

How to write a (bioanalytical) report

Bioanalysis (FA-BA319/FA-CPS337)

Irene van den Broek (i.vandenbroek@uu.nl)

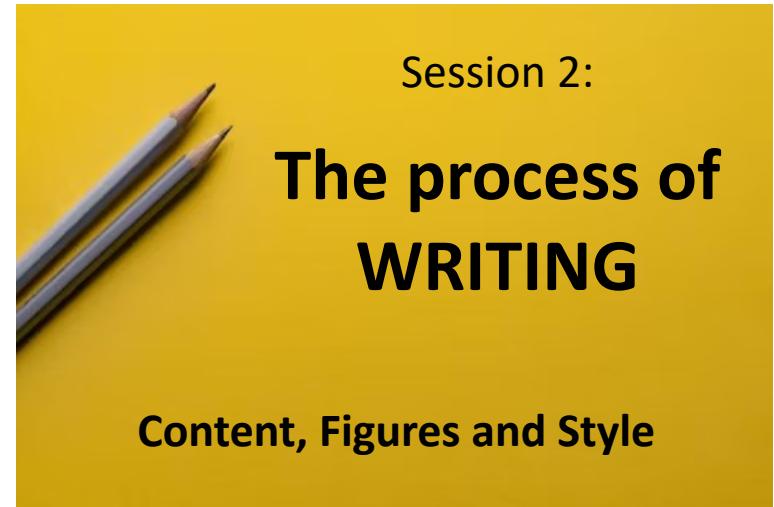


Session 1:

The process of THINKING

Context, Structure and Story

How to write a report seminars



Based on the *ideal* situation:

- Set the context
- Structure your content
- Sketch your (ideal) poster
- Sell your story

Based on *reality*:

- Introduction
- Method
- Results
- Discussion

Seminars: Make it useful!



Individual



Project Team



Peers

- Be prepared
- Make use of the dedicated time
- Different levels of experience:
 - ★ adapt the depth of the exercises to your needs
- Make use of your peers
- Help each other
- Ask questions
- Give feedback (also to me ☺)

Preparation

- Read **Writing Tip Series**: How to get started (part 1), Title and abstract (part 2), Introduction (part 3)



Tip 1 - How to get started: choose the optimal environment!



Tip 2 - Title and abstract: sell your paper!



Tip 3 - Introduction: work on that funnel shape!

- **Summarize relevant literature** related to your project.

Schedule Session 1 (the process of thinking)

Introduction and Warm-up



Context

Why? What? Who?

Development vs. validation vs. application

Exercise: Formulate the goal of your project

----- break -----



Structure

Ideal vs. reality

Exercise: Create an outline

Exercise: Sketch your ideal poster

-----break-----



Story

Story and flow

Exercise: Sell your story

Homework and survey

Report BA319/ CPS337



*All students write an **individual report** about the developed method and its validation. The introduction and materials & methods may be a team effort, although every student needs to write **individually** the results & discussion section (containing the conclusions) – course guide.*

Instructions: see BB

Rubric: see BB

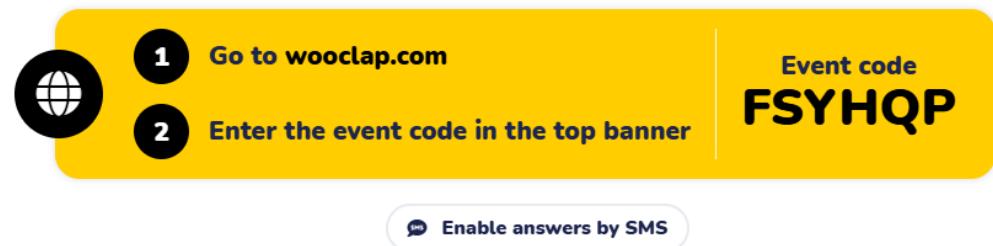
Deadline: last day of the course (**June 28th**) at 17:00

A: Report: Content					
1 Introduction (context and importance of project)					
2 Method (development and final method explained)					
3 Validation (well explained and sound calculations; other results)					
4 Conclusion (clear conclusion truly based on the results)					
5 Discussion (results in perspective; future plans)					

B: Report: Style					
1 Figures					
2 Connections and coherence					
3 Language					
4 Target group					
5 Length					

Warm-up: Some questions

wooclap



How do you feel about writing a report?



Share experiences



Discuss in pairs ([like/dislike](#) and [BA319/CPS337](#)):

- *What difficulties do you experience in writing a report?*
- *What helps you to make writing easier?*

➤ List *at least* :



3 challenges



3 tips

How to write: The process



Content

Structure

Process of thinking

Draft

Report

Process of writing

Story

Process of thinking

Story: start with the end in mind



Story

Know your **context**:

Who is your reader?

What is the bigger picture?

Why is your research relevant?

Who is your reader?



➤ **Think of yourself and your peers!**



- What was your background knowledge before you started this project?
- What additional information did you need?
- What information can you skip?
- If you were an outsider to this research, what would you want to know?

Bigger-picture vs. research goal



Bigger-picture goal:

Application of a bioanalytical method for ... in ...



Research goal:

Development and validation of a bioanalytical method for ... in ... using ...



RESEARCH ARTICLE

OPEN ACCESS



Development and qualification of an LC-MS/MS method for quantification of MUC5AC and MUC5B mucins in spontaneous sputum

Weiwen Sun ^a, Si Mou^a, Catherine Huntington ^b, Helen Killick^c, Ian Christopher Scott^c, Aoife Kelly^c, Monica Gavala^d, Jessica Larsson^c, Mani Deepika Vakkalanka^e, Neil E. Alexis^f, Walter Wiley^e, Aaron Wheeler^e, Kumar Shah^e, Moucun Yuan^e, William R. Mylott Jr. ^e, Kévin Contrepois ^{a*} and Anton I. Rosenbaum ^{a*}

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ABSTRACT

Aim: Airway mucins in sputum are promising respiratory disease biomarkers, despite posing substantial analytical challenges due to their physicochemical properties and rare and heterogenous nature of the matrix. We aimed to identify a suitable sputum collection and processing method, and qualify a bioanalytical method for MUC5AC and MUC5B quantification in clinical samples.

Method: Mucins were quantified in induced and spontaneous sputum collected from the same COPD patients, following various sample processing procedures. LC-MS/MS method used truncated recombinant mucins as surrogate analytes in surrogate matrix.

Results: Frozen spontaneous sputum was found to be a suitable and convenient matrix for mucin quantification and fit-for-purpose method qualification was performed.

Conclusion: Our methodology provides accurate and reliable MUC5AC and MUC5B quantification and facilitates multi-site clinical sputum collection.

ARTICLE HISTORY

Received 8 October 2024

Accepted 21 January 2025

KEYWORDS

MUC5AC; MUC5B; LC-MS/MS; bioanalytical method; sputum collection; sputum processing; method qualification

Development and qualification of an LC-MS/MS method for quantification of MUC5AC and MUC5B mucins in spontaneous sputum

Title

Bigger-picture goal

Aim: Airway mucins in sputum are promising respiratory disease biomarkers, despite posing substantial analytical challenges due to their physicochemical properties and rare and heterogeneous nature of the matrix. We aimed to identify a **suitable sputum collection** and processing method, and qualify a bioanalytical method for **MUC5AC and MUC5B quantification** in **clinical samples**.

Research goal



PRELIMINARY COMMUNICATION



Determination of drugs of abuse and metabolites in plasma microsamples by LC-MS/MS after microQuEChERS extraction

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Campinas (UNICAMP), Rua Vital Brasil 251, Campinas, CEP 13083-888, Brazil

ABSTRACT

Aim: Identifying drugs of abuse and their metabolites in plasma is vital in both forensic and clinical toxicology. While the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method offers an efficient approach to sample preparation, its application is complex due to the wide-ranging properties of target analytes and the challenges posed by biological matrix interferences. This study aims to develop a microQuEChERS approach for the quantification of 14 drugs of abuse and metabolites utilizing minimal sample and solvent volumes.

Methods: The microQuEChERS method involved using 10 µl plasma samples, 25 mg of a salt mixture and 150 µl of acetonitrile. Extracts were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), with a 7.5 min run. The assay was validated according to bioanalytical guidelines.

Results: The accuracy was 96.8–112.4%. The within-assay precision was within 2.0–8.9% and the between-assay precision was within 3.2–8.2%. Matrix effects were found to range from -5.7 to 13.5%. The extraction yield was higher than 74.7%.

Conclusion: This study described a microQuEChERS sample preparation approach for determining drugs of abuse and metabolites using plasma microsamples and LC-MS/MS. The approach is efficient, environmentally friendly and suitable for scenarios with limited amounts of biological samples.

ARTICLE HISTORY

Received 1 August 2024

Accepted 11 September 2024

KEYWORDS

drugs of abuse; LC-MS/MS;
MicroQuEChERS; plasma
microsamples

Determination of drugs of abuse and metabolites in plasma microsamples by LC-MS/MS after microQuEChERS extraction

Title

Bigger-picture goal

Aim: Identifying drugs of abuse and their metabolites in plasma is vital in both forensic and clinical toxicology. While the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method offers an efficient approach to sample preparation, its application is complex due to the wide-ranging properties of target analytes and the challenges posed by biological matrix interferences. This study aims to develop a **microQuEChERS** approach for the **quantification** of **14 drugs of abuse and metabolites** utilizing **minimal sample and solvent volumes**.

Research goal



RESEARCH ARTICLE



Development and application of a multi-sugar assay to assess intestinal permeability

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^aGSK, Biomarker Platforms, Precision Medicine, GSK, 1250 S Collegeville Rd, Collegeville, PA 19426, USA; ^bGSK, Gunnels Wood Road, Stevenage, SG1 2NY, United Kingdom

ABSTRACT

Aim: Bioanalytical assays to measure rhamnose, erythritol, lactulose and sucralose in human urine and plasma were developed to support an indomethacin challenge study for intestinal permeability assessment in healthy participants.

Methods: The multi-sugar assays utilized 5-μl sample matrix and a simple chemical derivatization with acetic anhydride, followed by RPLC-MS/MS detection.

Results: Rhamnose and erythritol quantification was established between 1.00–1,000 μg/ml in urine and 250–250,000 ng/ml in plasma. For lactulose and sucralose, dynamic ranges of 0.1–100 μg/ml (urine) and 25–25,000 ng/ml (plasma) were applied for biological measurements.

Conclusion: This work helped overcome some of the common analytical challenges associated with the bioanalysis of mono- and disaccharides and achieved improved limits of quantification.

ARTICLE HISTORY

Received 16 April 2024

Accepted 26 June 2024

KEYWORDS

ammonium adducts; bioanalysis; carbohydrates; derivatization; LC-MS/MS; saccharides

Development and application of a multi-sugar assay to assess intestinal permeability

└ Research goal

Aim: Bioanalytical assays to **measure rhamnose, erythritol, lactulose and sucralose in human urine and plasma** were developed to support an indomethacin challenge study for intestinal permeability assessment in healthy participants.

└ Bigger-picture goal

RESEARCH ARTICLE

SPECIAL FOCUS: DRIED BLOOD SPOTS

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Dried blood spot punches for confirmation of suspected γ -hydroxybutyric acid intoxications: validation of an optimized GC–MS procedure

Background: γ -hydroxybutyric acid (GHB), notorious as a club- and date-rape drug, was quantified in dried blood spots (DBS) by punching out a disc, followed by 'on-spot' derivatization and analysis by GC-MS. **Results:** A homogenous distribution in DBS was demonstrated and accurate results were obtained when analyzing a disc punched out from a 20–35 μl spot, regardless the hematocrit of the blood sample. Validation based on US FDA and European Medicines Agency guidelines was performed, with a calibration range covering 2–100 $\mu\text{g/ml}$. **Conclusion:** A sensitive GC-MS method for GHB analysis in DBS was successfully optimized and validated. The successful analysis of DBS collected from GHB abusers suggests the routine applicability of the DBS sampling technique for GHB analysis in toxicological cases.

Dried blood spot punches for confirmation of suspected γ -hydroxybutyric acid intoxications: validation of an optimized GC–MS procedure

Conclusion: A sensitive GC–MS method for GHB analysis in DBS was successfully optimized and validated. The successful analysis of DBS collected from GHB abusers suggests the routine applicability of the DBS sampling technique for GHB analysis in toxicological cases.

Validation of a quantitative assay for human neutrophil peptide-1, -2, and -3 in human plasma and serum by liquid chromatography coupled to tandem mass spectrometry

Irene van den Broek^{a,*}, Rolf W. Sparidans^a, Jan H.M. Schellens^{a,b}, Jos H. Beijnen^{a,c}

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ARTICLE INFO

Article history:

Received 21 January 2010

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Available online 15 March 2010

Keywords:

Human neutrophil peptides

Quantification

LC–MS/MS

ABSTRACT

A quantitative assay for simultaneous measurement of individual human neutrophil peptide-1, -2 and -3 concentrations will aid in exploring the potential of these antimicrobial peptides as biomarkers for various diseases. Therefore, a liquid chromatography–tandem mass spectrometry method has been developed and validated to allow separate quantification of the three human neutrophil peptides in human plasma and serum. Plasma and serum samples (100 μ l) were deproteinized by precipitation, followed by chromatographic separation on a Symmetry 300 C18 column (50mm x 2.1mm I.D., particle size 3.5 μ m), using a water-methanol gradient containing 0.25% (v/v) formic acid and human alpha-defensin 5 as internal standard. Tandem mass spectrometric detection was performed on a triple quadrupole mass spectrometer equipped with electrospray ionization. Despite low fragmentation efficiency of the antimicrobial peptides, multiple reaction monitoring was used for detection, though selecting the quaternary charged ions as both precursor and product. The method was linear for concentrations between 5 and 1000 ng/ml with a limit of detection around 3 ng/ml for all peptides. Intra- and inter-assay precisions were 14.8 and 19.1%, respectively, at the lowest measured endogenous concentration (6.4 ng/ml of HNP-1 in plasma), representing the lower limit of quantification of the assay. Recoveries of HNP-1, -2 and -3 from plasma and serum ranged between 85 and 128%. Analysis of serum samples from intensive care patients showed average concentrations of 362, 570 and 143 ng/ml for HNP-1, -2 and -3, respectively.

Validation of a quantitative assay for human neutrophil peptide-1, -2, and -3 in human plasma and serum by liquid chromatography coupled to tandem mass spectrometry

A quantitative assay for simultaneous measurement of individual human neutrophil peptide-1, -2 and -3 concentrations will aid in exploring the potential of these antimicrobial peptides as biomarkers for various diseases. Therefore, a liquid chromatography–tandem mass spectrometry method has been developed and validated to allow separate quantification of the three human neutrophil peptides in human plasma and serum.

Bigger-picture vs. research goal



Bigger-picture goal:

Application of a bioanalytical method for ... in ...



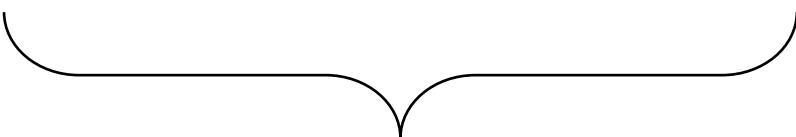
Research goal:

Development and validation of a bioanalytical method for ... in ... using ...



Report goal:

Development of a bioanalytical method for ... in ... using ...



Today: *ideal* situation



Set the context

Begin with the end in mind

Discuss with your group:

What is the bigger picture of your topic?

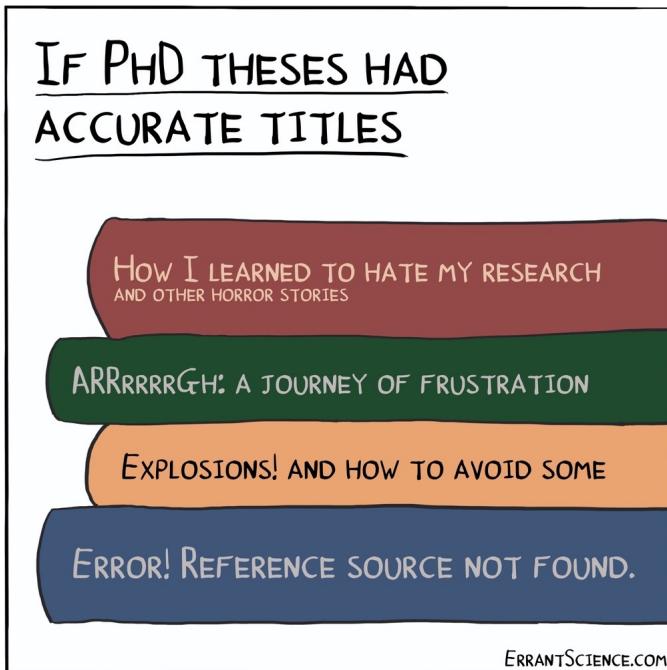
Why is *your* research relevant?

- Formulate your **bigger-picture goal**.
- Formulate a **clear** and **SMART research goal**
- ❖ Formulate a (preliminary) **title** for your report
 - Or think of various alternatives and choose later ☺



- List your goals and title(s) in the **Teams chat** (Report writing channel) – add your group number.

