

## Stability studies of amoxicillin/clavulanic acid combination in polyolefin infusion bags

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### ABSTRACT

**Study objectives:** To study the physical compatibility and chemical stability of amoxicillin, a  $\beta$ -lactam antibiotic combined with clavulanic acid, a  $\beta$ -lactamase inhibitor, using a stability-indicating high-performance liquid chromatographic (HPLC) assay.

**Methods:** The study samples were prepared by adding amoxicillin/clavulanic acid to 0.9% sodium chloride solution in polyolefin bags. The contents of the bags were studied after storage under the following conditions: at ambient temperature without protection from light and at 4°C with protection from light for 72 hours. Both compounds were considered stable if they retained  $\geq 90\%$  of the baseline drug concentration. Three dosages (two adult forms: 2 g/200 mg and 1 g/200 mg, and one paediatric form: 0.5 g/50 mg) were examined under laboratory conditions simulating those used routinely in hospitals. Evaluations for physical compatibility and chemical stability were performed initially and during the storage period. The physical compatibility was assessed using visual observation for signs of discoloration and precipitation at each sampling interval. The optical density was measured to give a measurable reading of the colour; pH values of solutions were also measured. The chemical stability of the drugs was evaluated by using a stability-indicating HPLC assay.

**Results:** When compared with ambient or refrigerated storage conditions, amoxicillin, whatever the studied preparation, was stable for a longer duration than clavulanic acid. The mixture was only stable for four hours at ambient temperature and for eight hours at 4°C. However, we noticed a colour change (from light to dark yellow) of the various reconstituted solutions, undoubtedly because of the pH variations.

**Conclusion:** We recommend that these solutions be kept refrigerated whenever possible.

### KEYWORDS

Amoxicillin, clavulanic acid, co-amoxiclav, HPLC, polyolefin bags, stability

### INTRODUCTION

The aim of the study was to assess the stability of amoxicillin combined with clavulanic acid (adult and paediatric forms) in 0.9% sodium chloride solution stored in polyolefin bags at room temperature without protection from light, and at 4°C with protection from light for 72 hours.

Amoxicillin is an antibiotic belonging to the penicillin class. It is a moderate-spectrum antibiotic active against

a wide range of Gram-positive, and a limited range of Gram-negative organisms. The inactivation of amoxicillin by  $\beta$ -lactamases produced by Gram-negative anaerobic bacteria can be circumvented by the addition of clavulanic acid, a  $\beta$ -lactamase inhibitor [1].

Clavulanic acid is a  $\beta$ -lactam, structurally related to the penicillins, which possesses the ability to inactivate a wide range of  $\beta$ -lactamase enzymes commonly found in micro-organisms resistant to penicillins and cephalosporins [2, 3]. It has good activity against the clinically important plasmid-mediated  $\beta$ -lactamases frequently responsible for transferred drug resistance [4].

Hence, the formulation of amoxicillin with clavulanic acid (co-amoxiclav) protects amoxicillin from degradation by  $\beta$ -lactamase enzymes and effectively extends the antibiotic spectrum to include many bacteria normally resistant to amoxicillin.

Co-amoxiclav is effective against susceptible bacteria causing infections of the middle ear, tonsillitis, throat infections, laryngitis, bronchitis, sinusitis and pneumonia. It is also used to treat urinary tract infections or skin infections [3].

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To reduce expenses, hospitals, as well as private practitioners, frequently use generic drugs. So, we studied the stability and the compatibility of the generic co-amoxiclav (Merck Generics, Lyon, France) in polyolefin infusion bags (Macoflex N, MacoPharma, Mouvoux, France) under various conditions. Co-amoxiclav can be given orally or by IV injection. Because this drug combination is often administered intravenously to hospital patients, advance preparation of the IV solutions can be useful to improve safety, time management and speed of drug delivery. Indeed, papers about long-term storage of drugs (either by freezing or at 4°C) with advance reconstitution have been published [5-10]. However, interactions can occur between drugs and containers, with the possible adsorption of drugs on to the inner plastic surface of the container, leading to loss of drug and thereby a decrease in concentration [11-13]. Therefore, for each plastic material used, and each drug, it is necessary to carry out some tests for compatibility and stability. Amoxicillin and clavulanic acid determinations have already been described [14-16]. The method proposed here is also applicable for routine analysis as well as quantitative determinations. The method has also proved to be suitable as a rapid and reliable quality control method.

