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## **Production and stability study of a hospital parenteral nutrition solution for neonates**

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1           **Title:** Production and stability study of a hospital parenteral nutrition solutions<sup>s</sup> for neonates

2

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## ABSTRACT

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Standard parenteral nutrition solutions are mixtures comprising interacting components that may degrade themselves over time. The objective of this study was to investigate the physicochemical and microbiological stability of a hospital preparation for parenteral nutrition in neonatology. The analysis were performed throughout the storage of the preparations at 2°C - 8°C (up to 4 months). The extent of stability was based on the determination of amino acids dosage, visual and physicochemical properties (glucose and electrolytes concentrations, pH and osmolality measurements, particle counting) and microbiological analysis (sterility test). A thermal degradation of ascorbic acid was conducted to evaluate the antioxidant properties of the parenteral mixture. Physicochemical and microbiological controls were found to comply with the specifications. Amino acids showed a good stability throughout the 4-months storage excepted for cysteine which was progressively degraded to cystine conferring a yellow coloration to parenteral solutions. Parenteral nutrition standards solutions remain stable for 4 months at 2°C - 8°C insuring safe administration in preterm infants.

**Keywords:** Stability-indicating method study; Parenteral nutrition; Neonatology; Amino acids; High-performance liquid chromatographic.

43 **1. Introduction**

44

45 The management of premature infants requires specific parenteral nutrition [1]. Some nutrients are  
46 essential to their development and the quality of parenteral nutrition can condition their future [2]. The  
47 prescription of parenteral nutrition in neonatology is standardized according to the recommendations  
48 of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and  
49 the European Society for Clinical Nutrition and Metabolism (ESPEN) [3,4]. Several parenteral nutrition  
50 formulations (industrial, standardized or individualized formulations) are available [5]. The  
51 standardized formulations might be prepared in-house by hospital pharmacists to meet rapidly the  
52 nutritional needs of a large number of premature infants. At hospital, standardized solutions are at  
53 high-risk of microbiological contamination and the stability of the preparation must be ensured. After  
54 development and production by hospital pharmacists, the preparation is checked before release and  
55 must remain stable throughout its period of use [6]. The amino acids present in the parenteral nutrition  
56 solutions may degrade over time, in particular by an oxidation phenomenon. Therefore, it is mandatory  
57 to verify the stability of the amino acids during the storage of the solutions to respond to protein needs.  
58 The ratio between essential and non-essential amino acids must also be preserved to promote  
59 nitrogen balance and anabolism of premature infants. In 2016, the Council of Europe specified the  
60 quality and safety requirements for hospital preparations [7]. To our knowledge, no data on the long-  
61 term stability of amino acids in hospital standardized solutions is available in the literature. Different  
62 methods for amino acids assay are currently available: including automatic amino acids analyser [8-  
63 11], **high-performance** liquid chromatographic (HPLC) coupled with **mass spectrometric** [12-15] or  
64 fluorescence detection [16, 17]. However, there is no specific HPLC method coupled with ultraviolet  
65 (UV) detection for amino acids in parenteral nutrition mixture assay.

66 After the development and the validation of a stability-indicating reversed phase-HPLC method, the  
67 main objective of this study was to evaluate the stability of a hospital standardized formulation for  
68 parenteral nutrition. Amino acids, physicochemical and microbiological analysis were carried out  
69 simultaneously to ensure the safety of the final preparation.

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71

72

73 **2. Methods**

74

75 **2.1. Sterile manufacture of neonatal parenteral nutrition solutions**

76 Parenteral nutrition solutions for neonates were prepared aseptically in a class 100 isolator (ISO 5) by  
77 using an automated pumping system (ExactaMix<sup>®</sup> 2400, Baxter Healthcare Corporation, Deerfield, IL,  
78 USA) and in compliance with Good Preparation Practices [18]. The composition of the parenteral  
79 nutrition solution is shown in Table 1. A commercial amino acid solution (Primene<sup>®</sup> 10%, Baxter  
80 Healthcare Corporation, Deerfield, IL, USA) was used to bring essential and non-essential amino  
81 acids to hospital preparation. Primene<sup>®</sup> 10% is initially indicated in the parenteral nutrition of the  
82 newborn or premature infants when oral or enteral feeding is insufficient or impossible. The  
83 parenteral nutrition solution was packaged in 250 mL EVA bags, (ExactaMix<sup>®</sup>, Baxter Healthcare  
84 Corporation, Deerfield, IL, USA). The bags were labelled and individually packaged (unique bag  
85 number and bar code).

86

87 **2.2. Stability study**

88 In order to prolong the duration of use initially set at 11 days after preparation, a stability study of the  
89 hospital preparation was carried out. Bags prepared and double wrapped were stored at 2°C - 8°C.  
90 The analysis were carried out after 1, 8, 15, 22, 29, 57, 85 and 118 days of storage at 2°C - 8°C.  
91 They were run from a pilot batch of 72 bags divided into 3 random groups. Each group consisted of  
92 24 bags and was tested independently (8 bags for sterility test, 8 bags for the physicochemical test  
93 series and 8 emergency bags). For each period of stability, 2 bags were taken from each of the  
94 groups:

95 - a 200 mL bag of parenteral nutrition was used for the determination of amino acids, glucose,  
96 phosphorus, sodium, potassium, calcium, pH, absorbance and osmolality measurements, particle  
97 counting. Each of these analysis was performed 3 times.

98 - a 200 mL bag of parenteral nutrition was used for sterility test.

99 Before analysis, the bags were removed from the cold room and maintained at 18°C - 22°C for  
100 24 hours to simulate the time of administration of a parenteral nutrition solution in the medical wards.  
101 The stability tests were conducted in accordance with the recommendations of the International  
102 Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for

103 Human Use (ICH) [19,20] and the European Pharmacopoeia [21]. The evaluation of stability was  
104 based on the determination of amino acids concentration, visual inspection, physicochemical  
105 parameters (glucose, phosphorus, sodium, potassium, calcium, pH, osmolality, absorbance and count  
106 of non-visible particles), and microbiological (sterility test). The number of bags and the analysis  
107 (physicochemical, microbiological) were determined according to the recommendations of the  
108 European Pharmacopoeia [21]. The specifications were defined according to the initial composition of  
109 the solution with an acceptance interval of  $\pm 15\%$ .

