DETERMINATION OF POTENTIALLY CARCINOGENIC COMPOUNDS IN FOOD

Trace analysis of vinylchloride, vinylidenechloride, acrylonitrile, epichlorohydrin and diethylpyrocarbonate

J.B.H. van Lierop

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 2 mei 1979
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

ISN 106770

Aan Ank

Aan Lars en Merel

S T E L L I N G E N

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EEG richtlijn van 30 januari 1978.

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Iedere analysemethode die dient om de wet te handhaven, zou in een gemeenschappelijk onderzoek volgens ISO-normen getest moeten worden.

Dit proefschrift, paragr. 3.3.7.

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Determination of carcinogenic compounds in food.

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Determination of carcinogenic compounds in food.

ACKNOWLEDGMENTS

I express my gratitude to Professor W. Pilnik for his guidance during the course of this work.

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SUMMARY

Toxicological evidence shows that some monomers present in packaging materials may be carcinogenic. These monomers, notably vinylchloride, vinylidenechloride, acrylonitrile and epichlorohydrin, may migrate from the packaging material into the food. Therefore, severe limits are set to the contents of these compounds. Very sensitive and specific methods are required for determining such contents. Moreover, a Food Inspection Service must be able to inspect large numbers of samples.

Gas chromatography, combined with headspace techniques and mass fragmentic detection, has proved to meet such requirements. Thus, vinylchloride and acrylonitrile could be measured down to 1 ppb in food and food simulants, vinylidenechloride down to 5 ppb in polymers, and epichlorohydrin as low as 6 ppb in food simulants. The potential carcinogenic compound diethylpyrocarbonate could be determined at the ppb level as diethylcarbonate.

Methods of analysis using gas chromatography with a flame ionization detector and a Hall detector developed at the beginning of our investigations, are now only used for screening purposes.

The method for determination of vinylchloride has been the subject of a collaborative study with sixteen participating laboratories.

Results of recent determinations are reported.

SAMENVATTING

Toxicologische gegevens tonen aan dat enkele monomeren, die in verpakkingsmaterialen aanwezig zijn, carcinogeen zouden kunnen zijn.

Deze monomeren, te weten vinylchloride, vinylideenchloride, acrylonitril en epichlorohydrine kunnen vanuit het verpakkingsmateriaal in het voedsel migreren. Daarom zijn er scherpe grenzen gesteld aan het gehalte van deze verbindingen.
Zeer gevoelige en specifieke methoden zijn nodig voor de bepaling van deze gehalten. Bovendien moet een Keuringsdienst in staat zijn grote aantallen monsters te analyseren.

Gaschromatografie in combinatie met top gas (headspace) technieken en massafragmentografische detectie bleek geschikt te zijn. Aldus konden vinylchloride en acrylonitril gemeten worden in voedsel en voedselsimulanten tot een gehalte van 1 µg/kg, vinylideenchloride tot een gehalte van 5 µg/kg in polymeren en epichlorohydrine tot 6 µg/kg in voedselsimulanten.
Het potentieel carcinogene conserveermiddel diethylpyrocarbonaat kon op het µg/kg niveau geanalyseerd worden als diethylcarbonaat.

Analysemethoden die gebruik maken van gaschromatografie met een vlamionisatie detector en een Hall detector werden ontwikkeld bij het begin van ons onderzoek. Deze worden nu alleen gebruikt voor een voorselectie.

De methode voor de bepaling van vinylchloride is het onderwerp geweest van een gemeenschappelijk onderzoek met zestien deelnemende laboratoria.

Resultaten van recente bepalingen worden gerapporteerd.

THE MATERIAL SUBMITTED IN THIS THESIS IS BASED ON THE FOLLOWING PUBLICATIONS (ADDED AS ANNEXES):

1975 van Lierop, J.B.H., Hogendijk, C.J. and Jongerius, Th.J.: Bepalingsmethoden van vinylchloride. De Ware(n)-Chemicus 5 40-47 (A)

1975 Ernst, G.F., and van Lierop, J.B.H.: A simple, sensitive determination and identification of vinylchloride with a Hall detector. J.Chromatogr. 109 439-440 (B)

1976 van Lierop, J.B.H. and Stek, W.: Analysis of vinylchloride in food simulants at low parts per billion level by mass fragmentography. J.Chromatogr. 128 183-187 (C)

1975 van Lierop, J.B.H., Hogendijk, C.J. and Jongerius, Th.J.: Bepalingsmethoden van vinylideenchloride De Ware(n)-Chemicus 5 157-162 (D)

1978 van Lierop, J.B.H.: De bepaling van acrylonitril met headspace gaschromatografie-massafragmentografie. De Ware(n)-Chemicus 8 178-182 (E)

1978 van Lierop, J.B.H.: A simple and rapid determination at the lower ppb level of epichlorohydrin by gas chromatography-mass fragmentography. J.Chromatogr. 166 609-610 (F)

1979 van Lierop, Ben H. and Nootenboom, Hans: Gas-Liquid Chromatographic-Mass Fragmentographic Determination of Low Levels of Diethylcarbonate in Beverages. J.Assoc.off.anal.Chem. 62 253-256 (G)

ABBREVIATIONS

DEC	diethylcarbonate
DEPC	diethylpyrocarbonate
DMA	dimethylacetamide
EEC	European Economic Community
EPCH	epichlorohydrin
FID	flame ionization detector
GC	gas chromatography
MS	mass spectrometry
ppb	µg/kg
ppm	mg/kg
PS	polystyrene
PVAC	polyvinylacetate
PVC	polyvinylchloride
PVDC	polyvinylidenechloride
VCM	vinylchloride
VDCM	vinylidenechloride

CONTENTS

1. INTRODUCTION

1.1 Dutch regulations on the packaging of food products.

The Food Inspection Service of Utrecht is one of the sixteen Food Inspection Services in The Netherlands, whose task is to enforce the Dutch Food and Commodities Law, *Warenwet*. The *Koninklijk Besluit* of April 20, 1972 assigned special fields of investigation to some of the Services, the aim being that these Services become specialized in that particular field.

The Food Inspection Service of Utrecht specializes in the investigation of packaging materials for food products. This specilization mainly involves the development of analytical procedures, resulting in field sample inspection.

The background of the proposed legislation of polymer food packaging *Ontwerp Verpakkingen en Gebruiksartikelenbesluit* (1977) is as follows:

> Plastic material and articles intended to come into contact with food products must be produced in compliance with sound manufacturing practices.
> They may not contain compounds which could endanger human health. They may not transfer their constituents to food products in a quantity which could
>
> -endanger human health
> -bring about an unacceptable change in the composition
> -change the odour, taste, colour, consistency or other essential characteristics of the food product (Aldershoff 1976, van Battum 1974, Halbesma 1977, Rossi 1977).

The Government sets the following limits to the migration from the packaging material into the food:

1. a global migration limit of 60 mg/dm^2 (6 x 10^{-3} kg/m^2)
2. a specific migration limit for some individual substances.

This thesis mainly deals with the development of tests to determine the amounts of migrated monomers in the food.

1.2 Purpose of this study

Recent toxicological evidence shows that some monomers (i.e. compounds which have not reacted during polymerisation) present in packaging materials may be carcinogenic (IARC 1974, FDA 1976). As a rule, the quantity of residual monomers decreases from a content of 10 per cent immediately after polymerisation to contents of 10^{-5} to 10^{-2} per cent (0.1 to 100 mg/kg) in the packaging material. The migration of these monomers into the food results in contents in the ppb range.

At this low level, the detection and quantitative determination of these monomers by standard analytical techniques does not yield the evidence required for an official method. In addition, the identity of the monomers must be assessed. With modern instrumentation, such as a mass spectrometer, identification and detection at very low levels can be obtained.

However, for the inspection of food it is also necessary to investigate large numbers of samples. The method must be fast therefore. The required speed can be achieved by applying headspace analysis as demonstrated in nearly all the methods described in this thesis. A further requirement is good reproducibility. The repeatibility can be measured within one laboratory, but for the determination of the reproducibility a collaborative study must be organized.

Summarizing: The analytical procedures should be specific and applicable at very low levels and suitable for routine inspection.

2. ANALYTICAL TECHNIQUES

2.1 Headspace gas chromatography

Gas chromatography is a suitable technique for trace analysis. Gas-liquid and gas-solid chromatography are both used in analytical methods for the determination of the monomers.

The sensitivity of the detection unit in gas chromatography largely determines the detection limit of a method. Three types of detectors are discussed in this thesis: a flame ionization detector, a Hall detector and a mass spectrometer. The sensitivity and selectivity of the method also depends upon parameters, such as type of column, method of injection, temperature and, last but not least, upon the amount and preparation of the sample.

Most methods described in this thesis use headspace gas chromatography. This is an indirect method for the determination of volatile constituents in liquids or solids by gas chromatographic analysis. The analysis of the vapour phase which is in thermodynamic equilibrium with the sample to be analyzed in a closed system, is a form of gas analysis (Drozd 1978, Haggenburg 1976, Shanks 1975).

An important advantage of this technique is that it does not involve a time-consuming clean-up of the sample. Furthermore, only volatile compounds are injected on the gas chromatographic column. This increases the life of the column and avoids tailing and ghost peaks. The disadvantage of injecting a large amount of air can be reduced by selecting a very specific column and detector.

Headspace gas chromatography has become a very important technique for the investigation of problems involving odours (Charamboulos 1978). Another important field of application is the ethanol analysis in blood (Kolb 1976). This headspace method is widely used for routine alcohol checking in West-Germany and Austria (Machata 1964).

In general headspace analysis looks very promising for the analysis of monomers in polymers and food.

2.2 Hall detector

The most widely used detector for gas chromatographic analysis is the flame ionization detector. The FID is sensitive and responds to nearly all the chemical compounds, but lacks specificity.

An electron capture detector is specific to halogen compounds. However, this detector had a lower sensitivity to VCM than the FID in our experiments. Therefore, no publications describing VCM analysis are found in the literature.

Hall (1974) developed a specific halogen detector having the same sensitivity as the FID. The Hall detector operates according to the same principle as the Coulson (1965) detector, which means: pyrolysis in a quartz tube of the compounds leaving the gas chromatograph, into hydrogen halogens which are trapped in a mixture of ethanol and water, causing an increase in electrical conductivity.
The Hall detector uses a small measuring cell. Hall claims that by incorporating other improvements, his detector has been made 20 to 25 times more sensitive than the Coulson detector. Wilson (1975), however, found only a factor of 4 for the increase in sensitivity.

The Hall detector was first applied in our laboratory to confirm the vinylchloride content in positive samples having a VCM content above 50 ppb (see 3.2). At a later stage, the Hall detector was used for determining VCM on a routine basis.

Dennison (1978) reports a poor day-to-day repeatibility of the Hall detector. During the first six months of our experience with the Hall detector, many commonplace problems had to be overcome; for example frequent damage to the quartz tube. A good day-to-day repeatibility was obtained, however, by incorporating certain technical improvements into the detector, notably levelling the flow of the carrier gas. Thus, a day-to-day repeatibility of 5 per cent was achieved. Repeatibility is, in this case, expressed in terms of the variation coefficient. (This result was obtained by injection of a standard solution of 0.03 ppm VCM in DMA for five successive days).

2.3 Mass fragmentography

The gas chromatograph mass spectrometer is one of the most powerfull analytical instruments available for tackling our problems. The separation of compounds is done by gas chromatography; the present interfaces for GC-MS are commercially available. The glass jet separator and membrane separator are the interfaces most used nowadays.

Applying capillary columns with a low flow velocity enables the direct coupling of a gas chromatograph with a mass spectrometer. In order to achieve a good separation, however, smaller amounts have to be injected on a capillary column than on a packed column. Although sharper peak shapes are obtained, they suffer from a serious loss of peak surface in the investigated field of headspace analysis. Because of this we did not apply capillary columns.

Full mass spectra can be measured in the low nanogram range with a mass spectrometer. A technique for measuring in the picogram range is mass fragmentography. Mass fragmentography is specific because detection is based on observing and measuring one or more of the ions formed by electron bombardment of the material to be analyzed. Almost all commercially available instruments can be equipped with systems for focussing the mass spectrometer on to a few specific fragments (Millard 1978).

We purchased a quadropole mass spectrometer after performing many test runs. For our investigations, the selection criteria were: sensitivity, stability and ease of operation.

2.4 General requirements

For this study, detectibility is one of the most important requirements. Because if the Delaney clause of the United States Federal Food, Drug, and Cosmetic Act (1958) is put into practice, the detection of extremely low concentrations is the dominating requirement. The Delaney clause states that a substance may not be present in food if it is found to induce cancer when ingested by man or animal. Avoiding the discussion as to whether this statement is fully applicable or not, it can be concluded that the task of the analytical chemist is to develop very sensitive and reliable methods and to provide qualified information (Kaiser 1974). The reliability of the methods should be stated in statistically justified terms, such as repeatibility and reproducibility (AOAC 1978). The definition of these terms can be found in the Draft International Standard of the ISO (1977). This standard also gives directives for organizing a collaborative study. In my opinion any official method should be tested by such a study.

3. TRACE ANALYSIS OF VINYLCHLORIDE

3.1 Literature

3.1.1 Introduction

The carcinogenicity of vinylchloride has been the subject of a large number of publications. Not only in the scientific journals, but also in the public press (Balemans 1974, BIBRA 1975, van Esch 1975, FDA 1975, Huber 1975, Koch 1978, WDR 1976). The main reason for this publicity was that the chemical industry produced 9.000 million kg of the carcinogen VCM annually (CEH 1976). Two points of view are given below: De Voogd (1977) wrote an article with the following title: "World-wide approach to the PVC problem". In this article, the danger of using PVC in food packaging material is described mainly as an emotional problem, caused by the influence of the press and because the industry was not active enough in providing proper information.

Reynders (1977), however, reports that in The Netherlands, consumer protection is bad, compared to the United States, because in The Netherlands the packaging of food in rigid and semi-rigid PVC is not forbidden as it is in the USA.

De Voogd explained that the industry had taken appropriate measures to avoid danger to the consumer. Reynders disagreed and suggested a total ban on the use of PVC for food packaging.

However, it may be assumed that sensitive analytical determinations will contribute to solving this problem.

3.1.2. Vinylchloride and polyvinylchloride

In 1835, vinylchloride or monochloroethylene $CH_2{=}CHCl$ was discovered by Regnault. The preparation was as follows:

$$\underset{\textit{1,1-dichloroethane}}{CH_3\text{-}CHCl_2} + KOH \longrightarrow \underset{\textit{VCM}}{CH_2{=}CHCl} + H_2O + KCl$$

1,2-dichloroethane may also be the starting material. Nowadays the industrial production of VCM is as follows:

$$\underset{\textit{acetylene}}{HC{\equiv}CH} + HCl \longrightarrow VCM$$

or

$$H_2C{=}CH_2 + 2\ HCl + \tfrac{1}{2}(O_2 + 4\ N_2) \longrightarrow C_2H_4Cl_2 + H_2O + 2\ N_2$$

ethylene *ethylenedichloride*

The ethylenedichloride is then thermally cracked to the monomer VCM and the by-product HCl (Bertram 1977, Truhaut 1978). The physical constants are given in Table 1.

Table 1

Physical constants of VCM

molecular weight	62.5
boiling point	- 13.9 °C
density of the liquid D^{20}_4	0.9121 kg/m^3
refractive index n^{10}_D	1.4066
density of the vapour	2.15 kg/m^3

VCM is slightly soluble in water (0.11 per cent w/w at 25°C), well soluble in ethanol and very soluble in diethylether, carbon tetrachloride and dimethylacetamide. It is also very soluble in fats, is inflammable and forms explosive mixtures with air.

The first limits to the vinylchloride content in the air in factories were set at such a level (500 ppm) that formation of these explosive mixtures could be avoided (OSHA 1974).

In 1835, Liebig observed the formation of polyvinylchloride (PVC) from vinylchloride (Schaffner 1975). In 1930 industrial production started in the United States:

$$CH_2{=}CHCl \longrightarrow -CH_2-\underset{\displaystyle Cl}{CH}-\left[\begin{array}{c}C-C\\ \\ Cl\end{array}\right]_n-CH_2-\underset{\displaystyle Cl}{CH}-$$

PVC, homopolymers and copolymers are generally made by one of the following polymerisation processes: in suspension; in a water emulsion with suitable surfactants; or by a solution polymerisation process.

Suspension polymerisation is effected by suspending droplets of VCM in water under controlled pressure and temperature. Each droplet contains small amounts of additives, such as initiators like benzoylperoxyde, and chain terminators like trichloroethylene. Plasticizers (phtalates), UV absorbers, stabilizers and so on are added to modify the properties of the polymer.

In 1976 the world production of the PVC was 8.5 million ton (CEH 1976). About 8 per cent is used for packaging. This includes food packaging material, coatings and components for food processing equipment, flexible tubing and water pipes (Schaffner 1974).

3.1.3 Toxicological aspects of VCM

For a long time VCM was considered safe. It was even used as an anaesthetic. Since the beginning of the seventies, VCM exposure has become increasingly associated with toxicological manifestations. For example the influence of VCM on the central nervous system, the liver, lungs and fingers (Cavigneaux 1975, Potter 1976, Thomas 1977).

In 1970, Viola extended these studies to investigations of carcinogenicity in rats.

Maltoni (1975) exposed rats to air containing 50 and 250 ppm VCM. This resulted in cases of angiosarcoma (a liver cancer).

Feron (1975) did rat experiments with oil solutions spiked with VCM, orally dosed, which also resulted in cases of angiosarcoma. The lowest effect level has not yet been determined.

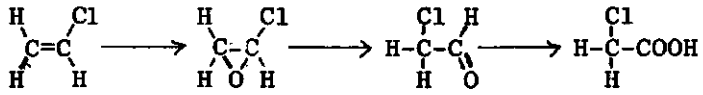
In 1974 the first human deaths related to VCM were reported by Creech (1974).

Bartsch (1975) published a paper describing 49 cases of angiosarcoma among workers exposed to a VCM atmosphere.

It is not clear whether it is the VCM itself or its metabolites that interact with the genetic material, because the exact *in vivo* metabolic route of VCM is unknown. The most likely hypothesis is the alkylation of the DNA molecule. This alkylation is probably caused by one of the metabolites of VCM such as chloroethyleneoxyde, chloroacetaldehyde or chloroaceticacid. Some *in vitro* genetic

studies give evidence of this mechanism.

According to Bartsch, the liver microsomal system from various animal species as well as from humans metabolizes the vinylchloride to chloroethyleneoxide.



metabolism of VCM (van Duuren 1973, Bartsch 1976)

The treatment of rats with drugs, known for their influence on the activity of microsomes, changed the toxicity and mutagenicity of vinylchloride in these rats. The chloroethyleneoxide rearranges to 2-chloroacetaldehyde. Direct proof of the activity of $ClCH_2CHO$ *in vivo* is not given in the literature (Hens 1977)

3.1.4 Polyvinylchloride as food packaging material

PVC has several properties that make it attractive as packaging material for food, for example its clarity, inertness and permeation control of gas and water vapour. PVC protects the contents against deterioration.

The main food packaging products made from PVC are containers, bottles, foil and flexible film.

Bottles are manufactured by blow-moulding a dry powder blend based on PVC polymer. They are used for liquids, such as fruit juice, cooking oil, wine and vinegar.

Foil is a rigid product ranging in thickness from 75 to 750 microns, produced by calendering or extrusion and based on low-molecular weight homopolymers or copolymers of vinylchloride and vinylacetate. The vinylchloride-vinylacetate is the PVC copolymer most widely used. It may contain up to 20 per cent of vinylacetate. PVC copolymers have better deep vacuum-drawing properties than the corresponding homopolymer. Such copolymers are therefore suitable for the packaging of biscuits, margarines, butter, salads, etc.

Flexible film is approximately 12.5 to 25 microns thick and is produced by extrusion blowing. It is used for wrapping cheese, meat, fruit and vegetables.

Other possibilities of food contact with PVC are bottle closure liners, lacquered food cans and heat-seal aluminium foil.

PVC polymers are also used as packaging materials for drug products, blood, cosmetics and also as components of medical appliances.

3.1.5. VCM migration into food and food simulants

Like most other polymerisation reactions, the reaction of VCM to produce PVC is not complete, and inevitably there is a residue of VCM left in the raw polymer produced. Some estimates have indicated that the residue in the polymer immediately after manufacturing may be up to 10 per cent. Most of this residual VCM is either vented to the atmosphere or recovered by vacuum stripping and used again.

This thesis deals with the determination of carcinogens in food and food packaging material. Therefore, the regulations and determinations concerning the presence of VCM in the air in factories are not discussed. However, it must be mentioned that the strict limits set to the presence of VCM in the atmosphere in factories made it necessary for the industry to develop methods for producing PVC with a lower VCM content. The content of VCM in the food packaging material is now in 1978 much lower, therefore, than three years before. See 3.3.6

With the introduction of PVC for food packaging, i.e. in the early sixties, one of the main obstacles was considered to be the possible migration of its plasticizers.

Plasticizers are high molecular weight, nonvolatile liquids which solvate and soften the polymer and are present up to 40 per cent in PVC (Koleske 1969). Early investigatory work done by Lehman (1956) indicated that PVC was insoluble in various solvent systems. Consequently, there was little concern about the safety of the food-contact articles made from PVC. Therefore, in 1968, even the bottling of distilled spirits in PVC was permitted.

In early 1973, Schenley distillers noted significant organoleptic differences between alcoholic beverages packed in glass and those packed in PVC. The cause appeared to be migrated VCM (Fed.Regist. 1974).
Because at that time carcinogenicity of VCM was already being discussed, regulations for food packaging in PVC were started and various laboratories began with VCM studies.

In 1973, the Food Inspection Service of Utrecht started investigations in very close cooperation with the Central Institute for Nutrition and Food Research (CIVO) in The Netherlands. The first regulations in The Netherlands gave a limit of 50 ppb for the VCM content in food *(Hoofdinspectie 1974)*.

Food simulants are used to facilitate the migration tests. They are well-defined solutions, covering the range from aqueous solutions to fat. Experiments can be done with food simulating solvents such as water, 15 per cent ethanol in water, 3 per cent acetic acid in water and vegetable oil. These four food simulants are recommended in The Netherlands. In the USA 50 per cent ethanol in water and n-heptane are also used (Fed. Regist. 1977). It is not always necessary to use a food simulant. Vinegar and salad oil were often used in our laboratory for migration experiments.

In the literature, several studies describe the relationship between the residual VCM contents in the PVC packaging material and the amount that can migrate into the food or food simulant (vom Bruck 1976, Chan 1978, Tester 1976).
Daniels (1975) presented a model that provides the relationship between VCM in PVC bottles and the maximum concentration in the food. He calculated that if 1 ppm in the VCM is present in the PVC compound, the maximum VCM concentration in the food is lower than 50 ppb. His migration conditions are comparable with those described in the Dutch regulations.

The packaging material is filled with the food or food simulant and kept at 40°C for 10 days.
Gilbert (1975), Morano (1975), Kashtock (1977) and Biran (1977) carried out extensive studies on the migration of VCM.
These studies mainly concern the hypothesis that below a certain level of the VCM the effective migration is zero.

However, Pfab (1977) reports the rate of migration to be proportional to the monomer concentration and the time of contact. His results also imply, that the level of 50 ppb in food cannot be reached when the monomer in the packaging material is below 1 ppm. The ratio packaging material to food is the generally assumed ratio of 1 kg per 6 dm^2.

Our own experiments confirm these results, with the exception of food packed in small *individual* portions. For example about 14 g butter or jam is packed in 0.4 dm^2 packaging material. The ratio packaging material to contents becomes 0.2 kg per 6 dm^2. Thus, five times more packaging material is used for the same unit of weight. In these cases only analysis of the food gives satisfactory information.

3.1.6 Methods for determining VCM

In October 1978 a computer search, going back two years in the Chemical Abstracts, was carried out by the library of the University of Utrecht. The key words were vinylchloride, analytical determination, gas chromatography and food.

