Composition profile and purity of analgesics

Project work

NMR spectroscopy and analytical chemistry

- guidelines and focus points to be elaborated -

Analytical Chemistry II

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The aims of this practical are:
1) To teach students the skills for developing a determination method. The different tasks to be executed will emphasize their associative thinking - based on literature knowledge and their chemical studies. They will learn how to consider the advantages and disadvantages of an instrumental/laboratory technique, also the multipurpose usage of a methodology. Last, how to put the results into context, and how to formulate an outlook, or further work.
2) To achieve these goals, the task is to develop a method for the characterization and quantitative determination of commercially available analgesics tablets, focusing on metamizole (MET) and caffeine (CAF) components. The main, non-invasive technique to be used is NMR spectroscopy. Several aspects will be covered: structure elucidation; quantitative determination (qNMR); characterization of MET decomposition to the active metabolite methylaminoantiyrine (MAA) under physiological and digestive pH model conditions, as well as determination of kinetic constants. Besides NMR spectroscopy, iodometric titration is also used for the determination of MET. The laboratory practice leans on the organic chemistry, analytical, physico-chemical chemistry, as well as NMR spectroscopy knowledge from previous studies. In the light of all these, the following topics will be covered, and discussed:

I. A thorough literature search has to be conducted reporting about existent determination procedures for MET and CAF.

Student work: Literature search from PubMed, collection of papers and patents.

Result: Presentation and discussion of alternative techniques and procedures. Pro and con arguments for the applied NMR determination.

II. NMR spectroscopy measurements: the basics of 1D, and 2D homo- and heteronuclear measurements: ^{1}H, $^{13}C\{^{1}H\}$, ^{1}H-^{1}H COSY, TOCSY, NOESY; ^{1}H-^{13}C HSQC and HMBC. Peak assignment strategies.

Student work: Preparation of neat MET and CAF NMR samples with concentrations corresponding to values that will be obtained from the dissolution of the tablet. Discussion regarding the choice of the solvent, solubility issues. Acquisition of spectra with supervision at 500MHz and 400MHz instruments. Evaluation of spectra.

Result: Hands-on knowledge in work with Bruker NMR instruments, deepening knowledge how spectra acquisition can be performed also automatized – mimicking industrial environment. Spectral evaluation using the TopSpin software. Peak assignment for each ^{1}H and ^{13}C environment of MET and CAF.

III. qNMR determination of MET and CAF from drug tablet using 1D ^{1}H measurements. Two methods need to be tested: (i) external calibration using known amounts of the neat compounds, and (ii) internal calibration using reference standard. Requirements of a qNMR spectra.

Student work: Sample preparation skills, dilution series, contemplating on the choice of a proper internal reference standard. For real quantification, to use a proper delay time, the T_1 longitudinal relaxation time values have to be determined.

Result: Quantification of the amounts of MET and CAF in the tablet. Additional information: solubility studies, and the effect of dissolution time. Discussion of the accuracy and precision.

IV. Monitoring the MET decomposition via 1D ^1H measurements at different pH values. Measurements performed at physiological pH and under acidic conditions, that mimic the environment in the stomach.

Student work: In-situ measurements at given time intervals. Integration of chosen peaks, graphical representation of the time dependency of these values. Evaluation of the kinetics, determination of the pseudo-first order kinetic constant for the decomposition.

Result: Reaction kinetics, explanation of the decomposition, correlation to the biological function.

IV. Determination of the MET content based on European Pharmacopoeia (Ph. Eur.) directives via iodometric titration.

Student work: Preparation of stock solutions, determining their exact concentration. Performing the titration with standard MET. Analyzing the effect of CAF on the result. Determining the MET content of the tablet.

Result: Hands-on skills for classical laboratory test planning, executing, and evaluating.

Requirements

All the above enumerated topics must be performed. The development of the work will be presented 3 times during the term in form of 15-20 min talks. The first two presentations are progress reports, while the final presentation must give a full overview of the practical, focusing on the main results and the outlook.

