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► **To cite this version:**

Catherine Creff-Froger, Mélaine Bessiral, Marie-Pierre Fourmond, Céline Hedou, Agnès Perrin-Guyomard. Certificates of analysis: a challenge to interpret . EuroReference - Les Cahiers de la Référence, 2017, 3, pp.27-34. 10.5281/zenodo.1172022 . anses-01787685

HAL Id: anses-01787685

<https://anses.hal.science/anses-01787685>

Submitted on 7 May 2018

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Certificates of analysis: a challenge to interpret

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Abstract

The active pharmaceutical ingredient (API) in a medicinal product is the component that is responsible for the therapeutic effect. The personnel at the ANSES Fougères Laboratory routinely handle medicinal analytical standard and calculate their API content using the certificates of analysis provided by the manufacturer. However, meaningful data in these documents are not always easy to determine or may sometimes be absent: interpretation of these data can therefore result in assessment errors. Having identified this problem, the ANSES Laboratory has put forward an approach aimed at harmonising interpretation of certificates of analysis.



Keywords

- ★ Certificate of analysis
- ★ Data interpretation
- ★ Veterinary drugs
- ★ Active pharmaceutical ingredient

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DOI: <http://doi.org/10.5281/zenodo.1172022>

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Introduction

Medicinal products, both for human and veterinary use, contain a single drug substance or multiple drug substances, and excipients. The drug substance (DS), or active pharmaceutical ingredient (API), is the compound that underlies the pharmacological and therapeutic effects.

Given the various national and European mandates of the ANSES Fougères Laboratory as a reference laboratory in the area of veterinary medicinal products, its personnel handle medicinal analytical standards on a daily basis. This work is done as part of testing carried out during the development and validation of methods, official controls, proficiency testing, and research projects. It is therefore essential for the personnel to be able to calculate the amount of API in the analytical standards on the basis of the certificates of analysis (CoAs) supplied by the manufacturers.

However, CoAs are not all formulated in the same way and may be incomplete: interpretation of data may therefore lead to errors in evaluation.

The ANSES Fougères Laboratory has identified this problem and is therefore putting forward an approach to harmonise the interpretation of CoAs.

Certificates of analysis: essential but potentially problematic documents

The CoA is a document that defines the analytical standards (name, CAS number, molecular formula, molecular weight, etc.) and indicates the required specifications, including appearance, purity, solubility, and water content. It provides the results of the identification and quality testing performed by the manufacturer for a batch, on the basis of the criteria cited in a pharmacopoeia. Therefore, the CoA is an essential document for any user of an analytical standard to determine the API content.

There is, however, no standard certificate of analysis: each manufacturer presents the specifications of the analytical standard and the analytical results on the basis of their own criteria. It may happen that important indications for the calculation of the API content are missing or imprecise or, more rarely, incorrect. Interpreting CoAs may thus be a sensitive task, potentially leading to assessment errors by the operators, especially if the operator is not a chemist by initial training.

As an example, the ANSES Fougères Laboratory carried out a survey among two groups of operators regularly using antibiotics to test the interpretation of various “critical” CoAs, with the users asked to determine the API content in the sample analytical standards. The first group was made up of 14 users from a single laboratory (1 CoA for ampicillin sodium), the second included 23 users mostly working in different laboratories (3 different CoAs). In both cases, the conclusion was clear: whenever there were doubts on interpreting the CoA, or if the chemical substance was somewhat complex, the results of API content calculations were highly heterogeneous. For instance, in the first group of 14 users, 8 different values were obtained, from 873 to 939 µg ampicillin/mg of ampicillin sodium. For the second group of users, the variability of results was of greater concern, specifically for the values obtained from data in the CoA for spectinomycin dihydrochloride pentahydrate, where the API content values ranged from 363 to 878 µg API/mg of analytical standard.

These tests show unequivocally that there is a paradox in ensuring careful metrological oversight of measurement methods if we are less rigorous downstream.



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From the medicinal analytical standard to the active pharmaceutical ingredient content

The medicinal analytical standard is a powder containing a chemical substance, impurities, and often residual water. Indications in the CoA concerning the purity and the residual water content of the analytical standard can be used to calculate the chemical substance content. The indications concerning the identification of the chemical substance will be used to calculate the API content.

When the CoA is incomplete, calculating the API content of each analytical standard requires reference documentary resources that must be made available to the user.

These documents include, on the one hand, pharmacopoeias: they contain various monographs that indicate the criteria for purity of analytical standards and the analytical methods to use for testing purposes. These monographs are authoritative references for any substance or formula included in the pharmacopoeia. They provide a recognised framework and are regularly updated. On the other hand, the Merck Index has been a reference source for chemical products for more than 100 years and presents more than 10,000 monographs, including those for medicinal chemical substances. Testers can consult this Index to supplement the information indicated in the CoA when the available pharmacopoeias are not sufficient. Likewise, there are several databases for chemical compounds on the internet that are easily accessible and comprehensive.