The Title: First impressions matter



- **Informative** and **attractive**:
 - Explains what your paper is about
 - Makes the reader want to read your paper
- **Short** and **concise**:
 - Less words is better
 - Describes the content accurately
 - Avoid vague titles; be specific.
- **Focused** and **clear**:
 - Describe the main result, if possible.
 - Key point plays leading role (describe bigger picture elsewhere)
- Contain the right **key words** or **search terms**:
 - Consider search engines and online search

Break



How to write: Linear vs. modular



Linear

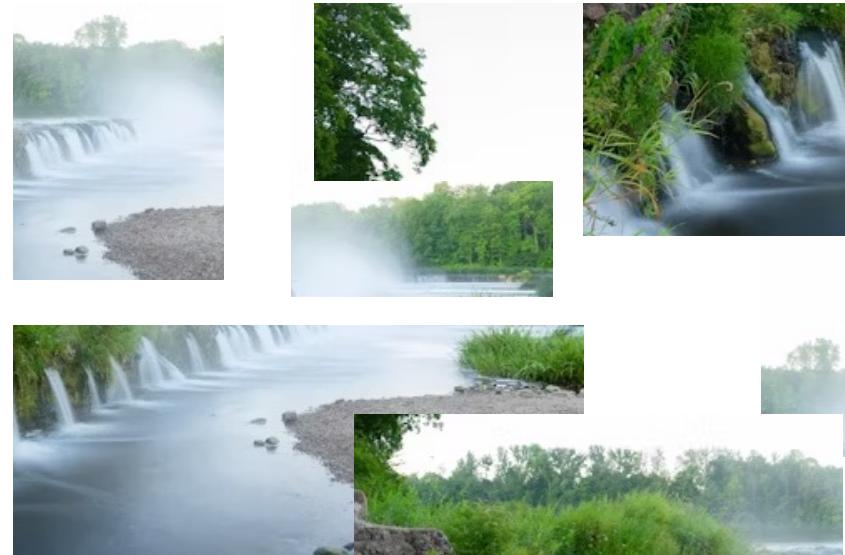


Modular

How to write: Linear vs. modular



Linear



Modular

How to write: Linear vs. modular



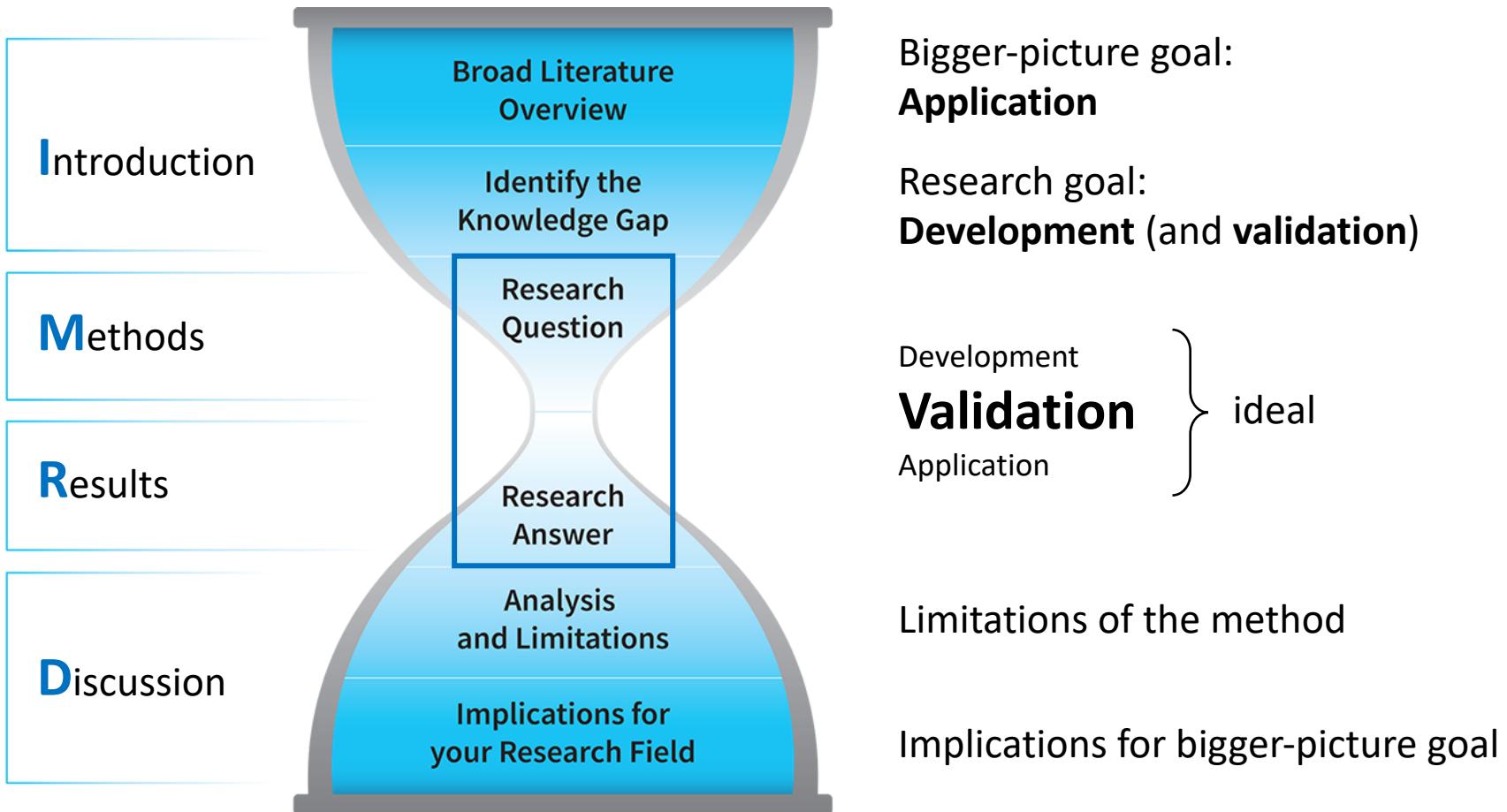
Linear



Modular

- Consider big picture before spending time on details
- No “*blank page syndrome*”
- Write easily and quickly at anytime (whenever there’s a gap in your schedule!)

Modular writing: The power of outlining



Example: Bioanalytical paper

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1087

before the clear solution was transferred into glass vials. A volume of 10 µl was injected into the LC–MS system.

2.6. Method validation

Validation was based on the FDA guidelines for Bioanalytical Method Validation [26].

2.6.1. Linearity

Ten calibration standards were prepared by serial dilution in concentrations of 0; 5; 10; 20; 50; 100; 200; 400; 700 and 1000 ng/ml above the endogenous concentration of HNP-1, -2 and -3, quantified as 7.4, 41.3 and 5.8 ng/ml, respectively. All standards were analyzed in each run, calculating target peptide: IS ratios of the peak area for each concentration level. Standard curves were constructed by least-squares linear regression analysis using a weighting factor of $1/x^2$ (with x as the concentration in ng/ml).

2.6.2. Precision and accuracy

Precisions and accuracies of the method for plasma and serum samples were determined by analysis of samples with endogenous concentrations and QC samples spiked at three different concentration levels in three separate runs ($n = 18$ at each level). Furthermore, one run contained plasma and serum spiked above the ULQ level at 5000 ng/ml, analyzed after 5-fold dilution with the same human plasma batch as used for the calibration standards to validate dilution of the samples. The accuracies of the recovered analytes were calculated as $\{[(\text{overall measured concentration} - \text{initial concentration})/\text{added concentration}] \times 100\%\}$. Intra- and inter-assay precisions were expressed as relative standard deviations (RSD).

2.6.3. Selectivity

Human serum and plasma samples from six different sources were spiked at concentrations of 100 ng/ml and analyzed in triplicate. The plasma samples consisted of samples with EDTA, lithium heparine or citrate–phosphate–dextrose as anti-coagulant. For all sample batches, endogenous concentrations were mea-

were assessed after six months storage at –80 °C. Deviations were calculated by comparing MS response ratios to freshly prepared samples at identical concentrations.

All stability tests were performed in triplicate and at three different concentration levels (LQC, MQC and HQC). The analytes were considered stable when 85–115% of the initial concentration was found.

2.7. Analysis of serum samples from intensive care patients

HNP-1, -2 and -3 concentrations were quantified in 24 serum samples obtained from 11 different patients at the intensive care unit. One serum sample of each patient was collected at 6.00 p.m., while additional samples of some patients were drawn on different days. Samples with concentrations beyond the ULQ of the assay were re-analyzed after 2- or 5-fold dilution of the sample with plasma from the same batch as used for preparation of the calibration standards. QC samples of all concentration levels were measured along with the patient samples to monitor the analytical performance.

3. Results and discussion

3.1. Method development

3.1.1. Sample pre-treatment

The protein precipitation procedure offered rapid sample preparation, facilitating high sample throughput. It furthermore provided better recoveries, especially for HNP-2, than solid-phase extraction (SPE) on different types of reversed-phase cartridges, although SPE is most commonly applied for sample clean-up of peptides from complex matrices [27]. Acidification of the organic solvent was required to prevent co-precipitation of the analytes. Addition of 200 µl instead of 250 µl acetonitrile resulted in substantially clearer extracts. Furthermore, evaporation of the supernatant appeared beneficial as interferences which were insoluble in the reconstitution solvent could be removed after reconstitution and subsequent centrifugation. However, as no solid precipitate was

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before the clear solution was transferred into glass vials. A volume of 10 µl was injected into the LC-MS system.

2.6. Method validation

Validation was based on the FDA guidelines for Bioanalytical Method Validation [26].

2.6.1. Linearity

Ten calibration standards were prepared by serial dilution in concentrations of 0, 5, 10, 20, 50, 100, 200, 400, 700 and 1000 ng/ml above the endogenous concentration of HNP-1, -2 and -3. Standards were prepared in three different batches. Standards were analyzed in each run, calculating target peptide: IS ratios of the peak area for each concentration level. Standard curves were constructed by plotting the ratio of the peak areas against a weighting factor of $1/\lambda^2$ (with λ as the concentration in ng/ml).

2.6.2. Precision and accuracy

Precision and accuracy of the method for plasma and serum samples were assessed by analysis of samples with endogenous concentrations and QC samples spiked at three different concentrations levels (0.5, 10 and 100 times the endogenous level). Furthermore, one run contained plasma and serum spiked above the ULQ level (500 ng/ml). All samples were analyzed in triplicate. Furthermore, plasma batch as used for the calibration standards to validate dilution of the samples. The accuracies of the recovered analytes were calculated as follows: $(\text{recovered concentration}/(\text{added concentration} + \text{endogenous})) \times 100\%$. Intra- and interassay precision were expressed as relative standard deviation (RSD).

2.6.3. Selectivity

Human serum and plasma samples from six different patients were spiked at concentrations of 100 ng/ml and analyzed in triplicate. The plasma samples consisted of samples with EDTA, lithium heparin or citrate-phosphate-dextrose as anti-coagulant. For ^{31}P imaging batches, endogenous concentrations were used.

2.6.4. Recovery and ion suppression

2.6.5. Stability

2.7. Analysis of serum samples from intensive care patients

1. Introduction

2. Experimental

2.1 Chemicals and reagents

2.2 Instruments

2.3 LC-MS/MS conditions

2.4 Preparation of standards and quality control samples

2.5 Sample preparation

2.6 Method validation

2.6.1 Linearity

2.6.2 Precision and accuracy

2.6.3 Selectivity

2.6.4 Recovery and ion suppression

2.6.5 Stability

3. Results and discussion

3.1 Method development

3.1.1 Sample pre-treatment

3.1.2 LC-MS/MS analysis

3.2 Method validation

3.2.1 Linearity

3.2.2 Accuracy and precision

3.2.3 Selectivity

3.2.4 Recovery and ion suppression

3.2.5 Stability

3.3. Internal standard and matrix selection

3.4 Analysis of serum samples from intensive care patients

4. Conclusion

Example: Bioanalytical paper

RESEARCH ARTICLE
SPECIAL FOCUS: DRIED BLOOD SPOTS

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Dried blood spot punches for confirmation of suspected γ -hydroxybutyric acid intoxications: validation of an optimized GC-MS procedure

Background: γ -hydroxybutyric acid (GHB), notorious as a club- and date-rape drug, was quantified in dried blood spots (DBS) by punching out a disc, followed by 'on-spot' derivatization and analysis by GC-MS. **Results:** A homogenous distribution in DBS was demonstrated and accurate results were obtained when analyzing a disc punched out from a 20–35 μ l spot, regardless the hematocrit of the blood sample. Validation based on US FDA and European Medicines Agency guidelines was performed, with a calibration range covering 2–100 μ g/ml. **Conclusion:** A sensitive GC-MS method for GHB analysis in DBS was successfully optimized and validated. The successful analysis of DBS collected from GHB abusers suggests the routine applicability of the DBS sampling technique for GHB analysis in toxicological cases.

The short chain fatty acid γ -hydroxybutyric acid (**GHB**) was synthesized in the early sixties as a structural analogue of γ -aminobutyric acid and also occurs naturally in blood, urine and peripheral and brain tissue [1,2]. Although the function of endogenous GHB has not completely been revealed yet, evidence suggests that it may act as a neuromodulator or neurotransmitter [2]. As a legal substance (sodium oxybate), GHB has a role as an anesthetic agent in the treatment of narcolepsy with cataplexy and in alcohol and opiate withdrawal. In addition, it has also been sold as a substance of nutritional supplements to induce sleep and increase muscle mass. Currently, illegal GHB (liquid ecstasy) as well as its precursors, γ -butyrolactone and 1,4-butanediol, are popular as club drugs and appear occasionally in drug-facilitated sexual assaults [3]. In those toxicological cases, the interpretation of a positive analytical result is a real challenge, owing to its endogenous presence and reported *in vitro* production [4,5]. Therefore, **cut-off levels** have been proposed by several authors and these are currently set at 4 or 5 μ g/ml for blood (serum) samples [2]. In addition, the detection window is very limited as GHB is rapidly metabolized and eliminated after oral ingestion (**plasma half-life** <1 h), so blood samples must be taken within 6 h after ingestion [6]. Consequently, a sampling delay may result in blood levels below the established cut-off level, no longer resulting in a positive case [7].

Blood sample collection may be facilitated by using **dried blood spot (DBS) sampling**. A DBS

is capillary whole blood obtained by a finger or heel prick and collected on a filter paper card. Advantages over a venipuncture are the easy and rapid way to collect a representative sample and the less specific sample transport and storage requirements [8]. Previously, DBS sampling has generally been used for newborn screening, however, more recently, this alternative sampling strategy is increasingly gaining interest in the context of therapeutic drug monitoring and (pre-)clinical studies, as well as in toxicology [9,10]. We recently reported on the development and validation of a new procedure for GHB determination in DBS, using '**on-spot**' derivatization and **GC-MS** [11]. Similarly, other drugs of forensic interest have been determined in DBS, such as 3,4-methylenedioxymethamphetamine, morphine and 6-acetylmorphine or cocaine [12–14].

To obtain a DBS on filter paper card, a drop of blood can be spotted directly on the filter paper or with the aid of a precision capillary [8]. In our previous study, we used the second sample collection technique, and spotted a drop of blood with a fixed volume onto the filter paper card, followed by analysis of the complete DBS [11]. However, as correct sampling in this case ideally requires the presence of trained staff and in routine practice, it is more convenient to collect the drop of blood directly on the filter paper card, we therefore modified our procedure accordingly. As we did not wish this simplification to be at the expense of sensitivity (LLOQ of 2 μ g/ml), we readjusted several sample pretreatment steps.

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Dried blood spot punches for confirmation of suspected γ -hydroxybutyric acid intoxications: validation of an optimized GC-MS procedure

Background: γ -hydroxybutyric acid (GHB), notorious as a club- and date-rape drug, was quantitated in dried blood spots (DBS) by punching out a disc, followed by 'on-spot' derivatization and analysis by GC-MS. Results: A homogeneous distribution in DBS was demonstrated and accurate results were obtained when analysing a 20–35 μ l spot, regardless the hematocrit of the blood sample. Validation based on US FDA and European Pharmacopoeia guidelines showed acceptable precision and accuracy for GHB analysis. Conclusion: A sensitive GC-MS method for GHB analysis in DBS was successfully optimized and validated. The successful analysis of DBS collected from GHB abusers suggests the routine applicability of the DBS sampling technique for GHB analysis in toxicological cases.

The short chain fatty acid γ -hydroxybutyric acid (GHB) was synthesized in the early sixties as a stress marker analogue of β -hydroxybutyric acid and also occurs naturally in muscle, liver, heart and brain tissue (1,2). Although the function of GHB in the body is not fully understood, evidence suggests that it may act as a modulator of the GABAergic system (3). In legal substance (ethanol) ethanol, GHB has a role as an anesthetic agent, in the treatment of narcolepsy and as a sedative-hypnotic after its withdrawal. In addition, it has also been used as a hallucinogen and as a迷幻剂 (hallucinogen) (4). Currently illegal GHB (liquid extract as well as in capsules, tablets and powder form) is often sold as club drugs and appear occasionally in drug facilitated sexual assault cases. In these cases, the interpretation of a positive analytical result is a real challenge, owing to its endogenous presence in human blood (5,6).

Therefore, cut-off levels have been proposed by several authors (7–10). However, a detection limit of 5 μ g/l for blood (serum samples) is, in addition to the detection of metabolites, which is rapidly metabolized and eliminated after oral ingestion (plasma half-life = 1 h), as blood samples are often taken at least 12 h after the last dose. Consequently, a sampling delay may result in blood samples with a low concentration of GHB, no longer resulting in a positive case (cut-off level).

A blood sample collection may be facilitated by using dried blood spots (DBS) sampling. A DBS

is capillary whole blood obtained by a finger or heel prick and collected on a filter paper card. The advantages over a venipuncture are the easy and rapid sample collection, the preservation and the less specific sample transport and storage. DBS sampling has been used for newborn screening, but generally been used for newborn screening, drug monitoring and toxicology analysis. The sampling strategy is increasingly gaining interest in the context of therapeutic drug monitoring and toxicology analysis (11–14). In addition, DBS sampling has recently reported on the development of a sensitive GC-MS method for the determination of GHB in DBS, using 'on-spot' derivatization and GC-MS (15). Similarly, other drugs of forensic interest such as cocaine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, morphine and its metabolites have been analyzed in DBS (16–19).

To obtain DBS on filter paper card, a drop of blood can be spotted directly on the filter paper card. However, this is not always feasible. In our previous study, we used the second sample collection technique, where a drop of blood is applied with a fixed volume onto the filter paper card. However, as correct sampling in this case ideally requires the presence of trained staff and an instrument to measure the volume of blood to be applied, a drop of blood directly on the filter paper card, we developed a simple alternative sampling technique. As we did not wish with this simplification to be at the expense of sensitivity (LLQC of 2 μ g/ml), we conducted several sample preparation steps.

1. Introduction

2. Experimental

2.1 Reagents

2.2 DBS sampling

2.3 Sample preparation and analytical procedure

2.4 DBS method validation

2.4.1 Influence of the punch localisation

2.4.2 Influence of the blood spot volume

2.4.3 Influence of the hematocrit

2.4.4 Validation

2.5 Determination of GHB in DBS collected at the emergency department

3. Results and discussion

3.1 DBS method validation

3.1.1 Influence of the punch localisation

3.1.2 Influence of the blood spot volume

3.1.3 Influence of the hematocrit

3.1.4 Validation

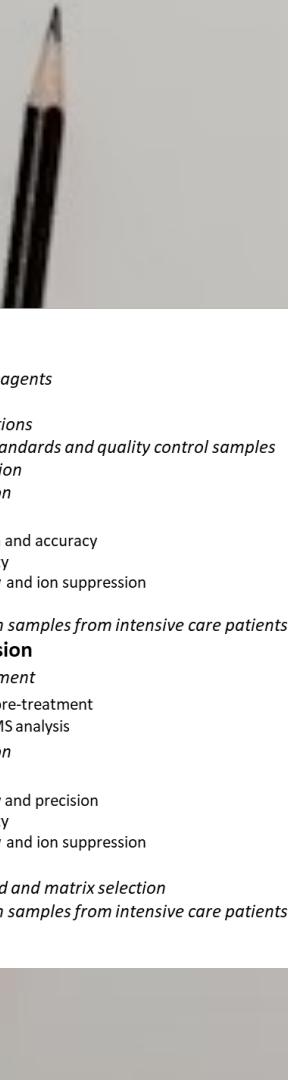
3.2 Determination of GHB in DBS collected in the emergency room

4. Conclusion

Structure your content

Divide the report in sections and stick to it!