Non-steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs are a family of drugs of "over the counter" that prevent blood clots, decrease fever, reduce pain, and consumed in large doses, they decrease fever. NSAIDs are on the first rung of the analgesic ladder (Janczura, 2021). As painkillers, they are indicated, among others, in post-traumatic and muscular pains, pains after surgery or tooth extraction, neuralgia, root syndromes, discopathy, renal and hepatic colic, cancer pain, migraine, and menstrual pain (Morelli, 2018). NSAIDs that are widely known include naproxen, ibuprofen, and aspirin. Salicylic acid, pyrazolone, and phenacetin (or acetophenetidin) form a group of three compounds from which most anti-inflammatory analgesics are derived.

Metamizole-Na (MET)

The pyrazolone derivative metamizole is chemically known as sodium [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) (methyl)amino] methanesulfonate monohydrate and belongs to the family of NSAIDs. It is mainly used for severe pain or fever. It was introduced into pharmacotherapy in 1922 and it is still used in several countries, due to its strong analgesic effect and relatively low cost (Weinert 2007). Metamizole-Na is taken orally, and it is rapidly and nearly completely absorbed, approaching 100% bioavailability (Vlahov, 1990). In the gastrointestinal system, it is non-enzymatically hydrolyzed to 4-methylaminoantipyrine (MAA), which is rapidly and nearly completely absorbed, reaching peak levels in 1 or 2 hours. The decomposition reaction of metamizole (Velagaleti, 2003) is depicted in Figure 1:

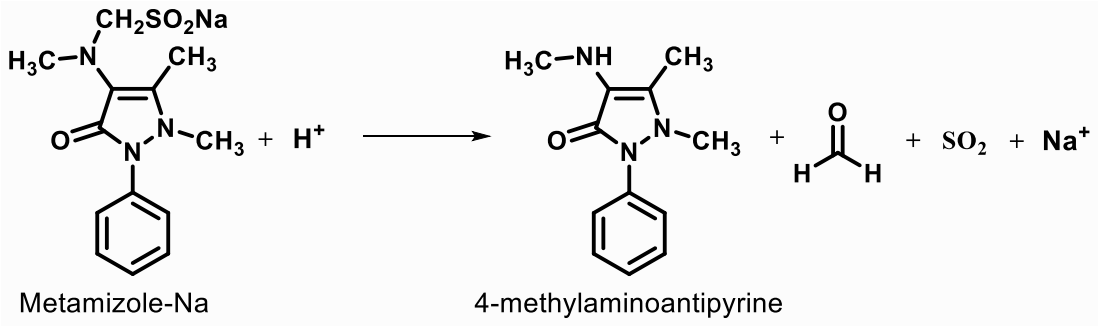


Figure 1. Decomposition reaction of MET to MAA

Combination of analgesics

Combination of pain-relieving drugs is advantageous, as it minimizes the potential drug-related side effects and low doses of individual drugs can be used. Combinations, on the other hand, boost therapeutic effect since the active ingredients function synergistically (Raffa 2012; Todd 2017). A combination of analgesics must contain ingredients which target different pain receptors, mediators and pain transmission pathways peripherally and centrally (Msolli 2021). A classic example is the combination of a NSAID with paracetamol. The two drugs exert their analgesic effect through different mechanisms of action; mainly in the periphery for NSAID and at the central nociception pathway for the paracetamol (Miranda 2006). They are

