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Reducing environmental impacts of pharmaceutical sector: some opportunities in quality control

Master's thesis in organic chemistry

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Content

1 Introduction

Since two decades there has been increasing environmental concern regarding pharmaceuticals. A lot of attention has been paid to the investigation of residues of pharmaceutically active substances in the aquatic environment from healthcare activities. Similarly to other industries pharmaceutical industry attempts to minimise its environmental footprint by increasing efficiency of resource usage across all aspects of its business. In recent years overall sustainability of the pharmaceutical sector and healthcare system has become an issue. The need of balancing the health and well-being of society with the environmental impacts caused by design, usage and practices surrounding pharmaceuticals has been realised.

A number of advancements are applied already bringing sustainability in healthcare closer to reality. Regarding environmental aspects, the key words are green chemistry applied to drug design, formulation, manufacturing and packaging, environmental risk assessment for new pharmaceuticals, minimising greenhouse gas emissions in distribution chain, etc. But so far almost no attention has been paid to the environmental aspects of quality control methods in the sector.

Recent changes in the European Union chemicals legislation by adoption of REACH Regulation have raised some concerns. The main exemption for pharmaceuticals only covers substances that are “used in” medicinal products, i.e. active substances and excipients, but does not cover processing aids, solvents or any other chemicals involved in the manufacturing cycle, and also in quality control. As quite a number of reagents used in analytical methods fall in the category of “substances of very high concern”, they supply is not guaranteed in the long-term perspective. Thus substitutes should be identified and the “dirty” substances phased out, but regarding the requirements of pharmaceutical legislation, it is not a straightforward process. When a medical product receives approval for marketing, it relates to the medicine, the method by which it was manufactured and also the quality control methods. Besides quality control methods are often defined by pharmacopoeias, including the European Pharmacopoeia prescribing specific standard methods for active substances, excipients, etc.

Technical guidance documents for the elaboration of European Pharmacopoeia monographs include general recommendation to avoid the use of reagents that are acknowledged to be

extremely toxic or otherwise hazardous (e.g. carcinogenic), or prohibited or restricted in one or more of the States party to the European Pharmacopoeia Convention. But there are only a few systematic practices to be mentioned. Some member States have expressed concern to include more environmental considerations while elaborating the monographs.

Taking into account that introduction of changes into validated standard methods might take years, it is ultimate time to start wider discussions on "greening" of analytical methods applied in quality control of pharmaceuticals.

Purposes of the current thesis are as follows:

a) introducing concept of green analytical chemistry and assessment its potential to become a concept systematically applied while developing standard methods in pharmaceutical analysis;
b) assessment of usage of hazardous chemicals in European Pharmacopoeia monographs and current practices regarding substitution of hazardous substances.

Taking into account problems faced in the Laboratory of State Agency of Medicines, Estonia, while working with official methods for pharmaceutical analysis, and information received while participating in the work of the European Pharmacopoeia Commission, following test objects were selected:

- ethanol to demonstrate "greening" potential of a pharmacopoeia monograph, including importance of clear definitions in official methods;
- chloroform to illustrate substitution practices in the European Pharmacopoeia.

Considering the scope of a master (MSc) thesis, it was not attempted to give comprehensive overview of all aspects to consider in "greening" of analytical methods nor providing full green concept to be considered by the European Pharmacopoeia. More detailed comparative evaluation, e.g. by using cleaner production methodology, which is taking into account simultaneously technical applicability, economic and environmental performance, could be done in further works.

2 Literature

2.1 General background: reduction of environmental impact of chemical processes.

Chemicals are present in all spheres of human life, among other numerous applications, they are used as pharmaceuticals, food additives, pesticides. In the long term, synthetic chemicals end up in the environment, and in many different ways. The chemical industry is a point source of emissions having impact mainly around that point. In everyday life the constituents and ingredients of consumer of household products and other open applications emit chemicals into the environment as non-point sources. Very often the degradation of substances in the environment is incomplete, and also transformation products can have toxic effects to environmental organisms and humans. The first and most influential description of the dangers related to chemicals in the environment is found in Rachel Carson's *Silent Spring*, published in 1962.

Polychlorinated biphenyls (PCBs) are a classic example of persistent pollutants. PCBs were synthesized for the first time in 1887, and as early as 1899 severe health problems associated with the handling of PCBs were reported. Since then their neurotoxic effects, carcinogenicity, bioaccumulation and effects to biota due to accumulation in food chain have been described in detail. Despite this knowledge, it was not until 1999 that PCBs were completely banned within the European Union (EU). This example cleary demonstrates that it is not only the time lag of the impact of the chemicals on environmental processes, but also the time lag of economic and political systems which has a significant effect.

There are other examples of long-term introduction of persistent organic chemicals into the environment resulting in need for global action to avoid deadly consequences: organochlorine pesticides (e.g. DDT, aldrin), industrial and combustion processes by-products polychlorinated dioxins and furans (PCDD/F). Even non-toxic chemicals can cause global concern - inert organohalogens called freons were developed in the early 20th century to be used as refrigerants. But in the 1970s scientific evidence showed that these human-produced chemicals are responsible for observed depletions of the ozone layer.

Growing social consciousness has lead to adoption of new environmental and chemical regulations. Attempts to achieve sustainable development, taking equally into account the ecological, economic and social dimensions, have created a new framework in which pollution prevention is the central consideration. The mind-set of technological progress must be changed from treatment to the prevention [1].

There are at least three options for decreasing the input of chemicals into the environment:

- the technical approach: advanced treatment (short- to medium-term effort);
- the education and training of users, *e.g.* retailers and consumers (medium-term effort);
- the substitution of critical compounds with benign ones (long-term effort).

The traditional short- to medium-term approach for the prevention and reduction of the input of chemicals into the environment during and after their use is containment. This is not an option for chemicals used in everyday life. In this case, the treatment if emissions, *e.g.* effluent, air or waste treatment – or "end of pipe technology" – is applied. It is difficult, if not impossible, to take this approach if the chemicals enter the environment *via* non-point sources. The treatment of emissions is often not satisfactory and can create new problems. Furthermore, it is often the case that not all trace compounds are fully removed by such treatment.

In any case, the treatment of emissions results in increased energy demand and additional costs, and may also create new wastes to be treated. "End-of-pipe" treatment can be viewed as an opportunity to buy time in order to develop more sustainable approaches. However, if a big investment has been made in providing one particular solution, *e.g.* advanced wastewater treatment, this technology has a tendency to persist even if the problems have changed, the technology has advances, or it is no longer adequate for a particular problem.

Some progress has been made with regard to medium-term strategies and containment at source. Responsible care and product stewardship have been created and implemented within a number of industries, which has contributed to the reduction of emissions. In the case of chemicals used in open systems, this approach has its limitations and is not always very efficient.

The third and long-term approach is the objective of Green Chemistry – to make chemistry itself more sustainable, and the key to sustainability is the design of chemicals and pharmaceuticals which are not harmful to the environment. This calls for a different understanding of the functionality of a chemical: its manufacturing and use, as well as its fate after use. [1]

2.1.1 Principles of green chemistry

Designing chemicals to meet both the requirements of their application and environmental considerations, throughout their life cycle, is ambitious and quite new approach. Green chemistry, also called sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances. Safety for humans and the environment, as well as the economical use of resources must be of utmost consideration in the development of new chemical processes. In the U.S., the Pollution Prevention Act of 1990 encouraged the US EPA to pursue alternative pathways for chemical synthesis in line with the above-mentioned principle. In 1993, this approach was formalized as the Green Chemistry Program [2].

The similar preventive approach is recognised in EU (more details given in Chapter 2.2.1). Guidance documents adopted in the integrated pollution prevention and control framework consider green chemistry as a key feature of best available techniques [3].

In 1998 were defined 12 main principles of Green Chemistry to ensure that “chemical products should be designed so that at the end of their function they do not persist in the environment and do break into innocuous products” [4]:

1. **Prevention:** It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy:** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less Hazardous Chemical Syntheses:** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. **Designing Safer Chemicals:** Chemical products should be designed to effect their desired function while minimizing their toxicity.
5. **Safer Solvents and Auxiliaries:** The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. **Design for Energy Efficiency:** Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. **Use of Renewable Feedstocks:** A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. **Reduce Derivatives:** Unnecessary derivatisation (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. **Catalysis:** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. **Design for Degradation:** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. **Real-time analysis for Pollution Prevention:** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. **Inherently Safer Chemistry for Accident Prevention:** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

These principles must become an essential part of the core chemistry as well as pharmacy. They illustrate the areas where the most intensive study is needed: catalysis and catalysts, prevention of chemical derivatisation, replacement of solvents and auxiliary substances, use of renewable resources, and saving energy. The influence of these principles can be illustrated best by the number of publications referring to Green Chemistry: ca 120 in 1999, ca 720 in 2009 [1].

Green chemistry will play a central role in reducing the environmental footprint of pharmaceuticals and in striving to make drug-based medical care more sustainable. Opportunities for the application of green chemistry span the entire lifecycle of the pharmaceutical, ranging from drug discovery and design, manufacture, formulation, delivery and packaging, to the treatment of waste. Progress in any of the following, for example, can serve to reduce the footprint of active pharmaceutical ingredients: [5]

- streamlining drug discovery, for example by capitalizing on ethnobiology, which in turn can catalyze the protection of endangered geographic locales; computational approaches for developing candidate leads;
- synthetic routes which have less reliance on hazardous reactants, reduced production of hazardous waste, or lower energy consumption, such as use of biocatalysis;
- optically pure active pharmaceutical ingredients that eliminate non-therapeutic isomers and reduce overall dose;
- chemical structures which are more amendable to microbial or physico chemical structural degradation, which lead to shorter environmental half lives and reduced potential for bioconcentration in non-target organisms, and structural transformation to more innocuous end products;
- structures or delivery formulations that facilitate the active ingredient in selectively reaching this biological target, thereby reducing dosage without the need to increase potency;
- packaging that promotes a longer shelf life or provides accurate real-time indications of expiry status, reducing the need for disposal, something that is especially important for those drugs sensitive to light, moisture or oxygen;
- waste treatment approaches for destruction that can be adopted by existing waste and drinking water treatment facilities or even by health care and consumers.

Regarding sustainability, there are also concerns that a narrow interpretation of Green Chemistry as “the use of chemistry for pollution prevention”, might lead to doing the same things better, rather than rethinking solutions altogether. For example, a less-polluting alternative to a synthetic organic pesticide could be organic farming, which may obviate the need for the pesticide completely [1].

2.1.2 Green analytical chemistry

The activity of analytical chemists in laboratories, i.e. through uncontrolled disposal of chemical waste, used reagents, etc., may also exert (however to a lesser extent) a negative influence on the environment. Analytical chemistry is considered to be a small-scale activity, but this is not always true in the case of controlling and monitoring laboratories whose number of runs performed is high. This makes an analytical laboratory comparable with the fine chemicals or pharmaceutical industry [6]. For example, in environmental monitoring a paradoxical situation has emerged because most of the analytical methodologies employed to investigate environmental problems generate chemical wastes, resulting in an environmental impact. In some circumstances, the chemicals employed are even more toxic than the species being monitored [7].

Therefore, further development of green chemistry should also comprise the development of green analytical chemistry. Analytical chemistry has not often been in a focus of green chemistry as illustrated by quite a few numbers of annual publications: about 5 in1995, about 25 in 2005 [8].

Green analytical methods would meet one or more of the twelve principles of green chemistry. Considering those it is easy to indicate the directions which will decide about the "green" character of analytical chemistry. The following should be treated as the top priorities:

- eliminating or minimizing the use of chemical reagents, particularly organic solvents, from analytical methods,
- eliminating from analytical procedures chemicals with high toxicity and ecotoxicity,
- reducing steps demanding much labour and energy, in particular analytical methods (per single analyse).
- reducing the impact of chemicals on human health [9].

Green Analytical Methodology definition “The use of analytical chemistry techniques and methodologies that reduce or eliminate solvents, reagents, preservatives, and other chemicals that are hazardous to human health or the environment and that also may enable faster and more energy efficient analyses without compromising required performance criteria” encompasses three key concepts:

- Primary consideration for selecting or modifying an analytical method is that it be able to meet specified performance criteria. These criteria may be referred to as “measurement quality objectives”. To use a method that fails to meet them would result in wasted time and money because the analytical data produced by it would not be able to be used.
- Second key concept is to use less toxic or hazardous solvents or chemicals in sample preparation and analytical measurements.
    - If possible replace hazardous chemicals with less hazardous chemicals;
    - If hazardous chemicals can’t be replaced then use smaller amounts of them;
    - Sample preservation and/or preparation steps are best places to look for opportunities.
- Third key concept is to decrease the amount of time and/or energy required to perform an analysis. It is accomplished by using smaller samples *e.g.* by making method more sensitive so less sample is needed for analysis, or by using *in-situ* measurements - replacing a method requiring sample preparation (e.g., atomic absorption) with *in-situ* analysis (e.g., x-ray fluorescence) [8].

Successful green analytical methodology also results in financial (more efficient resource use, saving time and labour by using faster techniques) and sociological advantages (reducing health and safety hazards for the analysts). The development of instrumental methods to replace wet chemistry in sample preparation and treatment is a general trend in analytical chemistry. Here, the main analytical result is related to an increase of analysis reliability, higher precision, and time saving, which very positively combines with a substantial reduction of waste. In most cases, the result of instrumental methods in analysis is a decrease in sample volume needed for analysis. In general, it leads also to an efficient use of energy, especially when the method is highly automated [6].

In clinical tests the need for automatic methods rose already in the 1950s, and the segmented flow analysis (SFA) was developed, which afforded not only substantially increased throughput, but also substantial savings in samples and reagents. SFA laid the foundations for modern computer-controlled flow techniques, which further dramatically decreased the needed sample and reagents volumes [7, 10].

In the pharmaceutical area Process Analytical Technology (PAT) has a good potential to meet the criteria of green chemistry. PAT has a history of more than 70 years. The early process analysis methods involved taking samples from various process streams and transporting them for tests in a central analytical laboratory. Then it was understood that real-time measurements result in more valuable data about a process. Currently PAT is mostly comprised of analytical technologies applicable to process with remote, real-time and high-throughput measurements. Data received enable development of sophisticated process models to substantially improve the process control [1, 11].

2.1.3 Green analytical chemistry in chromatography

The main demands for analysts using chromatographic methods wishing to implement the principles of green analytical chemistry are as follows:

- utilisation - as much as possible – "direct" chromatographic analysis, which permits us to determine analytes in a sample without any pretreatment or sample preparation;
- reduction of labour and energy consumption, e.g. reducing sample preparation time when direct chromatographic analysis is not possible;
- elimination or reduction of the amount of solvent from sample preparation steps applied before final chromatographic analysis;
- conducting all operations with solvents in a hermetic systems;
- reducing matrix interferences;
- reducing chromatographic run time;
- reducing the need for re-analysis;
- integration of steps of analytical procedures, i. e. by using hyphenated techniques [9].

In gas chromatography, first of all, eliminating or minimizing the amount of solvent in sample preparation techniques before final chromatographic analysis is highly recommended. Therefore, techniques using gas and supercritical fluids for extraction of many pollutants are very popular [12]. In environmental analysis preconcentration of air pollutants followed by thermal desorption has become a standard method. Advantages of thermal desorption versus conventional solvent extraction include 1000-fold improvement in detection limits, no chromatographic interference from solvent or solvent impurities, enhanced sample throughput

and lower cost per analysis. Another benefit is that thermal desorption is a very straightforward gas extraction process [13].

Since the introduction of gas chromatography in 1952, there have been unceasing efforts in improve separation speed as reduction of overall analysis time provides significant savings in time and money. The use of high speed or fast chromatography can be especially attractive for laboratories where many routine samples are analyzed on a daily base. It can also be beneficial in situations where short time-to-result is needed. The main routes toward faster separation include, amongst others:

- decreasing the inner diameter of the capillary columns
- fast temperature programming;
- application of shorter columns;
- working at turbulent flow;
- vacuum outlet operation;
- working above optimal carrier gas velocities [13].

One of the possibilities to achieve higher speed separations is using *porous layer open tubes* or PLOT columns introduced in 1990s. They are capillary columns where the stationary phase is based on an adsorbent or a porous polymer. PLOT columns are currently widely used in many applications, their main advantages are that they are highly selective and can be operated at much higher temperatures compared with liquid type stationary phases. However because the absorbent layer is built up from particles this can result in detector contamination from the particles and some cases non reproducible flow [14].

Coupling of gas chromatography with techniques having high identification ability, e.g. with mass spectrometry, (GC/MS) has gained prominence because confirmation can be achieved in the same step as analysis with second dimension of information. This provides increased confidence in the result in conjunction with increased effectiveness [9].

In HPLC one of the options is the replacement of solvent in method (*e.g.* acetonitrile for HPLC analysis with alternative solvents such as ethanol – more detailed preferences are given in Table 1.), and solvent reduction through the use of narrow-bore columns [15].

Table 1. Green Chemistry Solvent Selection Guide [15]

Preffered	Usable	Undesirable
Water	Cyclohexane	Pentane
Acetone	Heptane	Hexane(s)
Ethanol	Toluene	Di-isopropyl ether
2-Propanol	Methylcyclohexane	Diethyl ether
1-Propanol	t-Butyl Methyl Ether	Dichloromethane
Ethyl Acetate	Isooctane	Dichloroethane
Isopropyl acetate	Acetonitrile	Chloroform
Methanol	2-MeTHF	DMF
MEK	THF	N-Methylpyrrolidone
1-Butanol	Xylenes	Pyridine
t-Butanol	DMSO	Dimethylacetamide
	Acetic Acid	Dioxane
	Ethylene Glycol	Dimethoxyethane
		Benzene
		Carbon Tetrachloride

2.2 Substitution as a key preventive approach

Chemicals risk management concepts and related guidance documents suggest a large range of measures, ranging from elimination, modification or replacement of processes or products, control of emissions to exposure reduction by personal protection measures. Preventive approaches, including green chemistry, consider hazard reduction by substitution, which means “the replacement or reduction of hazardous substances in products and processes by less hazardous or non-hazardous substances, or by achieving an equivalent functionality via technological or organisational measures [16]. The key aspect in this definition is the functional equivalence, i.e. the achieving of the same functionality by lesser hazards, leading to a reduction of the quantitative input and/or enable the use of less hazardous chemicals.

The other key aspect is how to assess whether a chemical is “hazardous” and which one is “less hazardous”. A requirement for industry to classify substances and preparations according to standard criteria has long been a feature of the EU’s chemicals legislation. Currently Regulation No 1272/2008 on classification, labelling and packaging of chemical substances and mixtures (CLP) will replace after a transitional period current system for dangerous substances (Directive 67/548/EEC, or DSD) and for preparations (Directive 1999/45/EC). CLP Regulation takes over the classification criteria and labelling rules agreed

at United Nations level, the so-called Globally Harmonised System (GHS). GHS is based on the principle that the same hazards should be described in the same way all around the world, and it is expected to facilitate trade and to contribute towards global efforts to protect humans and the environment from hazardous effects of chemicals [17]. In EU, harmonised classifications and classifications received from manufacturers and importers of substances will be included in Classification & Labelling (C&L) Inventory. It is a database which is established and maintained by European Chemicals Agency (ECHA), and will available for public late 2010 [18].

