Investigating Y-site compatibility of intravenous drugs using Probe drum as analytical instrument

Degree Project in Pharmaceutical Formulation, KLGM16 – 30 credits

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Abstract

There is a lack of data concerning compatibility between intravenously administered drugs, especially for doses administered in neonatal care. Co-administrating drugs or other substances used in hospital care, for instance parenteral nutrition that are incompatible with each other can, in worst case scenarios, lead to fatal outcomes. It is not only important to study drug incompatibility at different conditions, but also of great importance to develop analysis methods that are efficient and easy for hospital personnel to use. The aim of this study is to investigate drug incompatibility between Ceftriaxone and Trastuzumab together with calcium and glucose and evaluate if Probe Drum is a suitable analytical instrument for this purpose. A validation of Probe Drum was done in the beginning of the project and the instrument was compared to NanoDrop and 50 Bio spectrophotometers. The results from the validation showed that the Probe Drum was sensitive and precise in a wide concentration range but not as sensitive as 50 Bio spectrophotometer.

To study the compatibilities between the drugs and the components selected from parenteral nutrition (calcium and glucose), clinically relevant concentrations were chosen. Concentration and running time in the Probe Drum were factors altered once incompatibility in terms of precipitations were shown. Ceftriaxone and calcium were incompatible when mixed forming a precipitation that could be detected from a visual inspection, this was not the case regarding Trastuzumab and glucose. Calcium concentrations of 10mM, 15mM and 20mM were titrated against a fixed concentration of Ceftriaxone. The results showed that there is a relationship between an increasing signal and longer running time at concentrations closer to equilibrium when mixing Ceftriaxone and calcium together. A calcium concentration of 10mM showed no formation of precipitation. Trastuzumab and glucose showed no incompatibility from a visual inspection and no subvisual particles were detected when analyzing the mixture under a light microscope. Lastly, it could be concluded that Probe drum is a suitable tool for drug incompatibility studies.

Acknowledgements

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Populärvetenskaplig artikel - Utvärdering av Probe Drum som analysinstrument för kompatibilitetsstudie av intravenösa läkemedel

För tidigt födda och sjuka barn hamnar på neonatal avdelning där de ofta behandlas med kombinationer av flera olika intravenösa läkemedel. Att kombinera intravenösa läkemedel innebär dock en risk för inkompatibilitet i form av fällningar och subvisuella partiklar, vilket kan riskera i livshotande tillstånd så som till exempel funktionssvikt i flera organ samt allvarliga hjärt- och kärlproblem.

Som en konsekvens av ett flertal rapporterade fall där Ceftriaxon, en form av antibiotika, och kalcium bildat skadlig fällning i lungor och njurar hos nyfödda gick den amerikanska Food and Drug Administration (FDA) 2007 ut med en varning gällande co-administration av denna läkemedelskombination. I dagsläget finns det en stor brist vad gäller data kring kompatibilitet mellan läkemedel inte minst för doser och koncentrationer som administreras till nyfödda. Det finns idag ett stort behov av mer data för neonatalvård och syftet med denna studie är därför att utvärdera lämpligheten att använda Probe Drum som analysinstrument för att snabbt och effektivt detektera inkompatibilitet mellan intravenösa läkemedel.

I studien gjordes en inledande validering där LABBOT, en uppdaterad version av Probe Drum, och två andra analysinstrument, NanoDrop och 50Bio, jämfördes. Från resultaten kunde man dra slutsatsen att LABBOT hade hög känslighet men att 50 Bio var ännu lite bättre, särskilt vid analys av lägre koncentrationer.

De två läkemedel som undersöktes i denna studie var Ceftriaxon samt Trastuzumab. Ceftriaxon är, som tidigare nämnt, en typ av antibiotika som är förekommande inom neonatal vård och Trastuzumab är en antikropp som används för behandling av bröstcancer. Trastuzumab används inte i neonatal vård men användes i denna studie då det fanns ett intresse för detta läkemedel där studien genomfördes. Dessa läkemedel har undersökts i kombination med kalcium respektive glukos då kalcium och glukos ingår i parenteral nutrition som ofta används i kombination med dessa läkemedel. Medan det finns rapporterade fall av inkompatibilitet mellan Ceftriaxon och kalcium finns det väldigt lite information kring kompatibiliteten mellan

Trastuzumab och glukos. På “process and life science”, en avdelning på kemicentrum som tillhör Lunds Tekniska Högskola där detta examensarbete genomförts, har studier gett skäl att misstänka inkompatibilitet mellan dessa substanser och därför har det varit av intresse att undersöka även denna kombination.

Parenteral nutrition värmesteriliseras ofta för att inte äventyra patienternas säkerhet. Denna process kan bilda nedbrutna produkter av glukosen. Dessa produkter är väldigt reaktiva och kan orsaka toxiska effekter efter administration av parenteral nutrition. Vid kompatibilitetsstudien av Trastuzumab och glukos undersöktes därför både steriliserad glukos samt icke-steriliserad glukos för att fastställa huruvida det finns någon skillnad mellan dessa.

Probe Drum är försedd med en pump som titrerar den ena komponenten till den andra samtidigt som provet analyseras för att detektera eventuell fällning vilket indikerar inkompatibilitet mellan komponenterna. Vid analys av Ceftriaxon och kalcium varierades koncentrationen av kalcium mellan 10, 15 och 20 mM och den totala titreringstiden mellan 2, 4 och 10 h. Ingen inkompatibilitet detekterades vid 10 mM, varken för 2, 4 eller 10h. Däremot detekterades fällning vid både 15 och 20 mM för alla tider. För att styrka resultatet från Probe Drum utfördes dessutom en visuell inspektion som bekräftade tidigare nämnda resultat.

Vid analys av Trastuzumab och glukos detekterades ingen inkompatibilitet vid analys med Probe Drum. För att fastställa att instrumentet inte missat några subvisuella partiklar undersöktes provet med hjälp av mikroskop, inga subvisuella partiklar kunde detekteras.

Utifrån resultaten presenterade ovan kan slutsatsen dras att Probe Drum är ett tillförlitligt analysinstrument för detektering av inkompatibilitet vid de koncentrationer som har används i denna studie. För lägre koncentrationer krävs det dock att studien utvecklas ytterligare. En fortsatt utvecklad studie är av intresse då de undersökta läkemedlen ibland ges i ännu lägre doser än de undersökta.

Resultatet av denna studie visade att Probe Drum är ett lämpligt analysinstrument då syftet är att screena för läkemedelsfällningar. Det finns däremot förbättringspotential, genom att öka pumpens kapacitetsvolym kan man enklare undersöka läkemedel och substanser där man av någon anledning inte kan öka koncentrationen av titranten. I denna studie tillät inte pumpens volym oss att nå vår önskade slutkoncentration av glukos i provet, dvs kyvetten. En större pump skulle även underlätta i de fall där man använder sig av titranter av låg löslighet. I framtiden kanske Probe Drum är ett analysinstrument som används av både sjukhuspersonal och farmaceuter.

Contents

1.Aim

The aim of this Master thesis is to investigate if Probe Drum can be utilized to detect drug incompatibilities among commonly used intravenous drugs. In this report Ceftriaxone and Trastuzumab are combined with calcium respectively glucose and further analyzed using Probe Drum.

A validation of LABBOT in comparison with Nanodrop and 50 Bio spectrophotometer will be carried out, with the additional objective to determine Limit of quantification (LOQ) and Limit of detection (LOD) of LABBOT analytical instrument.

2. Introduction and theoretical background

2.1 Pediatric care and drug incompatibility

Neonatal patients placed in intensive care units (ICU) often require several drugs administered simultaneously via a Y-site connection. According to Kumar et al, during one admission these patients can be administered up to 8.5 (+/- 8.3 SD; range 1-62) medications at the same time. Cases where children in the ICU have received up to 49 IV drugs have been reported.[1] Keeping track of the physical compatibility of these drugs is of great importance to avoid infusion of subvisible particles. Infusion of these particles might lead to life threatening conditions such as pulmonary dysfunction, cardiovascular arrest and multiorgan failure, as the particles impair the microcirculatory system among these infants. [2, 3]

There is a lack of data regarding compatibility available for commonly used IV medications. The limited data available are mainly applicable to doses administered to adults. Factors such as concentration, duration and administered ratio of the drugs can have an impact on the compatibility. These are factors that vary significantly between medications intended for children compared to adults. [4]

Drugs in neonatal care can be divided into different categories. In the article, *Compatibility of drug infusions in the NICU,* co-infusions were divided into three groups, drug-drug, drug-nutrition and drug-albumin; the compatibility of these combinations were further investigated using available literature. The conclusion was that 74% of administered drug-drug combinations in neonatal care are either incompatible or have not been tested. For the drug-nutrition combinations 89% were found to be incompatible or not studied and for the majority drug-albumin combinations no data was found. In the cases where the drug combinations were compatible 93% had restrictions concerning infusion fluid, contact time or concentration. And of those 93% further limitations regarding duration of stability had to be considered. Major differences in pH were stated in the article as a reason for instability that might lead to precipitation to form. [5]

In neonatal care infants are often dependent on parental nutrition combined with other IV medications for survival. To avoid the risk of mixing incompatible medications, the children are exposed to several access points. Apart from the difficulties when establishing access points, due to infants' veins being smaller and harder to spot, the access points also increase the risk of infections. [4] There are problems associated with several vascular lines, in addition to the risk of infection, other risks such as hypervolemia, clots, perfusion dysregulation and extravasation as a result of several access points. In cases where the patients require continuous infusion it is sometimes necessary to stop the administration of medications or the parental nutrition in order to infuse other medications. This can lead to side effects such as malnutrition.[2, 4]

Parenteral nutrition is categorized based on the content provided. All parental nutrition mixtures contain minerals, electrolytes, vitamins and carbohydrates. Depending on the protein and fat level, the mixture is called 3- in 1 or 2- in 1 parenteral nutrition. The difference between these two mixtures is presence of fat in 3- in 1 and absence of fat in 2- in 1.[16] High concentrations of calcium phosphate is the predominant cause for precipitation in parenteral nutrition co-administered with other drugs.[6] Calcium can be administrated via parenteral nutrition at various concentrations but can also be given at higher concentrations through injection, concentrations given to children under the age of four varies from.

