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Godet, Marie; Simar, Joanna; Closset, Mélanie; Hecq, Jean-Daniel; Braibant, Maximilien; Soumoy, Laura; Gillet, Patricia; Jamart, Jacques; Bihin, Benoît; Galanti, Laurence

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Marie Godet, Joanna Simar, Mélanie Closset, Jean-Daniel Hecq*, Maximilien Braibant, Laura Soumoy, Patricia Gillet, Jacques Jamart, Benoît Bihin and Laurence Galanti

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**Abstract**

**Background:** Vancomycin is increasingly administrated by continuous infusion. But the treatment of patient in intensive care need restricted volume to prevent fluid overload. The aim of the study was to evaluate the physical and chemical stability of solutions of a high concentration of vancomycin hydrochloride in 5 % glucose or 0.9 % NaCl.

**Methods:** Eight syringes of 50 mL, containing 41.66 mg/mL of vancomycin hydrochloride four syringes in 5 % glucose and four in 0.9 % NaCl were prepared and stored at ambient temperature during 48 h. Immediately after preparation and during 48 h, vancomycin hydrochloride concentrations were measured by a high-performance liquid chromatography (HPLC). Spectrophotometric absorbance at different wavelengths, pH measurement and microscopic observations were also performed.

**Results:** All solutions were physico-chemically stable during the whole period storage at ambient temperature: no color change, turbidity, precipitation or opacity, no significant pH variations or optic densities were observed in the solutions. Any crystals were seen by microscopic analysis. Solutions are considered chemically stable as the lower limit of the 95 % unilateral confidence interval on the mean remained above 90 % of the initial concentration for at least 48 h.

**Conclusions:** Solutions of vancomycin hydrochloride 41.66 mg/mL in syringe of 5 % glucose or 0.9 % NaCl are physically and chemically stable for at least 48 h when stored in syringes at ambient temperature.

**Keywords**: vancomycin infusions, concentrated solutions, high performance liquid chromatography, physicochemical stability, syringe, intensive care units

## Introduction

Vancomycin, a glycopeptide antibiotic, is often used as antibiotherapy for hospitalized patients [1]. The most common usage of vancomycin is to treat methicillin-resistant Staphylococcus aureus and epidermidis infections [2, 3] and for patients who are allergic to penicillin and cephalosporin. A temporary association with aminoglycoside or rifampicin can be used to obtain a synergic effect in case of endocardite [3].

Vancomycin is increasingly administrated by continuous infusions [4]. As reported in the literature, the infusions of vancomycin (2.5 g/250 mL) can be prepared by the centralized intravenous admixture service (CIVAS) [5–7] of the Pharmacy, according to the stability of the solutions [8–21].

Vancomycin could also be infused by syringe, particularly in intensive care to reduce the perfusion volume. Some data are available about this containers showing that the syringes of low dose vancomycin can be stored at 4 °C for 6 months [12] or at least 84 days [19] and once brought to 25 °C it must be used within 48 h [12]. Nevertheless, the stability of high concentrated solutions are not often investigated. Allen and Stiles [7] demonstrated a stability for 96 h at room temperature for a 40 mg/mL vancomycin while Barbault et al., a stability of vancomycin eye drops at 50 mg/mL for 15 days.

Furthemore, the production of vancomycin (GSK) was stopped requiring the use of another supplier (Mylan). The