Various determinations are described in the literature for analyzing vinylchloride in ambient air, in factories, in water, in PVC and in food and food simulants.
During the last five years the necessity of reliable and sensitive methods of analysis became urgent.

A group of investigators described methods to concentrate VCM on an absorbent. Ahlstrom (1975) used charcoal, Hill (1976) studied 20 different kinds of charcoal, Ives (1975) trapped VCM on Tenax cooled with dry ice, Dressman (1977) and Bellar (1976) concentrated VCM on a porous polymer after transferring the VCM from the aqueous phase to the gas phase.
After desorption the VCM was then determined by gas chromatographic methods.

Some of the gas chromatographic conditions applied for VCM determinations in various kinds of samples are given in Table 2.

TABLE 2

Methods for determination vinylchloride

author	Eckert Puschman	Puschman Eckert	Berens	Breder	Breder	Breder Diachenko	Fuchs	Dennison	Williams
year of publishing	1975	1975	1975	1975	1975	1977	1975	1978	1976
suitable for	PVC	oil	PVC	PVC	food & foodsim.	foodsim.	oil	PVC	alc.beverages vinegars
headspace injection	yes	yes	yes	no	no	yes	yes	yes	yes
amount of injection	0.3 ml	0.3 ml	0.3 ml	20 µl	20 µl	4 ml	1 ml	2 ml	1 ml
detector	FID	FID	FID	FID	FID	FID	FID	MS	FID
sensitivity	0.1 ppm	0.01 ppm	0.5 ppm	1 ppm	0.05 ppm	1 ppb	2 ppb	0.3 ppb	5-10 ppb
remarks	suitable for automation	suitable for automation	only for PVC powder	disadvantage is the large amount of liquid on col.		improvement of Breder of the method of 1975	internal standard cis-2 butene	time consuming	see next page Williams

TABLE 2 (continued)

author	Williams	Page	Pu	Fujii	Rosen
year of publishing	1975	1977	1977	1977	1975
suitable for	vinegars, alc.bever.	oil vinegar	PVC	water	air
headspace injection	no	yes	no	no	yes
amount of injection	5 µl	1 ml	50 µl	1000 µl	10 ml
detector	FID	Hall or FID microcoulometer		MS	MS
sensitivity	10-15 ppb	0.05-0.02 ppm	5 ppb	0.1 ppb	0.3 ppb
remarks	head-space method is better	no blank given in the method	large amount of in-jection	idem	first MS publication

Details of the headspace methods, for determining VCM in PVC, such as equilibrium time and temperature and type of column are extensively reported in the collaborative study. See 3.3.7, see Table 15.

In addition the following results of investigation are worthwhile mentioning:

1. Krishen (1976) eliminates disturbances caused by acetaldehyde in gas chromatographic analysis of low levels of VCM in air by using a pre-column with sodiumbisulphite.
2. Hoffman (1976) describes a quantitative determination of VCM in tobacco smoke. VCM is trapped on charcoal and is then converted to 1,2-dibromo 1-chloroethane, which can be analyzed by an electron capture detector.
3. Gilbert (1976) obtained a detection level of 5 ppb in PVC samples. However, the analyses were performed after major servicing of the MS equipment.

3.2 Limits for vinylchloride

As soon as the carcinogenicity of VCM was known, a limit of 50 ppb VCM in food was set in The Netherlands *(Hoofdinspectie 1974)*. This limit was chosen because at that time, it was the detection limit of the most sensitive analytical method.

In several meetings of experts, the VCM problem was discussed in Brussels in order to agree upon an EEC directive. The starting point was that VCM was a carcinogen, and carcinogens were not allowed to be present in food. This agrees with the Delaney clause used in the USA. It has become common practice now to provide an EEC directive, to control a limit, with a method having an analytical detection limit. In accordance with this practice, the admissible concentration of a carcinogenic compound has been established as the lowest amount that can be determined by the appropriate analytical method. So, the skill of the analytical chemist fixes the amount of VCM that may be present in food.

In practice the problem is even more complex because the analytical determination should be carried out by a "normal" laboratory. This often means that even laboratories with poor equipment must be in agreement with the decision.

Another problem is that for a reliable method it is necessary to determine not only the repeatibility but also the reproducibility. Therefore, the ISO standard prescribes that at least nine laboratories are needed for a collaborative study. This also means that the drafting of a directive for VCM takes a lot of time.

The proposed EEC directive contains two limits:

- for the packaging material
- for the food

See the collaborative study 3.3.7.

3.3 Experimental section

3.3.1 Gas chromatographic determination with an FID

A gas chromatographic determination with an FID is described by van Lierop (1974) (Annex A). The detection limits obtained with the methods described were sufficient at that time, because the contents of VCM in the PVC were high.
Nevertheless the need for a more specific method was already obvious. Some results of VCM determination are given in 3.3.5 and 3.3.6.

3.3.2 Gaschromatographic determination using a Hall detector

A gas chromatographic determination using a Hall detector is described by van Lierop (1974) (Annex B). This was the first identification method. Later routine measurements were made using this detector. The mass spectrometer method was then used for inspection and identification. Later the method described in Annex B was changed to a headspace method.

3.3.3 Headspace GC-MS determination of VCM with mass fragmentation in food and food simulants

The determination of VCM with headspace GC mass fragmentation in food and food simulants is described by van Lierop (1974) (Annex C). This method enabled us to carry out quantitative determinations at the 1 ppb level in the food. This method was used to confirm the presence of vinylchloride in positive

samples according to the method described in Annex B. Later, the method was used to determine the amounts of vinylchloride present below the limit of 50 ppb. These determinations were made to collect information on the presence of VCM in the food.

The results of this method were compared with the results of methods described in Annex B. Results are given in Table 3.

Table 3

Comparison of VCM determinations obtained with Hall and MS

	Hall	MS
diabetic jam	0.7 ppm	0.7 ppm
peach jam	20 ppb	20 ppb
strawberry jam	0.9 ppm	0.9 ppm
hazelnut butter	10 ppb	8 ppb
vinegar	6 ppb	1 ppb
peanut butter	4.3 ppm	4.1 ppm

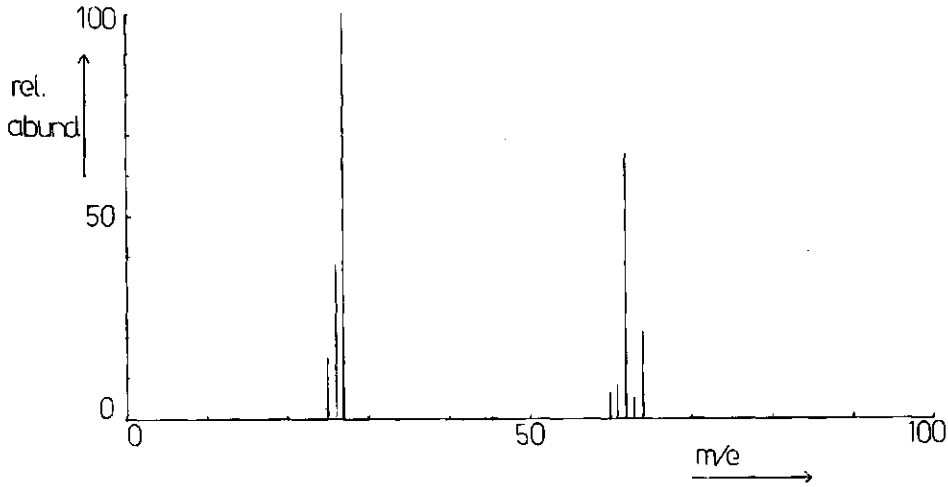
3.3.4 Headspace GC-MS determination in polyvinylchloride packaging material

The determination of VCM in the packaging material itself, in the homopolymer or in the copolymer polyvinylchloride-polyvinylacetate, was primarily used as a screening method. The determination of VCM in food was more difficult. The determination in food was only made in the case of a high VCM content in the PVC. The VCM determination could be made according to the method in Annex A as long as the VCM content in the polymer was in the ppm range. The enormous decrease of the VCM content in the polymer forced our laboratory to develope a more sensitive method for the determining of VCM in the polymer, a headspace GC method.
The mass spectrum of VCM is shown in Fig. 1. It can be concluded from this spectrum that using a headspace method, -which means injection of air containing nitrogen, oxygen and carbon dioxide, with masses of 28, 32 and 44- the masses of

VCM below 45 are not suitable for mass fragmentography due to lack of specificity. Therefore, the masses m/e 62 and m/e 64 were chosen.

Fig. 1

Mass spectrum vinylchloride



Different solvents, such as tetrahydrofuran, dimethylformamide and dimethylacetamide (DMA), are suitable for the dissolution of PVC. Ethylacetate, that swells the polymer, is also appropriate. This solvent has been used in our laboratory for several years. As advised by an EEC committee, headspace analysis of a polymer dissolved in DMA is now practiced. The method is principally as follows:

Weigh the polymer sample in an infusion flask, add DMA, close, equilibrate in a water bath and inject the headspace into a GC-MS apparatus. See Annex C for details.

A calibration is given in the low ppb region, see Table 4:

Table 4

Peak heights of VCM as a function of content

Amount VCM in ppb	Peak heights in mm at m/e 62
0.0	0
0.7	12
1.7	21
3.3	57
4.9	74
6.5	102
12.0	184

The calculated calibration curve from Table 4 is:

$$A = 15.44\ B + 0.27$$

A is the peak height in mm, B is the amount of VCM in µg/kg. Other calibration curves were made for higher ppb ranges.

The sensitivity of the method is shown in Fig. 2. An injection of 0.5 ml headspace of a standard solution of 0.1 ppb VCM in DMA and a blank are given.

If necessary, a lower detection limit can be obtained by injecting 2 ml sample and by raising the temperature of the water bath.

The repeatibility of the method was tested by the following procedure: Approximately 19 g PVC polymer film was cut into pieces each weighing 0.12 g. These pieces were mixed together and totals of 1.5 g of these pieces were dissolved in 15 g DMA. Twelve determinations were done. The results of these determinations are given in Table 5.

Fig. 2

Sensitivity of the VCM determination: mass fragmentograms of a blank (a) and a standard solution of DMA with 0.1 ppb VCM (b).

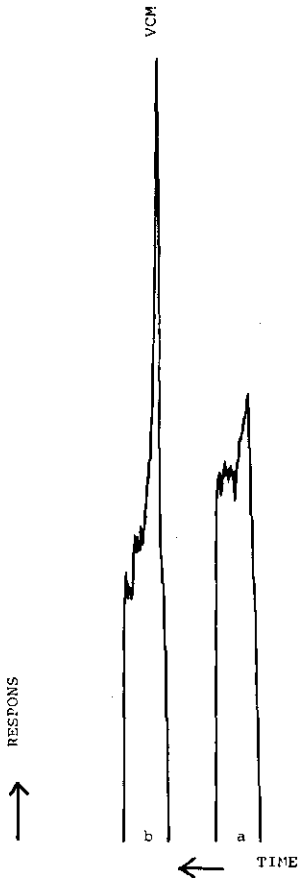


Table 5

Repeatibility of VCM analysis in polymers

sample	content in ppb
1	42
2	41
3	40
4	42
5	39
6	43
7	50
8	41
9	43
10	34
11	44
12	38

The mean is 41 ppb. The standard deviation is 4 ppb. The variation coefficient is 10 per cent.

Some measurements were made to determine absolute amount of VCM in the vapour under analysis conditions.

Liquid injections were made of four different VCM standard solutions (10 per cent VCM in 15 ml DMA) and the vapours of these solutions were also injected after equilibrium at 70°C.

The same response was obtained for injections of 5 µl solution and for 0.14 ml vapour with contents of 5.1 ppm. If a liquid injection of 2.5 µl and a 1 ml headspace (the normal amounts) are used, the response of the headspace is about 14 times greater.

Other results are given in Table 6.

Table 6

Comparison of liquid- and headspace injections

amount VCM in DMA in ppm	response in mm: inj. 5 µl liquid	0.1 ml headspace	% VCM in headspace
0.3	128	110	6.1
0.5	114	72	5.3
0.8	268	222	5.9
5.1	122	86	5.9

The percentage VCM in the vapour was calculated as follows:

5 µl of 5.1 ppm VCM in the liquid gives the same response as

$$\frac{122}{86} \times 0.1 = 0.14 \text{ ml gas}$$

5 µl contains the same amount of VCM as 0.14 ml gas. Assume this amount to be a g. The total amount in the liquid is:

$$\frac{15 \times 10^3}{5} \times a \text{ g} = 3.000 \text{ a g}$$

Because the volume in the infusion flask (vial) is 40 ml, the amount in the vapour is:

$$\frac{25}{0.14} \times a \text{ g} = 178.6 \text{ a g}$$

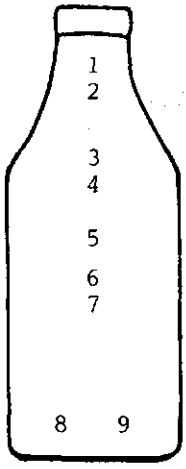
The percentage in the gas phase is:

$$\frac{178.6}{3.000} = 5.9 \text{ per cent}$$

The variations of the VCM content depending on the sampling spot was also investigated. For this purpose samples were taken from nine different positions of a 2 l PVC bottle, used commercially for the bottling of lemonade. The sampling positions are indicated in Fig. 3.

Fig. 3

Sampling spots on a 2 liter PVC bottle



The VCM content in these sample was determined. The results are given in Table 7.

Samples 8 and 9, taken from the thick bottom of this bottle, gave a content 3 times greater than a sample taken from position 3 or 4, the normal sampling area.

Table 7

VCM content as a function from sampling spot and thickness

sample	content in ppb	thickness in mm
1	44	0.4
2	30	0.4
3	33	0.4
4	53	0.4
5	44	0.4
6	46	0.6
7	37	0.6
8	146	1.9
9	145	1.8

3.3.5 Vinylchloride contents in food

Many investigators have analyzed the VCM content in food. Results from Canada, Norway, Australia, England and Switzerland are given to show this.

Williams (Canada 1975) found the following levels of VCM in foods packed in PVC bottles: for alcoholic beverages 0 to 1.6 ppm, for vinegar 0 to 8.4 ppm, for peanut oil 0.3 to 3.3 ppm.

Ehtesman (Norway 1976) found in 66 samples of various foods a mean of 79 ppb, while no VCM could be detected in sixteen additional samples.

The results of the Australian (1975) and British investigators (Ministry of Agriculture 1978) were combined to obtain Table 8, summarizing a large number of samples.

Table 8

VCM contents in various food samples (1974-1977)

VCM range in ppm	number of samples	%
< 0.01	278	69
0.01 - 0.05	72	18
0.05 - 0.20	37	9
> 0.20	15	4
total	402	

Rösli (Switzerland 1975) reported that from 41 vegetable oil samples thirteen had contents above 50 ppb with 1.75 ppm as the highest content. In 1977 the same laboratory found in 38 samples only two samples with a VCM content above the limit of 50 ppb.

Results obtained at the Food Inspection Service Utrecht (Annex A to C):

Each year since 1974 about 600 samples of PVC packaging material and food have been investigated.

In 1975 eight oil samples had contents above the 50 ppb limit. One salad dressing had a content of 0.25 ppm, four margarines had contents of 0.06, 0.10, 0.20 and 0.25 ppm. A whole batch of ready-made salads from one supplier was above the limit. A great quantity of wine contained 0.76 ppm VCM.

In 1976, the number of positive samples had considerably decreased; only one low-fat content margarine with 0.06 ppm, one fish-sample with 0.09 ppm and three biscuit-samples with 0.19, 0.13 and 0.07 ppm were found.

In 1977 amounts of VCM above 0.05 ppm were only found in the very small *individually*-wrapped portions. For example 4.1 ppm in peanut butter.

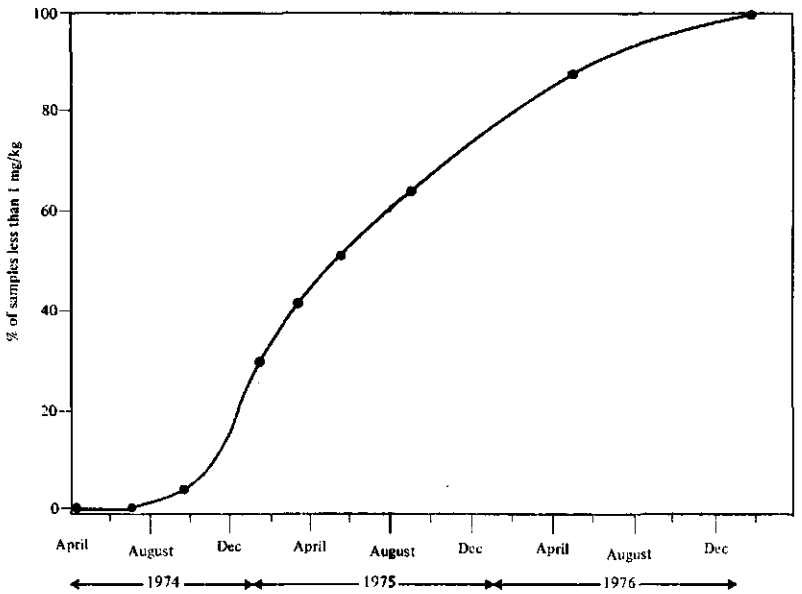
In 1978, in a study of 67 samples, analyzed using mass fragmentography (detection limit 0.1 ppb) VCM could be detected in only three samples. These contents were: 0.3 ppb in a soya oil, 0.6 ppb in vinegar and 2 ppb in a salmon salad.

3.3.6 VCM contents in polymers in PVC packaging materials

The decrease of the VCM content in the packaging material has been very marked in the last years. British investigations show this decrease in VCM content in the PVC material from April 1974 to February 1977 (Fig. 4).

Fig. 4

Proportion of PVC bottles having less than 1 mg/kg VCM



German investigators report a similar result (Table 9). In the first part of 1977 only one of the 119 bottles investigated had a content higher than 1 ppm (vom Bruck 1978).

Table 9

VCM content of PVC food packaging materials

VCM content in mg/kg	number of samples	
	$<$ 500ppb	$>$ 500ppb
second half year of 1975	52	14
1976	102	22
1977	472	9

In 1978, Dennison (USA) reported that 11 of 13 PVC food packing materials had VCM contents under 5 ppb.

An Australian report of 1975 concluded that in eighteen months the VCM content had been reduced by 25.

Results obtained at the Food Inspection Service Utrecht:

In 1974 contents of 10 to 864 ppm VCM were found in an investigation of 121 samples. The mean was 127 ppm.

In 1976 the contents were below 11 ppm. The results of an investigation with 67 samples carried out in October 1978, are given in Table 10. The mean of this investigation was 77 ppb. The mean VCM content of the food packaging material is reduced by about 1500 $(\frac{127.000}{77})$.

Table 10

VCM content in polymers mainly used for the packaging of biscuits, lemonades, vinegars and salads.

Type of polymer	Thickness in mm	VCM content in ppb
PVC	0.10	< 1
PVC-PVAC	0.15	< 1
PVC-PVAC	0.10	< 1
PVC-PS	0.45	< 1
PVC-PVAC	0.10	< 1
PVC-PVAC	0.10	< 1
PVC	0.30	< 1
PVC-PS	0.10	4
PVC-PVAC	0.10	5
PVC-PVAC	0.10	6
PVC-PVAC	0.10	6
PVC-PVAC	0.10	6
PVC	0.15	6
PVC-PS	0.40	8
PVC-PS	0.15	9
PVC-PVAC	0.10	9
PVC-PVAC	0.20	10
PVC-PS	0.15	10
PVC-PS	0.40	11
PVC-PS	0.40	11
PVC-PS	0.40	12
PVC-PVAC	0.40	12
PVC-PS	0.40	12
PVC-PS	0.50	13
PVC-PS	0.35	14
PVC	0.15	15
PVC-PS	0.40	15
PVC-PS	0.35	16
PVC-PVAC	0.15	17
PVC-PS	0.35	18
PVC	0.40	19
PVC-PVAC	0.10	25
PVC	0.10	26
PVC-PS	0.45	28
PVC-PS	0.45	28

Table 10 (continued)

Type of polymer	Thickness in mm	VCM content in ppb
PVC-PVAC	0.10	29
PVC-PVAC	0.20	29
PVC-PS	0.50	31
PVC-PS	0.35	36
PVC-PS	0.55	38
PVC-PVAC	0.10	40
PVC-PS	0.35	44
PVC-PS	0.50	45
PVC-PS	0.20	46
PVC-PVAC	0.40	50
PVC-PS	0.10	50
PVC-PVAC	0.10	50
PVC-PVAC	0.40	54
PVC-PS	0.50	60
PVC-PS	0.50	63
PVC-PS	0.40	63
PVC-PS	0.45	70
PVC-PS	0.35	90
PVC-PS	0.45	92
PVC-PVAC	0.10	93
PVC-PS	0.15	97
PVC-PS	0.15	110
PVC-PS	0.30	110
PVC-PVAC	0.10	120
PVC	0.20	120
PVC-PS	0.50	130
PVC-PVAC	0.10	140
PVC	0.20	170
PVC-PS	0.50	180
PVC-PS	0.30	410
PVC-PS	0.45	720
PVC	0.20	1200

3.3.7 Collaborative study

3.3.7.1 Introduction

The directive of the Council of the EEC of January 30, 1978, concerning VCM, contains the following provisions:

1. Maximum content of VCM in materials and articles 1 mg/kg
2. Criteria concerning the method for the determination of the amount of VCM in materials and articles and for the determination of the VCM content migrated from the materials and the articles.

a. The determination of the amount of VCM in materials and articles and the determination of VCM migrated from materials and articles into food, is carried out by gas chromatography with headspace.

b. The detection limit of the amount of VCM present in foodstuffs is 0.01 mg/kg.

c. The determination of the VCM migrated from materials and articles should in principle be carried out in the food. When this is impossible because of technical reasons, the Member States may allow determination with simulants.

The committee of experts has the task of developing the analytical methods.
In the meeting of February 21 and 22, 1978 of this committee it was agreed to start with the determination of VCM in materials and articles. It was also agreed that the German delegation should organize a collaborative study with polymers containing about 1 ppm vinylchloride.

As a preliminary investigation to that collaborative study, the Dutch delegation proposed to make such a study using standard solutions of vinylchloride in dimethylacetamide (DMA). The Food Inspection Service of Utrecht was requested to organize this project.

3.3.7.2 Set up

VCM is very volatile. Precautions were therefore taken to avoid the loss of VCM during dispatch. Each laboratory was required to send its own empty headspace vials to Utrecht. There they were filled with two concentrations of VCM in DMA in the range of 0.05 to 0.20 mg/kg. The exact amount was not known to the participants.

The vials were closed in Utrecht and sent to the participating laboratories. These vials could be used directly in the determination that was carried out according the method given by the commission. This method is not exhaustively described. The participants were free in the choice of the column, in the amount of sample used, the use of automatic or manual apparatus and in the use of an internal standard.

To meet the requirements of the participants using an internal standard, diethylether was added in a concentration which was indicated to the participants.

Two samples were made of each concentration: A1 and A2, B1 and B2. It was not revealed that A1 and A2 and B1 and B2 contained exactly the same concentration of VCM.
To all these standards, the internal standard diethylether was added in a concentration of 1.963 mg/kg (ppm). These samples were given not only to adjust the GC conditions, but also to estimate the quality of the DMA, present in the participating laboratory.

3.3.7.3 Preparation of the sample

VCM was liquefied from a lecture bottle of Matheson Gas Products, containing VCM-gas with a purity of 99 per cent. This gas was condensed into a reagent tube cooled with dry ice in acetone. The liquid VCM was added to a pre-weighed amount of DMA in an infusion flask. This DMA was also cooled with dry ice in acetone and distilled because commercial DMA (Merck no. 803235) contained interference peaks on our gas chromatogram. Diethylether had already been added to this DMA in a concentration of 1.963 mg/kg. All further dilutions were done with this DMA.