This paper describes the stability and compatibility of co-amoxiclav injection in a ready-to-use 0.9% saline solution after storage at ambient temperature without protection from light, and at 4°C with protection from light. In the same way, after preparation, the reconstituted solutions were subjected to aggressive treatments to reproduce certain unfavourable conditions (heating, freezing, acidic or alkaline treatments) in order to determine if the stability of the mixture would be compromised. Samples were visually inspected, tested for pH, and the concentrations of each component in the binary mixture were determined by a stability-indicating HPLC method.

## MATERIALS AND METHODS

### Chemicals and drugs

HPLC-grade acetonitrile was obtained from Prolabo (Paris, France). Analytical grade orthophosphoric acid was purchased from SDS (Peypin, France). The water used to prepare aqueous buffers and dilutions was de-ionised and purified (Milli-Q, Millipore, Saint-Quentin-Yvelines, France).

Three strengths of co-amoxiclav injection were studied:

- Co-amoxiclav vials of 2 g/200 mg sterile powder for IV use (adult form)
- Co-amoxiclav vials of 1 g/200 mg sterile powder for IV use (adult form)
- Co-amoxiclav vials of 500 mg/50 mg sterile powder for IV use (paediatric form)

Paracetamol was chosen as an internal standard; the commercial product Perfalgan (Bristol-Myers Squibb, Rueil-Malmaison, France) of clinical standard was used.

### Materials and chromatographic conditions

HPLC, a spectrophotometer and a pH-meter were used to conduct the analyses. Chromatographic analyses were performed using an HPLC system (Kontron Instruments, Saint-Quentin-Yvelines, France) equipped with a pump and a UV-VIS detector operating at suitable wavelengths. Solutions of the compounds to be studied were prepared with 0.9% sodium chloride and samples were introduced by manual injection with a Hamilton Rheodyne Syringe (250 µL) in a valve of 20 µL loop. Retention times and peak areas were determined by a computer connected to an Epson LX 800 terminal printer. Analyses of the drugs were performed on a 5 µm Symmetry C18 column (150 mm × 4.6 mm) (Waters, Milford, Massachusetts, USA) operating at room temperature.

Amoxicillin and clavulanic acid concentrations were determined by a stability-indicating HPLC assay. Separation of the compounds was based on an isocratic method using a mobile phase consisting of acetonitrile and aqueous buffer (orthophosphoric acid pure solution 0.06%) mixture (10:90, v/v). After being degassed in a helium stream and filtered (0.45 µm, Millipore), the mobile phase was pumped through the column at a flow rate of 1 mL/minute. A total of 20 µL of each sample was injected into the analytical column and the detector was set at 215 nm.

Amoxicillin and clavulanic acid calibration curves were constructed at concentration ranges of 20–80 µg/mL and 4–16 µg/mL respectively. Standard stock solutions (20 mg/mL and 4 mg/mL) of both amoxicillin and clavulanic acid were prepared in 0.9% sodium chloride. A set of dilutions at the desired concentrations were prepared for injection into the column. The internal standard (paracetamol) was prepared with a concentration of 20 µg/mL. The precision of the assays were validated by establishing the within-day and the between-day coefficients of variation. Discoloration of the various reconstituted solutions was investigated using a double-beam UV/Vis Uvikon 923 spectrophotometer (Kontron Instruments, Saint-Quentin-Yvelines, France) operating at room temperature. The detector was set at 420 nm.

The pH-meter, used to measure the pH of the solutions during storage, was a model HI 8520 N microprocessor equipped with a Micro pH electrode HI 1083 (Hanna Instruments, Lingolstein, France) and calibrated with standard buffer solutions at pH 4.0, 7.0 and 10.0.

## Preparation of solutions and storage conditions

PVC-free polyolefin infusion bags (Macoflex N), containing 0.9% sodium chloride injection, were kindly provided by MacoPharma Laboratories (Mouvoux, France).

Infusion bags containing 50 mL and 100 mL of 0.9% sodium chloride solution were used and a known amount of drug was added to achieve the desired concentrations.

