Temporal Stability Evaluation of Drugs in Plasma on Primary Sampling Tube

To the Editor:

The concentrations of several drugs in serum, plasma, or to a lesser extent in whole blood, are routinely monitored in clinical toxicology laboratories as a guide to therapeutic drug-dosing regimens and management of toxicity. These toxicological analyses are important parts of patient care, especially for drugs with a narrow therapeutic window. Therapeutic drug monitoring provides a valid clinical tool for the optimization of overall therapy.[1,2]

Laboratories performing the measurement of drugs must take into account stability issues to ensure the correct handling conditions of samples before and after analysis. This is a quality requirement for the accreditation of analyses according to ISO 15189 European norm.[3] Stability studies of drugs are most often conducted either in spiked biological matrices drawn from untreated patients, or in serum or plasma obtained after centrifugation and separation from treated patients. However, laboratory practice can be different. Plasma and serum samples sent to the toxicology laboratory are often stored in the primary sampling tube after centrifugation, without separation.

In this study, the preanalytical characteristics of frequently monitored drugs—acetaminophen, amikacin, digoxin, lithium, phenobarbital, phenytoin, salicylate, theophylline, valproic acid, and vancomycin—were investigated under different storage conditions of the samples. To do this, stability testing was performed to mimic real-time situations during sample processing and measurement. Whole blood from patients with prescribed drugs was collected into gel-free heparin collection tubes. After sampling and centrifugation, each sample was immediately analyzed at D0 to obtain drug concentration. For each drug, 6 blood samples with low-drug concentrations and 6 blood samples with high-drug concentrations were included in the study. Low values were defined as being close to either the lower limit of assay quantification or the lower limit of recommended therapeutic range. High values were defined as being close to either the upper limit of assay quantification or the upper limit of recommended therapeutic range.

After analysis at D0, half of the samples with low- and high-drug concentrations were stored between 2 and 8°C and were analyzed again 2 days, 3 days, and 7 days after the first assay (ie, at D2, D3, and D7). The other half of samples were stored at room temperature and were analyzed again 24 hours after the first testing (ie, at D1).

All tests were performed on the Architect Ci4100 analyzer platform (Abbott Laboratories, Chicago, IL). The following drugs were determined by chemiluminescence microparticle immunoassay: digoxin, phenobarbital, phenytoin, theophylline, valproic acid, and vancomycin. Acetaminophen and salicylate were determined by enzymatic colorimetric assay. Amikacine and lithium assays were performed by immunoturbidimetric (PETINIA) and colorimetric methods, respectively. Each assay result was validated by the analysis of commercial quality controls purchased from Bio-Rad Laboratories (Liquicheck TDM controls, Irvine, CA). All results were analyzed graphically and interpreted according to the international recommendations for bioanalytical method validation.[4] Samples were considered stable if the deviation of measured concentrations at $D > 0$ was less than 15% compared with measured concentration at D0.

For all samples stored between 2 and 8°C, the drug concentrations measured at D2, D3, and D7 were within the tolerance range of ±15% of the concentrations at D0. For samples stored at room temperature, all drug concentrations measured 24 hours after collection were also within the 15% tolerance range relative to the concentrations measured on the day of collection. These results were in agreement with those obtained previously during the analytical validation of Abbott assays, for which the variation coefficients of interassay imprecision are less than 15%. No significant change in concentrations was observed for any drug during the study period. Representative results obtained for digoxin and valproic acid are presented in Figure 1.

Drug assays in biological fluids (serum, plasma, or whole blood) are widely used for a variety of therapeutic agents. Therapeutic drug monitoring allows for optimizing the drug therapy for the cure or prevention of disease based on the individual patient characteristics.[1,2] To perform drug assays properly, the stability of drugs in biological fluids under routine conditions must be known. Many studies published in scientific literature looked at drug stability in serum or plasma samples, but for the most part, drug stability was conducted either in enriched biological matrices, or in plasma or serum of patients with drug treatment and previously removed from the primary sampling tube. This does not reflect our current practice. In most laboratories, the plasma sent to the toxicological laboratory is kept in the primary tube after centrifugation, without separation. Also, the Abbott kit insert of most drug assays performed on the Architect mentions the stability of drugs in samples after blood centrifugation and decantation in a secondary tube.[5] To store samples for drug testing in primary tubes after centrifugation, however, has clear advantages as it significantly reduces risk of sample mix-up and processing time.

