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Validation, analysis and comparison of two chromatographic methods developed for analysis of amines in peat soil-water samples

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Acknowledgement

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Abstract

The work described in this thesis was based on the results from a research project called *"Experimental Study Investigating Risks of Selected Amines"* (ExSIRA). The aim of the project was to gain knowledge on amines released from a carbon capture and storage (CCS) plant and their toxicity on a vulnerable nitrogen sensitive environment. Aqueous solutions of low mass aliphatic amines like monoethanolamine (MEA), diethanolamine (DEA) and 2-amino-2-methyl-1-propanol (AMP) are commonly used in post combustion capture technology, to remove CO_2 released from a fossil fuel fired power plant. The ExSIRA project simulated a worst case scenario release from a CCS plant by adding amines to experimental plots of an ombrotrophic raised bog at Smøla, Norway. The aim was to collect water and calculate a recovery of amines. However, no amines were found in the soil-water. The goal of the study in this thesis was to investigate two hypotheses connected to the fate of the amines, and validate two liquid chromatography mass spectrometry (LC-MS) methods developed by the Norwegian Institute for Air Research (NILU).

Method validation involved finding limits of detection (LOD) and quantification (LOQ), linearity, accuracy and precision of two analytical methods, in addition to determine the stability of analytes. One method analyzed the amines directly, while the other method analyzed amines indirectly through a derivative of dansyl chloride. None of the methods fulfilled all criteria's set in the method validation plan. In general, the direct method was best suited for analysis of high concentration samples due to a greater linear range, while the indirect method was best suited for analysis of lower concentrations due to higher sensitivity and smaller linear range. Both methods contained some uncertainties regarding accuracy and precision. Despite these uncertainties, the validation of DEA was better with the direct method, while MEA and AMP gave better results with the indirect method. The stability test indicated that only MEA showed instability and possible degradation over time.

A leaching experiment in soil columns was conducted by Line Tau Strand at the Department of Environmental Sciences at NMBU in order to simulate the ExSIRA project at Smøla. The soil columns were added amines and drained by gravity. The leachate collected contained 11-23 % of the same amines added to the columns. The two hypothesis developed from the ExSIRA project said that amines could not be detected because of too much noise in the sample matrix, and/or because all amines were adsorbed to the soil solid phase. Both of these hypotheses were rejected.

Sammendrag

Arbeidet beskrevet i denne oppgaven er basert på resultater fra et forskningsprosjekt som heter *"Experimental Study Investigating Risks of Selected Amines"* (ExSIRA). Målet med dette prosjektet var å få kunnskap om aminer som slippes ut i forbindelse med CO_2-fangst og deres påvirkning på et sårbart nitrogensensitivt miljø. Vandige løsninger av aminer som monoetanolamin (MEA), dietanolamin (DEA) og 2-amin-2-metyl-1-propanol (AMP) brukes i *"post-combustion"*-fangst for å fjerne CO_2 som slippes ut av et kraftverk drevet av fossilt brensel. ExSIRA prosjektet etterlignet et utslipp av aminer fra et slikt kraftverk med CO_2 rensing ved å tilsette disse aminene til et forsøksfelt på en ombrogen myr på Smøla, Norge. Målet med forsøket var blant annet å samle vann fra forsøksfeltet og beregne en gjennfinning av aminer i jordvannet, men ingen aminer ble funnet. Målet med studien i denne opgaven var å undersøke to hypoteser som forklarer hvorfor ingen aminer ble gjennfunnet i jordvannet i ExSIRA prosjektet, samt å validere to væskekromatografi-massespektrometriske (LC-MS) metoder utviklet av Norsk Institutt for Luftforskning (NILU).

Metodevalideringen innebar å finne deteksjons- og kvantifiseringsgrenser (LOD og LOQ), linearitet, nøyaktighet og presisjon til de to analytiske metodene, i tillegg til å se på stabilitet til aminene over tid. Den ene metoden analyserte aminene direkte, mens den andre metodene analyserte aminene indirekte gjennom et derivatiseringstrinn med dansylklorid. Ingen av metodene oppfylte alle kriteriene i metodevalideringsplanen. Generelt sett var den direkte metoden best egnet til analyse av aminer ved høyere konsentrasjoner enn den indirekte metoden, grunnet et større lineært område. Den indirekte metoden var imidlertid bedre egnet til analyse av aminer ved lavere konsentrasjoner, grunnet høyere sensitivet og et mindre lineært område. Begge metodene hadde noe usikkerhet i forbindelse med metodens nøyaktighet og presisjon. Til tross for det, viste valideringen at den direkte metoden var best egnet for analyse av DEA, mens den indirekte metoden var best egnet for analyse av MEA og AMP. Stabilitetstesten indikerte at MEA var ustabilt med en mulig nedbrytning over tid.

Et kolonneforsøk ble utført av Line Tau strand ved Institutt for Miljøvitenskap ved NMBU, for å simulere ExSIRA prosjektet på Smøla. Jordkolonnene ble tilsatt aminer og drenert ved hjelp av tyngdekraften. Eluatet samlet fra kolonnen inneholdt mellom 11-23 % aminer. De to hypotesene utviklet for å forklarer hvorfor ingen aminer ble funnet i ExSIRA prosjektet gikk ut på at alle aminene ble adsorbert til jord, og/eller at det var for mye støy i jordvannet til at aminene kunne detekteres. Begge disse hypotesene ble forkastet.

Abbreviations

AMP	2-methyl-2-amino-1-propanol/methylaminepropanol
CCS	Carbon Capture and Storage
DEA	Diethanolamine
DMA	Dimethylamine
DNS-Cl	Dansyl chloride
ES	External standard
ExSIRA	Experimental Study Investigating Risks of Selected Amines
HPLC	High pressure liquid chromatography
IR	Infrared
IS	Internal standard
LC-MS	Liquid chromatography mass spectrometry
LLE	Liquid liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MEA	Monoethanolamine
MP	Melting point
MQ	Milli-Q water
NMR	Nuclear magnetic resonance
NILU	Norwegian Institute for Air Research
NMBU	Norwegian University of Life Sciences
Q-TOF	Quadrupole time of flight
RSD	Relative standard deviation
SD	Standard deviation
SOM	Soil organic matter
SM	Sample matrix
TOC	Total organic carbon
UV	Ultraviolet-visible

Contents

1. Introduction

The use of fossil energy for the production of power is the largest source of greenhouse gases (Oljedirektoratet 2013), where carbon dioxide (CO_2) is the main contributor to global warming (Karl et al. 2008). An important tool to reduce CO_2-emissions to the atmosphere from for example fossil fuel combustion is carbon capture and storage (CCS). CCS is a process of capturing CO_2 generated from for example fossil fuel combustion, and storing it underground away from the atmosphere (Intergovernmental Panel on Climate Change 2005). A popular CCS-technique is called post combustion capture, where the CO_2 is removed after combustion of fossil fuels (Figueroa et al. 2008). This technique uses aqueous solutions of amines as a part of the process and are often called amine technology (Rochelle 2009). In association with amine technology, there is a risk of release of amines to the environment. Many comprehensive studies have been done in recent years on the toxicology of amines released to the environment from a CCS plant, but there are still gaps of knowledge in this field. The goal of this study was to validate, compare and perform analysis on two chromatographic methods developed for analysis of amines in soil water rich in organic matter. The analytical methods were developed by the Norwegian Institute for Air Research (NILU).

1.1 Background

In 2008 CCS was proposed for two Norwegian gas-fired power plants, Kårstø and Mongstad. The planning of a CCS facility at Kårstø was stopped in 2009, due to a fluctuating operating pattern, but the planning at Mongstad continued (Gassnova 2014). At Mongstad the plan was to develop CCS technology in two stages, where the first stage was to create a CO_2-capture technology center. The second stage was to construct a full-scale CCS plant (Oljedirektoratet 2013). One of the tasks of the technology center was to test different technologies for CO_2-capture (TCM 2010).

There are several CCS technologies, depending on if the CO_2 is captured directly from large point sources or from the atmosphere. When capturing CO_2 directly from point sources there are different technologies depending on when in the combustion process the CO_2 is removed (Benson & Orr 2008). One of these techniques is called post combustion capture. In this process the CO_2 is removed after combustion of fossil fuels (Figueroa et al. 2008). At

Mongstad, one of the technologies tested and chosen by the technology center was a post-combustion capture technique using amines as a part of the process. This amine technology has been known for several years, and has been used to separate CO_2 from natural gas and hydrogen since the 1930s (Rochelle 2009). In amine technology, CO_2 is captured by an amine solvent which is a liquid composed of water and amines. This liquid is used to absorb CO_2 from the flue gas in a process called gas sweetening/processing (TCM 2010). This technology is robust and has in recent years been tested for use on a larger scale for CO_2-capture from fossil fuel fired power plants (Rochelle 2009). This is now the most advanced and accepted technology for fossil fuel fired power plants (Reynolds et al. 2012).

According to the Norwegian Water Resources and Energy Directorate (NVE) the emission of amines to the environment from a CCS plant using amine technology is estimated to be between 40-160 tons per year. Additional emissions of nitrogen compounds like nitrogen oxides (NO_x) and ammonia (NH_3) will also be present (Falk-Pedersen et al. 2006). Nitrogen emissions have the potential of causing acidification of surface water and contribute to the eutrophication of terrestrial ecosystems. There are critical negative environmental consequences that arise due to excess nitrogen leached into water bodies like lakes, groundwater, rivers etc. This can lead to an increased growth of algae that can be harmful to both animals and humans. However, as an essential nutrient for plants and as a constituent in proteins, nitrogen is essential to animals (vanLoon & Duffy 2005).

In the planning stage of the CO_2-capture plant at Mongstad (and Kårstø) there was not enough knowledge about the toxicology of amines emitted from a CO_2-capture plant on human and animal health, vulnerable ecosystems and the environment (Karl et al. 2008). Secondary and tertiary amines can be transformed to nitramines and nitrosamines, which are known to be carcinogenic. These gaps of knowledge led to the initiation of several comprehensive studies about amines and toxicity. One of these projects is known as the ExSIRA project.

1.2 Previous work

In 2010 a project called *"Experimental Study Investigating Risks of Selected Amines"* (ExSIRA) was initiated. The project investigated amine emissions during carbon capture on different environments. The objective of the ExSIRA project was to study emissions of primary amines to the atmosphere from a CO_2-capture plant and their secondary photo oxidant products (produced in the atmosphere, after emission), on the environment. A

subproject of ExSIRA dealt with terrestrial ecology, and the main goal was to study possible effects of amine emissions from a CO_2-capture plant on a vulnerable nitrogen sensitive ecosystem. The main focus of the subproject was effects on vegetation, soil water and soil fauna (ExSIRA 2009).

The research of the terrestrial ecology subproject was performed on an ombrotrophic raised bog which is a vegetation type that has extremely poor access to nutrients (Aarrestad & Bruteig 2006). Ombrotrophic means that the bog is dependent on nutrients from the atmosphere, as wet or dry deposition. This makes them especially vulnerable to nitrogen pollution from air (Bobbink et al. 2003). Amines emitted from a CCS plant will easily dissolve in water because of high solubility and deposited as wet deposition (Karl et al. 2008). Norway has a wet and humid climate, but the deposition pattern will depend on the local climate. The vegetation and organisms living on these bogs are adapted to low access of nitrogen, and airborne nitrogen pollution can have a negative effect (Aarrestad & Bruteig 2006). Studies from the Netherlands and Britain have shown eutrophication and absence of characteristic species on ombrotrophic raised bogs exposed to nitrogen pollution. Bogs are wet areas that are acidic. The rate of decomposition of organic material is slow and peat is formed. In Norway ombrotrophic raised bogs are common due to the climate and high northern latitudes. Bog-mosses, sedges and heathers are typical plant species that can be found here (Bobbink et al. 2003).

The research done in this thesis builds on some results from the terrestrial ecology subproject where three amines used in CO_2-capture; monoethanolamine (MEA), diethanolamine (DEA) and 2-amino-2-methyl-1-propanol (AMP) were added to experimental plots of ombrotrophic bog at Smøla, Møre and Romsdal, Norway. A concentration equivalent to worst case scenario emissions from a CO_2-capture plant were added. The aim of the project was to see if these amines were leached through vegetation and peat soil and into the water phase on the bog, where they can contribute to eutrophication. The experiment was conducted over a long period of time with several additions of amines to the experimental plots (ExSIRA 2009). Soil water from these plots where collected continuously and the samples were sent to NILU for quantification and calculation of recovery of the amines. The results showed that none of the amines added to the experimental plot could be recovered from the water samples (Tau Strand 2014). These findings suggested the need for further investigations and the development of six hypotheses connected to the fate of the three amines:

1. MEA, DEA and AMP were absorbed or taken up by the above ground vegetation
2. MEA, DEA and AMP were absorbed in the soil solid phase and none followed the soil water extracted from the ombrotrophic raised bog at Smøla.
3. MEA, DEA and AMP entering the soil could not be detected because the soil water contained so much noise that they cover their signal.
4. The sampling equipment used was not suitable for sampling the amines as they most likely would be sorbed to equipment surfaces.
5. The sampling was not done at the right time and place. Hydrology, breakthrough curves were not known for the soils and two weeks after addition the dilution and movement of the amines could have reduced the likelihood of recovering any of the amines
6. Microbial degradation of the amines.

The study in this thesis does not aim at investigating all these hypothesis, but focuses on hypothesis 2 and 3 (Tau Strand 2014).

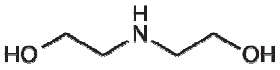
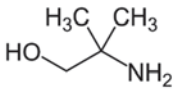
1.3 Amines

Amines are a functional group and organic compounds that are relatives of ammonia and are numerous in nature. They can be classified as primary, secondary or tertiary depending on the number of organic groups that are attached to the nitrogen (Hart et al. 2007). Natural sources of amines are among others degradation products from animals and plants. Anthropogenic sources vary from chemical industries, textile industries, agriculture, pharmaceutical industry, paints and adhesive industries to mention a few examples (Fournier et al. 2008). They are also commonly used in amine technology, as adsorbents for CO_2 and hydrogen sulfide (H_2S) (Reynolds et al. 2012). A group of amines called alkanolamines are frequently used for this purpose.

Alkanolamines are a group of organic chemicals that contains both an amine ($-NH_2$) and hydroxyl (-OH) functional group. They have properties typical for amines and alcohols, and can therefore undergo reactions characteristic for both functional groups. The amine part of the molecule is mildly alkaline, and the alcohol part is hygroscopic. This makes alkanolamines suitable for use in a number of different applications, such as surfactants, cosmetics, toiletry products, metalworking fluids, textile chemicals, agricultural chemical intermediates and cement grinding aids. Alkanolamines can be divided in three groups:

ethanolamines, isopropanolamines and butanolamines depending on the length of the hydrocarbon chain(s) in the molecule. According to Davis & Carpenter (1997) the ethanolamines and isobutanolamines are considered to be relatively nonvolatile. Table 1.1 shows the structure of MEA, DEA and AMP which are frequently used in amine technology.

Table 1.1. Physical and chemical properties of MEA, DEA and AMP.

	Molar mass	**Formula**	**Classification**	**Melting point (°C)**	**Chemical structure**
MEA	61.09	C_2H_7NO	Primary ethanolamine	10-11	
DEA	105.14	$C_4H_{11}NO_2$	Secondary ethanolamine	24-28	
AMP	89.14	$C_4H_{11}NO_2$	Primary isobutanolamine	28	

MEA is a primary ethanolamine and has a simple chemical structure and a low molar mass. It occurs naturally in both animals and humans as a constituent of phospholipids known as phosphatides. These lipids are composed of glycerol, two fatty acid esters, phpsophoric acid and MEA, and they are the building blocks of bio-membranes in animals. MEA is an important part of human and animal metabolism and is part of the process of making the essential vitamin choline (Knaak et al. 1997). This means that MEA can be found in the environment as a breakdown products, and according to Stevenson (1994) ethanolamine have been detected in trace amounts in soil/soil-extracts (Stevenson 1994). At high concentrations MEA is known to be an irritant to the skin, eyes and respiratory tract (Laag et al. 2009).

DEA is a secondary amine and does not occur naturally in phospholipids, like MEA. But at high concentrations DEA may substitute for MEA in phospholipids. The toxicity of DEA is similar to other amines. It is an irritant to skin, eyes and airways. However, it is less irritating than MEA (Laag et al. 2011). DEA is a secondary amine and can react with a nitrosating agent to form nitrosamines, which is carcinogenic. AMP is a primary amine classified as an isobutanolamine. Other than being used in gas processing, AMP is widely used in cosmetics. The function of AMP in this industry is to adjust pH, act as an emulsifying agent, and to regulate solubility and flexibility of various creams, lotions, soaps etc. In non-cosmetic products AMP has been used in leather dressing, cleaning compounds and polishes, insecticides, paints, antibacterial agent and as an indirect food additive. AMP is classified as an irritant to skin and eyes, but less toxic than MEA (Laag et al. 2009).

1.3.1 Chemical properties and reactions

DEA is the most polar molecule due to the two OH-groups, followed by MEA and AMP. The alkalinity of these compounds range from a pH of 10-12.5, where MEA is the most alkaline (Davis & Carpenter 1997). MEA, DEA and AMP will form basic aqueous solutions, and at this pH they will be in their anionic form, figure 1.1. The oxygen will have a negative charge. If the pH changes to neutral, the molecule will be a zwitterion, which means that it will have a negative and positive charge. The oxygen will have a negative charge, and the nitrogen a positive charge. At acidic pH, the molecules will be in cationic form due to the positive charge on the nitrogen atom. Figure 1.1 shows the charge of MEA at different acidic, neutral and basic pH.

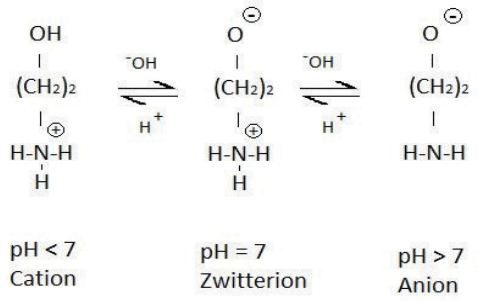
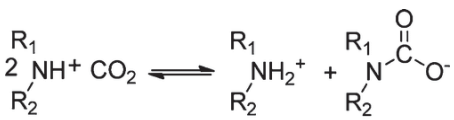


Figure 1.1. Charge of MEA at different pH.

Amines like MEA, DEA and AMP have been carefully investigated considering the optimization of amine solvents for carbon capture. Aqueous solvents are most commonly used, and they should ideally have properties like high CO_2 absorption capacity and low toxicity. CO_2 is an acidic compound that reacts reversibly and rapidly with an alkaline amine solution to form carbamate and protonated amine. This reaction is illustrated in figure 1.2. Both primary and secondary amines are used. The stoichiometry of the reaction is approximately two mole of amine per mole of CO_2, for primary and secondary amines like MEA, DEA and AMP (Reynolds et al. 2012).



