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Evaluation of pre-concentration techniques for gas chromatographic determination of low amounts of volatile contaminants and degradation products

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Summary

The project investigated the compared two pre-concentration methods, Thermal Desorption System (TDS) coupled to a Thermal Extractor (TE) and Multiple Headspace Injection (MHI) using a Multiple Purpose Sampler (MPS) for detecting volatile amines in solid pharmaceutical products and synthesis precursor chemicals samples. The TDS-TE and MHI extractions are directly injected into a Cold Injection System (CIS). The CIS captures the analytes using cold temperatures to condense the vapours. The sample is then injected into a gas chromatograph-mass spectrometer system. The resulting mass spectrum extracted from the chromatogram can then be used in coordination with a mass spectral library with search program to identify amines present in the samples.

The two pre-concentration methods were compared based on sensitivity to detect odorous amines, overall analyses time and general ease of use. The TE-TDS method was more labour intensive and required longer analyses times but resulted in higher sensitivity, ability to detect most amines and ability to adapt to smaller sample sizes and lower concentrations in the sample. MHI by contrast is easier to use as the MPS is largely automated and faster but the sensitivity might not be sufficient for detecting volatile amines in solid pharmaceutical products. The TDS-TE method is preferable due to greater sensitivity, and further work should be done to increase the automation of this pre-concentration method.

Contents

Introduction

Aim of study

This project aims to compare two different pre-concentration techniques for analysing volatile compounds, amines, at low concentrations in a gas chromatograph system in pharmaceutical products and synthesis precursor chemicals. The two techniques are, Multiple Headspace Injection (MHI) and Thermal Desorption Sampler (TDS) coupled to a Thermal Extractor (TE). These are injected into a Cold Injection System (CIS) that traps and concentrates the analytes. Of the evaluation parameters that can be assessed in this comparison, the most important are sensitivity and overall analyses time.

Background

This project was commissioned by Astra Zeneca and performed at their laboratories in Mölndal, Sweden. AstraZeneca is a Science driven pharmaceutical company that develops and delivers medicines to patients. The pharmaceutical industry is heavily regulated to ensure patient safety and proper use of the products. These regulations are continually changing and evolving to consider new knowledge, in order to both incorporate new information about the risks of certain compounds, and their potential degradation products in the human body.

A few years ago, a batch of pharmaceuticals in the Philippines was recalled after reports of a strong odour from the tablets and their container [1], [2]. This was allegedly due to a problem with the cooling system in the production plant for the active ingredient in the formulation, causing an increase of a known impurity. In the future, government agencies might require a complete analysis of the odour from drugs to ensure that any potentially harmful compounds are not above any threshold of concern. AstraZeneca therefore wishes to create and establish methods to analyse these odours before it potentially becomes a problem in the production or a health hazard for the patients. These concerns are the reason for this project as it will act as a foundation for further studies within this area of research and development.

Techniques

Techniques that can rival the human nose's sensitivity for detection of molecules must be very sensitive with low detection limits as the human nose is capable of detecting very small amounts of a substance. A form of gas chromatography coupled to a detection unit, often a mass spectrometer is used for detecting volatile compounds. The very low concentrations of volatile compound, in the case of this project, amines, in the samples does however necessitate additional concentration of the sample to achieve necessary sensitivity. These techniques used are described below with a brief description of how they work.

Gas Chromatography (GC)

Martin and Synge together published a paper regarding gas liquid chromatography in 1941, they were later also awarded the Nobel prize in chemistry for this [3]. In 1950 Gohlke published the coupling of a Gas Chromatograph (GC) and a Mass Spectrometer (MS) [3]. The combination of these newly invented techniques allowed for a good separation detection and identification of the same sample. The same combination is today, with considerable improvements, an invaluable analytical detection and quantification technique.

The basic principle of a GC is separation of volatile compounds. The separation is performed by using a column with a material inside which interacts with the sample, while not irreversibly binding it. This interaction will separate the components of the sample depending on how much they interact with the

stationary phase [4]. The column is only efficient if the sample is appropriate for the columns function. The column type most commonly used today is a long thin open tubular column. They have a narrow inner diameter, the inside is coated with a layer of stationary liquid phase film to which the sample in the gas phase can interact [4]. The column type used in the past was often a packed column, which is a column with larger diameter, filled with small particles coated with non-volatile stationary phase. They allow for a larger sample capacity but have poorer resolution, broader peaks and longer retention times [4]. GC is a powerful separation tool for analysis of volatile samples, however non-volatile compounds cannot be separated in a GC as they are not gaseous, unless they are chemically modified to make them volatile at high temperatures (for example up to 300 °C). GC uses a highly controlled oven to change the temperature to allow molecules to be separated based on both their relative volatility and interaction with the stationary film phase of the column. An additional advantage of GC is the very high chromatographic resolution that is possible, which allows separation of very similar compounds that may not be possible using other chromatographic techniques.

Mass Spectrometry (MS)

In 1910, J.J Thompson invented the first MS and used it to prove the existence of isotopes of elements [3]. This technique improved over the years to the advanced and sensitive method it is today. MS is now one of the most important detection methods available and is used in tandem with many chromatographic methods such as liquid- and gas chromatography.

An MS can have many different configurations and utilize different techniques for achieving the same goal. However, there are a number of common denominators. Firstly, the molecules injected are ionized, then directed through a magnetic field, filtered and directed into a detector [3]. The configuration for the MS used in this project is one that is suited for a continuous stream of analyte, as is required for a GC-MS setup. The ionization is done by an EI (electron impact). The filtering through a quadrupole that use four conductive rods to create an oscillating frequency in its electric field that guide only specific ions through depending on their mass divided by ion charge value (m/z) [4] The oscillation in the electric field is predetermined before the analysis by selecting a scanning range that limit what m/z values will at all be allowed through into the detector.

Multi Headspace Injection (MHI)

To perform MHI an MPS system is used. It is an automatic system used primarily for injection of samples into the GC with high precision and reliability compared to manual injection. Some systems can also perform other simpler tasks such as performing dilutions and mixing chemicals as preparation for injection. The system used in this project is capable of liquid injection and multiple headspace sampling with incubation and agitation of the samples before extraction [5].

Thermal Desorption System (TDS)

A TDS heats and extracts volatiles from liquid or solid samples. It consists of a number of components. First a TDS tube loaded with a sample or a tube with sorbent that already have adsorbed analyte, is slotted into place. The sample can be a solid or a small amount of liquid. The tube is inserted into a heater that heats the tube in order to facilitate release of volatile molecules. A helium gas stream carries the molecules forward [6]. This stream of molecules are then, depending on their boiling point, condensed into liquid inside the Cooled Injection System (CIS). The advantage of using a TDS instead of a

traditional headspace sampler is that, in theory, a higher degree of analyte in the sample can be extracted. The TDS and CIS techniques operate in tandem therefore allow for a high degree of pre-concentration of the desired analyte molecules before injection, which should increase the limit of detection on smaller sample volumes [6].

Thermal Extractor (TE)

A TE is a system utilized to gather analyte molecules in desorption tubes that are filled with sorbent materials such as TENAX and/or carbonized molecular sieves [7]. These substances are specifically made to adsorb analyte molecules of different sizes and reactivity. Thermal extractors are often used as the pre-treatment step to concentrate the analyte molecule from a larger quantity of sample in the form of either liquid, solid or gas [7]. A potential weakness with this technique is however that the sorbent must be made specifically to suit the sample. A too weak sorbent might not bind the smaller molecules and a too strong sorbent might adsorb too much of non-desired molecules.

Compared to a more direct injection of sample through liquid or headspace injection, the sorbent tube coupled to a TDS is, in theory, is at a risk of not being able to adsorb certain molecules. This is especially true if the sample holds many different components that need be analysed. That would then require repeated analyses with a number of different sorbent tube types.

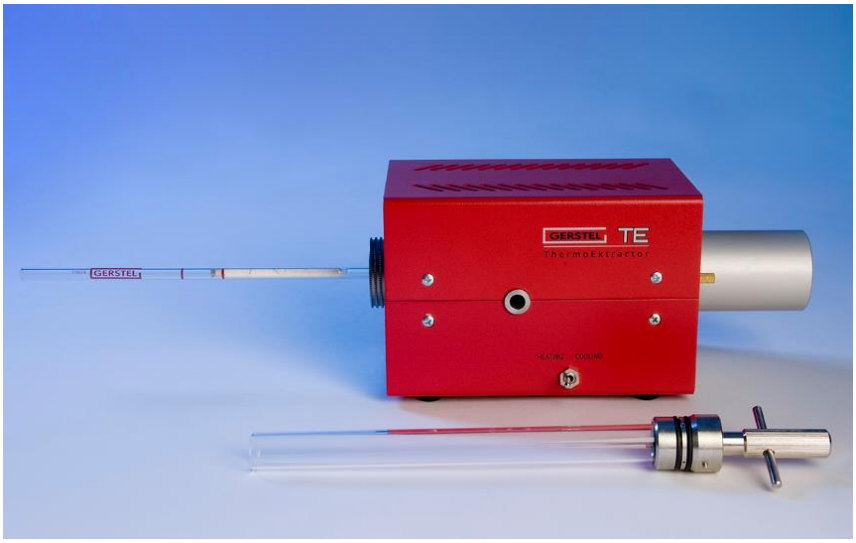

Figure 1: A picture showing the TE device used in this project. The large tube in the foreground is the TE-tube that is filled with sample and plugged with glass wool. This is inserted into the red box which heats the sample and inert gas is directed through the TE-tube into the small TDS tube filled with sorbent.

Cold Injection System (CIS)

A Cold injection system or “Cold trap”, is a component installed before the GC column to collect and concentrate analytes. The injection ports of GC’s have liners, which are glass tubes. The liner is where the analytes in the sample are condensed, trapped and concentrated in the case of multiple or a prolonged injection [5]. The liner can also have different sorbent materials inside or baffles to more efficiently contain the analyte. Condensation of the analyte is achieved by cooling the liner using the CIS system. The temperature can be adjusted and set depending on what analyte is anticipated, albeit with limits depending on the cooling used. Ethanol cooling, which is what this project used, can at most handle about

-40°C, which should be sufficient for most amines as DMA which is a small amine with a low boiling point (7°C) [8]. When the sample injection is complete, the CIS stops cooling and instead rapidly heats up to vaporize the condensed substances and inject them into the GC-column.

Amines

Amines are organic or inorganic compounds containing a nitrogen atom with a lone pair of electrons. The amines described in this report are all organic molecules.

Dimethylamine

Dimethylamine (DMA) is an organic secondary amine that smells like fish and ammonia, is colourless and flammable in its natural state [8]. The molecular weight of DMA is 45.085 g/mol. The DMA in this project was either dissolved in methanol to stabilize it or in a salt form with hydrochloride (HCL). This is required since DMA in its natural state is flammable and dissolves in water forming flammable and corrosive solutions [8]. The DMA dissolved in methanol is stable but still smells strongly of amines due to evaporation of the DMA. The DMA salt is used as a component in the synthesis of the drug metformin. The salt is however prone to adsorb water from the atmosphere and must therefore be stored in dry conditions [9].

Triethylamine

Triethylamine (TEA) is a clear colourless liquid with a strong fish-like ammonia odour, the molecular weight of TEA is 101,193 g/mol. The vapours are unpleasant and can irritate mucous membranes and eyes [10].

Other amines

N,N-Dimethyl butylamine (BAD) has a molecular weight of 101.193 g/mol [11]. Dimethyl propylamine (DMPA) have a molecular weight of 87.166 g/mol [12]. Dimethyl chloramine (CDA) has a molecular weight of 79.527 g/mol [13]. Ethyl dimethylamine (EDMA) has a molecular weight of 73.139 g/mol. Trimethylamine (TMA) has a molecular weight of 59.1 g/mol. All these amines have smells that resemble ammonia or fish. N,N,N,N-Tetramethyldiaminomethane (MDATM) have a molecular weight of 102.181 g/mol is a solid or solution at room temperature [14]. Nitro dimethylamine (NDMA) has a molecular weight of 74.083 g/mol. It is a yellowy oily liquid with a supposedly characteristic odour that occurs when the molecule decomposes due to exposure to direct sunlight. NDMA is suggested to be a carcinogen and is highly toxic [15].

