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RESEARCH ARTICLE



## Physicochemical and microbiological stability of two new oral liquid formulations of clonidine hydrochloride for pediatric patients

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### ABSTRACT

Pediatric patients present changing physiological features. Because of the lack of a suitable formulation for commercial management, pediatric specialties very often need to prepare extemporaneous formulations to improve the dosage and administration of drugs for children. Oral liquid formulations are the most suitable for pediatric patients. Clonidine is widely used in the pediatric population for opioid withdrawal, hypertensive crisis, attention deficit disorders and hyperactivity syndrome, and as an analgesic in neuro-pathic cancer pain. The objective was to study the physicochemical and microbiological stability and determine the shelf life of an oral solution containing 20 µg/mL clonidine hydrochloride in different storage conditions (5 ± 3 °C, 25 ± 3 °C, and 40 ± 2 °C). Using raw material with excipients safe for all pediatric age groups, two oral liquid formulations of clonidine hydrochloride were designed (with and without preservatives). Solutions stored at 5 ± 3 °C (with and without preservatives) were physically and microbiologically stable for at least 90 days in closed containers and for 42 days after opening. Two oral solutions of clonidine hydrochloride 20 µg/mL were developed for pediatric use from raw materials that are readily available and easy to process, containing safe excipients that are stable over a long period of time.

### ARTICLE HISTORY

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### KEYWORDS

Clonidine; compounding; stability; HPLC; microbiology

## 1. Introduction

The physiological characteristics of pediatric patients change according to gestational age and postnatal development and certain congenital or acquired conditions that may be unique to this period of life or shared with adults (Bartelink et al. 2006). The population of sick children is a small one, representing a very small percentage of consumption of medicines. Few pediatric clinical trials are performed (Muro-Brussi 2004), which means that many of the drugs marketed for adults are not approved for children or do not exist in dosage forms suitable for a particular age range (Nahata and Allen 2008). The lack of a suitable formulation for commercial management means that pediatric specialties very often need to prepare, as well as provide, extemporaneous preparations to prevent medication errors at the time of administration (Stephenson 2005). Compounding is often a last resort for such patients receiving drug treatment, especially when there is no active pharmaceutical ingredient (Turner et al. 1998; Feal et al. 2003). Oral liquid formulations are the most appropriate for pediatric patients because they enable safe, accurate dose administration to neonates, infants, and young children unable to swallow solid oral forms (Bauters et al. 2012). However, a serious problem with many liquid preparations is the lack of information about their suitability, bioavailability, and stability (Allen 2008). They are less stable than solid forms, easier to contaminate, and it is more

difficult to mask undesirable organoleptic characteristics that may hinder compliance. Stability studies published in the literature and on pediatric referral forms are often found to contain a specialty pharmaceutical, so that the time period for which the formula is valid cannot be applied when raw material is used, and vice versa. Furthermore, for many formulas needed in everyday clinical practice, there is no choice and no published studies of physicochemical and microbiological stability to provide the information needed to manage the drug safely in the patient's home over a reasonable period of time (American Academy of Pediatrics 1997; Haywood and Glass 2013). With respect to excipients (Whittaker et al. 2009), in order to obtain a formulation that is both stable and palatable, it is relatively common to resort to the use of excipients that are not always safe for all age ranges (Breitkreutz and Boos 2007). In 2006, the Committee for Medicinal Products for Human Use (CHMP) of the EMEA, following the advice of the pediatric working party, published formulations of choice for the pediatric population (EMA 2005). Since then, initiatives have appeared to optimize the use of excipients in this population; some examples are the Safety and Toxicity of Excipients for Pediatrics (STEP) database (Salunke et al. 2012) and the European Study of Neonatal Exposure to Excipients (ESNEE) initiatives (Turner and Storme 2012). For these reasons, the design and formulation of various forms of pediatric dosage should be well defined, including the physical, chemical, and biological

properties of the active substance and all other pharmaceutical ingredients used (Ernest et al. 2007). The ultimate goal is to obtain a stable and effective drug product that is easy to administer, well tolerated, and also meets the quality assurance standards required for preparations of this type (Brion et al. 2003; Wening and Breitreutz 2011).

Clonidine is an imidazoline derivative that lowers pressure in the central nervous system; it acts by reducing sympathetic tone, which lowers systolic and diastolic blood pressure and reduces the heart rate. The exact mechanism is unclear, but it seems that clonidine stimulates the alpha-2 adrenoceptors and imidazoline receptors at the central level. It also acts peripherally, which may be responsible for the transient increase in blood pressure after a bolus injection, and is a possible contributor to the hypotensive effect during chronic use. Clonidine is widely used in the pediatric population for opioid withdrawal, hypertensive crisis, attention deficit disorders and hyperactivity syndrome, and as an analgesic for neuropathic cancer pain (Brayfield 2014). The incidence of the neonatal abstinence syndrome (NAS) has increased substantially in the past decade (Patrick et al. 2012; Patrick et al. 2015; Tolia et al. 2015). In 2012, the syndrome was diagnosed in 21 732 infants in the United States (Patrick et al. 2015), which represents an increase by a factor of 5 during the previous 12 years (Patrick et al. 2012). This is consistent with the increased prevalence of the NAS in other locations, including England, Canada, and Western Australia (Davies et al. 2016), and reflects an increasing global problem. The increase in cases of the NAS corresponds with the reported rise in opioid use during pregnancy (Epstein et al. 2013; Krans et al. 2015; Stover and Davis 2015), which is attributed to the more liberal use of prescribed opioids for pain control in pregnant women (Ailes et al. 2015; Yazdy et al. 2015; Warren et al. 2015), illicit use of opioids such as oxycodone and heroin (Cicero et al. 2015; Gomes and Juurlink 2016), and a dramatic increase in opioid-substitution programs for the treatment of opioid addiction (Jansson and Velez 2012). The data in Spain show that the prevalence of NAS increased from 60% to 68% in heroin addicts and from 77% to 85.7% in methadone addicts between 1982 and 2008 (Ortigosa et al. 2011). Different authors have studied other pharmaceutical forms of clonidine (Levinson and Johnson 1992; de Goede et al. 2012; Ma et al. 2014; Ensom and Decarie 2014; Sauberan et al. 2016; Potier et al. 2017). Nevertheless, the current literature has no data on the physicochemical stability of formulations of clonidine solution 20 µg/mL based on raw material in noncommercial vehicles, and there are no data on microbiological stability for up to 90 days without preservatives. The analytical technique used to perform all these

studies was high performance liquid chromatography (HPLC), which is the reference method of the United States Pharmacopeia (USP-40) and the European Pharmacopoeia (Ph.Eur. 2016) for quantification of clonidine hydrochloride. The objective therefore was to develop a study of the physicochemical and microbiological stability of an oral solution of clonidine hydrochloride (20 µg/mL) under different storage conditions and to determine its shelf life in accordance with the USP.

## 2. Material and methods

### 2.1. Reagents

All the components of the formulation (clonidine hydrochloride, potassium sorbate, sucrose, and citric acid monohydrate) were Ph.Eur. grade, purchased from Acofarma (Barcelona, Spain). All reagents (clonidine hydrochloride, 2,6-dichloroaniline, sodium 1-octanesulfonate, o-phosphoric acid, 85% v/v, methanol, sodium hydroxide, hydrogen peroxide, and hydrochloric acid) were analytical or HPLC grade, purchased from Sigma-Aldrich (Darmstadt, Germany). All solutions were prepared using Milli-Q™ grade deionized water.

### 2.2. Standard operating procedure (SOP)

Two formulations (Table 1) were proposed that could be readily prepared by any pharmacist with the available raw materials. Following the SOP, two formulations were developed with a solution of 20 µg/mL clonidine as the starting drug substance, one with potassium sorbate as a preservative and the other without preservative. The preservative was selected and adjusted in accordance with the limitations on pediatric use determined by the regulatory authorities (EMA 2012).