For MEA, R_1 = H and R_2 = CH_2OH

Figure 1.2.Reaction of MEA with CO_2 (Reynolds et al. 2012).

1.3.2 Degradation

Alkanolamines are known to be unstable in presence of water at elevated temperatures or in presence of reactive metals (Davis & Carpenter 1997). A common problem with the use of aqueous solutions of MEA as an absorbent for CO_2 is degradation of the solvent due to irreversible side-reactions with CO_2 and other components of the flue gas. The degradation of alkanolamines in context with CO_2 capture has been the topic of several studies, but there are still reactions and mechanism of degradation that are poorly understood (Strazisar et al. 2003). There are three main paths of degradation of alkanolamines; oxidative degradation, thermal degradation and atmospheric degradation. The degradation products will depend on the type of amine. Oxidative degradation takes places in the presence of oxygen and is catalyzed by iron. This reaction produces organic acids and NH_3. The exact mechanisms of this reaction are not known, but a possible pathway is illustrated in figure 1.3. As illustrated the degradation is believed to be initiated by the reaction between ions such as Fe^{3+}, Fe^{2+} or Cu^{+} which will generate an oxide radical. The reaction can continue with or without oxygen present. With oxygen present, a reaction between oxygen and the oxide radical will form a peroxide radical. This radical will further react with amines to form iminies and hydrogen peroxide, which will finally form the final degradation products through processes like hydrolysis and oxidative fragmentation. The final degradation products of MEA would be ammonia and organic acids (Shao & Stangeland 2009).

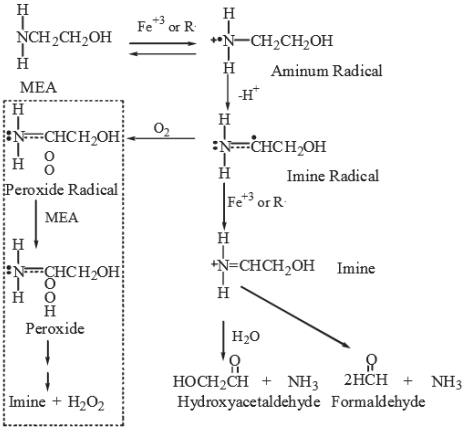


Figure 1.3. Possible mechanism of oxidative degradation of MEA (Chi & Rochelle 2001).

Thermal degradation occurs at temperatures higher than 205 ºC and is not very common (Chi & Rochelle 2001). Atmospheric degradation involves many complex processes that may form a number of different products. Degradation of amines will generally be initiated by reactions with OH-radicals to create amine radicals. Thereafter, reactions with ozone (O_2) and nitrate (NO_3) will initiate further degradation. Other degradation reactions will also take place.

Chemical and physical processes like absorption, adsorption and photolysis will also play important roles amines emitted to the atmosphere (Shao & Stangeland 2009).

1.4 Adsorption of organic chemicals on soil

The adsorption of organic chemicals by soil depends on the composition of the soil and the characteristics of the compound. Sorption is any removal of a compound from solution to a solid phase, and is one of the most important chemical processes that control the retention of pollutants and other chemicals in soil. The most important solid phases in soils are layer silicate clays, metal oxides and soil organic matter (SOM). The composition of these three elements in the soil depends on the type of soil and the sampling place. Layer silicate clays usually have a negative charge and do often represent the largest source of negative charge. Their charge is very little pH dependent. Metal oxides can have a variable charge, due to the hydroxylation of the surface when it comes in contact with water. Depending on the degree of hydroxylation, they can have an anionic ($M\text{-}O^-$), neutral (MOH) or cationic form (MOH_2^+), where M is the metal. The adsorption to metal oxides is highly pH dependent. The metal oxides will have a net positive surface charge at low pH and a net negative surface charge at high pH. In other words, the capacity to adsorb cations will be greater at high pH and the capacity to adsorb anions will be greater at low pH (Thompson & Goyne 2011).

SOM consists of materials in the soil that are living, non-living and partially decayed, in addition to biomolecules and humic matter (Thompson & Goyne 2011). Humic matter (also known as humus) is a group of organic matter that consists of humic acids, fulvic acids, hymatomelanic acids and humins. Compounds classified as humic matter has high molecular weights and have been formed in the soil in a process called humification (Tan 1994). Organic materials contain typically as much as 40-50% carbon (Brady & Weil 1999). In SOM there are many anionic, neutral and cation reactive sites, as well as non-polar regions of the soil solid phase. Typical anionic sites are hydroxyls (R-OH) and carboxylic(R-COOH) functional groups, while amino ($R\text{-}NH_2$) and sulfhydryl (R-SH) groups usually are cationic. Aromatics and aliphatic parts of a molecule are un-charged and non polar parts of the soil solid phase. The adsorption to SOM is highly pH dependent. High pH favors adsorption of cations, while low pH favors a lower capacity for adsorption of cations. The adsorption of an organic compound in soil, will depend on the presence of these three sorbent in the soil (Thompson & Goyne 2011).

The adsorption of organic compounds to soil depends on properties like (McBride 1994):

- pH in the soil
- functional groups
- acidity or basicity of functional groups
- polarity and charge of the molecule
- molecular size and shape

Molecules that contains functional groups like –OH, -NH_2 and –NHR will favor adsorption to SOM (Brady & Weil 1999). Alkanolamines like MEA, DEA and AMP are basic compounds that are all completely miscible in water. The amine part of the molecule will be protonated and become cationic when the pH is low (figure 1.1). This will give the nitrogen group a positive charge and make it more water-soluble. Cationic molecules will adsorb on soil organic matter, which has a net negative charge. The strength of the adsorption will be pH-dependent (McBride 1994). They will also strongly adsorb to silicate clays. Low pH will give several positive charges on the molecule and a stronger adsorption. The ability to form hydrogen bonds will also play an important role in adsorption, especially for compounds containing both amine and hydroxyl functional groups, like alkanolamines. Figure 1.4 shows the adsorption of the herbicide glyphosphate on a negatively charged clay mineral called kaolinite at different pH. The figure shows that the more acidic the clay mineral is, the more adsorption there is of the compound. At basic pH the adsorption is low. Glyphosphate contains both hydroxyl and amino functional groups, like the alkanaolamines (Brady & Weil 1999).

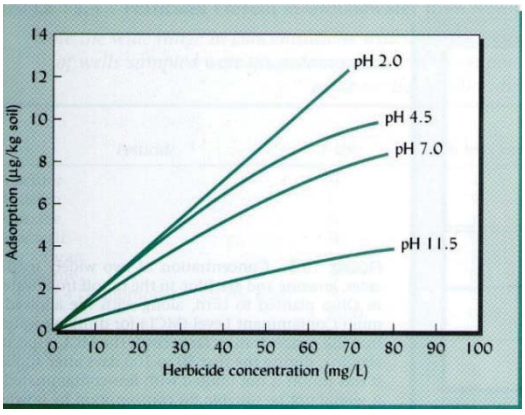


Figure 1.4. Adsorption of glyphosphate to kaolinite at different pH (Brady & Weil 1999, p 728).

Another important process that can affect the adsorption of chemicals in a water-saturated soil is leaching. The leaching of organic chemicals in soils is closely related to their potential for adsorption and their solubility in water. The solubility of a compound varies according to

structure and functional groups. Low mass aliphatic amines are water soluble. High water-solubility favors loss of the compound through leaching. Compounds that are strongly adsorbed to the soil, and not completely water soluble will not likely be leached. Different types of soil will have different potential for leaching of organic compounds. Soil that are highly permeable, sandy and low in organic matter will have a high potential for leaching. Another important factor is the amount of rainfall, where high rainfall will promote leaching and runoff losses. This threat will depend on the climate of the area (Brady & Weil 1999). The more water-soluble the compounds are, the more leaching will be favored.

Peat soil

Peat soil, also known as histosols, is an organic soil that is typically formed in areas that are saturated with water, and they can be found in areas with tropical temperature, temperate zones and tundra (Tan 1994). *"Not all wetlands contains histosols, but all histosols occur in wetland environments"* (Brady & Weil 1999). Organic soils are defined as soils that contain 20% or more organic material (Deckers et al. 1998), and are formed by the accumulation of partially decomposed organic material (Brady & Weil 1999). The decomposition of the organic material is slow due to anaerobic conditions in the soil. Complete decomposition of organic matter will produce products like CO_2 (vanLoon & Duffy 2005). In areas with cold climate the temperature will also have an inhibiting effect on the decomposition (Deckers et al. 1998). The amount of decomposed organic matter in the soil can be determined in the field by using a system called Von Post scale. This is a field test where properties like structure, color and composition of the soil gives an indication of the amount of decomposed organic matter. Peat soil does normally have black to dark brown color, due to the high content of organic matter (Brady & Weil 1999). The pH range of most organic soils is between 2.7 and 8.6. In general organic soils are acidic and this acidity is due to the presence of organic acids, exchangeable hydrogen, iron sulfide and silica acid (Miller & Donahue 1990).

1.5 Chromatographic analysis of amines

Methods used for determination of low mass amines such as alkanolamines, are well documented in matrices like water and food samples. Analytical methods includes gas chromatography (GC), thin layer chromatography (TLC), spectrofluorometry, high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) (Cao et al. 2005). Most common are methods were amines are derivatized with dansyl chloride (DNS-Cl) prior to analysis on HPLC (Silva 2005), however other derivatizing agents can also be used.

HPLC is a reliable, sensitive and rapid method, which can be coupled to different detectors (Cao et al. 2005). Amines like MEA, DEA and AMP does not have any UV absorption or fluorescent, hence they are derivatized or labelled with a compound that has these properties. Most HPLC separations are carried out using UV absorption detection due to its easiness to use. Dansyl amides (the product of the reaction between an amine and DNS-Cl) absorb lights in the UV-region. Figure 1.5 shows the UV absorption of a 0.01 and 0.1 mM dansyl glycine aqueous solution. The absorption maxima are observed at 214, 246 and 325 nm, and the absorption at 214 nm is the strongest (Takeuchi 2005).

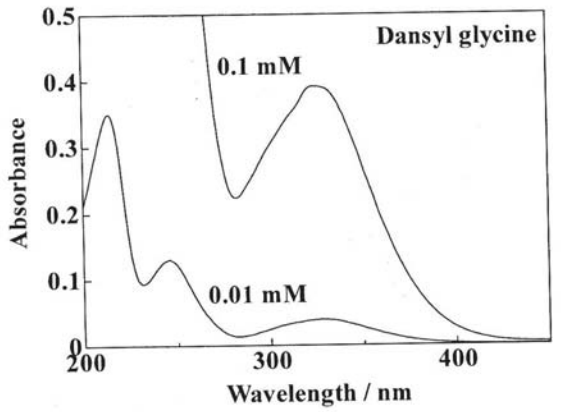


Figure 1.5. UV spectra of dansyl glycine (Takeuchi 2005, p. 233).

The poor detection limits of the UV detector makes it not suitable for the detection of environmental and food samples which usually contain trace amounts of amines. Thus HPLC combined with fluorescents detection is generally preferable due to its high sensitivity (Cao et al. 2005). Mass spectrometry is also an alternative for a very sensitive detector that can be used in combination with UV.

1.6 Dansylation

Dansyl chloride (DNS-Cl) or 5-dimethylaminonaphthalene-1-sulfonyl chloride was introduced in 1952 as reagent for preparation of fluorescent derivatives of proteins. Since that time, it has become a popular derivatisation agent for amino acids, amines and compounds containing amines (Seiler 1993). The reaction of DNS-Cl with amines generates aromatic sulfonamides (Fournier et al. 2008), figure 1.6. This reaction is often used prior to analysis on HPLC-UV. The structure of the DNS-Cl molecule contains two aromatic rings, which are highly fluorescent and can easily be detected with a UV-detector. It also contains a reactive group (sulfonyl chloride). This group reacts with analytes with a nucleophilic substitution

reaction (Silva 2005). Low mass amines such as MEA, DEA and AMP do not contain any conjugated double bonds, and cannot be detected by UV. When these compounds are labelled with DNS-Cl, they can be detected with a UV-detector. The sulfonamides created in this reaction are also easy to protonate in the ion source of a MS, hence a better MS-detection (Fournier et al. 2008).

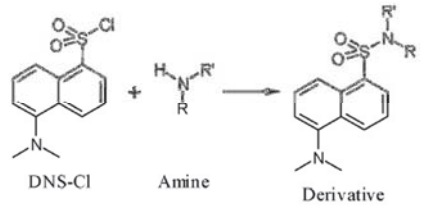


Figure 1.6. Structure of dansyl chloride and the chemical reaction between DNS-Cl and an amine (Fournier et al. 2008).

When DNS-Cl reacts with primary and secondary amines it forms dansyl amides. Tertiary amines may react with DNS-Cl at high pH and elevated temperatures, but it is most common to use primary and secondary amines. DNS-Cl is weakly soluble in water, while low mass aliphatic amines are completely soluble in water. A mixture of acetone and water is therefore used for the reaction. The reaction is most effective at basic pH, around 9,5-10 (Seiler 1993). pH is the most significant factor affecting the reaction. Another important parameter is temperature. High temperatures on the reaction make it go faster, and the reaction time is decreased. However, high temperatures will decrease the stability of the dansyl-derivatives, which will reduce the final yield. At room temperature the reaction should stay overnight, which is the traditional way for dansylation (Silva 2005). After the reaction, toluene can be used to extract excess reagent and remove side products from the reaction. The dansyl derivatives have a pale yellow color and are usually crystalline solids. They are also light sensitive and should be stored in a dark place (Seiler 1993). The dansyl reaction between MEA, DEA and AMP is illustrated in figure 1.7.

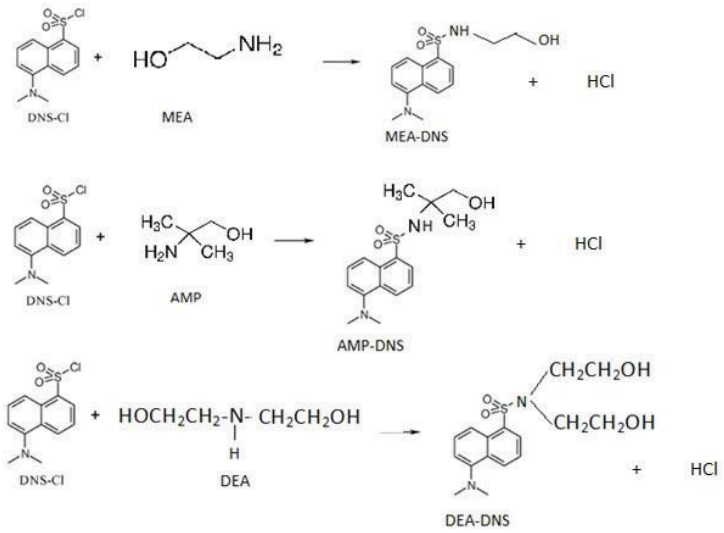


Figure 1.7. Reaction of MEA, AMP and DEA with DNS-Cl to form MEA-DNS, AMP-DNS and DEA-DNS. Adapted from Fournier et al. (2008).

For separation of MEA-, DEA- and AMP-DNS from the organic phase a technique called liquid liquid extraction (LLE) can be used. Table 1.2 list the molecular formula, mass and melting point of the amines, and amine derivatives that are illustrated in figure 1.7.

Table 1.2. Molecular formula and mass of DNS-Cl and amine derivatives.

Compound	Molecular formula	Molecular Mass (g/mol)	Melting point (ºC)
DNS-Cl	$C_{12}H_{12}ClNO_2S$	269,9	72-74
MEA-DNS	$C_{14}H_{18}N_2O_3S$	294,104	104-105*
AMP-DNS	$C_{16}H_{22}N_2O_3$	322,135	No data available
DEA-DNS	$C_{16}H_{22}N_2O_4S$	338,130	No data available

*(Stenstrøm 2014)

Side-reactions and breakdown of products

The dansyl reaction is easily done on primary amines like MEA. Branched primary amines like AMP has more steric hindrance. But the reaction is still very favorable for these compounds. Secondary amines, like DEA, have more steric hindrance than branched primary amines, which makes the reaction more challenging. Steric hindrance favors decomposition of products, which can lead to unwanted side reactions. Side reactions are unusual for primary amines, but can occur for secondary amines if conditions like pH and temperature are not optimal (equation 1 and 2). The reaction rate can be increased by increasing the pH, but this can again lead to an increased rate of hydrolysis of DNS-Cl, equation 1 (Silva 2005).

$$H_2O + DNS - Cl \rightarrow DNS - OH + HCl$$ Equation 1

$$R - NH - DNS + DNS - Cl \rightarrow DNS - NH_2 + other\ products$$ Equation 2

Both side reactions in equation 1 and 2 are favored at high pH-values, in addition to the dansyl reaction with amine in figure 1.5. Side reaction 2 can decompose dansylated amines if excess DNS-Cl is not used up during the reaction. In other words, this reaction can undo the amine dansylation (Stephens 1986). The side reactions can produce side products that are highly fluorescent and can interfere with chromatographic separation (Silva 2005).

1.7 Method validation

Method validation is the process where an analytical method is tested and proved to be acceptable (or unacceptable) for its intended use (Harris 2010). The goal of the validation is to determine the quality, reliability and consistency of the analytical results. The purpose of the method decides which parameters that are tested, and the limits of acceptance. Various parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy are usually tested. Another important parameter to test is the stability of analytes. The first point in the validation process in to define the scope of the method. Is the method quantitative or qualitative? A quantitative method will have higher acceptance limits for accuracy and precision than a qualitative method, hence they have to be validated differently. Secondly, a validation plan including method scope, validation test and acceptance criteria are made. Table 1.3 gives an indication of which parameters that should be tested depending on the purpose of the method (Huber 2007b).

Table 1.3. Validation parameters for an analytical method (Huber 2007b).

	Major compounds	Major compounds and traces	Traces	Traces
	Quantitative	Quantitative	Qualitative	Qualitative
Limit of detection	No	No	Yes	No
Limit of quantification	No	Yes	No	Yes
Linearity	Yes	Yes	No	Yes
Range	Yes	Yes	No	No
Precision	Yes	Yes	No	Yes
Accuracy	Yes	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
Ruggedness	Yes	Yes	No	Yes

Instrument linearity

The linearity of the instrument is a measure on the correlation between analyte response and the concentration of the analyte. In a linear method the analyte response and concentration must be proportional (Huber 2007b). As a measure on linearity, the square of the correlation coefficient, r^2, is frequently reported. This value must be very close to 1 to represent a linear fit (Harris 2010). The linearity is found by making solutions of the current analytes in different concentrations. The concentration span should cover the anticipated concentration in the samples Real samples can only be quantified in the area of linearity of the method (Huber 2007b). The calibration curve should contain at least 5-6 values/points. Linear regression is performed on the curve to obtain a regression equation. The equation should have a y-intercept not significantly different from zero (Huber 2007a). The place where the curve rounds of is outside of the linear range. Figure 1.8 shows an example of a linear calibration curve. Another criterion for linearity is that the y-intercept of the calibration curve (after the response of the blank has been subtracted from each standard) should be very close to 0 (Harris 2010).