2.2.1 EU legal framework as driver for substitution

Key drivers and barriers towards substitution are identified particularly with regard to economic factors, technical functionality, communication and social factors, risk information and the regulatory framework, that is set by legislation and standardisation. In any case, enterprises need clarity about strategic policy goals and legislation on substitution of hazardous substances, as well as trust in the enforcement capacity of authorities. [16]

In the EU and its Member States efforts towards substitution of hazardous substances can be found in numerous pieces of legislation and government programmes. As a general rule, substitution applies for workplaces in all EU Member States: if a less hazardous substance is available and can be introduced with reasonable means it has to be introduced [19]. For substances with very hazardous properties like carcinogenic or mutagenic substances, the legal requirements to substitute are more stringent on the protection of workers from the risk related to exposure to carcinogens at work for example, includes an obligation to substitute carcinogen substances if technically possible. Consequently, if the use if the carcinogen is maintained, the employer must provide justification to the authority upon request. If replacement is technically not possible a closed system is required. If this is not possible either, obligations to decrease the exposure as much as possible and some kind of monitoring must be applied [20].

For industrial processes having major pollution potential, the Integrated Pollution Prevention and Control (IPPC) system has established Best Available Techniques (BAT) based environmental permitting system. Definition of BAT includes options to prevent pollution by ”the use of less hazardous substances” in the processes [21]. The Directive on volatile organic

compounds (VOC) emissions includes an obligation to substitute the most hazardous solvents[1] [22]. For pharmaceutical industry VOC Directive is applicable if solvent consumption is exceeding 50 tonnes per year. Recently developed guidance documents provide sector specific information on VOC reduction and substitution measures, e.g. use of solventless film coating of tablets [23].

One of the strategies to facilitate risk prevention or risk reduction is setting up lists of (hazardous) substances. The Water Framework requires the establishment of a priority substance list. The substances of EU-wide of concern were selected in a monitoring and modelling based risk ranking procedure. Presently 33 priority substances (or substance groups) and 8 other pollutants are subject to cessation or phasing out of discharges, emissions and losses by 2020 as latest [25]. The measures may also include substitution of these substances in products and processes.

2.2.2 Substitution requirements in REACH regulation

Quite recently EU legal framework of chemicals management consisted of more than 40 directives and regulations developed since 1967. In the Strategy for a Future Chemicals Policy [26], the Commission laid the basis for a new strategy for ensuring a high level of chemicals safety and a competitive chemicals industry. One of the main reasons for developing new strategy was that a large number of substances had been manufactured and placed on the market in Europe for many years, sometimes in very high amounts, and yet there was insufficient information on the hazards that they pose to human health and the environment.

Regulation No 1907/2006 on registration, evaluation, authorisation and restriction of chemicals (REACH) entered into force on 1 June 2007. The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. Manufacturers and importers will be required to gather information on the properties of their chemical substances, which

[1] Similar approach is taken by medicinal products regulations while controlling amount of residual solvents in the product. Class 1 solvents (known human carcinogens, strongly suspected human carcinogens, or having deleterious environmental effects) should not be employed in the manufacture of drug substances, excipients, and drug products [24].

will allow their safe handling. REACH is very wide in its scope covering all substances whether manufactured, imported, used as intermediates or placed on the market, either on their own, in preparations or in articles, unless they are radioactive, subject to customs supervision, or are non-isolated intermediates. Waste is specifically exempted. Member States may exempt substances used in the interests of defence. Other substances are exempted from parts of REACH, where other equivalent legislation applies, e.g. pharmaceuticals [27].

Among other issues, the REACH Regulation calls for the progressive substitution of the most dangerous chemicals when suitable alternatives have been identified. For the substances which effects on humans and the environment are very serious and normally irreversible, REACH has introduced term 'substances of very high concern' (SVHC). These are:

- carcinogenic, mutagenic and reprotoxic substances of category 1 and 2 (CMRs);
- persistent, bioaccumulative and very toxic (PBT), very persistant and very bioaccumulative (vPvB) chemicals;
- identified from scientific evidence as causing probable serious effects to humans or the environment equivalent to those above on a case-by case basis, such as endocrine disrupters [28].

These substances have hazardous properties of such high concern that it is essential to regulate them centrally through a mechanism that ensures that the risks related to their actual uses are assessed, considered and then decided upon by the Community. Their uses will not be banned by default, but an **authorisation** is required for their use and their placing on the market. The authorisation procedure consists of two steps: in a first step, a candidate list is established. This list is published and periodically updated by the ECHA (see Annex I for the latest version). The interested parties can give comments about the candidate list. A decision is taken via comitology as to which substances will be included in the system and which deadlines will have to be met, or which uses will be exempted (e.g. because sufficient controls established by other legislation are already in place).

Once a substance is included in the system, in the second step of the procedure, those using or making available such a substance will need to apply for an authorisation for each use of the substance within the deadlines set including an analysis of possible substitutes. If this analysis shows that suitable alternatives are available then the application must also include a substitution plan. If not, information on relevant research and development activities must be

provided, if appropriate. An authorisation will be granted if the applicant can demonstrate that the risk from the use of the substance is adequately controlled. If not then it may also be granted if the socio-economic benefits outweigh the risks and there are no suitable alternative substances or processes. Substances, for which a safe level cannot be defined, cannot be authorised based on adequate control of risk. All authorisations will be reviewed after a certain time-limit which will be set on a case-by-case basis [1, 29].

Chemicals with go to authorisation does not have to belong to the registration. All substances which belong to authorisation may also go under the restriction process. If the substance is added to the authorisation it may not go to restriction list, if the risk to human health and environment is based on PBT and vPvB characteristics. The same time there might be restrictions to the field of use, which may cause risks to human health and environment by other properties and that is why their use is added to the restriction list. Authorisation cannot be granted to the uses which are brought out in restriction list [29].

The benefits of the REACH system will come gradually, as more and more substances are phased into REACH. The transitional periods and amount of information in the registration dossier depend on the manufactured or imported substance quantities and its properties – see Figure 1.

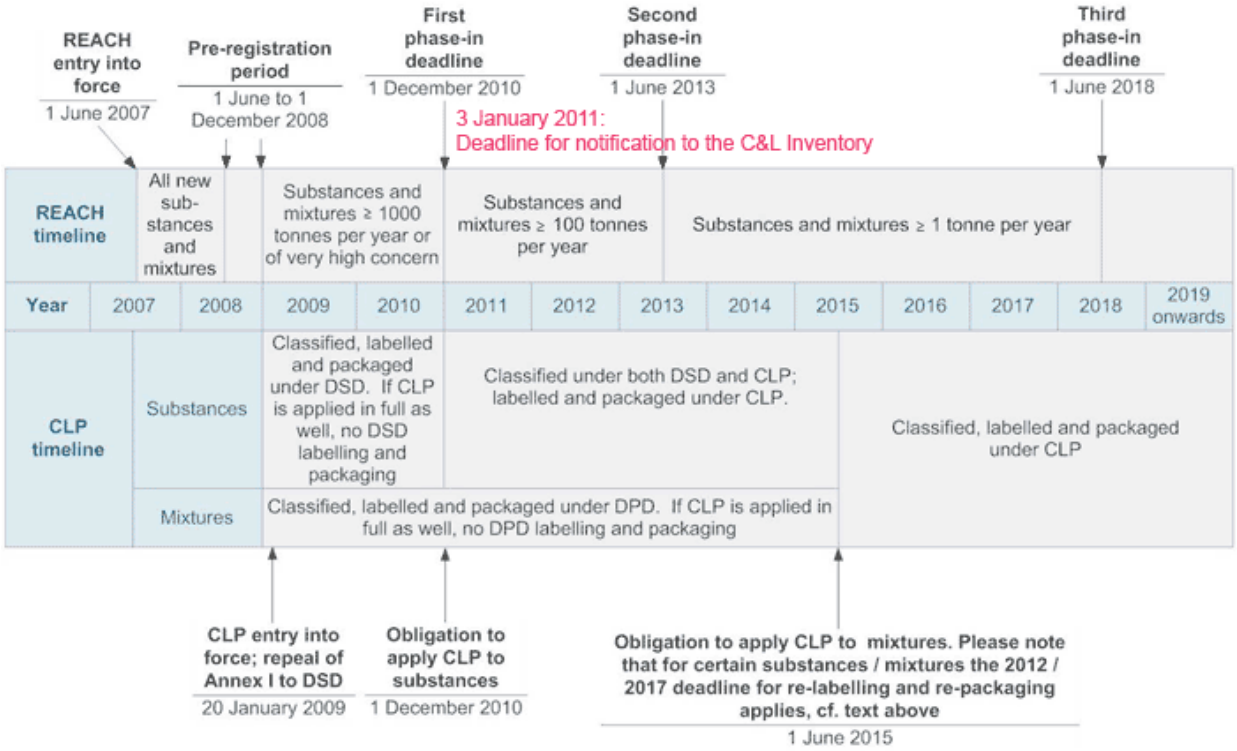

Figure 1. Transitional period in EC legal framework of chemicals management [30].

2.2.3 Practical considerations regarding substitution

Although legal framework is in place and huge number of guidance documents promoting substitution are available, the reality is quite poor. The main barriers are often short term economic considerations based on tendency to stick to well-established conventional substances, rather than undergoing any risks with respect to quality and liability by experimenting with less hazardous substitutes. In several cases, there is a considerable imbalance between the available risk information on a substance subject to substitution efforts, and its potential substitutes for which less information is available, partly due to the fact that they have never been used on a similar scale as the conventional substance. Besides, many of the guidances and case-studies are restricted to a specific group of products in a certain narrow application [16].

Even where functionally equivalent substitutes are readily available, economically viable and proven to be less hazardous, their introduction in a certain process or product is often hampered by the fact that complex communication along the supply chain is a prerequisite for implementation. It concerns especially pharmaceutical sector as manufacturers of active substances shall follow Good Manufacturing Practice requirements and any process modifications (including modifications in quality control methods - some further details are provided in Chapter 2.3.2) can be only carried out after approval by regulatory authority of medicinal products [3].

Substitution should be based on comparative assessment of alternatives which requires reliable information on the hazard of substitutes and the associated risk of using them. But products and processes are often labelled „green“ for advertising purposes without any solid evidence. An objective reason for this is the lack of measurable characteristics that can be applied to the greenness of a product or process. This is also the case for analytical chemistry, where the main emphasis is on the metrological quality of the data, and only recently has attention been directed to the environmental friendliness of the way the data are obtained [1].

There are several methods available to assess environmental performance of industrial processes at company level:

- Concrete risk assessments for the substances used focusing on hazardous properties and technical measures for reducing risk from product and production;

- Strategic instruments for the support of decision makers at companies prior to an investment in new products / production sites based on life cycle analysis (a technique to quantify and assess the inputs and outputs affecting the environmental performance of a product through its life cycle, from production, through use and, finally, disposal).
- Assessment tools used in procedures for awarding eco-labels or the adjacent criteria can be used as a guideline and a benchmark for companies' products. At the same time they might be an indicator for substances regulated in the future.
- Assessment tools used in the procedure for classification and labelling of chemical products [16].

Unfortunately most of these tools are not directly applicable for analytical methods or require a considerable amount of background knowledge. One of a few systematic approaches to assess greenness of analytical methods seems to be National Environmental Methods Index (NEMI), which is free internet-searchable database of over than 1 000 methods used in environmental analysis [31]. User can compare selected methods by performance characteristics (sensitivity, precision, bias, cost) and also by greenness profiles. Latter include the sample size that is worked up for analysis, chemicals used and amounts (to which the PBT and two hazardousness criteria are applied), pH (as corrosive criterion), and waste amount generated [32].

For simpler comparative assessments is applicable The Column Model, an informational tool on chemical hazards developed by the Institute for Occupational Safety (BIA) [33]. The model provides a simple practical aid for the identification of possible substitutes by using the information in the Safety Data Sheets or on the package labelling. The Column Model presents data on chemical hazards in a tabular format (see Annex II). The columns display different types of hazards: a) acute and chronic human health hazards, environmental hazards, fire and explosion hazards determined primarily by classification (assigned risk phrases [34]), b) exposure potential and liberation properties (mainly by aggregate state and liquid vapour pressure), and c) risks by technology mainly depending if a open, semi-closed or closed equipment is used. The rows give ranking by risk levels: very high, high, medium, low, and negligible risks. The Column Model is designed to compare alternatives only within a column and not across rows – in other words, substances can be compared for similar hazards only. Users must determine which potential hazards are of greatest relevance in their processes. For

more in-depth analysis, especially if dominance of a potential substitute across all six columns is low, dominance analysis and/or positional analysis can be used to compare the data [29].

More detailed assessment of analytical methods seems also possible by using cleaner production methodology, which in a project implementation feasibility analysis is taking into account simultaneously technical applicability (i.e. level of achievement of equivalent functionality), economic and environmental performance [35]. Cleaner production can be defined as a continuous implementation of an integrated preventive environmental strategy to processes, products and services to enhance the efficiency. It should lead to improved environmental performance, cost savings and the reduction of risks to humans and the environment. Cleaner production is achieved by applying know how, improving technology, and/or changing attitudes. It is worthwhile to mention that successful implementation of preventive strategies is dependent on presence of an environmental management system in an organisation [36].

2.3 Pharmaceuticals as a specific group of chemicals

Pharmaceuticals are a particular group of chemicals which have there own regulations, directives and guidelines, and therefore they are excepted from the scope of REACH regulation. It is considered that procedures applied by Directive 2001/83/EC for human medicines and Directive 2001/82/EC for veterinary medicines ensure at least similar safety.

The current EU pharmaceutical legislation clearly states that pharmaceuticals can have negative effects on the environment and that these must be reported. The elaboration of EU documents on the environmental risk assessment of human and veterinary pharmaceuticals began more than two decades ago; currently valid guidances were adopted in 2006 for human medicinal products [37], and in 2007 for veterinary pharmaceuticals [38]. Still, authorisation of a human medicines product, can explicitly not be denied if an environmental risk is identified. For veterinary pharmaceuticals the legal situation is different – authorisation may be denied, or may be limited to certain application areas, if evidence for an environmental risk exists. Besides, the risk assessments consider environmental exposure from the use of the medicinal product only, it does not consider the risk arising from the drug production and /or the disposal of used on unused pharmaceuticals [39].

2.3.1 Increasing environmental concern regarding pharmaceuticals

Approximately 550 peer-reviewed articles published on the issue "pharmaceuticals in the environment" in 1991-2008, but 65 % published rather recently, 2006 and onwards [40].

In principle, environmental issues have to be considered in design, manufacture, use and waste phases of pharmaceuticals. The objective of the design phase is to produce a regular stream of new medicines. A new drug will take from 8-12 years to develop from initial concept to marketable product. The companies have 50 to 150 potential drugs under research, but only few of them reach the market, e.g. in 2007 only 17 molecular entities [41].

From drug production there are risks for release of chemicals into surface and ground water, but also pharmaceuticals are a part of this release. The production is actually carried out in two stages. The first part produces the active ingredient, whilst secondary manufacture converts this active ingredient into a medicine that can be taken by the patient. Although these steps are supposed to be covered by requirements of other legislation, e.g. the eventual manufacturing plant will need to meet the consent conditions imposed by the local environmental regulator [39], there have been cases that relatively high concentrations of pharmaceuticals have been detected in surface, ground, and drinking water near production area, but the problem is minor compared to the situation is developing countries. Actually the global generic active pharmaceuticals ingredients (API) market is dominated by India and China. In 2004 42 % of APIs originated from these countries, but by 2010 already 60 % share is expected [42]. For example, in India is a common waste water treatment plant, which receives process water form ~90 bulk drug manufacturers. The “treated” effluent contained exceptionally high concentration of different pharmaceuticals, e.g. 28-31 mg/L of ciprofloxacin (fluoroquinolone antibiotic), 2,4-2,5 mg/L of cetirizine (H_1-receptor antagonist) [43]. The sampling program performed in nearby surface water bodies revealed up to 6.5 mg/L of ciprofloxacin and up to 1.2 mg/l of cetirizine, and in drinking water wells the concentrations were respectively up to 14 µg/L and 28 µg/L [44].

The use phase is considered to have the largest contribution to release APIs to the environment: a large proportion of produced finished medicinal products enter the sewers by excretion from patients. Not all of this is subsequently removed during wastewater treatment. In veterinary practice, veterinary drugs are released from treated animals to the terrestrial

environment. Large and increasing use of both human and veterinary pharmaceuticals has lead to the situation that about 160-180 different active pharmaceutical ingredients have been identified in the aquatic environment. In general, concentrations are in the range of ng to mg per litre of water, and it is believed that these concentrations are far too low to pose any threat to human beings and no immediate threat to wildlife [45]. But pharmaceuticals are designed to specifically and potently interact with biological molecules i.e. have an effect at low concentrations. So far, only few active pharmaceutical ingredients have been conclusively shown to cause adverse effects in organisms in the environment - there is scientific evidence that environmental exposure to steroidal estrogens from birth control pills caused harm to the reproductive systems in wild fish. Another example concerns terrestrial organisms – diclofenac (an anti-inflammatory agent) residues in the cattle meat has proven to be highly toxic to the kidneys of vultures, thus dramatically declining vulture populations in India and Pakistan. In addition, recent investigations have shown that several anti-psychotic and anti-depressant pharmaceutical substances could raise high environmental concerns [46].
Another potential source of pharmaceutical residues is unused and out-of-date medicines. Even if these pharmaceuticals may seem perfectly usable, their quality cannot be guaranteed and they should therefore not be reused. There are international regulations which declare that a pharmaceutical that has been in the possession of one customer should never be allowed back into the chain in order to be given to another customer. Unused medicines should, where possible, always be returned to a pharmacy which can ensure that such material is properly destroyed The return collection systems used by pharmacies was at first intended as a safety measure system, but nowadays it is mainly for the environmental reason [47].

The environmental impact from pharmaceuticals is not only caused by biologically active substances entering land and water. Energy consumption of the production processes and transportation in the distribution chain generate greenhouse gases. Although most major pharmaceutical companies have set themselves targets to dramatically reduce their energy demand as well as cutting their emissions of greenhouse gases, there is quite a space for improvement. In 2007 AstraZeneca reported that business travel by car amounted to 730 million kilometres, 90 percent of which was associated with sales and marketing (and only 10 percent of AstraZeneca’s total greenhouse gas emissions come from freight transport). This distance is equivalent to 18,300 times around the world and produced 150,000 tonnes of greenhouse gas emissions [47].