Individually:

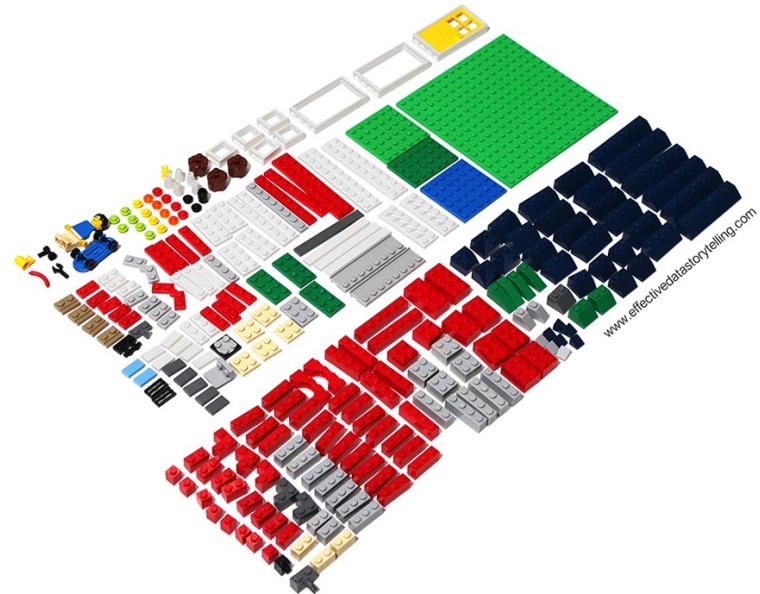
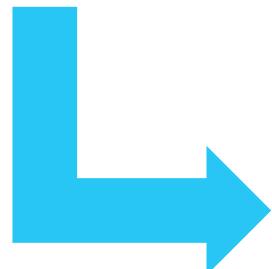
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1. **Introduction**
 2. **Experimental**
 - 2.1 Chemicals and reagents
 - 2.2 Instruments
 - 2.3 LC-MS/MS conditions
 - 2.4 Preparation of standards and quality control samples
 - 2.5 Sample preparation
 - 2.6 Method validation
 - 2.6.1 Linearity
 - 2.6.2 Precision and accuracy
 - 2.6.3 Selectivity
 - 2.6.4 Recovery and ion suppression
 - 2.6.5 Stability
 - 2.7 Analysis of serum samples from intensive care patients
 3. **Results and discussion**
 - 3.1 Method development
 - 3.1.1 Sample pre-treatment
 - 3.1.2 LC-MS/MS analysis
 - 3.2 Method validation
 - 3.2.1 Linearity
 - 3.2.2 Accuracy and precision
 - 3.2.3 Selectivity
 - 3.2.4 Recovery and ion suppression
 - 3.2.5 Stability
 - 3.3. Internal standard and matrix selection
 - 3.4 Analysis of serum samples from intensive care patients
 4. **Conclusion**

- Create an **outline** listing all sections
- Add **subheadings** to Methods and Results & Discussion, based on:
 - Expected experiments (ideal situation)
 - What you've already done
- ★ Look at the outline of other research articles for **inspiration**
- ★ Add **notes** and **ideas**: *What should be included? What is missing?*
- ★ Decide which sections you can write already (and when).

From outline to draft

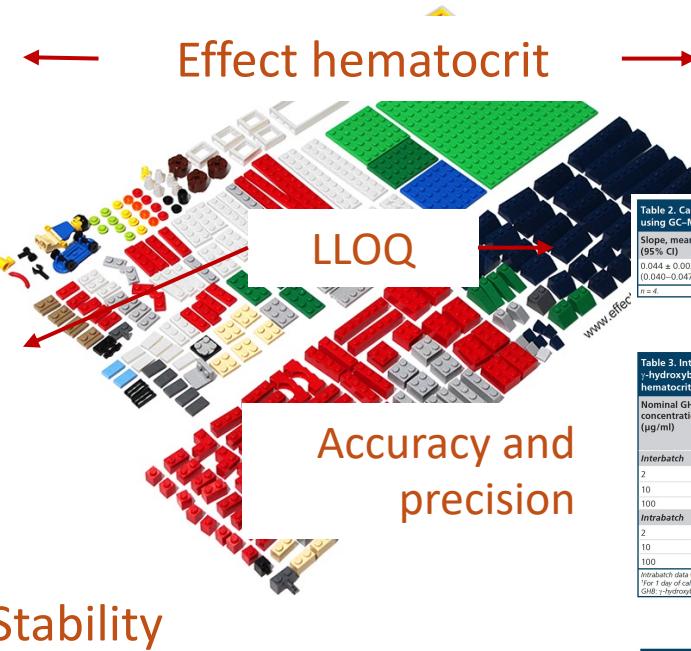
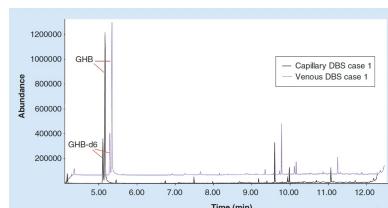
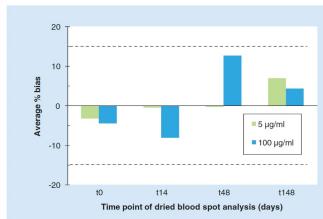
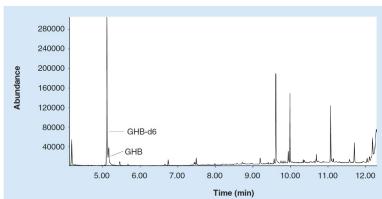
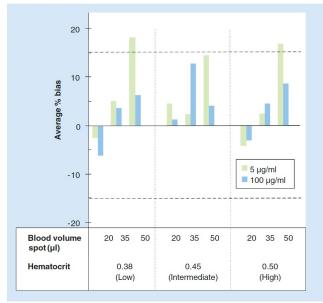


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Draft: Lay-out the results



Stability

ULQ

Application

Venous vs.
capillary

Table 1. Influence of hematocrit on the γ -hydroxybutyric acid concentration measured in dried blood spot samples using GC-MS in selected ion monitoring mode.		
Hematocrit	Low GHB concentration (5 $\mu\text{g}/\text{ml}$; n = 5)	High GHB concentration (100 $\mu\text{g}/\text{ml}$; n = 5)
0.34	-15.0	-5.17
0.39	-3.45	-2.73
0.44	Normalized	Normalized
0.46	-2.32	3.85
0.51	-0.910	1.18
0.56	11.1	10.9

Values indicate the % deviation from the γ -hydroxybutyric acid concentration obtained for the samples with a hematocrit of 0.44, which was used for normalization, given the reference interval of 0.37 to 0.51 for healthy women and men, respectively.

GHB: γ -hydroxybutyric acid.

Table 2. Calibration and sensitivity data for the determination of γ -hydroxybutyric acid in dried blood spots using GC-MS in selected ion monitoring mode.						
Slope, mean \pm SD (95% CI)	Intercept, mean \pm SD (95% CI)	R ²	Weighting factor	Range ($\mu\text{g}/\text{ml}$)	LOD ($\mu\text{g}/\text{ml}$)	LLOQ ($\mu\text{g}/\text{ml}$)
0.044 \pm 0.003 (0.040–0.047)	0.000 \pm 0.007 (-0.007–0.007)	0.999	1/ λ^2	2–100	1	2

n = 4

Table 3. Inter- (n = 4; in duplicate) and intra- (n = 6) batch precision and accuracy data for the QC of γ -hydroxybutyric acid determination in dried blood spot samples prepared in whole blood with the indicated hematocrit value.									
Nominal GHB concentration ($\mu\text{g}/\text{ml}$)	GHB concentration measured ($\mu\text{g}/\text{ml}$)			Precision (% RSD)			Accuracy (% nominal concentration)		
	Low (0.38)	Int. (0.45)	High (0.50)	Low (0.38)	Int. (0.45)	High (0.50)	Low (0.38)	Int. (0.45)	High (0.50)
<i>Interbatch</i>									
2	2.00	2.05	2.04	6.46	13.1	10.1 ^a	99.8	103	102
10	9.55	9.80	9.77	14.2	7.17 ^b	7.88	95.5	98.0	97.7
100	112	106	109	15.0	12.0	8.50	112	106	109
<i>Intrabatch</i>									
2	1.96				4.93			97.9	
10	9.03				6.13			90.3	
100	101				4.10			101	

Intrabatch data were obtained from blood with intermediate hematocrit.

^aFor 1 day of calibration, single analysis was performed of these QC's because of sample loss during sample preparation.

^bGHB: γ -hydroxybutyric acid; LOD: Limit of detection.

Table 4. Results of the dilution experiment of samples with a γ -hydroxybutyric acid concentration exceeding the highest point (100 $\mu\text{g}/\text{ml}$) of the calibration curve (nominal value of 200 $\mu\text{g}/\text{ml}$).			
Hematocrit	Back-calculated GHB concentration ($\mu\text{g}/\text{ml}$; n = 3; in duplicate)	Inter-batch precision (% relative standard deviation; n = 3; in duplicate)	Accuracy (% nominal concentration; n = 3; in duplicate)
Low (0.38)	191	6.74	95.6
Intermediate (0.45)	210	6.36	105
High (0.50)	207	5.82	103

The deviated extract was diluted 1/10 with ethyl acetate prior to analysis by GC-MS. The measured GHB concentration was back-calculated, taking the dilution factor into account.

GHB: γ -hydroxybutyric acid.

Table 5. γ -hydroxybutyric acid level ($\mu\text{g}/\text{ml}$) in paired venous and capillary dried blood spots and venous whole blood samples in seven patients presenting at the emergency department with a suspected γ -hydroxybutyric acid intoxication.			
GHB-positive case No.	Venous DBS (μg GHB/ml)	Capillary DBS (μg GHB/ml)	Venous WB (μg GHB/ml)
1	81.4	78.8	88.5
2	170	150	173
3	153	169	154
4	56.3	91.7 ^c	44.0
5	118	142	126
6	107	116	90.5
7	120	132	97.0

A DBS single analysis was analyzed under non-optimal denaturation and GC-MS operating in selected ion monitoring mode, while venous whole blood (in duplicate) was analyzed according to the procedure of Van Hees et al. [20].

^aWe suspect this higher value to be due to contamination of the finger tip.

DBS: Dried blood spot; GHB: γ -hydroxybutyric acid; WB: whole blood.

Draft: Lay-out the results

Peptide	MW (Da)	As	Sequence	Disulfide bridges
HNP-1	3442.1	29	ACYCBPAGTACERPPNCTQVGRWAWCC	2-30; 4-18; 9-26
HNP-2	3771.1	29	CYCYPACIGERVVTCCTQGRWAWFPC	1-29; 3-18; 8-28
HNP-3	3486.1	30	DCYCPACIGERVVTCCTQGRWAWFPC	2-30; 4-19; 9-29
HNP-4	3824.1	34	VAVCQVDPVTCCTQGRWAWFPC	2-34; 4-19; 9-29
HD-5	3582.2	32	ATCYCIGTCATRESSGCVGEGRVLRCR	3-31; 5-20; 10-30
HD-6	3708.3	32	ATFCCHCRSCSSTEYTSYCTVMGINHRVCL	4-31; 6-20; 10-30

	Sample	Average measured concentration (ng/ml) ^a	% Recovery	Intra-assay precision (%)	Inter-assay precision (%)
Plasma	HNP-1	Blank	6.4 ± 1.2	14.8	19.1
	LQC	18.7 ± 2.2	-1.8	6.2	14.7
	MQC	208.0 ± 2.5	-0.6	8.7	10.5
	HQC	804.2 ± 8.2	-0.3	10.0	10.2
	ULQ ^b	836.8 ± 2.51	-16.3	3.5	
HNP-2	Blank	10.0 ± 0.0	9.8	10.5	
	LQC	142.8 ± 12.1	15.0	7.5	8.5
	MQC	312.4 ± 3.49	2.3	8.4	11.2
	HQC	856.5 ± 5.5	0.7	10.4	11.2
	ULQ ^b	893.9 ± 3.10	-10.6	3.5	
HNP-3	Blank	9.1 ± 1.0	10.1	10.7	
	LQC	21.1 ± 2.1	-4.5	11.2	14.7
	MQC	208.2 ± 2.28	-7.5	8.6	9.5
	HQC	800.7 ± 9.7	-1.1	10.3	11.7
	ULQ ^b	856.8 ± 2.68	-14.3	3.4	
Serum	HNP-1	Blank	92.1 ± 9.1	5.6	9.9
	LQC	154.8 ± 14.4	7.5	5.3	12.6
	MQC	343.2 ± 28.3	0.4	4.7	8.2
	HQC	869.8 ± 66.7	-2.8	5.8	7.7
	ULQ ^b	860.0 ± 60.0	-10.3	5.4	
HNP-2	Blank	105.0 ± 10.9	6.5	10.3	
	LQC	161.3 ± 15.7	14.8	6.7	9.6
	MQC	342.7 ± 24.7	4.8	5.1	8.1
	HQC	900.8 ± 58.5	0.3	6.0	6.4
	ULQ ^b	884.5 ± 41.4	-11.5	4.7	
HNP-3	Blank	24.0 ± 1.8	6.7	7.3	
	LQC	74.2 ± 4.2	-1.7	5.9	5.7
	MQC	208.3 ± 14.4	-2.7	5.2	5.4
	HQC	804.5 ± 7.4	-3.8	5.5	9.2
	ULQ ^b	804.3 ± 13.3	-15.1	6.0	

^a Measured concentrations include endogenous and spiked peptides. Expected LQC concentrations in plasma were 12.5 ng/ml above the "blank" concentration for HNP-1 and -3 and 75 ng/ml for HNP-2. In serum, LQC samples were spiked with 50 ng/ml. MQC and HQC samples were spiked with 250 and 800 ng/ml, respectively in both matrices.

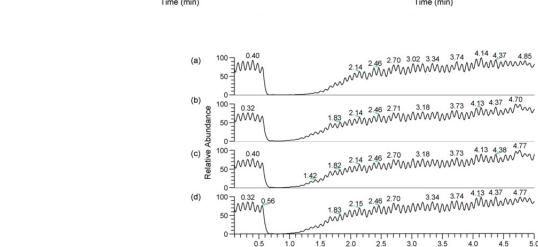
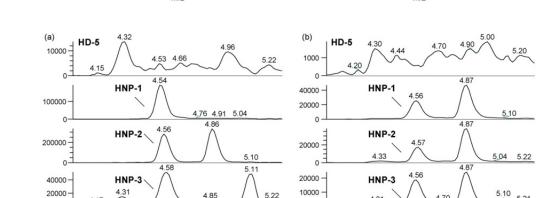
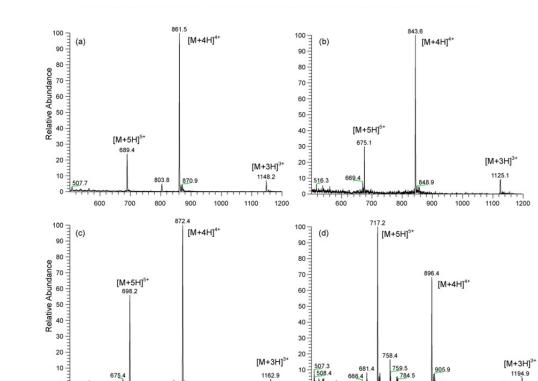
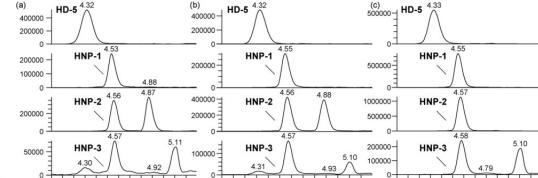
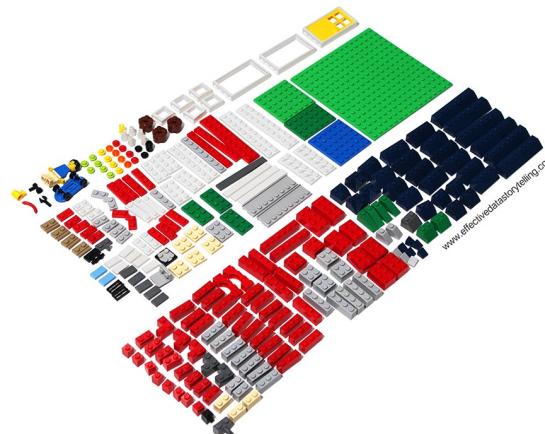
^b ULQ samples were spiked at 5 ng/ml in both matrices and measured after 5-fold dilution with blank human plasma from the same batch as used for preparation of the calibration standards.

	LQC	MQC	HQC
Plasma			
HNP-1	102.4 ± 10.1	95.1 ± 6.3	86.7 ± 11.5
HNP-2	121.4 ± 8.6	104.7 ± 5.5	88.6 ± 11.1
HNP-3	106.7 ± 15.3	96.4 ± 10.0	85.2 ± 10.8
HD-5			88.2 ± 12.3
Serum			
HNP-1	106.5 ± 7.7	123.5 ± 10.8	85.4 ± 10.9
HNP-2	128.3 ± 8.2	121.7 ± 10.3	90.2 ± 10.3
HNP-3	90.7 ± 8.6	114.7 ± 11.4	85.2 ± 11.3
HD-5			87.0 ± 10.0

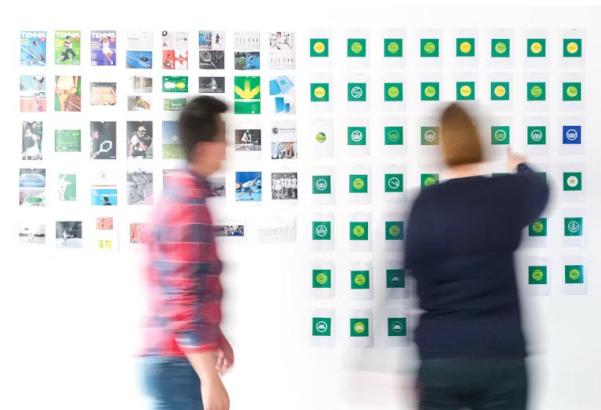
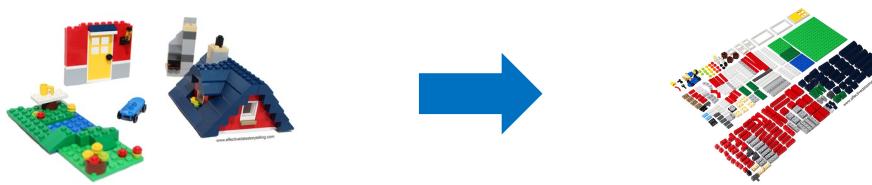
Peptide	Matrix	Condition	LQC	MQC	HQC	
			% DEV	RSD	% DEV	RSD
HNP-1		Freeze-thaw	8.7	6.4	0.2	9.9
		-3.5	9.7	-4.6	13.9	7.0
		-0.1	12.0	-0.6	13.0	9.1
		-12.0	-0.6	-0.6	-0.6	13.2
HNP-1		Short-term	-1.8	5.6	5.3	5.8
		2.5 h	0.7	5.7	2.4	7.0
		RT	-0.4	5.9	7.0	-2.7
		-20 °C	-0.0	5.3	5.4	4.9
HNP-2		Two months	10.2	8.6	8.7	5.2
		-80 °C	0.3	5.3	5.4	5.5
HNP-1		Autosampler	-6.9	4.9	-0.1	8.3
		5 °C; 7 days	-4.1	5.7	-0.3	10.0
		-24	5.5	2.1	10.0	-10.3
HNP-3		Final extract	135.8	568.2	659.2	558.0

Individual	Days between first sample	Concentration	HNP-1 (ng/ml)	HNP-2 (ng/ml)	HNP-3 (ng/ml)
#1	-	26.2	94.9	187.7	35.1
1	1	110.5	105.4	214.9	42.8
2	1	200.8	193.5	317.9	61.6
10	7.9	213.9	397.1	83.9	
11	7.2	91.4	245.4	32.2	
#2	-	135.8	568.2	659.2	558.0
1	1	172.2	374.0	257.8	293.0
8	8	NM	579.0	587.0	376.3
10	84.6	368.2	409.7	236.0	
#3	-	100.8	181.5	303.0	33.3
6	6	106.4	312.4	1143.0	127.9
8	8	20.6	343.1	1144.7	125.3
#4	-	26.9	282.6	332.7	0.0
1	1	23.7	208.8	330.0	4.0
#5	-	221.3	22.0	107.5	182
2	2	172.7	42.3	492.0	31.1
#6	-	307.5	152.7	566.0	465.0
1	1	220.6	25.3	284.9	94.3
#7	-	288.8	576.0	104.0	100.3
2	2	320.1	679.0	857.4	137.2
#8	-	49.7	64.3	292.9	14.3
#9	-	54.8	123.7	924.7	173.1
#10	-	17.0	84.8	267.9	193.2
#11	-	19.8	105.0	259.8	72.6

NM: not measured.



