ANALYSIS OF MALODOROUS NITROGEN-CONTAINING COMPOUNDS IN PAPER AND BOARD PRODUCTS

Master's Thesis and
Experimental Research Study
University of Jyväskylä
Department of Chemistry
Applied Chemistry
May 15th 2007
Aino Karvinen

ABSTRACT

This study deals with malodorous nitrogen compounds and possible ways of analysing these volatile compounds with a very distinctive and fish-like odour. The previous sensory tests have indicated that the harmful and reclamations causing odour in paper and board products used for packaging would mainly be originated from nitrogen-containing compounds. However, so far there have been no analysis practices suitable for the confirmation of this observation.

In literature part of this study, general properties and possible origins of malodorous nitrogen compounds in papermaking were described. The compounds mainly include amines, aromatic heterocyclic nitrogen compounds and ammonia, and then possible sources are, in addition to the process itself, papermaking chemicals and machine microbiology. Different analysis methods for individual compounds were also reviewed.

In the experimental part of this study a proper analytical method based on the extraction stage with HS-SPME and separation and identification stage with GC-MS was developed for low-molecular-mass amines. The method was mainly tested with standard solutions and paper and board samples. In addition, some process chemicals were analysed. This simply and rapid method is of practical importance, since it does not require any expensive investments.

ACKNOWLEDGEMENTS

This Master's Thesis was carried out at Stora Enso Oyj Research Centre Imatra between September 16th and March 30th 2007.

I would like to express my gratitude for my instructors Ph.D Tiina Räsänen and Ph.D Jari Räsänen from Stora Enso for their support and guidance throughout this thesis. I would likewise like to thank Prof. Raimo Alén who acted as my supervisor on the behalf of the University of Jyväskylä.

The whole personnel at Research Centre were very helpful throughout this thesis and I would like to thank everybody. Especially I would like to thank Salla Surma-Aho and Hannu Timperi. Their both help with the GC device was priceless.

Finally, I would like to thank my family and friends for supporting and encouraging me throughout my studies.

Imatra, May 15th, 2007

Aino Karvinen

CONTENTS

LITERATURE REWIEW *1*

1 INTRODUCTION *1*

2 CLASSIFICATION OF ODOROUS NITROGEN COMPOUNDS *2*

2.1 Organic nitrogen compounds *3*

2.1.1 Amines *3*

2.1.2 Aromatic heterocyclic nitrogen compounds *4*

2.1.3 Amides *6*

2.1.4 Other compounds *7*

2.2 Inorganic nitrogen compounds *7*

2.2.1 Ammonia *7*

2.2.2 Other compounds *8*

3 SOURCE OF NITROGEN COMPOUNDS IN PAPERMAKING *8*

3.1 Process *9*

3.2 Nitrogen-containing chemicals *11*

3.2.1 Biocides *11*

3.2.2 Fluorescence whitening agents *13*

3.2.3 Dyes *14*

3.2.4 Organic pigments *15*

3.2.5 Cationic starch *16*

3.2.6 Coating binders *17*

3.2.7 Crosslinkers *17*

3.3 Microbiological activity *18*

4 COMMONLY USED ANALYTICAL METHODS FOR EVALUATING TASTE AND ODOUR 20

4.1 Sensory evaluation 20

4.1.1 Sensory panel 21

4.1.2 Testing samples 21

4.2 Analytical equipment 22

4.2.1 Gas chromatography 22

4.2.2 High-performance liquid chromatography 24

4.2.3 Mass spectrometry 26

4.2.4 Infrared spectrometry 27

4.3 Multivariate instruments 30

4.3.1 Electronic nose 30

4.3.2 Sniffing port 31

4.4 Extraction methods 31

4.4.1 Supercritical fluid extraction 31

4.4.2 Solid-phase extraction 33

4.4.3 Solid-phase micro extraction (SPME) 34

5 ANALYSIS OF MALODOROUS NITROGEN-CONTAINING COMPOUNDS 36

5.1 Extraction 37

5.2 Analysis of compounds 38

5.2.1 Amines 38

5.2.2 Aromatic heterocyclic nitrogen compounds 40

5.2.3 Ammonia 40

EXPERIMENTAL PART *41*

6 AIMS OF EXPERIMENTAL STUDY *41*

7 METHODS *42*

7.1 Preparation of standards and samples *42*

7.2 Extraction with SPME fibre *44*

7.3 Analytical methods *46*

7.3 Sensory testing *47*

8 RESULTS AND DISCUSSION *48*

8.1 Pre-testing of method *48*

8.2 Testing with standard solutions *50*

8.3 Dipping tests *52*

8.4 Real paper and board samples *54*

8.5 Process chemicals *58*

9 CONCLUSIONS *60*

REFERENCES *61*

APPENDICES *66*

ABBREVIATIONS

AZC	Ammonium zirconium carbonate
BAs	Biogenic amines
BCDMH	Bromo-chloro-5,5-dimethylhydantoine
BEHPA	Bis-2-ethylhexylphosphate
BSC	Benzenesulfonyl chloride
CAR	Carboxen
CC	Column concentration
CE	Capillary electrophoresis
CI	Chemical ionisation
CMC	Carboxymethyl cellulose
DAD	Diode array detector
DBNPA	2-dibromo-3-nitrile propionamide
DMA	Dimethylamine
DNFB	Dinitrofluorobenzene
DVB	Divinylbenzene
ECD	Electron capture detector
EI	Electron impact ionisation
FIA	Flow injection analysis
FID	Flame ionisation detector
FTIR	Fourier transform infrared spectrometer
FTIRD	Fourier transform infrared detector
FWAs	Fluorescence whitening agents
GC	Gas-liquid chromatography
GSC	Gas-solid chromatography
HPLC	High performance liquid chromatography
HS	Headspace sampling
IBCF	Isobutyl chloroformate
IR	Infrared
LC	Liquid chromatography
LLE	Liquid-liquid extracti

MA	Methylamine
MF	Methyl formaldehyde
MS	Mass spectrometry
MSD	Mass selective detector
NAC	N-acetyl-L-cysteine
NCD	Nitrogen chemiluminescence detector
NIT	Naphthylisothiocyanate
NPD	Nitrogen phosphorus detection
O	Olfactometry
OBA	Optical brightening agent
OPA	*o*-phtalic aldehyde
PARC	Pattern recognition
PDMS	Polydimethylsiloxane
PLOT	Porous-layer open tubular column
PTFE	Poly(tetrafluoroethylene)
PTV	Programmed temperature vaporising injections
PVAc	Polyvinyl acetate
PVOH	Polyvinyl alcohol
SCC	Soil column chromatography
SCOT	Support-coated open tubular column
SDE	Simultaneous distillation and extraction
SFC	Supercritical fluid extraction chromatography
SFE	Supercritical fluid extraction
SHA	Static headspace analysis
SIM	Selected ion monitoring
SPE	Solid-phase extraction
SPME	Solid-phase micro extraction
TBA	Tributylamine
TCMTB	2-(thiocyanomethylthio)-bentzothiazole
TLC	Thin layer chromatography
TMA	Trimethylamine
UF	Urea formaldehyde
UHP	Ultra high purity
UV	Ultraviolet

UV/Vis	Ultraviolet/Visible light
WCOT	Wall-coated open tubular column

LITERATURE REWIEW

1 INTRODUCTION

For many years paper and board have been used for primary and secondary food packaging /1/. European Community and the member states specify that food packaging should not adversely affect organoleptic properties of packaged foods. Paper and board materials are often coated with adhesives, varnishes and plastics because of their multilayer structure. All these substances can cause odours and taint to final products.

Generally food packaging has some specific requirements such as the protection of products from outside dirt, contamination, moisture, odour and mechanical damages /2/. With appropriate packaging, the preservation of food products can be improved and changes in product quality can be prevented. To improve safety of packaging, there is legislation concerning the limit values of some materials and additives used for the production of paper and board packaging. In addition, paper used for packaging must be microbiologically appropriate.

Human nose is very sensitive to malodorous nitrogen compounds because these compounds are indicators of deterioration. Often while testing sensory properties with the human panel, odour and flavour that refer to deteriorated product are found. Whit this respect, analytical instruments do not sensitively detect deviant situations. The aim of this study was to find out, based on the present literature, the suitable methods for analysing these kinds of compounds in the final products and the possible sources of these compounds.

2 CLASSIFICATION OF ODOROUS NITROGEN COMPOUNDS

Nitrogen occurs in nature mainly as dinitrogen, an inert diatomic gas /3/. Nitrogen is one of the most electronegative elements and it forms various compounds, most of which can be considered organic rather than inorganic. Nitrogen has four available orbital for bond formation and maximum of four bonds is possible, by coordination as in donor-acceptor complexes or in amine oxides, or by a loss of an electron as in ammonium ions.

In all nitrogen compounds where the atom forms two or three bonds remain respectively two pairs or one pair of nonbonding or lone-pair electrons /3/. The lone pairs are responsible of donor properties of nitrogen and affect on stereochemistry of compound. If nitrogen has three covalent bonds, molecule type is pyramidal except in special cases. This kind of molecules executes an inversion in which the nitrogen atom oscillates through the plane of the three groups attached like an umbrella. Nitrogen can form multiple bonds just as its neighbours in periodic table, carbon and oxygen, can. It can form triple bonds with nitrogen or with other elements that include carbon, sulphur and some transition metals. When nitrogen forms one double bond and one single bond, the grouping is nonlinear. Structure of compounds is planar in few cases when π bonding is involved. In condensed phases there might be slight deviations in nonplanarity.

The formation of tetrahedral bonds to nitrogen occurs principally in ammonium cations (R_4N^+), amine oxides ($R_3N^{+-}O$) and in Lewis acid-base adducts (e.g., $R_3N^{+-}BX_3$) /3/. In these amine oxides and adducts, the bond must have considerable polarity. Nitrogen has a little tendency for chaining, primarily because of the weakness of single nitrogen bond. Nitrogen participates into hydrogen-bond formation both as a proton donor and as a proton receptor.

Nitrogen chemistry has some very specific features /4/. Formal oxidation state of nitrogen can range from +5 to -3. Nitrogen behaves differently in acidic and basic conditions. It can be concluded that the relative stability of oxidation states of nitrogen is dependent on pH.

2.1 Organic nitrogen compounds

2.1.1 Amines

An amine is a compound that contains nitrogen atom that has from one to three alkyl or aryl groups attached /5/ For this reason, the formula of amine is RNH_2, R_2NH or R_3N. If a compound contains four groups attached to the nitrogen it is an amine salt or a quaternary ammonium salt. In plants and animals, amines are widely distributed, and many of them have physiological activity. Volatile amines have very distinctive and usually offensive odours, e.g., methylamine has ammonia like odour and trimethylamine smells like a dead saltwater salmon. Aryl amines have not as unpleasant odour as alkyl amines have.

Small volatile amines are used in the manufacturing of industrial chemicals such as rubbers, plastics and other polymers, dyestuffs and corrosion inhibitors /6/. In addition, amines are formed and emitted also during metabolism of microorganisms, plants and animals.

Biogenic amines (BAs) are organic bases with aliphatic (putrecine, cadavirine, spermine and spermidine), aromatic (tyramine and phenylethylamine) or heterocyclic (histamine and tryptamine) structures that are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines /5/. Chemical structures of these BAs are presented in Figure 1. BAs are indicators of the degree of freshness or spoilage of food. Many of them have powerful physiological effects and have an important biological activity. They have also toxicological effects but the exact toxicity threshold values for compounds are different, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each compound.

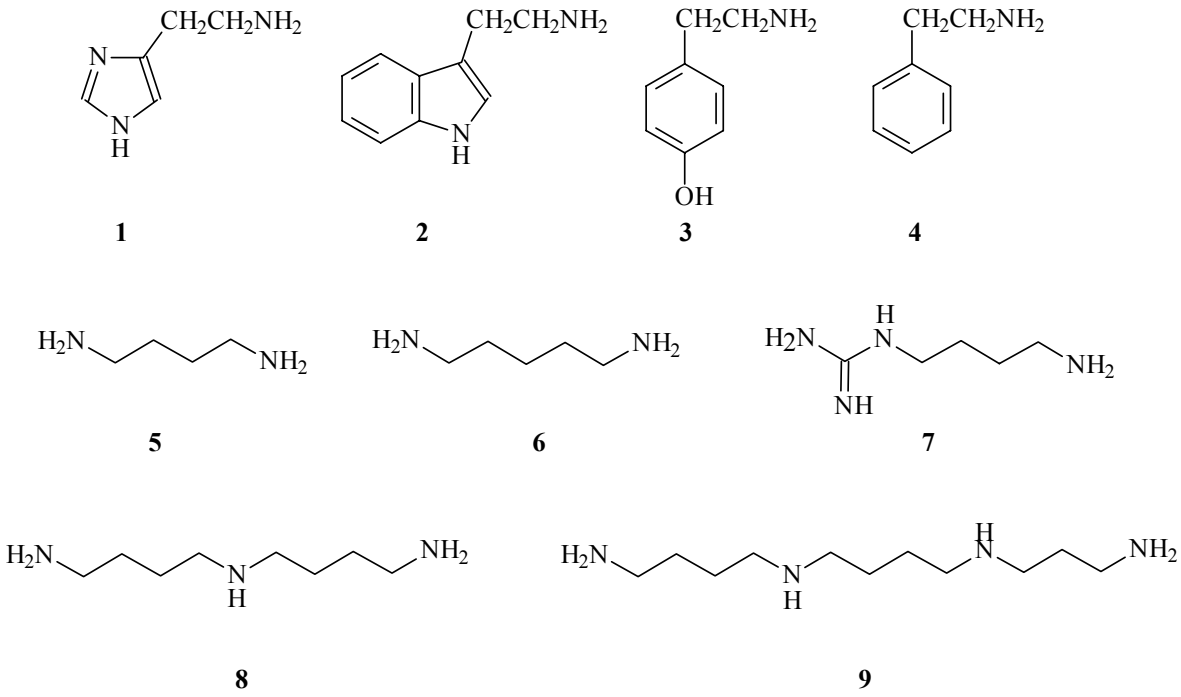


Figure 1. Some biogenic amines chemical structures /5/: (1) Histamine, (2) tryptamine, (3) tyramine, (4) 2-phenylethylamine, (5) putrecine, (6) cadaverine, (7) agmatine, (8) spermidine and (9) spermine.

2.1.2 Aromatic heterocyclic nitrogen compounds

A heterocyclic compound is a cyclic compound in which the ring atoms are of two different elements /7/. Heterocyclic compounds can be aromatic like a benzene ring. Of the common six-membered heterocycles, only the ones containing nitrogen are stable aromatic compounds. For a five-membered heterocycles to be aromatic, heteroatom must have two electrons to donate to the π cloud. Figure 2 presents composition of compounds discussed in this chapter. Heterocyclic compounds are important volatile components of many foods /8/. They are desirable flavouring ingredients because of their odour strength and complexity. Heterocyclic compounds are formed mainly through the thermal interactions of reducing sugars and amino acids.

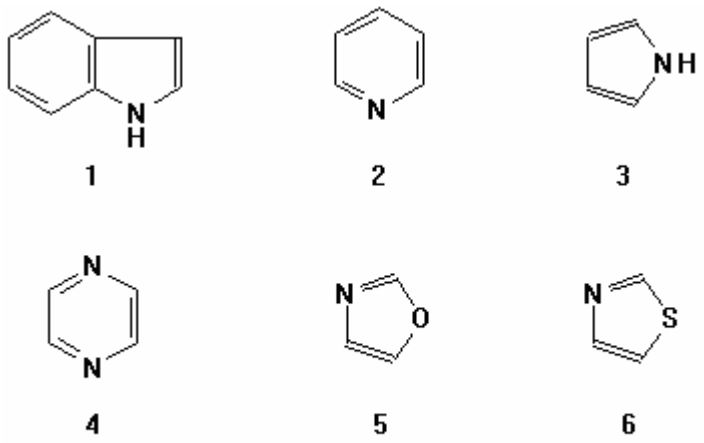


Figure 2. Heterocyclic nitrogen compounds: (1) Indole, (2) pyridine, (3) pyrrole, (4) pyrazine, (5) oxazole and (6) thiazole.

Typical alkaloid is a basic compound containing one or more heterocyclic nitrogen atoms /9/. Indole alkaloids are the largest group of alkaloids. Their structures are often very complex.

Pyridine is a six-membered aromatic heterocyclic compound containing one nitrogen atom /10/. Pyridine has a characteristic disagreeable nauseating odour. It is detectable at less than 1 ppm and its odour threshold value is 0.021 ppm. Compounds containing a pyridine ring play an important role in metabolism. Pyridine derivatives are important insecticides and herbicides because of their high bioactivity. They are also used as chemicals, solvents, catalysts and adhesives for textiles.

Pyrrole has a five-membered aromatic ring /7/. Pyrrole is not basic under the usual conditions, because nitrogen atom contributes two electrons to the aromatic π cloud. Thus, the pyrrole ring is partially negatively charged.

Pyrazine is a six-membered aromatic heterocyclic compound containing two nitrogen atoms /11/. Pyrazine and its derivatives, pyrazines, contribute significantly to the unique roasted flavour of many heated food products. Odour descriptions of pyrazines are usually nutty, roasted and earthy.

Oxazoles have a five-membered ring with oxygen and nitrogen in the 1 and 3 positions /8/. Oxazoles have a wide range of characteristic aromas. One oxazole has aged meat-like odour,

and other have flowery-like odour. Thiazoles comprise a sulphur-containing five-membered ring with sulphur and nitrogen in the 1 and 3 positions. Thiazoles can form through the degradation of cystine or cysteine, or by the interactions of sulphur-containing amino acids and carbonyl compounds.

2.1.3 Amides

Amides are compounds that have trivalent nitrogen bonded to a carbonyl group /7/. Besides hydrogen, alkyl substituents can also be attached to the nitrogen atom. Amides are synthesised from derivatives of carboxylic acids with ammonia or the appropriate amine (Figure 3).

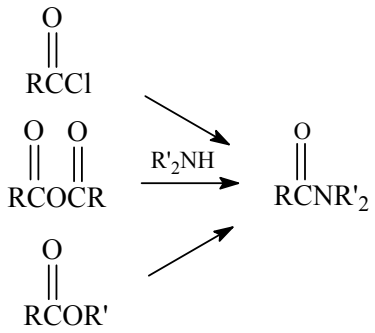


Figure 3. Syntesisation of amides /7/.

Proteins are the most important polyamides /7/. Plant and animal proteins are composed of twenty amino acids, which can be combined in a variety of ways.

2.1.4 Other compounds

In nitriles nitrogen is bonded to carbon with a triple bond /12/. Nitriles can form amines or ammonia by hydrolysation and reduce to amines with $LiAlH_4$. Amino acids act as building blocks of protein. Amino acids contain both a basic amino group and an acidic carboxyl group. In protein a large group of amino acids are linked together by forming amide bonds between the $–NH_2$ of one compound and the –COOH of another. Primary amines react with nitrous acid, HNO_3, to yield diazonium salts. Azo compounds are form when aryl diazonium salts undergo a coupling reaction with activated aromatic rings. Azo compounds are brightly coloured and they are used in dyes.

2.2 Inorganic nitrogen compounds

2.2.1 Ammonia

Ammonia, NH_3, is a colourless, poisonous gas with a very strong characteristic pungent smell /3,13/. Ammonia is produced in nature when any nitrogen containing organic material decomposes in the absence of air. Ammonia is a weak base. In its physical behaviour liquid ammonia resembles water. Both are highly associated because of the polarity of the molecules and strong hydrogen bonding. Liquid ammonia is a better solvent than water for organic compounds but poorer one towards ionic inorganic compounds. This is due to the fact that water has a higher dielectric constant. Hydrates are generated when ammonia is dissolved in water.

The most important use of ammonia is supply of vital agricultural nitrogen for crops /14/. In industry, ammonia is used in the manufacture of plastics and fibres like nylon, urethane and melamine. Other important use is the manufacture of chemicals, explosives and other nitrogen compounds serving as intermediates for dyes and pharmaceuticals.

2.2.2 Other compounds

In nitrogen oxides nitrogen has oxidation states from +1 to +5. Nitrogen oxides are gases, liquids and solids /13/. Nitrous oxide is a colourless gas possessing a mild, pleasing odour and a sweet taste. Nitrogen oxides exhibit extensive oxidation-reduction behaviour.
Nitrogen halides are usually very reactive /3/. They are used in explosives.