Another problem is overall use of chemicals. The following describes a pharmaceutical industry manufacturing process: the normal manufacturing yield from a single stage ranges between 35 and 95 %, with an average yield of 86 %. The typical primary manufacturing process involves six stages with an overall yield of 30-40 %. Overall yield does not capture the use of reagents, solvents and catalysts. If these are included, the average total material use is 16 kg/kg of stage product (intermediate). Even with a 100 % yield at each stage, a 16 kg/kg ratio of materials use would result in an overall mass productivity of about 1 %. It demonstrates that a large amount of the materials are not part of the product and could possibly end up in the environment as a result of the process [1].

All this issues raise the concern that the various roles played by pharmaceuticals in healthcare are currently not in balance with the needs of the ecological environment, and arguably with the needs of public health and wellbeing. The environmental footprint of healthcare could be substantially reduced and the overall effectiveness improved if we implement any number of a wide array of measures across the many facets of practice and administration of healthcare [5]. It is important to note that individual and societal overall health is determined by multiple of factors, including lifestyle considerations and public health measures. In the absence of such measures the availability of pharmaceuticals alone will not suffice to create a healthy society, even if the price of pharmaceuticals is affordable [41].

The new pharmaceutical strategy document that the European Commission presented in the autumn of 2008 points out that measures to prevent a pharmaceutical from having negative environmental effects may need to be incorporated also into other EU regulations and directives [48].

2.3.2 Quality control aspects

Very important aspect of pharmaceutical manufacturing is quality control. When a medicine receives approval for marketing, the authorisation relates to both the medicine, including its quality requirements, and to the method by which it was manufactured[2], including quality control methods. Simple product sampling techniques are insufficient to ensure the quality

[2] The manufacture of a pharmaceutical product is actually carried out in two stages. The first part produces the active ingredient, whilst secondary manufacture converts this active ingredient into a medicine that can be taken by the patient. It is necessary to control the pharmaceuticals quality and analytical methods at the both stages.

that is needed and manufacturers are required to follow strict Good Manufacturing Practice guidelines, GMP. There is a requirement for extensive and rigorous qualification and validation of equipment and procedures, together with comprehensive documentation of every aspect of the process [47].

Method validation is a process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. It is implicit in the method validation process that the studies to determine method performance parameters are carried out using equipment that is within specification, working correctly, and adequately calibrated. Method validation is usually considered to be very closely tied to method development. Many of the method performance parameters that are associated with method validation are in fact usually evaluated, at least approximately, as part of method development [49].

Procedure validation is a process, which purpose is to find out, if the procedure responds to the purpose, e.g. if it fits to the demands of concrete analysis defined in the scope. In addition, often demands on the repetitiveness and repeatability are there. Procedure characteristics and qualifications/restrictions are determined during the validation and what kind of factors and in which rate can affect them. The validation allows to demonstrate, if the procedure results are reliable and if the procedure suits to this analysis. Based on the validation data it's possible to improve procedure: to find the potential error sources and to eliminate them [49, 50].

During the validation it is determined some or all the following parameters according to the demands: selectivity, scope of application, limit of detection and quantitation, linear range, trueness, accuracy, sensitivity, robustness, measurement uncertainty, recovery, done job speed and labour intensity. [50]

A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem, for example to demonstrate the equivalence between approved standard method and a new one. The extent of validation or revalidation required will depend on the nature of the changes made in reapplying a method to different laboratories, instrumentation, operators, and the circumstances in which the method is going to be used. Some degree of validation is always appropriate even when using apparently well-characterised standard or published methods. And in most cases the speed and labour intensity which goes to the validation influences also the analysis cost – used sertified

materials, chemicals, etc. For example, where a method has been validate by a standard approving organisation, the user will normally need only to establish performance data for their own use of the method to transfer a method. On a transfer of a method it is assumed that a certain amount or elements of validation data for this analysis is already available and validated, that's why it would be necessary to take into account only few particularly validation properties. In some cases it can be done by comparison of the results of two laboratories performed on the same sample. But there are cases where there is not need to do additional validation, because adequate characteristics have already been taken into account by others. But it is always necessary to control the performance of available system [51].

To ease the workload related to ensuring safety of medicinal products, harmonisation of composition, quality specifications and quality control methods of pharmaceuticals and their raw materials has been target since centuries. A set of reference documents providing a legal and scientific basis for quality control during the development, production and marketing processes is called **pharmacopoeia,** and pharmacists and manufacturers of pharmaceuticals must comply with its quality requirements within a given political entity [52].

The European Pharmacopoeia (Ph. Eur.) has existed since 1964. Countries that wish to join the must sign the Convention on the Elaboration of a European Pharmacopoeia and make Ph.Eur. standards obligatory on their territories. So far 36 European countries and the European Union are signatory to the Convention. 8 European countries, 14 non-european countries and the World Health Organization are observers. Thus Compliance with Ph. Eur. requirements is one of the obligatory criteria for granting a marketing authorisation for a medicine in the EU. The texts of the Ph. Eur. cover active substances, excipients, substances or preparation for pharmaceutical use of chemical, animal, human or herbal origin, homoeopathic preparations and homoeopathic stocks, antibiotics, as well as dosage forms and containers. There are also texts applying to biologicals, blood and plasma derivatives, vaccines, radiopharmaceutical preparations [53].

One of the core activities of the European Pharmacopoeia is an on-going process to add and revise quality standards. At the moment from the 1 January of 2008 there is in use the Ph. Eur. 6th edition. To illustrate the related workload - there were 19 permanent expert groups supplemented by 47 "*ad hoc*" specialised working parties with more than 800 experts from all over the Europe establishing the texts of the Ph. Eur. With the Japanese and United States

Pharmacopoeias, the Ph. Eur. participates in the Pharmacopoeial Discussion Group with the aim of harmonising pharmacopoeial requirements worldwide. The draft texts prepared by the groups of experts are published in their entirety in a periodical called “Pharmeuropa”, and readers have three months to submit comments on these texts. The respective group of experts then analyses the comments, revises the text if necessary and submits it to the Commission for adoption. The Ph. Eur. Commission adopted 34 general chapters and 255 individual quality standards for ingredients in 2009 [54].

The analytical procedures described in a monograph of a pharmacopoeia for an active substance and other ingredients are considered to be validated, i.e. no further formal validation is not required for identification, assay and testing for listed impurities. In these cases method transfer is sufficient, i. e. it should be made sure that all reference materials needed are available and the required system suitability tests are performed [51].

2.4 Ethanol and related quality control methods

2.4.1 Ethanol and its properties

In current work ethanol was chosen as a test object, that is why is given a short overview about ethanol’s properties. Ethanol or ethylalcohol, CH_3CH_2OH, which is described as the one of the most exotic synthetic organic chemical which contains of oxygen by its unic properties combination. Ethanol might be a solvent, pesticide, drink, antifreeze, fuel, inhibitor and because of its variety it is used as an intermediate product to other chemicals. It is used to manufacture alcoholic beverages, medical products, and in chemical reaction as an intermediate substance [55].

Ethanol is volatile, combustible, clear, unstable colourless liquid, which is produced by microbial fermentation, by distillation or by ethylene dehydration. Ethanol’s formula is bought on Figure 2.

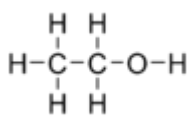

Figure 2. Formula of ethanol.

Ethanol's relative density is at 20°C 0,805 – 0,812 g/cm^3. Ethanol is miscible in all proportions with water. Soluble in alcohols, in diethyl ether and in most organic solvents. Ethanol is hygroscopic and forms explosive mixtures with air. When released to the atmosphere it will photodegrade in hours (polluted urban atmosphere) to an estimated range of 4 to 6 days in less polluted areas. Rainout should be significant. Ethanol poses no essential risk to aquatic flora or fauna – it is non-bioaccumulative, rapidly biodegradable and volatilised substance [56].

Ethanol causes skin and eye irritation through repeated contact. Ingestion can cause nausea, vomiting and inebriation; chronic use can cause serious liver damage. Note that "absolute" alcohol, which is close to 100% ethanol, may nevertheless contain traces of 2-propanol, together with methanol or benzene. The latter two are very toxic, while "denatured" alcohol has substances added to it which make it unpleasant and possibly hazardous to consume. Ethanol presents no danger under normal conditions of industrial hygiene. Irritation of respiratory tracts with headaches, vertigo, nausea, drowsiness in case of prolonged exposure by inhaling vapours. In an industrial environment, risk of accidents due to problems of concentration and possible interaction with the toxic effects of other chemical products, notably chlorinated solvents, amides, oxides and thiurames [57, 58].

2.4.2 Analytical methods

The most used method for determination of the ethanol content is calculation from the relative density using the alcoholimetric tables. This method is used in Ph. Eur. (method reference no. 01/2008:50500, the general formula agreed in 27 July 1976 Directive on alcohometry), as well as in Russian, British and United States pharmacopoeias.

One of the potential ways to determine ethanol's content is by acetylation. This method consists from 3 steps. At first ethanol and acetic anhydride is heated at the presence of anhydrous pyridine in sandbath and under tilt reflux (we will get esther and pyridine acetate). The second step is the hydrolysis of excess of the acetic anhydride:

$$(H_3C\text{-}CO)_2O + H_2O \xrightarrow{H^+} 2CH_3COOH.$$

In final step the formed acetic acid is titrated back by sodium hydroxide solution:

$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$. [59].

The most common method used for analysis of related substances in ethanol is gas chromatography (GC). In detection it is used flame ionisation detector. GC analysis is also applied for ethanol used in production of medicinal products as described in monographs European Pharmacopoeia [53] and United States Pharmacopoeia [60]. The Ph. Eur. method is further described in the chapter 3.2. In addition to GC, UV-absorbance is determined to ensure detection of non-volatile impurities, other tests to be performed are appearance, acidity or alkalinity, and residue of evaporation. It is worthwhile to mention that the method is in pharmacopoeia from 1989 and it has not been changed so far.

3 Experimental part

Taking into account problems faced in the Laboratory of State Agency of Medicines, Estonia, while working with official methods for pharmaceutical analysis, ethanol was selected as a test object to demonstrate "greening" potential of a pharmacopoeia monograph. Most of the analytical work was performed in autumn 2008 to spring 2009. Although the aim was not method validation, main conclusions were confirmed by independent runs in autumn 2009.

As a complimentary result, data on ethanol quality used in different applications in the Estonian pharmaceutical sector were received.

3.1 Materials

3.1.1 Samples taken

In Estonia until 2009 there was one major producer of ethanol from grain, but at the moment this factory is closed and there are no other major ethanol producers. Still, some stocks are still in use. In 2009 a pilot plant was started in a farm to produce ethanol, using sugar beet as a raw material. Pharmaceutical producers also use imported batches.

Altogether samples from 4 different suppliers of ethanol were taken:

1) USA ethanol 79.6%
2) Estonian ethanol 1 96.2%
3) Latvian ethanol 96.3%
4) Estonian ethanol 2 96.7%.

3.1.2 Materials used

- Ethanol, C_2H_5OH (same as substance to be tested, *i.e.* samples)
- 4-methylpentane-2-ol, for synthesis, $C_6H_{14}O$, Aldrich
- Anhydrous methanol, >99,9%, CH_3OH, Riedel-de-Häen
- Acetaldehyde, 99,5%, C_2H_4O, Sigma-Aldrich
- 1, 1- Diethoxyethane (acetal), 99%, $C_6H_{14}O_2$, Aldrich
- Benzene, for chromatography, C_6H_6, Merck KGaA

- Purified water: it was used the *Milli-Q*-water cleaning system (*Milli-RO*$^{6}_{plus}$ and *Milli-Q*$_{plus}$), Millipore Corporation
- Chromatographic syringes: 5 µL and 10 µL
- Volumetric flasks for preparation of sample solutions:
  5 ml flask (±0.06 mL),
  10 ml flask (±0.06 mL),
  20 ml flask (±0.06 mL).

3.2 Methods applied

Following test described in Ph. Eur. monograph for ethanol [61] were performed (test methods are not described in detail - reference is given to Ph: Eur. text, prescribed quality requirements are shown):

Appearance (visual method in Ph. Eur. 01/2008:20201): 1.0 ml of ethanol was diluted to 20 ml with water. After standing for 5 min, the dilution remained clear when compared with water.

Acidity or alkalinity. To 20 ml of ethanol was added 20 ml of carbon dioxide-free water and 0.1 ml of phenolphthalein solution. The solution was colourless. After adding 1.0 ml of 0.01 M sodium hydroxide, the solution changed colour and is now pink (30 ppm, expressed as acetic acid).

Relative density (pycnometric method): 0.805 to 0.812

Absorbance (by UV/VIS absorption spectrophotometry, Ph. Eur. 01/2008:20225): measured in 5-cm cell using water as the compensation liquid. Absorbance maxima: 0.40 at 240 nm, 0.30 between 250 nm and 260 nm and 0.10 between 270 nm and 340 nm. The absorption curve is smooth.

Volatile impurities (by gas chromatography, Ph. Eur. 01/2008:20228): preparation of test and reference solutions and relevant chromatographic conditions are described further in Chapters 3.2.2 and 3.2.3. Specified impurities are acetal (1,1-diethoxyethane), acetaldehyde, acetone, benzene, cyclohexane, methanol, butan-2-one (methyl ethyl ketone), 4-methylpentan-2-one (methyl isobutyl ketone), propanol, propan-2-ol (isopropyl alcohol), butanol, butan-2-ol, 2-.methylpropanol (isobutanol), furane-2-carbaldehyde (furfural), 2-

methylpropan-2-ol (1,1-dimethylethyl alcohol), 2-methylbutan-2-ol, pentan-2-ol, pentanol, hexanol, heptan-2-ol, hexan-2-ol, hexan-3-ol, in total 22 substances. Limits are set as follows:

- methanol: not more than 100 ppm V/V (0.1 µl/ml);
- acetaldehyde+acetal: maximum 10 ppm V/V (0.1 µl/ml);
- benzene : maximum 2 ppm V/V (0.002 µl/ml); if necessary the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with different polarity);
- total of other impurities: not more than 300 ppm (0.3 µl/ml) by 4-methylpentan-2-ol peak ;
- disregard limit: 9 ppm (0.009 µl/ml) by 4-methylpentan-2-ol peak.

3.2.1 Apparatus

Gas-chromatograph Trace Ultra GC with flame ionisation detector and Autosampler TriPlus AS, THERMO Electron Corporation.
UV-Vis spectrophotometer, UV1601 Shimadzu.

3.2.2 Making solutions for GC analysis of volatile impurities

All the solutions volumes have been essentially reduced as compared to original pharmacopoeia method – as the diluents is substance to be examined, to perform the analysis is needed 870 ml of sample for the 1st run, and additional 500 ml if parallel test is performed. 5-10 ml of the substance to be examined was taken for preparation of each solution, because the actual volume needed for the analysis is 3 mL. All the original concentrations were taken into account, thus leading also to substantial reduction in volume of substances used to prepare reference solutions. This is the main reason why validation was not performed.

Test solution (a). The substance to be examined, i.e. ethanol.

Test solution (b). It was taken 1 5 µL of 4-methylpentan-2-ol and added to 5.0 ml of the substance to be examined. From there it was taken 1.0 mL of the solution diluted to 10.0 mL with the substance to be examined. Concentration 0.3 µl/ml or 300 ppm.

Reference solution (a). 10 µL of anhydrous methanol was diluted to 5.0 ml with the substance to be examined. From there 1.0 ml of the solution was diluted to 10.0 ml with the substance to be examined. Concentration 0.2 µl/ml or 200 ppm.

Reference solution (b). 10 µL of anhydrous methanol and 10 µL of acetaldehyde were diluted to 10.0 mL with the substance to be examined. From the solution 100 µL was taken to 10.0 mL flask and was diluted with the substance to be examined. Concentration of both impurities 0.01 µl/ml or 10 ppm.

Reference solution (c). 60 µL 1, 1- diethoxyethane (acetal) was diluted to 20.0 mL with the substance to be examined. From there 100 µL of the solution was taken to 10.0 ml flask and was diluted with the substance to be examined. Concentration 0.03 µl/ml or 30 ppm.

Reference solution (d). 5 µL benzene was diluted to 5.0 mL with the substance to be examined. From the solution 10 µL was taken to 5.0 mL flask and was diluted with the substance to be examined. Concentration 0.002 µl/ml or 2 ppm.

System suitability: reference solution (b)

Reference solution (b) is used to validate the system suitability. The resolution between acetaldehyde (the first peak) and anhydrous methanol (the second peak) should be at least 1.5.

3.2.3 Chromatographic system

Different capillary columns (described further, monograph foresees fused silica column with stationary phase poly[(cyanopropyl)(phenyl)][dimethyl]siloxane (contains 94 per cent of dimethylsiloxane groups, film thickness 1.8 µm, 30 m x 0,32 mm)
Carrier gas: helium for chromatography (purity 5.0 was used)
Linear velocity: 35 cm/s, or about 1.6 ml/min
Split ratio: 1:20
Detection: flame ionisation
Injection volume: 1 µL

For analysis the temperature programme (given in Table 2.) was used.

Table 2. Used temperature set-up.

	Time, min	Temperature, ˚C	Linear velocity, ml/min
Column	0 – 12	40	1.6
	12 – 32	40 → 240	1.6
	32 – 42	240	1.6
Injection port		200	
Detector		280	

While selecting columns for performing test on volatile impurities, adjustment of column parameters was considered as allowed by Ph.Eur. method of analysis 04/2009 20246 "Chromatographic separation techniques". There is defined the extent to which the various parameters of a chromatographic test may be adjusted to satisfy the system suitability criteria without fundamentally modifying the methods. Changes other than those indicated require revalidation of the method. In gas chromatography following adjustments are allowed: stationary phase film thickness in capillary columns: − 50 per cent to + 100 per cent; column dimensions: length: ± 70 per cent, internal diameter: ± 50 per cent; flow rate: ± 50 per cent, temperature: ± 10 per cent.

In principle, all the columns selected were matching with parameters given in Ph. Eur. monograph. Rtx-1301 by Restek had film thickness -20 %. Two other columns were decided to be of same producer (Supelco), but of different nature: Supel-Q PLOT (particles layer thickness -20 %) and OVI-G43 column (film thickness + 67 %). The column descriptions are given below:

1. Column: Rtx®-1301, 30 m x 0.32 mm, d_p = 1.5 μm.

   The Rtx-l301 columns are fused silica columns coated with crossbonded 6% cyanopropylphenyl – 94% dimethyl polysiloxane stationary phase. It is having low to mid-polarity. It is a general purpose column fro residual solvents, alcohols, oxygenates and other volatile organic compounds, equivalent to USP G43 phase. Operation temperature range is -20...280 ^{o}C. The column is considered to ensure long-term reproducibility and low bleed even with sensitive detector types. [62]

2. Column: Supel-Q ™ PLOT, 30 x 0.32 mm, d_p = 1.5 μm, stationary phase 6% cyanopropylphenyl – 94% dimethyl polysiloxane.