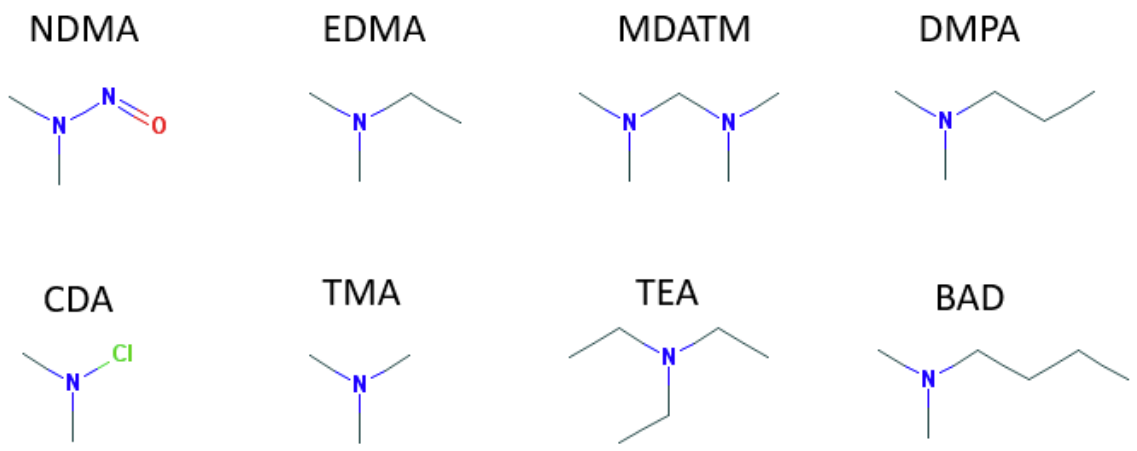

Figure 2: The structure of the eight amines that were detected in the samples analysed. The lines without a letter signifies a bond to a methyl group (CH_3). [8]–[17]

Metformin-Hydrochloride

Metformin is a drug primarily used for treating type 2 diabetes mellitus and is one of the most prescribed drugs in the world. The drug works by improving glucose uptake and utilization, inhibiting production of hepatic glucose and reducing intestinal glucose absorption [18]. Metformin has also been used for the treatment of polycystic ovary syndrome and for general health improvement such as weight loss [18]. The metformin molecule is synthesized by the fusion of dimethylamine and dicyandiamide. Metformin is therefore rich in nitrogen and a hypothetical degradation of metformin runs the risk of forming foul smelling and potentially harmful amines. The metformin molecule has been shown to be unstable at higher temperatures [19].

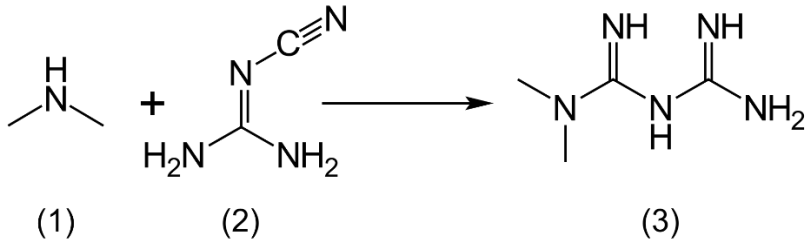

Figure 3: The synthesis of metformin (3) from the two components Dimethylamine (1) and Dicyandiamide (2) [20].

Metformin hydrochloride is not completely thermally stable over room temperature; at 60°C metformin is not heavily affected by thermal degradation over a 24-hour period with an observed recovery percentage of 99.98% [19]. Higher temperatures close to metformin's melting point (ca 223 °C) do however induce considerable thermal instability, even over short durations, causing decomposition. Exposing metformin to 220°C for 1-2 minutes causes the metformin to degrade and metformin-HCl recovery is only 86% [19]. This makes using high temperatures while handling or desorbing metformin substances very impractical as many amines might be formed as by-products and therefore make the analysis searching for amine impurities inaccurate.

Experimental

Instruments

The gas chromatograph used was an Agilent 6890 with a CP-Volamine Agilent CP7448 column (Agilent, Santa Clara, California, USA). The mass spectrometer used was Hewlett Packard 5973 Mass Selective Detector (Palo Alto, California, USA). The Multipurpose sampler used was a Gerstel Multipurpose Sampler MPS 2 (Gerstel GmbH & Co.KG, Mülheim an der ruhr, Germany). The TDS was a Gerstel TDS 3 system (Gerstel GmbH & Co.KG). The Cooled Injection System (CIS) was a Cooled injection system 4 with the controller C505 (Gerstel GmbH & Co.KG). A spectral library, NIST02.L (National Institute of Standards and Technology, Gaithersburg, Maryland, USA), was used to reference and identify molecules,

Three types of thermal extraction tubes were used and tested during the project. Carbotrap 300, Tenax TA, and Carboxene/Tenax (all from Gerstel GmbH & Co.KG).

Materials

Table 1: The four types of samples used to obtain the results presented in this report. The samples have been given a simple designation, a short odour description and their origin. Grolman and Shougang are the "codenames" for the synthesizer that created the sample batch. Sandoz is the company that made the tablets.

Sample designation	Sample odour	Sample origin or synthesizer
Tablet1	Strong amine odour	Metformin Sandoz® 500 mg, Vnr 091457, purchased in local drug store.
Metformin-HCL1	Strong amine odour	Shougang, finely ground Metformin powder
DMA-HCl1	Strong amine odour	Grolman, finely ground DMA powder
Tablet2	plastic odour, probably from the bottle. Considered as odourless reference against Tablet1	Metformin Sandoz® 500 mg, Vnr 484928, purchased in local pharmacy.

A solution of 2 mol/L Dimethylamine (DMA) in methanol, Tributylamine (TBA), Ethyl dimethylamine (EDMA) and Triethylamine (TEA) were bought from Sigma Aldrich (Darmstadt, Germany).

Methods

Two principal methods are used in this project, the TDS-TE method where both the Thermal Extractor (TE) and thermal desorption system (TDS) are used in series, called TDS-TE. The other method is the multipurpose sampler (MHI) that prepares and injects the headspace sample automatically. The results are presented in Total ion current (TIC) or extracted ion chromatogram, where a single ion is isolated and counted.

TDS-TE

The material or sample that was to be analysed was first weighed using a balance with two decimal points of accuracy for grams. The sample was moved using a metallic spoon into the large TE tube and subsequently weighed. The TE tube used was then plugged with a large piece of glass wool at one end, a few centimetres from the inlet. When the appropriate amount had been transferred into the tube and weighed, two plastic Erlenmeyer flask stoppers covered in plastic wrapper was used to plug each end to minimize air exchange with the exterior. An appropriate filtered desorption tube was selected and inserted into the TE device along with the sample filled TE tube. The thermal extractor was then started with a

desired program. After TE completion the filtered desorption tube was transferred to the TDS loader and an appropriate method started. After the analysis was complete the desorption tube was conditioned using a conditioning method that reached higher TDS temperatures.

The TDS analysis method used "171107 TDS_230C_15min.M": GC settings, oven heating ramps was started at 35°C, held for 1.5 minutes then ramp 1 was started at 4°C/min and ended at 135°C. Ramp 2 had a rate of 40°C/min and ended at 230°C, this adds up to 33.38 min total runtime. The inlet flow was set to 16.6 ml/min with a pressure of 17.33 psi. The CIS was chilled to -30°C during pre-concentration and a heating ramp of 12°C/min to 230°C and held for 1 min for injection. TDS settings, the TDS initial temperature was set to 80°C, this was then ramped up by 55°C/min to 230°C and held for 15 min. The MS scan range was between 30-120 m/z.

The conditioning method used to condition the desorption tubes between each use is performed as a normal sample desorption by placing the tube in the TDS loader and starting a method with the following settings. GC settings, the oven heating ramps was started at 35°C, held for 1.5 minutes then ramp 1 was started at 110°C/min and ended at 230°C and held for 4 minutes. The total run time is 5 minutes. The inlet flow was set to 16.6 ml/min with a pressure of 17.33 psi. CIS settings, the CIS was not chilled but set to 60°C during pre-concentration and a heating ramp of 12°C/min to 230°C and held for 1 min for injection. TDS settings, the TDS initial temperature was set to 80°C, this was then ramped up by 55°C/min to 320°C and held for 15 minutes. The MS scan range was between 30-120 m/z.

Multiple Headspace Injection (MHI)

The sample was weighed exactly and transferred into a 20 ml GC vial. The vials were then sealed with a manual crimper and placed on MPS vial holder before the experiment started.

The method "171019 HS 26min heating, 25 ext 50 inc 30scan.M" is the standard MHI analysis method used and all settings that can are the same as in the TDS method "171107 TDS_230C_15min.M".

Method "171019 HS 26min heating, 25 ext 50 inc 30scan.M". GC settings, the oven heating ramps was started at 35°C, held for 1.5 minutes then ramp 1 was started at 4°C/min and ended at 135°C. Ramp 2 had a rate of 40°C/min and ended at 230°C, this adds up to a total runtime of 33.38min. The inlet flow was set to 16.6 ml/min with a pressure of 17.33 psi. CIS settings, the CIS was chilled to -30°C during pre-concentration and a heating ramp of 12°C/min to 230°C and held for 1 min for injection. MPS settings, the MPS syringe and incubator temperatures was set to 80°C and 50°C respectively. The syringe injection volume was set to 2400µl with a total of 25 injections. The setting "pressurization" was also used which filled the sample vial with air between each extraction. The MS scan range was between 30-120 m/z.

An alternate MHI analysis method that was used before the latest version was created is "171019 HS 26min heating, 25 ext 50C incubation.M". This method has set the lower MS scan range to 12 instead of 30 and thereby have not removed the large water peak in the chromatogram. The other settings are the same.

Data treatment

Data treatment were performed with Agilent's software MSD Chemstation. This displays the chromatograms from both MS and FID detection. The MS is however almost exclusively used as it allows for identification of amines using library searches for mass spectra using the library NIST02.L. Once amines have been identified the peak areas of each amines will be recorded by manual integration for both total ion chromatogram and extracted ion chromatogram for each identified amine.

Results and discussion

Response of amine directly added into TDS tubes

The TDS equipment was initially tested using amines in standard solution mixtures. The amines were, dimethylamine (DMA), Ethyl dimethylamine (EDMA), Triethylamine (TEA) and Tributylamine (TBA). These solutions were analysed to obtain an estimate of the limit of detection and the retention time for amines that were expected to be found in the samples. The limit of detection of the following experiment with the planned samples might be lower as the split between FID and MS after the GC-column was adjusted due to these results. The MS capillary was shortened to increase the gas flow going to the MS compared to the FID. Removing flow to the FID was logistically unfeasible since the GC-MS device for other analyses too.

The results of these standard tests for each amine can be seen in table 2-5.

Table 2: The peak area and amount of DMA added to the 1µL standard solution that was analysed. The standard was diluted into parts of the original standard to create a standard curve.

Samples (dilution %)	Weight (µg)	Peak Area (10^6)
Standard 100%	18	19.8
Standard 60%	10.8	21.1
Standard 30%	5.41	10.7
Standard 15%	2.70	10.3
Standard 3%	0.54	6.57
Standard 1.5%	0.27	4.27
Standard 0.6%	0.11	not visible

Table 3: The peak area and amount of EDMA added to the 1µL standard solution that was analysed. The standard was diluted into parts of the original standard to create a standard curve.

EDMA in standard	Weight (µg)	Peak Area (10^6)
Standard 100%	14.0	56.0
Standard 60%	8.40	34.0
Standard 30%	4.20	21.0
Standard 15%	2.10	12.4
Standard 3%	0.420	4.93
Standard 1.5%	0.210	5.73
Standard 0.6%	0.0840	1.29

Table 4: The peak area and amount of TEA added to the 1µL standard solution that was analysed. The standard was diluted into parts of the original standard to create a standard curve.

TEA in standard	Weight (µg)	Peak Area (10^6)
Standard 100%	14.5	325
Standard 60%	8.71	212
Standard 30%	4.35	100
Standard 15%	2.18	76.0
Standard 3%	0.435	38.6
Standard 1.5%	0.218	31.1
Standard 0.6%	0.0871	6.20

Table 5: The peak area and amount of TBA added to the 1µL standard solution that was analysed. The standard was diluted into parts of the original standard to create a standard curve.

TBA in standard	Weight (µg)	Peak Area (10^6)
Standard 100%	31.2	443
Standard 60%	18.7	220
Standard 30%	9.36	70.1
Standard 15%	4.68	27.1
Standard 3%	0.936	5.10
Standard 1.5%	0.468	3.91
Standard 0.6%	0.187	not detectable

Linearity of the response against added amount amines were plotted into four standard curves. The four-different amine standard curves are shown in figure 4-7.

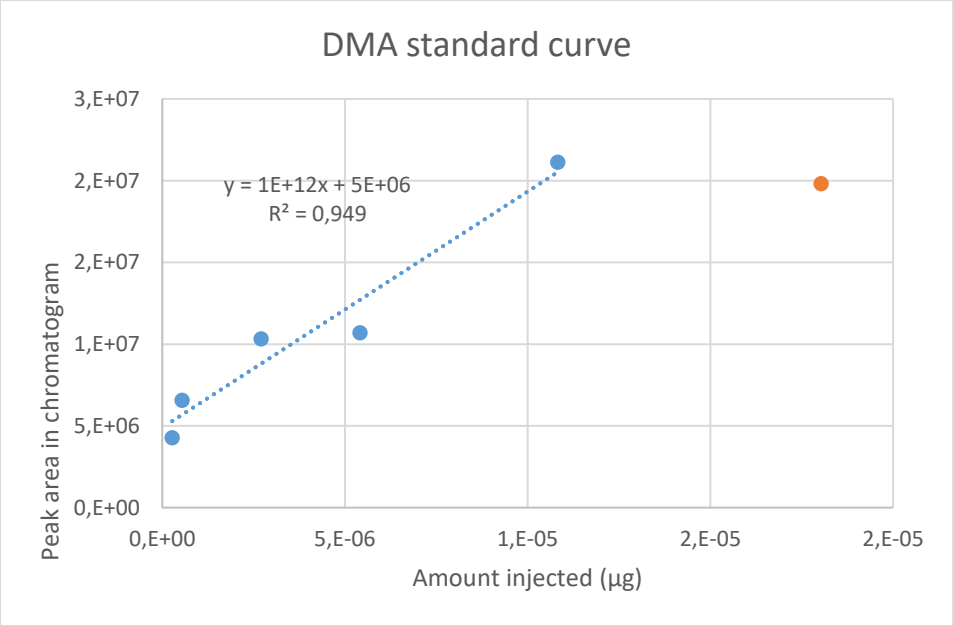

Figure 4: Standard curve for DMA where the x-axis shows amount (µg) of amine inserted into the TDS for analysis. The y-axis shows the peak area for that specific amine in the chromatograms. The orange data point is the last point and is suspected to be outside the linear range. No further experiment has been done to reveal the reason due to limited time in this study.

