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Article

Extended stability study of an extemporaneously analgesic solution of clonidine, ropivacaine and fentanyl

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The aim of this work was to perform an extended stability study for the analgesic containing fentanyl, clonidine and ropivacaine in physiological saline solution 0.9% at different infusion sites, such as infusion bags, epidural infusion sets and syringes. The extended stability was assessed by an HPLC system equipped with a photodiode array detector set at 210 nm. The separation was conducted on a C18 column maintained at 40˚C and using an isocratic mobile phase consisted of buffer solution-methanol-acetonitrile (45:45:10, v/v/v). The presence of particulate matter and the pH of each solution were also investigated. Twenty-four hours after the preparation, the formation of one suspected product was observed and for all drugs, in 24 hours it was observed the concentration decrease in different sets (PVC infusion bags, syringes and epidural infusion administration sets). The pH values of each solution varied no more than 5% during the study and no particle was observed. Conclusion: The extended stability study was applied to the analgesic solution and promoted the detection of an unexpected peak in 24 hours. Based on it, further stability studies are necessary to determine the extended stability data.

Keywords: Cancer. Clonidine. Extended stability. Fentanyl. Ropivacaine.

INTRODUCTION

Patients with lung cancer undergo a procedure called thoracotomy to remove all or part of the lung through a cut on one side of the thorax. It is associated with a post-thoracotomy pain syndrome (PTPS), which can last for months after the surgery (Gotoda *et al.,* 2001; Wildgaard et al., 2011) reducing the patient´s quality of life (Kehlet, Jensen, Woolf, 2006; Cregg, Anwar, Faquhar-Smith, 2013; Haroutiunian *et al.,* 2013), and increasing the morbidity. For its management, there is a great number of methods available, like epidural analgesic infusions with opioids with local anesthetics (Peeters-Asdourian, Gupta, 1999; Guay, Nishimori, Kopp, 2016) associated with clonidine that improves and prolongs the analgesia (Tryba, Gehling, 2002) with effective pain relief.

Preemptive thoracic epidural analgesia (TEA) preserves pulmonary function, reduces pain severity and analgesic requirements (Arm *et al.,* 2010) and it is associated with a better control of postoperative pain and a decrease in epidural medications or supplemental intravenous treatment (Sentürk *et al.,* 2002).

The pharmaceutical mixture of fentanyl citrate (FENT CIT)5.0 µg/mL, hydrochloride clonidine (CLO HCl) 1.0 µg/mL and hydrochloride ropivacaine (ROP HCl) 1000 or 2000 µg/mL (Figure 1) in sodium chloride (NaCl) 0.9% is an analgesic solution that can improve analgesia in post- thoracotomized oncologic patients from the Brazilian National Cancer Institute. This triple combination was used previously. It increased analgesia

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in continuous lumbar epidural infusion after total knee arthroplasty and, though the increase in clonidine dose is related to hypotension, it improves the quality of the pain management regimen (Forster, Rosenberg, 2004).

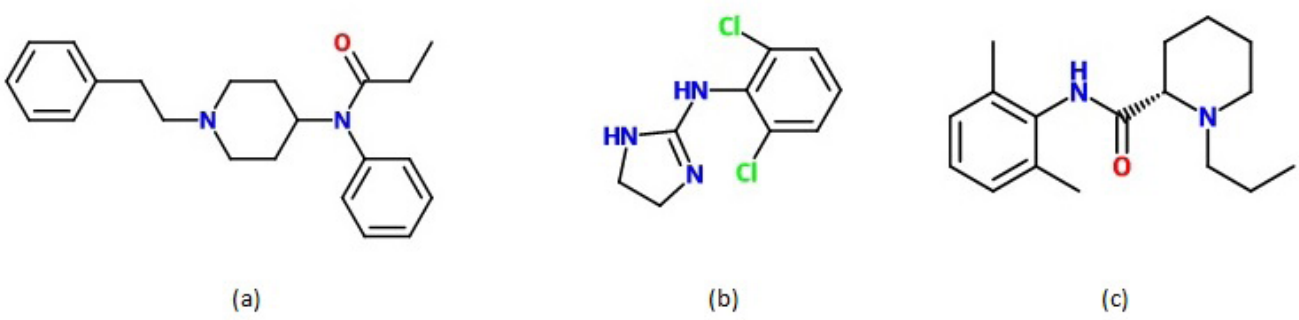

FIGURE 1 - Molecular structures of the following compounds: (a) fentanyl; (b) clonidine; (c) ropivacaine.