***Corresponding author: Jean-Daniel Hecq,** Department of Pharmacy, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, E-mail: jean-daniel.hecq@uclouvain.be
**Marie Godet,** Medical Laboratory, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, E-mail: marie.godet@uclouvain.be
**Joanna Simar, Mélanie Closset,** Medical Laboratory, CHU UCL Namur, Yvoir, Belgium, E-mail: joanna.simar@uclouvain.be; melanie.closset@student.uclouvain.be
**Maximilien Braibant, Laura Soumoy, Patricia Gillet,** Department of Pharmacy, CHU UCL Namur, Yvoir, Belgium, E-mail: maximilien.braibant@uclouvain.be; laura.soumoy@uclouvain.be; patricia.gillet@uclouvain.be
**Jacques Jamart, Benoît Bihin,** Scientific Support Unit, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, E-mail: jacques.jamart@uclouvain.be; benoit.bihin@uclouvain.be
**Laurence Galanti,** Medical Laboratory, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, E-mail: laurence.galanti@uclouvain.be

stability of this new vancomycin was studied at 12.5 mg/ml but not at higher concentrations such as 40 mg/mL.

The aim of the study is to determine the physical and chemical stability of a higher concentration of vancomycin hydrochloride (Mylan) (41.66 mg/mL) in 5 % glucose or 0.9 % NaCl solutions.

# Materials and methods

## Solution preparation

Commercially-available vancomycin hydrochloride solutions (Vancomycine® 1 g Mylan, Hoeillart, Belgium lot B2173) were prepared in a vertical laminar-airflow hood with sterile water-for-injection (Baxter, Lessines, Belgium, lot 14IO2T1E) as recommended by the manufacturers. These solutions were added to polypropylene syringes (Terumo, Haasrode, Belgium; lot 1412283) containing glucose 5 % (Baxter, Lessines, Belgium, lot15A14E46) or NaCl 0.9 % (Baxter, Lessines, Belgium, lot14L01660) to obtain a final volume of 48 mL and then a 41.66 mg/mL concentration solution. The syringes were stored at ambient temperature, without protection from light.

## Standard and quality control solutions

A standard solution of 0.5 g of vancomycin hydrochloride (Vancomycine Mylan® Mylan, Hoeilaart, Belgium lot B2174) were prepared in 10 mL of purified water. This 50 mg/mL solution was diluted in purified water to obtain four standard solutions (40, 30, 20, 10 and 5 mg/mL) and two quality controls (40 mg/mL and 10 mg/mL)

## Chromatographic conditions

As previously described [20], an Alliance Waters high-performance liquid chromatographic (Alliance, model 2695, Waters Association, Milford, MA, USA) system was used with a DAD detector (model 996, Waters Association) and a data acquisition and processing module (Empower 2 Software, Waters Association, Milford, MA, USA).

A reversed phase column was used with associated guard column (Prevail C18 5 µm 150 mm × 4.6 mm, ref 99209 with Prevail guard column C18 7.5 mm × 4.6 mm, ref 99286, Alltech associates, Deerfield IL 60015).

The mobile phase was constituted of 15 % acetonitrile (ref C03C11X, Lab-scan Ltd, Dublin, Ireland) and 85 % $KH_2PO_4$ buffer (pH 3.00; 0.025 M) ($KH_2PO_4$, ref 1.04873.1000, Merck, Darmstadt, Germany and $H_3PO_4$, ref 10G090501, VWR International, Fontenay-sous-Bois, France).

The flow rate was set at 1 mL/min, the column temperature at 35 °C, the Wavelenght (DAD detector) at 210 nm.

## Validation of high-pressure liquid chromatographic method [22]

### Precision

Two control solutions of 10 and 40 mg/mL of vancomycin hydrochloride were undertaken in triplicate to calculate the within (n = 10) and between (n = 9) day reproducibility.

### Linearity of analytical response

Linearity was evaluated by 7 dilutions of the solution (2.5, 5, 10, 20, 30, 40, and 50 mg/mL of vancomycin hydrochloride) injected in triplicate.

### Stability indication

The stability indicating capability of the chromatographic method was assessed using decomposed solutions of drugs. Degraded samples of vancomycin hydrochloride were then assayed to confirm separation of the parent antibiotic from its degradation products. Vancomycin solutions at natural pH (3.72), alkaline pH (11.54) by adding NaOH 5 M (Merck, Darmstadt, Germany), and acidic pH (1.60) by adding HCl 12 M (Merck, Darmstad, Germany) were heating at 100 °C during 30 and 60 min. Ten µL of these solutions was injected in the HPLC system before and after heating.