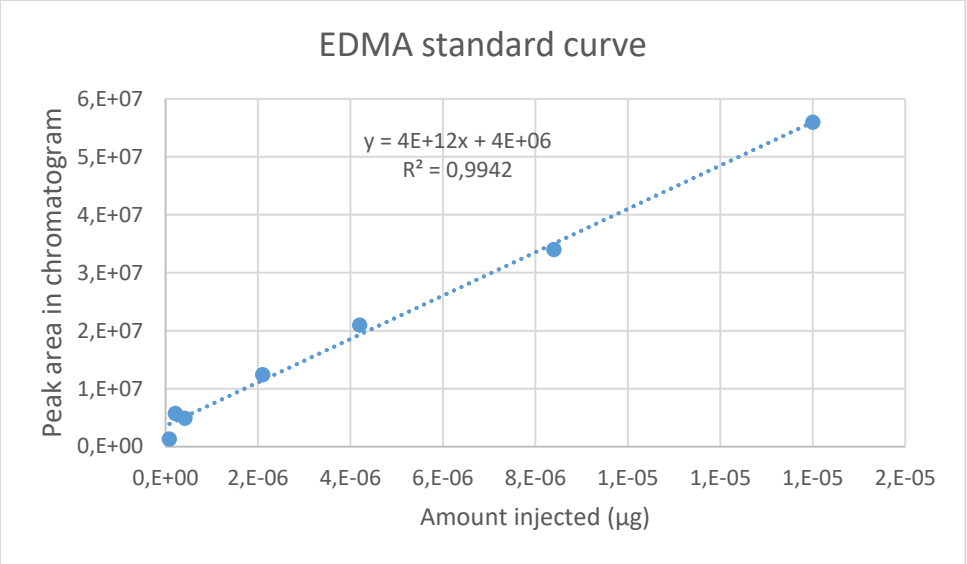

Figure 5: Standard curve for EDMA where the x-axis shows amount (µg) of amine in 1 µl of solution, which is also the amount inserted into the TDS for analysis. The y-axis shows the peak area for that specific amine in the chromatograms.

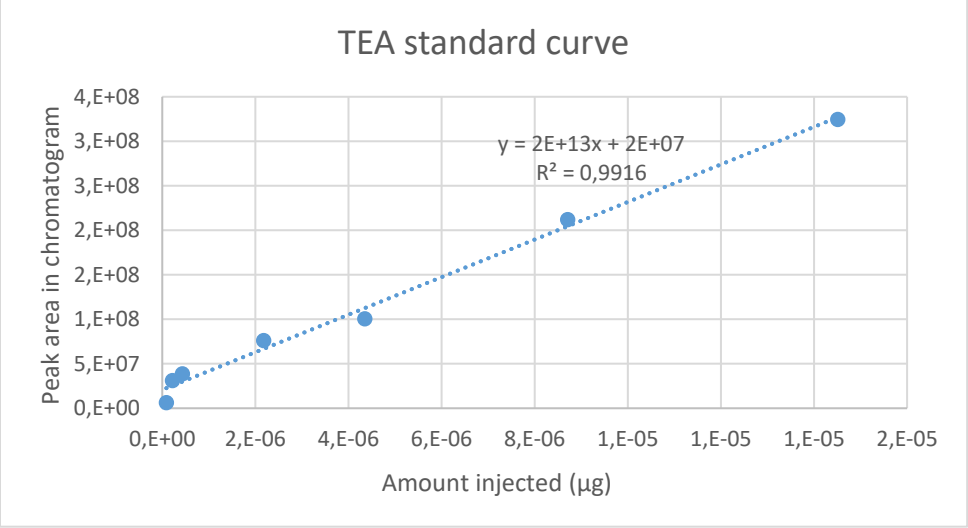

Figure 6: Standard curve for TEA where the x-axis shows amount (µg) of amine in 1 µl of solution, which is also the amount inserted into the TDS for analysis. The y-axis shows the peak area for that specific amine in the chromatograms.

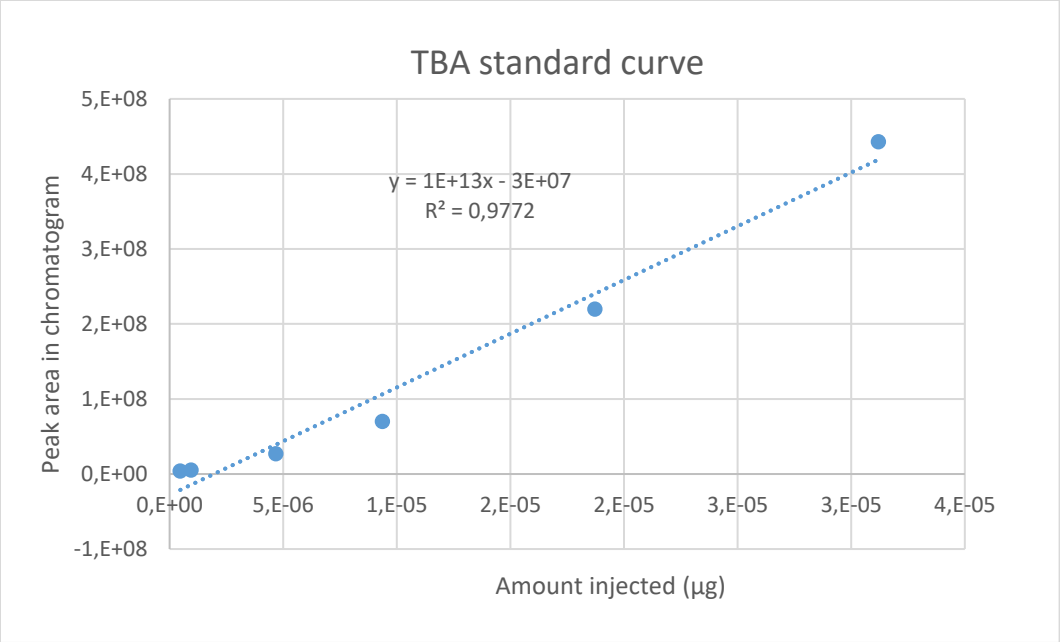

Figure 7: Standard curve for TBA where the x-axis shows amount (µg) of amine in 1 µl of solution, which is also the amount inserted into the TDS for analysis. The y-axis shows the peak area for that specific amine in the chromatograms.

The results above, show a linear response of the amines in the studied range. The sample amount provides a predictable response following a linear equation in this range. The changes made to the split ratio between the FID and MS detection units ensures that roughly half of the sample after the GC column enters the MS and FID respectively. The DMA curve also shows that at higher concentrations of amine the curve is no longer linear, this might be due to saturation. Further study is needed to reveal reason for the result. Due to limited time, no further experiment has been done in current study.

Choice of TDS-TE Tube

Different adsorption tubes could be used in the TDS-TE for GC analysis of volatile samples to collect the desired analytes. There are several types available, optimized for different molecules based on characteristics such as molecule size in terms of carbon chain length, hydrophobicity etc. A series of test has been performed to find a proper tube for amines used in this study. The results in the form of peak area for relevant amines for the comparison between the tubes “Carbotrap 300” (T1, T2), “Carboxene/Tenax” (T3) and “Tenax TA” (T5) using 15 tablet 1, run for 90minutes in the TE device at 50°C then analysed using method “171107 TDS 230C_15min.M” can be seen in table 6 and 7. Table 6 shows the area from the TIC (Total ion current) chromatogram and table 7 from extracted ion chromatograms for the specific parent ion of the molecule.

Table 6: Peak area (TIC) for each amine detected for the tubes T1/T2, T3, T5.

TIC	DMA	TMA
Carbotrap300 (T1, T2)	2.49E+07	2.99E+06
Carboxene/Tenax (T3)	2.09E+07	3.11E+06
Tenax TA (T5)	3.56E+06	4.08E+05

Table 7: Peak area for each amine detected from the extracted ion chromatograms for tubes T1/T2, T3, T5.

Extracted ion	DMA (45 m/z)	TMA (59 m/z)
Carbotrap300 (T1, T2)	7.08E+06	7.00E+05
Carboxene/Tenax (T3)	5.76E+06	7.42E+05
Tenax TA (T5)	1.38E+06	6.43E+04

The results seen in table 6 and 7 show that T5 (Tenax TA) have a lower efficiency in trapping amines. The other two materials show similar adsorptive qualities, however Carbotrap 300 would be preferred for DMA adsorption and since it is one of the synthesis components for the metformin it is suspected to be the reason for the amine smell. The Carbotrap300 (T1, T2) was chosen as the adsorption tube and used in the TDS-MHI comparisons later.

MHI number of extractions

The Multipurpose sampler equipment (MPS) is capable of extracting a number of times from the same sample via by compensating for the withdrawn sample by adding air between each extraction gain of response as an effect to increased number of extractions was investigated in the metformin samples tablets1 and tablets2 but also in the Metformin-HCl1. These samples were all extracted 1, 4 and 25 times. The overall analyses time for these were 50, 55 and 120 minutes respectively, where one extraction takes approximately a minute. The relevant chromatograms, 32-40, can be viewed in appendix A.

Table 8 details the peak area for each detected amine using TIC and specific extracted ions for relevant amines, for Metformin-HCl1 for each sample in table 8.

Table 8: Peak area for the amines detected in 2grams of Metformin-HCl1 using 1, 4 or 25 extractions in the MPS equipment. The extracted ion peak area is also shown in the latter 3 rows.

MHI (TIC)	DMA	TMA
1 extraction (2g Metformin-HCl1)	Not detectable	1.83E+05
4 extractions (2g Metformin-HCl1)	4.52E+05	5.22E+05
25 extractions (2g Metformin-HCl1)	1.97E+06	2.16E+06

MHI (Extracted ion area)	DMA (45 m/z)	TMA (59 m/z)
1 extraction (2g Metformin-HCl1)	Not detectable	1.09E+04
4 extractions (2g Metformin-HCl1)	1.02E+05	9.33E+04
25 extractions (2g Metformin-HCl1)	4.90E+05	5.03E+05

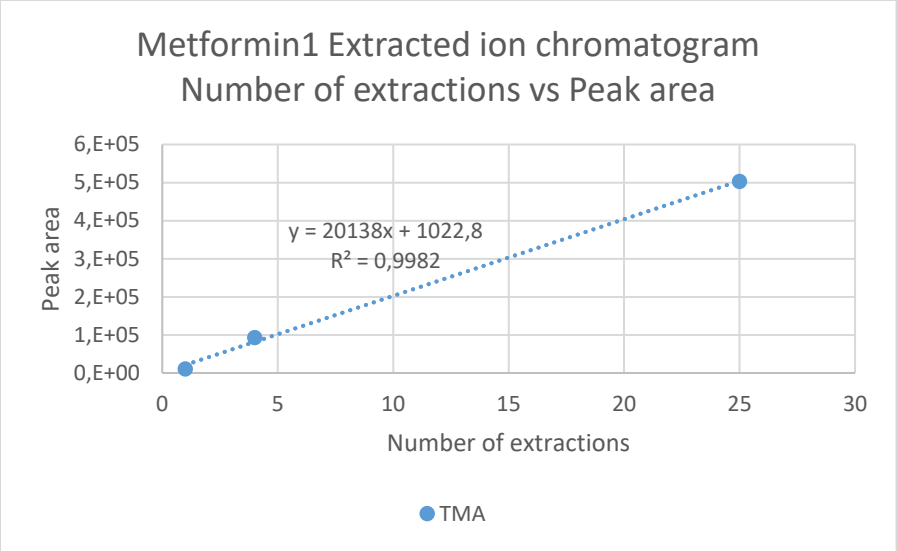

Figure 8: Metformin-HCl1 extracted ion chromatogram peak area against number of extractions for the amine TMA. A linear relationship can be observed between peak area and the number of extractions.

Table 9 and 10 shows the peak area (TIC) and specific extracted ions for tablets1 for each sample and amine found. The method used was "171019 HS 26min heating, 25 ext 50C inc 30scan.M".

Table 9: Peak area (TIC) for the amines detected in 15 tablets1 using 1, 4 or 25 extractions in the MHI equipment

MHI (TIC)	TMA	CDA
1 extraction (15 tablets1)	4.97E+05	Not detectable
4 extractions (15 tablets1)	4.64E+06	8.33E+04
25 extractions (15 tablets1)	3.72E+07	1.12E+06

Table 10: Peak area (Extracted ion area) for the amines detected in 15 tablets1 using 1, 4 or 25 extractions in the MHI equipment.

MHI (Extracted ion area)	TMA 59 m/z	CDA 79 m/z
1 extraction (15 tablets1)	9.83E+04	Not detectable
4 extractions (15 tablets1)	9.35E+05	8.54E+03
25 extractions (15 tablets1)	7.40E+06	1.35E+05

Table 11 details the peak area with and without specific extracted ions for tablets2 for each sample. The method used was "171019 HS 26min heating, 25 ext 50C inc 30scan.M"

Table 11: Peak area for the amines detected in 15 tablets2 using 1, 4 or 25 extractions in the MPS equipment. The extracted ion peak area is also shown in the latter 3 rows.