110

### 111 **2.2.1. Amino acid stability**

112 A stability-indicating reversed phase-HPLC method was developed to determine the concentration of  
113 the amino acids contained in the parenteral nutrition solution (i.e. L-aspartic acid, L-glutamic acid, L-  
114 serine, L-histidine, L-glycine, L-threonine, L-arginine, L-alanine, L-aurine, L-tyrosine, L-cystine, L-va-  
115 line, L-methionine, L-tryptophan, L-phenylalanine, L-isoleucine, L-ornithine, L-leucine, L-lysine and L-  
116 proline).

117 Concentrations of two amino acids (L-cysteine, L-aurine) were not or partially determined during the  
118 study. L-cysteine was not detected in this study because its assay required a different derivatization  
119 method. However, the concentration of cystine, the degradation product of L-cysteine, was monitored  
120 during the study. Specifically, an additional cystine assay was performed after 330 days of storage to  
121 model the rate of cystine formation in standard parenteral nutrition solution.

122

#### 123 **2.2.1.1. Chemicals and reagents**

124 Amino acids standards (> 99% purity) (Sigma Aldrich®, Saint-Louis, Missouri, USA) were used for  
125 identification and quantification by HPLC. Chemicals for buffer solution were all analytical grade:  
126 disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$  anhydrous), sodium tetraborate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot$   
127  $10 \text{ H}_2\text{O}$ ), sodium azide ( $\text{NaN}_3$ ), hydrochloric acid 37% (HCl), phosphoric acid 85% ( $\text{H}_3\text{PO}_4$ ) (Thermo  
128 Fisher Scientific®, Waltham, Massachusetts, USA). The reagents for derivatization of amino acids (3-  
129 mercaptopropionic acids, 3-MPA, ortho-phthalaldehyde, OPA, 9-fluorenylmethylchloroformate,  
130 Fmoc), borate **buffer** (0.4 M in water, pH 10.2) were obtained from Agilent® technologies (Santa  
131 Clara, California, USA) and were stored at 4°C. Acetonitrile (ACN), methanol (MeOH) were HPLC  
132 grade and received from Thermo Fisher Scientific® (Waltham, Massachusetts, USA). Water was

133 purified through a Purelab® Option Q7 purifying system (Veolia® water technologies, Saint-Maurice,  
134 France). The buffer solution was filtered through polyvinylidene fluoride (PVDF) at 0.22 µm (Thermo  
135 Fisher Scientific®, Waltham, Massachusetts, USA) and all samples were filtered through regenerated  
136 cellulose (RC) at 0.20 µm (Thermo Fisher Scientific®, Waltham, Massachusetts, USA).

137

#### 138 **2.2.1.2. Instrumentation**

139 An HPLC system (Agilent® 1290 Infinity Quaternary LC System, Santa Clara, California, USA) was  
140 equipped with a quaternary pump with integrated vacuum degasser, a thermostated column  
141 compartment, an autosampler and a multiple wavelength detector. The results were collected and  
142 evaluated statistically using HP ChemStation® software (Agilent®, Santa Clara, California, USA).

143

#### 144 **2.2.1.3. Stock solution and standard solutions for chromatographic analysis**

145 A stock solution containing all of twenty amino acids was prepared (Table S1) with dissolution of  
146 amino acids in HCl 0.1 **N M** (100 mL) submitted to magnetic agitation at 4°C over 12 hours. From the  
147 stock solution, standard solutions of amino acids were prepared in 20-1000 µM range (Table S2).

148

#### 149 **2.2.1.4. Preparation of sample solution**

150 To quantify amino acids in parenteral nutrition bags, samples were diluted 1:50 (v:v) in HCl 0.1 M,  
151 then filtered through a 0.20 µm RC membrane (Dutscher®, Brumath, France) and injected to the  
152 column.

153

#### 154 **2.2.1.5. Amino acids chromatographic assay**

155 Amino acids might be assayed with pre-column derivatization and UV detection. The primary amino  
156 groups reacted with OPA in the presence of 3-MPA (pH 10) to form an isoindole derivative (Fig. S1A).  
157 The secondary amino groups reacted with FMOC (pH 10) to form a secondary amide (Fig. S1B). The  
158 products of OPA derivatization were detected at 338 nm while products of FMOC derivatization were  
159 detected at 262 nm. The autosampler allowed online derivatization by a program injection below:  
160 2.5 µL of borate buffer and 1 µL of sample were drawn successively, then mixed five times. After  
161 0.2 min, 0.5 µL of 3-MPA/OPA reagents was added and mixed ten times, then 0.4 µL of FMOC  
162 reagent was taken and mixed ten times. Finally, 32 µL of diluent was drawn, and then 12 µL of the

163 mixture was injected. The chromatographic separation of amino acids was obtained by using a C<sub>18</sub>  
164 column for high pH, 2.7 μm, 3.0 mm x 150 mm (Poroshell® HPH, Agilent® Technologies, Santa Clara,  
165 California, USA) thermostated at 40°C. Mobile phase flow rate was 0.64 mL/min. The separation was  
166 achieved with gradient using two eluents. Eluent A contained 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 10 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 5  
167 mM NaN<sub>3</sub> adjusted to pH 8.2 with concentrated HCl and eluent B included 45% of ACN, 45% of MeOH  
168 and 10% of ultra-pure water. The initial mobile phase composition was 98% A, 2% B, which changed  
169 linearly from 0.53 min to 20.10 min to the composition of 43% A, 57% B. The second linearly gradient  
170 started from 20.10 min to 20.25 min, where composition was 100% B and this composition was  
171 maintained for 3.3 min. Subsequently, the mobile phase returned at the initial composition in 0.15 min  
172 and was remained during 3.3 min to be balanced the column.