However, the absence of stability data is an important issue and such gap should be filled to ensure safety and quality during the treatment, which can be obtained through further investigation addressing stability studies. Physical-chemical, environmental, personnel and operational factors can affect the integrity, stability and sterility of sterile preparations. Parenteral drugs are often diluted and administered in different manufacturer's conditions, which can affect their stabilities. Extended stability expiration date is the maximum period that the minimum of 90% of a labeled drug is measurable in solution in storage conditions in a specified recipient. These stability data assure the beyond-use dating to dispensing the sterile preparations during the overall treatment duration (Bing *et al.,* 2005). ROP and CLO or ROP and FENT have been shown stable for up to 30 days after preparation (for up to 30 °C in Mark II Polybags®) (Svedberg, McKenzie, Larrivee-Elkins, 2002). However, the infusion of these three drugs in the same infusion bag was not determined.

FENT (N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl] propanamide) is a potent synthetic narcotic analgesic, lipophilic with a rapid onset and 75-100 times more potent than morphine. Its metabolites, norfentanyl and despropionylfentanyl are inactive and without pharmacologic effects (Sakata, Issy, 2008). It is commonly used in epidural treatment for postoperative analgesia and, when combined with local anesthetics, it minimizes the dosage of both drugs and reduces side effects (Gustein, Akil, 2005).

CLO (N-(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine) is an imidazoline used in anesthetic practice, a lipid-soluble drug which crosses the blood-brain barrier (Khan, Fergunson, Jones, 1999). Spinal CLO associated with sufentanil 7.5 µg, added to bupivacaine 2.5 mg, prolongs analgesia without producing serious adverse reactions (D'Angelo *et al.,* 1999). CLO increases motor block duration and analgesia when it is administered with ROP, prolongs postoperative analgesia and increases sedation (Alves, Braz, 2002).

ROP ((S)-N (2,6-dimethylphenyl)-1-propylpiperidine-2carboxamide) is a S-enantiomer of 1-propyl-2',6'- pipecoloxylidine that interacts directly with voltage-gated sodium channels and promotes increase in the threshold for electrical excitability. It has a reduced potency in the production of anesthesia but is less cardiotoxic and more motor-sparing than bupivacaine (Catterall, Mackie, 2005).

The aim of this work was to perform an extended stability study for the analgesic containing FENT CIT, CLO and ROP at different infusion sites, such as infusion bags, epidural infusion sets and syringes.

MATERIAL AND METHODS

Chemical and reagents

The secondary standards and the commercially available formulations of FENT CIT, CLO HCl and ROP HCl were obtained from Cristália Produtos Químicos

Farmacêuticos Ltda. (Rio de Janeiro, Brazil) High-Performance Liquid Chromatography (HPLC) grade acetonitrile and methanol from Tedia® (Rio de Janeiro, Brazil), sodium acetate monobasic from ProQuimios® (Rio de Janeiro, Brazil) glacial acetic acid from Merck® (São Paulo, Brazil), triethylamine from Vetec® (Fortaleza, Brazil) and ultra-pure water were used to prepare the mobile phase.

Equipment

The method was developed in a HPLC system, comprising a Jasco® from Cromatec® (São Paulo, Brazil), HPLC LC 2000 equipped with a MD-2018 photo diode array detector (DAD), a ChromNav data acquisition system, two pumps with a PU-2089s degasser, AS-2059 autosampler, a LC-NetII/ADC interface system and a CO-2060 column oven.

Development of Chromatographic conditions

The analytes FENT CIT, CLO HCl, and ROP HCl in the samples and standard solutions were injected (40 µL) and eluted isocratically through a C18 Kromasil Asko Nobel® column (250 mm X 4.6 mm, 5 µm) maintained at 40˚C using sodium acetate monobasic buffer solution (pH= 5.0; 5 mM)-methanol-acetonitrile (45:45:10, v/v/v) as the mobile phase at a flow rate of 1.0 mL/min. The detection occurred at 210 nm and peak area responses were used to quantify the drugs. Under these conditions, the chromatographic parameters were obtained and described in the Table I. The retention times (TR) obtained for FENT CIT, CLO HCl and ROP HCl were 10.23, 2.67 and 8.29, respectively. All drugs had a peak purity index of 100%. The asymmetry values obtained, related to chromatographic efficiency, for FENT and CLO (1.0355 and 1.4980) were satisfactory. The asymmetry value obtained for the ROP (0.8810) was below the recommended lower value, probably because it is still a large injected mass. However, all values are below 2. The number of theoretical plates for each drug was respectively for FENT CIT, CLO HCl and ROP HCl 8655, 6319 and 5219.

TABLE I - Chromatographic parameters developed for the method of quantification of FENT CIT, CLO HCl and ROP HCl by HPLC/DAD

Chromatographic parameters	FENT CIT	CLO HCl	ROP HCl
Retention Time (RT) (min)	10.23	2.67	8.29
Theoretical Plates	8655	6319	5219
Assimetry Factor	1.0355	1.4980	0.8810
Peak Purity Index (%)	100.0	100.0	100.0
Mass Ratio Distribution	4.1476	0.3420	3.1713

‘Source: Elaborated by the authors

Method Validation

The method described above was validated according to the Brazilian Guideline RE n°899/2003 (Brasil, 2003). Validation was done during three days for the following validation parameters: specificity, linearity, range, accuracy, precision and robustness.