MHI (TIC)	TMA (59 m/z)	BAD (101 m/z)	CDA (79m/z)
1 extraction (15 tablets2)	1.62E+05	1.70E+05	Not detectable
4 extractions (15 tablets2)	-	1.94E+06	Not visible
25 extractions (15 tablets2)	1.81E+07	5.29E+07	4.72E+05
MHI (Extracted ion area)			
1 extraction (15 tablets2)	2.44E+04	6.82E+02	Not visible
4 extractions (15 tablets2)	9.35E+05	1.11E+05	Not visible
25 extractions (15 tablets2)	3.75E+06	3.11E+06	6.53E+04

As expected the higher number of extractions produce a significantly higher peak. with a linear relationship, as can be seen in figure 9.

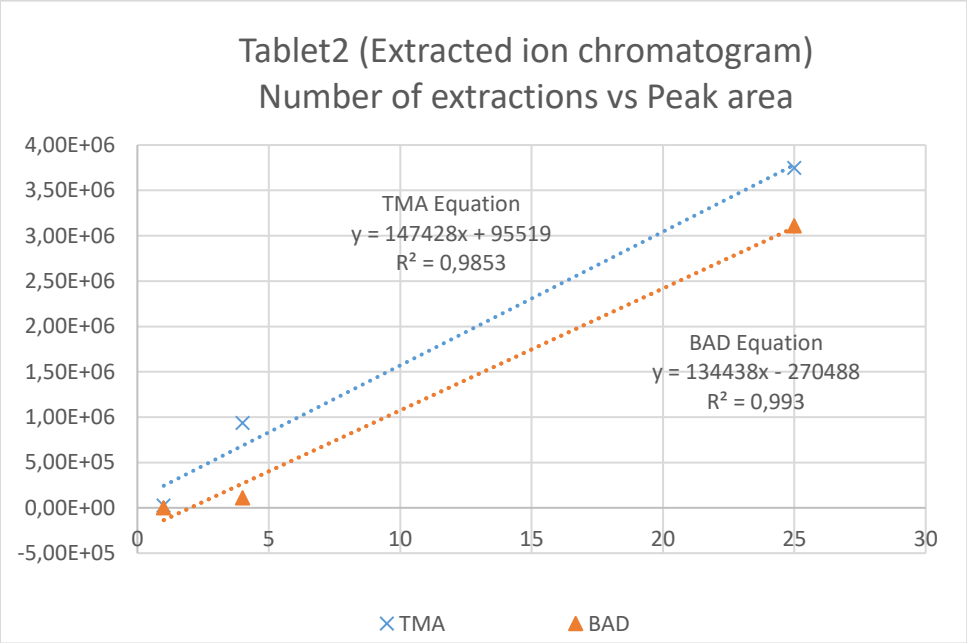

Figure 9: Tablet2 extracted ion chromatogram peak area plotted against number of extractions for the amines TMA and BAD. Where the upper blue dots are for TMA and the lower red dots for BAD.

Number of extractions while using the MHI method is a trade-off between analyses time and sensitivity. It can easily be seen that more extractions give higher sensitivity and it does scale linearly up to 25 extraction, as seen in figure 8 and 9. The efficiency of each consecutive extraction is expected to be lower as the amine concentration in the air is lower after each extraction, the amine concentration does recover quickly as the plot is linear but only up to a point as the amines will sooner or later be exhausted. Up to 25 extractions, there has not been any indication that a drop in the extracted amount of the analytes per extraction. It is however quite clear from the results in section “MHI number of extractions” that 4 extractions are inadequate, more extractions are required, and at least 25 extractions are preferable, especially the fact that some amines were not detectable.

TDS-TE and MHI comparisons

The general experimental setup and waiting time between the samples presented in this section is the same for all MHI and TDS analyses respectively. The first two MHI runs are performed in direct series then a 4 hour wait time is implemented after which run 3 and 4 is conducted. TDS-TE runs 1 and 2 are performed as close as possible in a series with approximately a 30minute wait time between every TE run as the GC-MS analysis is more time demanding. The third 15-hour run is however delayed for 2 hours as only two Carbotrap 300 desorption tubes are available and conditioning a tube is required before thermal extraction can be started.

Tablet 1 comparison

Tablet 1 were tested in both the TDS-TE and the MPS equipment with as close as similar as possible methods. The method used for the MHI analysis is "171019 HS 26min heating, 25 ext 50C incubation.M" and for TDS "171107 TDS_230C _15min.M".

The below figure 10 shows the chromatogram for MHI Tablets1 (run1). Upper chromatogram shows DMA and TMA peaks zoomed in with a picture in picture of the whole chromatogram. The lower chromatogram is zoomed in on the CDA peak. Subsequent runs chromatograms can be viewed in Appendix A, figure 17-21.

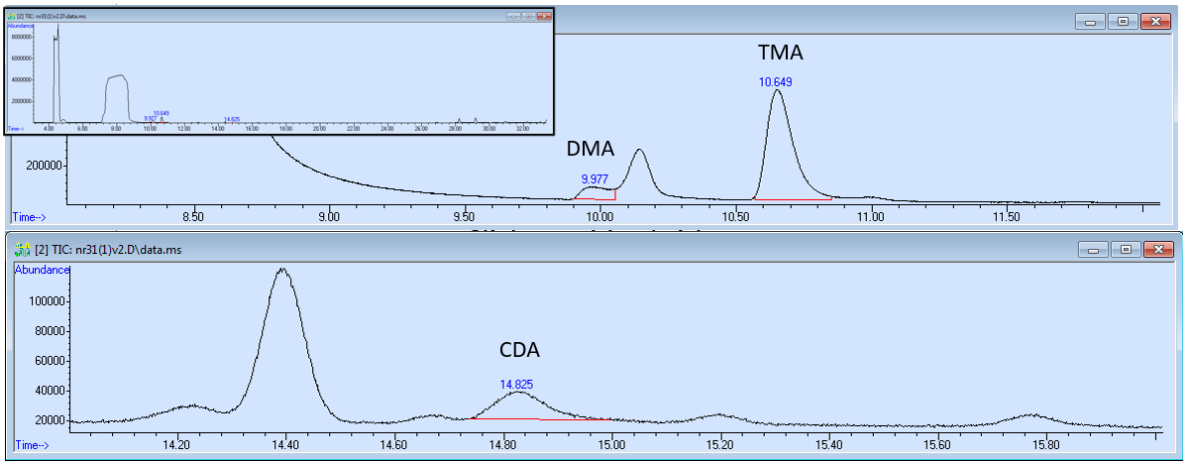

Figure 10: The chromatogram for 15 tablets1 (run1) using MHI with first the zoom in on DMA and TMA peaks with the whole chromatogram as a picture in picture. The second chromatogram is a zoom in on the CDA peak.

Figure 11 show the chromatogram for TDS Tablets1 (run1) with a zoom in on the DMA and TMA peaks and a picture in picture of the whole chromatogram.

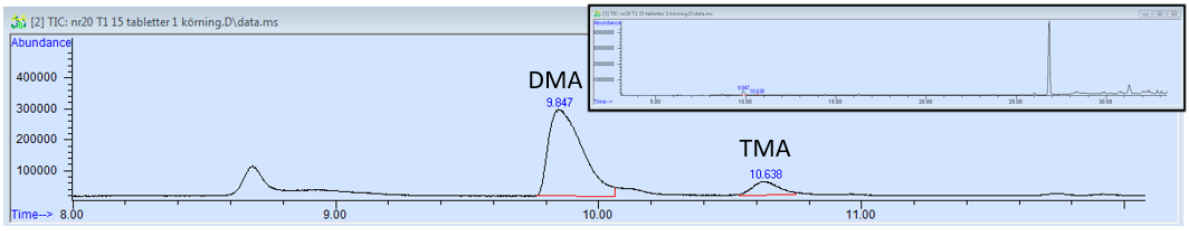

Figure 11: The chromatogram for 15 tablets1 (run1) in using TDS-TE with the zoom in on DMA and TMA peaks with the whole chromatogram as a picture in picture.

The results in terms of peak area for each amine for the chromatograms can be seen in table 12 and 13 for the extracted ion peak are.

Table 12: The peak areas for the amines detected for runs of tablets1. All MHI runs was extracted 25 times and analysed with the method "171019 HS 26min heating, 25 ext 50C incubation.M". All TDS runs were analysed with the method "171107 TDS_230C_15min.M"

MHI (TIC)	DMA	TMA	CDA
15 tablets1 (run 1)	4.50E+06	3.72E+07	1.12E+06
15 tablets1 (run 2)	Not visible	2.45E+07	7.18E+05
15 tablets1 (run 3)	Not visible	1.73E+07	6.94E+05
15 tablets1 (run 4)	Not visible	1.32E+07	3.18E+05
TDS-TE (TIC)			
15 tablets1 (run1) (90min)	2.49E+07	2.99E+06	Not visible
15 tablets1 (run2) (90min)	1.22E+07	4.80E+05	Not visible
15 tablets1 (run3) (900min)	2.47E+07	2.81E+06	Not visible

Table 13; The extracted ion peak areas for the amines detected for runs of tablets1. All MHI runs was extracted 25 times and analysed with the method "171019 HS 26min heating, 25 ext 50C incubation.M". All TDS runs were analysed with the method "171107 TDS_230C_15min.M"

MHI (Extracted ion area)	DMA (45 m/z)	TMA (59 m/z)	CDA (79 m/z)
15 tablets1 (run 1)	2.00E+06	7.40E+06	1.35E+05
15 tablets1 (run 2)	1.69E+06	5.34E+06	7.82E+04
15 tablets1 (run 3)	1.08E+06	3.70E+06	8.05E+04
15 tablets1 (run 4)	7.36E+05	2.73E+06	6.16E+04
TDS-TE (Extracted ion area)			
15 tablets1 (run1) (90min)	7.08E+06	7.00E+05	Not visible
15 tablets1 (run2) (90min)	3.60E+06	1.33E+05	Not visible
15 tablets1 (run3) (900min)	6.97E+06	6.08E+05	Not visible

Tablet1 comparisons between the TDS-TE and MHI methods, show that TDS-TE is unable to detect CDA in the tablets. The MHI do however have a significantly higher proficiency at detecting TMA both on the TIC chromatogram and the extracted ion chromatogram. The chromatograms are of comparable quality in terms of peak visibility. This suggests that the MHI method is in this case the preferred method as it is both able to detect two amines with higher sensitivity and is a considerably less labour-intensive technique that is the faster of the two.

Metformin-HCl 1 comparison

Metformin-HCl 1, the type that smells strongly of amines, were tested using both the TDS-TE and the MHI methods, which are made as similar as possible. The method used for the MHI analysis is "171019 HS 26min heating, 25 ext 50C inc 30scan.M" and for TDS "171107 TDS_230C _15min.M".

In figure 12, the chromatogram for MHI Metformin-HCl1 (run1) is shown. The main picture shows the DMA and TMA peaks zoomed in and the inserted picture gives a view of the whole chromatogram. Subsequent runs chromatograms can be viewed in Appendix A, figure 20-24.

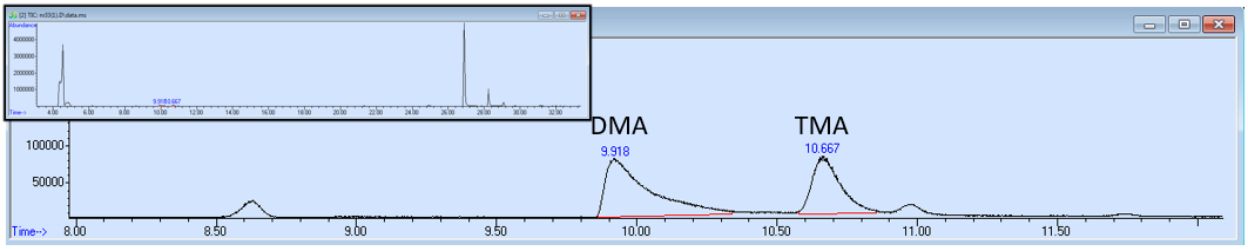

Figure 12: The chromatogram for 2 grams of Metformin-HCl1 (run1) using MHI with the zoom in on DMA and TMA peaks with the whole chromatogram as a picture in picture. Analysed using "171019 HS 26min heating, 25 ext 50C inc 30scan.M".

Figure 13 show the chromatogram for TDS Metformin-HCl1 (run1) with a zoom in on the DMA and TMA peaks and a picture in picture of the whole chromatogram.

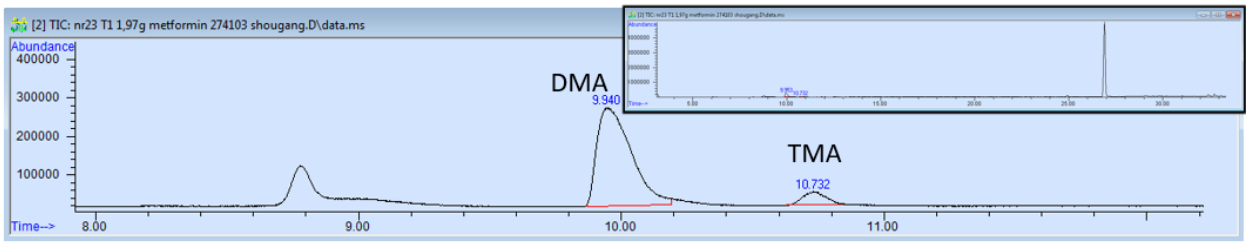

Figure 13: The chromatogram for 2 grams of Metformin-HCl1 (run1) in TDS with the zoom in on DMA and TMA peaks with the whole chromatogram as a picture in picture. Analysed using "171019 HS 26min heating, 25 ext 50C inc 30scan.M".