A Macoset transfer system (accessories facilitating and making perfusion safer) was provided by MacoPharma Laboratories (Mouvoux, France). The system is available with two diameters (20 mm and 30 mm) for easy adjustment to the bottle and to make reconstitution of medicine easier in a closed system. The injection site combined with the Macoset transfer system provides a closed reconstitution system. It prevents the penetration of air from the outside and therefore reduces the risk of contamination by the environment.

Three commercially available strengths of powdered co-amoxiclav were studied under various conditions. The reconstitution of the drugs was carried out under laboratory conditions, simulating those used routinely in hospitals, using a laminar-airflow hood.

- The commercially available vial of 2 g amoxicillin/200 mg clavulanic acid (adult form) was added to 100 mL of 0.9% sodium chloride in a polyolefin bag using the Macoset transfer system to produce a solution containing 20 mg/mL of amoxicillin and 2 mg/mL of clavulanic acid.
- The commercially available vial of 1 g amoxicillin/200 mg clavulanic acid (adult form) was added to 50 mL of 0.9% sodium chloride in a polyolefin bag using the Macoset transfer system to produce a solution containing 20 mg/mL of amoxicillin and 4 mg/mL of clavulanic acid.
- The commercially available vial of 0.5 g amoxicillin/50 mg clavulanic acid (paediatric form) was added to 25 mL of 0.9% sodium chloride in a polyolefin bag using the Macoset transfer system to produce a solution containing 20 mg/mL of amoxicillin and 2 mg/mL of clavulanic acid.

For the paediatric form, it was first necessary to withdraw 25 mL of sodium chloride 0.9% from a 50 mL polyolefin bag before reconstitution. In all cases, the final concentration of amoxicillin was identical.

The bags containing the drug solutions were agitated by flexing, massaging and shaking to simulate the agitation that a bag can undergo during preparation, transportation and administration.

The stability of drugs was studied after storing the bags at room temperature (20–25°C) without protection from light, and at 4°C with protection from light, for 72 hours by a stability-indicating HPLC method.

## Effects of freezing, heating, acidic and alkaline treatments on the stability of co-amoxiclav

Long-term storage of drugs (by freezing or under refrigeration) prepared in advance has been studied [5–9]. Amoxicillin is an antibiotic which degrades easily and, as a consequence, any manipulation of the active drug can lead to the formation of different degradation products [16].

Our study was carried out using the adult dosage (co-amoxiclav 2 g/200 mg). Reconstituted solutions were made as described previously. Several situations were studied in order to determine if the stability of the preparation would be compromised.

- Three bags (each with 20 mg and 2 mg per mL of amoxicillin and clavulanic acid respectively) were prepared as described above. Immediately, samples were removed to determine the initial concentration ( $T_0$ ). The bags were agitated and stored at -20°C for 72 hours. One by one, the bags were thawed at specified time intervals (24 h, 48 h and 72 h) and samples were withdrawn for HPLC analysis.
- Three other bags were prepared as described above. Samples (10 mL) were withdrawn from each bag and treated either with hydrochloric acid 1 N (v/v) or sodium hydroxide 1 N (v/v) in polypropylene tubes and kept at room temperature for two hours. Then, at specified time intervals (30 min, 60 min, 120 min) an aliquot of solution (1 mL) was withdrawn from the tubes up to the end of the storage period for HPLC analysis.
- As described previously two other bags were prepared. Samples (5 mL) were withdrawn from each bag and placed in polypropylene tubes. Tubes containing the solutions were placed in a water bath at 60°C for one hour. An aliquot of solution (1 mL) was withdrawn from the tubes at specified time intervals (10 min, 30 min, 60 min) throughout the study period for HPLC analysis.

## Visual, optical density and pH determinations

Immediately after preparation of the solutions, and at specified time intervals, the bags were agitated and samples were withdrawn and placed in clear glass tubes. Samples were visually inspected for clarity, precipitation and colour change. In the same way, the optical density was measured to determine the intensity of colouring; pH values of solutions were also measured.

## RESULTS AND DISCUSSION

### Chromatography

Amoxicillin and clavulanic acid concentrations were determined by using a stability-indicating HPLC assay. All assays were performed isocratically at ambient temperature. The compounds were resolved with satisfactory baseline

separation under developed conditions. Another assay checked the absence of interference by the excipients used in the pharmaceutical preparation of co-amoxiclav as well as the absence of interference of the degradation products.