3 SOURCE OF NITROGEN COMPOUNDS IN PAPERMAKING

Identifying the source of odour in finished product can be difficult, because there are many possible aspects that can cause the odour /15/. However, complaints related to odour can be costly, so it is important to find the source. The origin of malodours in paper and board products can be seeding from factors like water system closure, the use of recycled fibre, neutral to alkaline pH papermaking conditions, and high landfill costs. Offensive odours are often caused by microbial spoilage, but still non-biological sources cannot be excluded. Also chemicals and contaminants can be source of off-odour and off-taste.

Production lines in process are linked to each other /16/. In Figure 4 a pulp and water flow system is described. In the process system there are numerous material flows, e.g., furnish components, water, fine and filler materials and dissolved and colloidal materials.

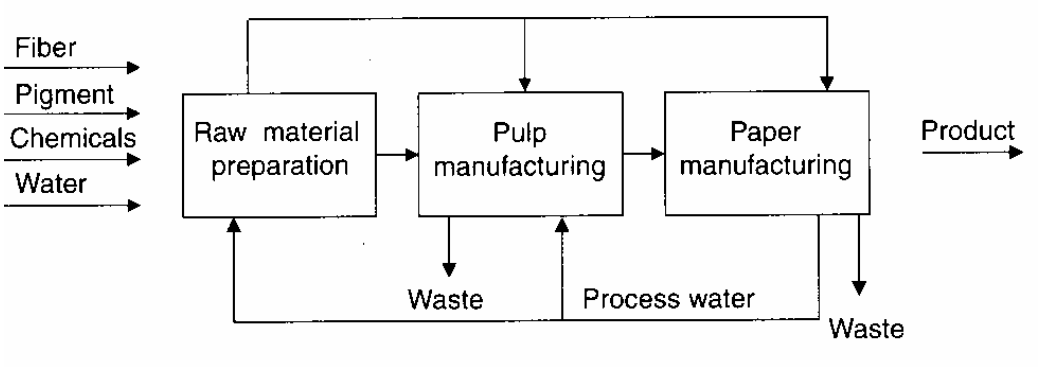


Figure 4. Pulp and water flow arrangement /16/.

3.1 Process

Water is one of the main raw materials used in papermaking process /17/. Water is used as an important transport medium, as a solvent for raw materials and chemicals and as for building hydrogen bonds between fibres. The white water (circulation water system of paper machine) contains several different substances with different characteristics and behaviour. It is hard to predict their influence and interactions in papermaking process.

Particles in the water phase and their origin are listed in Table 1 /17/. White water contains dissolved and dispersed polymers, surface-active agents, and other organic substances and inorganic salts.

Table 1. Particles occurring in the water system of a paper machine /17/

Type	Chemical composition	Origin
Fibres	Cellulose, hemicelluloses, lignin, extractives	Mechanical and chemical pulps
Fines	Cellulose, hemicelluloses, lignin, extractives	Mechanical and chemical pulps
Minerals	Metal silicates calcium carbonate Silicate bentonite particles	Fillers, coating pigments deinked pulp Retention aids
Surface active agents	Fatty acids and their soaps Resin acids and salts Non-ionic surface active agents Alkyl sulphates, sulphonates Cationic polymers Silicates	Mechanical and chemical pulps Deinked pulp Mechanical pulp Rosin sizes Dispertants, coated broke Coated broke Retention aids Bleaching chemicals
Disperced particles	Insoluble fatty and resin acids Styrene-butadiene, acrylate and PVAc latexes Emulgated oil	Extractives, sizes, deinked pulp Coated broke, deinked pulp Defoamers, extractives
Inorganics	Metal cations, various anions	Raw material, minerals Alum Pulps
Gas bubbles	Air Carbon dioxide	Ambient air Calcium carbonate

The paper machine always produces a certain amount of broke that consist of trimmings, clippings, bottoms of machine rolls and unusable product in connection with changing paper grades /18/. Broke is also produced if the paper machine does not run properly that cause problems with product quality. The broke is used again by adding it back to the process. Typically the amount of broke is 10 % to 30 % of substances in the headbox. Broke can be coated, uncoated, dried or undried. Coated broke may produce problems in wet end chemistry due to the coating colours and binder latexes.

Nitrogen compounds can also be from the wood itself /19/. Amino acids, which are free or linked as proteins are found from some hardwood species, e.g., oak, birch and eucalypts, from the xylem and other tissues, e.g., leaves and phloem. The proportion of proteins in wood is very low and the nitrogen content is lower than 0.1 %. The nitrogen content cannot be attributed merely to the proteins because particularly many tropical woods can also contain alkaloids.

3.2 Nitrogen-containing chemicals

In papermaking there are many possible nitrogen chemicals that may cause mal odour to the product. Fluorescence whitening agents, dyes, organic pigments, cationic starches, coating binders and crosslinkers belongs to this group.

Chemical additives may contain biocides and its by-products. These can cause off-odour and off-taste to the product /20/.

3.2.1 Biocides

Biocides, chemical antimicrobial agents, are used to prevent microorganisms' growth and numbers /21/. Microorganisms can develop resistance to industrial biocides if their use is constant and repeatable. Some industries may switch biocides so that organisms' resistance to a particular biocide does not emerge, and others simply increase the concentration of biocide they use. The mean of biocide increase is that microorganisms become overwhelmed and after that process can lower the dose to usual level. When biocide is changed, it is necessary to change it to one that has a different antimicrobial mechanism of action from the former one microorganism that became resistant. Biocides are used as antimicrobial agents and preservatives that may be used as components of paper and board in contact with aqueous and fatty food or dry food. Biocides are also used as antimicrobial agents and preservatives that may be used in defoaming agent in coating, slime-control and to control or suppress microbial

growth and activities in paper coating additives and non-fibrous materials. For the use of biocides there are regulations concerning, e.g., their maximum levels, possible applications and using conditions.

Nitrogen is present in most of the biocides /22/. The most common active nitrogen compounds in biocides are listed in Figure 5.

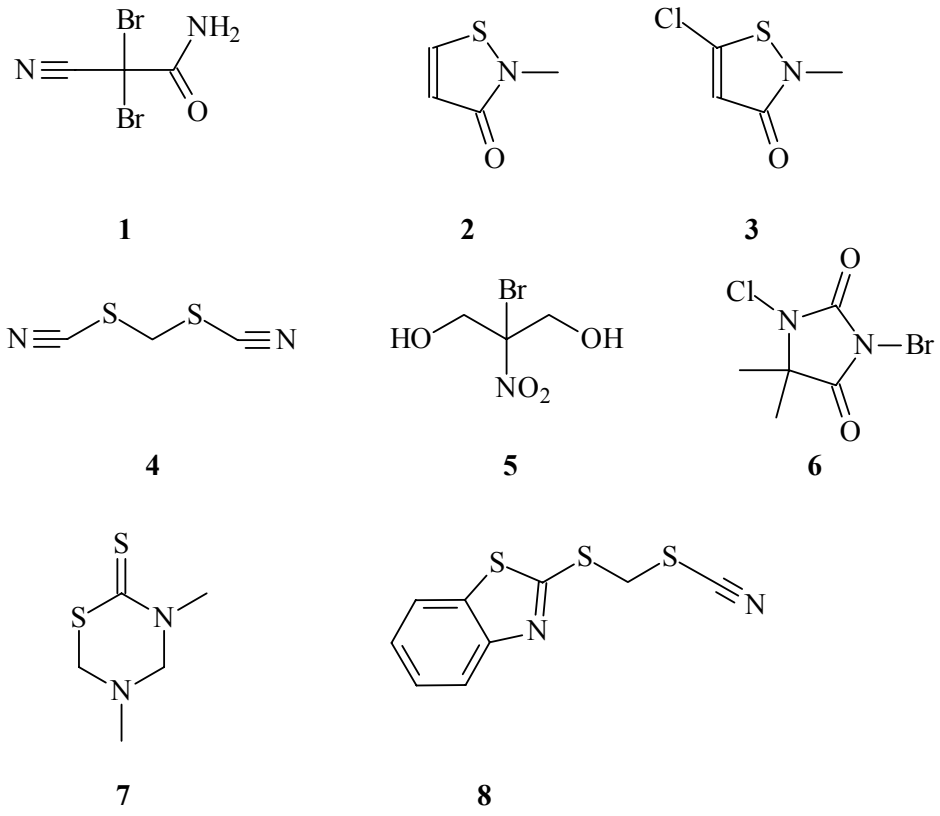
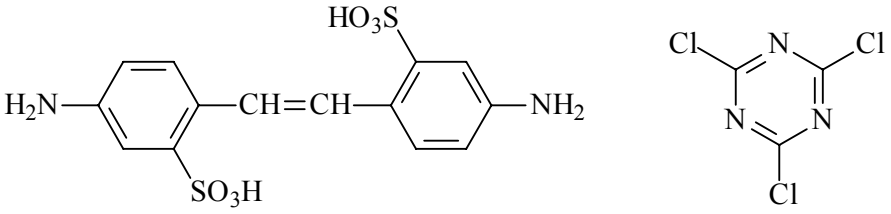


Figure 5. Nitrogen containing biocides: (1) 2-Dibromo-3-nitrile propionamide (DBNPA), (2) 2-methyl-4-isothiazolin-3-one, (3) chloro-2-methyl-4-isothiazolin-3-one, (4) methylene-bis(thiocyanate), (5) bromo-2-nitropropane, (6) bromo-chloro-5,5-dimethylhydantoine (BCDMH), (7) dimethyl-1,3,5-2*H*-tetrahydro-thiazine-2-thione and (8) 2-(thiocyanomethylthio)-bentzothiazole (TCMTB).

These compounds have their own action mechanisms /22/. Mechanism of 2-dibromo-3-nitrile propionamide (DPNBA) is prohibition of the nutrient supply, especially the supply of glucose. 2-Methyl-4-isothiazolin-3-one and chloro-2-methyl-4-isothiazolin-3-one acts probably as an analogue to thiamine (vitamin B1). For methylene-bis(thiocyanate), mechanism is binding of Fe^{3+} ions to complexes to prevent Fe supply. Bromo-2-nitropropane, also known as Bronopol, is an enzyme inhibitor and bromo-chloro-5,5-dimethylhydantoine (BCDMH) is a cell membrane protein oxidiser. The mechanism of dimethyl-1,3,5-2*H*-tetrahydro-thiazine-2-thione, also known as Datsomet, is to release formaldehyde and methyl carbamate while decomposing. After that formaldehyde reacts with amino groups of enzymes and proteins. 2-(Thiocyanomethylthio)-bentzothiazole (TCMTB) inactivates metal-enzyme complexes and reacts with some cell components.

3.2.2 Fluorescence whitening agents

Fluorescence whitening agents (FWAs) are also called as optical brighteners (OBAs) /23/. Mostly FWAs that are currently used in paper industry are derivatives of condensation product of diaminostilbene disulphonic acid with cyanuric chlorides in a 1:2 molar ratio. Structures of these starting compounds are presented in Figure 6. The triazine rings are further derivatised usually with aniline, *p*-sulphanilic acid or dimetanilic acid. The final position in triazine ring is usually derivatised with secondary alkylamines. Diethanolamine is the most common alkylamine used. FWAs are negatively charged when they are added at the wet end and they attach to the surface of the cellulose fibre. Attaching take place via ion pairing with metal ions present in the water.



1 **2**

Figure 6. Structures of diaminostilbene disulphonic acid (1) and cyanuric chloride (2).

FWAs are used in wet end as an additive of sizing agent and fillers /23/. FWAs can be added with surface addition via a size press. FWAs have the ability to absorb long-wave UV light in the range of 300-400 *n*m and then remit light at longer wavelengths in the range of 420-500 *n*m. The result of addition of FWAs into the product is that it enhance the blue portion of the reflectance spectrum of the paper leading to a whiter appearance.

3.2.3 Dyes

Dyes used in papermaking can be divided to acid, basic and direct dyes /24/. In Figure 7 are typical examples from these groups presented. Acid dyes are all water-soluble salts of coloured organic acids, which are dissociated in water. Most acid dyes are azo dyes. Basic dyes are the salts of colour bases and they are soluble in aqueous acids. They differ from other dyes, because they are soluble to methyl-, ethyl-, or isopropyl alcohols, as well as compounds that have similar solvent characteristics, among with oils and waxes. Direct dyes are sodium salts of dye acids. Chemically they resemble acid dyes, but they differ in their affinity for cellulose fibres. In contrast to direct dyes, acid dyes have no affinity towards cellulose. However, here is no clear boundary between acid and direct dyes. Acid dyes have usually more acid groups than direct dyes have.

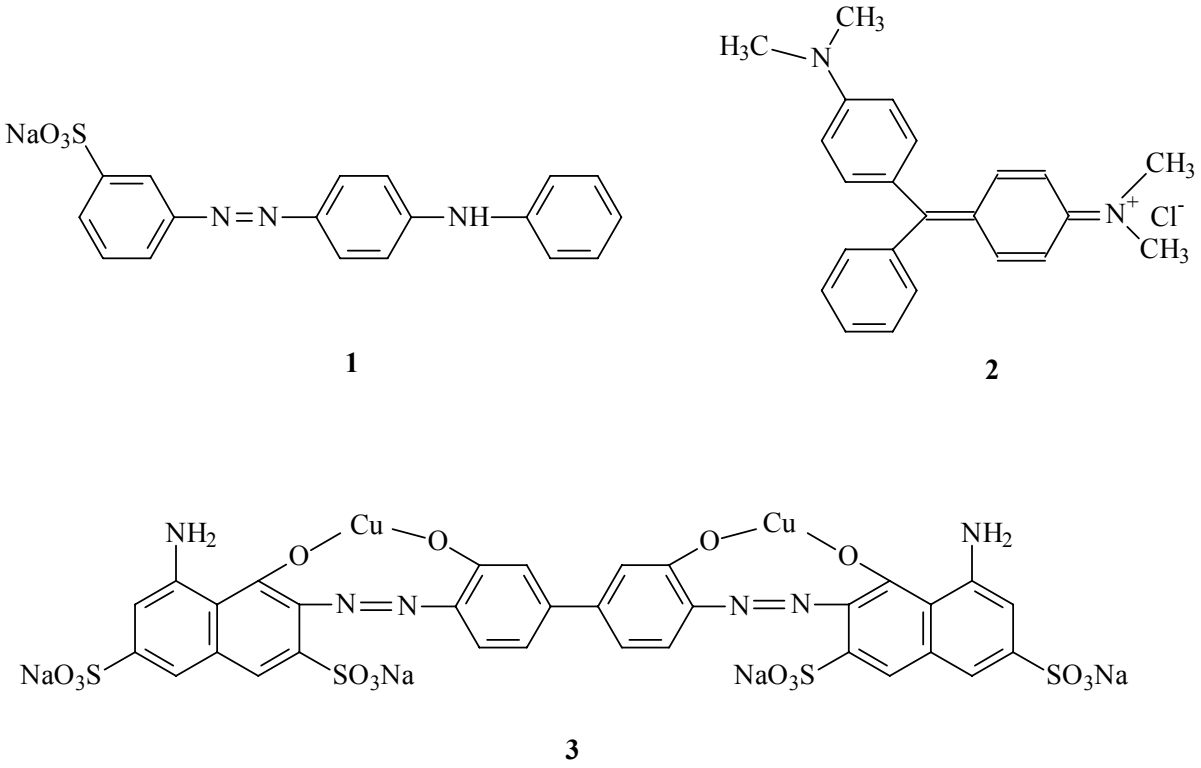


Figure 7. Typical dyes utilised for paper: Acid yellow 36 (acid dye) (1), basic green 4 (basic dye) (2) and direct blue 218 (direct dye) (3).

3.2.4 Organic pigments

In papermaking process, pigments are used in paper pulp coloration and in paper surface coatings /25/. These pigments are used as a colouring agent. In contrast to soluble dyes, pigments are practically insoluble during their application. Organic pigments can be divided into two groups: Azo pigments and non-azo pigments, also known as polycyclic pigments. Azo pigments have azo group (-N=N-) and they are divided further into monoazo and diazo pigments. Polycyclic pigments have a condensed aromatic or heterocyclic ring system dome of which contains nitrogen. As an example, in Figure 8 is presented the structure of pigment yellow 1.

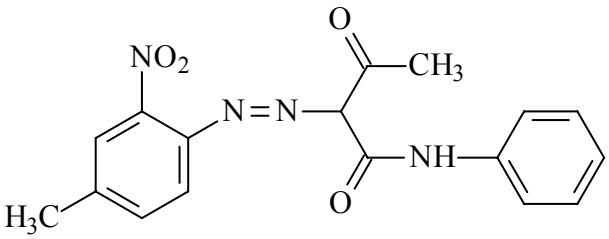


Figure 8. Pigment yellow 1 /25/.

Azo pigments are typically formed by diazotisation and coupling and it involves primary aromatic amine and a nucleophilic aromatic or aliphatic compound with active methyl group /25/. Diazonisation of an aromatic amine yields a compound that reacts with a coupling component. Pigments may contain traces of aromatic amines.

If pigment is used in paper filling it is the most abundant component of coating /26/. Pigment forms 80-90 % of the coating by weight. There is a synthetic urea-formaldehyde (UF) pigment developed some years ago for paper filling /27/.

3.2.5 Cationic starch

Starch is among the most used dry-strength additive in papermaking /28,29/. Starch is used in papermaking to improve paper strength. There are also many starch modifications. Modified starches can be divided into chemical and rheological modifications.

Cationic starches are starch ether products made by an etherification reaction at raised pH and temperature (Figure 9). Cationic starch is tested for the use as filler pigments /29/.

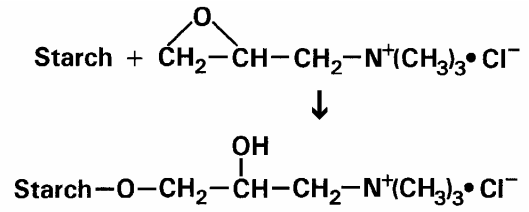


Figure 9. Reaction between starch and a cationisation agent /28/.

3.2.6 Coating binders

Binders are component of the coating colour /30/. Binders are added to the coating colours to bind pigment particles to base paper and to each others, for partly filling of voids between pigment particles to get porous coating system and for affecting viscosity and water retention of coating colour. Binders are either derivatives of natural polymers or fully synthetic. Latexes are a big group of fully synthetic binders. Derivatives of natural polymers are starches, proteins, cellulose derivatives and carboxymethylcellulose (CMC).

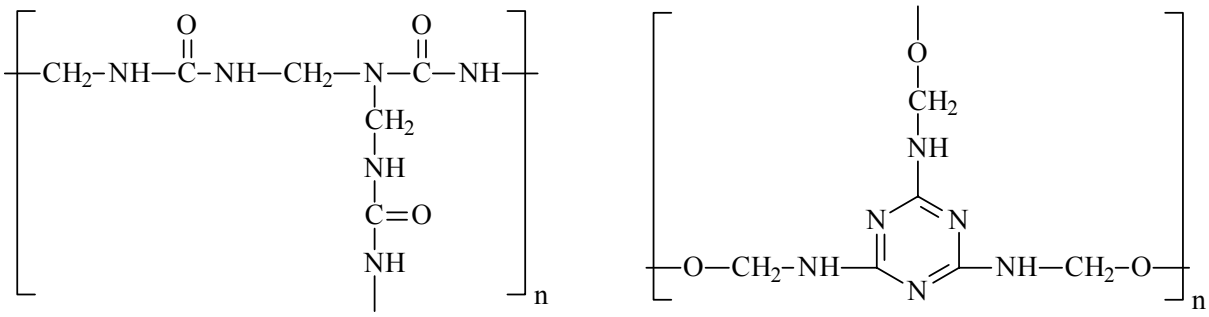
Soy protein has been used as a co-binder for several decades in paper and board industry /31/. Soy protein is obtained from soybeans. Soy protein is composed of 20 well known amino acids, it has many functional groups such as amino, carboxyl, hydroxyl, phenyl and sulfhydryl groups, in which amino is the most abundant. Other protein used in binders is casein /32/. It is derived from milk, and it was widely used before 1950s.

3.2.7 Crosslinkers

Crosslinkers are also referred to insolubilisers /32/. Their portion of coating is small, but they have a big effect on the final performance of the coated paper sheet. Traditionally, crosslinking agents have been used to render the insolubility of coating or surface treatment applied to paper. Coating is required to be more resistant to moisture when water content

increases in inks and fountain solutions. Crosslinkers have also a role in improving other properties of coating in wet and dry conditions. A good crosslinking helps the coating to keep the runnability requirements of the machine while meeting the needs of the end application. Crosslinkers react with coating binders and other relevant elements.

Nitrogen-containing crosslinkers are amino resins and ammonium zirconium carbonate (AZC) /32/. Amino resins include melamine-formaldehyde (MF) and UF crosslinkers (Figure 10). AZC is the most commonly used metallic ion in crosslinking paper coatings.