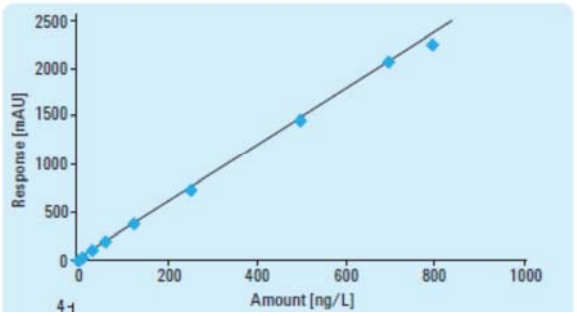


Figure 1.8. Linear calibration curve (Huber 2007b).

Detection- and quantification limit

Limit of detection is the lowest amount of analyte that gives a signal that is statistically different from background noise. Typically, in mass spectroscopy, limit of detection is where the signal is three times the background noise (S/N=3). At this level, the analyte cannot be quantified; it is limited to qualitative analysis. In order to enable reporting of amount of analyte in a sample, the signal to background noise ratio should be at least 10 (Huber 2007b). This minimum signal/background noise ratio is referred to as the limit of detection (Hoffmann & Stroobant 2007). Equation 3 and 4 can be used to calculate the LOD and the LOQ, where s is the standard deviation of the blank sample and m is the slope of the linear calibration curve (Harris 2010).

$$\frac{3S}{m} = LOD$$ Equation 3

$$q\frac{10S}{m} = LOQ$$ Equation 4

Accuracy and precision

Accuracy is a measure of analyte test results agreement with the true value in the analyzed samples. In other words, a measure of how well test results from the method agree with the true value of the samples that was tested (Huber 2007b). The accuracy of a method can be determined in different ways, but the most common way is to perform a recovery test of spiked samples. Blank samples with the same sample matrix as the unknown samples are spiked with a known concentration of analyte (Harris 2010). The samples should be prepared the same way as unknown samples and using the same laboratory equipment. At least three parallels should be made. The expected recovery depends on the sample matrix, the sample processing procedure and the analyte concentration. The lower the concentration of analyte, the bigger interval around 100% recovery is expected (Huber 2007b). An indication of expected analyte recovery at different concentrations can be found in table 1.4.

Table 1.4. Analyte recovery at different concentrations (Huber 2007b, p146).

Active ingredient (%)	**Analyte ratio**	**Unit**	**Mean recovery (%)**
100	100 %	1	98-102
≥ 10	10 %	10^{-1}	98-102
≥ 1	1 %	10^{-2}	97-103
≥ 0.1	0.1 %	10^{-3}	95-105
0.01	100 ppm	10^{-4}	90-107
0.001	10 ppm	10^{-5}	80-110
0.0001	1 ppm	10^{-6}	80-110
0.00001	100 ppb	10^{-7}	80-110

The precision is usually expressed as a standard deviation, and say something about how well replicate measurements agree with one another (Harris 2010). There are many ways of measuring the precision of a method depending on the resources available and the scope of the method. Different ways to demonstrate precision includes instrument precision, repeatability, intermediate precision and inter-laboratory precision. Instrument precision (also called injection precision) is the reproducibility obtained when a sample is injected 5-10 times times into the instrument (Harris 2010). Repeatability is a measure on reproducibility when the same method is repeated several times. Repeatability can be divided in two categories: within assay repeatability and between assay repeatability. Within assay repeatability is the reproducibility measured when three to six parallels of a sample of sample matrix and a

known concentration of analyte is analyzed on one day by the same person and the same instrument and laboratory equipment. This test gives information on the reproducibility of the method under the same operating conditions on one day. Errors during the sample preparation should be minimized by using an experienced analyst for the sample preparation. Between assay repeatability is the reproducibility measured when six parallels of a sample is analyzed six days by the same person and the same instrument and laboratory equipment. The between assay tells us how reproducible the method is from day to day under the same operating conditions. Intermediate precision (also known as ruggedness) is the variation observed between different people, instruments and days in the same laboratory. Inter-laboratory precision (also called reproducibility) is a measure on the variation between different laboratories and different people (Harris 2010).

1.8 Quantification

1.8.1. The internal standard method

The internal standard method can be used to quantify the amount of analyte in a sample. An RFF (relative response factor) value is calculated from standards with a known concentration of analyte, equation 5. The RRF value calculated from the standards are used to calculate the concentration of analytes in the sample, equation 6. If the blank samples contains signals of the analyte, the areas should be corrected for the blank (Alltech Associates 1998).

$$RFF = \frac{area\ IS \times amount\ analyte}{amount\ IS \times area\ analyte} \qquad \text{Equation 5}$$

$$amount\ analyte = \frac{aount\ IS \times area\ analyte \times RFF}{area\ IS} \qquad \text{Equation 6}$$

1.8.2. The external standard method

In the external standard method a sample with a known amount of analytes are analyzed. A response factor is calculated, equation 7. This method assumes that there is a linear response on different concentrations (Alltech Associates 1998). The response factor is then used to calculate the amount of analyte in a sample, equation 8.

$$response\ factor = \frac{peak\ area}{sample\ amount} \qquad \text{Equation 7}$$

$$amount\ of\ analyte = \frac{peak\ area}{response\ factor} \qquad \text{Equation 8}$$

1.9 Goals of this study

The present study has three main goals:

1. Validate and compare two different analysis methods developed by NILU for analysis of amines, and say something about the suitability of the methods in this analysis. The method validation includes synthesis of external standards used in one of the two methods.
2. Analyse soilwater samples from a leaching experiment in order to test the hypothesis that peat soil adsorbes all amines added.
3. Analyse soilwater samples from the leaching experiment in order to test the hypothesis that there are too much noise in peat soil to be able to analyze amines in this matrix.

The idea behind this study was to compare two different analysis methods for HPLC-MS, one direct and one indirect method for analysis of low mass amines. These two methods could then be used for analysis of the amines from the leaching experiment. Two of the six hypothesis to why there were not found any recovery of amines in the ExSIRA project were tested in this master thesis. The field work at Smøla and leaching experiment was conducted by Line Tau Strand, NMBU. The method validation and synthesis of amine derivatives where conducted at the laboratory at NILU, and finally the check of purity of the synthesized products were done at the Department of Chemistry, Biotechnology and Food Science at NMBU.

2. Materials and methods

The materials that have been used for the experimental part of this thesis can be found in appendix I. It includes a list of chemicals and instruments.

2.1 Preparation of mobile phases and solutions

A list of mobile phases and solutions used in this thesis are presented in table 2.1. The table describes the contents of these solutions.

Table 2.1. Content of mobile phases and solutions.

Solution	**Content**
Channel A mobile phase	500 ml MQ 500 µl Formic acid
Channel B mobile phase	100 % methanol
Reference mass solution	95% methanol 0.2% acetic acid 5% Reference stock solution
Reference mass stock solution	95 % methanol 5 % MQ 5 µM Purine 1.25 µM HP-0921 125 µM $TFANH_4$
Tuning solution	25 ml undiluted tuning mix 75.25 ml acetonitrile 3.75 ml MQ
Washing solution (1:1:1)	1/3 methanol 1/3 MQ 1/3 ACN
0.1 % Hydrochloric acid (HCl)	HCl MQ
10 % Sodium hydroxide (NaOH)	1 g NaOH 10 ml MQ
1% Dansyl chloride DNS-Cl) -solution	1% DNS-Cl 90 % ACN 10% MQ
0.2 M Sodium bicarbonate ($NaHCO_3$) -buffer	0.02 mol $NaHCO_3$ 100 ml MQ 2.95 ml 10 % NaOH

2.2 Sampling of peat soil

Peat soil was sampled from an ombrotrophic raised bog at Smøla, Møre and Romsdal, Norway in October 2012 by Line Tau Strand. The soil was later used in a leaching

experiment. The properties of the soil collected were determined at NMBU and can be found in table 2.2.

Procedure:

25-30 litres of soil were collected from a depth of 15-40 cm under the root matt of the vegetation on the bog. The peat soil consisted of weakly decomposed fibric material of mixed moss and sedge. The soil was packed in four separate black garbage bags with as little air as possible. The soil was saturated with water at the time of the sampling. The bags were taped and transported in Zarges cases. The samples were at the Department of Environmental Science at NMBU in a cold storage room at 4 ºC.

Table 2.2. Properties of peat soil collected at Smøla.

Property	**Value**	**standard deviation**
Bulk density (g cm^{-3})	0.060	0.012
Total porosity (% v/v)	96	1
0.1 bar H_2O content (% v/v)	34	6
Hydraulic conductivity (cm h^{-1})		
Fibric (remains after washing through 1mm)	5.29	1.13
Sapric (all material sieved trough 4 mm)	0.07	0.01
Fiber content (% v/v)	42	2
Decomposition classified in the von Post scale	4/5	-
Pyrophosphate index	5/6	-
pH (fresh)	4.54	0.05
pH (dry, 1:2.5 v/v)	4.27	0.02
CEC (cmolc kg-1)	109	15
Base saturation (%)	23	3
C (kg 100kg-1)	50.1	2.3
N (kg 100kg-1)	1.12	0.22
CN	46	9

2.3 Leaching experiment in soil columns

A leaching experiment of peat soil in soil columns was carried out to see if amines used in CO_2 capture plants are leached or retained in peat soils. The experiments described in this chapter were performed by Line Tau Strand at the Department of Environmental Science at NMBU. The experiment followed the procedure in the OECD guideline (OECD/OCDE 2004). A number of tests to find a preparation method for soil columns with correct hydraulic conductivity were performed prior to the leaching experiment. The soil columns needed a hydraulic conductivity close to the natural conductivity of peat soil in an ombrotrophic raised bog, in order to simulate the ExSIRA project. A preparation method for soil columns were developed at NMBU.

2.3.1 Preparation of soil columns

The soil columns were prepared according to the method developed at NMBU to obtain a hydraulic conductivity close to that of an ombrotrophic raised bog.

Procedure:

1. All the soil was pressed through a 12.5 mm sieve. The material that did not pass through the sieve was not used in the experiment. After sieving the material was thoroughly homogenized and split in two equally big samples. One sample was washed through a 1 mm sieve until the water running through was clear in order to wash out most of the humified material. The fibrous material left on the sieve was mixed with the second samples of 12.5 mm sieved soil. This was done to obtain an acceptable hydraulic conductivity. Some of the fibrous material was put aside for use in the bottom and top of the peat columns.
2. The pre-treated soil was packed in glass columns with an inner diameter of 4.2 cm. A 1 cm layer of fibrous peat material was put in the foot of the column.
3. The soil samples were added to the column to make a length of 16 cm. Then two centimeters of the fibrous peat material was added to the top of the column, making the total length 18 cm. The soil density was equivalent to natural soil density for peat soil at Smøla (Dry soil density 0.06 g/cm^3, moist 0.85 g/cm^3). Twenty soil columns were prepared with this method.
4. A long glass stick was used to remove air bubbles from the column. After the column had been prepared it was stored in a cool and dark storage room until the experiment was performed.

2.3.2 Leaching experiment

The twenty soil columns prepared from peat soil were added samples of amines and artificial precipitation that was leached through by gravity.

Procedure:

1. The twenty soil columns were coated with aluminum foil to prevent light from coming in. Firstly, the columns were saturated with artificial precipitation (0.01 M $CaCl_2$) to remove all air, by submerging them in a bucket. Secondly, they were left to drain by gravitation.
2. 200 ml artificial precipitation (0.01 M $CaCl_2$) was added to the columns prior to treatment with amines. This was done twice in order to ensure well-functioning

columns. After addition of artificial precipitation the columns were left to drain by gravity. The amount of leachate collected after each round of addition was between 200-250 ml. All leachate was collected and stored, but only the leachate from the second round of addition of artificial precipitation was sent for analysis (second leachate). This sample was split in two; one for analysis at NMBU and the other one was immediately frozen and subsequently sent to NILU for analysis. At NMBU physical and chemical properties of the soil-water from the second leachate such as pH, total organic carbon (TOC) and total amount of nitrogen (TOT N) was determined. These properties can be found in table 2.2.

3. The twenty columns were divided into two series (1-10 and 11-20). In each series MEA, DEA and AMP were added to 3 columns each and one column in each series was kept as a control (no amines added). This made 3 parallels of each amine in each series. Concentrations equivalent to *"worst case scenario"* emissions from a CO_2 capture plant was added to the columns. These concentrations can be found in table 2.3.
4. 551.3 µl of each amine solution was added evenly on the surface of three columns in each series. Each column was saturated with approximately 235 ml water, and a theoretical concentration of amines in the columns could be calculated, table 2.3.

Table 2.3. Concentration of amine solution (= worst case scenario), volume added to columns and theoretical concentration in soil columns after dilution.

	Concentration of solution added (mg/l)	**Volume added to column (µl)**	**Theoretical concentration in column (mg/l)**
MEA	872	551.3	1.105
DEA	1501	551.3	1.902
AMP	1273	551.3	1.613

5. The columns rested for one hour before 200 ml of artificial precipitation was added (0.01 $CaCl_2$). The columns were left to drain by gravity. This provided between 200 - 250 ml leachate. This sample was split in two, one for analysis at NMBU and one for analysis at NILU. At NMBU, properties like TOC, TOT N and pH was determined, table 2.4. The samples sent to NILU were collected in glass bottles and frozen immediately. The other samples were placed in a cold storage room.

Table 2.4. List of columns used in the leaching experiment and their physical and chemical properties. The columns were divided in two series; 1 and 2. Each series contained 10 columns divided in three groups; A, B and C, and one control. The second leachate were not added any amines. For the third leachate group A was added MEA, B was added DEA and C was added AMP.

	A	**B**	**C**	**Control**
Series 1	Column 1-3	Column 4-6	Column 7-9	Column 10
Series 2	Column 10-13	Column 14-16	Column 17-19	Column 20
Second leachate				
pH	3.56	3.48	3.52	3.50
TOC (mg/l)	4.86	4.35	4.45	4.40
TOT N (mg/l)	1.95	1.55	1.70	1.63
Hydraulic conductivity (cm h^{-1})	4.90	5.97	5.60	5.30
Third lachate	MEA	DEA	AMP	-
pH	3.31	3.33	3.30	3.34
TOC (mg/l)	4.01	4.15	4.05	3.98
TOT N (mg/l)	1.72	1.55	1.55	1.60
Hydraulic conductivity (cm h^{-1})	3.92	3.92	4.83	4.50

2.4 Analytical methods

Two analytical methods were validated and used for analysis, one direct and one indirect. The direct method analyzed amines directly without any special techniques for sample preparation. The indirect method analyzed amines labeled with DNS-Cl prior to analysis. Common for the two methods was the use of an Agilent 1290 Infinity LC-system coupled to an Agilent 6500 series Q-TOF LC/MS system. The instrument was located at NILU Kjeller, Norway. Both methods were developed by NILU.

2.4.1 Instrumentation

The LC-system was equipped with an auto-sampler, UV detector and a termostatted column compartment. Different analytical columns were used for the direct and indirect method as well as different instrument settings. In the direct method a reversed phase HPLC-column with a pentafluorophenyl (F5) phase bonded to a silica based stationary phase, was used. The F5-phase acted as a strong Lewis acid because the five fluorine groups had an electron withdrawing effect. This column was suitable for analysis of small, water soluble and basic compounds like MEA, DEA and AMP (Sigma-Aldrich Co 2014). In the indirect method a reversed phase HPLC-column with a silica based C18 stationary phase (T3), was used (Waters 2007). This column was suitable for analysis of polar compounds like MEA-, DEA- and AMP-DNS. The temperature of the columns was set to 40 ºC. The mobile phase running

through the columns was identical for both methods, with unequal mobile phase flow. The direct method used a flow of 0.200 ml/min with a maximum column pressure of 390 bar, while the indirect method used a flow of 0.400 ml/min with a maximum column pressure of 1200 bar. The T3 column was more robust than the F5-column, with an analysis time 10 minutes. In comparison, the F5 column had an analysis time of 21 minutes. LC-settings for both methods can be found in table 2.5.

A gradient was used during analysis for both methods. The instrument was coupled to two mobile phases, channel A and B. Channel A contained a solution of 0.1 % formic acid, and channel B contained 100 % methanol. The gradient started with a composition of 90% A and 10% B in both methods. The solvent composition of the mobile phase gradient in both methods can be found in table 2.5. Common for both methods, was an automatic sample injection by an auto-sampler. Between each injection the needle was flushed with washing solution (table 2.1) ten seconds between each injection. All samples were stored at 0.8 ºC.

Table 2.5. LC-settings for the direct and indirect method.

	Direct method	**Indirect method**
Injection volume (µl)	10	3
Mobile phase flow (ml/min)	0.200	0.400
Column	Discovery HS-F5	T3 acquity
Column temperature (ºC)	40	40
Analysis time (min)	21	10
Mobile phase gradient	0-13 min: 90 % A, 10 % B 13-15 min: 10 % A, 90 % B 15-16 min: 10 % A, 90 % B 16.5-18.5 min: 90 % A, 10 % B	0-2 min: 90 % A, 10 % B 2-7 min: 1 % A, 99 % B 7-8 min: 1% A, 99 % B 8.00-8.10 min: 90 % A, 10% B 8.10-10 min: 90 % A, 10 % B

A mass spectrometer was used as a detector for the LC-separation. This is a very sensitive detector and is commonly used together with HPLC. This mass spectrometer had an electro-spray ion source, and a Quadrupole Time of Flight (Q-TOF) mass filter. Both methods scanned for positive ions with an m/z of 25-1100. The ion source settings of the two methods can be found in table 2.6. A reference mass solution containing compounds with an m/z of 121.05 an 922.01 was used parallel to analysis (table 2.1).

Table 2.6. Ion source settings of the direct and indirect method

Parameter	Direct method	Indirect method
Ion polarity	Positive	Positive
Mass range (m/z)	25-1100	25-1100
Gas temperature (^{o}C)	125	290
Gas flow (l/min)	15 l/min	14 l/min
Nebulizer (psig)	35	35
Sheat gas temp (^{o}C)	350	350
Sheat gas flow (l/min)	11	11

2.4.2 Method validation plan

A method validation plan was developed for the validation of the direct and indirect method according to their intended purpose. The purpose was to analyze soil-water samples from peat soil to see if amines added to soil columns could be detected and quantified by HPLC-MS. The aim was to be able to quantify the amines detected in samples in order to calculate a recovery. For this purpose, the methods were quantitative. The following parameters were common for the validation of both methods: finding the detection limit (LOD), quantification limit (LOQ) and linearity of the instrument. In the direct method the accuracy of the method should be validated as a recovery test, and precision of the instrument tested in a within and between assay repeatability test. In the indirect method LOD, LOQ and linearity had to be found on the DNS-derivatives to avoid error due to yield of the reaction. Even though the analysis is performed on DNS-derivatives, converted results for the amines are presented in this study in order to compare the two methods. The indirect method included a derivatization step of amines to form derivatives of DNS-Cl, which means that the accuracy and repeatability of this reaction should be validated. The accuracy should be validated by calculating the reaction yield, and the precision of the reaction should be tested as within and between assay repeatability test. For quantification of samples in the indirect method, the external standard method was used. MEA-, DEA- and AMP-DNS was used as externals standards. These compounds are not commercially available, and as a part of the validation, they had to be synthesized and purified. Table 2.7 contains the plan over which parameters that should be tested for each method and the acceptance limits.