   Supel-Q Plot columns contain a porous divinylbenzene polymer that effectively resolves carbon dioxide and C1-C4 hydrocarbons at above ambient temperatures. It is also suitable for analyses of other gases, such as sulphur gases, and alcohols, ketones, aldehydes, and many polar other compounds. Gasoline and other petroleum fractions can be analyzed as well. This column exhibit very little bleed, even at the maximum temperature. Supel- Q ™ PLOT column offers better resolution in less time. A proprietary procedure fixes the polymer to the fused silica tubing and ensures the particles will not be dislodged from the tubing in normal use [63].

3. Column: OVI-G43, 30 x 0.53 mm, d_p = 3.0 μm, stationary phase 6% cyanopropylphenyl – 94% dimethyl polysiloxane.

   OVI-G43 column is specially prepared and tested to meet the requirements of USP and Ph.Eur. general method for determining residual organic solvents in pharmaceutical preparations. This column is used to separate organic volatile impurities for research purposes or qualitative analysis [63].

4 Results and discussion

4.1 GC analysis of volatile impurities in ethanol

4.1.1 Detection limits of the chromatographic system

At first method as provided in European Pharmacopoeia was performed with three different commercially available capillary columns. In the Annex III are presented the retention times and detector responses at different concentrations of reference solutions. For the peaks detection were initially used the same concentrations as in Ph.Eur. monograph, but for some substances peaks were not detectable: methanol (0.01 µl/ml) and 4-methyl-2-pentanol (0.3 µl/ml) with Q-Plot column, benzene (0.002 µl/ml) with Rtx-1301 column, 1,1-diethoxyethane (0.03 µl/ml) with OVI-G43 and Rtx-1301 column. So the concentration was increased at least hundred times and the impurity peaks what were not found earlier were now detected (Annex IV, Chromatogram 1).

As for flame ionisation detector (FID) is highly sensitive, and systems functions properly (at least 3-4 substances at prescribed concentration detected within the same setup, i.e. no fault connections to be assumed, sensitivity check performed and passed), the only reasonable explanation seems to be different column performance for each substance. As OVI-G43 and Rtx-1301 coatings are considered equivalent to DB 624 (found to be suitable during monograph development according to the Ph.Eur. knowledge database), the problematic issue might be residual activity, which causes irreversible adsorption, thus diminishing the amount of substance reaching the detector at smaller concentrations [64].

4.1.2 Fulfilment of system suitability criteria with different columns

Ph.Eur. monograph for ethanol [61] sets following system suitability criterion for GC analysis of volatile impurities in ethanol - the resolution between acetaldehyde and methanol (respectively peak 1 and peak 2 on Chromatogram 1 provided in Annex IV) has to be better than 1.5, i.e. baseline resolution has to be achieved.

Resolution was calculated as follows:

$R_s = [1.18(t_{R2} - t_{R1})] / W_{h1} + W_{h2}$

where:

R_s – is the resolution between peaks of 2 components,

t_{R1} and t_{R2} - retention times (time or volume) along the baseline from the point of injection to the perpendiculars dropped from the maxima of 2 adjacent peaks ($t_{R2} > t_{R1}$)

W_{h1} and W_{h2} the respective widths of each peak at its half-height [53].

Ph. Eur. ethanol has a specific feature regarding preparation of reference solutions – ethanol to be tested is used as diluent, including for system suitability solution. Thus it was decided to run ethanol from each supplier on each column (except for 2 samples on Q-plot column due to lesser volume of the sample taken from the supplier). Results are presented in Table 3.

Table 3. Resolution (R_s) between acetaldehyde and methanol peaks

Sample	Rtx®-1301	OVI-G43	Q Plot
USA ethanol (80 %)	1,59	2,16	11,5
Estonian ethanol 1	1,06	1,33	Not tested
Latvian ethanol	2,03	1,83	Not tested
Estonian ethanol 2	2,03	1,90	6,71

It can be concluded that the system suitability of the specified test is directly dependant on the substance to be tested. In case of Estonian ethanol 1 it was failed to achieve required separation on both of the columns used – mainly due to elevated “noise” level in the segment of the chromatogram where acetaldehyde and methanol should be eluted (Chromatogram 2 in Annex IV).

All the other peaks were well baseline separated on Rtx-1301 and OVI-G43 columns. On Q-PLOT column benzene and 4-methyl-2-pentanol were retained quite close (retention times respectively 18.5 and 20.8 minutes).

4.1.3 Modification of column temperature for PLOT column

Taking into account that the resolution achieved between acetaldehyde and methanol peaks was much larger with the Q-Plot column than with the other columns, and last peak was

eluting 8-10 minutes later, it was considered to find out, is it possible to shorten analysis time by modifyng the temperature gradient of the Ph. Eur. method (provided in Table 2).

At first it was skipped 12 minutes period of holding initial column temperature at 40 ^{o}C and temperature was raised to 240 ^{o}C within 10 minutes. The resolution between methanol and acetaldehyde was 7.78, also benzene was shifted away from 4-methyl-2-pentanol, but acetal and 4-methyl-2-pentanol peaks were not well separated – resolution was only 0.31 (see Annex IV Chromatogram 3).

Further different temperature programming options were tested to achieve better separation of acetal and 4-methyl-2-pentanol peaks, all starting from column temperature 40 ^{o}C and ending at 240 ^{o}C. No changes were observed when the temperature was hold at 90 degrees for 5 minutes. Holding temperature at 110 ^{o}C for 5 minutes resulted in retention time shift of 30 seconds for both substances. Also further attempts to have additional holds, e.g. 125 degrees for 3 minutes (in the chromatogram between 18-20 min), and at 160 ^{o}C did not improve the peak separation.

Finally it was tried to use the higher starting temperature – 70 ^{o}C. Using temperature programming without intermittent holds as given in Table 4 resulted in following retention times: methanol 2.2 minutes, acetaldehyde 2.4 minutes, ethanol 2.46 minutes, benzene 10.4 minutes, acetal 12.82 minutes, and 4-methyl-2-pentanol 13.1 minutes (Annex IV Chromatogram 4). Improved separation was achieved: the resolution between methanol and acetaldehyde was 8.72, between acetal and 5-methyl-2-pentanol 0.45. Also acetaldehyde was better resolved from ethanol peak, practically achieving baseline resolution (Annex IV Chromatogram 5). With this temperature set-up time-saving is approximately 8 minutes compared to the original temperature set-up.

Table 4. Modified temperature set-up while using Q-PLOT column

	Time, min	Temperature, ˚C	Linear velocity, ml/min
Column	0	70	1.6
	0-10	70 → 240	1.6
	10-34	240	1.6
Injection port	-	200	-
Detector	-	280	-

Thus by using a different type of column than originally specified, can be saved about 20 % of time required for analysis. But this also means savings in labour, resource (e.g. carrier gas) and energy costs, especially in case the analysis is performed as a routine. More efficient use of resources means also reducing environmental burden of the method.

As the similar chromatographic conditions are applied in Ph. Eur. method for analysis of residual solvents (01/2008:20424) in active substances, similar greening improvement might be available for this method (assumedly being in use at much more extent than ethanol analysis, and thus having also somewhat larger environmental relevance).

4.2 UV absorbance curve and limits

The absorbance of ethanol samples were studied in wavelength region between 230 nm and 350 nm, in a 5 cm cell using water as the reference. Results are provided in Table 5.

Table 5. Absorbance of ethanol samples from different sources.

Wavelenght (nm)	Limit value	USA ethanol (80 %)	Estonian ethanol 1	Latvian	Estonian ethanol 2
340.00	0.1	-0.005	-0.002	0.002	0.002
270.00	0.1	0.031	0.357	0.062	0.03
260.00	0.30	0.039	0.338	0.111	0.056
250.00	0.30	0.077	0.675	0.199	0.115
240.00	0.40	0.183	1.827	0.357	0.255

The main discussion point here is meaning of ‘smooth’ absorbance curve of the standard method as no reference is provided in the Ph.Eur. monograph. From the previous laboratory practice an ethanol sample was analysed which met the absorbance criteria, but the spectrum obtained was somewhat similar to the one provided on Figure 3 - the UV spectrum of USA ethanol (80 %) shows plateau between 260...290 nm, and also in this case the absorbance limits are not exceeded. Is this still satisfying the specified quality requirements? In personal communication with EDQM scientific administrators UV spectrum of non-compliant sample was provided (see Figure 4) with the remark that while during draft monograph was published in Pharmeuropa 7.2 following comment was made:

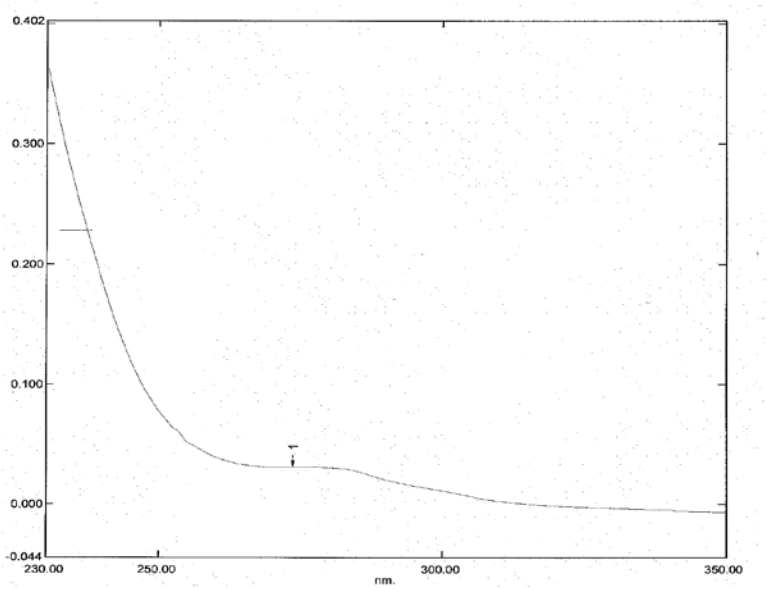

Figure 3. UV spectrum of USA ethanol (80 %)

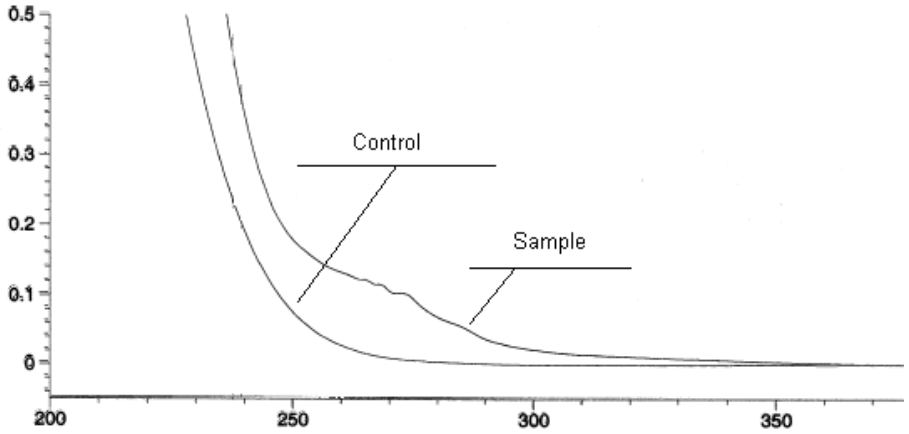

Figure 4. Reference spectrum and out-of-specification sample spectrum [provided by EDQM]

"The presence of aromatic impurities may give the UV-curve a rough appearance in the region 250-280 nm. Bitrex (denatonium benzoate, a bittering substance which could be added to alcohol in order to denature it), *e.g.*, gives rise to small peaks at 260-270 nm. Therefore add "the curve is smooth", which is also in accordance with the present USP monograph."

Still, if the sample curve provided in Figure 4 is clearly rough, it is not so evident for the curve presented on Figure 3, especially taking into account that 'smooth' besides 'free from unevenness of surface' means also 'not rough', 'free from abrupt curves or bends' [65]. Besides, perception of 'smooth' in different languages might be different thus creating

additional problems for non-native speakers of English. And there are no evident absorbance peaks at the curve on Figure 3.

Clear non-conformity appears to be with the Estonian ethanol 1 having in UV spectrum a relatively strong bump in region of 260-300 nm and with quite evident peaks (Figure 5.). At the same time the absorbance criteria are not met: instead of being 0.4 at 240 nm, it is about 1.8. The reason might be raw materials used and also the production procedure. This ethanol is not made from grain (e.g. wheat or corn), but it is manufactured from sugar beet, and practically in a pilot plant. In contrast, the Estonian ethanol 2 gives a very smooth absorbance (Figure 6), which clearly satisfies the requirements of the standard method.

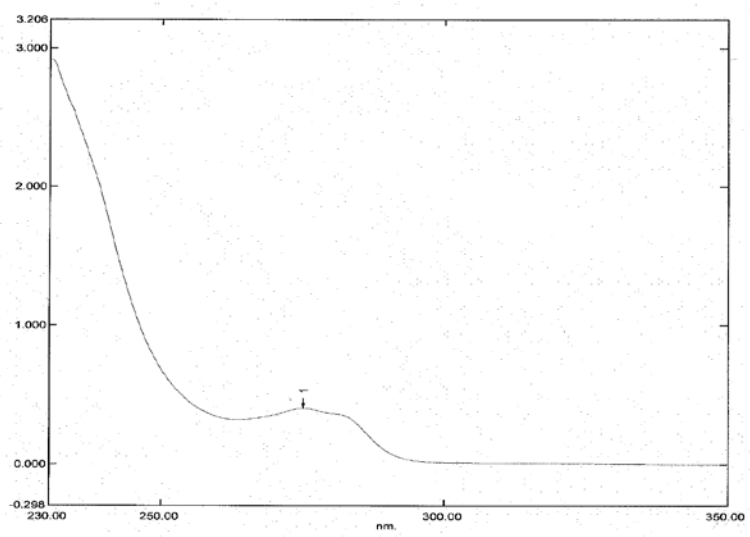

Figure 5. UV spectrum of Estonian ethanol 1.

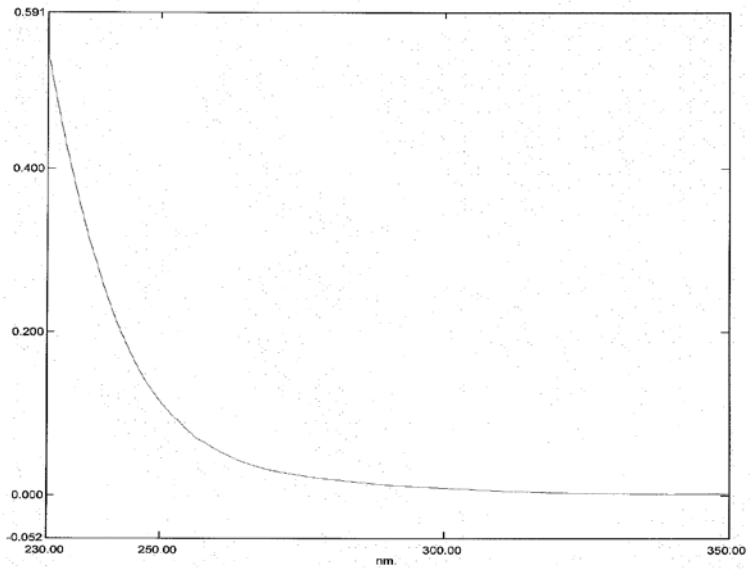

Figure 6. UV spectrum at ethanol from Estonian ethanol 2.

4.3 Considerations for quality control from ethanol results

The above mentioned observations are creating problems especially for laboratories performing tests on non-routine basis, including official medicines controlling laboratories (OMCLs). Transfer of the gas-chromatographic (GC) method for a specific sample maybe analysed next time after 5 years might require testing of several columns and still lengthy fine-tuning of the chromatographic system. Each attempt failing to meet the specified performance criteria while using would result in wasted money and resources thus also having a certain environmental burden.

Taking into account quite the number of requests for revision received each year by Ph.Eur. Commission Secretariat, and again previous personal experiences while working with pharmacopoeial methods, it raises question about sufficient robustness of the pharmacopoeial methods for laboratories performing non-routine tests. It seems that the method validation to test robustness by performing analysis in two laboratories on the same sample (as described in Chapter 2.3.2) might not always be sufficient.

An hint how to reduce need for GC testing comes from absorbance analysis. The same Estonian ethanol 1 which has problems in absorbance requirements fulfilment has also problems with gas chromatographic resolution. The presence of unidentified contaminants seems to be the reason why the required resolution is not achieved – instead of proper peaks there are small humps at acetaldehyde and methanol peaks (see Annex IV Chromatogram 2), and it is the same with three different columns. Any attempt to improve the situation failed.

From here rises question, is it necessary to continue with other tests when quite simple and straightforward instrumental test already gives non-compliance, as it is in case of UV-absorbance in current work. If at first is done the absorbance test and the results does not comply with the specified requirements, then from here we can assume that it is not necessary to continue with further more complex analysis, e.g. as GC, but just consider the sample failing the quality (assuming that quality criteria in the method are better defined, e.g. by providing a reference spectra in the monograph or knowledge database). Avoiding attempts to satisfy performance criteria while transferring the method could save a lot of resources in quality control laboratories doing non-routine work.

The topic initiates wider discussion on extent the sustainability or environmental issues are considered while elaborating Ph.Eur. monographs and performing the quality control of medicines.

Regarding applicability of modifications of the GC method, *e.g.* by using PLOT columns, or possibility to skip GC analysis at all, if absorbance fails, still needs further evaluation, e.g. by arranging market surveillance study on ethanol in States party to the European Pharmacopoeia Convention.

4.4 Environmental considerations in European Pharmacopoeia

A number of advancements are applied already bringing sustainability in healthcare closer to reality. Regarding environmental aspects, the key words are green chemistry applied to drug design, formulation, manufacturing and packaging, environmental risk assessment for new pharmaceuticals, minimising greenhouse gas emissions in distribution chain, etc. But so far it seems almost no attention has been paid to the environmental aspects of quality control methods in the sector.

Technical guidance documents for the elaboration of Ph.Eur. monographs [66] include general recommendation to avoid the use of reagents that are acknowledged to be extremely toxic or otherwise hazardous (e.g. carcinogenic), or prohibited or restricted in one or more of the States party to the European Pharmacopoeia Convention. But there are only a few systematic practices to be mentioned, e.g. avoidance of chloroform (further in Chapter 4.5). Some member States have expressed concern to include more environmental considerations while elaborating the monographs.

