

Improving the stability of potassium clavulanate in admixture with amoxicillin

By Elena M. Vega, BSc, PhD, Ruben H. Manzo, BSc, PhD and Nancy Sola, BSc, PhD

- OBJECTIVE — To improve the shelf-life of the intravenous solution of amoxicillin and clavulanic acid, by lowering the pH to an extent that does not affect the solubility of amoxicillin.
- METHODS — Hydrochloric acid 0.1M was used to lower the pH of intravenous co-amoxiclav. The limit of pH at which the admixture remained clear was determined. The shelf-life (t_{90}) at 25C of both an acidified admixture (pH 7.70–7.80) and a non-modified admixture (Augmentin, pH 9.08–9.19) was determined by direct high performance liquid chromatography measurement of the concentration of the potassium salt of clavulanic acid over 12 hours, in both the presence and absence of light.
- RESULTS — Acidified admixture appeared to be more stable ($t_{90} = 12\text{h}$) than that of non-modified Augmentin ($t_{90} = 5\text{h}$). Acidified admixture also exhibited lower pH variation over time and retained visual clarity for a longer period.
- CONCLUSION — The incorporation of 1ml of sterile 0.1M hydrochloric acid per 10ml of final admixture volume appears to be a good strategy to increase the stability of intravenous co-amoxiclav.

Co-amoxiclav is a fixed combination of the sodium salt of amoxicillin (amoxiNa) and the potassium salt of clavulanic acid (clavK). Although clavulanic acid has weak antibacterial activity when used alone, its combination with certain penicillins results in a synergistic effect which expands the spectrum of activity of the penicillin against many strains of beta-lactamase producing bacteria.

Preformulation studies indicate that clavulanic acid undergoes acid-base catalysed reactions depending on pH and the presence of buffer salts.¹ Maximal stability of clavulanic acid is reported to be at pH 6.3. Although amoxicillin is subject to similar degradation pathways it appears to be more stable than clavulanic acid. In fact, evaluation of the stability of this combination in intravenous form² as well as in oral suspensions^{3,4} showed that amoxicillin was markedly more stable than clavulanic acid. Therefore, in this paper, the stability of the admixture is assessed, in terms of clavK stability only.

The pH of the reconstituted intravenous mixture of amoxiNa and clavK is in the range 8.5–9.5. The low stability of clavK at this pH range can affect the efficacy and safety of therapy. Since visible degradation (apparent by a colour change) has been observed before its administration, it is considered of interest to improve the stability of the mixture.

Method

Amoxicillin degrades rapidly under neutral or acidic conditions, therefore the pH limit at which co-amoxiclav can be kept without its constituents degrading needed to be determined. This was done by the addition of 0.1M hydrochloric acid (HCl) in 0.2ml increments up to 3ml, to amoxiNa and amoxiNa/clavK solutions. A pH value for each resultant solution was obtained and the clarity of each solution was assessed by visual inspection.

Nominal concentrations used in this study were amoxicillin 10mg/ml and clavulanic acid 2mg/ml. Intravenous Augmentin (amoxicillin and clavulanic acid) was reconstituted to a final volume of 100ml to obtain this concentration. All solutions were

prepared from the same batch (BN 59668H766 Ch-B, GlaxoSmithKline) with high performance liquid chromatography (HPLC) grade water, immediately before use.

Lithium clavulanate (reference standard, United States Pharmacopoeia [USP]), amoxicillin trihydrate (reference standard Instituto Nacional del Medicamento [INAME]) and Augmentin IV 1.2g were used.

HPLC was used to measure clavK concentration over a period of 12 hours. This determined the shelf-life of the admixtures in the following conditions:

- Presence of light at unchanged pH
- Absence of light at unchanged pH
- Presence of light with 1ml 0.1M HCl added
- Absence of light with 1ml 0.1M HCl added

The shelf-life indicates the time taken for the concentration of drug in the admixture to reduce to 90 per cent of its initial concentration (t_{90}).