The linearity range for both methods should cover the theoretical concentration in columns (table 2.3) in case no amines are adsorbed and the recovery is 100%. Since these methods are quantitative, and the expected concentrations are so high, there is no expected value for LOD, but the LOQ should be greater than 5µg/l. The precision of the instrument is expected to be smaller than the precision of the dansyl reaction. The acceptable value for precision depends

on the sample matrix the analyte is located in. The within assay repeatability test is expected to have a lower RSD than the between assay repeatability test.

Table 2.7. Method validation plan and acceptance limits for different parameters tested.

Direct method	**Acceptable**	**Indirect method**	**Acceptable**
LOD	-	**LOD**	-
LOQ	< 5 µg/L	**LOQ**	< 5 µg/L
Linearity: Calibration curve	MEA: - 1,2 mg/l DEA: - 2.0 mg/l AMP: - 1,7 mg/l	**Linearity:** Calibration curve	MEA: - 1,2 mg/l DEA: - 2.0 mg/l AMP: - 1,7 mg/l
Accuracy: Recovery test	80 – 110 %	**Accuracy:** Reaction yield	< 80 %
Precision: Repeatability of the instrument (%RSD)	Within assay: <5 Between assay: <15	**Precision:** Repeatability of the reaction (%RSD)	Within assay: <10 Between assay: <20
Stability of analytes: Short term stability test	-	**Stability of analytes:** Long term stability test	-
-	-	**Synthesis of external standards**	Pure products

2.5 Preparation of samples and standards

2.5.1 Direct method

The samples analyzed with the direct method were analyzed directly on the HPLC-instrument. However, samples were diluted due to the addition of internal standards.

Procedure:

1. Standards were made from freshly made stock solution. Stock solutions were made by weighing chlorides of MEA, DEA and AMP, and diluting in MQ to a concentration of 250 mg/l. A mix solution of the three amines with an appropriate concentration was made in MQ or in sample matrix (SM, column water from the second leaching). Standards of 50 and 100 µg/l were made directly in vial. 300 µl of a 500 µg/l internal standard solution of ^{13}C-MEA and ^{13}C-dimethylamine (^{13}C-DMA) were added. The samples were mixed and analyzed.
2. Samples were prepared by adding 1.2 ml sample and 300 µl internal standard solution in LC-vials. The samples were mixed and analyzed. The samples were diluted 20 % in this procedure. Method blanks were made by adding MQ instead of sample in LC-vials.

2.5.2 Indirect method

Samples and standards were derivatives with DNS-Cl prior to analysis on HPLC-MS, with a method developed by NILU. External standards were analyzed directly.

Samples and standards

Samples were added a higher concentration of internal standard than standard samples due to higher noise in sample matrix.

Procedure:

1. A 1 % DNS-Cl solution and 0.2 M sodium bicarbonate buffer adjusted to pH 9.8 was prepared (chapter 2.1). The DNS-solution was made fresh and used the same day it was prepared. After preparation it had to rest for minimum 1 hour before use.
2. Internal standard solutions in MQ were made of ^{13}C-MEA and ^{13}C-DMA. A 500 µg/l mix solution was made. This solution was made fresh before each analysis.
3. Table 2.8 lists the procedure of the method for standards and samples. Samples were added a higher concentration of internal standards than standard samples. The components of the reaction were added in the order described in the table. The 1% DNS-solution was the last component to be added, because it will initiate the reaction.. The concentration of IS in standards were 8.3 µg/l, and in samples 16.7 µg/l. Samples and standards were diluted three times with this method.
4. LC-lids were washed, dried and put on top of the vials (not attached). The vials were place in an ultrasound bath for 5 minutes in order to catalyze the reaction. The temperature of the bath was 20 ºC. Lids were attached and samples were ready for analysis.
5. Method blanks were made according to the standard sample procedure. The addition of standard was dropped, and the concentration of internal standards was 12.5 µg/l.

Table 2.8. Preparation of standards and samples.

	Standard (µl)	**Sample (µl)**
Standard/Sample	500	500
ISTD (500 µg/l)	25	50
MQ	25	-
Acetonitrile	300	300
0.2 M buffer	350	350
1% DNS	300	300
Total	1500	1500

Preparation of external standards

Samples analyzed with the indirect method were quantified with the external standard method. External standards were prepared from dansyl amides of MEA, DEA and AMP synthesized at NILU.

Procedure:

1. Stock solutions of MEA-, DEA- and AMP-DNS were made by weighing crystals of the derivatives and diluting them in an appropriate amount of acetonitrile and MQ (50:50-solution) to obtain a concentration of 100 mg/l.
2. Standard mix solutions, with concentrations of 10 and 50 µg/l were made by diluting stock solutions. 1.5 ml of each standard solution was transferred to LC-vials.
3. Blank samples were made by transferring 1.5 ml of the 50:50-solution of acetonitrile and MQ into LC-vials.

2.6 Validation of the direct method

2.6.1 Instrument linearity

The instrument linearity was tested by making calibration curves of MEA, DEA and AMP. Dilutions of amine in MQ were made from 1 µg/l to 5 mg/l.

Procedure:

1. A 250 mg/l stock solution of MEA, DEA and AMP diluted in MQ, was made by weighing the chlorinated amines. The amines were made the same day of the analysis.
2. Dilutions of 1, 3, 6, 10, 25, 50, 100, 250, 500 750, 1000 and 5000µg/l was made in MQ.
3. Samples were analyzed by adding 1.2 ml sample and 300 µl internal of a 500 µg/l standard solution in a LC-vial. Method blanks were made from MQ and added IS.

2.6.2 Detection- and quantification limits

The LOD and LOQ for each amine were found in different ways. The limits of MEA were found experimentally with standards of different concentrations, DEA was found by calculating the LOD and LOQ from equation 3 and 4 in chapter 1.7. Ten blank samples of MQ and IS were made to calculate a standard deviation of the blanks. The limits of AMP was found by calculating the signal to noise ratio in MassHunter, and based on this value,

calculate the concentration when the signal/noise is 3 and 10. The LOD and LOQ were also calculated at injection volumes of 5 and 15 µl.

2.6.3 Accuracy

The accuracy of the method was checked by performing a recovery test. The recovery test was done in sample matrix at 25 µg/l and 300 µg/l. These concentrations were chosen because 25 µg/l was close to the LOQ of MEA, and 300 µg/l lied close to the expected value of the samples from the leaching experiment. The test was performed in sample matrix, where a mix of the first leachate of all 20 columns was used as sample matrix. The accuracy of the method was also calculated at 5 and 15 µl injection volume. At 5 µl injection volume the accuracy was calculated at 50 and 300 µg/l.

Procedure:

1. Chlorinated salts of MEA, DEA and AMP were weighed and a stock solution with a concentration of 250 mg/l was made by dissolving the salts in MQ. Three parallels of each amine solution were made.
2. Two mix solutions of all three amines were made for each parallel with a concentration of 300 µg/l and 25 µg/l in sample matrix. These solutions were made directly in vial from appropriate intermediary mix solutions in sample matrix, to avoid errors in concentration due to dilution when internal standard solution was added.
3. Standards of MEA, DEA and AMP were made by diluting the 300 µg/l mix solution and making a standard with concentration of 100 and 50 µg/l in MQ, directly in vial.
4. All samples were added 300 µl of a 500 µg/l internal standard solution containing ^{13}C-MEA and ^{13}C-DMA.
5. Blank samples of sample matrix for correction of recovery test samples, and blank samples of MQ for correction of standards were prepares with 300 µg/l internal standard solution.

2.6.4 Precision

The precision of the method was checked by performing a test of repeatability. Within and between assay repeatability was tested in sample matrix in order to say something about the reproducibility of the method. The within assay repeatability test was analyzed from the same samples as the recovery test to save time. This test followed the same procedure as the recovery test. The between assay repeatability test was conducted over 6 days, where fresh

stock solutions of amines were made each day, in addition to fresh IS solution. The within assay repeatability test counted as the first day in the between assay repeatability test.

Procedure:

1. Chlorinated salts of MEA, DEA and AMP were weighed and stock solutions with a concentration of 250 mg/l were made by dissolving the salts in MQ.
2. A mix solution of all three amines in sample matrix, were made with an appropriate intermediary concentration. Two parallels of a concentration of 300 µg/l and 25 µg/l were made directly in vials, together with 300 µl of a 500 µg/l IS solution. The total volume in vial was 1500 µl, and the end concentration of amines should be 300 and 25 µg/l.
3. Standards of MEA, DEA and AMP were made by diluting the intermediary mix solution of all three amines, and making standards with concentration of 100 and 50 µg/l in MQ, directly in vial. 300 µl IS were added.
4. Blank samples of sample matrix and MQ were made.
5. This procedure was repeated 5 times.

2.7 Synthesis of dansyl derivatives

Dansyl derivatives were synthesized according to a method described by Fournier et al. (2008). This method was adapted in order to get successful synthesis and pure products that could be used as external standards. The synthesis was performed at NILU, and the purity of the compounds was determined at the Department of Chemistry, Biotechnology and Food Science at NMBU.

2.7.1 Primary amines

Primary amides that was synthesized was MEA-DNS and AMP-DNS. MEA was available as both chlorinated salt and pure amine, whereas AMP was only available as chlorinated salt.

Procedure:

1. 1.5 g of DNS-Cl was dissolved in 30 ml of acetone. Two parallels were made, one for the synthesis of MEA-DNS (parallel 1) and one for the synthesis of AMP-DNS (parallel 2).
2. 1.022 g of pure MEA was dissolved in 3 ml of MQ water, and 1,499 g of AMP-Cl was dissolved in 3 ml of MQ water. According to Fournier et al. (2008), a 3:1 molar excess of amine was used in order to make sure that all DNS-Cl was used during the reaction.

3. 3 ml of a saturated solution of sodium bicarbonate in MQ was added to parallel 1. 3 ml of the 0.2 M $NaHCO_3$ buffer (see table 2.1) was added to parallel 2.
4. The MEA-solution was added to parallel 1 and the AMP-Cl solution was added to parallel 2. A magnet was put in each glass bottle and the mixtures were put on a magnetic stirrer overnight. The bottles were covered with aluminum foil to prevent light from degrading the amines.
5. After completion of the reaction the solution was evaporated to dryness (close to dryness) on a turbovap. The bath temperature of the turbovap held 35 ºC.
6. 30 ml of water was added to the precipitate to dissolve the sodium bicarbonate and the solution was transferred to a separation funnel. The aqueous phase was acidified to pH 2 with 1M HCl.
7. 30 ml of toluene was added to the separation funnel and the the dansyl derivatives were extracted by liquid liquid extraction (LLE). Three extractions were made to obtain optimal recovery efficiency.
8. The organic phase was collected and dehydrated with anhydrous magnesium sulphate to remove any water from the sample. Anhydrous magnesium sulphate was added until the liquid turned clear. The organic phase was decanted in a turbovap glass without transferring any of the magnesium sulphate in the bottom of the glass bottles.
9. The organic phase was evaporated to dryness with nitrogen vaporization on turbovap glasses. The bath temperature held 35 ºC.
10. Pale yellow crystals of MEA-DNS were found in the parallel 1 bottle. The crystals were scraped of the glass walls, and collected in a small glass vial. In the parallel two bottles, the reaction of AMP and DNS-Cl had formed yellow/orange syrup of AMP-DNS. The syrup was scraped off and collected in a glass vial.
11. The derivatives were put in a vacuum-desiccator for 5 days to remove any solvent left in the glass. Thereafter they were stored in a dark place at – 18 ºC.

2.7.2 Secondary amines

The secondary amine DEA was available as both salt and non salt. The synthesis was done several times with both the salt and the non-salt, and some modifications were done on the method.

Procedure:

1. 1.39 g of DNS-Cl was dissolved in 30 ml of acetone. 1.499 g of DEA was dissolved in 3 ml of MQ in a glass bottle.
2. The solution with DNS-Cl in 30 ml acetone was added about one spatula of sodium bicarbonate to make it saturated. In addition 2 ml of the 0.2 M $NaHCO_3$ buffer was added. The DEA solution was added 1 ml of 0.2M $NaHCO_3$ buffer.
3. The DEA solution was added to the dansyl solution and a magnet was added. The solution was put on a magnetic stirrer overnight. The reaction solution was kept dark under aluminum foil at room temperature, to avoid degradation of DEA.
4. After completion of the reaction, the solution was transferred to an extraction funnel with 150 ml toluene.
5. Excess amine was extracted with 30 ml of MQ water that was added 0.01 M sodium hydroxide (0.01 M) to make the water phase slightly basic. The extraction was repeated 3 times. The pH of the water phase was measured to be between 7 and 9 for the three extractions.
6. The organic phase was dehydrated with anhydrous magnesium sulfate until the liquid turned clear. The color of the liquid was pale yellow.
7. The organic phase was collected in a turbovap bottle, and dehydrated to dryness on a turbovap (zymark). The water bath of the turbovap held a temperature of 35 ºC for the evaporation of toluene. The organic phase was dehydrated to dryness.
8. The DEA-DNS syrup that was collected was put in a vacuum-desiccator for 5 days.
9. The derivatives were store in a dark place at – 18 ºC. 1.375 g DEA-DNS was left after vacuum evaporation.

2.7.3 Confirmation of purity

The purity of the synthesized derivatives was determined by ^{1}H NMR, ^{13}C NMR and IR. These analyses were conducted at the Department of Chemistry, Biotechnology and Food Science at NMBU by Simen Gjelseth Antonsen. Melting point was also determined here. UV spectra of the synthesized amides were found at NILU by using the UV-detector on the HPLC-instrument.

Ultraviolet-Visible-spectroscopy

The synthesized amine-derivatives were scanned with a UV-detector in order to check purity. The UV-detector coupled to the chromatographic system was used. The products were

scanned for absorption of UV-light at wavelengths between 190-400 nm. Only compounds with conjugated double bonds give UV absorption. It is the chromophore of the molecule that absorb UV light, the chromophore of the products are the dansyl-part.

Melting point

The melting point of MEA- and AMP-DNS was determined on a melting point apparatus to check the purity of the products. DEA-DNS was not determined because it was a liquid at room temperature.

Procedure:

1. Crystals of MEA-DNS and AMP-DNS were put in capillary glass tubes. The glass tube was placed in the melting point apparatus.
2. Expected melting point for MEA-DNS was used as a reference. For AMP-DNS there was no reference available, so different temperatures was tested.

2.8 Validation of the indirect method

2.8.1 Instrument linearity

The instrument linearity of MEA-, DEA- and AMP-DNS was tested by analyzing the dansyl derivatives that had already been synthesized. Solutions of 0.1 - 6000 µg/l was made and dissolved in a solution of 50:50 acetonitrile and MQ.

Procedure:

1. Stock solutions of MEA-, DEA- and AMP-DNS were made by weighing crystals of the derivatives. The crystals were dissolved in a 50:50 mixture of acetonitrile and MQ with an appropriate volume to give concentrations of 100 mg/l.
2. A mix solution of all three derivatives was made with a concentration of 6 mg/l. This solution was used to make dilutions of 2500, 1000, 500, 100, 50, 10, 5, 1, 0.25, 0.1 µg/l. Blank samples of acetonitrile and MQ solution was made.
3. The samples were analyzed directly with the indirect method.

2.8.2 Detection- and quantification limits

The limit of detection and limit of quantification for MEA- AMP-DNS was calculated by using equation 3 and 4 in chapter 1.7. 10 blank samples of the method were made by adding all the reagents of the sample method in table 2.8, except for 500 µl sample, to LC-vials. The

samples followed the same procedure as real samples, and were put on an ultrasound bath. These standards were used to calculate a standard deviation of the blanks. The LOQ and LOD of DEA-DNS were calculated by finding the signal to noise ratio 10 parallels of a 10 µg/l standard. The LOQ and LOD for the amines were calculated from the LOQ and LOD of the derivatives.

2.8.3 Accuracy

The reaction yield of the dansylation in the indirect method, described in chapter 2.5.2, table 2.8, was tested in order to say something about method accuracy. The reaction yield was tested in both sample matrix and MQ to see if there was any difference between the two matrices.

Procedure:

1. Chlorinated salts of MEA, DEA and AMP were weighed and a stock solution with a concentration of 250 mg/l was made by dissolving the salts in MQ. Three parallels of each amine solution were made.
2. Two mix solutions of all three amines were made from each parallel, with a concentration of 1 mg/l in MQ. Solutions with a concentration of 100, 50 and 10 µg/l were made directly in vial from intermediary solutions in sample matrix and MQ with appropriate concentrations. The samples containing MQ were treated as “standards” and samples containing sample matrix were treated as “sample” in table 2.8. The samples were treated with the same procedure as described in chapter 2.5.2.
3. Blank samples of sample matrix and MQ were made.
4. The accuracy of the method was calculated by quantifying all samples in sample matrix and MQ, and calculating a percent recovery. The average recovery of the three concentrations in each sample matrix was used to say something about the method accuracy.

2.8.4 Precision

The precision of the method was determined by measuring the reproducibility of the dansyl reaction with a within and between assay repeatability test. The precision was determined in both sample matrix and MQ. The within assay repeatability test was analyzed from the same samples as the accuracy test to save time. This test followed the same procedure as the accuracy test described in chapter 2.8.3. The between assay repeatability test was conducted over 6 days, where fresh stock solutions of amines were made each day, in addition to fresh IS

solution. The first day of the between assay repeatability test, used data from the within assay repeatability test, as these two test were the same. A RSD value was calculated based on the average of the recoveries calculated each day.

Procedure:

1. Chlorinated salts of MEA, DEA and AMP were weighed and a stock solution with a concentration of 250 mg/l was made by dissolving the salts in MQ.
2. A mix solution of all three amines was made with a concentration of 1 mg/l in MQ. Solutions with concentrations of 100, 50 and 10 µg/l were made directly in vial from intermediary solutions in sample matrix and MQ with appropriate concentrations. The samples containing MQ were treated as "standards" and samples containing sample matrix were treated as "sample" in table 2.8. The samples were treated with the same procedure as described in chapter 2.5.2.
3. Blank samples of sample matrix and MQ were made.
4. This procedure was repeated 5 times.
5. The precision of the method was calculated by finding the reaction yield at 10, 60 and 100 µg/l, and calculate a relative standard deviation based on the average recovery of these samples.

