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Microbiological and Physico-Chemical Stability of Ketamine Solution for Patient-Controlled Analgesia Systems

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Research Article

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ABSTRACT

Ketamine solution is regularly used along with opioids to treat patient suffering from refractory cancer pain. The aim of this study was to determine the expiry date of solutions of ketamine, by conducting microbiological and physicochemical stability assay. Five series of six ketamine solutions at 1 mg/mL in the portable PCA systems were stored at room temperature or at +33°C for respectively 28 days and 7 days. They were sampled immediately after preparation, and some day of the following weeks. According to European Pharmacopoeia VIIIth edition, microbiological stability was assessed by the research of antimicrobial activity of the product and sterility tests. The physico-chemical study was performed by determining aspect, pH, osmolality and loss of weight evolution. All samples were tested to follow variation of drug concentrations by high performance liquid chromatography coupled to an ultraviolet detector. A study of degradation forced was realized to separate degradation product. There were changes in pH and loss of weight values after 28 days. No precipitation or change in color was observed in any of the sample solutions. There was no significant loss of ketamine over 28 days at +20°C and over 7 days at +33°C. No degradation product was found during the study. This study indicates that ketamine solutions in the PCA systems are stable for a minimum of 28 days at room temperature.

INTRODUCTION

The management of pain is a real public health challenge. In oncology, prevalence of pain increases as metastatic patients have a longer life expectancy. In a European survey realised in 2008, 72% of cancer patients suffered pain [1]. Pain treatment is centred on strategies aimed at ensuring a better quality of life for patients, and several guidelines exist [2-4]. Despite the existence of many treatments, it was shown that in 30 to 80% of cases, patients' pain is inadequately treated. In France a recent study shown that this rate increased over the last ten years [5]. To answer this issue, the French National Cancer Plan 2009-2013 focuses on improvement of pain management and therefore pain relief.

The management of cancer severe pain justifies the use of level III WHO ladder analgesics, in example strong opioids, like morphine, oxycodone, hydromorphone, methadone, fentanyl and sufentanil, usually by oral route. When the pain is not relieved or adverse events are uncontrolled, we can use additional administration routes such as endovenous or subcutaneous routes using a patient-controlled analgesia system (PCA) [6].

Originally developed for postoperative pain, PCA is frequently used for the treatment of cancer pain. This system allows

releasing a constant base flow for the treatment of continuous pain and bolus injections, self-administered by the patient for treatment of pain peaks. PCA provides autonomy to the patient, to reach stable analgesia and rapid response to pain peaks, and adjust administered doses to patients opioids needs.

When pain is still insufficiently relieved, analgesics adjuvants like ketamine **(Figure1)** can be added and is recommended in refractory cancer pain by French health authorities and WHO [7,8]. Indeed ketamine, which antagonize N-methyl-D-aspartate (NMDA) receptor, seems to increase the opioid-induced analgesia through an opioid sparing strategy, even if strong data from large randomized controlled studies is still lacking [9]. Its effectiveness has been studied in the management of cancer pain in paediatrics [10-12] but also in adults [13-16]. Interests of ketamine are these anti-allodynic and antihyperalgesic effects.

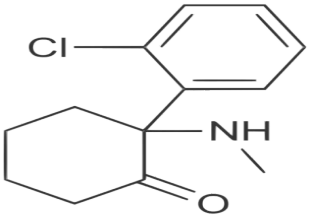

Figure 1. Chemical structure of ketamine.

The use of ketamine in patients with intravenous opioids administered through PCA systems raised the concern of using also ketamine in PCA systems.

But data is lacking on ketamine stability according to storage conditions and time between preparation and administration. Despite the availability of publication, no time of expiry corresponding to delivery systems for PCA was applied [17-19]. This lack of data led us, to proceed to a microbiological and physicochemical stability study of ketamine solutions prepared at 1mg/mL in polyvinyl chloride (PVC) bags for PCA devices. This study provide the determination of an expiry date in order to schedule campaigns of production in connection with the clinical staff from the Pain Unit, to afford already available preparations for urgent requests, prepared in good manufacturing practices process with qualitative and quantitative analytical control.

MATERIALS AND METHOD

Materials

The pharmaceutical solutions used were prepared from ketamine hydrochloride (10 mg/mL, Panpharma, Fougères, France). For this study 5 mL vials containing 50 mg ketamine hydrochloride base were required.

As a diluent, isotonic 0.9% sodium chloride was used (0.9% NaCl 500 mL, Freeflex®, Fresenius-Kabi, Sevres, France).

