Determination of Elemental Impurities in Pharmaceutical Products using Inductively Coupled Plasma Emission Spectrometry

Master's Thesis
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Tiivistelmä

Tässä Pro Gradun kokeellisessa osassa kehitettiin kattava analyysimenetelmä alkuaine-epäpuhtauksien analysoimiseen lääkevalmisteista ICP-OES -laitteella, ja lääkeaineanalyysit tehtiin Fermion Oulun laboratoriossa. Kehitetty analyysimenetelmä täytti ICH Q3D ohjeistuksen, Amerikan Yhdysvaltojen farmakopean ja lääkeaineiden hyvän toimintatavan (GMP) vaatimukset. Yhteensä 92 eri lääkevalmistetta analysoitiin. Cd, Pb, As, Hg, Co, V, Ni, Tl ja Pd pitoisuudet lääkevalmisteissa kvantitoitiin käyttäen ulkoista kalibrointia, jossa oli yhteensopiva näytematriisi, ja Au, Ir, Rh, Ru, Se, Ag, Pt, Li, Sb, Ba, Mo, Cu, Sn ja Cr määritettiin rajakokeella. Menetelmän varmennus tehtiin käyttäen standardilisäysnäytteitä, kalibroinnin oikeellisuutta seurattiin QC-näytteillä ja menetelmälle laskettiin määritysrajat kullekin alkuaineelle. Lopuksi lääkevalmisteen käyttäjän päivittäiset altistusmäärät kullekin epäpuhtaudelle laskettiin, ja näitä tuloksia verrattiin sallittuihin päivittäisiin altistusrajoihin.

Menetelmä oli riittävän tarkka, robusti, selektiivinen ja luotettava täyttämään lääkealan ohjeistukset alkuaine-epäpuhtauksien määrittämisessä. Kokeellisen osan tulokset osoittavat, että alkuaine-epäpuhtauksien määrä kaikissa lääkevalmisteissa aiheuttaa pienemmän altistuksen, kuin sallitut päivittäiset altistukset kullakin epäpuhtaudella. Alkuaine-epäpuhtauksista kaikista eniten huomiota tuli kiinnittää lyijyyn, sillä sen pitoisuudet olivat useassa lääkevalmisteen näytematriisissa yli määritysrajan, ja lyijyn sallitut pitoisuudet olivat alhaisimmat kaikista määritettävistä alkuaineista. Muita kehittämiskohteita havaittiin näytteenhajotuksessa, jossa oli välillä haasteita, sekä palladiumin määrityksen luotettavuudessa, sillä sen standardinlisäysnäytteiden saannot olivat vaihtelevan alhaisia eri näytteillä.

Tämän tutkielman kirjallisessa osassa käsitellään ICH Q3D ohjeistuksen ja Amerikan Yhdysvaltojen farmakopean alkuaine-epäpuhtauksista kertovia osioita, miten näitä ohjeistuksia sovelletaan kätännössä eri laadunvalvontalaboratorioissa, sekä miten siirtyminen vanhoista analyysimenetelmistä uusiin on tapahtunut eri tieteellisten artikkelien perusteella. Kirjallisessa osassa käsitellään myös ICP-OES tekniikkaa yleisesti ja lääketeollisuuden alkuaine-analytiikassa, ja miten menetelmän varmennusta ja validointia tehdään lääketeollisuuden ja GMP:n mukaisesti.

Abstract

An extensive method for analysing elemental impurities with ICP-OES in pharmaceutical products was developed and conducted in Fermion Oulu laboratory in the experimental part of this thesis. The method was developed to fulfill requirements of ICH Q3D Guideline on elemental impurities, US Pharmacopoeia and current good manufacturing practises in pharmaceutical industry. A total amount of 92 different products were analysed. Cd, Pb, As, Hg, Co, V, Ni, Tl and Pd were quantified from samples using matrix matched external calibration and a limit test for Au, Ir, Rh, Ru, Se, Ag, Pt, Li, Sb, Ba, Mo, Cu, Sn and Cr were conducted. Method verification was done with spike samples, continuous calibration verification and quality control samples and calculating method quantification limits. Daily exposures of each element were finally calculated from the concentration and limit test data and compared to the permitted daily exposures.

The method showed sufficient accuracy and reliability to the pharmaceutical guideline requirements. The results showed that elemental impurity levels were low throughout all sample matrices, and impurity levels were not over the permitted daily exposures in any of the pharmaceutical products analysed. Of all the elemental impurities, lead had to be taken most into consideration in this project, because low concentrations of lead over quantification limit was found in several products, and lead had the lowest PDEs of all impurities. Some challenges were encountered in the sample digestion, and the method was found to be unreliable in analysing palladium in several sample matrices, yielding low spike recoveries.

The theoretical part covers the ICH Q3D guideline and the new US Pharmacopoeia chapters about elemental impurities, and how they are applied in quality control laboratories, and how the transition from the old heavy metal tests to the new ones been done in pharmaceutical industry according to scientific articles. Also ICP-OES technique in pharmaceutical analytics is discussed and how method verification and validation are done with ICP-OES technique accorfing to GMP and pharmaceutical industry guidelines.

Preface

This Master's thesis was done in co-operation with Fermion, an affiliate of finnish pharmaceutical company Orion in order to develop analysis method according to ICH Q3D Guideline on elemental impurities. The focus of the theoretical part was confined to elemental impuriry guidelines and how they are applied in pharmaceutical industry using ICP-OES instruments, as well as ICP-OES method validation.

Ari Väisänen from University of Jyväskylä was the supervisor for the theoretical part. The experimental part of this Master's Thesis was performed in the quality control laboratory of Fermion Oulu plant. The lab work was done in timespan of three and a half months starting September and ending December of 2017, and the theoretical part was written between October of 2017 and May of 2018. A separate analysis report, not included in this thesis, was written and send to Orion for further use in the risk assessment process. Supervisor of experimental part of this thesis was QC chemist team leader Antti Kivilahti. Work was done largely in co-operation with QC chemist Ari Turpeinen, who helped and guided me with analytical procedures and method development. The bibliography sources for this work were gathered from analytical chemistry and pharmaceutical industry scientific journals and literature, using mainly Google Scholar and Jyväskylä University Library literature JYKDOK search engines.

I would like to thank deeply Ari Väisänen for great guidance and help on the subject and writing process of this thesis. I would like to thank Antti Kivilahti for great supervision, and Ari Turpeinen for great outlook on elemental analysis, their preliminary work on the subject made many aspects of my job quite a bit easier and convenient. I would like to thank Juho Leikas for introducing be to this challenging project, it turned out to be at least as interesting as I imagined at the beginning. I would also like to thank my spouse, family and friends for support in the process of making this thesis, without you this paper would not be finished in ages.

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Sampo Pakkanen

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Abbreviations

AAS Atomic Absorption Spectrometry

ANOVA Analysis of Variance

API Active Pharmaceutical Ingredient

CCD Charge-Coupled Device

CGMP Current Good Manufacturing Practice

CID Charge Injection Device

CRM Certified Reference Material

EMA European Medicines Agency

FDA United States Food and Drug Administration

FIMEA Finnish Medicines Agency

FINAS Finnish Accreditation Services

GMP Good Manufacturing Practice

ICH The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

ICP-OES Inductively Coupled Plasma Optical Emission Spectrometry

ICP-MS Inductively Coupled Plasma Mass Spectrometry

IDL Instrument Detection Limit

ISO International Organization for Standardization

J Target Concentration

LO(A)EL Lowest-Observed-(Adverse)-Effect Level

LOD Limit of Detection

LOQ Limit of Quantification

MCC Microcrytalline Cellulose

MDL Method Detection Limit

MQL Method Quantification Limit

NO(A)EL No-Observed-(Adverse)-Effect Level

OECD Organisation for Economic Co-operation and Development

PDE Permitted Daily Exposure

ppm parts per million

ppt parts per trillion

PTFE Polytetrafluoroethylene, “Teflon”

Q3D ICH Guideline for Elemental Impurities

QA Quality Assurance

QC Quality Control

RF Radio Frequency

RSD Relative Standard Deviation

S Standard Deviation

SOP Standard Operating Procedure

SRC Single Reaction Chamber

STD Standard Solution

TUKES Finnish Safety and Chemicals Agency

USP United States Pharmacopeia

VIM International Vocabulary of Metrology

XRF X-ray Diffraction Fluorescence Spectrometry

I Theoretical part

1 Introduction

Pharmaceutical products, also known as medicines or drugs, have a big role in modern society. Safe and functional pharmaceuticals are commonly the foundation of healthcare in modern western medicine, treating medical conditions such as inflammation, pain, cancer and so on. Pharmaceutical spendings in Organisation for Economic Cooperation and Development member (OECD) countries are shown in Figure 1. In year 2015 statistics by OECD, the share of pharmaceuticals of the total healthcare spendings in Finland were 12.5 percent. The percentage is moderately low compared to some countries such as Hungary and Mexico, where use of pharmaceuticals has even greater impact on total healthcare costs. The percentages in these countries were the highest among OECD countries: 29.2% and 27.2% of total spendings, respectively.[1] The same statistical data also shows that an average finnish citizen spends 501 USD, or approximately 400 euros, per year on pharmaceutical products.

The safety of pharmaceutical products is a great concern in public health, because of wide use of pharmaceutical products of the whole population, and over the whole lifetime of individuals. In Finland, medicinal product regulations are supervised over the entire life cycle finnish medicines agency FIMEA. It also monitors distribution, pharmacovigilance (study of medicinal adverse effects) and medicines marketing promotion.[2] National and international pharmacopoeias and other regulatory bodies also govern the specifications of drugs, and international harmonisation organisations bring pharmaceutical companies and national lawmakers together to unify the pharmaceutical industry.

Elemental impurities in pharmaceuticals had risen to discussion in late 2010s after the reforming actions taking place in pharmacopoeias and other guidelines on elemental impurities around the globe. More and more spectrometrical analysis techniques, such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and X-ray fluorescence

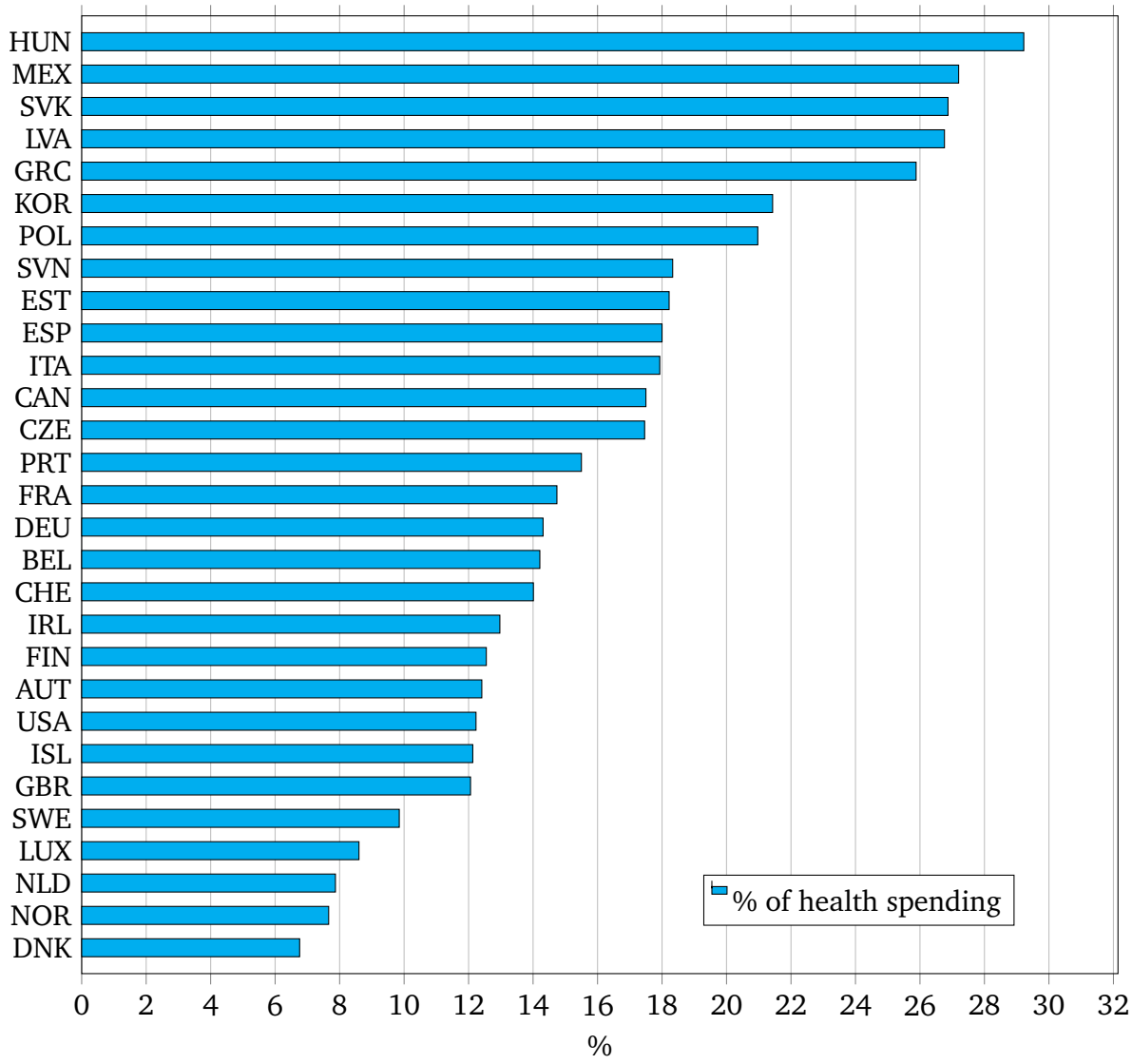


Figure 1: Percentage of pharmaceutical products in total healthcare spending in OECD countries from year 2015.[1]

spectrometry (XRF), are used for analysis of inorganic impurities in samples in many different fields of chemistry, and they have constanlty been developed to be even reliable, robust, precise and achieving lower detection limits in the recent centuries.[3] Also, the sample digestion procedures have come a long way since the days of dissolving material in acid baths in open containers on a hot-plat. Many companies have been supplying powerful closed vessel microwave- or ultrasound-assisted digestion systems, which provide minimal loss of analytes and solvents.[4] Some of these applications have recently been gradually introduced to pharmaceutical industry in several different recent pharmacopoeia, to become a new ”industry standard”.

The theoretical part of this Master's Thesis will be focusing on the content and requirements of the ICH Q3D guideline and the new USP chapters <232> and <233>, and how the transition from the old pharmacopoeia limits of heavy metal impurities and analysis method to the new ones been done in pharmaceutical industry. We will be focusing on the ICP-OES techniques, because of it's wide applicability and robustness in analysing different kinds of samples. In the experimental part an ICP-OES method for elemental impurities in pharmaceutical products such as tablets and capsules is developed and performed, and the daily exposures of the impurities are calculated.

2 Elemental Analysis in QC laboratories

2.1 QC-laboratories in Pharmaceutical Industry

Quality control, or QC laboratories in pharmacological industry do chemical and physical analysis on raw materials, intermediate and final pharmaceutical products. According to World Health Organization, the term quality control "*refers to the sum of all procedures undertaken to ensure the identity and purity of a particular pharmaceutical*".[5] In Fermion, QC laboratory is usually part of a larger quality management organization, and co-operates with quality assurance (QA) systems, which ensure that no mistakes or flaws are made in manufacturing process, and that final pharmaceutical products are up to the accepted criteria. QC produces information of the analysed samples organization analyses the required samples for quality assurance systems. In finnish systems, quality organizations are always separate from the product development and manufacturing organizations by legislation, to avoid bias in analysis results.[6]

QC laboratories are strictly directed and supervised by national law of Finland and international laws of countries, where the pharmaceutical company operates. In Finland, FIMEA confirms the principles of good manufacturing practises (GMP) which are laid down by the European Commission directive 2003/94/EY.[6] GMP or current GMPs (CGMP) basically affect all drug manufacturing plants and institutions manufacturing drugs for clinical research. Standard operating procedures, or SOPs, are implemented in QC laboratories, to assure reliability of work in laboratory environment. The analytical methods, systems and instruments, such as GC- and HPLC-chromatographs and spectrometers used in pharmaceutical laboratories are also validated for their intended use, and must also be compliant with the CGMP and applied pharmacopoeias, such as US Pharmacopoeia and ICH guidelines, which pose a big role how the pharmaceutical quality control and assurance are carried out, describing

quality management, personnel, utilities and facility requirements. One of the important guidelines is ICH Q7 *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*, which is applied almost worldwide in pharmaceutical industry.[7]

2.2 Pharmaceutical Elemental Analysis

Several potentially harmful and toxic elements may be found in the processes of manufacturing drug products, and their concentrations must be monitored during the process and in the final product.[3,8] Presence of the metal ions may also affect the stability of the formulation, making the drug product's shelf life shorter. They may come from excipients, package material, water or other solvents, leeched from manufacturing equipment or used in the synthesis of the active pharmaceutical ingredients (abbreviated as API). Many of these elements may be added on purpose due to their functional features. For example, palladium is excellent catalysts in many organic syntheses, barium, gadolinium, iron, manganese and sodium are used as imaging agents, and platinum compounds have many applications in cancer treatment.[9]

However many of the ICH Q3D elements are not added on purpose. They may come for example from impurities of geological materials in tablet excipients.[8] As, Cd, Hg, Pb, Sb, Tl and U are classified as non-essential elements, there are no known biological significance for animals or plants, they are not needed at all in a biological sense. Non-essential elements have a toxic effect on organisms, if their available concentrations are too high.[3,10]

2.3 Elemental Impurity Guidelines

Pharmacopoeias are official publications published by authorities or governments which include identification of medicinal drugs and their effects and use. They may contain quality monographs of the medicinal drugs, which are descriptions of preparation and quality aspects of certain drugs. There are several guidelines and pharmacopoeia entries published by monitoring organisations about elemental analyses.[3] Perhaps the most influential in pharmacological industry are ICH Q3D elemental impurities guideline[8], US Pharmacopoeia chapters <232> and <233> elemental impurities limits and procedures[11,12], and EMA Guideline on

Specification Limits for Residues of Metal Catalysts or Metal Reagents.[13] The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, abbreviated as ICH, is a non-profit worldwide organisation based in Switzerland. ICHs mission is to bring together regulatory authorities and pharmacological industry to discuss scientific and technical aspects of drug production and development.[14] One of ICHs main functions is to provide recommendations and guidelines, in order to achieve better harmonisation in the pharmacological industry, thus achieving safer and more consistent procedures and criteria for developing drug products. It has a role of unifying and harmonising previously mentioned organisation's guidelines.