Exercise: Sketch your poster



Individually

1

Project teams

2

Peers

3

Sketch your (ideal) poster

What's needed to achieve your research goal?



Individually:

- **Sketch a poster** that shows *how* you have accomplished your research goal.
 - Use pen + paper, Powerpoint, or sticky notes
- Think of **the most ideal results** and experiments:
 - Ignore results gained in the past weeks.
 - Include experiments you still need to do.
- Use the (sub)headings of your **outline**.
- ★ Make captions for the required tables and figures.
- ★ Indicate the *flow* of your story: how does each figure build on the previous one?

Sketch your (ideal) poster

What's needed to achieve your research goal?



Project teams:

- Discuss the **differences** and **similarities** between your ideas and try to reach a consensus on what to put on the poster.
- Make a **sketch** of your new poster
 - Use pen + paper, Powerpoint, sticky notes or whiteboard

Discuss:

- ★ Which results on the poster have you obtained already?
- ★ Which results / experiments are still needed?
- ★ Make an action plan and prioritize your planned experiments.

Sketch your (ideal) poster

What's needed to achieve your research goal?



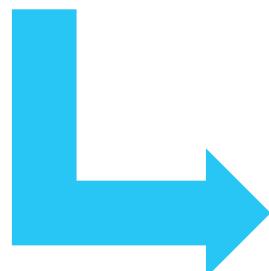
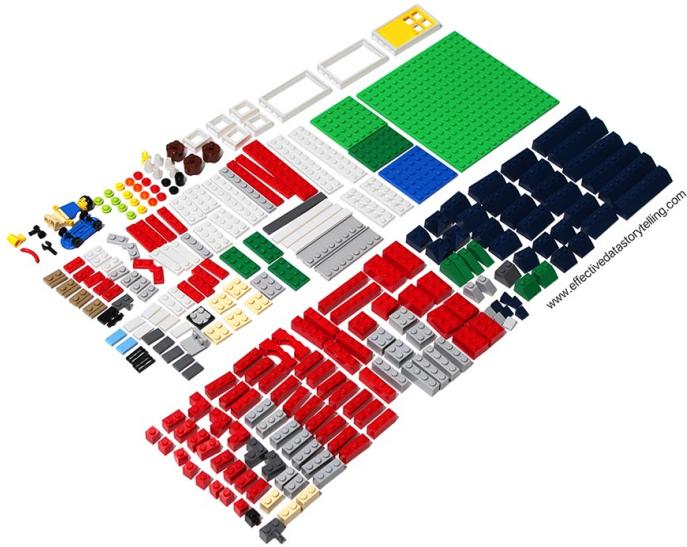
Peers:

- Look (as group) at the poster of another group and try to understand the **goal**, the **results** and the **flow** (storyline).
- Write on a sticky note:
 - **TOP:** What's clear?
 - **TIP:** What needs more clarification / confirmation?

Break



How to write: Sell your story



The introduction is a STORY...



A story that answers 5 questions



Bigger picture:

1. Why is your research important?

Literature survey:

2. What is known? *What has already been done?*
3. What is unknown? *What needs to be improved?*

Your research: *Reference studies with common goal(s)*

4. How does your research fill these discrepancies?
5. What are your most important findings?

➤ *Encourage the reader to read on!*

The introduction in 5 sentences

1. Why is your research important?

Compound X can be used as a biomarker for...

2. What is known? What has already been done?

Different analytical techniques (e.g.,....) have been developed to quantify X in.... Extraction of X from ... often includes

3. What is unknown? What needs to be improved?

However, previous methods showed poor sensitivity and recovery, whereas commonly applied extraction methods are laborious and time-consuming.

4. How does your research fill these discrepancies?

Technique Y enables ... and can allow...

5. What are your most important findings?

Using technique Y, we demonstrated ... when applied to

The introduction is a STORY

RESEARCH ARTICLE

SPECIAL FOCUS: DRIED BLOOD SPOTS

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Dried blood spot punches for confirmation of suspected γ -hydroxybutyric acid intoxications: validation of an optimized GC-MS procedure



Background: γ -hydroxybutyric acid (GHB), notorious as a club- and date-rape drug, was quantified in dried blood spots (DBS) by punching out a disc, followed by 'on-spot' derivatization and analysis by GC-MS. **Results:** A homogenous distribution in DBS was demonstrated and accurate results were obtained when analyzing a disc punched out from a 20–35 μ l spot, regardless the hematocrit of the blood sample. Validation based on US FDA and European Medicines Agency guidelines was performed, with a calibration range covering 2–100 μ g/ml. **Conclusion:** A sensitive GC-MS method for GHB analysis in DBS was successfully optimized and validated. The successful analysis of DBS collected from GHB abusers suggests the routine applicability of the DBS sampling technique for GHB analysis in toxicological cases.

The short chain fatty acid γ -hydroxybutyric acid (**GHB**) was synthesized in the early sixties as a structural analogue of γ -aminobutyric acid and also occurs naturally in blood, urine and peripheral and brain tissue [1,2]. Although the function of endogenous GHB has not completely been revealed yet, evidence suggests that it may act as a neuromodulator or neurotransmitter [2]. As a legal substance (sodium oxybate), GHB has a role as an anesthetic agent, in the treatment of narcolepsy with cataplexy and in alcohol and opiate withdrawal. In addition, it has also been sold as a substance of nutritional supplements to induce sleep and increase muscle mass. Currently, illegal GHB (liquid ecstasy) as well as its precursors, γ -butyrolactone and 1,4-butanediol, are popular as club drugs and appear occasionally in drug-facilitated sexual assaults [3]. In those toxicological cases, the interpretation of a positive analytical result is a real challenge, owing to its endogenous presence and reported *in vitro* production [4,5]. Therefore, **cut-off levels** have been proposed by several authors and these are currently set at 4 or 5 μ g/ml for blood (serum) samples [2]. In addition, the detection window is very limited as GHB is rapidly metabolized and eliminated after oral ingestion (**plasma half-life** <1 h), so blood samples must be taken within 6 h after ingestion [6]. Consequently, a sampling delay may result in blood levels below the established cut-off level, no longer resulting in a positive case [7].

Blood sample collection may be facilitated by using **dried blood spot (DBS) sampling**. A DBS

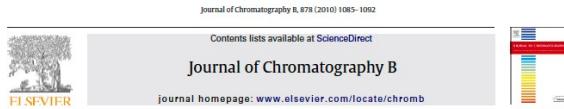
is capillary whole blood obtained by a finger or heel prick and collected on a filter paper card. Advantages over a venipuncture are the easy and rapid way to collect a representative sample and the less specific sample transport and storage requirements [8]. Previously, DBS sampling has generally been used for newborn screening; however, more recently, this alternative sampling strategy is increasingly gaining interest in the context of therapeutic drug monitoring and (pre-)clinical studies, as well as in toxicology [8,10]. We recently reported on the development and validation of a new procedure for GHB determination in DBS, using 'on-spot' derivatization and **GC-MS** [9]. Similarly, other drugs of forensic interest have been determined in DBS, such as 3,4-methylenedioxymethamphetamine, morphine and 6-acetylmorphine or cocaine [12–14].

To obtain a DBS on filter paper card, a drop of blood can be spotted directly on the filter paper or with the aid of a precision capillary [8]. In our previous study, we used the second sample collection technique, and spotted a drop of blood with a fixed volume onto the filter paper card, followed by analysis of the complete DBS [11]. However, as correct sampling in this case ideally requires the presence of trained staff and in routine practice, it is more convenient to collect the drop of blood directly on the filter paper card, we therefore modified our procedure accordingly. As we did not wish this simplification to be at the expense of sensitivity (LLOQ of 2 μ g/ml), we readjusted several sample pretreatment steps.

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1. The interpretation of a positive analytical result for illegal GHB in toxicological cases is a real challenge, owing to its endogenous presence and reported *in vitro* production.
2. Therefore, cut-off levels have been proposed.
3. However, a sampling delay may result in blood levels below the established cut-off level as GHB is rapidly metabolized and eliminated after oral ingestion.
4. Blood sample collection may be facilitated by using dried blood spot (DBS) sampling.
[short overview DBS literature]
5. In this study, we demonstrated applicability by analyzing DBS collected from patients with a suspected GHB-intoxication at the emergency department.

The introduction is a STORY



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Journal of Chromatography B
journal homepage: www.elsevier.com/locate/chromb

Validation of a quantitative assay for human neutrophil peptide-1, -2, and -3 in human plasma and serum by liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT
A quantitative assay for simultaneous measurement of individual human neutrophil peptide-1, -2 and -3 concentrations will aid in exploring the potential of these antimicrobial peptides as biomarkers for various diseases. Therefore, a liquid chromatography-tandem mass spectrometry method has been developed and validated allowing the simultaneous quantification of the three human neutrophil peptides in human plasma and serum. Plasma and serum samples were deproteinized by trichloroacetic acid, followed by liquid chromatographic separation on a Symmetry 300 C₁₈ column (50 mm × 2.1 mm I.D., particle size 3.5 µm), using a water–methanol gradient containing 0.25% (v/v) formic acid and human alpha-defensin 5 as internal standard. Tandem mass spectrometric detection was based on a triple quadrupole mass spectrometer equipped with electrospray ionization. Despite low fragmentation efficiency of the three antimicrobial peptides, multiple reaction monitoring was used for detection, though selecting the quaternary charged ions as both precursor and product. The method was linear for concentrations between 5 and 100 ng/ml with a detection limit around 3 ng/ml for all peptides. The detection limits for HNP-1, -2 and -3 were 14.4 and 19.13, respectively, at the lowest measured endogenous concentration (6.4 ng/ml of HNP-1 in plasma), representing the lower limit of quantification of the assay. Recoveries of HNP-1, -2 and -3 from plasma and serum ranged between 85 and 128%. Analysis of serum samples from intensive care patients showed average concentrations of 362, 570 and 143 ng/ml for HNP-1, -2 and -3, respectively.

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1. Introduction

Human neutrophil peptides (HNP-1, -2, -3 and -4) are antimicrobial molecules, belonging to the alpha-subfamily of human defensins and are stored in the granules of neutrophils. Two other alpha-defensins are localized in Paneth cell granules and are named human alpha-defensin 5 and 6 (HD-5 and -6)[1]. All alpha-defensins contain a conserved C-terminal heptapeptide with four disulfide bonds, as shown in Table 1. HNPs make up about 5–7% of the total cellular protein content in the azurophil granules [2–4]. Compared to HNPs 1–3, HNP-4 shows less amino acid sequence homology and a 100-fold lower concentration in the neutrophils [2].

The clinical relevance of HNPs 1–3 has been studied relatively extensively, whereas less is known about the other alpha-defensins, partly due to the lack of available reference materials [1,5]. In these studies, elevated concentrations of HNPs 1–3 in various matrices have been related to different types of diseases, suggesting high diagnostic potential. Infections have shown to result in up-regulated levels of HNPs 1–3 in plasma, blood, and bronchoalveolar lavage fluid (BALF) of patients with various types of lung diseases [3,8–12]. Besides infections and lung diseases, raised HNPs have been related to gastrointestinal diseases like Crohn's disease, ulcerative colitis, and colorectal cancer [13–19]. Additionally, HNPs 1–3 have been related to renal cell carcinoma [20,21] and have been found up-regulated in saliva samples of patients with oral diseases [22–24].

To further explore the role of HNP-1, -2 and -3 as diagnostic markers for specific diseases, a timely application of an LC-MS/MS assay would be beneficial. Previous quantification of HNPs 1–3 has mainly been performed by radio- or enzyme immunoassays. How-

1. Elevated concentrations of HNPs 1-3 in various matrices have been related to different types of diseases, suggesting high diagnostic potential.
2. Previous quantification of HNPs 1-3 has mainly been performed by radio- or enzyme immunoassays.
3. However, the specificity of immunoassay techniques does not allow distinction between the homologous HNP subtypes.
4. The high specificity of mass spectrometric detection offers the benefit to enable separate quantification of HNP-1, -2, and -3.
5. Here, an LC-MS/MS assay is presented as the first method to allow simultaneous quantification of the individual concentrations of HNP-1, -2, and -3 in human plasma and serum.

[reference to 1 earlier LC-MS study in saliva samples]

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[doi:10.1016/j.jchromb.2010.03.014](https://doi.org/10.1016/j.jchromb.2010.03.014)

The introduction is a STORY

Clinical Chemistry 62:1
188–197 (2016)

Proteomics and Protein Markers

Automated Multiplex LC-MS/MS Assay for Quantifying Serum Apolipoproteins A-I, B, C-I, C-II, C-III, and E with Qualitative Apolipoprotein E Phenotyping

Irene van den Broek,¹ Fred P.H.T.M. Romijn,¹ Jan Nouta,¹ Arnoud van der Laarse,^{1,2} Jan W. Drijfhout,³ Nico P.M. Smit,¹ Yuri E.M. van der Burgt,^{1,4} and Christa M. Cobbaert^{1*}

BACKGROUND: Direct and calculated measures of lipoprotein fractions for cardiovascular risk assessment suffer from analytical inaccuracy in certain dyslipidemic and pathological states, most commonly hypertriglyceridemia. LC-MS/MS has proven suitable for multiplexed quantification and phenotyping of apolipoproteins. We developed and provisionally validated an automated assay for quantification of apolipoprotein (apo) A-I, B, C-I, C-II, C-III, and E and simultaneous qualitative assessment of apoE phenotypes.

METHODS: We used 5 value-assigned human serum pools for external calibration. Serum proteins were denatured, reduced, and alkylated according to standard mass spectrometry-based proteomics procedures. After trypsin digestion, peptides were analyzed by LC-MS/MS. For each peptide, we measured 2 transitions. We compared LC-MS/MS results to those obtained by an immunoturbidimetric assay or ELISA.

RESULTS: Intraassay CVs were 2.3%–5.5%, and total CVs were 2.5%–5.9%. The LC-MS/MS assay correlated ($R = 0.975\text{--}0.995$) with immunoturbidimetric assays with Conformité Européenne marking for apoA-I, apoB, apoC-II, apoC-III, and apoE in normotriglyceridemic ($n = 54$) and hypertriglyceridemic ($n = 46$) sera. Results were interchangeable for apoA-I $\leq 3.0 \text{ g/L}$ (Deming slope 1.014) and for apoB-100 $\leq 1.8 \text{ g/L}$ (Deming slope 1.016) and were traceable to higher-order standards.

CONCLUSIONS: The multiplex format provides an opportunity for new diagnostic and pathophysiological insights into types of dyslipidemia and allows a more personalized approach for diagnosis and treatment of lipid abnormalities. © 2015 American Association for Clinical Chemistry

Apolipoproteins are centrally involved in lipid metabolism and atherosclerotic processes and fulfill an important role as biomarkers for cardiovascular disease (CVD)⁵. Risk. Apolipoprotein B-100 (apoB-100), for example, accurately reflects the number of potentially atherogenic LDL particles, whereas apoA-I reflects atheroprotective HDL particles. The apoB/apoA-I ratio is therefore a well-established CVD risk factor (1). In addition, apoB-48 is a specific measure of chylomicrons and their remnants (2,3). Other apolipoproteins are widely distributed across the lipoprotein subclasses and offer important information about underlying diagnosis and cardiovascular risk (3). ApoC-I, apoC-II, and apoC-III are involved in the uptake or degradation of triglycerides, chylomicron remnants, and cholesterol-rich lipoproteins by regulation of lipoprotein lipase (4–7). Similarly, apoE is a determinant of CVD risk and atherosclerosis. A standard tool for diagnosing dysbetalipoproteinemia is apoE phenotyping on the basis of immunostaining of 3 major isoforms (apoE2, apoE3, and apoE4) or apoE genotyping (3, 8, 9). Moreover, apoE polymorphisms are associated with other lipid abnormalities and neurological disorders such as Alzheimer disease (3, 8, 9).

Despite their key role in pre-, pro-, and antiatherosclerotic processes, apolipoprotein measurements for CVD risk assessment are not generally embedded in clinical practice. Although well-standardized clinical immunoassays are available for serum apoA-I and apoB, CVD risk assessment is traditionally based on HDL and LDL or non-HDL cholesterol concentrations. All homogeneous and calculated lipid parameters can suffer from analytical inaccuracy in the context of specific dyslipidemic states, particularly those associated with marked hypertriglyceridemia or abnormal lipoproteins (10). Moreover, in the case of apoC-I, apoC-II, apoC-III, and apoE, standardized clinical immunoassays are generally not

1. Apolipoproteins are centrally involved in lipid metabolism and atherosclerotic processes and fulfil an important role as biomarkers for cardiovascular disease (CVD).
2. Although well-standardized clinical immunoassays are available for serum apoA-I and apoB, CVD risk assessment is traditionally based on HDL and LDL or non-HDL cholesterol concentrations.
3. However, all homogeneous and calculated lipid parameters can suffer from analytical inaccuracy, whereas standardized clinical immunoassays for apoC-I, apoC-II, apoC-III, and apoE are not available, impeding the discovery of pathophysiological clues for correct clinical diagnosis and adequate treatment of dyslipidemia.
4. LC-MS/MS is a powerful approach for highly multiplexed protein quantification and isoform differentiation and could support clinical studies to elucidate the role of apolipoproteins and their variants in CVD as well as the implementation of apolipoprotein measurements in clinical practice.
5. Here, we describe the development of an LC-MS/MS method for multiplexed quantification of serum apoA-I, apoB, apoC-I, apoC-II, apoC-III, and apoE, including various validation steps toward clinical implementation.

[reference to earlier LC-MS/MS assays for apolipoproteins]

Get your 5 answers and mold this into a **STORY**



Bigger picture:

1. ✓ Why is your research important?

Literature survey:

2. ✓ What is known?

What has already been done?

3. What is unknown?

What needs to be improved?

Your research:

4. How does your research fill these discrepancies?

5. What are your most important findings?

Sell your story

Create your unique selling point

Group:

- Take your **summary of relevant literature** and discuss:
 - What is **unknown**? What are current limitations?
 - How does **your research** fill this discrepancy?
- Think of your ideal situation (**poster**) and discuss:
 - What **details** do you want to share to get the reader curious and encourage the reader to read on?



- **Write your *sales pitch* (5 sentences):**
- post your *sales pitch* in the Teams channel 😊
 - Read the sales pitches and share with a **reaction** how the story makes you feel: 👍 😲 ❤️ 💡 🏆 😮 💯 ✌️ !! 🙌
 - Write a \uparrow_{TOP} in the replies

Literature review in the introduction



Bigger-picture goal:

Application of a bioanalytical method for ... in ...



Research goal:

Development and validation of a bioanalytical method for ... in ... using ...

