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Long-Term Stability of an Infusion Containing Morphine, Ketamine and Lorazepam in Syringe at 5±3˚C

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Abstract

Background and Objective: Patients in palliative care are injected with a sedation infusion containing morphine, ketamine and lorazepam in order to relieve pain. This infusion is prepared by the nursing staff according to demand, but the awareness of its long-term stability could allow a preparation in batch and in advance by a Centralized Intravenous Additive Services (CIVAS). The aim of this study was to evaluate the physicochemical stability in syringes at 5±3°C of the injectable with two different levels of concentration commonly used.

Method: Five syringes were prepared under aseptic conditions for each level of concentration (low concentration: morphine 1.5 mg/ml, ketamine 1.5 mg/ml and lorazepam 0.1 mg/ml; high concentration: morphine 6 mg/ml, ketamine 5 mg/ml and lorazepam 0.3 mg/ml). Solutions were stored at 5±3°C for 25 days and the stability was periodically investigated (each day the first week, then 3 times a week). Particle appearance or colour change were checked by visual inspection while crystals were searched under the microscope. pH was measured to assess its stability and absorbance at 350, 410 and 550 nm were monitored to estimate the solution turbidity. The molecules concentrations were measured by Ultra-High Performance Liquid Chromatography (UHPLC) – diode array detection.

Results: Crystals were visible to the naked eye after 23 days in syringes of low concentration and after 11 days in syringes of high concentration. Some crystals were already observed under the microscope after 7 days in high concentration solutions. pH and absorbance at 410 and 550 nm were stable. Absorbance at 350 nm showed a decrease after 11 and 7 days in low and high concentration infusion respectively, suggesting the degradation of a compound absorbing at this wavelength. Solutions were considered chemically stable while the lower one-sided prediction limit at 95% remains superior to 90% of the initial concentration. Morphine and ketamine were stable for 25 days while lorazepam was unstable from day 0.

Conclusion: Infusions containing morphine, ketamine and lorazepam used in palliative care are unstable at 5±3°C immediately after preparation. Consequently, the mixture of these three components should be prevented, even extemporaneously.

Keywords: Drug Stability; Ketamine; Lorazepam; Morphine; Palliative Care

Glossary of Terms

CIVAS : Centralized IntraVenous Additive Services

HC : High concentration

ICH : International Conference on Harmonisation

LOD : Limits of detection

LOQ : Limits of quantification

LC : Low concentration

PDA : Photodiode array

RSD : Relative standard deviation

RT : Room temperature

SD : Standard deviation

UHPLC : Ultra-High Performance Liquid Chromatography

1. Introduction

Patients suffering from incurable or terminal disease resort to palliative treatment in order to relieve pain, reduce awareness and anxiety [1,2]. The management of the terminal stage of disease is a complex issue usually requiring the combination of active principles with different analgesic and sedative mechanisms [2,3]. A mixture constituted of morphine, ketamine and lorazepam with different concentrations is commonly used in palliative care.

Morphine seems to be the gold standard opioid for relieving moderate and severe pain that is the most common symptom of terminal diseases [1]. Ketamine is usually used in combination with opioid in case of refractory pain in order to avoid an increase of doses and linked side-effects [4]. Lorazepam, a benzodiazepine, allows to reduce patients' anxiety and agitation [1].

The combination of the three molecules in one syringe is advantageous for patients who are injected only once. It could be also beneficial for the hospital in terms of preparation costs and time management if the mixture could be reconstituted in advance by a Centralised Intravenous Additive Service (CIVAS) [5]. For this purpose, mixture should be stable in defined storage conditions. Some studies about visual compatibility and stability of morphine mixed with ketamine at different concentrations showed different compatibility results: for at least 4 days in different containers at room temperature (RT) [3,6], 10 days in syringes at RT and in refrigerator and for 91 days in syringes at RT and in refrigerator [7,8]. Morphine and lorazepam mixture was also studied and was compatible for 24 hours in glass tubes at RT [9]. However, a mixture of lorazepam 4 mg/ml and ketamine 50 mg/ml was observed on a 4 hours' period and showed an incompatibility between these drugs due to the turbidity of the solution [10]. A mixture containing morphine, ketamine and lorazepam was already assessed for physical stability during 4 hours but the compatibility could not be determined [10].

