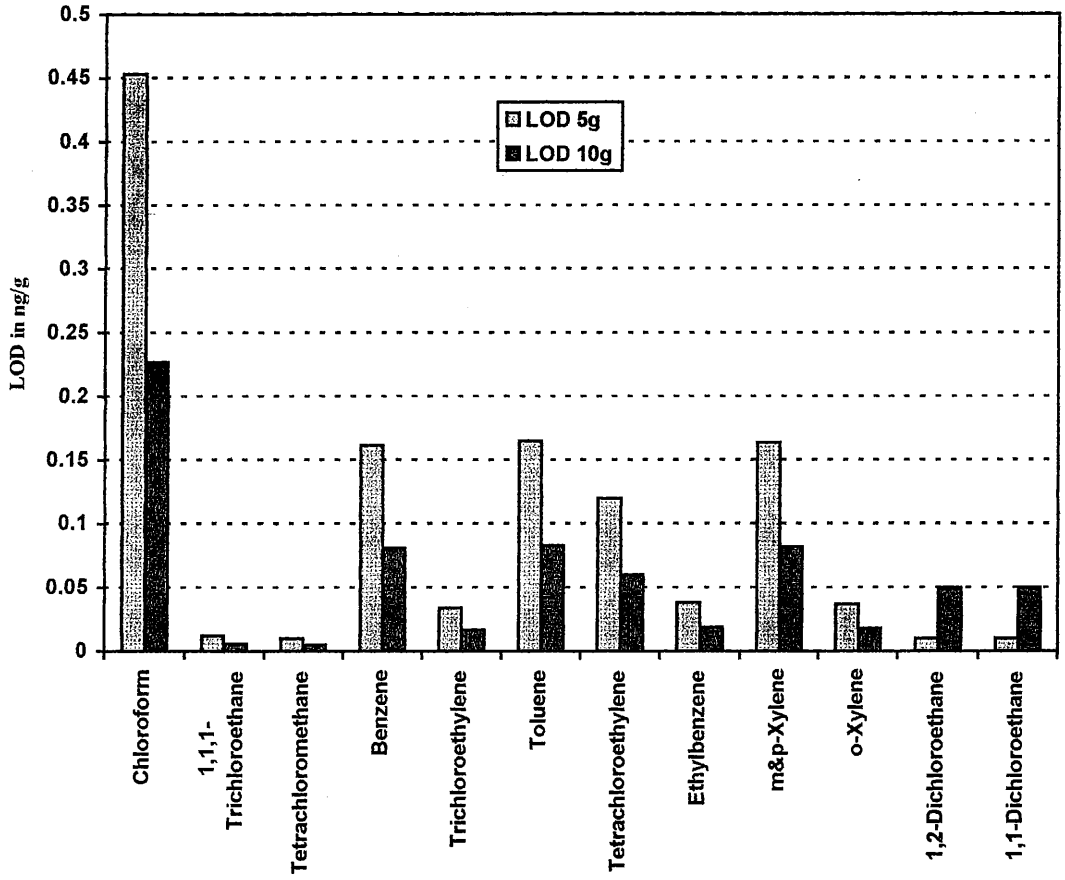


Figure II.4.9: LODs for the analysis of VOCs in marine biota.

II.4.2.7 ANALYTICAL QUALITY ASSURANCE

II.4.2.7.1 Introduction

Analytical quality assurance (QA) for the determination of well known organic contaminants such as CBs has been extensively discussed and documented on several occasions (QUASIMEME). Current practice consists of the routine analysis of internal reference materials (IRMs), certified standard materials (CRMs), procedural blanks and recovery analysis. Participation in intercalibration exercises is also

recommended. The analysis of IRMs and CRMs allows the construction of a control chart that is a monitor for the analytical quality of the analysis.

Unfortunately, there is at present no CRM available for the analysis of VOCs in marine biota or any other tissue. To monitor the reproducibility of the method over a long period of time an IRM could be developed. This would in turn allow the construction of a control chart and thus control of the analytical quality. The development of an IRM was therefore considered. However, the behaviour of the target compounds in biological tissue is largely unknown. It would in any case require a homogenous sample of sufficient quantity. The practice thus far was to prepare a homogeneous batch of fish oil. In the case of VOCs, the preparation of such a batch would already result in (possibly complete) losses of the VOCs. One possibility might be to spike a fish oil with the VOCs, preferably with labelled compounds. However, a fish oil is not to be compared with fish tissue. A IRM or CRM should ideally be the same matrix as the one that will be analysed. Such work is currently being undertaken in the Netherlands (de Boer, 1996) for the analysis of organochlorines in fish tissue. A batch of homogenised fish tissue was stored in sterilised conserves. The results indicated no effects of the conservation process on the analysis of the CBs and OCPs. Whether this would be the case for VOCs is extremely doubtful. The sterilising process will more than likely lead to losses of the VOCs. The only alternative to this would be to prepare a batch homogenised tissue that is stored in the freezer. However, the research for the proper storage conditions such as minimum temperature and container used is a study on its self and was not feasible during the framework of this project. The latter was largely due to the considerable analytical difficulties that were associated with this type of analysis. However, a number of QA measures was taken during the analysis of the environmental samples.