Method

Preparation of the solution: According to the Good Manufacturing Practices, solutions were prepared in aseptic conditions and under a laminar-airflow hood (type IIB). Sixty vials of ketamine hydrochloride doses of 50 mg/5 mL were needed to prepare five series of six polyvinyl chloride (PVC) bags (Rythmic pump®, Micrel medical devices, Vence, France). The bags were complemented by 100 mL of isotonic NaCl. After homogenisation, the residual air was removed. All bags were stored at ambient temperature (+25°C +/-2°C) [20] to mimic storage conditions. During the administration of analgesic solution, patients can keep the bag close to their bodies. In these conditions, solution might achieve +33°C. So every new week, a new series of six bags were placed at +33°C to mimic conditions of use.

Microbiological study

Investigation on the antimicrobial activity specific to the product: The determination of the absence or the presence of specific antimicrobial activity of ketamine hydrochloride was obtained by preliminary microbial growing test described in the "general methods" of the European Pharmacopoeia (8th edition) [21]. This test compares the fertility of thioglycolate broth (TGB) and trypticase soya broth (TSB) with or without the studied active substance. Pharmacopoeia recommends using 10 to 100 colony forming units (CFU) of the four strains: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231) and *Clostridium sporogenes* (ATCC 19404).

The first three strains were seeded in TSB (100 mL broth, ref: AEB611506M, AES laboratory, Bruz France). Anaerobic bacteria were seeded in TGB (100mL broth, ref: AEB611406M, AES laboratory, Bruz France). Two series were prepared, without solution of ketamine and the second by adding 5 mL of ketamine solution 1 mg/mL. Inoculated culture media were incubated at +37°C or +20°C [22], respectively for bacteria and yeast for 72 hrs. The test is based on the comparison of fertility environments in the presence and absence of ketamine.

Sterility test

The sterility test is carried under aseptic conditions. The test is performed by direct inoculation of the culture broth with studied active substance. TGB is primarily intended for research of anaerobic bacteria, and TSB for research yeasts and aerobic

bacteria. A volume of 5 mL of six bags each series was aseptically sampled on days D0, D7, D14, D21 and D28 and directly seeded into TGB and TSB. Negative controls were performed with distilled water to guarantee the absence of interference caused by the handling conditions. The culture media were incubated at minimum fourteen days at +37°C and +20°C, respectively for bacteria and yeasts. Several times during the incubation, the media were examined to detect signs of macroscopic microbial growth.

Physicochemical study [23,24]

pH, weight and osmolality evolution: For each sample, change of color and appearance of turbidity were monitored. Then, pH and osmolality were respectively measured with a calibrated pH meter and an osmometer (Osmometer Automatic®, Roebling, Berlin Germany) calibrated by a glucose solution to 300 mOsmol/L (Roebling, Berlin Germany). Finally, one series of six bags was weighed throughout the study to identify a potential loss of mass.

Analytical validation [25]

In conformity with ICH Q2 (R1), analytical method used was validated by linearity, precision, and limits of detection and quantification.

Linearity: The linearity of an analytical procedure is its ability to obtain peak area directly proportional to the concentration of the analyte in the sample. The determination of linearity is obtained by the results of six calibration range validated by appropriate quality controls (QC).

Fidelity: Fidelity expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same sample. Fidelity can be considered at two levels: repeatability and intermediate precision. Repeatability represents the precision under the same operating condition for a short time interval. For this test each QC sample were analyzed ten times. The intermediate precision evaluates the inter-laboratory variability and reliability of different methods in an environment. The determination of intermediate precision consists of several measures of each QC under the same conditions at six different days and with three different analysts.

Accuracy: The accuracy or correctness is the systematic error (bias) of a value obtained from a value considered true. The accuracy is given by the errors between the theoretical values and the analytical controls dosed 6 times by 3 different manipulators.

Detection limit and quantification limit: The detection limit of an analytical procedure is the lowest amount of analyte in a sample which can be detected but no necessarily quantified The quantification limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The detection and quantification limits are based on the standard deviation of the response and the slope of the calibration curve.