### 2.3. Storage conditions

The study took into account the conditions of use and storage of different solutions in real-work environments. All solutions were packaged in amber glass bottles and produced in batches of three for each storage condition, according to ICH guidelines (ICH 2003).

The different storage conditions were:

- Refrigeration ( $5 \pm 3^\circ\text{C}$ )
- Room temperature ( $25 \pm 2^\circ\text{C}$ )
- $40 \pm 2^\circ\text{C}$

**Table 1.** Clonidine hydrochloride 20 µg/mL compounding standard operating procedure.

Clonidine hydrochloride 20 µg/mL oral solution with preservative	Clonidine hydrochloride 20 µg/mL oral solution without preservative
Formulation one (F1):	Formulation two (F2):
Clonidine HCl stock sol. 1 mg/mL .....2 mL	Clonidine HCl stock sol. 1 mg/mL .....2 mL
Purified water .....48 mL	Purified water .....48 mL
Potassium sorbate .....150 mg	Simple Sirup Q.S. to .....100 mL
Simple sirup Q.S. to .....100 mL	Monohydrate citric acid q.s. to adjust pH = 4–5
Monohydrate citric acid q.s. to adjust pH = 4–5	
1. Make a stock solution of clonidine hydrochloride 1 mg/mL, weighing on an analytical balance 100 mg clonidine hydrochloride powder (raw material), and dilute to 100 mL with purified water in volumetric flask.	1. Make a stock solution of clonidine hydrochloride 1 mg/mL, weighing on an analytical balance 100 mg clonidine hydrochloride powder (raw material), and dilute to 100 mL with purified water in volumetric flask.
2. Weigh 150 mg of potassium sorbate and diluted with 48 mL of purified water in a beaker with stirring.	2. Take 2 mL of stock solution of clonidine and add to the mixture.
3. Take 2 mL of stock solution of clonidine and add to the mixture (step 2).	3. Add simple sirup (64g sucrose/36g water) sufficient quantity to make 100 mL of final volume and mix.
4. Add simple sirup (64g sucrose/36g water) sufficient quantity to make 100 mL of final volume and mix.	4. Adjust pH to 4–5 with citric acid 5% w/v.
5. Adjust pH to 4–5 with citric acid 25% w/v.	5. Fill into amber glass bottle.
6. Fill into amber glass bottle.	

The stock solution of clonidine 1 mg/mL was studied for 90 days under refrigeration ( $5 \pm 3^\circ\text{C}$ ).

## 2.4. Physicochemical studies

### 2.4.1. Validation of the stability-indicating HPLC method

The reference method proposed to demonstrate chemical stability for 90 days was HPLC, in accordance with the official clonidine hydrochloride product monograph (USP-40). The remaining clonidine concentration was measured and the stability limits were established with a recovery range of 90–110% with respect to the initial concentration. The HPLC system used was the Agilent® 1260 Infinity liquid chromatography system (Waldbronn, Germany), equipped with a quaternary pump, an autosampler, thermostatted column compartment, and an ultraviolet detector diode array. Chromatographic conditions were as follows: flow rate: 1.5 mL/min; the mobile phase was prepared by dissolving 1.1 g of sodium 1-octanesulfonate in 500 mL of ultrapure water, then adding 500 mL of methanol and 1 mL of orthophosphoric acid to the solution, and adjusting to pH 3 with 1 N NaOH. Injection volume was 50  $\mu\text{L}$ , the temperature in the column compartment was  $25^\circ\text{C}$ , and the detection wavelength was 220 nm. The column used was the Zorbax Eclipse XDB-C8  $4.6 \times 150$  mm,  $5\ \mu\text{m}$  (Agilent®, USA). System suitability tests were performed every day of analysis and after the acceptance criteria had been approved, the clonidine samples were analyzed. The system suitability solution was composed of 2  $\mu\text{g}/\text{mL}$  of clonidine hydrochloride and 2.4  $\mu\text{g}/\text{mL}$  2,6-dichloroaniline. Chromatographic parameters monitored during the days of analysis included column efficiency (USP acceptance limit:  $>3500$  theoretical plates), tailing factor (USP acceptance limit:  $<1.5$ ), and relative retention times (0.5 for clonidine and 1.0 for 2,6-dichloroaniline).

The validation method for the quantification of clonidine was in accordance with ICH guidelines (ICH 2005). To demonstrate the linearity of the method, a calibration line was constructed with six standard clonidine solutions for the calibration range 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5  $\mu\text{g}/\text{mL}$ , and the results plotted to obtain a calibration curve. Analysis of variance (ANOVA) was then performed. All statistical tests were performed at the 95% significance level. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy was expressed as a percentage and calculated as  $[\text{measured concentration}/\text{nominal concentration}] \times 100$ . The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as percentage relative standard deviation (% RSD), calculated according to the following equation:  $[\text{standard deviation measured concentration}/\text{mean measured concentration}] \times 100$ . Intra-day precision was expressed as RSDs at low, medium, and high concentrations (five replicate injections at each level of concentration) within the range of linearity over one working day (intra-day) and after five consecutive working days (inter-day). The limit of detection (LOD) and limit of quantification (LOQ) were calculated, based on the standard deviations of the response and the slope. 2-D and 3-D UV spectral analysis of clonidine was used to test the specificity and selectivity of the method (v4.03b Agilent® Chemstation software). Extraction and analysis of samples were carried out on days 0, 2, 6, 10, 14, 20, 30, 40, 50, 70, and 90. On days of analysis, 1 mL of each sample was extracted from each container and a 1:10 dilution made with mobile phase, resulting in a nominal concentration of 2  $\mu\text{g}/\text{mL}$ . In the same way, 1 mL of stock solution of clonidine hydrochloride 1 mg/mL was extracted and a dilution

was made with mobile phase to obtain a final concentration of 2  $\mu\text{g}/\text{mL}$ . About 50  $\mu\text{L}$  was then injected into the HPLC system for analysis.

### 2.4.2. Accelerated degradation studies

Each formulation was subjected to accelerated stress testing in acidic (2 N HCl), basic (2 N NaOH), and oxidizing ( $\text{H}_2\text{O}_2$  12% v/v) media and heat ( $80^\circ\text{C}$ ) for 12 h, and then analyzed by HPLC.

### 2.4.3. pH measurements

The pH of both formulations was measured in triplicate using a Crison GLP21 pH meter (Crison Instruments S.A., Barcelona, Spain) calibrated at pH 4.01, 7.00, and 9.21. Measurements were performed on days 0, 14, 30, 40, 60, and 90.

### 2.4.4. Osmolality measurements

The osmolality of the two formulations was measured in duplicate with an OM-6050 OsmoSTATION® ARKRAY osmometer (Kyoto, Japan) on days 0 and 90, calibrated with two standard solutions in the range of 300–1000 mOsm/kg- $\text{H}_2\text{O}$ . Each sample was diluted (1:5) with ultrapure water for measures that fell within the calibration range.

### 2.4.5. Visual inspection

The physical properties of the samples stored at each temperature were visually inspected in order to determine whether parameters such as odor, color, or a tendency to precipitate spontaneously could be detected visually. In accordance with the Real Farmacopea Española (RFE chapter 2.9.20 2014), 3 mL of each formulation were taken and transferred to a transparent test tube fitted with a stopper, inverted, and observed for 5 s against a black background and 5 s against a white background under a very bright light. Measurements were performed on days 0, 14, 30, 40, 60, and 90.

### 2.4.6. Data analysis

The objective was to obtain 90–110% recovery of clonidine in each formulation throughout the study up to 90 days. Agilent® ChemStation software v4.03b (Agilent Technologies®, Waldbronn, Germany) was used for data processing.