A co-infusion is considered to be compatible when there is "a lack of visible changes in the solution/intravenous lines and/or physicochemical stability of the components during the test period".[5] Additionally, no more than 10% decomposition of one or more of the substances was also stated as a criteria for compatibility. Drug incompatibility is often investigated from two standpoints, physical and chemical incompatibility. Physical incompatibilities include color change, precipitation and formation of bubbles and can be investigated with a simple visual inspection. However, in the case of subvisual particles a visual inspection is not sufficient. Changes in potency and stability are factors to consider when analyzing the chemical compatibility of co-infusions. [4, 5]

Particle size and count are important factors when determining drug compatibility. Up to 85 000 particles/day can be infused in children at neonatal intensive care units even when the infusion rates are very slow.[3] The US pharmacopeia <788> has established two methods for determination of compatibility by defining the limits of particle size and count. [7, 15] Method one aims to investigate particle count using light obscuration (LO), this can further be combined with flow imaging (FI) microscopy. [2] Method two is a microscopic particle count test hence backgrounded membrane imaging (BMI) can be used as an analytical method in subvisual particle analysis.[2, 8] In table 1, the defined limits of particle count and size in accordance with the USP <788> are shown. The analytical methods can be inconclusive with each other thus giving different results in regard to if the co-infusion is compatible or not. [2, 8, 15]

Table 1: Limits for subvisual particle exposure according to US pharmacopeia <788>

Particle size	Method 1	Method 2
10 µm	≤ 25 particles/mL	≤ 12 particles/mL
25 µm	≤ 3 particles/mL	≤ 2 particles/mL

2.2 The history of Ceftriaxone

Ceftriaxone is a third-generation cephalosporin used to treat bacterial infections and was first approved by the United Drug and Food administration (FDA) in 1984. The drug is mainly administered intravenously but can also be given intramuscular to patients. Compared to the earlier generations of cephalospors, Ceftriaxone is more effective against Gram-negative bacteria. However, activity against Gram-positive bacteria is also included in Ceftriaxone's activity spectrum against bacteria.[9] Children in neonatal care are often given Ceftriaxone against meningitis and pneumonia but the drug can also be used to treat other infections caused by bacteria such as skin infections, Gonorrhea and Syphilis. Commonly reported side effects according to FASS are eosinophilia, leukopenia, thrombocytopenia, diarrhea, rashes and an increase in liver enzymes.[13]

There is a risk of precipitation when administering Ceftriaxone together with calcium, especially when given to premature and full-term newborns less than 28 days old mainly due to their low blood volume. In 2007, the FDA together with Rocephin came out with a warning regarding co-administration of Ceftriaxone and calcium. Several cases of precipitates of Ceftriaxone and calcium in the lungs and kidneys of diseased premature and full-term infants had been reported. The statement given in July 2007 by the FDA prohibited healthcare personnel to administer Ceftriaxone and calcium simultaneously even via separate infusion lines, furthermore calcium containing products should not be provided to the patients within 48 hours after a Ceftriaxone treatment. In 2009, this statement was revised by the FDA after two vitro studies including blood plasma from both adults and neonatal showed no direct correlation between the risk of precipitation together with various concentrations of calcium. The new recommendations read as follows, “calcium containing products may be sequentially administered in patients older than 28 days if the infusion lines are thoroughly flushed between infusions with a compatible fluid”. [10]

The relation between Ceftriaxone and calcium in a solution can be illustrated by a precipitation equilibrium, see equation 1 below.

$$Ceftriaxone + Ca^{+2} \leftrightarrow Ceftriaxone - Ca\ complex\ (l) \leftrightarrow Ceftriaxone - Ca\ complex\ (s) \quad (1)$$

The amount of reactants determines how much complex you get and then the fact that the complex becomes insoluble drives the reaction towards complex formation.

2.3 Trastuzumab and glucose

Trastuzumab is an IgG1 humanized monoclonal antibody used to treat early stages of breast cancer and advanced gastric cancer. The drug is given intravenously and mostly in combination with chemotherapy. [11] Trastuzumab selectively targets the human epidermal growth factor receptor 2 (HER2). These receptors are found to a great extent on the surface of certain cancer cells and stimulate cell growth. By binding to the receptor Trastuzumab stops the cell growth which leads to apoptosis. [14]

Possible incompatibilities between Trastuzumab and glucose have been discovered at the department of process and life science where this master's thesis is conducted hence an interest of a further investigation. Parenteral nutrition has a high glucose content and is often administered to cancer patients which further increases the interest of investigating the compatibility of Trastuzumab and glucose.

Parental nutrition is often heat sterilized to not compromise the safety of the patients. However, heat sterilization can lead to formation of glucose degradation products (GDPs). Oxidation, hydrolysis and dehydration are the main reasons for GDP formation. The problem with GDPs is their high reactivity which can cause toxic effects after parenteral administration.[12]

In this study we will investigate the compatibility of non-sterile glucose dissolved in phosphate buffer and compare it with sterile glucose dissolved in sodium chloride, together with Trastuzumab. Clinically relevant concentrations of glucose will be used when conducting the experiment using heat sterilized glucose.

Trastuzumab is not used in neonatal care however this was available to us at the department where this master thesis was conducted.

2.4 Analytical instruments – LABBOT and Probe Drum

LABBOT is a multi-analytic tool which measures fluorescence, absorbance, light scattering as well as pH if an external pH-meter is connected. Fluorescence can be measured at wavelengths between 290 to 840 nm and absorbance at wavelengths between 225-770 nm. It is also provided with a pump facilitating titration which can be followed in real time. In addition to this the machine provides temperature control from 6°C to 85°C keeping the sample constant at the desired temperature as well as providing adjustable mixing using a magnetic stirrer at the desired speed. As fluorescence, absorbance and light scattering can be analyzed to determine compatibility among different injectabilities, LABBOT is suitable for this purpose. In this study we will use the LABBOT's predecessor Probe Drum due to its availability at the department. The difference between LABBOT and Probe Drum is mainly the size of the pump used for titration. The pump in LABBOT holds a maximum of 100ul compared to Probe Drum where it holds a maximum of 250ul. Probe Drum cannot do individual measurements which LABBOT can.

3. Method and materials

3.1 Validation of LABBOT

To validate the sensitivity of the LABBOT a comparative study was conducted. Three instruments were compared to each other in terms of Limit of detection (LOD) and Limit of quantification (LOQ). LABBOT, NanoDrop and 50 Bio Spectrophotometer were chosen as analytical instruments. The absorbance of Bovine serum albumin (BSA) was measured at different concentrations and are presented in the result section.

3.1.1 Preparation of phosphate buffer & BSA

The first step of the validation was to prepare a phosphate buffer which was prepared as followed:
15.6g NaH_2PO_4 x $2H_20$ and 14.2g Na_2HPO_4 was dissolved in 1000ml H2O each in separate beakers, using a magnetic stirrer. 423 ml NaH_2PO_4 solution and 577 ml Na_2HPO_4 solution was then added to a 1000 ml beaker and mixed. pH was measured at 4.5 using pH paper and then stored in 50 ml plastic tubes at –18°C until usage.

0.004g Bovine Albumin Serum (BSA) was dissolved in 4 ml phosphate buffer using a magnetic stirrer and stored at 5°C until usage.

3.1.2 Validation of equipment

For each instrument the same sample was used to minimize sources of error. 2ml of stock solution was first added to a cuvette and measured using spectrophotometer followed by LABBOT. The same cuvette was used for both instruments on the same day. The dilution of the BSA was conducted in the same cuvette throughout the entire process by substituting a part of BSA with phosphate buffer. The removed volume of BSA was saved and transferred into Eppendorf tubes and later used for analysis with the NanoDrop.

3.1.3 50 Bio Spectrophotometer

A wavelength of 280 nm was chosen followed by blanking of the instrument by adding 1 ml phosphate buffer to a cuvette and measuring the absorbance. 1 ml of 1 mg/ml BSA solution was then added to a new cuvette and placed in the spectrophotometer followed by 10 measurements of the absorbance. The BSA solution in the cuvette was then diluted by replacing a calculated amount of BSA solution with phosphate buffer, keeping the total volume in the cuvette constant at 1 ml. The absorbance was then measured 10 times before diluting again and this procedure was repeated to obtain measurements at all concentrations presented in the result section.

3.1.4 LABBOT

The same procedure as with validation of 50 Bio were carried out here with the exception that blanking using phosphate buffer was done between each concentration and not only in the beginning.

3.1.5 NanoDrop

The wavelength was set to 280 nm. In accordance with the instructions given by NanoDrop a drop of milli q water was added to the measuring space using a 20 µl pipette with the purpose of calibrating the instrument.

After this measurement the measuring space was cleaned using a Kimtech wipe, this was repeated after every measurement throughout the whole study. A drop of phosphate buffer was added to the measuring space in order to blank the instrument. A new drop of BSA was added prior to each measurement and the measuring space was cleaned after each measurement. This procedure was repeated ten times for each concentration and the BSA was collected from the saved Eppendorf tubes as mentioned in section 3.1.2.

3.2 Drug incompatibility study

This study was divided into two parts with each part assigned a drug together with a substance expected to precipitate when mixed. The substance was titrated against the drug and analyzed using Probe Drum or manually mixed in a cuvette. The factors altered were the concentration of titrant and the time between additions of titrant when using the Probe Drum. Details about the method of each part are further presented below. A flow cell was also built and used for further compatibility studies.

3.2.1 Ceftriaxone and calcium

Ceftriaxone diluted in sodium chloride was used as titrand with a constant concentration of 40mg/ml throughout all experiments. Three different samples with a calcium concentration of 10mM, 15mM and 20mM were also diluted in sodium chloride and used as titrants. These samples were all derived from a 1M calcium stock solution. When not actively used all of the prepared samples were stored in fridge at 2-8°C.

A total of nine experiments were carried out in this part. As previously mentioned, the concentration of calcium and the time between additions of the titrant was varied. The experimental set up resulted in a total running time of either 2h, 4h or 10h. See table 2 for the experimental scheme used.

Table 2: Experimental setup

2h (900s) 10mM	2h, (900s) 15mM	2h, (900s) 20mM
4h, (1600s) 10mM	4h, (1600s) 15mM	4h, (1600s) 20mM
10h (4300s) 10mM	10h (4300s) 15mM	10h (4300s) 20mM

Lower concentrations of calcium were tested manually in cuvettes before conducting the experimental set up in table 2. However, concentrations lower than 10 mM of calcium together with the commonly administered concentration 40mg/ml of Ceftriaxone did not give any precipitation that the LABBOT could detect.

3.2.1.1 Program set up

The volume of the titrand was set to 2 ml each run and transferred into clear PMMA cuvettes. The diluted calcium was added in steps of 25µl until a total volume of 200µl had been added to the cuvette containing the Ceftriaxone solution. A stirring magnet was used in the cuvette during the whole program and the stirring speed was set to 4. The temperature was set to 25°C. Equilibrium time was adjusted in accordance with the wanted total running time, the Probe Drum program and was set to respectively 900s (2h), 1600s (4h) or 4300s (10h), the equilibrium time is the time between additions of the titrant. Sodium chloride was used as the blank prior to each run and was added to a clear PMMA cuvette in volumes of 2 ml each run.

The absorbance was measured at a wavelength spectrum of 280-720nm. A second detector measured the light scattering with a laser at 637nm. After each analysis a visual inspection was performed in order to further confirm the formation of precipitation, this was done by holding the cuvette against a black surface.