Before autumn of 2017, US pharmacopoeia requirement of elemental impurity analysis was an old precipitation method, which indicated total amount of heavy metals in the sample. The potentially toxic heavy metal ions (M^{n+}) were precipitated as monovalent, trivalent or pentavalent sulfide ions according to chemical equations (R1) and (R2),

$$m\,M^{n+} + n\,H_2S\,(aq) \longleftrightarrow M_mS_n\,(s) + 2\,n\,H^+\,, \tag{R1}$$

$$m\,M^{n+} + \frac{1}{2}\,n\,H_2S\,(aq) \longleftrightarrow M_mS_{n/2}\,(s) + n\,H^+\,. \tag{R2}$$

then the colored precipitation was visually compared to the color of reference sample. If the sample color was not darker than reference, the test was intepreted as passed – heavy metal concentrations were determined lower than accepted limit concentration in the reference sample[15]. The poor performance of the heavy metals sulfide precipitation test was known widely in the late 1990s and early 2000s.[16] The shortcomings – reliability and specificity – of the test were questioned and in the pharmaceutical and analytical chemistry industry, since the test didn't give clear indication which heavy metals were present in the sample, or what their concentrations were, heavy metal cations such as Cu^{2+} and Hg^{2+} having the lowest equilibrium sulfide ion concentrations, therefore they precipitate more easily than *e.g.* Pb^{2+} sulfides. Also In some cases, the use of excess sulfide, tends to form complex sulfide ions which may remain in solution, therefore adding more possibilities for errous results.[17] The color formation in the sulfide precipitation was also not consistent in all occasions.[16] The method was finally brought up and discussed by USP and EMA in late 1990s, which mobilised the reform of the elemental impurity guidelines and methods.[18]

Development of modern guidances started roughly in the beginning of the 21st century. USP initiated workshops and forums between years 2000 and 2008 to revise their pharmacopoeia chapter *Heavy Metals*. In 2008 EMA published their own specification limit guideline which introduced the permitted daily dose approach (PDE) based on toxicity data of the potential impurities, instead of concentration limits of the elements in drug products.[19] USP chapters <232>–<233> development was finished in 2013 with two new chapters on elemental impurity tests and procedures, replacing old heavy metals test. In 2009 ICH joined in the development of elemental impurity analysis. Q3D Guideline for Elemental Impurities is an attempt to bring together and harmonise the elemental analysis methods proposed by EMA and USP in three regions: The US, Europe and Japan. Q3D guideline was first released for public consultation by ICH steering committee in June 2013. The final guideline was drafted in December 2014. Final Q3D guideline implemented the PDE approach used by EMA, and USP chapters were aligned to be as compactible with Q3D as possible.[20]

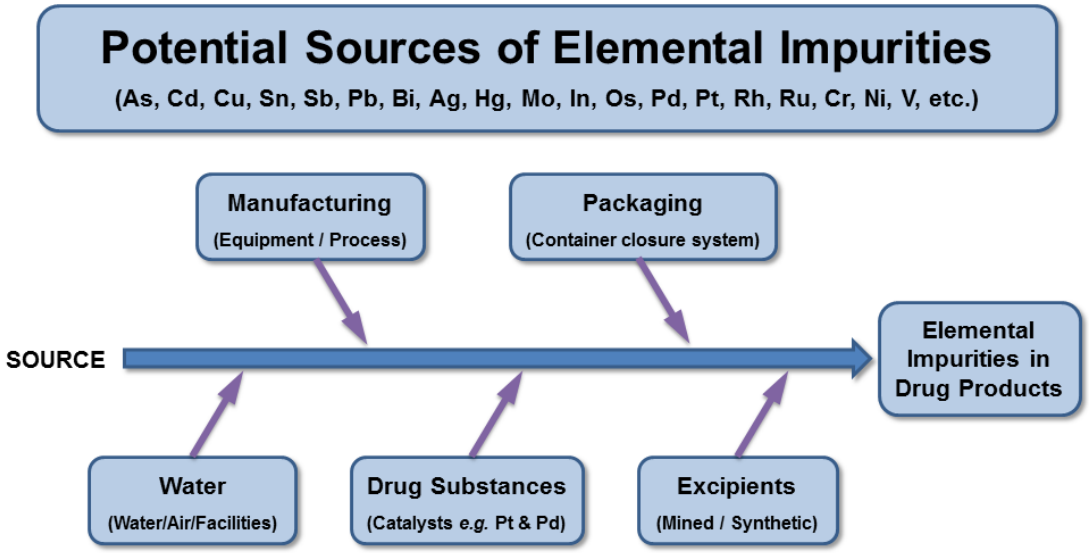


Figure 2: Sources of elemental impurities in manufacturing pharmaceutical products according to ICH Q3D, modified after Balaram *et. al*[3] and Pohl *et. al*[20].

2.4 Permitted Daily Exposures

The ICH Q3D guideline introduces permitted daily exposures (PDEs) to assign toxic element limits in pharmaceuticals. PDEs are calculated based on toxicity data of each element.

PDEs represent the maximum safe daily doses, or exposures, of each elemental impurity in pharmaceutical products, for the whole population.[8]

The basis of calculating PDEs are the the No-Observed-(Adverse)-Effect Levels (NO[A]EL) or the Lowest-Observed-(Adverse)-Effect Levels (LO[A]EL) of each element. NO[A]EL and LO[A]EL are mainly calculated based mainly on experimental data on human and animal tests (short and long term studies), which are then extrapolated with ”modifying” or ”safety” factors. The route of administration is also considered; toxicity of elements in humans is relative to bioavailability of toxic components, and it is affected by which way element enters the body. PDEs are calculated separately for oral, parenteral and inhalation products. For example, route specific toxicity of the elements is observed with chromium; it has PDE of 3 μg/day by inhalation, over 3600 times lower than oral PDE. The usual proposed pharmacopoeia analysis methods (ICP-OES, ICP-MS, AAS, ...) do not differentiate between different species of chemical impurities, such as oxidation state – they just measure the total concentrations of each element. The chemical speciation is important property in toxicity.[15,21] Therefore some assumptions of impurity speciations must be also factorised in PDE calculations. The modification factors include variables such as:

- Extrapolation between data between animal species
- Variability between individuals
- Weighting studies lasting over one half lifetime
- Reproductivity and maternity studies
- Carcinogenic effects
- Chemical speciation studies
- Using LO[A]EL instead of NO[A]EL

Equation for calculating permitted daily exposures is as follows:

$$\mathrm{PDE} = \mathrm{NO[A]EL} \times M/F \quad , \tag{1}$$

where M is mass adjustment of mass of arbitrary adult human, and $F = [F1 \times F2 \times ... \times F5]$ is total of modifying factors, characteristic for each element. The toxicity data is scaled to assume the mass of adult human body of 50 kg. Using this scaling we can represent the daily

limits of the impurities (Table 1). It can be discussed, that permitted daily exposures are set quite low for a regular person, by using really low mass compared to a typical masses of 60 kg or 70 kg used in exposure guidances in pharmaceutical industry. The low mass scaling is justified by PDEs applying also to pediatric patients who are considered the most sensitive population. Therefore the built-in safety factors must be set accordingly to this population.[8]

Table 1: Permitted daily exposures of elements by oral adminstration considered in risk assessment of elemental impurities

Element	Risk Class	PDE µg/day	Element	Risk Class	PDE µg/day
Cd	1	5	Rh	2B	100
Pb	1	5	Ru	2B	100
As	1	15	Se	2B	150
Hg	1	30	Ag	2B	150
Co	2A	50	Pt	2B	100
V	2A	100	Li	3	550
Ni	2A	200	Sb	3	1200
Tl	2B	8	Ba	3	1400
Au	2B	100	Mo	3	3000
Pd	2B	100	Cu	3	3000
Ir	2B	100	Sn	3	6000
Os	2B	100	Cr	3	11000

2.5 Applying Guidelines in QC-laboratories

For practical use in chemical laboratory, it is required to convert the µg/day units to $\mu g\, g^{-1}$ or µg/l, which requires information on maximum daily amount of pharmaceuticals products ingested. ICH suggests three different ways to approach the risk assessment. Drug manufacturers may analyse the products as they are sold – tablets, capsules, injection solutions, and so on. Either maximum dose of 10 grams for each product may be used, or the maximum dose for each pharmaceutical may be individually estimated, to convert the PDE to concentration unit. Third approach is to analyse the components and combine their elemental impurity concentrations to match the final product.[8] It may be argued, that the most true and accurate results for each product's impurities are acquired using the second approach –

it estimates the true dose more accurately than assuming 10 g dose, and takes unknown and random impurity sources (in manufacturing, packaging, and so on, see Figure 2) better into account than analysing only the components. The individual maximum daily dose approach is also the way which Orion uses in their elemental impurity risk assessment. The role of PDEs of analytes in risk assessment is to act as a control threshold. In Q3D guideline it is said that if the elemental impurity levels are consistently less than 30% of each PDE, no additional controls are required.[8] The consistency of low enough impurity levels must be assured, and the more assurance must be done the closer the impurity levels are to the 30% PDEs. This may drive the analysis laboratories to calculate the target concentrations of the calibrations and sample dilutions near the 30%PDE values.

The Q3D guideline document leaves the choice of analysis method somewhat open, giving option to use the applied pharmacopoeia methods in the risk assessment. The US Pharmacopoeia / National Formulary are influential documents worldwide, which govern the analytics in the industry, especially when doing business in North America. USP Chapter <233> suggest ICP-OES and ICP-MS as principal analysis techniques for elemental impurities in pharmaceuticals. In the chapter it is stated that the elements amenable to detection by emission spectrometry should be analysed by ICP-OES, and the elements amenable to mass spectrometry should be analysed by ICP-MS.[12] The elements suitable for each technique are not specified in the USP chapter, rather suitability must be demostrated by analysis verification. Both of techniques are great for trace analysis of elements thanks to their selectivity, sensitivity and robustness.[22]

Other requirements in USP <233> chapter are closed vessel digestion with concentrated acids on samples. However the use of hydrogen fluoride is not required, although in many cases it is necessary for complete digestion (see Section 4.2). Use of internal standard is not required either. Use of appropriate reference materials is required in ICP-OES and ICP-MS methods. There are many important reasons to use reference materials in trace analysis, such as impact of the sample matrix on recoveries, and performance of sample digestion can be controlled easily. Finding suitable certified reference materials (CRM) for inorganic impurities, which match with sample matrix of pharmaceutical tablets, pills and capsules are not found easily. Therefore many laboratories doing the risk assessment analysis are using inhouse quality control samples prepared from commercial standard solution to matched matrix, instead of CRMs.[3]

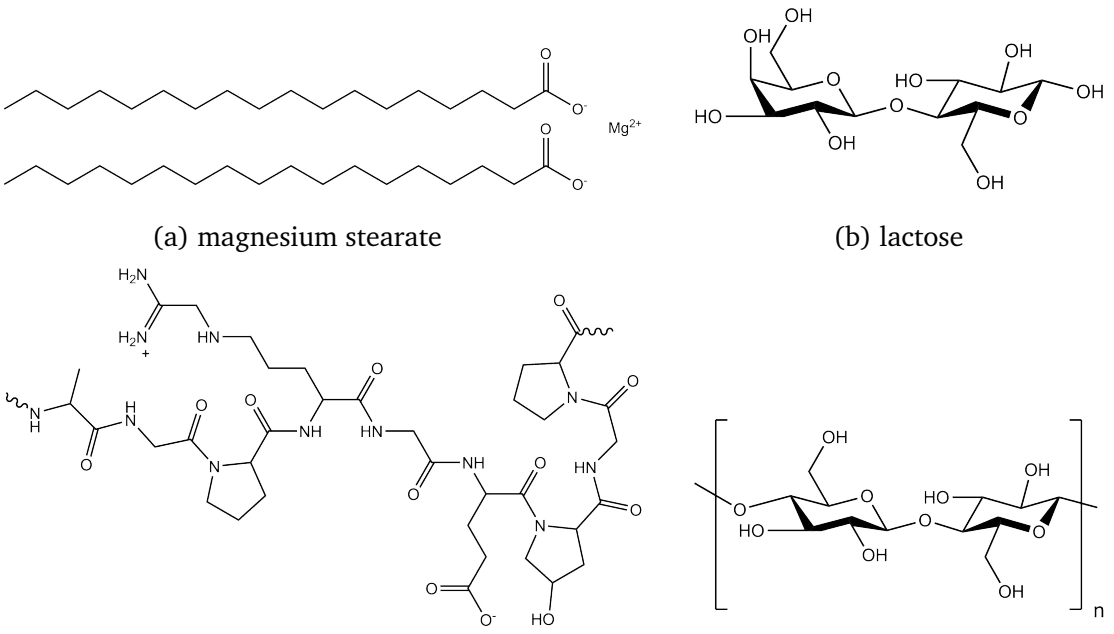
3 About Pharmaceutical Products

3.1 Active Pharmaceutical Ingredients (API)

According to United States Food and Drug Administration (FDA), the definition for active pharmaceutical ingredient is: *"Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function of the body."*[23] In other words, APIs are the chemical substances, usually manufactured in specialised drug factories, added in the medicinal tablets, pills, capsules, suspensions and solutions. Many ways of manufacturing APIs may be used, they include chemical manufacturing, deriving APIs from animal sources, extractions from plant and herbal sources, biotechnological manufacturing such as fermentation and cell culture, and so on.[7] There is a wide variety of chemical substances used as APIs, they include for example inorganic and organic salts. Fermion manufactures and develops several new API molecules[24], which of many are made using organic syntheses.

3.2 Excipients

All of the mass in pharmaceutical dosage forms, *e.g.* tablets or capsules are in most cases not the active pharmaceutical ingredient, but mixture of APIs and excipients. Traditional understanding of excipient is that the substances used are chemically inert and act as a "filler". However, in modern pharmacology excipients can be viewed more as adjuvant agents. In most cases many helping APIs to carry out its activity, by helping and regulating



(a) magnesium stearate

(b) lactose

(c) gelatin

(d) microcrystalline cellulose (MCC)

Figure 3: Chemical structures of some common organic excipients in pharmaceutical pills, tablets and capsules. (c) is a representative structure formed of amino acids (*Ala-Gly-Pro-Arg-Gy-Glu-4Hyp-Gly-Pro*)[26].

their release from the formulation.[25] They may be necessary in some pharmaceuticals to aid manufacturing processess of the product. They have a big role in the efficiency and mechanism of action, because different excipients alter the properties of the formulation in many senses. Excipients chosen have a great effect on stability and bioavailability of the formulation, and also their interactions with APIs and each other. Excipients may also be added to increase patient acceptability, *e.g.* making them easier or pleasant to ingest by using coatings and sweeteners.[9]

Many common excipients are organic substances, some chemical structures shown in Figure 3. Carbohydrates such as cellulose and lactose may be added to make drug product manufacturing easier, and fatty acid salts such as magnesium stearate are common in coatings. Also bigger compounds like gelatin are present in many drugs. The most popular inorganic substances in pharmaceuticals are several oxides and silicates of different metals (Fe, Mg, Al, Ti, ...) originated from minerals. Inorganic substances and salts are abundant in APIs and excipients – Approximately content of drug products is 40 % basic and 10 % acidic salts, leaving only half of the drug with other substances.[15] Various chemical classifications and roles of excipients are shown in Table 2.

Table 2: Chemical classifications and roles of excipients in pharmaceuticals, modified after Pifferi *et. al*[27]

Chemical classification	Roles to affect
water, alcohols	compliance
esters, ethers, carboxylic acids	dose precision and accuracy
stearates	dissolution, dose release
glycerides and waxes	stability
carbohydrates	manufacturability
hydrocarbons and halogen derivatives	tolerability
natural and synthetic polymers	disaggregation
minerals	dissolution
proteins	controlled release
dyes, sweeteners	patient acceptibility
various: preservatives, surfactants,...	absorption

Naturally, every drug type has it's own characteristic excipients. Even apparently similar drugs, for example analgesics such as Orion's Burana® and Para-Tabs® have different excipient list (Table 3). This may result from different chemical properties of the APIs in each product, requiring different functional excipients to regulate *e.g.* bioavailability, stability, or other properties. Also, the development of the drugs may have been done in different time and place where the starting point of choosing excipients had been different, resulting in use of different ingredients for each drug.

3.3 Effects on ICP-OES Analysis

Wide variety of different ingredients in drugs alters the elemental analysis resuls compared to analysis of water based samples. Excipients add mass to the samples, and the sample matrix becomes difficult to handle with ICP-OES without sample digestion and dissolution. Many excipients and APIs are virtually insoluble in acidic water solutions, such as minerals, glycerides and waxes. Therefore it is common in elemental impurity analysis to use powerful digestion methods and concentrated acid matrices to digest organic material and inorganic minerals, so that the metals are found as aquaous ions in the sample solution, as discussed

Table 3: Comparison of excipients in two common analgesics in Finland, excipients in bold are found in both drugs. Items not in order of amount (source: `www.laakeinfo.fi`, accessed 14.3.2018)

Burana® 600mg	Para-Tabs® 1g
Magnesium stearate	**Magnesium stearate**
Microcrystalline cellulose	**Microcrystalline cellulose**
Waterless colloidal silicon dioxide	**Waterless colloidal silicon dioxide**
Titanium oxide	**Titanium oxide**
Macrogol	**Macrogol**
Gelatin	**Gelatin**
Polyethene glycol	Sodium starch glycolate (type C)
Sucrose	Talc (magnesium silicate)
Hypromellose	Partly hydrolysed polyvinyl alcohol
Crosslinked sodium carboxy methyl cellulose	
Polysorbate 80	
Glycerol	
Lactose monohydrate	

in section 4.2. Even though "complete" digestion is achieved, sample matrix effect can be challenging, as it effects sample transport and excitation state in the plasma, as discussed in section 4.5.

In addition to matrix effect, many excipients and APIs are salts of organic and inorganic substances. One of the most abundant salts in excipients are magnesium stearate, magnesium silicate, titanium oxide, and sodium salts of cellulose or starch derivatives. Elements with strong emission lines such as aluminum, iron and titanium are also commonly found in excipients. Especially titanium and red iron oxide are popular film coating ingredients.[28]

Different formulations challenge ICP-OES method development and validation for pharmaceuticals, because using the same method for different drugs, validation characteristics such as robustness of the system or trueness of results and so on, may change from sample to sample. Method validation should then be done for each element analysed, and also for each different sample matrix.