II.4.2.7.2 QA measures

Blank analyses

First of all, a daily blank analysis accompanied each batch of samples. The latter is extremely important in view of the omnipresence of these contaminants. This is the first warning signal for the proper functioning of the analytical system. Very high

blank values prohibit the analyses of real samples in which low concentrations are expected.

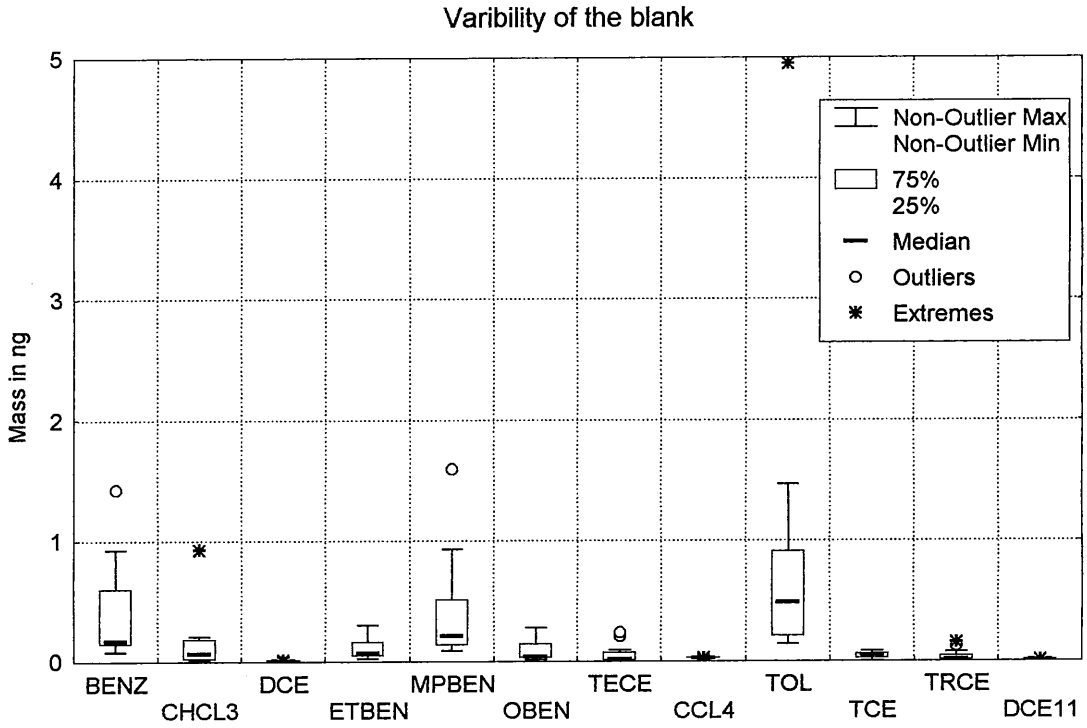


Figure II.4.10: Box and whisker plot representing the variability of the blank values of the different VOCs during the analysis of the environmental samples (BENZ = benzene, CHCL3 = chloroform, DCE = dichloroethane, ETBEN = ethylbenzene, MPBEN = m&p-xylene, OBEN = o-xylene, TECE = tetrachloroethylene, CCL4 = tetrachloromethane, TOL = toluene, TCE = trichloroethane, TRCE = trichloroethylene, DCE11 = 1,1-dichloroethane).

As a rule blank values should be at least ten times below those of the calibration solution. If not, a number of measures need to be undertaken to clean the system. The blank values are further used to calculate the LOD (see earlier). The long term variability of the blank during the analysis of the actual samples is illustrated in figures II.4.10 to II.4.12.

With the exception of some extremes (defined as 3 times the difference between the 75th and 25th percentile) and outliers (defined as 1.5 times the difference between the 75th and 25th percentile) (figure II.4.10) the mass of the blank was generally below 1 ng. There was a substantial difference between the MAHs and the CHCs as far as the blanks are concerned. Where the mass of MAHs was on average below 1 ng with the exception of toluene (figure II.4.11), the mass of the CHCs was below 0.2 ng for

chloroform and below 0.1 ng for most other CHCs. For 1,1-dichloroethane, 1,2-dichloroethane and tetrachloromethane blank values were mostly below the detection limit of the MS (figure II.4.12).