The results in terms of peak area and extracted ion peak area corresponding to the 2 grams of the metformin that was analysed using both methods can be seen in table 14 and 15 respectively.

Table 14: The peak areas for the amines detected for runs of Metformin-HCl 1. All MHI runs was extracted 25 times and analysed with the method "171019 HS 26min heating, 25 ext 50C incubation.M". All TDS runs were analysed with the method "171107 TDS_230C_15min.M".

MHI (TIC)	DMA	TMA	MDATM
2g Metformin-HCl (run 1)	8,42E+06	5,06E+06	Not visible
2g Metformin-HCl (run 2)	4,13E+06	2,07E+06	Not visible
2g Metformin-HCl (run 3)	2,37E+06	1,17E+06	Not visible
2g Metformin-HCl (run 4)	1,82E+06	1,06E+06	Not visible
TDS-TE (TIC)			
2g Metformin-HCl (run1) (90min)	2,42E+07	2,15E+06	Not visible
2g Metformin-HCl (run2) (90min)	2,81E+07	2,48E+05	Not visible
2g Metformin-HCl (run3) (900min)	8,60E+07	1,28E+06	5,12E+07

Table 15: The extracted ion peak areas for the amines detected for runs of tablets1. All MHI runs was extracted 25 times and analysed with the method "171019 HS 26min heating, 25 ext 50C incubation.M". All TDS runs were analysed with the method "171107 TDS_230C_15min.M"

MHI (Extracted ion area)	DMA (45 m/z)	TMA (59 m/z)	MDATM
2g Metformin-HCl (run 1)	1,97E+06	9,76E+05	Not visible
2g Metformin-HCl (run 2)	9,97E+05	4,65E+05	Not visible
2g Metformin-HCl (run 3)	6,18E+05	2,50E+05	Not visible
2g Metformin-HCl (run 4)	4,22E+05	2,25E+05	Not visible
TDS-TE (Extracted ion area)			
2g Metformin-HCl (run1) (90min)	6,13E+06	4,97E+05	Not visible
2g Metformin-HCl (run2) (90min)	7,54E+06	5,66E+04	Not visible
2g Metformin-HCl (run3) (900min)	2,43E+07	3,23E+05	1,84E+06

Metformin-HCl1 was analysed using both MHI and TDS-TE methods and in the results section table 14 and 15 show that TDS-TE have the capability of detecting MDATM as a result of the longer adsorption times in the TE device. DMA also shows a higher concentration using TDS-TE compared to MHI analysis. TMA concentration is however generally higher in the MHI analysis. These results suggest that TDS-TE is the preferred method for sensitivity and ability to detect amines in the Metformin-HCl sample, while having a longer analyses time as primary drawback.

The sample Metformin-HCl1 was also used to run a very long TE run for 64,5 hours at 50 °C. The method used for the TDS run was "171107 TDS_230C _15min.M". The chromatogram is shown below in figure 14.

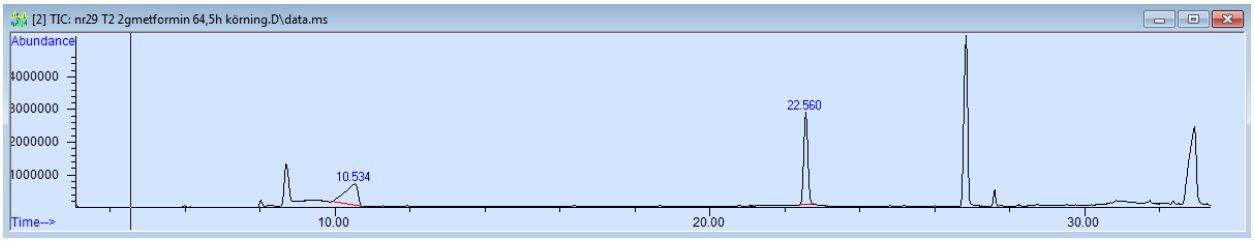

Figure 14: The extra-long 64,5h TE run of Metformin-HCl1, analysed using method "171107 TDS_230C _15min.M". The first peak (blue number and red bottom line) is a very broad and large DMA peak and the second is MDATM. Analysed using "171019 HS 26min heating, 25 ext 50C inc 30scan.M".

The peak area results for both the peak area and extracted ion peak area for all three amines can be seen in table 16.

Table 16: Peak area and extracted ion chromatogram peak area for DMA, TMA and MDATM for the 64,5-hour long TE run using Metformin-HCl1. The method used was "171107 TDS_230C_15min.M"

TDS-TE analysis	DMA (45 m/z)	TMA (59 m/z)	MDATM (102 m/z)
Peak area (TIC)	1,51E+08	(inside DMA peak)	2,13E+08
Peak area (extracted ion chromatogram)	4,55E+07	1,18E+06	9,73E+06

DMA-HCl1 comparison

The DMA-HCl salt powder were tested using both the TDS-TE and the MHI methods, which are as similar as possible. The method used for the MHI analysis is "171019 HS 26min heating, 25 ext 50C inc

30scan.M" and for TDS "171107 TDS_230C _15min.M", which settings can be viewed in the methods section above.

The below figure 15 shows the chromatogram for MHI DMA-HCl1 (run1). The picture shows DMA and TMA peaks zoomed in with a picture in picture of the whole chromatogram. Subsequent runs chromatograms can be viewed in Appendix A.

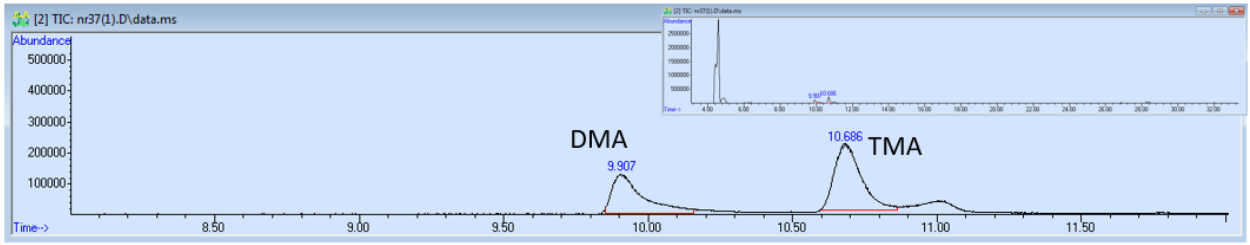

Figure 15: Chromatogram of MHI DMA-HCL1 (run1) zoomed in on the DMA and TMA peaks, with a picture in picture of the whole chromatogram. Analysed using method "171019 HS 26min heating, 25 ext 50C inc 30scan.M"

Figure 16 show the chromatogram for TDS DMA-HCl1 (run1) with a zoom in on the DMA and TMA peaks and a picture in picture of the whole chromatogram. The second picture is a zoom in on the peaks for the amines, DMPA, MDATM, TEA and NDMA in that order.

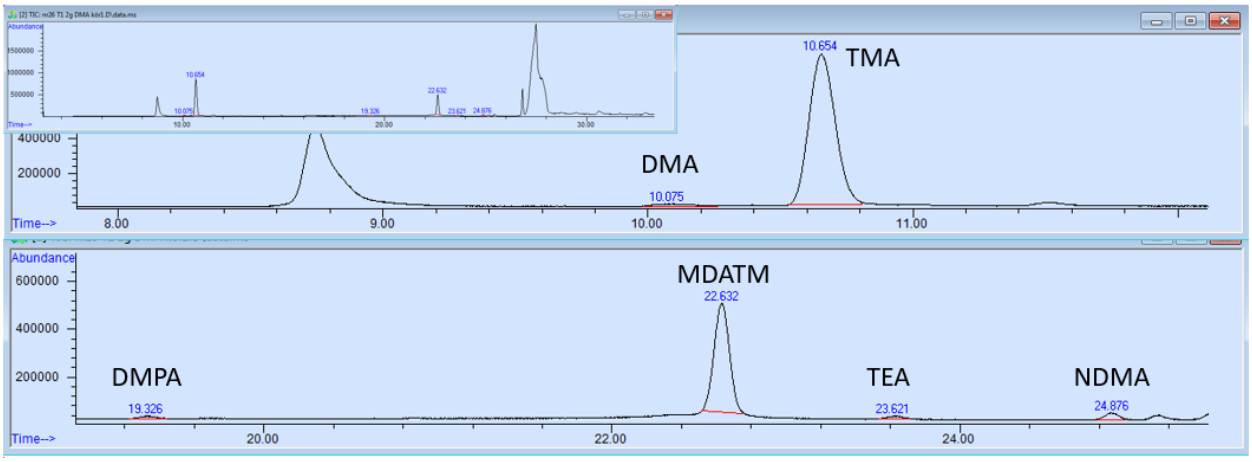

Figure 16: Chromatogram of MHI DMA-HCL1 (run1) zoomed in on the DMA and TMA peaks, with a picture in picture of the whole chromatogram. The second picture is zoomed in on the DMPA, MDATM, TEA and NDMA peaks. Analysed using "171019 HS 26min heating, 25 ext 50C inc 30scan.M".

The results in terms of peak area and extracted ion peak area pertaining the 2 grams of DMA-HCl that was analysed using both methods can be seen in table 17 and 18 respectively.

Table 17: The peak areas for the amines detected for runs of 2grams of DMA-HCl1. All MHI runs was extracted 25 times and analysed with the method "171019 HS 26min heating, 25 ext 50C inc 30scan.M". The TDS-TE analyses were desorbed in the TE for 90minutes except for the third run which is desorbed for 900minutes. All TDS runs were analysed with the method "171107 TDS_230C_15min.M".

MHI	DMA	TMA	DMPA	MDATM	TEA	NDMA
2g DMA-HCl1 (run 1)	8,42E+06	5,06E+06	Not visible	Not visible	Not visible	2,87E+05
2g DMA-HCl1 (run 2)	4,13E+06	2,07E+06	Not visible	Not visible	Not visible	1,78E+05
2g DMA-HCl1 (run 3)	2,37E+06	1,17E+06	Not visible	Not visible	Not visible	1,23E+05
2g DMA-HCl1 (run 4)	1,82E+06	1,06E+06	Not visible	Not visible	Not visible	1,35E+05
TDS-TE						
2g DMA-HCl1 (run1) (90min)	2,11E+06	5,89E+07	8,09E+05	2,98E+07	9,14E+05	1,65E+06
2g DMA-HCl1 (run2) (90min)	5,98E+06	3,34E+07	8,33E+05	1,35E+07	1,12E+06	<LOD
2g DMA-HCl1 (run3) (900min)	4,04E+07	1,99E+08	4,51E+06	2,01E+08	4,68E+06	5,24E+05

Table 18: The extracted ion peak areas for the amines detected for runs of 2grams of DMA-HCl1. All MHI runs was extracted 25 times and analysed with the method "171019 HS 26min heating, 25 ext 50C inc 30scan.M". The TDS-TE analyses were desorbed in the TE for 90minutes except for the third run which is desorbed for 900minutes. All TDS runs were analysed with the method "171107 TDS_230C_15min.M".

MHI (Extracted ion area)	DMA	TMA	DMPA	MDATM	TEA	NDMA
2g DMA-HCl1 (run 1)	8,42E+06	5,06E+06	Not visible	Not visible	Not visible	2,87E+05
2g DMA-HCl1 (run 2)	4,13E+06	2,07E+06	Not visible	Not visible	Not visible	1,78E+05
2g DMA-HCl1 (run 3)	2,37E+06	1,17E+06	Not visible	Not visible	Not visible	1,23E+05
2g DMA-HCl1 (run 4)	1,82E+06	1,06E+06	Not visible	Not visible	Not visible	1,35E+05
TDS-TE (Extracted ion area)						
2g DMA-HCl1 (run1) (90min)	2,11E+06	5,89E+07	8,09E+05	2,98E+07	9,14E+05	1,65E+06
2g DMA-HCl1 (run2) (90min)	5,98E+06	3,34E+07	8,33E+05	1,35E+07	1,12E+06	<LOD
2g DMA-HCl1 (run3) (900min)	4,04E+07	1,99E+08	4,51E+06	2,01E+08	4,68E+06	5,24E+05

DMA-HCl1 comparison show that there is no DMPA, MDATM or TEA detected at all in any of the MHI analyses. The reason for this is unknown but it might be due to the fact that all these amines are in gaseous form heavier than air and therefore do not rise to the top in the MPS-vials used. The auto sampler needle used to extract the headspace sample only penetrates a short distance and therefore do not retrieve any DMPA, MDATM or TEA that are situated at the bottom of the vial. The vial with the sample is heated to 50°C and agitated between extraction, the results suggest that this might not be sufficient for proper mixing. The peak area for the other amines are also higher while using the TDS-TE method. This strongly suggests that the TDS-TE method is preferred to the MHI method in this case.

All three direct comparisons of the MHI and TDS-TE pre-concentration methods for Tablet1, Metformin-HCl1 and DMA1 have differing results that do not point towards one of the two methods having higher sensitivity for a specific amine in either case. However, the inability of the MHI method to detect the

three amines, DMPA, TEA and MDATM is a severe weakness that is more serious than the inability of the TDS-TE method of detecting one amine, namely CDA. The sensitivity is in general better when using the TDS-TE method and it can easily be improved by adsorbing amines in the TE device for a longer duration. However, the effectiveness of longer TE sessions diminish over longer desorption times as can be seen in table 15 where the peak area results for a 64,5 hours TE desorption is only approximately a magnitude higher than the normal 90-minute desorption as seen in table 13 and 14. This can be due to the fact that the total amount of amine in the metformin was depleted or simply that the tube was saturated with amines.