theoretically complementary, and their association represents what is commonly called multimodal analgesia. Caffeine has been reported as adjuvant drug when combined to analgesics and anti-inflammatory drugs to control tension pain and migraine (Straube 2011, Derry 2014). Caffeine is a naturally occurring molecule present in many plants' seeds, leaves, and fruit, where it is thought to act as a natural insecticide. It has a long history of being consumed by humans in the form of liquids like tea and coffee, as well as foods like chocolate. Coffee (100 to 150 mg/mug), tea (75 mg/mug), cola drinks (up to 40 mg/drink), energy drinks (about 80 mg/drink), plain chocolate (up to 50 mg/bar), and caffeine tablets (100 mg/tablet) are all common sources of caffeine today. Some "high-energy" drinks contain as much caffeine as five or six cups of coffee. Caffeine is a methylxanthine that acts as a stimulant to the central nervous system. It is a psychostimulant with a wide range of physiological effects in humans, including enhanced wakefulness, alertness, endurance, heart rate, and blood pressure (Vlahov 1990). Paracetamol, ibuprofen, aspirin, and metamizole-Na are examples of analgesics that exist in combination with caffeine. The chemical structure of CAF is shown in Figure 2.

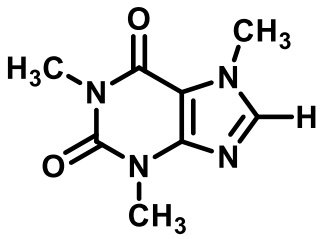


Figure 2. Chemical structure of caffeine

Because of their simplicity of administration and improved stability, solid state pharmaceuticals are the most recommended formulations for oral use. Furthermore, tablets have the advantage of preserving the drug's chemical integrity for a longer period and allowing the correct administration of the specified dose. These medications, however, may have bioavailability issues. Bioavailability refers to the speed and extent to which a drug or its therapeutic group is absorbed from a pharmaceutical form and becomes available at the site of action. The importance of quality control studies increases for drugs exempt from medical prescription (or “over-the-counter drugs”), in solid pharmaceutical forms containing aspirin, acetaminophen, metamizole or ibuprofen; in such cases, interchangeability is determined only by comparing the dissolution profiles and in vitro studies are the only ones that grant bioequivalence of these drugs. Deviations from a drug's recommended quality can have major effects for patients and even the public health system. Because of the low quality and wide variance in the concentration of primaquine in different medications, there have been reports of resistant Plasmodium vivax strains. Despite the present strict legislation governing good manufacturing practice (GMP) and quality control, it is still possible to find over-the-counter medications that fail to meet quality standards (OTC). Therefore, introduction of methods aiming the quantitative determination of the components, especially if they are non-invasive and can characterize several components from one sample are of particular interest.

Pharmaceutical analysis

Chemistry, pharmacology, and biochemistry play big role in the discovery of new drugs. Identification of a drug molecule that has therapeutic value to battle, control, check, or cure diseases is the first step in the process of drug development. The synthesis and characterization of such molecules which are also called active pharmaceutical ingredients (APIs) and their analysis to create preliminary safety and therapeutic efficacy data are prerequisites to identification of drug candidates for further detailed investigations (Velagaleti 2003).
Analytical techniques play a great role in understanding the physical and chemical stability of the drug impact on the selection and design of the dosage form, quantitation of the impurities and identification of those impurities which are above the set limits, to evaluate the toxicity profiles of these impurities to distinguish these from that of the API, when applicable and assessing the content of drug in the marketed products.

NMR spectroscopy

The execution of quantitative experiments using NMR spectroscopy is quite simple. However, in order to achieve accurate and reproducible results using integration-based determination, certain key factors relating to both acquisition parameter settings and post-acquisition processing must be considered and carefully adjusted. The basic NMR measurements have to be recapitulated (Berger 2004) and measurements will be performed together with an instructor. The necessary measurements include:
^{1}H: *zg*, water suppression with excitation sculting *zgesgp*; T_1 relaxation time determination
^{1}H-^{1}H 2D correlations: COSY, NOESY, ROESY (different types of pulse sequences)
^{13}C{^{1}H}: *zgpg, zgig*
^{1}H-^{13}C 2D correlations: HSQC, HMBC (different types of pulse sequences).