1 **2**

Figure 10. Amino resins: (1) UF and (2) MF.

3.3 Microbiological activity

Pulping process and papermaking provides a favourable environment for the growth of microorganisms /21/. There are many reasons why problems due to microorganisms in modern papermaking have been increased severity and frequency. Paper machines have become larger, faster and more complex, white water reuse and increasing use of chemical additives, etc.

Microbiological problems can be classified in paper and board industry into those that disrupt the process, those that threats production safety and those that have an adverse effect on the quality of the end product /22/. Microorganisms can cause odour or taste defects by producing compounds during microbial metabolism. Microorganisms in paper mill systems mainly include aerobic and anaerobic bacteria, fungi and slime. Algae, nematodes and protozoa can

also cause microbiological problems. Factors that are relevant in microorganisms growth are listed in Table 2.

Table 2. Factors relevant for the growth of microorganisms /22/

Micro-organisms	Optimal temperature °C	Light	Oxygen	Optimal pH	Water
Bacteria (0.5–1 μm)	Psycrophiles 15 Mesophiles 35 Thermophiles 55	No effect	Aerobic > 4 ppm Anaerobic < 4 ppm Facultative: no effect	6.5–7.5	Essential
Fungi (10 μm)	22–30 Moulds T_{max} = 65 Yeast T_{max} = 45	Could affect spore germination	Moulds > 4 ppm Yeast: no effect	5.0–6.0 4.0–5.0	Nonessential
Algae (> 100 μm)	< 35 for green algae	Essential	O_2 needed to start photosynthesis	4.5–9.0	Essential
Protozoa (10–1000 μm)	20	No effect	Aerobic > 2 ppm Anaerobic < 2 ppm	6.5–7.5	Nonessential

The main sources of microbial contamination are water, cellulosic raw material, broke and papermaking chemicals /22/. Water has proved to be the main source of algae and filamentous bacteria entering the system. When water system becomes more closed, the microflora associated with the wet end become more complex. Cellulosic raw material usually contains plenty of microorganisms, especially, in case where recycled fibre is used. The broke can be microbiologically rich because it has many different types of organisms flourishing in it. The total microbial numbers can be quit high depending on the contamination of dilution waters, original contamination and especially storage time. Especially coated broke is critical, because coating is a good nutrient for microorganisms.

Papermaking chemicals are mostly biodegradable, so microbial contamination may affect their technical performance /22/. Modified starch is used in surface sizing, as a wet-end additive and in coating. Wet-end and size-press starches represent ideal nutrition for bacterial growth. Moulds and aerobic endospore-forming bacterias produce and excrete exocellular starch-depolymerising enzymes. Microorganisms may contaminate end product either during the manufacturing process when microorganisms are within the product or during recolonisation, when microorganisms are found on the surface of product. In the drying

section heat and steam usually kill bacterial vegetative cells. But the surviving free spores can remain dormant until they reach external conditions that are favourable for germination and development to vegetative cells (microbiological growth). The growth of fungi on paper and paperboard may be common and it can produce odorous metabolites.

Because of the paper mill complex ecosystem, its mixed microbial flora can metabolise various products that can be further metabolised by other microorganisms /22/. Hence the source of the off-flavour is difficult to trace. In most cases, off-odour is caused by actions of specific bacteria in suitable organic substrata. Compounds that cause malodour belong mainly to the categories of sulphurated and nitrate substances.

When protein is spoiled, it produces a foul, nauseating odour /15/. Putrescine is formed when amino acid arginine is breaking. The proteins of paper chemicals and additives may taint e.g, protein is a component of certain coatings and also certain starches may have high protein content. For starch made of potato the protein content is 1-4 %, corn 7-12 %, barley 7-10 %, wheat 9-15 % and tapioca 1 % /28/.

Biogenic amines are generated by amino acid enzymatic decarboxylation /33/. Microorganisms usually use this pathway of amino acid metabolisation.

4 COMMONLY USED ANALYTICAL METHODS FOR EVALUATING TASTE AND ODOUR

4.1 Sensory evaluation

Different people have different perception to taste and odour, e.g., a preference of person is a highly individual characteristic /34/. Thus, to get reliable results of sensory properties one has

to use sensory tests with trained assessors called panellists. When analysing affective assessments, a sensory panel must consist of a large number of persons, typically between hundred and a thousand people. In this case, panellist should not receive any training for reliable results. The odour of material does not necessarily correlate with the off-flavour that might transfer from the material to the food.

4.1.1 Sensory panel

Sensory analysis uses human senses to evaluate off-odour and off-flavour /34/. Sensory tests can be performed in several ways, depending on what kind of information is to be looked for. Panel size, panel composition, analytical procedure and statistical analysis are selected according the purpose of the analysis. Sensory panel is used instead of instrumental analysis in situations when sensory properties are of interest. Sensory panel assessors must be chosen, trained and tested before analyses. Humans have different kind of ability to taste and smell, and 1/3 of the persons will typically be rejected because their fail the taste tests. Humans have four taste receptors, which detect sweet, salt, sour and bitter, respectively. When testing appropriate sensory panel candidates these four flavours are given to detect. A person suitable for odour panel is dependent on person's detection thresholds and the ability to remember odours and articulate the odour sensations. For testing these abilities can also familiar samples from environment been given to smelling test. Panellist must be trained before to gain reliable results. In sensory analysis, the assessors of panel typically evaluate one or several samples. Human senses can quickly provide detailed information about the samples.

4.1.2 Testing samples

The difference test is a procedure that determines whether there is any difference between two samples /34/. A typical test is a triangle test having two equal samples and one odd sample. Difference tests are so called forced choice tests which panellists must choose one to be the odd sample. This is shown to increase the accuracy of the difference tests. Ranking tests are

also difference tests where panellists are asked to rank the samples, for example,according to their odour intensity.

Scoring tests tell us how big the difference between samples by numbers is /34/. The scale could be continuous or discrete. When the scale is used, two or more points must to be defined as reference samples. No odour is often used as one point.

The odour profiling is a method where the sensory panel describes the odour by using a set of attributors or descriptors /34. Panellists score sample odour. One method is to develop descriptors during the panel training, so that all panellists use the same words describing odours. Another method is that panellists use the words they think are appropriate.

4.2 Analytical equipment

4.2.1 Gas chromatography

In gas chromatography the gaseous mobile phase is gas, called a carrier gas, and the stationary phase is either liquid, called gas-liquid chromatography (GC), or solid, called gas-solid chromatography (GSC) /35/. Currently GC is more used than GCS. GC is suitable for separation of volatile and thermally stabile compounds. Separation process can be divided into three stages. First, compounds move in a gas phase, then they diffuse in liquid phase and third, compounds detach from the surface of stationary phase. The separation of compounds is dependent on quantity and frequency of contacts between the analyte and the stationary phase. Diffusion rate of the analyte is dependent on the gas quality, temperature and molar mass of the analyte. The most determine factor for separation is how components evaporate from the stationary phase. This is dependent on the interactions between analyte and stationary phase.

Operation principle is that carrier gas is directed to the injector with a proper pressure /35/. Sample is injected either to the injector or directly to the column and it is vaporised if necessary. In column analyte components are dispersed between the mobile phase and the

stationary phase. Column is typically in the convection oven, which temperature is easy to change and that can be programmed. Compounds disperse between phases, and they separate while moving with different rates through the column to the detector. This is caused by different chemical and physical properties of analyte components. At the detector, components cause electric signal, which is filed. Carrier gas is usually hydrogen, helium or nitrogen and its purity should be at least 99.995 %. Carrier gas affects significantly to the columns efficiency, resolution, analyse time and response.

Injection to the GC is typically made with a syringe /35/. There are several sample injection methods. Split injection, splitless injection and different programmed temperature vaporising injections (PTV) are vaporising injection methods. In these kinds of injections sample is vaporised at the injector and is then conducted to the column. On-column injection is an injection method where the sample is injected directly to the column with a syringe. Sample is vaporised with the carrier gas flow. Analytes can also be determined with headspace sampling (HS) from the liquids or solids. Sample is usually first collected in the sample loop and is then conducted to the GC. Headspace can be either static or dynamic. Static headspace is good for determine the main components. It is especially a good choice for qualitative screening for its rapidity. Dynamic headspace is a proper method for determine content of small components.

Generally used columns are long, narrow open tubular columns made of fused silica (SiO_2) and coated with polyimide /36/. Typical column lengths are from 15 m to 100 m, and inner diameters are between 1.10 mm and 0.53 mm. The wall-coated open tubular column (WCOT) features a 0.1-5 μm thick film of stationary liquid phase on the inner wall on the column. The support-coated open tubular column (SCOT) has solid particles coated with stationary liquid phase attached to the inner wall of the column, and in the porous-layer open tubular column (PLOT) the inner particles are the active stationary phase. Packed columns contain a fine solid support coated with a non-volatile liquid stationary phase. Solid layer itself can also be the stationary phase. Packed columns are usually made of stainless steel, nickel, or glass and their typical measures are 1-5 m in length and 3-6 mm in diameter.

For quantitative analyses most used detectors in gas chromatography are a flame ionization detector (FID) and an electron capture detector (ECD), for qualitative analysis detectors are a mass spectrometer detector (MSD) and a Fourier transform infrared detector (FTIRD) /35,36/. Principles of MSD and FTIRD are discussed later in this chapter. In qualitative analysis

comparing with a computer its spectrum to library data determined earlier identifies a chromatographic peak. Quantitative analysis is based on the area of chromatographic peak, so that the area of the peak is proportional to the quantity of the compound. In FID the elute is burned in a mixture of H_2 and air. When organic compounds are burned in an oxidising flame, CH radicals are formed and they are thought to produce CHO^+ ions in a flame. Flames electrical conductivity is linearly dependent on the molarity of organic compounds. The FID is insensitive to non-hydrocarbons.

In ECD, when gas enters the detector, it is ionised by high energy electrons emitted from a foil containing a radioactive radioisotope /35/. Formed electrons are attracted to the anode and they produce a small steady current. When analytes enter the detector it responses by varying the frequency of voltage pulses between the anode and the cathode to maintain a constant current. The electron capture detector is particularly sensitive to halogen-containing molecules, conjugated carbonyls, nitriles, nitro compounds and organometallic compounds. It is relative insensitive to hydrocarbons, alcohols and ketones.

4.2.2 High-performance liquid chromatography

In liquid chromatography (LC) the mobile phase is liquid and the stationary phase is either solid or liquid /35/. Sample components have an active interaction with both mobile and stationary phases. Equipment for high-performance liquid chromatography (HPLC) contains eluant containers, pumps system, injector, column and detector. The eluant is pumped through the column with a known flow rate. During injection the sample is transported with the eluant to the column. Sample particles interact with the eluant and stationary phase, which causes separation and it is detected at the detector. There are several different techniques to use in LC. In the normal phase technique, the stationary phase is more polar than the mobile phase, and in the reverse phase technique eluant polarity is much bigger than stationary phase polarity. Reverse phase chromatography is the most used technique in LC. Other often-used techniques are ion exchange, ion pair, ion, size exclusion and affinity chromatography.

The pump system must be able to pump with different flow rates and pressures /35/. The flow must be steady and reproducible, because pulses may disturb the function of the detector. A typical flow rate in analysis is 0.05-5 mL/min. When capillary columns are used the flow rate may go under 0.1 μL/min. Sample injection is made with an injection valve. The injection valve has usually interchangeable sample loops with well-defined volumes. The sample is extracted with an eluant, if possible, and then injected with a syringe to the sample loop. When the vent is shifted, the flow direction change, and the sample is pumped to the column with the eluant.

HPLC columns are usually made of steel or plastic, and their lengths are 5-30 cm /35,36/. Columns inner diameters are 1-5 mm and they are packed with the stationary phase having a particle size of 3-10 μm. In preparative chromatography columns with a larger inner length and a particle size of 10-40 μm are used. A short guard column containing the same stationary phase as the main column to prevent degradation of dust or small particles and sample impurities protects entrance to the main column. The stationary phase material can be non-porous, pellicular, porous or perfusion. The shape of particles can be spherical, lump or block. The most common stationary phase contains highly pure, spherical, microporous particles of silica that are permeable to solvent and have a surface area of several hundred square meters per gram. Bonded stationary phases can be covalently attached to the silica particles surface by chemical reactions.

HPLC detection is based on compounds chemical and physical properties /35/. HPLC detectors can be divided into the categories: spectrophotometric, electrochemical and other detectors. Spectrophotometric detectors are a UV/Vis detector that gives response to compounds, or derivatives that can absorb ultraviolet or visible light, and a fluorescence detector that gives response to compounds, or derivatives that absorb fluorescence light. The UV/Vis detector is one of the most used detectors, because it is relatively sensitive, its linear response area is large and changes of samples temperature and composition of eluants does not much interferes detection. Electrochemical detectors give response to compounds that oxidise or reduce easily. Function of these detectors is based on conductance, conductivity, potential or carrying current. The most used electrochemical detector is an amperometric detector. A conductivity detector is used in ion chromatography. Other detectors are refractive index detectors, MS detector and evaporative light-scattering detector. Mass spectrometry is discussed in more details in the next chapter. The refractive index detector is thought to be a

general detector of HPLC because it gives response to all compounds. This response is based on changes in eluant reflection index caused by sample molecules. Light-scattering detectors are based on interactions between light and molecules. When light hits molecules, it induces momentary dipole to the molecules and makes them oscillate at the frequency of original light.

4.2.3 Mass spectrometry

In mass spectrometry (MS), gaseous molecules are ionised, accelerated by electronic field, and then separated according to their mass /35,36/. Analysing can be proceeded either directly with MS or it can be connected to some analytic chromatographic or electrophoresis instrument that acts as a sampling system. MS is used as a detector of both quantitative and qualitative analysis in GC and LC. In the ionisation chamber compounds are ionised. Electron impact ionisation (EI) and chemical ionisation (CI) are the most used methods for volatile compounds. In EI electrons bombard gaseous molecules entering the ionisation source. This ionises molecules ($M + e^- \rightarrow M^+ + 2e^-$) and breaks them into smaller fragments. CI is a gentler technique that yields less fragmentation. The ionisation source is filled with methane. Energetic electrons convert methane to variety of reactive products.

Separation of ions can be made according to energy of ions, momentum or velocity /35,36/. For the determination of mass/charges (m/z) ratio, using two of these separation methods is enough. The most used analysator is a quadrupole mass spectrometer, which composition is presented in Figure 11. In a quadrupole mass spectrometer, eluate passes through a heated connector into the electron impact ionisation chamber, which is pumped rapidly to maintain a good vacuum. Ions are accelerated through a potential of 5-15 V before they enters the quadrupole mass separator. The separator consists of four parallel metal rods, which are applied with a constant voltage and a radio-frequency oscillating voltage. Electronic field deflects ions and allows only an ion with one particular m/z-ratio to enter the detector. By varying rapidly the applied voltages, the ions of different masses reach the detector. Quadrupole mass spectrometer is best for the quantification of analytes. Other much used ion separation techniques include a time-of-flight MS and an ion trap MS. The time-of-flight MS

is based on ions velocities caused by their same kinetic energies and different m/z values. The ion-trap MS has a cavity that is controlled by gate electrode that take care of that ions are entering to the detector one by one.

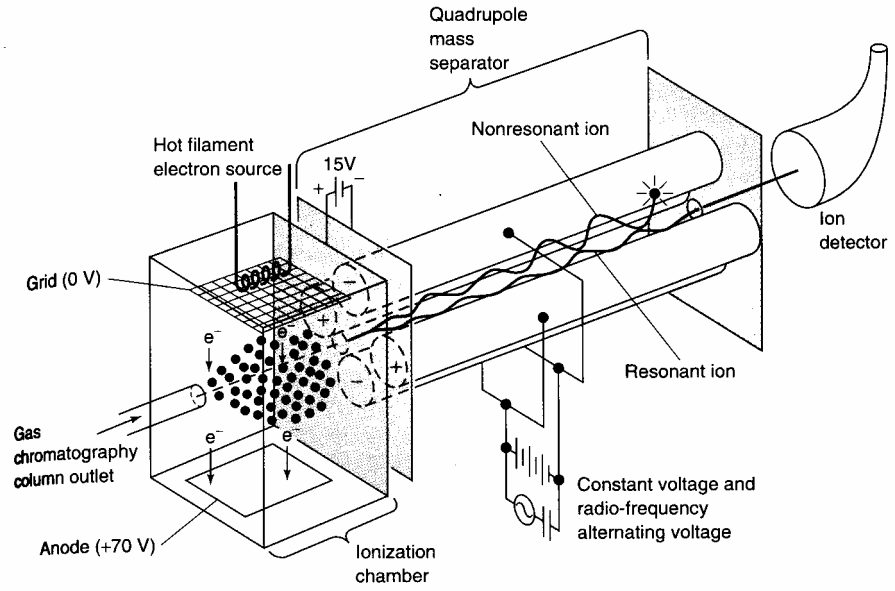


Figure 11. Quadrupole mass spectrometer /36/.

Mass spectrometer records the total current from all ions of all masses over a wide, selected range /36/. Another, selected ion monitoring (SIM) can also be used. SIM is used to look for one particular compound or class of compounds. A mass spectrum is a graph of peak from the chromatogram that shows the relative abundance of each fragment that strikes the detector of MS. Analytes are then identified from spectrum by comparison with spectral library of known compounds.

4.2.4 Infrared spectrometry

Infrared (IR) spectrometry is based on molecules vibration state transitions that cause energy absorption in the infrared area /35/. These absorption frequencies correlate with compounds functional groups and molecules composition. When IR radiation hits molecule, it absorbs radiation in wavelengths that causes change the dipolemoments of molecules. The

wavelengths are characteristics for every functional group. The bigger change vibration produce, the stronger is the absorption. The infrared area of an electric-magnetic spectrum extends from the visible light red spectrum area to the micro wavelength area (14 000-10 cm^{-1}). Traditional IR devices are too slow to use as a detector. Fourier transform infrared (FTIR) spectrometer is the method that rapidly shows the whole spectrum.

In Fourier analysis a curve is decomposed into a sum of sine and cosine terms /36/. Fourier series:

$$y = \sum_{n=0}^{\infty} \left[a_n \sin(n\omega x) + b_n \cos(n\omega x) \right], \quad (1)$$

where, $\omega = \frac{2\pi}{x_2 - x_1}$, a and b are the Fourier coefficients and x_1-x_2 is the interval.

The more sine and cosine terms are taken to the procedure; the more accurate approximation it gives to the curve.

The most important part of the FTIR device is the interferometer /36/. The sample is usually placed between the output of the interferometer and detector (Figure 12).

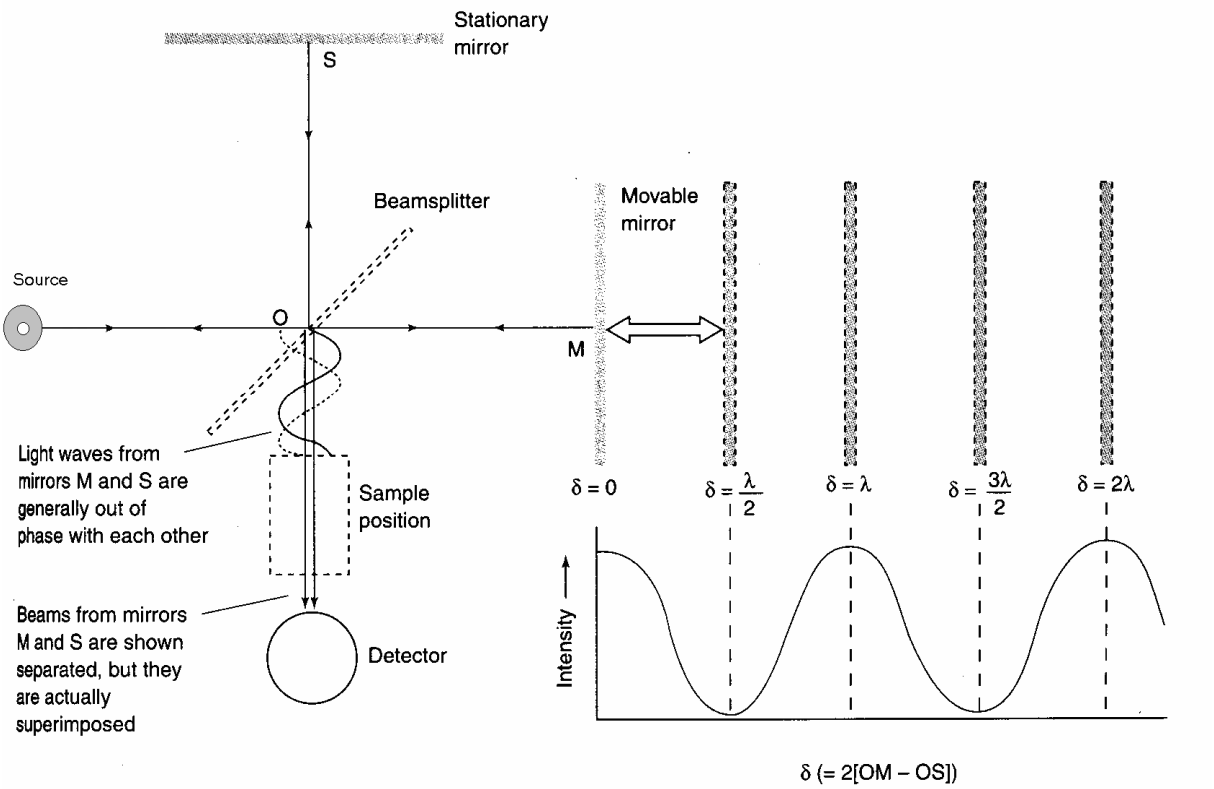


Figure 12. Diagram of an interferometer /36/.

