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P. VAN EENOO, A. SPAERKEER, F.T. DELBEKE:
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Stability studies of selected doping substances in methanolic solution

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1. Introduction

Recently papers on the stability of some doping agents in urine, including caffeine (1) and salbutamol (2), have been published.

One of the prerequisites for obtaining an accreditation by the World Anti-Doping Agency is that the doping control laboratory has also obtained IS017025 accreditation (3). Paragraph 5.6.3.2 and 5.6.3.3 of the ISO17025 accreditation guidelines state that reference materials must be controlled and subjected to intermediate controls (4). Hence, the absence of documentation on the stability of reference solutions could be regarded as a shortcoming of a laboratory to these guidelines, eventually leading to loss of accreditation. Moreover, instability of reference compounds can be an important factor in the overall estimation of uncertainty of measurement. Indeed, a significant contribution of this factor will lead to bias of the measured urinary concentrations.

For several anabolic steroids data on the stability as a methanolic solution is available in literature (5). For most other substances no stability data as a methanolic solution is available, therefore the stability of selected doping substances was investigated.

2. Methods and materials

In order to minimize the workload the literature and certificates of the reference materials were checked for the presence of this type of data, prior to start with the stability studies.
In collaboration with the Association of Official Racing Chemists (AORC) a protocol was established to determine the stability of the methanolic solutions (6).
For stability testing purposes, the solutions were stored under different conditions, namely at 40°C, at room temperature, in a refrigerator (4°C) and at -20°C. The temperature of each storage condition was checked daily.
In order to minimize the effects of evaporation the vials were tightly closed using screw caps and seals. Moreover the volume of air above the methanolic solution was kept as small as possible. All vials were weighed before and after storage and vials with significant weight loss (> 2 %)due to evaporation (stored at room temperature and 40°C) were discarded. The percentage of vials that needed to be discarded depended on the storage condition and storage time. This percentage varied from as little as 5 % (storage for 6 months at 4°C) to as high as 50% after 6 months at 40°C, indicating the importance of evaporation in the overall process.
The stored solutions were tested in triplicate every three months following the protocol shown in Fig. 1 and compared with three aliquots of the same solution that were stored at -20°C. When necessary, the solutions stored at -20°C were compared with freshly prepared solutions. After addition of an internal standard and evaporation the samples were analyzed using the routine methods (7-10).

The signals (relative to the internal standard) of the reference compounds were compared using a t-test (α=0.02 or α=0.05 for 2% and 5 % differences, respectively) are shown in Table 1.

Table 1. Interpretation of the data from stability studies

Difference (%)	Interpretation
< 2	Stable, difference due to random effects
2-5	warning level, the solution is still regarded as stable
> 5	the solution is regarded as not stable

3. Results and discussion

Several assumptions were made in the testing protocol:

- the stability of a substance is a physicochemical property, not depending on the manufacturer
- the stability is not concentration dependent
- the stability is temperature dependent and the Q_{10}-factor is 2. This is similar to the one used by Westwood et al. (5) and is a generally well accepted value in chemical analysis.
- the substance is assumed to be stable at the reference condition (-20°C)

Theoretically, the ISO17025 guidelines do not distinguish between substances intended for quantitative and qualitative testing. However, ILAC-G17 (11) states that only uncertainty in quantitative testing is considered for the time being. Hence, it can be concluded that stability testing for substances in qualitative testing is not of major importance as long as their presence can be established with a sufficient degree of certainty. Traditionally, GC- and LC-MS have been regarded as appropriate stand-alone techniques for this type of identification purposes (12, 13).

For substances intended for quantitative testing however, instability could be a significant component in the overall estimation of measurement uncertainty and needs to be evaluated. A 5 percent tolerance seems acceptable within the framework of uncertainty of measurement and was also previously used (5).

The certificates of analysis of our reference substances were checked. Only the certificate of 11-nor-Δ9-tetrahydrocannabinol.9-carboxylic acid, which is purchased as a methanolic solution, had an indication of the stability of the reference substances in methanol. Based upon the date of purchase, the criteria and storage conditions mentioned on the certificate of analysis, 5 years storage of a stock solution in a refrigerator should be within the tolerance ranges (14).

A summary of the stability periods according to each storage condition for the different substances tested is given in Table 2.