Many papers and NMR textbooks present in detail the critical parameters that must be considered for qNMR experiments. In addition to optimal shimming, tuning, matching, and setting of processing parameters, a couple of additional requirements must be met before a precise quantification can be performed. The signal used for quantification should be clearly separated from all other signals and assigned to an atom of the substance to be quantified (Holzgrabe 2010). A singlet is more appropriate than a multiplet, as it is simple and easy to integrate. Precise integration depends on the application of phase-, baseline- and drift-corrections in order to produce a suitable line shape – all these factors need to taken into consideration, and tested while evaluating the NMR spectrum (Görög 2000). The corrections can be done automatically or manually. Especially in the latter case, the integration will differ from one operator to the next, which cannot be avoided because software routines cannot solve all problems. Furthermore, the integration limits should be set to 64 times the full width at half signal height (fwhh) to ensure that >99 percent of the total signal intensity (the signal is a Lorentzian line) is obtained. However, due to adjacent signals, this may be difficult, and a

compromise must be reached. 32 times or 16 times the full width at half signal height especially for singlets can be enough.

To determine absolute amounts of major component(s) or related substances, a reference material of definite purity unrelated to the target analyte must be added to the analyte solution and measured simultaneously; this procedure is known as the internal standard method.

In this case, the ratio of peak areas (*A*) of the analyte and the reference substance (*cal*) can be used directly to calculate contents while taking into account the molecular weights (*M*), weights (*m*), number of nuclei representing the integrated resonances (*N*) of the analyte and the standard, and standard purity (*P*), respectively.

The internal standard method's uncertainty is determined by the uncertainties in the weights of standard and sample, the purity of the reference material and the uncertainties in the signal areas, which are significantly affected by the operator precision of phasing, baseline correction, and integration. To reduce the uncertainty of sample preparation, greater masses of analyte and internal standard should be weighed, or stock solutions should be used, even if an additional preparation/dilution step is required (Al-Deen 2004).

Many publications have stated that a suitable internal standard should be readily available in a highly pure form, inexpensive, stable and chemically inert, nonvolatile and non-hygroscopic, soluble in most of the NMR solvents, easily weighable and optimal molecular weight (Beyer 2010a). To avoid distortion of integrated signal intensity due to relaxation effects, the next pulse must be applied five times the longest T_1 relaxation time (90° pulse) to ensure complete relaxation of the excited nuclei (99.3 percent of the equilibrium magnetization is measured after $5T_1$). However, it has been proposed elsewhere that internal standards with a short T_1 relaxation time and several signals in the NMR spectrum, such as ethyl-4-(dimethylamino) benzoate, thymol, and nicotine amide, are suitable for routine work (Beyer 2010b).

The most frequently applied NMR commands:

To check the parameters:
ased
eda
sw – spectral window
TD – time domain
aq – acquisition time
d1 – delay between scans
NS – number of scans (transients/repetitions)
p1 – hard pulse (typical value for 90º is about 8-10 µs, depending on the probehead)
plW1 – the power level in Watt corresponding to the p1 hard pulse
pldB – the power level in dB corresponding to the p1 hard pulse
For acquisition:
zg – start the experiment

For processing and evaluation:
SI – size
em – exponential multiplication
ft – Fourier transformation
pk – phase correction
efp – *em, ft and pk* in one
apk – automatic phase correction
bas – baseline correction
pp – peak picking
dcon – deconvolution
int - integration
xfb – spectral processing in 2D

Materials and Instrumentation

The reference standards, MET (99-101%) and CAF (98.5%) purity as per the European pharmacopeia) are purchased from the local pharmacy. Deuterated water (D_2O) of 99.9v/v% purity, sodium trimethylsilylpropanesulfonate (DSS, 99.95% purity) and 10X phosphate-buffered saline (PBS) are available in the analytical chemistry laboratory. DSS (internal calibrant) and PBS solutions with the desired concentrations need to be prepared. Tablets containing a mixture of 400 mg MET and 60 mg CAF as active pharmaceutical ingredients (APIs) are bought from the local pharmacy. Precision weighing scale (0.00001g) is used for weighing of the drug samples. Spectra are recorded on 500 MHz Bruker Avance III NMR spectrometer, and on 400 MHz Bruker Avance Neo NMR spectrometers. Processing and evaluation of the spectra is done in TopSpin 3.6 and TopSpin 4.0 softwares.