Specificity

Specificity was determined from the analysis of samples of solutions prepared without true triplicates samples of CLO HCl, ROP HCl and FENT CIT, at concentrations corresponding to 80%, 100% and 120% of the theoretical concentration of the test, from the stock solutions prepared. The samples were injected into a chromatograph coupled to a diode array detector, through which peak purity and degradation tests were used, to attribute the chromatographic peak obtained to only one component.

Analgesic solution degradation

The degradation study was carried out by the methodology proposed by Singh & Bakshi (2000). From it, a classification system was created, in which the drug is exposed to different conditions (acidic, basic and oxidation hydrolysis). For the present study, the conditions for classifying the drug as stable were chosen. Under these conditions, the drugs were exposed to: 1. Acid (HCl 1 N) and alkaline (NaOH 1 N) hydrolysis reactions with reflux at 90 ºC for 12 h 2. Oxidative degradation reaction (H_2O_2 3%) at room temperature for 12 h. If the drug does not degrade, it is considered stable. To carry out this assay, solutions were prepared in high concentrations of the drugs CLO HCl (25 µg/mL), ROP HCl (500 µg/mL) and FENT CIT (25 µg/mL) in saline solution of 0.9 NaCl %, isolated and mixed, and also from 0.9% NaCl saline solution (white) prepared from ampoules and secondary standards, in order to evaluate their stability properties and the specificity of the method in acidic, basic media and oxidative.

Linearity

Five-point analysis was determined for each day of validation, corresponding to 80%, 90%, 100%, 110% and 120% of the theoretical concentration (FENT CIT 30 µg/mL, CLO HCl 4 µg/mL and ROP HCl 100 µg/mL of the test. The solutions were prepared from the stock solution of the secondary standards of FENT CIT, CLO HCl and ROP HCl for the three days of validation, with a total of nine replicates. The test results were treated with the determination of the correlation coefficient (minimum acceptable value = 0.99).

Range

For the intended application of the method, the range determined was 80% - 120% and linearity was evaluated.

Accuracy

It was performed at two levels: 1. Repeatability (intra-assay precision) and 2. Intermediate precision (inter-assay precision). To verify the repeatability, we used 9 determinations (corresponding to 3 triplicates) of the solutions at 85, 95 and 115% of the theoretical concentration of the test (low, medium and high concentrations). Solutions were prepared from the available secondary standards of FENT CIT 1.0. mg/ml, CLO HCl 1.0 mg/ml and ROP HCl 10.0 mg/ml in 0.9% NaCl saline solution. All analyzes were performed on the same day with the same instrumentation and analyst.

To verify the intermediate precision, 9 determinations (85%, 95% and 115%) were used, based on the available standards. All analyzes were performed on three different and consecutive days, with the same instrumentation and the same analyst.

Precision and Robustness

It was determined from stock solutions of drug standards that are components of the analgesic solution, at concentrations corresponding to 85%, 95% and 115% of the theoretical concentration of the test. The solutions were manipulated in true triplicate, with a total of 9 determinations for each day of validation.

For the robustness test, on two different days after the third day of validation, the conditions of the chromatographic run were modified: 1. Run temperature from 40°C to 35°C (first day) and 2. Change of flow 1, 0 ml/min to 0.8 ml/min. Modifications were performed with samples at the theoretical concentration of the test (100%) in true triplicate. From the results obtained, the accuracy and precision values for each variation of the proposed method were calculated.

Determination of a particulate matter, pH and extended stability assays

Solution preparation

The manipulation of sterile polyvinyl chloride (PVC) bags and syringes of the analgesic solution was made in a hospital pharmacy-controlled atmosphere area. To the particulate matter and pH assays, PVC bags of the analgesic solution were prepared in their respective concentrations: CLO HCl 1.0 µg/mL, ROP HCl 2000.0 µg/mL and FENT CIT 5.0 µg/mL in NaCl 0.9%. To this purpose, 3.33 mL of CLO HCl in 150.0 µg/mL ampoule, 50.0 mL of ROP HCl in 10.000 µg/mL ampoule and 25.0 ml of FENT CIT in 50.0 µg/mL ampoule were used and mixed to 171.67 mL of NaCl 0.9%.

To the extended stability assay, PVC bags of analgesic solution were also prepared in their corresponding concentrations: CLO HCl 4.0 µg/mL, ROP HCl 100.0 µg/mL and FENT CIT 30.0 µg/mL in NaCl 0.9%. To this purpose, 2.67 mL of CLO HCl in 150.0 µg/mL ampoule, 1.0 mL of ROP HCl in 10.000 µg/mL ampoule and 60.0 mL of FENT CIT in 50.0 µg/mL ampoule were used and mixed to 36.33 mL of NaCl 0.9%. Each PVC bag was connected to filled epidural infusion administration sets. Syringes were filled with 10.0 mL of analgesic solution.