## Physical stability

Visual compatibility was defined as the absence of particulate formation, haze, precipitation, color change and gas evolution [23]. At each time of the study, various tests were conducted to detect any particle contamination: visual and microscopical inspection and optical density measurements. The samples were then visually inspected

with unaided eye, in front of a black and white background, the pellet obtained after centrifugation at 3000 rpm for 8 min was observed with a microscope 10 × (Carl Zeiss, Germany), looking for crystals.

The potential presence of subvisible particles was investigated by spectrophotometry. The optical densities were then measured by a spectrophotometer (Genesys 10 UV, Spectronic Unican) at 350 nm, 410 nm and 550 nm [24].

The pH of the solutions were measured with a pH-meter (Inolab WTW Weilhein, Germany) equipped with a glass electrode (Biotrode Hamilton Bonaduz, Switzerland) calibrated with two standard solutions at pH4 and pH7 (CertiPur, Merck, Darmstadt, Germany).

## High-pressure liquid chromatographic assay

The five standard solutions, the two quality controls and the samples were all diluted 40-fold and 10 µL of each were injected into the chromatograph.

Results were automatically calculated by interpolation of a five-level calibration curve (linear through zero), performed by the Empower 2 software (Waters Association, Milford, MA, USA) using peak areas versus standard concentrations.

## Stability study

Four syringes of 2 g infusion per 48 mL in 5 % glucose and four syringes of the solutions in 0.9 % NaCl were prepared as described above and stored until 48 h at ambient temperature (range: 18–25 °C). The concentrations of vancomycin hydrochloride were determined in triplicate for each of them immediately after preparation and after 2, 4, 6, 8, 22, 24, 26, 28, 30 and 48 h of storage.

## Statistical analysis

As recommended by the US Food and Drug Administration, solutions were considered stable if the 95 % one-sided lower confidence limit of the concentration remains superior to 90 % of the initial concentration [25] or 95 % of the initial concentration when any signs of physical instability exist [26].

# Results

## Validation of the method

The within and between-day repoducibilities realized on two concentrations (40 mg/mL and 10 mg/mL) are shown in Table 1 and could be considered as acceptable.

**Table 1:** Precision of the assay.

| | | Within-day variation (n = 10) | Between-day variation (n = 9) |
|---|---|---|---|
| 40 mg/mL | Mean (mg/mL) | 41.14 | 39.61 |
| | SD (mg/mL) | 0.98 | 0.9 |
| | CV (%) | 2.4 | 2.3 |
| 10 mg/mL | Mean (mg/mL) | 10.26 | 10.60 |
| | SD (mg/mL) | 0.20 | 0.29 |
| | CV (%) | 2.0 | 2.8 |

Linear-regression analysis of peak area yielded a determination coefficient $r^2 > 0.999$ in the range of 2.5 mg/mL to 50 mg/mL.

The degradation assay showed that the decomposition product peaks were resolved from the peak corresponding to the intact drug in all conditions (Figure 1).

## Physical stability

All solutions were physically stable during the whole period storage at ambient temperature: no color change, turbidity, precipitation or opacity, no significant variation in pH values in 5 % glucose (mean ± SD: 3.09 ± 0.01; minimum: 3.07- maximum: 3.12) and in 0.9 % NaCl (mean ± SD: 3.17 ± 0.01; minimum: 3.15 –maximum: 3.19) or optical densities were observed in the solution. Any crystals were seen by microscopic analysis.

## Chemical stability

Concentrations of vancomycin hydrochloride during storage expressed as percentage of the initial dosage are shown in Table 2. The concentrations of the solutions remain stable with a lower confidence limit superior to 90 % of the initial concentration until 48 h. They are no difference between the solutions of 5 % glucose and 0.9 % sodium chloride.