4.4.1 Activities of EDQM

In closer look at the activities of the European Directorate for the Quality of Medicines and Health Care (EDQM, established in 1996), administering the work related to Ph.Eur., it is possible to find a number of ongoing efforts to achieve better sustainability:

The Biological Standardisation Programme (BSP) pursues the following goals in the area of standardisation of biologicals: development and validation of new analytical methods and validation of alternative methods based on the 3R concept (i.e. the **R**efinement, **R**eduction and **R**eplacement of animal experiments). Since the programme's start in 1992, 112 BSP projects have been initiated, and in the year 2009 21 projects were pursued in different fields. Amongst these, 5 projects were devoted to the establishment of alternatives to animal experiments. Three projects were concluded and proposals were made to the relevant Ph. Eur. group of experts for revision of the monographs. [54]

European Pharmacopoeia Commission contributed to the reduction of animal testing in the Ph.Eur. related work by revising a set of quality standards concerning human blood products. Although the Limulus amebocyte lysate assay (LAL) has replaced the rabbit pyrogen test quite long ago, further work to replace *in-vivo* tests with *in-vitro* tests has been performed. Still many formulations, e.g. vitamins, steroids and hormones are applied intramuscularly using a lipid carrier, masking LAL assays. To avoid further pyrogen testing in rabbits, *in vitro* pyrogen test (IPT) based on human whole blood was validated in a collaborative study. [67]

Network of Official Medicines Control Laboratories (OMCL). More than 40 European countries have been participating in the activities of the OMCL network since 1994. The role of this network is to ensure the consistent quality of medicines marketed in the member states and to contribute to the mutual recognition of the results of quality control testing of medicines by these states. There are two levels of collaboration within the network:

- general activities involving all of the member states and the observer states covering work in the area of quality management systems, such as audits and proficiency testing studies (PTS) as well as market surveillance studies (MSS).
- activities restricted to the European Union (EU) and the European Economic Area (EEA) concerning products with a centralised marketing authorisation (CAP), products authorised according to the mutual recognition or the decentralised procedure (MRP/DCP) and the Official Control Authority Batch Release (OCABR) system for biological products (human and veterinary). [54]

Such activities save public money and environment thanks to resource pooling. For the competent national authorities, it shares, thus avoids duplication of, work. Networking gives access state-of-the-art technology and selective analytical procedures.

4.4.2 Sustainable trends with analytical methods

There are following recent publications clearly showing sustainability considerations regarding Ph.Eur. analytical methods and also related regulatory procedures.

Herbal preparations: time-lag in legal procedures

In pharmacopoeial monographs for herbal drugs and herbal preparations, conventional assay methods such as colorimetry or spectrophotometric assays are often replaced by modern, more specific and more reliable methods, e.g. liquid chromatography. However, existing dosage recommendations in the monographs on efficacy and safety of herbal medicinal products which are an important basis for licensing procedures do not refer to the mandatory new methods but to the existing photometric methods. The laboratory comparison of the determination of silymarin of Milk Thistle extract shows that a conversion factor can be calculated which allows a correlation between the new and the existing method. The solution to use a conversion factor should also be applied to other herbal drugs and herbal preparations monographs in the European Pharmacopoeia to avoid a conflict between the pharmacopoeial monographs and those used by The Committee on Herbal Medicinal Products (HMPC). [68]

Heavy metal limit test

About 100 years ago a general test for heavy metals was introduced into the United States Pharmacopoeia (USP) based on the precipitation of metal sulphides from both alkaline and acidic media. The test was to demonstrate the absence of ‘undesirable metallic impurities’ such as antimony, arsenic, cadmium, copper, iron, lead and zinc in pharmaceutical substances. The test complies if there was no visible precipitate observed in a given time. Similar tests were later introduced in the other pharmacopoeias. In 1940’s the method evolved towards its present form my introducing a lead standard so that the method become a limit test against reference solution. At the time the method was considered to be particularly useful to limit concentrations of lead and/or copper which were widely used for water pipes and in factory equipment, respectively. Lead was also potentially present if sulphuric acid, produced by the lead chamber process, was employed in the synthesis of substances for pharmaceutical use. Nowadays the test has been retained despite of the fact that sulphuric acid is no longer produced via the lead chamber process. The aim of the test is to control metal contaminants potentially coming from reagents, solvents, electrodes, reaction vessels and rubber seals. But the following metals could not be detected under the specified test conditions: chromium,

cobalt, manganese, thallium, titanium, tungsten and zinc. The heavy metals test is a non-specific test. Despite it has been argued that element-specific instrumental methods such as X-ray fluorescence, atomic absorption/emission spectrophotometry or induced coupled plasma emission would be more appropriate for control of toxic metals, the limit test is still considered to be indicative of the overall quality of production. In recent years, a considerable amount of work has been devoted to the simplification and the improvement of the test in the European Pharmacopoeia. Efforts have been successfully made to increase its robustness and reliability by reducing the use of ignition methods (methods C and D) as there is always a risk of loss of analyte. Progress has been made by facilitating the comparison and increasing the sensitivity of the test by the introduction of a filtration step. [69]

Purity testing of pharmaceutical substances: change of detection method

The purity of a substance for pharmaceutical use is generally controlled by liquid chromatography using UV detection. In the absence of a chromophore other detection systems are employed, i.e. refractometry (ex. sugars, polymers), pulsed amperometry (ex. Aminoglycosides) and derivatisation followed by UV detection (ex. amikacin, gentamicin). However, any of the above alternatives has limitations like being not sufficiently sensitive, requiring experienced operators and required frequent cleaning of the electrode due to poisoning, derivatisation is not recommended for impurity testing as difficult to validate. Evaporative light-scattering detector (ELSD) is a promising alternative in all these applications. It is a robust powerful technique capable of detecting a solute which is less volatile than the mobile phase, insensitive to mobile phase variations permitting the use of gradient systems. ELSD can also be used to set up MS-compatible methods. [70]

There are also some interesting findings regarding earlier practices, e.g. while controlling amount of residual solvents in the product. In case of Class 1 (solvents to be avoided) and Class 2 solvents (solvents to be limited) there is need to determined using chromatographic techniques such as gas chromatography. Any harmonised procedures for determining levels of residual solvents as described in the pharmacopoeias should be used, if feasible. Otherwise, manufacturers would be free to select the most appropriate validated analytical procedure for a particular application. If only Class 3 solvents are present, a nonspecific method such as loss on drying may be used. [24]

Another quite long-term practice is replacement of thin-layer chromatography (TLC) with LC method in identification or test for related substances. In 2008, European Pharmacopoeia Commision sessions adopted 5-10 revised/corrected monographs with the relevant replacements per session. [71, 72]

Althoug several examples are available, there is no evidence on systematic approaches, i.e. there is no common driving force like an environmental management system behind .Still, as already previously mentioned, legislative requirements are the most efficient driving force for substitution. EDQM is paying more and more attention to substitution issues due to REACH regulation.

4.5 Substitution of hazardous substances in monographs

4.5.1 Implications of REACH regulation to European Pharmacopoeia

REACH objectiveness is to ensure a high level of protection of human health and the environment through elimination or control of risks, underpinned by precautionary principles. Manufacturers, importers, suppliers and downstream users have burden of proof for the absence or control of risks:

1. Analyse risks of substances, including exposure from uses,
2. Find alternatives or register, notify and/or apply for authorisation,
3. Communicate identified risks to downstream users (e.g. by safety data sheets).

These above-mentioned reasons have also consequences on European Pharmacopoeia. Suppliers are likely to remove substances of very high concern from the market in the EU, when Annex XIV applies [73]. For European Pharmacopoeia it is necessary to find alternative methods with new substances for substance analysis or to justify requesting exemptions for European Pharmacopoeia use from ECHA [74]. ECHA is still at early stages of REACH implementation and the full impact on the European Pharmacopoeia from the very high concern chemicals removal from the market is not yet clear – especially from the perspective of the Ph. Eur. suppliers and users. But EDQM has made steps for implementation of REACH, starting in 2005 with the request to the European Commission to exclude notifications for pharmacopoeial substances as „unintended use” (but with no result). In 2008 the EDQM requested the European Commission to prevent withdrawal of essential reagents

and raw materials and to exclude pharmacopeial use of substances either under the article 56(3) or 58(2). But the answer was that it would be assessed on case-by-case basis. And in 2009 the EDQM reviewed the use in Ph. Eur. of the first ECHA consultation for annex XIV substances. The Ph. Eur. has considered the use and replacement of:

- Dibutyl phthalate (DBP)
- Bis (2-ethyl(hexyl)phthalate) (DEHP)
- Diarsenic trioxide
- Cobalt dichloride. [75]

The ECHA Member States Committee included dibutyl phthalate and di(2-ethlhexyl) phthalate into their Annex XIV recommendation to the European Commission. Cobalt dichloride and diarsenic trioxide were excluded. EDQM submitted the requests to ECHA on April 2009 with justification on regulatory grounds. As a result EDQM got permission for scientific research and development under certain conditions. Ph. Eur. has new intention for Annex XIV in process and exemptions are not easily granted. For consideration to Ph. Eur. it to reinforce the existing approach of avoiding the use of substances of very high concern in the European Pharmacopoeia, also seek for alternative substances to those (potentially) listed for Annex XIV and, when feasible, revise the existing monographs. If no alternatives are available to Annex XIV substances, justify exemptions on scientific grounds in order to continue their use in the monographs [74].

The Secretariat had commented on the publication of a first REACH-annex by the ECHA and the groups of experts would now be searching alternatives where possible. The discussion included a list of substances submitted by the Finnish delegation, dealing with hazardous substances regulated by labour protection authorities. It was stressed that the potential non-availability of certain chemicals in future e.g. for calibration purposes could be a serious technical problem and therefore the subject was very important. EDQM started to seek a legal advice also for this problem [76].

In addition, the European Commission has published a new act 2009/C 132/12 which aims to restrict laboratory use of ozone depleting substances (e.g. carbon tetrachloride, 1,1,1 trichloroethane and methyl bromide) in order to comply with Montreal protocol in the upcoming amendment of Regulation (EC) No 2037/2000. As there is still lacking information to thoroughly analyse the impact of all these regulations on the Ph. Eur., all existing

regulations/impacted substances/impacts on Ph. Eur. shall be compiled and be made available as an annex to the proposed policy paper. In principle, by June 2010 the ECHA candidate list includes 38 substances or group of substances (listed in Annex I), but EDQM has identified the future strengthening of REACH may affect up to 1194 substances [77].

4.5.2 Hazardous substances used in European pharmacopoeia

In Annex VI is presented a preliminary assessment, which hazardous substances defined by various frameworks are used in the European Pharmacopoeia. As the work was performed prior publication of 1st candidate list for authorisation by ECHA in June 2009, references used are related to pre-REACH legislation (priority lists according to EU-wide risk assessments, the basis laid down by Article 8(1) of Council Regulation (EEC) 793/93).

In the priority substances list there are some of the cells coloured violet, some yellow and some orange and some of them have no colour at all. The different colours indicate that the information is found out from different sources. If the cell has no colour then is the information about classification got from Merck safety data sheet, but if the cell is yellow then is the classification information got from Acros safety data sheet and in orange cell is the information got from European Chemicals Bureau (ECB) harmonised classifications. In the priority substance list were by CAS number determined what kind of chemicals you could find in Ph. Eur. These CAS numbers were painted violet.

First it is important to find out how many substances are used in Ph. Eur. what are in priority lists and is it possible to change the dangerous substance to less dangerous. It was found out that Ph. Eur. 5.0 contains about 69 of dangerous substances in which 29 of them are cancerogenic and mutagenic substances, thus being primary target for authorisation.

4.5.3 Assessment of substitution efficiency

As already indicated in Chapter 4.4, the general policy of Ph. Eur. working groups is avoidance of chloroform. Thus it is replaced mainly by dichloromethane as having similar properties relevant for analytical methods - polarity of the substance is almost the same, also

it has almost the same density (see Annex VI) thus the replacement is not requiring extensive revalidation , and because it is considered as less hazardous substance.

In Ph. Eur. monographs chloroform is one of the most used hazardous substances. In 6.0 European Pharmacopoeia there is still 119 monographs with contain chloroform in assay, identification, related substance and other methods. At the moment lot of monographs in Ph. Eur. which contain chloroform are substituted with dichloromethane. In 6.0 European Pharmacopoeia is about 708 monographs with contain dichloromethane.

In Table 6 is presented comparison of classifications of chloroform and dichloromethane. In Table 7 is presented comparison based on the Column model (basis given in Annex II).

Table 6. Classification of chloroform and dichloromethane.

Classification	**Chloroform** Xn; R22-48/20/22 Xi; R38 Carc. Cat. 3; R40 (Xn) Harmful if swallowed. Irritating to skin. Limited evidence of a carcinogenic effect. Harmful: danger of serious damage to health by prolonged exposure through inhalation and if swallowed.	**Dichloromethane** Carc. Cat. 3; R40 (Xn) Limited evidence of a carcinogenic effect.

Table 7. Column model rating:

Criterion in Column model	**Chloroform**	**Dichloromethane**
2a. Acute health hazards	Medium: Xn; R22	Low. not classified
2b. Chronic health hazards	High: Carc. Cat 3	High: Carc. Cat 3
3. Environmental hazards	Very high: WKG 3, WFD List I substance	Medium: WKG 2
4. Fire and explosion hazards	Neglible: inflammable	Neglible: inflammable
5. Exposure potential	High: vapour pressure 209 hPa	Very high: vapour pressure 475 hPa

At first it seems that there is no difference between the two substances in working environment, but still dichloromethane is less dangerous to the nature than chloroform.

But for future considerations: also dichloromethane is a chlorinated solvent, and there is increasing pressure for substitution. Thus other alternatives should be considered.

5 Summary

Technical guidance documents for the elaboration of European Pharmacopoeia monographs include general recommendation to avoid the use of reagents that are acknowledged to be extremely toxic or otherwise hazardous (e.g. carcinogenic), or prohibited or restricted in one or more of the States party to the European Pharmacopoeia Convention. But there are only a few systematic practices to be mentioned, e.g. reduction of animal tests used in European Pharmacopoeia monographs for biological preparations. Some member States have expressed concern to include more environmental considerations while elaborating the monographs.

Taking into account that introduction of changes into validated standard methods might take years, it is ultimate time to start wider discussions on "greening" of analytical methods applied in quality control of pharmaceuticals.

Purposes of the current thesis were as follows:

a) introducing concept of green analytical chemistry and assessment its potential to become a concept systematically applied while developing standard methods in pharmaceutical analysis.
b) assessment of usage of hazardous chemicals in European Pharmacopoeia monographs and current practices regarding substitution of hazardous substances.

Taking into account problems faced in the Laboratory of State Agency of Medicines, Estonia, while working with pharmacopoeial methods, and information received while participating in the work of the European Pharmacopoeia Commission, following was performed:

Based on ethanol monograph "greening" potential of a pharmacopoeia was demonstrated. As a result of introducing use of a PLOT column in GC analysis was demonstrated at least 20 % reduction potential of time and resource usage, being in good accordance with green analytical chemistry principles and being especially beneficial for laboratories performing routine analysis. As the similar chromatographic conditions are applied in official methods for analysis of residual solvents in active substances, similar greening improvement might be available with method assumedly being in use at much more extent than ethanol analysis.

At the same time UV-absorbance measurements of ethanol revealed importance of clear definitions in official methods, i.e. when the absorbance curve is considered as “smooth”.

Analysis of different batches of ethanol with both methods revealed that avoiding attempts to satisfy performance criteria while transferring the method could save a lot of resources in quality control laboratories doing non-routine work. If at first is done relatively simple instrumental test, e.g. absorbance test and the results does not comply with the specified requirements, then from here we can assume that it is not necessary to continue with further more complex analysis, e.g. as gas chromatography, but just consider the sample failing the quality.

Regarding applicability of modifications of the GC method, *e.g.* by using PLOT columns, or possibility to skip GC analysis at all, if absorbance fails, still needs further evaluation, e.g. by arranging market surveillance study on ethanol in States party to the European Pharmacopoeia Convention.

The second part of the work was analysing current avoidance and substitution practices of hazardous chemicals in the European Pharmacopoeia. Although The European Pharmacopoeia Commission has initiated a comprehensive study to evaluate and mitigate REACH Regulation impact on already approved analytical methods and some chemicals are avoided while elaborating / re-defining the monographs, there is still much of potential to improve. E.g. substitution of chloroform with dichloromethane is not an environmentally friendly option, and still the number of monographs using chlorinated solvents, both chloroform and dichloromethane, are growing in time.

Considering the scope of a master (MSc) thesis, it was not attempted to give comprehensive overview of all aspects to consider in “greening” of analytical methods nor providing full green concept to be considered by the European Pharmacopoeia. More detailed comparative evaluation, e.g. by using cleaner production methodology, which is taking into account simultaneously technical applicability, economic and environmental performance, could be done in further works.

6 Kokkuvõte

Euroopa farmakopöa monograafiate väljatöötamise tehniline juhend sisaldab üldiseid soovitusi, et vältida reagentide kasutamist, mis on tunnistatud äärmiselt toksiliseks või muidu ohtlikuks (nt. kantserogeensed ained), või keelatud või piiratud ühes või mitmes Euroopa farmakopöa konventsiooni liikmesmaades. Hoolimata töös toodud muudest näidetest nagu loomkatsete mahu vähenemine, on siiski raske välja tuua süstemaatilist lähenemist farmakopöa analüüside keskkonnamõju vähendamisele Mõned liikmesmaad on väljendanud muret, et monograafiate väljatöötamisse peaks hõlmama rohkem keskkonna kaalutlusi.

Võttes arvesse, et muutuste kasutusele võtmine valideeritud standard meetodisse võib võtta aega aastaid, siis on viimane aeg, et alustada laiemat diskussiooni ravimite kvaliteedi kontrolliks kasutatavate analüütiliste meetodite keskkonnamõju vähendamiseks.

Käesoleva töö eesmärgid olid järgmised:

a) rohelise analüütilise keemia mõiste tutvustamine ja tema potentsiaali hindamine, et saada süstemaatiliselt kasutatavat ideed/mõistet, samal ajal arendades standardseid meetodeid farmaatsia analüüsides/uurimuses.
b) ohtlike kemikaalide kasutamise hindamine Euroopa Farmakopöa monograafiates ja praegune tava ohtlike ainete asendamise suhtes.

Võttes arvesse Ravimiameti laboril tekkinud probleeme standardse meetodiga töötamisel ja Euroopa farmakopöa komisjoni töös osalemise käigus saadud informatsioonist, tehti järgmist.

Etanooli monograafia põhjal demonstreeriti farmakopöa potentsiaali “rohelikustamisel”. PLOT kolonni tutvustamise tulemusel GC analüüsil demonstreeriti vähemalt 20% aja ja ressurssi kasutamise vähenemise potentsiaali, olles heas vastavuses rohelise analüütilise keemia põhimõtetega ja olles eriti kasulik rutiinanalüüse teostavatele laboritele. Kuna sarnased kromatograafilised tingimused on rakendatud Euroopa Farmakopöas jääksolventide analüüsi meetodis ja see meetod on eeldatavalt ulatuslikumas kasutuses kui etanooli analüüsi-meetod, siis tasuks kaaluda sarnaste muudatuste tegemist - rohelikustamise tulemuslikkus on tunduvalt suurem.