Since difficulties in interpreting CoAs are found repeatedly, but almost never addressed in the literature (Brown, 2008), the ANSES Fougères Laboratory decided to take a pragmatic approach and create a guide for the interpretation of CoAs, within the framework of the laboratory's quality assurance system. This approach is described below, step by step.

Content of chemical substance in the analytical standard: selecting the value to use

In order to determine the chemical substance content of their analytical standards, manufacturers carry out an assay by referring to a reference standard. The results of this testing are expressed in the CoA in different ways: either in international units/mg (IU/mg), $\mu\text{g}/\text{mg}$, $\mu\text{g}/\text{ml}$, or as a percentage.

On CoAs, some analytical standards with antibiotic activity still have their content expressed in IU/mg (bacitracin, colistin, penicillins, sometimes streptomycin and tylosin, etc.), but it is not always easy, specifically for chemist users, to understand the notion of conversion coefficients IU/mg (originating from WHO standards). At the ANSES Fougères Laboratory, where most testing is performed in the framework of reference activities on veterinary medicinal product residues, the data of interest are related to the maximum residue limits (MRLs) and are therefore expressed in $\mu\text{g}/\text{kg}$. It was therefore decided within this context, excluding microbiological testing, that since CoAs for these antibiotics never indicate the level of impurities, this level would be considered to be equal to 0%, and therefore that the chemical substance content of the analytical standard is equal to 100%.

If, on the basis of the CoAs, the only quantitative indication that can be used to determine the chemical substance content of the analytical standard is purity, expressed in %, we need to assimilate this value to a chemical substance content in the analytical standard by default. For example, a CoA for cefquinome sulphate indicates: "Purity (HPLC): 99.4%". This figure indicates that the batch of analytical standard has a purity of 99.4% in cefquinome sulphate, *i.e.* 994 μg of cefquinome sulphate/mg of analytical standard.



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When the manufacturer indicates on the CoA a value in $\mu\text{g}/\text{mg}$, this complicates matters. In general, this value represents the content in API and not the content in chemical substance. To be sure, we need to check this information in a pharmacopoeia. In this example of a CoA for amikacin sulphate, it is the API content that is indicated: "Potency: 776 μg amikacin base/mg (anhydrous basis)". When we check the data in the United States Pharmacopoeia (USP), we find that the result of the assay must be between 691 and 806 μg amikacin/mg. Therefore, the value indicated clearly corresponds to the content in amikacin and not in amikacin sulphate.

More rarely, a value expressed in $\mu\text{g}/\text{mg}$ can reflect the chemical substance content. This is the case for tetracycline HCl, for example, since it is always mixed with its epimer, 4-epi-tetracycline. Checking in the USP shows that the content in tetracycline HCl, without taking into account the epimer, must not be less than 900 $\mu\text{g}/\text{mg}$.

On some CoAs, the chemical substance content is associated with the expression "as is". This means that the manufacturer assayed the chemical substance content in the analytical standard as it will be presented in its final packaging, without transformation (specifically loss on drying). As a result, the content indicated is the value that must be retained. We must not consider the water content of the analytical standard, even if this value is indicated elsewhere.

Some analytical standards are supplied in solution. In most cases, to prepare the solution, the supplier took into account the chemical substance content of the analytical standard; the chemical substance content of the solution is therefore 100%.

Sometimes, however, a CoA may indicate two values. It then becomes essential to refer to the pharmacopoeia to find out whether this is a value for chemical substance content or API content. An example from a CoA for ampicillin sodium shows the rationale to follow: "Assay (HPLC Weight%): 93.3% - Potency: 878 μg ampicillin/mg": 93.3% represents the % of ampicillin sodium (or chemical substance) in the analytical standard, 878 μg represents the API content (ampicillin) in the analytical standard (after verification in the USP35: from 845 to 988 μg ampicillin/mg). It is easier to retain directly the API content, *i.e.* the value 878 $\mu\text{g}/\text{mg}$.

If a CoA indicates two values for content expressed in the same way (*i.e.* both in % or both in $\mu\text{g}/\text{mg}$), we should always opt for the value obtained with the most specific method. If, for example, we can choose between a value obtained using a titrimetric method and a value obtained using a chromatographic method, the second value should be selected.

Understanding the water content of the analytical standard

On the CoAs, manufacturers mostly indicate the water content in analytical standard.

