the critical concentrations required to produce the metastable foam would also have remedied the problem, but would have affected analytical sensitivity.

How might this problem affect flame atomic absorption analysis? Imprecise and drifting absorbance readings are usually attributed to analysis of samples high in solids, which block the burner slots, or to nebulizer failures. The possibility of blocking the spray chamber would seem remote because of its large interior volume. Additionally, to identify this problem, one would have to shut down the flame during an analytical run and disassemble the spray chamber for inspection. Thus the presence of an interfering foam would probably pass unnoticed.

Butanol has been used in the past (2) as a signal enhancer; this enhancing effect may have been partly due to the inhibition of foam formation. Overly noisy magnesium assays in protein-containing solutions of lanthanum chloride (3) may have resulted from interference by foam. Thus the possibility of metastable foam production in spray chambers should be considered as a possible cause of degraded analytical performance.

References


In our laboratory we modified an enzymatic creatinine kit (Boehringer Mannheim, for use in the Hitachi 705) to measure creatine in serum. In this enzymatic creatinine PAP (p-aminophenazone) kit, creatinine is enzymatically converted by creatininas (EC 3.5.2.10) to creatine, which is then measured by a sequence of three enzymatic steps. For measurement of creatine, we omit the creatininas, which is supplied in the kit in separate tablets, and make use of the remaining reaction sequence:

Creatine + H₂O \( \rightarrow \) creatinase (EC 3.5.3.3) + sarcosine + urea

Sarcosine + H₂O + O₂ \( \rightarrow \) glycine + HCHO + H₂O₂

H₂O₂ + phenol derivative + 4-aminophenazone peroxidase (EC 1.11.1.7) \( \rightarrow \) red benzoquinone-imine dye

To obviate possible interferences, one must include a sample blank, which consists of the reagent plus added p-chloromercuribenzoic acid (3.6 mg/10 mL of reagent), to inactivate the enzymes.

For the creatine standard we use aqueous creatine monohydrate. The standard curve for our method is linear to at least 250 μmol/L.

Comparison of the results by our method (y) with those by the method in which creatine is converted chemically to creatinine and assayed by a standard Jaffé creatinine method (x) gave the following linear regression equation: y = 0.97x + 1.4; n = 36; \( r^2 = 0.973 \). We found less day-to-day variation by our method (CV 6.1% at 30.3 μmol/L, n = 19) than for the Jaffé method (CV 15.4% at 40.7 μmol/L, n = 19). Analytical recovery of creatine added to serum was 96.1%. Values for creatine are slightly increased (4–10%) by hemolytic sera. The method is not suitable for use with lipemic or icteric sera.

Simple Method for Extracting Vitamin D Metabolites from Biological Samples, F. Risco, M. Babé, and M. L. Traba (Lab. Unidad Metabólica, Fundación Jiménez Diaz, Avd. Reyes Católicos 2, Ciudad Universitaria, 28040 Madrid, Spain)

Several procedures have been reported for extracting metabolites of vitamin D from serum. These involve various organic solvents such as ethanol, isopropyl ether, chloroform, diethyl ether, ethyl acetate, or acetone. However, extractions from tissue samples are performed mainly by the Bligh and Dyer method (Can J Biochem Physiol 1959;37:911–7). We compared the classical Bligh/Dyer extraction procedure (first with chloroform/methanol, followed by separation in chloroform/water) and a much simpler extraction with acetone. The figure below shows schematically the steps we used in the two methods to extract vitamin D metabolites from samples of rat-kidney homogenate and dog-tibia perfusate. Radioactivity recovered was used as a measure of the extraction efficiency.

Analytical recovery obtained for [³H]25(OH)D₃ extraction from kidney homogenate was 75.2 ± 5.6 by the Bligh/Dyer procedure vs 81.3 ± 4.1 with acetone-tol (P < 0.005, n = 6). The recovery for perfusate samples was 85.1 ± 8.5 and 96.3 ± 3.3 by the Bligh/Dyer and acetone-tol procedures, respectively (P < 0.0025, n = 6). Therefore, the extraction was better with acetone-tol than with the Bligh/Dyer procedure.

Comparing recovery of [³H]25(OH)D₃ in kidney homogenate by the two methods, we found no significant differences (82.5 ± 5.6 by Bligh/Dyer and 84.0 ± 3.2 by acetone-tol, n = 18), but the precision was better with acetone-tol, and the procedure is easier and quicker.

Thus we suggest that acetone-tol be used to extract vitamin D metabolites from biological samples.