173 The range of calibration was adapted to theoretical concentration of twenty amino acids in parenteral  
174 nutrition bag. The validation was realized applying in the first instance standards solutions of amino  
175 acids and then dosage from parenteral nutrition bags. The analytical validation was performed  
176 according to the recommendations of the ICH Q2B [20] including the assessment of system suitability,  
177 specificity, linearity, accuracy, precision (repeatability, intermediate precision), limits of detection and  
178 quantification (see more details in supplementary information file) and capability. The system  
179 suitability was determined from replicate injections of the system suitability standard before and during  
180 sample analysis. Retention time, numbers of theoretical plates, capacity factor were the  
181 chromatography parameters selected for the system suitability test. The capability allowed to assess  
182 the risk of false negatives that can be generated by a validated analytical method and was calculated  
183 as follows:  $C_p = [\text{difference between the highest and lowest limits of confidence interval}] / 6\sigma$ . A simple  
184 calculation of the capability of the method allowed to estimate the risk of false negative results and  
185 performance of the method [22].

186

### 187 **2.2.2. Visual controls**

188 Before any analysis, a macroscopic examination of the bags was carried out. Visual inspection of the  
189 bags included the colour and clarity of the solution, as well as the absence of visible particles and  
190 precipitates.

191

### 192 **2.2.3. Physicochemical stability**

193 The glucose concentration was determined using a UV photometric enzymatic method (DiaSys® Dia-  
194 gnostic Systems, Glucose GOD FS, Holzheim, Germany). The glucose reacting with oxygen was  
195 converted into gluconic acid and hydrogen peroxide by the action of glucose oxidase dehydrogenase.  
196 Hydrogen peroxide was then converted by peroxidase to quinone imine after reaction with  
197 4-aminoantipyrine and phenol. Following the enzymatic cascade, quinone imine was measured at  
198 500 nm with a UV/Visible spectrophotometer (UV-2101 PC®, Shimadzu®, Kyoto, Japan). The  
199 technical characteristics of this assay were as follows: the detection limit was 0.06 mM, the linearity  
200 was satisfactory between 0.06 and 22.2 mM and the maximum coefficient of variation of the fidelity  
201 did not exceed 0.87%. Glucose assays were performed on parenteral nutrition solutions diluted 1:200  
202 (v:v) in distilled water. Phosphorus was measured using a UV photometric method with end-point  
203 measurement (DiaSys® Diagnostic Systems, Glucose GOD FS, Holzheim, Germany). The phosphate  
204 reacted with sulfuric acid and ammonium molybdate to form a long inorganic phosphorus molybdate  
205 complex. The maximum absorption of the complex was determined at 340 nm. The detection limit  
206 was 0.065 mM, the linearity was satisfactory between 0.06 and 9.69 mM and the maximum  
207 coefficient of variation of the fidelity did not exceed 2.22%. Phosphorus assays were performed on  
208 parenteral nutrition solutions diluted 1:10 (v:v) in distilled water. The absorbance between 200 and  
209 800 nm of the parenteral nutrition solution was measured with a UV/Visible spectrophotometer (UV-  
210 2101 PC®, Shimadzu®, Kyoto, Japan) after 1:20 (v:v) dilution in distilled water. The pH was measured  
211 using a HQ30d multi-parameter meter (Hach Lange®, Marne-la-Vallée, France) thermostated and  
212 calibrated before each measurement with buffer solutions (buffer pH 4.01 and 7.00). Osmolality  
213 (mOsm/kg) was measured by freezing point depression using an osmometer (Model 2020®,  
214 Advanced Instruments®, Norwood, Massachusetts). Since the expected specifications were given in  
215 osmolarity (mOsm/L), a conversion of the measurements was carried out. Particle counting was  
216 carried out in accordance with the European Pharmacopoeia (monograph 2.6.1 "Sterility") [21] using  
217 an automatic optical counter (Hiac/Royco 9103®, Beckman Coulter®, Brea, California, USA).  
218 Electrolytes (sodium, calcium, potassium) were assayed by atomic absorption spectrophotometry  
219 (SAA240FS Agilent Varian®, Santa Clara, California, USA). The quantification limits of sodium,  
220 calcium and potassium were respectively 0.03 µM, 0.1 µM and 0.06 µM.

221

#### 222 **2.2.4. Microbiological stability**

223 The sterility test was carried out on the parenteral nutrition bags by the membrane filtration technique  
224 recommended by the European Pharmacopoeia (monograph 2.6.1 "Sterility") [21]. This test was  
225 carried out in a closed system under a horizontal laminar flow hood (ISO 5) with a Steritest Equinox®  
226 pump (Millipore®, Molsheim, France). The filtered volume was 200 mL. The sterility test required two  
227 liquid culture media (Trypcase-Soja and Thioglycolate, Biomérieux®, Lyon, France). The culture media  
228 were observed after 24h, 48h, 7 and 14 days of incubation.

229

### 230 **2.3. Anti-oxidant properties**

231 The antioxidant properties of parenteral nutrition solution were evaluated by studying the thermal  
232 degradation of ascorbic acid. Ascorbic acid oxidizes to dehydroascorbic acid under the effect of an  
233 increase in temperature according to first-order kinetics [23]. Glucose and some amino acids are likely  
234 to reduce the thermal oxidation of ascorbic acid [24, 25]. Four ascorbic acid solutions were prepared  
235 by diluting a commercial injectable solution of ascorbic acid (Laroscobine® injectable, Bayer  
236 Healthcare, Lyon, France, ascorbic acid 1 g - 5 mL, excipients: water for injection,  
237 *p*-methylhydroxybenzoate, propyl-*p*-hydroxybenzoate, sodium hydroxide) in (A) water for injection,  
238 (B) glucose solution, (C) an amino acid solution (Baxter Healthcare®, Deerfield, IL, USA), (D) a binary  
239 glucose and amino acid solution (Primene® 10%, Baxter Healthcare®, Deerfield, IL, USA). A binary  
240 solution of glucose and amino acids (Primene® 10%, Baxter Healthcare®, Deerfield, IL, USA) was  
241 prepared without the addition of ascorbic acid (E). The qualitative and quantitative composition of the  
242 A-E solutions is shown in Table 2. Each solution was maintained at 40°C for 150 min and then at 55°C  
243 for 30 min. After the heat treatment, the absorption spectrum of the A-E solutions was determined  
244 between 200 and 800 nm. Finally, a visual comparison of the solutions was carried out using  
245 photographs.