3.2.2 Y-site flow cell

Each liquid used in this part was assigned a beaker, to each beaker a silicone tube with an inner diameter of 2 mm was attached. These tubes were connected to a peristatic pump each, with a flow rate at 0.4 ml/min each. At the outlet of the pumps the tubes were joint together with a Y-site connection where the two liquids continue together in one joint tube, with a flow rate of 0.84 ml/min. A plastic tube with an inner diameter of 1 mm was attached to the joint silicone tube with a plastic connection. The smaller tube was then added to the bottom of a PMMA cuvette through a plastic lid, see figure 3. Two holes, 2 mm each, were drilled in the lid of the cuvette using a drilling machine.

The purpose of the second part of the flow cell was to keep the liquid level constant in the cuvette. To do so a second tube with a 1 mm inner diameter was placed at the surface of the

desired liquid level in the cuvette, connected to a bigger silicone tube also attached to a peristaltic pump. The pumping rate of this pump was set to 20 ml/min in order to minimize the risk of overflow in the cuvette. A schematic drawing of the flow cell is illustrated in figure 1 furthermore a picture of the flow cell used is included as well, see figure 2.

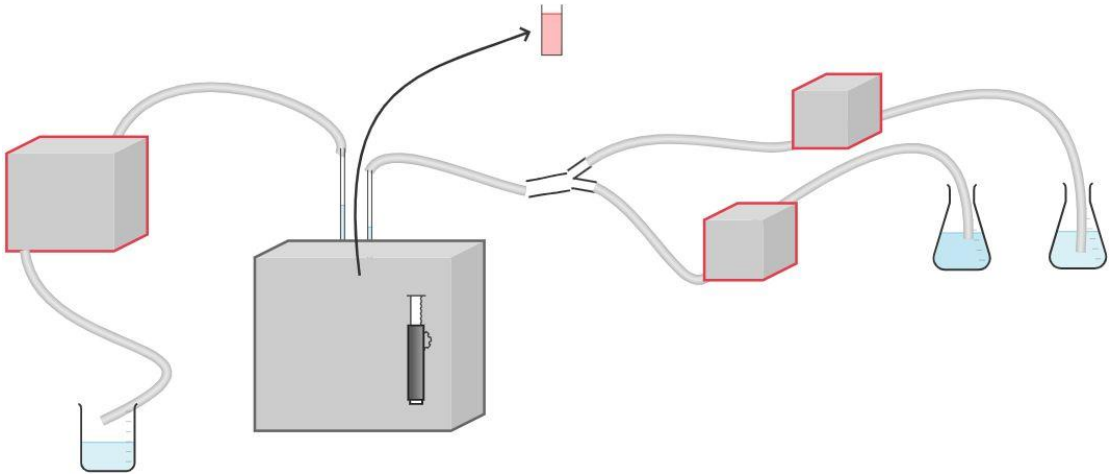

Figure 1: Schematic drawing of flow cell. The cubes with the red border are representing the peristatic pumps used. The beakers to the right are the beakers containing Ceftriaxone respectively calcium. The small beaker with the red content represents the cuvette used furthermore the arrow is illustrating where in the Probe Drum the cuvette is placed.

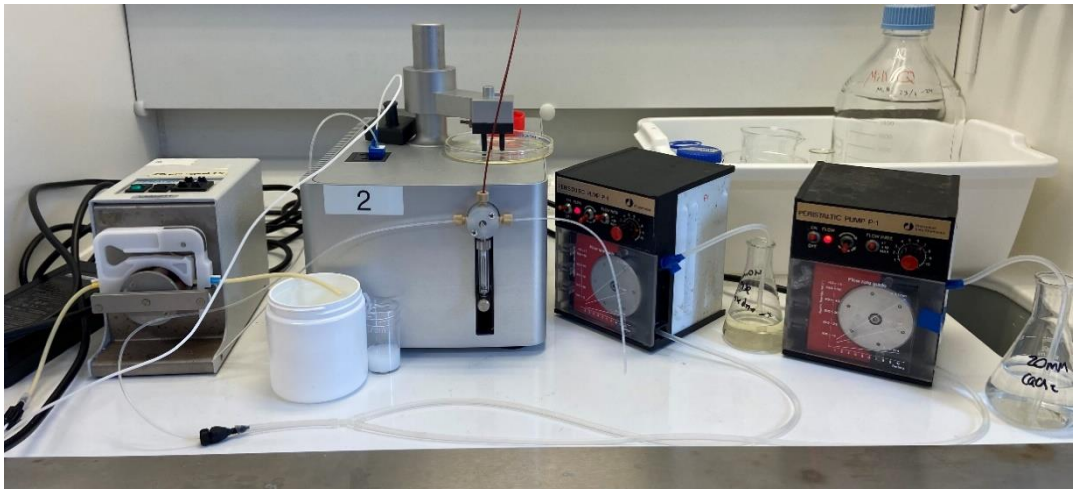

Figure 2: Picture of the real flow cell with all the components used. The machine marked with "2" is the Probe Drum.

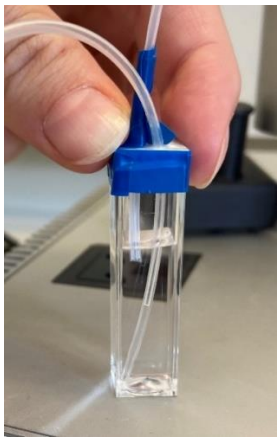

Figure 3: Picture of the cuvette used in the flow cell where the right tube is connected to the Y-site providing the cuvette with the drug mixture and the left tube representing the tube pumping out the liquid.

The cuvette was first filled with 3 ml sodium chloride and placed in the Probe Drum followed by blanking. The flow cell was then started, and any possible precipitation formed was analyzed for 158 min using the Probe Drum. The beakers were initially filled with 58 ml each of the original solutions of the drugs or substances analyzed. The calcium solution had a concentration of 20mM and was prepared in accordance with section 3.2.1.

3.2.2.1 Program set-up for Probe Drum

The sample volume in the cuvette was set to 3 ml and initial temperature to 25°C. In order to adjust the total running time, the baseline was set to 14 400 s. Spectra during equity time was turned on and the signal was measured every 144 s.

Similar to the set up in section 3.1.1.1, the absorbance was measured at a wavelength spectrum of 280-720nm. A second detector measured with a laser at 637nm. After each analysis a visual inspection was performed in order to further confirm the formation of precipitation, this was done by holding the cuvette against a black surface.

3.2.3 Trastuzumab and glucose

The objective of this part was to investigate the compatibility between Trastuzumab and glucose. Trastuzumab was combined with both sterile and non-sterile glucose, therefore this method description will be divided into two parts. The Trastuzumab that was used had a concentration of 10 mg/ml.

3.2.3.1 Non-sterile glucose

1.1M glucose was prepared by diluting 0.25 g glucose in phosphate buffer, see section 3.1.1 for description of the buffer. 200µl 10mg/ml Trastuzumab was added to a PMMA cuvette containing 3 ml non-sterile glucose for 2 hours using Probe Drum. The cuvette was then visually inspected followed by microscopic analysis. The microscopic analysis was carried out directly after the visual inspection and repeated the day after.

3.2.3.2 Sterilized glucose

200 µl 10 mg/ml Trastuzumab was added to a cuvette containing 3ml sterilized glucose and mixed gently. A visual inspection was done after 2 hours followed by microscopic analysis. The microscopic analysis was carried out directly after the visual inspection and repeated the day after.

4. Results & Discussion

4.1 Validation

4.1.1 Linear regression and determination of LOD and LOQ

Table 3 below shows a summary of the calculated values of LOD and LOQ for respective instruments. A more thorough description of how the calculations were carried out is provided in the appendix section of this report under Appendix B.

Table 3: Calculated LOD and LOQ values for respective analytical instruments.

	LOD [mg/ml]	**LOQ [mg/ml]**
50 Bio	0.0026	0.0080
LABBOT	0.0137	0.0416
NanoDrop	0.2134	0.6467

As seen in table 3 above, both LOD and LOQ are the lowest for 50 Bio, approximately ten times higher for LABBOT compared to 50 Bio, and the highest for NanoDrop. This indicates that 50 Bio has the highest sensitivity followed by LABBOT and lastly NanoDrop. As seen in table 4 in Appendix, the lowest concentration measured was 0.015mg/ml meaning that we could have continued the measurements using 50 Bio. The calculated LOD and LOQ for NanoDrop is in accordance with the literature.[17]

In figure 4 below it is seen that the measured absorbances using 50 Bio, LABBOT and NanoDrop plotted against the BSA concentrations create 3 different calibration curves, one for each instrument. The R^2 gives an indication of how well the linear regression fits the data, with an R^2 value of 1 indicating perfect fit.

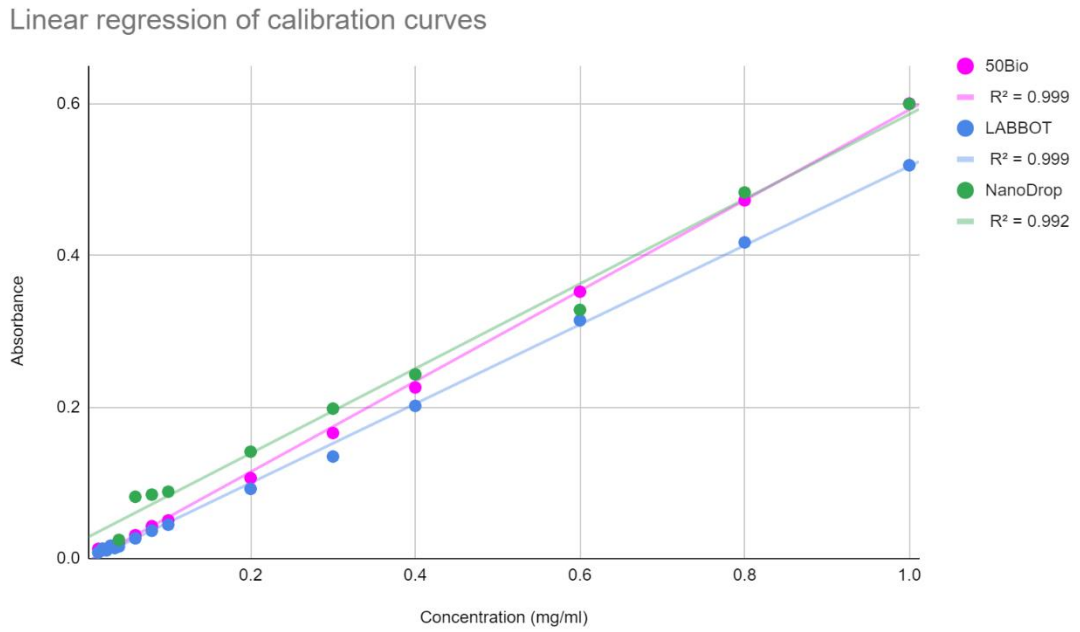

Figure 4: Calibration curves for each instrument with measured absorbance plotted against BSA concentration. Separate calibration curves are presented in Appendix C.