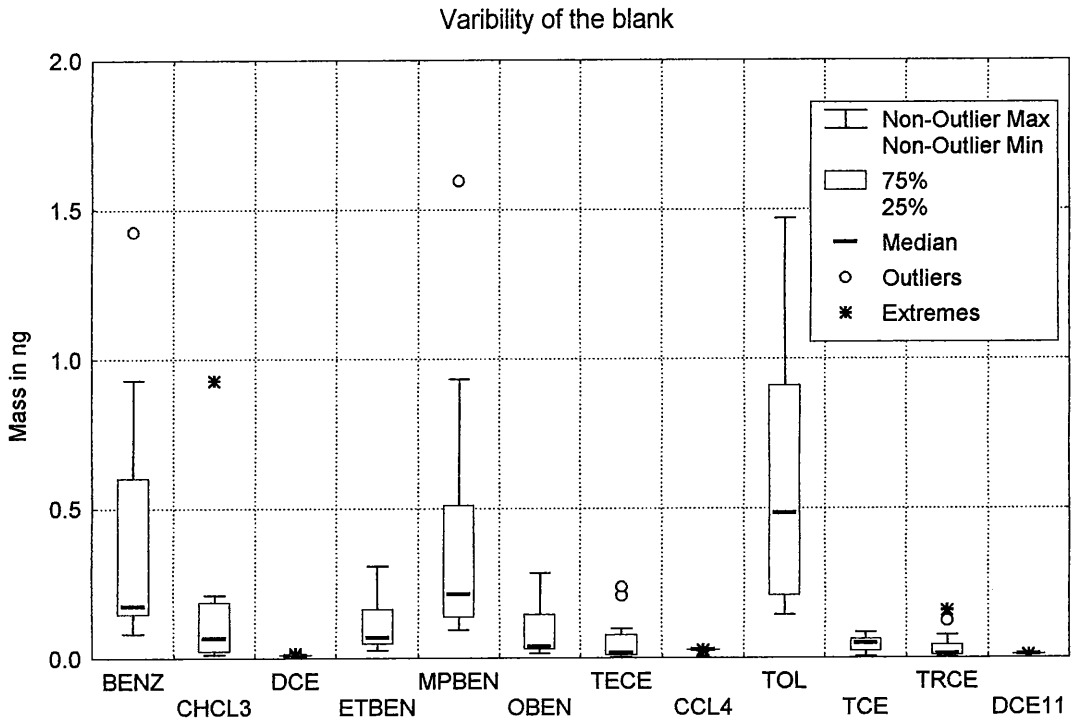


Figure II.4.11: Box and whisker plot representing the variability of the blank values of the different VOCs without the extreme outlier for toluene during the analysis of the environmental samples (BENZ = benzene, CHCL3 = chloroform, DCE = dichloroethane, ETBEN = ethylbenzene, MPBEN = m&p-xylene, OBEN = o-xylene, TECE = tetrachloroethylene, CCL4 = tetrachloromethane, TOL = toluene, TCE = trichloroethane, TRCE = trichloroethylene, DCE11 = 1,1-dichloroethane).

The high values for the MAHs originated mainly from the Tekmar P&T apparatus. The higher values of chloroform compared to the other CHCs are attributed to the frequent use of this solvent in other parts of the building. Comparing the median values of both the standard and the blank shows that the ratio between both was generally above 10 and thus complied with the rule mentioned above (table II.4.6). For the outliers illustrated in figures II.4.10 to II.4.12 the analyses were halted and the system was cleaned at the first instance, by purging it with He at elevated temperatures. The blank values were further used to determine the LODs for each batch of samples (see earlier).

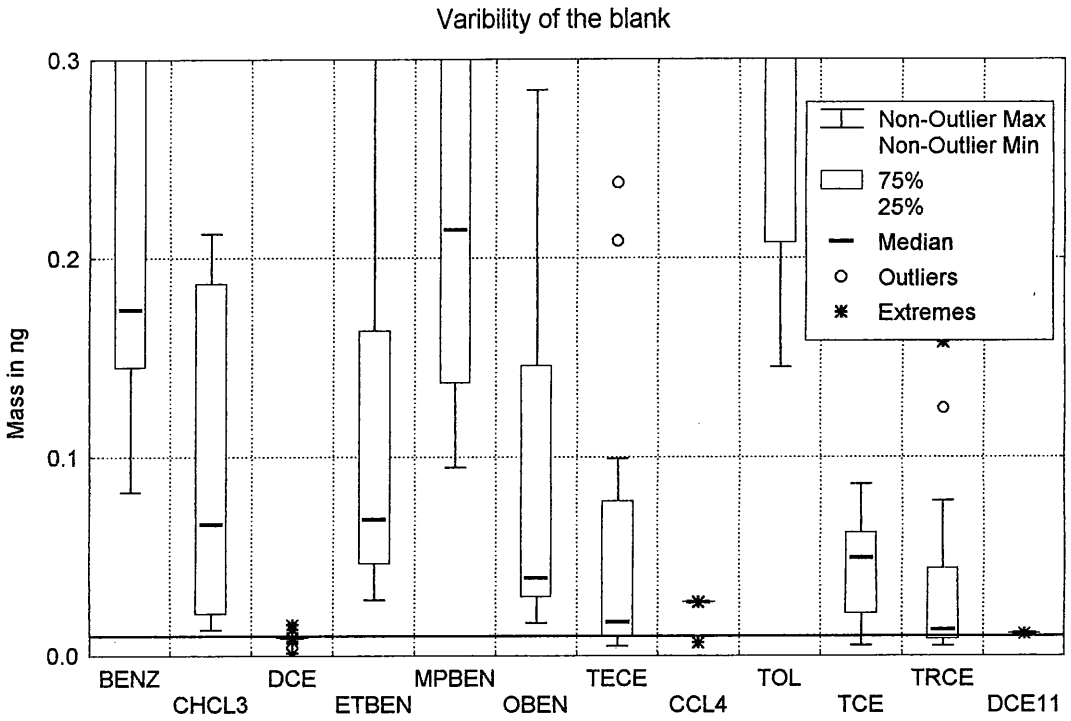


Figure II.4.12: Detail of the box and whisker plot representing the variability of the blank values for the different VOCs during the analysis of the environmental samples. The line above the x-axis represents the LOD of the MS for most compounds (BENZ = benzene, CHCL3 = chloroform, DCE = dichloroethane, ETBEN = ethylbenzene, MPBEN = m&p-xylene, OBEN = o-xylene, TECE = tetrachloroethylene, CCL4 = tetrachloromethane, TOL = toluene, TCE = trichloroethane, TRCE = trichloroethylene, DCE11 = 1,1-dichloroethane).