Stress testing (According to ICH Q1A) [26]**:** Finally, for determination of degradation pathway of the drug, substance was submitted to study of the forced degradation. The stress testing was analyzed by HPLC on another system equipped with Diode Array Detector (DAD L-2455, Merck Hitachi, Tokyo, Japan). The mobile phase and chromatographic conditions are the same as below. Acid and base stress testing was respectively obtained by adding hydrochloric acid solution (HCl 37%, Normapur, VWR, Fontenay sous bois, France) to pH 2 and sodium hydroxide solution (NaOH 2 M, Normapur, VWR, Fontenay sous bois, France) to reach pH 12. The oxidation was obtained by the addition of a hydrogen peroxide solution (H_2O_2 30%, Normapur, VWR, Fontenay sous bois, France). In reduction conditions, HCl with zinc dust (Zn 98%, Sigma Aldrich, St Louis, USA) were added to solution. For each condition, two series were prepared and placed one at +20°C, the other at +40°C during 48 hours.

Concentration evolution study: Variations in concentration of ketamine were investigated for 28 consecutive days at room temperature. Samples of 2 mL were collected sterilely. The six bags of the first series were analyzed at length of the study on D0, D1, D2, D3, D7, D10, D14, D17, D21, D24, and D28. Every new week D7 and D14 and D21 and D28 each bag of a new series were also analyzed and placed seven days at +33°C. Each bag was tested after 3 and 7 days at +33°C.

The assay samples was analyzed by high performance liquid chromatography (HPLC) coupled to an ultraviolet detector. HPLC system consist of an isocratic pump (pump L-7100, Lachrom, Merck Hitachi, Tokyo, Japan), an auto sampler (auto sampler L-2200 Elite Lachrom, Merck Hitachi, Tokyo, Japan), oven at +40°C (T-6300 column thermostat, Merck, Darmstadt, Germany), an non-polar Agilent HPLC column Zorbax® SB-C18, 5 µm, (150 mm length x 4.6 mm i.d.) (Massy, France) and a UV-visible detector (Waters 2487 dual λ Absorbance detector, Guyancourt, France). The integration of the results is provided by the software (Multi-HSM Manager, Merck Hitachi, Tokyo, Japan).

The mobile phase was a mixture of 25% of acetonitrile (Hypersolv , VWR, Fontenay sous bois, France), 75% of ultrapure water (Millipore simplicity, Guyancourt, France) containing 1.03 g of sodium heptanesulfonate (Lichropur, Merck, Darmstadt, Germany) and 4 mL of acetic acid 100% (Ph.Eur., VWR, Fontenay sous bois, France).

The flow rate was set to 1.25 mL/min. Injection volumes were 10 µL and ketamine hydrochloride is detected at 215 nm for a retention time of 6.2 min. **(Figure 2)**

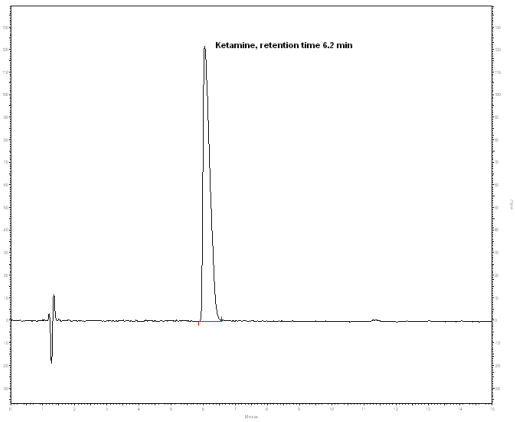

Figure 2. Ketamine solution chromatogram.

All samples were diluted to one-tenth in the mobile phase. A range of six-point calibration (0, 25, 50, 100, 250, 500 µg/mL) was validated by quality control QC_1, QC_2, QC_3, QC_4 respectively adjusted to 40, 90, 110, 450 µg/mL. To overcome the variability of the mechanical injector and the analyst, the assays were always carried out twice.

RESULTS

Microbiologic study: Validation of the absence of antimicrobial activity: Culture media supplemented with ketamine had revealed growth of the four reference strains tested. Between the positive controls and the ketamine hydrochloride solutions, no difference was detected after incubation. Therefore the solution of ketamine hydrochloride at 1 mg/mL had no antibacterial activity.

Sterility test: All samples were incubated under the same conditions on D0, D7, D14, D21 and D28 at +37°C and +20°C. After fourteen days incubation, no bacterial growth was detected. Negative controls also revealed no bacterial growth.

Physicochemical study: Evolution of pH, weight and osmolality: In storage conditions, the osmolality of solutions did not change. The appearance of solutions remained clear and without precipitate. As shown in the **Table 1**, a decrease of 5.6% of pH values was observed after 28 days at +20°C. In the same way and over 28 days, the weight was reduced of 0.76% as shown in **Table 1.** However, variations of osmolality at +20°C were not significant.