The results obtained in this study prove that all tested drugs are stable in plasma at low and high concentrations in the primary sampling tube stored up to 7 days between 2 and 8°C and 1 day at room temperature. Acceptance criteria used to prove sample stability were based on clinical relevance. For all drugs, a maximum deviation of ±15% from the initial value was accepted, which is considered to be a clinically nonsignificant difference.

The limitation of this drug sample stability study is that the results are based on small sample size of 12

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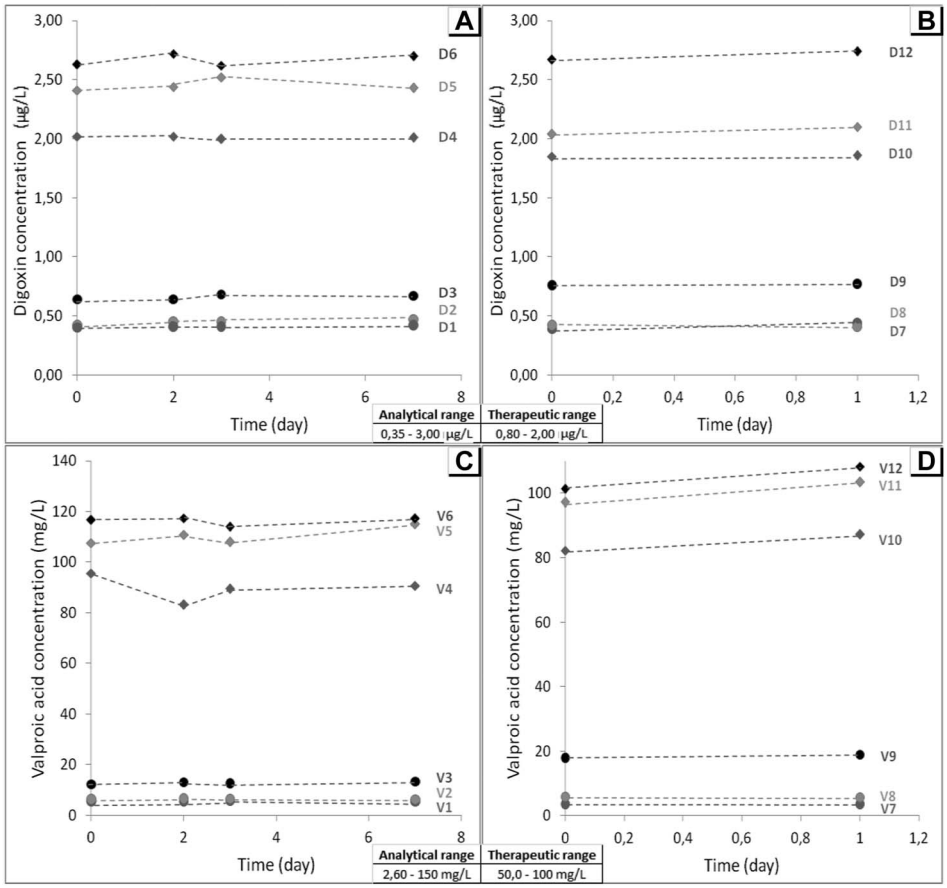

FIGURE 1. Graphical analysis of high and low concentrations of digoxin and valproic acid in samples stored between 2 and 8°C (A, C) and at room temperature versus time (B, D).

specimens per drug tested. Second, all measurements were taken only once for economic and practical reasons. Third, this study addresses the stability of the drugs in samples that are centrifuged immediately after collection, but some facilities do not centrifuge samples before sending them to the laboratory, which is another parameter to be tested.

In conclusion, this study demonstrates that acetaminophen, amikacin, digoxin, lithium, phenobarbital, phenytoin, salicylate, theophylline, valproic acid, and vancomycin are stable over time in human plasma under the usual conditions of sample storage. Their stabilities ensure the quality of toxicological analysis performed on the Architect analyzer platform per the practices of our laboratory, which is essential for the accreditation of these analyses and useful despite the automation used.

Virginie Mistretta, PhD
Raphaël Denooz, PhD
Corinne Charlier, PhD
Laboratory of Clinical, Forensic,
Environmental and Industrial Toxicology,
CHU Sart-Tilman,
University of Liège,
Liège, Belgium.

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