Etanooli UV neeldumise mõõtmine tõi esile standardse meetodi selgete definitsioonide tähtsuse, nt. millal peetakse neeldumise kõverat “tasaseks”.

Erinevate etanooli partiide analüüsil ilmnes, et vältides asjatuid katseid rahuldada teostamise kriteeriume meetodi ülevõtmisel võib säästa palju ressursse, seda eriti mitterutiinsel põhimõttel töötavates kvaliteedikontrolli laborites. Kui algul on tehtud üldiselt lihtne instrumentaalne test, nt. neelduvuse määramine ning testi tulemused ei vasta etteantud nõuetele, siis võime siit eeldada, et ei ole vaja edasi jätkata rohkem komplekssete analüüsidega, nt. gaaskromatograafiaga, vaid lihtsalt tuleb tõdeda, et proov ei ole kvaliteetne.

Kas etanooli monograafias toodud metoodikat on otstarbekas muuta, nt. tuua sisse PLOT kolonni kasutamine, või teatud juhtudel loobuda gaaskromatograafilisest analüüsist üldse – sellele võiks vastuse saada üle-euroopalise etanooli turu-uuringu kaudu.

Töö teises osas analüüsiti kehtivat ohtlike kemikaalide kasutamise vältimise ja asendamise praktikat Euroopa Farmakopöa töös. Kuigi Euroopa Farmakopöa Komisjon on algatanud ulatusliku uuringu, et hinnata ja leevendada REACH määruse mõju juba heaks kiidetud analüütilistele meetoditele ja mõningaid kemikaale välditakse monograafiate väljatöötamisel / ümbermääratlemisel, on seal siiski palju paranemise potentsiaali, nt. kloroformi asendamine diklorometaaniga ei ole keskkonnasõbralik variant. Lisaks tuleb märkida, et nii kloroformi kui diklorometaani kasutatavate monograafiate arv ajas kasvab.

Arvestades magistritöö ulatusega, ei üritatud anda ulatuslikku ülevaadet kõikidest võimalikest analüütilise meetodi rohelikustamise võimalustest, samuti ei olnud eesmärgiks luua täielikku alusdokumenti Euroopa Farmakopöa töö keskkonnasäästlikumaks muutmiseks. Edasistes töödes tuleks konkreetsemalt võrrelda varasemate ja muudetud/muudetavate meetodite olemust, nt kasutades puhtamat tootmise metoodikat, mis võtab arvesse samaaegselt tehnilise rakendatavuse, majandusalase ja keskkonna-alase tulemuslikkuse.

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Annexes

Annex I. Candidate list for substances of very high concern for authorisation (as of 18.06.2010)

[http://echa.europa.eu/chem_data/authorisation_process/candidate_list_table_en.asp]

Substance name	EC No. (CAS No.)	Date of inclusion	Reason for inclusion
2,4-Dinitrotoluene	204-450-0	13.01.2010	Carcinogenic
4,4'- Diaminodiphenylmethane (MDA)	202-974-4	28.10.2008	Carcinogenic
5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene)	201-329-4	28.10.2008	vPvB
Acrylamide	201-173-7	30.03.2010	Carcinogenic and mutagenic
Alkanes, C10-13, chloro (Short Chain Chlorinated Paraffins)	287-476-5	28.10.2008	PBT and vPvB
Aluminosilicate Refractory Ceramic Fibres *having Al_2O_3 and SiO_2 concentration ranges Al_2O_3: 43.5 – 47 % w/w (or 45.5 – 50.5 % w/w), SiO_2: 49.5 – 53.5 % w/w (or 48.5 – 54 % w/w),* *and fibres have a length weighted geometric mean diameter less two standard geometric errors of 6 or less µm.*	-	13.01.2010	Carcinogenic
Ammonium dichromate	232-143-1	18.06.2010	Carcinogenic, mutagenic and toxic for reproduction
Anthracene	204-371-1	28.10.2008	PBT
Anthracene oil	292-602-7	13.01.2010	Carcinogenic[1)], PBT and vPvB
Other anthracene oil based products, *e.g.* anthracene oil-anthrancene paste	292-603-2	13.01.2010	Carcinogenic[2)], mutagenic[3)], PBT, vPvB
Benzyl butyl phthalate (BBP)	201-622-7	28.10.2008	Toxic for reproduction
Bis (2-ethylhexyl)phthalate (DEHP)	204-211-0	28.10.2008	Toxic for reproduction
Bis(tributyltin)oxide (TBTO)	200-268-0	28.10.2008	PBT
Boric acid	233-139-2 / 234-343-4	18.06.2010	Toxic for reproduction
Cobalt dichloride	231-589-4	28.10.2008	Carcinogenic
Diarsenic pentaoxide	215-116-9	28.10.2008	Carcinogenic
Diarsenic trioxide	215-481-4	28.10.2008	Carcinogenic
Dibutyl phthalate (DBP)	201-557-4	28.10.2008	Toxic for reproduction
Diisobutyl phthalate	201-553-2	13.01.2010	Toxic for reproduction
Disodium tetraborate, anhydrous	215-540-4	18.06.2010	Toxic for reproduction
Hexabromocyclododecane (HBCDD), and all major diastereoisomers identified (α-,β-, γ-)	247-148-4	28.10.2008	PBT
Lead chromate	231-846-0	13.01.2010	Carcinogenic and toxic for reproduction
Lead chromate molybdate sulphate red (C.I. Pigment Red 104)	235-759-9	13.01.2010	Carcinogenic and toxic for reproduction

Lead hydrogen arsenate	232-064-2	28.10.2008	Carcinogenic and toxic for reproduction
Lead sulfochromate yellow (C.I. Pigment Yellow 34)	215-693-7	13.01.2010	Carcinogenic and toxic for reproduction
Pitch, coal tar, high temp.	266-028-2	13.01.2010	Carcinogenic, PBT and vPvB
Potassium chromate	232-140-5	18.06.2010	Carcinogenic and mutagenic
Potassium dichromate	231-906-6	18.06.2010	Carcinogenic, mutagenic and toxic for reproduction
Sodium chromate	231-889-5	18.06.2010	Carcinogenic, mutagenic and toxic for reproduction
Sodium dichromate	234-190-3 (7789-12-0 and 10588-01-9)	28.10.2008	Carcinogenic, mutagenic and toxic for reproduction
Tetraboron disodium heptaoxide, hydrate	235-541-3	18.06.2010	Toxic for reproduction
Trichloroethylene	201-167-4	18.06.2010	Carcinogenic
Triethyl arsenate	427-700-2	28.10.2008	Carcinogenic
Tris(2-chloroethyl)phosphate	204-118-5	13.01.2010	Toxic for reproduction
Zirconia Aluminosilicate Refractory Ceramic Fibres *following concentration ranges of* Al_2O_3*: 35 – 36 % w/w, and* SiO_2*: 47.5 – 50 % w/w, and* ZrO_2*: 15 - 17 % w/w, and fibres have a length weighted geometric mean diameter less two standard geometric errors of 6 or less (µm).*	-	13.01.2010	Carcinogenic

1) The substance does not meet the criteria for identification as a carcinogen in situations where it contains less than 0.005 % (w/w) benzo[a]pyrene (EINECS No 200-028-5)
2) The substance does not meet the criteria for identification as a carcinogen in situations where it contains less than 0.005 % (w/w) benzo[a]pyrene (EINECS No 200-028-5) and less than 0,1 % w/w benzene (EINECS No 200-753-7).]
3) The substance does not meet the criteria for identification as a mutagen in situations where it contains less than 0,1 % w/w benzene (EINECS No 200-753-7).]

Annex II. The Column Model

[http://www.dguv.de/ifa/en/pra/spalte/spaltmod.pdf]

1 Risks	2a Acute health hazards (single affection, e.g. accident with chemicals)	2b Chronic health hazards (repeated affection)	3 Environmental hazards[1)]	4 Fire and explosion hazards[2)]	5 Exposure potential	6 Hazards caused by procedures
Very high risk	♦ Very toxic substances/preparations **(R26, R27, R28)** ♦ Substances/preparations which may liberate very toxic gases when in contact with acids **(R32)**	♦ Carcinogenic substances of categories 1 or 2 **(Carc.Cat.1, K1, Carc.Cat.2, K2, R45, R49)** ♦ Mutagenic substances of categories 1 or 2 **(Mut.Cat.1, M1, Mut.Cat.2, M2, R46)** ♦ Preparations containing carcinogenic or mutagenic substances of categories 1 or 2 in concentrations ≥ 0.1%	♦ Substances/preparations with the warning symbol **N** and hazards indications **R50, R51, R53, R54, R55, R56, R57, R58, R59** ♦ Substances/preparations of the German water pollution class **WGK 3**	♦ Explosive substances/ preparations **(R2, R3)** ♦ Extremely flammable gases and liquids **(R12)** ♦ Spontaneously flammable substances/preparations **(R17)**	♦ Gases ♦ Liquids with a vapour pressure > 250 hPa (mbar) (e.g. dichloromethane) ♦ Dust producing solids ♦ Aerosols	♦ Open processing ♦ Possibility of direct skin contact ♦ Application on large area
High risk	♦ Toxic substances/preparations **(R23, R24, R25)** ♦ Substances/preparations causing severe burns (highly corrosive) **(R35)** ♦ Substances/preparations which may liberate toxic gases when in contact with water or acids **(R29, R31)** ♦ Skin sensitizing substances **(R43, Sh)** ♦ Substances sensitizing the respiratory tract **(R42, Sa)** ♦ Preparations containing skin or respiratory tract sensitizing substances in a concentration ≥ 1% (in case of gases ≥ 0.2%)	♦ Substances toxic to reproduction of categories 1 or 2 **(Repr.Cat.1, R_t1, R_f1, Repr.Cat.2, R_t2, R_f2, R60, R61)** ♦ Preparations containing substances toxic to reproduction of categories 1 or 2 in concentrations ≥ 0.5% (in case of gases ≥ 0.2%) ♦ Carcinogenic substances of category 3 **(Carc.Cat.3, K3, R40)** ♦ Mutagenic substances of category 3 **(Mut.Cat.3, M3, R68)** ♦ Preparations containing carcinogenic or mutagenic substances of category 3 in concentrations ≥ 1% ♦ Substances which can accumulate in the human body **(R33)**		♦ Highly flammable substances/preparations **(R11)** ♦ Substances/preparations, liberating extremely flammable gases when in contact with water **(R15)** ♦ Oxidizing substances/ preparations **(R7, R8, R9)** ♦ Substances/preparations with specific properties **(R1, R4, R5, R6, R7, R14, R16, R18, R19, R30, R44)**	♦ Liquids with a vapour pressure of 50 ... 250 hPa (mbar) (e.g. methanol)	
Medium risk	♦ Substances/preparations harmful to health **(R20, R21, R22)** ♦ Substances, which may accumulate in breast milk **(R64)** ♦ Substances/preparations causing burns (corrosive) **(R34, pH ≥ 11.5, resp. ≤ 2)** ♦ Substances harmful to eyesight **(R41)** ♦ Non toxic gases; may cause suffocation by air displacement (e.g. nitrogen)	♦ Substances toxic to reproduction of category 3 **(Repr.Cat.3, R_t3, R_f3, R62, R63)** ♦ Preparations containing substances of category 3 toxic to reproduction in concentrations ≥ 5% (in case of gases ≥ 1%)	♦ Substances/preparations without warning symbol **N**, but with hazards indications **R52, R53** ♦ Substances/preparations of the German water pollution class **WGK 2**	♦ Flammable substances/ preparations **(R10)**	♦ Liquids with a vapour pressure of 10 ... 50 hPa (mbar), except water (e.g. toluene)	♦ Closed processing but exposure possibilities e.g. when filling, sampling or cleaning
Low risk	♦ Irritant substances/preparations **(R36, R37, R38)** ♦ Skin affections when working in wet environment ♦ Substances/preparations which may cause lung damage if swallowed **(R65)** ♦ Skin affecting substances/preparations **(R66)** ♦ Vapours causing drowsiness and dizziness **(R67)**	♦ Otherwise chronically affecting substances (no R-phrase, but nonetheless a hazardous substance!)	♦ Substances/preparations of the German water pollution class **WGK 1**	♦ Hardly flammable substances/preparations (flashpoint 55 ... 100 °C)	♦ Liquids with a vapour pressure of 2 ... 10 hPa (mbar) (e.g. xylene)	
Negligible risk	♦ Harmless substances by experience (e.g. water, sugar, paraffin and similar)		♦ Not water polluting substances/preparations (**NWG**, formerly **WGK 0**)	♦ Inflammable or very hardly flammable substances/preparations (for liquids flashpoint > 100 °C)	♦ Liquids with a vapour pressure < 2 hPa (mbar) (e.g. glycol) ♦ Solids releasing no dusts	♦ Tightly closed equipment ♦ Closed equipment with exhaust facilities at points of emission

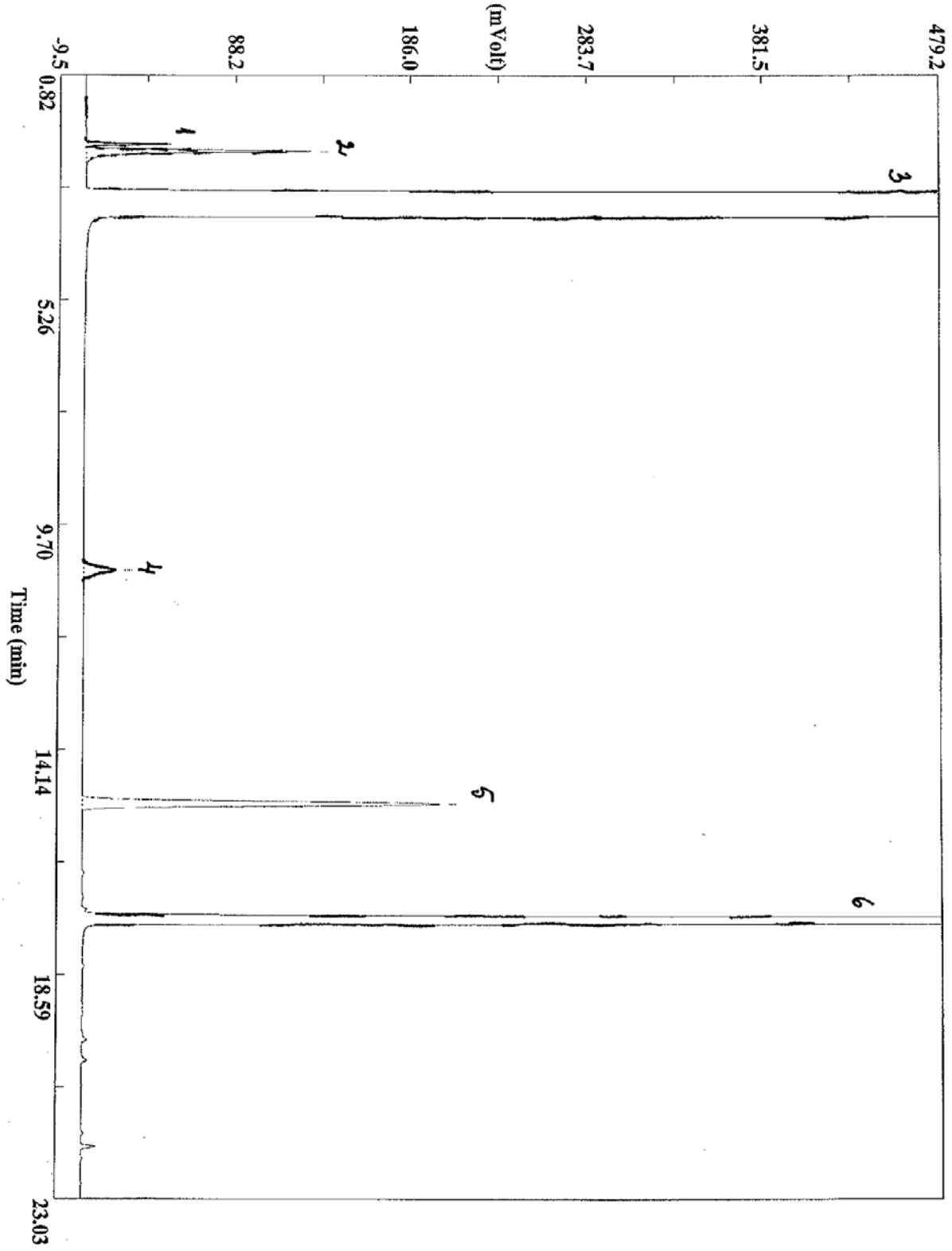
Annex III. Retention times and responses for reference solutions

Column		OVI-G43	Supelco-Q Plot	Rtx-1301		OVI-G43	Supelco-Q Plot)	Rtx-1301		OVI-G43	Supelco-Q Plot)	Rtx-1301
Substance	**Conc.** (µl/ml)	RT	RT	RT		Area	area	area		height (mVolt)	height (mVolt)	height (mVolt)
acetaldehyde	**1**	1,177	7,35	2,14		12623620	1113677	331163		726790	9183	27801
	0,01	1,192	7,84	2,04		130662	11619	6128		6607	223	834
methanol (dry)	**0,01**	1,232	ˇND	3,02		30031	ND	3440		2001	ND	389
	0,2	1,243	4,92	3,04		1686964	174317	224771		101630	1387	29585
	1	1,215	4,56	3,02		2831836	2561761	1041243		164504	25618	149405
benzene	**1**	5,66	18,46	8,97		44702600	9857284613	134933		434940	1373305	3323
	0,002	5,65	18,84	ND		153282	33801782	ND		1521	282862	ND
1,1-diethoxyethane	**3**	9,91	25,27	14,19		64526579	1173362	22826		158418	63082	426
	0,03	9,90	25,43	ND		ND	51167	ND		ND	1028	ND
4-methyl-2-pentanol	**30**	14,70	20,88	17,23		289959113	4523820	12147340		1422647	490295	244190
	0,3	14,66	ND	17,09		7947408	ND	356374		118411	ND	10915