2.9 Stability of analytes

The analyte stability was tested as a part of the method validation. Long and short term stability was tested.

2.9.1 Long-term stability

The long term stability of MEA, DEA and AMP was tested over a period of three months. The amines were dissolved in MQ and sample matrix, and stored in a freezer. The aim of the test was to see if the amines were stable under these storage conditions in MQ and sample matrix.

Procedure:

1. Chlorinated salts of MEA, DEA and AMP were weighed and a stock solution with a concentration of 100 mg/l was made by dissolving the salts in MQ. Three parallels of each amine solution were made.

2. A mix solution of all three amines was made for each parallel with a concentration of 1 mg/l in MQ. This was an intermediary solution, used to make further dilutions. Solutions with a concentration of 50 µg/l were made by diluting 1.25 ml 1 mg/l mix solution in 25 ml MQ, and 600 µl 1 mg/l mix solution in 11.4 ml sample matrix. Three solutions of amines in MQ and three in sample matrix with a concentration of 50 µg/l were obtained.
3. Two parallels of pure MQ, and two parallels of pure sample matrix were made.
4. The samples were analyzed according to the indirect method described in chapter 2.5.2. Samples containing MQ was prepared as "standard", and samples containing sample matrix were prepared as "samples", table 2.8. The samples were diluted three times, so the end concentration of amines was 16.67 µg/l.
5. This procedure was repeated four times, and the samples were quantified in order to say something about the stability of each analyte.

2.9.2 Short-term stability

The short-term stability of analytes was tested over 7 days, and analyzed with the direct method.

Procedure:

1. Chlorinated salts of MEA, DEA and AMP were weighed and a stock solution with a concentration of 100 mg/l was made by dissolving the salts in MQ. 3 parallels of each amine solution were made.
2. Mix solutions of all three amines were made with a concentration of 4.5 mg/l in MQ. One mix solution was made for each parallel. This was an intermediary solution, used to make further dilutions. A 300 µg/l solution was made directly in vial by adding 100 µl of 4.5 mg/l mix solution, 200 µl of a 750 µg/l IS solution of ^{13}C-DMA and ^{13}C-MEA, and 1.2 ml MQ.
3. Standards were made from fresh solution of amines each day, with a concentration of 50 and 100 µg/l. Blank samples of MQ added 200 µl IS solution was used.
4. This procedure was repeated 6 times. Fresh solutions of internal standards were made each day.

2.9.3 Effect of ultrasound treatment

Aqueous solutions of MEA, DEA and AMP were tested to see if the ultrasound bath treatment in the indirect method had any effect on stability. The same procedure as described in chapter 2.9.1 was followed, and three parallels of each amine were made in MQ. The 50 µg/l mix solutions (three parallels), and two blank samples of MQ were treated as "standards" in table 2.8 and the concentration in vial was 16.67 µg/l. Two series of each parallel was made. One series was put on an ultrasound bath and the other series was analyzed directly. The area of the two series was compared.

3. Results

3.1 Chromatogram peaks

Chromatogram peaks were identified by searching for mass and retention time in MassHunter. Extracted ion chromatograms of a 50 µg/l amine standard analyzed with the direct method can be found in figure 3.1. Retention times can be found in table 3.1.

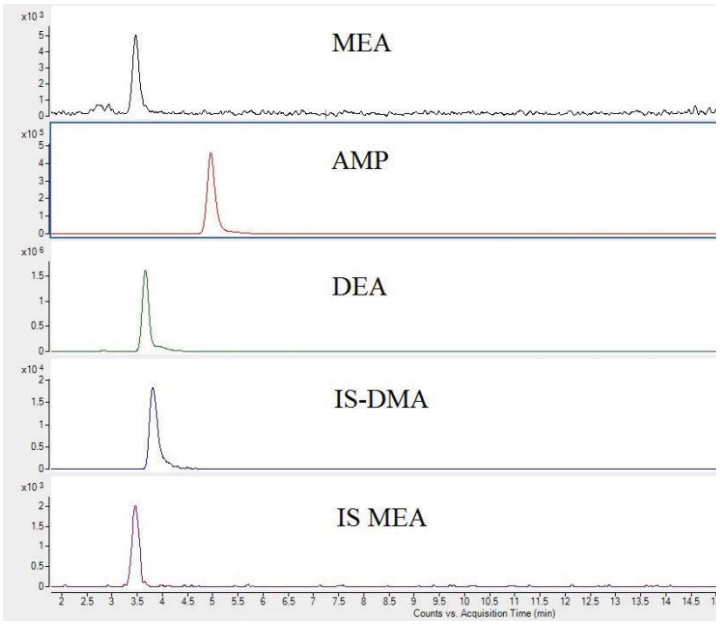


Figure 3.1 Chromatographic peaks of MEA, AMP, DEA, IS-DMA and IS-MEA from 50 µg/l standard analyzed with the direct method.

Extracted ion chromatogram of a 10 µg/l amine standard analyzed with the indirect method can be found in figure 3.2. Retention times can be found in table 3.1.

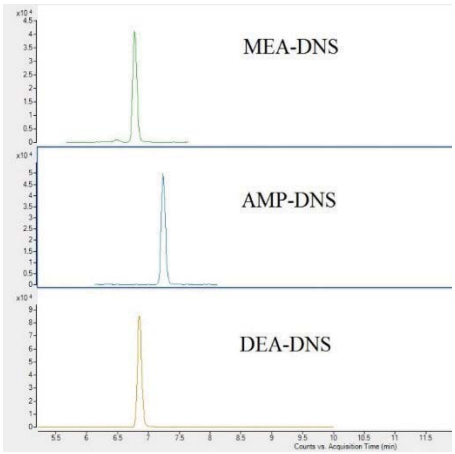


Figure 3.2 Chromatogram peaks of MEA-DNS, DEA-DNS and AMP-DNS from a 10 µg/l standard analyzed with the indirect method.

All peaks had retention time in the range of 3.4-3.8 in the direct method, except for AMP. The peaks of MEA and DEA overlapped. AMP had a much higher retention time than the other analytes. The retention time of MEA and DEA analyzed with the indirect method did also overlap.

Table 3.1. Retention time of amines and amine derivatives analyzed with the direct and indirect method.

Compound	Direct method (min)	Compound	Indirect method (min)
MEA	3.46	MEA-DNS	6.77
DEA	3.67	DEA-DNS	6.85
AMP	4.96	AMP-DNS	7.23
IS-MEA	3.47	IS-MEA-DNS	-
IS-DMA	3.80	IS-DMA-DNS	-

3.2 Synthesis of amine derivatives

MEA-, DEA- and AMP-DNS was synthesized and crystallized. The crystals of MEA-DNS were pale yellow, and the product of DEA-DNS was a light yellow liquid. The synthesis of AMP-DNS was not successful. According to table 3.2 the reaction yield of MEA-DNS was 31 % and DEA-DNS was 36 %. However, this was only calculated from the crystals collected from turbovap-tubes, hence the real reaction yield was probably greater. Melting point was determined at NMBU. According to table 3.2 the melting point of MEA-DNS lied between 101-102 ºC, while AMP-DNS lied between 83-84 ºC. The melting point of DEA-DNS could not be determined.

Table 3.2. Melting point and reaction yield of MEA-, DEA- and AMP-DNS.

	Product	Melting point (ºC)	Yield (%)
MEA-DNS	Pale yellow crystals	101-102	31 %
DEA-DNS	Pale yellow liquid	-	36 %
AMP-DNS	Pale yellow crystals	83-84	-

The purity of the products was examined by UV-, IR- and ^{1}NMR- and ^{13}C NMR-spectra. UV-spectra were found at NILU by using the UV-detector coupled to the chromatography system. MEA-, DEA and AMP-DNS gave three ʎmax; ʎ215, ʎ250 and ʎ335. ʎ215 was the most intense and ʎ335 was very small. IR, ^{1}H NMR-, ^{13}C NMR-spectra confirmed the presence of DNS-products. Spectra can be found in appendix III-VI.

3.3 Method validation

The purpose of the direct and indirect method was to quantify soil water samples of amines, and they were validated according to this purpose. The methods were validated mostly in sample matrix, but also in MQ where it was necessary. The calibration curves were made in a solution of MQ (direct method) and MQ and acetonitrile (indirect method). The two methods were different in quantification of analytes, and were therefore validated differently. Calibration curves, LOD and LOQ were found the same way for both methods. In the direct method, accuracy of the method and precision of the instrument were found by calculating recovery and reproducibility within and between assays. In the indirect method accuracy and precision of the method was found by calculating the reaction yield of the DNS-reaction, and within and between assay of the DNS-reaction. The data from the method validation can be found in table 3.3.

Direct method

The calibration curve of MEA was linear from 10-500 µg/l, AMP from 1-500 and DEA had a linear range from 1-750 µg/l (appendix II). The calibration curve of MEA was corrected for IS-MEA, and DEA and AMP were corrected for IS-DMA. The LOD and LOQ were calculated by calculating the signal to noise of the peak in MassHunter. A blank sample containing sample matrix was used to correct all samples. The recovery of amines in sample matrix at 25 and 300 µg/l was over 50% for MEA and DEA, but under 25% for AMP. The repeatability test showed a within assay repeatability below 20 RSD at 25 µg/l and 10 RSD at 300 µg/l. The between assay repeatability was under 20 RSD for all compounds at 25 and 300 µg/l.

Indirect method

The linear range of MEA-DNS was from 1-1000 µg/l, AMP-DNS from 0.25-1000 µg/l and for DEA-DNS from 1-1000 µg/l (appendix II). The LOQ and LOD of the amines was calculated by using equation 3 and 4, the standard deviation of a blank sample and then correct for the mass difference between amines and amine derivatives. Reaction yield was calculated in both MQ and sample matrix to see if there was a difference between the two matrices. The reaction yield is a little higher in MQ than in sample matrix. The reaction yield is lower for DEA, than for MEA and AMP. Repeatability of the reaction was done within and between assay in both MQ and sample matrix to see if there was a difference. There is no significant difference between MQ and sample matrix, but the % RSD is higher for the

between assay samples than the within assay samples. DEA has a higher % RSD both in within assay and between assay than MEA and AMP. MEA and AMP are not very different.

Table 3.3. Method validation data.

	Direct method			**Indirect method**		
	MEA	**DEA**	**AMP**	**MEA**	**DEA**	**AMP**
LOD (µg/l)	8	0.2	0.4	0.03	0.09	0.48
LOQ (µg/l)	24	0.7	1	0.10	0.20	1.60
Linearity (R^2)	0.998	0.998	0.998	0.999	0.996	0.999
Recovery (%)						
25 µg/l	77	74	7	-	-	-
300 µg/l	79	51	22			
Reaction yield (%)						
MQ	-	-	-	65	34	58
SM				50	40	66
Reproducibility of method (%RSD)						
Within assay:						
25 µg/l	16	9	3			
300 µg/l	4	5	9	-	-	-
Between assay:						
25 µg/l	15	19	16			
300 µg/l	13	18	17			
Reproducibility of reaction (%RSD)						
Within assay:						
MQ				13	24	12
SM	-	-	-	12	27	22
Between assay:						
MQ				29	56	25
SM				15	59	35

Injection volume - direct method

A higher injection volume was used in hope of getting a lower LOD and LOQ in the direct method. A higher injection volume did not give a lower LOD and LOQ for MEA and DEA, but for AMP it did give a lower LOD and LOQ than the 10 µl injection volume. Injection volumes of 5 µl were also tested, but gave a bigger variation in LOD and LOQ between the three amines. The LOD and LOQ for MEA and DEA were higher than the 10 and 5 µl injection, but for AMP they were lower. Injection volume of 10 µl proved to give the lowest LOD and LOQ. The LOD and LOQ were calculated from the signal to noise ratio calculated in MassHunter.

Table 3.4. LOD and LOQ at different injection volumes.

	5 µl injection	10 µl injection	15 µl injection
MEA (µg/l)			
LOD	63	14	39
LOQ	190	41	117
DEA (µg/l)			
LOD	1	0.2	0.4
LOQ	3	0.7	1
AMP (µg/l)			
LOD	3	10	6
LOQ	8	29	19

The injection volume in the direct method was changed to 5 µl in hope to get a better recovery of the amines, because it will reduce the noise in the sample. The recovery of MEA could not be calculated because the standards and internal standards had a concentration of 100 µg/l, which was lower than the quantification limit (table 3.7). The recovery was calculated at 50 and 300 µg/l. DEA gave a recovery of 73 % at 300 µg/l and AMP gave a recovery of 62% at the same concentration. At 50 µg/l, the recovery of DEA was 76 % and AMP was 56 %.

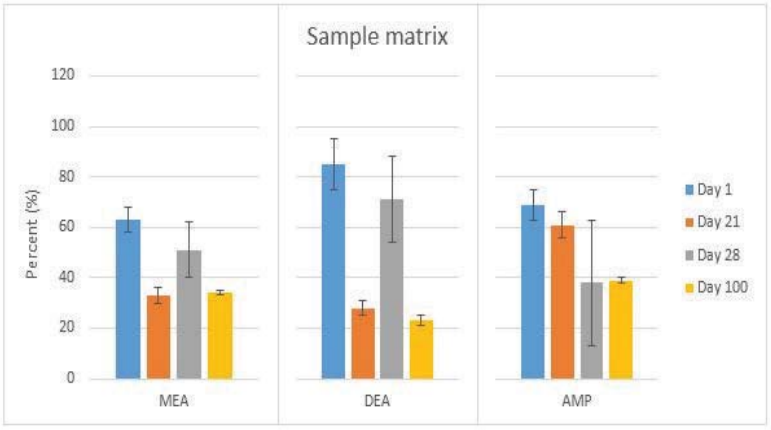
3.4 Stability of amines

Long term stability of amines

The long term stability of amines was tested over a 3 month period on standard samples with a concentration of 50 µg/l in sample matrix and MQ. The stability was tested in sample matrix to see if the amines are degraded over a long period of time. A parallel test was performed in MQ to see if a possible degradation will be different in MQ. This test will also give an indication of how long standard stock solutions can be stored. Samples from the long term stability test were analyzed with the indirect method. A percent recovery was calculated each day of analysis and the results were presented in column diagrams, figure 3.3 and 3.4.

The stability of MEA, DEA and AMP in sample matrix can be found in figure 3.3. All amines had a decreasing trend, with some variations in the four days of analysis. Day 1 of the analysis of MEA is significantly different than three other days, with a recovery of 63 %. Day 21 and 100 of the experiment gave recoveries of 33 and 34 % respectively, while day 28 gave a higher recovery of 51 %. The analysis of DEA shows great variations between days. Day 1 gave a recovery of 85%, while day 21 and 100 gave a recovery of 28 and 23 % respectively. The analysis on day 28 gave a recovery of 71 %. AMP had the clearest decreasing trend. The recoveries calculated decreased according to number of storage days. Day 1 gave a recovery

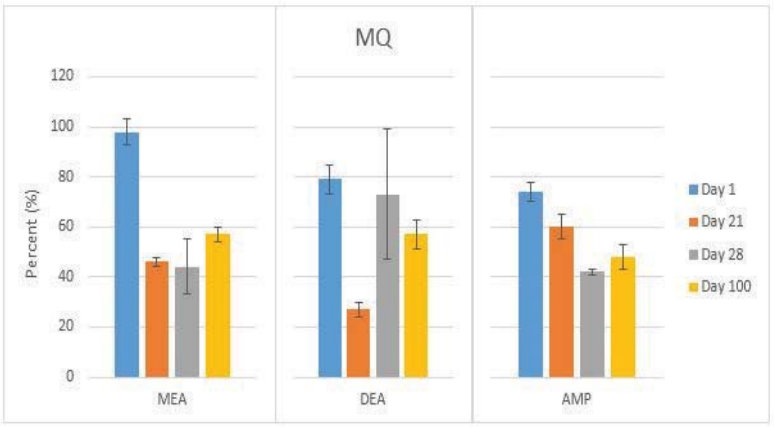
of 69 % and day 100 a recovery of 39%. The relative standard deviations calculated between the analyses performed during the whole period can be found to the right in figure 3.3.



	RSD (%)
MEA	32
DEA	60
AMP	30

Figure 3.3. Stability of amines in sample matrix measured over a 100-day period. Percent recovery was calculated each day of analysis, and presented with standard deviations. A table with the relative standard deviation (RSD) between each day of analysis can be found to the right in the figure.

The stability of MEA, DEA and AMP in MQ can be found in figure 3.4. MEA and AMP had a clear decreasing trend, while DEA had more variable recoveries. The day 1 analysis of MEA stands out from the three remaining days. The recovery of day 1 was 98%, and the recoveries of day 21, 28 and 100 lied between 44 and 57 %. DEA had a great gap between the day 1 and day 21 analysis, where day 1 gave 79 % and day 21 gave 27 % recovery. Day 28 and 100 gave recoveries of 73 and 57 % respectively. The trend in MQ follows the trend in sample matrix. The analysis of AMP in MQ is very similar to the analysis performed in sample matrix. The recovery of day 1 was calculated to 74% and day 100 was calculated to 48%. The relative standard deviations calculated between the analysis performed during the whole period can be found to the right in figure 3.4.



	RSD (%)
MEA	41
DEA	39
AMP	25

Figure 3.4. Stability of amines in MQ measured over a 100 day period. Percent recovery was calculated each day of analysis, and presented with standard deviations. A table with the relative standard deviation (RSD) between each day of analysis can be found to the right in the figure.

Short term stability

A short term stability test was conducted in order to investigate the stability from day to day in MQ. This test was based on the results from the long term stability test, because the gap between day 1 and 21 was quite big for MEA and DEA in both MQ and sample matrix, figure 3.3 and 3.4. The stability was tested over 7 days, and all amines were tested with the direct method. DEA and AMP gave relative standard deviations between days of 17 and 9 % respectively. These values were not significantly different from the relative standard deviations presented between assay in table 3.3 for MEA and DEA, hence the figures were not presented in this thesis. MEA was a difficult compound to analyze, because of the low intensity of the peak, dirty samples with interfering compounds, and unstable retention time. MEA gave a significantly different relative standard deviation than the one presented in table 3.3 in MQ, but the results were not presented because it was not 100 % sure that the compound really was MEA.

Ultrasound treatment

In the indirect method, samples and standards were placed in an ultrasound bath to catalyze the derivatization. Standards of 300 µg/l in MQ were analyzed before and after placement in ultrasound bath, to see if this step could degrade analytes. The samples did not have a significantly different area after ultrasound bath than before.

3.5 Leaching experiment

The second leachate and amine treated leachate from the soil column experiment was collected and analyzed on HPLC-MS with the direct and indirect method. The samples analyzed with the direct method were quantified with the internal standard method, and samples analyzed with the indirect method were quantified with the external standard method. The average of six parallel columns was calculated. The concentration of the second leachate is presented, and concentration and percent recovery is presented for the amine treated leachate. In some cases the amine could be detected, but not quantified because the concentration was lower than the LOQ. The results of the quantification can be found in table 3.5.