## 2.5. Microbiological stability

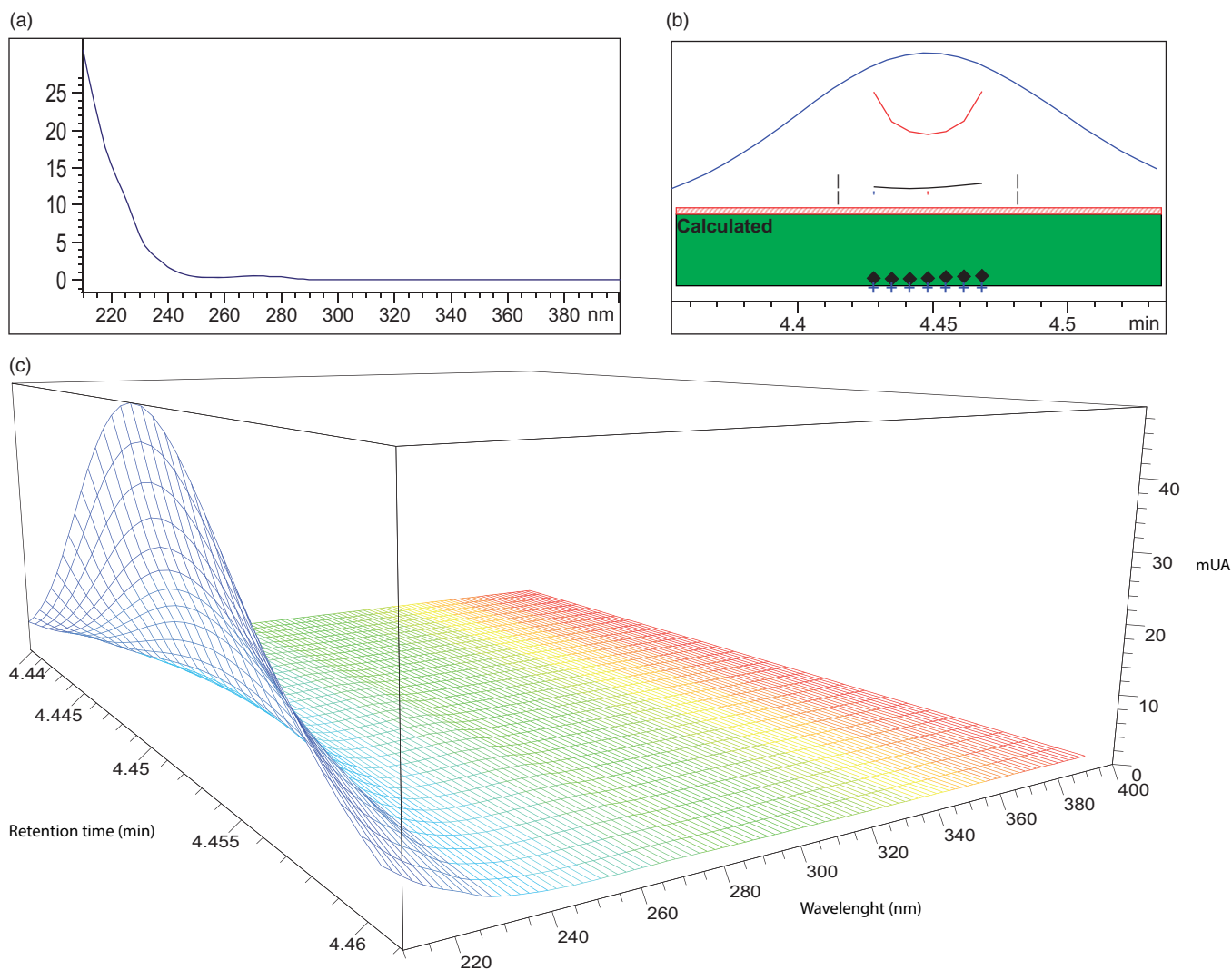
The microbiological stability of the closed and open bottles kept in the storage conditions established by the study was determined in accordance with USP-40 (USP, chapters 61 and 62). Closed bottles were analyzed on days 0, 7, 14, 28, 42, 60, and 90 and open bottles on days 0, 7, 14, 28, and 42 after preparation. Open bottles were opened three times a day in the pharmacy laboratory to simulate typical drug dosage and 1 mL of solution was extracted on each occasion.

In order to determine the ability of microorganisms to grow in the studied formulation, several standard strains were selected: *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 9027™), *Bacillus subtilis* subsp. *spizizenii* Nakamura et al. (ATCC® 6633™), *Candida albicans* (Robin) Berkhout (ATCC® 10231™), *Aspergillus brasiliensis* Varga et al. (ATCC® 16404™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 8739™), *Salmonella enterica* subsp. *enterica* (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC® 14028™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 6538™), and *Clostridium sporogenes* (Metchnikoff) Bergey et al. (ATCC® 19404™; LGC Standards, S.L.U., Barcelona, Spain).

**Table 2.** Repeatability ( $n = 15$ ) and intermediate precision ( $n = 15$ ) at low, medium, and high level concentrations.

Concentration	Repeatability (intra-day) $\mu\text{g/mL}$			Intermediate precision (inter-day) $\mu\text{g/mL}$		
	RSD (%)			RSD (%)		
	Low	Medium	High	Low	Medium	High
Clonidine hydrochloride	0.992 (0.43)	1.998 (0.44)	3.504 (0.74)	0.994 (0.95)	1.996 (0.86)	3.507 (0.93)

RSD: relative standard deviation.

**Figure 1.** Clonidine spectral analysis: (a) UV specter, (b) peak purity, and (c) 3-D spectral analysis.

A general-purpose culture medium of trypticase soy agar (TSA), tryptic soy broth (TSB), and sabouraud glucose agar (SAB; Soria Melguizo, S.A., Madrid, Spain) was used to perform the assay.

This test was conducted by taking a 1:10 dilution of the clonidine sirup and adding 100  $\mu\text{L}$  of the ATCC strains in a  $1.5 \times 10^3$  cfu/mL solution, which was then spread across the general culture medium using the surface-spread method. The same concentrations of bacteria were simultaneously inoculated into the same media without product. All strains were inoculated in duplicate into each medium. To determine the sterility of the product, two further tests were performed: the plate count method and tests for specified organisms.

For the plate-count method, a 1:10 dilution of product was prepared for testing in TSB. One mL of this dilution was spread

across TSA and SAB using the surface-spread method. The TSA plates were incubated at 35–37  $^{\circ}\text{C}$  for 48 h; SAB plates were incubated at 20–25  $^{\circ}\text{C}$  for 5 days. The results were expressed as total aerobic microbial count (TAMC) and total combined yeasts and molds count (TYMC). The acceptance criteria for microbiological quality of product were  $10^2$  cfu/mL for TAMC and  $10^1$  cfu/mL for TYMC, in accordance with USP-40 (USP, chapter <1111>).