Question 2: What is known?

- Literature **demonstrates the knowledge gap** that has led to your research question.

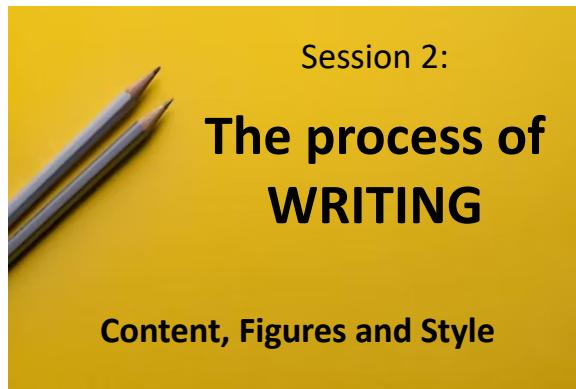
Previous quantification of HNPs 1-3 has mainly been performed by radio- or enzyme immunoassays [...].

Question 4: How does your research fill these discrepancies?

- Literature **provides an account of what others** with (somewhat) related research questions **have done**.

One quantitative assay for HNP-1, -2 and -3 with MS detection has been reported [...], using LC-MS for saliva samples.

Next session: be prepared



In addition:

- Read **Writing Tip Series** part 4-7
 - Fill up your outline with notes and descriptions
 - Pick some of your favorite papers (literature review!) and analyse how the introduction is written
 - Divide which sections can be written, when and by whom.
- Start writing! ☺**

Individual:

- Pick 1 result and make a **visualization**
- **Write** a self-explanatory **caption** for this figure/table

Group:

- **Write** (2 paragraphs of) the **introduction**:
 - Use your literature summary and sales pitch
 - Include at least 5 references, and 5-10 sentences.
 - The **first paragraph**: focus on your **bigger-picture goal** and what is known (**Q1 & Q2**)
 - An overview of what other studies with a related **research goal** have done (**Q4**)
 - ★ Write the connecting paragraph(s) (**Q3**)
-
- **Bring** an example of 1 **unexpected result**

Feedback session 1



<https://forms.gle/LvWjsToRhMYoYuS9A>

- What did you expect from the report writing seminar(s)?
- To what extend was your expectation met in session 1?
- List at least 3 things from session 1 that were helpful for you
- What could be improved in the **structure** (exercises, interaction, time management) of session 1?
- What could be improved in the **content** (topics) of session 1?
- What would you like to see covered in session 2?
- Any additional comments or feedback are welcome!



How to write a (bioanalytical) report

Bioanalysis (FA-BA319/FA-CPS337)

Irene van den Broek (i.vandenbroek@uu.nl)



Session 2:

The process of WRITING

Content, Figures and Style

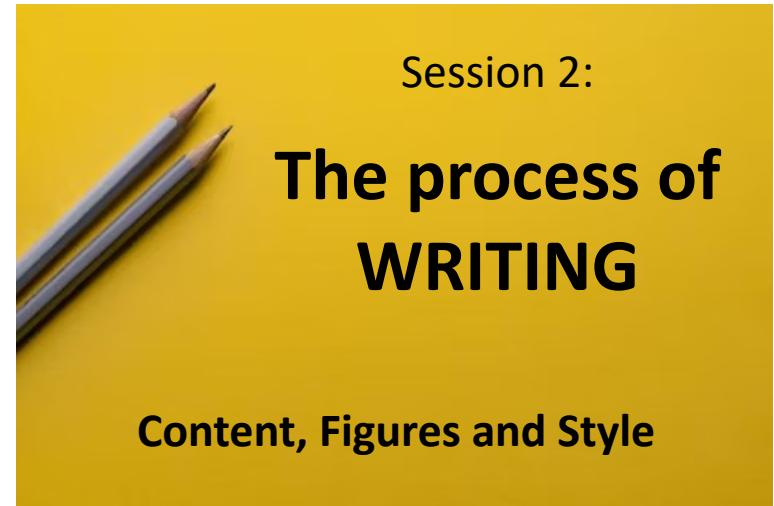
How to write a report seminars



Session 1:

The process of **THINKING**

Context, Structure and Story



Session 2:

The process of **WRITING**

Content, Figures and Style

Based on the *ideal* situation:

- Formulate your goal
- Structure your content
- Sketch your ideal poster
- Sell your story

Based on *reality*:

- Introduction
- Method
- Results: tables and figures
- Discussion

Preparation

- Read **Writing Tip Series:** Methods (part 4), Results (part 5), Discussion (part 6), Tables and figures (part 7).



- **Individual:** Visualize 1 result and write a self-explanatory caption
- **Group:** Write a literature review (2 paragraphs) related to bigger-picture and research goal
- **Group:** Bring an example of 1 unexpected result (as food for discussion)

Schedule Session 2 (the process of **writing**)

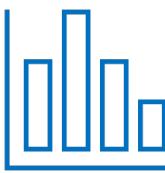


Style

Language, structure, editing

Exercise: Literature review

----- break -----

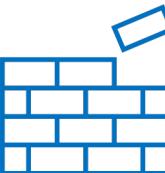


Figures

Visualizing results

Exercise: What's in this figure?

-----break-----



Content

Introduction, Methods, Results, Discussion

Exercise: An unexpected result

Abstract, References, Appendix

Writing Tips and Survey

How to write: The process



Content

Structure

Process of thinking

Draft

Report

Process of writing

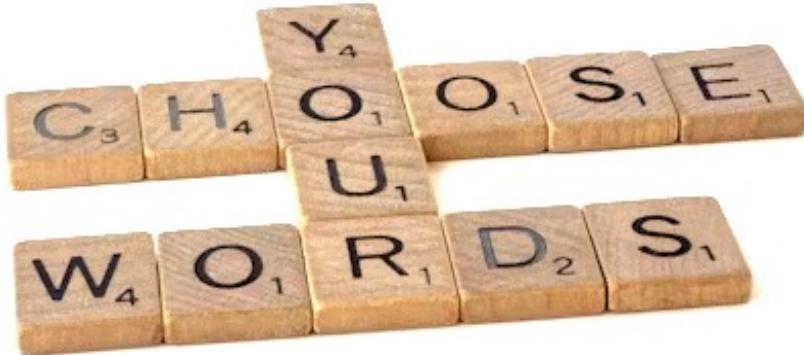
Story

Process of thinking

**Academic
Style**



Academic **style**



Objective:

avoid **superlatives** (i.e., *groundbreaking*) and **exaggerations** (i.e., *extremely, amazing, enormous*).

Impersonal:

use **first person** sparingly

Accurate:

avoid **indefinite** words (i.e., *frequently or a lot*), list exact specifications.

Unambiguous:

be careful with **linking words** such as “this” or “that”.

Formal:

no metaphors or **slang**

Getting **tense**

Nog opmaken



- Introduction and Discussion/Conclusion → present tense
- Methods and Results → past tense
- Quoting *previously published work* → present tense
- Describing *your own work* → past tense

Balance Active and Passive voice

Passive voice:

Wordy
Abstract
Impersonal
Distanced

- ✓ Emphasize the *action*
- Methods

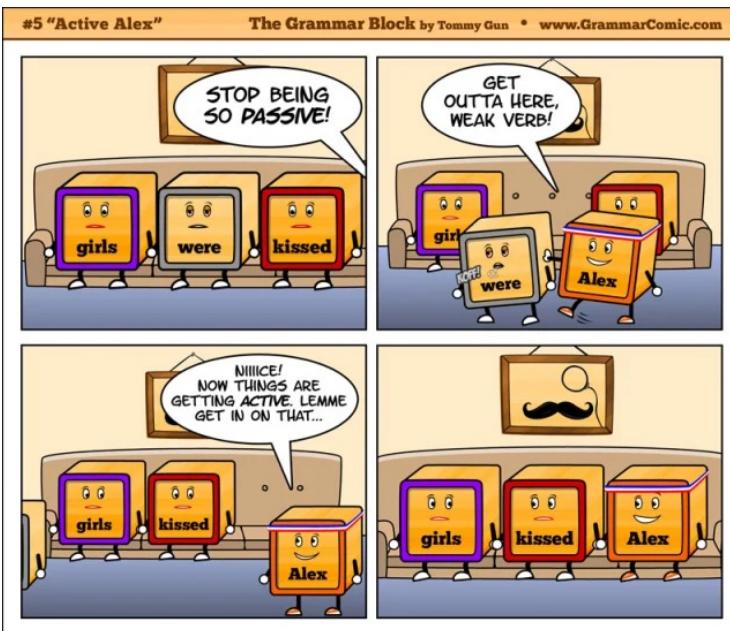


Active voice:

Direct
Clear
Concise
Engaging

- ✓ Emphasize the *subject*
- Introduction
- Results
- Discussion

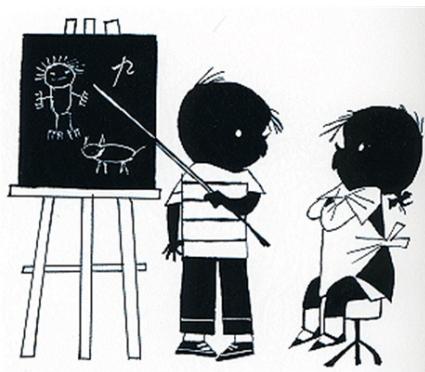
Balance Active and Passive voice



Introduction	Use active voice to state the purpose and objective	<i>"We aim to investigate..."</i>
Methods	Use passive voice for standard procedures	<i>"The samples were prepared..."</i>
Results	Use active voice to report key findings	<i>"We found a significant correlation..."</i>
Discussion	Use active voice to interpret results and state conclusions	<i>"Our findings suggest that..."</i>

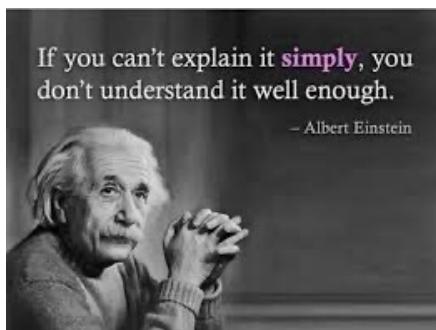
Keep it

S₁ I₁ M₃ P₃ L₁ E₁



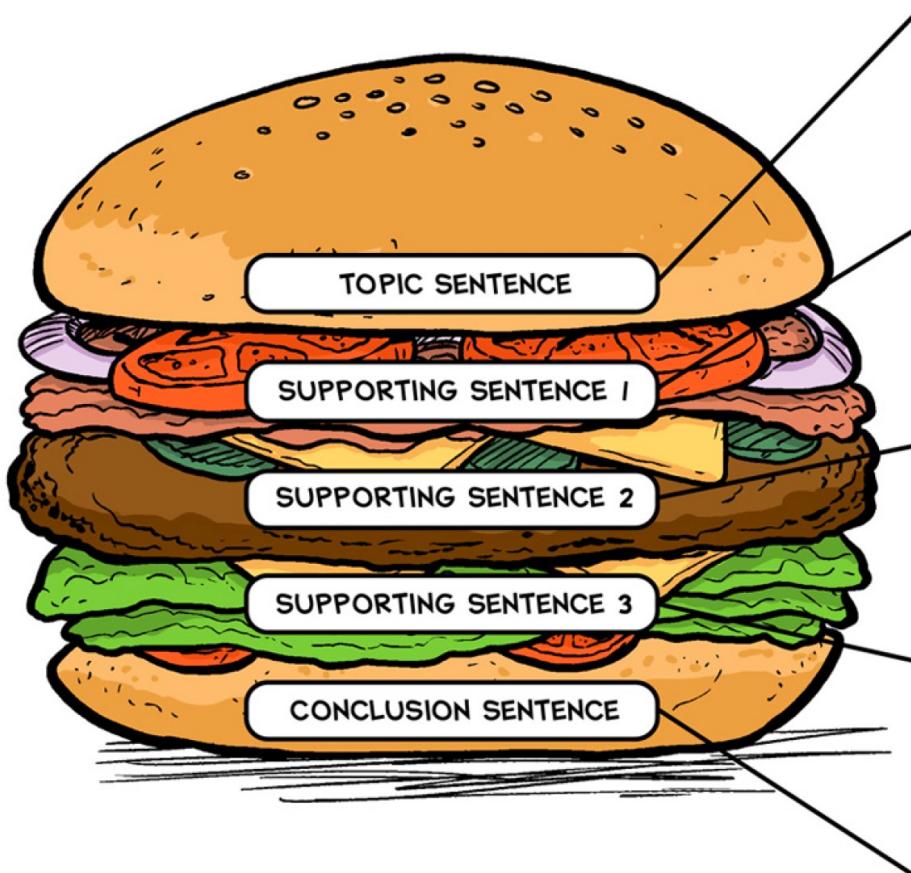
Academic ≠ Complex

- ✓ Use short sentences
- ✓ Don't use unnecessarily difficult words
- ✓ Avoid redundancy



<i>As a consequence of</i>	~	Because
<i>Despite the fact that</i>	~	Although
<i>In order to</i>	~	To
<i>By means of</i>	~	By
<i>In case of</i>	~	If
<i>With regard to</i>	~	About

Paragraph structure



State the main idea

- Choose it well!

**Explain the main idea in more detail
(optional)**

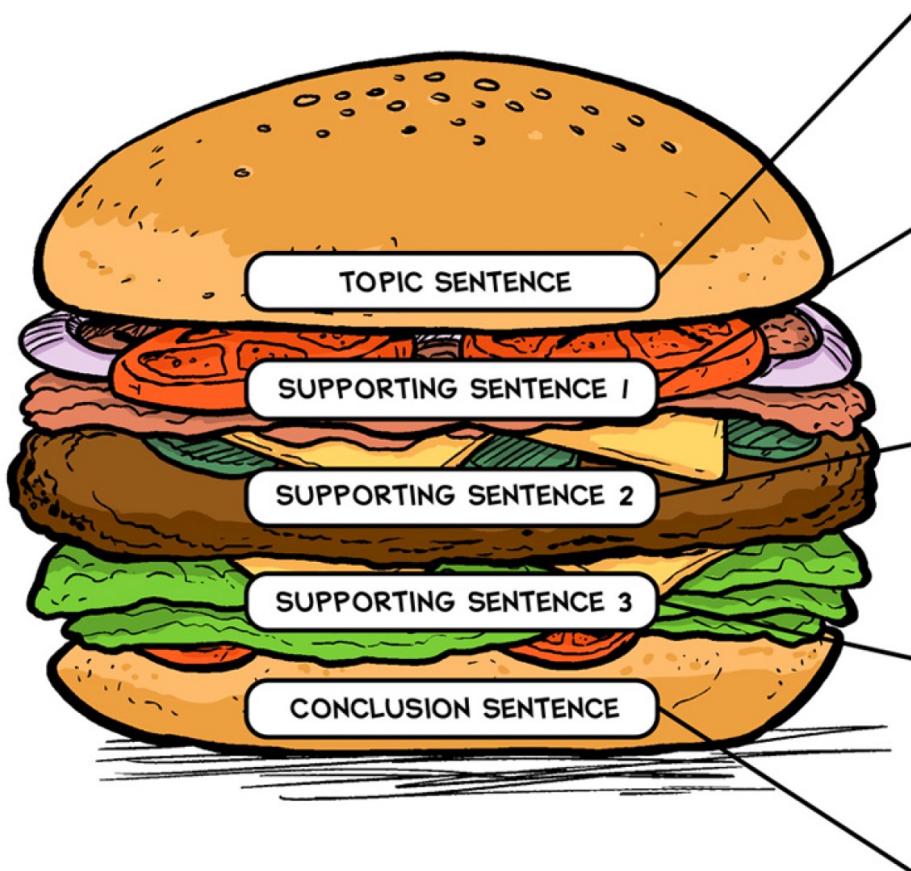
**Support the main idea with evidence
(facts, examples)**

Discuss the evidence

Summarise the main point

- Link to next paragraph

Paragraph structure



To further explore **the role of HNP-1, -2 and -3 as ...**

Previous studies show ... [refs]

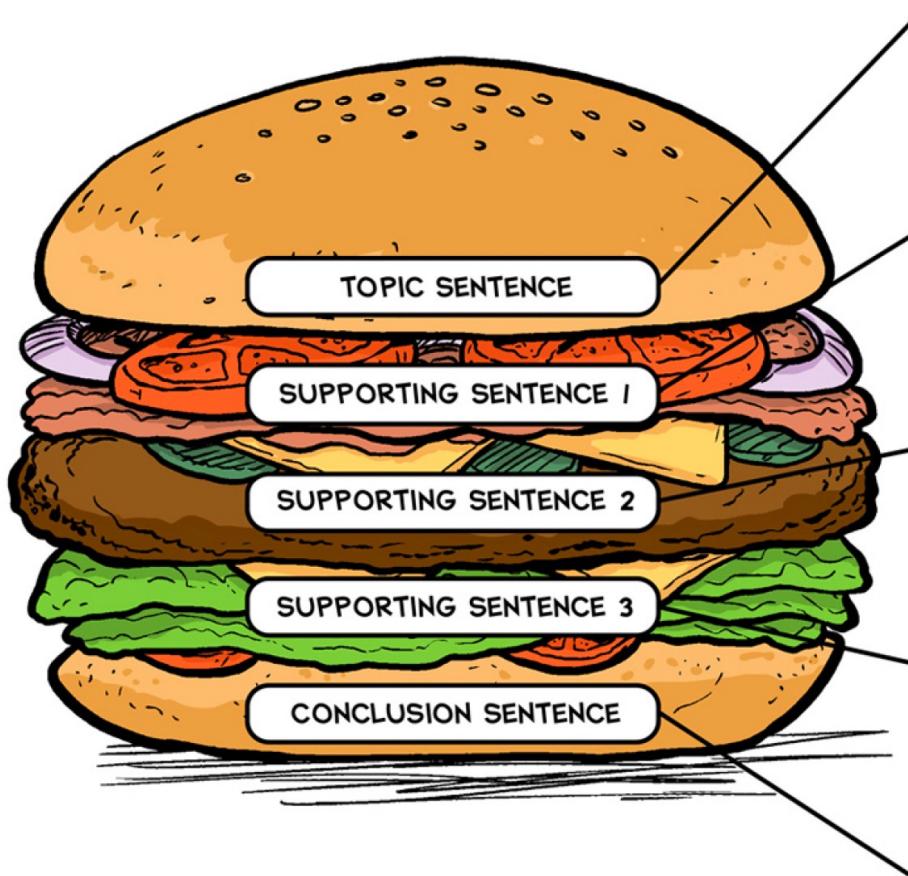
Nevertheless, it remained unclear...

Mass spectrometric detection offers...

Link paragraphs: Transition words

Additions	Additionally – also – moreover – furthermore – again – further – then – besides – too - similarly
Alternatives	Whereas – conversely - in comparison - by contrast; alternatively – although – otherwise - instead
Reasons	Therefore – accordingly - as a result of – consequently – thus – hence - for this reason
Contrast	However – nonetheless – furthermore - despite x - notwithstanding x - in spite of x – although - though
Examples	For example - for instance – namely - such as – including - particularly
Sequences	First(ly) - second(ly) - third(ly) – another – additionally – subsequently – next - then
Summary	In conclusion – therefore - to conclude – altogether - overall

Link paragraphs: Transition words



GHB is a ... and occurs in ... [refs].