Radiation from the source strikes a beamsplitter that transmits and reflects some light to the mirrors /36/. Beamslitter is typically a layer of germanium evaporated on to KBr plate when the spectrum region is (4 000-400 cm^{-1}). For longer wavelengths (< 400 cm^{-1}), a film of the organic polymer Mylar is used. The interferometer has a stationary mirror in a constant distance and a movable mirror. From the mirrors the reflected rays travel back to the beamsplitter where half of each ray is transmitted and other half is reflected. One recombined ray goes towards the detector and other travels back to the source. Two waves from the mirrors arrive to the detector usually in a different phase because the paths from the mirrors are different. The difference in the pathways followed by the by the two waves is called the retardation, δ. Constructive interference occurs whenever the retardation is an integral multiple of the wavelength. A graph of output light intensity versus retardation is called an interferogram. It is the sum of contributors from all source of light.

In FTIR the entire interferogram is recorded in a few seconds and stored in a computer /35/. Comparing it to the spectrum library then identifies the spectrum. Collecting interferograms and averaging them improve the signal-to-noise ratio.

4.3 Multivariate instruments

Multivariate instruments relay between analytical equipment and sensory evaluations. Electronic nose and sniffing port are generally used for analysing sensory properties.

4.3.1 Electronic nose

Artificial olfaction research in late 1980s and early 1990s led to the launch of commercial instruments called electronic noses for needs of variety industries /37,38/. The research was inspired by the mechanisms involved in human olfaction. Mechanism of electronic nose tries to simulate human sensory system. Electronic noses have many potential applications such as checking the raw materials quality, off-flavours and taints, process monitoring for odour quality during processing and product quality of foods, drinks, cosmetics, chemicals and pharmaceuticals. An electronic nose is an instrument, which consist an array of chemical sensors, data pre-processor and pattern recognition (PARC) engine. Each sensor has only partial specificity to a wide range of odorant molecules. Electronic nose have two main odour sampling methods: static headspace analysis (SHA) and flow injection analysis (FIA). In SHA the sample is placed in the container, which is placed to the sensor array chamber, where sensor array is saturated with the sample vapour. In FIA background gas in constantly pumped to the sensor array chamber and gas containing the odour is injected into the background gas before it reaches the sensors. It is usually computer automated.

Electronic nose is like a gas chromatography without a column, with an injector connected straight to the detector /38/. Operation with an electronic nose is based on adsorption of volatile compounds to the surface of gas-selective sensors that can be made of various materials. Sensors made of semiconducting metal oxides are sensitive to combustible gases and are operated at high temperatures (100-600 ºC). Compounds induce change in metal oxides electric conductivity, which depends on compounds properties. The ones made of electric conducting polymers are also based on changes in electric conductivity. They respond to polar compounds and can be operated at near room temperatures. Piezo acustic and quartz crystal sensors responds are based on changes in sensors oscillating frequency. Sensory

groups responds forms specific fingerprint profile to the volatile compound or group of compounds. Electronic nose can be taught by saving fingerprints in the odour-library and unknown odours can then be identified with it.

4.3.2 Sniffing port

Analytical system can be equipped with an olfactometry (O) which is called the sniffing port /1/. Sniffing port enables odour detection to be monitored by human nose.

O is attached to GC, and it provides a sensory profile of the odour active components present in aroma extract by sniffing /39/. GC-O is used for determine key odorants from food products. This is made to understand food aroma composition or off-flavour identification. GC-O methods differ from one another in the technique used. Techniques can be based on dilute extraction, time-intensity or the frequency of odour detection determined by an untrained panellist.

4.4 Extraction methods

Instrumental analysing usually requires that compounds have to be extracted from the sample matrices. Usually odorous compounds are volatile and for those, most used methods are presented in this chapter.

4.4.1 Supercritical fluid extraction

In supercritical fluid extraction (SFE) a pressurised solvent, a supercritical fluid, is pumped through a heated extraction vessel /36/. Sample is in the vessel and fluid can be left in there

for a moment, or it can be pumped through it constantly. After the vessel, the fluid flows through the capillary tube to release pressure. Extracted analytes goes through the capillary tube to the collection vessel, where the fluid is evaporated, or analytes can be dissolved from the collection vessel to get solution of analytes. SFE system is presented in Figure 13. Supercritical fluid appears after the critical point of the temperature and the pressure is reached, and it has a density and a viscosity between those of the gas and liquid, same as its ability to act as a solvent. Carbon dioxide, CO_2, is the most commonly used as a supercritical fluid because it is relatively inexpensive and because by using it, there is no need for costly disposal of organic solvents. CO_2 has a low critical temperature (31.3 ºC) and it is a non-toxic, but it is not a good solvent for highly polar or high-molecular-mass solutes. If a second solvent such as methanol, CH_3OH, is added, the solubility of polar analytes increases. SFE can dissolve also non-volatile compounds.

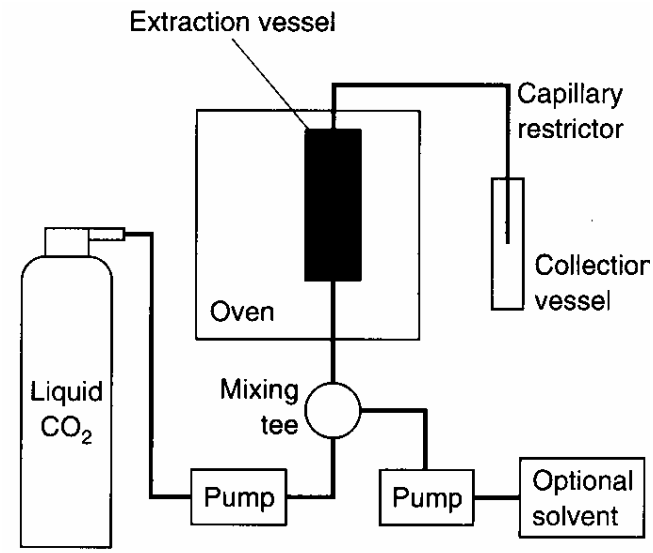


Figure 13. Apparatus for SFE /36/.

SFE can also be contacted to the chromatographic instrument /35,36/. This technique is between GC and HPLC, because of the solvent properties that lye between those of gas and liquid. SFE chromatography (SFC) allows analysis for a wide scale of compounds, because also relatively polar compounds can be separated contrary to GC. SFC can be contacted to a GC, HPLC or another SFC device.

4.4.2 Solid-phase extraction

Solid-phase extraction (SPE) is a technique that was developed as a complement or as a replacement for liquid-liquid extraction (LLE) /40/. SPE has three principle goals: Sample concentration, sample clean up and to transfer analytes from the sample matrix to a different solvent or to the gas phase. SPE is widely used for the isolation of analytes from a mobile phase such as gas, fluid or liquid. During the sampling process analytes are transferred to the solid phase that is then isolated from the sample, and the analytes are eluted from it using liquid or fluid or by thermal desorption into the gas phase. The sampling process is presented in Figure 14.

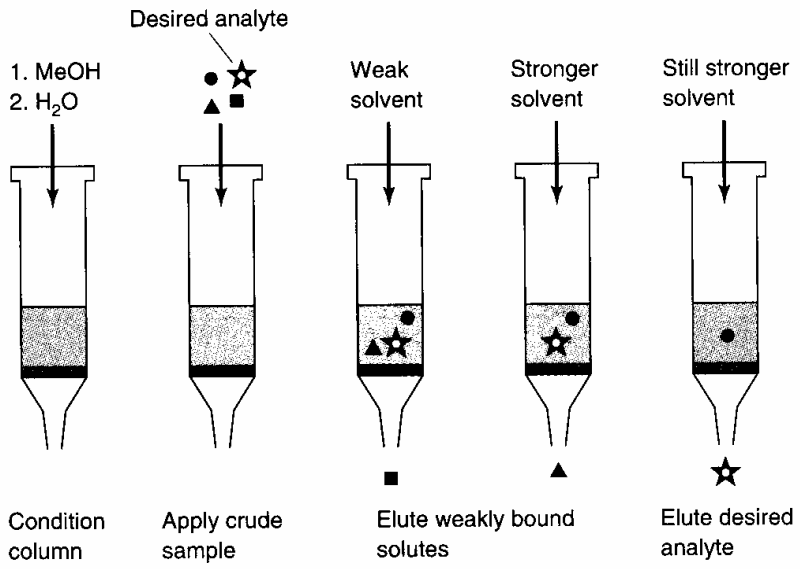


Figure 14. Steps in SPE /36/.

A high level of automation is possible using robotics or on-line interfaces to separation and spectroscopic instruments, but manual sample processing is a more common practice in most laboratories /40/. In SPE there is a disposable cartridge containing silica-based chemically sorbents of a suitable size for sample processing by a gentle suction. Typical cartridge device consist of short columns containing sorbent packed between a porous plastic or a metal frits. Sorbents nominal particle size is 50-60 µm.

SPE discs were developed to get higher sample processing rates for large sample volumes and to minimise plugging by suspended particles and matrix components /40/. Discs are also used for handling small-volume samples because small-diameter discs are easy to prepare. Today, several different disc formats are offered. Discs has particle loaded membrane containing sorbent particles of 8-12 μm diameter immobilised in a web of short poly(tetrafluoroethylene) (PTFE) fibrils. Discs are flexible and superficially resemble filter paper discs with some supportive structure, made of porous glass or plastic.

Cartridges and discs use the same sorbent technology /40/. SPE sorbents can be divided into three groups: Inorganic oxides, low-specifity sorbents and compound- and class-specific sorbents. Silica gel, alumina, synthetic magnesium silicate and diatomaceous earth are inorganic oxides and are the most important sorbent materials for SPE. They are used primarily as a filter aid and dispersant for solvent extraction. Low-specifity sorbents like chemically bonded silicas, porous polymers and carbon are commonly used for the isolation of contaminants from the aqueous solution. Compound-specific and class-specific sorbents are based on ion exchange, bioaffinity, molecular recognition and restricted access materials. Sorbent selection is important, and it is made by consideration. Decision is dependent on sample solvent and the type of the analyte.

4.4.3 Solid-phase micro extraction (SPME)

Solid phase micro extraction (SPME) is a simple method which provides extraction without solvent /36/. Compounds can be extracted from liquids, air or sludge. The main component of SPME is a fused silica fibre, which is coated with 10-100 μm thick film. The film is similar of non-volatile liquid GC stationary phases. The SPME syringe is presented in Figure 15.

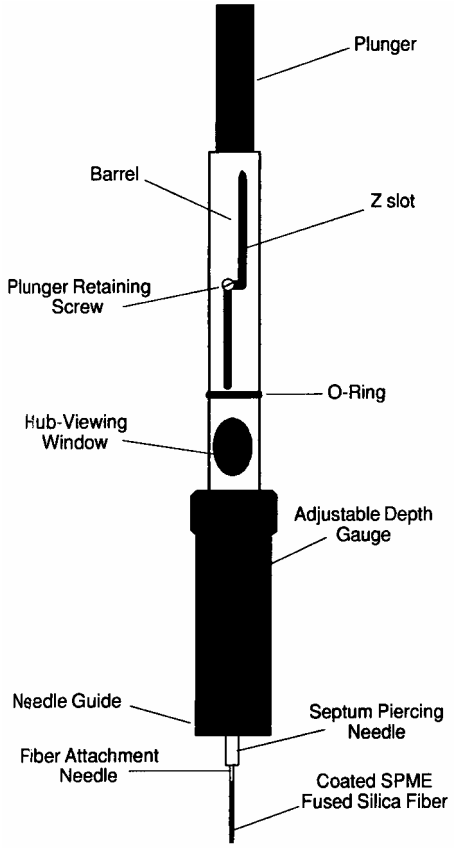


Figure 15. The SPME syringe /41/.

When extracting with SPME, first the fibre is exposed to the sample solution or the gaseous headspace of the liquids with stirring in a vial. The sample may be heated during the procedure. Only fraction of the analyte in the sample is extracted into the fibre. After a fixed time fibre is retracted and needle is withdrawn. Syringe is then injected to the GC inlet and analyte is thermally desorbed from the fibre in the splitless mode.

The mass m (µg) of the extracted analyte in the coated fibre is

$$m = \frac{KV_f C_0 V_s}{KV_f + V_s}, \tag{2}$$

where, V_f (mL) is the volume of film on the fibre, V_s (mL) the volume of solution being extracted, C_0 (µg/L) analytes initial concentration in the solution being extracted and K the partition coefficient for solute between the solution and the film /36/. $K=C_f/C_s$, where C_f is the analyte concentration in the film and C_s the analyte concentration in the solution.

SPME is a method that can be utilised with both GC and HPLC /42/. There are different fibres for various uses. For choosing the fibre, the first step is to identify the molecular mass range of the analytes to be extracted. Every fibre has the recommended maximum, operating and conditioning temperature. Fibres stationary phases are different. Non-bonded phases, e.g., polydimethylsiloxane (PDMS), are stable with some water-miscible organic solvents. These cannot be used with or rinsed with nonpolar organic solvents. Bonded phases are stable with all organic solvents. Compound with a higher molecular mass desorbs easier from the surface of the 7 μm or 30 μm PDMS absorption fibre coating. Smaller molecules are retained in the pores of the fibres containing adsorbents in the coating.

5 ANALYSIS OF MALODOROUS NITROGEN-CONTAINING COMPOUNDS

This section concentrates on analysis methods for nitrogen compounds that causes mal odour. Interest is focused on organic compounds. Analysis concerns amines, heterocyclic nitrogen compounds and ammonia. These compounds were selected because they have often very distinctive malodours and some of them are present in material spoilage.

When studying literature, the most used analysing instrument was GC-MS for all compounds of interest. HPLC was also often used when analysing amines. In addition, some other instruments were used, e.g., UV spectrophotometer, IR and gas sensors. All methods found from literature and discussed in this chapter are listed in Appendix 1.

Critical steps for analysis of these compounds are extraction, separation and finally detection and possibly quantification.

5.1 Extraction

Nitrogen compounds are often analysed from food beverages, because they can act as indicators of spoilage. In literature, there are also many articles about the analysis of these compounds from environmental waters. When analysing waters, SPE is the most used extraction method.

Molins-Legua and Campins-Falcó /43/ have summarised some of amine extraction procedures from literature during the years 2000-2005 from several matrices. Amines are usually extracted in basic conditions independently of their pK_a values or if they are aliphatic or aromatic. Often samples are alkalinized in the sample preparation step. Some works used acidic conditions especially for aliphatic amines and BAs. Traditionally aqueous amine samples have been extracted by LLE, despite the disadvantages, e.g., matrix interference, emulsion formation, large volumes of hazardous solvents and time consumption. To prevent this problem, an ion-pairing agent can be added to the analyte to help the solvent extraction. To extract aliphatic and biogenic amines, highly polar solvents (e.g. $HClO_4$, TCA, butanol and ethylacetate) have been used and for aromatic amines more non-polar solvents (e.g., $CHCl_3$, $CHCl_3$ and isopropanol). SPE, SFE and recently SPME are other techniques, which include sample clean-up and/or sample enrichment. These techniques can be applied to multiple biological samples either naturally liquid, as biological fluids or beverage or after acidic extraction from solid material. SFE has been used mainly for aromatic amine extraction and SPME to aromatic and aliphatic amines.

Önal /5/ has reviewed on extraction methods used for quantification of biogenic amines in food samples. For extraction, many different solvents have been used, such as hydrochloric acid, trichloroacetic acid, perchloric acid, methanesulfonic acid, petroleum ether and other organic solvents. Also SPE have been used to extract components.

Chan *et al.* /44/ have used SPME fibres for extraction of volatile alkyl amines from fish. The sample treatment of fibre was made in headspace of sample vial. Namieśnik *et al.* /45/ extracted also volatile aliphatic amines from air with SPME. Akyüts and Ata /46/ have extracted aromatic and aliphatic amines from sediment and environmental water samples by ion-pair extraction. Mishra *et al.* /47/ have extracted ammonia, aliphatic amines and aromatic

amines from environmental waters simultaneously by SPE of their benzoyl derivatives. The method consisted of pre-column formation of benzoate esters and benzamides with benzoyl chloride and SPE of the derivatives. Claeson *et al.* /48/ have extracted primary and secondary amines from the air with solid sorbent, XAD-2, impregnated with naphthylisothiocyanate (NIT). Kush *et al.* /49/ have extracted long-chain primary alkyl amines from boiler water with SPE using methanol and water in each extraction.

O'Connor *et al.* /50/ have extracted biosolids and filtrate samples from pulp and paper mill to determine indoles. Extraction procedure depends of the sample consistency. Samples were extracted by using methyl *t*-butyl ether.

5.2 Analysis of compounds

5.2.1 Amines

Molins-Legua and Campins-Falcó /43/ have also summarised analyse devices while summarising some of amine extraction procedures from literature during the years 2000-2005. According to their study, GC-MS and HPLC techniques are much used to determine amines. Especially GC-MS device is used when analysing aromatic amines and HPLC for biogenic amines.

Önal /5/ has reviewed of various analytical methods developed for quantification of BAs in food samples. Analyses are mainly based on chromatography: Thin layer chromatography (TLC), capillary electrophoresis (CE), GC and HPLC. For detection, most used are fluorescence, UV and electrochemical detectors. The one dimensional, double development TLC technique is used for the separation and determination of dansyl derivatives of BAs. The one-dimensional TLC technique is also used to separate some BAs. CE with conductometric detector is used as a sensitive and quick method that does not require sample clean up or derivatization. GC is usually connected to MS or tandem MS for analyses.

The most used analysis method is HPLC with pre- or post-columns. HPLC device is used to analyse lots of different BAs from several food matrices. Derivatisation or HPLC was usually made with OPA and in most cases detectors like diode array detector (DAD) and UV detector were used.

Chan *et al.* /44/ have determined volatile amines with GC-MS from fish. They used a Supelco PTA-5 column (30 m × 0.25 mm). Detection limits were from 0.10 to 0.15 μg/mL for methyl-, dimethyl-, and trimethylamines. GC-MS was made in a SIM mode. Namieśnik *et al.* /45/ determines volatile amines with GC-FID. A stabilwax-DB/KOH mega bore column (60 m × 0.53 mm × 1.5 μm) was used.

Akyüts and Ata /46/ have used GC-MS method to analyse aliphatic and aromatic amines. First compounds were extracted with ion-pair extraction with bis-2-ethylhexylphosphate (BEHPA), derivatised with isobutyl chloroformate (IBCF), and then analysed with GC-MS device because of its superiority in selectivity and sensitivity. Their column was a ZB-5 ms (30 m × 0.25 mm × 0.25 µm). Other derivatisation techniques for GC analysis of amines that are most popular are acylation, silylation, carbamate formation, sulphonamide formation and phosphonamide formation. Mishra *et al.* /47/ determines aliphatic and aromatic amines with GC-MS device. The column they have used was a HP-5 column (30 m × 0.25 mm × 0.25 μm).

Claeson *et al.* /48/ have developed a HPLC method with tandem MS to analyse volatile primary and secondary amines. Tamim *et al.* /51/ have analysed biogenic amines from poultry carcasses with HPLC with a dual pump solvent system.

Kush *et al.* /49/ have determined long-chain alkyl amines from boiler water with GC combined simultaneously with FID and nitrogen phosphorus detection (NPD) and also GC-MS was used. Compounds were derivatised with acylation before analyse. Column used was a DB-5 ms (60 m × 0.25 mm × 0.25 μm) from J&W Scientific Folsom.