Finally, tests for specified microorganisms were performed, which included tests for bile-tolerant Gram-negative bacteria, *E. coli*, *Salmonella*, *P. aeruginosa*, *S. aureus*, Clostridia, and *C. albicans*, and using the medium specified for each microorganism: enterobacteria enrichment broth mossel for gram-negative bacteria, xylose lysine deoxycholate agar for *Salmonella*, columbia agar with 5% sheep blood for *S. aureus*, MacConkey agar for *E. coli* and *P. aeruginosa*, reinforced clostridial medium for

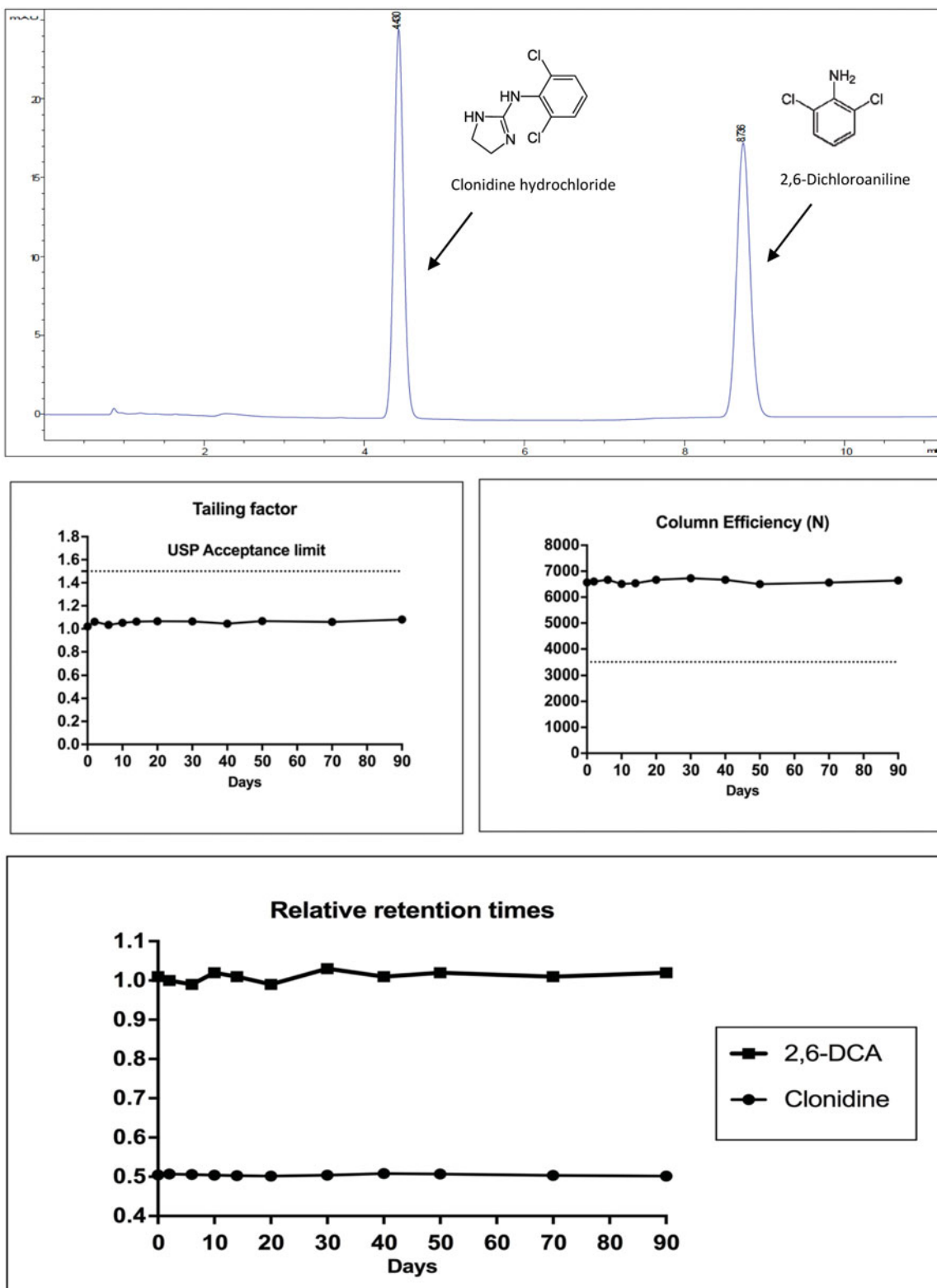


Figure 2. Chromatogram and chromatographic parameters of suitability test (2,6-DCA = 2,6-dichloroaniline).

Clostridia, and SAB for *C. albicans*. Each medium was inoculated and incubated in accordance with USP-35. Identification of isolates was by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. The product complied with the test if no colonies of the specified microorganisms were detected.

### 3. Results

#### 3.1. Physicochemical stability

##### 3.1.1. HPLC analysis

ANOVA of the linear regression confirmed the linearity of the method by rejecting the null hypothesis of deviation from linearity

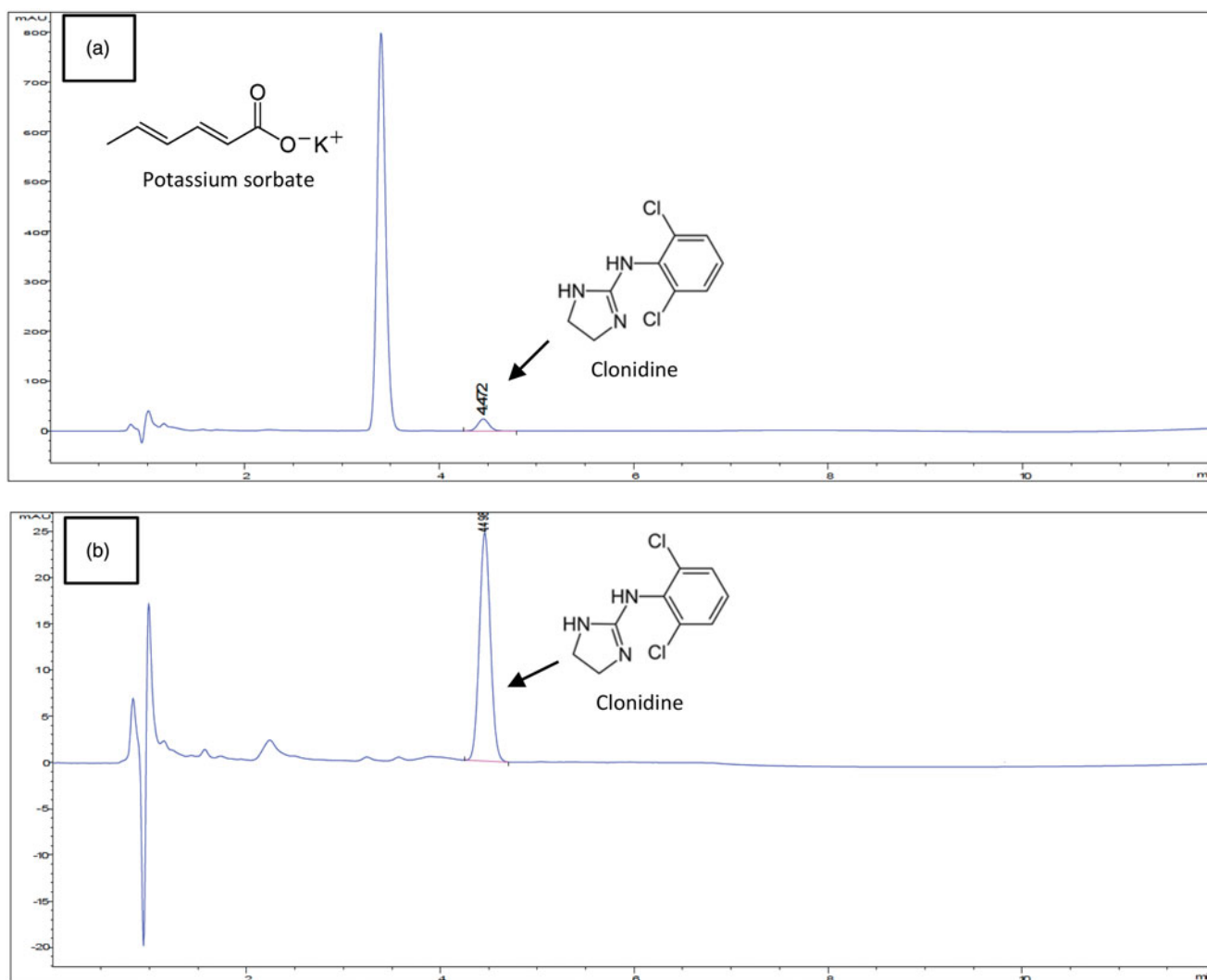


Figure 3. Chromatograms at day 0 of (a) the formulations with preservatives (F1) and (b) without preservatives (F2).