Table 2. Results of stability testing, indicating the number of months (m) during which no significant degradation (<5%) was observed for stock solutions under different storage conditions

Substance	40°C	Room temperature	Fridge	-20°C
Caffeine	3 m	≥ 12 m	NT	NT
Pseudoephedrine	3m	6 m	9 m	≥ 12 m
Methylephedrine	< 3m	3 m	12 m	NT
Ephedrine	3 m	6 m	12 m	NT
Cathine	3 m	3 m	6 m	≥ 12 m
Phenylpropanolamine	3 m	6 m	12 m	NT
Morphine	< 3m	3 m	6 m	≥ 24 m
Salbutamol	< 3m	3 m	6 m	12 m

NT: not tested

As shown in Table 2, several substances can only be stored for a limited period in a refrigerator without significant degradation effects and marked differences in stability are observed between substances with similar structures, e.g. cathine and phenylpropanolamine. The data also suggests that for some substances, e.g. salbutamol, instability can be an important factor in the estimation of uncertainty. Appropriate storage and renewal of stock solutions of these compounds is of utmost importance to achieve adequate quantitative results. Moreover, it is important to realize that the data in this study concern stock solution. In contrast to stock solutions, working solutions are subjected to multiple cooling-down and warming up cycles and are therefore presumably less stable. Hence, it is necessary to reduce the shelf-life of working solutions considerably compared to stock solutions

4. Conclusion

For several threshold substances in doping control degradation of the substance in a methanolic solution can significantly contribute to the overall uncertainty of measurement. In these cases, appropriate storage conditions and renewal schedules need to be implemented within the laboratory to guarantee sufficient accuracy and to minimize the effect of this parameter in the uncertainty.

5. References

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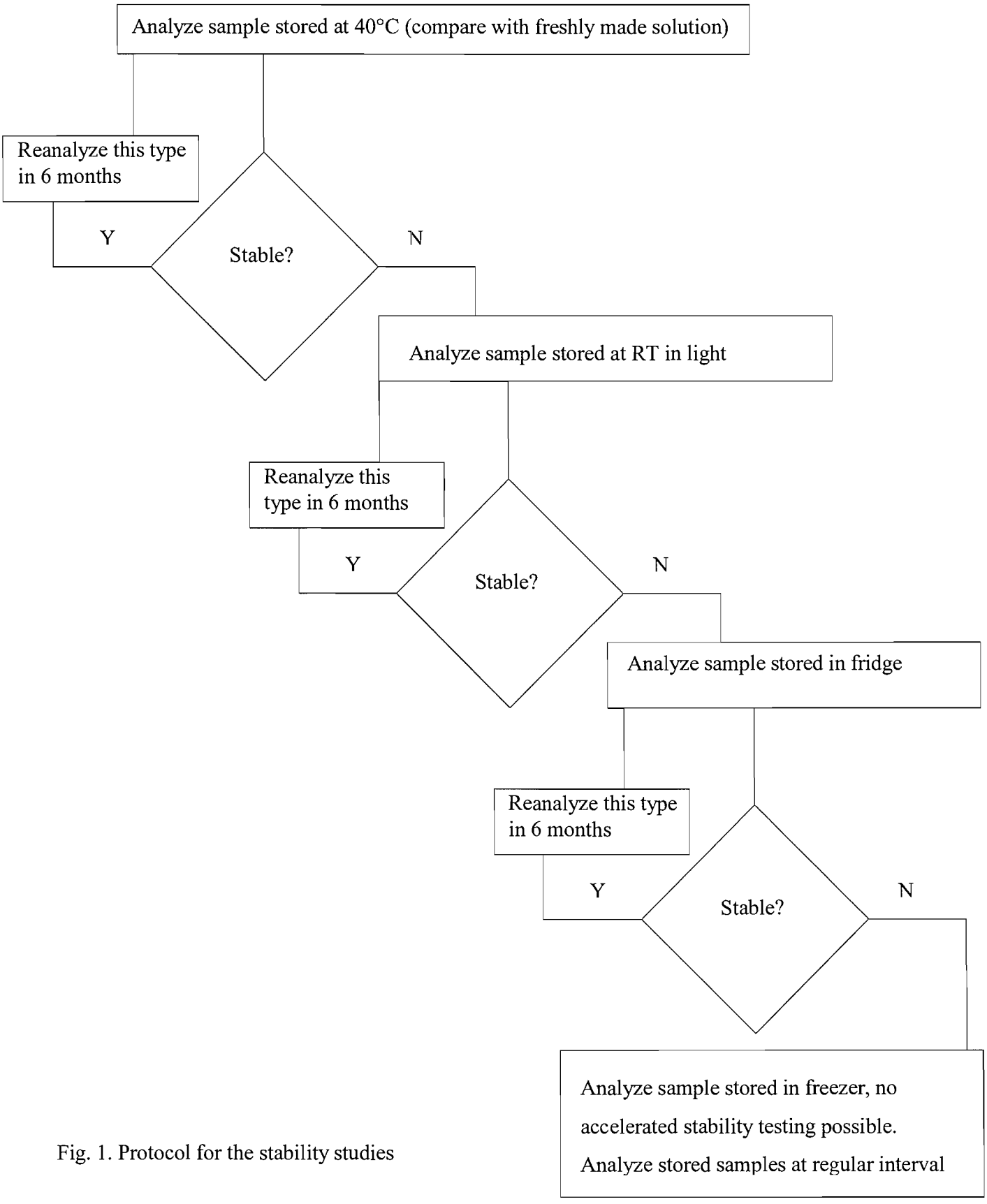

Fig. 1. Protocol for the stability studies