246

### 247 **2.4. Statistical analysis**

248 Statistical tests for the validation of the amino acids method were performed by the Excel® software  
249 (Microsoft Office®, Redmond, Washington, USA, 2007) with a risk  $\alpha$  of 5%. The linearity was evaluated  
250 by a linear regression model that was estimated by the method of least squares. The verification of the  
251 homogeneity of variance was performed by *Cochran's* test. The test for determining the existence of a  
252 significant slope was made by a *Fischer-Snedecor's* test. To validate the study of the accuracy, a

253 *Cochran's* and an *ANOVA* tests were performed. The confidence interval of the average recovery was  
254 determined by *Student's* test. Finally, the precision was validated by *Cochran's* test. The coefficients  
255 of variation were calculated for the intermediate precision and the repeatability.

256 Statistical analysis of the stability results was carried out using KaleidaGraph® software (Synergy®  
257 Software, Reading, PA, USA) with a risk  $\alpha$  of 5%. A *Wilcoxon's* test was performed to test the  
258 significance of the results between day #1 and day #118 of storage for the analysis of cystine  
259 concentration.

260

### 261 **3. Results**

262

#### 263 **3.1. Amino acids chromatographic assay**

##### 264 **3.1.1. Analytical development and optimization**

265 In the first condition, the mobile phase was composed of eluent A (phosphate buffer pH 7.82) and  
266 eluent B included 45% of ACN, 45% of MeOH and 10% of ultra-pure water through a column Zorbax  
267 Eclipse®-AAA 4.6 mm x 150 mm, 3.5  $\mu$ m. In the second condition, the mobile phase was composed of  
268 eluent A (10 mM Na<sub>2</sub>HPO<sub>4</sub>, 10 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 0.5 mM NaN<sub>3</sub> adjusted to pH 8.2) and eluent B (45% of  
269 ACN, 45% of MeOH and 10% of ultra-pure water) through a column Poroshell® HPH C18 2.7  $\mu$ m, 3.0  
270 mm x 150 mm (Table S3). The most accurate separation was obtained in the second condition (Fig.  
271 S2).

##### 272 **3.1.2. Method validation**

273 After developing the method, a validation was conducted in accordance with ICH guidelines (Q2B)  
274 before practical use [20]. The system suitability tests checking the performance of analysis systems  
275 before, and during routine analysis, were performed automatically for each analysis. Three types of  
276 tests performed automatically for each analysis met the acceptance criteria (Table S4-S3). Table S5  
277 S4 reported that retention time and area under the curve remained unchanged even in the presence of  
278 glucose. Fig. S3 showed similar retention time and a decrease of amino acids peak area in normal  
279 and forced degradation conditions, respectively.

280

281

282 The validation parameters of amino acid assay by HPLC are reported in Table S6 S5. A  
283 significant correlation between amino acid peak areas and concentrations was found for each  
284 amino acids ( $r \geq 0.991$ ). The precision, determined by repeatability and intermediate fidelity,  
285 was performed from solutions ranged between 80 - 600  $\mu\text{M}$  according to the amino acids and  
286 was found satisfactory (table S5;  $\text{CV} < 5.0\%$ ). The limits of detection (LOD) and quantification  
287 (LOQ) demonstrated that the method was highly sensitive. The capability of each amino acid  
288 was greater than 1.33. Therefore, the performance of the method was considered as  
289 satisfactory and the risk of false negative results was estimated  $< 0.01\%$ . The validation of the  
290 chromatographic amino acids assay according to the ICH guidelines allowed ensuring the  
291 reliability, accuracy and precision of amino acids assay for daily analysis. The mean recovery  
292 of each amino acid was obtained with a confidence interval ranging from 95% to 105%  
293 demonstrating the high accuracy of the method.

294

#### 295 **3.1.1.2. Assays of the amino acids in parenteral nutrition**

296 The results of amino acids assays were reported in Table 3. Fig S4 showed the chromatogram of the  
297 amino acids contained in nutrition parenteral solutions. The concentrations of the amino acids were  
298 considered to be consistent throughout the stability study for all the amino acids. The ratio of  
299 essential to non-essential amino acids was maintained throughout the stability study. Cystine was  
300 found in increasing concentrations during the study while this amino acid was absent or in small  
301 quantities initially. Fig. 1 showed cystine concentrations over time and the modelling of the cysteine  
302 degradation. Cysteine formation from L-cysteine degradation was followed a zero order kinetic.

303

#### 304 **3.1.2. Visual controls**

305 Visual inspection of the bags showed neither precipitates nor visible particles. During the storage of  
306 the bags, a progressive yellow colouring of the solution was observed.

307

#### 308 **3.1.3. Physicochemical and microbiological stability**

309 The physicochemical and microbiological parameters between 1 and 118 days of storage at  $2^\circ\text{C}$  -  
310  $8^\circ\text{C}$  were conformed to the specifications. The results of the physicochemical parameters were  
311 shown in Table 4. Changes in phosphorus and glucose concentration from day #1 ranged between

312 95% - 105% for phosphorus and 88% - 101% for glucose. The results of the sodium, potassium and  
313 osmolality measurements showed results in accordance with the specifications. The pH  
314 measurements were in accordance with the acceptance criteria. The calculated measurement error  
315 for physicochemical parameters was less than 15%. The maximum absorption of nutrition solutions  
316 was determined at 226 nm over time. The results of the non-visible particle counts were in  
317 accordance with the recommendations of the European Pharmacopoeia. The sterility tests confirmed  
318 the absence of microorganism contamination through the time of storage, in conformity with the  
319 European Pharmacopoeia.