The infusion flask was closed with an aluminium screw cap, with a rubber septum, protected by a teflon disc.

For this experiment, the first infusion flask had a volume of 130 ml and contained 100 ml DMA, approximately 2 g VCM was added. The dilutions werde done with disposable syringes (trade name B-D Discardit of polypropene - polyethylene). A certain amount of liquid was taken from the infusion flask through the septum. The exact amount was determined by weighing. A concentration of about 10 mg VCM/kg DMA was obtained in two stages. From this solution the standard solutions A and B were prepared in infusion flasks of 500 ml. These flasks were almost

filled with the amount of solution needed for this collaborative study. The following procedure for filling the vials given to the partcipants was chosen:

1. All the samples were taken through the septum using a suitable disposable syringe.
2. The vials were immediately closed after filling.
3. If necessary supplementary air was injected into the flasks.
4. The sample was carefully added to the vial.

3.3.7.4 General Statistics

The Draft International Standard ISO/DIS 5725 of 1977 - Precision of test methods - Determination of repeatibility and reproducibility - has been followed for the calculation of the results.

The repeatibility r is the value below which the absolute difference between two single test results obtained with the same method of identical test material, under the same conditions (same operator, same apparatus, same laboratory and a short interval of time) may be expected to lie with a specified probability; in the absence of other indications the probability is 95 per cent.

The reproducibility R is the value below which the absolute difference between two single test results obtained with the same method on identical test material, under different conditions (different operators, different apparatus, different laboratories and different time) may be expected to lie with a specified probability; in the absence of other indications the probability is 95 per cent.

Some other tables of the Statistical Manual of the AOAC (Youden 1975) have also been used.

3.3.7.5 Calculation of the results

The results received from the 16 participating laboratories are given in Table 11. The results of laboratory 16 were not used, because this laboratory received vials that where incorrectly closed. Laboratories 2 and 4 were considered as statistical outliers (Youden 1975).

Table 11

Results of vinylchloride measurements

Contents of vinylchloride in µg/kg x 1000

Laboratory number	A1	A2	B1	B2
1	46	54	130	133
2	42	44	117	126
3	55	52	133	133
4	70	74	223	222
5	54	53	139	144
6	51	52	133	133
7	72	67	143	140
8	45	47	136	139
9	54	56	148	138
10	52	53	138	137
11	49	49	148	148
12	51	50	139	140
13	50	50	129	132
14	57	57	146	146
15	51	52	143	130
16	0	47	116	116

For 15 laboratories the sum of ranking the values of Table 11 must be between the limits of 8 and 56 (95 per cent confidence). For laboratory 2 the score is 4, for laboratory 4 the score is 59. We received a letter from laboratory 4 stating that there was some doubt about their standard preparation.
Laboratory 2 received a very large sample, see Table 15, part 1, which may explain the low result. The measurement of laboratory 7 at level A, with a value of 72, is also considered to be a

statistical outlier. A criterium for rejecting outlying measurements with a 1 in 20 probability of a wrong decision is given. (Youden 1975). In this Dixon test with 13 measurements a measurement is an outlier if, using the following formula

$$\frac{X_n - X_{(n-1)}}{X_n - X_1} > 0.52$$

Applied to the value of 72 reported by laboratory 7 for sample A the calculation is:

$$\frac{72 - 57}{72 - 46} = 0.58$$

So the value reported by laboratory 7 for sample A is no longer considered.
After rejecting the outliers, the results given in Tables 12 and 13 could be calculated. On the basis of these two tables, Table 14 was made according to page 24 of the ISO/DIS 5725.

For the mean of level A 0.052 mg/kg is found and for the mean of level B 0.138 mg/kg is found. These are the exact amounts of VCM added by our laboratory, for A 0.052 and for B 0.138 mg VCM/kg DMA.
The repeatibility and reproducibility are for the level A 0.005 and 0.009 resp. and for the level B 0.010 and 0.017 resp.
If we assume that r and R are proportional with m, we can express r and R in a level independent way.

The repeatibility in % is:

$$\tfrac{1}{2}\left(\frac{0.0054}{0.0517} + \frac{0.0101}{0.1384}\right) \times 100\ \% = 9\ \%$$

The reproducibility in % is:

$$\tfrac{1}{2}\left(\frac{0.0091}{0.0517} + \frac{0.0172}{0.1384}\right) \times 100\ \% = 15\ \%$$

Table 12

number of laboratory	ranges per laboratory	
	level A	level B
1	8	3
3	3	0
5	1	5
6	1	0
7	-	3
8	2	3
9	2	10
10	1	1
11	0	0
12	1	1
13	0	3
14	0	0
15	1	13

Table 13

mean per laboratory	
level A	level B
50.0	131.5
53.5	133.0
53.5	141.5
51.5	133.0
-	141.5
46.0	137.5
55.0	143.0
52.5	137.5
49.0	148.0
50.5	139.5
50.0	130.5
57.0	146.0
51.5	136.5

3.3.7.6 A two sample chart

A two sample chart as described by Youden (1975) has been made with the results given in Table 11. Along the X axis, the difference between the value of a laboratory for level A minus the mean for A are plotted. The same is done for the value of a laboratory for the level B.

The two results provide a pair of coordinates that determine a point for each laboratory.

If all the differences are caused by random errors, the points are scattered among the four quadrants, ++ , - , -+ , +- .

TABLE 14

	level A	level B
Number of laboratories p	12	13
Number of replicates n	2	2
Coding constants a.b	a = 0, b = 1	a = 0, b = 1
$S_1 = \sum x_i$	$S_1 = 620$	$S_1 = 1799$
$S_2 = \sum x_i^2$	$S_2 = 32126.50$	$S_2 = 24.9319$
$S_3 = \sum w_{ci}$	$S_3 = 86$	$S_3 = 332$
$s_r^2 = \frac{S_3}{2p} \times \frac{1}{b^2}$	$s_r^2 = \frac{86}{24} = 3.58$	$s_r^2 = \frac{332}{26} = 12.78$
$s_i^2 = \frac{1}{b^2}\left[\frac{pS_2 - S_1^2}{p(p-1)}\right] - \frac{s_r^2}{2}$	$s_i^2 = \frac{12 \times 32126.50 - 620^2}{12 \times 11} - \frac{3.58}{2} = 6.68$	$s_i^2 = \frac{13 \times 249319 - 1799^2}{13 \times 12} - \frac{12.78}{2} = 24.00$
$m = a + \frac{S_1}{b.p}$	m = 51.67	m = 138.4
$r = 2.83 \sqrt{s_r^2}$	$r = 2.83 \sqrt{3.58} = 5.36$	$r = 2.83 \sqrt{12.78} = 10.11$
$R = 2.83 \sqrt{s_i^2 + s_r^2}$	$R = 2.83 \sqrt{6.68 + 3.58} = 9.06$	$R = 2.83 \sqrt{24.00 + 12.78} = 17.17$

Usually the points are found to be dominant in two quadrants, the ++ upper right quadrant and the -- or lower left quadrant. This tells us that if a laboratory gets a result that is too high with one material (level A), it is almost sure to be similarly high with the other material (level B). The same holds for low results. In general, the points form an elliptical pattern with the major axis of the ellipse running diagonally at an angle of 45° to the X axis.
This is true of the pattern shown in Fig. 6.

3.3.7.7 Comparison of methods

The participants gave some details concerning the method used. In Fig. 7 the gas chromatograms are given. The numbers correspond with the number of the participant in Table 11. Table 15 gives relevant data. The vial type is given by a number in Table 15, part 1. These numbers correspond with those given in Fig. 5.

The volume of vial type 1 is: 10 ml, of 2: 25 ml, of 3: 25 ml, of 4: 40 ml, of 5: 320 ml.