Table 1. Evolution of pH, osmolality and weight.

	pH values		**Osmolality values**		**Weight values**	
	Mean pH	Values ± RSD	Mean	Values ± RSD	Mean	Values ± RSD
Ketamine at +20°C (n=6)	D0	4.94 ±0.05	D0	269	D0	133.2 ± 0.3
	D28	4.66 ± 0.05	D28	269	D28	132.2 ± 0.3
	Δ (Variation %)	-5.6%	Δ (Variation %)	0.0%	Δ (Variation %)	-0.76%
Ketamine at +33°C	D0	4.59 ± 0.05	D0	269		
	D7	4.50 ± 0.03	D7	269		
	Δ (Variation %)	-1.96%	Δ (Variation %)	0.0%		

Cconcerning the study at +33°C, no change in osmolality values was observed. However a reduction in pH averaged 1.96% was notable. This decrease was not significant.

Analytical validation

Linearity: Linearity was studied over a measurement interval between 0 and 500 µg/mL. The target concentration was 100 µg/mL. This method was linear, the average equation of linear regression was: y=13287 x 26018 with a mean r^2 calculated at 0.9997.

Fidelity: Repeatability is expressed by coefficient of variation of repeatability (CV_r) and fidelity intermediate precision (CV_i). As show in **Table 2**, the analytical method is precise; no CV exceeds 1.5% for repeatability and 1.7% for intermediate precision.

Table 2. Results of the precision study of the method.

CV_r (%) (n=10)	QC_{40}	1.4548
	QC_{90}	0.0822
	QC_{110}	0.3176
	QC_{450}	0.1451

CV_i (%) (n=6)	QC_{40}	1.7067
	QC_{90}	1.0538
	QC_{110}	1.3630
	QC_{450}	0.9745

Accuracy: The analytical method is accurate in terms of mean values and the relative standard deviation values calculated from the six analyses for each QC **(Table 3).** For each quality control, variation on the observed values did not exceed 3.0%. The analytical technique was considered exact.

Table 3. Results of the accuracy study of the method.

QC_{40}	**Mean (n=6)**	**39.56**
	Variation (%)	-1.11
QC_{90}	Mean (n=6)	92.18
	Variation (%)	2.43
QC_{110}	Mean (n=6)	112.93
	Variation (%)	2.66
QC_{450}	Mean (n=6)	448.92
	Variation (%)	-0.24

Detection limit and quantification limit: The limits of detection (LD) and quantification (LQ) were determined using the slope and standard deviation (SD) of the intercept of the regression lines. The LD and LQ calculated are respectively: 3.5 µg/mL (LD=3.3 x SDb/a) and 10.6 µg/mL (LQ=10 x SDb/a). The analytical method was successfully validated and is suitable for quantitative measurements of solutions of ketamine.

Stress testing: In acidic and alkaline conditions, no product degradation was detected. However, a decrease of 18% of the peak area of ketamine was observed between the sample kept under alkaline conditions and the control sample. This decrease can be explained by the appearance of visible precipitate after 24 h at +40°C in basic medium.

In oxidizing conditions a degradation product was detected. The determination of this degradation product was permit using a diode-array detector (UV-DAD) to explore the peak purity. Chromatographic analysis coupled to UV detection allowed to determine retention time at 4.6 min for the degradation product and 6.2 min for ketamine **(Figure 3).** The peak purity was 99.4%

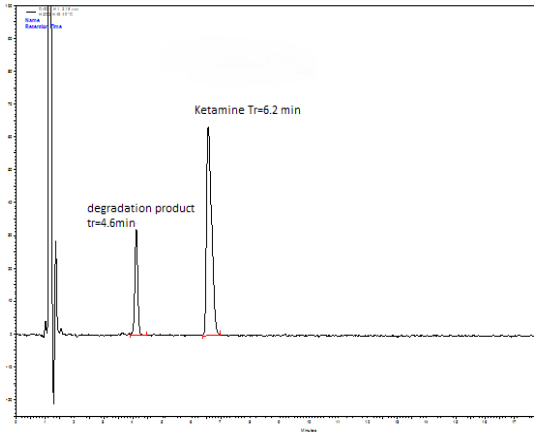

Figure 3. Degradation product in oxidizing conditions chromatogram.