Solutions were prepared and kept in stoppered Erlenmeyer flasks in a bath at 25C. A sample was withdrawn immediately after preparation of the solution, diluted with cold water to yield the analysis concentration and maintained at 4C until performing the assay. The time at which this sample was taken was considered zero time. The same procedure was used to take samples at appropriate time intervals up to 12 hours. The experiment was carried out in a laminar airflow hood type A.

Drug concentrations were determined using a HPLC assay method adapted from USP 24, using a pump (Spectra Systems P2000) ultraviolet light detector (Thermo Separator Products SC100, fixed wavelength) at 230nm and a C18 reverse phase column (LiChrosorb RP 18 [10 μm] 250 \times 4 mm).

The mobile phase consisted of 4 per cent methanol and 96 per cent 0.05M sodium phosphate adjusted to pH 4.47 \pm 0.01 and was delivered isocratically at a flow rate of 1ml per minute. The injection volume was 20 μl . Under these conditions, clavK and amoxiNa eluted at 4.2 and 9.0 minutes, respectively.

Elena Vega is a research teaching assistant and Ruben Manzo is a professor in the pharmacy department at the University of Córdoba, Argentina. At the time the research was started, Nancy Sola was a professor in the pharmacy department at the University of Córdoba

Table 1: Variation of pH after addition of increasing volumes of HCl to solutions of amoxicillin and amoxicillin/clavulanic acid

Added volume HCl 0.1M (ml)	pH *	pH *
	AmoxiNa 10mg/ml (10ml)	AmoxiNa 10mg/ml and clavK 2 mg/ml (10 ml)
0.0	8.92	8.90
0.2	8.64	8.60
0.4	8.33	8.30
0.6	8.12	8.07
0.8	7.93	7.91
1.0 [†]	7.78	7.70
1.2	7.61	7.55
1.4	7.49	Not determined
1.6	7.41	7.46
1.8	7.20	7.22
2.0	7.02 [‡]	7.05 [‡]
2.2	6.83 [‡]	6.86 [‡]
2.4	6.54 [‡]	6.68 [‡]
2.6	6.10 [‡]	6.34 [‡]
2.8	4.57 [‡]	5.61 [‡]
3.0	3.55 [‡]	3.91 [‡]

* Average of two solutions
[†] pH chosen for stability studies
[‡] Turbidity was observed

Samples were diluted with HPLC grade water to obtain a concentration of 25µg/ml of clavulanic acid for analysis. At this concentration the relative standard deviation, determined from six replicate injections, was 0.11 per cent. The calibration curve was linear within the range 0–36.7µg/ml ($r^2 > 0.9994$).

The analytical method was proved to be stability-indicating by accelerated degradation of clavK. A volume of 5ml 0.1M

sodium hydroxide was mixed with 10.65mg of clavK, and 5ml 0.1M HCl was mixed with 10.45mg clavK. The resulting solutions were subjected to reflux for two hours. Decomposition product peaks appeared in the chromatograms before three minutes; no intact drug was observed. There was no interference among degradation product peaks with the peak of intact drug.

The peak area method was used to calculate drug concentration. The

concentration of clavK at zero time (100 per cent) was used as reference to calculate the remaining percentage of clavK in samples taken within the 12 hours.

Results

The results of the the addition of 0.2ml increments of HCl 0.1M to 10ml of amoxiNa and amoxiNa/clavK solution, are shown in Table 1. In both cases, addition of 1.8ml of 0.1M HCl yielded the last visually clear solution (visual clarity only includes particles that are greater than 70 microns in size — smaller particles cannot be seen by the naked eye). The pH values were 7.2 and 7.16, respectively. Higher volumes produced turbidity.

Table 2 shows the percentage of clavK remaining in solution under different conditions of pH and light, at time intervals of 0, 0.5, 1, 4, 6, 8, 10 and 12 hours after reconstitution.

Shelf-life (t_{90}) is determined from estimating the time at which the concentration of clavK drops below 90 per cent of the concentration at time zero. This estimate is also reported in Table 2.