Fig. 5

Different vials used in the collaborative study

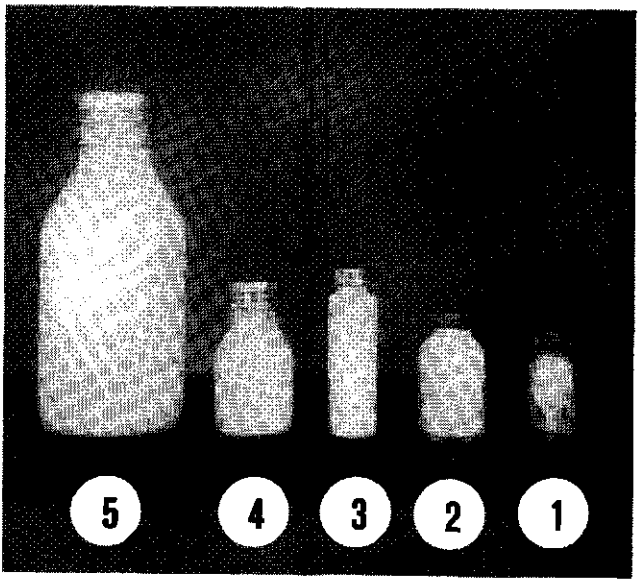


Fig. 6, A two sample chart

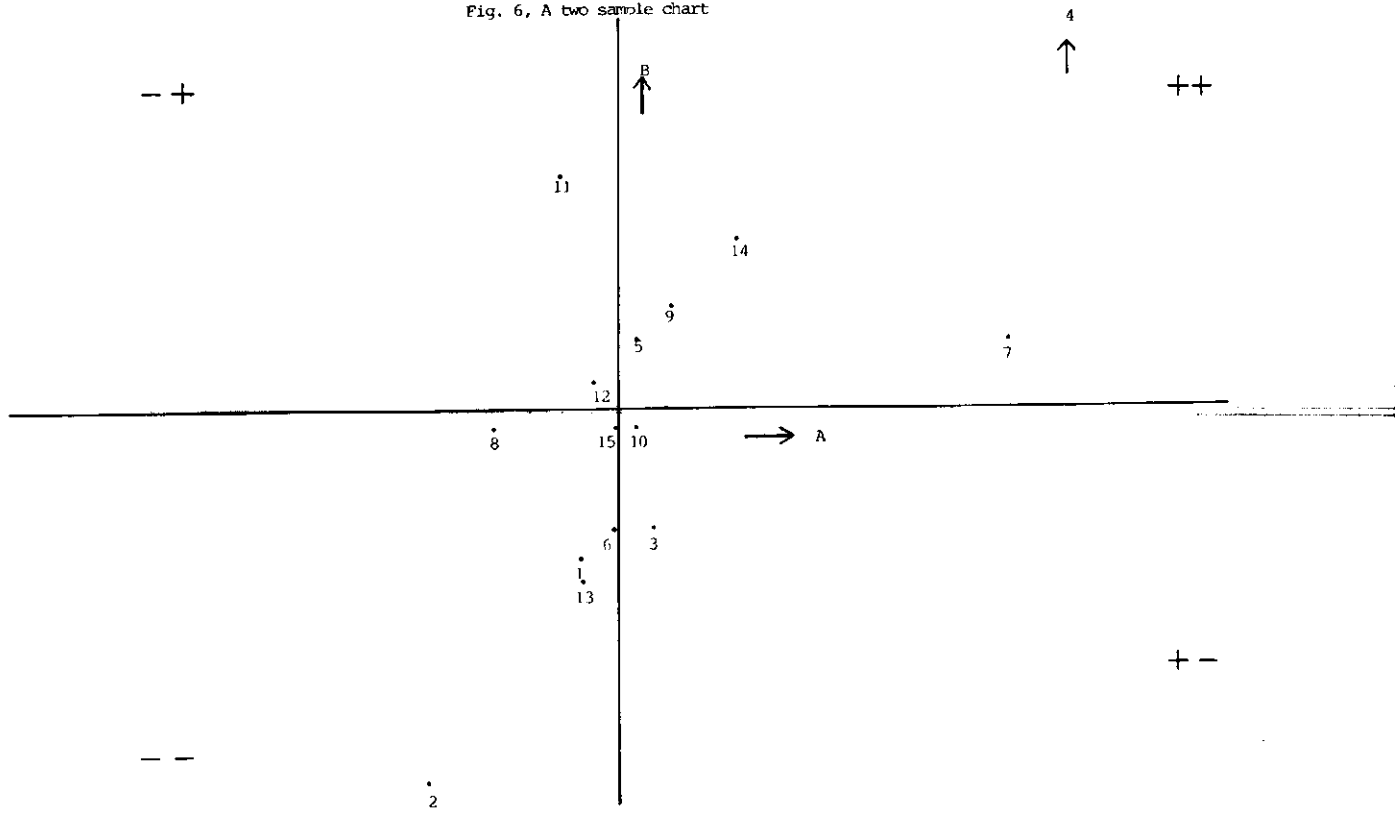


Fig. 7, part 1
Gaschromatogram of the partici- pants 1, 2, 3 and 15

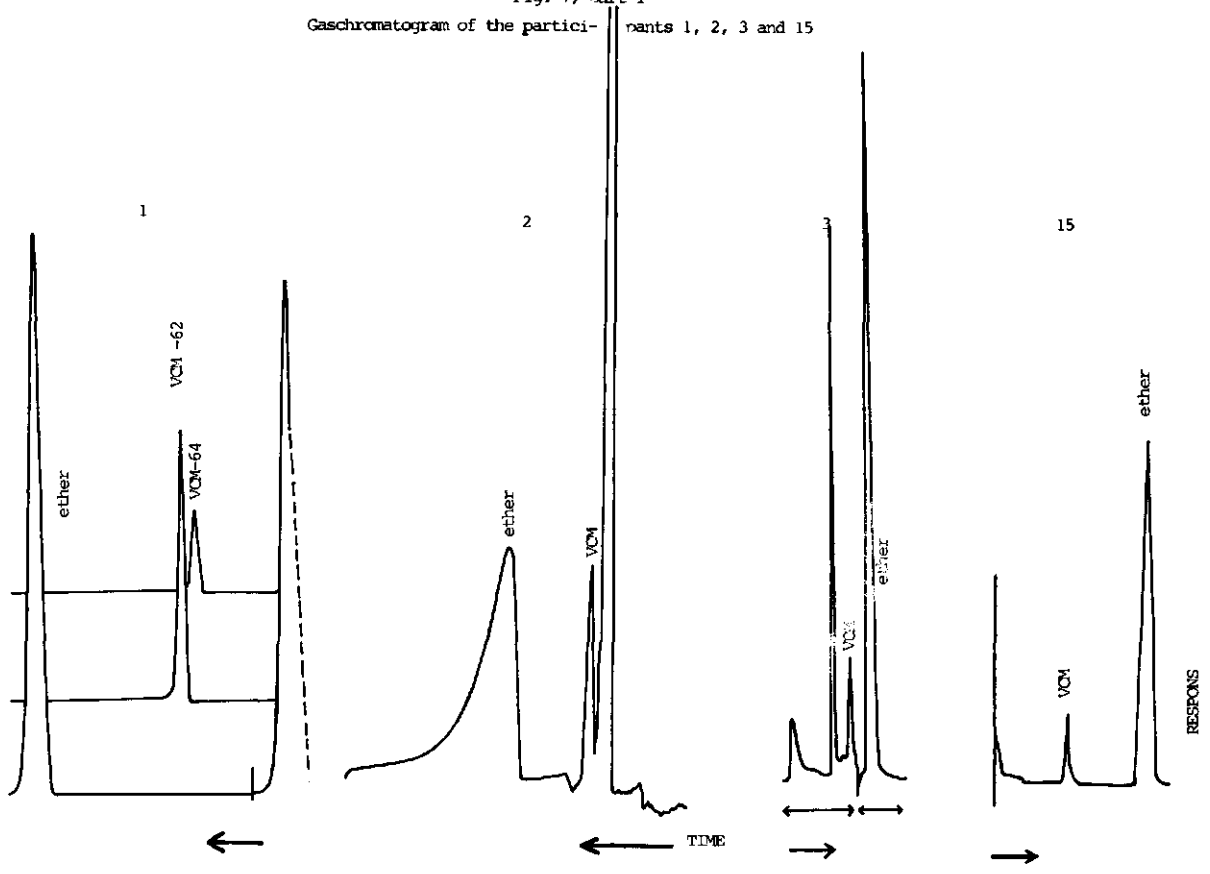


Fig. 7, part 2
Gas chromatograms of the participants 16, 7, 12 and 8

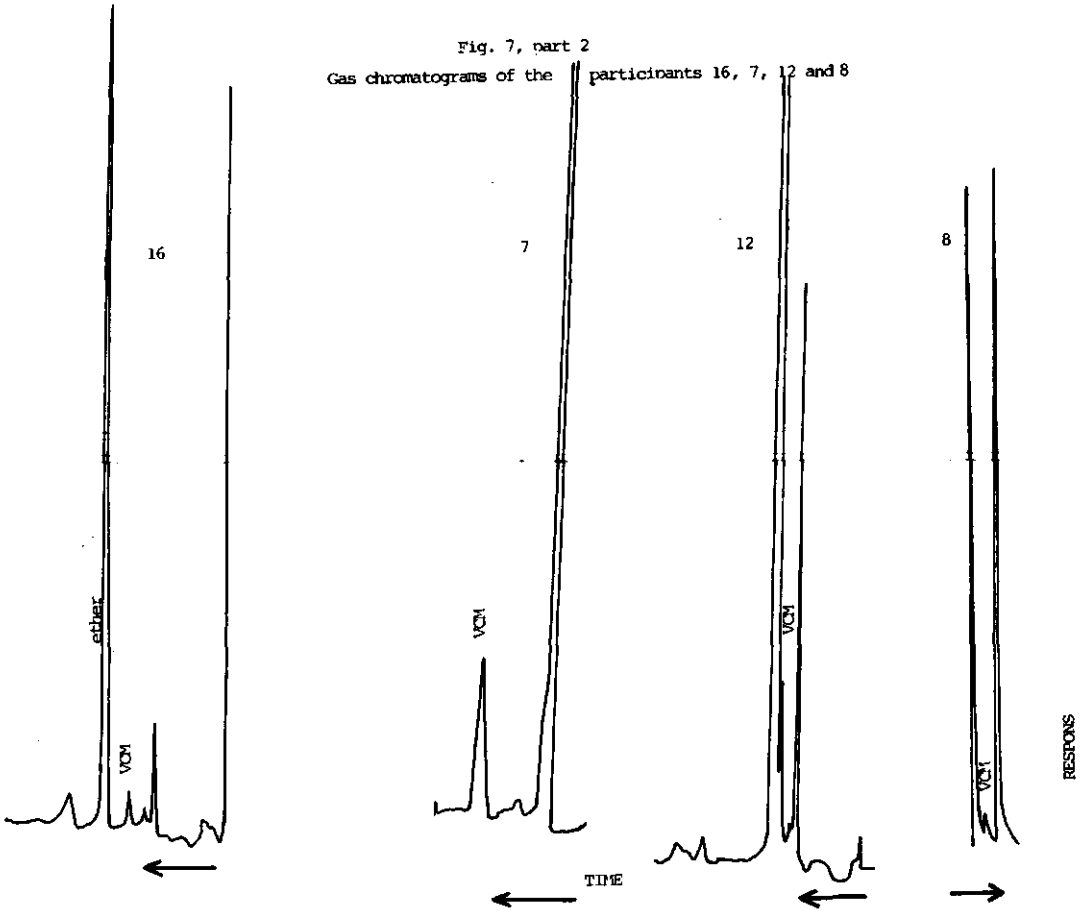


Fig. 7, Gas chromatograms of the part 3 participants 6, 9 and 10

Fig. 7, part 4
Gas chromatograms of the participants 13, 11 and 14

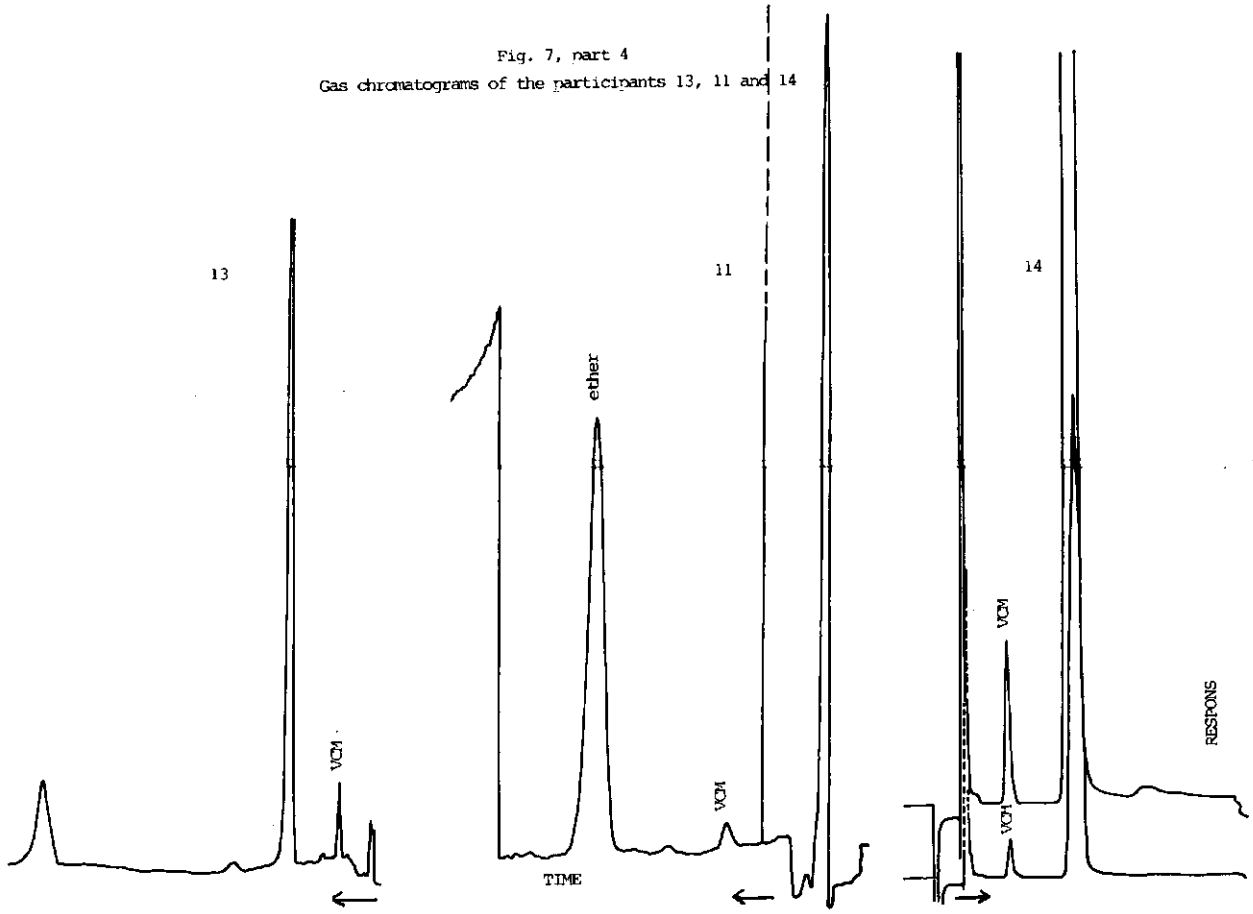


Fig. 7, part 5

Gas chromatograms of the participants 4 and 5

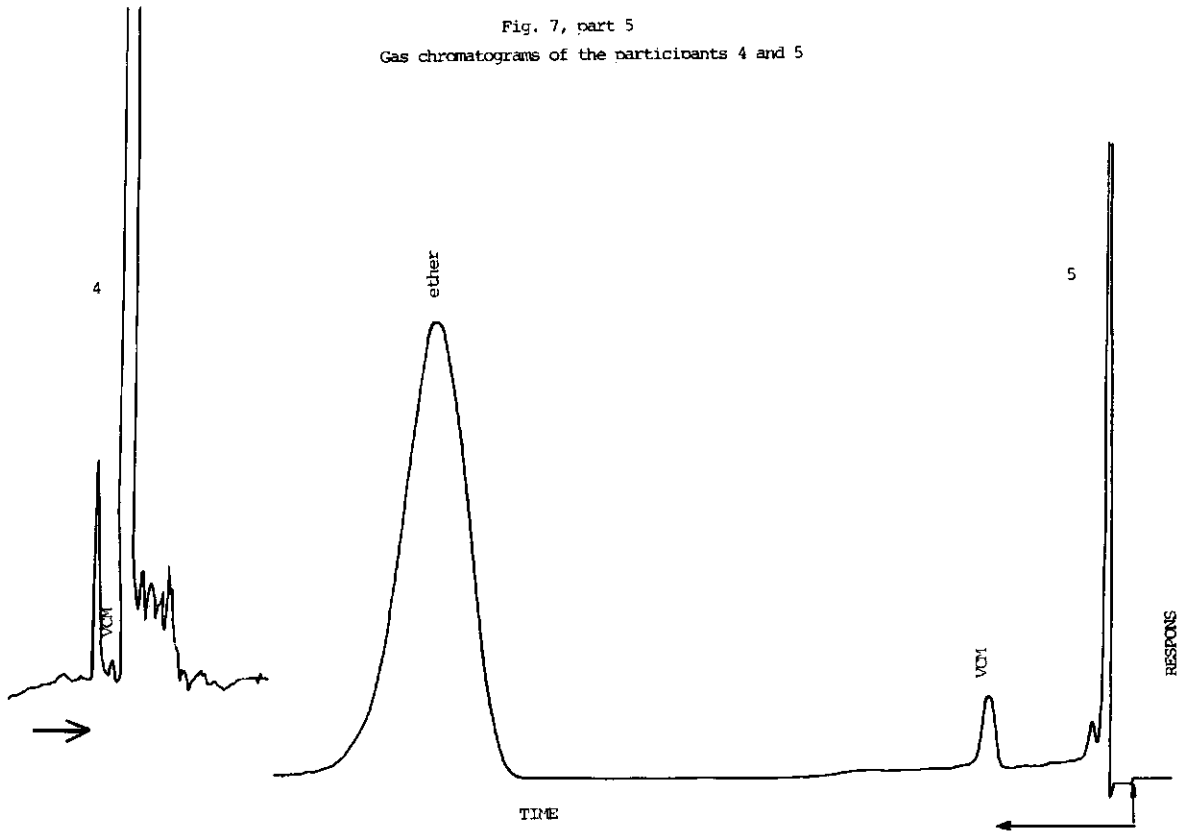


Table 15, part 1

number	sample volume	vial type	thermostate temperature	time in thermostate	GC apparatus	manual or automatic	amount of injection
1	15 ml	4	70°C	1 hour	Finnigan 4000	manual	1 ml
2	50 ml	5	70°C	2 hours	HP 5710 A	manual	1 ml
3	10 ml	3	60°C	2 hours	Girdel 3000	manual	2 ml
4	5 ml	1	60°C	3 hours	Tracor 560	manual	0,4 ml
5	6 ml	1	55°C	2 hours	Perkin Elm.F22	manual	0,5 ml
6	10 ml	2	50°C	2 hours	Enraf ABA Nonius	automatic	2 ml
7	10 ml	3	50°C	2 hours	P E F 42	manual	2,5 ml
8	20 ml	4	50°C	2 hours	Carlo Erba GB	manual	1 ml
9	5 ml	2	55°C	15-20 hours	Packard 427	manual	1 ml
10	6 ml	2	50°C	2 hours	Varian 3700	manual	1 ml
11	10 ml	3	35°C	2 hours	P E F 42	automatic	5 sec
12	10 ml	3	60°C	2 hours	P E F 40	automatic	5 sec
13	10 ml	3	50°C	2 hours	P E F 40	automatic	5 sec
14	10 ml	3	50°C	2 hours	P E F 40	automatic	5 sec
15	10 ml	2	60°C	3 hours	Girdel 3000	manual	2 ml
16	2 ml	3	70°C	30 min.	P E F 40	automatic	5 sec

Table 15, part 2

number	material	length	diameter	carrier gas	support loading
1	stainless steel	2 m	1/8 inch	helium	0,2 % Carbowax 1500 on Carbopack C 80–100 mesh
2	stainless steel	6 m	1/8 inch	nitrogen	5 % SE 30 on Chromosorb GAW DMCS 80–100 m
3	stainless steel	3 m	1/4 inch	nitrogen	Halcomid 5 % + 5 % PEG 400 on WAW DMCS 60–80 m
4	glass	50 m	0,5 mm	nitrogen	SE 52 capillair
5	stainless steel	1 m	1/8 inch	nitrogen	Porapak QS 80–100 m
6	Cr/Ni	3&5 m	2 mm i.d.	helium	1, 2, 3-tris (2 cyanoethoxy) propaan 20–100 Chrom PAW 60–80
7	stainless steel	90 cm	1/8 inch	nitrogen	Chromosorb 102 100–200 m
8	glass	1,8 m	3 mm i.d.	nitrogen	15 % Ucon LB 550 X on Chromosorb WAW 60–80 m.
9	glass	6 m	2 mm i.d.	nitrogen	15 % SE 30 on Chromosorb WAW DMCS 60–80 m
10	glass	6 m	2 mm i.d.	nitrogen	15 % SE 30 on Chromosorb W DMCS 80–100 m
11	stainless steel	1&½ m	1/8 inch	nitrogen	25 % tritolylphosphate on Chromosorb P 80–100 m
12	stainless steel	3 m	3 mm	nitrogen	Carbowax 1540, 0,5 % Atpet 80 on Chromosorb W 60–85 m
13	stainless steel	3 m	1/8 inch	nitrogen	25 % di-isodecylphtalate on Chromosorb WAW 60–80 m
14	stainless steel	3 m	1/8 inch	nitrogen	25 % di-isodecylphtalate + 0,5 % Atpet on Diatomite 60–72m
15	stainless steel	6 m	1/4 inch	nitrogen	10 % Halloomid + PEG 400 10 % on WAW DMCS 60–80
16	steel	4 m	2 mm	nitrogen	15 % Ucon LB-550 X on Chromosorb W-HP 100–120 m

Table 15, part 3

number	column temp.	detection	calculation graph	calculation internal stand.	recorder speed
1	70 °C	MS 62-64		yes	1 cm/min
2	65 °C	FID	yes		1 cm/min
3	50 °C	FID		yes	0,5 cm/min
4		FID	yes		
5		FID		yes	
6		FID	yes		
7	110 °C	FID	yes		0,5 cm/min
8		FID	yes		
9	20-25 °C	FID		yes (cis-2-but.)	1 cm/min
10	25 °C	FID		yes (cis-2-but.)	1 cm/min
11		FID	yes		
12		FID	yes		
13		FID	yes		
14		FID	yes		
15	50 °C	FID		yes	0,5 cm/min
16		FID		yes	

3.3.7.8 Remarks

1. A second collaborative investigation with VCM in solution was carried out between the laboratories with statistical outliers. Small changes in their method of analysis improved the results. One change was for instance the use of a smaller sample for participant 1.
2. This reproducibility study forms the basis for a collaborative investigation with polymer containing VCM.

4. APPLICATIONS OF HEADSPACE GC-MS METHODS

4.1 Vinylidenechloride

4.1.1 Introduction

The use of VDCM polymers and copolymers in food packaging is low compared to the use of PVC and copolymers (Hollifield 1978). About 80 million kg Saran, a copolymer of VDCM and VCM, were manufactured in three years, compared to about 8.500 million kg PVC and copolymers for packaging in one year (CEH 1976). According only a few articles about VDCM are published in journals dealing with food investigations.

VDCM is a compound structurally related to VCM. It has been shown to be mutagenic by microbiological tests, and possibly carcinogenic in rats (Bartsch 1976). Some properties of VCM and VDCM are compared in Table 16.

Table 16

Comparison of VCM and VDCM

Names	vinylchloride chloroethylene 1-chloroethene VC or VCM	vinylidenechloride dichloroethylene 1,1-dichloroethene VDC or VDCM
Structure		
Bruto formula	C_2H_3Cl	$C_2H_2Cl_2$
Molecular weight	62.5	96.9
Boiling point	-13.9 °C	+31.5 °C

The most widely used VDCM polymer in food applications is Saran. This a copolymer of VCM (15 - 20 per cent) and VDCM, which is used in films, coatings and laminates, which have good printing properties. Another application is an artificial gut for meat sausages.

Methods of determination:

Motegi (1977) dissolved PVDC in the mixed solvent carbon tetrachloride-tetrahydrofuran and injected this solvent into a gas chromatograph, fitted with an FID. The limit of detection of this method was 1 ppm VDCM in the PVDC film.

Birkel (1977) developed a method to determine VDCM in Saran food packaging films by using electron capture gas solid chromatography. He can detect 5 ppm in VDCM in films using this method.

Hollifield (1978) combined these experiences of several authors of VCM determinations and those of Birkel and published a headspace method for the determination of VDCM in food simulants.

Motegi (1977) could not detect VDCM in 14 specimens of household-wrap PVDC films. In five casing films for fish-sausage, the VDCM content was below 1 ppm.

Birkel (1977) found levels of 6.5 to 10.4 ppm in six household films with an average of 8.8 ppm. The industrial films, investigated by Birkel showed levels ranging from 20.8 to 26.2 ppm, increasing from the beginning to the end of the roll.

Hollifield (1978) found VDCM contents in Saran films of 1.6 to 13 ppm.

4.1.2 Experimental section

4.1.2.1 GC determinations using an FID and a Hall detector

Van Lierop (Annex D 1975) describes a gaschromatographic determination of VDCM in m-xylene. The samples were extracted with m-xylene and this extract was analyzed by gas chromatography using a Hall detector. This determination had sufficient sensitivity to check the limit of 50 ppb VDCM in the food.

The method described above was, however, only applicable to aqueous food and beverages.

Van Lierop (Annex D 1975) developed a headspace gas chromatographic method using the Hall detector. This method was applicable to oil and was able to check the 50 ppb limit.

4.1.2.2 VDCM determination with headspace GC-MS

Headspace GC-MS made it possible to develop a very sensitive method for the determination of VDCM in the polymer.

The polymer is dissolved in DMA; after equilibrium a headspace sample is injected to a GC-MS apparatus. The same column as for the VCM determination can be used, with the oven temperature raised to 110°C. A mass spectrum of VDCM is shown in Fig. 8. The mass fragment m/e 61 was chosen. While measuring this one fragment no interferences were found. In case of doubt, or when interferences are expected, other fragments such as m/e 63, 91 or 98 can also be measured.

Calibration curves in the low ppb regions were linear. The detection level was 5 ppb. The variation coefficient was 10 per cent at the 40 ppb level. This result was obtained by repeated injection of the same 10 per cent solution of a commercial sample. A mass fragmentogram of this sample and of a blank is shown in Fig. 9.

4.1.2.3 Results of VDCM determination

In 1975, 79, 110 and 11 ppm VDCM were found in Saran films. In a sample of Krehalon (a PVDC copolymer), 5 ppm was found and in an artificial gut 6 ppm.

In 1978, polymers such as cellophane coated with PVC, were analyzed. The contents are given in Table 17. They are calculated on the total amount of polymer.

Table 17

Amounts of VDCM in polymers, coated with PVC

Range of VDCM content in ppb	Amount of samples
< 5	6
5 - 50	22
50 - 100	4
> 100	0
total	32

Fig. 8, Mass spectrum of vinylidenechloride

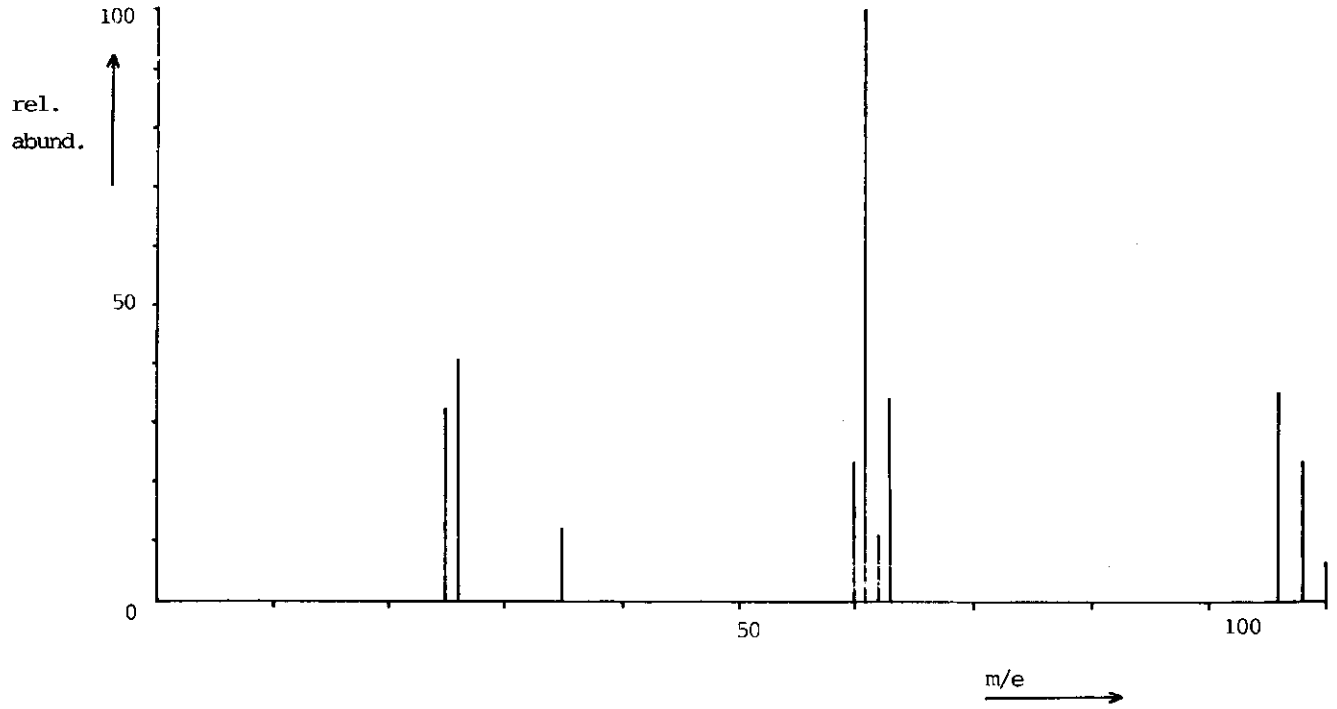
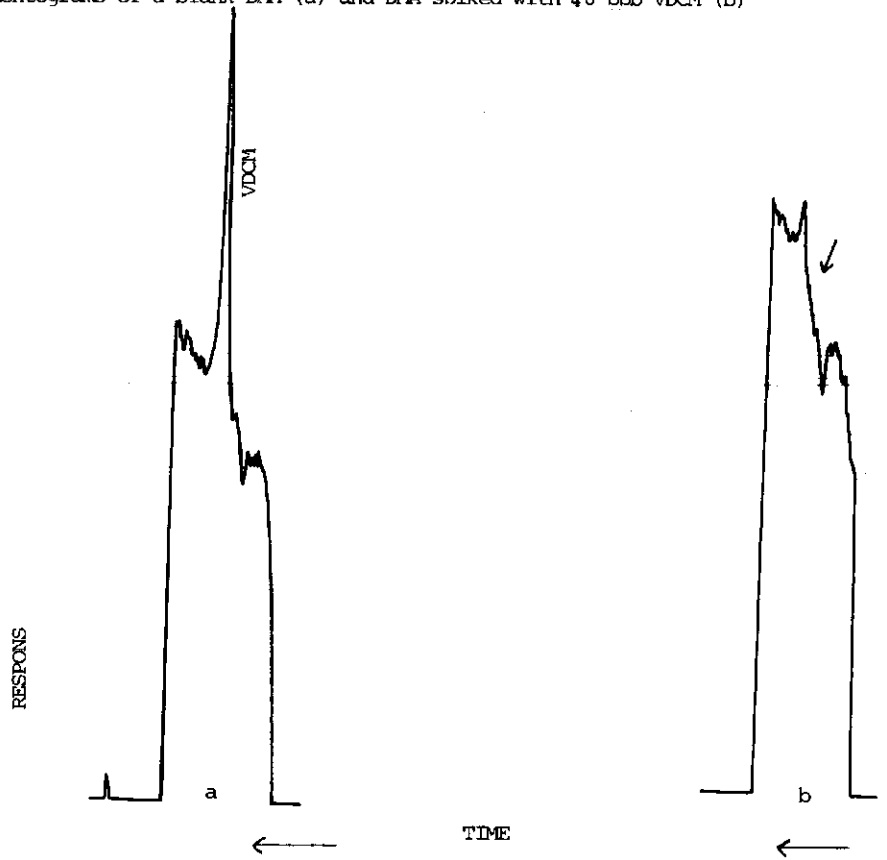


Fig. 9, Sensitivity of VDCM determination

Mass fragmentograms of a blank DMA (a) and DMA spiked with 40 ppb VDCM (b)



4.2 Trace analysis of acrylonitrile

4.2.1 Introduction

Copolymers such as polyacrylonitrile-butadiene styrene and polystyrene-acrylonitrile have good gas barrier properties. They are, therefore, suitable as containers for carbonated beverages such as beer and soft drinks. Other applications are camping articles for food use. So far these are the only applications on the market in The Netherlands.

The structure of acrylonitrile is given below:

$$CH_2=\overset{H}{C}-C\equiv N$$

It has a boiling point of 78°C.

Studies in the United States have shown that acrylonitrile is a powerful teratogen and suspected carcinogen (Fed.Regist. 1977).

Residual acrylonitrile may migrate from the packaging material into the food. The Food and Drug Administration in the USA has regulations for the permissable level of acrylonitrile migration into food simulants. In West Germany the *Bundesgesundheitsamt* has set a limit of 5 ppm acrylonitrile in acrylonitrile polymers and copolymers and a limit of 10 ppb in food. In The Netherlands similar or even more severe limits are being considered. These limits will be based on results obtained using the method described in 4.2.2.

Methods of detection for acrylonitrile are described in the literature. Infra red spectroscopy, polarography and gas chromatography, using an FID or a specific nitrogen detector, were published.

Brown (1978) has just published a GC method for acrylonitrile using a nitrogen phosphorous detector, and he has also reported results of migration experiments. These results are in line with our results given in 4.2.2.

4.2.2 Experimental section

4.2.2.1 Headspace GC-MS determination

Van Lierop (Annex E 1978) describes a headspace GC-MS determination of acrylonitrile in food and food simulants. This method is sensitive, has a detection limit of 1 ppb, and is

also very specific because three mass fragments of acrylonitrile m/e 51, 52 and 53 are measured simultaneously. The same column as used for VCM and VDCM could be employed.

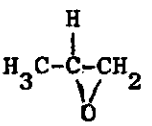
4.2.2.2 Results of acrylonitrile determinations

Migration experiments were carried out on bottles manufactured from acrylonitrile copolymer. The food and food simulants used in these investigations were lemonade, water, 3 per cent acetic acid and 15 per cent ethanol-water mixture.
After standard migration times 1 to 4 µg/kg acrylonitrile were determined (Annex E).

4.3 Trace analysis of epichlorohydrin

4.3.1 Introduction

Epichlorohydrin, 1-chloro 2,3-epoxypropane (EPCH) and diphenylol propane can be used as reagents in the manufacture of epoxyresins.



EPCH ; boiling point 116 °C

Epoxy resins are macro molecular materials belonging to the class of thermosetting resins. They differ from thermoplastics such as PVC with respect to chemical reactions before acquiring their final properties. The reaction causes the molecules to be interconnected by crosslinks changing the molecular structure irreversibly. An important field of application is the paint industry. Because of their excellent adhesive properties, chemical resistance, flexibility and toughness given to the paint film, epoxy resins are being used for high performance coatings for a variety of applications ranging from food cans to crude oil tanks. One of the food packaging applications is the coating of cans for beer, vegetables, juices and aerosols. It has recently been assumed that EPCH may be a mutagenic compound (Kučerová 1977).

A possible route by which EPCH may reach foodstuffs is via the packaging material. Residual EPCH may still be present in the epoxyresins used as packaging material.

No sensitive determinations of EPCH in food or food simulants have been found in the literature. Some information concerning EPCH determinations in epoxy resins at the ppm level was, however, obtained from industry.

To investigate the possible migration of EPCH from can coatings to the food, our Government required a sensitive EPCH determination. Regulations setting limits to EPCH contents in food could be considered using the results of these determination, followed by migration studies.

4.3.2 Experimental section

4.3.2.1 GC-MS determination of epichlorohydrin

Van Lierop (Annex F 1978) describes a determination of EPCH based on mass fragmentography, which provides the highest sensitivity and detection with high specificity.The determination of m/e 49 in diethylether is possible at the level of 6 ppb. Headspace was not used because the boiling point is rather high compared to that of VCM, VDCM and acrylonitrile. Concentrations of EPCH which had migrated from packaging materials into the food or food simulant were obtained by extraction with diethylether.

4.3.2.2 Results of EPCH determinations

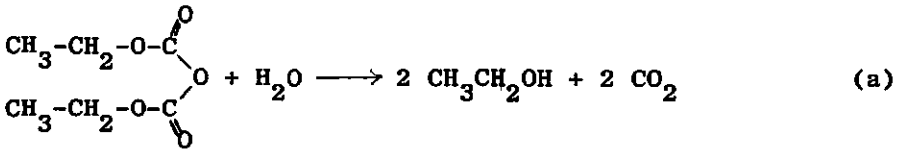
Migration experiments were done with some cans and sheets coated with epoxy resins. The resulting aqueous solutions were extracted using diethylether. No EPCH was found in the extracts. On the basis of these results it is possible to conclude that the maximum amount of EPCH that could have migrated is less than 3 ppb.

4.4 Trace analysis of diethylpyrocarbonate

4.4.1 Introduction

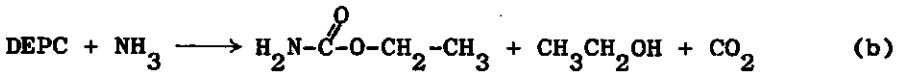
Diethylpyrocarbonate is used as a preservative in beverages. DEPC is discussed in this thesis, because the method of analysis shows the versatility of the headspace GC-MS technique

outside the field of monomers, it is also a potential carcinogenic by formation of urethan. When DEPC is added to water a reaction (a) occurs:



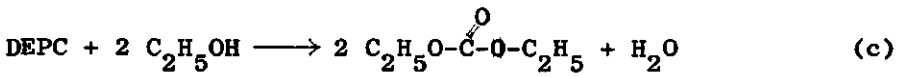
(a)

DEPC seemed an ideal preservative because it eliminates many micro-organisms. However, it can be considered as carcinogenic, because it reacts with NH_3 to ethylcarbamate (urethan) according to reaction (b):



(b)

The rapid reaction of DEPC in water (reaction a) makes a sensitive determination of DEPC after 24 hours impossible in non-alcoholic beverages. If DEPC is added to beverages containing alcohol, reaction (c) occurs:



(c)

Methods are described to determine this diethylcarbonate and, by using the alcohol content, the amount of added DEPC can be calculated.
DEPC has been forbidden as an additive to beverages in The Netherlands since January 1976.

4.4.2 Experimental section

4.4.2.1 Headspace GC-MS determination of low levels of diethylcarbonate in beverages

Van Lierop (Annex G 1979) describes a sensitive headspace gas chromatographic method for the determination of low levels of DEC in beverages.
During the elution of DEC, an impurity of DEPC, the column is vented to a mass spectrometer with a multiple ion detection system and operated in the electron impact mode. The ions m/e 63 and m/e 91 are monitored. DEC is not present in beverages in concentrations higher than 5 ppb. Thus, the presence of

DEC in higher amounts is proof for the use of DEPC. Our method could detect 1 ppb DEC.