Determination of particulate matter and pH in analgesic solution

Particulate matter in the analgesic solution of FENT CIT 5.0 µg/mL, CLO HCl 1.0 µg/mL and ROP HCl 2000.0 µg/mL was determined by the microscope particle count test (United States Pharmacopeia, 2008). Samples were removed from the analgesic solution into a Class II laminar flow cabinet, using aseptic techniques and observing biosafety precautions. A binocular microscope coupled to an illuminator was used to determine the appearance of the solution. For this, an ocular micrometer - a circular diameter graticule (MF-830 Microforge NARISHIGE®) - was coupled to the microscope. Analgesic solutions were filtered in a gridded membrane filter of 0.8 µm. Membranes were observed with the binocular microscope at 100±10x magnification. To count the particles, the technique of partial count was chosen. To determine the pH, a pHmeter (Digimed®) was used. For both assays, aliquots of 225 mL and 25 mL from twelve PVC bags of the analgesic solution were used to particulate matter and pH assays. They were performed at times 0, 24, 48 and 72 hours (h) after manipulation. The one-way analysis of variance (ANOVA) was performed, followed by Bonferroni's posttest to identify the differences at the studied times.

Extended stability study of the analgesic solution

To the extended stability study solutions of FENT CIT 30.0 µg/mL, CLO HCl 4.0 µg/mL and ROP HCl 100.0 µg/mL in NaCl 0.9% stored in five PVC infusion bags, five peridural infusion administration sets and five syringes were analyzed by HPLC/DAD at times 0 h and 24 h after manipulation through the developed method. In this study, stability was assumed if the loss was less than 10% of the initial concentrations (Bing, *et al.,* 2005) The hypothesis test (t-test) was conducted to compare percentage decrease in 24 h.

RESULTS AND DISCUSSION

Method validation

Specificity

Due to the unavailability of impurity standards or degradation products, a degradation test was performed in order to compare the RT of 2.658, 8.283 and 10.197 minutes for CLO HCl, ROP HCl and FENT CIT (Figure 2), respectively, with the RT of compounds obtained from samples stored under stress conditions for drug solutions isolated and mixed in 0.9% NaCl, in this study. It was not possible to carry out the oxidation test of the analgesic solutions prepared from the ampoules and the secondary standard due to the loss of the respective samples during the degradation test. It is observed that under the described conditions, no chromatographic peak elution was observed in the RT corresponding to the study drugs, that is, 2.6683, 8.2883 and 10.2283 minutes. Purity testing was also used to demonstrate the specificity of the chromatographic peak.

For all drugs, 100% high peak purity values were observed with high sensitivity and resolution. When compared, the RT of the chromatographic peaks of each drug separately, in solution prepared from the ampoules and the standard; the results demonstrate similarity between the RT obtained, confirmed by the injections performed in the extended stability study, through the dosage of drugs in bag, syringe and infusion set.