320

### 321 **3.2. Anti-oxidant properties**

322 After thermal stress of the ascorbic acid, ascorbic acid – glucose – amino acid based solutions  
323 showed variable colouring depending on the presence of glucose and/or amino acids. Solution E  
324 (glucose-amino acid solution) remained colourless. Fig. 2A showed the UV-visible absorption spectra  
325 of the solutions and Fig. 2B corresponded to the photographs of the solutions after thermal stress.  
326 The addition of amino acids and / or glucose to ascorbic acid resulted in a smaller shift of the  
327 absorption spectrum to the visible range. These results were confirmed by a visual inspection  
328 showing that solutions C, D were less coloured than solutions A, B.

329

### 330 **4. Discussion**

331 Parenteral nutrition solutions are complex mixtures with many components that can interact with each  
332 other. A Maillard reaction may develop over time when glucose solution is brought into contact with  
333 an amino acid mixture. This reaction is favoured by high temperature, an alkaline pH and high water  
334 content [26]. Some amino acids (e.g., lysine, glycine and methionine) are more involved in the  
335 Maillard reaction. The low temperature storage and acid pH of the studied parenteral solution  
336 prevented potential Maillard reaction [27]. The toxicity of the Maillard reaction products is not known  
337 but recent study showed the antioxidant role of products formed between glucose and cysteine during  
338 the Maillard reaction [28-30]. However, in the present parenteral solution stored at 4°C – 8°C or  
339 heated, no browning of the solution was noted. The yellow colouring would not be due to a Maillard  
340 reaction but rather to the cysteine degradation. The amino acids were not degraded and all amino  
341 acid concentrations were retained throughout the stability study. The use of synthetic amino acids

342 and not protein hydrolysates might explain the stability of the amino acid content. After storage, the  
343 parenteral nutrition solution remains adapted to premature infants. The respective compositions of  
344 essential and non-essential amino acids were maintained in order to preserve the ratio of essential  
345 amino acids to non-essential amino acids.

346 The relatively high lysine content was maintained and allows sufficient neonatal input. Taurine, an  
347 essential amino acid in children and not in adults, was preserved during the preservation of the  
348 solution in sufficient quantities for premature infants. The oxidation of cysteine, whose concentration  
349 was not determined during the study, was increasing over time and led to the formation of cystine by  
350 binding two molecules of cysteine. Moreover, in the presence of glucose, the formation of  
351 D-glucocysteine was described in the literature [31]. The increasing amounts of cysteine confirmed  
352 the oxidation reaction. The cysteine oxidation led to the formation of cystine which is metabolically  
353 active but not very soluble in an aqueous medium [6]. The order 0 kinetics of cysteine appearance in  
354 the solution imparted a yellow coloration to the preparation and represented a limit for long-term  
355 storage. Filling under nitrogen during manufacture, already carried out for commercial solutions,  
356 might avoid the degradation of amino acids. The most important losses during the storage of nutrient  
357 mixtures were reported for the oxidation-sensitive amino acids (alanine, proline, threonine,  
358 methionine, glycine and arginine) contained in polyvinylchloride bags [32]. This observation was  
359 partially found in our study with a decrease of methionine, threonine, alanine and arginine  
360 concentrations. Some amino acids (glycine, leucine and tryptophan) were sensitive to the intense  
361 luminosity which accelerated their degradation [33]. Refrigerated storage protected from light limited  
362 this phenomenon.

363 The energy intake by carbohydrates was retained allowing the preferential use of amino acids for  
364 protein synthesis. The bacterial endotoxins were not carried out because the slightly yellow colour of  
365 the solution did not allow the colorimetric determination of the endotoxins.

366 The thermal degradation test showed the protective effect of a glucose-amino acid solution in the  
367 ascorbic acid oxidation. The ascorbic acid oxidation in aqueous solution appeared to be limited in the  
368 presence of amino acids and glucose. The literature showed that sucrose at pH 5.0 was protective  
369 against acid ascorbic oxidation [34]. A supplementation of ascorbic acid in the parenteral nutrition  
370 solution would be possible and would stabilize ascorbic acid during the period of administration of the

371 solution. However, further studies will be needed to demonstrate the 24-hour stability of ascorbic acid  
372 supplemented parenteral nutrition mixture.

373

#### 374 **5. Conclusion**

375 This formulation of standard parenteral nutrition solution remained stable for four months at 2°C -  
376 8°C. The stability of the solution was demonstrated with a validated method to assay amino acids in  
377 parenteral nutrition solution. This study presented the first method for the assay of twenty amino  
378 acids in complex mixture. The importance of the amino acids assays in hospital standardized  
379 solutions and the properties of degradation products should be considered.

380

381 **Declaration of interest:** the authors have declared that they have no conflict of interest in relation to  
382 this article.

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402 [\\_and\\_safety\\_assurance\\_requirements\\_for\\_medicinal\\_products\\_prepared\\_in\\_pharmacies.pdf](https://www.edqm.eu/sites/default/files/resolution_cm_res_2016_1_quality_and_safety_assurance_requirements_for_medicinal_products_prepared_in_pharmacies.pdf).
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466 **Legends**

467

468 **Fig.1. A: Linear profiles of (●) cystine ( $r = 0.936$ ,  $p < 0.0001$ ) and (○) cysteine ( $r = 0.984$ ,  $p < 0.0001$ ) concentrations**  
469 **in parenteral solution as a function of time of storage at 2°C - 8°C ( $n = 9$  experimental determinations). Profile of**  
470 **cysteine concentration was calculated as the difference of initial concentration of cysteine in the parenteral**  
471 **solution and the experimental cystine concentrations determined throughout the time of storage. B: Box Plot:**  
472 **Cystine concentration for 118 days of storage at 2°C - 8°C ( $n = 9$  experimental determinations). \*:  $p < 0.05$ ; \*\*\*  $p$**   
473 **< 0.01 as compared to day #1 group (Wilcoxon's test).**