3.4 Typical Elemental Impurities

Q3D Class 1 elements As, Cd, Hg and Pb typically are introduced in final pharmaceutical products from excipients made from mined materials.[8] These trace elements are typically impurities in minerals, where they may replace certain metal ions in the lattice. They also are characteristic for each mineral. For example, As, Cd, and Pb are all chalcophilic elements, which form substances with sulfur in the soil. They also can be found as impurities in silicates.[10] The other classified elements in excipitients can be quite hard to predict. There are more than one thousand different excipients used in pharmaceutical industry, therefore the different impurities from excipients may also include unexpected elemental impurity, and it is usually harder to monitor the impurities. The excipient suppliers do not always provide all sufficient certificates of their products to easily do the ICH Q3D risk assessment – in the worst case scenario, the elemental impurity analysis and the responsibility of monitoring may be left somewhat entirely for the buyer. Quality control and assurance of separate excipients in pharmaceutical companies are usually not as well and throughoutly monitored as the APIs.[25,27]

Elemental impurities resulting from manufacturing of the API come most cases from common reagents, solvents and catalysts used in the process. One of the most monitored element in many API products is palladium, because of it's big role in organic synthesis – Palladium catalysed carbon-carbon and carbon-heteroatom coupling synthesis reaction steps have been popularised in many drug manufacturing plants. Even though catalysts are in most cases bound in carbon or polymer material, and the removal of the catalyst materials from the final synthesis products and other refinement processes, palladium levels in APIs and final pharmaceutical products are a great concern in pharmaceutical industry, because of it's low permitted daily exposure.[29] Also Ir, Os, Pt, Rh and Ru may be used as catalytes and must be considered in risk assessment. Cr, Cu, Mo, Ni and V are more amenable to contaminate the pharmaceutical products by contact with materials, where they are commonly used or found as imourities in coatings and materials. These include *e.g.* reaction vessels, mixing tanks, filters, fillings, containers and packaging.[20]

4 Determination of Elemental Impurities in Drug Products

4.1 Sampling

Sampling is defined as a process of collecting a representative sample for analysis[30] and it is one of the most critical parts of an analysis. Sampling must be done correctly to acquire reliable and correct results. It is extremely important that the sample for analysis is fit for its purpose. the whole analytical process is meaningless if the sample is not suitable or representative of the target material being analysed. In terms of pharamaceutical products, a *sample* can be solid and well defined object, for instance a tablet, pill or capsule. The samples are contained in some kind of container, package, bottle or blister. In this case, it is easy to define a *sample* to be one individual tablet, capsule or pill. Pharmaceutical products can also be in liquid form, which makes defining a *sample* a bit harder. Liquid products can be packed in single dose containers like ampoules, or bigger bottles where consumer can dose the amount of drug they need.

A system for sampling and sample management is used in analytical chemistry, what describes different steps in sampling process.[30] Flowchart for sampling is shown in Figure 4. A *lot* means the total material from which the sample is taken, like a single batch of drug product made in the factory. A *bulk sample* is taken from the *lot* and it is smaller but representative sample of the bigger *lot* for analysis. A laboratory sample for analysis is taken from the *bulk sample*. *Aliquots* can be taken from the laboratory sample as replicates for the measurements.

ICH Q3D guideline for elemental impurities does not offer a distinct definition for a size, or state of pharmaceutical *sample*.[8] In the *USP* chapters 232/233 a example is presented where sample size in wet-digestion technique is 0.500 grams.[12] In some research articles on

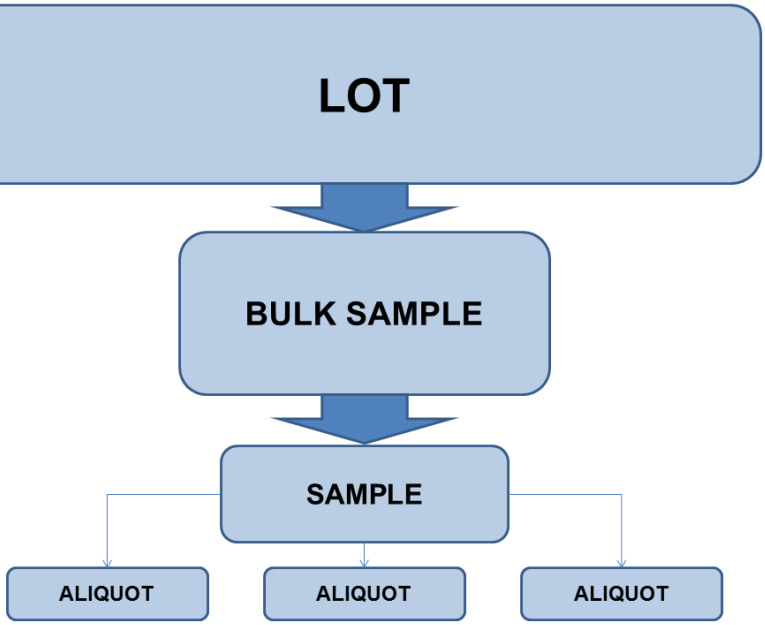


Figure 4: Flowchart of sampling procedure

analysing elemental impurities in drug products, sample is a whole tablet or capsule and several tablets or capsules are taken as replicate samples for analysis.[31] Different approach used by Schramek *et. al* is to homogenise a bigger amount of *bulk sample* and take a lab sample of standard amount of 0.500 grams from it.[32] Sampling for Orion's elemental analysis is done by analysing products as a whole. In Orion's elemental analysis it is pursued that the lab sample is as similar as the product that consumer uses.

The advantage of the method used by Schramek *et. al* is that sample is ensured representative of the material analysed. A big homogenous amout of the *bulk sample* is grounded, for example one hundred tablets, and a standard amount of that sample is taken for analysis. Representativeness is better with this method than taking a single or just few tablets as a sample without grounding them. However, as there are additional steps in sample preparation, the statistical uncertainty of analysis results become greater. Grounding the sample also increases sample contamination risk, for instance elemental contamination, cross contamination from other samples ground in the same system, container contamination and so on.[30] Grinding system should be able to homogenise the tablets, capsules and pills properly without leaving big chunks of different parts of the sample. Orion and Stürup *et. al* research group[31] use a different way of sampling as they use unground samples. In this method sampling and preparation has less steps, therefore making sample preparation quick and fluent.

4.2 Sample Preparation and dissolution

Sample preparation for ICP-OES usually requires the sample to dissolve to the used acid and solvent in order to the analysis be quantitative and reliable. Drug tablets and capsules are known to be challenging type of sample due to large amounts of excipients, coatings and active ingredients being poorly soluble to water based solutions (see chapter 3). It is common to aid the sample dissolution by transferring energy to the aliquot some way; raising temperature or pressure, mechanical strirring and so on. Most common and functional ways to assist sample dissolution are microwave and ultrawave digestion systems.

4.2.1 Microwave digestion

Microwave digestion is one of the most effective sample digestion techniques available in commercial analytical laboratiories.[30] In some cases it can be relatively slow and expensive digestion method, but especially coupled with closed high pressure reaction vessels, a complete digestion may be achieved with some sample matrices. Metal cations become dissolved in water thus being available for ICP-OES analysis. It is also recommended digestion method by US Pharmacopoeia.[12]

Digestion of pharmaceutical excipients is found to be effective with closed vessel microwave assisted methods. For example, in an article by Li *et. al* analyte recoveries of Q3D elements were very good and low detection limits were acquired.[18] Digestion method used mainly HNO_3 – HCl acid mixture in 1:1 ratio, but included H_2O_2 for some organic samples and HF to deal with poorly digesting compounds (e.g. talc, TiO_2, SiO_2).

A 2012 article by Niemelä *et. al* focused on comparing recoveries of Pt, Pd, Rh and Pb in CRMs by ICP-OES using different closed vessel microwave digestion methods.[33] The methods used mainly 1:3 HNO_3 – HCl (*aqua regia*) and HNO_3 – HCl – HF acid mixtures, and yielded generally good recoveries with both. Addition of HF improves the recovery if Si, but doesn't affect other elements. Furthermore, impact of the digestion temperature was studied with *aqua regia*, in temperatures greater than 160 °C recoveries were considered good. Under that temperature digestion was generally uncomplete, and going to higher temperatures than 160 °C the digestion efficiency was not significantly increased.

There are also several articles about analysing elemental impurities from finished, consumer drug products. A single reaction chamber (SRC) digestion system was used for analysing pharmaceutical products and compared to traditional multiple PTFE vessel using system in article by Muller *et. al.*[34] higher temperatures and pressures in SRC digestion resulted in lower carbon residues in analysis samples, which may translate to better analysis performance of ICP-MS and ICP-OES analysis. However, solid digestion residue were found in the vessels and vials when using HNO_3 or $HNO_3 - HCl$.

Figure 5: A single reaction chamber microwave assisted digestion system UltraWAVE equipped with control unit (right), manufactured by Milestone Srl. Samples in test tubes made of regular or quartz glass equipped with pressure balancing caps are placed inside pressurised PTFE container.[35]

4.3 Analytics of Drug Products with ICP-OES

4.3.1 Overview of ICP emission spectrometry

ICP stands for Inductively Coupled Plasma, and OES for Optical Emission Spectrometry, or Optical Emission Spectrometer.[30,36] ICP-OES is modern and multi-functional technique for

analysing elements in different samples, including environmental, industrial, plant, tissue and pharmaceutical samples with a big range of concentrations. Quantifically measurable elements with one particular ICP-OES instrument are shown in Figure 6.

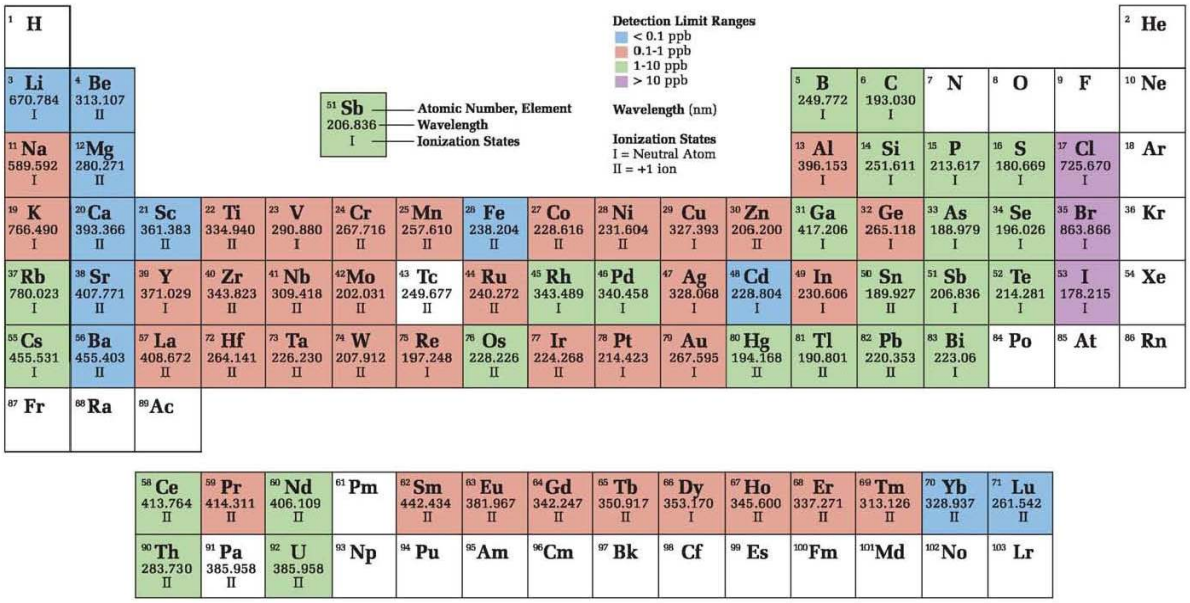


Figure 6: Colored elements measurable with Optima 7000DV ICP-OES -instrument (Source: Perkin-Elmer).

In ICP emission spectrometry, samples are introduced into inductively coupled argon plasma through sample injection system. Molecules are then atomised and ionised in the plasma and eventually excited. When the electrons return from excitation state to lower energ state, atoms and ions emit photons, which is called ionic or atomic emission. Emission is then collected to a detector using Echelle or Rowland circle optics, which separate different emission wavelenghts from each other.[37] The detector then measures the emission signal intensities for respective wavelenghts. Emission wavelenghts correspond to the difference in energy of the excited and ground state of the electron (see Figure 7). Every element has its characteristic emission spectrum and strong emission lines. Using this information and that the intensity of the emission signal is relative to the abundance of the element in the plasma, it is possible to determine the concentration of each element in the sample, which is done by using calibration by known standard concentrations.

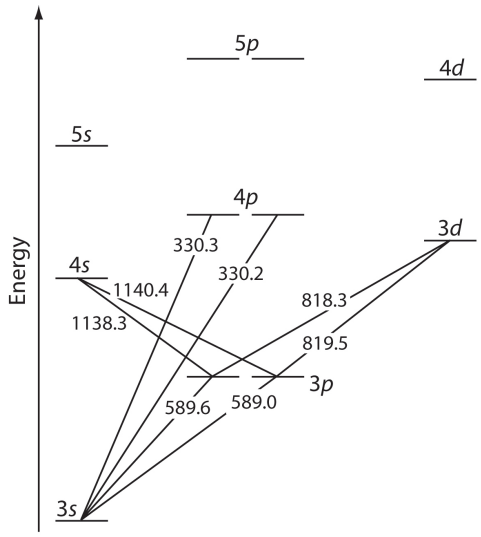


Figure 7: Portion of the valence electron structure of sodium. Lines and wavelength between orbitals correspond to differences of energy states between them.

4.3.2 Inductively Coupled Plasma

Inductively coupled plasma in ICP-OES instrument is argon gas ionised in a plasma torch.[36] A radio frequency (RF) generator creates alternating current in an induction coil or other inductive structure (for example a *flat plate*[38] as in Figure 8). Due to electromagnetic induction[39], a magnetic field is generated around the coil. An ignition spark initiates argon ionisation and the magnetic field around plasma torch accelerates the argon ions and electrons, which makes up the plasma.

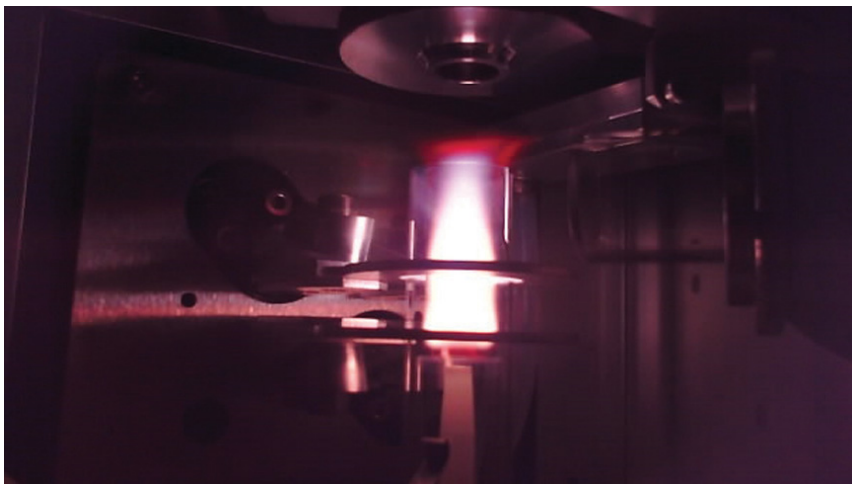


Figure 8: Avio 200 -spectrometer with *flat plate* plasma technique (Source: Perkin-Elmer `https://www.perkinelmer.com/corporate/stories/Introducing-Avio-200.html`).

The emission from plasma and analytes in it are collected using several optical apparatus, typically commercial ICP-OES instruments use two different plasma views, axial and radial (Figure 9. The emission signal is collected either from the side of the plasma (radially to the direction of the argon plasma flow) or from the top of the plasma (axially towards plasma source). In radial view, only a narrow volume of the plasma is viewed. In axial view the plasma is viewed through the central channel, which basically views the entire plasma from top to bottom. Radial view is considered less sensitive to the analyte emission signal than axial, because only a small part of the emission is seen. It has its advantages in dealing with interferences caused by excitation states in different parts of the plasma – unwanted regions of the plasma may be excluded from the signal. Radial view is useful analysing difficult matrix samples with high analyte concentrations and analytes with lines with high intensity, such as iron.[36]

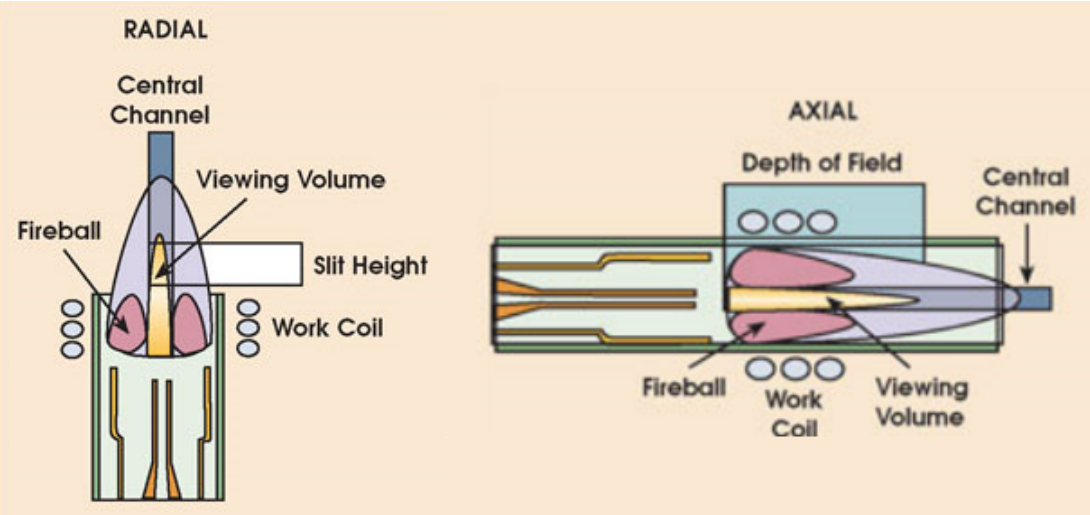


Figure 9: Plasma views used in ICP-OES instruments. In both figures, plasma is viewed from the right side (Modified after: `https://www.photonics.com/a18395/Inductively_Coupled_Plasma_Fuels_Elemental` visited 13.4.2018)

Performance of the inductively coupled plasma is a key factor of success on ICP-OES and ICP-MS analysis techniques and it can be measured using a term *plasma robustness*.[36,40,41] Plasma robustness describes ionisation and atomisation conditions in the plasma. Mermet with his research group defined that in robust plasma conditions any changes in sample matrix does not significantly affect the analysis line intensities. Generally in ICP-OES, measure of robustness is ratio of Mg II 280.270 nm/Mg I 285.213 nm ratios ionisation lines; plasma is robust, if the ratio is more than 10. Another way to measure robustness is comparing two different analyte lines with the same ionization and excitation energies. This method

should be considered especially with use of internal standards. This can be seen with Rh II 233.5 nm and Pb II 220.3 nm lines. Ionization energies are 7.46 and 7.42 eV; excitation energies are 7.40 and 7.37 eV, respectively. The Rh 233.5/Pb220.3 intensity ratio should stay relatively constant in robust conditions. If excitation state in plasma decreases, ratio should dip down, indicating non-robust conditions. This behaviour indicates that internal standardation should be done only in robust plasma conditions, because non-robustness may possibly cause this uncorrelated behaviour between analyte lines.[42]

The greatest factors affecting the plasma robustness are large RF power and low sample injection and nebuliser gas flows. A figure (Figure 10) from article by Silva *et. al* is shown below, which shows a way to discover robust conditions in elemental analysis with a dual view ICP-OES instrument. The vertical axis shows ratio of Mg II/Mg I lines, which indicates excitational state in the plasma – the higher, the better. The nebulation gas rate of sample injection system including nebulizer and nebulizing chamber leading to plasma is then increased in small increments, and intensity ratios are calculated.[41] Figure (a) shows measurement done in axial plasma view, showing narrower area with high ratio than in Figure (b), meaning nebulizer gas flow adjustment is more important in axial than radial view – in radial view ratio is constantly lower (less robust) than axial view, but clearly bigger room for maneuver with instrument parameters. This behaviour is observed with dual-view plasma instruments, where the radial view uses longer optical path than axial view, such as Perkin-Elmer Optima 3000 DV used in the article.