In addition, ... has been sold as ...
Currently, ... are popular as ...

Therefore, ... have been proposed
... and these are ... [ref].

In addition, ..., **so** blood samples
must be taken within 6 h after
ingestion [ref].

Consequently, a sampling delay
may result in ..., no longer
resulting in a positive case [ref].

The first draft is for your eyes only!



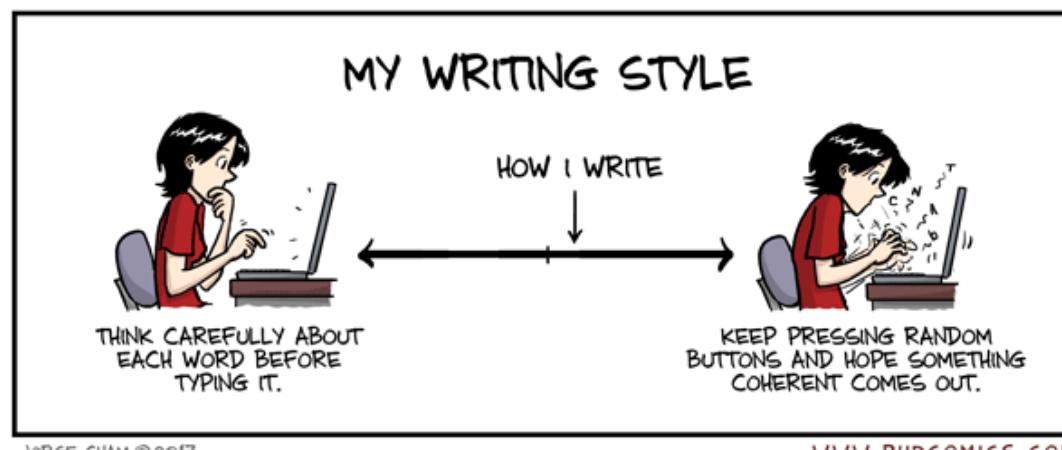
*Almost all good writing begins with terrible first efforts. You need to start somewhere. Start by getting something — anything — down on paper. A friend of mine says that the first draft is the **down draft** — you just get it down. The second draft is the **up draft** — you fix it up. You try to say what you have to say more accurately. And the third draft is the **dental draft**, where you check every tooth, to see if it's loose or cramped or decayed, or even, God help us, healthy.*

From the “Shitty First Drafts” chapter of **Anne Lamott’s *Bird By Bird***:

Writing ≠ editing



- (real) **Draft 1:** Write so **YOU** understand it
- **Draft 2:** Edit through the eyes of your **reader**
 - Grammar, style
 - Read between the lines: More elaboration needed on topics that are obvious to you?
- **Draft 3:** Let **someone else** read it
 - Structure, content, complex (long) sentences
 - Do they understand it?





With a little **AI**d from...

AI might help to **check language** and **improve style**,
but it can **not** write a scientific report!



AI Letter to Santa by Rick McKee, CagleCartoons.com

YOU can:

- **Understand** your own research results
- Develop **logical** arguments
- Reason and **think critically**
- Develop **creativity** and authenticity

- Don't use words that you don't understand or have never seen before!
- **Declare whether and how AI tools were used;** name the tool, the use case, and the scope.

More about Style: Skills boosters



- Develop your academic writing style

<https://www.youtube.com/watch?v=JjwXC2Bc8EQ>



- Improve your paragraph structure

<https://www.youtube.com/watch?v=WKpom8KFbf4>



- The art of Speedwriting

<https://www.youtube.com/watch?v=PIVlgDcivKE>

- De kunst van Speedwriting

<https://www.youtube.com/watch?v=JjwXC2Bc8EQ>

Literature review

Peer Feedback

In pairs:

- Exchange your literature review with a student from another group
- Provide each other with feedback on **Style**:
 - **Academic Style**: Look for words that are not: objective, impersonal, accurate, unambiguous or formal
 - Is **tense** used correctly?
 - Is **passive/active voice** well-balanced?
 - Are words/sentences **clear** and **simple**?
 - Are **paragraphs well-structured** and connected (e.g. **transition words**)
- ★ Provide feedback on **Storyline**:
 - Are message and limitations of current literature clear?
- Also provide **TOPs** when you like certain words/sentences!
- Discuss the use of **AI for Style and Grammar corrections**:
 - Did you use it? Where did it help you? Where not?

Literature review

Improve your introduction



With your project-group:

- Share the feedback you received on your Literature Review
 - Style
 - Storyline
- Discuss the changes you want to make (and how)
- Formulate your **key take-away** for this (and future) writing assignments.
- ★ Discuss / write connecting paragraphs

Break



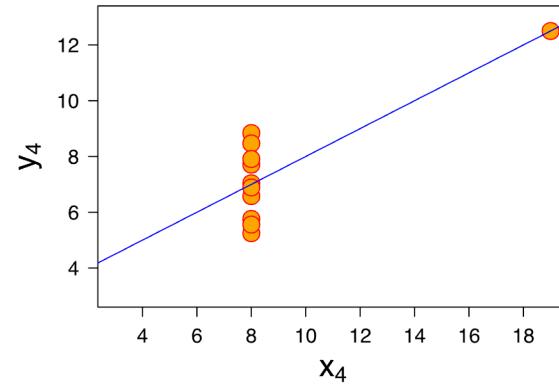
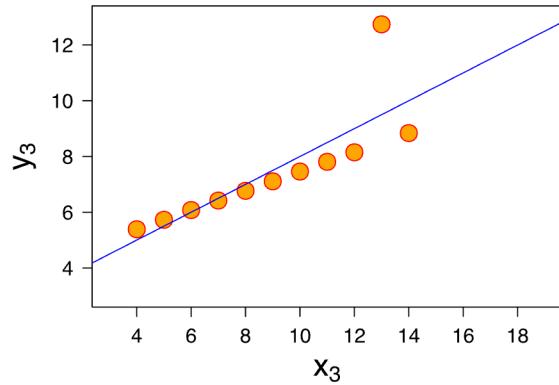
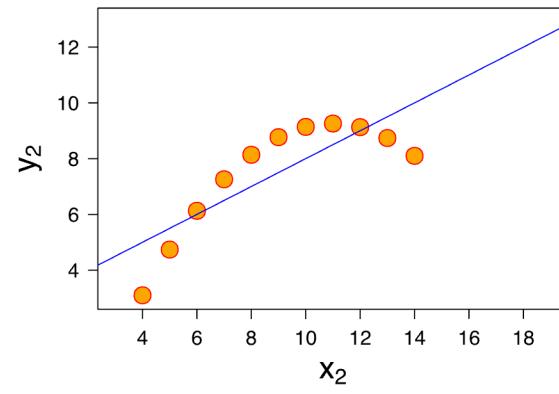
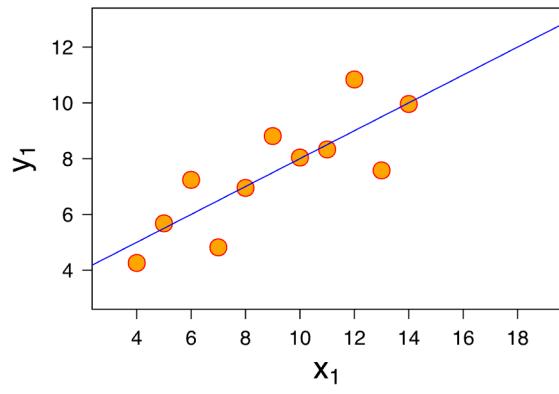
Figures



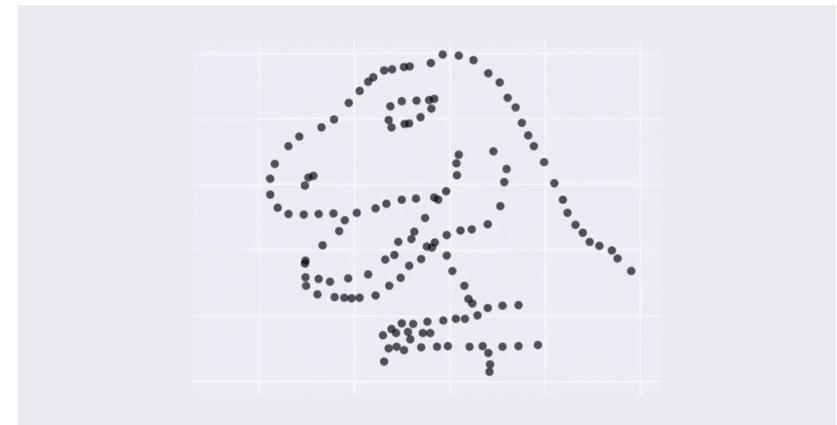
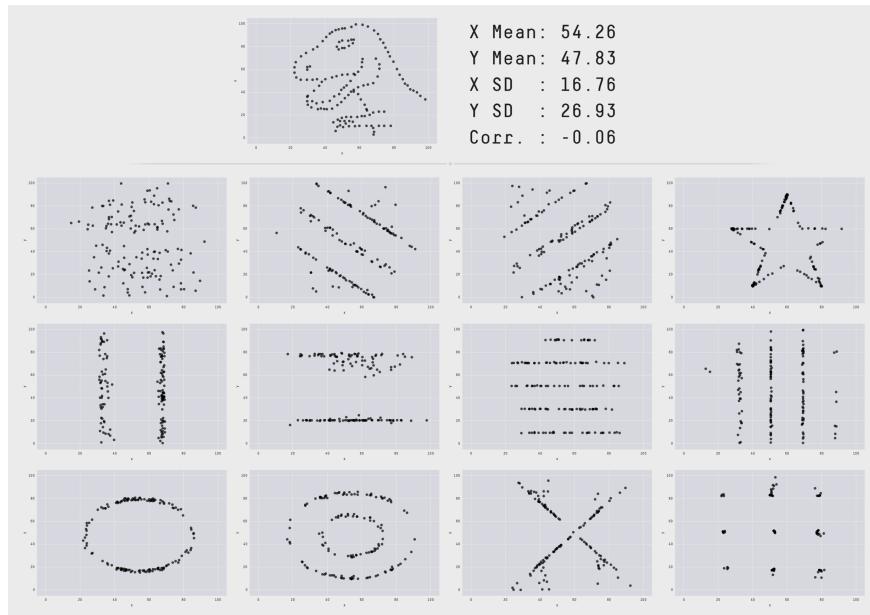
Why visualize results?

	I		II		III		IV	
	x	y	x	y	x	y	x	y
	10	8,04	10	9,14	10	7,46	8	6,58
	8	6,95	8	8,14	8	6,77	8	5,76
	13	7,58	13	8,74	13	12,74	8	7,71
	9	8,81	9	8,77	9	7,11	8	8,84
	11	8,33	11	9,26	11	7,81	8	8,47
	14	9,96	14	8,1	14	8,84	8	7,04
	6	7,24	6	6,13	6	6,08	8	5,25
	4	4,26	4	3,1	4	5,39	19	12,5
	12	10,84	12	9,13	12	8,15	8	5,56
	7	4,82	7	7,26	7	6,42	8	7,91
	5	5,68	5	4,74	5	5,73	8	6,89
SUM	99,00	82,51	99,00	82,51	99,00	82,50	99,00	82,51
AVG	9,00	7,50	9,00	7,50	9,00	7,50	9,00	7,50
STDEV	3,32	2,03	3,32	2,03	3,32	2,03	3,32	2,03

Why visualize results?



The Datasaurus Dozen 😊: Never trust summary statistics alone!



Figures: an example

CE-UV

The first measurement (phenylalanine standard) gave the following results:

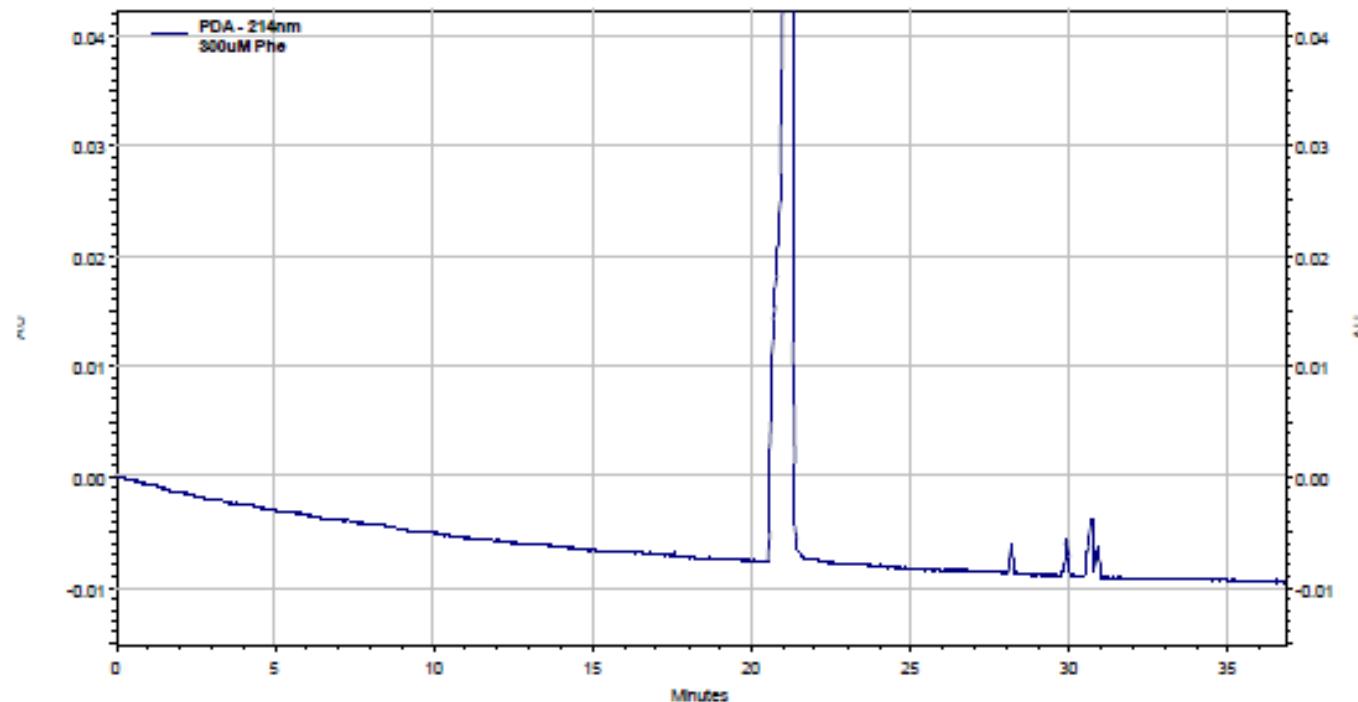


Figure 1: Electropherogram at 214 nm.

Figures: an example

CE-UV

CE-UV analysis of a ... ng/mL phenylalanine standard in ... showed a main peak with a retention time of ... minutes (**Figure 1**).

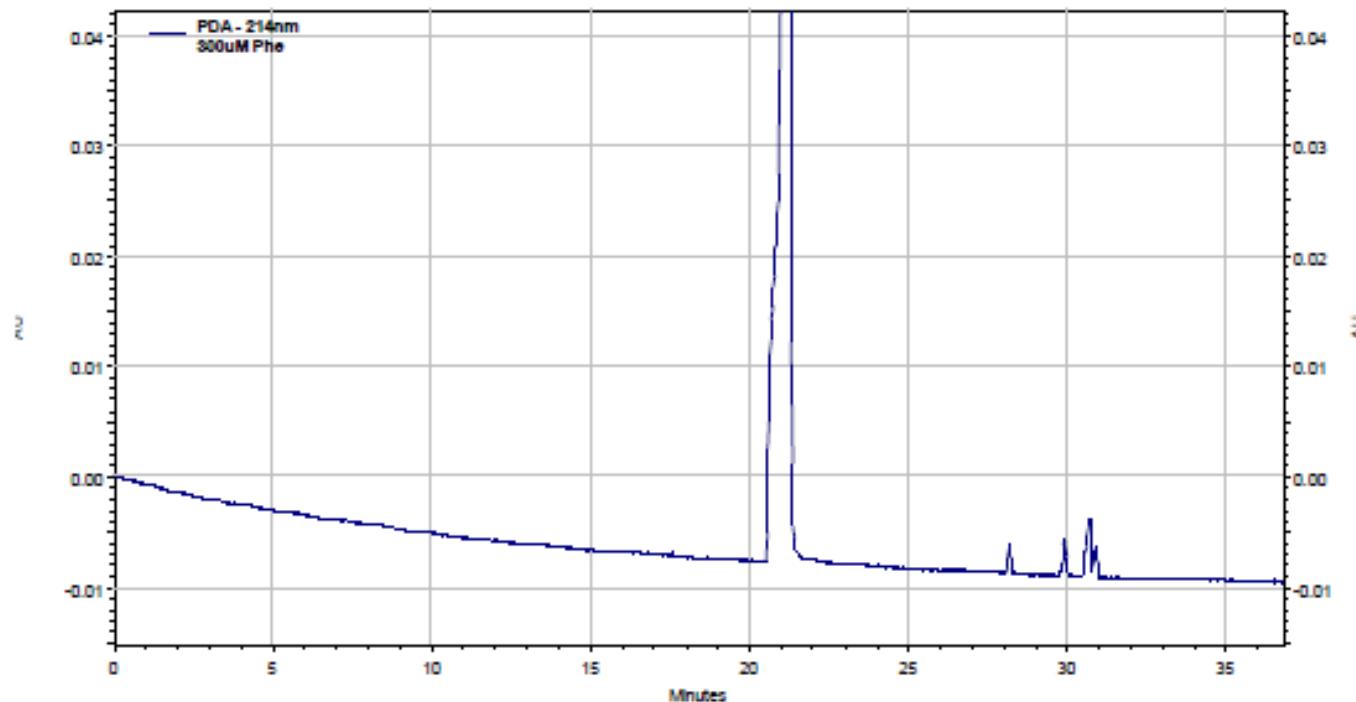
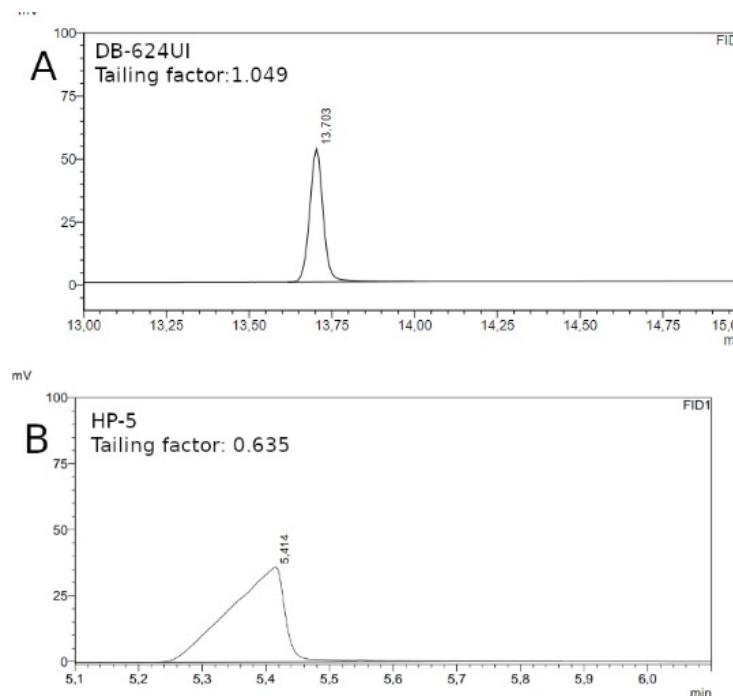
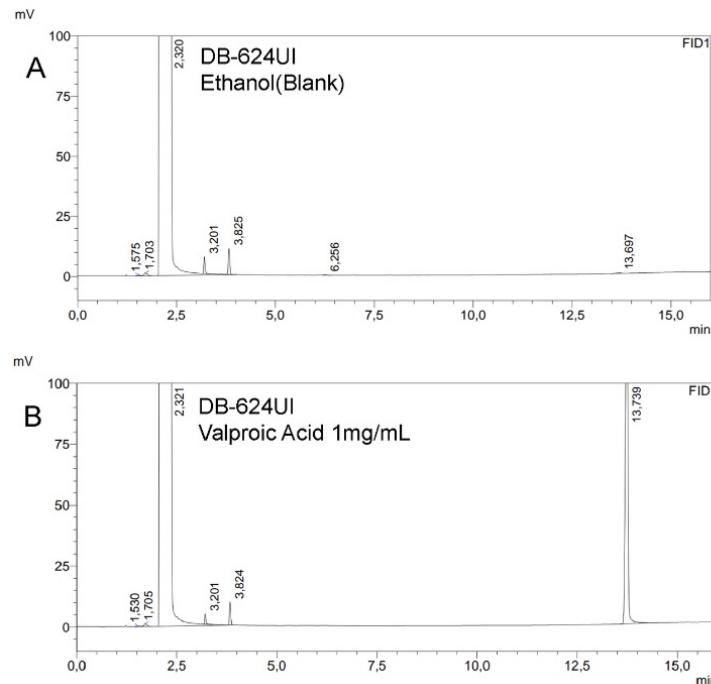


Figure 1: CE-UV electropherogram of phenylalanine (...ng/mL) at 214 nm.