with statistical significance of 0.05 ( $\alpha = 0.05$ ). The coefficient of variation of the method was 1.60%. The equation obtained by linear regression was:  $y = 96.558x + 6.8387$  ( $n = 18$ ;  $R^2 > 0.9995$ ) with residual standard error of 1.54. The repeatability and intermediate precision of the data are shown in Table 2. The LOD and LOQ based on the standard deviation of the responses and the slope of the calibration curve were  $0.07 \mu\text{g/mL}$  and  $0.20 \mu\text{g/mL}$ , respectively. 2-D and 3-D UV spectral analysis confirmed the selectivity of the method, with the absence of co-eluates within the same retention time window as clonidine. Peak purity confirmed the specificity of the technique throughout the study (Figure 1). The results of the suitability test were in accordance with USP acceptance criteria (Figure 2). Retention times for clonidine were 4.4 min with chromatograms showing degradation products in formulations with preservatives stored at  $40 \pm 2^\circ\text{C}$  and room temperature ( $25 \pm 3^\circ\text{C}$ ) (Figures 3, 4, and 5). There was no significant degradation of clonidine (less than 10%) throughout the study in all batches without preservatives, regardless of the storage conditions, and also in formulations with preservatives stored under refrigeration ( $5 \pm 3^\circ\text{C}$ ). However, the degradation of clonidine in bottles with preservatives stored at room temperature and at  $40 \pm 2^\circ\text{C}$  was more than 10% at 10 days (10.14% of degraded clonidine) and at 40 days (13.2% of degraded clonidine), respectively (Figure 6). The stock solution  $1 \text{ mg/mL}$  remained stable during the 90 days of the study.

### 3.1.2. Accelerated degradation testing

The chromatograms analyzed after the formulations were assayed for 12 h in stress conditions are shown in Figures 7 and 8. The results show that the method was stability-indicating, with complete separation of the degradation products from our drug peak of interest, in this case clonidine (retention time 4.2 min). The most destabilizing conditions for the formulas (with and without preservatives) were oxidizing and alkaline conditions.

### 3.1.3. pH measurement

pH did not vary by more than 0.5 unit in formulations with preservatives under any of the storage conditions, but increased by one pH unit in formulations with preservatives (Table 3).

### 3.1.4. Visual control

On day 0, both formulations were transparent, without coloration and absent from suspended particles. No change in color, odor, formation of gas, or suspended particles were observed in any of the formulations during the 90 days of the study.

### 3.1.5. Osmolality measurement

Osmolality at days 0 and 90 varied by less than 5%, except in the formulation with preservative stored at  $40 \pm 2^\circ\text{C}$ , where there was a difference of almost 10% between day 90 with respect to day 0. The results are shown in Table 4.

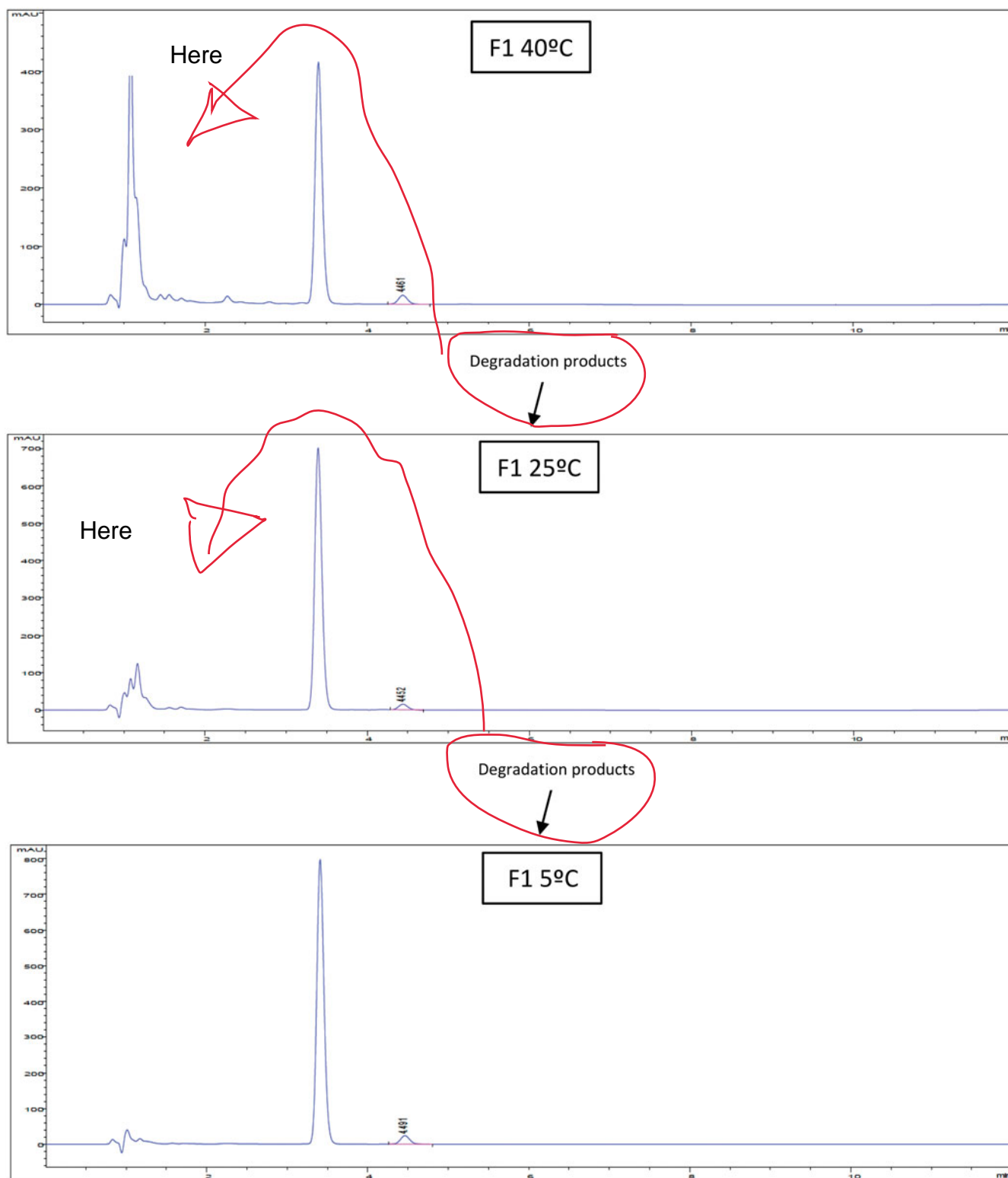


Figure 4. Chromatograms at day 90 of formulations with preservatives (F1) in the different storage conditions.

### 3.2. Microbiological stability

The ability of ATCC strains to grow in clonidine was confirmed under all study conditions. All closed bottles showed microbiological stability at day 90, regardless of the conservation conditions. Open bottles with preservatives were stable at day 42; bottles without preservatives stored at  $40 \pm 2^\circ\text{C}$  were microbiologically stable for up to 28 days, and those stored at  $25 \pm 3^\circ\text{C}$  up

to day 7. The tests for specified microorganisms were negative (growth was within the acceptance criterion for effectiveness) in all assays throughout the study period.