4.4.2.2 Results of DEC determinations

138 Samples of lemonade, fruit drinks, wine and beer were analyzed for DEC. Sixteen samples had 30 ppb DEC. Thus, DEPC had been added to these sixteen samples.

5. LITERATURE REFERENCES

Ahlstrom, D.H., Kilgour, R.J., Liebman, S.H.. Anal.Chem. 47 (1975) 1411-1412

Aldershoff, W.G.. Second International Symposium on Migraton. Hamburg, 1976

AOAC, Association of Offical Analytical Chemists. Anal.Chem. 50 (1978) 337A - 340A

Australian Report, 1975, Private communication

Balemans, A.W.M.. Chem.Weekbl. 70 (1974) 5-9

Bartsch, H., Montesanto, R.. Mutat.Res. 32 (1975) 93-114

Bartsch, H., Malaveille, C., Barbin, A., Bresil, H., Tomatis, L., Montesanto, R.. Environmental Health Perspectives 17 (1976) 193-198

Battum, D. van. Voedingsmiddelentechnol. 7 (1974) 13-16

Bellar, T.A., Lichtenberg, J.J., Eichelberger, J.W.. Environm Sci.Technol. 10 (1976) 926-930

Berens, A.R.. Angew.makromol.Chem. 47 (1975) 97-110

Bertram, C.G.. Environm.Sci.Technol. 11 (1977) 864-869

BIBRA, A.M.S.. Food Cosmet.Toxicol. 13 (1975) 275-284

Biran, D.. Ph.D. Thesis Xerox University Microfilms 77 - 24953

Birkel, T.J., Roach, J.A.G., Sphon J.A.. J.Assoc.off.anal.Chem. 60 (1977) 1210-1214

Breder, C.V., Dennison, J.L., Brown, M.E.. J.Assoc.off.anal.Chem. 58 (1975) 1214-1220

Brown, M.E., Breder, C.V., McNeal, T.P.. J.Assoc.off.anal.Chem. 61 (1978) 1383-1388

Bruck, G.C.vom, Eckert, W.R., Rudolph, F.B., Koch, J., Figge, K.. Fette Seifen Anstrichm. 80 (1976) 72-76

Bruck, G.C.vom, Eckert, W.R., Figge, K., Koch, J., Rudolph, F.B.. Mitt.Gebiete Lebensm.Hyg. 69 (1978) 20-41

Bundesgesundheitsamt, Bundesgesundhbl. 21 (1978) 121

Cavigneaux, C., Morel, C.. Cahiers de Notes Documentaires 79 (1975) 201-207

CEH, Chemical Economics Handbook, Stanford Research Institute California USA

Chan, R.K.S., Anselmo, K.J., Reynolds C.E., Worman, C.H.. Polym. Eng.Sci. 18 (1978) 601-606

Charamboulos, G.. Headspace analysis of food and beverages New York etc, Academic Press, 1978

Coulson, D.M.. J.Gas Chromatogr. 3 (1965) 134

Creech, J.L., Jonson, M.N.. J.occup.Med. 16 (1974) 150-151

Daniels, G.A., Procter, D.E.. Mod.Packag. 48 (1975) 45-49

Dennison, J.L., Breder, C.V., McNeal, F., Snijder, R.C., Roach, J.A., Sphon, J.A.. J.Assoc.off.anal.Chem.61 (1978) 813-819

Diachenko, G.W., Breder, C.V., Brown, M.E., Dennison, J.L.. J.Assoc.off.anal.Chem. 60 (1977) 570-575
Dressman, R.C., McFarren, E.F.. J.Chromatogr.Sci. 15 (1977) 69-72
Drozd, J., Novak, J.. J.Chromatogr. 152 (1978) 55-61
Dutch Food and Commodities Law (Warenwet), Ministerie van Volksgezondheid en Milieuhygiëne, The Haque
Duuren, B.L.van. Ann.N.Y.Acad.Sci. 246 (1973) 258-267
Eckert, W.R.. Fette Seifen Anstrichm. 77 (1975) 319-323
EEC, Directive of the EEC Working Party on materials and articles intended to come into contact with foodstuffs, January, 1978
Ehtesmann-Ud Din, A.F.M., Nordb, E., Underdal, B.. Lebensm. Wiss u Technol. 10 (1975) 33-35
Ernst, G.F., Lierop, J.B.H.van, J.Chromatogr. 109 (1975) 439-440
Esch, G.J., Logten, M.J. van, Food Cosmet.Toxicol. 13 (1975) 121-124
FDA Consumer dec 1975 - jan 1976 5-7
FDA Consumer sept 1976 18-21
FDA Fed.Regist. 40 (1975) 40529
Fed.Regist. 39 (1974) 194
Fed.Regist. 42 (1977) 13546
Feron, V.J., Speek, A.J., Willems, M.J., Battum, D.van, Groot, A.P.de.. Food Cosmet Toxicol 13 (1975) 633-638
Fuchs, G., Gawell, B., Albanus, L., Slorach, S.. Var Foeda 27 (1975) 134-145
Fujii, T.. Anal.Chem. 49 (1977) 1985-1987
Gilbert, S.G., Giancin, J.R., Morano, J.R., Rosen, J.D., Package Development 1975 20-24
Gilbert, S.G.. J.Food Sci. 41 (1976) 958-953
Haggenburg, H., Scmidt, A.P.. Gas chromatographic headspace analysis, London etc, Heyden, 1976
Halbesma, L.H.. Voedingsmiddelentechnol. 10 (1977) 15-22
Hall, R.C.. J.Chromatogr.Sci. 12 (1974) 152-160
Hall, M.E., Stevens, W.J.. Anal.Chem. 49 (1977) 2277-2281
Hens, L., Verschaeve, L.. Cahiers voor Arbeidsgeneeskunde XIV (1977) 259-271
Hill Jr, R.H., McCammon, C.S., Saalwachter, A.T., Teass, A.W., Woodfin, W.J.. Anal.Chem. 48 (1976) 1395-1398
Hoffmann, D., Patrianakos, C., Brunneman, K.D., Gori, G.B.. Anal.Chem. 48 (1976) 47-50
Hollifield, H.C., McNeal, T.. J.Assoc.off.anal.Chem. 61 (1978) 537-544
Hoofdinspectie van Volksgezondheid, (Headinspection of Public Health) 1974

Huber, H.. Chem.Ing.Techn. 19 (1975) 803-808
IARC, International Agency for Research on Cancer. Lyon Monographs volume 7, 1974 (A new edition will be published in 1979)
ISO, International Organization for Standardization. Precision of test methods-Determination of repeatibility and reproducibility ISO/DIS 5725
Ives, N.F.. J.Assoc.off.anal.Chem. 58 (1975) 457-460
Joe, F.L., Kline, D.A., Miletta, E.M., Roach, J.A.G., Roseboro, E.J., Fazio, Th.. J.Assoc.off.anal.Chem. 60 (1977) 509-516
Kaiser, R.E.. Z.Anal.Chem. 27 (1974) 186-195
Kashtock, M.E.. Ph.D.Thesis, Xerox University Microfilms 78-5091
Koch, E.R., Vahrenholt, F.. Seveso ist Ueberall. Köln, Kiepenheuer & Witsch (1978) 210-226
Kolb, B.. J.Chromatogr. 122 (1976) 553-568
Koleske, J.V., Wartman, L.H.. Poly(vinylchloride), New York etc Gordon and Breach Science Publishers, 1969
Krishen, A., Tucker, R.G.. Anal.Chem. 48 (1976) 455-456
Kučerová, M., Zhurkov, V.S., Polivková, Z., Ivanova, J.E.. Mutat.Res. 48 (1977) 355
Kunitake, N.. Bull.Brew.Sci. 15 (1969) 1-4
Lehman, A.J.. Food Packaging 20 (1956) 159
Lierop, J.B.H.van, Hogendijk, C.J., Jongerius, Th.J., De Ware(n)Chemicus 5 (1975) 40-43
Lierop, J.B.H.van, Hogendijk, C.J., Jongerius, Th.J., De Ware(n)Chemicus 5 (1975) 157-162
Lierop, J.B.H.van, Stek, W., J.Chromatogr. 128 (1976) 183-187
Lierop, J.B.H.van, J.Chromatogr, 166 (1978) 609-610
Lierop, J.B.H.van, De Ware(n)Chemicus 8 (1978) 178-182
Lierop, J.B.H.van, Nootenboom, H., J.Assoc.off.anal.Chem. 62 (1979) 253-266
Löfroth, G., Gejvall, T.. Science 174 (1971) 1248-1250
Machata, G.. Mikrochim.Acta 262 (1964) 2-4
Maltoni, C., Lefemine, G.. Ann.N.Y.Acad.Sci. 246 (1975) 195
Millard, B.J.. Quantitative Mass Spectrometry, London, Heyden 1978
Ministry of Agriculture, Fisheries and Food Survey of vinyl chloride content of polyvinylchloride for food contact and of foods. London,1978
Motegi, S., Ueda, K., Tanaka, H., Ohta, M.. Bulletin of the Japanese Society of Scientific Fisheries 42 (1976) 1387-1394
Morano, J.R.. Ph.D.Thesis 1965, Xerox University Microfilms 75-24727
Morano, J.R., Giacin, J.R., Gilbert, S.G.. J.Food Sci. 42 (1977) 230-234

Ontwerp Verpakkingen en Gebruiksartikelenbesluit. Warenwet Bijlage deel B, Methoden en Onderzoek, The Hague Ministerie van Volksgezondheid en Milieuhygiëne, 1977
OSHA, Department of Labor.Fed.Regist. 39 (1974) 35890
Ough, S.C.. J.Agric.Fd.Chem. 24 (1976) 228-231
Page, D.P., O'Grady, R., J.Assoc.off.anal.Chem. 60 (1977) 576-579
Pfab, W., Mücke, G.. Verpack.Rundsch. 28 (1977) 49-52
Potter, H.R.. Food Cosmet.Toxicol. 14 (1976) 347-354
Pu, H.H., Hicks, S.Z.. J.Chromatogr. 132 (1977) 495-501
Puschmann, J.. Angew.makromol.Chem.Die 47 (1975) 29-41
Reynders, L.. Het plastic PVC, Rotterdam, Ordeman, 1977
Rosen, M.D., Morano, J.R., Pareless, S.R., Giancin, J.R. Gilbert, S.G.. J.Assoc.off.anal.Chem. 58 (1973) 700-702
Rösli, M., Zimmerli, B., Marek, B.. Mitt.Geb. Lebensm. unt.Hyg. 66 (1975) 507-511
Rösli, M., Marek, B.. Mitt.Geb. Lebensm.unt.Hyg. 68 (1977) 550-553
Rossi, L.. J.Assoc.off.anal.Chem. 60 (1977) 1282-1291
Schaffner, R.M., Lombardo, P.. J.Assoc.off.anal.Chem. 58 (1976) 12-11-1213
Shanks, R.A.. Scan 1975 20-21
Stijve, T., Kalsbach, R., Eyring, G.. Trav.Chim.Aliment.Hyg. 67 (1976) 403-427
Tester, D.A.. J.Soc.Cosmet.Chem. 49 (1976) 459-466
Thomas, J.C.. J.Macromol.Sci.Chem. A11 (1977) 1553-1556
Truhaut, R., Berrod, J.. Cahiers de Notes Documentaires 80 (1978) 365-385
Viola, P.L., Bigotti, A., Caputo, A.. Cancer Res. 31 (1971) 516-522
Voogd, P.de Ned.Chem.Ind. 19 (1977) 179-183
WDR, Westdeutscher Rundfunk Köln, television program, october, 1976
Williams, F.M., Umstead, M.E.. Anal.Chem. 40 (1968) 2232-2234
Williams, D.T., Miles, W.F.. J.Assoc.off.anal.Chem. 58 (1975) 272-275
Williams, D.T.. J.Assoc.off.anal.Chem. 59 (1976) 30-32
Wilson, B.P., Cochrane, J.. J.Chromatogr. 106 (1975) 174-179
Wunderlich, H.. J.Assoc.off.anal.Chem. 55 (1972) 557-558
Youden, W.J., Steiner, E.H.. Statistical Manual of the Association of Offical Analytical Chemists, Washington, 1975

ANNEX A

De Ware(N)-Chemicus, 5 (1975), 40

BEPALINGSMETHODEN VAN VINYLCHLORIDE. - INLEIDING.

Keuringsdienst van Waren, Utrecht.
J.B.H.van Lierop, C.J.Hogendijk en Th.Jongerius.

Maart 1975.
Kode:

1. INLEIDING.

Uit meerdere publikaties betreffende inhalatie van vinylchloride monomeer (VCM) blijkt, dat VCM carcinogeen is (2.1.).
Ten behoeve van het Verpakkingen- en Gebruiksartikelenbesluit van de Warenwet werden bepalingsmethoden voor vinylchloride (VCM) ontwikkeld. De toelaatbare migratie van VCM in levensmiddelen is door de Hoofdinspektie van de Volksgezondheid gesteld op 0,05 mg uit 6 dm^2 verpakkingsmateriaal (2.2.). Dit komt overeen met een gehalte van 5 ppm in het levensmiddel, omdat 1 dm^3 eet- of drinkwaar geacht wordt te zijn verpakt in 6 dm^2 materiaal.
Twee analysevoorschriften worden gegeven voor een gaschromatografische bepaling van vinylchloride VCM. Deze methoden zijn geschikt voor de bepaling van VCM in PVC, waarbij gebruik wordt gemaakt van xyleen als extractiemiddel voor de migratieproef. De gaschromatografische methoden kunnen uitgevoerd worden op de onderstaande manieren, te weten:

1. met een vlam-ionisatiedetector (voorschrift A; zie 2.3.).
2. met een detector volgens Hall (voorschrift B; zie 2.4. en 2.5.).

De tweede methode was noodzakelijk, omdat retentietijden alleen niet voldoende waren voor een identifikatie van een verbinding. Een Hall detector is in principe een geleidbaarheidsmeter. De uit de kolom stromende gassen worden door een kwartsbuis geleid en vermengd met waterstofgas bij 820°C. Indien een chloorverbinding aanwezig is, vindt omzetting in zoutzuur plaats. Dit zoutzuur verhoogt de geleidbaarheid in de meetcel zeer sterk en is zodoende een maatstaf voor de chloorverbindingen.
Gekonkludeerd kan worden, dat in 1974, 58% van het onderzochte verpakkingsmateriaal een gehalte van meer dan 100 ppm aan VCM had, hetgeen een overschrijding van de migratielimiet met zich meebracht (2.6. en 2.7.).

2. LITERATUUR.

2.1. American Fed. Regist. 39 (1974), 194.

2.2. Brief van de Hoofdinspekteur van de Volksgezondheid van 27-5-1974.

2.3. J.B.H.van Lierop, C.J.Hogendijk en Th.Jongerius, De Ware(N)-Chemicus, 5 (1975), 41 - 43.

2.4. J.B.H.van Lierop, C.J.Hogendijk en Th.Jongerius, De Ware(N)-Chemicus, 5 (1975), 44 - 47.

2.5. G.F.Ernst en J.B.H.van Lierop. J.Chromatogr. (accepted for publication 1975).

2.6. C.J.Hogendijk, J.B.H.van Lierop en Th.Jongerius, Bepalingsmethoden van Vinylchloride R 68 16174 dec. 1974.

2.7. J.B.H.van Lierop, C.J.Hogendijk en Th.Jongerius, Overzicht van de resultaten van vinylchloridebepalingen R 68 10174 febr. 1975.

De Ware(N)-Chemicus, 5 (1975), 41 - 43

DE GASCHROMATOGRAFISCHE BEPALINGSMETHODE VAN VINYLCHLORIDE M.B.V. DE VLAMIONISATIE DETECTOR - VOORSCHRIFT A.

Keuringsdienst van Waren, Utrecht.
J.B.H.van Lierop, C.J.Hogendijk en Th.Jongerius.

Maart 1975.
Kode:

1. DOEL.

Deze methode beschrijft hoe men vinylchloride VCM kan bepalen in m-xyleen.

2. SAMENVATTING.

Het monster wordt geanalyseerd met gaschromatografie over een SE 30 kolom op Chromosorb G AW DMCS met een vlamionisatiedetector.

3. APPARATUUR EN REAGENTIA.

3.1. Een geschikte gaschromatograaf met toebehoren.

3.2. m-Xyleen P.A. (Merck).

3.3. Vinylchloride (Baker).

3.4. Maatkolven.

3.5. Pipetten.

3.6. Maatcylinders.

3.7. SE 30 (Chrompack).

3.8. Chromosorb (Chrompack).

4. GASCHROMATOGRAFISCHE KONDITIES.

4.1. Gaschromatograaf: Hewlett Packard 5750 B

4.2. Kolom: Materiaal: roestvrij staal
Lengte : 6 meter
Inw.diam.: 1/8 inch.
Vulling : 5% SE 30
op 80-100 mesh Chromosorb G AW DMCS

4.3. Detectorsysteem: Vlamionisatie.
waterstof gassnelheid ± 30 ml/minuut
lucht gassnelheid ± 350 ml/minuut

4.4. Temperaturen: Injektieblok 210°C
Detectieblok 320°C
Ovenprogramma: 2 Min. isotherm op + 50°C, daarna met 60°C/min.
verwarmen tot 300°C, vervolgens 4 min. op 300°C en laten afkoelen.
Bij het sluiten van het deksel weer injekteren en programma starten.

4.5. Dragergas: stikstof, gassnelheid 35 ml/minuut.

4.6. Injektiehoeveelheid: 4 µl.

4.7. Gevoeligheid: Injektie van 4 µl sterkte 6 ppm, absoluut 24 nanogram, geeft bij een versterking van 10 x 4 een uitslag van 164 mm op een 1 mV recorder met volle schaal uitslag van 20 cm.

4.8. Recordersnelheid: 1 cm/minuut.

5. WERKWIJZE.

5.1. Het maken van een ijklijn.

Uit een vinylchloridecylinder vangt men vloeibaar vinylchloride op. Dit giet men over in een maatkolf waarin een gekoelde hoeveelheid xyleen. De toegevoegde hoeveelheid VCM wordt gewogen. Men vult de maatkolf aan bij kamertemperatuur met m-xyleen.
Van deze oplossing worden de verdunningen gemaakt.
De oplossingen moeten in het vriesvak van de koelkast (ca. -15°C) worden bewaard en zijn dan gedurende enige tijd houdbaar, mits de nodige voorzorgen in acht worden genomen bij het maken van verdunningen zoals het gelijkmatig brengen op kamertemperatuur en het snel weer sluiten van de maatkolf na het uitpipetteren.
Het verdunnen van de standaard dient zoveel mogelijk onder gekoelde omstandigheden te gebeuren.
De ijklijn wordt gemaakt van tenminste 4 punten, exclusief de blanko xyleen.

5.2. Bepaling.

Het als extractiemiddel van een simulant of migratievloeistof gebruikte xyleen kan direkt worden geinjekteerd.
Gemeten werden de piekhoogten.

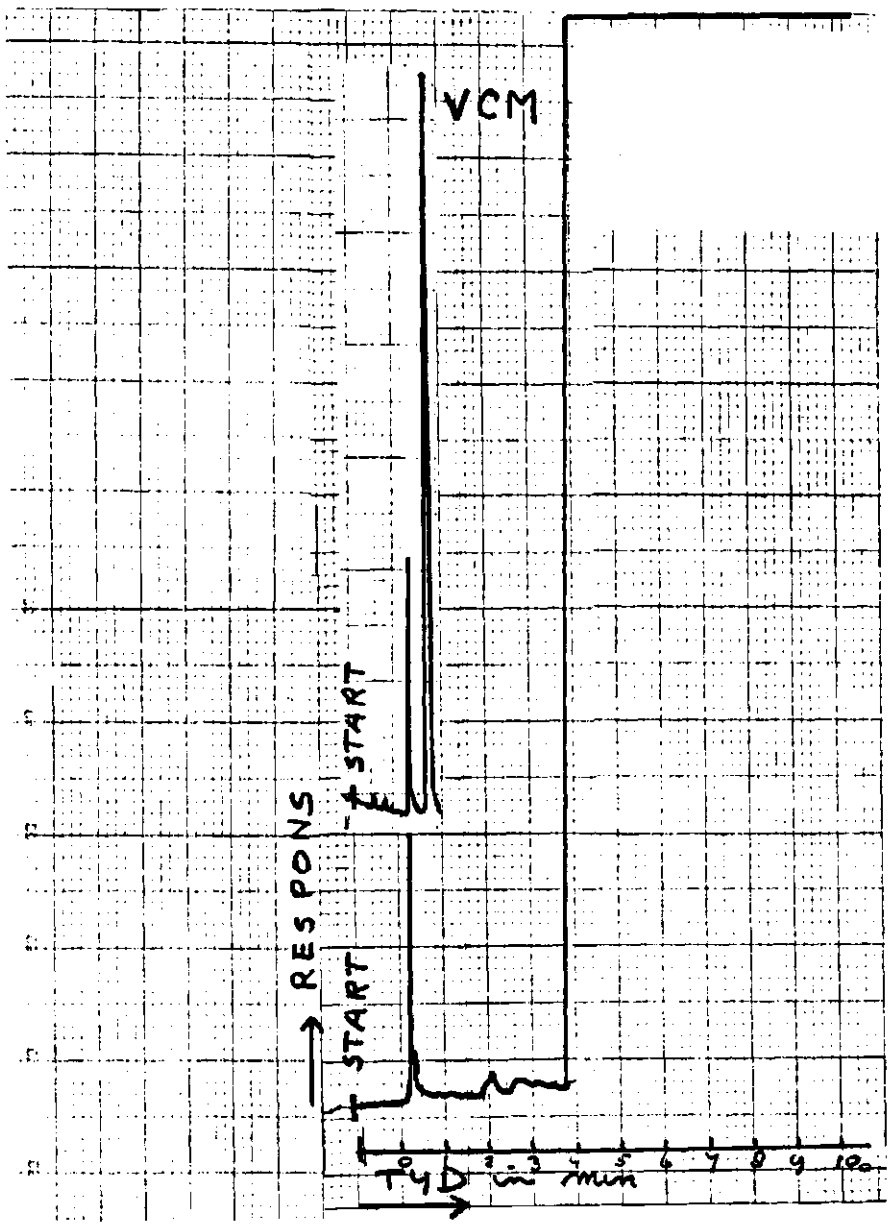
6. OPMERKINGEN.

6.1. Bovenstaande methode voor het maken van een ijklijn werd ook gevolgd bij de oplosmiddelen tetrahydrofuraan-isobutylalkohol en ethylacetaat.

6.2. De door ons gebruikte HP 5750 is uitgerust met 2 injektiepunten en 2 gescheiden vlam-ionisatiedetectoren, echter met één elektrometer.
Door nu 2 vrijwel gelijk gemaakte kolommen te monteren, kan men het aantal uit te voeren analyses bijna verdubbelen, door na het verschijnen van de vinylchloridepiek op kolom A de elektrometer om te schakelen op B, te injekteren en dan te starten met de programmering.
De soms noodzakelijke basislijnkorrektie is niet van invloed op de piekhoogte.

6.3. Indien het VCM-gehalte in het voedingsmiddel of simulant 0,05 ppm is, bereikt men met deze methode een piekhoogte van 11 mm.
Een versterking van 10 x 1 in plaats van 10 x 4 blijkt nog goed toepasbaar.

6.4. In figuur 1 wordt een gaschromatogram van blanko m-xyleen gegeven en daarin een gedeelte van een gaschromatogram van m-xyleen met VCM.



FIGUUR 1:
Gaschromatogram van blanko m-xyleen en van m-xyleen met VCM.

De Ware(N)-Chemicus, 5 (1975), 44 -47

DE GASCHROMATOGRAFISCHE BEPALINGSMETHODE VAN VINYLCHLORIDE M.B.V. DE HALL - DETECTOR. - VOORSCHRIFT B.*)

Keuringsdienst van Waren, Utrecht.
J.B.H.van Llerop, C.J.Hogendijk en Th.Jongerius.