The volume that can be used for analysis is greater for the TDS-TE method, where the 2 grams used can easily be increased at least 3 times which should not make the process significantly less efficient. Rate of gas flow through the TE tube is also variable and can be increased. The same flexibility is not true for the MHI method where the vial faces severe limitations in terms of headspace volume available. If too much sample is added the ratio between the sample volume and the gas-solid interface area, that allow for release of volatiles from the solid, will decrease and therefore decrease the volatile release significantly.

The overall analyses time is also to be considered for both techniques and the TDS-TE method are made up of more factors that are necessary for analysis but not actual analysis steps, which include conditioning of tubes, approximately 50 minutes, TE adsorption, usually 90 minutes but can be longer if required and the desorption in TDS and GC-MS analysis step, approximately 120 minutes. This means that the total analysis time is approximately 260 minutes for the TDS-TE method if one sample is analysed. The MHI method's total analysis time is much shorter at approximately 120 minutes. The preparation steps that include filling vials or tubes with sample takes the same amount of time for both methods.

Chiefly due to these long analysis times, analytical chemistry basics such as performing replicates and quantitative measurements and establishing the limit of detection/quantification of the amines in the samples was not performed. The experiments that are described in the report were prioritised as the focus was to get an overall assessment of which method was better, rather than to develop a final method. Future experiments should perform these experiments to validate the method for use.

The TDS-TE method's more numerous steps also make the methods implementation more labour intensive as the tube must first be conditioned using the TDS and GC-MS. Then amines are adsorbed in the TE device, which is manually operated, and each tube must be individually loaded. The tube must then be moved back to the TDS loader and analysed. The MPS is fully automatic after loading the vial with sample into the MPS device.

Conclusion

In conclusion, both methods are viable for this specific analysis. It can be said that the MHI method is simpler and less time and labour intensive. The TDS-TE method is however more flexible in terms of potential sensitivity, since the TE device can be used to desorb samples for longer durations if necessary. The overall analyses time is variable for both methods, MHI can use more extractions to increase sensitivity at the expense of time of analyses and the same can be said for TDS-TE and the TE desorption time. Overall the TDS-TE method is preferable, for detecting amines discussed in this report, if available but the MHI method is viable if not.

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Appendix

Tablet1 Comparison Chromatograms

Tablet1 MHI chromatograms run 2, 3 and 4. With figure 15 showing chromatogram for run 2

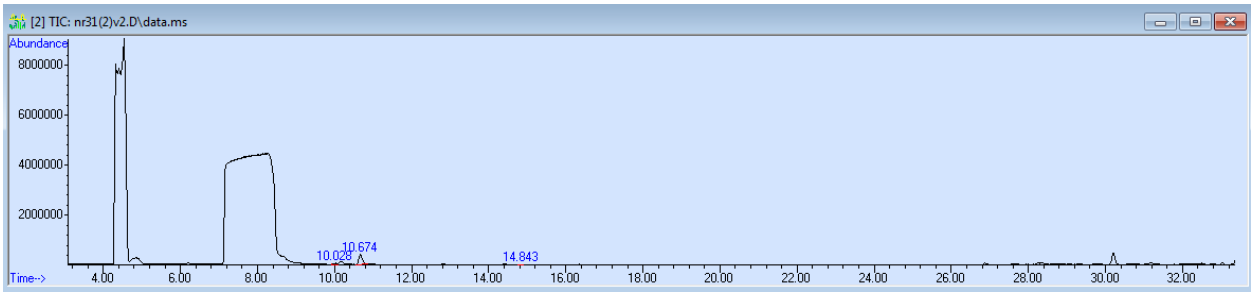

Figure 17: Tablet1 chromatogram MHI (run2) entire chromatogram with DMA, TMA and CDA peaks respectively marked in blue retention times. The two large peaks at 4,5 and 8 minutes are carbon dioxide and water respectively.

Figure 16 shows chromatogram for MHI Metformin-HCl1 run 3

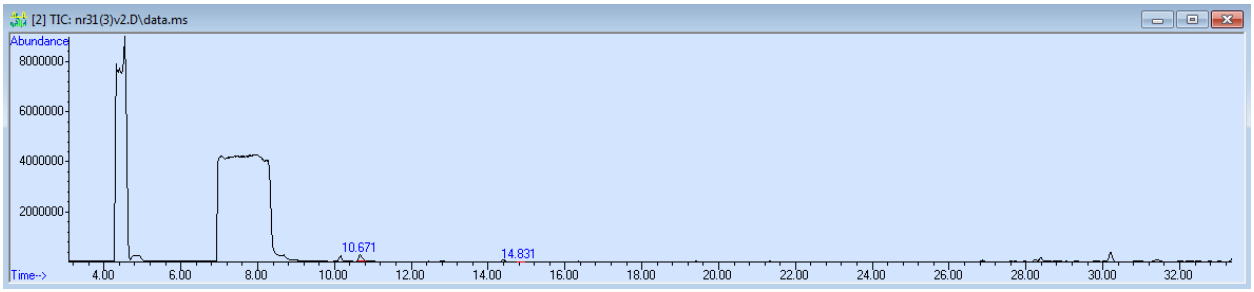

Figure 28: Tablet1 chromatogram MHI (run3) entire chromatogram with TMA and CDA peaks respectively marked in blue retention times. The two large peaks at 4,5 and 8 minutes are carbon dioxide and water respectively.

Figure 17 shows chromatogram for MHI Metformin-HCl1 run 4

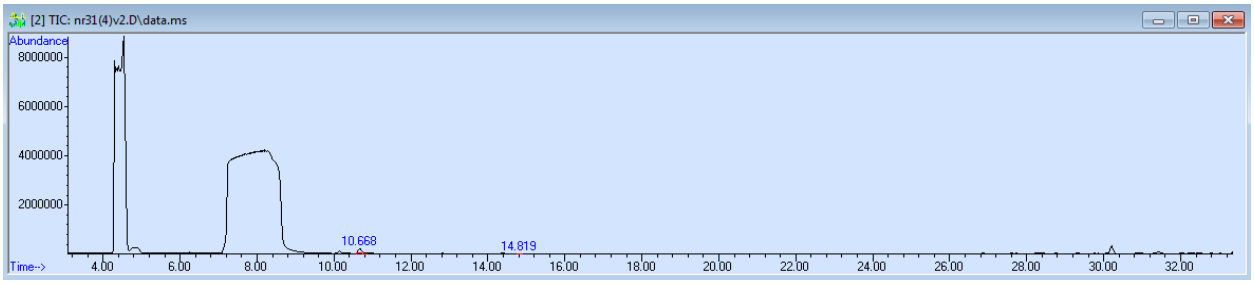

Figure 19: Tablet1 chromatogram MHI (run4) entire chromatogram with TMA and CDA peaks respectively marked in blue retention times. The two large peaks at 4,5 and 8 minutes are carbon dioxide and water respectively.

Tablet1 TDS chromatograms run 2 and 3. With figure 18 showing run 2 with first the entire chromatogram followed by a zoom in on the DMA and TMA peaks.

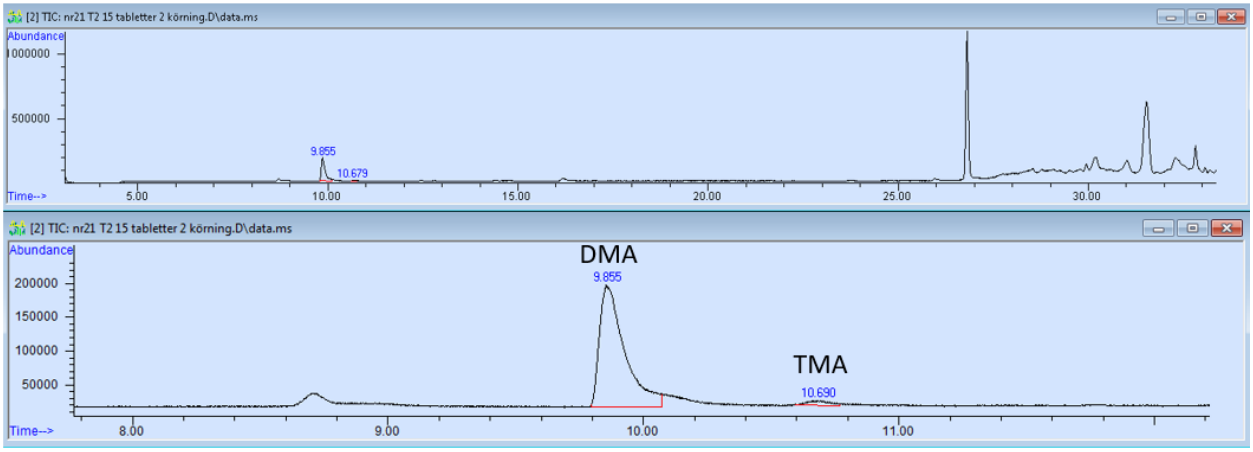

Figure 20: Tablet1 chromatogram TDS (run2) entire chromatogram with DMA, TMA peaks respectively marked in blue retention times and a zoom in on the DMA and TMA peaks.

figure 19 showing run 3 with first the entire chromatogram followed by a zoom in on the DMA and TMA peaks.

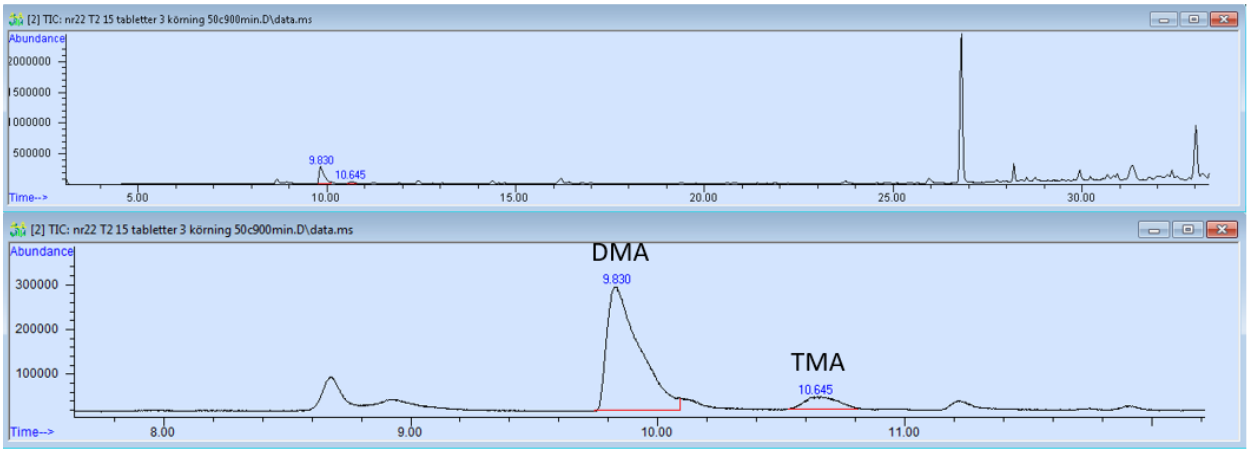

Figure 21: Tablet1 chromatogram TDS (run3) entire chromatogram with DMA, TMA peaks respectively marked in blue retention times and a zoom in on the DMA and TMA peaks.

Metformin-HCl1 comparison chromatograms

Chromatograms for Metformin-HCl1 MHI, run 2, 3 and 4 respectively. Peak area can be viewed in table 13 and 14 for both amines.

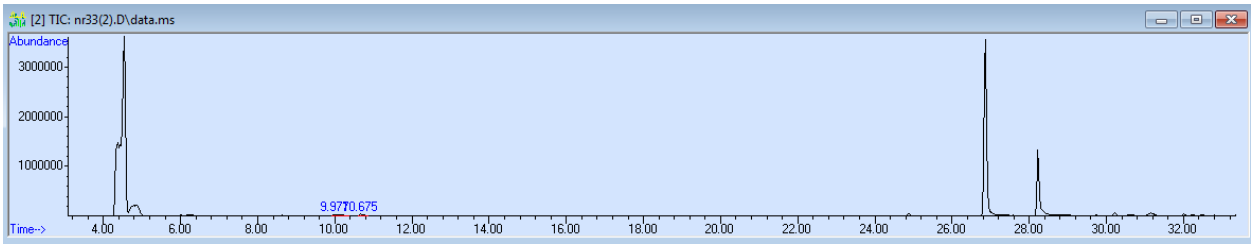

Figure 22: Metformin-HCl1 chromatogram MHI (run2) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

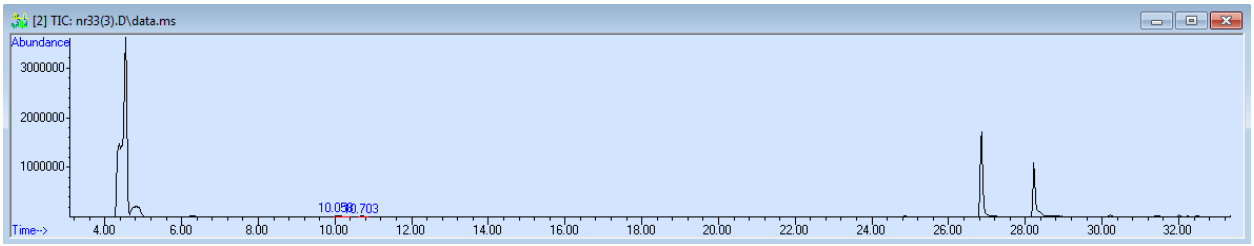

Figure 23: Metformin-HCl1 chromatogram MHI (run3) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

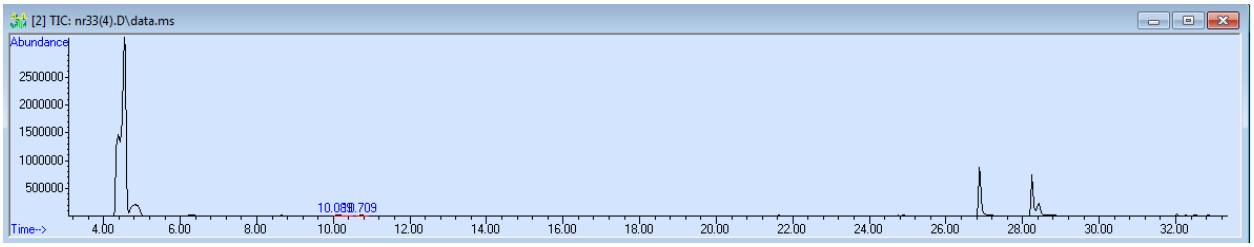

Figure 24: Metformin-HCl1 chromatogram MHI (run4) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

Chromatograms for Metformin-HCl1 TDS-TE, run 2 and 3. Peak area can be viewed in table 15 and 16 for both amines.