In figure 4 the R^2 value is presented for each instrument, as seen in the figure all R^2 values are close to 1 indicating that the regression is a good fit of the data presented. However, the R^2 value of the NanoDrop is lower compared to the other instruments validated. This could be an indication that the NanoDrop has a lower precision than LABBOT and 50 Bio. This is further confirmed by the Standard deviation values presented in Appendix A, as NanoDrop has significantly higher standard deviations compared to the other instruments.

4.1.2 Relative standard deviation

In the figures below the standard deviation of the measured absorbances at each concentration divided by the mean value of the measured absorbances is plotted against the concentration. Unlike looking at just the standard deviation, which says how much the measurements vary from the mean absorbance, we now get a value relative to the absorbance. This gives a clearer picture as a standard deviation does not take the value of absorbance into account. As seen in all three figures below, the precision is the highest at higher concentrations as the SD/mean abs ratio is smaller. This indicates that the instruments' ability to analyze deteriorates as the concentration approaches LOD, as expected.

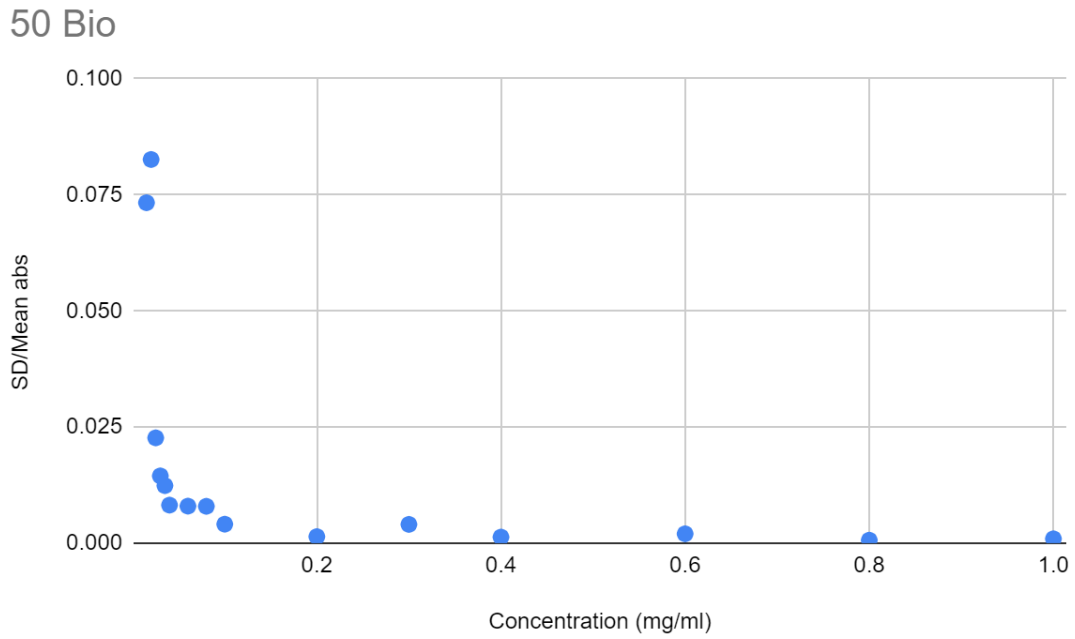

Figure 5: Standard deviation through mean absorbance plotted against concentration for measurements using spectrophotometer.

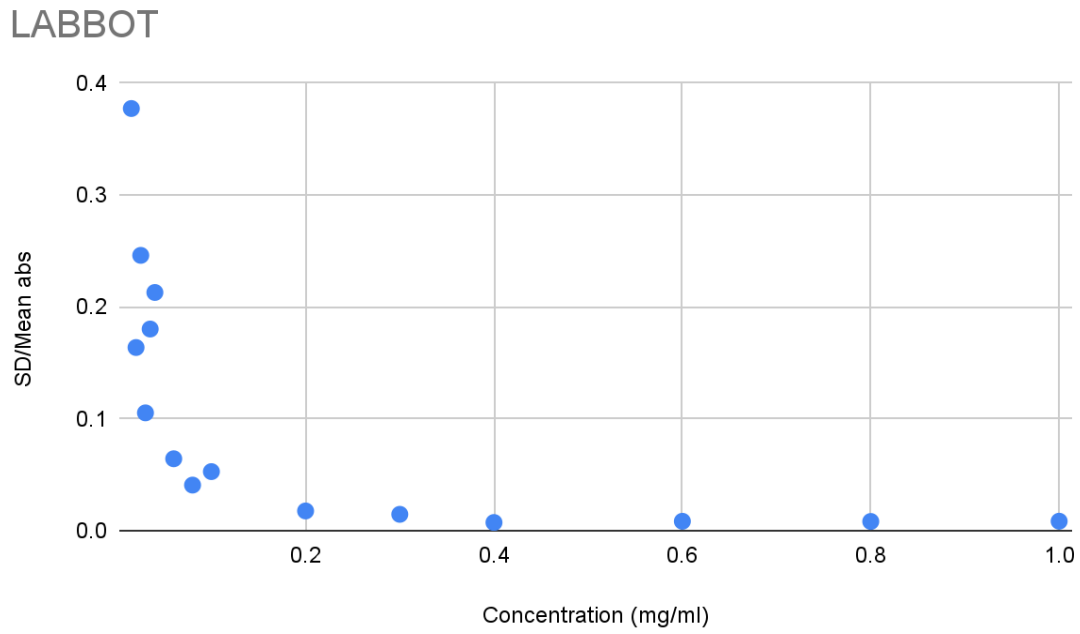

Figure 6: Standard deviation through mean absorbance plotted against concentration for measurements using LABBOT.

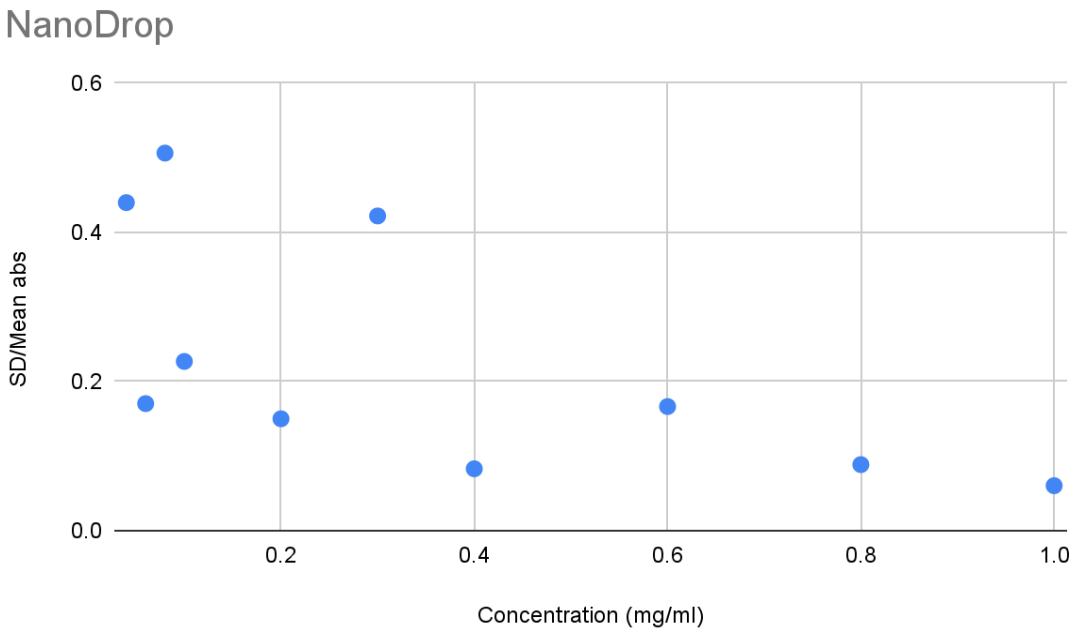

Figure 7: Standard deviation through mean absorbance plotted against concentration for measurements using NanoDrop.

The figures above illustrate that the relative standard deviation is the lowest for 50 Bio, meaning that the instrument has the highest precision of the instruments. Figure 5 (50 Bio) and 6 (LABBOT) show that the relative standard deviation for both instruments approaches 0 as the concentration is increased. This makes 50 Bio more suitable especially at low concentrations as this is where the relative standard deviation alters the most between the instruments. For concentrations approaching 1mg/ml the precision of the instruments does not differ as much, though 50 Bio still has higher precision.

The relative standard deviation is higher for NanoDrop compared to 50 Bio and LABBOT at low concentrations and it does not approach 0 as the concentration is increased. This indicates that NanoDrop is the least suitable instrument for measurements at both low and high concentrations. For the NanoDrop we were not able to measure at concentrations lower than 0.04 mg/ml before receiving negative results, unlike LABBOT and 50 Bio where the measurements were carried out at lowest 0.015 mg/ml. However, as limit of quantification for NanoDrop were calculated at, 0.6467mg/ml measured absorbances of concentrations lower than this are unreliable.

4.2 Results & discussion of Ceftriaxone

4.2.1 Results of Ceftriaxone titrated against 10mM calcium

In the figure below the light scattering signal obtained when titrating 10mM calcium to 40mg/ml Ceftriaxone for 2, 4 respectively 10 hours using the probe drum is plotted against the titrant concentration in the cuvette. The signals reached during 2 and 10h are considered too low to indicate precipitation.