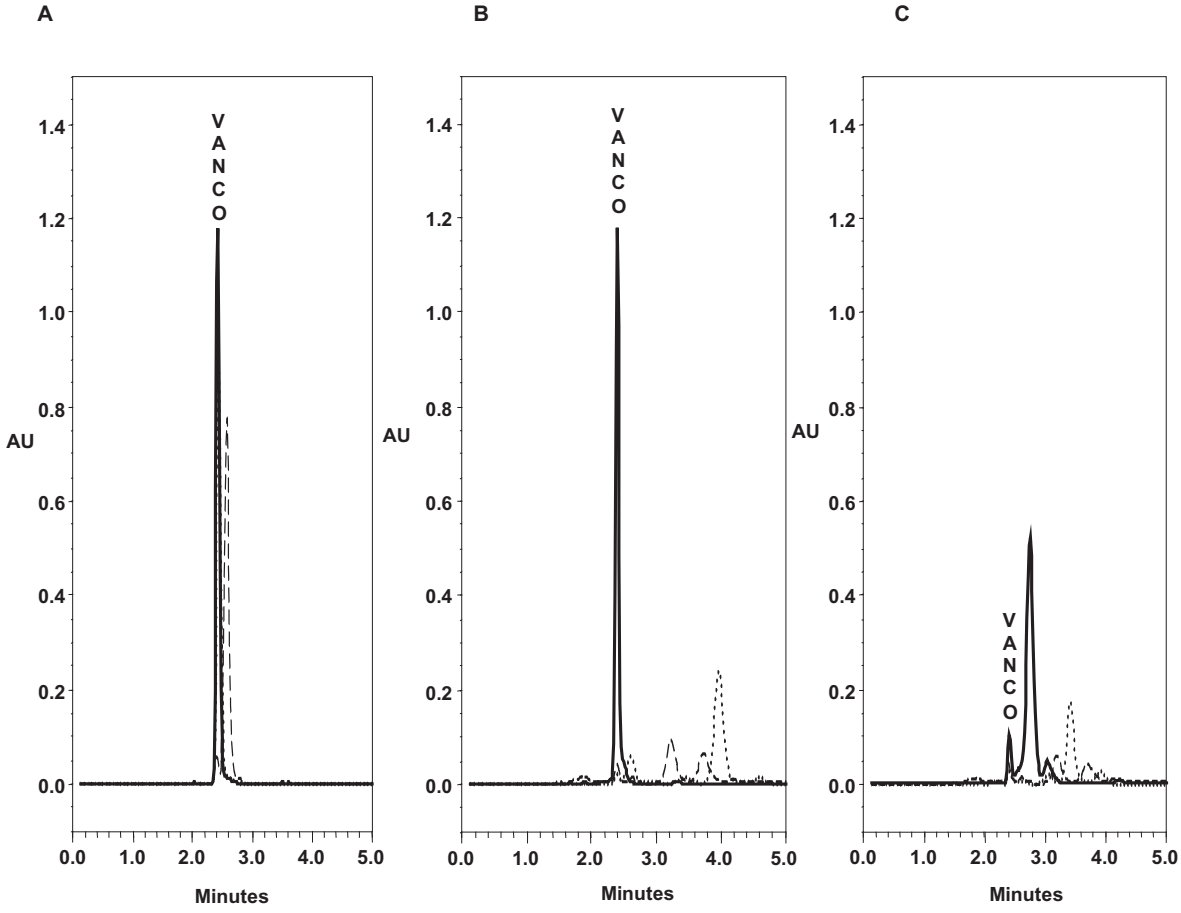


**Figure 1:** Chromatograms showing degradation test. (A) No heating at natural pH(3.72), at acidic pH(1.60) and at alkaline pH (11.54). (B) Heating at 100 °C for 30 min at natural pH(3.72), at acidic pH(1.60) and at alkaline pH (11.54). (C) Heating at 100 °C for 60 min at natural pH (3.72), at acidic pH(1.60) and at alkaline pH (11.54).