Table II.4.6: Median relative height of the standards and blanks and the ratio between standard and blank.

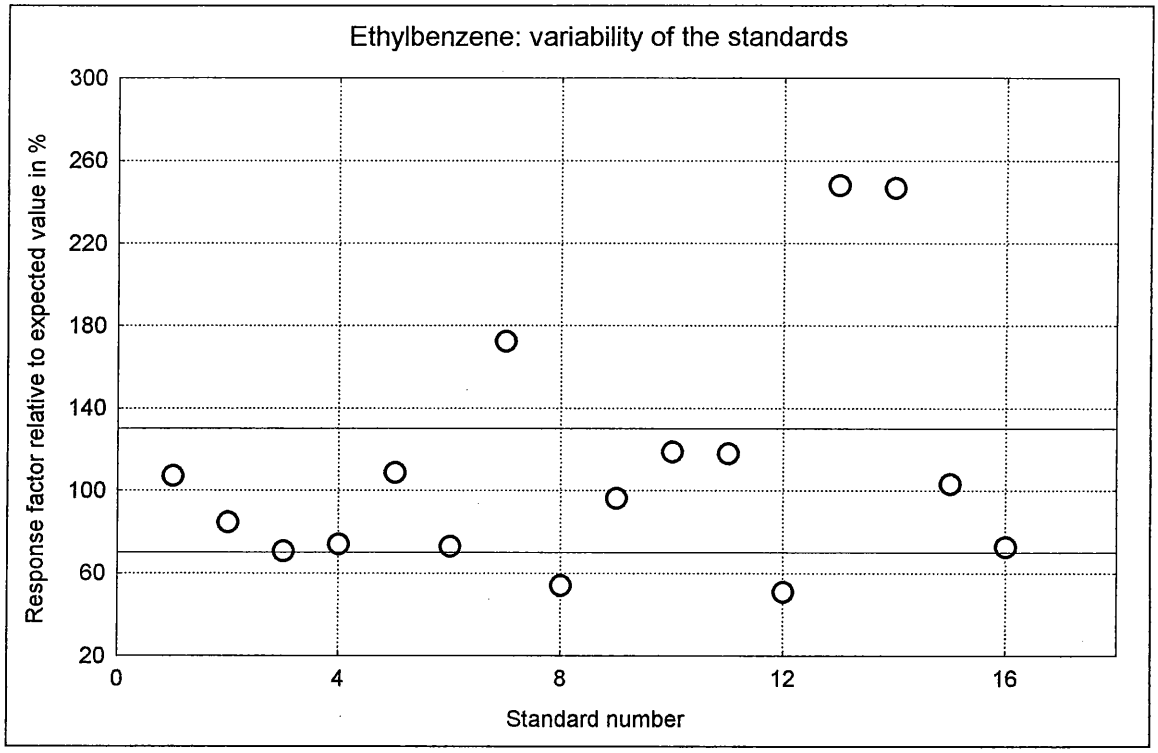
Compound	Median standard	Median blank	Ratio
1,1-Dichloroethane	1.05	0.00	na
Benzene	1.24	0.22	5.59
Chloroform	1.37	0.06	22.07
1,2-Dichloroethane	0.51	0.00	na
Ethylbenzene	1.51	0.06	24.02
m&p-Xylene	2.25	0.14	15.85
o-Xylene	1.33	0.05	24.24
Tetrachloroethylene	1.12	0.02	58.22
Tetrachloromethane	0.28	0.00	na
Toluene	1.56	0.37	4.23
Trichloroethane	1.45	0.01	132.49
Trichloroethylene	1.20	0.02	79.10

na = not applicable

Variability of the standard and standard analysis

A second QA measure is to monitor the relative height of the different VOCs during the analysis of the standard solution used for calibration. If the system is working properly no deviations higher than 30 % from the median relative height are expected. The latter is twice the worst case RSD for standard solutions in water. Figures II.4.13. illustrate the long term variability for the relative height of the standard solutions expressed as the percentage of the median height. The warning limits shown on the graphs are set at 30 %. In the case that the warnings limits were exceeded, the analysis was halted and the system was evaluated for proper functioning. Very low values could often be attributed to a leak in the system and on some occasions to a reduced performance of the MS. Very high values were mostly due to high system contamination and a change in the response of the MS.

On certain occasions a standard solution was treated as a sample and thus analysed as a sort of IRM. The latter is indicative for the proper calibration and function of the system but should in no way be compared with the analysis of a real IRM. In the advent of a continued monitoring programme for these compounds an IRM would eventually become indispensable. The results of these analyses are illustrated in figure II.4.14. This measure was only undertaken when the results of the previous test were out of control. The test then provides a way to determine whether the problem was MS or P&T related.