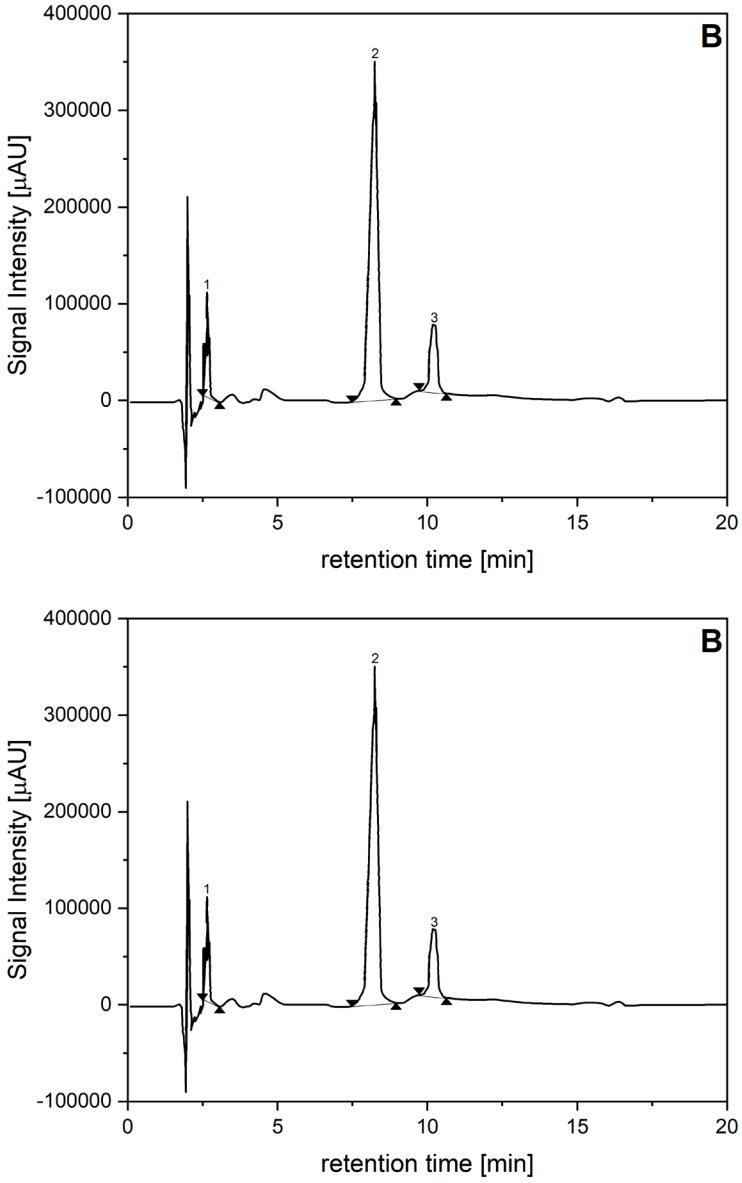

FIGURE 2 - Chromatograms showing: A. Physiologic solution of NaCl 0.9%; B. Analgesic solution of 1. CLO HCl 4.0 µg/mL; 2. ROP HCl 100.0 µg/mL and 3. FENT CIT 30.0 µg/mL.

Linearity

To quantify the analyte in the pharmaceutical form, a calibration curve was analyzed in the range of 80%-120% of the theoretical concentration of the three analytes, through the determination of five concentration levels, corresponding to the points 80, 90, 100, 110 and 120%, respectively. Through the data obtained, it was possible to establish the correlation coefficient (r) of the calibration curve for each drug component of the mixture, for each

day of validation. Table II shows that the correlation coefficient was greater than 0.99 (Brasil, 2003) for the three drugs in the study and the analytical methodology remained linear within the range of 80% to 120%.

TABLE II - Linearity parameters of the analytical method

Linearity parameters	FEN CIT Mean ± Standard Deviation	CLO HCl Mean ± Standard Deviation	ROP HCl Mean ± Standard Deviation
Angular Coefficient (a)	34245.57 ± 742.6613	156682.3 ± 23995.93	55912.67 ± 3612.837
Linear Coefficient (b)	-18155.2 ± 15754.53	2457.943 ± 13543.75	-13425.4 ±32697.99
Correlation Coefficient (r)	0.991658 ± 0.000207	0.994457 ± 0.001381	0.993297 ± 0.002328

Accuracy and Precision (Intraday and Interday)

The averages of the results of inter-assay and intra-assay precision are found, respectively, in Tables III. To determine the inter- and intra-assay accuracy, the proposed analytical methodology was applied to a reference standard of known purity. Accuracy was calculated through the percentage difference between the mean of the obtained concentrations and the true value, plus the confidence intervals (Brasil, 2003). The susceptibility of the method to analytical variations was determined by varying the temperature (35°C) and mobile phase flow (0.8 mL/min). Accuracy and precision were calculated for the three drugs. The results of the robustness test obtained in table 18 show that the method is robust to variations in the conditions of the analytical parameters, whose values were not greater than 5%. The analysis of samples stored in a tray (short term stability) for 10 hours demonstrated that the drugs remain stable during the proposed running period for the extended stability test. The results (Table III) demonstrate that the accuracy and precision values obtained also do not exceed a variation above 5% (Brasil, 2003).

TABLE III - Results of interday and intraday accuracy and precision of the analytical methods (n=3 for three concentrations levels)

Drugs	Interday (Mean %)		Intraday (Mean %)	
	Accuracy	Precision	Accuracy	Precision
FENT CIT	98.03	2.99	98.03	1.44
CLO HCl	99.80	2.66	99.78	1.11
ROP HCl	100,05	3.43	100.08	1.51

Source: Elaborated by the authors.
n=9

Robustness

The results of the chromatographic parameters (Table 4) are concerned with the acceptability criteria for the validation of analytical methods proposed by the Guide for Validation of Analytical and Bioanalytical Methods (Brasil, 2003).

TABLE IV - Short term robustness and stability test results

Parameters	FENT CIT	
	Accuracy (%)	Precision (%)
Temperature 35°C	98.13	3.94
Flow 0.8 mL/min	105.00	4.49
Tray Stability	96.95	4.84
	CLO HCl	
Temperature 35°C	102.92	0.09
Flow 0.8 mL/min	98.13	1.81
Tray Stability	96.49	3.07
	ROP HCl	
Temperature 35°C	104.26	2.56
Flow 0.8 mL/min	101.89	0.01
Tray Stability	95.00	1.60

Source: Elaborated by the authors.

The method was suitable for the quantification of the drugs FENT, CLO and ROP in the extended stability study.

Particulate matter and pH assays

In solutions for parenteral infusion with a nominal content of 250 mL, the average number of particles equal or greater than 25 µm does not exceed 2 per mL, whereas those equal or greater than 10 µm does not exceed 12 per mL (United States Pharmacopeia, 2008). During the overall period of 0, 24, 48, and 72 h, there was no evidence of particulate material. Amorphous and indistinct material were observed at times 0 h and 24 h but it cannot be considered particulate material (Table V).