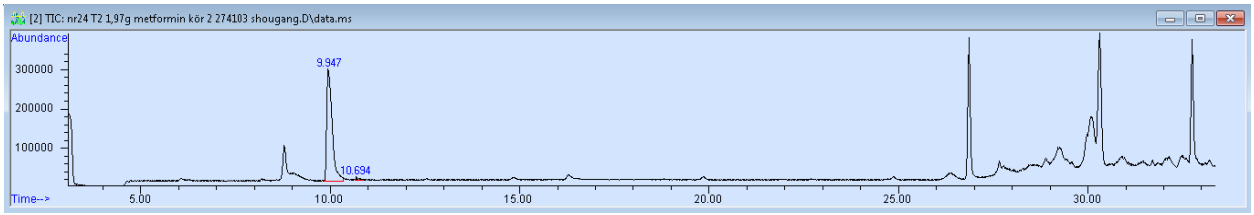

Figure 25: Metformin-HCl1 chromatogram TDS-TE (run2) (90 minutes) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

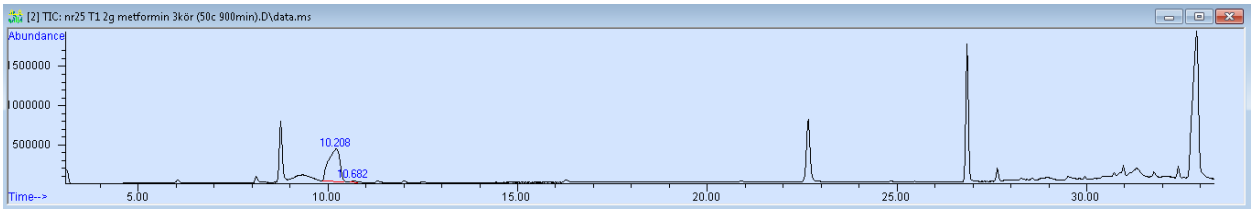

Figure 26: Metformin-HCl1 chromatogram TDS-TE (run3) (900 minutes) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times. Note that the DMA peak is "malformed" and very large.

DMA1 comparison chromatograms

Chromatograms for Metformin-HCl1 MHI, run 2, 3 and 4 respectively. Peak area can be viewed in table 17 and 18 for both amines.

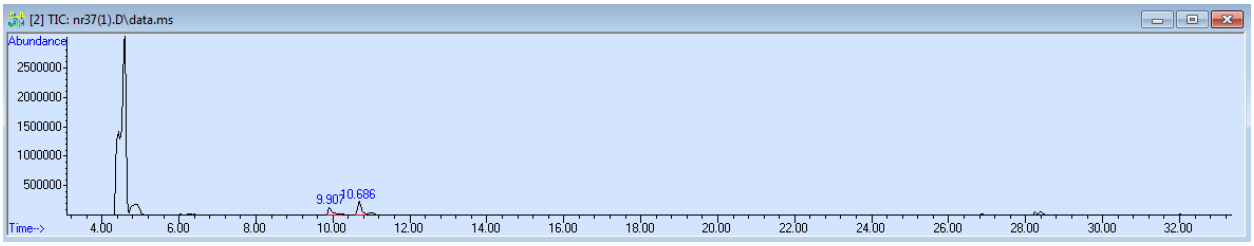

Figure 27: DMA-HCl1 chromatogram MHI (run2) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

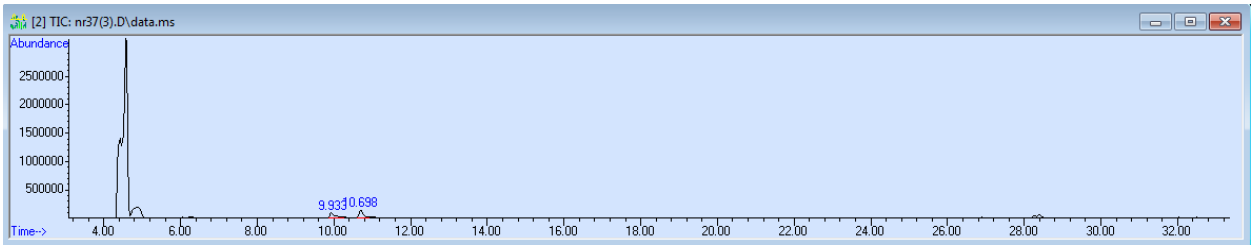

Figure 28: DMA-HCl1 chromatogram MHI (run3) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

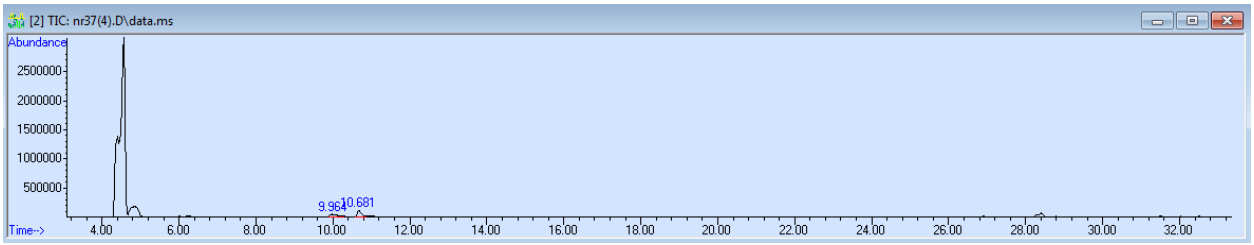

Figure 29: DMA-HCl1 chromatogram MHI (run4) entire chromatogram with DMA and TMA peaks respectively marked in blue retention times.

Chromatograms for DMA-HCl1 TDS-TE, run 2 and 3 respectively. Peak area can be viewed in table 19 and 20 for all amines.

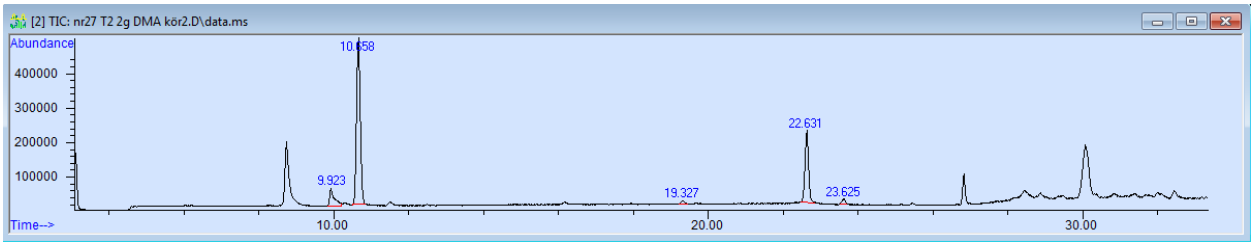

Figure 30: DMA-HCl1 chromatogram TDS-TE (run2) (90 minutes) entire chromatogram with DMA, TMA, DMPA, MDATM, TEA and NDMA peaks respectively marked in blue retention times.

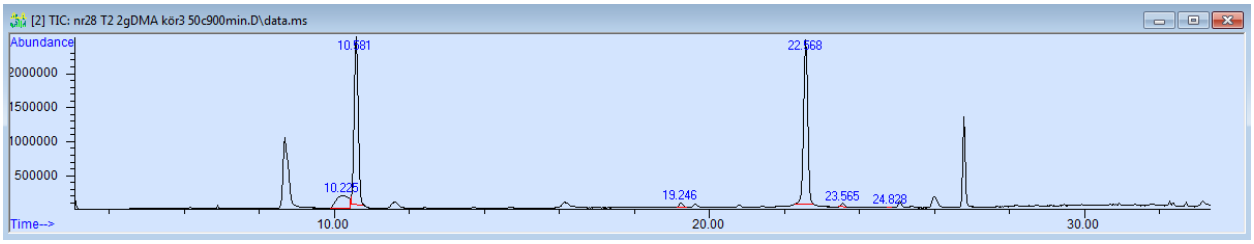

Figure 31: DMA-HCl1 chromatogram TDS-TE (run3) (900 minutes) entire chromatogram with DMA, TMA, DMPA, MDATM, TEA and NDMA peaks respectively marked in blue retention times.

Number of extractions chromatogram

Metformin-HCl1 1 extraction chromatogram in figure 32.

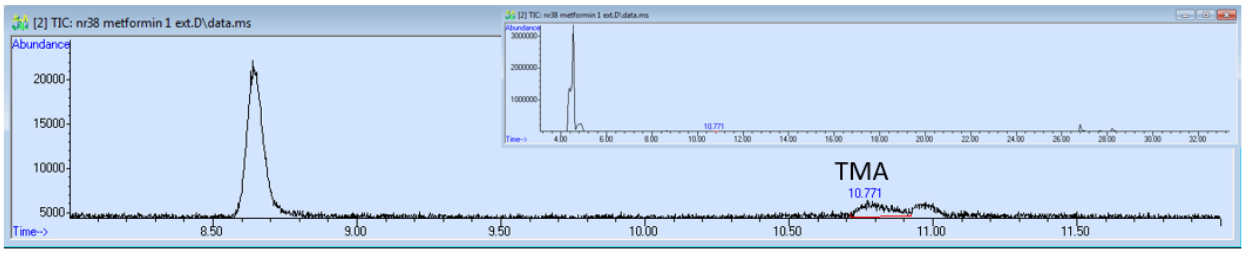

Figure 32: The chromatogram for 2 grams of Metformin-HCl1 with 1 extraction suing MHI with the zoom in on the TMA peak with the whole chromatogram as a picture in picture.

Metformin-HCl1 4 extractions chromatogram in figure 33.

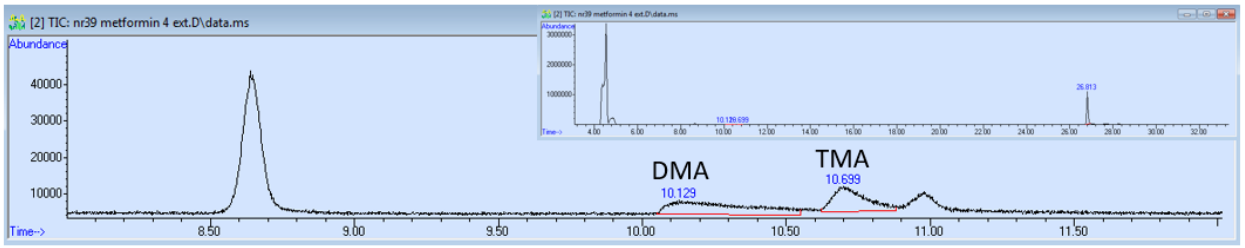

Figure 33: The chromatogram for 2 grams of Metformin-HCl1 with 4 extractions using MHI with the zoom in on the DMA and TMA peaks with the whole chromatogram as a picture in picture.

Metformin-HCl1 25 extractions chromatogram see figure 34.

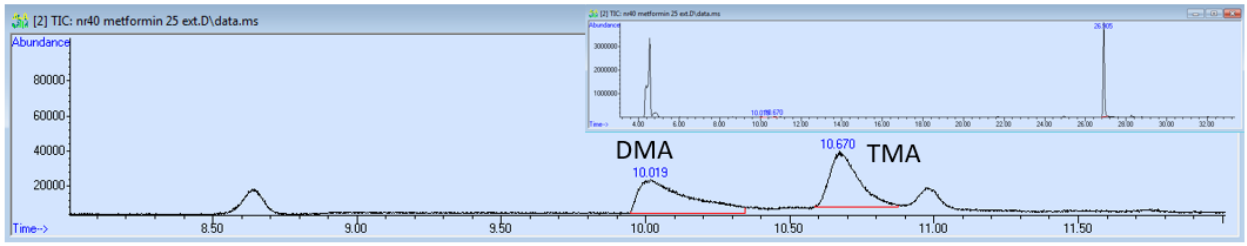

Figure 34: The chromatogram for 2 grams of Metformin-HCl1 with 25 extractions using MHI with the zoom in on DMA and TMA peaks with the whole chromatogram as a picture in picture.