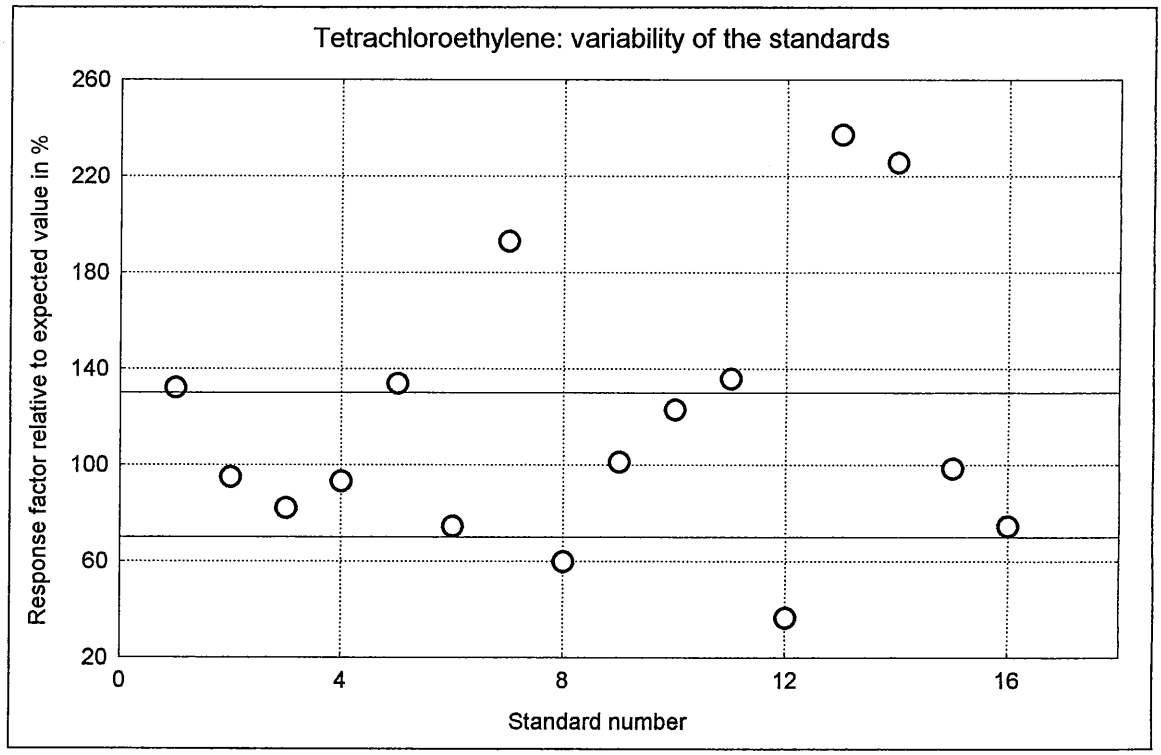
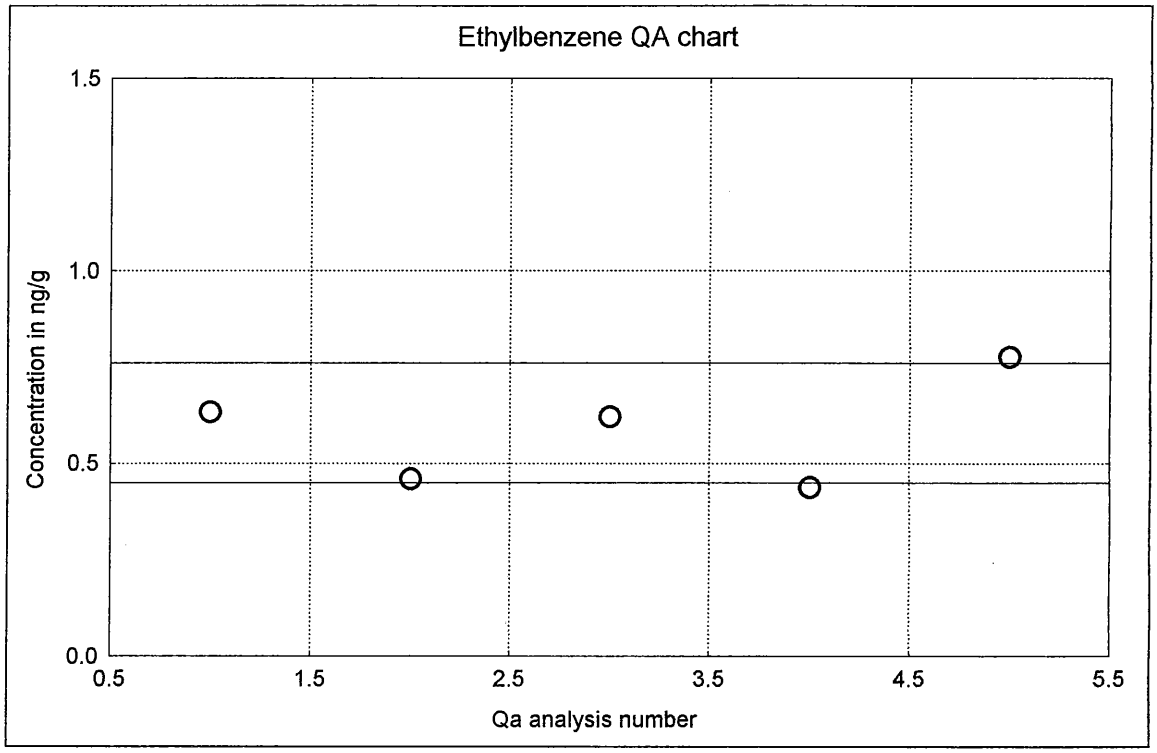


Figure II.4.13: Quality control charts using the relative heights of calibration solutions for ethylbenzene (upper) and tetrachloroethylene (lower). The lines represent the control limits.