The aim of this study is to investigate the physical and chemical stability of a mixture containing morphine, ketamine and lorazepam in syringes at 5±3°C with two levels of concentration commonly used in palliative care.

2. Materials and Methods

2.1 Solutions Preparation

Five syringes (Becton Dickinson, lot 1612224P, Madrid, Spain) containing 20 ml of the Low Concentration (LC) infusion were prepared under aseptic conditions by adding 3 ml of morphine (10mg/1ml, lot 180338, Sterop, Anderlecht, Belgium), 0.6 ml of ketamine (Ketalar® 500mg/10ml, lot 816076, Pfizer, Brussels, Belgium) and 0.5 ml of lorazepam (Temesta® 4mg/1ml, lot 4084, Pfizer, Brussels, Belgium) to 15.9 ml of NaCl 0.9% (lot 18032014, B. Braun, Melsungen, Germany). LC solutions contained 1.5 mg/ml morphine, 1.5 mg/ml ketamine and 0.1 mg/ml lorazepam. Five syringes (Becton Dickinson, lot 1612224P) of 20 ml of the High Concentration (HC) infusion were prepared by injecting 3 ml of morphine (40mg/1ml, lot 180171, Sterop), 2 ml of ketamine (Ketalar® 500mg/10ml, lot 816076, Pfizer) and 1.5 ml of lorazepam (Temesta® 4mg/1ml, lot 4084, Pfizer) in 13.5 ml of NaCl 0.9% (lot 18032014, B. Braun). HC solutions contained 6 mg/ml morphine, 5 mg/ml ketamine and 0.3 mg/ml lorazepam.

2.2 Stability Study Design

Solutions were stored at 5±3°C for 25 days and periodically analysed (after 0, 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25 days). Physical stability was assessed immediately while chemical stability was evaluated at the end of the study by analysing aliquots frozen at -80°C each day of analysis. It allowed to limit results variability due to experiment conditions.

2.3 Physical Stability

Visual inspections of the solutions in front of black and white backgrounds were performed to detect any colour change or particle appearance. Solutions were centrifuged at 2150 g for 5 minutes (Heraeus multifuge 1S, Thermo Scientific, USA) in order to observe the pellet under the microscope 80X (Jenamed, Carl Zeiss, Germany), searching for crystals. Turbidity of solutions was evaluated by absorbance measurements at 350, 410 and 550 nm [11]. pH was measured with a glass electrode pH meter (InoLab, WTW GmbH, Germany) to check its stability.

2.4 Chemical Stability

2.4.1.Chromatographic conditions

Molecules concentrations were measured by Ultra-High Performance Liquid Chromatography (UHPLC) (Acquity UPLC H-Class, Waters, Milford, USA) coupled with a Photodiode Array (PDA) detector (Acquity, Waters). Data were acquired and processed by the Empower 3 software (Waters). The separation was performed on a reverse phase column (Acquity UPLC BEH C18 130Å 1.7 µm 2.1 mm X 100 mm, lot 0311380032, Waters) connected to a pre-column (Acquity UPLC BEH C18 VanGuard Pre-column 130Å 1.7 µm 2.1 mm X 5 mm, lot 0307372572, Waters) and heated at 30°C. An elution gradient was carried out with ammonium formate 5 mM (lot BCBX5107, Sigma-Aldrich, Saint-Louis, USA) adjusted to pH 3 with formic acid (lot 1198871, Biosolve, Dieuze, France) and 0.1% of formic acid in acetonitrile (lot 1268011, Biosolve). The run started with 7% of organic phase before being gradually increased to 70% in 2 minutes. The column was washed with 97% of organic phase for 2 minutes and then reequilibrated with 7% of organic phase for 2 minutes before the next run. The flow rate was fixed at 0.5 ml/min. Chromatograms were obtained at 285 nm (morphine), 268 nm (ketamine) and 250 nm (lorazepam).

2.4.2.Standard and control solutions

A calibration curve was performed each day of UHPLC analysis based on 5 standard solutions and a 1/X regression procedure. 3 control solutions were used (Table 1). All solutions were prepared from a fresh mixture of morphine, ketamine and lorazepam.