**Table 2:** Variation of the vancomycin hydrochloride concentrations.

| Hours | Vancomycin hydrochloride in Glucose 5 % | | Vancomycin hydrochloride NaCl 0.9 % | |
|---|---|---|---|---|
| | Initial concentration ± SD (mg/mL):43.91 ± 7.5 | | Initial concentration: ± SD (mg/mL):42.11 ± 3.73 | |
| | Mean percentage of initial concentration | 95 % lower one-sided confidence limit of mean percentage | Mean percentage of initial concentration | 95 % lower one-sided confidence limit of mean percentage |
| 0 | 100.0 | 99.0 | 100.0 | 97.5 |
| 2 | 99.9 | 98.9 | 100.1 | 97.8 |
| 4 | 99.8 | 98.9 | 100.2 | 98.0 |
| 6 | 99.6 | 98.8 | 100.3 | 98.3 |
| 8 | 99.5 | 98.7 | 100.4 | 98.5 |
| 22 | 98.7 | 98.0 | 101.1 | 99.5 |
| 24 | 98.6 | 97.9 | 101.2 | 99.5 |
| 26 | 98.5 | 97.7 | 101.3 | 99.5 |
| 28 | 98.3 | 97.6 | 101.4 | 99.5 |
| 30 | 98.2 | 97.4 | 101.5 | 99.4 |
| 48 | 97.2 | 95.7 | 102.4 | 98.7 |

## Discussion

Vancomycin is often used as antibiotherapy for hospitalized patients [1] and is increasingly administrated by continuous infusions [4].

The total volume of infusion administrated to patients admitted into intensive care units is often important [27], the importance of fluid balance and the prevention of fluid overload in many disease states remaining a prevalent discussion in this unit [28]. One possible way to avoid such type of adverse effects is to reduce the global volume of infusion by using drugs solution within higher concentrations. Concentrated solutions (2 g of vancomycin in 48 mL of solution) are then often needed to be administrated to the critical ill or injured patients. High concentrations of vancomycin being deleterious for venous system, particularly in case of extravasation, the solution must be administrated through a central line, the most often present in hospitalized patients in intensive care.

Data on the stability of a new commercial vancomycin (Mylan, Hoeilaart, Belgium) were poorly investigated, particularly for concentrated solutions [29, 30].

According to these results, solutions of vancomycin hydrochloride (Mylan, Hoeillart, Belgium) 41.66 mg/mL in 5% glucose or 0.9% NaCl are physically and chemically stable at least for 48 h when stored in syringes at ambient temperature, without protection from light. The final volume of 48 mL was preferred to 50 mL as it adjusts easily the hourly flow rate of the syringe pump.

These results are in accordance with previous results with other manufacturers or solutions [8, 31, 32] adds to the list of our systematic study of the chemical stability of long-term intravenous medication solutions ready for use [33].

## Conclusion

According to this study, solutions of vancomycin hydrochloride 41.66 mg/mL in 5% glucose or 0.9% NaCl are physically and chemically stable at least for 48 h when stored in syringes at ambient temperature. This concentration may be used in ICU to treat patient and prevent fluid overload.

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## Bionotes

**Marie Godet**
Medical Laboratory, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium,
**marie.godet@uclouvain.be**

Marie Godet is PhD in agronomical sciences (2010). She was in charge of the chromatography sector (until 2017) at the department of clinical biochemistry of the medicine laboratory at CHU UCL Namur. She is collaborating on stability studies about injectable drugs in collaboration with the department of pharmacy.