Maart 1975.
Kode:

1. DOEL.

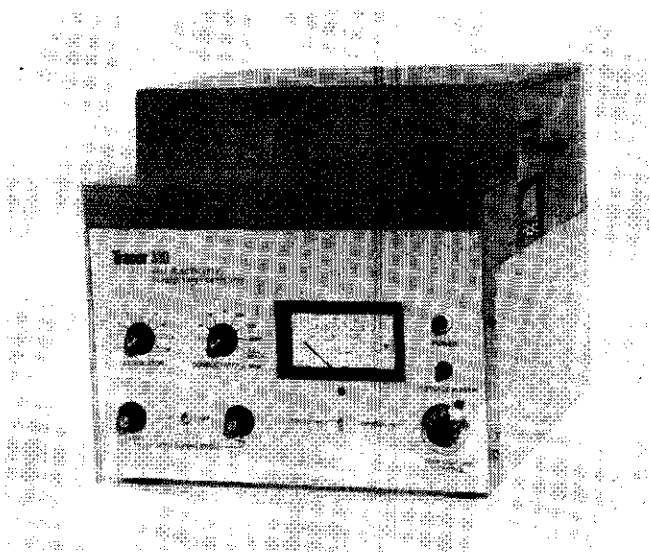
Deze methode beschrijft hoe vinylchloride bepaald en geidentificeerd kan worden in m-xyleen.

2. SAMENVATTING.

Het monster wordt geanalyseerd door gaschromatografie over een 5% SE 30 kolom op Chromosorb G AW DMCS.
Identifikatie vindt plaats met een specifieke halogeen detector volgens Hall.

3. APPARATUUR EN REAGENTIA.

3.1. Gaschromatograaf met Hall-detector (zie figuur 1).



FIGUUR 1:
De elektrolytische geleidbaarheidsdetector volgens Hall (HECD-detector).

3.2. m-Xyleen P.A. (Merck).

3.3. Vinylchloride (Baker).

3.4. Maatkolven.

*) Wordt t.z.t. gepubliceerd.

3.5. Pipetten.

4. GASCHROMATOGRAFISCHE KONDITIES.

4.1. Gaschromatograaf: Hewlett Packard

4.2. Kolom: Materiaal: roestvrij staal
Lengte : 4 meter
Inw.diam.: 1/8 inch
Vulling : 5% SE 30 op 80-100 mesh
Chromosorb G AW DMCS

4.3. Detectorsysteem: Hall-detector. Voor principe zie figuur 2.
Waterstof gassnelheid: Inlet pressure of 10 p.s.i.
Oventemperatuur: 820°C.
Detector: sensitivity x 10 attenuator x 1

4.4. Temperaturen: Injektieblok 80°C
Oven 80°C

4.5. Dragergas: argon, gassnelheid 5,5 ml/minuut

4.6. Injektiehoeveelheid: Injektie van 2,5 µl, sterkte 2,6 ppm, absoluut 6,5 nanogram, geeft een uitslag van 162 mm op een 1 mV recorder met volle schaal-uitslag van 200 mm.

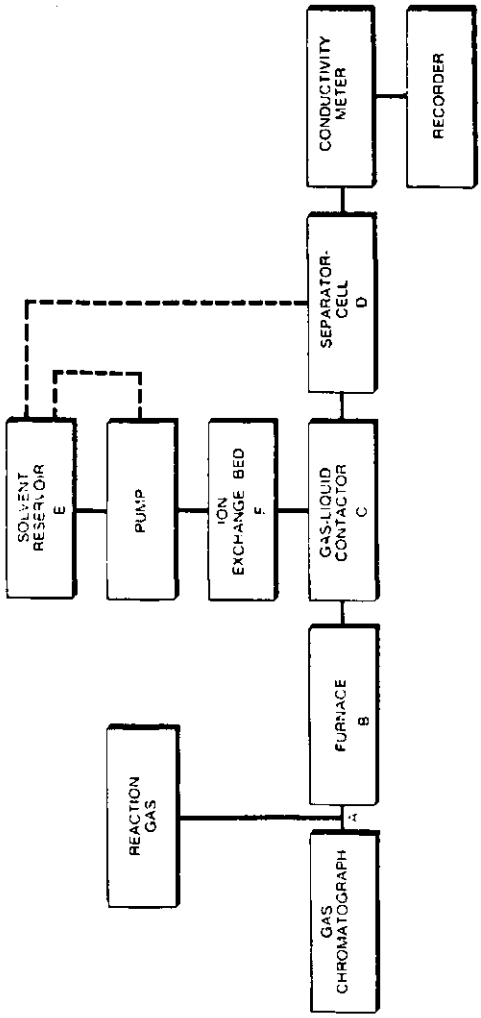
4.7. Recordersnelheid: 5 minuten per inch.

5. WERKWIJZE.

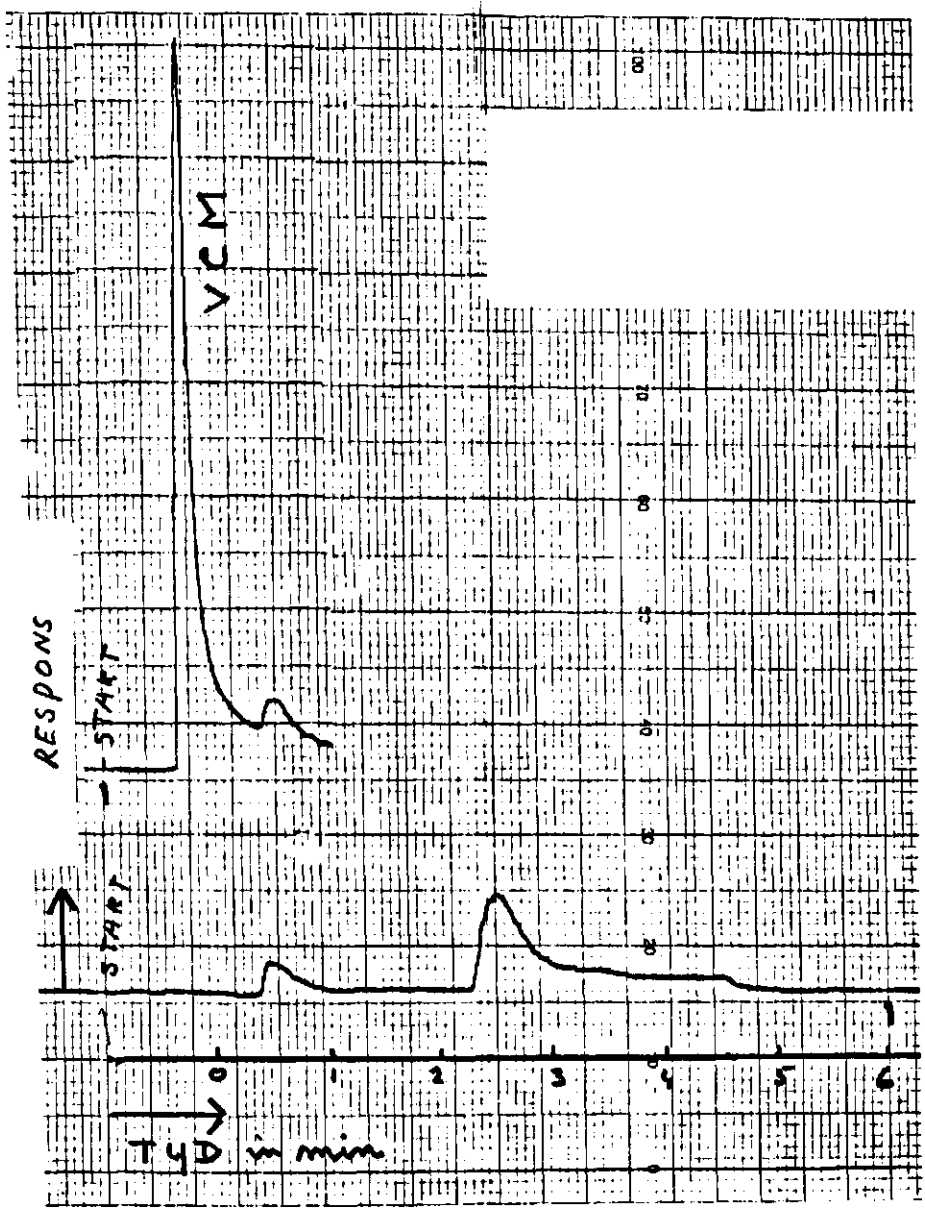
Zie voorschrift A m.b.v. de vlamionisatiedetector (De Ware(N)-Chemicus 5 (1975), 41 - 43.

6. OPMERKINGEN.

6.1. In figuur 3 wordt een gaschromatogram van blanko m-xyleen gegeven en daarin een gedeelte van een gaschromatogram van m-xyleen met VCM.



FIGUUR 2:
Blok diagram voor het HECD-systeem.



FIGUUR 3:
Gaschromatogram van blanko m-xyleen en van m-xyleen met VCM.

ANNEX B

Reprinted from
Journal of Chromatography, 109 (1975) 439–440

CHROM. 8228

Note

A simple, sensitive determination and identification of vinyl chloride by gas chromatography with a Hall detector

G. F. ERNST and J. B. H. VAN LIEROP
Food Inspection Service, Nyenoord 6, Utrecht (The Netherlands)
(Received January 9th, 1975)

Recent publications have emphasized the toxicity of vinyl chloride monomer (VCM)[1]. In The Netherlands the Public Health Authorities have ordered a limit to the amount of VCM in food and this method for the determination of VCM was therefore developed in order to monitor VCM in food products.

VCM may be present in polyvinyl chloride packaging materials, for instance bottles, and may migrate into the contents, such as wine, vinegar and soft drinks. The VCM can be removed from the food by extraction with *m*-xylene and this extract can be analyzed by gas chromatography.

Methods involving many different types of columns and detectors have been described. However, these methods use the retention time as the only proof of identity. Even the use of two different columns does not give sufficient evidence, because compounds extracted with *m*-xylene from wine, for instance, can have the same gas chromatographic behaviour as VCM.

A more specific detector than a flame ionization or thermal conductivity detector was needed. Williams and Umstead[2] used a Dohrmann microcoulometer for the determination of halogenated hydrocarbons. The selective microelectrolytic conductivity detector according to Hall[3] is specific for halogens. We analyzed *m*-xylene extracts from foods on apolar columns with this detector and could identify and determine VCM even in nanogram amounts. The sample, having passed through the column, is reduced with hydrogen in a quartz tube and the conductivity of the hydrogen chloride formed is measured.

EXPERIMENTAL AND RESULTS

A Hewlett Packard 5750A gas chromatograph was equipped with a 4 m × 1/8 in. O.D. stainless-steel column, filled with 5% SE-30 on 80–100 mesh Chromosorb G (AW, DMCS). The Tracor 310 detector according to Hall[3] was connected to the gas chromatograph by a quartz tube of length 15 cm and I.D. 2 mm. The carrier gas (argon) had a flow-rate of *ca.* 5.5 ml/min, and hydrogen was passed into the quartz tube in an oven at 820° at an inlet pressure of 10 p.s.i.

Samples of 2.5 μl were injected at 90° isothermally. The sensitivity for 0.5 full-scale deflection was 4.0 ng of VCM, using a 1-mV recorder with a speed of 5 min/in. at a detector sensitivity setting of 10, attenuation 1.

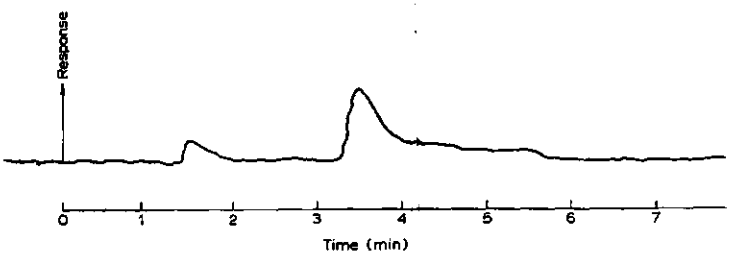


Fig. 1. Chromatogram of *m*-xylene.

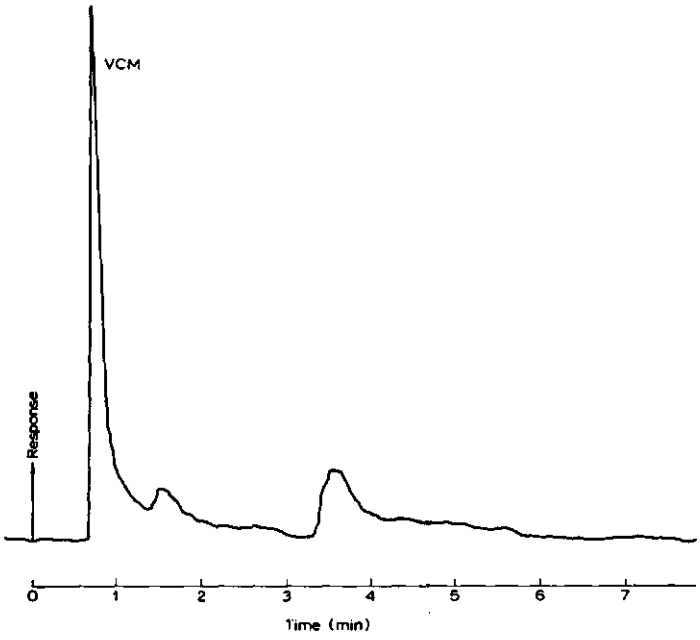


Fig. 2. Chromatogram of *m*-xylene containing 2.6 ppm (w/v) VCM.

Chromatograms of *m*-xylene and of *m*-xylene containing 2.6 ppm of VCM are shown in Figs 1 and 2.

The method was tested on *m*-xylene extracts from wine containing 0.1 ppm of VCM and the results will be published in this journal.

REFERENCES

1 Department of Labor, O.S.H.A., *Fed. Regist.*, 39 (1974) 35890.
2 F. M. Williams and M. E. Umstead, *Anal. Chem.*, 40 (1968) 2232.
3 R. C. Hall, *J. Chromatogr. Sci.*, 12 (1974) 152.

ANNEX C

Reprinted from

Journal of Chromatography, 128 (1976) 183–187

CHROM. 9336

Note

Analysis of vinyl chloride in food simulants at low parts per billion levels by mass fragmentography

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and
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(Received April 5th, 1976)

Poly(vinyl chloride) (PVC) is used as packaging material for many food and drink products, such as wine, olive oil, soft drinks and margarine. From this material vinyl chloride monomer may migrate into the food product. Since vinyl chloride is carcinogenic, there are already strict regulations regarding the amount of VC which may be present in the air in factories[1], and the preliminary results of oral administration of olive oil, containing vinyl chloride, to rats resulted in a proposed ban on the use of vinyl chloride plastics in rigid and semi-rigid packaging in the United States of America[2]. In the European Community a maximum limit of 50 ppb of vinyl chloride in food and drinks is proposed, also due in part to the lack of more sensitive methods of analysis. The present methods[3–5] are mainly based on the use of gas chromatography with flame ionization detection to determine the amount of vinyl chloride, migrated into food and simulants, as salad oil, acetic acid–water and alcohol–water mixtures. The sensitivity of these methods is limited and identification of the vinyl chloride may be difficult. A specific halogen detector according to Hall enables identification, but little gain in sensitivity is obtained[6].

Since the need for more sensitive methods of analysis is imminent, mass fragmentography seemed to be a promising technique to use, although reported detection levels in food and food simulants were still in the 50 ppb range[7]. We report here the determination of vinyl chloride in food simulants at the 1 ppb level by use of mass fragmentography[8].

EXPERIMENTAL

Materials

The following materials were employed: vinyl chloride (Matheson, East Rutherford, N.J., U.S.A.); pure salad oil packed in cans, 96% alcohol and acetic acid (E. Merck, Darmstadt, G.F.R.); and tap water.

Preparation of samples

Liquid vinyl chloride, obtained by condensing the gas in solid carbon dioxide–

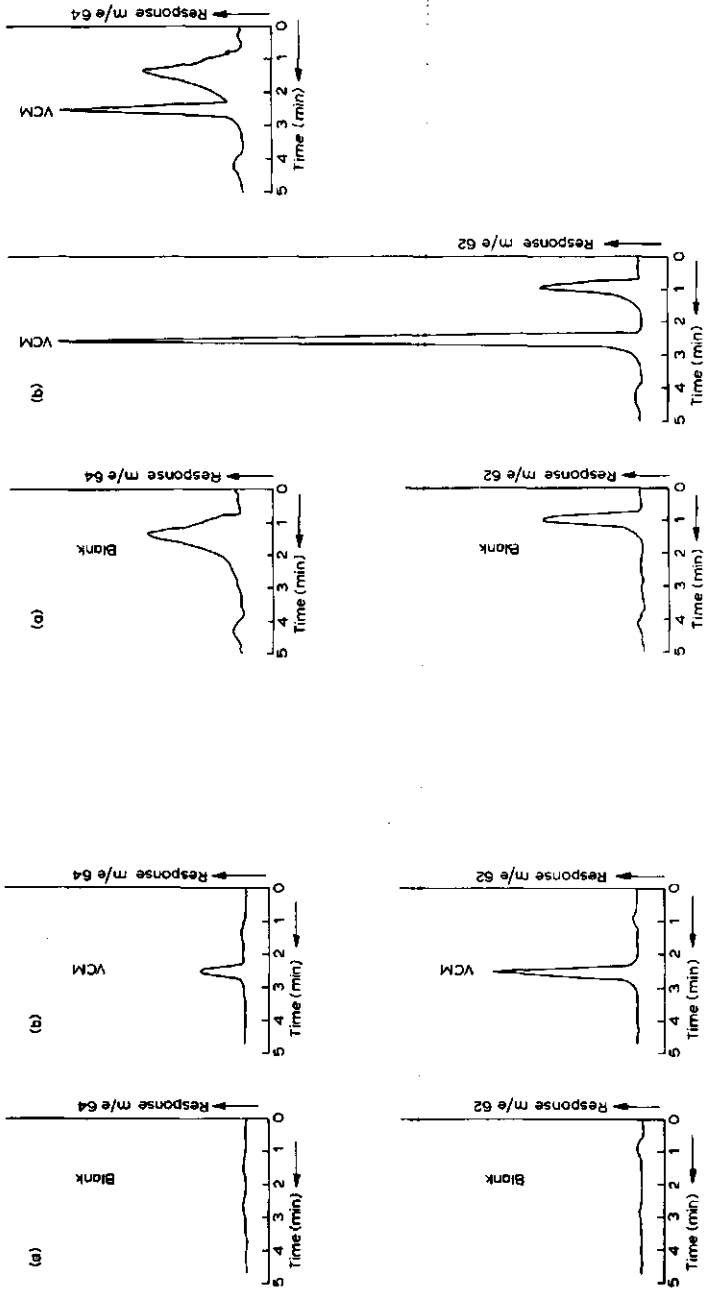


Fig. 1. Fragmentograms of salad oil (blank) (a) and of salad oil containing 98.6 ppb of vinyl chloride (VCM) (b).

Fig. 2. Fragmentograms of salad oil (blank, amplification 20 times higher than in Fig. 1) (a) and of salad oil containing 7.6 ppb of vinyl chloride (VCM) (b).

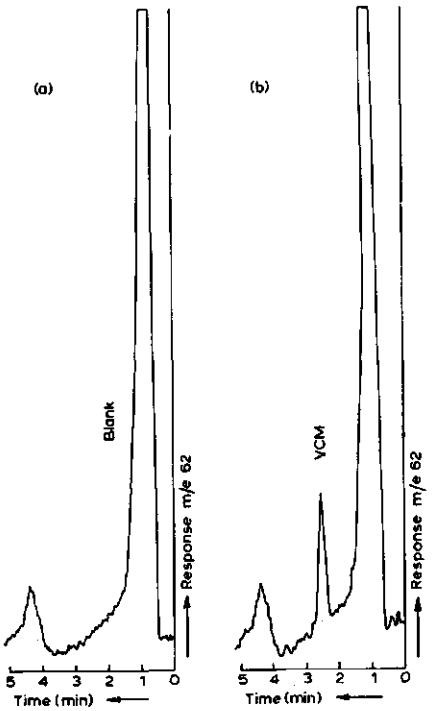


Fig. 3. Fragmentograms of salad oil (blank, amplification 10 times higher than in Fig. 2) (a) and of salad oil containing 0.3 ppb of vinyl chloride (VCM) (b).

acetone, was added to a known amount of simulant and the amount of vinyl chloride added was determined by weight. 100 g of the sample were placed in a 320-ml infusion flask which was then closed with an aluminium screw cap and silicone rubber septum. This flask was conditioned in a water-bath at 50° for at least 1 h before injection.

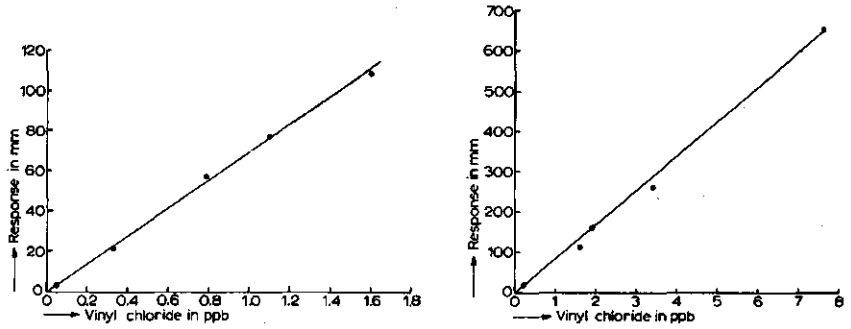


Fig. 4. Graphs of the response of the *m/e* 62 signal against the contents of vinyl chloride in salad oil.

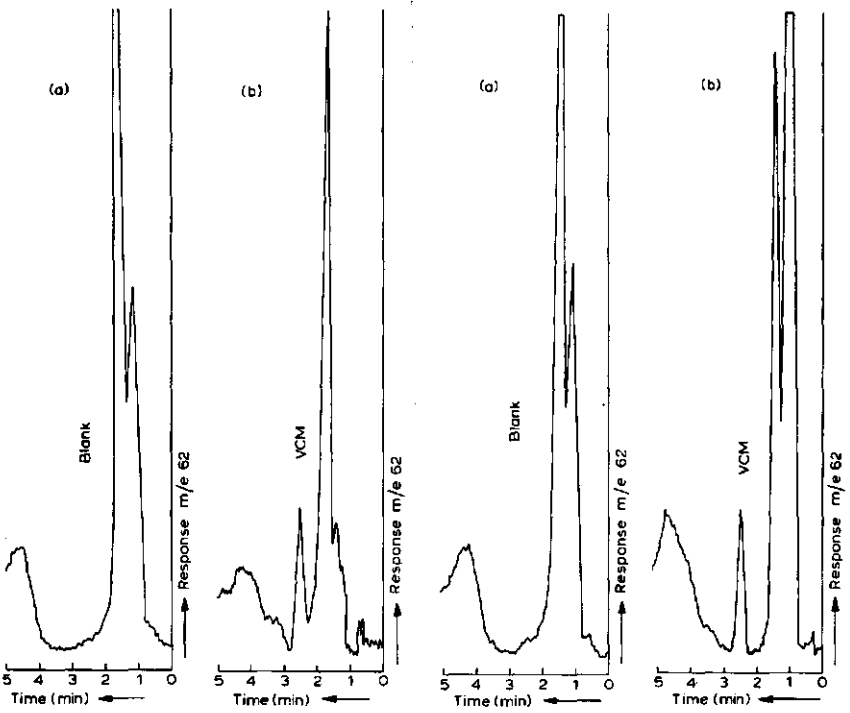


Fig. 5. Fragmentograms of 10% alcohol–water (blank) (a) and of 10% alcohol–water containing 0.1 ppb of vinyl chloride (VCM) (b).