TABLE V - Results obtained from microscopic particle testing

Time (hours)	Petri Dishes	Particle Number	Particle Size (µm)
0	1 (blank)	0	-
	2	1	6 (*)
	3	0	-
	4	0	-
24	1 (blank)	0	-
	2	1	30 (*)
	3	0	-
	4	1	30 (*)

TABLE V - Results obtained from microscopic particle testing

Time (hours)	Petri Dishes	Particle Number	Particle Size (µm)
48	1 (blank)	0	-
	2	0	-
	3	0	-
	4	0	-
72	1 (blank)	0	-
	2	0	-
	3	0	-
	4	0	-

(*) Particle with amorphous appearance
Source: Elaborated by the authors

Regarding the pH assay, for each time (0 h, 24 h, 48 h, and 72 h) difference in pH was compared to identify significant changes (p=0.05) (Table VI) Through Bonferroni's post-test, it was observed that there were significant differences in pH between the times 24, 48, and 72 h when compared to that at time 0 h (Figure 3). However, the variability remained between ± 5% from the initial value. Considering the pH values of the sterile injectable solutions, i. e., fentanyl: 4.0 – 6.5; clonidine: 5.0 -7.0 and ropivacaine (4.0 – 6.0) (EMC, FDA), the analgesic solution did not present pH outside the acceptable range for the drugs individually.

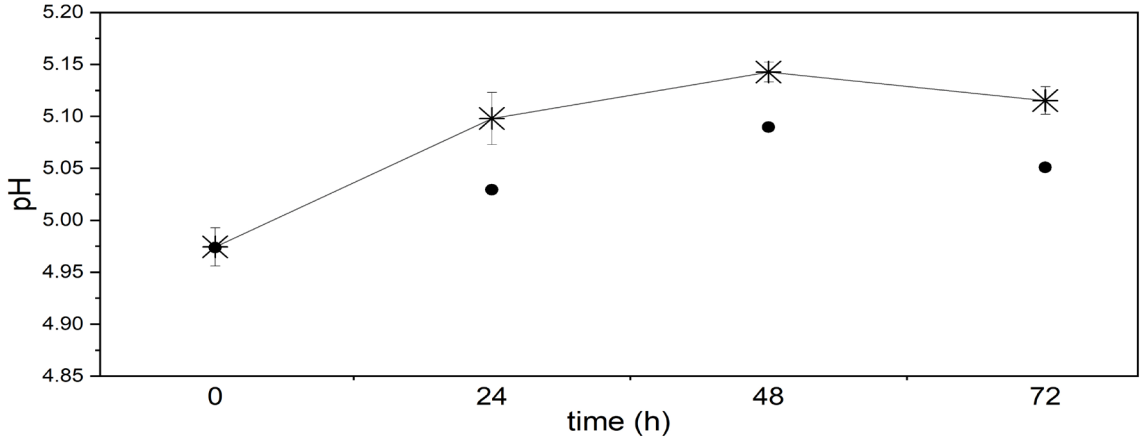

FIGURE 3 - Result of pH assay at times 0, 24, 48 and 72 h.

According to the studies carried out by the authors, no reference was found on the stability of the analgesic solution at different pHs. However, as the pH variation did not exceed the pH limits imposed by the manufacturers on each formulation separately, the 5% variation was accepted for the present extended stability study.

TABLE VI - pH values obtained at times 0 h, 24 h, 48 h and 72 h

Time (hours)	Infusion Bag	pH 1	pH 2	pH 3
0	1	5.04	5.00	4.96
	2	4.99	4.96	4.96
	3	4.97	4.96	4.94
	Mean ± SD	5.00 ± 0.04	4.97 ± 0.02	4.95 ± 001
24	1	5.16	5.11	5.11
	2	5.13	5.08	5.07
	3	5.11	5.07	5.06
	Mean ± SD	5.13 ± 0.04	5.09 ± 0.02	5.08 ± 0.03
48	1	5.18	5.17	5.16
	2	5.13	5.13	5.13
	3	5.13	5.13	5.13
	Mean ± SD	5.15 ± 0.03	5.14 ± 0.02	5.14 ± 0.02
72	1	5.16	5.13	5.13
	2	5.12	5.11	5.12
	3	5.11	5.10	5.09
	Mean ± SD	5.13 ± 0.03	5.11 ± 0.02	5.11 ± 0.02

SD: Standard Deviation
Source: Elaborated by the authors

Extended stability study

The analgesic solution of CLO HCl 4.0 µg/mL, ROP HCl 100.0 µg/mL and FENT CIT 30.0 µg/mL in NaCl 0.9% stored in five infusion bags, in five peridural infusion administration sets and in five syringes was manipulated at 20.4ºC for 1.5 h and stored at 2-8ºC. Analyses were conducted over a 24-h period.