In reducing conditions, two products of degradation are also highlighted by chromatography. These two compounds were separated from ketamine. The retention times for degradation products were 5.5 min and 5.9 min, respectively **(Figure 4).** For each of the peaks observed purity was on average of 99.3%.

Concentration-degradation evolution study: For each sample stored at +20°C or +33°C, no degradation product was detected during the study. Only the ketamine peak was observed with retention time at 6.2 min **(Figure 2).** From the first week, a higher concentration was observed under our storage conditions. There was not significant variation of concentration of ketamine during the study **(Table 4).** The variations observed were due to the variability of the analytical method.

At +33°C, the concentration of ketamine varied lightly: On average the decrease was of -0.03% over a period of 7 days **(Table 5).** These variations were not significant.

The normalized values (concentration=100 µg/mL for Day 0) show that ketamine concentrations remain in the 5% variation range.

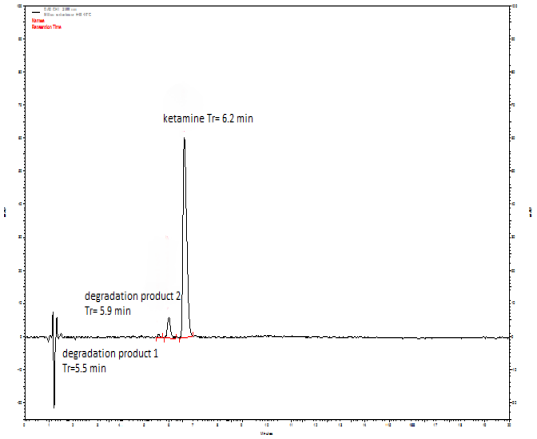

Figure 4. Degradation products in reducing conditions chromatograph.

Table 4. Evolution of the ketamine concentrations during the study at +20°C.

	D0	D1	D2	D3	D7	D10	D14	D17	D21	D24	D28
Mean (n=6)	97.086	97.728	97.817	98.412	98.641	98.377	98.064	99.068	98.191	98.586	97.840
RSD	2.07	0.79	1.44	1.069	0.70	1.40	0.98	1.27	0.99	1.49	0.93
Δ to D0 (variation %)		0.66	0.75	1.36	1.60	1.33	1.01	2.04	1.1	1.54	0.78
Normalized values (%)	100	100.7	100.8	101.4	101.6	101.3	101.0	102.0	101.1	101.5	100.8

Table 5. Evolution of the ketamine concentrations during 7 days at +33°C.

	D0	D3	D7
Mean (n=24)	97.41	98.23	97.38
RSD	1.29	1.44	1.09
Δ to D0 (variation %)		0.84	-0.03
Normalized values (%)	100	100.8	99.9

DISCUSSION

The development of quality assurance system gives a significant input to guarantee best health system at each step (prescription to dispensation and administration) for patients and to ensure security and good quality of care. This study was done with this aim.

To ensure the quality of the preparation delivered to patients it is important to study the microbiological issues. According to our results, ketamine do not have an anti-microbiological activity. This allows us to conclude on the sterility test. Indeed, no bacterial and yeast growth was observed. So, we are able to guarantee the microbiological stability of the ketamine solution.

The analytical method was performed and reliable for all criteria in conformity with the recommendations described in the ICH. This allows us to use these results and to define an expiry date of use for the solutions prepared.

As regard to physicochemical study, the loss of weight was concomitant with an increase of the drug concentration, thus we can postulate this may be due to slight evaporation of the solution in bags during the period studied. Moreover, in a study [28], no evidence of adsorption of ketamine was found with medical plastics commonly used. The modification of drug concentrations was insignificant after 28 days and remained below the official rate defined to 10%, and no degradation product was observed during the study. Conditions of storage have revealed no impact on stability of product. In the same way, different temperatures tested (+20°C and +33°C) have no impact on preservation of ketamine hydrochloride solution. Indeed, between +33°C and +20°C, no significant differences were showed.

All these results demonstrate that these ketamine hydrochloride solutions prepared in devices for patient administrations were stable for up to 28 days at room temperature +25°C +/- 2°C and for 7 days at +33°C.

To improve the quality and the security, development of post-production analytical control before delivery of therapeutic to patient was needed.

In practice, the Department of Clinical Pharmacy of the Institute Gustave Roussy is now able to product qualified batches of ketamine hydrochloride solutions for PCA devices with expiry date of 28 days at room temperature. This study allows the organisation of the production campaigns in collaboration with the Pain Unit. This enables us to decrease the risk of loss of product and to respond as quickly as possible to urgent prescription requests.