For amoxicillin, a good linear response was found between the peak area-ratio and the concentration, with a correlation coefficient better than 0.999 ( $y = 0.590x - 0.033$ ).

The within-day coefficients of variation for replicate analysis ( $n = 6$ ) of four different concentrations (20, 40, 50, 80  $\mu\text{g/mL}$ ) averaged 1.90%, 1.60%, 0.95% and 0.48% respectively. The between-day coefficient of variation ( $n = 8$ ) was less than 1.05%.

In the same way, a good linear response was found for clavulanic acid assays. The correlation coefficient was better than 0.999 ( $y = 0.0545x + 0.024$ ) for calibration curves (4–16  $\mu\text{g/mL}$ ).

The within-day coefficients of variation for replicate analysis ( $n = 6$ ) of four different concentrations (4, 8, 10 and 16  $\mu\text{g/mL}$ ) averaged 3.90%, 3.15%, 2.92% and 2.24% respectively. The between-day precision ( $n = 8$ ) averaged 2.98%.

The retention times for amoxicillin, clavulanic acid and the internal standard were 2.54 min, 3.45 min and 4.30 min respectively.

## Stability studies after preservation of bags at ambient temperature and at 4°C

### Stability of co-amoxiclav at ambient temperature

The analysis of each sample was performed by HPLC after suitable dilution in the mobile phase in order to fit the calibration curves. Paracetamol, the internal standard, was added just before injection into the Rheodyne loop.

At time zero, the initial concentration of co-amoxiclav was designated as 100% and all subsequent measured concentrations were expressed as percentages of the initial concentration. Where applicable, the stability studies followed the European guidelines on stability of existing active substances and related finished products [17]. The stability was defined as a concentration within 90–105% of the initial one. Therefore a decrease from mean initial concentration ( $T_0$ ) of more than 10% was considered to represent a significant loss of drug [18, 19]. Changes to these concentrations were assessed throughout the storage period (up to 72 hours). The values measured of both amoxicillin and clavulanic acid were expressed as a percentage of the initial drug concentrations.

Tables 1, 2 and 3 show the remaining concentrations of both amoxicillin and clavulanic acid in polyolefin bags after

storage at ambient temperature. For all the preparations studied, amoxicillin concentrations remained above 93% of the initial value, and most were near 100% during the first six hours of storage. Remaining concentrations of clavulanic acid after four hours' storage averaged 94%.

Losses in amoxicillin were observed after six hours of storage. They averaged 16% after eight hours of storage. During a 72-hour period of storage, remaining concentrations were 43% of the initial concentration in polyolefin bags.

After six hours' storage, about 17% of clavulanic acid concentration was lost. After 72 hours of storage, there was no trace of clavulanic acid in the various preparations.

No difference was noticed between both adult and paediatric preparations. Amoxicillin and clavulanic acid concentrations remained stable for up to six hours and four hours respectively in polyolefin bags, whichever the preparation studied.

For all samples, after visual inspection, there was no visible evidence of precipitation or gas formation throughout the storage period. However, we noticed a change in colour (from light to dark yellow) of the various reconstituted solutions after storage at room temperature without protection from light. During four hours of storage, preparations were clear and yellow when viewed in normal room light. The intensity of colour (measurement of the optical density) was monitored by a spectrophotometer operating at 420 nm (see Figure 1).

Average pH measurements are given in Figure 2. For all samples, the pH of the various solutions started to decrease after four hours of storage. During the same period of time,

**Table 1: Remaining concentrations of amoxicillin and clavulanic acid (2 g/200 mg/100 mL)**

Time (hours)	Amoxicillin %	Clavulanic acid %
$T_0$	100.00 +/- 0.00	100.00 +/- 0.00
$T_0 + 1$	100.50 +/- 1.70	102.30 +/- 4.80
$T_0 + 2$	100.90 +/- 1.10	98.10 +/- 3.00
$T_0 + 4$	98.30 +/- 2.85	95.20 +/- 3.70
$T_0 + 6$	93.00 +/- 2.70	78.60 +/- 2.90
$T_0 + 8$	87.00 +/- 4.00	64.40 +/- 3.10
$T_0 + 24$	65.90 +/- 1.20	0.00 +/- 0.00
$T_0 + 48$	54.00 +/- 2.20	
$T_0 + 72$	44.90 +/- 3.20	

Storage was in polyolefin bags at room temperature for 72 hours. Results are an average of five measurements.