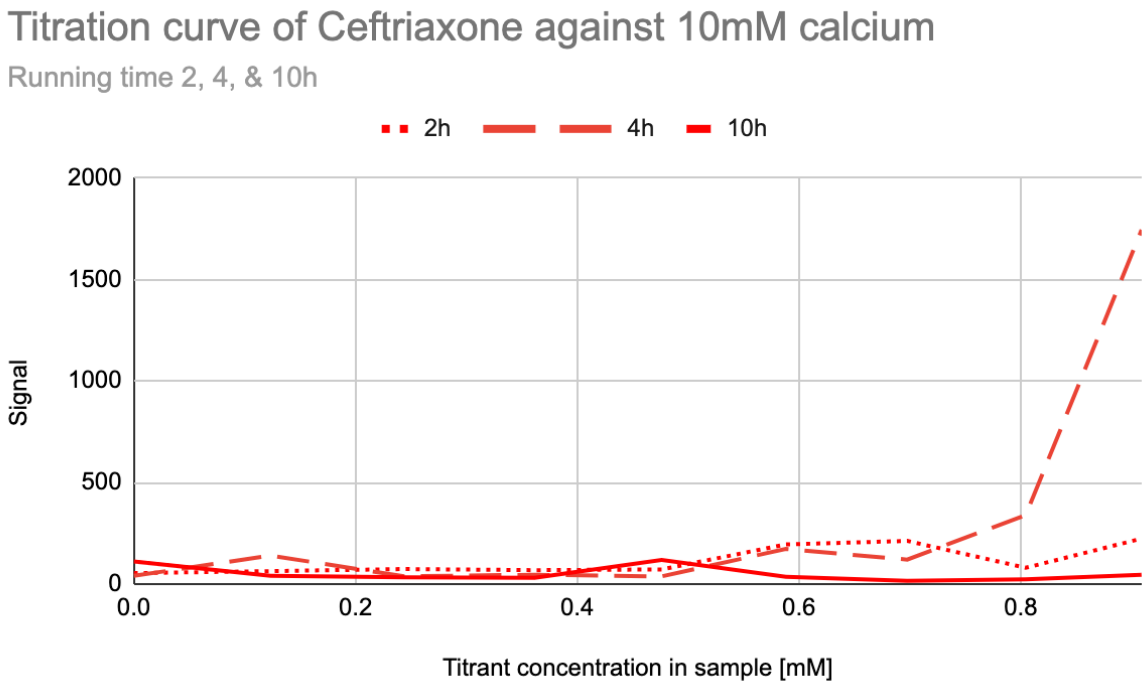

Figure 8: Signal plotted against concentration of titrant in sample with Ceftriaxone when titrating using 10mM calcium to 40 mg/ml Ceftriaxone for 2,4 and 10hours.

4.2.1.1 Discussion of result section 4.2.1

As seen in figure 8 above the signal formed when the titrant concentration in the sample reaches 0.6, 0.8 respectively 0.5mM is significantly lower compared to the signal in the remaining graphs below. This low signal indicates that precipitation has not occurred when titrating with 10mM calcium to 40mg/ml Ceftriaxone for 2, 4 or 10 hours. This is also confirmed by the visual inspection afterwards which did not show any precipitation in any of the samples. Furthermore, it can be concluded that there are not enough reactants present to form an insoluble complex.

However, the maximum signal obtained during the 4 hour run is significantly higher than the maximum signals obtained in the 2 respectively 10 hour runs, this is probably due to contamination in the sample, for example dust grains, as the signal still differs from the signals obtained when titrating with higher calcium concentrations as well as no precipitation is shown during the visual inspection.

4.2.2 Results of Ceftriaxone titrated against 15mM calcium

In the figure below the light scattering signal obtained when titrating 15mM calcium to 40 mg/ml Ceftriaxone for 2, 4 respectively 10 hours using the probe drum is plotted against the titrant concentration in the cuvette.

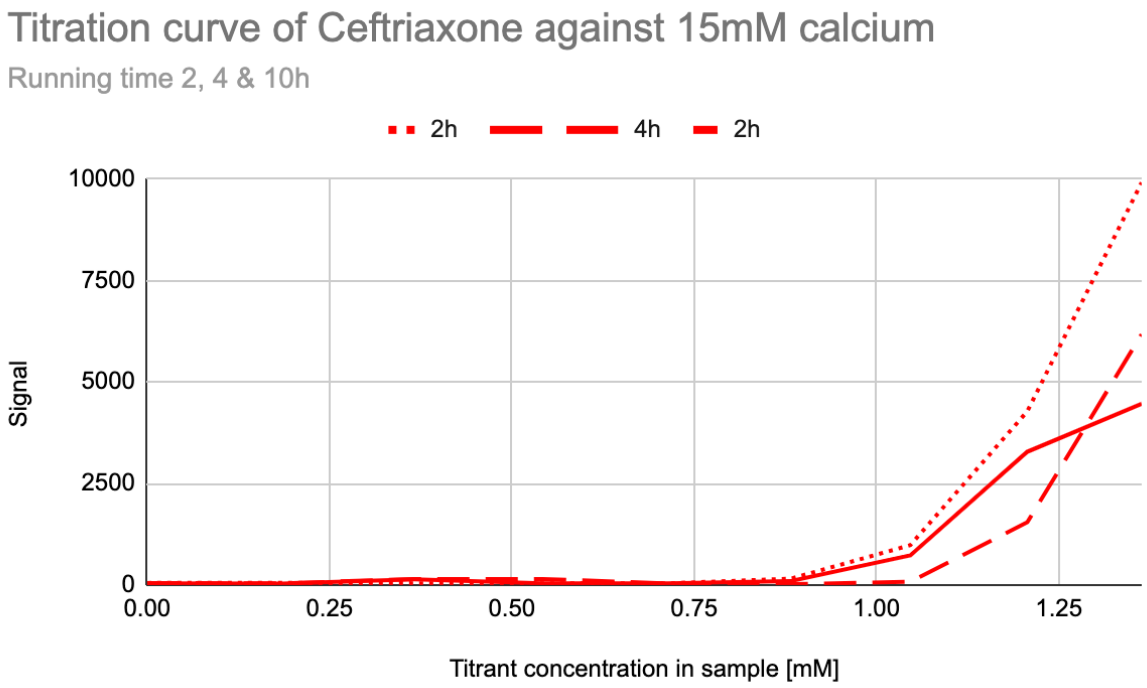

Figure 9: Signal plotted against concentration of titrant in sample with Ceftriaxone when titrating using 15mM calcium to 40 mg/ml Ceftriaxone for 2,4 and 10 hours.

4.2.3 Results of Ceftriaxone titrated against 20mM calcium

In the figure below the light scattering signal obtained when titrating 20mM calcium to 40 mg/ml Ceftriaxone for 2, 4 respectively 10 hours using the probe drum is plotted against the titrant concentration in the cuvette.

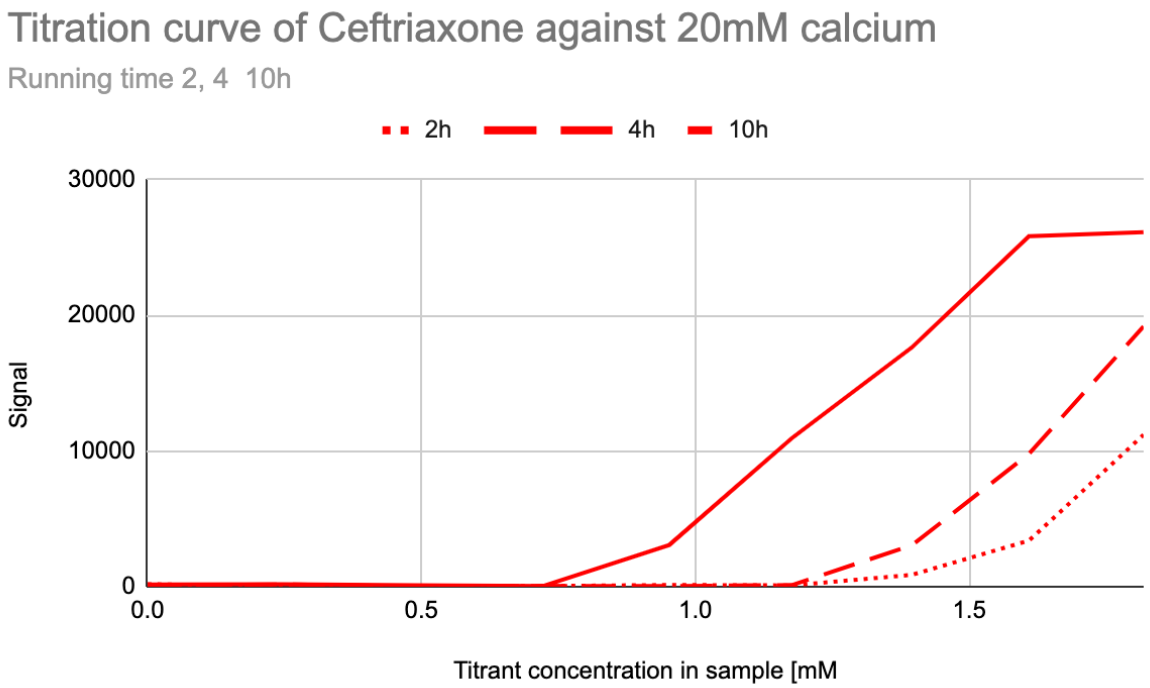

Figure 10: Signal plotted against concentration of titrant in sample with Ceftriaxone when titrating using 20mM calcium to 40 mg/ml Ceftriaxone for 2,4 and 10 hours.

4.2.4 Discussion of result sections 4.2.2 and 4.2.3

For calcium concentrations of 15mM and 20mM the results show that precipitation has occurred, see figure 9 and 10. The visual inspection also confirms these results.

The results also show that a higher signal is obtained when titrating with 20mM calcium compared to 15mM when comparing at the same running time. These results were expected as the size of the signal is dependent on the amount of precipitation as well as the size of the particles formed during precipitation. The latter depends on running time with shorter running time often resulting in bigger precipitation particles.

One of our objectives was to investigate if time between the additions of titrant, i.e. the total running time in figures, affects the amount of precipitation that could be formed. As seen in the result section, at 20mM the signal is increased with a longer running time. However, this is not the pattern when comparing the figures belonging to 15mM calcium, where the signal decreases with a longer running time.

A longer running time implies a longer time between addition and measurement, increasing the probability of precipitation dissolving. Furthermore, after an addition of the 15mM calcium a high concentration of complex is obtained locally in the sample which is then dissolved rapidly. This is a possible explanation for the pattern observed from the results obtained from the 15mM experiments. Another possible explanation for the decrease in signal with longer running time is that the particle size distribution is different for the experiments as the light scattering signal also is dependent on the size of the particles.

To be able to explain the results at 20mM, one must take the equilibrium equation into account, see equation 1. A fixed amount of Ceftriaxone is added to the cuvette from the start. After a certain amount of calcium is added, all of the free Ceftriaxone components will be occupied in the precipitation form. This means that the amount of precipitation will stay constant even if more calcium is added due to Ceftriaxone being the limiting reactant in this case. The reason that the precipitate does not dissolve again, hence the stagnation of the curve in figure 10, at 20mM compared to 15mM is because that the high concentration of free calcium ions keep the solution saturated.

4.2.5 Result of flow cell compatibility study using Ceftriaxone and calcium

In this section the result from the analysis of the flow cell using Probe Drum is presented below. However, there were some deviations from the methodology when conducting the experiment that can be considered sources of errors. Firstly, at one point during the first 20 minutes the tube connected to the beaker containing the Ceftriaxone solution was pulled above the liquid level causing air to be pumped in instead of Ceftriaxone.

The pump connected to the outlet of the cuvette was clogged by precipitation hence not fulfilling its purpose thus making the cuvette overflow. This was corrected by removing the cuvette and the belonging tubes and cleaning them with water before putting it back in its original set up. This happened numerous times throughout the whole experiment.

After 158 minutes the Ceftriaxone was finished, and the program was stopped earlier than expected.