	Morphine (mg/ml)	Ketamine (mg/ml)	Lorazepam (mg/ml)
Calibrator 1	0.5	0.5	0.025
Calibrator 2	0.9	0.9	0.045
Calibrator 3	1.8	1.8	0.090
Calibrator 4	4.5	4.5	0.225
Calibrator 5	9.0	9.0	0.450
Control 1	1.0	1.0	0.050
Control 2	3.0	3.0	0.150
Control 3	6.0	6.0	0.300

Table 1: Calibrator and control solutions.

2.4.3.Validation of the UHPLC method

The quantification method was validated according to the ICH Q2(R1) guidelines (ICH 2005). Linearity, precision, limits of detection and quantification and stability-indicating capability of the method were evaluated.

2.4.3.1. Linearity

The range of linearity was determined in duplicate by a twofold dilution series (14 dilutions) from a high concentrated mixture: 12 mg/ml morphine, 10 mg/ml ketamine and 0.6 mg/ml lorazepam.

2.4.3.2. Precision

Intra-assay variation was evaluated by the Relative Standard Deviation (RSD) of 10 successive injections of the same sample while inter-assay variation was determined by the injection of 10 samples prepared on different days. Both intra- and inter-assay variations were evaluated for 3 levels of concentration (corresponding to control solutions).

2.4.3.3. Limits of detection and quantification

Injectable water (lot 190258092, B. Braun) was injected 10 times as blank to calculate the Limits of Detection (LOD) and Quantification (LOQ) as follows: LOD = mean + 3SD and LOQ = mean + 10SD.

2.4.3.4. Stability-indicating capability

A forced degradation test was performed to evaluate the stability-indicating capability of the method. Main peaks were observed to verify their decrease in case of degradation and degradation products peaks were observed to check they were not interfering with main peaks [12]. This test was carried out on fresh mixtures of same concentrations as the LC and HC solutions and on solutions containing only one active principle: morphine 6 mg/ml, ketamine 5 mg/ml and lorazepam 0.3 mg/ml. This forced degradation design allowed to determine the origin of each degradation product and to verify that they were no interference on different main peaks.

Vials containing each studied solutions were prepared in natural, acid (HCl 0.1M), alkaline (NaOH 0.1M) and oxidation (H_2O_2 3% (w/w), lot STBH5484, Sigma-Aldrich) conditions. Samples were analysed after 0, 2 and 4 days at room temperature and 60°C. Samples were neutralised before the UHPLC injection.

2.4.4. Chemical stability study

Aliquots coming from a same syringe were defrosted at room temperature and analysed the same day by chromatography. 1 µl of samples was injected by the UHPLC system without any previous dilution and analyses were performed in triplicate.

2.4.5. Statistical analysis

Solutions were considered chemically stable while the lower one-sided confidence limit at 95% remains superior to an acceptance criterion, as recommended by the ICH Q1E guidelines (International Conference on Harmonization (ICH) 2004) [13,14].

Therefore, an one-sided 95% confidence interval on the mean was calculated to determine the time when the product concentration fell under 90% of the initial concentration [12,15].

A more recent approach based on the individual observations rather than the observations mean was also performed by using a prediction interval in lieu of the confidence interval in order to compare results [16].

3. Results and Discussion

3.1. Physical Stability

Crystals were detected under the microscope after 7 days in HC solutions (Figure 1). Some crystals were visible to the naked eyed after 11 days and 23 days in HC and LC solutions respectively.

Figure 1: Crystals observed under the microscope after 7 days in high concentration solutions.

No colour change and pH variation (mean ± SD: 5.1 ± 0,2) were observed. Absorbance at 410 nm (LC: 0.003 ± 0.001; HC: 0.012 ± 0.002) and 550 nm (LC: 0.000 ± 0.001; HC: 0.002 ± 0.001) remained stable along the study while absorbance at 350 nm showed a decrease after 11 and 7 days in LC (0.043 ± 0.009) and HC (0.113 ± 0.046) solutions respectively. This drop of optical density at 350 nm could be correlated with crystals appearance, suggesting that a compound absorbing at 350 nm had precipitated. Actually, lorazepam absorbs at 350 nm and is likely to precipitate due to its limited solubility in aqueous solution (« Notice: information de l'utilisateur Temesta 4 mg/ml solution injectable lorazépam » 2017) [17]. Furthermore, a previous stability study concerning lorazepam in the same container showed a rapid lorazepam crystallisation [18].