**Table 2: Remaining concentrations of amoxicillin and clavulanic acid (1 g/200 mg/50 mL)**

Time (hours)	Amoxicillin %	Clavulanic acid %
T <sub>0</sub>	100.00 +/- 0.00	100.00 +/- 0.00
T <sub>0</sub> + 1	102.20 +/- 2.90	100.40 +/- 2.85
T <sub>0</sub> + 2	98.30 +/- 1.40	95.80 +/- 3.30
T <sub>0</sub> + 4	99.10 +/- 1.70	95.70 +/- 5.20
T <sub>0</sub> + 6	95.00 +/- 2.80	86.00 +/- 3.10
T <sub>0</sub> + 8	81.25 +/- 3.20	72.40 +/- 1.80
T <sub>0</sub> + 24	54.90 +/- 3.80	27.30 +/- 5.80
T <sub>0</sub> + 48	46.20 +/- 4.70	0.00 +/- 0.00
T <sub>0</sub> + 72	37.40 +/- 4.20	

Storage was in polyolefin bags at room temperature for 72 hours. Results are an average of five measurements.

the preparations took on an intense yellow colouring. These findings indicated that the intensity of the colouring of the various solutions is directly related to the pH decrease.

We therefore recommend that when preparations are to be stored under ambient storage conditions without protection from light, they should be used in the first four hours after reconstitution whenever possible.

### Stability of co-amoxiclav after storage at 4°C

When the reconstituted solutions of amoxicillin combined with clavulanic acid were stored at 4°C with protection from light, the remaining concentrations of drug were determined by a suitable method discussed previously.

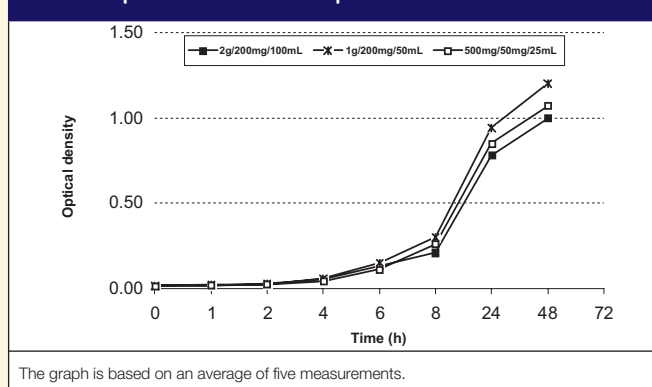
Tables 4, 5 and 6 show the changes to the remaining amounts of both amoxicillin and clavulanic acid in the

**Table 3: Remaining concentrations of amoxicillin and clavulanic acid (500 mg/50 mg/25 mL)**

Time (hours)	Amoxicillin %	Clavulanic acid %
T <sub>0</sub>	100.00 +/- 0.00	100.00 +/- 0.00
T <sub>0</sub> + 1	100.10 +/- 5.05	97.50 +/- 2.50
T <sub>0</sub> + 2	97.20 +/- 4.70	96.00 +/- 0.00
T <sub>0</sub> + 4	96.40 +/- 2.50	92.00 +/- 1.00
T <sub>0</sub> + 6	94.90 +/- 2.20	84.70 +/- 3.40
T <sub>0</sub> + 8	83.20 +/- 3.10	60.40 +/- 3.90
T <sub>0</sub> + 24	66.70 +/- 2.30	0.00 +/- 0.00
T <sub>0</sub> + 48	55.50 +/- 4.70	
T <sub>0</sub> + 72	46.50 +/- 2.10	

Storage was in polyolefin bags at room temperature for 72 hours. Results are an average of five measurements.