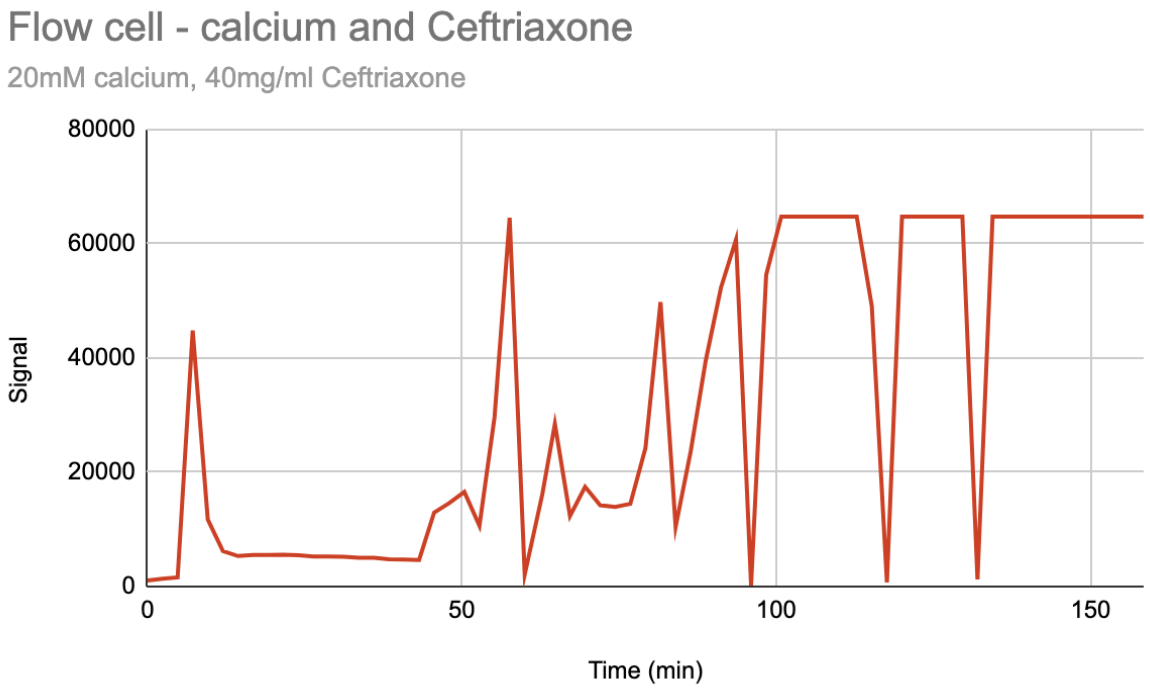

Figure 11: Signal plotted against time in minutes of calcium and Ceftriaxone joined together via y-site connection.

4.2.5.1 Discussion of results derived from the flow cell compatibility study

As seen in figure 11 in the section above, the signal increases during the first 100 minutes of the run. The flat curve between 10 and 50 minutes can be explained by the decreased flow of Ceftriaxone, due to the tube connected to the beaker containing Ceftriaxone, resulting in no precipitation being formed.

Between 50 and 100 minutes the results show an increase in signal and after 100 minutes the curve seems to stagnate. This is most likely due to reaching the maximum signal Probe Drum is capable of measuring.

4.3 Results of Trastuzumab titrated against glucose

4.3.1 Probe Drum analysis

In the figure below the light scattering signal obtained when titrating 10 mg/ml Trastuzumab against 50 mg/ml non-sterile glucose for 2 hours using Probe Drum.

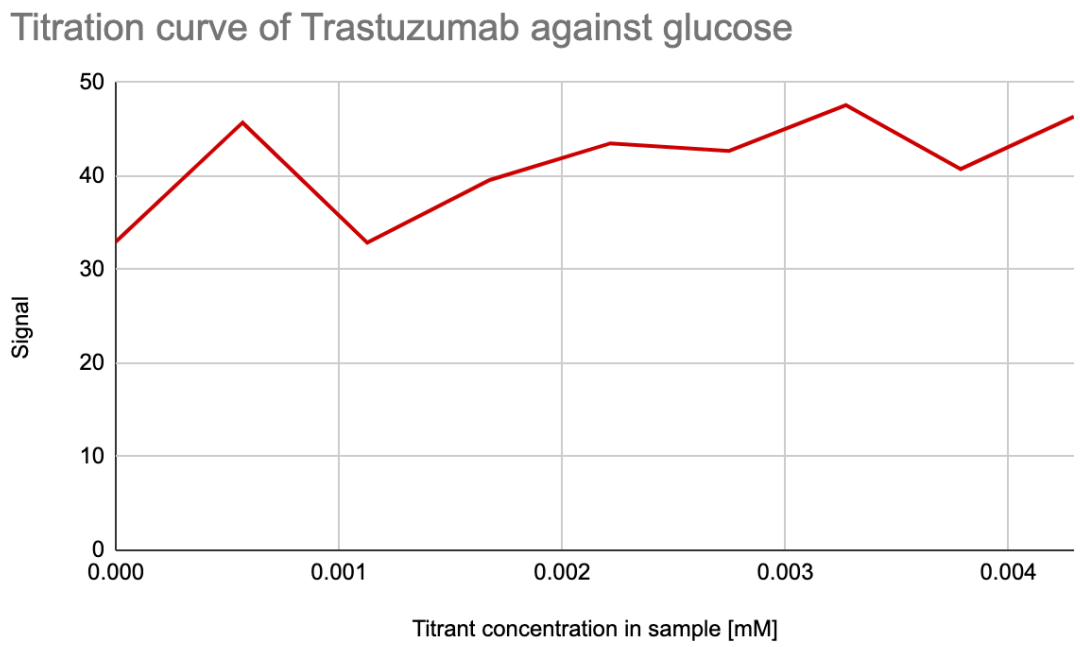

Figure 12: Calibration curve of 10 ml/mg Trastuzumab titrated against 50 mg/ml non-sterile glucose for 2 hours using Probe Drum.

4.3.2 Visual inspection

There were no signs of incompatibility formed from the visual inspection.

4.3.3 Microscopic inspection

The figures below show a microscopic picture of 50 mg/ml non-sterile glucose and sterile glucose, 10 mg/ml Trastuzumab along with 10 mg/ml Trastuzumab and 50 mg/ml sterile glucose after 2 respectively 24 hours as well as 10 mg/ml Trastuzumab and 50 mg/ml non-sterile glucose after 2 respectively 24 hours.

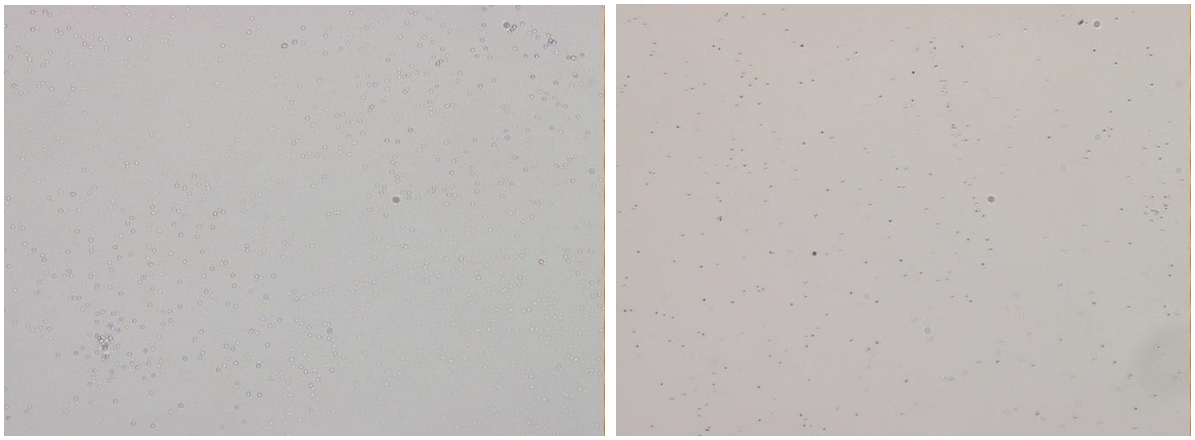

Figure 13 & 14: Microscopic picture of 50 mg/ml sterile glucose (left) and 50 mg/ml non-sterile glucose (right).

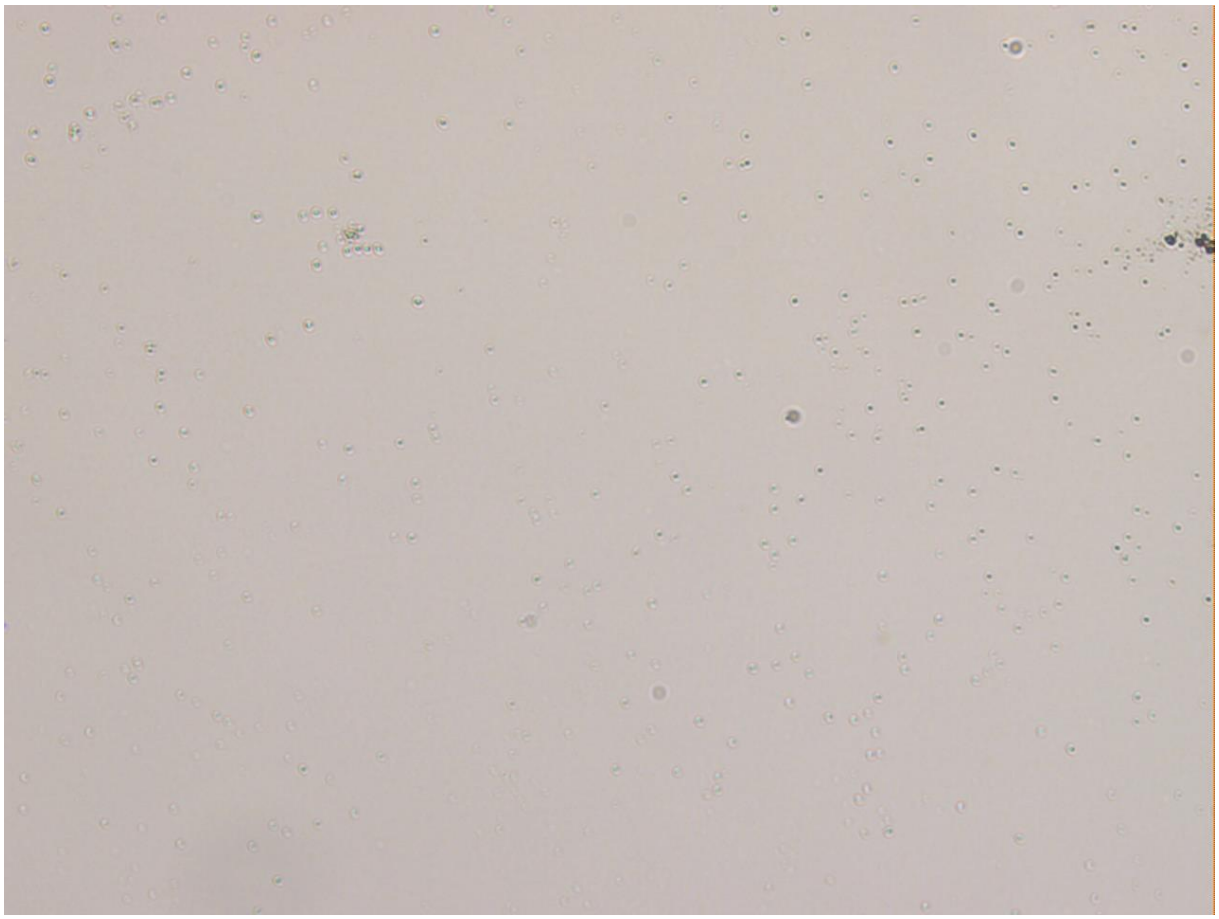

Figure 15: Microscopic picture of 10 mg/ml Trastuzumab.