Mere plasma robustness does not ensure good analysis sensitivity and performance. Firstly, the greatest available RF coil power should be used in the ICP-OES instrument. If sample and nebuliser flows are too low, signal to background noise ratio may be lowered and detection limits may get worse, which is not a desirable thing in trace analysis. Also random error of droplet formation increases variation between replicate measurements and samples may grow, thus making RSD% bigger.[40,41] Inductively coupled plasma is also used in other spectrometric techniques thanks to its robustness and good atomisation and ionisation potential. Maybe the most well-known technique is ICP -mass spectrometry, which is can get to even 100 to 1000 times lower detection limits than ICP-OES.[16,36] Instrument's plasma robustness correlates directly to the robustness of the analysis method, which is a method validation characteristic discussed in section 5.3.

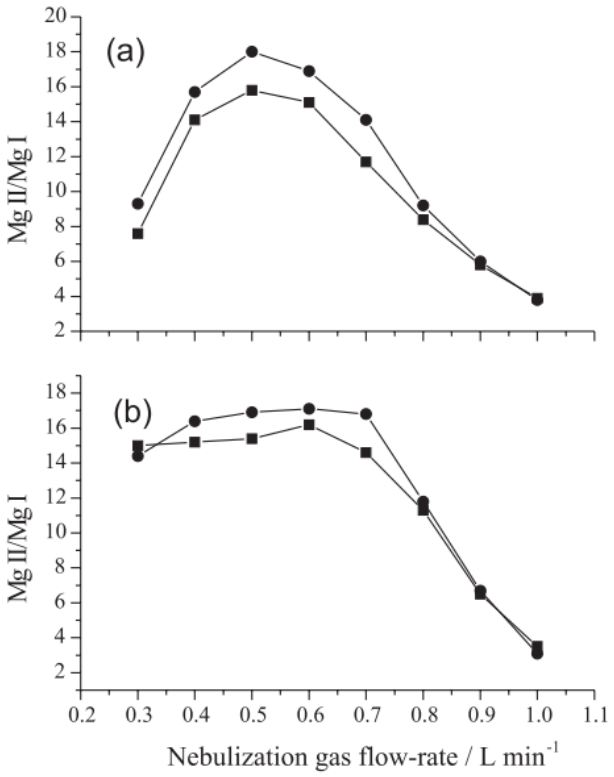


Figure 10: Effect of nebulization gas flow-rate on Mg II/ Mg I ratios using a dual-view instrument (a) axial ICP-OES (b) radial ICP-OES with applied power of 1,3kW. (■) 1% v/v HNO_3 and (●) 10% tertiary amine solution, cited from Silva *et. al*.[41]

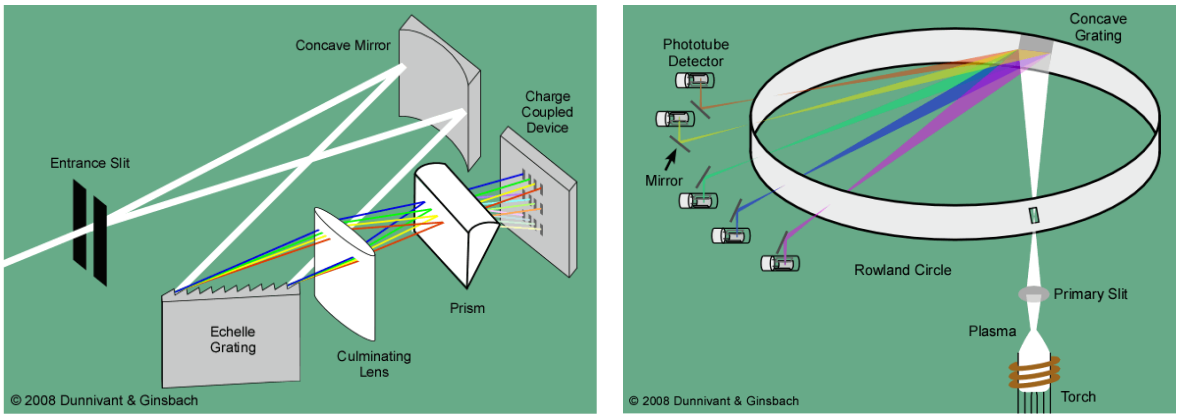
4.3.3 Emission Spectrometer

In ICP-OES instrument the spectrometer part consists of several optical systems and a detector. First the total electromagnetic radiation from the argon and analyte atoms/ions in plasma is collected using an entrance slit, mirrors and lenses. From this wide spectrum of visible light and other wavelengths the emission signals are then separated using polychromators. Two types of optical systems are widely used in commercial ICP-OES instruments (Figure 11).

Echelle grating is the most popular solution with good resolution power for most of analyte lines between approximately 200–450 nm. Echelle grating systems consist from concave mirror, which collects the signal to the echelle grating, where the separation of different wavelengths happen. Polychromated light is then directed to a prism using a culminating lens and then refracted to the detector.

The other, and less popular, system is Rowland circle optics. It uses an optic circle system where the signal from plasma is introduced trough a slit and refracted using concave grating.

This refracts the different wavelengths to different directions on the perimeter of the circle, where the detectors are positioned. Rowland circle optics are usually large thus instruments using these optics require more space, resulting in bulkier instruments than echelle spectrometers. However, the good feature is better resolution in sub 200 and higher than 450 nm wavelengths.



(a) Echelle optics

(b) Rowland optics

Figure 11: Typical optical system types in commercial ICP-OES instruments, in rowland optics (b) CCD/CID -type array detectors are used instead of phototubes these days. (Source: Dunnivant & Ginsbach[43] `http://people.whitman.edu/~dunnivfm/FAASICPMS_Ebook` visited 15.4.2018)

Both of these techniques use similar detector technology, namely coupled charge device (CCD) or charge injection device (CID) detectors. On the detector separate ”slices” of wavelengths, or analyte lines, are then collected on separate pixels on the doped silicon semiconductor, which translates the emission lines into electrical signal, which can be processed using a computer software.[30,36] The measurement process of the different emission lines may be done either sequentially or simultaneously, depending on how the spectrometer operates. Sequential measurement means that emission lines are collected one after another, and the optics in the spectrometer are adjusted between each line. In simultaneous measurement all of the wanted lines are collected at once. In many ICP-OES instruments with dual-view configuration, each line characteristic to a view is measured simultaniously, but radial and axial views are measured sequentially (semi-simultaneous instruments). Obviously, sequential instruments are slower than simultaneous instruments, especially doing multi-element assays. The resolution power of the instrument is lower than in simultaneous measurements, which lowers the selectivity of ICP-OES method. Also the drift of the analyte signals during sequential measurements is much greater. This may render the use

of an internal standard ineffective, because of optics adjustments between the analyte and internal standard measurements – the line of internal standard line should be always be measured simultaneously with the analyte line. Because of these factors causing uncertainty in measurements, should modern simultaneous (or at least semi-simultaneous) instruments be used in accurate elemental analysis.

4.4 Detection Limits in Trace Analysis

When measuring trace amounts of elemental impurities must detection and quantification limits taken into account. A lot of different ways to measure limit of detection (LOD) and limit of quantification (LOQ) are introduced, but there still is some debate which is the most accurate and good way to do it. An ICP spectrometry expert, Jean-Michel Mermet discussed about the industry standards and some alternative ways on acquiring detection limits in his 2007 article[44]. The most used method for determining LOQ is ten times the standard deviation of blank signal[45], and it was shown by Mermet that it suffers from severe limitations. For example, it assumes that signal noise is gaussian distributed, amount of measurements is insufficient , there is a possibility of outliers and it only considers instrumental limit of quantification and not the method itself.

There are several publications about development and validation of ICP-OES elemental impurity analyses which use the usual LOQ is equal to $10s'_0$ approach.[4,20,31,46] Sometimes additional confirmation of the LOQ is conducted using spiked samples near LOQ concentrations and relative standard deviation (%RSD) of replicate samples is determined.[47]

In some articles detection limits were determined using linear regression of spiked samples. Schramek *et. al* calculated limits with five samples spiked near expected LOD concentrations of analytes according to a german standardisation institute document DIN 32645.[32]

4.5 Interferences in Measurements

4.5.1 Matrix Effects

Matrix effects in ICP emission spectrometry stand for the non-spectral interferences in measurements. They are some variables, which cause physical differences in the samples with respect to "normal" behaviour of samples *e.g* pure water or different kinds of acid solutions. Matrix effects may alter the sample behavior in nebulisation, atomisation or ionisation processes.[48] Selection of acid used has a significant effect on the matrix effect, affecting both the nebulisation of the sample and excitation and ionisation processes in the plasma. The acid itself may also add more matrix into the sample. For example, using sulfuric acid H_2SO_4 may seem appropriate for digesting organic samples, but it's matrix effect is substantial, lowering all analyte intesities due to shifted baseline. Sulfuric acid makes the solution more viscous than other widely used acids, even in small quantities. This affects on the aspiration rate, aerosol generation and transport, and plasma temperature decrease, which leads to low recoveries.[49] Another example of matrix effect caused organic matrix in ICP based techniques is systematically excessive (greater than one hundred percent) spike recoveries on some elements, such as arsenic.[50] Abundant alkaline and earth-alkaline metal ions may also alter excitational state of the plasma, causing matrix effect.[15]

Dealing with the non-spectral interefences is usually routine for pharmaceutical elemental analysis with ICP emission spectrometry. Pharmaceutical samples are considered difficult sample matrices, due to varying ingredients of tablets and capsules, abundance of organic and inorganic excipients with low solublitity to acid water solutions *et cetera*. Using internal standards, standard addition method or matrix matched standard solutions are the usual ways to deal with matrix effect. Internal standard in ICP emission spectrometry means a standard addition of a analyte not present in the sample to all sample and standard aliquots. Then multi-variable evaluations are used to correct the matrix effect. Internal standard element for analysis method must not be abundant in the sample matrix.[30,36,48,51]

Using internal standards such as yttrium (Y 371.030 and 360.073 nm) or scandium (Sc 361.384 nm) have been succesful ways to deal with Na and Ca matrix effects, as regards Cd, Co, Cr, Ni, Pb, and V analytes. Also other Y and Sc lines have had succes as internal

standard.[48] Using yttrium internal standard and phosphate precipitation method has also been succesful with pharmaceutical trace analysis.[51]

4.5.2 Spectral Interferences

According to an article by Zachariadis and Sahanidou, titanium compounds in the sample matrix, *e.g.* TiO_2 in tablet coatings and excipients does not have significant effect on recovery of Al, Zn, Mg, Fe, Cu, Mn, Cr, Pb in elemental analysis of sunscreens, even in moderately high concentrations as 20 $mg\,l^{-1}$.[52] HNO_3 – HCl – HF acid mixture and microwave assisted digestion was used in the study. Zachariadis implies that this also applies to pharmaceutical samples, in his 2011 article.[28]

Iron's analyte lines may cause interferences in sample matrices rich in iron, since their high relative intensities. The analyte lines may widen and cause intensity baseline to shift around these lines. For example in 2001 article by Gouveia *et. al* claims that Fe ionic line II 247.857 nm caused positive interference in carbon 247.857 nm wavelenght, causing 117% carbon recovery when Fe concentration was 100 $mg\,l^{-1}$[53]

Main interferences determining lead are iron and aluminum. Iron has also other emission line with great intensity at 248.327 nm which may also cause positive interference, when iron is abundant in the matrix. Also aluminum gives interference to the most sensitive and widely used lead analyte line Pb 220.353 nm.[54]

5 Validation of Elemental Analysis

5.1 Error in analytical process

Uncertainty is always present in laboratory, and finding ways to deal with it in the analytical processes is one of the most common challenges in analytical chemistry. There is always some level of uncertainty in analytical methods, procedures and personnel conducting chemical analysis, therefore random error and systematic error must be taken into account while developing and doing chemical analysis.

Random error is caused by uncertainty in analysis, and it causes variation in results, individual results falling in both sides of the average. Systematic errors cause the results to be errous in the same sense, for example all the results being too high or too low. These both error types can arise in all the steps of the analytical procedure, causing uncertainty in the final results. Gross error describes the bigger errors, which are so critical, that require abandoning the experiment and redoing it. This can mean mishaps in the laboratory, such as dropping the sample, pipetting too much, and so on. The effects of error in analytical procedure is shown in figure 12. In this graph it is illustrated, how important the error is in different steps to the whole analysis. The importance escalates early in the process in sampling and sample handling, and how the result data is processed and interpreted in the end. The instrumental error is actually really small factor in the success of the whole analytical method.[36]

Analytical chemistry is by nature both quantitative and qualitative science. The quantitative nature is present in wide spectrum of applications, because usually the research question is in form of "*how much*", or "*what is the concentration*" of the analyte in question. Therefore the errors arising from the analytical processes may be tackled using statistical methods. These statistical methods may include using statistical repeated measurement data to estimate

confidence limits, propagation of random and systematic errors, doing significance tests such as t-test, F-test, analysis of variance, outlier tests, ANOVA, and so on. These statistical methods have a stable status in analytical method validation procedures, and they often are the underlying principles of them, even though all method validation guides do not include that much of statistical models in them.[55] Statistical methods often give reliable verification on the quantitive results, in addition to careful and accurate practical work in the laboratory, therefore being very popular tool among analytical chemists in all industries.[45]

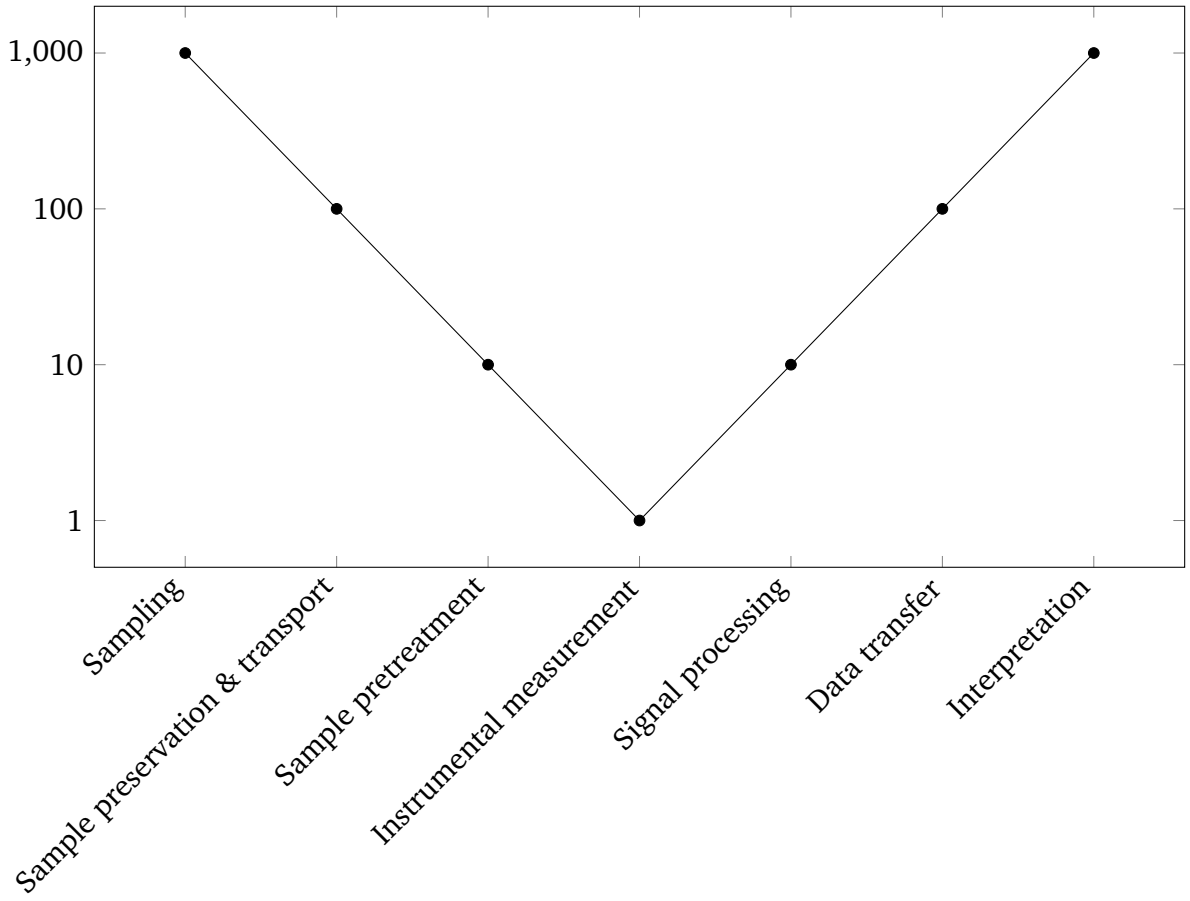


Figure 12: A rough illustration of the relative importance of typical analysis errors.[36]

5.2 What is Validation?

When developing and using different kinds of analytical methods in chemistry laboratory, it is usually taken as granted that the method is valid and good for its intended use. It kind of comes within the process of making the analysis method and many times it isn't thought out too much. Often this is the case, especially when the analysis results are meant only

for a scientist, a research group, or a student doing some experiments for their own use. Validation becomes highly important when the values obtained and quality of the analysis results are very significant, the results are sent to someone who has not participated in the analysis process, or both. In order that the receiver can trust that the process is valid and the results are correct, must a comprehensive ensurement be done. Ensuring the processes obtaining analytical results are called method verification and method validation, which respectivily mean a bit different kinds of quality ensurement processes. The connection between uncertainty and method validation is strong – all the method validation characteristics try to measure different aspects of gross, random and systematic error arising from analytical methods, and set limits and requirement for analytical methods in order to have sufficient control over uncertainty.

Verification is defined by VIM (*International vocabulary of metrology – Basic and general concepts and associated terms*) as: "Provision of objective evidence that a given item fulfils specified requirements"; validation is defined in ISO 9000 standard: "Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled ".[55] As defined, verification is contained in a validation procedure. Verification processess are done to get a certain process validated. Validation is a larger process and it ensures that a process is fit for a defined task, it can be applied to all kinds of processes, including instruments, work procedures, spreadsheets and so on.[56] The difference of verification from validation is that verification is an action to confirm something. One way to describe validation could be a more of a combination and review of different kinds of verifying actions.

When talking about validation in analytical chemistry, people usually mean analytical method validation. Validated method is proven to be reliable and suitable for its use in defined circumstances, and the uncertainty of the results estimated on a given level of confidence.[55]. Method validation does take some amount of work but it provides laboratories with solid information and evidence on the performance of methods, thus giving more confidence on their lab work. Validation process can be done using single-laboratory validation approach, when the method is developed and used for only one laboratory. If the procedure is used more widely or meant to be published as a standardised procedure, interlaboratory validation may be necessary. Then additional verification in different laboratories is done and the results compared.