### 4. Discussion

In this study, we proposed and studied from a physicochemical and microbiological point of view two new formulations of



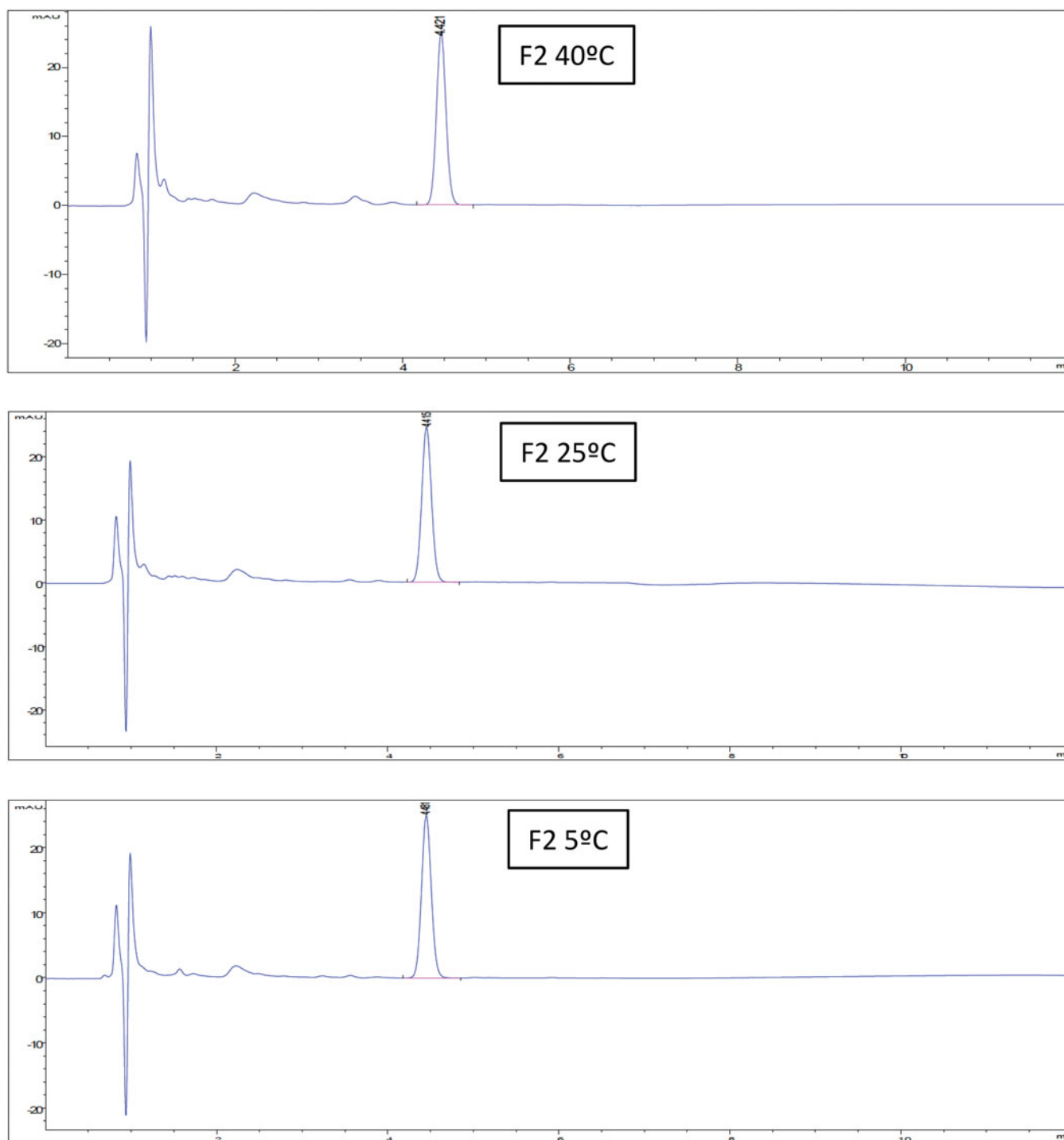
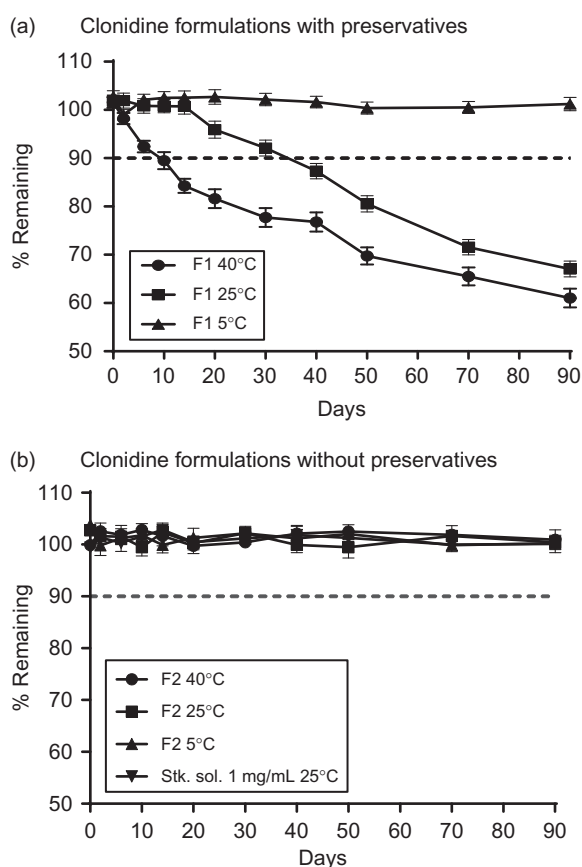


Figure 5. Chromatograms at day 90 of formulations without preservatives (F2) under different storage conditions.

clonidine 20 µg/mL (with and without preservative) for use in pediatric patients (all age groups). With respect to the physico-chemical assessment, the only formulation with preservative that remained stable throughout the 90 days of study was the one stored under refrigeration ( $5 \pm 3^\circ\text{C}$ ). All formulations without preservative were completely stable for 90 days. The standard solution of clonidine 1 mg/mL stored at  $5 \pm 3^\circ\text{C}$  was stable for 90 days. The degradation process, therefore, appeared to be related to the presence of preservative (potassium sorbate) and was temperature-dependent, as can be seen in Figures 4, 5, and 6. The stability of potassium sorbate has been widely studied in both the food industry (Arya 1980; Castro et al. 2005) and the

pharmaceutical industry (Yarramraju et al. 2007), although there are few data describing interactions with drugs used in pharmaceutical compounding. The reaction between sorbic acid and amines under extreme conditions (in an autoclave for 6 h at  $200^\circ\text{C}$ ) has been described by Kheddis et al. (1981), Verbiscar and Campbell (1964), and Khandelwal and Wedzicha (1990), who demonstrated the formation of dihydropyridones, derived from sorbic acid and various amines. Ferrand et al. (2000) investigated whether this reaction was more prominent in milder conditions ( $20^\circ\text{C}$  and  $50^\circ\text{C}$ ). It seems likely that the secondary amine of clonidine (located between the imidazole group and dichlorophenyl) reacts by nucleophilic attack (at  $25^\circ\text{C}$  and  $40^\circ\text{C}$ ) with the double



**Figure 6.** Evolution of the clonidine concentration: (a) formulations with preservatives and (b) formulations without preservatives.