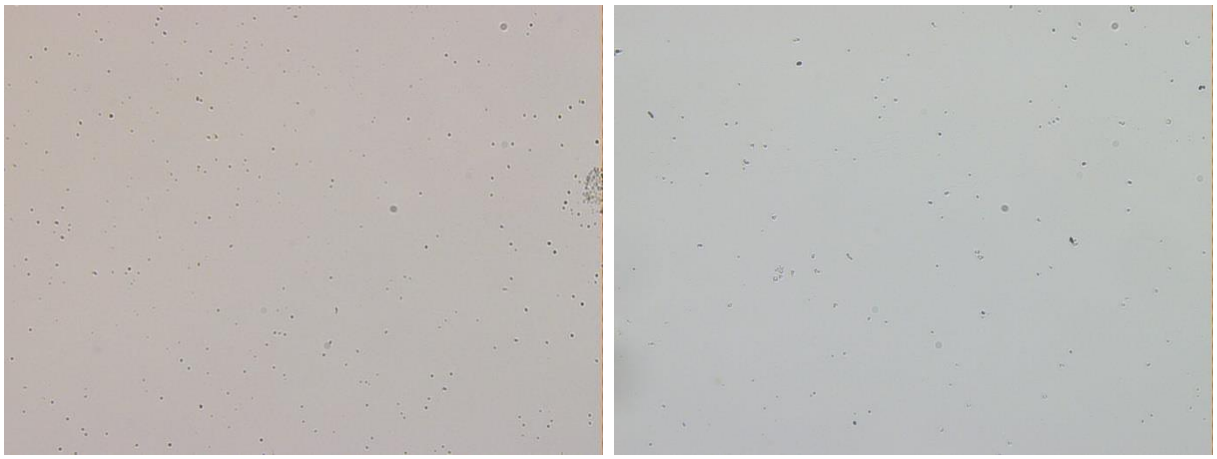

Figure 16 & 17: Microscopic picture of 10 mg/ml Trastuzumab and 50 mg/ml sterile glucose 2 hours after mixing (left) and 1 day after mixing (right).

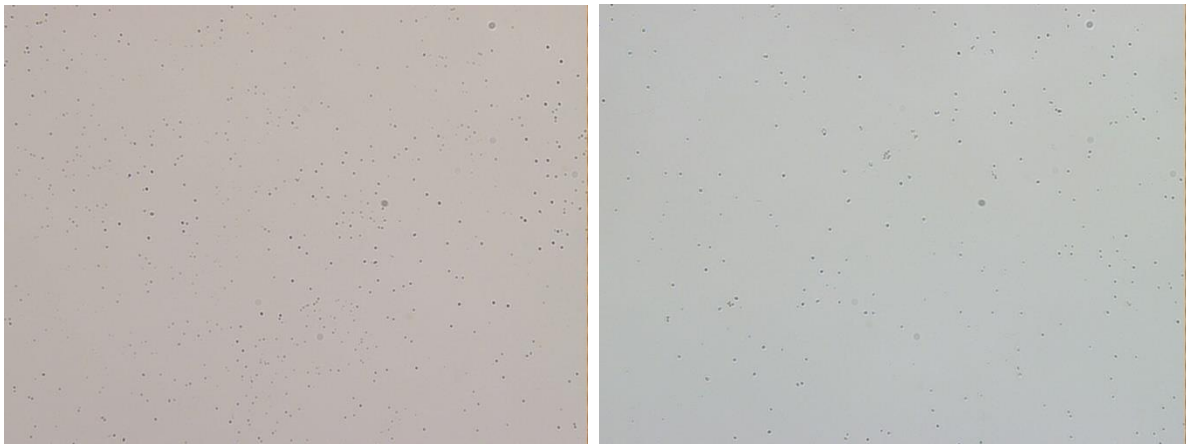

Figure 18 & 19: Microscopic picture of 10 mg/ml Trastuzumab and 50 mg/ml non-sterile glucose 2 hours after mixing (left) and 1 day after mixing (right).

4.4 Discussion of Trastuzumab titrated against glucose

As seen in the results section above there is no sign of incompatibility from either the Probe Drum analysis (figure 12), as the signal does not increase during the titration, nor the visual inspection. However, there might still be precipitation formed in terms of subvisual particles which is why the visual inspection was followed up using a microscope.

From the microscopic analysis, the results indicate that there is no significant difference in amount or size of particles between Trastuzumab combined with glucose (figure 16-19) and glucose and Trastuzumab on their own (figure 13-15). This is the case for both sterile and non-sterile glucose as well as when the analysis of combined Trastuzumab and glucose was done one day later.

The most probable explanation for the lack of precipitation regarding combination of Trastuzumab and sterile glucose might be that the glucose concentration that was reached in the sample was too low due to the low titrant concentration. A possible solution for this would be to increase the glucose concentration in the titrant. Unfortunately, this was not possible in

this study as the sterile glucose was delivered ready to use. However, an increased glucose concentration was used when non-sterile glucose was analyzed using Probe Drum resulting in no incompatibility. Here the most probable reason for lack of precipitation could be a too low glucose concentration in the sample, this time due to the added titrant volume being too low rather than that the titrant concentration is too low. Here it would have been useful to have a bigger pump in Probe Drum to reach a higher concentration of the titrant in the cuvette for the purpose of investigating drug incompatibility since many drugs are delivered at already desirable concentrations in liquid form.

5. Conclusion

50 Bio spectrophotometer showed the highest sensitivity followed by LABBOT and lastly the NanoDrop. The difference between 50 Bio and LABBOT is most significant at lower concentration. However, the 50 Bio lacks LABBOT's ability to titrate and keep temperature constant.

From this study it can be concluded that Probe Drum is a suitable tool for titrations studies aimed for detecting precipitations.

No drug incompatibility was discovered between Trastuzumab and glucose at the concentrations and volumes tested in this study. Due to limitations regarding the pump volume in the Probe Drum the desired concentration of glucose used in neonatal care, in the cuvette could not be reached. Making Probe drum not suitable for detecting drug-incompatibility between glucose and Trastuzumab.

References

1. Kumar, P., et al., Medication Use in the Neonatal Intensive Care Unit: Current Patterns and Off-Label Use of Parenteral Medications. Journal of Pediatrics, 2008. 152(3): p. 412-415.e1.

2. Ross, E.L., et al., Physical Compatibility of Y-site Pediatric Drug Administration: A Call for Question of US Pharmacopeia Standards? Journal of Pediatric Pharmacology and Therapeutics, 2023. 28(1): p. 84-92.

3. Perez, M., et al., Dynamic Image Analysis to Evaluate Subvisible Particles during Continuous Drug Infusion in a Neonatal Intensive Care Unit. Scientific Reports, 2017. 7(1).

4. Greenhill, K., E. Hornsby, and G. Gorman, Investigations of physical compatibilities of commonly used intravenous medications with and without parenteral nutrition in pediatric cardiovascular intensive care unit patients. Pharmaceuticals, 2019. 12(2).

5. Kalikstad, B., Å. Skjerdal, and T.W.R. Hansen, Compatibility of drug infusions in the NICU. Archives of Disease in Childhood, 2010. 95(9): p. 745-748.

6. Allwood, M.C. and M.C.J. Kearney, Compatibility and stability of additives in parenteral nutrition admixtures. Nutrition, 1998. 14(9): p. 697-706.

7. Tomczak, S., M. Stawny, and A. Jelińska, Co-administration of drugs and parenteral nutrition: In vitro compatibility studies of loop diuretics for safer clinical practice. Pharmaceutics, 2020. 12(11): p. 1-12.

8. Ross, E.L., et al., Physical compatibility of medications with concentrated neonatal and pediatric parenteral nutrition: A simulated Y-site drug compatibility study. Journal of Parenteral and Enteral Nutrition, 2023. 47(3): p. 372-381.

9. Richards, D.M., et al., Ceftriaxone: A Review of its Antibacterial Activity, Pharmacological Properties and Therapeutic Use. Drugs, 1984. 27(6): p. 469-527.

10. Steadman, E., et al., Evaluation of a potential clinical interaction between ceftriaxone and calcium. Antimicrobial Agents and Chemotherapy, 2010. 54(4): p. 1534-1540.

11. Boekhout, A.H., J.H. Beijnen, and J.H.M. Schellens, Trastuzumab. Oncologist, 2011. 16(6): p. 800-810.

12. Leitzen, S., et al., Quantification of degradation products formed during heat sterilization of glucose solutions by lc-ms/ms: Impact of autoclaving temperature and duration on degradation. Pharmaceuticals, 2021. 14(11).

13. Läkemedelsindustriföreningen. (2024). Ceftriaxone Fresenius Kabi. I FASS Allmänhet. Accessed 5 May 2024 from https://www.fass.se/LIF/product?nplId=20111210000040&userType=0.

14. Läkemedelsindustriföreningen. (2024). Herceptin. I FASS Allmänhet. Accessed 5 May 2024 from https://www.fass.se/LIF/product?userType=2&nplId=20000828000019#product-information.

15. The United States Pharmacopeial Convention (2012). US Pharmacopiea <788>. Accessed 13 May 2024 from https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisions/788_particulate_matter_in_injections.pdf

16. Slattery E, Rumore MM, Douglas JS, Seres DS. 3-in-1 vs 2-in-1 parenteral nutrition in adults: a review. Nutr Clin Pract. 2014;29(5):631-635.