* ND - not detected
RT - retention time

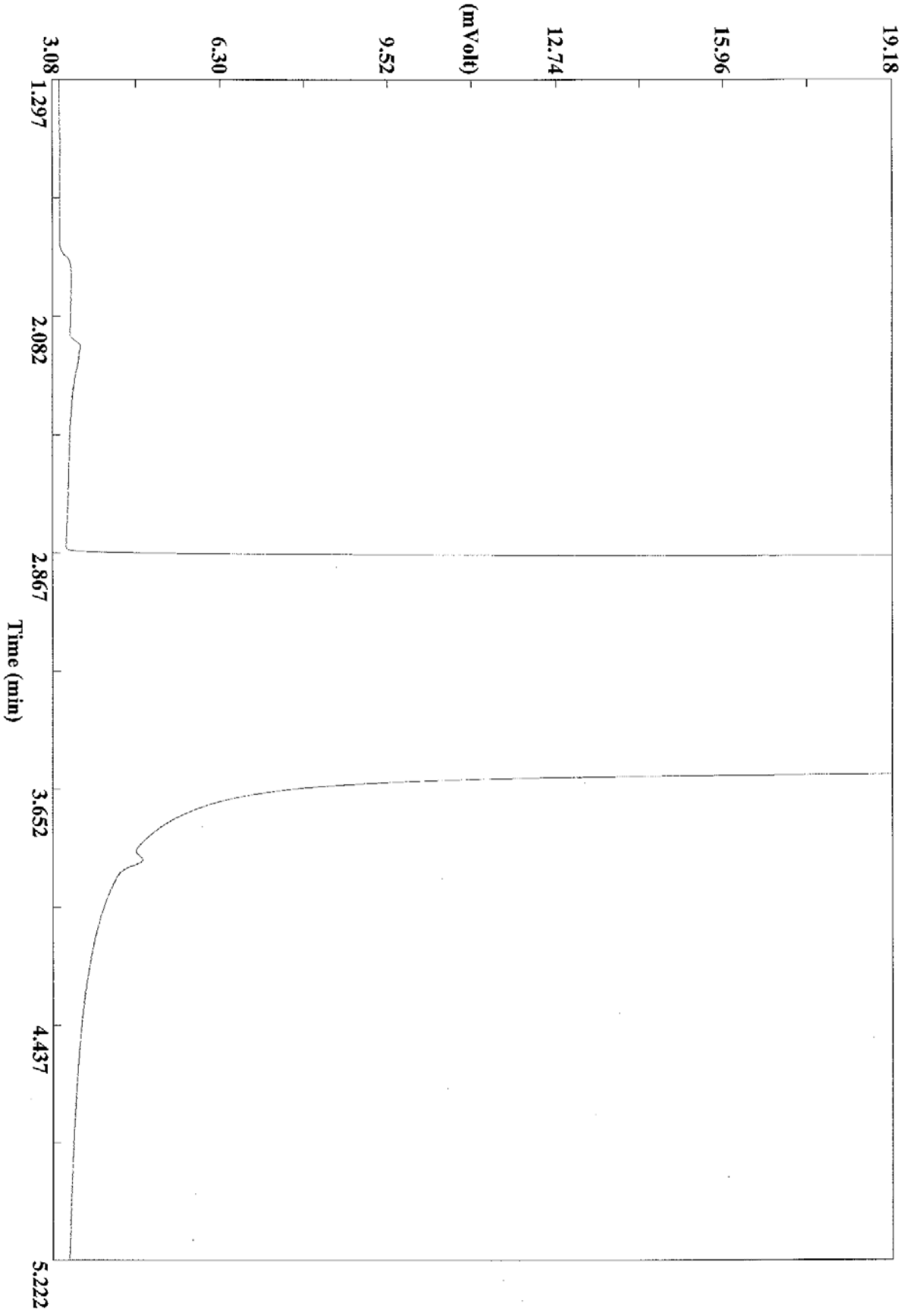
Annex IV. Chromatograms

Chromatogram 1. Ethanol's impurity mixture on Rtx®-1301 column.

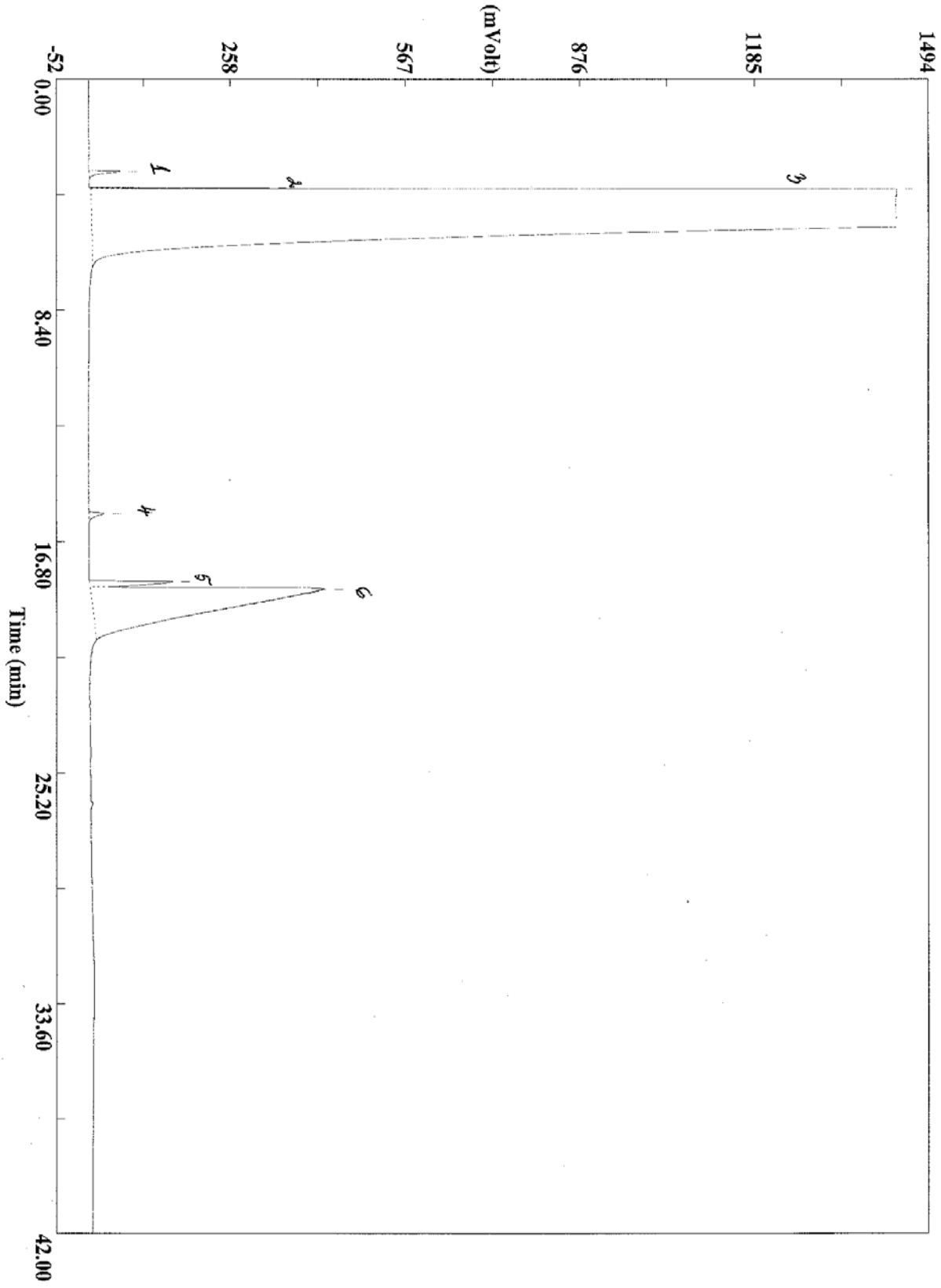


1. acetaldehyde
2. methanol
3. ethanol
4. benzene
5. 1,1-diethoxyethane (acetal)
6. 4-methylpenta-2-ol

Chromatogram 2. Estonian ethanol 1 on Rtx®-1301 column (extact)

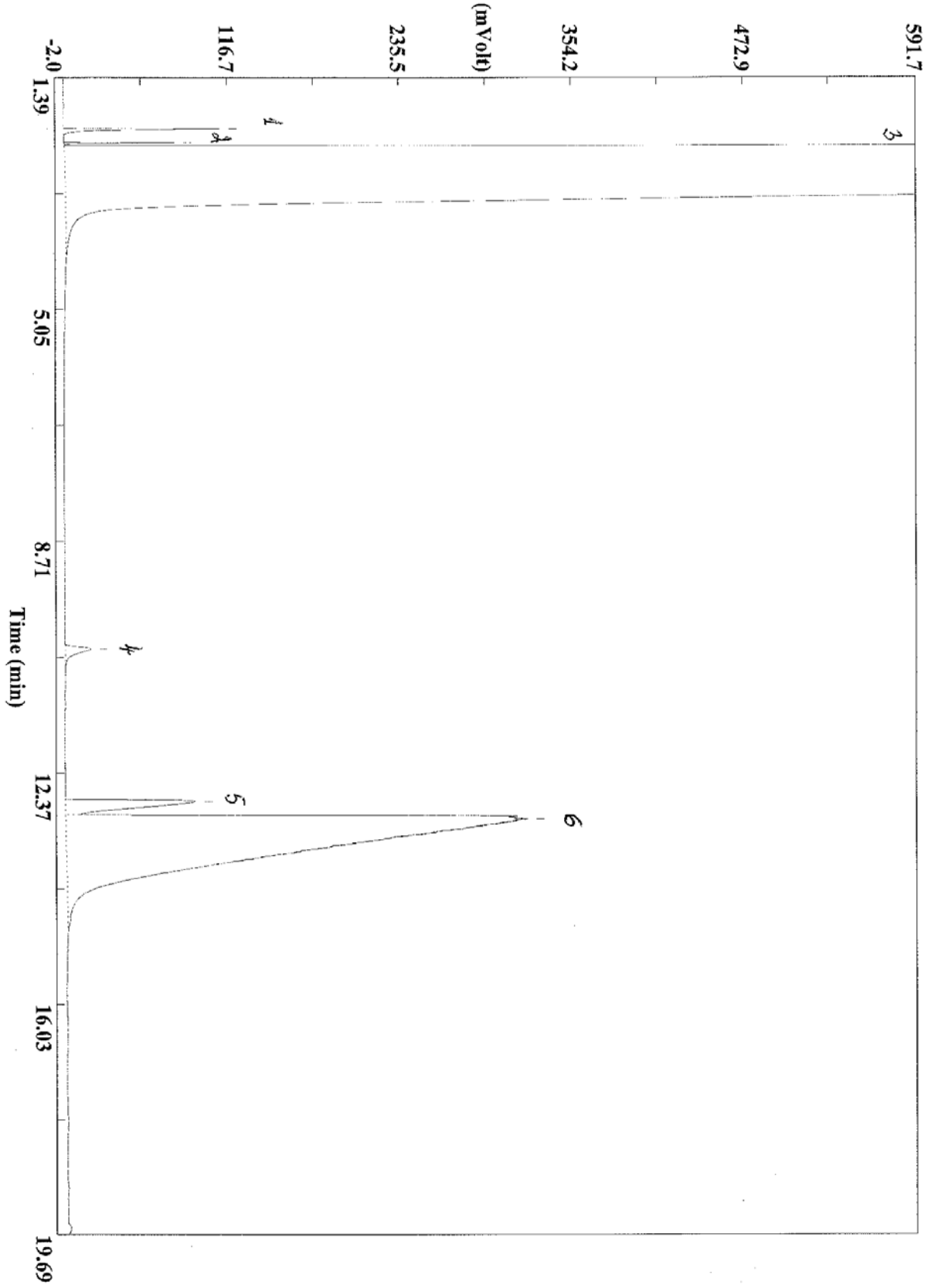


Chromatogram 3. Modified temperature set-up for Q-PLOT column (start at 40˚ C).



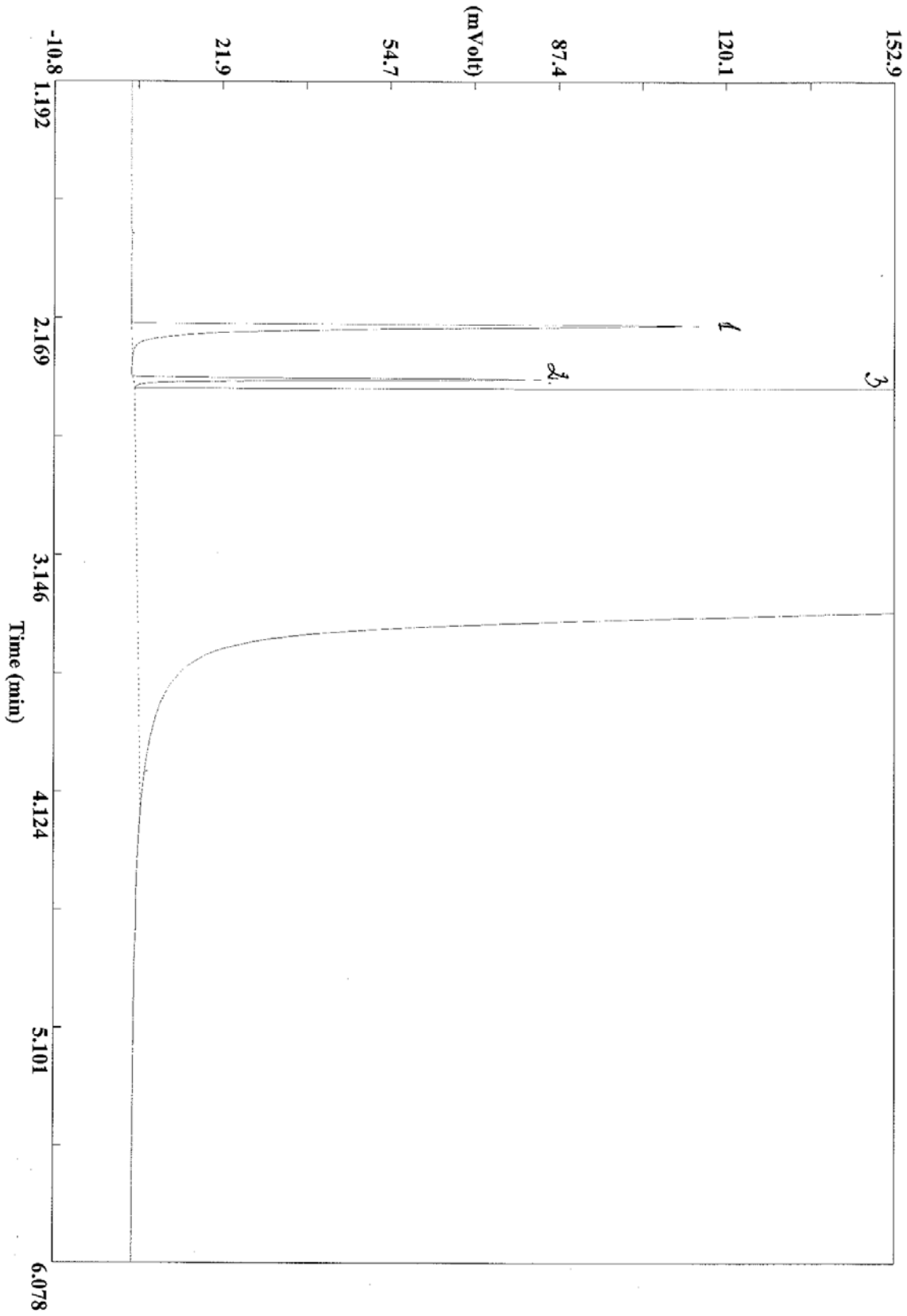
1. methanol
2. acetaldehyde
3. ethanol
4. benzene
5. 1,1-diethoxyethane (acetal)
6. 4-methylpenta-2-ol

Chromatogram 4. Modified temperature set-up for Q-PLOT column (start at 70˚ C).



1. methanol
2. acetaldehyde
3. ethanol
4. benzene
5. 1,1-diethoxyethane (acetal)
6. 4-methylpenta-2-ol

Chromatogram 5. Extract of Chrom 4. to illustrate separation between peaks



1. methanol 2. acetaldehyde 3. ethanol

Annex V. Substances in Ph.Eur . indicated in priority lists of different frameworks

First priority list [http://ecb.jrc.it/priority-setting/priolist01.php]

CAS nr	EC nr	Water class	Name	Classification	Danger indication
60-00-4	200-449-4	-	edetic acid	R 36-52/53	Xi, N
62-53-3	200-539-3	-	aniline	Carc. Cat. 3; R40 Muta.Cat.3; R68 T; R23/24/25-48/23/24/25 Xi; R41 R43 N; R50	T, N
64-02-8	200-573-9	-	tetrasodium ethylenediaminetetraacetate	Xn; R22 Xi; R41	Xn
71-43-2	200-753-7	2	benzene	R 45-46-11-36/38-48/23/24/25-65 M, K	T, F
75-05-8	200-835-2	-	acetonitrile	R 11-20/21/22-36	F, Xn, N
79-01-6	201-167-4	1	trichloroethylene	Carc. Cat. 2; R45 Muta. Cat. 3; R68 [Repr. Cat. 3; R63] Xi; R36/38 R67 R52-5	T
79-06-1	201-173-7	-	acrylamide	R 45-46-E20/21-E25-36/38-43-E48/23/24/25-62 M,K	T
79-10-7	201-177-9	-	acrylic acid	R10 Xn; R20/21/22 C; R35 N; R50	C, N
79-20-9	201-185-2	-	methyl acetate	F; R11 Xi; R36 R66 R67	F, Xi
79-41-4	201-204-4	-	methacrylic acid	R 21/22-35	C
80-62-6	201-297-1	-	methyl methacrylate	R 11-37/38-43	F, Xi
84-74-2	201-557-4	-	dibutyl phtalate	R 61-50-62	T, N
91-20-3	202-049-5	-	naphthalene	R 22-40-50/53 K	Xn, N
95-76-1	202-44-8-4	-	4,3-dichloroaniline	R 23/24/25-33-50/53	T, N
95-80-7	202-453-1	-	4-methyl-m-phenylenediamine	Carc. Cat. 2; R45 Muta. Cat. 3; R68 Repr. Cat. 3; R62 T; R25 Xn; R21-48/22 R43 N; R51-53	T, N
98-82-8	202-704-5	-	cumene	R 10-37-51/53-65	Xn, N
100-41-4	202-849-4	-	ethylbenzene	R 11-20	F, Xn
100-42-5	202-851-5	-	styrene	R10 [Carc. Cat.] [Muta. Cat.] [Repr. Cat.] Xn; R20 Xi; R36/37/38	Xn
101-77-9	202-974-4	-	4,4′-methylenedianiline	Carc. Cat. 2; R45 Muta. Cat. 3; R68 T; R39/23/24/25 Xn; R48/20/21/22 R43 N; R51-53	T, N
103-11-7	203-080-7	-	2-ethylhexyl acrylate	R 37/38-43	Xi, N
106-46-7	203-400-5	-	1,4-dichlorobenzene	Carc. Cat. 3; R40 Xi; R36 N; R50-53	Xn, N
106-99-0	203-450-8	-	buta-1,3-diene	-	-
107-02-8	203-453-4	-	acrylaldehyde	-	-
107-13-1	203-466-5	-	acrylonitrile	R 45-11-E23/24/25-37/38-41-43-51/53 K	F, T, N
107-64-2	203-508-2	-	dimethyldioctadecylammonium chloride	-	-
108-05-4	203-545-4	-	vinyl acetate	F; R11	F
108-95-2	203-632-7	-	phenol	Muta. Cat. 3; R68 T; R23/24/25 C; R34 Xn; R48/20/21/22	T
110-49-6	203-772-9	-	2-methoxyethyl acetate	R 60-61-E20/21/22	T
110-65-6	203-788-6	-	but-2-une1,4-diol	T; R23/25 C; R34 Xn; R21-48/22 R43	T