Validation is connected strongly to method development work. Developing new method or making changes to existing method is often based on or build around method validation criteria. The criteria for method validation depend on the nature of the method and are set by different guidelines. In pharmacological industry, most of all method validation criteria come from national and transnational pharmacopoeia, and other monitoring organisations, such as transnational ICH, United States based FDA, and finnish FIMEA. Orion's validation criteria and guidelines are written in documents called standard operation procedures (SOP), there are sevelar Orion and Fermion SOPs related to process, analytical laboratory, and method validation. SOP documents are put together from the previously mentioned organisation's applicable guidelines, mainly guided by ICH and USP guidelines.

5.3 Method Validation Characteristics

Method validation is typically build around certain characteristics, which will identicate the performance of the method. Typical characteristics are shown in table 4. Criteria for the method are set based on the performance characteristics for verification. Due to analytical chemistry being such a large field, there is a lot of different ways to understand and handle method validation, therefore there is a lot of characteristics on the list. Some analytical methods handle characteristics different ways, for example in ICP-OES working range always is within calibration and linearity, which why they are usually dealt with together in ICP-OES method validation. Some of the characteristics share a lot of features and can be described using other names, such as recovery sometimes meaning trueness/accuracy.

There are some characteristics which are almost always present in method validation and mentioned frequently. They are selectivity, linearity, accuracy, precision, working range, and detection and quantification limits. Lot of other characteristics are somehow connected to these characteristics, or describe the same thing different way.

Application of these characteristics for ICP-OES methods are discussed in section 5.4.

Table 4: Typical analytical method performance characteristics according to different bibliography sources

Characteristic	References
Applicability	[56]
Selectivity (or Specificity)	[55,56,57,58,59]
Calibration and Linearity	[56,57,58,59]
Accuracy (Recovery/Trueness)	[55,56,57,58,59]
Precision	[55,56,57,58,59]
Working Range	[55,56,57,58,59]
Limit of Detection	[55,56,57,58,59]
Limit of Quantification	[55,56,57,58,59]
Analytical Sensitivity	[55,56]
Ruggedness (Robustness)	[55,56,58]
Fitness for Purpose	[56]
Matrix Variation	[56]
Measurement Uncertainty	[55,56]

5.4 Applying Method Validation to ICP-OES

Validation of ICP-OES methods for elemental analysis includes some widely used standard procedures to measure method performace characteristics, validation procedures of pharmaceutical and trace element analysis methods are very similar in many articles and other literature.[11,15,16,36,45,55,60,61,62] Not all performance characteristics discussed earlier apply to all ICP-OES method validations, next we will discuss some method validation characteristics in pharmaceutical trace analysis.

Due to simultaniuous nature of ICP-OES analysis, every performance characteristic must be assessed for every desired analyte in the method. This results from different chemical and physical properties of each element in the sample matrix, and every analyte may behave different way in the sample matrix, sample introduction, plasma and spectrometer optics.

5.4.1 Specificity / Selectivity

Selectivity is the ability to accurately quantify analytes in presence of interferences and measurement conditions. In ICP-OES selectivity means that the method must separate or process spectral interferences and different analyte signals so that they don't affect the measurement of an analyte. Specifity is that one specific analyte gives one signal response, which is unique for that analyte. Sometimes in ICP-OES method validation articles, the term selectivity is used when talking about specificity, and vice versa. This is understandable, because the difference of these terms is very vague in ICP-OES method validation. They both basically describe the same thing, distinguish the intensity signal of wanted analyte, but selectivity can be understood as "ultimate specificity" – for method to be able to be specific for several analytes. In this thesis specificity is used when discussing about separating different analyte signals from interferences. Specificity can be demonstrated in method validation by measuring (matrix matched) blank samples on each analyte line used in analysis, and doing recovery tests on samples spiked with analyte elements. Doing this it is possible to detect interferences coimg from matrix effects or spectral sources. This also opportunity to use background correction or other multi-variable interference correction methods to get as clear analyte line intensity as possible.[55,56,57,58,59]

5.4.2 Working and Linear Range

Because of elemental analysis by ICP-OES is relative method, must linearity of the calibration be verified. Quantitative analysis is based on calibration by emission signal intensities of known standards, which are then assigned to known concentrations. The working range of quantitative analysis method is also based on the linear range of the method, therefore working range begins from the limit of quantification and ends in the concentration of the highest calibration standard. Linearity of the calibration is determined by linear regression of the calibration curve. Typically in quantitative trace analysis methods of pharmaceuticals linear regression must be r^2 at least ≥ 0.990 or ≥ 0.9990 depending on desired accuracy and precision, and relative standard deviation of the calibration standard replicates to be low.[55,56,57,58,59]

Linearity cannot be ensured above the highest calibration standard, so analysis results above the calibration curve are not considered reliable in many cases, especially in analysis of toxic elements, where it is important to assure concentrations to be under certain limit. Therefore working range must be taken into account during method development, so the calibration standard concentrations are not too low. However, if calibration standards are chosen to be too high or far apart from each other or there are too many of them, r^2 may get too low and linear calibration becomes unreliable.It is widely considered an industrial standard in ICP-OES methods to use three or two point calibration curve (in addition of blank sample calibration point).[11,15,16,62]

5.4.3 Precision

The most common measures of precision are repeatability, intermediate precision and reproducibility.[47] Repeatability is to be able to get similar results from analytes with several replicates (meaning %RSD between replicates is low) Intermediate precision is to be able to reproduce the same results in different circumstances, *e.g.* different day, by different person doing the analysis or different laboratory equipment (but still suitable for the method). Reproducibility may mean ability to do analyses in different laboratories, and should be considered if the method is meant to be used as a standard method in several different laboratories. Precision can be tested by doing sufficient amount of replicate analyses and conducting analyses in different occasions, and after that evaluating if results differ from one occasion to another. Evaluating Standard deviation, relative standard deviation and confidence intervals of the analysis results are one of the most used ways to do this.[55,56,57,58,59,60]

5.4.4 Trueness

Trueness is closeness, or fitness of the result to the true or hypothetical value which takes the systematic error of measurements into account. Sometimes the term accuracy is used to describe trueness, and there is some debate of using these terms, which is the correct way expressing this validation characteristic. In this thesis accuracy is described as total product of both trueness and precision.[55,56,57,58,59]

One of popular ways to measure trueness in ICP-OES is using certified reference materials (CRMs), and evaluating recoveries *i.e.* the difference of results to their certified values. There is a great value using CRMs in emission spectrometry due to vast amount of spectral interferences present all times in the measurement processes. CRMs also usually have suitable sample matrix in them which gives information on how well matrix matching is working on sample preparation.[11,15,16,45,62] If suitable CRMs are not available, known standard addition samples *e.g.* continuous calibration verification quality control samples (CCVs) can be used to verify that measured intesities do not shift during and between measurements. Trueness can be evaluated by comparing the results produced with an alternative analysis method, which has already proven trueness. Quality control samples may be prepared from commercial standard solutions or ready-made commercial quality control samples may be used. QC samples can give good reference to trueness of the results, but they don't give information about matrix effect on the samples.[47,58]

5.4.5 Detection and Quantification Limits

A lower limit for meaningful results must be defined in an analytical method. Terms limit of detection (LOD), limit of quantification (LOQ) are widely used to describe the ability of the procedure how small concentrations of analytes can be analysed reliably. Detection limits of different elements with ICP-OES are directly related to the relative intensity of the analyte line. Therefore, elements with weak emission lines (halogens, arsenic) have higher limits than ones with strong emission (*e.g* alkaline metals, alkaline earth metals).[30,36] Few more terms such as method detection limit (MDL) and intrument detection limit (IDL) are used in some laboratories to define limits for the instrument and different analysis methods separately. IDL is the lowest limits that an analytical instrument can achieve and usually acts as a basis for calculating MDL, which takes more of the uncertainties and interferences arising from actual analyses into account.[55,56,57,58,59]

One way estimating MDL is analysing seven replicates of reagent water (blank) samples fortified at a concentration of two to three times the instrument detection limit, and determining the standard deviation of the replicates.[63] Then MDL is calculated

$$\mathrm{MDL} = t_{n-1} \times S, \tag{2}$$

where t is the student's t-value for a confidence level of 99% with $n-1$ degrees of freedom, and S is standard deviation of the replicate analyses.

Most guidelines instruct laboratories to estimate detection limits by measuring signal of 10 replicates of blank samples or reagent blanks with no analyte concentrations, then calculating the standard deviation of these replicates.[55,56,57] When blank correction is not used routinely in the method, the equation for estimating LOD is

$$\mathrm{LOD} = 3s_0' = 3\left(\frac{s_0}{\sqrt{n}}\right), \tag{3}$$

where s_0 is standard deviation of the analyte blank signal and n is number of the replicates, and LOQ is estimated

$$\mathrm{LOQ} = 10s_0' = 10\left(\frac{s_0}{\sqrt{n}}\right), \tag{4}$$

and standard deviation for a statistical sample N is calculated as follows:

$$s_N = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2}\,, \tag{5}$$

where $\{x_1, x_2, ..., x_N\}$ are concentrations of the sample and $\bar{x}$ is the mean value of all sample concentrations. The $3s_0'$ LOD and $10s_0'$ LOQ methods have produced sub-ppm detection limits for trace analysis of Q3D elements in pharmaceuticals, ranging from LOQ of 0.83 $\mu g\,g^{-1}$ for zinc to 0.01 $\mu g\,g^{-1}$ for cadmium and manganese.[31]

Patel *et. al* calculated quantification limits of approximately 2.5 $\mu g\,g^{-1}$ for most of their trace elements lines (Ni, Cu, Zn, Fe, Mg, Sn, Pd, Pt, Ru) in their 2015 study.[62] The limits are relatively high compared to study by Støving *et. al*, due to more strict quantification limit calculations. The calculations involved signal to noise ratio and also additional recovery tests, to ensure that recoveries differ from 100% no more than ±8%. This way the possibility of the estimated quantification limit to be erroneously low is minimized. With Patel *et. al* method and confidence levels this high it is not possible to get as low quantification limits with ICP-OES as some Q3D elements such as lead requires, because of extremely low PDE of lead. ICH Q3D, USP chapters <232> and <233>, and other guidelines do not require

this kind of evaluation of quantification limits but use other measures to ensure the result verification.

More ways of estimating detection and quantification limits are discussed more profoundly in section 4.4.

5.4.6 Robustness

Robustness (or ruggedness) of the method is demonstrated as reliability of the analysis with respect to relatively small, but deliberate variations in method parameters.[55,57] Robustness indicates the reliability of the method in normal usage, where some method parameters may alter from day to day, *e.g.* as result of differences in temperature, atmospheric pressure, and humidity in the laboratory. Stages the most suspectible to parameter variations must be identified and tested during method developments using robustness (ruggedness) tests, which test the stability of each critical parameter. The usual test subjects in ICP-OES methods are power of the RF-generator, nebulizer gas and sample flows, and sample matrix variation.[15,16,36,45,62]

Robustness of the plasma and RF-generator can be tested by Mg I / Mg II intensity ratio as discussed in section 4.3.1. One example of robustness test of matrix variation is in 2007 article written by Zachariadis and Michos, where they compared slopes of the analytes using standard addition, with several different sample matrices (antibiotics and excipients). The slope should be near constant in different samples to demonstrate robustness. Comparison of standard deviation is shown in figure 13, and t he authors concluded that no major differences (greater than 10%) are found applying the method to different antibiotics and excipients.[64]

G.A. Zachariadis, C.E. Michos / Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 951–958

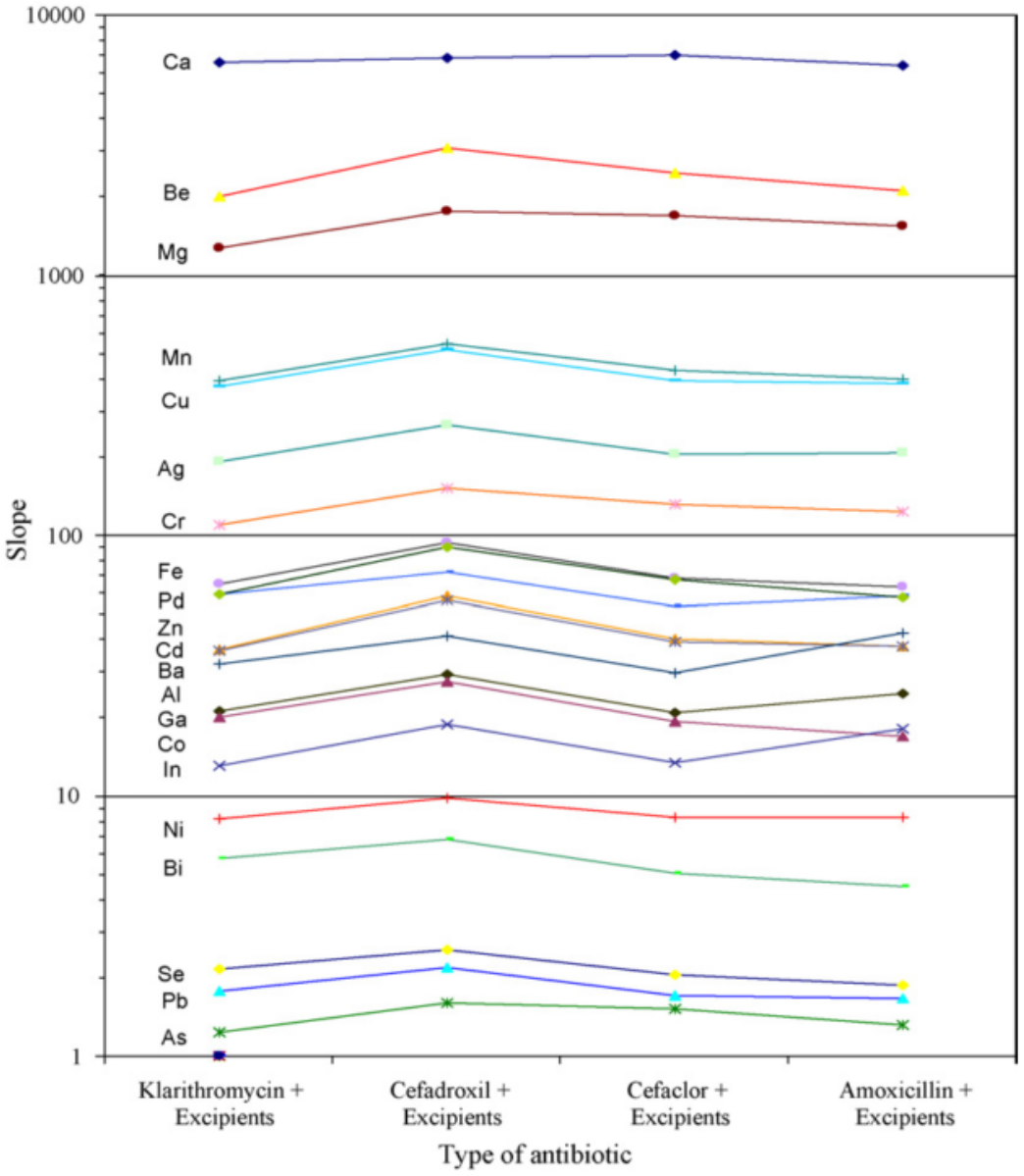


Figure 13: Example of a robustness test of the slope using standard addition, when a slurry analysis method is applied to different commercial antibiotics and several excipients.[64]

II Experimental part

6 Background and Objectives

The experimental part of this Master's Thesis was performed in the quality control laboratory of Fermion Oulu plant, which is an affiliate of finnish pharmaceutical company Orion. Fermion produces active pharmaceutical ingredients (API's) for Orion and other pharmaceutical manufacturers. This research project was inititated to fulfill the ICH Q3D guideline for elemental impurities in drug products. The guideline requires drug manufacturers to make risk assessments of harmful elemental impurities of their drug products which are on the market. The analyses were conducted and the results were evaluated and compared to permitted daily exposures of each analysed element.

The research objective was to develop a method for elemental analysis of pharmaceutical products. The method should be applicable for a wide range of pharmaceutical tablets, capsules, and so on. The method and it's development should be in accordance with good manufacturing practices of pharmaceutical products (GMP). Also a verification of the method performance and reliability was required.

In this lab work a comprehensive ICP-OES method for analysing elemental impurities in large amount of different type of drugs was developed and introduced. All of the products analysed were meant for oral administration except one product was for intravenous use. Main focus of the method development was to refine the method to work well for analysis of different kinds of tablets, capsules and oral suspensions, without loss of reliability and low quantification limits typical of ICP-OES. An actual method validation was not done according to Fermions SOP:s or cGMP:s but nececcary verification of the results were done to ensure reliability of analyses.

7 Reagents and Devices

In this work only pure and applicable reagents for trace analysis were used, presented in Table 5. Water was purified with ELGA water purifying system. All containers, bottles, sample tubes, volumetric flasks etc. were made of plastic, except disposable sample tubes used in microwave digestion were made of glass. All labware were rinsed, washed in a laboratory dishwasher, soaked in 20% nitric acid bath and rinsed again before use. Pipetting was done with Thermo FinnPipette 10 – 100 µl, 100 – 500 µl ja 1 – 5 ml micropipettes, which were calibrated regularly.

Table 5: Reagents used in the lab work

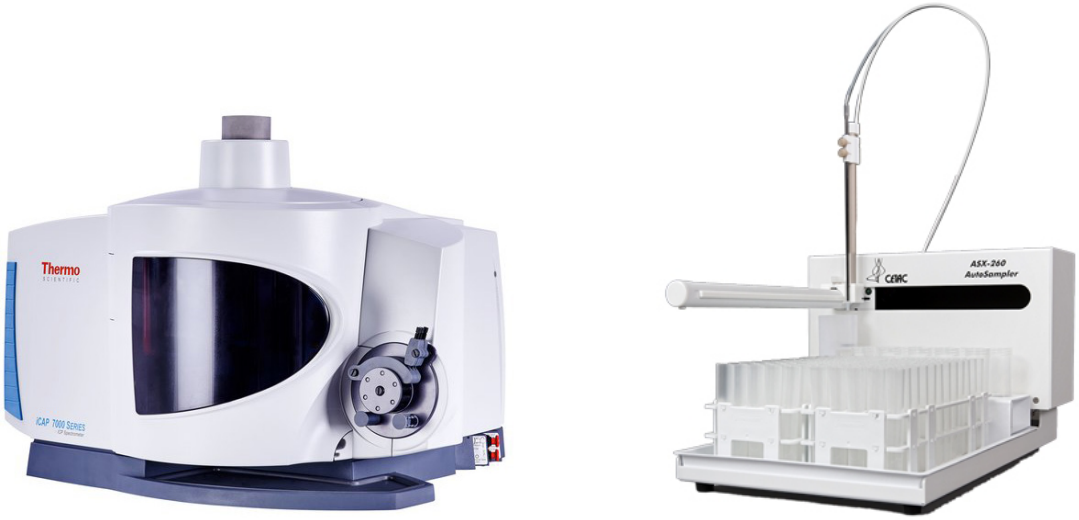
Manufacturer	Reagent
ROMIL	Nitric Acid SpA (Super purity Acid) (67 – 68 %)
Sigma-Aldrich	Periodic table mix 1 for ICP (33 elements[a], 10 mg/l)
Sigma-Aldrich	Periodic table mix 2 for ICP (17 elements[b], 10 mg/l)
Sigma-Aldrich	Hydrogen peroxide 30 v-%

[a] Al, As, Ba, Be, Bi, B, Ca, Cd, Cs, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mg, Mn, Ni, P, K, Rb, Se, Si, Ag, Na, Sr, S, Te, Tl, V ja Zn

[b] Au, Ge, Hf, Ir, Mo, Nb, Pd, Pt, Re, Rh, Ru, Sb, Sn, Ta, Ti, W ja Zr

FinnSonic ultrasonic bath with a timer and heating element was used in predigestion of the samples. Actual digestion was performed with Milestone UltraWAVE microwave acid digestion system. The device was a single reaction chamber digestion system (SRC), which means that capped sample tubes were located in the same PTFE vessel with each other within the reactor. SRC technique enabled several different materials and sample sizes to be digested at the same time. Temperature and pressure were stabilised between the sample tubes and the system monitored the conditions inside the chamber.