17. University of Rhode Island. Nanodrop. Accessed 27 May from https://web.uri.edu/riinbre/nanodrop-8000-2/

Appendix

Appendix A: Validation of LABBOT

Table 4: Obtained mean absorbances as well as calculated standard deviations, limit of quantification (LOQ) and limit of detection (LOD) from each concentration using 50Bio spectrophotometer

Concentration (mg/ml)	Mean absorbance	Standard Deviation	LOQ (mg/ml)	LOD (mg/ml)
0.015	0.01304	0.000955917242	0.008028123421	0.002649280729
0.020	0.01138	0.000940212741 9		
0.025	0.01328	0.000301109061 1		
0.03	0.01534	0.000222111083 3		
0.035	0.01787	0.000221359436 2		
0.04	0.02031	0.000166332999 3		
0.06	0.03118	0.000248551358 4		
0.08	0.04295	0.000340750805		
0.1	0.05065	0.000206827894 1		
0.2	0.10647	0.000149443411 8		
0.3	0.16593	0.000670074622 7		
0.4	0.22600	0.000294392028 9		
0.6	0.35241	0.000707813534 8		
0.8	0.47286	0.000298886823 6		
1.0	0.60065	0.000562237593 1		

Table 5: Obtained mean absorbances as well as calculated standard deviations, limit of quantification (LOQ) and limit of detection (LOD) from each concentration using LABBOT.

Concentration (mg/ml)	Mean absorbance	Standard Deviation	LOQ (mg/ml)	LOD (mg/ml)
0.015	0.00831	0.0031	0.0415646243	0.01371632602
0.020	0.01330	0.0022		
0.025	0.01107	0.002723988089		
0.03	0.01733	0.00182455474		
0.035	0.01413	0.002546479051		
0.04	0.01617	0.003442560417		
0.06	0.02691	0.001729129518		
0.08	0.03701	0.001507352351		
0.1	0.04490	0.002369716345		
0.2	0.09224	0.001622891795		
0.3	0.15389	0.002245217139		
0.4	0.20177	0.001468218876		
0.6	0.31454	0.00263236269		
0.8	0.41742	0.003421760431		
1.0	0.51918	0.004374115784		

Table 6: Obtained mean absorbances as well as calculated standard deviations, limit of quantification (LOQ) and limit of detection (LOD) from each concentration using NanoDrop.

Concentration (mg/ml)	**Mean absorbance**	**Standard Deviation**	**LOQ (mg/ml)**	**LOD (mg/ml)**
0.04	0.0249	0.0400	0.6467283387	0.2134203518
0.06	0.0818	0.0182		
0.08	0.0847	0.0344		
0.1	0.0885	0.0236		
0.2	0.1413	0.0186		
0.3	0.1981	0.0688		
0.4	0.2432	0.0211		
0.6	0.3284	0.0579		
0.8	0.4833	0.0430		
1.0	0.6002	0.0359		

Appendix B: Equations used to calculate LOQ and LOD

$LOQ = 10 \cdot \left(\frac{SD}{S}\right)$ (2)

$LOD = 3.3 \cdot \left(\frac{SD}{S}\right)$ (3)

LOQ = Limit Of Quantification

LOD = Limit Of Detection

SD = Standard Deviation

S = Slope of calibration curve

Appendix C: Calibration curves from validation

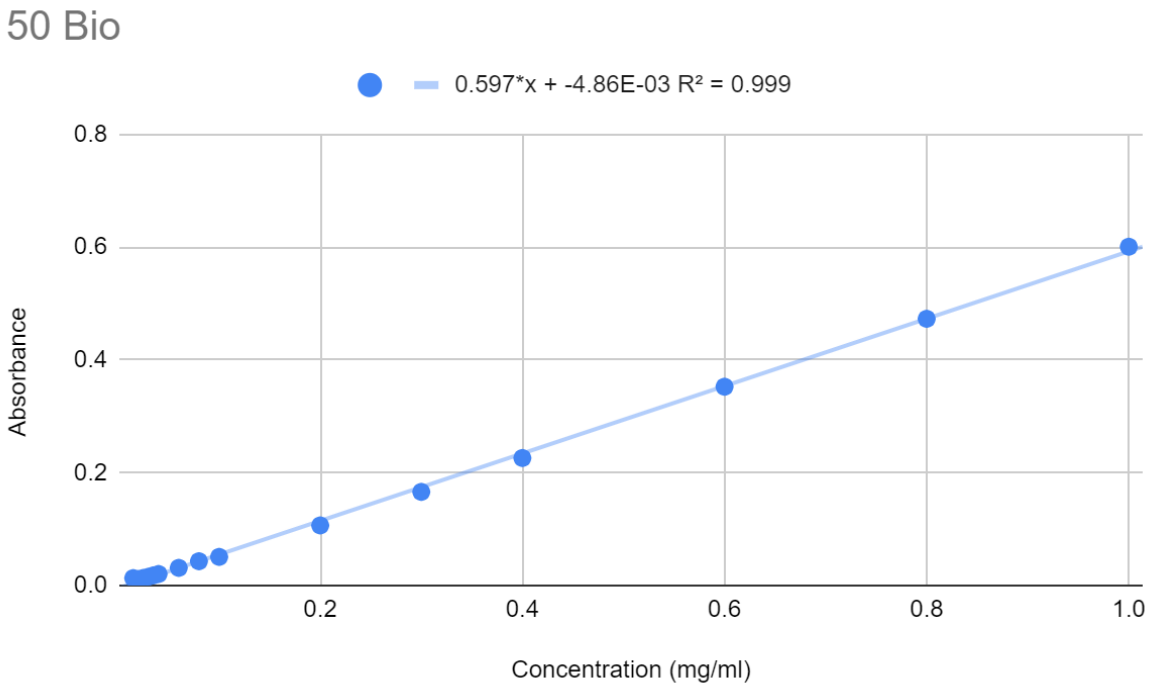

Figure 20: Calibration curve for the 50 Bio spectrophotometer. Concentration plotted against mean value of measured absorbances along with equation for the calibration curve.

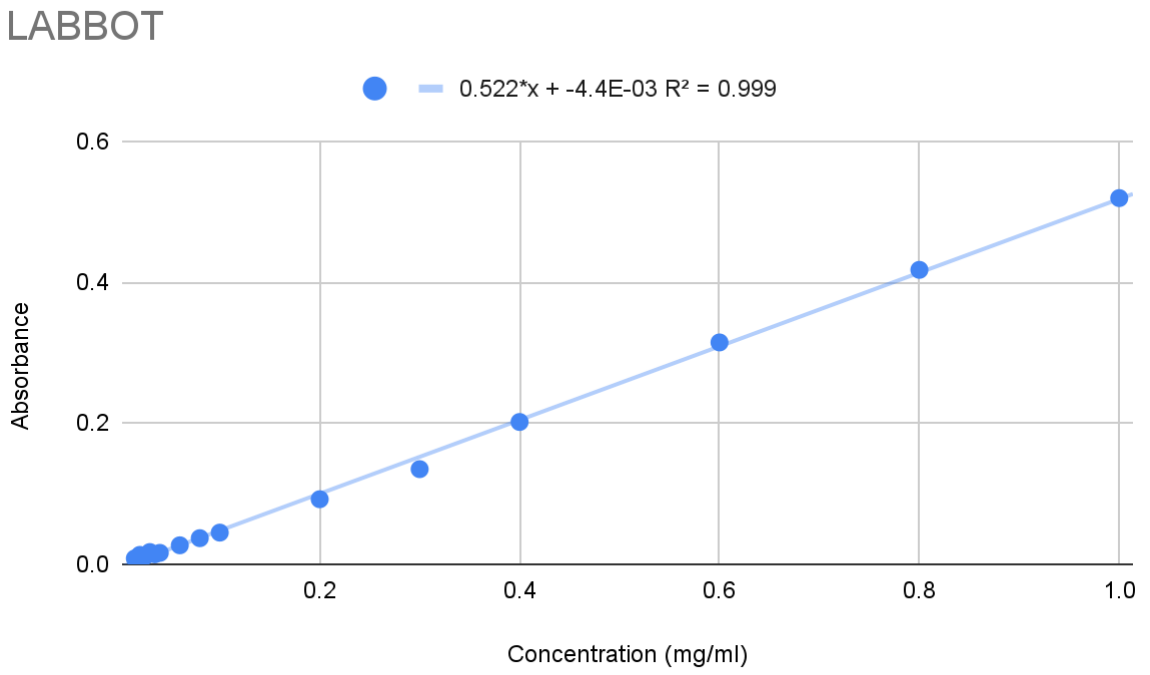

Figure 21: Calibration curve for LABBOT. Concentration plotted against mean value of measured absorbances along with equation for the calibration curve.

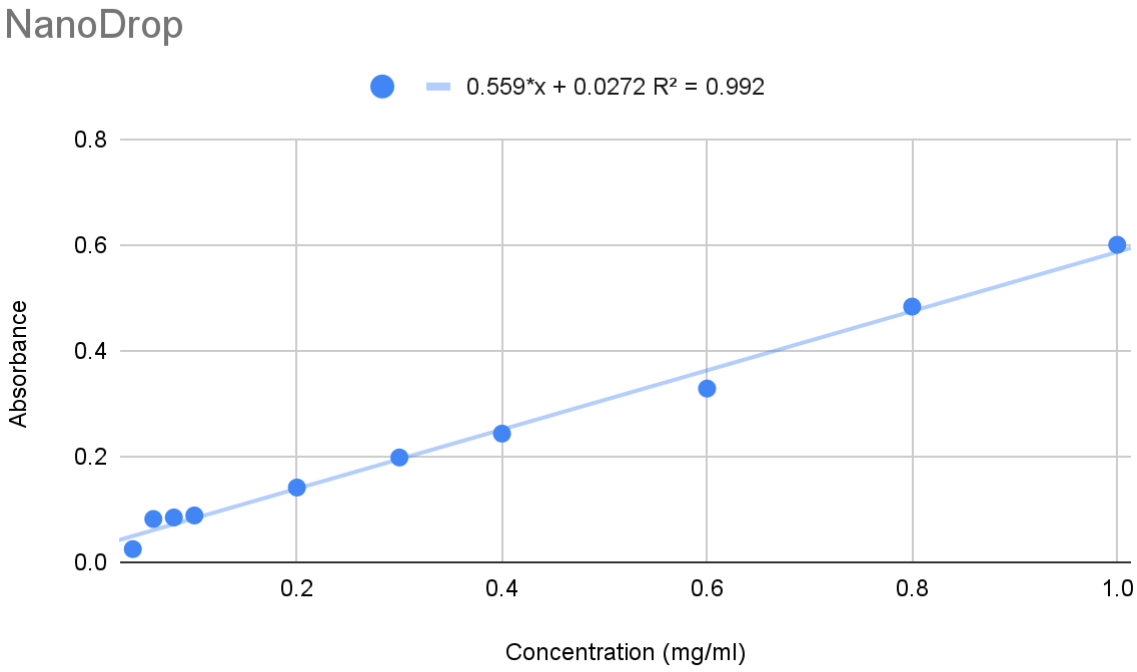

Figure 22: Calibration curve for NanoDrop. Concentration plotted against mean value of measured absorbances along with equation for the calibration curve.