Direct method

In the second leachate only DEA could be quantified, MEA and AMP were detected, but could not be quantified. After amine treatment a significant higher amount of amine was detected than in the second leachate, but the percent recoveries are low, all under 5%. DEA has the highest recovery of 5%, while AMP had the lowest of 1%.

Indirect method

In the second leachate only MEA could be quantified. AMP was detected, but at concentrations lower than LOQ. DEA could not be detected. After amine treatment a significant higher amount of amines were detected with recoveries between 9 and 23%. MEA had the highest recovery of 23%, and DEA had the lowest of 9%.

Table 3.5. Content of amines in second and third leachate from the leaching experiment in soil columns. Group A, B and C are calculated of the average of six parallel. Group A was added MEA, B added DEA and C added AMP.

	Direct method			**Indirect method**		
Column	**A**	**B**	**C**	**A**	**B**	**C**
Second leachate						
MEA (µg/l)	< LOQ	< LOQ	< LOQ	4.0 ± 4.4	4.4±5.6	3.9±3.8
DEA (µg/l)	4.1 ± 2.5	2.6 ± 0.7	3.3 ± 1.2	-	-	-
AMP (µg/l)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Amine treatment	**MEA**	**DEA**	**AMP**	**MEA**	**DEA**	**AMP**
MEA						
Conc. (µg/l)	67 ± 29	-	-	257 ± 70	-	-
Recovery (%)	6			23		
DEA						
Conc. (µg/l)	-	213 ± 118	-	-	170 ± 70	-
Recovery (%)		11			9	
AMP						
Conc. (µg/l)	-	-	72 ± 11	-	-	362 ± 147
Recovery (%)			4			22

The two analysis methods gave different quantifications in the second leachate, and different recoveries in the amine treated leachate. The indirect method gave higher recoveries of amines in the amine treated leachate than the direct method.

Two parallels of control columns were treated at the same time as the sample columns. The control columns were treated the same way as the sample columns, but they were not treated with any amines in the third leaching of artificial rain. The concentration of amines in the second and third leachate from the control columns can be found in table 3.6. In the direct method, MEA could not be quantified, but detected. Concentrations relatively close to the LOQ were quantified for DEA and AMP. There was no siginificant difference between the second and third leachate. In the indirect method MEA was quantified in the second and third leachate, but DEA and AMP could only be quantified in the third leachate. The difference between the second and third leachate is significant.

Table 3.6. Content of MEA, DEA and AMP in control samples from the second and third leachate.

	Direct method			**Indirect method**		
	MEA	**DEA**	**AMP**	**MEA**	**DEA**	**AMP**
Second leachate (µg/l)	< LOQ	3.2 ± 1.8	3.4 ± 3.9	4.3 ± 4.2	-	< LOQ
Third leachate (µg/l)	< LOQ	5 ± 4	1 ± 0.02	39 ± 4	10 ± 0.5	2 ± 3

4. Discussion

4.1 Analytical methods and validation

4.1.1 Direct method

Figure 3.1 shows the chromatographic peaks of MEA, DEA, AMP and internal standards analyzed with the direct method. MEA has a very low intensity compared to the other peaks. One reason may be ion suppression, but the analyte peak in figure 3.1 was dissolved in MQ, which is a very pure matrix with few interfering compounds. The MQ used, contained an amount of 4-5 ppb of total organic carbon, which was nothing compared to the sample matrix, table 2.4. The intensity of MEA was slightly better in MQ than in sample matrix, but no significant difference between the two matrices. This means that possible interfering ions had to be present in MQ as well as sample matrix. DEA and AMP had more intense peaks with less interfering compounds in the chromatogram. The sample matrix contained a lot of noise, which made the analysis more difficult, as the retention time had a tendency to change in sample matrix, up to one minute. Furthermore, MEA formed sodium adducts with a variable abundance from 5-40 % of the molecular ion peak. The other amines did also form sodium adducts, but with lower abundance. The sodium adduct and molecular ion was integrated together.

Method validation

The results from the method validation tests can be found in table 3.3. The method was validated according to the method validation plan in chapter 2.4.2. The linearity of the method was set to cover the theoretical recovery of the columns if no amines were adsorbed on the soil. However, the expected recoveries were lower than the theoretical concentrations from table 2.3. The linearity of MEA ranged from 10 µg/l-430 µg/l. MEA had a smaller linear range than DEA and AMP, which had a linear range from 1 µg/l-830 µg/l. None of the amines covered the theoretical concentration added to soil columns. However, an earlier test had shown that DEA and AMP were linear to 5 mg/l with an R^2 value ≤ 0.90. These results were not used because the leachate from the soil column experiment did not prove to contain concentrations of amines higher than 400 µg/l. This meant that samples from the leaching experiment did not have to be diluted before analysis. In the sample preparation of the direct method, all samples were 20% diluted. Calibration curves for MEA, DEA and AMP can be found in appendix II.

According to the method validation plan in table 2.7, there was no special requirement for the LOD of the method, nevertheless the LOQ should be at least 5 µg/l. The LOD and LOQ for MEA were calculated to be 8 and 24 µg/l respectively. These values were above the criteria in table 2.7. DEA and AMP had LODs and LOQs that were below the acceptance criteria set in table 2.7. The injection volume of the method was changed to 5 and 15 µl to see how this changed the LOD and LOQ. The reason for this test was to see if MEA could get a lower LOD and LOQ, since it had a significantly higher value than DEA and AMP. The hypothesis was that a higher injection volume will give a lower LOQ, and a lower injection volume will give a higher LOQ. Figure 3.4 shows the results from this test, and it shows that the LOQ is higher for MEA when the injection volume is increased and decreased. When the injection volume decreases to 5 µl, the amount of analyte on the column is reduced, so the LOD and LOQ will increase. But when the injection volume is increased to 15 µl, the amount of analyte on the column is greater. The LOD and LOQ should have decreased, but it did not. A reason why the LOD and LOQ also increase at 15 µl injection volume may be due to the noise in the samples. The samples contained many interfering compounds and TOC and TOT N values were high. When the injection volume increased more of these interfering compounds were injected on the column. Different injection volumes were only tested in sample matrix, and the result could have been different in MQ. DEA got a slightly higher LOD and LOQ at 5 and 15 µl injections, but there was no significant change. AMP had the highest LOQ at 10 µl, and the lowest at 5 µl injection. This test indicated that the optimal injection volume for MEA and DEA, when the aim is to get low LOD and LOQ values, was 10 µl. For DEA, the optimal injection volume was 5 µl.

Even though DEA and AMP had better LOD and LOQ values than MEA, MEA had the best method accuracy with a recovery of 77% at 25 µg/l and 79% at 300 µg/l. There was no significant difference between accuracy close to LOQ or in the upper part of the calibration curve. These values were below the values in the method validation plan where the ideal recovery should be between 80-110 % according to Huber (2007b), table 1.7. DEA had a recovery of 74% at 25 µg/l, which was similar to the recovery of MEA at that concentration. The recovery at 300µg/l was expected to be better, but was only 51%. These values were also below the acceptance criteria in table 2.7. AMP had bad recoveries that were not acceptable. At both concentrations the recoveries were below 25%. The recoveries of AMP should not have been this low. One explanation may be that the instrument had been down for approximately two months before the method validation started. The samples from the

leaching experiment were analyzed prior to method validation, and AMP gave nice and stable peaks at that time. Another explanation may be that the noise in the sample matrix made it difficult to find and integrate peaks of AMP. It is also possible that the column used was old and worn out.

The injection volume was changed to 5 µl to try to get a better recovery of the analytes, especially AMP. When the injection volume decrease so will the amount of dirt from the samples and the recovery may be better. One the other hand, when the injection volume is reduced, the LOQ and LOD usually increase. As a result, the recoveries were calculated at 50 and 300 µg/l. The internal standards in the sample had a concentration of 100 µg/l, which was below the LOQ for MEA (table 3.4), hence the recovery of MEA could not be calculated. At 50 µg/l DEA had a recovery of 76% and AMP 56 %, at 300 µg/L DEA had a recovery of 73 % and AMP at 62 %. These values were better than the recoveries found at 10 µl injection in table 3.3. The difference in recovery for AMP was great, and indicates that optimal injection volume for AMP should be 5 µl. At 5 µl injection AMP will have a better LOD, LOQ and accuracy. A 5 µl injection volume was also optimal for DEA regarding LOD, LOQ and method accuracy. Other validation parameters were not tested at 5 µl injection volume.

The precision of the instrument was best for the within assays sample, and significantly greater between assays. MEA had a better precision at 300 µg/l than at 25 µg/l at within and between assays. This is normal because 25 µg/l is closer to the LOQ of the method, and the analysis will be more unstable. DEA did also follow the same trends as MEA. The precision was better at 300 µg/l than at 25 µg/l. DEA had a much lower LOQ than MEA, so the reproducibility should have been approximately the same at both concentrations. For AMP the best RSD were at 25 µg/l for the within assay and between assay samples. AMP had a smaller LOD and LOQ than MEA and there is no significant difference between the two concentrations. According to the method validation plan in table 2.7, within assays RSD should be below 5% and between assay below 15%. At 300 µg/l, MEA and DEA fulfill the criteria for the within assay test, AMP fulfill these criteria at 25 µg/l. None of the amines fulfill the criteria for the between assay test.

Overall, DEA gave the best results of the validation of the direct method, considering LOQ, LOD, accuracy, precision, linearity and chromatogram peaks. However experiments with different injection volumes indicated that a 5 µl injection would give better LOD, LOQ and

method accuracy. In conclusion, DEA will probably give the most reliable information in analysis of soil-water samples.

4.1.2 Indirect method

Dansylation

Before the method could be validated, the external standards had to be synthesized and purified to make sure that there was a reference that gave exact peaks of the derivatives that could be used for quantification and validation. For the dansylation of amines to form dansyl amides, pure amines and chlorinated amines were used. MEA and DEA were available in both forms, but AMP was only available in the chlorinated form. MEA-DNS was only synthesized from the pure amine, DEA was synthesized from the pure amine and the chlorinated amine, and AMP was only synthesized from the chlorinated amine. The synthesis of MEA-DNS was successful with the method described by Fournier et al. (2008).

As mentioned in chapter 1.6, pH is the most significant factor affecting the reaction, and optimal pH conditions are around 9.5 – 10 (Seiler 1993). When the chlorinated amines were used, a problem with pH occurred. The pH of the reaction solution turned acidic and pH values of 2-3 were measured. This was due to the production of hydrochloric acid (HCl) during the reaction, figure 1.5. When chlorinated amines were used, a greater amount of HCl was formed, than when pure amines were used as reagents. Dansyl derivatives become unstable under acidic conditions, especially secondary amines (Silva 2005). According to the method described in Fournier et al. (2008), the reaction solution was added a saturated solution of sodium bicarbonate as a buffer prior to the reaction. This buffer was not strong enough to neutralize the acid produced in the reaction when amine salts of DEA and AMP were used, and the reaction could not complete due to acidic conditions and instability of DEA-DNS and AMP-DNS. As a result the reaction products were not pure enough to be used for quantification or validation. To overcome this problem a 0.2 M $NaHCO_3$ buffer with a pH of 9.8, was added instead of the sodium bicarbonate buffer. This made the end solution more basic, but the reaction products were still not pure enough. When the pH of the reaction increases so will the reaction rate. But increased pH may lead to side reactions that will undo the dansylation and may hydrolyze dansyl chloride, equation 1 and 2 (Silva 2005; Stephens 1986). This may have been the reason why AMP-DNS was not pure after a second try of dansylation with a more basic buffer. Another factor that affects the reaction is steric hindrance. Branched primary amines like AMP and secondary amines like DEA has steric hindrance. However, secondary amines will still have more steric hindrance than branched

primary amines. This makes the reaction more challenging and may also have been a contributing factor in the failed synthesis of DEA- and AMP-DNS from chlorinated amines.

DEA-DNS was also synthesized from pure the pure amine. The synthesis was not successful, and the end reaction solution was still acidic. DEA is a secondary amine and is unstable under acidic conditions. It does also have more steric hindrance which makes the reaction more challenging. A 0.2 M solution of $NaHCO_3$ with a pH of 9.8 was used instead of the saturated sodium bicarbonate buffer proposed by Fournier et al. (2008). This made the synthesis successful. In conclusion, only the synthesis of MEA-DNS and DEA-DNS were successful.

To verify that MEA-DNS and DEA-DNS were successfully synthesized, and that the products were pure, UV-, ^{1}H NMR-, ^{13}CNMR- and IR-spectra and melting point were tested. AMP-DNS was also tested even though the synthesis was not successful. The response values of the derivatives were first checked on LC-MS, and compared with response values of pure derivatives synthesized by analysts at NILU. The response values of the successful MEA- and DEA-DNS were more or less identical with the NILU-standards. The response value of AMP-DNS were only about half of the NILU AMP-DNS standard. All three derivatives gave UV-spectra with three characteristic peaks at ʎ215, ʎ250 and ʎ335 (Takeuchi 2005) illustrated for dansyl glycine in figure 1.5. These peaks proved that the sample contained dansyl-derivatives. IR-spectra proved the presence of the OH-group in the derivatives which is not present in dansyl chloride (figure 1.7). This functional group was present for all three dansyl amides. ^{1}H NMR and ^{13}C-NMR confirmed that the derivatives were synthesized by confirming the presence of functional groups characteristic for the dansyl amides.

Another important property that was found and used to verify the presence of dansyl derivatives was melting point. Only MEA- and AMP-DNS were tested because DEA-DNS were liquid at room temperature. The melting point of MEA-DNS was known to lie between 104-105 ºC (Stenstrøm 2014), table 1.2. No data on melting point were available for the two other compounds. The melting point found for MEA-DNS was measured to lie between 101-102 ºC, table 3.2. This value corresponded well with the melting point found in the literature. The melting point of AMP-DNS was measured to lie between 83-84 ºC, which is about 10 ºC higher than the melting point of dansyl chloride. Whether this is the real melting point for pure AMP-DNS or not cannot be determined, because there are no references available. To decide the purity of the products, response values calculated from area of the chromatographic peaks of NILU-standards were used. In addition the appearance of the products was

evaluated. The reaction yield was only calculated for MEA-DNS and DEA-DNS because AMP-DNS was not pure. Both compounds had a reaction yield between 30-35 %, but the real yield was probably much higher. It was impossible to collect all products of the walls of the turbovap glass.

Method validation

The dansyl amides had a tendency to form sodium adducts. These adducts were not as abundant as in the direct method, but they were abundant enough to make a significant difference on the area of the peaks. The adducts were integrated together with the molecular ion of MEA-, DEA- and AMP-DNS. The method was validated according to the method validation plan in chapter 2.4.2. The results from the method validation test can be found in table 3.3. The indirect method had the same criteria for linearity as the direct method. The linear range should cover the theoretical concentration of amines in the soil columns. MEA- and DEA-DNS were linear over a range of 1-1000 µg/l and AMP-DNS from 0.25-1000 µg/l. None of the amines were linear above 1 mg/l. This means that they did not cover the theoretical concentration of amines in the soil columns presented in table 2.3. However when samples from the leaching experiment was analyzed with the indirect method, they were diluted three times. This means that a dilution is made and samples originally containing concentrations of 1.2-2.0 mg/l could be analyzed within the linear range.

The LODs and LOQs for the indirect method were much lower, than for the direct method. When amines were derivatized with DNS-Cl, the dansyl amides created had a much higher mass which means that the amount of dansyl amide detected will be higher than the amount of amine in the molecule. The derivatization step increases the sensitivity of the method. MEA had a LOQ of 0.10, DEA of 0.20 and AMP of 1.60. These values are below the acceptance limit from table 2.7. An important factor to remember is that samples are diluted three times. This means that these LODs and LOQs calculated from standards does not apply for samples. If a sample contained a concentration close to the LOQ it would have been diluted three times. This means that the value would be closer to the detection limit and the concentration could not have been calculated. The real LOQs would be three times higher than the ones presented in table 3.3. However, the purpose of this method was to quantify samples from soil column experiments, which were known to contain an amount of amines higher than the LOQ, so this was not a big problem in this study.

The reaction yield of DEA was much lower than the reaction yield of MEA and AMP. DEA is a secondary amine and has more steric hindrance than MEA and AMP, and this makes the reaction more difficult or unstable. MEA and AMP had a reaction yield of 65 and 58 % in MQ and 50 and 66 % in sample matrix. For MEA, the reaction yield was greatest in MQ, but for AMP the reaction yield was greatest in sample matrix. The reaction yield was calculated as an average of samples with different concentrations. There was no significant effect of concentration on the reaction yield. According to the validation plan, the accuracy of the reaction were too low, were DEA had a reaction yield below 50% in both matrices. Because of these low reaction yields, the LOD and LOQs presented in table 3.3 will not apply for real samples. The limits will be higher, because a higher concentration of analyte is needed to reach the LOQ and LOD.

Another important factor is the precision of the reaction. The precision was measured as a within and between assay repeatability test. The repeatability of the dansyl reaction was measured within assay and between assay. According to the method validation plan it is expected that the within assay samples have a lower RSD than the between assay samples. This theory proved to be correct for all amines. However, the reaction yields were not within the limits in table 2.7. According to this table the within assay repeatability should be >10% and the between assay repeatability should be >20%. All amines have RSD values above this limit, except for MEA which has a between assay repeatability in sample matrix >20%.

4.1.3 Stability of amines

Long and short-term stability of analytes were tested in MQ and sample matrix in order to see how long an aqueous solution of analytes could be stored. The long-term stability test was analyzed and quantified with the indirect method. The short-term stability test was analyzed and quantified with the direct method.

Long-term stability

The stability of amines was tested over a period of 100 days. Long-term stability was tested because samples from the leaching experiment had been stored for nearly a year before analysis. The aim was to get an indication of the stability of the samples from the leaching experiment. Another aim was to see how long an aqueous solution of amines could be stored in MQ. The stability of MEA, DEA and AMP in sample matrix can be found in figure 3.3. The percent recovery of all amines is significantly smaller on day 100 than day 1. For MEA day 21 and 100 are approximately the same, and the recovery of day 28 is 50%. Day 1 had a

recovery above 60%. The relative standard deviation of the four days of analysis was 32%. This value is twice as high as the RSD from the between assay repeatability test in table 3.3, and indicates that there is a significant instability and possible degradation of MEA in sample matrix.

The analysis of DEA in sample matrix (figure 3.3) indicates that there is a great breakdown if day 28 is ignored. The RSD between the days of analysis was 60 %, which is about the same as the RSD from the method validation. This means that it is difficult to draw a conclusion on the stability of DEA, even if figure 3.3 can give an indication of degradation. AMP had a clear decreasing trend according to figure 3.3, although the variations between each day were not big. The RSD between the four analysis days was 30% which is below the RSD from the between assay repeatability test, were the RSD of the reaction was 35%. These data does not give enough information about the stability of AMP, and it is not possible to conclude that AMP is degraded over time.