Fig. 6. Fragmentograms of 3% acetic acid–water (blank) (a) and of 3% acetic acid–water containing 0.1 ppb of vinyl chloride (VCM) (b).

Gas chromatography–mass spectrometry (GC–MS) system

The analyses were carried out on a Finnigan 3200 GC–MS system, equipped with a multiple-ion detection (MID) unit and used in the electron-impact mode. The MID unit was tuned to the *m/e* 62 and 64 ions of vinyl chloride and the integrated signals were simultaneously recorded. 3-ml headspace samples were injected by means of a pre-warmed (50°) gas-tight syringe into a U-shaped glass column (1.5 m × 2 mm I.D.) which was loaded with Carbopack C (80–100 mesh) coated with 0.2% Carbowax 1500 (Supelco). The column was operated isothermally at 50°; injection block temperature, 180°. The carrier gas (helium) flow-rate was 20 ml/min. After elution of the vinyl chloride, the other components of the sample were vented to the atmosphere.

RESULTS AND DISCUSSION

In Figs. 1–3 we give some examples of mass fragmentograms for 98.6, 7.6 and 0.3 ppb of vinyl chloride in salad oil and the blanks. Although the influence of the air peak on the elution of lower concentrations of vinyl chloride is clear, the interference

is small due to the good separation of the Carbopack column. Other column fillings such as OV-1, SE-30, OV-17 and OV-207 on Chromosorb gave satisfactory results at the 50 ppb level, but at lower levels the better separation of the Carbopack column is essential.

Graphs of the peak heights of the *m/e* 62 response against the known vinyl chloride content in salad oil are given in Fig. 4, showing the good linearity of the method. Figs. 5 and 6 show the *m/e* 62 response for 0.1 ppb of vinyl chloride in the food simulants 10% alcohol–water and 3% acetic acid–water respectively.

On the basis of these results we can say that amounts of 1 ppb of vinyl chloride in food simulants can be easily detected and quantitated since we used food simulants of technical grade without further purification. We expect to have indicated a sensitive and specific method for the direct analysis of vinyl chloride in food and drink samples. Although definite conclusions can only be made after thorough evaluation of large series of analyses of various foods and drinks, so far this method is superior to existing ones using gas chromatography and flame ionization detection. Further experiments on food simulants and analyses of food and drink samples are in progress.

REFERENCES

1 *Department of Labor O.S.H.A. Fed. Regist.*, 39 (1974) 35890.
2 *Federal Drug Administration. Fed. Regist.*, 40 (1975) 40529.
3 C. V. Breder, J. L. Dennison and M. E. Brown, *J. Ass. Offic. Anal. Chem.*, 58 (1975) 1214.
4 J. Puschmann, *Angew. Makromol. Chem.*, 47 (1975) 29.
5 W. R. Eckert, *Fette, Seifen, Anstrichm.*, 77 (1975) 319.
6 G. F. Ernst and J. B. H. van Lierop, *J. Chromatogr.*, 109 (1975) 439.
7 D. T. Williams and W. F. Miles, *J. Ass. Offic. Anal. Chem.*, 58 (1975) 272.
8 J. D. Rosen, J. R. Morano, S. R. Pareles, J. R. Giacin and S. G. Seymor, *J, Ass. Offic. Anal. Chem.*, 58 (1975) 700.

De Ware(N)-Chemicus 5 (1975) 157

BEPALINGSMETHODEN VAN VINYLIDEENCHLORIDE

Keuringsdienst van Waren, Utrecht.
J.B.H.van Lierop, C.J.Hogendijk en Th.J.Jongerius.

November 1975.
Kode:

1. INLEIDING.

Ten behoeve van het Verpakkingen- en Gebruiksartikelenbesluit van de Warenwet werden bepalingsmethoden voor vinylideenchloride monomeer (VDCM) ontwikkeld (2.1.). De toelaatbare migratie van VDCM in levensmiddelen is door de Hoofdinspektie van de Volksgezondheid gesteld op 0,05 mg uit 6 dm^2 verpakkingsmateriaal (2.2.). Dit komt overeen met een gehalte van 0,05 ppm in het levensmiddel. Deze eis is gelijk aan die voor vinylchloride. VDCM kan aanwezig zijn in polyvinylideenchloride en zijn copolymeren (polyalkeenpolymeren, polyacrylaat, polyvinylchloride, gechloreerde polymeren). Er werden verschillende gaschromatografische methoden ontwikkeld, die een grote overeenkomst hebben met de bepalingen voor vinylchloride. Naast de screening methoden met een vlamionisatie detector werden ook twee specifieke methoden ontwikkeld (2.3., 2.4.):

a. bepaling van VDCM in de m-xyleen (waterige eet- en drinkwaren).
b. bepaling van VDCM in de headspace van olie.

Bij deze methoden wordt gebruik gemaakt van een halogeen detector volgens Hall, waarin in een kwartsbuis door middel van waterstof een reductie wordt uitgevoerd, waarna eventueel gevormde halogeenwaterstof wordt gedetecteerd.

2. LITERATUUR.

2.1. J.B.H. van Lierop, C.J.Hogendijk, Th.J.Jongerius; Bepalingsmethoden van vinylideenchloride — R 68/03/75 juli 1975.

2.2. Brief van de Hoofdinspekteur van Volksgezondheid van 17 december 1974.

2.3. G.F.Ernst, J.B.H.van Lierop, J.Chromatog. 109 (1975) 439-440.

2.4. J.B.H.van Lierop, C.J.Hogendijk, Th.J.Jongerius; De Ware(N)-Chemicus 5 (1975) 44-47.

De Ware(N)-Chemicus 5 (1975) 158 - 159

DE GASCHROMATOGRAFISCHE BEPALING VAN VINYLIDEENCHLORIDE IN M-XYLEEN (waterige eet- en drinkwaren)

Keuringsdienst van Waren, Utrecht.
J.B.H.van Lierop, C.J.Hogendijk en Th.J.Jongerius.

November 1975.
Kode:

1. DOEL.

Deze methode beschrijft hoe men VDCM kan bepalen in m-xyleen.

2. SAMENVATTING.

Het monster wordt geanalyseerd met behulp van gaschromatografie over een SE 30 kolom op Chromosorb G AW DMCS met een Hall detector.

3. APPARATUUR EN REAGENTIA.

3.1. Een geschikte gaschromatograaf met Hall detector.

3.2. m-Xyleen (p.a. Merck).

3.3. Vinylideenchloride (standaard oplossingen in m-xyleen).

3.4. SE 30 (Chrompack).

3.5. Chromosorb (Chrompack).

3.6. Standaard laboratorium glaswerk.

4. GASCHROMATOGRAFISCHE KONDITIES.

4.1. Gaschromatograaf: Hewlett Packard 5750 B.

4.2. Kolom:
Materiaal : roestvrij staal
Lengte : 4 meter
Inw.diameter : 1/8 inch
Vulling : 5% SE 30 op 80-100 mesh Chromosorb G AW DMCS.

4.3. Detector systeem:
Hall detector (voor principe zie literatuur).
Waterstofgassnelheid: inlet pressure 10 p.s.i.
Oventemperatuur: 820°C.
Detector: sensivity x 1 attenuator x 3.
Snelheid geleidbaarheidsvloeistof: ± 0,5 ml/min.

4.4. Dragergas: Argon 5,5 ml/min.

4.5. Kolomtemperatuur: 80°C.

4.6. Injektiehoeveelheid: 2 µl.

4.7. Recorder: 1 mV recorder volle schaaluitslag 20 cm.

4.8. Recordersnelheid: 5 min. per inch 20 cm.

4.9. Gevoeligheid: 0,05 ppm VDCM in de simulant resulteert in een signaal van 75 mm.

5. WERKWIJZE.

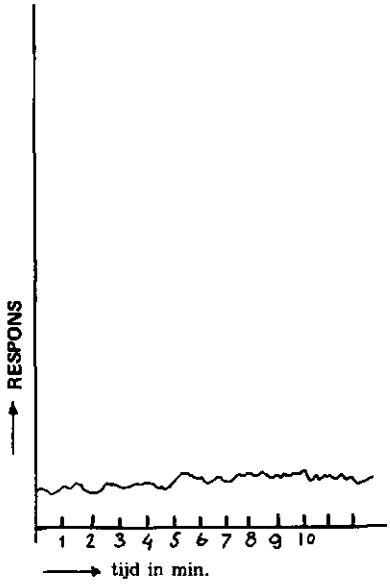
5.1. Ijklijn.
Breng in kolven van 300 ml, 250 ml van de waterige eet- of drinkwaar of de simulant. Voeg hieraan toe 25 ml xyleen + 0 ml van een verse standaard vinylideenchloride-oplossing met een sterkte van 15 mg/liter, 24 ml + 1 ml van deze standaardoplossing enz. Breng vervolgens de inhoud van de kolf met behulp van een magneetroerder met een zodanige snelheid in beweging dat de vloeistoffen zich net niet vermengen. Laat een uur roeren bij kamertemperatuur en daarna ontmengen. Injekteer de bovenstaande xyleen-oplossing. Meet in de chromatogrammen de hoogten van de vinylideenchloridepieken en zet deze uit tegen de vinylideenchloride-koncentraties.

5.2. Bepaling.
Het als extractiemiddel gebruikte xyleen van de te onderzoeken eet- of drinkwaar of simulant, wordt direkt geinjekteerd en de piekhoogte wordt gemeten.

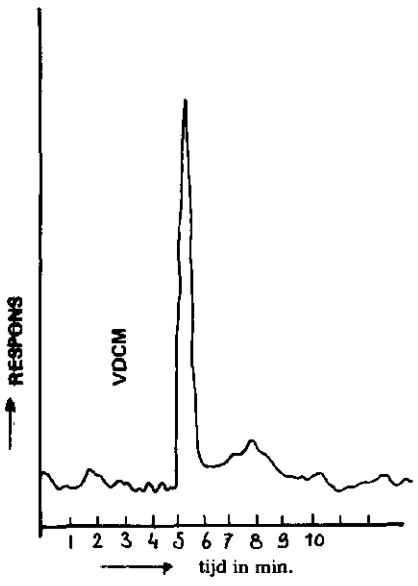
6. OPMERKINGEN.

6.1. In figuur 1 wordt een gaschromatogram gegeven van m-xyleen verkregen na extractie van een simulant (10% alkohol) die geen VDCM bevatte.
In figuur 2 wordt een gaschromatogram gegeven van een simulant (10% alkohol) die 0,05 ppm VDCM bevatte.

6.2. Gelijkwaardige chromatogrammen met die van 10% alkohol werden verkregen bij de simulanten azijn en 3% azijnzuur.



FIGUUR 1: Xyleen verkregen na extractie van de simulant (10% alkohol) die geen VDCM bevatte.



FIGUUR 2: Xyleen verkregen na extractie van de simulant (10% alkohol) die 0,05 ppm VDCM bevatte.

De Ware(N)-Chemicus 5 (1975) 160 - 162

DE GASCHROMATOGRAFISCHE BEPALING VAN VINYLIDEENCHLORIDE IN DE HEADSPACE VAN OLIE

Keuringsdienst van Waren, Utrecht.
J.B.H.van Lierop, C.J.Hogendijk, en Th.J.Jongerius.

November 1975.
Kode:

1. DOEL.

Deze methode beschrijf hoe men VDCM kan bepalen in olie.

2. SAMENVATTING.

De olie wordt in een infuusfles in een waterbad verwarmd. Daarna wordt het bovenstaande gas (topgas, headspace gas) bemonsterd en geanalyseerd op een gaschromatograaf met een Hall detector.

3. APPARATUUR EN REAGENTIA.

3.1. Een geschikte gaschromatograaf met Hall detector.

3.2. m-Xyleen (p.a. Merck).

3.3. Vinylideenchloride (standaard oplossingen in m-xyleen).

3.4. SE 30 (Chrompack).

3.5. Chromosorb (Chrompack).

3.6. Standaard laboratorium glaswerk.

3.7. Infuusflessen van 250 ml met aluminium schroefdop en rubber septum.

3.8. Gasdichte injektiespuit van 2,5 ml.

3.9. Waterbad van 70°C.

4. GASCHROMATOGRAFISCHE KONDITIES.

4.1. t/m 4.5.
Zie voorschrift A, behalve de volgende wijzigingen.
De Ware(N)-Chemicus, 5 (1975) 158-159.

4.6. Injektiehoeveelheid: 0,5 ml.

4.9. Gevoeligheid: Bij een versterking van 1 x 3 geeft 0,06 ppm in de olie een uitslag van 32 mm.

5. WERKWIJZE.

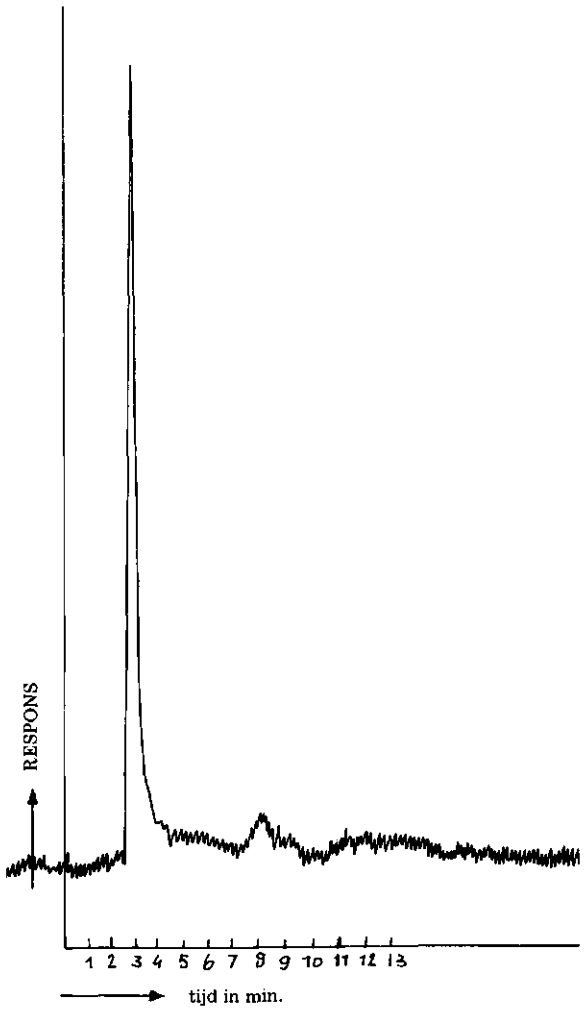
5.1. IJklijn.
Bereid uit de verdunde standaardoplossing van 3 mg VDCM op 1000 gram olie een ijkreeks in de infuusflessen van 250 ml door te nemen 100 gram olie, 99 gram + 1 gram verdunde standaardoplossing, 98 gram olie + 2 gram verdunde standaardoplossing. De olie bevat dan 0,00 ppm VDCM, 0,03 ppm VDCM, 0,06 VDCM enz. De flessen met olie worden gedurende een uur bij 70°C in een waterbad verwarmd alvorens in het bovenstaande gas het VDCM-gehalte wordt bepaald door 0,5 ml hiervan te injekteren.
Meet in de chromatogrammen de hoogten van de vinylideenchloride-pieken en zet deze uit tegen de VDCM-koncentratie in de olie.

5.2. Bepaling.

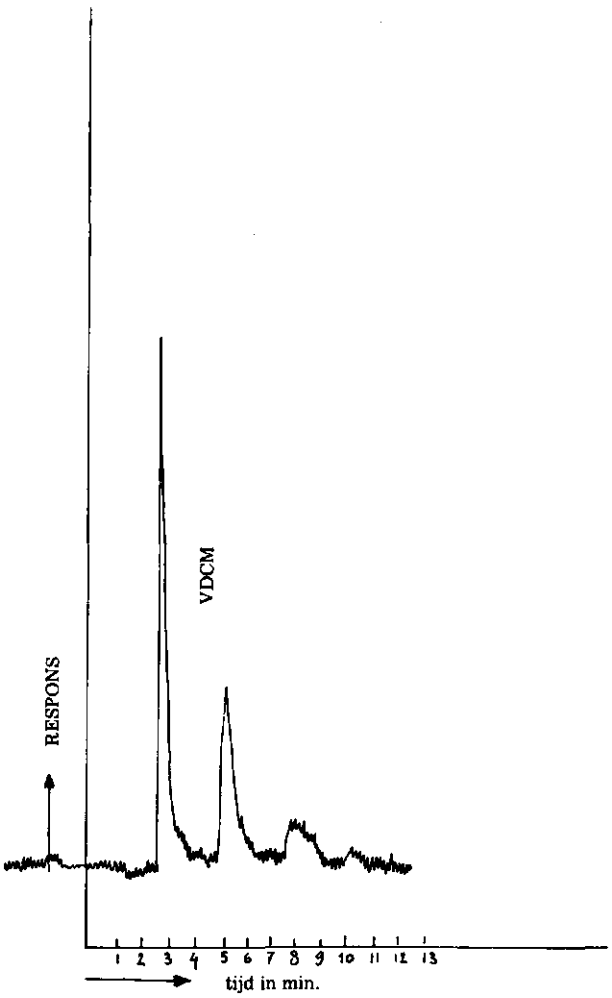
Breng 100 gram van de olie in een infuusfles van 250 ml. Sluit de fles af door middel van een schroefdop voorzien van een rubber septum. Plaats de fles gedurende een uur in een waterbad van 70°C. Bepaal het VDCM-gehalte in het bovenstaande gas door 0,5 ml in te spuiten. Meet in het chromatogram de hoogte van de VDCM-piek en leid uit de ijkgrafiek de hoeveelheid vinylideenchloride in de olie af.

6. OPMERKINGEN.

In figuur 1 wordt een chromatogram gegeven van olie zonder VDCM toevoeging.
In figuur 2 is aan deze olie 0,06 ppm VDCM toegevoegd.



FIGUUR 1: Olie zonder VDCM toevoeging.



FIGUUR 2: Olie met 0,06 ppm VDCM.

De Ware(n)-Chemicus 8 (1978) 178-179

ANNEX E

DE BEPALING VAN ACRYLONITRIL MET HEADSPACE GASCHROMATOGRAFIE-MASSAFRAGMENTOGRAFIE

Keuringsdienst van Waren, Utrecht
Drs. J.B.H. van Lierop

augustus 1978

SUMMARY

Title: Determination of acrylonitril by headspace gaschromatography-massfragmentography.
Author: J.B.H. van Lierop.
Address: Food Inspection Service, Nijenoord 6, 3552 AS Utrecht, The Netherlands.

Acrylonitril, a possible toxic compound, may migrate from food packaging material into food. Its presence in food and food simulating solvents was demonstrated by using gaschromatographic (GLC) headspace method coupled with massfragmentation detection. The described procedure consisted in headspace sampling followed by GLC analysis of vapour (column: stainless steel, length 1,6 m, i.d. 3 mm, packed with 0,2% Carbowax 1500 on Carbopack C 80-100 mesh; carrier gas He 20 ml/min flow; injection temp. 150°C, column temp. 70°C, detector interface temp. 150°C and mass fragmentation detection of the fragments m/e 51, 52 and 53).
The detection limit of acrylonitril is 1 µg/kg in food simulants and food.
In migrates from acrylonitril copolymer bottles 1-4 µg/kg acrylonitril has been found after migration under standard conditions (10 days 40°C).
The described procedure can also be used to determine the acrylonitril content in copolymers.

1. INLEIDING

De Food and Drug Administration in de Verenigde Staten heeft maatregelen afgekondigd tegen 'acrylonitril' flessen (2.1., 2.2.). Dit op grond van de toxiciteit, die uit dierproeven gebleken is.
Acrylonitril (zie figuur 1 voor structuurformule) kan als residu aanwezig zijn in copolymeren zoals polyacrylonitril-butadieen-styreen (ABS) en polystyreen-acrylonitril (SAN). Bij gebruik van deze copolymeren als verpakkingsmateriaal bestaat de mogelijkheid dat acrylonitril migreert uit de verpakking in het levensmiddel.
In het kader van de specialisatie Verpakkingen werden gevoelige methoden ontwikkeld om acrylonitril kwantitatief te bepalen en om de aanwezigheid in het voedsel te kunnen bevestigen.
In de literatuur zijn detectiemethoden beschreven met behulp van infrarood, polarografie en gaschromatografie met vlamionisatie (FID) en met specifieke stikstofdetectie.

$$CH_2 = CH - C \equiv N$$

Figuur 1: Structuurformule van acrylonitril.

Met de infrarood-methode (2.3.) worden bepalingen in het 5-30 mg/kg-gebied beschreven, de polarografische methode gaat tot 10 µg/kg (2.4.), de gaschromatografische methode met behulp van de specifieke stikstof-detector kan tot 50 µg/kg gaan in synthetische standaarden (2.5.).
In de simulanten water en 3% azijnzuur werd door ons een detectiegrens van 10 µg/kg bereikt met een gaschromatografische FID-methode. Moeilijkheden traden op bij de bepaling in de simulant 15% ethanol. Met behulp van headspace gaschromatografie, gekombineerd met massafragmentografie, werd een methode ontwikkeld met een detectiegrens van 1 µg/kg acrylonitril in simulanten. Deze methode heeft een grote specificiteit omdat drie fragmenten m/e 51, 52 en 53 tegelijk bepaald kunnen worden.
Er werden ijkreeksen gemaakt en geanalyseerd met de voedselsimulanten 3% azijnzuur en 15% ethanol-water en gazeuse.
Bij migratieproeven, 10 dagen 40°C, volgens 2.6. werden in de bovengenoemde simulanten waarmede de flessen gevuld waren, acrylonitril-gehaltes van 1-4 µg/kg gevonden.
De ontwikkelde GC-MS headspace-methode bleek ook geschikt voor een bepaling van acrylonitril in het copolymeer. Als oplosmiddel werd daarvoor dimethylacetamide gebruikt.

2. LITERATUUR

2.1. Fed. Regist., March 11, 1977.

2.2. *F.D.A. Consumer 1977*, 26.

2.3. M.E. Hall en J.W. Stevens, *Anal. Chem. 49* (1977) 2277-2281.

2.4. Bedrijfsvoorschrift.

2.5. Application Note Hewlett Packard ANGC B-76.

2.6. Ontwerp Verpakkingen- en Gebruiksartikelenbesluit, Methoden van Onderzoek, Hoofdstuk 1.

De Ware(n)-Chemicus 8 (1978) 180-182

DE BEPALING VAN ACRYLONITRIL MET HEADSPACE GASCHROMATOGRAFIE-MASSAFRAGMENTOGRAFIE

VOORSCHRIFT

Keuringsdienst van Waren, Utrecht
Drs. J.B.H. van Lierop

augustus 1978

1. DOEL

Dit voorschrift beschrijft de kwantitatieve bepaling van acrylonitril (C_3H_3N) in de voedselsimulanten water, 3% azijnzuur-water en 15% ethanol-water en in gazeuse.

2. DEFINITIE

Het acrylonitrilgehalte wordt opgegeven in µg/kg simulant.

3. GEBIED VAN TOEPASSING

De hier beschreven methode is toepasbaar voor de bepaling van acrylonitril in voedsel en voedselsimulanten (zie 10.2.).

4. BEGINSEL

De bepaling vindt plaats met behulp van headspace gaschromatografie met massafragmentografie als detectietechniek. De massafragmenten m/e 51, 52 en 53 worden tegelijkertijd bepaald via GC-MS.