5.2.2 Aromatic heterocyclic nitrogen compounds

Heterocyclic compounds are usually determined by GC-MS. In literature, heterocyclic nitrogen compounds are often determined simultaneously with other volatile and odorous compound groups.

Curioni and Bosset /52/ have reviewed pyrazines and indoles analysed from cheese with GC-O among with other odorants. O'Connor *et al.* /50/ have determined indoles with gas chromatography connected to mass detector or flame ionization detector, also GC-O was used. Bi *et al.* /53/ have analysed heterocyclic nitrogen compounds from water contaminated with coal tar. Analyse is proceeded with HPLC system equipped with a soil column. The method is called soil column chromatography (SCC). Wang *et al.* /54/ have analysed indoles from diesel fuel with two-dimensional GC-GC with nitrogen chemiluminescence detector (NCD). This equipment have major advantages of GC-GC, it has improved resolution and enhanced sensitivity.

5.2.3 Ammonia

For ammonia several analysing instruments are used, e.g., HS-GC, UV/VIS and HPLC. Ammonia is often analysed simultaneously with other compounds.

Mishra *et al.* /47/ have analysed ammonia simultaneous with amines with GC-MS. The detection limit for ammonia was 20 μg/mL. Column they have used was a HP-5 (30 m × 0.25 mm × 0.25 μm).

Claeson *et al.* /48/ have determined ammonia with amines by LC-MS/MS device. Extraction was made from the air with solid sorbent, XAD-2, impregnated with naphthylisothiocyanate (NIT).

EXPERIMENTAL PART

6 AIMS OF EXPERIMENTAL STUDY

The main aim of this study was to develop a method for analysing malodorous nitrogen compounds from the papermaking process and end products. At the moment there is no analytical method available to determine these compounds from paper and board samples even though human nose easily detects these malodorous compounds.

Volatile nitrogen compounds have very small threshold values and if these compounds end up to the final product customers can smell it. If paper and board are used for food packaging, the odour is very disturbing.

The goal was to develop a simple and rapid method for this purpose. It was also hoped that the principal sources of these malodorous compounds would be found in papermaking process. In addition, when tracing possible sources, some process chemicals were also analysed.

7 METHODS

The method which was applied was chosen based on the article of Chan *et al.* /44/. Their study concentrates on the analysis of methylamine (MA), dimethylamine (DMA) and trimethylamine (TMA) from fish with SPME and GC/MS. The method was chosen because it is suitable for the volatile amines, having the odour of spoiled fish. Also working with MS was important to ensure the detection of the right compounds. In their study Chan *et al.* /44/ have compared four different commercial fibres and found out that two of them have much better performance. These fibres were carboxen/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS) and polydimethylsiloxane/divinylbenzene (PDMS/DVB). The column used was Supelco PTA-5.

In this study PDMS/DVB fibres were selected. The column was different than that used by Chan *et al.* /44/. The used column was *Carbowax™ Amine* by Supelco and it was chosen because it is specially designed for amines.

In this chapter the method and analytical procedure are only generally reviewed and more detailed working instructions are described in Appendix 2.

7.1 Preparation of standards and samples

Different board and paper samples and process chemicals were analysed. Process chemicals were chosen according to the possibility of containing nitrogen compounds. All analytes needed in the determinations are listed in Table 3.

Table 3. Analytes used in this study

Analyte	Supplyer	Purity	M/(g/mol)	Other information
Trimethylamine HCl	Sigma-Aldrich	98 %	95.58	
Dimethylamine HCl	Sigma-Aldrich	99 %	81.55	
Methylamine HCl	Sigma-Aldrich	98+ %	67.52	
n-Propylamine HCl	Sigma-Aldrich	99+ %	95.57	
Dodecylamine	Fluka AG	puriss.	185.36	
Diethylamine	Jt.Baker	98 %	73.14	
Diethanolamine	Jt.Baker	99 %	105.14	
Ethylenediamine	Jt.Baker	98 %	60.10	
Triethanolamine	Jt.Baker	99 %	149.19	
HCl Ampul	Jt.Baker			Dilut-it 1 mol/l
Tributylamine	Acros organics	pro analysis	185.35	
N,N-diethylethanol-amine	Acros organics	99 %	117.19	
Cyclohexylamine	BDH	99 %	99.18	

Water used in the method was ultra high purity (UHP) water. Individual amine stock solutions of MA, DMA, TMA, *n*-propylamine and dodecylamine at concentration of 1000 μg/mL were prepared by dissolving quantitative amounts of chemicals in 0.5 M HCl. Stock solutions of analytes to the concentration levels of 0.5, 1.0, 0.2, 0.5 and 10.0 μg/mL were diluted in 0.5 M HCl. Mixtures of MA, DMA, TMA and *n*-propylamine containing two compounds were made in concentration levels of 5 μg/mL. Other amines used were liquids that were diluted in 0.5 M HCl to concentrations applied. Devices needed are shown in Table 4.

Table 4. Devices used in the preparation stage

General devices	Description
Magnetic stirrer	Jencons Gensis hotplate/stirrer Model Ch.3, 230V, 500W
Analytical balance	Mettler AT 400, max 405 g d=0.1 mg
Ultrapure water system	Millipore Milli-Q-plus
Automate pipette	Labsystem Finnpipette 4500, 200-1000 μL

Handmade, odour free paper sheets were dipped in the standard solutions with concentration of 5.0 and 10.0 μg/mL to ensure that the method would be suitable for paper and board. Sheets were cut in to pieces of approximately 2 g before dipping. After dipping sheets were left to dry at a hume hood for the next day. Dry sheets were cut to pieces approximately 1

cm^2, diluted in 20 mL of 0.5 M HCl for 15 minutes with slight stirring in a decanter glass with a cover class on the top of it. Then the decanter glass was left to rest for two minutes prior to the analysis.

The preparation of board samples was performed in the same way as that applied to handmade sheets in the dipping test.

Process chemicals were prepared as follows. Approximately 2 g of chemical was weighed and diluted in 20 mL of 0.5 M HCl for 15 minutes with slight stirring in a decanter class with a cover class on the top of it. Then the container was left to rest for two minutes prior to the analysis.

7.2 Extraction with SPME fibre

In this method SPME fibres with PDMS/DVB coating were used (Figure 15). SPME fibre had to be conditioned before use and after storage. In conditioning the injection port temperature had to be set according to the individual instructions and fibre inserted at the inlet with the help of inlet guide (Figure 16). For PDMS/DVB coated fibre the conditioning temperature was 250 ºC and recommended operating temperature 200-270 ºC. Conditioning time was half an hour, although a longer time did not damage the fibre. After conditioning the fibre, the GC column had to be conditioned for half an hour at the upper temperature of the oven program of the method.

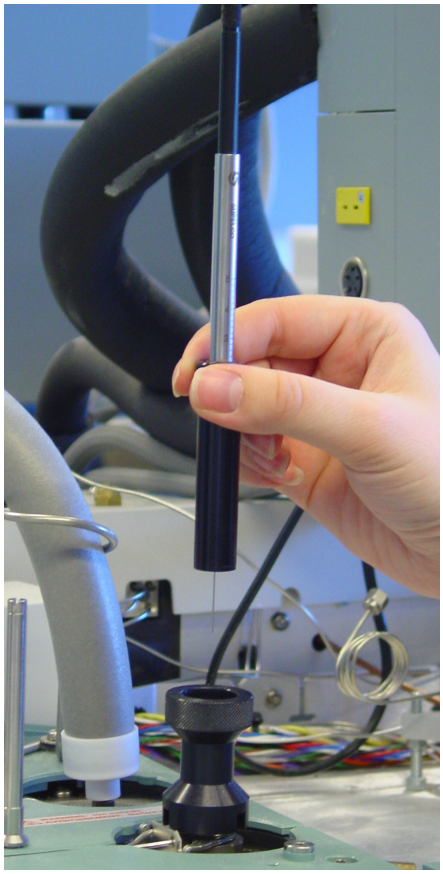


Figure 16. Inlet guide

Blank analysis was preceded after conditioning. Blank analyses should be made at the beginning of each day to ensure that the fibre and needle are not contaminated. If fibre was contaminated it had to be carefully cleaned. For cleaning there were two methods. First method was to heat the fibre to 20 ºC below its maximum temperature for one hour to overnight. A second alternative was to rinse the fibre in a water miscible solvent for one hour and then thermally desorb it for 30 minutes. PDMS/DVB have bonded phase and it is stable with all organic solvent. Suitable solvent for PDMS/DVB fibre cleaning was ethanol.

The fibre was consistently desorbed in the same depth in the injection port and for the same length of time for all standards and samples to ensure the reproducibility. Every day before sample analysis blank desorption was made to ensure that the needle and the fibre were free of contaminants. To ensure that fibre are in the same depth, in each analysis the needle was adjusted in the depth of 2 cm.

Extraction devices used are presented in Table 5. 0.5 mL of sample liquid was pipetted to the headspace vial (20 mL) with 0.5 mL of internal standard (concentration 5.0 μg/mL) chosen

together with 1 mL 15 M NaOH. A magnetic stirring bar was added and then vial was capped. The vial was put in a magnetic stirrer (400 rpm). SPME fibre was inserted into the inlet of GC in 200 ºC. After 15 minutes fibre was inserted inside the vial for headspace extraction carefully avoiding the spillage onto the fibre. Extraction time was 5 minutes and directly after extraction fibre was inserted to the inlet of GC and analysed. In cases of real paper and board samples and process chemicals, the internal standard was no longer added to the vial and sample amount pipetted in the vial was then 1 mL. In addition, it was tested that the magnetic stirring bar did not contaminate the sample.

Table 5. Description of the extraction device used

Extraction device	Description
SPME manual holder	Supelco™
SPME fibre	Supelco™ StableFlex™ 65 µm PDMS/DVB coating for manual holder
SPME inlet guide	Supelco™
Headspace vial	20 mL

7.3 Analytical methods

Analyses were made with a GC-MS device described in table 6. The inlet was set to 200 ºC. Column was initially kept in 40 ºC for 5 min and then ramped at 15 ºC/min to 200 ºC and kept for 3 min. The length of oven programme was 18.70 minutes. Flow was set at 0.9 mL/min. In MS electron impact (EI) mode was used and SCAN parameters were for low and high masses 30.0 and 650 m/z, respectively. Ion extraction was used to find out the compounds from the chromatograms and to compare their peak areas to each other's.

Table 6. Description of GC-MS

Analysis device	Description
GC	HP 6890 series system
Column	CARBOVAX™ AMINE FUSED SILICA Capillary Column 30 m x 0.53 mm x 1.0 μm film thickness
MS	HP 5973
Programme	ChemStation Version D.03.00.611

7.3 Sensory testing

Sensory testing was made at Stora Enso Research Centre Imatra in the odour and taste laboratory with their trained panel staff. To ensure the reliability of organoleptic analytics the panellist are trained regularly. Odour tests were made according to standard EN-1230-1:2001.

In the method panellist described the odour and evaluated the odour by points:

0 = No off-odour
1 = Weak off-odour
2 = Moderate off-odour
3 = Moderately strong off-odour
4 = Strong off-odour

8 RESULTS AND DISCUSSION

8.1 Pre-testing of method

Method was adjusted so that it would fit better for the analysing of paper, board and process chemical samples. Different kinds of oven programmes were tested to improve the separation of compounds. The oven temperature was raised slower so that also high-molecular-mass and more complex amine compounds could be detected better. One reason for it was also that all compounds would have enough time to go through the column so that it would not contaminate.

To find out what kind of compounds would be detected with the method, testing was made with standard solutions. The concentration levels of amines varied from 10 to 1000 μg/mL. First tests were made with low concentrations, and if compounds were not detected, then concentration was raised.

In Table 7 are listed the retention times for each amine detected. In other case, the retention time is marked *Not found*. With diethylamine the detection was difficult because the SCAN parameters started from ion 30 m/z, and this parameter was also the best detection ion for this compound. Small amines appear at the beginning of the temperature programme with the retention times below 3 min. The method was suitable for amines that were rather simple. If amine had other functional groups, the method did not suit well. The best results were gained with low-molecular-mass aliphatic amines. These amines are volatile and because of that they are more likely to give off-odour to product. The retention times presented for amines are not exact, because injections were made manually, although the results are still comparable to each other.

Table 7. Retention times for amines

Amine	Retention time (min)
MA	1.1
DMA	1.0
TMA	0.9
n-propylamine	1.6
Diethylaminoethanol	6.0
Diethylamine	2.0
TBA	9.0
Dodecylamine	11.0
Cyclohexylamine	9.0
Triethanolamine	Not found
Ethylenediamine	Not found
Diethanolamine	Not found

The best ion for each detected amines for identification and the molecule ions are listed in Table 8. Amines were detected and the areas of their peaks were measured from ion chromatograms. These ions were chosen according to the structure of amine and how characteristic they are for each amine so that compounds could be identified. Amines shared some characteristic ion peaks in their mass spectra; such as ions 30 and 58 m/z. Ions for identification were considered carefully so that the best result was gained.

Aliphatic amines undergo α-cleavage, which usually results a base peak at 30 m/z /47/. Because several amines share this peak it can cause problems in identification if SIM mode is used.

Table 8. Best ions for amines to extract ion chromatograms

Amine	Best ion (m/z)	Molecule ion (m/z)
MA	30	31
DMA	44	45
TMA	58	59
n-propylamine	59	59
Diethylamine	73	73
Diethylethanolamine	86	117
TBA	142	185
Dodecylamine	128	185
Cyclohexylamine	56	99

Amines that were detected with the method odour descriptions and threshold values are presented in Table 9. The threshold values of these compounds were very small. According to the odour descriptions small amines were of further interest.

Table 9. Odours and threshold values of detected amines /55,56/

Amine	Description of odour	Odour threshold value (ppm)
MA	Ammonia like, in low concentrations fish-like	3.2
DMA	Pungent, fish-like,ammonia like	0.22
TMA	Pungent, fish-like, ammonia like	0.00044
n-propylamine	Not found	Not found
Diethylaminoethanol	Weak ammonical	0.011
Diethylamine	Fish-like, ammonia like	0.13
TBA		Not found
Dodecylamine	Not found	Not found
Cyclohexylamine	Unpleasant, fishy	2.6

Other than nitrogen compounds that appear in GC chromatograms are mainly alcohols, ketones and aldehydes. Sometimes when large amounts of these compounds appear, it could reduce the amount of the nitrogen compound at the SPME fibre.

During the development SPME fibre was contaminated with the compounds tested. Compounds accumulate easily to the fibre so it had to be cleaned regularly. The best way to clean the fibre from amines was by first soaking the fibre to the ethanol for one hour and then thermally desorbing the fibre at 250 ºC for half an hour. This was a fast way to ensure that fibre was clean. The chromatograms of empty column and blank fibre are shown in Appendix 3.

8.2 Testing with standard solutions

Method was tested with standard solutions of known concentrations to see if the peak areas would correlate with the concentrations of compounds.

Making the calibration curve was unsuccessful. Peak area of the standard was calculated with the internal standard as follows.

$$A = \frac{A_S * 800000}{A_{iS}}, \quad (3)$$

where A_S = area of the standard, A_{iS} = area of the internal standard and number 800000 was chosen to get the areas of the internal standards equal.

As seen from Figure 17, calibration curve was not possible to make with TBA as an internal standard. Areas of parallel samples differ too much from each other. Also *n*-propylamine did not work as an internal standard, because its retention time was too close to those of MA, DMA and TMA. The consequence was, that the peaks of standards and internal standards were mixed. Internal standard should be a compound that does not have a retention time close to ones of analysed compounds, also it should not fragment during analyse. TBA seems to fragment and that distorts results. Repeatability seems to be better for small concentrations than for larger. The reason could be that the maximum capacitate of the fibre is crossed. Also other compounds extracted beside amines uses capacitate of the fibre.

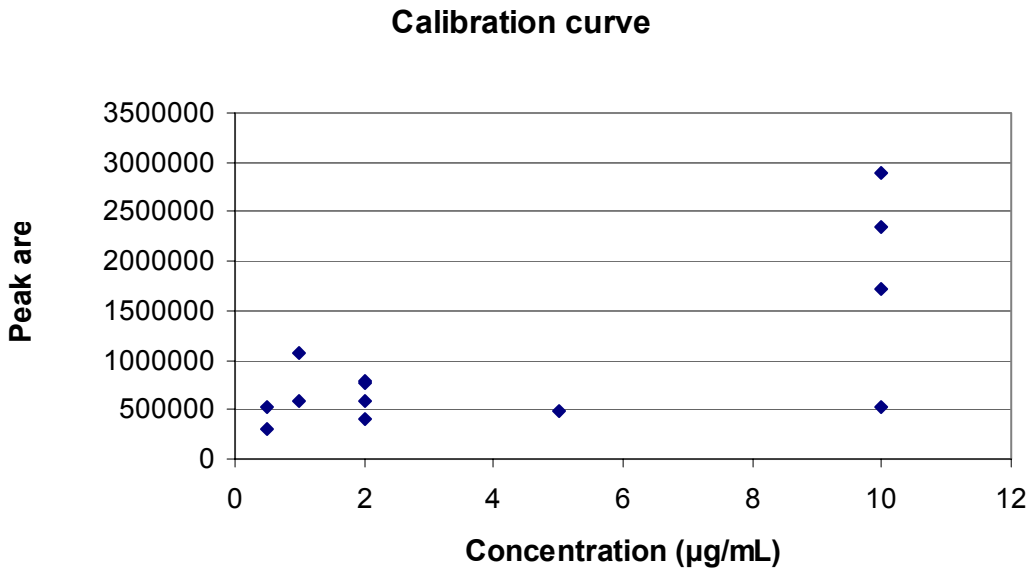


Figure 17. Calibration curve for TMA with TBA as an internal standard

Mixtures of MA, DMA, TMA and n-propylamine were made to see how the peaks would appear. As an example are in Figure 18 presented part of the chromatograms of DMA and TMA. The first peak in the chromatogram is solvent peak, next one is TMA and after that is

DMA. The compounds overlapped to some extend with each other, but the detection could still be done.

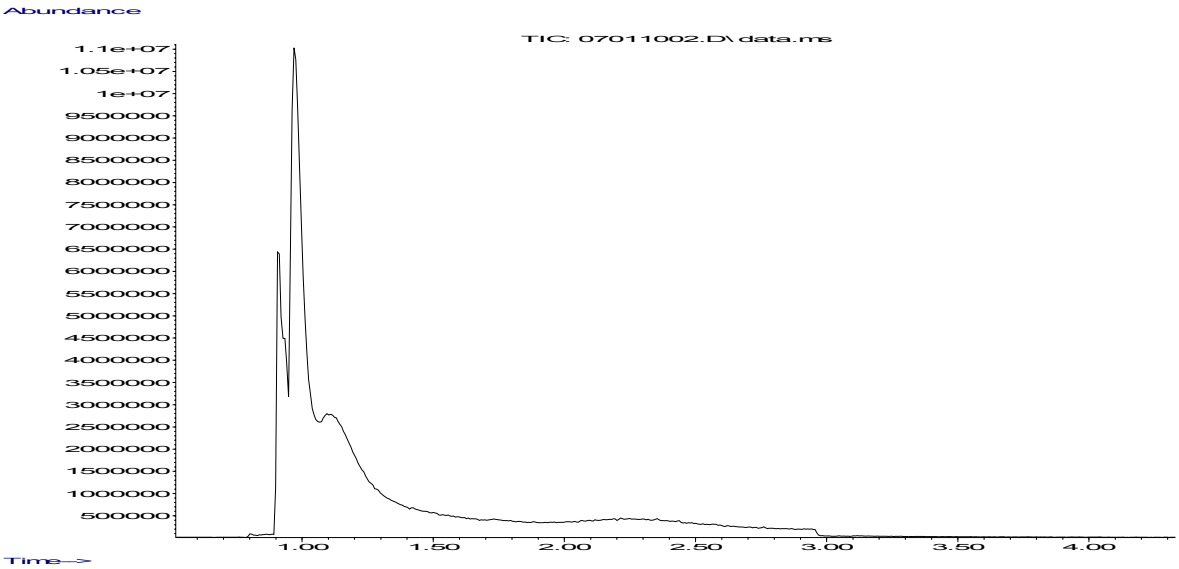


Figure 18. The chromatogram of DMA and TMA.

For determination the sensitivity of the method additional tests were made with DMA. The smallest concentration of DMA was 0.01 μg/mL.

The separation of compounds could be improved if samples were analysed with the SIM programme instead of SCAN.