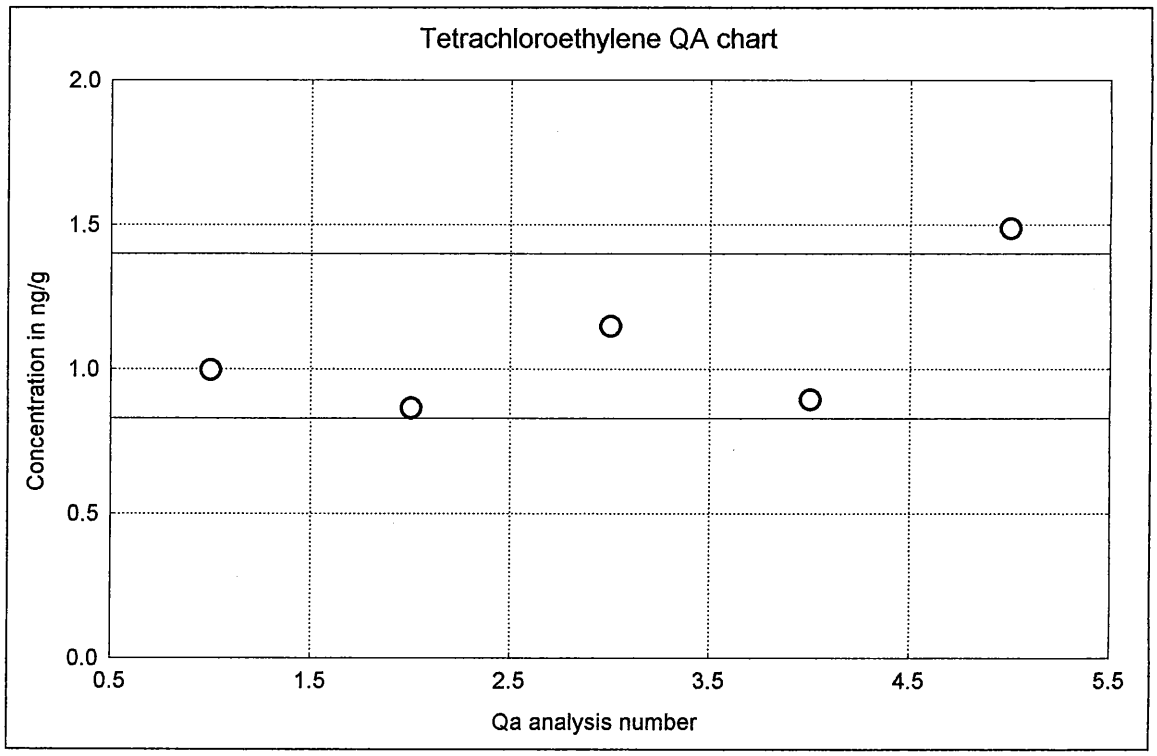


Figure II.4.14: Quality control charts using the relative heights of calibration solutions for ethylbenzene (upper) and tetrachloroethylene (lower). The lines represent the control limits.

Identification of the target compounds

Positive identification of the target compounds was performed using both the retention times and the MS spectra of the analytes. The high sensitivity of the ion-trap MS allows full scan spectra even at low concentrations. The practice for the identification and quantification of the target compounds was as follows: first the presence of the compound is verified by determining the presence of a peak in the retention window were the compound is expected, using the selected ions given in table II.4.8. If a peak is detected, the full scan mass spectrum is compared with the one stored in the library. Only when a positive identification is made, the peak is quantified using the selected masses mentioned earlier.

Table II.4.8: Retention windows and selected masses of the target compounds.

Compound	Retention window (min)	Selected mass
1,1-Dichloroethane	4:30-4:50	63,64
Chloroform	6:10-6:30	83,85
Trichloroethane	6:40-6:60	61,97,99
Tetrachloromethane	7:00-7:20	117,119
1,2-Dichloroethane	7:10-7:30	62
Benzene	7:10-7:30	78
Trichloroethene	8:00-8:20	60,130
Trifluorotoluene	8:15-8:35	94,129,166
Toluene	9:45-9:65	91
Tetrachloroethene	10:40-10:60	91,105
Ethylbenzene	12:00-12:20	91,106
m&p-Xylene	12:05-12:25	91,106
o-Xylene	12:45-12:65	91,106

II.4.3 EXPERIMENTAL SECTION

II.4.3.1 METHOD FOR THE DETERMINATION OF VOLATILE COMPOUNDS IN MARINE BIOTA

A method was developed that allows the simultaneous determination of the volatile organochlorines chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene and the volatile aromatics benzene, toluene, ethylbenzene and the xylenes (BTEX) in marine biota. The biological tissue is first homogenised using an Ultra-Turrax blender and transferred to a 24 ml sample vial. After addition of 10 ml water and internal standard the homogenate is treated in a ultrasonic bath to further disrupt the tissue. The glass vessel is then connected to a Tekmar LSC 2000 purge and trap apparatus coupled to a gas Finnigan-Magnum GC-MS. The volatiles are forced out of the tissue by purging with a stream of helium gas while heating at 70°C and trapped onto a Vocarb 4000 sorbent trap (Supelco). After purging the trap is backflushed while being rapidly heated and the analytes are desorbed into a cryofocusing module connected to the analytical. The analytes are injected into the column by rapidly heating the module.