DISCLOSURES AND ACKNOWLEDGMENT

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REFERENCES

1. Breivik H et al. Cancer-related pain: a pan-european survey of prevalence, treatment, and patient attitudes. Annals of oncology. 2009; 20 :1420-1433.
2. Portenoy RK. Treatment of cancer pain Lancet. 2011; 377(9784):2236-47.
3. Krakowski I et al. (2003) Summary version of the standards, Options and Recommendations for the use of medical analgesics for the treatment of nociceptive pain in adults with cancer (update 2002). Br J Cancer. 89:567-572.
4. Caraceni A et al. Use of opioid analgesics in the treatment of cancer pain: evidence-based recommendations from the EAPC. Lancet Oncol. 2012; 13:e58-68.
5. Welsch C et al. Données épidémiologiques sur la douleur du cancer en France. Evolution sur deux décennies de la prévalence et de l'intensité de la douleur chez les maladies atteints de cancer. Douleur Analg. 2013; 26:126-132
6. Lossignol DA, Obiols-Portis M, Body JJ. Successful use of ketamine for intractable cancer pain. Supportive care cancer. 2005; 13:188-193
7. ANSM. RBP. Recommandation de bonne pratique pour la prise en charge de la douleur cancéreuse rebelle chez l'adulte en situation palliative. 2010.
8. Hocking G et al. Ketamine: does life begin at 40? Pain Clinical Updates. 2007; 15:3.
9. Bell RF, Eccleston C, Kalso E. Ketamine as adjuvant to opioids for cancer pain. A qualitative systematic review. Journal of Pain and Symptom Management. 2003; 26(3):867-75.
10. Kajiume T et al. Continuous Intravenous Infusion of Ketamine and Lidocaine as Adjuvant Analgesics in a 5-Year-Old Patient with Neuropathic Cancer Pain. J Palliat Med. 2012; 15(6):719-22.
11. Klepstad P, Borchgrevink P, Hval B, Flaat S, Kaasa S. Ketamine as an adjuvant to opioids for cancer pain. Cochrane Database Syst Rev. 2003; (1):CD003351
12. Conway M, White N, Jean CS, Zempsky WT, Steven K. (2009) Use of continuous intravenous ketamine for end-stage cancer pain in children. J Pediatr Oncol Nurs. 26(2):100-6.
13. Lloyd-Williams M. Ketamine for Cancer Pain. Journal of Pain and Symptom Management. 2000; 19(2):79-80.
14. Fine PG. Low-Dose Ketamine in the Management of Opioid Nonresponsive Terminal Cancer Pain. Journal of Pain and Symptom Management. 1999; 17(4):296-300.
15. Salas S et al. Ketamine analgesic effect by continuous intravenous infusion in refractory cancer pain: considerations about the clinical research in palliative care. J Palliat Med. 2012; 15(3):287-93.
16. Salas S et al. Utilisation de la kétamine en soins palliatifs: revue de la littérature Med Pal; 2004; 3: 277-284.
17. Donnelly RF. Physical compatibility and chemical stability of ketamine-morphine mixtures in polypropylene syringes. Can J Hosp Pharm. 2009; 62(1):28-33.
18. Walter SE, Law S, DeAngelis C. Stability and compatibility of hydromorphone and ketamine in normal saline. Can J Hosp Pharm2001; 54:193-201.
19. Watson DG et al. Compatibility and Stability of Dexamethasone Sodium Phosphate and Ketamine Hydrochloride Subcutaneous Infusions in Polypropylene Syringes. Journal of pain and symptom management.2005; 30(1):80-6.
20. Schmid R et al. The stability of a ketamine-morphine solution. Anesth Analg. 2002; 94(4):898-900.
21. General methodes. 2.6 Biological methods. 2.6.1 Sterility 07/2010:20601. 2014 European Pharmacopoeia 8th edition.
22. Ensom MH et al. Stability of Hydromorphone-Ketamine Solutions in Glass Bottles, Plastic Syringes, and IV Bags for Pediatric Use. Can J hosp Pharm. 2009; 62(2):112-118.
23. Monograph of Ketamine 01/2008:1020. European Pharmacopoeia 7th edition
24. Stability testing of new drug substances and products. Q1A(R2) International conference on harmonisation ICH
25. Validation of analytical procedures: Text and Methodology Q2 (R1). International conference on harmonisation ICH.
26. Impurities in new drug products Q3B (R2). International conference on harmonisation ICH.