ICP-OES measurements were made with Thermo iCAP ICP-OES Duo -series spectrometer. Cylinderic plasma torch with radial window was installed in the instrument and plasma was produced with an RF-coil. The instrument had single CID-detector, which according to the manufacturer, enables better control over the measurement signal compared to more frequently used CCD-detector in ICP-OES instruments.[65] Emission was guided to detector using via optics using both axial view of the plasma. Cetac ASX-260 AutoSampler and peristaltic pump were used for sample introduction. (Figure 14). Instrument was operated and measurement data was processed using Thermo Qtegra Intelligent Scientific Data Solution -software (version 2.7).



(a) (b)

Figure 14: (a) Thermo iCAP ICP-OES instrument and (b) Cetac ASX-260 autosampler

8 Method Development

8.1 Preliminary tests

Osmium was decided to not be analysed at all, because of it's reaction with concentrated nitric acid, which produces toxic osmium tertroxide. It reduces stability of osmium in the solutions drastically, and is also a safety hazard for laboratory work. In scientific literature one article is found where stabilization of osmium with thiourea is used.[66] However, this method was not tested in this lab work because of time limitations.

A method for analysing elemental impurities in Fermion APIs was used as a basis for the new method to be used for the pharmaceutical products. The method development started from comparing the effectiveness of the sample digestion and dissolution, and different kinds of concetrated acid mixtures were used. These include nitric acid, hydrochloric acid, sulfuric acid, and they were diluted in lab water, to match 40% *(v/v)* acid concentration. For example, some of the recommended acid mixtures for pharmaceutical materials from the microwave digestion system manufacturer Milestone were used as a basis of the digestion method.[35] A variety of different sizes, digest the same tablets and capsules, and then an ICP-OES analysis was conducted, and the analytical lines of target elements were examined.

There was not found to be great visual difference in HNO_3 and the *aqua regia* $HNO_3 - HCl$ (1:1, 1:3 and 3:1 ratios) acid digestion power. Adding even small amounts of under 5% *(v/v)* of sulfuric acid to the acid mixture was found to increase the matric effect, causing baseline shifting or loss of sensitivity in many analytical ICP-OES line intensities. Baseline shifting is present in the lower wavelenghts (< 250 nm), for example when analysing lead, as seen in Figure 15.

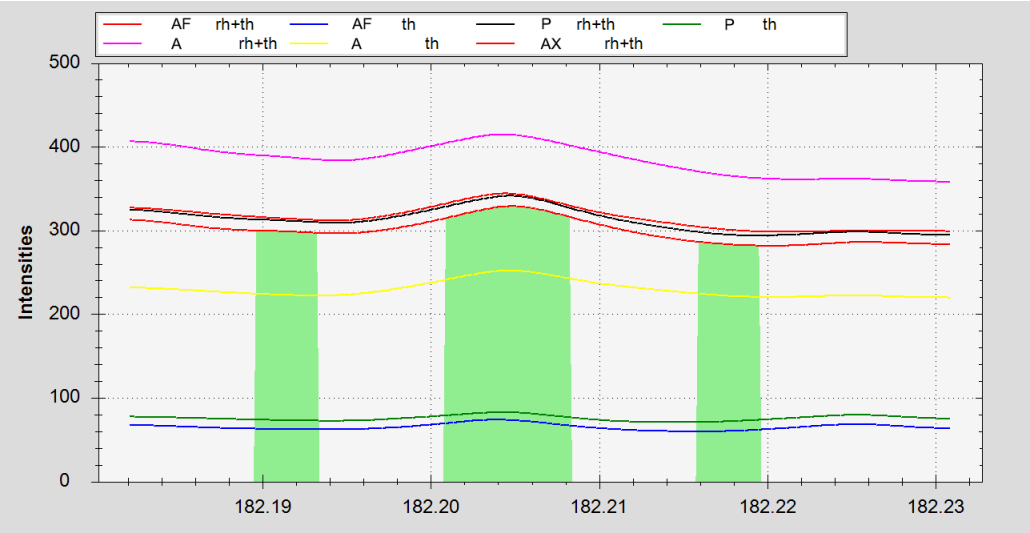


Figure 15: Comparison of nitric acid and sulfuric acid digestion with Pb 182 nm analyte line

The sensitivity loss caused by sulfuric acid can seen in Figure 16, where the respective analyte intensities (peak height from the baseline) of lithium 670 nm line for pharmaceuticals **AF**, **P** and **A** were systematically lower in $HNO_3 - H_2SO_4$ digestion (rh+th), compared to only HNO_3 (th). The 40% HNO_3 solution was chosen over *aqua regia* because of the potential corrosive effects of hydrochloric acid in the digestion system.[35]

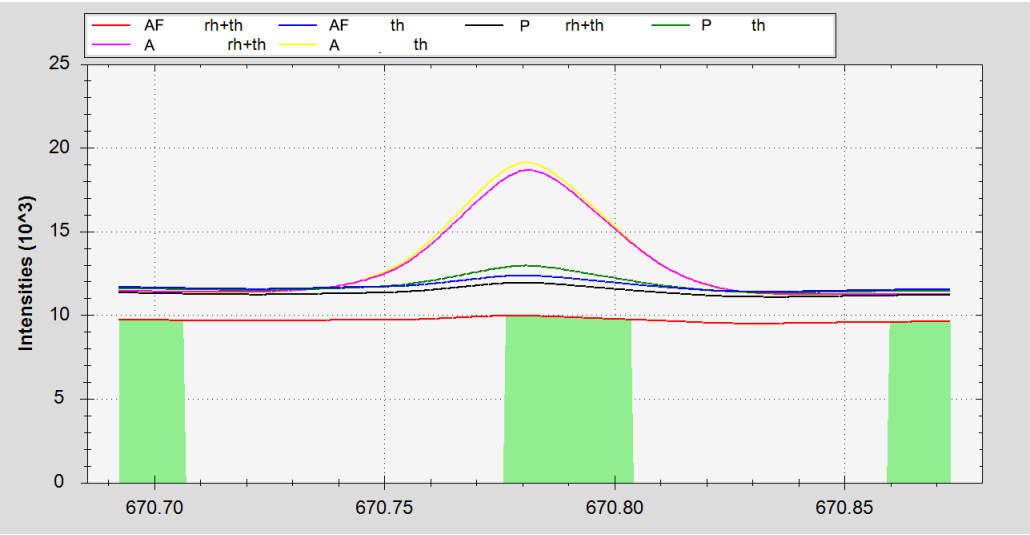


Figure 16: Comparison of nitric acid and sulfuric acid digestion with Li 670 nm analyte line

8.2 Target concentrations

The method used in the analysis of pharmaceutical products was based on several method developed for analysis method for analysing elemental impurities in API's. The API methods

were not as extensive as Q3D requires for pharmaceuticals, so the applicability of API methods were tested with wider variety of elements.

The target concentration J (in w/w unit) for each element were calculated to match 30 % of the permitted daily exposure. Used standard, spike, and QC solution concentrations in quantification were then assigned according to US Pharmacopoeia chapter <233>:

- Matched matrix blank
- Two standard solutions: $0.5J$ and $1.5J$
- Sample solution diluted to $< 1.5J$
- Drift of analyte signal during measurements < 20%
- Spike solutions $0.5J$ – $1.5J$ with 70 – 150 % recovery

This led to use of same external standard and quality control solution concentrations as with elemental analysis of APIs (guidance also provided by ICH and USP) . This choice of J also ensured that potential impurity concentrations near 30% PDE were easy to monitor. For example the calibration (working/linear) range matches the acceptable concentration range for elements with lowest permitted exposures with 10 g daily dose of pharmaceuticals. For limit tests, the limit concentrations were chosen to match the 30% PDE with the lowest exposure limit, therefore giving unusually strict limits for several elements, such as chromium, tin, copper and molybdene.

Target concentrations being set really low, the detection limits of all quantified elements had to be considered – The analysis results would have been meaningless, if the limit of quantification of certain element is greater than the threshold limit 30% of PDE! The greatest factors affecting quantification limits were identified to be plasma robustness, efficiency of digestion (matrix effect) and dilution factor. The ICP-OES instrument's plasma and sample digestion conditions being rather optimised, a maximum dilution factor (mass of diluent : mass of sample) of 50:1 was set. If the samples were diluted more than that, calculated quantification limits may have increased too much.

8.3 Workflow

It was decided early in method development process to analyse all the elements classified (1, 2A, 2B and 3) in Q3D guideline. Because of large amount of elements in the assay, elements were divided to three categories: The ones quantified with external calibration, ones analysed with limit test with one point limit ”calibration”. Also, if sample matrix was found to have large amount of spectral interferences or matrix effects, additional verification was done with standard addition method (see fig. 17).

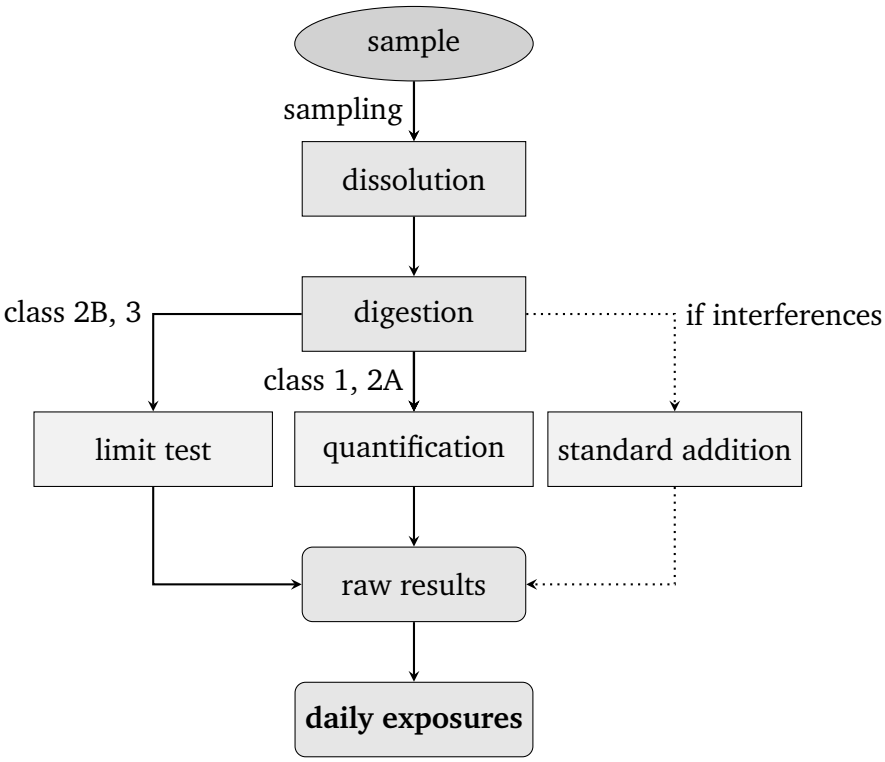


Figure 17: Flowchart of the analysis method procedure starting from original sample to final results

9 Experimental procedures

9.1 Sample preparation

Sample for analysis was sampled from original package. Drug product tablet or capsule was weighed whole in a glass vial for analysis and accurate mass was noted. Mass of the sample was at least 200 mg for small tablets, capsules or solutions, and at least 500 mg for big tablets. The sample mass was chosen thus the sample dilution factor was not more than 50. Weighing and dilution amounts are presented in Table 6. If the tablet was large and had a groove for splitting, it could have splitted if needed. One sample was analysed per a product lot and one spiked sample was prepared for each individual drug product.

Table 6: Weighing for sample digestion and dilution volumes

Sample m (mg)	40 % HNO_3 (v/v) (µl)	HNO_3 (ml)	Dilution volume (ml)
$200 \leq m < 500$	150	4	10
$m \geq 500$	375	10	25

Sample was suspended in 40 % (v/v) nitric acid and was predigested in ultrasonic bath for at least 10 minutes until surface of the drug had started to decompose. Standard addition of 1 $\mu g\,g^{-1}$ was made to the spike sample, the addition volume was calculated with formula

$$m_{spike} = \frac{m_{sample} \times c_{spike}}{c_{stock}}, \tag{6}$$

Where m_{spike} is mass of the spiked solution, m_{sample} is mass of the sample, c_{spike} is target concentration and c_{stock} is stock concentration. Spike concentration of 1.0 $\mu g\,g^{-1}$ and stock concentration of 10 $\mu g\,g^{-1}$ was used. If we approximate for water based solutions that $\rho \approx 1$, we can assume that 1.0 ml of the solution weights 1.0 gram. Standard addition

volumes were calculated quite simply in $\mu g\, g^{-1}$, or ppm weight ratio unit (*parts per million*) using this approximation. [i] In 20 °C, this approximation produces relative error of 0.18% which is marginal compared to other error sources in this analysis method, and therefore not evaluated.

Nitric acid was added into sample tubes and sample was predigested in ultrasonic bath for at least 15 minutes until sample was decomposed for the most part, or at least suspended in the acid. If the sample didn't decompose well, additional heating was done in the ultrasonic bath in approximately 50 °C for at least 10 minutes. Dissolved sample was then wet-digested in microwave acid digestion system. For each run, nitric acid baseload recommended by manufacturer was measured in PTFE vessel and 4 – 15 samples were arranged in sample rack inside the vessel in reaction chamber, pictured in Figure 18, which is taken after the digestion, the vessel is inserted in the microwave chamber. Chamber was closed and initial pressure and temperature was applied and samples were run with a microwave program. After the digestion samples were allowed to cool in room temperature and were diluted in 10 ml or 25 ml volumetric flasks to laboratory water. If any cloudyness in sample solution or solid deposit was found in the sample tubes, 0.45 µm syringe filtration was used to clear the samples before ICP-OES analysis. For each sample digestion run, a blank sample was prepared to keep track of sample contamination (for example cross-contamination or contamination from container). Blank samples were prepared in the same way that the actual samples.

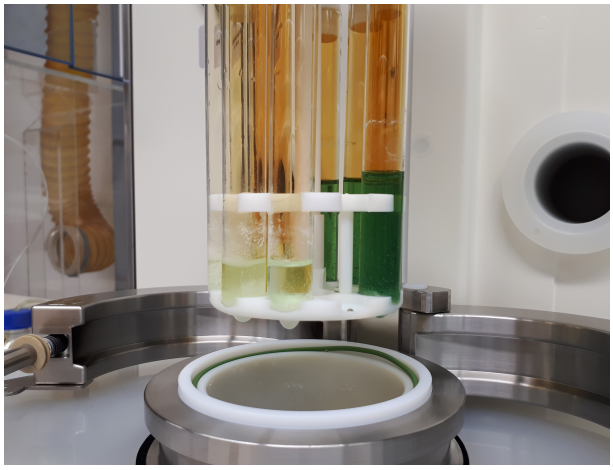


Figure 18: UltraWAVE digestion system with digested samples in the rack.

[i] Standard solution manufacturers and suppliers get ppm, mg/l ja $\mu g\, g^{-1}$ blissfully mixed in their products, which makes the author anxious from time to time.

9.2 Elemental Analysis with ICP-OES

Elemental analyses were done mainly on the same or the next day as sample digestion, however maximum of 4 days between digestion and analysis was allowed. Samples were transferred to plastic sample tubes in the autosampler. Quantified elements (Table 7) were run with ICP-OES against an external multielement calibration, shown in Table 8. Standard solution concentrations were chosen using target concentration from 0.01 to 0.20 $\mu g\,g^{-1}$. Standards were prepared into matched sample matrix background, 40% (*v/v*) nitric acid (400 ml HNO_3 diluted into 1000 ml laboratory water).

Not all the target elements were quantified, but limit test was done to them. These include all class 3 and most of class 2B elements, because of their high PDEs, and only the information of concentrations of analytes being lower than PDEs is required. In pharmaceutical limit test of trace elements an acceptable concentration limit is set. Then, the instrument using the analysis method should be able to distinguish prepared limit test intensity from 80% and 120% aliquots of the limit concentrations, so that 80% aliquot produces smaller intensity than the limit aliquot, and 120% produces bigger intensity than the limit aliquot accordingly.

Limit test standards were chosen according to estimated maximum daily dose of the product, divided to over 3 grams per day 0.20 $\mu g\,g^{-1}$ standard and 5 grams per day 0.16 $\mu g\,g^{-1}$ standard, as shown in Table 9. 80% and 120% versions were made for both of the standards to verify resolution power of the instrument. Limit test aliqots were analysed in the same run as samples and calibration standards. Sample emission intensities were compared to limit test intensities to determine, if analyte concentrations were under the limit concentrations.

A standard addition method was developed for one difficult sample matrix because of spectral interferences on Pb analyte lines 220.3 and 182.2 nm. Standard addition method was used to varify the results obtained with quantification method. A spike solution of 10 $\mu g\,g^{-1}$ Pb in 1% HNO_3 was prepared. Samples were sampled from three different lots, four aliquots per lot to make standard calibration. Sample preparation and digestion was done similarly to regular samples. After microwave digestion, a standard addition of Pb spike solution was done (Table 10) to aliquots and were filled to total volume of 25 ml. Then the elemental analysis was done similarly to these standard addition samples. The standard addition calibration curve was evaluated from the intesities and results were calculated.