The stability of MEA, DEA and AMP in MQ can be found in figure 3.4. The amines show the same trends as in sample matrix. MEA had a recovery of 91% on day 1, on the remaining days; the recovery lied between 46 and 57 %. MEA had a clear decreasing trend. The RSD between days was 41%, which was greater than the RSD value calculated for the reaction precision in the method validation in MQ (table 3.3). This indicates that there was a significant instability and degradation of MEA in MQ. The recoveries of DEA were variable, and did not indicate any clear degradation of DEA, such as in sample matrix. The RSD between days was 39 %, which was smaller than the RSD calculated from the method validation. This means that the variable recoveries of DEA were possibly not due to degradation over time. AMP had the same decreasing trend in MQ as in sample matrix. However the RSD between days was 25%. This value was the same as the value found in the method validation, hence the variability was probably not due to degradation over time.

The long-term stability test indicated that of the three amines tested, only MEA gave indications of being unstable and possibly degraded over time. The amount MEA quantified decreased over time in both sample matrix and MQ, although the trend was most clear in MQ. A lot of research has been done on degradation of aqueous solutions of MEA, especially in context with gas sweetening in CCS technology, and not all reactions and mechanisms are completely understood (Strazisar et al. 2003). The conditions of MEA in this thesis were completely different from the conditions found in a CO_2-capture plant. According to Chi &

Rochelle (2001) MEA degrades in the presence of CO_2 and oxygen. The sample matrix used was rich in organic matter, and possibly saturated with CO_2 (Tau Strand 2014). A possible explanation may be that MEA reacted with naturally occurring CO_2. According to this theory, there should have been a big difference between sample matrix and MQ. The sample matrix generally had a lower recovery than the MQ-samples. This theory can also be part of the explanation to the lack of amines in the water samples collected from Smøla in the ExSIRA project.

According to Reynolds et al. (2012) the stochiometry of the reaction between amine and CO_2 (figure 1.2) is approximately two moles amine per mole CO_2. This means that more MEA could be degraded, than the amount of CO_2 that was reacting. This reaction will reach equilibrium over time (Reynolds et al. 2012). However, the limiting reactant in this case is CO_2. In figure 3.3 and 3.4 the biggest difference is between day 1 and day 21. Thereafter the variations are less. According to Reynolds et al. (2012) an aqueous solution of MEA will react rapidly with CO_2, and this may be the explanation of the difference between day 1 and 21. Thereafter the reaction might have reached equilibrium. MEA does also react with other sour gases such as H_2S, COS and CS_2. These gases are common in a CCS plant, and may cause degradation problems (Islam et al. 2010). It is not known if these amines are present in trace amounts in the solutions used in this study, but probably not.

Other hypothesis for unstable quantification of MEA in sample matrix and MQ may be interference between amines because all amines were mixed in the same solution, undissolved external standards, or reaction with unknown interfering compounds. To summarize, the samples from the leaching experiment containing MEA will probably have had a decreasing trend like the MEA-samples in figure 3.3. Though these samples were stored in a mix solution, while the MEA-samples from the leaching experiment was not. MEA cannot be stored over a three month period and before further experiments have been conducted on MEA stability, fresh solution should be made before use.

Short-term stability

No conclusion could be drawn from the short-term stability test regarding stability.

4.2 Leaching experiment

The conclusion of the validation of the two analytical methods showed that MEA and AMP should be analyzed with the indirect method, and DEA should be analyzed with the direct method. Only the results of DEA-analysis with the direct method, and MEA- and AMP-analysis with the indirect method is further discussed in this chapter.

4.2.1 Second leachate

Samples from the second leachate were analyzed because they may give an indication of the content of amines and TOC in real samples of peat soil. The amount of TOC, TOT N and pH of the second leachate samples can be found in table 2.4. It was expected that samples from the second leachate contained more TOC, TOT N and natural content of amine (if there was any) than the third leachate samples. Table 2.4 show that the pH was slightly higher than in the third leachate, so was the TOC and TOT N. Table 3.5 show the content of amine in the second leachate samples. Only MEA and DEA were detected in all columns, and at concentrations high enough for quantification. AMP was detected, but at concentrations lower than the quantification limit.

There was no surprise that MEA was detected, because it is a normal constituent of phospholipids in animals and humans (Knaak et al. 1997), and has been detected in soil/soil extracts in other studies (Stevenson 1994). On the other hand DEA is not known to be a constituent of phospholipids, or occurring naturally in soil. The concentration of MEA lied between 3.9 and 4.4 µl and the concentrations of DEA lied between 2.6 and 4.1 µl. These concentrations were relatively high compared to the LOQ of MEA and DEA. The presence of DEA in samples may have been due to contamination in the column experiment, noisy samples interfering compounds with the same mass as analytes, instrument contamination on columns, ion source, or mobile phases. Other possible error sources were carry-over of samples or injection errors. Analytical columns and mobile phases were checked for contamination by running the method gradient (table 2.5) for each method, without injecting any sample. No analytes were detected for each method, which proved that the instrument could not be the source of contamination. Carry-over from one sample to the next was another possibility, but the injection needle was cleaned between each injection and a pre-run of 5 minutes were set up between each sample, so this hypothesis was not likely to be true. A possible explanation is contamination of samples during leaching experiment at NMBU or sample preparation at NILU.

Control samples, which were analyzed parallel to the second and third leachate samples, did also give an indication of the natural content of amines in the soil. Theoretically, the third leachate samples were expected to contain less amine than the second leachate sample, because of their water solubility and the favor of leaching of low mass aliphatic amines. The control samples in table 3.6 show that all amines were detected and that the concentration was higher in the second leachate samples, than the third leachate samples. There is a significant difference in MEA, where the concentration in the second leachate was 4.3 µg/l and in the third leachate 39 µg/l. The most likely explanation is carry-over of other samples containing a higher concentration of amines. When these samples were analyzed, long work-lists containing more than 100 samples were analyzed, which may have caused some carryover.

4.2.2 Third leachate

Hypothesis 2

The aim of the leaching experiment was to test hypothesis 2 and 3 from the ExSIRA project, presented in chapter 1.2. Hypothesis 2 says that amines were absorbed in the soil solid phase and none followed the soil water that was extracted. In other words all amines were adsorbed in peat soil. Table 3.5 shows the results from the leaching experiment after addition of amines (third leachate). In this experiment, error sources like absorption through vegetation, hydrology and breakthrough curves and sampling equipment were excluded. All amines from the leaching experiment were detected in the leachate and concentrations were high enough to be quantified. However, recoveries calculated according to theoretical concentrations were only 11% for DEA, and 23 and 22 % for MEA and AMP respectively. This means that over 75% of the amines were adsorbed to the soil.

Low recoveries indicate that there has been some retention in the soil. Peat soil is an organic and usually acidic soil (Miller & Donahue). The pH of the soil columns were all below 3.4 and at these conditions the alkanolamines will have a positive charge on the nitrogen atom (figure 1.1). The adsorption of alkanolamines to soil is very pH dependent (McBride), and low pH have a low capacity of cation adsorption (Thompson and Goyne). According to table 2.2 the content of carbon in the soil used in this experiment was 50.1 %. Organic material contains between 40-50% carbon (Brady & Weil 1999), which indicates that the soil contained almost 100 % organic material. This means that adsorption to clay or metal oxides mentioned in chapter 1.4, can be excluded. Even though the capacity of organic soil to bind cations will reduce with decreasing pH, its ability to bind cations will still be great. The ability will be even greater in decomposed organic material. The soil was classified to 4/5 on

Von Post scale (table 2.2). This means that the soil was weakly to moderately decomposed, hence a great adsorption of analytes to soil is possible.

An experiment using soil columns under laboratory conditions is far from natural conditions. The soil water extracted under field conditions had more uncertainties regarding dilution of amines, hydrologic conditions, extraction time and place etc. The soil used in this experiment had been sieved in order to get a hydraulic conductivity close to a natural bog. Samples from the ExSIRA project probably contained more TOC and TOT N, dirt and interfering compounds that could make the analysis more difficult. The fact, that the sampling took place at a bog gave many uncertainties regarding water currents and their effect on hydraulic conductivity. Other explanations to why no amines were found in the ExSIRA project may be that some of the other hypothesis in chapter 1.2 was true. Amines could have been absorbed or taken up by the above ground vegetation, sorption to sample equipment, microbial degradation, or that the sampling was not done at the right time and place.

In conclusion, the experiment showed that between 11 – 23 % of amines were leached through organic soil, when concentrations equivalent to the "worst case scenario" emissions from a CO_2-capture plant were added. This means that hypothesis 2, that says that all amines are adsorbed to peat soil, can be rejected. However, soil with properties described in table 2.2 will adsorb more than 75 % of the amines added, so a 100 % recovery cannot be expected. In order to be sure that the amines detected was the same amines that were added in the column experiment, isotope labeled nitrogen could have been used. The uncertainty of the two analytical methods regarding accuracy, indicates that the recovery of amines were probably higher than the values presented in table 3.3 (the accuracy of the method varies according to method and amine). This experiment shows that amines leach through soil and be washed out in ground-water, lakes etc. which may have negative effects on the environment. Especially dangerous are DEA, which is a secondary amine, and may form nitrosamines, which is carcinogenic.

Hypothesis 3

Hypothesis 3 says that amines entering the soil could not be detected because soil water contained so much noise that it would cover their signal. As already mentioned, the amines were detected in all columns where they had been added, consequently this hypothesis was rejected. However, the soil water matrix contained a lot of noise and interfering compounds, as discussed in chapter 4.1.1. This caused a lot of problems in the direct method. In ExSIRA

samples, the sample matrix had probably more noise, due to a higher content of TOT N and TOC. Although this is natural content for soil water, it makes the analysis difficult on a sensitive instrument like HPLC-MS. The leaching experiment with the direct method indicated that this noise caused big problems in the detection of MEA and AMP.

4.3 Comparison of methods

The analytical methods presented in this study were both developed by NILU for the same purpose; analysis of low mass amines. Advantages with the direct method was that it was easy to use (shorter sample preparation time, but relatively long analysis time), contained few steps in the sample preparation, direct analysis of amines and cheaper to use than the indirect method. Advantages with the indirect method was higher sensitivity towards analytes (lower LODs and LOQs), samples were less dirty due to a lower injection volume, short analysis time, robust column.

Disadvantages with the direct method in comparison with the indirect method, is that samples injected to the column are dirtier due to a higher injection volume and a lower dilution of sample. Samples were diluted 20% with the direct method and 67% in the indirect method. This caused some problems for two of the analytes, MEA and AMP, and made the internal standard IS-MEA difficult to use in some samples. Disadvantages of the indirect method were the low reaction yield, especially for DEA, and the low precision of the reaction. Based on the results from the method validation the direct method appears to be the best choice for samples with high concentrations of analytes, especially for DEA and AMP, which are linear to 5 mg/l. The indirect method is the best choice for analysis of analytes with lower concentrations as the LODs and LOQs are lower. To summarize, DEA should be analyzed by the direct method, while MEA and AMP should be analyzed by the indirect method, for the work conducted in this study.

Regarding the quantification of second and third leachate samples in table 3.5, the methods are not comparable. This is probably due to uncertainties of the methods for specific analytes. As already mentioned DEA was the most suitable analyte for the direct method, while MEA and AMP were the most suitable analytes for the indirect method. Therefore, the results of DEA quantified with the direct method, and MEA and AMP with the indirect method are the most realistic and accurate results, considering the uncertainties from the method validation. These results gave the highest recovery values of the amine treated leachate.

5. Conclusion and future perspectives

This thesis described the validation and comparison of two analytical methods developed for analysis of three low mass aliphatic amines, commonly used in post combustion capture in CCS technology. One method analyzed the amines directly, without any sample preparation procedure. This method proved to be the best choice for DEA, although the criteria's of the method validation plan was not fulfilled. The method accuracy lied between 50-75%, hence there was some uncertainty regarding quantification. The sample matrix turned out to be very rich in interfering compounds, thus the chromatographic peak of MEA and AMP were difficult to find and integrate. As a consequence, these analytes had poorer validation. A future challenge could be to experiment with lower injection volumes, hoping to get better accuracy and precision, without compromising LOD and LOQ too much.

The other method analyzed the analytes indirectly as dansyl amides. This method was best suited for analysis of MEA and AMP, as DEA gave a low reaction yield and poor reaction precision. However, none of the amines fulfilled the acceptance criteria of the validation plan. Reaction yields for MEA and AMP lied between 50 and 70 % in sample matrix, which gave the method some uncertainty regarding quantification. Another part of the validation of this method included the synthesis of dansyl derivatives used as external standards. The method originally used was described by Fournier et al. (2008), and proved to be most effective with the use of pure and primary amines as reagents, like MEA. Though, some amines were only available as salts, this method was optimized for these compounds with a successful synthesis of pure compounds. Future work on the indirect method could be done to investigate if the reaction yield could be optimized.

Degradation of aqueous solvents of amines are well known and a problem in CCS technology. The stability of MEA, DEA and AMP was tested as a part of the method validation, by using the indirect method. No conclusion could be drawn on the stability of DEA and AMP, whereas MEA showed instability and possible degradation. The RSD between days in the stability test of MEA was significantly higher than the RSD in the between assay repeatability test in the method validation. The instability of MEA could have been due to reaction with CO_2 present soil water (and air) which was probably saturated with CO_2, due to the high content of organic matter. The stoichiometry of the reaction between amine and CO_2 is approximately 2 mole amine per mole CO_2. The reaction happens rapidly and is an

equilibrium, which means that the amount of reagents and product should be equal after time. This theory corresponds well to the column diagram of MEA. On the other hand, the uncertainty of the reaction regarding precision and accuracy of MEA is significant. This test only gives indications of degradation. Future challenges could be further testing on the stability of MEA, as well as DEA and AMP.

Leaching experiment in soil columns of second leachate showed that MEA and DEA were present in sample matrix at concentrations below 4.5 µg/l. MEA was found by the indirect method, while DEA was found by the direct method. MEA has been reported to be present in soil by Stevenson (1994) and is also a constituent of phospholipids in animals and plants. The presence of MEA was no surprise, whereas the presence of DEA was more unexpected and may have been due to contamination. The third leachate contained all the amines added in the leaching experiment, though the recoveries were below 25% for both methods. Since the amines could be detected and quantified the hypothesis' that peat soil adsorbs all amines added and that the noise in the soil will cover the chromatographic signal, where both rejected. An experiment performed under laboratory conditions is far from natural conditions. Water samples from the ExSIRA project probably contained more TOC, TOT N, dirt and interfering compounds than the water samples in this study, which would have made these analysis more difficult.

All in all, the results from this study can give some explanation of the results from the ExSIRA project by eliminating two of the hypothesis developed in association with the lack of recoveries of amines in soil water from the field experiment at Smøla. To be able to conclude about this experiment, further work should be done on the remaining three hypotheses. This study have also showed two methods used for amine analysis and the uncertainty associated with them.

6. References

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Appendix I - Materials

Table A.1. List of instruments and suppliers.

Instrument	Supplier
Agilent 1290 Infinity Diode Array Detector	Agilent Technologies
Agilent 1290 Infinity Binary Pump	Agilent technologies
Agilent 1290 Infinity Autosampler	Agilent technologies
Agilent 1290 Infinity Thermostatted Column Compartment	Agilent technologies
Column: Discovery^{R} HS-F5-3, 15 cm x 2,1 mm, 3 µm	Sigma-Aldrich
Column: HSS T3 Acquity, 2.1 x 150mm, 1.7 µm	Waters
Agilent 6500 Series Q-TOF LC/MS System	Agilent Technologies
Ultrasonic Cleaner	VWR
TurboVap®	Zymark®
IMS Minishaker	IKA
Ascend^{TM} 400 (NMR)	Bruker
Spectrum BX FT-IR system	Perkin-Elmer
9100 Melting point apparatus	Electrothermal

Table A.2. List of chemicals and their molecular formula, cas-number, purity and supplier.

Chemical	Molecular formula	Cas-number	Purity (%)	Supplier
Acetonitrile	C_2H_3N	75-05-8	100	Merck
Acetic acid	CH_3COOH	64-19-7	100	Merck
API-TOF Reference mass solution kit	-	-	-	Agilent
Dansyl chloride (DNS-Cl)	$C_{12}H_{12}ClNO_2S$	605-65-2	>99.0	Sigma-Aldrich
Diethanolamine (DEA	$C_4H_{11}NO_2$	111-42-2	>98	Sigma-Aldrich
Diethanolamine hydrochloride	$C_4H_{12}NO_2{\cdot}HCl$	14426-21-2	>98	Sigma-Aldrich
Dimethyl-13C_2-amine hydrochloride	C_2H_8ClN	286012-99-5	>99	Isotec
ESI-Low Concentration Tuning Mix	-	-	-	Agilent
Ethanolamine (MEA)	C_2H_7NO	141-43-5	>99.5	Sigma-Aldrich
Ethanolamine ^{13}C hydrochloride	$C_2H_7NO{\cdot}HCl$	1173019-25-4	> 99	Sigma-Aldrich
Ethanolamine hydrochloride	$C_2H_7NO{\cdot}HCl$	2002-24-6	>99.0	Sigma-Aldrich
Hydrochloric acid	HCl	7647-01-0	32	Merck
Magnesium sulfate	$MgSO_4$	7487-88-9	>99	Sigma-Aldrich
Methanol	CH_3OH	67-56-1	100	Merck
Methylbenzene (toluene)	C_7H_8	108-88-3	>99.7	VWR
Nitrogen (compressed)	N_2	7727-37-9	100	Yara praxair
Propanone (acetone)	C_3H_6O	67-64-1	>99.7	VWR
Sodium bicarbonate	$NaHCO_3$	144-55-8	>99.5	Merck
Sodium hydroxide	NaOH	215-185-5	>99	Merck
2-amino-2-methyl-1-propanol -hydrochloride (AMP)	$C_4H_{11}NO{\cdot}HCl$	3207-12-3	>99	Sigma-Aldrich

Appendix II – Calibration curves

Calibration curves of MEA, DEA and AMP analyzed with the direct method, and MEA-, DEA- and AMP-DNS analyzed with the indirect method.

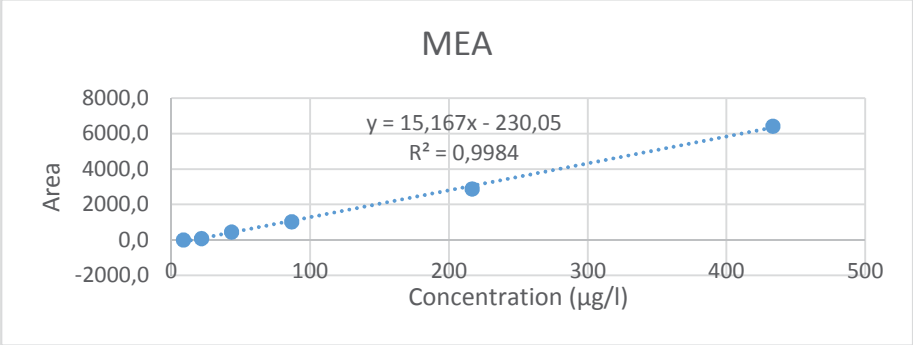


Figure A.1. Calibration curve of MEA.