8.3 Dipping tests

When developing the method suitable for real samples, dipping tests were made. The analysis of dipped samples in different ways was tried. First, sample was put in a capped vial and fibre was inserted to the headspace of the vial for five minutes. No results were gained with it. Second, the capped vial was heated in an oven at 80 ºC for 15 minutes and then fibre was inserted to the headspace of the vial for five minutes. Compounds in which sheets were

dipped were not detected from the chromatogram. These test were made with the TMA concentration of 1000 μg/mL.

After these trials, board sheets were tried to soak in water and in 0.5 M HCl before extracting with SPME fibre to find out if it would give response peaks. At first the concentration of standard solutions in which sheets were dipped was 1000 μg/mL. Because TMA was easily detected and gave high peak area, was concentration of 10 μg/mL tried. This also gave clear result. The peak area originated from 1000 μg/mL solution compared to peak area originated from 10 μg/mL solution was approximately 100 times higher. This indicated that some concentration approximations could be made according to peak area. Chromatogram peaks of these experiments are presented in Figure 19 and 20.

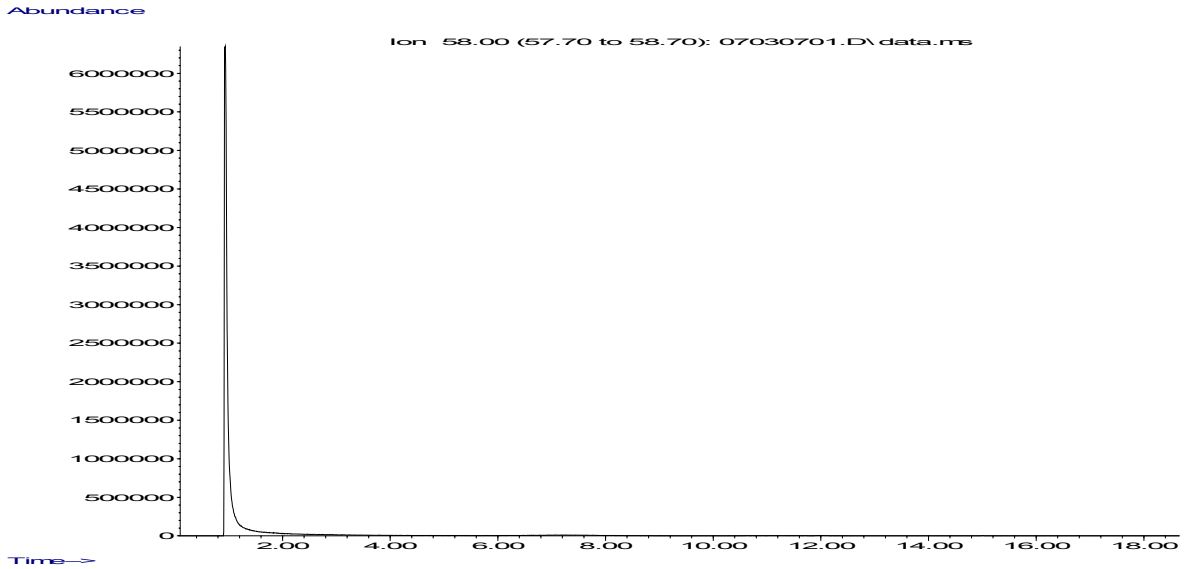


Figure 19. Chromatogram (ion 58 m/z) of the dipping test with a concentration of 1000 μg/mL.

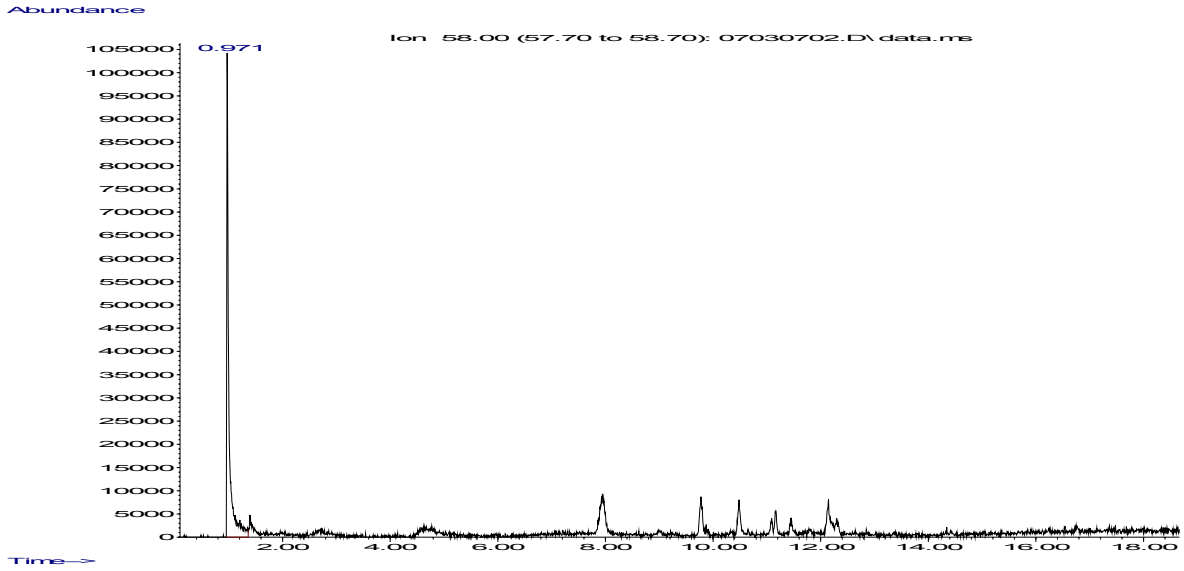


Figure 20. Chromatogram (ion 58 m/z) of the dipping test with a concentration of 10 μg/mL.

0.5 M HCl was found to be a better choice because it gave a better response and it was more likely to give results. Testing was made with handmade board sheets that were dipped in standard solutions of TMA and TBA.

8.4 Real paper and board samples

Some real board and paper samples that had an amine-like odour in the sensory tests were analysed. Odour test points were between 1.2 and 3.1 for the selected pilot-made samples 1-7. Samples 8 and 9 were reference samples and had the odour points 0.5. When sample gets points over 1 the odour is present, but is not still disturbing. When points of the sample are over 2 the odour is clear and easily detectable and with the points over 3 complaints from customers are likely to be come. Samples 1-7 were chosen to test the method because they had odour comments such as pungent, fish and rotten, all of which strongly refers to amines. Samples 8 and 9 were references and they did not have the similar odour descriptions to those of the previous samples.

Some chromatograms concerning these samples are enclosed (Appendix 3). As an example of the procedure of processing the results (sample 2) is presented in Figures 21-23.

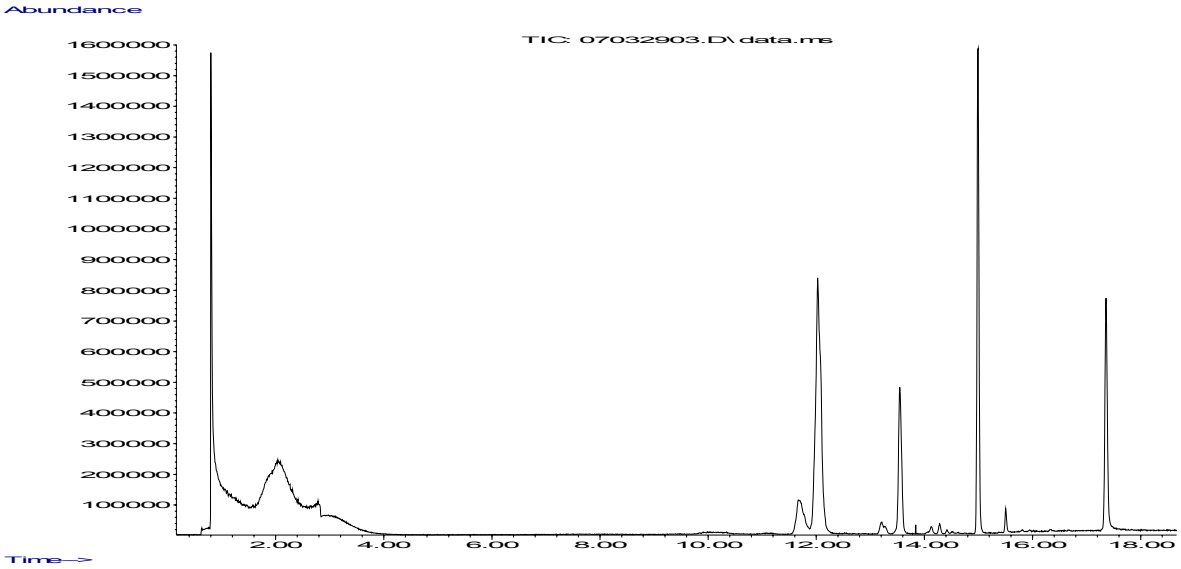


Figure 21. Chromatogram of sample 2.

Because TMA peak was often overlapped with the solvent peak, the extracted ion chromatogram was done with ion 58 m/z. As can be seen from Figure 18 the peak with the retention time 0.8 min was very clear.

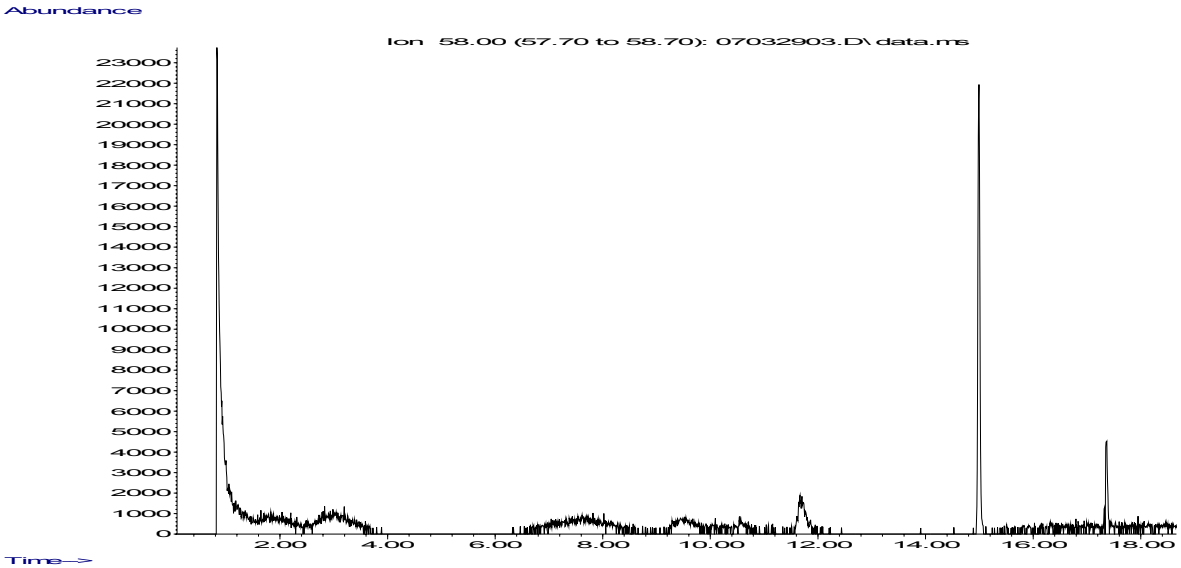


Figure 22. Extracted ion chromatogram of sample 2.

Figure 23 shows the detection of methylamine from the sample. Peak with 32 m/z derived from the solvent peak. Without it the correspondence of these two is high.

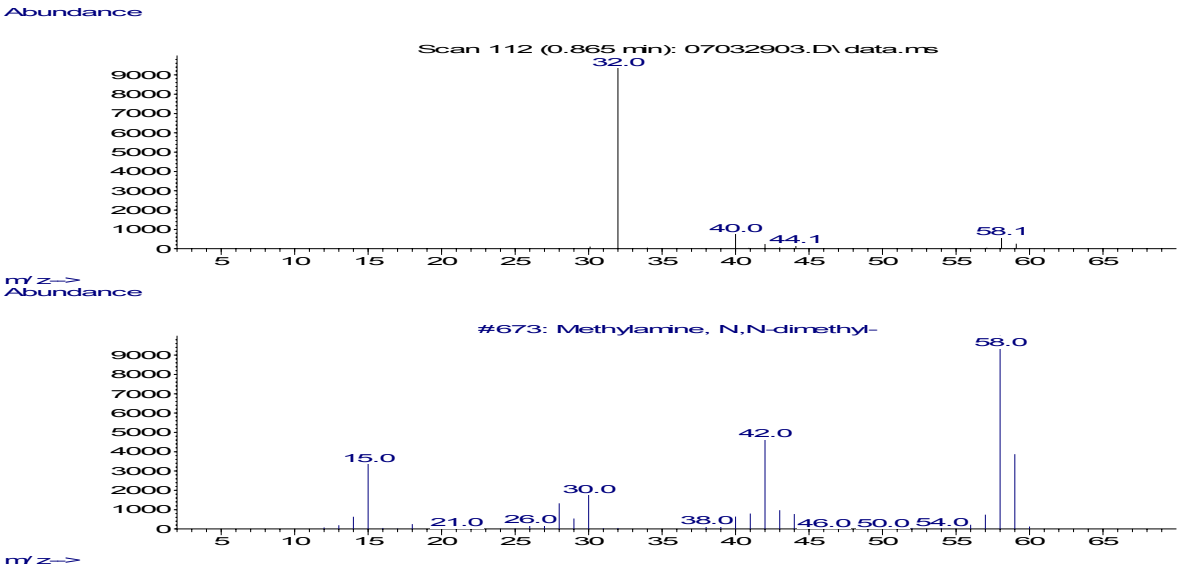


Figure 23. Identifying MS of sample 2.

Many samples contained TMA with different amounts. Areas of all these peaks gained are presented in Figure 24. Also similar samples that did not have the same kind of odour were tested for reference. Predictably, reference samples did not have TMA or any other nitrogen compound peaks. Sensory testing was made with fresh samples. Samples were stored at room temperature for few months and because TMA is very volatile compound, some of it could have been evaporated before analysis. This fact could explain why odour points and TMA peaks did not correlate. in addition, one reason could be the lack of internal standard.

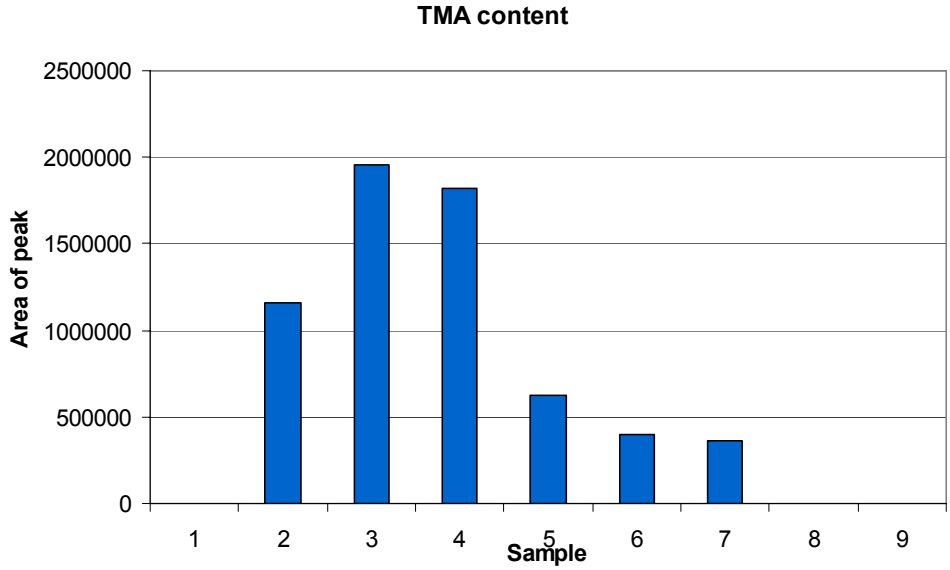


Figure 23. Area of TMA peaks in paper and board samples.

In Table 10 are given the results from the sensory tests and the peak area for comparison. The samples that have amine-like odour in the sensory test should be analysed immediately to ensure the same concentration levels of all compounds.

Table 10. Comparison of TMA peaks and sensory test

Sample	Nitrogen compound	Area of peak	Odour value	Description of odour
sample 1	Not found	0	2.4	latex, printing ink, pungent
sample 2	TMA	1157295	2.3	latex, printing ink
sample 3	TMA	1956552	2.4	solvent, printing ink, latex
sample 4	TMA	1817621	2.7	pungent, latex
sample 5	TMA	626965		no results
sample 6	TMA	401647	1.2	latex
sample 7	TMA	363653	3.1	latex, fish ammonia
sample 8	Not found	0	0.5	no comments
sample 9	Not found	0	0.5	pulp

8.5 Process chemicals

Different process chemicals were analysed and areas of these peaks are presented in Table 11. These samples were chosen because these chemicals are containing most likely nitrogen compounds. This conclusion was made according to the literature data.

Table 11. Areas of amine peaks in process chemical samples.

Sample	Nitrogen compound	Area of peak
latex 1	TMA	1397106
latex 2	Not found	
latex 3	Not found	
latex 4	Not found	
starch 1	Not found	
starch 2	Benzyl cyanide	576238
starch 3	Not found	
starch 4	Not found	
binder 1	Not found	
binder 2	Not found	
OBA 1	Pyridine	1243137
OBA 2	Aniline	428836140
dye	TMA	5169327
defoamer	Aniline	13533174
bioside	Not found	

TMA was found from both latex 1 and from dye. The content of TMA was much larger in dye. Cyanide was found from starch 2 and pyridine from OBA 1. Aniline was found from OBA 2 and from defoamer. Aniline is used in the process of making some OBAs (see Chapter 3.2.2) and it can be leftover as a residual process.

In Table 12 are listed the ions used for the identification of the compounds in chemical samples. Ions for extraction ion chromatograms were chosen according the same principal as that in the pre-testing method section (ions for TMA, see Chapter 8.1).

Table 12. Ions for detected compounds extraction ion chromatograms

Compound	Best ions (m/z)	Molecule ion (m/z)
Benzyl cyanide	90 and 117	117
Pyridine	52 and 79	79
Aniline	66 and 93	93

Odour descriptions of these compounds are listed in Table 13. Aniline has a rather high threshold value. Pyridine gives off-odour in smaller contents. For benzyl cyanide no odour description or threshold values were found.

Table 13. Odours and threshold values of detected compounds

Compound	Odour description	Odour threshold values (ppm)
Benzyl cyanide	Not found	Not found
Pyridine	Unpleasant	0.17
Aniline	Charasteristic distinctive	1.1

9 CONCLUSIONS

The method developed in this study is suitable for analysing low-molecular-mass amines from paper and boar samples and also for analysing these compounds from papermaking chemicals. The extraction method is very selective for volatile amines without other functional groups. The SPME fibre is a good method for extraction, because it does not extract many interfering compounds present in the sample.

The method is rather sensitive for detection of easily volatile amines, even though the exact concentrations of compounds cannot be determined. However, the method is a clear improvement to the earlier situation because now nitrogen-containing malodorous compounds can be identified and their amounts can be roughly approximated.

The results of real paper and board samples correlated well with those of sensory testing. The reference samples did not give any peaks of nitrogen compounds. Samples should be rapidly analysed if the sensory test gives amine-like odour and easily volatile compounds are still detectable.

In the forthcoming studies, a proper internal standard should be found to obtain a more accurate concentration value of each nitrogen-containing malodorous compounds. In addition, the system (sensory testing combined with chromatographic analysis) should be tested for a wide range of real samples including especially previous nitrogen-containing papermaking chemicals.