Preparation of standard and test solutions

Solutions needed for the determination:

Two sets of 0.01M PBS stock solutions with different pH. Measure 1 ml 0.1 M PBS into a 10 ml volumetric flask and top to the mark with double distilled water. The pH has to be adjusted to 7.00±0.02 and 2.00±0.02 with 1M NaOH and 1 M HCl.

DSS stock solution of pH 7.00 is prepared by accurately weighing 2.93 mg of DSS in a 25 ml Eppendorf. 18 ml of 0.01 M PBS and 2 ml of D_2O are added. The solution is vortexed till complete dissolution.

Preparation of Caffeine and Metamizole-Na standards

0.9 mg of caffeine powder is accurately weighed in a 1.5 ml Eppendorf. 1 ml stock solution of DSS is added using a micro pipette. The solution is thoroughly mixed using a vortex mixer till complete dissolution. These samples are prepared in triplicates. Note: no need for this exact

number given in the handout but calculate exactly the concentrations based on accurate weighing values. 500 ul of each replicate is transferred to a 5 mm NMR tube for ^{1}H NMR measurements. 1.39 mg of metamizole-Na monohydrate powder is accurately weighed in a 1.5 ml Eppendorf. 1 ml of the DSS stock solution is added using a calibrated micro pipette. The solution is thoroughly mixed using a vortex mixer till complete dissolution. The samples are also prepared in triplicates. 500ul of each replicate was used for ^{1}H NMR measurements. 1 mM of CAF and 2mM MET with 10% v/v D_2O and 90% v/v H_2O each are also needed.

Preparation of the tablet sample

Three tablets each containing a mixture of 60 mg CAF and 400 mg MET as per the manufacturer are weighed separately. Each tablet is crushed, thoroughly ground into a fine powder in a mortar with pestle. A portion of about 1.65 mg is weighed in 1.5 ml Eppendorf. 1ml of DSS stock solution is added to each portion. The solution is thoroughly mixed using a vortex mixer till complete dissolution. It is then centrifuged and 500 µl of the supernatant is transferred to NMR tube for measurements.

For testing the effect of dissolution time and pH on the decomposition of MET, a pure MET powder of approximately 1.78 mg is weighed and dissolved in 1 ml DSS stock solution of pH 7.02. The solution is vortexed and 500 µl of the supernatant is immediately transferred to NMR tube for measurements. One has to be quick as the first spectrum is needed to be acquired shortly after sample preparation. Thereafter, a series of spectra with varying time intervals are also acquired. Similar procedure is followed with approximately equivalent amount of pure MET sample in 1 ml DSS stock solution of pH 2.02.

Acquisition and processing of the NMR spectra

The quantitative ^{1}H-NMR spectra are recorded at 298K, with correspondingly optimizing the acquisition parameters. A proper delay time based on relaxation time measurements has to be chosen to ensure complete T_1 relaxation of protons for accurate quantification.

14 ppm spectral width for sufficient “empty” space on either side of the spectrum has to be set, in order to ensure that signals of interest are not affected by attenuation due to receiver filters.

All chemical shifts are reported in parts per million (ppm) relative to the DSS main signal at 0 ppm. ^{1}H-NMR spectra are manually corrected for phase and baseline distortions using TopSpin software. The manual corrections of the phase and baseline are essential to obtain a high-quality absorption line shape. To ensure correct, accurate, and repeatable integrations, this manual mode was used for signal integration. This also prevented excess area for the selected signal of the typical quantitative peaks.

Since NMR signals are Lorentzians, in order to capture approximately 95 to 99 % of the total intensity of the singlets in this work, the integration regions for all signals were set to 16 times the half-width of the signals. This is the highest integration limits that would be achieved without overlap.