During the manufacturing process of an analytical standard, the chemical substance may be combined with one or more H_2O molecules or water of crystallisation. A molecule associated with water of crystallisation is known by the name hydrate: monohydrate, dihydrate, etc. This phenomenon is common, particularly during crystallisation of carboxylic acids and molecules presenting in this form such as beta-lactams (penicillins, cephalosporins, carbapenems), quinolones and fluoroquinolones.

Various methods (thermogravimetric, chemical, spectrometric) can be used to determine the water content. The Karl Fischer (KF) method is a titration technique which is based upon the oxidation of sulphur dioxide by iodine in a methanolic hydroxide solution. The KF method assays both residual water in the analytical standard (moisture) and water of crystallisation. When the method to determine the water content of an analytical standard in the form of hydrate (cephalexin hydrate, for example) is the KF method, we must be careful not to take into account the H_2O molecule when calculating the API/chemical substance ratio. The loss



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on drying method (gravimetry) is only used to assay residual water in the analytical standard.

On CoAs, water content is indicated but the method that was used to calculate this value is not given. For instance, we may see "Water": in most cases, we can find the method used in the pharmacopoeia. However, if this is not the case, we should choose the "residual water" option, unless we have the CoA for another batch of the analytical standard from the same manufacturer, with a similar water content.

In general, the water content is expressed in %, but in some cases it is expressed in mole of water/mole of chemical substance: we then need to think in terms of molecular weight. For example, the CoA of a batch of apramycin sulphate shows that there are 0.3 moles of water per mole of apramycin sulphate. This way of indicating the water content does not simplify the calculations. Particularly since, in this case, the sulphate content is also expressed in mole/mole.

In short, it is essential to know which technique was used to determine the water content. To do this, it is very important to refer to the pharmacopoeias. This is particularly critical for chemical substances in the form of hydrates.

Identification of the chemical substance: uncertainties

Knowing the identity of the chemical substance will serve to calculate its content in active pharmaceutical ingredient (API).

The identity of a chemical substance is indicated in part by its name but it is chiefly determined by a unique registration number from the Chemical Abstracts Service (CAS) database. The CAS attributes these numbers to all chemical products that have been described in scientific documentation. About 30 million compounds have been attributed a CAS number to date.

Most CoAs indicate this CAS number but in some cases it is not given. This can pose a problem in the event that a chemical substance described has different names. For example, cloxacillin sodium is also cloxacillin sodium monohydrate (CAS Number: 7081-44-9), not to be confused with cloxacillin sodium which is implied but not indicated as being "anhydrous" (CAS Number: 642-78-4). Other examples include pyrantel pamoate and pyrantel embonate, which are the same chemical substance (CAS Number: 22204-24-6), as well as sulphame-thazine, sulphadimidine and sulfadimerazine (CAS Number: 57-68-1). There are many similar examples showing the importance of knowing the CAS number. A chemical substance is also defined by its formula, whether semi-structural or molecular, and its molecular weight.

All these characteristics can be used to determine whether a chemical substance is a hydrate, and whether it is in salt form. The semi-structural formula tells us about the precise structure of the molecule, and the number of ions or elements it is made up of, which is not clear solely on the basis of the chemical substance's name. For instance oxytetracycline hydrochloride is made up of 1 "oxytetracycline" and 1 "hydrochloride", sisomicin sulphate is 2 "sisomycins" and 5 "sulphates", malachite green, 1 "malachite green cation" and 1 "chloride anion". Here again, there are multiple examples.

The active pharmaceutical ingredient content in the analytical standard: some complexity

Once the chemical substance and its chemical structure have been identified, it is easy to calculate the active pharmaceutical ingredient:chemical substance ratio (API:CS ratio). For



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example, for the chemical substance trimethoprim, there is 100% trimethoprim; for emamectin benzoate, all we need is the ratio of the molecular weight of emamectin versus that of emamectin benzoate. This yields a content: in this case 86.2%.

However, we will not need a calculation if the CoA indicates the chemical substance content of the analytical standard in µg/mg (excluding exceptions, see above). The calculation will nonetheless be more complex if the sulphate content is expressed in mole/mole.

We must always exercise caution when dealing with chemical substances that are in ionic form. For instance, in the case of nafcillin sodium, nafcillin "base" has lost an H⁺ proton but gained an Na⁺ proton. Therefore, the difference in molecular weight of nafcillin sodium versus nafcillin "base" is 22 instead of 23, a change we need to take account of when calculating the nafcillin/nafcillin sodium ratio.

Likewise, we must be careful concerning CoAs for chemical substances in hydrate form, where the water content was calculated using the KF method: we must not take the H₂O molecules into account in duplicate.

Once we have determined the purity (or content of the analytical standard in chemical substance), the water content and the API content (if necessary), these data are multiplied with one other to obtain the API content of the analytical standard. This operation is of course to be repeated with each change of manufactured batch of the analytical standard.