5. REAGENTIA

5.1. Acrylonitril.

5.2. Voedselsimulanten zoals water, 3% azijnzuur, 15% alcohol en koolzuurhoudende limonade (gazeuse).

6. APPARATUUR

6.1. Laboratoriumglaswerk.

6.2. Gaschromatograaf-massaspectrometer, merk Finnigan 4000 of gelijkwaardig.
Een drie-pens recorder.
Kolommateriaal: metaal.
Kolomvulling: 80-100 mesh Carbopack C + 0,2% Carbowax 1500.
Kolomlengte: 1,60 m.
Kolomdiameter: 3 mm.
Dragergas: Helium, flow 20 ml/min.
Injectietemperatuur: 150°C.
Detector interface temperatuur: 150°C.
Afblaassysteem: gedurende 2,3 minuten na de inspuiting is de verbinding tussen gaschromatograaf en massaspectrometer gesloten, daarna gedurende 2 minuten geopend en tenslotte weer gesloten. Dit programma wordt via een microprocessor gestuurd.
De MID Promim is ingesteld als volgt: kanaal 1 op m/e 51, kanaal 2 op m/e 52, kanaal 3 op m/e 53.
De massaspectrometrische condities zijn uiteraard afhankelijk van het apparaat. Zie voor een voorbeeld 11.1.

7. WERKWIJZE

Van de migraten of van het te onderzoeken levensmiddel wordt ca 50 ml ingewogen in een infuusfles van 120 ml.
Dit infuusflesje wordt afgesloten met een aluminium schroefdop waarin een rubber septum. Dit flesje wordt in een waterbad van 70°C tenminste 1 uur verhit.
Van het topgas wordt 1 ml geïnjecteerd in de gaschromatograaf met een gasdichte injectiespuit.

8. IJKLIJNEN

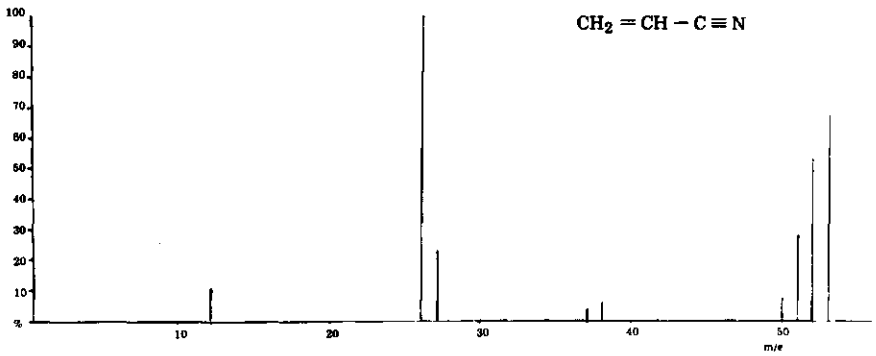
IJklijnen zijn gemaakt door vloeibaar acrylonitril in de simulanten of in het te onderzoeken voedsel in te wegen.

9. BEREKENING VAN DE GEHALTEN ACRYLONITRIL

Bepaal de piekhoogte driemaal, lees af op de ijklijnen en neem het gemiddelde.

10. OPMERKINGEN

10.1. Het massaspectrum van acrylonitril wordt gegeven in figuur 1. Gezien het feit dat er met headspace stikstof (M = 28) bevattende lucht wordt geïnjecteerd, is de m/e 27 piek niet geschikt. Daarom werden de m/e 51, 52 en 53 gekozen.



Figuur 1: Structuurformule en massaspectrum van acrylonitril.

10.2. In figuur 2 op de volgende bladzijde worden massafragmentogrammen gegeven van drie verschillende concentraties acrylonitril in 15% ethanol. De massafragmentogrammen van standaarden acrylonitril in 3% azijnzuur en in gazeuse zagen er gelijk uit. De simulant olie is nog niet onderzocht omdat de ontvangen acrylonitrilcopolymeer-flessen niet bedoeld zijn om olie in te verpakken.

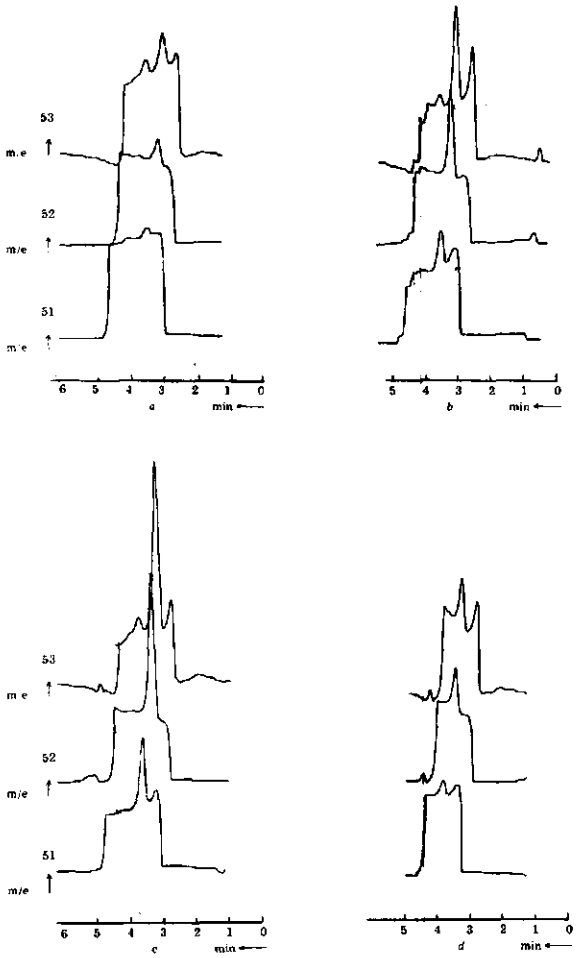
10.3. In figuur 2 d is een massafragmentogram gegeven van 15% alcohol, die gedurende 10 dagen bij 40°C in een Barex fles verpakt is geweest. De hoeveelheid acrylonitril in dit migraat bedroeg 3 µg/kg. De migraten 3% azijnzuur en gazeuse, afkomstig uit hetzelfde type fles, bevatten 2-4 µg acrylonitril per kg migraat.

10.4. Een verbetering van de piekvorm is waarschijnlijk te verwachten bij gebruik van een glazen kolom in plaats van een metalen.

10.5. De materiaalbepaling werd slechts met een beperkt aantal monsters uitgevoerd. De GC-MS condities waren dezelfde als bij de hier beschreven bepaling. Vóór de equilibratie in het waterbad werden de 10% copolymeer-oplossingen in DMA één nacht bewaard bij kamertemperatuur. In Barex flessen werd 1,8 µg/kg bepaald.

11. REFERENTIE

11.1. J.B.H. van Lierop en H. Nootenboom, *De Ware(n)-Chemicus 8* (1978) 33-49.



Figuur 2: Massafragmentogrammen van acrylonitril in 15% ethanol (a = 1,0 µg/kg; b = 5,4 µg/kg; c = 10,7 µg/kg; d = monster).

ANNEX F

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CHROM. 11,255

Note

Simple and rapid determination of epichlorohydrin at the lower parts per billion* level by gas chromatography–mass fragmentography

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It has recently been supposed[1] that epichlorohydrin (EPCH**; 1-chloro-2,3-epoxypropane) may be a mutagenic compound. A possible route by which EPCH may reach foodstuffs is via the packaging material. Epoxy resins are widely used as coatings for cans employed for packaging of food, and large containers for milk and juices are often coated with epoxy resins; one of the compounds used in the manufacture of the epoxy resins is EPCH. Residual epichlorohydrin may then be present in the can coating and migrate into the beer, juices, etc., contained in the can.

The method described here for the determination of EPCH is based on mass fragmentography, which provides the highest sensitivity of detection with high specificity. The determination of the fragment of *m/e* 49 in diethyl ether is possible at the level of 6 ppb.

EXPERIMENTAL AND RESULTS

A Finnigan 4000 gas chromatograph–mass spectrometer was equipped with a 1.5 m × 0.3 mm I.D. glass column, packed with 80–100-mesh Carbopack C (Supelco, Bellefonte, Pa., U.S.A.) loaded with 0.2% of Carbowax 1500 The carrier gas (helium) had a flow-rate of *ca.* 20 ml/min. The gas chromatograph was connected with the mass spectrometer through an all-glass separator. A vacuum diverter system was used to prevent the solvent contaminating the source of the mass spectrometer, which was used in the electron-impact mode with the multiple ion detection unit tuned at *m/e* 49.

Samples of 1 or 5 μl were injected at 100° (isothermal) and the signals were measured on a Rikadeni recorder with a chart speed of 10 mm/min. Standard solutions of EPCH in diethyl ether were prepared.

Fig. 1 shows a mass fragmentogram of the blank diethyl ether and of standards containing 6, 12 and 30 ppb of EPCH. Fig. 2 is a calibration graph for these low contents of EPCH in diethyl ether, obtained using standard solutions with concentrations of 6, 12, 30, 72 and 96 ppb of EPCH.

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* Throughout this article, the American billion (10^9) is meant.

** It is common to use the abbreviation ECH for epichlorohydrin in the polymer industry. However this may lead to confusion, because ECH has also been used for chloroethanol in the literature[2]. Therefore, epichlorohydrin is abbreviated to EPCH in this paper.

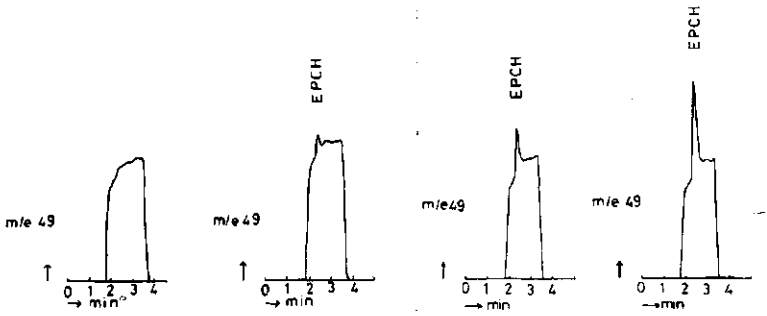

Fig. 1. Mass fragmentagram at *m/e* 49 of a blank of diethyl ether and of diethyl ether containing 6, 12 and 30 ppb of EPCH.

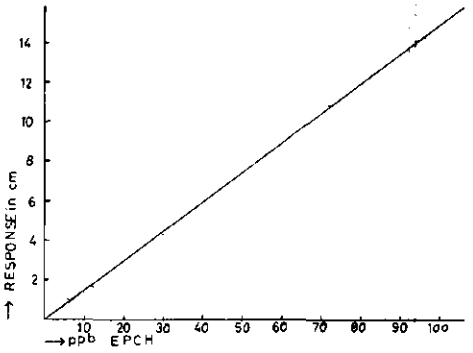

Fig. 2. Calibration graph for diethyl ether solution containing 6, 12, 30, 72 and 96 ppb of EPCH.

With standard solutions with EPCH concentrations of 10 and 20 ppb in water we established that after three extractions with diethyl ether after addition of sodium chloride the extraction of EPCH was quantitative.

Under standard conditions for migration[3] of immersion at 40° for 10 days, three types of cans and four sheets coated with epoxy resins were investigated for the migration of EPCH into water. The resulting aqueous solutions were extracted with diethyl ether, using 50 ml of ether for 100 ml of solution, and the extracts examined by the method described. No EPCH was found in the extracts.

On the basis of these results it is possible to conclude that the maximum amount of EPCH that could have migrated is less than 3 ppb into the water.

REFERENCES

1 M. Kucerova, V. S. Zhurkov, Z. Polivkova and J. E. Ivanova, *Mutat. Res.*, 48 (1977) 355.
2 T. Stijve, R. Kalsbach and G. Eyring, *Trav. Chim. Aliment. Hyg.*, 67 (1976) 403.
3 *Ontwerp Verpakkingen en Verbruiksartikelenbesluit Warenwet, Bijlage Deel B, Methoden van Onderzoek*, Ministerie van Volksgezondheid en Milieuhygiëne, The Hague, 1977.

ANNEX G

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FOOD ADDITIVES

Gas-Liquid Chromatographic-Mass Fragmentographic Determination of Low Levels of Diethylcarbonate in Beverages

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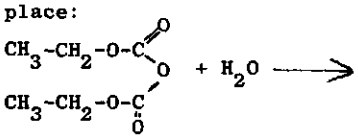
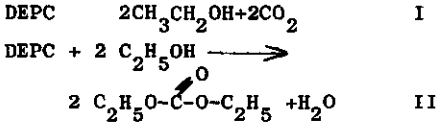
Sodium chloride and ethanol (omitted for samples with >10% alcohol) are added to the beverage sample and the sample is allowed to equilibrate in a 30°C water bath. An aliquot of the headspace is injected into a gas chromatograph containing a column packed with 0,2% Carbowax 1500 on 80-100 mesh Carbopack C. During the elution of diethylcarbonate (DEC) an impurity of diethylpyrocarbonate, the column effluent is vented to a mass spectrometer with a multiple ion detection system and operated in the electron impact mode. The ions at m/e 63 and 91 are monitored. Lemonade, fruit drinks, wine, and beer samples (138 total) were analyzed for DEC. Sixteen samples had >30 ppb DEC. Eight analyses of a lemonade sample gave a mean of 88 ppb with a coefficient of variation of 11%.

Diethylpyrocarbonate (DEPC) has been added to beverages (soft drinks, wine, and beer) to prevent fermentation generally at a level of 60-300 ppm. However, the carcinogen ethylcarbamate (urethan) may be formed (1). Therefore, the use of DEPC is forbidden in many countries.
The following reactions can take place:



DEPC $\quad 2CH_3CH_2OH + 2CO_2$ $\quad$ I



DEC

Wunderlich (2) developed a technique based on reaction II to measure the amount of DEPC added to beverages of known alcoholic content.
In a collaborative study, 1-10 ppm added diethylcarbonate was determined with good results; the method has been adopted as an official final action AOAC method (3). The method was based on extraction of DEC with carbon disulfide and determination by flame ionization gas-liquid chromatography (GLC).
McCalla (4) reported that a false positive result can be obtained with the method described and he suggested that infrared analysis be used to confirm the presence of DEC.
Kunitake (5) reported a gas phase analysis of DEC in beer. Gas phase analysis has advantages compared to GLC of a liquid sample. Only volatile components are injected into the gas chromatograph, so the lifetime of the column is prolonged. Moreover, often the gas phase technique is more sensitive.
Mass fragmentography provides high sensitivity with high specificity. The method presented here is based on headspace GLC, using a graphite column. This type of column was chosen because of the good separation between air and volatile components on this column.

METHOD

Apparatus

(a) *Combined gas chromatograph-mass spectrometer.*-Model 4000 with multiple ion detection (MID) (Finnigan Corp., Sunnyvale, CA 94086) unit and used in electron

impact mode.Gas chromatograph was interfaced to mass spectrometer with all-glass jet separator. MID unit was tuned to m/e 63 and 91 ions of DEC, and integrated signals were simultaneously recorded. Operating conditions: 1.5 m x 3 mm id glass GLC column packed with 0.2% Carbowax 1500 on 80-100 mesh Carbopack C (Supelco, Inc., Bellefonte, PA 16823); helium carrier flow 20 mL/min; temperatures (^{o}C)-injector 150, column 150, separator 200, transfer line 235; pressure mass spectrometer 1.2 x 10^{-7} torr; ionization voltage 80 v;emission current 150 uamp; preamplifier 10^{-9} amp/v; 4-pen recorder (Rikadenki) 1 v; chart speed 1 cm/min. A vacuum diverter was used to vent column effluent; column effluent was vented to mass spectrometer only during the time that DEC would elute from the column. i.e., for 1.5 min. after injection nearly all the column effluent was vented, then the diverter was opened for 1.5 min. and closed again.

(b) *Infusion flask.*-120 mL bottle with screw cap and rubber septum.

(c) *Gas syringe.*-5 mL pressure locked (Glenco Scientific,Inc.,Houston, TX 77007, or equivalent).

Reagents

(a) *Diethylcarbonate.*-Puriss (Fluka, or equivalent). Prepare solutions containing 7, 14, 34, 68, and 137 ppb DEC with ethanol.

(b) *Diethylpyrocarbonate.*-Purum (Fluka or equivalent).

Determination

Add 10 mL ethanol, 70 mL sample, and 32 g NaCl to infusion flask and stopper. Mix thouroughly 5 min. by shaking. Place flask in 30^{o}C water bath and let stand 30 min to reach equilibrium. Inject 1 mL headspace with gas-tight syringe and measure peak height on chromatogram. For beverages (wines) with >10% alcohol, use 80 mL sample and 32 g NaCl; do not add ethanol.

Mass Spectrometry

The mass spectrum of DEC is shown in Fig. 1. Since interferences from air and ethanol may be present in the region below m/e 50, the peaks m/e 63 and 91 were chosen for the MID of DEC. In a few lemonade samples, an interference was found at m/e 91. Therefore, the ratio 91:63 was used to confirm the presence of DEC in the beverages analyzed.

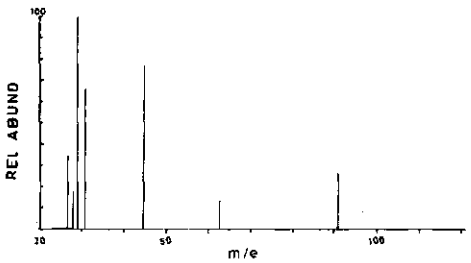


FIG. 1 - Mass spectrum of DEC.

Table 1. PEAK HEIGHTS FOR DIFFERENT AMOUNTS OF DEC.

	Peak height,mm,at	
DEC,ppb	m/e 61	m/e 91
7	3	5
14	8	14
34	18	29
68	34	56
137	66	106

Calibration curve

Prepare calibration curve from standard solutions of DEC in ethanol. The peak heights obtained are given in Table 1 and chromatograms for 7 and 14 ppb DEC are given in Fig. 2. The calculated regression lines were as follows: for m/e 63, $A = 0.49B + 0.65$; for m/e 91, $A = 0.77B + 2.21$, where A = mm peak height, and B = ppb DEC. The correlation coefficient for both lines was 1.00. The ratio of m/e 91:63 was 1.62

Method Study

One soft drink containing DEC was analyzed 8 times by the same analyst on the same day. Eight aliquots were taken from 1 flask to prepare 8 different headspace flasks. One 1 ml headspace injection was made for each flask.

One hundred and seven soft drink samples were purchased in local retail stores. Wine (23 samples) and beer (8 samples) were also analyzed.

RESULTS AND DISCUSSION

The 8 replicate determinations of the lemonade sample showed a mean of 88 ppm DEC ± 10%; *see* Table 2.

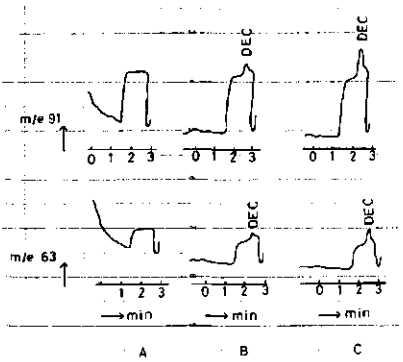


Fig.2-Mass fragmentograms of a blank (A) and standard solutions with 7 (B) and 14 ppb DEC (C) added.

Table 2. DETERMINATION OF DEC (ppb) CALCULATED ON BASIS OF THE 2 m/e SIGNALS*

Detn	At m/e 63	At m/e 91	Mean
1	88.5	84.1	86.30
2	84.4	75.1	79.75
3	78.3	69.9	74.10
4	106.8	103.6	105.20
5	102.8	94.5	98.65
6	90.5	85.4	87.95
7	88.5	80.3	84.40
8	86.4	81.6	84.00
Mean	90.78	84.31	87.54(88)
Std dev.	9.5	10.6	10
%, Coeff. of var.,%	10	13	11

* Eight aliquots frome one lemonade sample.

Of the 107 soft drink samples analyzed, 15 samples had >15 ppb DEC. The results for these samples are given in Table 3, along with the m/e 91:63 ratio. Figure 4 is a mass fragmentogram of Orangeade 1. Two lemonade samples had a peak ratio of >3.00; these samples were considered to be negative for DEC. One wine sample, a white German Rhine wine of a 1970 vintage, showed 6000 ppb DEC. In 1970 the use of DEPC was permitted in Germany and The Netherlands. A complete mass spectrum was obtained to verify that the substance was DEC. All of the other 22 wines contained <5 ppb DEC. No DEC was found in 8 samples of beer analyzed.

Table 3. AMOUNTS OF DEC CALCULATED FROM 2 m/e SIGNALS 63 AND 91 AND THE PEAK RATIO 91/63

Sample	At m/e 63, ppb	At m/e 91, ppb	Ratio of m/e 91:63 Peaks
	Orangeade		
1	91	92	1.67
2	44	43	1.59
3	153	155	1.64
4	94	96	1.65
5	142	141	1.59
6	226	232	1.66
7	61	62	1.60
8	127	130	1.63
9	92	99	1.73
10	38	38	1.63
	Other Beverages		
Grape-fruitdrink	119	118	1.60
Lemonade 1	82	82	1.62
Lemonade 2	84	96	1.85
Lemonade 3	162	174	1.71
Black current drink	82	84	1.65

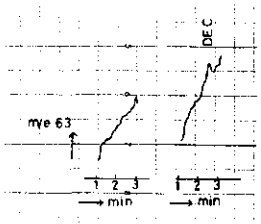


FIG. 3-Mass fragmentograms of a blank and of a standard solution with 1 ppb DEC. A higher attenuation is used than in Fig. 2

The method can detect as little as 1 ppb DEC, as shown in Fig. 3. For this chromatogram, 20 mv instead of 1 v was used for the recorder voltage. The signal at m/e 91 is not included because the signal-to-noise ratio was poor.

If we assume that the added DEPC has completely disappeared, according to reaction I, and that the DEC found is only the impurity of the added DEPC, the amount of DEPC added can be estimated. We assume that the content of the DEC in the DEPC used is 0.1%. If 100 ppb DEC were found, 100 ppm DEPC was added.

We also attempted to determine DEC by flame ionization GLC. However, only a small peak is produced for about 50 ppb DEC and this peak is not sufficient for the quantitation. The response can be improved by concentrating the sample, but this would require more time. An inexperienced analyst can carry out 50 analyses in one day, using the method described here. This includes both the sample preparation steps and the mass spectrometric determinations.

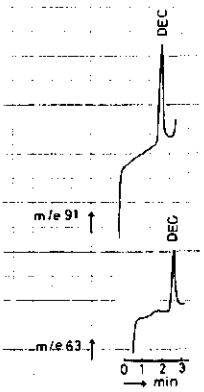


FIG. 4-Mass fragmentograms of an orangeade sample with a DEC content of 91 ppb (based on m/e 63) and 92 ppb (based on m/e 91).

References

(1) Löfroth,G., & Gejvall, T. (1971) *Science* 174, 1248-1250

(2) Wunderlich, H. (1972) *J.Assoc.Off. Anal.Chem.* 55 557-558

(3) *Official Methods of Analysis* (1975) 12th Ed., AOAC, Washington,DC, secs 11.066-11.068

(4) McCalla, M.A. (1977) *J.Assoc.Off. Anal.Chem.* 60, 237-238

(5) Kunitake, N. (1969) *Bull.Brew.Sci.* 15, 1-4

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CURRICULUM VITAE

Johannes Bernard Hendrik van Lierop werd op 26 december 1935 te Amsterdam geboren.
Hij behaalde het getuigschrift Gymnasium B in 1954 aan het Gereformeerd Gymnasium te Amsterdam. In datzelfde jaar begon hij zijn studie in de scheikunde aan de Vrije Universiteit aldaar. Het kandidaatsexamen richting f werd afgelegd in september 1958, het doctoraal examen in juni 1962 met als hoofdvak organische scheikunde (o.l.v. Prof.Dr.Ir. J.J. Coops).

Vanaf september 1962 tot en met februari 1967 was de auteur werkzaam op de afdeling synthetische polymeren van de AKU te Arnhem. Van april 1967 tot juli 1974 was hij quality control manager bij Polak Frutal Works te Amersfoort.
Sinds juli 1974 is hij werkzaam als scheikundige bij de Keuringsdienst van Waren te Utrecht. Na een begin op de afdeling Verpakkingen werd het hier beschreven onderzoek voortgezet op de afdeling Instrumentele Analyse.