Peak assignment and calculations from the NMR spectra

Based on the chemical structural formula each NMR resonance peak has to be assigned based on the acquired spectra. The result is given in Table 1.:

Table 1. Assignment of the MET and CAF resonances

Signal No.	δ (ppm)	Assignment	Multiplicity

The integral of an NMR signal is directly proportional to the number of corresponding spins, described by the following equation (1):

$$I = k \times n \quad (1)$$

where *n* represents relative number of the spins, which cause the resonance and *k* is an unknown constant to all proton signals in the same ^{1}H-NMR spectrum because these proton signals are running the same single-pulse. Although different signals correspond to different numbers of nuclei, their relationship can be described by Equation (2):

$$\frac{I_1}{I_2} = \frac{n_1}{n_2} \quad (2)$$

To calculate the molar concentration of MET remaining in solution after decomposition into 4-MAA equation (3) is applied.

$$C_{MET} = \frac{I_{MET}}{I_{cal}} \times \frac{N_{cal}}{N_{MET}} \times C_{cal} \quad (3)$$

in which *I* and *N* denote the integral areas of singlets and number of protons of MET and internal calibrant (DSS). C_{MET} and $C_{\mathrm{cal.}}$ denote the concentration of MET and the concentration of the calibrant (DSS).

To determine the purity of MET and CAF standards, equation 4 is applied. Equation 5 is used to determine the weight of CAF and MET per tablet.

$$P_x = \frac{A_x}{A_{cal}} \frac{N_{cal}}{N_x} \frac{M_x}{M_{cal}} \left(\frac{m_{cal}}{m_{powder}}\right) (P_{cal}) \quad (4)$$

$$m_x(\mathrm{g}) = \frac{A_x}{A_{cal}} \frac{N_{cal}}{N_x} \frac{M_x}{M_{cal}} \left(\frac{m_{cal}}{m_{powder}}\right) (P_{cal})\ T \quad (5)$$

Where, m_x is the mass of CAF or MET. A_x is the integral area value of the chosen signal for quantitative calculation of CAF or MET. A_{cal} is the integral value of the ^{1}H signal of DSS. N_x and N_{cal} indicate the number of protons for the integrated signal of the analyte and the quantification standard, respectively. In our special case, N_x = 3 and N_{cal} = 9; M_x and M_{cal} are the molecular mass of CAF=194.19 g/mol, MET=351.35 g/mol and DSS (196.34g/mol),

respectively; m_{cal} and m_{powder} are the weighted mass (mg) of (DSS) calibrant and weighted mass of tablet powder sample taken for analysis, respectively; P_{cal} is the purity of the DSS calibrant (99.95%), and T is the weighted mass of a tablet (g).

Final values are collected in Table 2. A similar table has to be presented for CAF as well.

Table 2. NMR determination of MET.

***m* /mg**	***m* MET (mg/tablet) ± SD**	**MET nominal mass (mg/tablet)**	**% MET/tablet**
Mean ± SD			

Decomposition studies at different pH values will lead to the data collected in Table 3.

Table 3. Kinetics of MET decomposition at different pH values.

pH	**Reaction time /h**	**Integrated intensity of the chosen signal for MET /a.u.**	**cMET/ mM**	**Integrated intensity of the chosen signal for MAA /a.u.**	**cMAA/ mM**

Data need to be evaluated based on the Int = f(concentration) graphical representation. The reaction taking place can be written as:

$$\mathrm{MET^-} + \mathrm{H^+} \rightleftharpoons \text{products}$$

As [H+] can be considered constant during the reaction, the rate of MET decomposition is:

$$\frac{d[\mathrm{MET}]}{dt} = -k \times ([\mathrm{MET}] - L)$$

where k is the pseudo-first order rate constant, and L is the limit concentration of MET to which MET concentration converges at equilibrium, or, equivalently, at t=∞. Considering this the resulting concentration – reaction time dependence will be:

$$c = (c_0 - L)e^{-kt} + L$$

where, c is the concentration of MET and MAA, c_o is the concentration of MET and MAA at t = 0 and t is the reaction time. Fitting the experimental data to this equation will yield the rate constant.