II.4.3.2 MATERIALS

All materials used for the various experiments and analyses were of research grade quality. The CHCs chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene were obtained (Merck) and the MAHs benzene, toluene, ethylbenzene and the xylenes (Merck) were used, without further purification, as standards in the experiments. Methanol (Baker, VOC) was used as solvent for the preparation of standard solutions. As internal standard (IS) α,α,α-trifluorotoluene (Aldrich) was used. Vocarb 4000 traps (8.5 cm Carbopack C, 10 cm Carbopack B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001) were obtained from Supelco and used as adsorption traps (1/8" OD). Water used for the preparation of blanks and standards was obtained from Baker. Antifoam (Vel), phosphor wolframic acid (Merck), sodium chloride (Merck) and 1-octanol (Merck) were used to test the reduction of sample foaming.

II.4.3.3 APPARATUS

A microprocessor controlled P&T system, Tekmar LSC-2000 (Tekmar, Cincinatti, USA) was coupled with a gas chromatograph - mass spectrometer system (Finnigan Magnum Ion trap MS, Finnigan, San Jose, USA), by a heated transfer line ending in a cryogenic focuser at the GC. The P&T system was provided with a 25 ml fritted sparger and a moisture control module (MCM) as wet trap. The internal lines of the P&T are glass-lined stainless steel. The transfer line and internal lines are connected with a heated 6-port switch valve. The samples were purged using an impinger (Alltech) connected to purge gas outlet and the 25 ml frit sparger of the Tekmar (Tekmar, Cincinatti, USA). Samples were stored prior to analysis in 24 ml sample vials (Alltech) that were for analysis coupled to the impinger with a Wheaton connector (Wheaton). Samples were homogenised with a Janke&Kunkel sharing blender and the tissue was further disrupted in a Bransonic ultrasonic bath.

II.4.3.4 SAMPLING AND STORAGE OF THE SAMPLES

II.4.3.4.1 Sampling

No special methods are described in literature regarding the collection of biological material for the analysis of VOCs. The major concerns when sampling for VOCs are losses due to volatilisation of the compounds or contamination of the samples through the sampling equipment or through contact with the surrounding air. Whether contamination occurs during sampling is questionable, since it would involve a rapid migration of the VOCs through the outer epidermis of the biota during the process. Similarly, losses would require the same but reverse process. Further, it seemed unfeasible to design conditions which would minimise both processes when collecting biological material, especially for the commonly used fishing techniques.

The samples were collected with the Belgian Oceanographic vessel 'Belgica' using beam-trawling and processed as swiftly as possible to avoid contamination and losses. Sampling is done in accordance to the guidelines of OSPARCOM (Oslo and Paris Commisions, 1990).

II.4.3.4.2 Sample storage

As for sampling the two major concerns are contamination and losses due to volatilisation. A number of measures are reported to prevent both from happening. Dickson and Riley (1973) stored their sample in a Dewar vessel with solid carbon dioxide. Hiatt (1983) used fish stored under dry ice. Easely *et al.* (1981) stored their fish wrapped in aluminium foil in the freezer. Ferrario *et al.* (1985) packed the samples immediately in ice and stored them at -5 °C.

Therefore, the most practical approach seemed to store the undissected fish immediately after sampling at -28 °C in closed containers. Both contamination and volatilisation would imply migration of the analytes through the skin of the fish. As there are no direct sources of VOCs in the storage room contamination would have to originate from the surrounding air. Yet the freezers are air tight. It therefore seems unlikely that this form of contamination would significantly contribute to the observed concentrations. The latter has not been investigated during this project, although it would become imperative in the prospect of prolonged monitoring of these compounds. The practical considerations are difficult at best. Testing this contamination would involve comparing undisturbed fish tissue (or better a whole animal) over a time interval. This would be unfeasible with homogenised tissue as the structure is drastically changed. Yet a homogenous sample is necessary as the VOCs are unevenly distributed in the tissue. Testing losses during storage would again involve a homogenous sample to which a known amount of preferably labelled VOCs is added. This would imply adding labelled standard to a whole fish. A way to simulate both adverse affects would be to use a fish oil in stead of whole fish. However, the behaviour of the compounds is unlikely to be similar in both matrices.

II.4.3.5 ANALYTICAL PROCEDURE

II.4.3.5.1 Preparation of blanks and standard solutions

Preparation of blanks

Water specially prepared for the analysis of VOCs (Baker) was used to prepare blanks and standard solutions (see further). The water was treated by heating it to a temperature of 90°C and simultaneous purging with helium (N 7.0, l'Air Liquide) or

N_2 (N 6.0, l'Air Liquide) in a glass sparger. As a routine, water used for preparations, was continuously purged during storage with the gasses mentioned above.

For the preparation of blank samples 15 ml of the treated water was inserted in a 100 ml syringe and 4 µl of a known concentration of internal standard (α,α,α-trifluorotoluene, Aldrich) was added to the water in the syringe. The latter was done by inserting a 10 µl HPLC syringe in the opening of the 100 ml syringe. The water sample was then run through the entire analytical procedure starting with the homogenisation step, followed by treatment in the ultrasonic bath and finally on-line P&T concentration and analysis.