Discussion

According to the solubility-pH profile of amoxicillin, the visual compatibility of the admixture on addition of HCl (as outlined in Table 1) was limited by the low solubility of amoxicillin at neutral pH. The instability of amoxicillin became apparent after 1.8ml of HCl had been added. It was therefore considered practical and appropriate to incorporate 1ml of HCl 0.1M per 10ml of admixture final volume for comparing clavK concentration by HPLC, as this

Table 2: Percentage of initial clavulanic acid (clavK) remaining in solution over time, with and without acidification of the mixture, under different light conditions

Time (hours)	Percentage clavK remaining			
	Acidified pH		Non-modified pH	
	Light	Dark	Light	Dark
0	100.00 ± 1.98	100.00 ± 2.49	100.00 ± 5.47	100.00 ± 2.54
0.5	98.18 ± 1.96	98.80 ± 2.46	96.40 ± 5.39	101.24 ± 2.49
1	98.18 ± 1.93	100.44 ± 2.43	100.70 ± 5.32	101.36 ± 2.45
4	97.39 ± 1.85	98.09 ± 2.33	99.78 ± 5.21	97.67 ± 2.39
6	94.40 ± 1.84	96.11 ± 2.34	93.48 ± 5.12	94.50 ± 2.38
8	92.64 ± 1.88	94.73 ± 2.43	85.81 ± 5.22	90.10 ± 2.52
10	91.28 ± 1.96	Not determined	86.43 ± 5.49	87.31 ± 2.78
12	87.86 ± 2.08	89.41 ± 2.76	81.63 ± 5.91	Not determined
Starting pH	7.80	7.78	9.19	9.08
Final pH	7.70	7.70	8.71	8.70
Shelf-life (hours)	11.2	12.0	5.5	5.2
Colour of admixture	None (after 12 hours)	None (after 12 hours)	Yellow (after six hours)	Yellow (after six hours)

produced a pH level that did not affect the stability of amoxicillin. The pH of the modified admixture was in the range 7.70–7.80. This solution remained clear and the pH level did not change significantly for at least 48 hours.

Acidified admixtures (pH 7.70–7.80) appeared to be more stable than those of non-modified Augmentin (pH 9.08–9.19) under both light and dark conditions.

Shelf-life (t_{90}) obtained with the non-modified admixture of Augmentin agreed with that reported by Ashwin *et al.*,² which was in the range 4.8–5.2 hours when reconstituted with water for injections BP, and kept at 25°C. The shelf life of the acidified admixture was increased to around 12 hours.

Haginaka *et al.*¹ reported that clavK in aqueous solution undergoes complicated acid-base catalysed reactions depending on pH and the presence of buffer salts such as borate and carbonate. As shown in Table 2, the improvement in stability of the admixture achieved through the addition of HCl was also accompanied by a lower variation in pH over time, reducing from a drop of 0.4 to a drop of 0.1.

In addition, the development of a yellow colour that was observed in the non-modified admixture was not apparent for at least 12 hours after adding HCl.

With regards to the stability of amoxiNa, its “degradation rate versus pH” profile is a U-shaped curve.^{5–7} Therefore, the shelf life of amoxiNa might also increase at the reduced pH used in this study. However this is outside the scope of this paper.

Finally, it should be noted that HCl solution monographs are included in pharmacopoeias and that a pharmacy service could potentially prepare sterile ampoules of 0.1M HCl.

Conclusion

The short shelf-life (five hours) of reconstituted Augmentin is a critical constraint, which implies that if the admixture is prepared by centralised intravenous additives services, this must be done immediately before use. If it has not been used within this period it must be disposed of.

If the pH is adjusted to 7.8 with HCl, its shelf-life increases significantly, from five hours to 12 hours, without affecting the compatibility of the admixture. The acidified admixture also exhibits less pH variation over time and retains visual clarity.

Addition of sterile solution of HCl in the way described here appears to be a good strategy to increase the stability of the admixture and therefore increase the safety of its administration.

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