474 **Fig.2. A: UV-visible spectra of aqueous solutions of glucose, amino acids, glucose and amino acids, supplemented**  
475 **or not with ascorbic acid. B: Photographs of aqueous solutions of glucose, amino acids, glucose and amino acids,**  
476 **supplemented or not with ascorbic acid.**

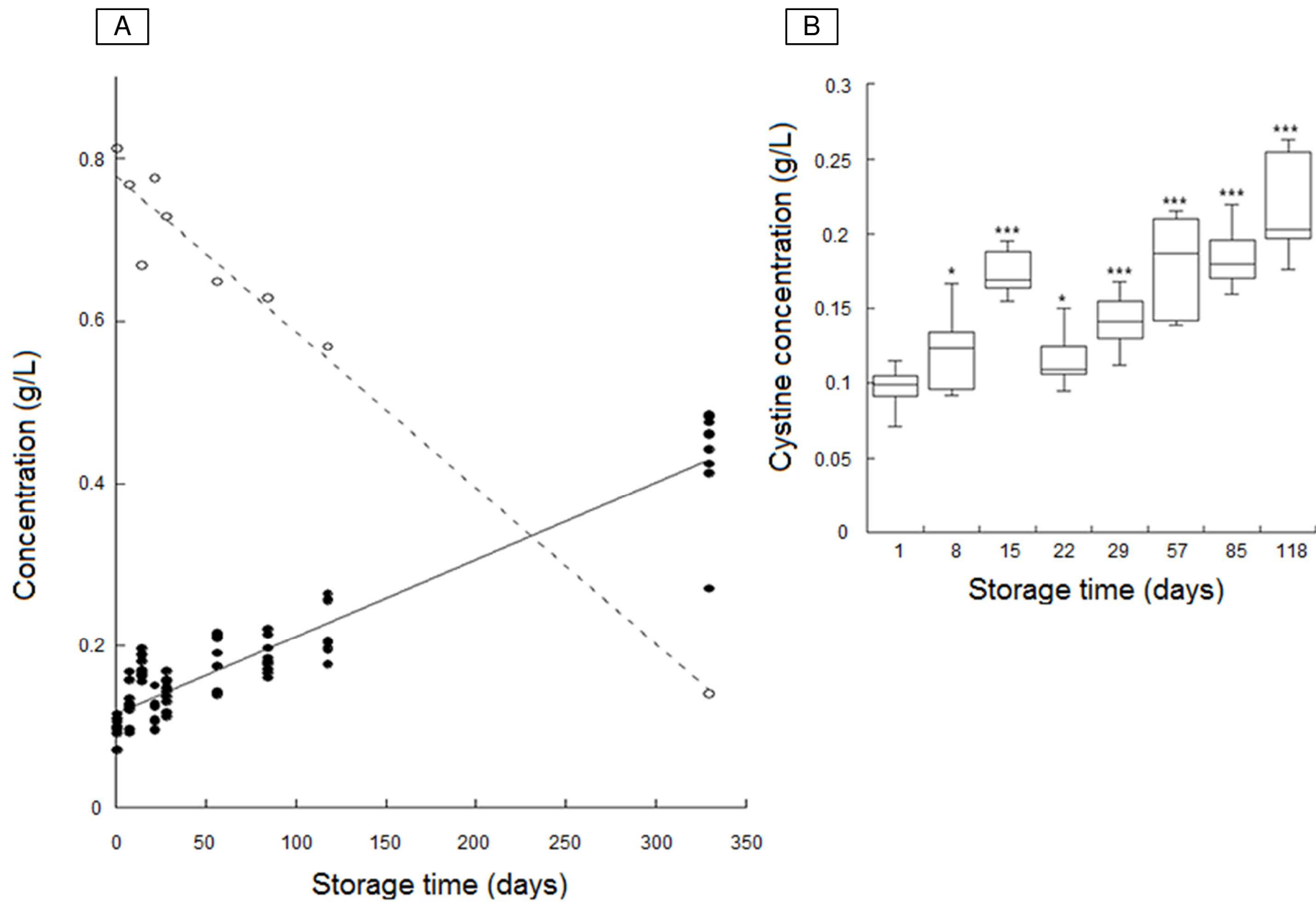


Figure 1

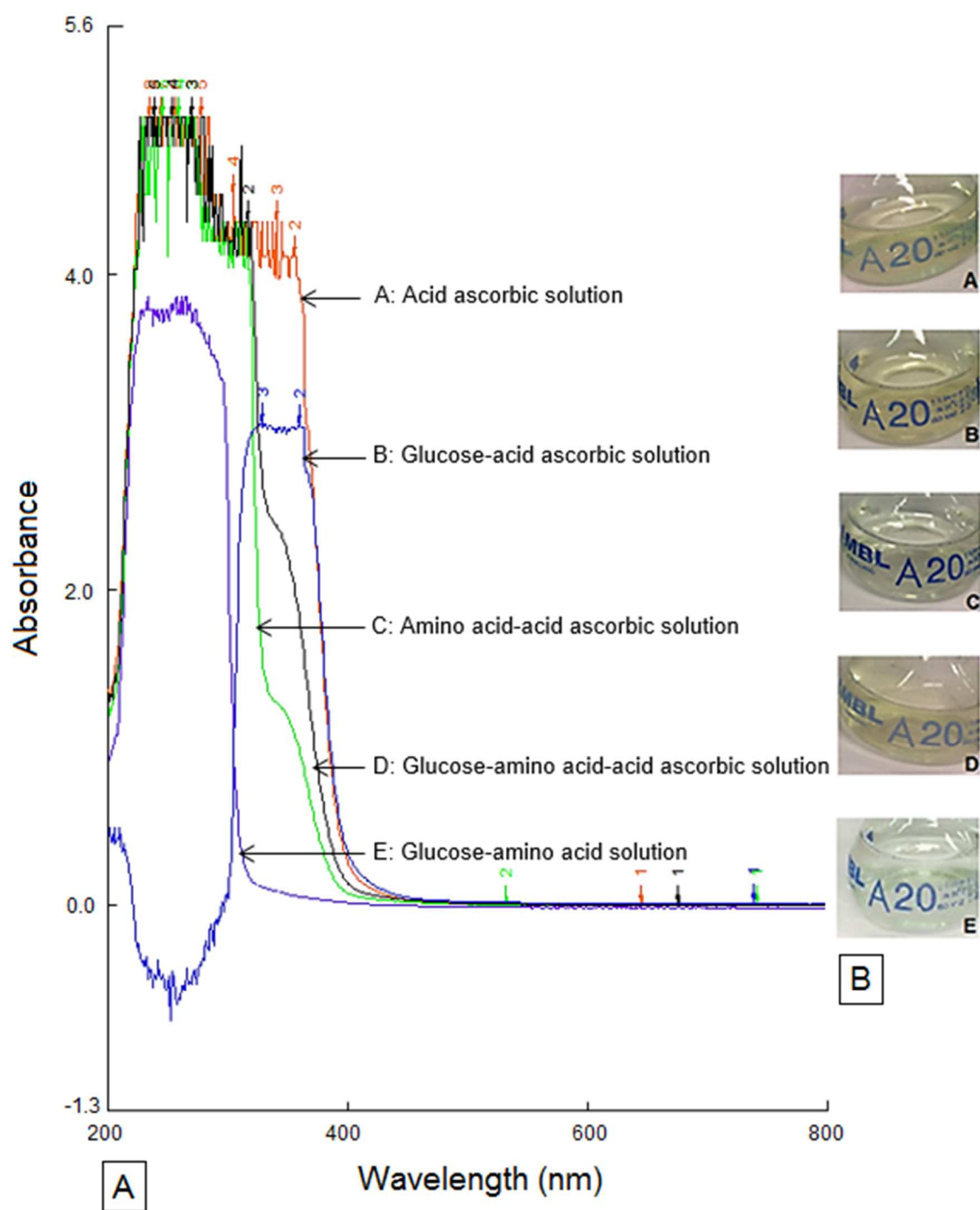


Figure 2

**Table 1: Composition of the standard parenteral nutrition solution.**

<b>Components</b>	<b>Concentrations</b>
<b>Sodium</b>	34 mM
Sodium lactate	
<b>Potassium</b>	20 mM
Monopotassium phosphate	
<b>Calcium</b>	20 mM
Calcium gluconate	
<b>Phosphorus</b>	20 mM
Monopotassium phosphate	
<b>Glucose</b>	944 mM (170 g/L) 680 kcal/L
<b>Amino acids</b>	436 mM (48 g/L) 192 kcal/L
<b>Nitrogen</b>	7.2 g/L
<b>Total energy</b>	872 kcal/L
<b>Glucose/nitrogen energy</b>	95 kcal/g
<b>Protein-energy ratio</b>	5.5 g/100 kcal

**Table 2: Composition of aqueous solutions of glucose, amino acids, glucose and amino acids, supplemented or not with ascorbic acid.**

<b>Solutions</b>	<b>Components g/L (mM)</b>		
	Ascorbic acid	Glucose	Amino acids*
<b>A</b>	87 (494)	-	-
<b>B</b>	87 (494)	74 (411)	-
<b>C</b>	87 (494)	-	42 (382)
<b>D</b>	87 (494)	74 (411)	42 (382)
<b>E</b>	-	74 (411)	43 (390)

\*cysteine concentration was about 0.79 g/L (6.5 mM).

**Table 3: Qualitative and quantitative amino acid composition of standard parenteral nutrition solution during storage at 2°C - 8°C.**