3.2. Quantification Method Development

A C18 column was chosen because of the hydrophobic nature of the 3 mixture components (partition coefficient (*log P*): morphine 0.9, ketamine 3.35, lorazepam 3.53). pH-distributions of species showed that a unique species for each molecule was in solution at pH below 4. pH 3 was fixed in order to neutralise residual silanols and limit secondary interactions while morphine and ketamine are positively charged at acidic pH. Therefore, ammonium formate (pKa = 3.75) adjusted at pH 3 with formic acid was used to buffer at this pH. 0.1% acid formic in acetonitrile as organic phase allowed a suitable separation and a rapid elution. The analyse of the molecules spectra showed that the maximum absorption wavelengths were 285 nm (morphine), 265 nm (ketamine) and 230 nm (lorazepam). Chromatograms were extracted at these wavelengths, except for lorazepam at 250 nm to limit solvent interferences. Several dilutions and injection volumes of samples were tested before choosing an injection of 1 µl of sample without any dilution, thereby decreasing results variability due to the operator. Finally, the developed method allowed the quantification of the 3 mixture components simultaneously.

3.3. Quantification Method Validation

The Table 2 shows the linearity range determined for each molecule based on the mean calculated for the duplicates. It shows also LOD and LOQ obtained by calculation from the background noise. Intra- and inter-assay variations are displayed as the mean of variations obtained for the 3 levels of concentration tested.

	Linearity			LOD (µg/ml)	LOQ (µg/ml)	Intra-assay RSD (%)	Inter-assay RSD (%)
	Range (µg/ml)	Equation	R^2				
Morphine	1.465 – 12 000	452.28x	0.9997	0.087	0.206	0.26	1.18
Ketamine	1.221 – 10 000	289.04x	0.9998	0.260	0.617	0.14	1.14
Lorazepam	0.073 – 600	4244.3x	0.9997	0.257	0.670	0.14	2.22

Table 2: Linearity, limits of detection and quantification, intra- and inter-assay variations for each molecule.

Forced degradation study proved that the method was stability-indicating given that a decrease of each main peak was observed in the different conditions tested. These decreases were quantified and showed that degradation occurred for all molecules and achieved the recommended degradation range (5-20%) (Rawat et Pandey 2015). Moreover, some degradation products could be detectable on chromatograms and were not interfering with main peaks. Forced degradation chromatograms after 4 days at room temperature and 60°C for solutions containing one active principle are showed on Figure 2.