bonds of the sorbate at positions 2 and 4 to form dihydropyridones and other adducts. Potassium sorbate was used as a preservative after the European Medicines Agency (EMA) published safety warnings about the possible endocrine-disrupting effects and estrogenic activity of human exposure to parabens (EMA 2012) and the risk to neonatal patients exposed by benzoates (EMA 2014). Accelerated degradation tests showed that the most destabilizing stress conditions for both formulas were the oxidizing and alkaline conditions, demonstrating in turn that the method was stability-indicating. The most notable variation in pH occurred in formulations without preservatives, particularly those stored at  $40 \pm 2^\circ\text{C}$ , which increased by more than one pH unit during the study. The pH remained most stable in formulas with preservative, probably enhanced by the buffer action exerted by the potassium sorbate and citric acid used to adjust pH. Nevertheless, there was no further degradation of clonidine in these formulations, which were the most stable from a physicochemical point of view. Osmolality increased (by approximately 10%) in the formula with preservatives stored at  $40 \pm 2^\circ\text{C}$ , probably related to the increase in the number of molecules of degraded clonidine and potassium sorbate. The osmolality of formulations with or without preservative exceeds the limit recommended by the American Academy of Pediatrics, which is set at 400 mOsm/kg- $\text{H}_2\text{O}$  for neonates (American Academy of Pediatrics 1976). This is important because some authors have reported problems of digestive intolerance or necrotizing enterocolitis associated with hyperosmolar feeds in neonatal patients (Le Guennec et al. 1983; Aceti et al. 2009). From a microbiological point of view, no microbial growth was found in closed bottles during the 90-day study, regardless of the storage conditions or use of preservatives. However, growth was observed at day 90 in the stock

solution without preservative stored at  $5 \pm 3^\circ\text{C}$ . There was no growth in open formulations with preservatives during the 42 days analyzed, irrespective of the storage conditions, although growth was found on days 42 and 14 in formulations without preservative stored at  $40^\circ\text{C}$  and  $25 \pm 3^\circ\text{C}$ , respectively. Hence, the microbiological stability of these two formulations could be set at 28 and 7 days, respectively. The use of potassium sorbate as preservative explains the lack of growth observed in open bottles with preservative. Potassium sorbate has been used for this purpose in the food industry and has demonstrated its ability to inhibit growth of several pathogenic bacteria, including *E. coli* and *S. aureus*, among others, as well as yeasts and molds (Carlsson et al. 2001; Kyungwha and Azlin 2004; Amin Zare et al. 2014). In the stock solution, bacterial growth above the permitted limit was observed on day 90, and an increment in pH value was also observed. When the pH falls to very low levels, bacterial growth is delayed, indicating the direct effect of acidity on inhibition of bacterial growth. Increasing the pH in the stock solution would be a factor involved in bacterial growth and would require the addition of a preservative to have antibacterial effect (Carlsson et al. 2001). It is known that antimicrobials are more effective in lower pH values because of the cellular changes and damage associated with these conditions (Oh and Marshall 1994; Lieberman et al. 2006). The stability of clonidine was recently studied by Ma et al. (2014) and Emson et al. (2014). They demonstrated that formulations of clonidine suspensions using tablets in commercially available vehicles and stored in syringes and plastic bottles remained stable for 91 days. Sauberan et al. (2016) demonstrated that a formulation of clonidine suspension  $20 \mu\text{g}/\text{mL}$ , prepared by crushing clonidine hydrochloride, and adding simple sirup, remained stable for 35 days. De Goode et al. (2012) demonstrated physicochemical stability for 9 months in a formulation of clonidine hydrochloride  $50 \mu\text{g}/\text{mL}$  with methylparaben as preservative. More recently, Potier et al. (2017) carried out a study similar to the one developed by our research team, demonstrating a shelf life of 60 days in refrigeration (30 days in use) with a less concentrated clonidine hydrochloride solution ( $10 \mu\text{g}/\text{mL}$ ) in a commercial vehicle (Inorpha®). In our study, we started with clonidine drug substance and developed two solutions of clonidine  $20 \mu\text{g}/\text{mL}$  (with and without preservatives) and demonstrated physical, chemical, and microbiological stability for 90 days. All the raw materials used (drug substance and excipients) can easily be obtained at any community pharmacy or hospital pharmaceutical service, which means that any patient who needs this medicine can obtain it in optimal oral dosage form (solution) from any pharmacy or pharmaceutical service. Important information is also provided about physical, chemical, and microbiological stability at different temperatures, up to  $40 \pm 2^\circ\text{C}$ , as well as the minimum quality, safety and efficacy for administering the medication when the storage conditions are less than optimal, such as those often found among disadvantaged social groups, the homeless, and others who live on the streets, for example. For these patient groups, the formulas can be prepared without preservatives because their stability has been demonstrated and they do not spoil. More stability studies of formulations with a high impact on clinical practice are needed, especially those involving pediatric patients, since this is the most vulnerable age group. For generations, it has been considered that young children are best treated with oral liquid formulations because they are easy to swallow and provide adequate dosing flexibility (van Riet-Nales et al. 2015). There are, however, numerous problems associated with use of oral liquids, including a bad taste, limited chemical, physical and/or microbial stability, the need for a dosing device, risk of

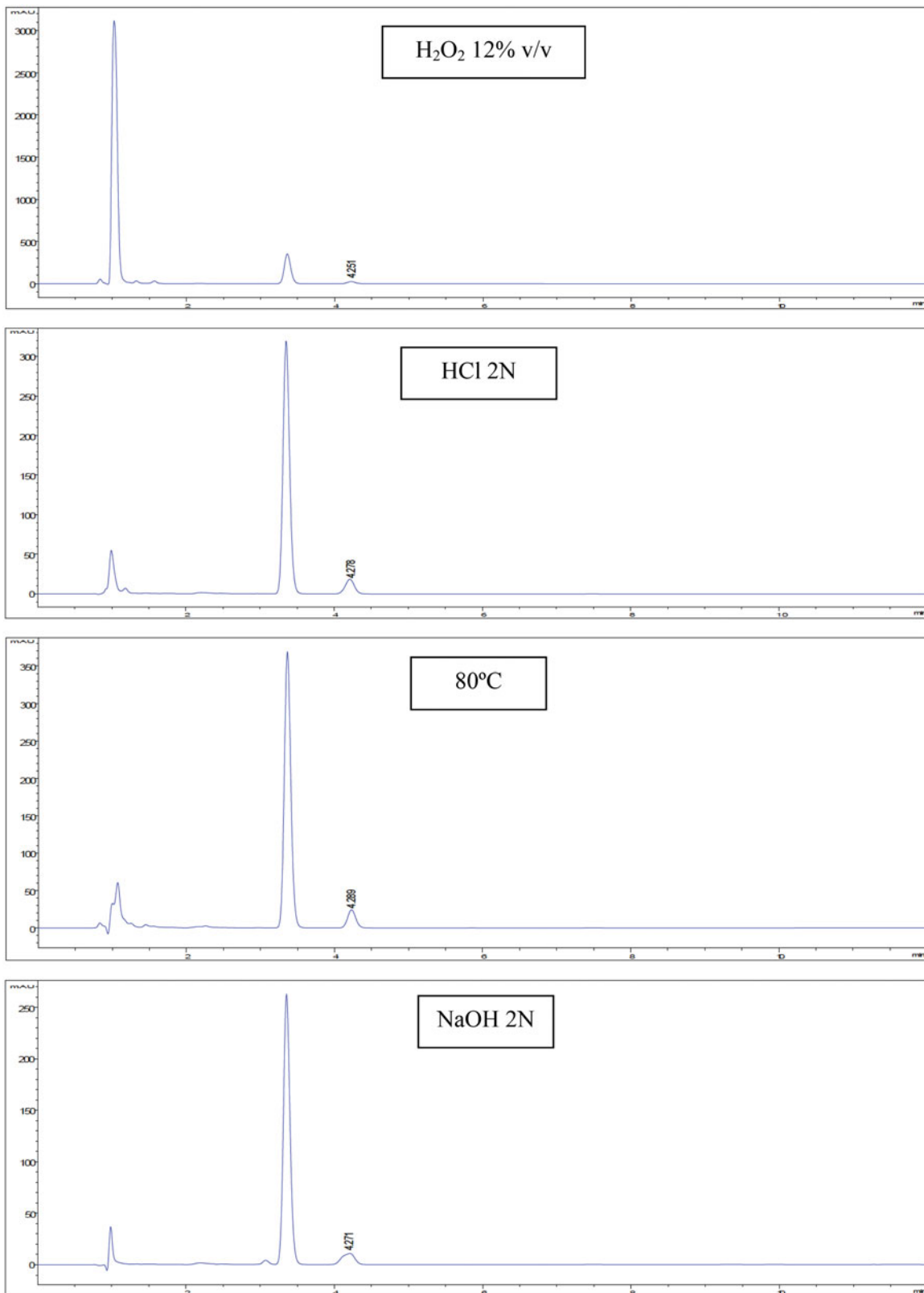


Figure 7. Chromatograms stress conditions of the formulation with preservative.

errors when measuring the dose, sedimentation and non-homogeneous distribution of drug substance, emulsion breaking, bulky volume, and portability issues. Most of these problems can be avoided with conventional solid oral flexible pediatric formulations such as powders or granules, which may not, on the other hand, be well accepted. For these reasons, newer oral flexible

dosage forms, such as minitables or orodispersible films may be more appropriate (Ivanovska et al. 2014). New approaches, such as the oral solid dosage pen, whose dose can adjusted by cutting tablet-like slices of the drug inside the pen to the required length, are expected to introduce greater flexibility and convenience to pediatric dosing approaches (Wening et al. 2012).