Amino acids (g/L)	Specifications (g/L)	Storage time (days)							
		1	8	15	22	29	57	85	118
<b>Essentials</b>									
L-histidine	1.55-2.09	1.93 ± 0.04	1.94 ± 0.03	1.92 ± 0.08	1.88 ± 0.04	1.89 ± 0.15	1.96 ± 0.05	1.86 ± 0.03	2.01 ± 0.03
L-isoleucine	2.74-3.69	3.34 ± 0.07	3.36 ± 0.05	3.32 ± 0.11	3.24 ± 0.05	3.24 ± 0.24	3.29 ± 0.09	3.22 ± 0.06	3.33 ± 0.04
L-leucine	4.08-5.52	5.12 ± 0.12	5.14 ± 0.08	5.07 ± 0.17	4.93 ± 0.08	4.96 ± 0.37	5.05 ± 0.14	4.93 ± 0.09	5.10 ± 0.07
L-lysine	4.49-6.07	6.08 ± 0.17	5.99 ± 0.12	5.93 ± 0.22	5.80 ± 0.10	5.80 ± 0.44	6.02 ± 0.17	5.69 ± 0.23	6.06 ± 0.12
L-methionine	0.98-1.32	1.16 ± 0.03	1.14 ± 0.05	1.08 ± 0.05	1.03 ± 0.03	1.09 ± 0.12	1.05 ± 0.07	1.05 ± 0.03	1.07 ± 0.07
L-phenylalanine	1.72-2.31	2.13 ± 0.04	2.11 ± 0.03	2.09 ± 0.07	2.02 ± 0.03	2.04 ± 0.16	2.09 ± 0.06	2.01 ± 0.04	2.13 ± 0.03
L-threonine	1.51-2.04	1.84 ± 0.04	1.85 ± 0.03	1.82 ± 0.05	1.77 ± 0.03	1.79 ± 0.13	1.83 ± 0.05	1.78 ± 0.03	1.85 ± 0.02
L-tryptophan	0.82-1.10	1.10 ± 0.02	1.06 ± 0.03	1.05 ± 0.06	1.00 ± 0.03	0.99 ± 0.09	1.02 ± 0.04	0.93 ± 0.03	1.03 ± 0.05
L-valine	3.11-4.19	3.90 ± 0.10	3.91 ± 0.05	3.85 ± 0.13	3.70 ± 0.06	3.78 ± 0.28	3.80 ± 0.13	3.75 ± 0.07	3.87 ± 0.04
<b>Total [1]</b>	21.00-28.33	26.72 ± 0.63	26.62 ± 0.47	26.29 ± 0.94	25.48 ± 0.45	25.73 ± 1.88	26.28 ± 0.80	25.40 ± 0.61	26.67 ± 0.44
<b>Non-essentials</b>									
L-alanine	3.27-4.41	4.29 ± 0.10	4.30 ± 0.06	4.25 ± 0.13	4.12 ± 0.06	4.24 ± 0.35	4.01 ± 0.11	3.96 ± 0.07	4.06 ± 0.06
L-arginine	3.43-4.63	4.30 ± 0.09	4.22 ± 0.31	4.22 ± 0.11	4.10 ± 0.06	4.10 ± 0.33	4.19 ± 0.12	4.07 ± 0.08	4.22 ± 0.05
L-aspartic acid	2.45-3.31	2.99 ± 0.07	3.01 ± 0.07	2.98 ± 0.08	2.92 ± 0.04	2.93 ± 0.22	3.00 ± 0.08	2.94 ± 0.05	3.02 ± 0.04
L-glutamic acid	4.08-5.52	5.21 ± 0.12	5.29 ± 0.10	5.19 ± 0.16	5.05 ± 0.08	5.11 ± 0.39	5.18 ± 0.14	5.09 ± 0.09	5.25 ± 0.07
glycine	1.63-2.20	2.02 ± 0.06	2.01 ± 0.04	2.00 ± 0.07	1.92 ± 0.03	1.97 ± 0.15	2.02 ± 0.06	1.95 ± 0.04	2.02 ± 0.04
L-ornithine	1.29-1.76	1.55 ± 0.06	1.50 ± 0.05	1.48 ± 0.05	1.47 ± 0.03	1.41 ± 0.12	1.56 ± 0.05	1.45 ± 0.05	1.55 ± 0.05
L-proline	1.23-1.65	1.12 ± 0.16	1.26 ± 0.16	1.34 ± 0.18	1.27 ± 0.08	1.43 ± 0.09	1.27 ± 0.11	1.59 ± 0.04	1.29 ± 0.05
L-serine	1.64-2.20	2.01 ± 0.05	2.03 ± 0.04	2.01 ± 0.06	1.94 ± 0.03	1.97 ± 0.14	2.02 ± 0.06	1.97 ± 0.04	2.03 ± 0.03
L-taurine	0.24-0.34	0.34 ± 0.02	0.33 ± 0.01	0.34 ± 0.02	0.33 ± 0.01	0.25 ± 0.05	0.27 ± 0.01	0.24 ± 0.01	0.28 ± < 2%
L-tyrosine	0.18-0.25	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.21 ± < 2%	0.21 ± 0.02	0.22 ± 0.01	0.21 ± < 2%	0.22 ± < 2%
<b>Total [2]</b>	19.44-26.26	24.05 ± 0.74	24.17 ± 0.85	24.02 ± 0.87	23.33 ± 0.46	23.62 ± 1.86	23.74 ± 0.97	23.47 ± 0.51	23.94 ± 0.39
<b>Total [1+2]</b>	44.44-54.59	50.77 ± 1.37	50.79 ± 1.32	50.31 ± 1.81	48.48 ± 0.91	49.35 ± 3.74	50.02 ± 1.77	48.87 ± 1.12	50.61 ± 0.83
<b>Ratio [1/2]</b>	0.80-1.46	1.13 ± 0.06	1.10 ± 0.06	1.11 ± 0.08	1.11 ± 0.04	1.09 ± 0.17	1.11 ± 0.06	1.08 ± 0.05	1.11 ± 0.04

Values are expressed as: mean concentration ± standard deviation or ± < coefficient of variation (%).

ND: not determined

**Table 4: Characterization of the physicochemical properties of the standard parenteral nutrition solution during storage at 2°C - 8°C.**

Parameters and specifications	Storage time (days)							
	1	8	15	22	29	57	85	118
<b>pH</b> : 4.08-5.52	4.99 ± 0.03	5.05 ± 0.01	5.11 ± 0.03	5.02 <sup>a</sup> ± 0.02	5.06 ± 0.04	5.06 ± 0.08	4.92 ± 0.02	5.05 ± 0.03
<b>Osmolarity</b> : 1552-2098 mOsm/L	1698 ± 33	1724 ± 21	1723 ± 39	1758 <sup>a</sup> ± 30	1715 ± 16	1704 ± 17	1698 ± 17	1708 ± 33
<b>Glucose</b> : 802-1085 mM	992 ± 28	965 ± 35	981 ± 60	873 <sup>b</sup> ± 81	961 ± 17	997 ± 58	912 ± 22	974 ± 28
<b>Absorption maximum wavelength (nm)</b> : not specified	226	226	226	226	226	226	226	226
<b>Number of particles/mL</b>								
> 10 µm : ≤ 25	3 ± 1	3 ± 2	1 ± 1	3 ± 2	3 ± 1	4 ± 2	1 ± 1	4 ± 5
> 25 µm : ≤ 3	1 ± 1	0	0	0	1 ± 1	1 ± 1	0	1 ± 1
<b>Sodium</b> : 29-39 mM	37 ± 1	37 ± < 1%	ND	39 ± < 1%	39 ± < 2%	38 ± 1	38 ± 1	38 ± 1
<b>Potassium</b> : 17-23 mM	20 ± 1	20 ± < 1%	22 ± 1	21 ± 1	21 ± < 2%	20 ± 1	20 ± < 2%	20 ± 1
<b>Calcium</b> : 17-23 mM	18 ± < 2%	18 ± < 1%	20 ± < 2%	20 ± < 1%	19 ± < 2%	19 ± < 2%	18 ± < 2%	19 ± < 2%
<b>Phosphorus</b> : 17-23 mM	21 ± 1	21 ± 1	20 ± 1	22 <sup>b</sup> ± 1	20 ± 1	20 ± 1	21 ± 1	21 ± 1

Values are expressed as: mean concentration ± standard deviation or ± < coefficient of variation (%).

ND: Not determined

<sup>a</sup>: 3 experimental measurements.

<sup>b</sup>: 6 experimental measurements.