Iodometry – analytical titration and calculations

Preparation of the necessary stock solutions:
a) 0.1 M sodium thiosulfate solution is prepared by dissolving 25 g of sodium thiosulphate in a 1-litre volumetric flask containing 100 ml distilled water. The solution is topped up to 1000 ml with distilled water and thoroughly mixed**.** To determine the exact concentration – factor -, 0.01 M potassium bi-iodate is prepared by dissolving 3.9000 g of potassium bi-iodate in a 1-litre volumetric flask containing 500 mL of distilled water. This is diluted to the mark with distilled water. This solution was titrated against the thiosulfate. 0.1 M $Na_2 S_2O_3$ is used to clean a 12 ml burette by topping up twice. The burette is filled with 0.1 M $Na_2S_2O_3$. 0.4 g of potassium iodide (KI) is separately added into a 250 ml Erlenmeyer flasks containing 20 ml of distilled water. 4 drops of 20% HCl is added. 5.0 ml of 0.01 M potassium bi-iodate solution is pipetted and titration with the thiosulfate titrant is started immediately. When the solution becomes pale yellow, 4-6 drops of aqueous starch solution (indicator) are added and the titration process continues while swirling the flask until the blue colour changed to colorless. At least 3 parallel titrations are performed. Calculation of the exact sodium thiosulfate concentration.

b) 0.05 M iodine solution is prepared by dissolving 25 g of potassium iodide in 100 ml of distilled water in 1000 ml volumetric flask. 12.7 g of iodine is added followed by three drops of 0.1 M hydrochloric acid. The solution is kept in the dark for three weeks – this is necessary as dissolution of iodine is very slow. Finally, it is diluted to 1000 ml with distilled water. In the next step the exact concentration must be determined. For this the previously prepared 0.1 M sodium thiosulfate solution is used.
Steps: a 12 ml burette is filled with 0.1 M sodium thiosulfate solution with known concentration. 10 mL of iodine solution is pipetted and transferred into 250 ml Erlenmeyer flask. The solution is titrated while swirling the flask, until a pale-yellow color appeared. 4-6 drops of starch solution are added and titration is continued until the blue color disappears. At least 3 repetitions are needed. From the volume of thiosulfate used the iodine solution concentration is calculated.
The following reactions take place:

$$KH(IO_3)_2\,(aq) + 10\,KI\,(aq) + 12\,HCl\,(aq) \rightarrow 6\,I_2\,(aq) + 6\,H_2O\,(l) + 10\,KCl\,(aq) + KHCl_2\,(aq) \qquad (i)$$

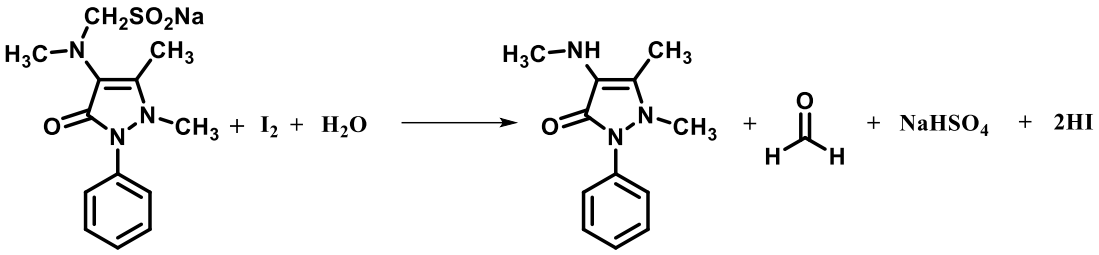
$$6\,I_2\,(aq) + 12\,S_2O_3^{2-}\,(aq) \rightarrow 12\,I^-\,(aq) + 6\,S_4O_6^{\,2-}\,(aq) \qquad (ii)$$

Once the stock solutions are readily available the tablets containing MET are titrated.