Table 7: Used emission wavelengths and was line quantified or analysed by limit test

Element	Q3D class	Primary line (nm)	Secondary line (nm)	quantified (x)	limit test (x)
Cd	1	226.5		x	
Pb	1	220.3	182.2	x	
As	1	189.0		x	
Hg	1	184.9		x	
Co	2A	238.8		x	
V	2A	292.4		x	
Ni	2A	221.6		x	
Tl	2B	190.8		x	
Au	2B	242.7			x
Pd	2B	340.4		x	
Ir	2B	212.6			x
Rh	2B	343.4			x
Ru	2B	240.2			x
Se	2B	196.0			x
Ag	2B	328.0			x
Pt	2B	265.9			x
Li	3	670.7			x
Sb	3	217.5			x
Ba	3	455.4			x
Mo	3	202.0			x
Cu	3	327.3			x
Sn	3	189.9			x
Cr	3	276.6			x

Table 8: Concentrations of multi-element calibration solutions

Standard	Elements and concentrations
blank	40% *(v/v)* nitric acid
STD 1	0.01 $\mu g\,g^{-1}$ As, Cd, Hg, Pb, Ni, V, Co, Pd, Tl, Au
STD 2	0.04 $\mu g\,g^{-1}$ As, Cd, Hg, Pb, Ni, V, Co, Pd, Tl, Au
STD 3	0.20 $\mu g\,g^{-1}$ As, Cd, Hg, Pb, Ni, V, Co, Pd, Tl, Au
QC	0.02 $\mu g\,g^{-1}$ As, Cd, Hg, Pb, Ni, V, Co, Pd, Tl, Au

Table 9: Limit test solutions

Standard	Concentration	Elements
Limit test 3 g standard	0.20 $\mu g\,g^{-1}$	Cu, Li, Sb, Pt, Au, Sn, Cr, Ba, Ru, Mo, Ir, Rh, Se, Ag
Limit test 3 g 80 %	0.16 $\mu g\,g^{-1}$	Cu, Li, Sb, Pt, Au, Sn, Cr, Ba, Ru, Mo, Ir, Rh, Se, Ag
Limit test 3 g 120 %	0.24 $\mu g\,g^{-1}$	Cu, Li, Sb, Pt, Au, Sn, Cr, Ba, Ru, Mo, Ir, Rh, Se, Ag
Limit test 5 g standard	0.120 $\mu g\,g^{-1}$	Cu, Li, Sb, Pt, Au, Sn, Cr, Ba, Ru, Mo, Ir, Rh, Se, Ag
Limit test 5 g 80 %	0.096 $\mu g\,g^{-1}$	Cu, Li, Sb, Pt, Au, Sn, Cr, Ba, Ru, Mo, Ir, Rh, Se, Ag
Limit test 5 g 120 %	0.144 $\mu g\,g^{-1}$	Cu, Li, Sb, Pt, Au, Sn, Cr, Ba, Ru, Mo, Ir, Rh, Se, Ag

Table 10: Determination of lead with standard addition, target concentration $J = 1{,}0\ \mu g\,g^{-1}$, $m_{tabl.}$ = sample mass in mg (only done for **CM** and **CN** tablets)

STD conc. (µg/g of sample in final vol.)	STD addition (µl)	Sample solution vol. (ml)	Final volume (ml)
0.0	0.00	10	25
0.5	$m_{tabl.} \times 0.05$	10	25
1.5	$m_{tabl.} \times 0.15$	10	25
2.0	$m_{tabl.} \times 0.20$	10	25

The ICP-OES instrument plasma parameters are presented in Table 11. Axial plasma view was used for better sensitivity in trace metal analysis and long exposure time was used to lower the deviation of replicate measurements. The gas flows and RF power settings were the same settings as used for API analysis to acquire robust conditions. Sample introduction parameters are shown in Table 12, which were optimised for the 40% nitric acid matrix, for maximum efficiency in sample transportation and nebulisation. Background correction was performed with Qtegra ISDS -software using two correction points on both sides of an analytical line, where the background signal is its local minimum. If large spectral interferences or rising of baseline are detected on the other side of the analytical line, two background correction were chosen only on the other side.

Table 11: Plasma parameters

Parameter	Value
Plasma view	Axial
Exposure time	30 s
RF power	1350 W
Nebulizer gas flow	0.5 $\mathrm{l\,min^{-1}}$
Coolant gas flow	12 $\mathrm{l\,min^{-1}}$
Auxialiry gas flow	0.5 $\mathrm{l\,min^{-1}}$

Table 12: Sample introduction parameters

Parameter	Value
Introduction method	Autosampler
Sample uptake time	40 s
Rinse time	At least 20 s
Pump speed (analysis)	45 rpm
Pumpu speed (rinse)	45 rpm
Nebulizer Chamber	Cyclonic
Nebulizer	Concentric
Amount of replicates	2
Background correction	1 or 2 points

10 Results and Discussion

10.1 Sample Digestion

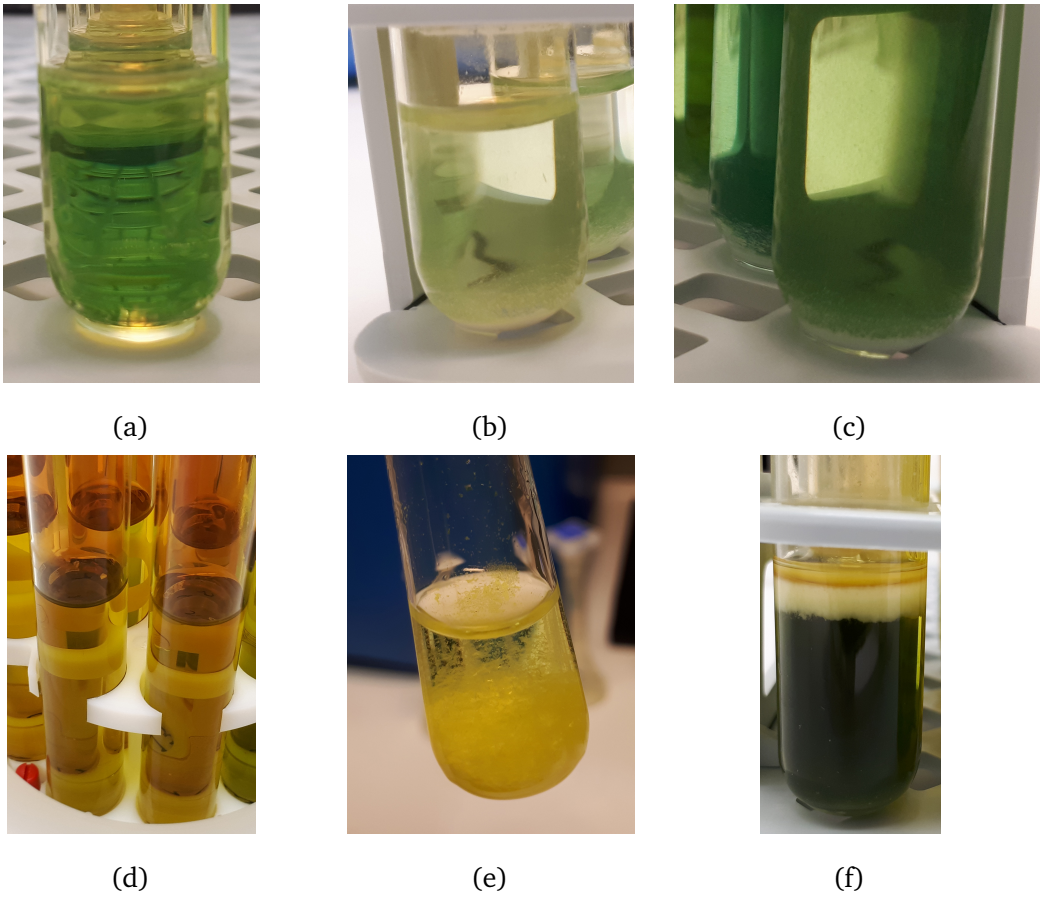
The pre-digestion was chosen to be done by adding a small volume of 40% nitric acid on the samples because several drug tablets and capsules did not start to decompose fast enough by just adding pure nitric acid on them. Surface of the tablet or the capsule had to break before the decomposing could start properly. The other reason to use more dilute nitric acid was the unexpected reactions of samples with nitric acid. With some samples the decomposition reaction was very keen and exothermic. If the sample reacted with nitric acid too furiously, it would have started boiling and coming out of the sample tube. To minimize the risk, pre-digestion was wanted to start slowly enough to keep sample decomposition in control, and break the surface or capsule as much as possible before addition of concentrated acid.

Typically after addition of concentrated nitric acid, samples started to decompose slightly faster than during predigestion. Usually samples produced nitric oxides in the solution and also releasing them from the solution as brown gas. Using ultrasonic bath the decomposition time reduced from several hours to approximately 10 minutes. Water based oral suspensions, oils and injection solutions dissolved almost immediately in nitric acid without help of ultrasonic bath. Rate of decomposition differed greatly among drug products. Products containing a great amount of excipients from geological sources were hardest to decompose. Capsules decomposed very well in predigestion, probably because capsule surface is usually made from cellulose or similar organic material.

Performance of the pre-digestion was not found to have a significant effect on performance of wet-digestion with UltraWAVE, for the most part. Most important thing was that the surface of the sample was sufficiently decomposed, so the hot acid could dig in the sample. Especially big, bulky tablets which were not decomposed very well in pre-digestion might

stick inside the sample tube or float on the acid, which caused uneven heating of the tube. Sometimes the floating sample tablet formed a plug, which caused the pressure in tube to become too big. These events caused to some tubes to break and ruin the digestion run.

After digestion sample solutions were green or yellow and mostly clear. Digested samples were categorised to six types, according to their appearance after digestion program was completed, see Figure 19. Insoluble material was on average in the bottom of the tube, some rare cases it floated on top of the water phase. The residue in the bottom of the tubes was usually white powdery residue. The floating phase was often liquid after the digestion, but solidified in room temperature if left untouched for a while.



(a) (b) (c)

(d) (e) (f)

Figure 19: Different types of samples after microwave digestion. (a) clear green-hued solution without residue, (b) almost colorless clear solution with two visible phases, (c) green-hued clear solution with residue in the bottom, (d) clear solution with an orange hue, (e) yellow flakes in clear yellow-hued solution, and (f) dark clear solution with two visible phases, organic phase on top was solidified after cooling.

The entire contents of sample tubes were transferred into volumetric flasks and some clear sample solutions became cloudy when water was added into sample solution, and later the insoluble material floated to the bottom of the flasks, as shown in Figure 20. This indicates that samples contain compounds and/or ions which are soluble in nitric acid but not in water. The residues in digested samples were not analysed in this lab work but hypothesis is that they are mainly inorganic silicon and/or titan oxides. Silicon and titan oxides are soluble only to hydrogen fluoride or mixtures of hydrogen fluoride and other acids.[20] Even using hydrogen fluoride, perfect digestion results are not acquired. In addition, usage of hydrogen fluoride in laboratories has big safety and health risks, and may require special equipment and procedures to use.[4] The floating residues in seperate phase were most definately organic substances found in excipients, such as glycerol, because they were found exclusively in products containing a large amount of glycerol as an excipient.

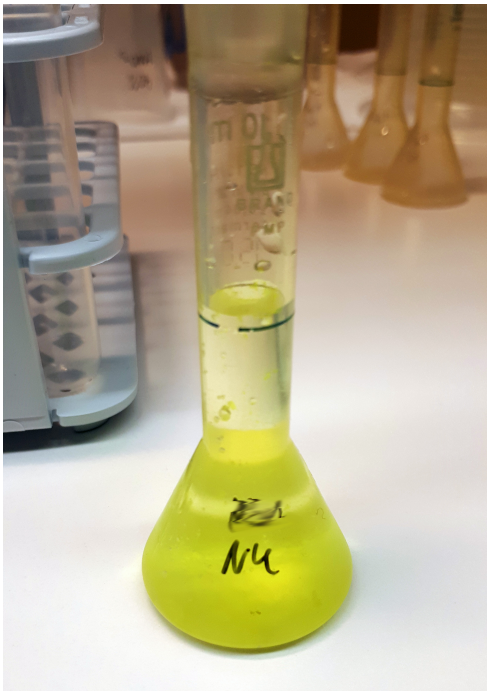


Figure 20: Digested sample transferred in volumetric flask, undigested yellow residue can be seen in the bottom

10.2 Elemental analysis

10.2.1 Quantification of the Target Elements

92 different pharmaceutical products were successfully analysed, and ICH Q3D class 1 and 2A as well as Pd and Tl were quantified from the pharmaceutical products. A review of the concentration data of the elemental impurities is presented in this chapter, in addition of couple of examples. Error margins of quantified results are estimated with 95% confidence limits. The concentrations of three replicates and the mean value, as well as respective quantification and detection limits for each element is presented in Table 13.

Table 13: Quantified analyte replicate concentrations and quantification limits of elemental impurities in pharmaceutical product **Z**, sample size 665 mg diluted into 25 ml of acidic water solution. Mean is calculated with 95% confidence level.)

Elements	1. lot ($\mu g\,g^{-1}$)	2. lot ($\mu g\,g^{-1}$)	3. lot ($\mu g\,g^{-1}$)	mean ($\mu g\,g^{-1}$)	LOD ($\mu g\,g^{-1}$)	LOQ ($\mu g\,g^{-1}$)
As	0.204	0.152	0.140	0.20±0.09	0.054	0.170
Cd	0.019	0.019	0.021	0.020±0.002	0.006	0.020
Hg	-0.028	-0.008	-0.007	-0.01±0.03	0.020	0.063
Pb	0.797	0.663	0.759	0.8±0.2	0.044	0.140
Ni	0.094	0.081	0.085	0.10±0.02	0.116	0.370
V	0.005	0.010	0.006	0.007±0.01	0.007	0.022
Co	-0.025	-0.015	-0.014	-0.020±0.015	0.011	0.034
Pd	-0.043	-0.016	-0.025	0.03±0.04	0.088	0.280
Tl	-0.011	0.069	0.052	0.04±0.10	0.030	0.096

The data from elemental analyses were compared to the PDEs and presented in a spreadsheet one product per sheet. The results were then inspected by a quality control chemist and then sent to Orion for further use in risk assessment processes. Daily exposures of each element was calculated from the concentration data of each pharmaceutical product and a worst case scenario approach was used in the presentation of the results. This means that the highest result of the three replicates of each analyte was used in calculating daily exposures. The final quantified results are shown in Table 14, side by side with 30% of the permitted concentrations in this particular pharmaceutical, and 30% of the PDE, which were used as a threshold level in the risk assessment. This data of pharmaceutical product **Z** is chosen,

because it is one of the only samples with several elemental impurities over the detection limits.

Table 14: Worst case exposures of elements in pharmaceutical product **Z** and their permitted daily exposures. PDE's are calculated using maximum daily dose of 2.0 g of pharmaceutical product **Z**.

Quantified	Worst case		Permitted daily exposures	
elements	conc. ($\mu g\,g^{-1}$)	exposure (μg/day)	30% PDE ($\mu g\,g^{-1}$)	30% PDE (μg/day)
As	0.204	**0.406**	2.256	**4.5**
Cd	0.021	**0.041**	0.752	**1.5**
Hg	<LOQ	**<LOQ**	4.511	**9.0**
Pb	0.797	**1.590**	0.752	**1.5**
Ni	<LOQ	**<LOQ**	30.075	**60.0**
V	<LOQ	**<LOQ**	15.038	**30.0**
Co	<LOQ	**<LOQ**	7.519	**15.0**
Pd	<LOQ	**<LOQ**	15.038	**30.0**
Tl	<LOQ	**<LOQ**	1.203	**2.4**

Quantification results matched the expectations pretty well, almost every quantified element concentration was under the method detection limits, and well below permitted concentrations. Challenging sample matrices were present at all times but thanks to good sample preparation, intruduction and instrument resolution, matrix effects were handled well. This is shown with good spike recoveries on the standard addition samples on all elements analysed except palladium. Because of large amount of elemental impurities in pharmaceuticals were analysed and the results are quite similar, only few examples of the samples analysed are presented in the experimental part of this thesis. Complete results of all concentrations of quantified elemets are presented in Appendix 1.

10.2.2 Limit Tests

Limit test was done to all (N= 92) the samples to test if the intensities of class 2B and 3 analyte lines exceed the intensities of the limit solutions. Not a single element concentration was found to exceed the limit test concentration in any of the limit tests for any pharmaceutical products. An example of results for pharmaceutical product **Z** is shown in Table 15, where the intensities are shown side by side. The limit test concentration was set to 7.5 $\mu g\,g^{-1}$ for every element analysed, which is quite low for the sample size of 665 mg. This concentration is well below the calculated 30% PDE value for most of the elements.

Table 15: Limit tests of elemental impurities in pharmacutical product **Z**, intensities of analyte lines were compared to known limit test line intensities.

Limit test	Sample intensities			Limit test solution		
emission line(nm)	lot 1 (cps)	lot 2 (cps)	lot 3 (cps)	I (cps)	conc. ($\mu g\,g^{-1}$)	exposure (μg/day)
Cu 327.3	17	16	16	4249	7.5	15.0
Li 670.7	3618	3496	4136	229198	7.5	15.0
Sb 217.5	-1	0	0	245	7.5	15.0
Pt 265.9	-6	-5	-7	230	7.5	15.0
Au 242.7	-1	1	3	889	7.5	15.0
Sn 189.9	3	2	2	327	7.5	15.0
Cr 276.6	32	24	24	3345	7.5	15.0
Ba 455.4	9663	10178	12139	231283	7.5	15.0
Ru 240.2	-17	-13	-14	717	7.5	15.0
Mo 202.0	6	5	5	1117	7.5	15.0
Ir 212.6	-20	-16	-17	240	7.5	15.0
Rh 343.4	-22	-23	-20	1599	7.5	15.0
Se 196.0	3	2	2	88	7.5	15.0
Ag 328.0	-33	-32	-32	4446	7.5	15.0

10.3 Method Verification

Verification of the results obtained with this analysis method was done according to verification plan. Verification plan was worked with our cooperative partners at Orion and approved before beginning of the analyses, and it was worked to be constistent with good manufacturing practices in pharmaceuticals, ICH Q3D guideline, and USP chapters <232> and <233>.

10.3.1 Calibration

For quantification, the calibration solutions were prepared from stock solutions made from verified commercial standard solutions. All used calibrations were verified to fulfill the limits set. Instrument calibration was done daily before each measurement. The lower limit for calibration linearity was set to $r^2 > 0.992$ and calibration stability was continuously monitored with matrix matched quality control (QC) samples, allowed recovery was set to 80–120 %.