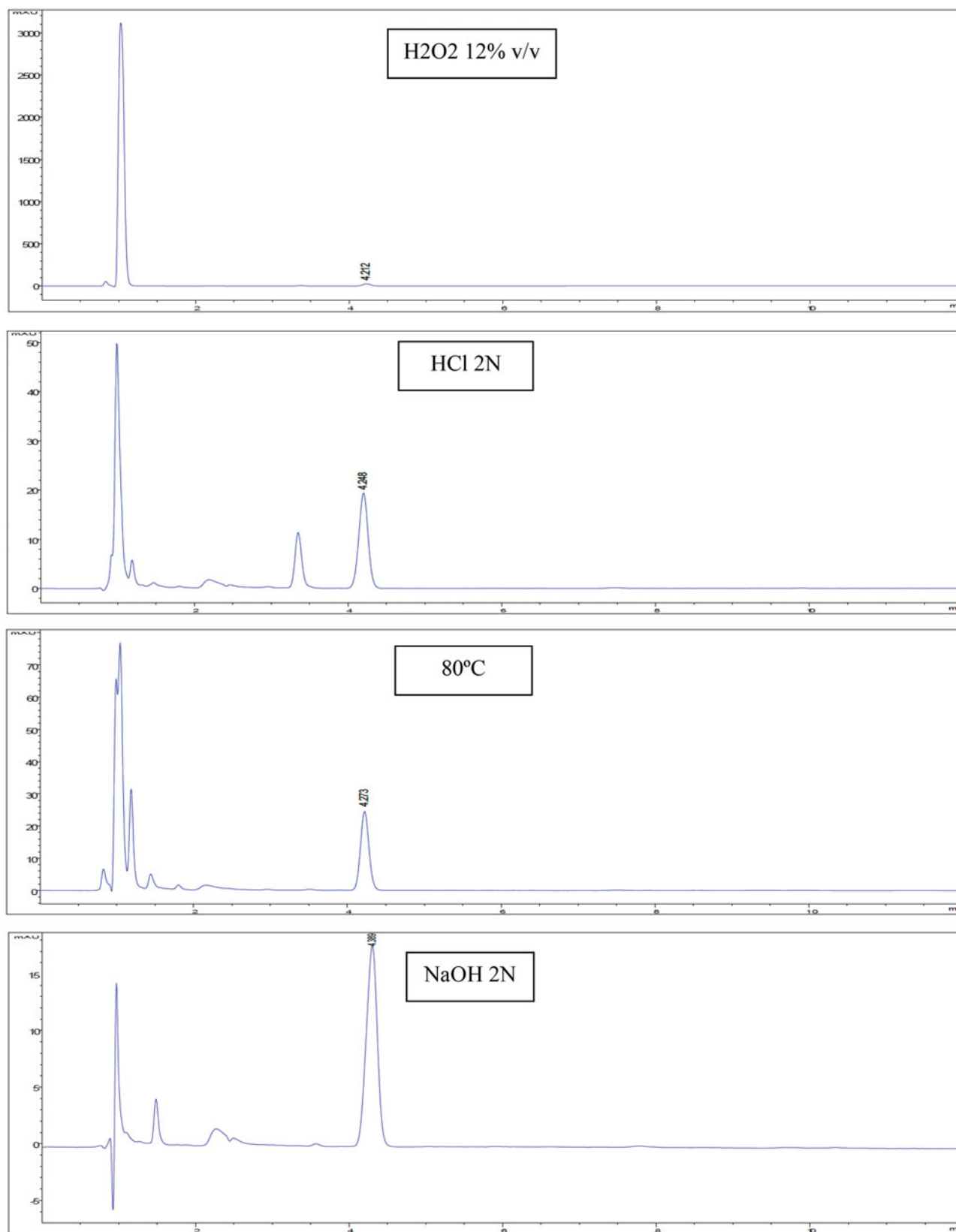


Figure 8. Chromatograms stress conditions of the formulation without preservative.

## 5. Conclusion

Two oral clonidine solutions of 20 µg/mL were developed from readily available drug substance and excipients that demonstrated physicochemical and microbiological stability for 90 days in both

formulations (with and without preservative) and under all storage conditions (closed formulations). Physicochemical and microbiological stability in use (open formulations) were demonstrated for at least 42 days under refrigeration ( $5 \pm 3^\circ\text{C}$ ). The standard

**Table 3.** Changes in pH in the formulations according to storage conditions.

Formulation/pH	Day 0	Day 14	Day 30	Day 40	Day 60	Day 90
F1 40°C	4.56 ± 0.005	4.60 ± 0.010	4.63 ± 0.010	4.53 ± 0.030	4.54 ± 0.010	4.37 ± 0.042
F1 25°C	4.57 ± 0.005	4.56 ± 0.021	4.64 ± 0.005	4.56 ± 0.030	4.57 ± 0.005	4.43 ± 0.031
F1 5°C	4.57 ± 0.005	4.58 ± 0.010	4.64 ± 0.011	4.61 ± 0.015	4.56 ± 0.005	4.38 ± 0.022
F2 40°C	4.52 ± 0.015	4.99 ± 0.036	5.44 ± 0.081	5.39 ± 0.063	5.48 ± 0.051	5.53 ± 0.062
F2 25°C	4.51 ± 0.028	4.67 ± 0.010	4.86 ± 0.058	4.77 ± 0.005	4.76 ± 0.062	4.90 ± 0.010
F2 5°C	4.51 ± 0.028	4.60 ± 0.005	4.61 ± 0.071	4.62 ± 0.070	4.63 ± 0.036	4.65 ± 0.005
Stk. Sol. 1 mg/mL	5.76 ± 0.028	5.70 ± 0.010	5.75 ± 0.005	5.72 ± 0.042	5.96 ± 0.070	6.07 ± 0.042

F1: formulations with preservative; F2: formulations without preservative.

**Table 4.** Osmolality of the formulations in different conditions.

Osmolality	<sup>a</sup> Mean ± SD (mOsm/kg-H <sub>2</sub> O)	
	Day 0	Day 90
F1 40°C	1350.63 ± 7.07	1483.46 ± 65.25
F1 25°C		1360.49 ± 70.21
F1 5°C		1366.32 ± 24.66
F2 40°C	1327.24 ± 3.53	1376.23 ± 20.20
F2 25 °C		1313.17 ± 46.45
F2 5°C		1325.45 ± 21.79

<sup>a</sup>Duplicate measures.

F1: formulations with preservative; F2: formulations without preservative.

solution of clonidine hydrochloride 1 mg/mL has a shelf life of 60 days in use.

This study shows that it was possible to develop two formulations of clonidine adapted to pediatric patients, not in commercial vehicles, starting from readily available drug substances and excipients, with a shelf life (90 days in refrigeration and 42 days in use) that provides the patient with greater comfort and permits the optimization of resources in hospital pharmacy services and community pharmacies.

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No potential conflict of interest was reported by the authors.

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