Preparation of standard solutions

Methanol was chosen as solvent for the preparation of standard solutions. An initial standard solution (stock solution) was made by diluting 1 ml of the different target compounds in 100 ml of methanol as follows: a small quantity (approx. 20 ml) of solvent was introduced in a volumetric flask and the weight was recorded. One ml of each of the target compounds was added to the methanol and at each occasion the weight is recorded. Finally, the volume was brought to 100 ml and the weight was again recorded. This allows correction for eventual weight losses. This method allows calculation of the concentration on both a volume and a weight basis. Reporting and using standard solutions on a weight basis is recommended for analytical purposes (Wells *et al.*, 1992) but by using accurate volumes at the same time, a rapid calculation of concentrations and of dilution series is possible. In any case, standards were used on a weight basis for analytical purposes. From the stock solution, dilution series are made by dissolving known quantities in methanol, again on a weight basis. Frequent renewal of standard solutions is recommended due to the high volatility of the analytes. The dilutions were continuously monitored for concentration differences. As a rule, no concentration differences should be allowed that exceed the analytical variability.

For calibration of the system 4 µl methanolic solution with a known concentration (generally between 0.4 and 0.8 ng/µl) of the various target compounds was injected

with a 10 µl syringe in an 100 ml syringe containing 15 ml of blank water (see above). Afterwards, another 4 µl of a methanolic solution containing the internal standard (about 0.4 ng/µl) was also introduced into the 100 ml syringe with a different 10 µl syringe. The water is then injected into a 24 ml sample vial (Alltech) and thee sample vial is connected to the on-line P&T set-up, pre-concentrated and analysed with the GC-MS.

II.4.3.5.2 Sample pre-treatment and P&T concentration of the sample

The frozen fish sample were thawed in their recipients and edible tissue and liver were isolated out of the fish. The biological tissue was first homogenised at 0° C using an ultra-turrax blender (Janke and Kunkel) and transferred to a 24 ml sample vial (Alltech). After addition of 10 ml organic free water and internal standard (1,1,1-trifluorotoluene) the vial is closed with a Teflon lined screw cap and the homogenate was treated in a ultrasonic bath (20 min at 0°C) to further disrupt the tissue. The glass vessel was then coupled to an impinger connected to the P&T. The volatiles were forced out of the tissue by purging the sample for 30 min with a stream of helium gas at 10 ml/min while heated at 70°C in a water bath. The analytes were trapped onto a Vocarb 4000 sorbent trap (Supelco) mounted in the P&T apparatus. The operational parameters of the P&T apparatus are given below:

Tekmar: Trap standby temperature: 45 °C, purge temperature: 35 °C, mount temperature: 40 °C, oven temperature: 250 °C, line temperature: 250 °C, cryo union standby temperature: 250 °C.

II.4.3.5.3 Desorption and cryofocussing

After purging the trap was backflushed while being rapidly heated at 250 °C and the analytes were desorbed into a cryofocusing module cooled to -120°C and connected to the analytical column.

II.4.3.5.4 Injection, chromatographic separation and detection

The analytes were injected into the column by rapidly heating the cryofocusing module from -120°C to 200 °C in 0.75 min. Separation was done on a Restek, RTx-502.2, 60 m, 0.32 mm i.d., 1.8 µm film. With the following GC conditions:

Carrier: helium, inlet pressure 16 psi.

Temperature program: 40°C for 2 min, from 40 ° C to 200 °C at 10° C/min, hold 5 min.

The target compounds are detected on a Finnigan Magnum Ion trap detector on the basis of retention times and mass spectra and quantified using the total mass of selected ions (table II.4.8). The operational parameters of the mass spectrometer are given below:

Detector: Magnum Ion Trap Detector (ITD), Electron Impact mode (EI), multiplier voltage: 2550 V, manifold temperature: 220 ° C, emission current: 13 µA, Axial Modulation (A/M) amplitude: 4.0 V, mass range: 50 - 250 amu (atomic mass units), scan rate: 1000 ms, filament delay: 180 s, mass defect: 50 mmass / 100 amu, background mass: 45 amu.

II.4.3.6 CONCLUSIONS

After considerable difficulties, an on-line method was developed that allowed the determination of VOC in biological tissue with a good recovery, acceptable repeatability and sufficiently low limits of detection. The on-line method was preferred over the off-line approach for reasons of repeatability. The method can however be further improved. Both methods are hampered by the presence of background concentrations of the contaminants. Detection limits were therefore based on the presence of background concentrations in such a way that an analyte is considered detectable when its relative response is higher than that of the blank plus three times the daily variability of the blank. LODs could therefore be further reduced by eliminating or reducing the presence of these blank values. As an alternative larger sample intakes, yielding lower LODs, could be studied. It was determined that the background values are related to the use of the P&T apparatus and background levels

in the laboratory environment. Current other methodologies like Solid Phase Micro Extraction (SPME) could perhaps eliminate the use of a P&T altogether. Finally, further reduction of sample foaming at elevated flow rates and reduced sample handling, could lead to shorter analysis times. The latter is of paramount importance as the current methodology does not allow a large sample throughput.

II.4.3.7 REFERENCES

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