The extended stability was examined by the recovery data in 0 and 24 h for the three drugs in different recipients. A decrease in the concentration of all the drugs was observed in all studied recipients in 24 h. The percentage decreases in 24 h for the drugs CLO HCl, ROP HCl and FENT CIT in PVC infusion bags, syringes and epidural infusion administration sets were calculated, respectively (Table VII). For all drugs, in 24 hours it was observed the concentration decrease. Moreover, a further finding was observed: the least recuperation rates were in peridural infusion administration sets for CLO HCl and FENT CIT: 66.34 % and 83.14%, respectively. For ROP HCl, the least concentration rate was found in syringes (75.49%). Simultaneously, a peak in samples of 24 h with retention time equal to 4.838 ± 0.087 minutes was observed in the three analyzed recipients (A, B and C) (Figure 4).

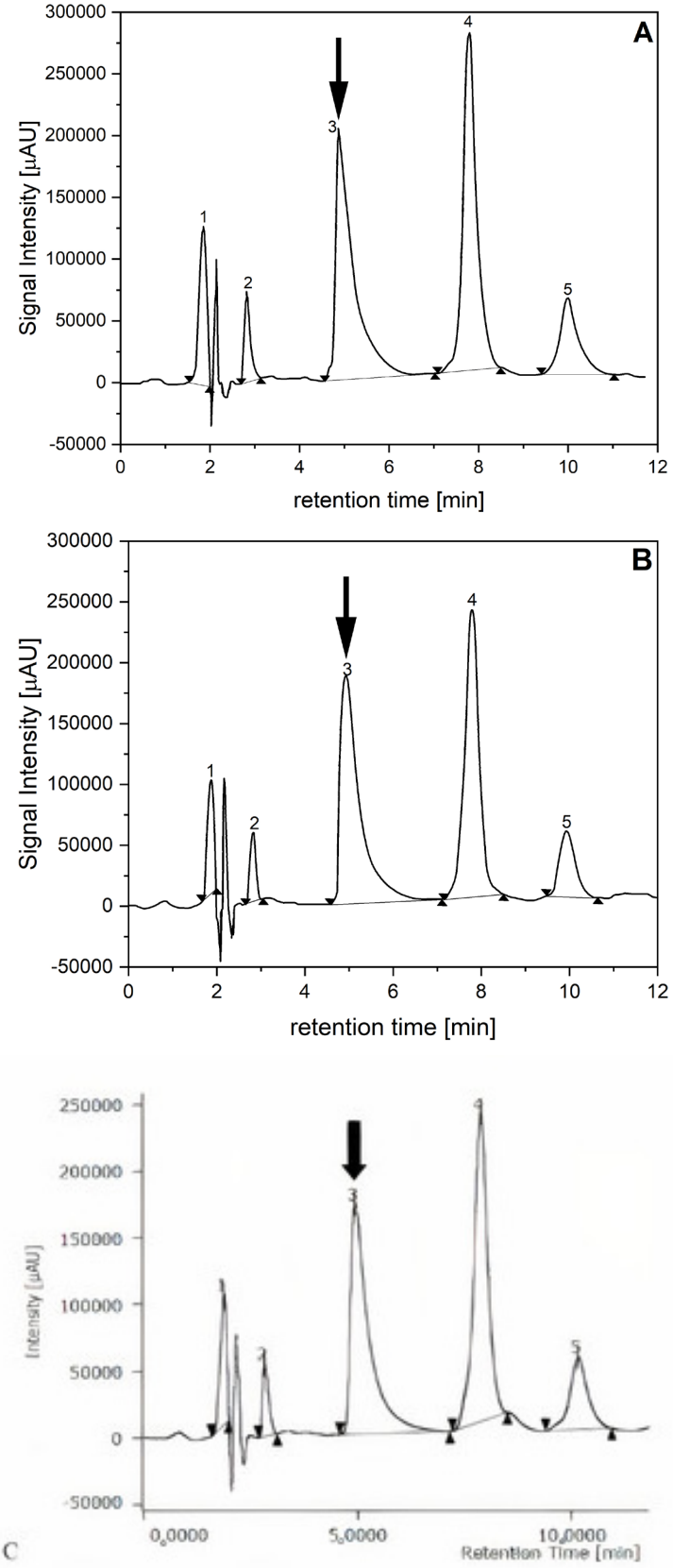

FIGURE 4 - Chromatograms from the extended stability study in A. infusion bag; B. peridural infusion administration sets and C. syringes. 1. Compound of fentanyl ampoule; 2. CLO HCl; 3. Suspect peak 2; 4. ROP HCl and 5. FENT CIT. In detail (black arrow) to identify the suspect peak.