Tablet1 1 extraction chromatogram see figure 35.

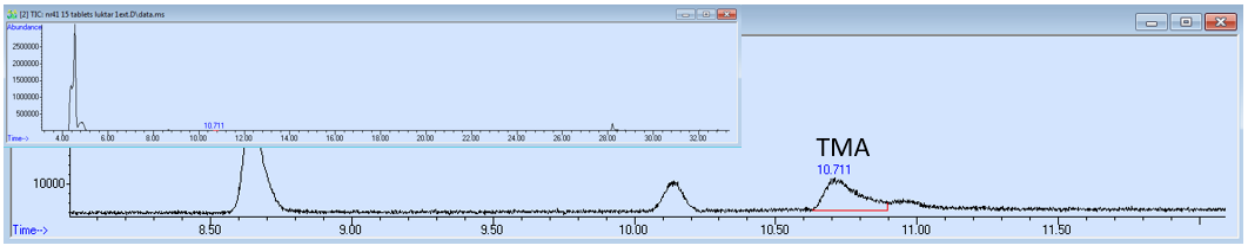

Figure 35: The chromatogram for 15 tablet1 with 1 extraction using MHI with the zoom in on the TMA peak with the whole chromatogram as a picture in picture.

Tablet1 4 extractions chromatogram see figure 36.

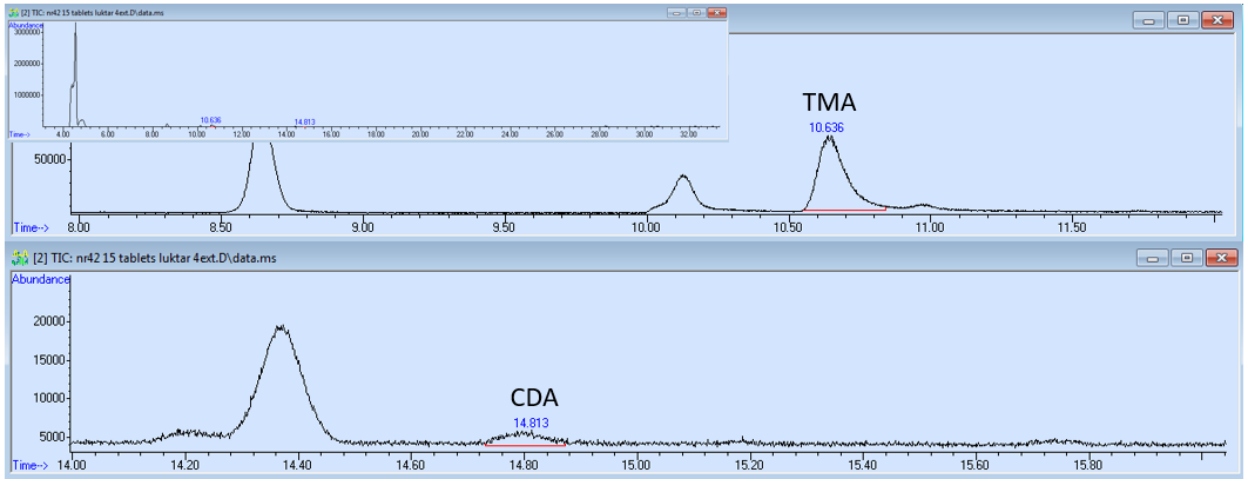

Figure 36: The chromatogram for 15 tablet1 with 4 extractions using MHI with the zoom in on the TMA peak with the whole chromatogram as a picture in picture. The second picture is a zoom in on the CDA peak.

Tablet1 25 extractions chromatogram see figure 37, note that this is the same chromatogram as shown in figure 8 in tablet1 comparsion.

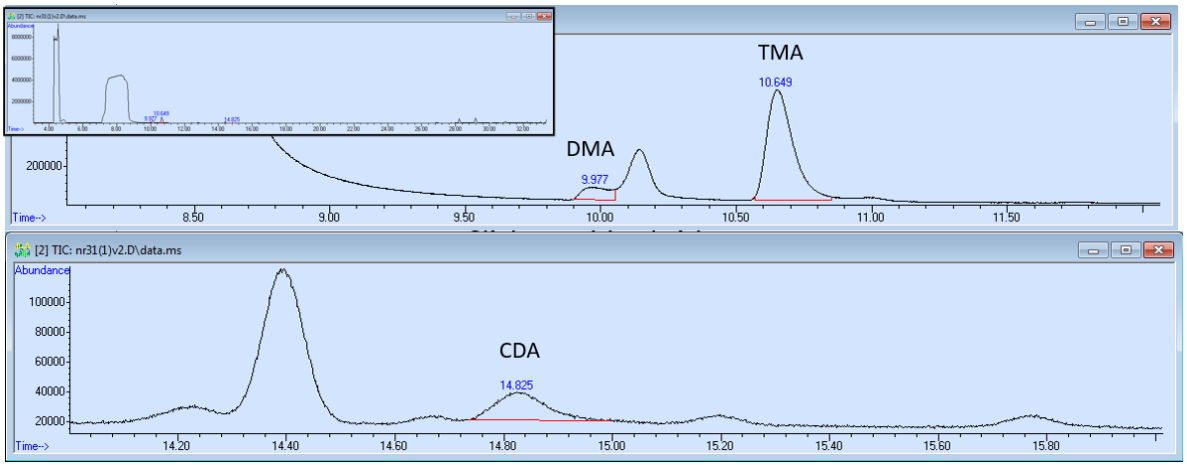

Figure 37: The chromatogram for 15 tablets1 with 25 extractions using MHI with first the zoom in on DMA and TMA peaks with the whole chromatogram as a picture in picture. The second chromatogram is a zoom in on the CDA peak.

Tablet2 1 extraction chromatogram see figure 38.

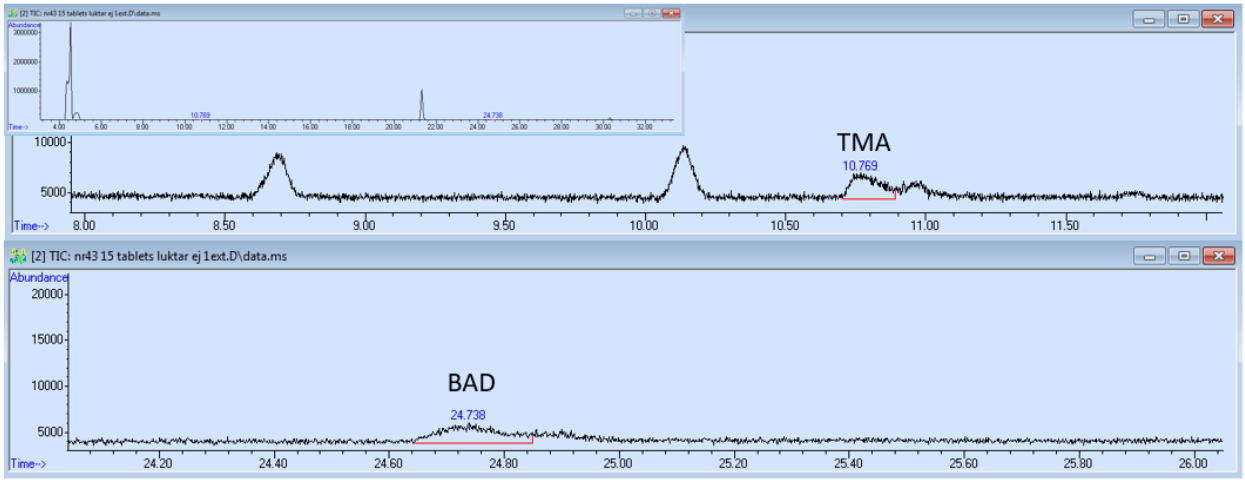

Figure 38: The chromatogram for 15 tablets2 with 1 extractions using MHI with first the zoom in on the TMA peak with the whole chromatogram as a picture in picture. The second chromatogram is a zoom in on the BAD peak.

Tablet2 4 extractions chromatogram see figure 39.

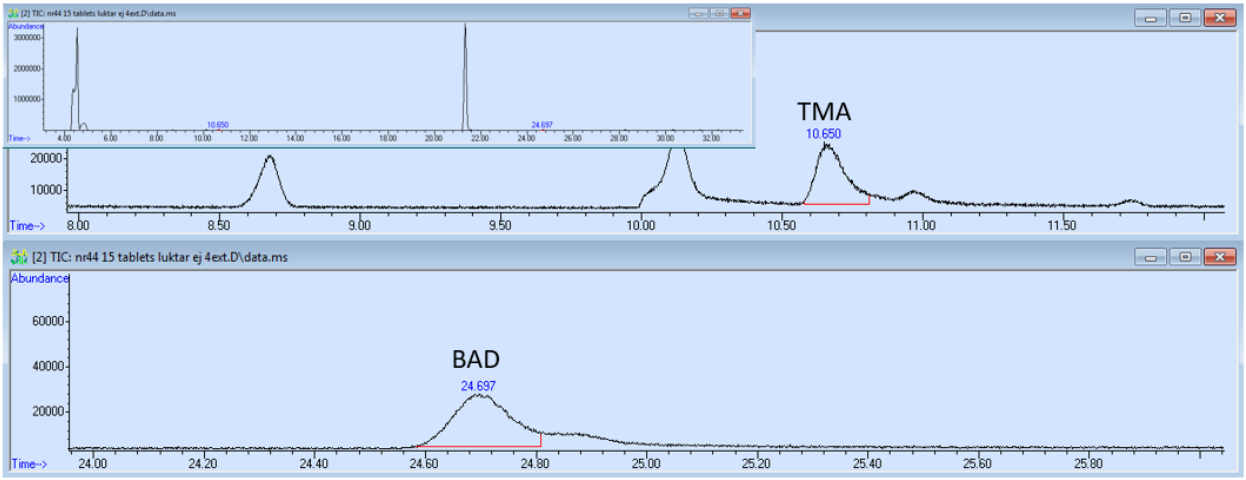

Figure 39: The chromatogram for 15 tablets2 with 4 extractions using MHI with first the zoom in on the TMA peak with the whole chromatogram as a picture in picture. The second chromatogram is a zoom in on the BAD peak.

Tablet2 25 extractions chromatogram see figure 40.

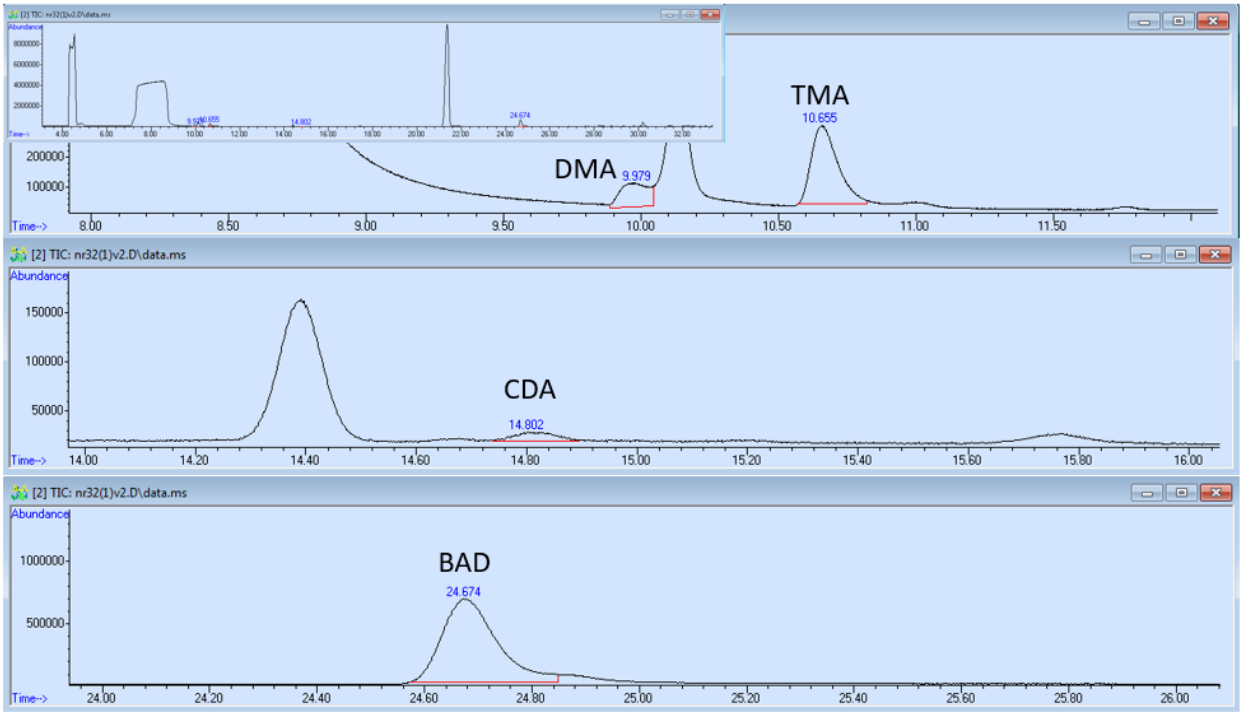

Figure 40: The chromatogram for 15 tablets2 with 25 extractions using MHI with first the zoom in on the DMA and TMA peaks with the whole chromatogram as a picture in picture. The second chromatogram is a zoom in on the CDA peak. The third is a zoom in on the BAD peak.