CAS nr	EC nr	Water class	Name	Classification	Danger indication
110-82-7	203-806-2	-	cyclohexane	F; R11 Xn; R65 Xi; R38 R67 N; R50-53	F, Xn, N
111-77-3	203-906-6	-	2-(2-methoxyethoxy)ethanol	R 63	Xn
112-34-5	203-961-6	-	2-(2-butoxyethoxy)ethanol	R 36	Xi, N
117-84-0	204-214-7	-	dioctyl phthalate	-	-
127-18-4	204-825-9	-	tetrachloroethylene	Carc. Cat. 3; R40 Repr. Cat. 2; R61 Xi; R38 R67 N; R51-53	T, N
141-97-9	205-516-1	-	ehtyl acetoacetate	R 36	Xi
1163-19-5	214-604-9	-	bis(pentabromophenyl)ether	R 53	N
1570-64-5	216-381-3	-	4-chloro-o-cresol	-	-
7664-39-3	231-634-8	-	hydrogen fluoride	R 26/27/28-35	T+, C
32536-52--	251-087-9	-	diphenyl ether, octabromo derivative	-	-

Substance in European Pharmacopoeia
Harmonised classification of the substance from ex-European Chemicalas Bureau
Hazard data from Acros safety data sheets
Hazard data from Merck safety data sheets

Second priority list [http://ecb.jrc.it/priority-setting/priolist02.php]

CAS nr	EC nr	Water class	Name	Classification	Danger indication
67-66-3	200-663-8	1	chloroform (trichloromethane)	R 22-38-40-48/20/22 K	Xn
71-23-8	200-746-9	-	propan-1-ol	R 11-41-67	F, Xi
75-45-6	200-871-9	-	chlorodifluoromethane	-	-
75-56-9	200-879-2	-	methyloxirane	R 45-46-12-E20/21/22-36/37/38 M,K	F+, T
77-78-1	201-058-1	-	dimethyl sulphate	Carc. Cat. 2; R45 Muta. Cat. 3; R68 T+; R26 T; R25 C; R34 R43	T+
88-12-0	201-800-4	-	1-vinyl-2-pyrrolidone	R 20/21/22-40-41-48/20 K	Xn
90-04-0	201-963-1	-	o-anisidine	Carc. Cat. 2; R45 Muta. Cat. 3; R68 T; R23/24/25	T
95-33-0	202-411-2	-	N-cyclohexylbenzothiazole-2-suphenamide		-
98-01-1	202-627-7	-	2-furaldehyde	Carc. Cat. 3; R40 T; R23/25 Xn; R21 Xi; R36/37/38	T
100-97-0	202-905-8	-	methenamine	F; R11 R43	F, Xi
108-88-0	203-625-9	-	toluene	F; R11 Repr.Cat.3; R63 Xn; R48/20-65 Xi; R38 R67	F, Xn
109-66-0	203-692-4	-	pentane	R 12-51/53-65-66-67	F+, Xn, N

110-80-5	203-804-1	-	2-ethoxyethanol	R 60-61-10-E20/21/22 Rep	T
111-15-9	203-839-2	-	2-ethoxyethyl acetate	R10 Repr. Cat. 2; R60-61 Xn; R20/21/22	T
115-96-8	204-118-5	-	tris(2-chloroethyl)phosphate	Carc. Cat. 3; R40 Repr. Cat. 2; R60 Xn; R22 N; R51-53	T, N
117-81-7	204-211-0	-	bis(2-ethylhexyl)phthalate	R 60-61	T
120-82-1	204-428-0	-	1,2,4-trichlorobenzene	R 22-36/38-50/53	Xn, N
123-91-1	204-661-8	-	1,4-dioxane	R 11-19-36/37-40-66 K	F, Xn
557-05-1	209-151-9	2	zinc distearate	Not hazardous	-
1314-13-2	215-222-5	2	zinc oxide	R 50/53	N
7440-66-6	231-175-3	2	zinc	N; R50-53	N
7646-85-7	231-592-0	2	zinc chloride	C; R34 Xn; R22 N; R50-53	C, N
7681-52-9	231-668-3	-	sodium hypochlorite	R 31 34	C
7722-84-1	231-765-0	-	hydrogen peroxide	R 22-41	Xn
7733-02-0	231-793-3	2	zinc sulphate	Xn; R22 Xi; R41 N; R50-53	Xn, N
7779-90-0	231-944-3	-	trizinc bis(orthophosphate)	-	-
25154-52-3	246-672-0	-	nonylphenol	-	-
25167-70-8	246-690-9	-	2,4,4-trimethylpentene	-	-
25637-99-4	247-148-4	-	hexabromocyclododecane	N; R50-53	N
26761-40-0	247-977-1	-	di-isodecyl phthalate	Not hazardous	-
28553-12-0	249-079-5	-	di-isononyl phthalate	-	-
32534-81-9	251-084-2	-	diphenyl ether, pentabromo derivative	-	-

Third priority list [http://ecb.jrc.it/priority-setting/priolist03.php]

CAS nr	EC nr	Water class	Name	Classification	Danger indication
75-91-2	200-915-7	-	tert-butyl hydroperoxide	R 7-21/22-23-34-44-52/53	O, T
79-11-8	201-178-4	-	chloroacetic acid	T; R23/24/25 C; R34 N; R50	T, N
80-05-7	201-245-8	-	4,4-isopropylidenediphenol	Repr. Cat. 3; R62 Xi; R37-41 R43 R52	Xn
81-14-1	201-328-9	-	4-tert-butyl-2,6-dimethyl-3,5-dinitroacetophenone	Carc. Cat. 3; R40 N; R50-53	Xn, N
81-15-2	201-329-4	-	5-tert-butyl-2,4,6-trinitrio-m-xylene	E; R2 Carc. Cat. 3; R40 N; R50-53	E, Xn, N
85-68-7	201-622-7	-	benzyl butyl phthalate	Repr. Cat.2; R61 Repr. Cat.3; R62 N; R50-53	T, N
98-95-3	202-716-0	-	nitrobenzene	R 23/24/25-40-48/23/24-51/53-62 K	T, N
110-85-0	203-808-3	-	piperazine	Repr. Cat. 3; R62-63 C; R34 R42/43	Xn, C
120-12-7	204-371-1	-	anthracene	N; R50-53	N

122-39-4	204-539-4	-	diphenylamine	R 23/24/25-33-50/53	T, N
1306-19-0	215-146-2	1	cadmium oxide	Carc. Cat. 2; R45 Muta. Cat. 3; R68 Repr. Cat. 3; R62-63 T; R48/23/25 T+; R26 N; R50-53	T+, N
1333-82-0	215-607-8	-	chromium trioxide	O; R9 Carc. Cat. 1; R45 Muta. Cat. 2; R46 Repr. Cat. 3; R62 T+; R26 T; R24/25-48/23 C; R35 R42/43 N; R50-53	O, T+, N
1634-04-4	216-653-1	-	tert-butyl methyl ether	R 11-38	F, Xi
3033-77-0	221-221-0	-	2,3-epoxypropyltrimethylammonium chloride	Carc. Cat. 2; R45 Muta. Cat. 3; R68 Repr. Cat. 3; R62 Xn; R21/22-48/22 Xi; R41 R43 R52-53	T
3327-22-8	222-048-3	-	(3-chloro-2-hydroxypropyl)timethylammonium chloride		-
5064-31-1	225-768-6	-	trisodium nitrilotriacetate	-	-
7440-02-0	231-111-4	2	nickel	Carc. Cat. 3; R40 T; R48/23 R43	T
7440-43-9	231-152-8	1	cadmium	F; R17 Carc. Cat. 2; R45 Muta. Cat. 3; R68 Repr. Cat. 3; R62-63 T+; R26 T; R48/23/25 N; R50-53	F, T+, N
3-11-7775	231-889-5	2	sodium chromate	R 45 46 60 61 21 25 26 34 42/43 48/23 50/53 8	T+, N
7778-50-9	231-906-6	2	potassium dichromate	O; R8 Carc. Cat. 2; R45 Muta. Cat. 2; R46 Repr. Cat. 2; R60-61 T+; R26 T; R25-48/23 C; R34 Xn; R21 R42/43 N; R50-53	O, T+, N
7782-50-5	231-959-5	-	chlorine	T; R23 Xi; R36/37/38 N; R50	T, N
7786-81-4	232-104-9	2	nickel sulphate	Carc. Cat. 1; R49 Muta. Cat. 3; R68 Repr. Cat. 2; R61 T; R48/23 Xn; R20/22 Xi; R38 R42/43 N; R50-53	T, N
5-09-7789	232-143-1	-	ammonium dichromate	E; R2 O; R8 Carc. Cat. 2; R45 Muta. Cat. 2; R46 Repr. Cat. 2; R60-61 T+; R26 T; R25-48/23 Xn; R21 C; R34 R42/43 N; R50-53	E, T+, N
10039-54-0	233-118-8	-	bis(hydroxylammonium) sulphate	E; R2 Carc. Cat. 3; R40 Xn; R21/22-48/22 Xi; R36/38 R43 N; R50	E, Xn, N
10588-01-9	234-190-3	-	sodium dichromate	O; R8 Carc. Cat. 2; R45 Muta. Cat. 2; R46 Repr. Cat. 2; R60-61 T+; R26 T; R25-48/23 C; R34 Xn; R21 R42/43 N; R50-53	O, T+, N
11138-47-9	234-390-0	-	perboric acid, sodium salt	-	-
13775-53-6	237-410-6	-	trisodium hexafluoroaluminate	-	-
15096-52-3	239-148-8	-	trisodium hexafluoroaluminate	R 20/22-48/23/25-51/53	T, N
26447-40-5	247-714-0	-	methylenediphenyl diisocyanate	-	-
30899-19-5	250-378-8	-	pentanol (1-pentanol)	R10 Xn; R20 Xi; R37 R66	Xn

Fourth priority list (as of 24.10.2006). [Official Journal of the European Communities]

CAS nr	EC nr	Water class	Name	Classification	Danger indication
77-47-4	201-029-3	-	hexachlorocyclopentadiene	-	-
79-94-7	201-236-9	-	2,2,6,6-tetrabromo-4,4-isopropylidenediphenol	R 36/37/38	Xi
88-72-2	201-853-3	-	2-nitrotoluene	R 45-46-E22-62-51/53 K	T, N
98-54-4	202-679-0	-	4-tert-butylphenol	[Repr. Cat. 3; R62-63] Xi; R37/38-41 N; R51-53	Xn, Xi, N
98-73-7	202-696-3	-	4-tert-butylbenzoic acid	R 20/21/22-48/20/21/22-51/53-62	Xn, N

107-98-2	203-539-1	-	1-methoxypropan-2-ol	R10 R67	-
108-65-6	203-603-9	-	2-methoxy-1-methylethyl acetate	R10	-
111-76-2	203-905-0	-	2-butoxyethanol	Xn; R20/21/22 Xi; R36/38	Xn
112-07-2	203-933-3	-	2-butoxyethyl acetate	R 20/21	Xn
112-90-3	204-015-5	-	(Z)-octadec-9-enylamine	R 22 34	C
121-14-2	204-450-0	-	2,4-dinitrotoluene	Carc. Cat. 2; R45 Muta. Cat. 3; R68 Repr. Cat. 3; R62 T; R23/24/25 Xn; R48/22 N; R50-53	T, N
124-30-1	204-695-3	-	octadecylamine	R 38-41-51/53	Xi, N
994-05-8	213-611-4	-	2-methoxy-2-methylbutane	R 11-22	F, Xn
1222-05-5	214-946-9	-	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran		-
1309-64-4	215-175-0	-	diantimony trioxide	Carc. Cat. 3; R40	Xn
1310-73-2	215-185-5	-	sodium hydroxide	R 35	C
1330-43-4	215-540-4	-	disodium tetraborate, anhydrous	Repr. Cat. 2; R60-61	T
1506-02-1	216-133-4	-	1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-heamethyl-2-naphthyl)ethan-1-one	Xn; R22 N; R50-53	Xn, N
3333-67-3	222-068-2	2	nickel carbonate	Carc. Cat. 1; R49 Muta. Cat. 3; R68 Repr. Cat. 2; R61 T; R48/23 Xn; R20/22 Xi; R38 R42/43 N; R50-53	T, N
7718-54-9	231-736-0	2	nickel dichloride	R 25-43-50/53	T, N
7784-18-1	232-051-1	-	aluminium fluoride	R 36/37/38	Xi
7789-75-5	232-188-7	-	calcium fluoride	Not hazardous	-
10043-35-3	233-139-2	-	boric acid, crude natural	Repr. Cat. 2; R60-61	T
11113-50-1	234-343-4	-	boric acid	Repr. Cat. 2; R60-61	T
13138-45-9	236-068-5	2	nickel dinitrate	O; R8 Carc. Cat. 1; R49 Muta. Cat. 3; R68 Repr. Cat. 2; R61 T; R48/23 Xn; R20/22 Xi; R38-41 R42/43 N; R50-53	O, T, N
13674-84-5	237-158-7	-	tris(2-chloro-1-methylethyl) phosphate	-	-
13674-87-8	237-159-2	-	tris[2-chloro-1-(chloromethyl)ethyl] phosphate		-
26523-78-4	247-759-6	-	tris(nonylphenyl) phosphite	R43 [N; R50-53]	Xi
38051-10-4	253-760-2	-	2,2-bis(chloromethyl)trimethylene bis(bis(2-chlotoethyl)phosphate)		-

Fifth priority list. Water environmental substances (as of 24.10.2006) [http://lex. Andmevara.ee/estlex]

CAS nr	EC nr	Water class	Name	Classification	Danger indication
319-86-8	-	-	delta-hexachlorocyclohexane	-	-
319-84-6	-	-	alfa-hexachlorocyclohexane	-	-
319-85-7	-	-	beta-hexachlorocyclohexane	-	-
107-06-2	203-458-1	-	1,2-dichloroethane	R 45-11-E22-36/37/38	T, F

789-02-6	-	-	o,p - DDT	-	-
50-29-3	200-024-3	-	p,p - DDT	-	-
309-00-2	206-215-8	-	aldrine	-	-
60-57-1	200-484-5	-	dieldrine	-	-
72-20-8	200-775-7	-	endrine	-	-
465-73-6	207-366-2	-	isodrine	26/27/28 - 50/53	T+, N
118-74-1	204-273-9	-	hexachlorobenzene	-	-
79-01-6	201-167-4	-	trichloroethylene	Carc. Cat. 2; R45 Muta. Cat. 3; R68 [Repr. Cat. 3; R63] Xi; R36/38 R67 R52-53	T
56-23-5	200-262-8	-	carbontetrachloride	Carc. Cat. 3; R40 T; R23/24/25-48/23 N; R59 R52-53	T, N
95-48-7	202-423-8	-	o-kresole	R 24/25-34	T
108-39-4	203-577-9	-	m-kresole	R 24/25-34	T
106-44-5	203-398-6	-	p-kresole	R 24/25-34	T
15972-60-8	240-110-8	-	alachlore	Carc. Cat. 3; R40 Xn; R22 R43 N; R50-53	Xn, N
75-09-2	200-838-9	-	dichloromethane	R 40	Xn
7664-41-7	231-635-3	-	ammonia	R10 T; R23 C; R34 N; R50	T, N
71-43-2	200-753-7	-	benzene	R 45-46-11-36/38-48/23/24/25-65	F, T
-	231-768-7	-	phosphorus and it derivates	F; R17 T+; R26/28 C; R35 N; R50	F, T+, C, N
76-44-8	200-962-3	-	heptachlorine	-	-
91-20-3	202-049-5	-	naphthalene	R 22-40-50/53	Xn, N
-	-	-	silicon compounds	-	-
92-87-5	202-199-1	-	benzidine and it salts	Carc. Cat. 1; R45 Xn; R22 N; R50-53	T, N

Annex VI. Comparison of chloroform and methylene chloride properties

	Cloroform	**Methylene chloride**
Synonyms	Trichloromethane; Methyl trichloride; Methane trichloride	MC; Dichloromethane (DCM); Methylene dichloride; Methylene bichloride; Methane dichloride
CAS no:	67-66-3	75-09-2
Molar mass, g/mol	119.38	84.93
Chemical formula	$CHCl_3$	CH_2Cl_2
Description	Clear colourless liquid	Clear colourless liquid
Odour	Ether-like odour	With a penetrating ether-like odour
Solubility in water	0,8g/100g in water at 20°C	1.32g/100g in water at 20°C
Volatility	100%	100%
Density	1.48 g/cm^3 (20 ^{o}C) *	1.33 g/cm^3 (20 ^{o}C)
Boiling point, °C	62	39.8
Melting point, °C	-63.5	-97
To avoid	Light, heating, air	Heating, humidity, closeness to the heater
Dangerous degradation products	By heating secedes carbonoxide, carbondioxide, hydrogenchloride, phosgene vapors	By heating secedes hydrogenchloride, phosgene vapors. May secede carbonoxide.
Avoid contact with following substances	Strong plastics and chemically active metals like aluminum, magnesium powder, sodium and potassium; acetone, fluorine, methanol, sodiummetoxide, dinitrogentetraoxide, triisopropylphosphine.	Strong oxidants, strong cauterizes, plastics, rubber, nitric acid, water+heat, chemically active metals; like aluminum, magnesium powder, sodium and potassium, lithium. Avoid open flame and electric arc. Liquid methylchloride reacts with some plastics, rubber and coating.
Toxity	Cancerogenic	Cancerogenic
Water hazard	WKG 3 (higly water endagering)	WGK 2 (water endangering)
Is used in	Is used in fluorocarbon production to fridges and as aerosole reactive fuel; is used in fire extinguishers to decrease carbon tetrachloride freezing point, in fluorocarbon waxes, in plastics and thermaly stable polymers production, in synthetic silk, floor polishing, paints and pesticides production. Is used as extractive solvent for rubber, sterols, essential oils and alkaloids industry. Is used in chemical analysis and in determination of active substances	Is used as solvent, especially when high volatility is needed. Good solvent for oils, fats, vaxes, resins, bitumen, rubber and cellulose acetate and is a good paint and fat remover. It is used in content of reactive fuel mixtures in aerosole containers like plastics solvent, extractive component in pharmaceutical industries. The solvent properties are increased sometimes by mixing it with methanol,

	and in photographic processing. Early usage in anesthesia is finised and in human medicine. It is allowed in cosmetic as a solvent in final products manufacturing processes, which contains chloroform only in minute amount.	petroleum oil or tetrachloroethylene.
Storage	Store in well closed conteiner in cool dry well ventilated place (less than 30° C).	To avoid physical damage. Store in a cool dry well ventilated place in containers with are covered with zinc or lead, because of the substance high vapor pressure and corrosive characteristics. Store away from straight sunlight, heater.

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