Description: an example

The retention time (RT) of VPA with this method was determined to be 13.74 minutes (see figure 3B). In this chromatogram, a large peak was seen at around 13.74 minutes in the spiked sample that was not visible in the blank (see figure 3A). At 13.70 min there was a small peak visible in the blank sample, but this was not seen again after the further optimization of the GC protocol, and therefore did not interfere with the VPA signal. The tailing factor increased substantially for the DB-624UI column (see figure 4). Therefore, this column was used in all following experiments.

The retention time (RT) of VPA with this method was determined to be 13.74 minutes (see figure 3B). In this chromatogram, a large peak was seen at around 13.74 minutes in the spiked sample that was not visible in the blank (see figure 3A). At 13.70 min there was a small peak visible in the blank sample, but this was not seen again after the further optimization of the GC protocol, and therefore did not interfere with the VPA signal. The tailing factor increased substantially for the DB-624UI column (see figure 4). Therefore, this column was used in all following experiments.



The retention time (RT) of VPA with this method was determined to be 13.74 minutes (see figure 3B). In this chromatogram, a large peak was seen at around 13.74 minutes in the spiked sample that was not visible in the blank (see figure 3A). At 13.70 min there was a small peak visible in the blank sample, but this was not seen again after further optimization of the GC protocol, and therefore did not interfere with the VPA signal. The tailing factor increased substantially for the DB-624UI column (see figure 4). Therefore, this column was used in all following experiments.

Analysis of *a standard with 1 mg/mL VPA in ethanol* showed that the retention time of VPA was 13.74 minutes (Figure 3b). Analysis of *blank ethanol* showed a peak at 13.70 minutes with *an AUC that was xxx% of the AUC of the 1 mg/mL VPA standard* (Figure 3a). *[if it was not seen again, may be no need to mention it? Or explain what has been optimized?]*

The use of a *HP-5 column* resulted in an asymmetrical (*fronting*) peak *with a tailing factor of 0.635* (Figure 4). Changing to a DB-624UI column resulted in a symmetrical peak *with a tailing factor of 1.049* (Figure 4). Therefore, the DB-624UI column was selected.

Sketch the Figure (1)



In pairs:

- Exchange your **description** (*without table/figure*) with a student from another group
- Try to **recreate each other's result** based on this description.
- **Evaluate:**
 - Is all the relevant information depicted in the reconstructed image?
 - What information is missing in the description?
 - ★ Is any information missing in the caption?

What is *in* this Figure?(2)

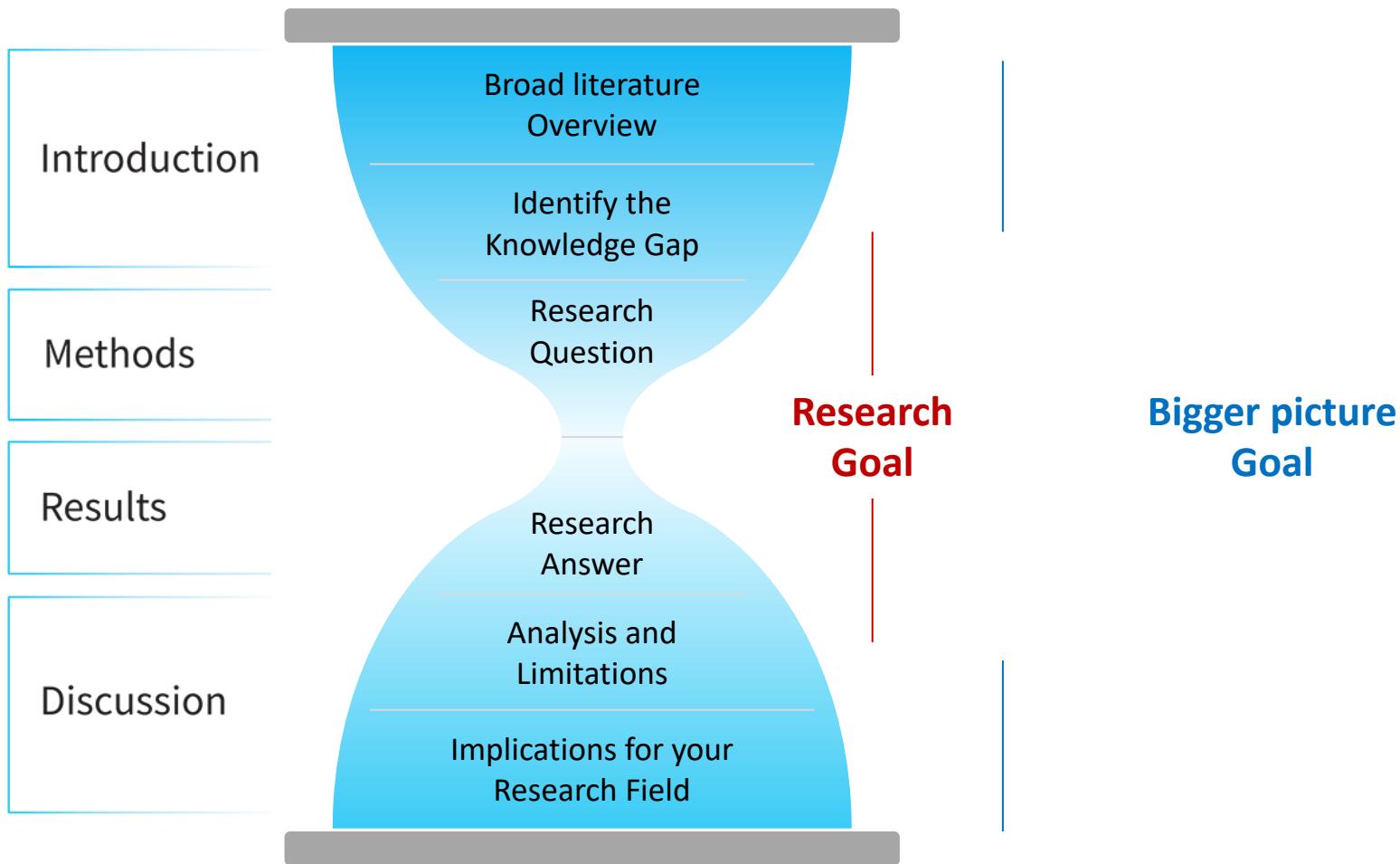
In pairs:

- Exchange your **table/figure** (without description – caption is ok!) with a (another) student from another group
- **Write down your conclusion** based on each other's table/figure.
- **Evaluate:**
 - Compare your conclusion with the written description
 - What are the differences in interpretation?
 - What information is needed in the caption to correctly interpret the result?
 - ★ Is any information missing in the description?

Break



Content: the hourglass structure



Introduction: A story that answers 5 questions



Tip 3 - Introduction: work on that funnel shape!

Bigger picture:

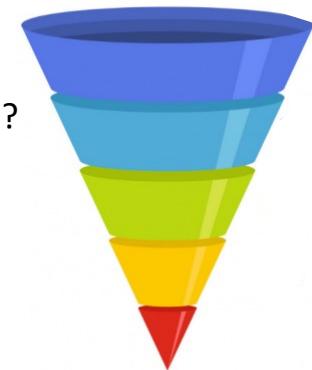
1. Why is your research important?

Literature survey:

2. What is known?
3. What is unknown?

Your research:

4. How does your research fill these discrepancies?
5. What are your most important findings?



Materials and Method: couples the reason to the proof



Tip 4 - Methods: provide a cookbook with the study's ingredients!

- This is where you make your story **believable**:
 - Formulate systematically *all* experiments
 - Someone should be able to repeat the experiments and get the same results
- **Not** a lab book!!! So:
 - No protocols / bullet-points / imperative
 - Be **concise**: leave-out standard labware
 - Use references (but not to your lab book)
- Keep it factual (yes, that means it's a bit *boring*...)
- Use **subsections**
- Check **examples**!

Methods: Function above Form

2. Experimental

2.1. Chemicals and reagents

Human neutrophil peptide-1, -2 and -3 as well as human alpha-defensin 5 were obtained from Peptanova (Sandhausen, Germany) in portions containing exactly 0.1 mg. Acetonitrile (gradient grade), methanol (HPLC grade) and LC-MS grade water were from Biosolve (Valkenswaard, The Netherlands), formic acid (p.a.) from Merck (Darmstadt, Germany) and trifluoroacetic acid (99.5%) from Acros Organics (Geel, Belgium). Blank human plasma and serum were obtained from the Sanquin Bloodbank (Utrecht, The Netherlands).

2.2. Instruments

The LC-MS/MS analyses were performed using an Accela high-speed chromatographic system coupled to a TSQ Quantum Ultra triple quadrupole mass spectrometer equipped with a heated electrospray ionization (H-ESI) probe (all from Thermo Fischer Scientific, San Jose, CA, USA). The analytical column, a Symmetry 300 C₁₈, 50 mm × 2.1 mm I.D., with 3.5 μm particle size (Waters Chromatography, Milford, MA, USA) was protected by a Polaris C₁₈-A guard column, 10 mm × 2.0 mm I.D. with 3 μm particle size (Varian Inc., Palo Alto, CA, USA).

2.3. LC-MS/MS conditions

Formic acid (0.25%, v/v) in water was used as eluent A and methanol as eluent B. The flow rate was set to 300 μl/min and column temperature to 30 °C, while autosampler temperature was maintained at 5 °C. During 3 min after injection eluent B was increased from 20 to 30%, followed by an increase to 90% in 1.8 min, both via a linear gradient. Eluent B was maintained at 90% for 0.7 min, followed by a rapid return to the initial conditions (25% B), which were kept during 1 min for re-equilibration. Total run time was 6.5 min.

- Describe settings / conditions / procedure for **THE** method = *the validated method (end-product)*
- List other (relevant) protocols under different subsections, e.g., method development
- List (relevant) conditions with only minor changes from **THE** method in Results and Discussion

Table 1
MS/MS-parameters for all analytes

Compound	Tube lens off set	Parent ion (<i>m/z</i>)	Product ion (<i>m/z</i>)
Enfuvirtide/M-20	124	1124.0	1343.5
Tifuvirtide	102	1008.4	1219.0
d60-Enfuvirtide	131	1139.0	1363.5
d50-Tifuvirtide	113	1018.4	1231.5

Results: The heart of your report!

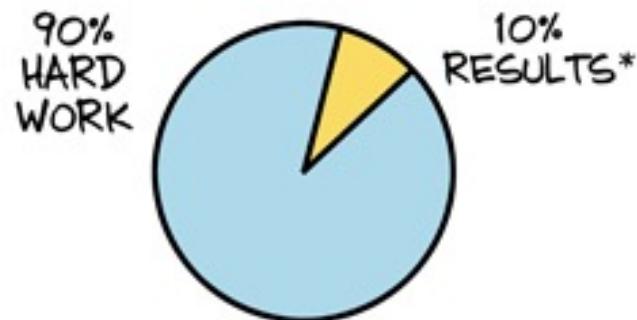


Tip 5 - Results: present findings without interpretation!

- The results section is a **STORY** as well:
 - Present tables/figures **NOT** in the order as performed: focus on making a point!
 - Each figure/table builds on the previous one
- The text should **guide the reader**:
 - It should **NOT** simply repeat the information in the tables and figures
 - **NOT** a series of tables with no explanation
 - Refer to tables and figure in the text
 - Use clear section headings
- Be **selective!** **Never present** the (same) data in more than one way!

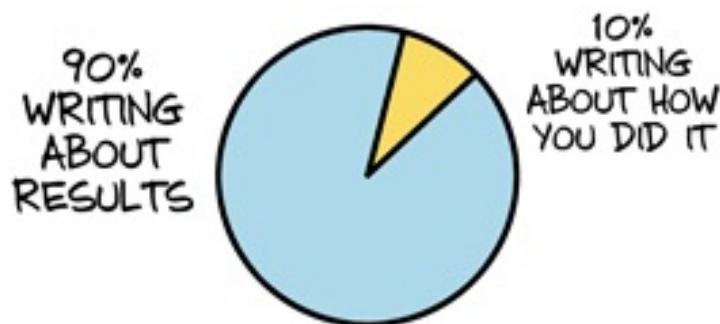
Results: The heart of your report

DOING RESEARCH:



* BEST CASE SCENARIO

WRITING ABOUT RESEARCH:



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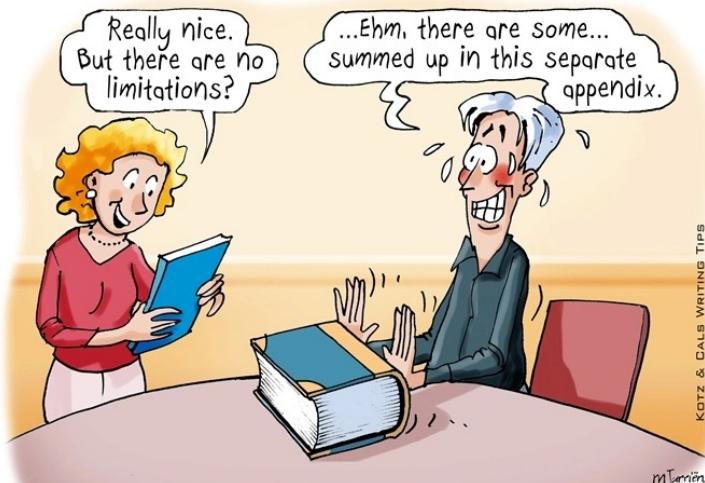
Tables and Figures: a picture is worth a thousand words



Tip 7 - Tables and figures: make them self-explanatory!

- Consider your **storyline**: Each figure/table builds on the previous one
- Use tables when **exact numbers** are important or too much / complex to describe.
- the reader should be able to understand the figure/ table without reading the text!
- Provide a **descriptive title**
- Avoid redundancy
- **Refer** to tables and figure in the text

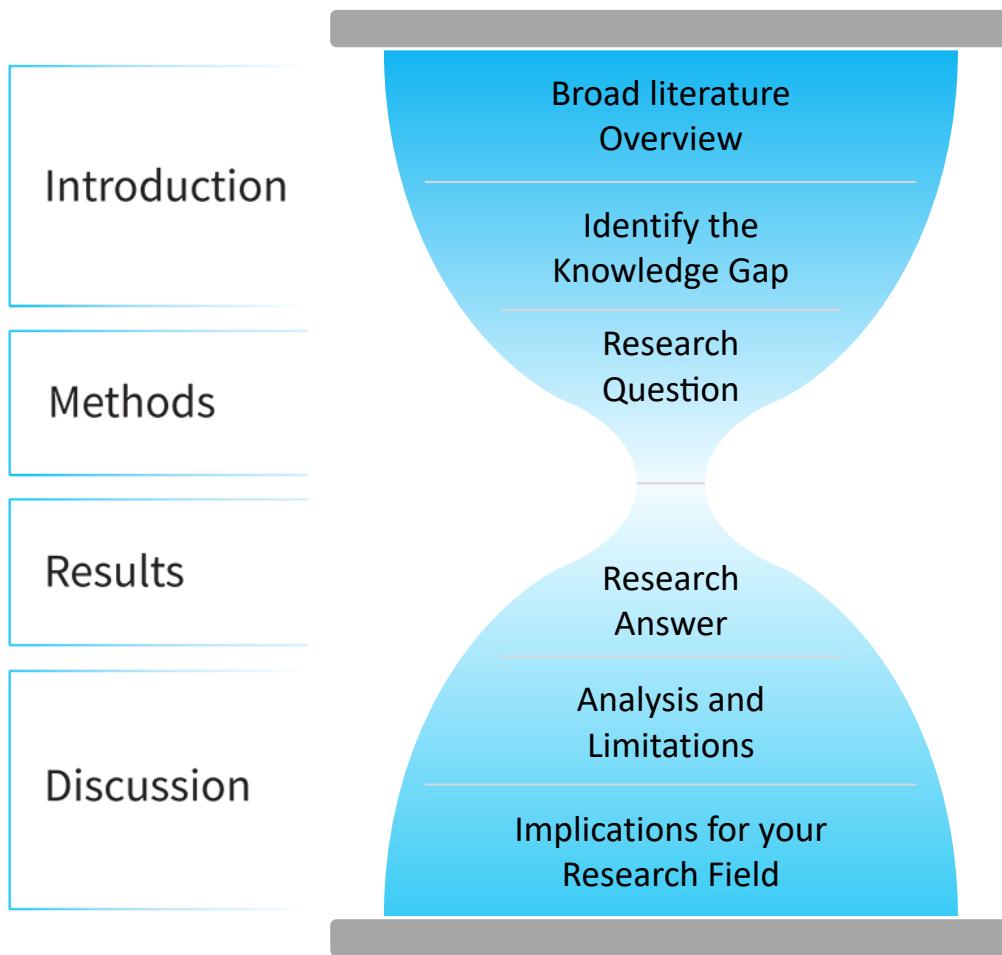
Discussion: The interpretation and significance of your results



Tip 6 - Discussion: be frank in acknowledging limitations!