**Figure 1: Evolution of optical density during the studied period at room temperature**



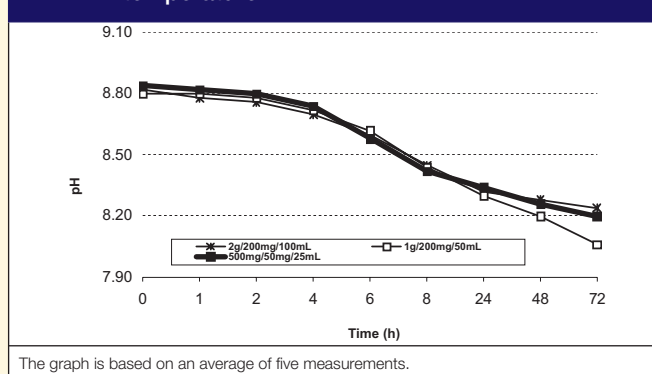
mixture at various time points. With regard to the results obtained previously, storage at 4°C allows an increase in the duration of the stability of the preparations. Whatever the studied preparation, amoxicillin concentrations remained above 94% of the initial value, and most were near 100% during the first 10 hours of storage. Similarly, the remaining concentrations of clavulanic acid after eight hours' storage averaged 97%.

When compared with ambient storage conditions, refrigeration allows the shelf life of the mixture to double.

Beyond eight hours and 10 hours of storage, a respective decrease in the concentrations of clavulanic acid and amoxicillin occurred. During a 72-hour period of storage, remaining concentrations of amoxicillin averaged 69%. However, no clavulanic acid remained in solution.

During the same period, we observed a colour change in the various preparations. This change is correlated to the decline of pH values (see Figures 3 and 4). There was no visible evidence of precipitation or gas formation throughout the storage period. No difference was noticed between

**Figure 2: Change in pH during the studied period at room temperature**



**Table 4: Remaining concentrations of amoxicillin and clavulanic acid (2 g/200 mg/100 mL) at 4°C**

Time (hours)	Amoxicillin %	Clavulanic acid %
T <sub>0</sub>	100.00 +/- 0.00	100.00 +/- 0.00
T <sub>0</sub> + 1	102.10 +/- 3.30	98.20 +/- 3.65
T <sub>0</sub> + 2	101.00 +/- 2.40	100.30 +/- 0.60
T <sub>0</sub> + 4	98.40 +/- 1.90	99.20 +/- 3.05
T <sub>0</sub> + 6	100.40 +/- 2.70	98.90 +/- 2.80
T <sub>0</sub> + 8	100.20 +/- 1.30	96.30 +/- 3.60
T <sub>0</sub> + 10	97.30 +/- 2.40	84.10 +/- 2.20
T <sub>0</sub> + 14	89.80 +/- 1.50	70.20 +/- 1.30
T <sub>0</sub> + 24	85.70 +/- 3.30	58.80 +/- 5.20
T <sub>0</sub> + 48	80.25 +/- 2.90	0.00 +/- 0.00
T <sub>0</sub> + 72	74.10 +/- 2.60	

Storage was in polyolefin bags at 4°C for 72 hours. Results are an average of five measurements.

both adult and paediatric preparations. According to the results obtained, the various preparations can be kept at 4°C in polyolefin bags and used within eight hours.

During the chromatographic analyses, no additional peak corresponding to degradation products was observed in the chromatograms. Therefore, losses of both drugs were not because of degradation, since the assay method used is specific and can detect degradation compounds. Losses of the drugs would doubtless be because of a phenomenon occurring in the bag. The main problem is the possible adsorption or absorption of drugs on to the

**Table 5: Remaining concentrations of amoxicillin and clavulanic acid (1 g/200 mg/50 mL) at 4°C**

Time (hours)	Amoxicillin %	Clavulanic acid %
T <sub>0</sub>	100.00 +/- 0.00	100.00 +/- 0.00
T <sub>0</sub> + 1	102.70 +/- 3.05	101.60 +/- 1.70
T <sub>0</sub> + 2	102.00 +/- 2.60	100.80 +/- 2.90
T <sub>0</sub> + 4	99.50 +/- 2.90	101.10 +/- 2.60
T <sub>0</sub> + 6	97.60 +/- 1.60	100.00 +/- 3.50
T <sub>0</sub> + 8	99.40 +/- 5.50	98.50 +/- 2.90
T <sub>0</sub> + 10	94.20 +/- 2.20	82.50 +/- 1.20
T <sub>0</sub> + 14	85.70 +/- 2.80	67.20 +/- 1.80
T <sub>0</sub> + 24	82.60 +/- 0.90	56.00 +/- 4.80
T <sub>0</sub> + 48	71.00 +/- 4.50	0.00 +/- 0.00
T <sub>0</sub> + 72	64.00 +/- 3.20	

Storage was in polyolefin bags at 4°C for 72 hours. Results are an average of five measurements.