TABLE VII - Results of extended stability study for CLO HCl, ROP HCl and FENT HCl in different sets

Analyzed Sets	Drugs	CLO HCl		RR (%)	ROP HCl		RR (%)	FENT CIT		RR (%)
Time (h)		0	24	CLO HCl	0	24	ROP HCl	0	24	FENT CIT
Infusion bags	Mean ± SD	3.97 ± 0.25	3.00 ± 0.22	75.55	140.43 ± 18.12	126.08 ± 20.98	89.79	52.03 ± 7.52	51.35 ± 6.72	98.70
Syringes		3.97 ± 0.48	3.16 ± 0.46	79.75	132.74 ± 11.82	100.20 ± 9.20	75.49	52.16 ± 5.48	45.25 ± 5.28	86.74
Peridural infusion administration sets		4.39 ± 1.08	2.91 ± 0.88	66.34	132.50 ± 13.58	104.06 ± 15.03	78.54	49.06 ± 6.40	40.79 ± 4.63	83.14

SD: Standard Deviation: SD
RR: Recuperation rate (%)
Elaborated by the authors

The purpose of this study was to determine the extended stability of an analgesic solution of the drugs CLO HCl, ROP HCl and FENT CIT in saline solution. Because of their widely use, many HPLC methods have been developed to analyze these drugs in other analgesic admixtures (Wolf, Poklis, 2005; Sálmeron-García *et al.,* 2009). The association between local anesthetics and opioids improves the thoracic epidural analgesia in patients who had undergone pulmonary resection (Mahon *et al.,* 1999). The addition of CLO 2 μg/mL to a continuous lumbar epidural infusion admixture of FENT 5 μg/mL and ROP 2 mg/mL improves analgesia in patients after total knee arthroplasty (Forster, Rosenberg, 2004). Previous methods have not identified the three drugs in the same solution. Combinations of CLO and ROP as well as admixtures of ROP and FENT were analyzed by High-Performance Liquid Chromatography – Ultraviolet (HPLC/UV) detector separately and were stable for up to 30 days (Svedberg, McKenzie, Larrivee-Elkins, 2002). A HPLC/UV method based on European Pharmacopeia was carried out to identify the stability of a ROP/FENT solution which was described as stable for 51 days in glass containers and in ethylene-vinyl acetate (EVA) bags, unprotected from light (Hartmann *et al.*, 2003).

The High-Performance Liquid Chromatography – Diode Array Detector (HPLC/DAD) was chosen because these drugs have chromophore groups that can be identified with more advantages when compared to the HPLC/UV to the extended stability study, like the analysis of peak purity, detection of multiple wavelengths and the acquisition of the scan spectrum. Assays to identify incompatibility among the ingredients of the formulation or changes in solutions caused by environmental factors were performed in solutions in PVC bags. The analyzed pH values have not varied more than 5%, despite the statistically significant difference between time 0 h and others. Signs of incompatibility and precipitation have also not been evidenced by the microscope particle count test during the whole 72-h study period.

Parenteral drugs are often compounded, diluted prior to administration and stored under different conditions than the established for bulk ingredients (Bing *et al.,* 2005). For this reason, studies have been conducted to evaluate the stability and compatibility of analgesic solutions in different recipients (Svedberg, McKenzie, Larrivee-Elkins, 2002; Hartmann *et al.*, 2003; Peterson *et al.*, 1998; McCluskey *et al.*, 2009; Lee *et al.*, 2005). These results were not specific to a packaging material or drug, as reported by a previous study (Hartmann *et al.*, 2003), focusing on the instability of FENT in PVC bags by adsorptive losses. The decrease was concomitant with the emergence of a new chromatographic peak with high

absorbance at 210 nm. This result was not observed in any stage of the method development in solutions prepared from ampoules or from the standards of the drugs isolated or in combination under laboratory conditions. In spite of the previous assays results which indicated no instability related to pH changes and precipitation formation, it is evident that the solution of the drugs FENT CIT, CLO HCl and ROP HCl in NaCl 0.9% is not stable, and the reasons are the unknown suspect peak and the loss of chemical potency in 24 h. In addition, a compound related to FENT CIT commercial formulation was observed in all stages of the development method (data not shown). From these results, it can be concluded that this peak corresponds to an excipient or a possible impurity/degradation product from FENT CIT (Garg *et al.*, 2010). The structural elucidation of both compounds is beyond the scope of this study. However, it is important to highlight that the absorbance was very low (in FENT solution) when it is compared to the results of the analgesic solution. Due to these results, further extended stabilities studies are necessary for the identification of the suspected peak.

CONCLUSION

The extended stability study was successfully applied to the analgesic solution that was efficient to detect an unexpected peak in 24 h. Based on the physicochemical study, we conclude that the analgesic solution of FENT CIT 5.0 µg/mL, CLO HCl 1.0 µg/mL and ROP HCl 1000 or 2000 µg/mL needs additional extended stability studies.We weren't capable to identify/quantify suspected peaks (products and degradation, formulation components or generation of new products) to determine the extended validity of the extemporaneous solution in the period of 24 h. So, further studies are necessary to identify/quantify the suspected peaks in different times and this way, to determine the extended stability data.

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