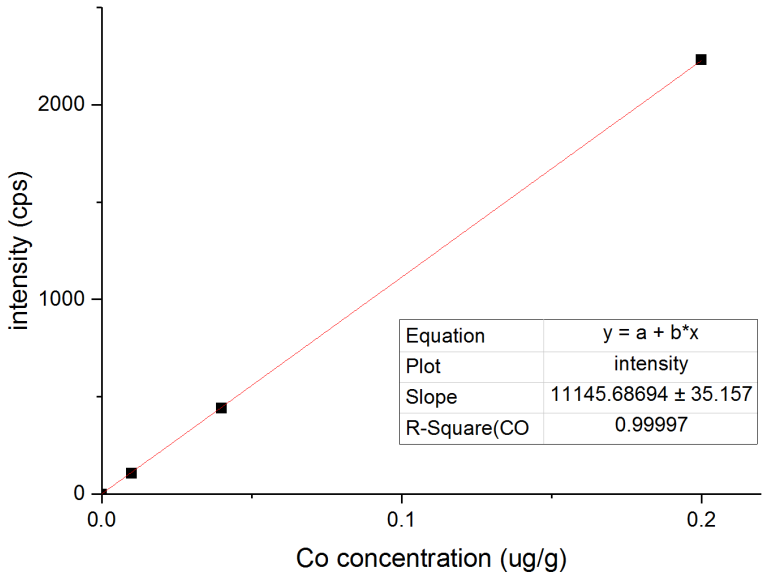
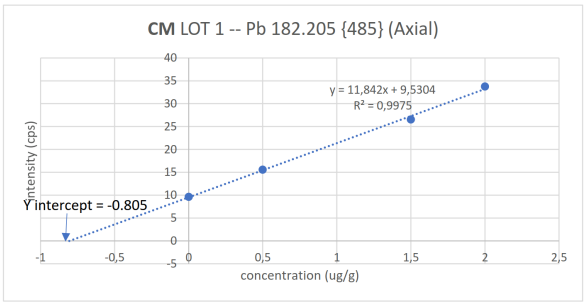
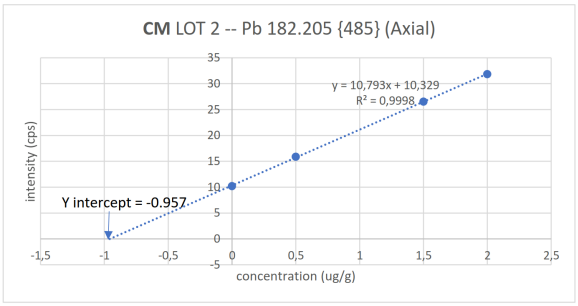


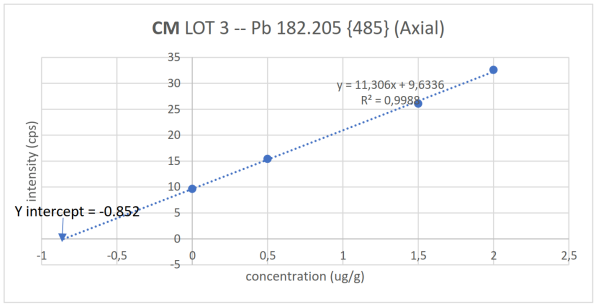
Figure 21: An example of external calibration: Calibration of cobolt 228.616 nm line using matrix matched standard solutions for pharmaceutical **CM**

Limit test solutions were prepared and calibration done similarly to the quantification. Verification on the resolution power of limit test was done with 80% and 120% concentrations of the actual limit test solution, and all of the limit test verifications were inspected to fulfill the applicability limits.

Standard addition calibration was used in two occasions for additional verification on the lead concentrations. The standard addition calibration resulted in similar concentration results as external calibration (Figure 22), where calibrations are shown for pharmaceutical **CM**. Standard addition was done for three different lots of the same pharmaceutical products. Standard addition calibration for **CM** resulted in mean Pb concentration of 0.871 $\mu g\,g^{-1}$, and external calibration gave 0.861 $\mu g\,g^{-1}$. Calculated %RSD for three replicate calibrations for the same sample matrix was quite high, but the single calibrations were in acceptable linearity range, with r^2 varying from 0.9954 to 0.9998.







lot	sample conc. ($\mu g\,g^{-1}$)
1	0.805 ± 0.417
2	0.957 ± 0.098
3	0.852 ± 0.278
mean	**0.871**
S	0.078
%RSD	8.931

Figure 22: Standard addition calibration and calculated concentrations of **CM**

10.3.2 Detection limits

Method detection limit (MDL) was estimated using EPA standard method[63], shown in Table 16. It was made sure that we acquired low enough quantification and detection limits on each element, so the concentrations could be verified to be lower than the PDEs. First instrument detection limit was calculated using blanks, and then fortified blanks spiked 1 to 3 times IDL

were analysed, and standard deviation was calculated, equation (2) was then used with t_{n-1} value 2.8214 to acquire the detection limits.

Table 16: Detection limits used in elemental analysis

				MDL		MQL	
Element	Emission line (nm)	IDL ($\mu g\,ml^{-1}$)	IQL ($\mu g\,ml^{-1}$)	($\mu g\,g^{-1}$)	($\mu g\,ml^{-1}$)	($\mu g\,g^{-1}$)	$\mu g\,ml^{-1}$)
Cd	226.5	0.0001	0.0002	0.008	<0.001	0.027	0.001
Pb	220.3	0.0010	0.0032	0.059	0.001	0.186	0.004
As	189.0	0.0023	0.0076	0.071	0.001	0.226	0.005
Hg	184.9	0.0006	0.0019	0.026	0.001	0.084	0.002
Co	238.8	0.0004	0.0012	0.014	<0.001	0.045	0.001
V	292.4	0.0002	0.0008	0.009	<0.001	0.029	0.001
Ni	221.6	0.0003	0.0008	0.155	0.003	0.492	0.010
Tl	190.8	0.0019	0.0062	0.040	0.001	0.127	0.003
Au	242.7	0.0010	0.0035	0.042	0.001	0.134	0.003
Pd	340.4	0.0014	0.0046	0.117	0.002	0.372	0.007
Ir	212.6	0.0013	0.0044	0.039	0.001	0.125	0.002
Rh	343.4	0.0013	0.0044	0.050	0.001	0.158	0.003
Ru	240.2	0.0010	0.0033	0.082	0.002	0.262	0.005
Se	196.0	0.0028	0.0093	0.080	0.002	0.254	0.005
Ag	328.0	0.0006	0.0018	0.031	0.001	0.099	0.002
Pt	265.9	0.0034	0.0113	0.049	0.001	0.157	0.003
Li	670.7	<0.0001	<0.0001	0.009	<0.001	0.029	0.001
Sb	217.5	0.0026	0.0087	0.069	0.001	0.218	0.004
Ba	230.4	<0.0001	<0.0001	0.017	<0.001	0.053	0.001
Mo	202.0	0.0003	0.0011	0.029	0.001	0.093	0.002
Cu	327.3	0.0005	0.0017	0.020	<0.001	0.064	0.001
Sn	189.9	0.0005	0.0016	0.008	<0.001	0.027	0.001
Cr	276.6	0.0004	0.0012	0.213	0.004	0.678	0.014

The MDL calculations were repeated in the end of the project to verify detection and quatification limit robustness (Figure 23). The critical quantification limits of class 1 elements were very similar thoughout the laboratory project, but some elements' limits changed from the beginning to the end, this behavior was seen in nickel, palladium and chromium with decreasing quantification limit, and thallium, ruthenium, selenium and platinum with increasing limit. Clear explanation of the changes in MQL was not found in this research project.

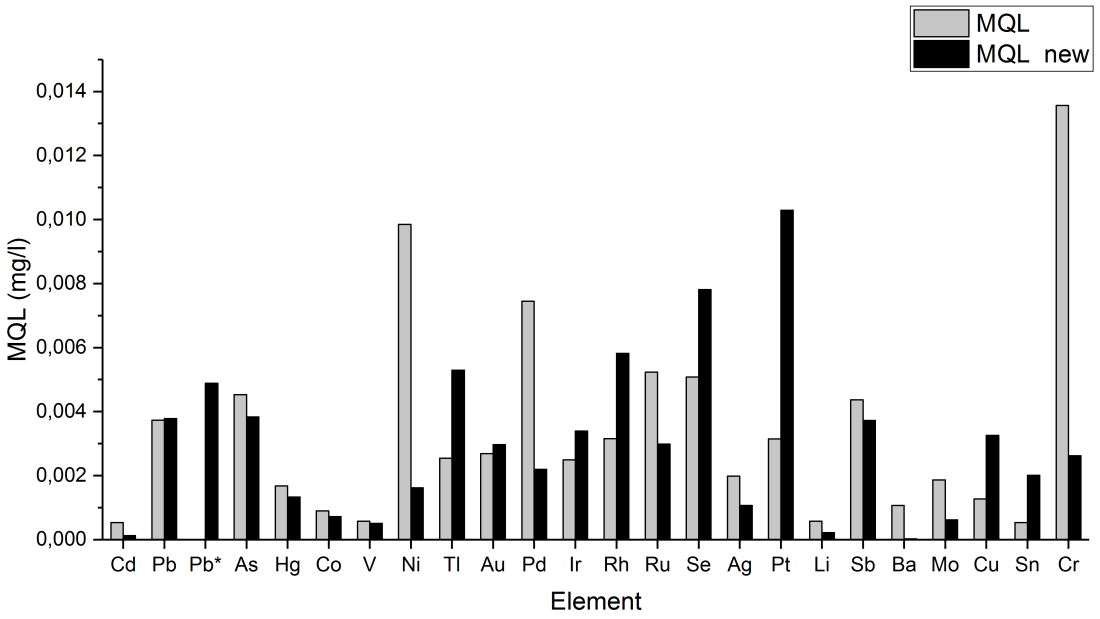


Figure 23: Comparison of method quantification limits (* MQL of the secondary Pb 182.2 analyte line).

10.3.3 Recovery Tests

Recovery tests were done to verify trueness and accuracy, since there were no suitable CRM available. Spiked samples were analysed beside the actual samples, in which standard addition of analytes were done. Recoveries of the analytes were calculated and recovery percents from 70% to 150% were allowed, consistent with the USP chapter <233>[11]. Our recovery-% data in Table 17 shows, that spike recoveries are inside allowed limits.

In spite of results being acceptable, there is a lot of variance in the results as seen in recoveries ranging from near allowed minimum recovery to maximum. This can be partially explained with strong matrix effect in samples.

Mercury, cobalt and arsenic have the most consistent recoveries in class 1 and 2A Q3D elements, with relative standard deviation being under 9.0% among samples. Arsenic and mercury also have the highest mean recovery percents, and have over 90% recovery in all samples. Palladium had inconstistent spike recoveries from time to time. Low recoveries of around 70 percent and deviation between preparated replicate samples made palladium challenging to measure quantitatively. A constistent and clear connecting factor was not

found between the samples with low spike recoveries, and it seemed to be random from product to product.

Table 17: Recoveries of quantified analytes in all spiked pharmaceutical samples (N = 92), with the exception of Pd, where (N = 86).

Analyte	mean recovery (%)	max recovery (%)	min recovery (%)	*S*	RSD%
As	111.8	148.6	93.8	9.8	8.8
Cd	106.6	139.1	82.6	9.6	9.0
Hg	110.4	146.8	96.4	8.6	7.8
Pb	104.5	147.3	74.8	11.4	10.9
Ni	104.2	138.1	80.4	9.8	9.4
V	99.9	134.6	77.1	9.9	9.9
Co	103.9	138.6	82.3	9.1	8.7
Pd	100.3	133.5	70.1	9.6	9.6
Tl	103.6	134.9	74.6	10.8	10.5

10.3.4 Interferences

ICP-OES is a technique sensitive to interferences caused by spectral interferences or matrix effect, therefore all analyte lines used were visually inspected and suitable correction was done using Thermo's Qtegra -software for ICP-OES instrument. The computational correction used was baseline correction, where smooth and low background pixels left and right to the analyte peak were chosen, and at the center where the pixels where the peak integration was done. The resolution of CID detector was found to be sufficient to separate the analyte signal and the unwanted interferences. If interferences such great where the computational correction was not enough were found, was the secondary emission line used, to avoid errous results.

Using this method of verifying emission lines was found very successful. Overall, small amount of spectral or matrix interferences were detected in the timespan of analysis of pharmaceuticals, and only few of them severely interfered with the analyte lines used. With only few of total 92 samples, secondary emission lines were required to quantify elements. The most interferences arised in determination of lead in the samples. In Figure 24 an

unidentified interference from the sample matrix near secondary Pb line 182.2 nm is seen in all the **S** sample replicates and spiked sample, but not in the matched matrix calibration standards, which makes the analyte line unreliable in quantification. However, the primary Pb 220 nm line was free of interferences, which was then used.

Another example of interferences seen in the analysis was baseline shifting. Baseline shifting means uneven, ascending or descending emission signal throughout the observed wavelength area of each element. In Figure 25, primary lead analyte line is covered by very high background emission. The emission of spiked sample (yellow) can barely be seen. The baseline correction can not be done on both sides, which result in unusable analyte line. In this case, secondary analyte was found to be too unsensitive, and a standard addition method was used to verify the results.

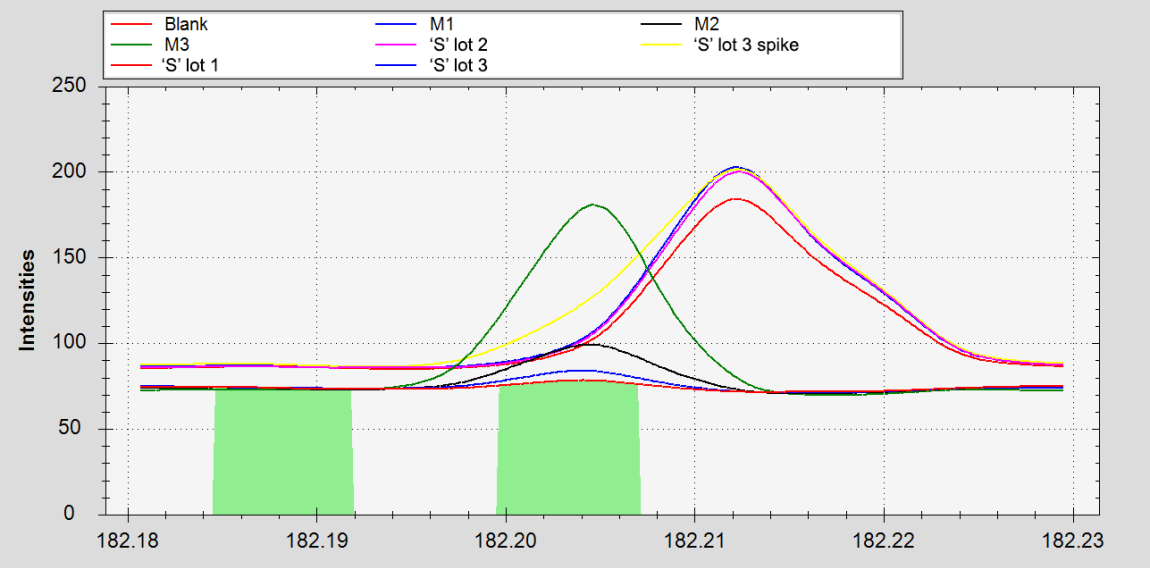


Figure 24: Interference near Pb182.2 analyte line in pharmaceutical **S**, blank is matrix matched, M1-M3 matched matrix calibration standards, spike sample is 1 $\mu g\,g^{-1}$ Pb.

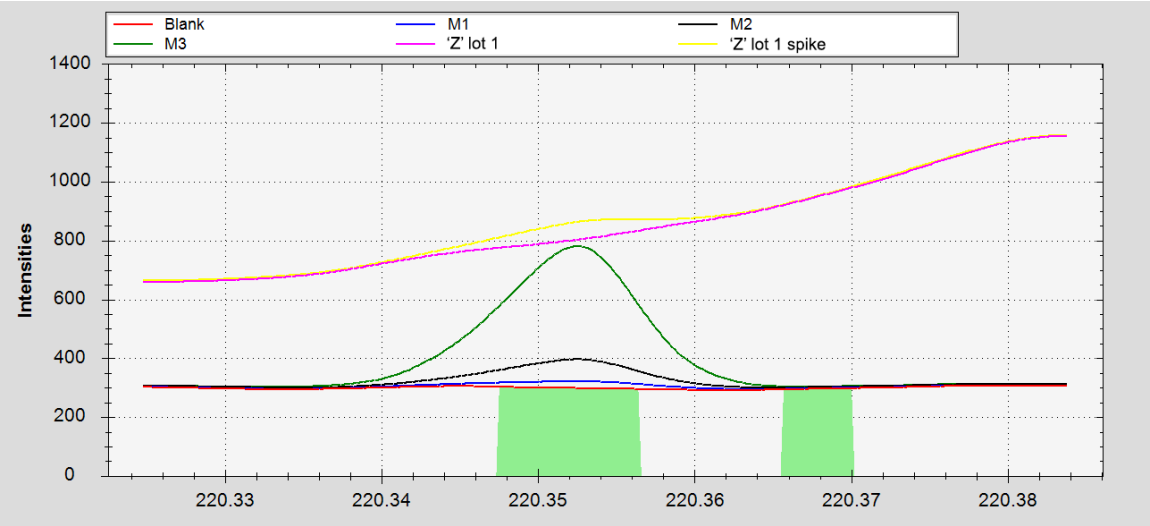


Figure 25: Baseline shift near Pb220.0 analyte line in pharmaceutical **Z**, blank is blank is matrix matched, M1-M3 matched matrix calibration standards, spike sample is 1.0 $\mu g\, g^{-1}$ Pb.

11 Conclusions

An extensive method for analysing elemental impurities in pharmaceuticals was developed and tested in a quality control laboratory applying good manufacturing practices. Sufficient method verification was conducted to show trueness, accuracy and robustness of the method. Method showed success analysing small (< 500 mg) tablets, digestion of bigger tablets was found to be more challenging. Bigger tablets resulted in more sediment in digested samples, which had to be filtered out.

The method is decent for screening a large number of elements in the sample matrix. If better accuracy and quantitativity is wanted, must the matrix effect and spectral interferences be handled some way in the sample digestion. The deficiency of certified reference materials raised some uncertainty in the quantified concentrations, because the sample matrix variation between each sample and also the matrix matched calibration, quality control and spike samples. However, the additional verification with standard addition method showed, that the variance in matrix effect was not that great, both external matched matrix calibration and standard addition giving same results. The use of hydrogen fluoride in the acid mixture is advised to digest the mineral material more completely. Precipitation of some matrix substances may also be necessary to lower detection limits on some elements.

Method was also found to be efficient with chemical use – small tablets requiring only 4ml of concentrated HNO_3 per aliquot, and stock solutions for calibration, limit tests and quality and calibration verification samples, lasted for several months without need to be redone frequently. This analysis method was found to be moderately quick, microwave digestion and ICP-OES analysis requiring the longest times to do. Fastest way was to do multiple (up to three different) sample digestions simultaneously. This way up it was possible to do analyse all elemental impurities from up to six products per day.

Palladium was suprisingly hard element to accurately quantify, beacuse of inconsistent very low spike recoveries in some samples, and the reason for this behavior was left unsolved. However, limit tests were sufficient to indicate levels below 30% PDE.

The accuracy Q3D guideline requires is tough to achieve in such low concentrations and complex sample matrix as drugs. However, powerful sample digestion and other preparation paired with robust plasma in ICP-OES yielded in acceptable results. There was some deviation between replicates in all samples, and error margins in the final results might be high. However, the worst case scenario -approach was used in handling the analysis results, and the highest concentration of the replicates were used in comparison to permitted daily exposures of each element. After calculating the final exposures using corrected maximum daily doses of the products reported by Orion, none of the 92 pharmaceutical products exceeded the permitted daily exposure of any of the elemental impurities. 30% value of the PDEs were used as a limit for risk assessment process: If an elemental impurity in pharmaceutical product exceeds 30% of PDE, must the risk of exposure to be assessed in the manufacturing process. Lead was only impurity which was found in concentrations around 30% PDE in few products.

I hope that the results of this research project will help to promote the modernization of the elemental analysis in pharmaceutical industry. Spectrometric techniques such as ICP-OES and ICP-MS are still not broadly used in quality control chemistry, but may be in few years, as the laboratories learn how to apply the ICH Q3D guideline in their own laboratories. There might be some elaboration or changes in the guidance of the elemental analyses, such as mandatory use of internal standards, or certified reference materials, which are absent in the industry at the moment of writing this thesis.

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Appendices