**Table 6: Remaining concentrations of amoxicillin and clavulanic acid (500 mg/50 mg/25 mL) at 4°C**

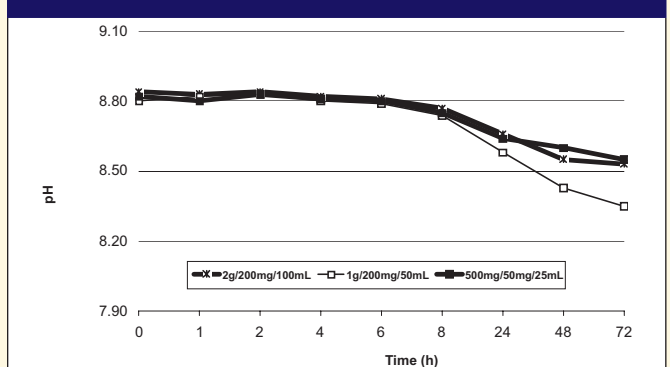
Time (hours)	Amoxicillin %	Clavulanic acid %
T <sub>0</sub>	100.00 +/- 0.00	100.00 +/- 0.00
T <sub>0</sub> + 1	101.80 +/- 3.90	100.60 +/- 2.20
T <sub>0</sub> + 2	102.00 +/- 3.20	98.70 +/- 3.20
T <sub>0</sub> + 4	100.00 +/- 3.10	99.50 +/- 2.80
T <sub>0</sub> + 6	100.00 +/- 2.40	100.00 +/- 0.90
T <sub>0</sub> + 8	99.90 +/- 1.20	97.20 +/- 4.20
T <sub>0</sub> + 10	98.00 +/- 0.00	86.40 +/- 1.50
T <sub>0</sub> + 14	91.10 +/- 1.50	72.80 +/- 2.20
T <sub>0</sub> + 24	87.90 +/- 2.20	63.20 +/- 2.60
T <sub>0</sub> + 48	80.20 +/- 2.90	0.00 +/- 0.00
T <sub>0</sub> + 72	70.20 +/- 3.40	

Storage was in polyolefin bags at 4°C for 72 hours.

inner surface of the container leading to a loss of drugs and a decrease in the injection concentrations. This could lead to therapeutic consequences because patients would not receive the prescribed dose [11-13].

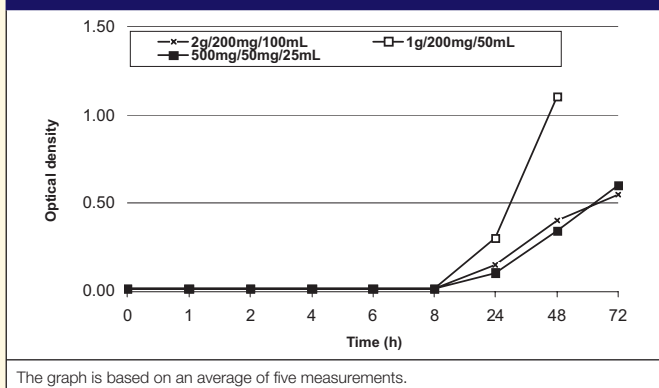
That is why, for every plastic material intended for use as a container, and for each drug to be contained in it, it is necessary to carry out compatibility and stability tests. Indeed, the nature of the plastic material is the most important factor, because it determines the nature and the amount of drug binding. However, it is recognised that PVC causes most interactions with drugs, whereas polyolefin material is considered more compatible [13]. Therefore, polyolefin bags are a good alternative in the preparation and the storage of co-amoxiclav formulations. With regard to the results obtained, other phenomena (e.g. oxidation and hydrolysis), which should be determined, would be the cause of drugs losses in polyolefin bags.

**Figure 3: Change in pH during studied period at 4°C**



The graph is based on an average of five measurements.