REFERENCES

1. Tice, P. and Offen, C., Odors and taints from paperboard food packaging, *Tappi J.*, 77(1994)149-154.
2. Järvi-Kääriäinen, T. and Leppänen-Turkula, A., (Ed.), *Pakkaaminen, perustiedot pakkauksista ja pakkaamisesta*, Helsinki, Finland, 2002, pp. 44-62.
3. Cotton, F.A. and Wilkinson, G., *Advanced Inorganic Chemistry*, 5th ed., John Wiley & Sons, New York, USA, 1988, pp. 305-381.
4. Rayner-Canham, G., *Descriptive Inorganic Chemistry*, 2nd ed., W.H. Freeman and Company, New York, USA, 2000, pp. 296-321.
5. Önal, A., A review: Current analytical methods for the determination of biogenic amines in foods, *Food Chem.* (2006), doi:10.1016/j.foodchem.2006.08.028.
6. Claeson, A.S., Östin, A. and Sunesson, A.L., Development of a LC-MS/MS method for the analysis of volatile primary and secondary amines as NIT (naphthylisothiocyanate) derivatives, *Anal. Bioanal. Chem.*, 278(2004)932-939.
7. Fessenden, R., Fessenden, J. and Logue, M., *Organic Chemistry*, 6th ed., Brooks/Cole Publishing Company, Pacific Grove, USA, 1998.
8. Ho, C.-T. and Carlin, J., Formation and aroma characteristics of heterocyclic compounds in foods, In book: Teranishi, R., Buttery, R. and Shahidi, F. (Eds.), *Flavour Chemistry-Trends and Development*, Third Chemical Congress of North America (195th National Meeting of the American Chemical Society), Toronto, Ontario, Canada, June 5-11,1988, American Chemical Society, Washington, USA, 1989.
9. Kametani, T., Honda, T., Fukumoto, K. and Ihara, M., Alkaloids, In book: Gerhartz, W. (Ed.), *Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 1*, 5th ed., VCH, Weinheim, Germany, 1985, pp. 353-402.
10. Shimizu, S., Watanabe, N., Kataoka, T., Shoji, T., Abe, N., Morishita, S. and Ichimura, H., Pyridine and pyridine derivatives, In book: Elvers, B. (Ed.), *Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 22*, 5th ed., VCH, Weinheim, Germany, 1993, pp. 399-429.
11. Edams, A. and De Kimpe, N., Formation of pyrazines and 2-acetyl-1-pyrroline by *Bacillus cereus*, *Food Chem.*, 101(2007)1230-1238.
12. McMurry, J., Fundamentals of organic chemistry, 4th ed., Brooks/Cole publishing Company, USA, 1998.

13. Robinson, W., Odom, J. and Holtzclaw, H., *General Chemistry*, 10th ed., Houghton Mifflin Company, USA, 1997.
14. Bakemeier, H., Huberich, T., Krabetz, R., Liebe, W., Schunck, M. and Mayer, D., Ammonia, In book: Gerhartz, W., (Ed.), *Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 2*, 5th ed., VCH, Weinheim, Germany, 1985, pp. 143-233.
15. Robertson, L. and Rice, L., Odour in paper and board, Proc., *60th Appita Annual Conference and Exhibition*, Melbourne, Australia, 3.-5.4.2006, TAPPI Press, Atlanta, USA, pp. 435-441.
16. Sundholm, P., Mill operations in production of main paper and board grades, In Book: Paulapuro, H., (Ed.), *Papermaking Part 1, Stock Preparation and Wet End,* Fapet Oy, Helsinki, Finland, 2000, pp. 11-55.
17. Holmberg, M., Paper machine water chemistry, In book: Neimo, L., (Ed.), *Papermaking Chemistry*, Fapet Oy, Helsinki, Finland, 1999, pp. 204-221.
18. Palonen, H., Influence of coated broke on wet end chemistry, In book: Neimo, L., (Ed.), *Papermaking Chemistry*, Fapet Oy, Helsinki, Finland, 1999, pp. 240-251.
19. Fengel, D. and Wegener, G., *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, Germany, 1983, pp. 182-226.
20. Robertson, L., Odor source, location determines approach to finished product issues, *Pulp Pap.*, 80(2)(2006)38-42.
21. Eagon, R., Paper, pulp and food grade paper, In book: Rossmoore, H., (Ed.), *Handbook of Biocide and Preservative Use*, Blackie Academic & Professional, Suffolk, Great Britain, 1995, pp. 83-132.
22. Alakomi, H.-L., Kujanpää, K., Partanen, L., Suihko, M.-L., Salo, S., Siika-aho, M. Saarela, M., Mattila-Sandholm, T. and Raaska L., VTT research notes 2152, *Microbiological Problems in Paper Machine Environments*, Espoo, Finland, 2002, 97p.
23. Foster, K., The chemistry and applications of fluorescent whitening agents for the paper industry, Course notes, *Dyes, Fillers & Pigments Short Cource*, Chicago, USA, 26.-28.4.1995, Tappi Press, Atlanta, USA.
24. Scott, W., *Principles of Wet End Chemistry*, Tappi press, Atlanta, USA, 1996, pp. 45-48.
25. Herbst, W. and Hunger, K., *Industrial Organic Pigments: Production, Properties, Applications*, 2nd ed., WCH, Weinheim, Germany, 1997.
26. Lehtinen E., Coating pigments-general, In book: Lehtinen E., (Ed), *Pigment Coating and Surface Sizing of Paper*, Fapet Oy, Helsinki, Finland, 2000, pp. 61-67.

27. Krogerus, B., Fillers and pigments, In book: Neimo, L., (Ed.), *Papermaking Chemistry*, Fapet Oy, Helsinki, Finland, 1999, pp. 117-149.
28. Ketola, H. and Andersson, T., Dry-strength additives, In book: Neimo, L., (Ed.), *Papermaking Chemistry*, Fapet Oy, Helsinki, Finland, 1999, pp. 268-287.
29. Krogerus, B., Modified starch pigments as paper additives, Proc. *Minerals in Papermaking-Scientific and Technological Advances in Fillers,* Stockholm, Sweden, 9-19.2.1999, Pira, 1999.
30. Lehtinen, E., Coating binders – general, In book: Lehtinen E., (Ed), *Pigment Coating and Surface Sizing of Paper*, Fapet Oy, Helsinki, Finland, 2000, pp. 189-195.
31. Tran, T., Soy protein, In book: Lehtinen E., (Ed), *Pigment Coating and Surface Sizing of Paper*, Fapet Oy, Helsinki, Finland, 2000, pp. 189-195.
32. Wolff, B., Guidelines for crosslinker selection, Cource notes, *1998 Coating Materials: Pigments, Binders and Additives Short Cource*, Norfolk, VA, 16.-18.3.1998, Tappi Press, Atlanta, USA, 1998, pp. 701-717.
33. Granvogl, M., Bugan, S. and Schieberle, P., Formation of amines and aldehydes from parent amino acids during thermal processing of cocoa and model systems: New insights into pathways of the Stecker rection, *J. Agric. Food Chem.*, 54(2006)1730-1739.
34. Wiik, K., *Studies on Odour in Paper Containing TCF Bleached Sulphite Hardwood Pulp*, doctoral thesis, Norwegian University of Science and Technology, Oslo, Department of Chemical Engineering, 1999.
35. Riekkola, M.-L. and Hyötyläinen, T., *Kolonnikromatografia ja kapillaarielektromigraatiotekniikat*, 2nd ed., Yliopistopaino, Helsinki, Finland, 2002.
36. Harris, D., *Quantitative Chemical Analysis*, 5th ed., W.H. Freeman and Company, New York, USA, 1998.
37. Craven, M., Gardner, J. and Bartlett, P., Electronic noses – development and future prospects, *Trends in Analytical Chemistry*, 15(9)(1996)486-493.
38. Kaipainen, A., Raatikainen, O. and Pitkänen, M., Elektroninen nenä haistaa laadun, *Kemia-Kemi*, 25(2)(1998)112-115.
39. Hanaoka, K., Sieffermann, J.-M. and Giampaoli, P., Effects of the sniffing port air makeup in gas chromatography-olfactometry, *J. Agric. Food Chem.*, 48(2000)2368-2371.
40. Poole, C., New trends in solid-phase extraction, *Trends in Anal. Chem.*, 22(6)(2003)362-373.
41. Instructions for the Supelco™ solid phase microextraction fiber holder for manual use, Sigma-Aldrich co, Bellefonte, 1998.

42. Solid phase microextraction fiber assemblies, Sigma-Aldrich co, Bellefonte, 1999.
43. Molins-Legua, C. and Campins-Falcó, P., Solid phase extraction of amines, *Anal. Chim. Acta*, 546(2005)206-220.
44. Chan, S., Yao, M., Wong, Y., Wong, T., Mok, C. and Sin, D., Evaluation of chemical indicators for monitoring freshness of food and determination of volatile amines in fish by headspace solid-phase microextraction and gas chromatography-mass spectrometry, *Eur. Food Res. Technol.*, 224(2006)67-74.
45. Namieśnik, J., Jastrzębska, A. and Zygmunt, B., Determination of volatile aliphatic amines in air by solid-phase microextraction coupled with gas chromatography with flame ionisation detection, *J. Cromatogr. A*, 1016(2003)1-9.
46. Akyüts, M. and Ata, Ş., Simultaneous determination of aliphatic and aromatic amines in water and sediment samples by ion-pair extraction and gas chromatography-mass spectrometry, *J. Chromatogr. A*, 1129(2006)88-94.
47. Mishra, S., Singh, V., Jain, A. and Verma, A., Simultaneous determination of ammonia, aliphatic amines, aromatic amines and phenols at μg l^{-1} levels in environmental waters by solid-phase extraction of their benzoyl derivatives and gas chromatography-mass spectrometry, *Analyst*, 126(2001)1663-1668.
48. Claeson, A.S., Östin, A and Sunessons, A.L., Development of a LC-MS/MS method for the anaysis of volatile primary and secondary amines as NIT (napthylisothiocyanate) derivatives, *Anal. Bioanal. Chem.*, 378(2004)932-939.
49. Kusch, P., Knupp, G., Hergarten, M., Kozupa, M. and Majchrzak, M., Solid-phase extraction-gas chromatography and solid-phase extraction-gas chromatography- mass spectrometry determination of corrosion inhibiting long-chain primary alkyl amines in chemical treatment of boiler water in water-steam system of power plants, *J. Chromatogr. A*, 1113(2006)198-205.
50. O'Connor, B., Buchanan, B. and Kovacs, T., Compounds contributing to odors from pulp and paper mill biosolids, *Pulp Pap. Can.*, 101(2)(2000)57-60.
51. Tamim, N., Bennett, L., Shellem, A. and Doerr, J., High-performance liquid chromatographic determination of biogenic amines in poultry carcasses, *J. Agric. Food Chem.*, 50(2002)5012-5015.
52. Curioni, P. and Bosset, J., Review: Key odorants in various cheese types as determined by gas chromatography-olfactometry, *Int. Dairy J.*, 12(2002)959-984.

53. Bi, E., Schmidt, T. and Haderlein, S., Sorption of heterocyclic organic compounds to reference soils: column studies for process identification, *Environ. Sci. Technol.*, 40(2006)5962-5970.
54. Wang, F., Robbins, W. and Greaney, M., Speciation of nitrogen-containing compounds in diesel fuel by comprehensive two-dimensional gas chromatography, *J. Sep. Sci.*, 27(2004)468-472.
55. Amoore, J.E. and Hautala, E. Odor as an aid to chemical safety: Odour thresholds compared with threshold limit values and volatilities for industrial chemicals in air and water dilution, *J. of Appl. Toxicol.*, 3(6)(1983)272-290.
56. Sittig, M., *Handbook of Toxic and Hazardous Chemicals*, Noyes Publication, New Yersey, USA, 1981.

APPENDICES

Appendix 1: Table of methods for analysing malodorous nitrogen compounds.

Appendix 2: Working instructions for analysing malodorous amines from paper and board samples and process chemicals (6 pages).

Appendix 3: Chromatograms (6 pages).

Table of the methods for analysing malodorous nitrogen compounds

Method	Nitrogen compounds: Amines	Arom heter.	Ammonia	Other inorg.	Sample	Pretreatment	Detection limit
HS-GC	x				Oligonucleotide	ammonia solution	0.9-50 µg/L
GC-MS	x		x		environ waters	SPE, derivatization	0.1-100 µg/L
UV/Vis			x		water and air	SPE+OPA/NAC	0.015-0.50 µg/L
Fiber optic spec	x	x			amine vapors	flows with N	140-28 000 ppb
GC-FID	x				air, stand. solution	SPME	2-150µg/L
LC-MS/MS	x		x		air	derivatised with NIT	0.12-0.25 ng/µL
LC-spectrofluoro detector	x				food	0.6M percloric acid extraction	70-200 µg/L
HS-GC/MS	x	x			fish sauce	pH as is & pH11 20%NaOH	
GC-MS		x			fish sauce	CC+ fractionation,SDE	
GC-MS		x			earth-almond	distillation,extraction	
LC-MS-MS	x				cocoa beans	adding dansyl chloride in acetone	
GC-MS	x				water	dichloromethane,derivatised 2,4-DNFB & BSE	
HPLC	x				poultry carcasses	extracted percloric acid, derivatised	
gas sensors	x		x		mouth air, urine	no pretreatment	
GC-O		x			cheese	direct extraction, SFE,distillation or HS	>0,5 µg/kg
HRGC, -MS, -O				Cyanates	marine sponge	dynamic headspace extraction	>0,4 mg/kg
IR		x				matrix-isolation technique	
GC-MS	x				fish	HS-SPME	0.10-10 µg/mL
GC-MS	x				environmental samples	ion-pair extraction with BEHPA +IBCF	0.07-0.50 ng/L
GC-MS	x		x		environmental waters	derivatisation with benzoyl chloride, SPE	7-39 µg/L

Working instructions for analysing malodorous amines from paper and board samples and process chemicals

This method is based on the article of Chan *et al.* /1/.

1 Needed materials and devices for analyse

Analytes needed

- NaOH pellets for 15 M NaOH solution
- 0.5 M HCl ampull

Before starting analysing prepare 15 M NaOH and 0.5 M HCl solutions.

List of needed devices

- Magnetic stirrer
- Analytical balance
- Ultrapure water system
- Automate pipette (200-1000 μL)
- SPME manual holder
- SPME fibre (65 µm PDMS/DVB coating for manual holder)
- SPME inlet guide
- Mangnetic bars
- Decanter class
- HS vial (20 mL)

Install the SPME fibre inside the SPME manual holder.

Analyses are made with GC-MS device described in Table 1. The inlet was set to 200 ºC. Column is initially kept at 40 ºC for 5 min and then ramped at 15 ºC/min to 200 ºC and kept for 3 min. The length of oven programme is 18.70 minutes. Set the flow at 0.9 mL/min. Use MS electron impact (EI) mode and set SCAN parameters for low mass 30.0 and high mass 650 m/z. Ion extraction is used to find the compounds from the chromatograms and to compare their peak areas to each other's.

Table 1. Analysing instrument

Analysis device	Description
GC	HP 6890 series system
Column	CARBOVAX™ AMINE FUSED SILICA Capillary Column 30 m x 0.53 mm x 1.0 μm film thickness
MS	HP 5973
Programme	ChemStation Version D.03.00.611

2 Analysing samples

Paper and board samples

Weigh approximately 2 g of sample and cut it into peaces approximately 1 cm^2. Put the weighed sample in a decanter class with magnetic bar and 20 mL of 0.5 M HCl with a cover class on the top of it and place it in a decanter class together with a magnetic stirrer for 15 minutes with slight stirring (200-400 rpm). Then take the decanter glass to the fume hood and leave it to rest for two minutes.

Process chemicals

Weigh approximately 2 g of chemical and dilute it in 20 mL of 0.5 M HCl for 15 minutes with slight stirring (200-400 rpm) in a decanter class with cover class on top of it. Then leave the container rest for two minutes.

Extracting

Put a clean magnetic barrel into a HS vial and take 1 mL of sample liquid and 1 mL 15 M NaOH with an automate pipette into the vial. Cap the vial. Put the vial in a decanter glass together with a magnetic stirrer (400 rpm) for 15 min and insert the needle of SPME holder with the help of inlet guide (Figure 1, pp. 4) through the injection port of GC and expose the fibre. Meantime you can insert the sample information to the GC. After 15 minutes, remove the fibre from the inlet and put it in the headspace of vial for 5 minutes.

Analysing sample

Start the analysing method (Aino.m) and because injection is made manually push the prerun button of GC. When everything is clear, put the needle inside the inlet, expose the fibre and and start the program by pushing start button. Remove the fibre from the inlet after two minutes.

After running the GC programme Seek nitrogen compounds from the chromatogram. Use the extract ion chromatograms to look for compounds. Best ions for this are listed for tested compounds in table 2.

Table 2. Best ions for extract ion chromatograms and molecule ions

Amine	Best ion (m/z)	Molecule ion (m/z)
MA	30	31
DMA	44	45
TMA	58	59
n-propylamine	59	59
Diethylamine	73	73
Diethylethanolamine	86	117
TBA	142	185
Dodecylamine	128	185
Cyclohexylamine	56	99

3 Note when extracting with SPME needle

There are some instructions when using SPME fibres. In this method SPME fibres with PDMS/DVB coating are used. SPME fibre must be conditioned before use and after storage. In conditioning the injection port temperature must be set according to the instructions and fibre inserted at the inlet with the help of inlet guide (Figure 1). For PDMS/DVB coated fibre the conditioning temperature is 250 ºC and recommended operating temperature 200-270 ºC. Conditioning time is half an hour, but longer time will not hurt the fibre. After conditioning the fibre must the GC column be conditioned for half an hour at the upper temperature of the program.

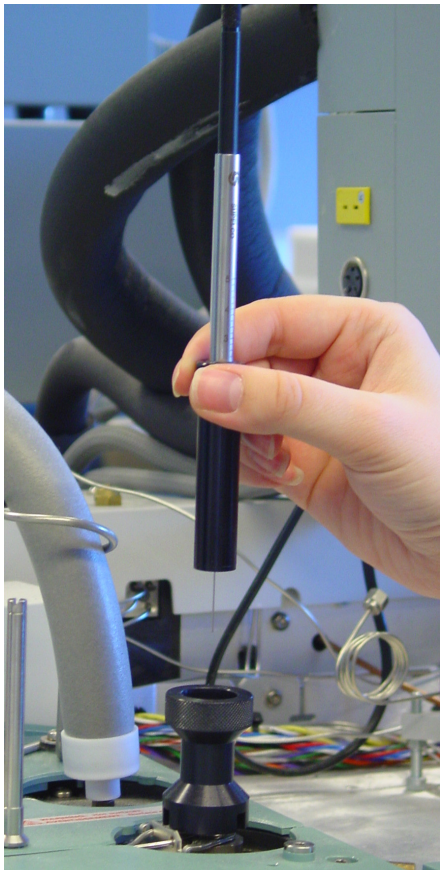


Figure 1. Inlet guide.

Blank analysis is preceded after conditioning. Blank analyses should be made at the beginning of each day to ensure that the fibre and needle are not contaminated. If fibre is contaminated it can be severely cleaned. For cleaning there are two methods. First method is to heat the fibre to 20 ºC below its maximum temperature for from one hour to overnight. A second alternative is to rinse the fibre in a water miscible solvent for one hour and then thermally desorb it for 30 minutes. PDMS/DVB has bonded phase and it is stable with all organic solvent. Suitable solvent for PDMS/DVB fibre cleaning is ethanol. The manufacturer promises that fibre can be reused 50-100 times. When fibre does not get free of contaminants or you notice that fibre does not work properly it have to be changed.

The fibre should be consistently desorbed at the same depth in the injection port and for the same length of time for all standards and samples to ensure the reproducibility. Every day before sample analysis blank desorption should be made to ensure that the needle and the fibre are free of contaminants.

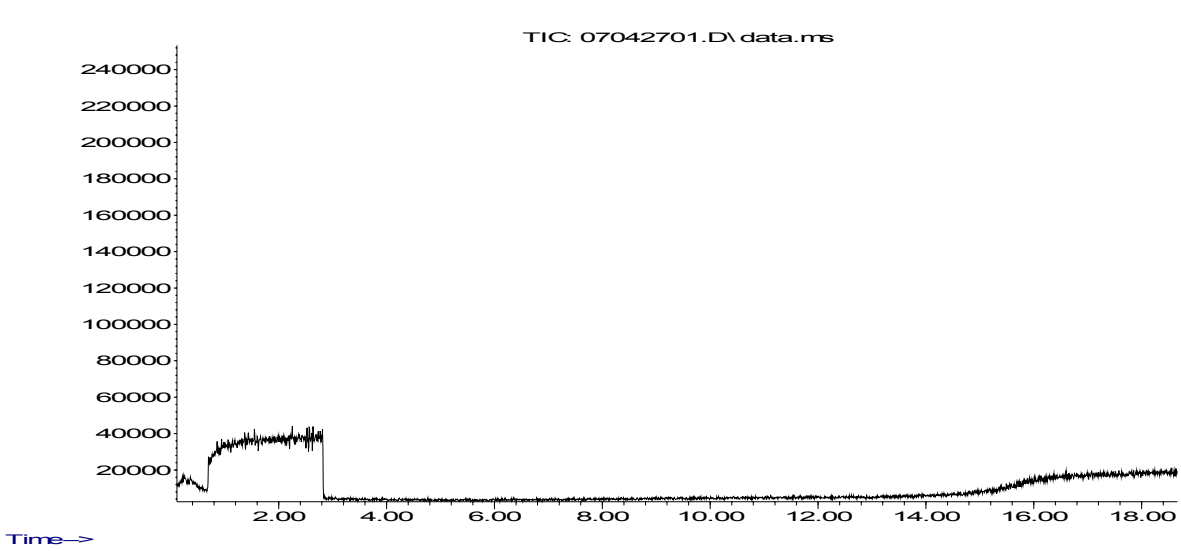
Extraction devices used are presented in Table 5. 0.5 mL of sample liquid was pipetted to the headspace vial (20 mL) with 0.5 mL of internal standard (concentration 5.0 μg/mL) chosen and 1 mL 15 M NaOH. A magnetic stirring bar was added and then vial was capped. The vial was put in a magnetic stirrer (400 rpm). SPME fibre was inserted into the inlet of GC in 200 ºC. After 15 minutes fibre was inserted inside the vial for headspace extraction carefully avoiding the spillage onto the fibre. Extraction time was 5 minutes and directly after extraction fibre was inserted to the inlet of GC and analysed. With real paper and board samples and process chemicals internal standard was no longer added to the vial and sample amount pipetted in the vial was then 1 mL.