**Joanna Simar**
Medical Laboratory, CHU UCL Namur, Yvoir, Belgium, **joanna.simar@uclouvain.be**

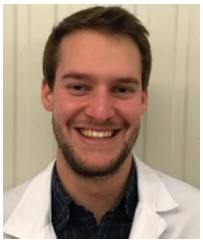
Joanna Simar is a medical doctor (2012). She spent one year (2014) working at the department of clinical biochemistry of the medicine laboratory at CHU UCL Namur. She has collaborated with the department of pharmacy on stability studies about injectable drugs.

**Mélanie Closset**
Medical Laboratory, CHU UCL Namur, Yvoir, Belgium,
**melanie.closset@student.uclouvain.be**

Mélanie Closset is a medical doctor (2009) with a master degree in ethic. She's working at the department of clinical biochemistry of the medicine laboratory at CHU UCL Namur. She is collaborating on stability studies about injectable drugs in collaboration with the department of pharmacy.

**Jean-Daniel Hecq**
Department of Pharmacy, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, **jean-daniel.hecq@uclouvain.be**

Professor Jean-Daniel Hecq is Hospital pharmacist and doctor in pharmaceutical sciences. He works at the CHU UCL Namur since 1979 and is chief of the department of Pharmacy since 1989. He is also the coordinator of the Drug Stability Research Group in the same CHU and the author since 35 years of a Belgian electronic database so entitled “Stability of injectable drugs in infusion”.



**Maximilien Braibant**
Department of Pharmacy, CHU UCL Namur, Yvoir, Belgium,
**maximilien.braibant@uclouvain.be**

Maximilien Braibant is a hospital pharmacist. He has been working at the CHU UCL Namur since 2017. He is working as a clinical pharmacist in orthopedic surgical wards and in monitoring patients under new direct oral anticoagulants (DOACs).

**Laura Soumoy**
Department of Pharmacy, CHU UCL Namur, Yvoir, Belgium, **laura.soumoy@uclouvain.be**

Laura Soumoy is a hospital pharmacist since 2014. She has been working at the CHU UCL Namur since 2014 and is now chief of Production sector, sterile (chemotherapy, parenteral nutrition and antibiotherapy) and non-sterile (2017). Since 2017, she is also a member of the Drug Stability Research Group in the same CHU.

**Patricia Gillet**
Department of Pharmacy, CHU UCL Namur, Yvoir, Belgium, **patricia.gillet@uclouvain.be**

Patricia Gillet is hospital pharmacist. She has been working at the CHU UCL Namur since 1994. She was in charge of Production sector, sterile (chemotherapy, parenteral nutrition and antibiotherapy) and non-sterile since 2009 to 2017. Currently, she teaches pharmacology to pharmacy assistants in technical school.

**Jacques Jamart**
Scientific Support Unit, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, **jacques.jamart@uclouvain.be**

Jacques Jamart is medical doctor (1972) and surgeon (1978) then received a master degree in statistics (1984). He worked in experimental surgery mainly in the field of organ preservation for transplantation and more recently as biostatistician and head of Scientific Support Unit (1993) of University Hospital of Mont-Godinne. He is also professor at Catholic University of Louvain (2009) and at University of Namur (2010).

**Benoît Bihin**
Scientific Support Unit, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium, **benoit.bihin@uclouvain.be**

Benoît Bihin is biologist. PhD Student at the university of Namur since 2011, he is also lecturer of biostatistics courses. He works at the CHU UCL Namur as biostatistician since 2014 and is a member of the Drug Stability Research Group.

**Laurence Galanti**
Medical Laboratory, CHU UCL Namur, Yvoir, Belgium; Drug Stability Research Group, CHU UCL Namur, Yvoir, Belgium,
**laurence.galanti@uclouvain.be**

Professor Laurence Galanti, MD, PhD is co-director of the department of laboratory medicine at CHU UCL Namur, in charge of clinical biochemistry on the site of Mont-Godinne. She holds several positions in national and international scientific organizations. Her research focused on clinical application of biomarkers of exposition to tobacco smoke and on the stability of injectable drugs in collaboration with the department of pharmacy.