**Figure 4: Changes in optical density during the studied period at 4°C**

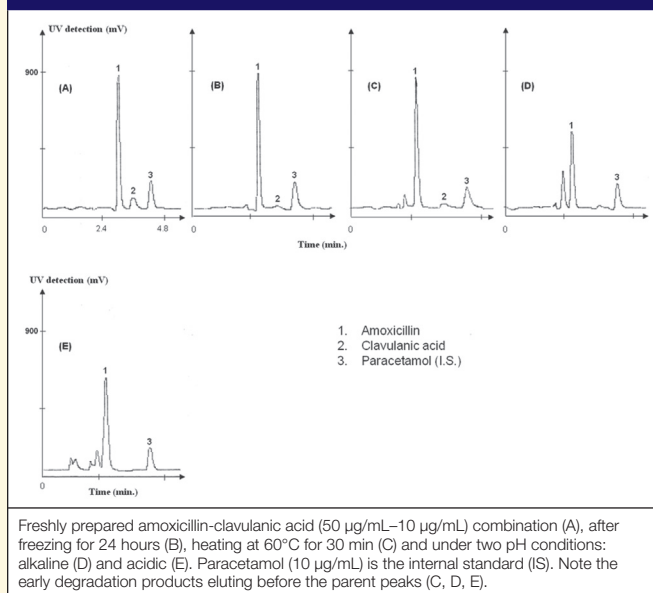


This type of study is important for all pharmaceutical products reconstituted in bags and could be made for all drugs stored either at the ambient temperature or at 4°C after reconstitution.

### Effects of freezing, heating, acidic and alkaline treatments on the stability of co-amoxiclav mixture

Losses in drug concentrations are important and variable according to the treatment used. Whatever the stress-testing study (heating, acidic or alkaline treatment), we noted a loss in concentration of both amoxicillin and clavulanic acid within 30 minutes, and consequently a decrease in the stability of the preparation (see Table 7). At the same time, we observed a colour change of the various preparations treated. During chromatographic analyses, additional peaks corresponding to breakdown products appeared on chromatograms, but none of these peaks interfered with the peak corresponding to

**Figure 5: Chromatograms of amoxicillin-clavulanic acid under various conditions**



the active drugs (see Figure 5). Freezing also caused a loss in concentration of both compounds within 24 hours, but no colouring occurred in the preparation and no additional peaks were observed on chromatograms.

The results obtained showed that co-amoxiclav in saline solution should not be prepared and frozen in advance, or mixed with any other drug causing either an increase in pH (>8.80) or a decrease (<8.80). Similarly, the preparation should never be exposed to temperatures exceeding 50°C. It seems that pH variations are not the only factor responsible for discolouration of preparations and therefore, the loss of the stability of the mixture. Daylight must also be taken into account.

### CONCLUSION

Co-amoxiclav in 0.9% sodium chloride solution stored in polyolefin bags at room temperature without protection from light, and at 4°C with protection from light for 72 hours, showed a marked difference in stability. Stored at ambient temperature, the mixture remained stable for four hours. On the other hand, at 4°C the mixture remained stable for up to eight hours. However, we noted a colour change (from light to dark yellow) in the various reconstituted solutions, undoubtedly because of the variation in pH.

**Table 7: Remaining concentrations of amoxicillin and clavulanic acid after extreme treatment**

Time	Reconstituted solution treatments							
	Heating		Acid		Alkaline		Freezing	
	Amox/clav (%)	Amox/clav (%)	Amox/clav (%)	Amox/clav (%)	Amox/clav (%)	Amox/clav (%)	Amox/clav (%)	
T <sub>0</sub>	100	100	100	100	100	100	100	
T <sub>0</sub> + 10 min	95	98						
T <sub>0</sub> + 30 min	85	70	96	0	63	30		
T <sub>0</sub> + 60 min	78	50	88	-	49	16		
T <sub>0</sub> + 2 h			74	-	33	13		
T <sub>0</sub> + 24 h							70 0	
T <sub>0</sub> + 48 h							64 -	
T <sub>0</sub> + 72 h							44 -	

Amox: amoxicillin; clav: clavulanic acid; T: time; min: minutes; h: hours

No difference was observed between both adult and paediatric preparations.

With regard to the results obtained, we recommend the use of the reconstituted preparations within four hours, and

within eight hours after storage, at ambient temperature and at 4°C, respectively. However, when reconstituted preparations are subjected to aggressive conditions, we noted a loss of stability of the mixture within 30 minutes. Thus, these conditions must be avoided in clinical practice.

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