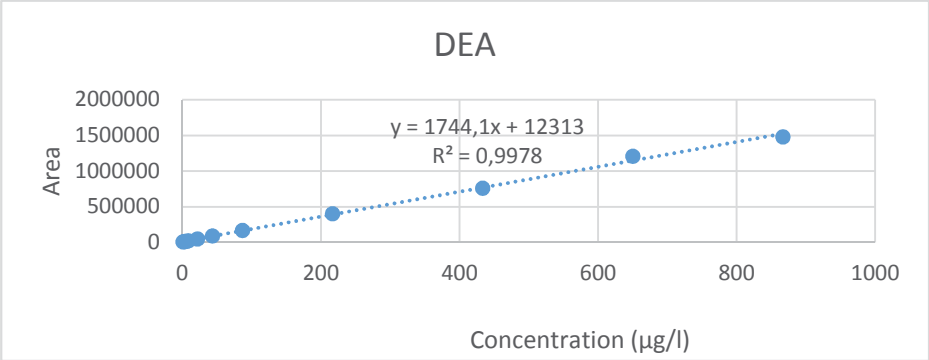


Figure A.2. Calibration curve of DEA.

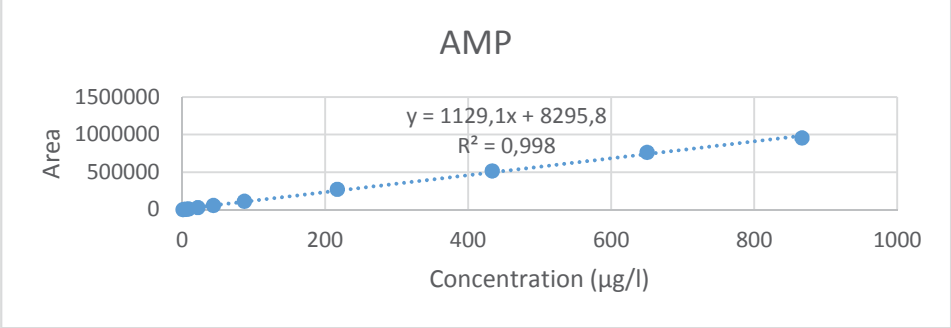


Figure A.3. Calibration curve of AMP.

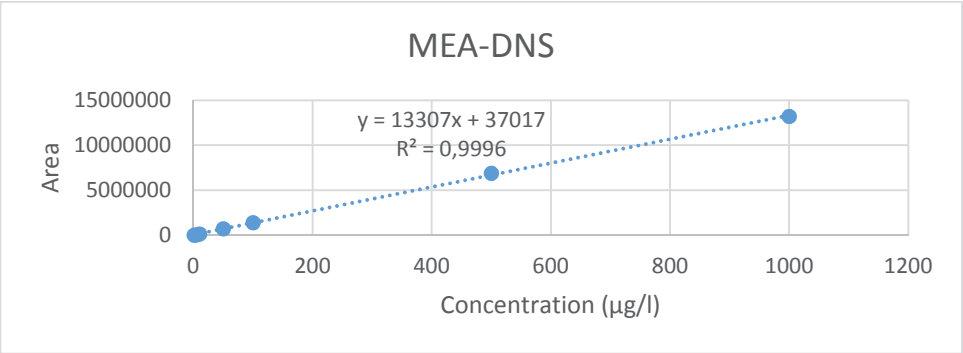


Figure A.4. Calibration curve of MEA-DNS.

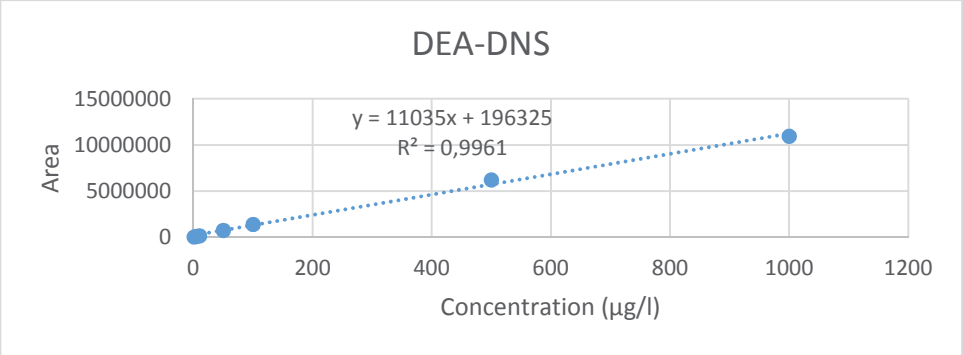


Figure A.5. Calibration curve of DEA-DNS.

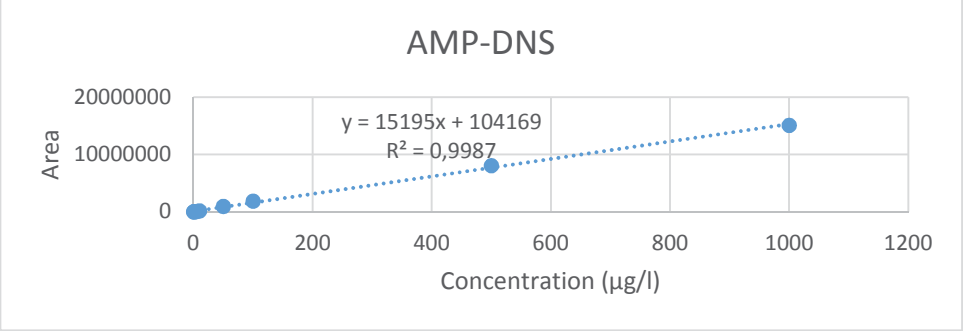


Figure A.6. Calibration curve of AMP-DNS.

Appendix III – UV-spectra

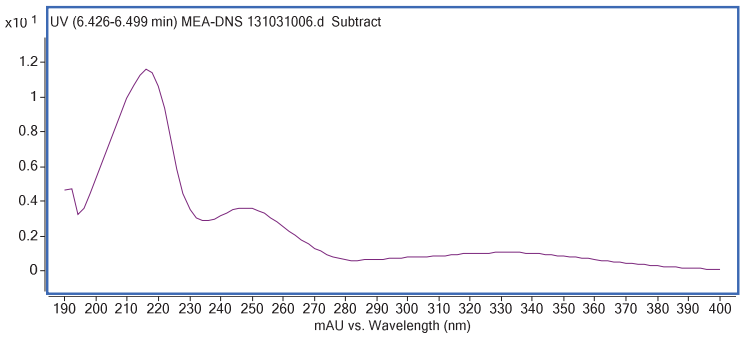


Figure A.7. UV-spectra of MEA-DNS.

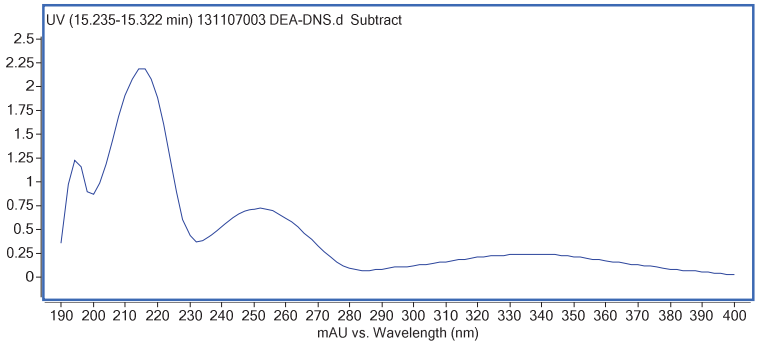


Figure A.8. UV-spectra of DEA-DNS.

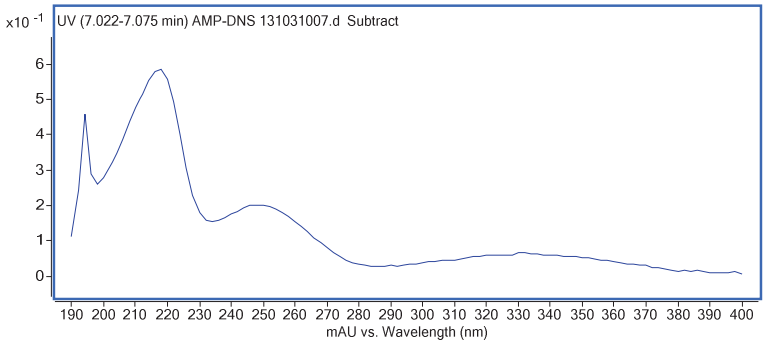


Figure A.9. UV-spectra of AMP-DNS.

Appendix IV – IR-spectra

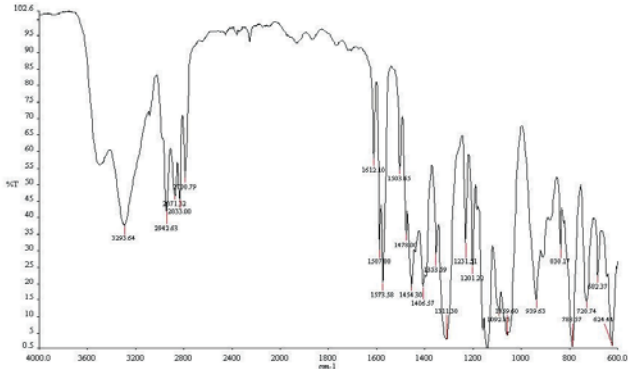


Figure A.10. IR-spectra of MEA-DNS.

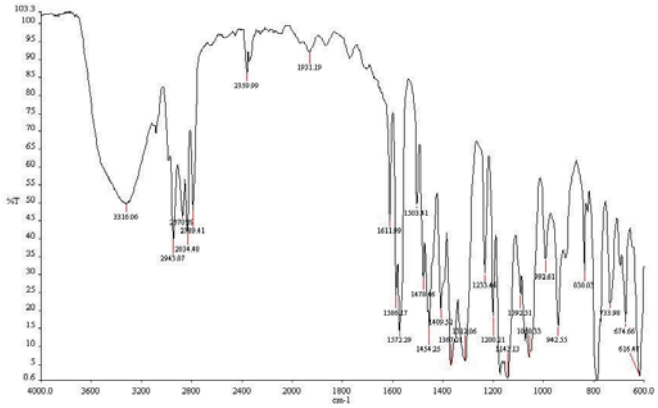


Figure A.11. IR-spectra of DEA-DNS.

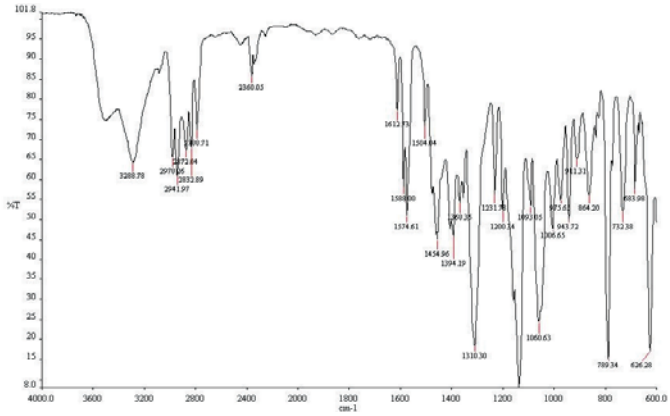


Figure A.12. IR-spectra of AMP-DNS.

Appendix V – ^{1}H NMR-spectra

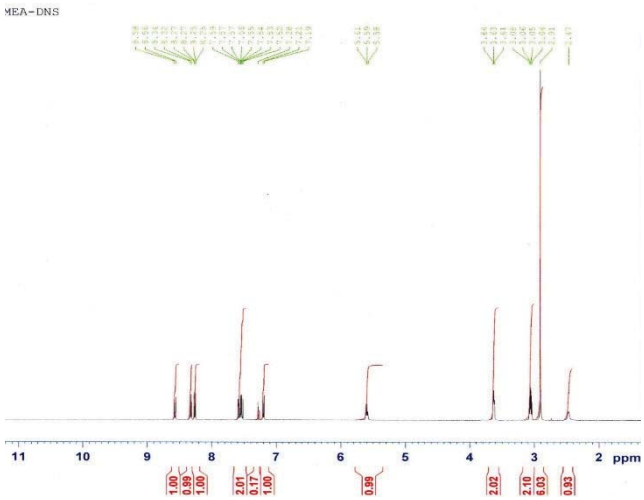


Figure A.13. ^{1}H-NMR spectra of MEA-DNS.

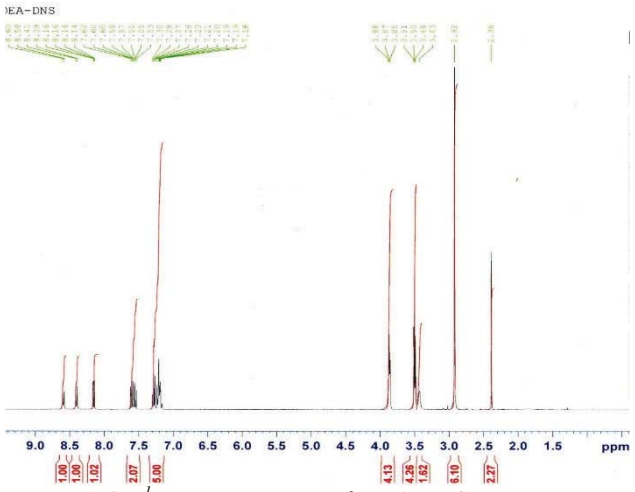


Figure A.14. ^{1}H-NMR spectra of DEA-DNS.

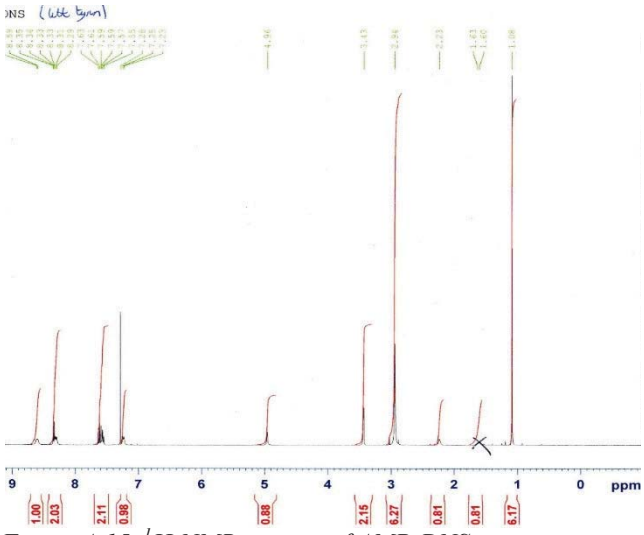


Figure A.15. ^{1}H-NMR spectra of AMP-DNS.

Appendix VI – ^{13}C NMR-spectra

^{13}C NMR (100 MHz, $CDCl_3$):

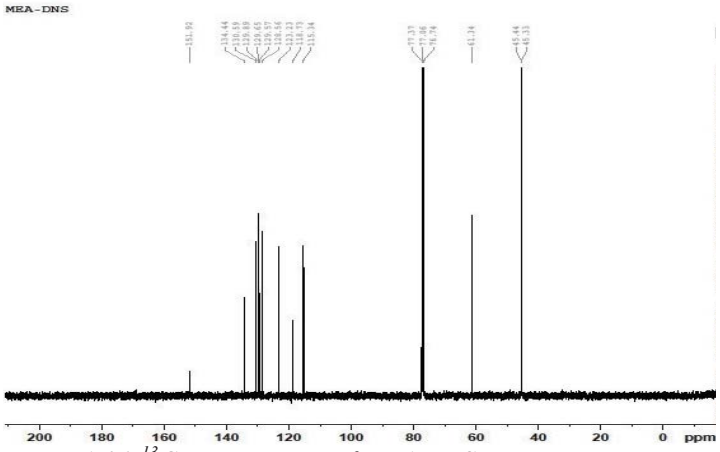


Figure A.16. ^{13}C-NMR spectra of MEA-DNS.

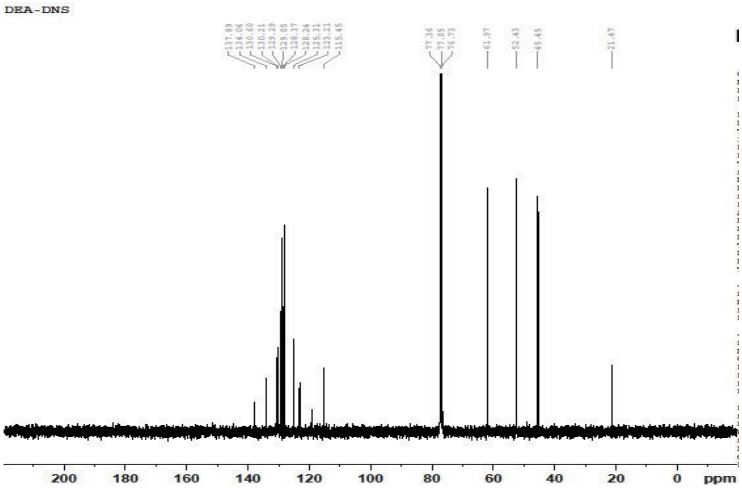


Figure A.17. ^{13}C-NMR spectra of DEA-DNS.

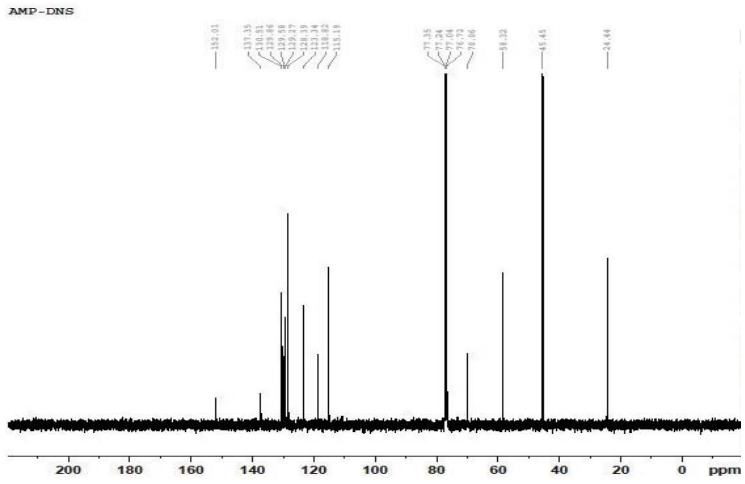
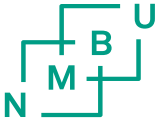


Figure A.18. ^{13}C-NMR spectra of AMP-DNS.



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