Metamizole sodium is determined from separate individual tablet samples and also from a mixture of three tablets. From single tablet samples, 4 tablets were separately crushed in a mortar with pestle by different individuals. 3 replicates of approximately 0.2 g from each tablet powder are weighed and transferred into 250 ml Erlenmeyer flasks. Note: in most cases the

third replicate mass is less than 0.2 g since each tablet weighed about 0.650 g and some powder stuck on the wall of the mortar and pestle. 12 ml burette is washed twice with distilled water, then with 0.05 M iodine solution before filling it up. 10 ml of 0.01 M ice cooled HCl is added into one of the flasks containing MET powder. Immediate titration is done while swirling the flask. When the color of the solution changed to orange, 15 drops of starch solution were added. Titration continues until a dark green color appears indicating the end point. The same procedure is repeated with the other replicates from individual tablets. The titres are tabulated and MET content is calculated from each titre.

The reaction taking place is:



Metamizole-Na 4-methylaminoantipyrine (iii)

The following tables and calculations can be used for the determination:

Table 4. Standardization of 0.1 M $Na_2 S_2O_3$

Titres	Volume of $S_2O_3^{2-}$ /ml
I	
II	
III	
Average	**6.05**

Volume of bi-iodate solution used in each titration = 5.0 ml

$$n_{bi-iodate} = c_{bi-iodate} \times v_{bi-iodate}$$

From reaction (i) : $n_{I_2,liberated} = 6 \times n_{bi-iodate}$

From reaction (ii): $n_{S_2O_3^{2-}} = \frac{12}{6} \times n_{I_2}$

$$c_{S_2O_3^{2-}} = \frac{n_{S_2O_3^{2-}}}{V_{S_2O_3^{2-}}}$$

$$Factor, f = \frac{c_{S_2O_3^{2-}}}{c'_{S_2O_3^{2-}}}$$

where, $c_{S_2O_3^{2-}}$ is the actual molar concentration of the thiosulfate solution and $c'_{S_2O_3^{2-}}$ is the nominal molar concentration of the thiosulfate solution.

Actual concentration of the iodine solution is calculated from the stoichiometry reaction iv.

$$2\ S_2O_3^{2-}\ (aq) + I_2\ (aq) \rightarrow S_4O_6^{2-}\ (aq) + 2I^-\ (aq) \qquad (iv)$$

Table 5. Standardization of the 0.05 M iodine solution

Titres	Volume of $S_2O_3^{2-}$ /ml
I	
II	
III	
Average	

Volume of Iodine solution used in each titration = 10.0 ml

$$c_{I_2} = \frac{n_{I_2}}{V_{I_2}} \qquad Factor, f = \frac{c_{I_2}}{c'_{I_2}}$$

where, c_{I_2} is the actual molar concentration of the thiosulfate solution and c'_{I_2} is the nominal molar concentration of the thiosulfate solution.

Finally MET content is determined from reaction (iii), and data are summarized in Table 6.

Table 6. MET determination from different tablets, and at least 3 titrations/tablet:

Tablet	***Vol.*** **Iodine /ml**	m **Powder /g**	**Calculated** m **MET/g**	m **MET/tablet (mg)**	**% MET/tablet**
1					
2					
3					
4					
Mean ± SD					

Still, one has to test, whether the CAF present in the drug will influence this determination. This can be found out performing the titrations with standard MET and CAF samples, with addition of exact values from each component alone and next to each other.

Comparison of the qNMR and the iodometric methods; conclusions and outlook

The comparison of the results of qNMR with those obtained from the iodometric titration method recommended for MET determination by the European Pharmacopeia will be done based on the summarization shown Table 7.

Table 7. MET and CAF content by qNMR and iodometric titration methods.

Active Ingredient	qNMR	Iodometry	Expected content
$m_{\text{MET tablet}}$/mg	±	±	
$m_{\text{CAF tablet}}$/mg	±	-	

Proposed viewpoints for comparison: accuracy, precision, sample preparation and measurement time, wealth of information. The possible error sources need to be also discussed and highlighted.

References:

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