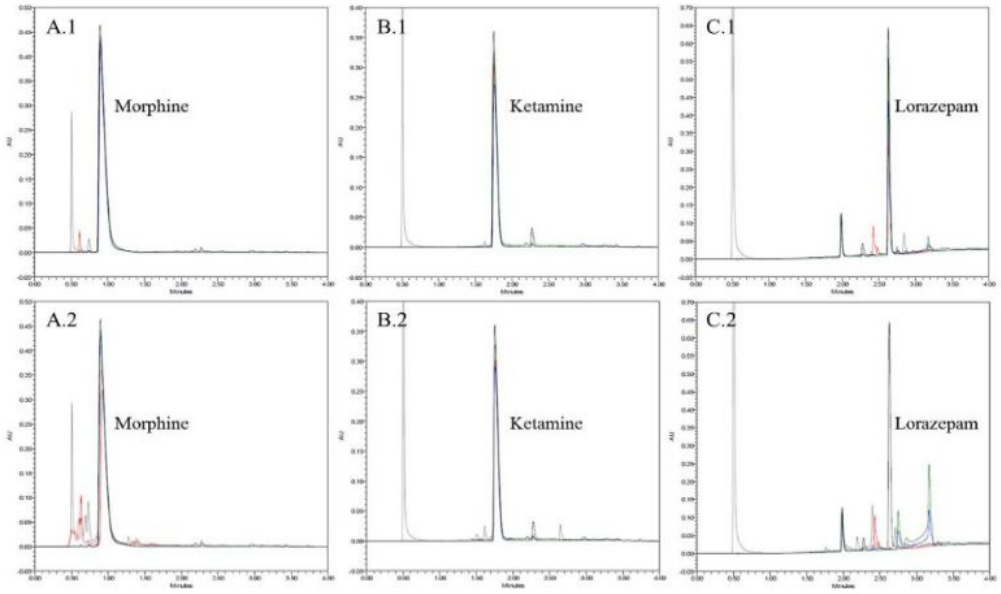

Figure 2: Forced degradation chromatograms of morphine 6 mg/ml (A), ketamine 5 mg/ml (B) and lorazepam 0.3 mg/ml (C). 1: T=4 days at room temperature in natural (green), acid (blue), alkaline (red) and oxidant (grey) conditions. 2: T=4 days at 60°C in natural (green), acid (blue), alkaline (red) and oxidant (grey) conditions. Natural condition at T=0 day is showed in black.

3.4. Chemical Stability

Morphine and ketamine were stable in LC and HC solutions during the 25 days of analysis given that the lower limit of the one-sided 95% confidence interval remained superior to 90% of the initial concentration (Figure 3 A.1, A.2, B.1 and B.2). However, a drop of lorazepam concentration below 90% was observed after 3 days and 1 day in LC (Figure 3 C.1) and HC (Figure 3 C.2) solutions respectively. It strengthens the supposition that crystals observed in the physical stability study was lorazepam precipitates.

Based on the use of a 95% unilateral prediction interval, lorazepam was considered unstable at day 0 for LC and HC solutions (Figure 3 C.1, C.2). Ketamine in LC solutions (Figure 3 B.1) was also considered unstable at day 0 due to an initial variability in the syringe preparation, impacting the prediction interval that is based on individual observations.

A limit of both methods is the use of a linear regression to model the concentrations evolution versus time. This model does not fit for lorazepam analysis as showed in Figure 3 C.1 and C.2. However, the stability time obtained with this linear model is more conservative in this case, therefore, there is no risk of considering stable a solution that is not. In the same way, the stability results obtained with the use of the prediction interval were taken on and solutions were judged chemically unstable from day 0.

Even though a lorazepam degradation was observed, no degradation product peak was detected on chromatograms. Microbiological aspects were not investigated.

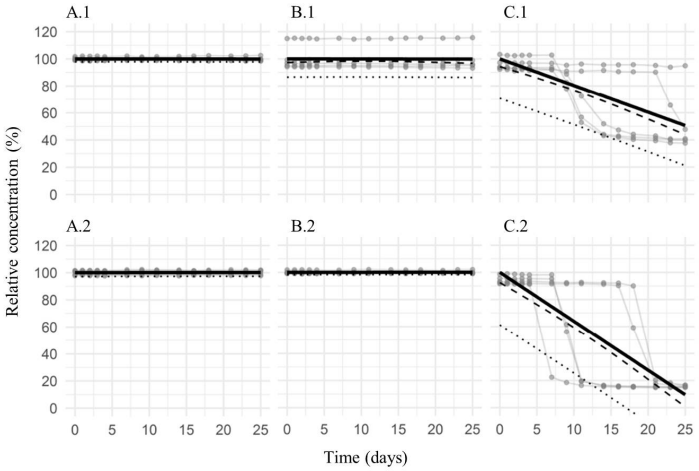

Figure 3: Relative concentration versus time for morphine (A), ketamine (B), lorazepam (C) in low (1) and high (2) concentration solutions. Grey = observations in each syringe, black line = fitted mean, dashed line = 95% unilateral confidence interval, dotted line = 95% unilateral prediction interval.

4. Conclusion

Patients in palliative care are injected with morphine, ketamine and lorazepam to manage pain and anxiety. The physicochemical

stability of a mixture containing the three components with two levels of concentration was investigated at 5±3°C in order to consider its preparation in advance by a CIVAS. Unfortunately, results showed that mixture was unstable immediately after preparation for low and high concentration solutions. The instability is due to crystals appearance and a drop of the lorazepam concentration. Consequently, the three components should not be mixed before the injection to the patient, even extemporaneously.

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