Example of calculating the API content based on the certificate of analysis

FIGURE 1/ Certificate of analysis for a batch of apramycin sulphate

| Certificate of Analysis | | |
|------------------------------|--|---------------------------|
| Product Name: | APRAMYCIN SULFATE SALT ~ 95 % TLC | |
| Product Number: | A2024 | |
| Batch Number: | BCBP2820V | |
| CAS Number: | 65710-07-8 | |
| Formula: | $C_{21}H_{41}N_5O_{11} \cdot xH_2SO_4$ | |
| Formula Weight: | 539.58 | |
| Storage Temperature: | 2-8 C | |
| Quality Release Date: | 09 JAN 2015 | |
| TEST | SPECIFICATION | RESULT |
| APPEARANCE (COLOR) | WHITE TO LIGHT YELLOW | LIGHT YELLOW |
| APPEARANCE (FORM) | POWDER | POWDER |
| PURITY (TLC AREA %) | APPROX. 95% | 99 % |
| SOLUBILITY (COLOR) | COLORLESS TO LIGHT YELLOW | SLIGHTLY YELLOW |
| SOLUBILITY (TURBIDITY) | CLEAR (< 3.5 NTU) | CLEAR (<3.5 NTU) |
| SOLUBILITY (METHOD) | 200 MG PLUS 8 ML OF WATER | 200 MG PLUS 8 ML OF WATER |
| WATER | ≤ 5 MOL/MOL | 0.3 MOL/MOL |
| SULFATE | ≤ 3 MOL/MOL BASED ON SULFUR | 2.0 MOL/MOL |

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The CoA used as an example (Figure 1) is for a batch of apramycin sulphate. The CoA is complete, but we must note that the molecular weight indicated is that of apramycin and not that of apramycin sulphate, which is important for the subsequent calculation.

Also, the CoA indicated that the purity of the analytical standard is equal to 99%, and that the water content is equal to 0.3 mole/mole (*i.e.* of apramycin sulphate) and its sulphate content 2 moles/mole (of apramycin sulphate).

Firstly, we need to calculate the water content of the analytical standard, expressed as a percentage. We must work with the molecular weight because the result is expressed in moles:

$$\frac{(0.3 \text{ mole water } (18.01 \times 0.3 = 5.4))}{1 \text{ mole apramycin } (539.58) + 2 \text{ moles sulfate } (2 \times 98.08 = 196.16) + 0.3 \text{ mole water } (5.4)} = \frac{5.4}{741.14} = 0.73\%$$

We then need a formula to obtain the API:CS ratio (active pharmaceutical ingredient:chemical substance ratio), also expressed as a percent (%):

$$\frac{1 \text{ mole apramycin } (539.58)}{1 \text{ mole apramycin } (539.58) + 2 \text{ moles sulfate } (2 \times 98.08 = 196.16)} = \frac{539.58}{735.74} = 73.3\%$$

As explained above, the data obtained are multiplied by one another to reach the API content of the analytical standard:

$$99\% (\text{purity}) \times 99.27\% (100\% - 0.73\% \text{ water}) \times 73.3\% \left(\text{ratio} \frac{\text{API}}{\text{CS}} \right) = 72.04\%$$

The API content of the analytical standard is equal to 72.04%, *i.e.* 720.4 µg active apramycin per mg of analytical standard.



The choice of the ANSES Fougères Laboratory: centralisation of calculations

To counter the risk of errors when interpreting CoAs, the ANSES Fougères Laboratory opted for a centralised approach: all the API contents of medicinal analytical standards used at the laboratory are evaluated by a reference person, assisted by deputies. When a CoA is received, and using a specific Excel spreadsheet and an internal interpretation guide for CoAs, which is constantly updated, the reference person carries out the necessary calculations. This person then records the API content of the analytical standard in the Laboratory Information Management System (LIMS) as a unique and traceable value that is accessible to all users of the analytical standard. The deputies, who are trained in performing these calculations, check the data and the record in succession, which also has the effect of ensuring that their skills are maintained.

Conclusion

The CoA is an essential document for users of medicinal analytical standards because it contains key data to calculate the API content. However, these data are not always easy to determine and/or interpret: sometimes, they may be missing or imprecise. To avoid errors in interpretation, which are possible among different users, the ANSES Fougères Laboratory recommends a standardised approach with data processing referred only to trained personnel who can consult a guide on the interpretation of CoAs. In this way, for each batch of medicinal

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analytical standard, a single value for API content that is verified and traceable is available to all laboratory personnel.

Reference

Brown P. 2008. Certificates of Analysis. *Nutraceuticals World*, November 2008: 30-32. https://www.bcit.ca/files/appliedresearch/pdf/certificates_of_analysis_nov_08_nw.pdf