References

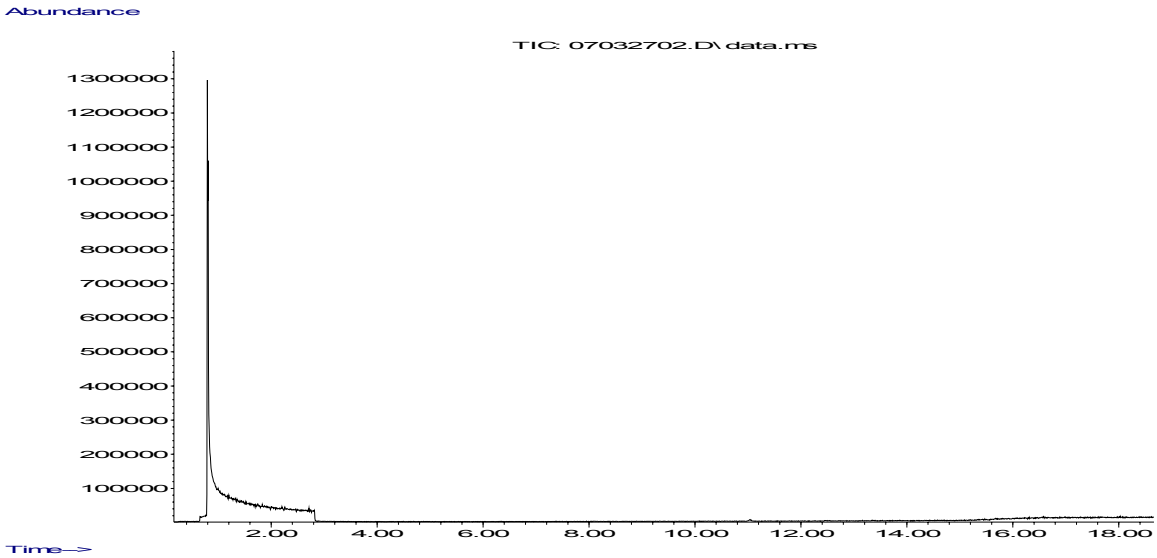
1. Tice, P. and Offen, C., Odors and taints from paperboard food packaging, *Tappi J.*, 77(1994)149-154.

Chromatograms

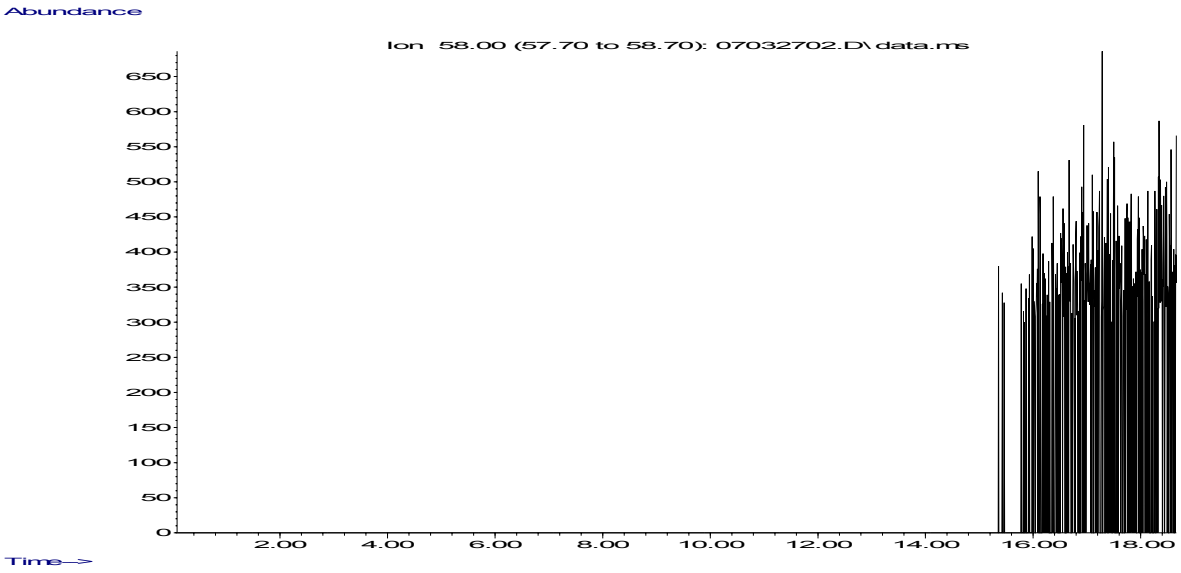
Empty column



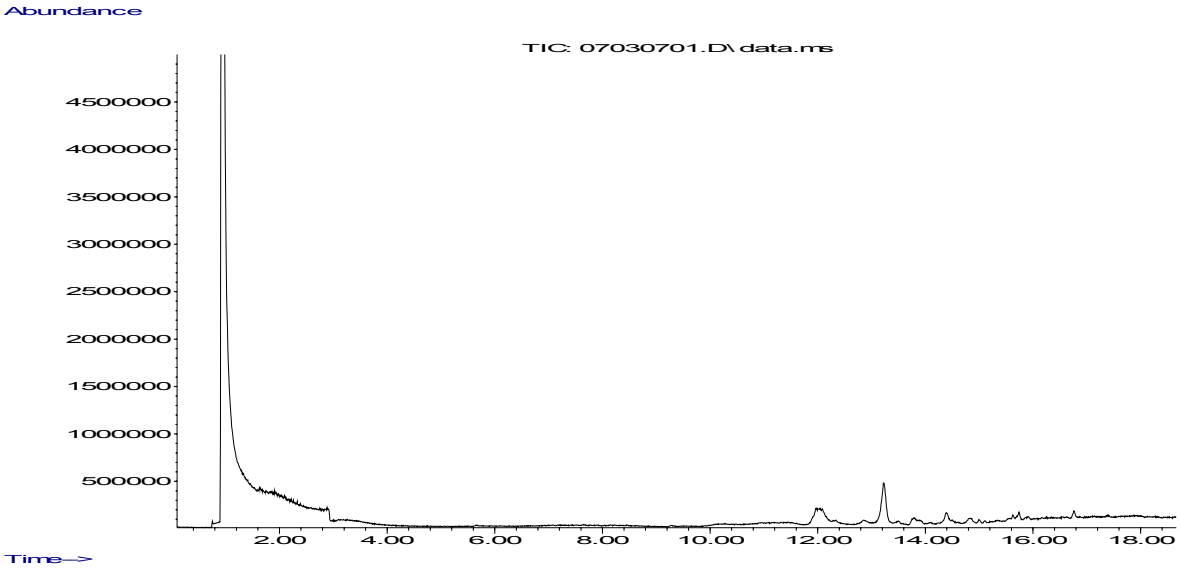
Blank fibre

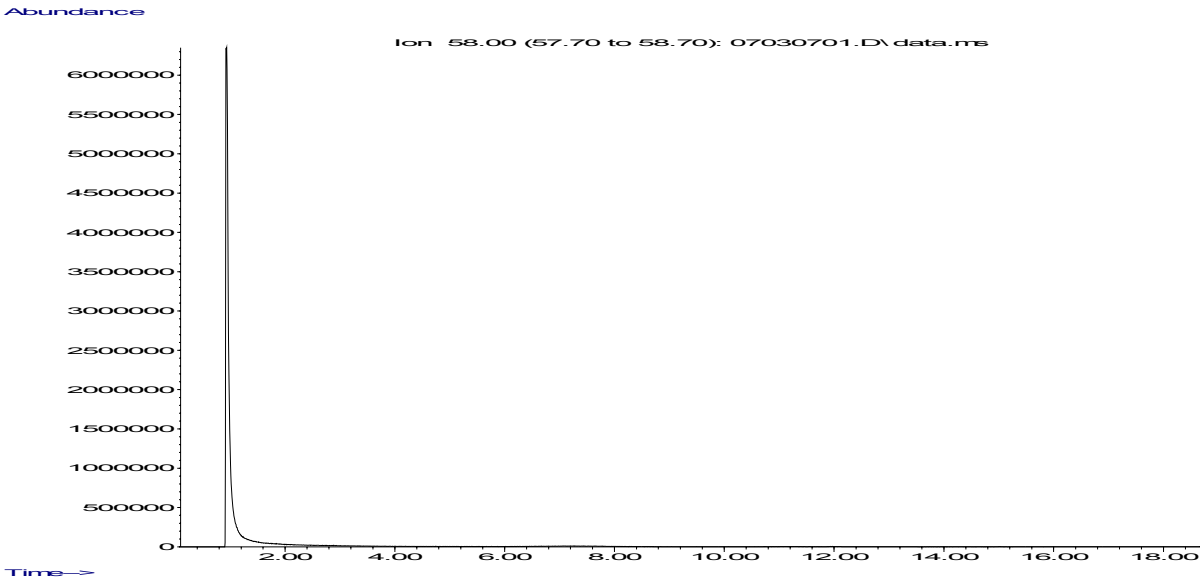


Extracted ion chromatogram of blank fibre with ion 58.

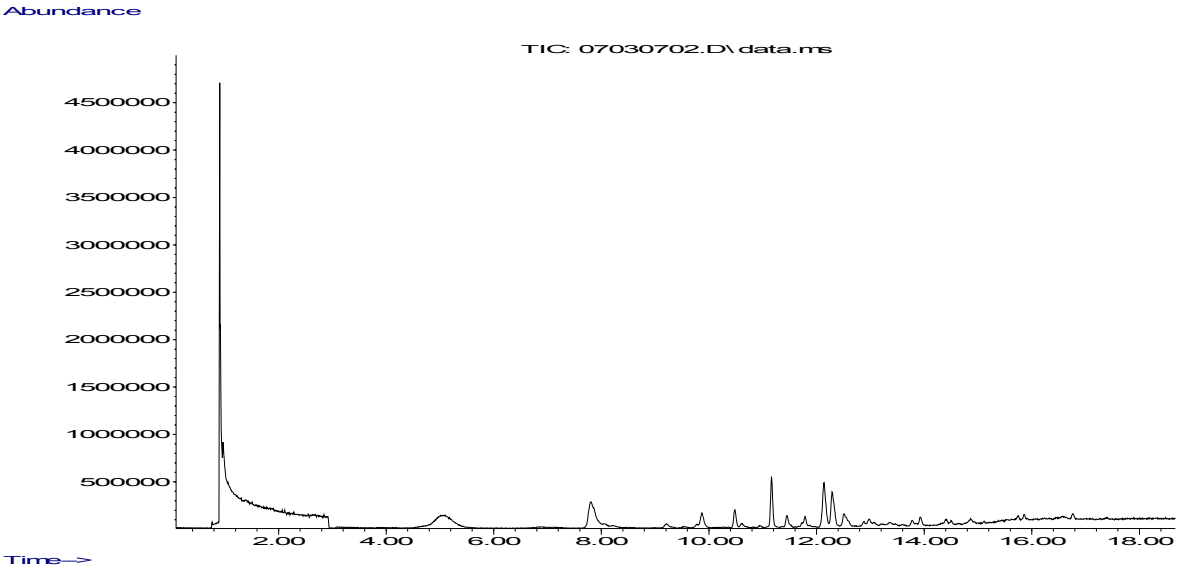


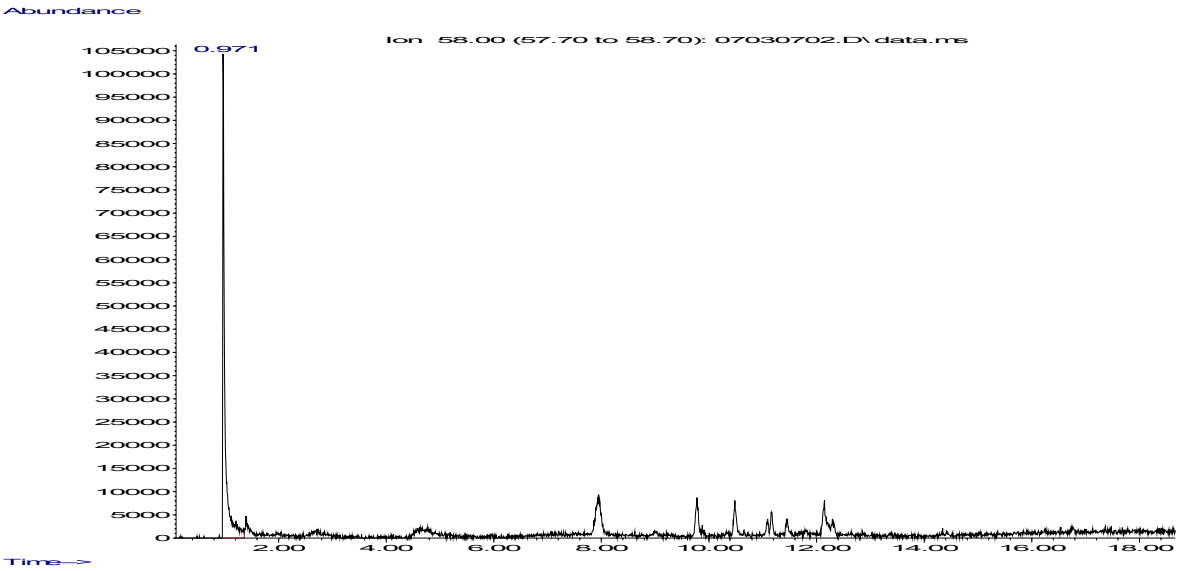
TMA sheet 1000 ug/ml (dipping test)



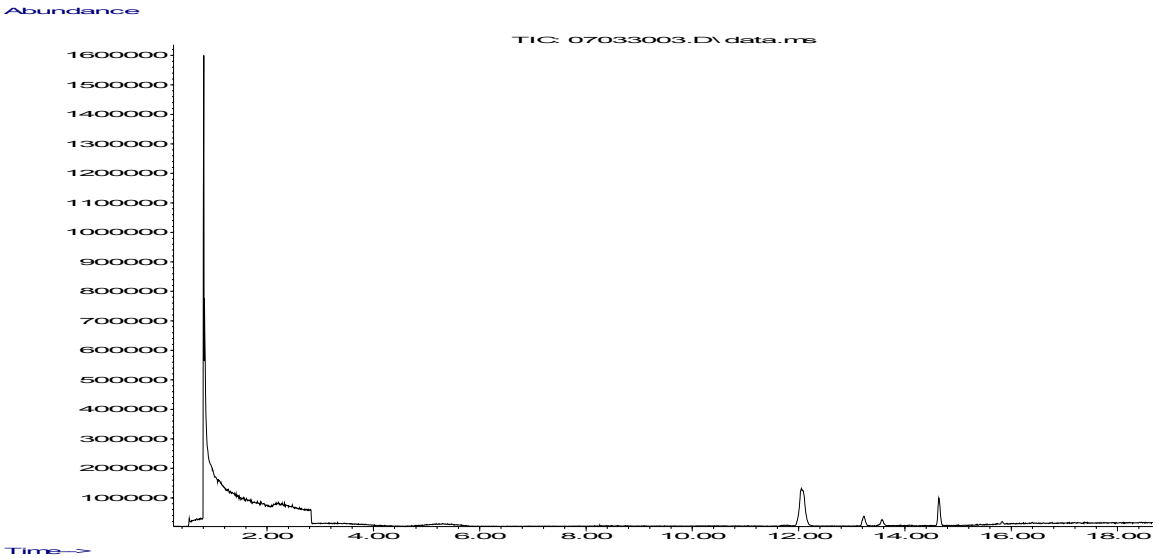


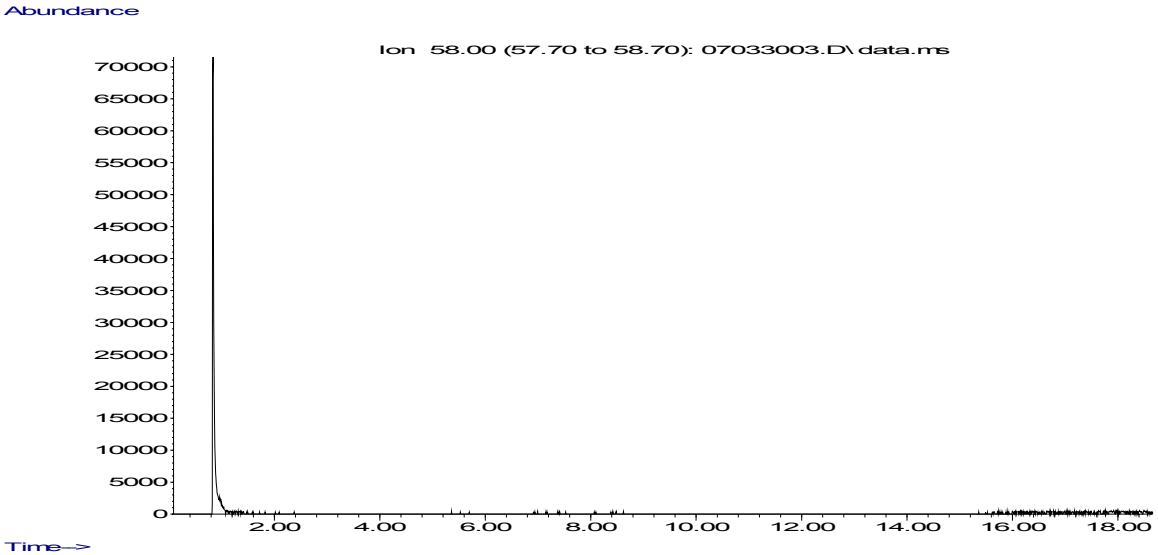
TMA sheet 10 ug/ml (dipping test)

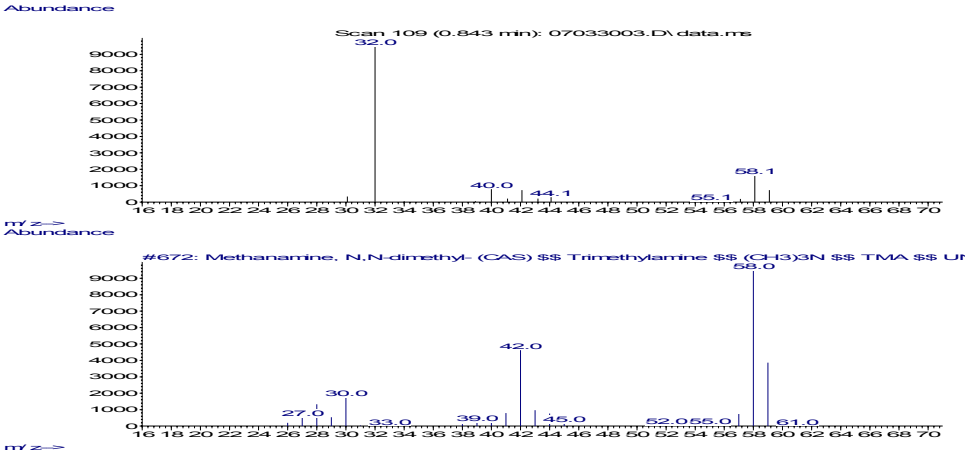




Latex 1


Abundance
TIC: 07033003.D\ data.ms
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time-->


Abundance
Ion 58.00 (57.70 to 58.70): 07033003.D\ data.ms
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time-->


Abundance
Scan 109 (0.843 min): 07033003.D\ data.ms
32.0
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
40.0
44.1
55.1
58.1
16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70
m/z-->

Abundance
#672: Methanamine, N,N-dimethyl- (CAS) $$ Trimethylamine $$ (CH3)3N $$ TMA $$ Ut
58.0
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
42.0
30.0
27.0
33.0
39.0
45.0
52.055.0
61.0
16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70
m/z-->