- Do the results **match your expectations?**
- Try to **explain unexpected results**
- Place individual results in a **wider context** (combine results)
- Discuss possibilities for **improvement** and suggest possible **follow-up experiments**
- Be **critical!**

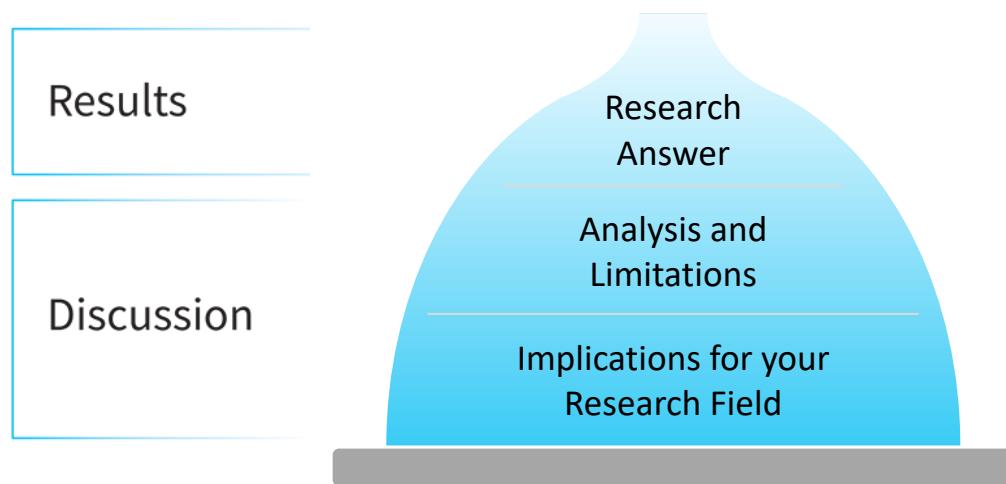
Discussion: Inverted funnel-shape



Structure: Opposite to the Introduction!



Discussion: Be critical!



Explain the **similarities, differences and inconsistencies**:

- between the different experiments? **Your opinion**
- compared to groups that performed the same experiments? **Peer opinion**
- compared to peer-reviewed literature on the same topic? **Expert opinion**

An unexpected result

Get help from your peers!

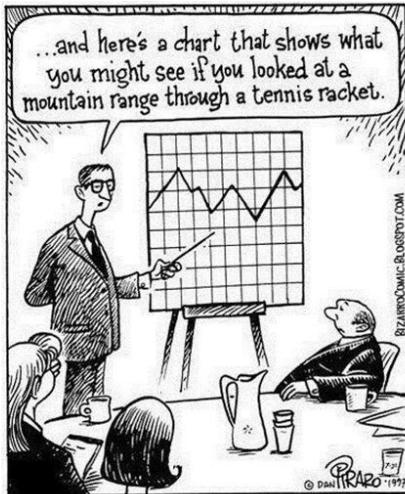
Group: Present a result that is difficult to interpret in 3 rounds:

- 1. Own opinion:** Discuss with your group what this result means
How do you interpret these data? What are the limitations and implications? What did you expect before the experiment was performed, are the results similar or different?

- 2. Peer opinion:** Present your result. Only explain the problem, do not share your own opinion yet. What questions and suggestions do you have for each other?

- 3. Expert opinion:** Answer the following question: What additional information is needed to verify your results. Where/how can you find or obtain that information?

Discussion vs. Conclusion



Discussion = The **interpretation** of your results

Explain the similarities, differences and inconsistencies with results (**Results and Discussion**):

- More coherent
- Easier for you and for the reader
- Avoid that discussion is written as a summary of the results

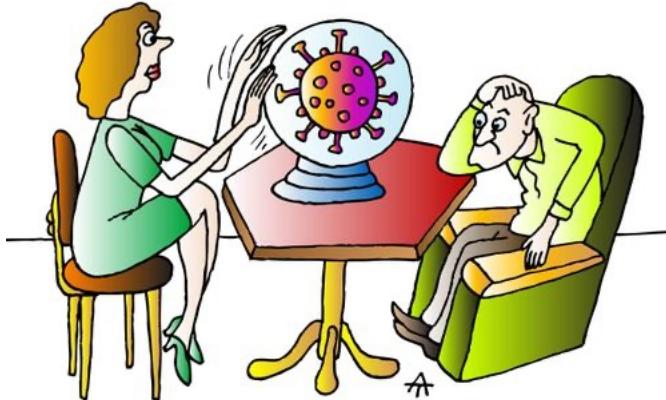


Conclusion = The **significance** of your results
(**general discussion**)

NOT a summary of results and discussion, instead:

- Generalise: most plausible **explanation**
- Consider the bigger picture: (global) **implications**
- Think about the next steps: **recommendations**

Recommendations: Think about next year's students



Covid future | Alexei Talimonov, www.cartoonstock.com

- What will be the logical next steps in this research?
- How would you plan and perform these next steps?
- What are the shortcomings in your own research and how would you solve these?
- What data do you need to complete the entire story?
- What does the next person in the line of this project need to do?

Summary / abstract: first impressions matter



Tip 2 - Title and abstract: sell your paper!

- **Attract the attention** of the reader in a haystack of other papers
- Be short, compact and **concise**
- Use IMRD structure:
 1. *What* have you been doing (or *why* did you do this) (**goal**)
 2. What did you actually *do* (**method**)
 3. What did you *find* (**results**)
 4. What does this *mean* (**discussion/conclusion**)

Summary - example

GOAL

Fludarabine and cyclophosphamide are anticancer agents mainly used in the treatment of hematologic malignancies. We have developed and validated an assay using high-performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrometry for the quantification of fludarabine in combination with cyclophosphamide in human heparin and human EDTA plasma. Sample pre-treatment consisted of a protein precipitation with cold acetonitrile (-20°C) using 250 μL of plasma. Separation was performed on an Extend C18 column (150 \times 2.1 mm i.d.; 5 μm) with a stepwise gradient using 1 mM ammonia solution and acetonitrile at a flow rate of 400 $\mu\text{L}/\text{min}$. The analytical run time was 12 min. The triple quadrupole mass spectrometer was operated in the positive ion mode and multiple reaction monitoring was used for drug quantification. The method was validated over a concentration range of 1 to 100 ng/mL for fludarabine and cyclophosphamide in human heparin and human EDTA plasma. The coefficients of variation were <13.9% for inter- and intra-day precisions. Mean accuracies were also within the designated limits ($\pm 15\%$). The analytes were stable in plasma, processed extracts and in stock solution under all relevant conditions. Copyright © 2005 John Wiley & Sons, Ltd.

Summary - example

METHOD

Fludarabine and cyclophosphamide are anticancer agents mainly used in the treatment of hematologic malignancies. We have developed and validated an assay using high-performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrometry for the quantification of fludarabine in combination with cyclophosphamide in human heparin and human EDTA plasma. Sample pre-treatment consisted of a protein precipitation with cold acetonitrile (-20°C) using 250 μL of plasma. Separation was performed on an Extend C18 column (150 \times 2.1 mm i.d.; 5 μm) with a stepwise gradient using 1 mM ammonia solution and acetonitrile at a flow rate of 400 $\mu\text{L}/\text{min}$. The analytical run time was 12 min. The triple quadrupole mass spectrometer was operated in the positive ion mode and multiple reaction monitoring was used for drug quantification. The method was validated over a concentration range of 1 to 100 ng/mL for fludarabine and cyclophosphamide in human heparin and human EDTA plasma. The coefficients of variation were <13.9% for inter- and intra-day precisions. Mean accuracies were also within the designated limits ($\pm 15\%$). The analytes were stable in plasma, processed extracts and in stock solution under all relevant conditions. Copyright © 2005 John Wiley & Sons, Ltd.

Summary - example

RESULTS

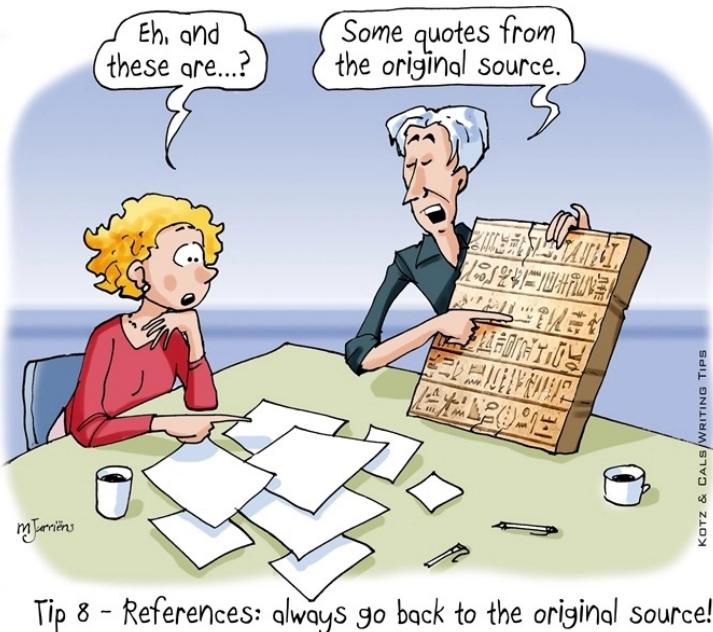
Fludarabine and cyclophosphamide are anticancer agents mainly used in the treatment of hematologic malignancies. We have developed and validated an assay using high-performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrometry for the quantification of fludarabine in combination with cyclophosphamide in human heparin and human EDTA plasma. Sample pre-treatment consisted of a protein precipitation with cold acetonitrile (-20°C) using 250 μL of plasma. Separation was performed on an Extend C18 column (150 \times 2.1 mm i.d.; 5 μm) with a stepwise gradient using 1 mM ammonia solution and acetonitrile at a flow rate of 400 $\mu\text{L}/\text{min}$. The analytical run time was 12 min. The triple quadrupole mass spectrometer was operated in the positive ion mode and multiple reaction monitoring was used for drug quantification. The method was validated over a concentration range of 1 to 100 ng/mL for fludarabine and cyclophosphamide in human heparin and human EDTA plasma. The coefficients of variation were <13.9% for inter- and intra-day precisions. Mean accuracies were also within the designated limits ($\pm 15\%$). The analytes were stable in plasma, processed extracts and in stock solution under all relevant conditions. Copyright © 2005 John Wiley & Sons, Ltd.

Summary - example

CONCLUSION

Fludarabine and cyclophosphamide are anticancer agents mainly used in the treatment of hematologic malignancies. We have developed and validated an assay using high-performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrometry for the quantification of fludarabine in combination with cyclophosphamide in human heparin and human EDTA plasma. Sample pre-treatment consisted of a protein precipitation with cold acetonitrile (-20°C) using $250\text{ }\mu\text{L}$ of plasma. Separation was performed on an Extend C18 column ($150 \times 2.1\text{ mm i.d.}; 5\text{ }\mu\text{m}$) with a stepwise gradient using 1 mM ammonia solution and acetonitrile at a flow rate of $400\text{ }\mu\text{L/min}$. The analytical run time was 12 min. The triple quadrupole mass spectrometer was operated in the positive ion mode and multiple reaction monitoring was used for drug quantification. The method was validated over a concentration range of 1 to 100 ng/mL for fludarabine and cyclophosphamide in human heparin and human EDTA plasma. The coefficients of variation were $<13.9\%$ for inter- and intra-day precisions. Mean accuracies were also within the designated limits ($\pm 15\%$). The analytes were stable in plasma, processed extracts and in stock solution under all relevant conditions. Copyright © 2005 John Wiley & Sons, Ltd.

References



Tip 8 - References: always go back to the original source!

- Use references as used in literature
- Number references and use numbers to refer
- Only list references you actually refer to in the text
- Use references in your discussion too

See: <https://libguides.library.uu.nl/citing>

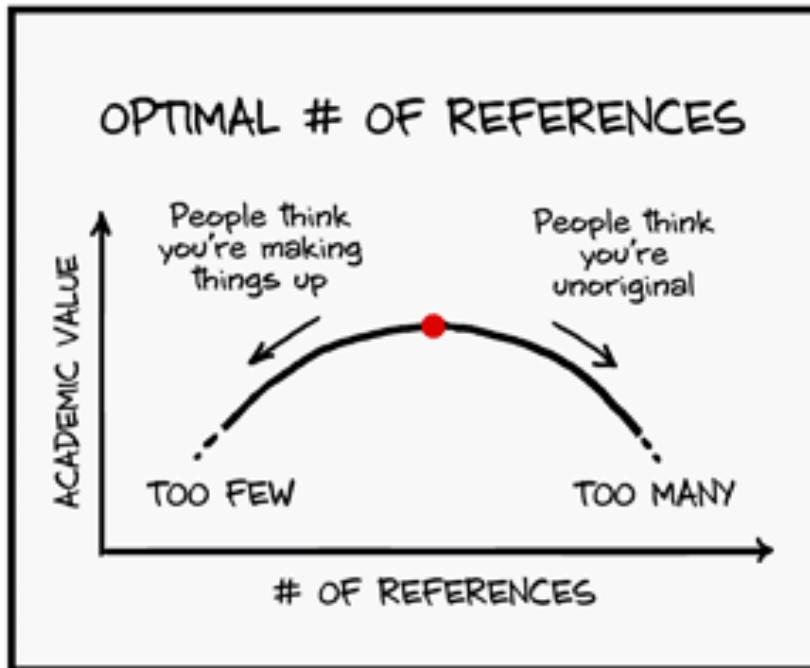
Appendices



Tip 6 - Discussion: be frank in acknowledging limitations!

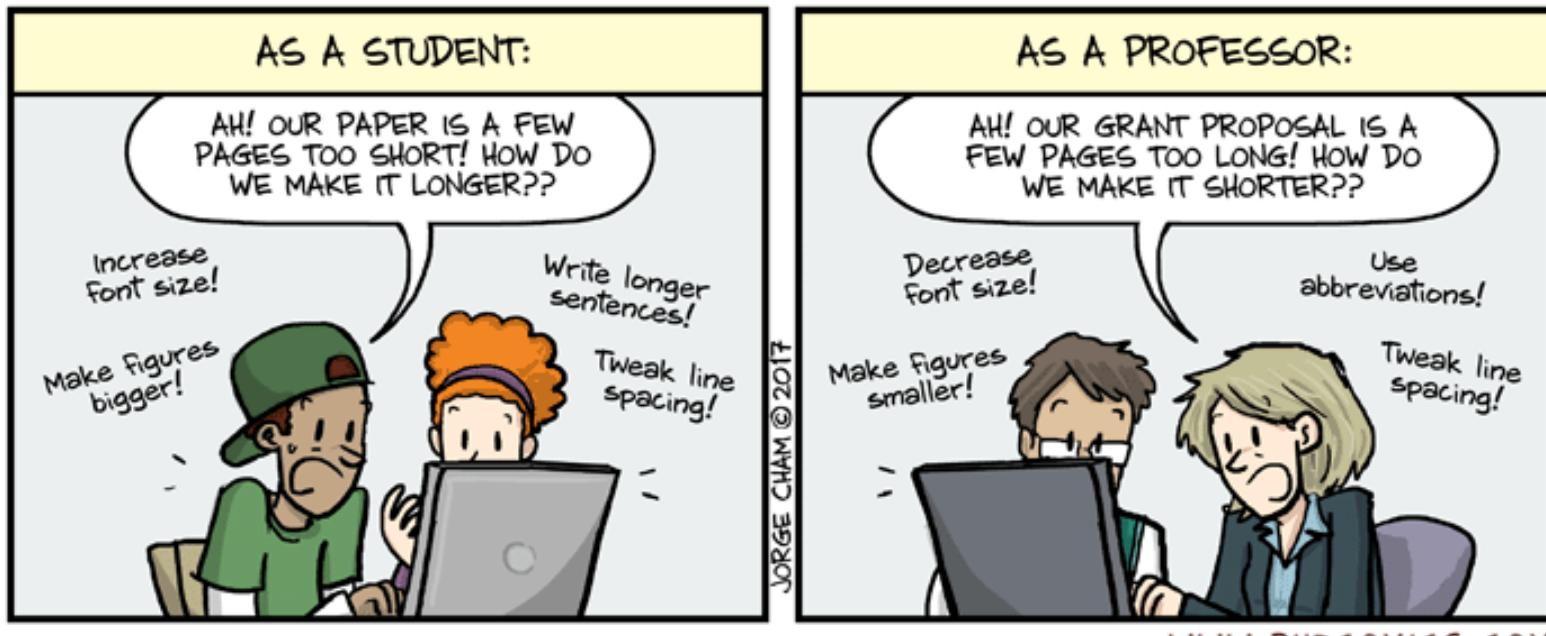
- Only add appendices when relevant
- Only add appendices you refer to in the text
- **Number** appendices
- Appendices should also come with a **title** and **legend**

How many references?



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How many pages?



Don't rely solely on your spell checker!



"Why can't somebody make a spell-checker that knows how to check spells?!"

Ode to my spell checker (author unknown):

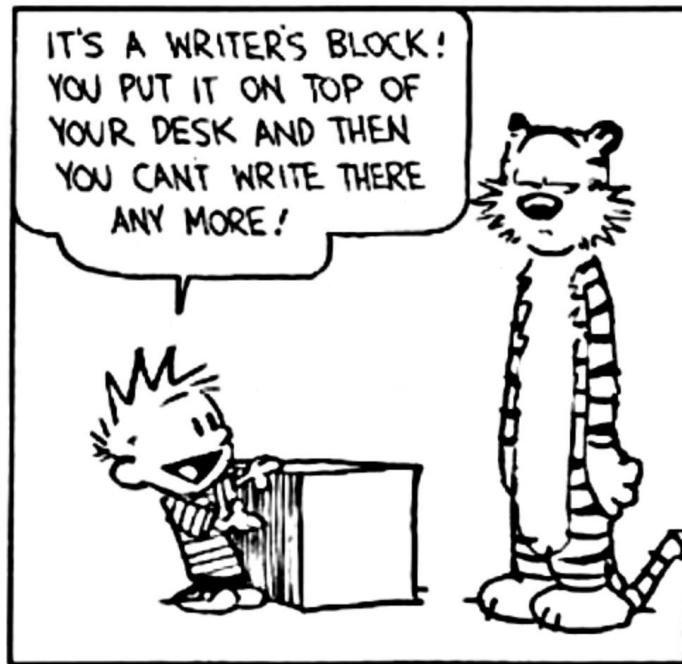
Eye halve a spelling checker
It came with my pea sea.
It plainly marks four my revue
miss steaks eye kin knot sea.

Eye strike a quay and type a word
and weight for it to say
Weather eye yam wrong oar write.
It shows me strait a weigh

As soon as a mist ache is maid.
It nose bee fore two long
and eye can put the error rite.
Its rare lea ever wrong.

Eye have run this poem threw it,
I am shore your pleased to no.
Its letter perfect awl the way.
My checker told me sew

Don't worry about a writer's block

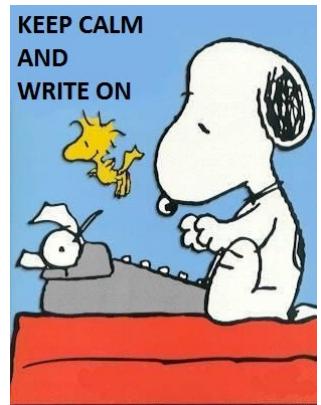


Just get started! ☺



Despite what most students think this is NOT
how you write papers

Resources



Good luck!



Feedback session 2

<https://forms.gle/EdJt9F67WLyDWVGd9>

- What did you expect from the report writing seminar(s)?
- To what extend was your expectation met in session 2?
- List at least 3 things from session 2 that were helpful for you
- What could be improved in the **structure** (exercises, interaction, time management) of session 2?
- What could be improved in the **content** (topics) of session 2?
- What topics did you miss in session 1 and 2?
- Any additional comments or feedback are welcome!

