Phytosterol determination in lipid emulsions for parenteral nutrition

Abstract
Objective: The presence of phytosterols in vegetal lipid emulsions has been associated with alterations of liver function tests. Determination of phytosterols content, currently undeclared, would allow the development of strategies to prevent or treat these alterations.

Method: 3-4 non-consecutive batches of 6 lipid emulsions from different providers (Clinoleic®, Intralipid®, Lipofundina®, Lipoplus®, Omegaven® and Smoflipid®) were analyzed. Differences in total phytosterol assay between providers and batches were statistically studied by a one-way ANOVA and Kruskal-Wallis non-parametric approximation and post hoc Scheffé test (p < 0.05).

Results: The absence of phytosterols was confirmed in Omegaven®, emulsion based on fish oil. The highest assay of phytosterols (422.4 ± 130.5 µg/mL) has been related with the highest percentage of soya bean oil in Intralipid. In the remaining emulsions, concentrations were from 120 to 210 µg/mL related to the percentage of soya bean oil. Statistically significant differences of phytosterol content in lipid emulsions were observed among different providers (F = 23.59, p = 0.000) as well as among non-consecutive batches. Clinoleic™ (F = 23.59, p = 0.000), Intralipid™ (F = 978.25, p = 0.000), Lipofundina™ TLC/TCM (F = 5.43, p = 0.045), Lipoplus™ (F = 123.53, p = 0.000) and Smoflipid™ (16.78, p = 0.000). Except for Lipofundina™ TLC/TCM, the differences between batches were marked.

Conclusions: Lipid emulsions, registered on Spanish pharmaceutical market, contain variable quantities of phytosterols dependent on commercial brand and batch.

KEYWORDS
Phytosterols; Lipid emulsions; Parenteral nutrition; Soybean oil; Liver function tests.

PALABRAS CLAVE
Fitosteroles; Emulsiones lipídicas; Nutrición parenteral; Aceite de soja; Parámetros de función hepática.

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Introduction
Lipid emulsions (LEs) are routinely used in parenteral nutrition (PN). Prior to the inclusion of LEs in these formulas, PN required high amounts of glucose, which was associated with a range of problems\(^{1}\). The high energy efficiency of lipids led to a reduction in the use of glucose.

In Spain, the use of LEs in PN became routine practice in the 1980s. Initially, all LEs were based on soybeans, but since then a range of formulations has been developed. Currently, 5 LEs are registered for the Spanish pharmaceutical market. They are based on soybeans, olives, medium-chain triglycerides (MCTs), and fish oil in different concentrations and combinations.

Although LEs were initially used as an energy substrate, the anti-inflammatory effect of fish oil\(^{2,3}\) and the lower lipid peroxidation effect of olive oil\(^{4}\) has led to these lipids being proposed as pharmacotherapeutics.

Parenteral nutrition-associated liver disease is one of the most relevant complications of PN. Parenteral nutrition-associated liver disease has a multifactorial component\(^{4,5}\), and the quantity and type of lipid\(^{6}\) have clearly been established as among the factors associated with the disease. Therefore, it is relatively common in clinical practice to reduce doses or to even temporarily stop the administration of lipids altogether\(^{6,7}\). For several years, it was hypothesised that these complications were associated with the use of plant-based LEs. Since the time of the study by Clayton in the paediatric population\(^{8}\), this possibility has been attributed to the presence of phytosterols, which hypothesis was subsequently confirmed in adult patients by our study group\(^{9}\). The phytosterol content of LEs is currently undeclared, and thus does not appear in the Summary of Product Characteristics or on the label. Currently, all emulsions available on the Spanish pharmaceutical market contain variable amounts of plant-based lipids and therefore contain phytosterols. This means that LE use entails their erratic administration.

Phytosterols occur in plants and are considered to be equivalent to cholesterol due to their similar structure and similar functions in cell membrane regulation. There has been a recent increase in their clinical importance due to their demonstrated beneficial effects on cholesterol re-duction when orally administered\(^{10,11}\). Due to their potential hepatotoxicity, the determination of phytosterol content in LEs would improve the management and prevention of hepatic complications in PN.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) analytical methods, particularly for the analysis of food and plant extracts, are available for the qualitative and quantitative determination of phytosterols. Gas chromatography can simultaneously determine phytosterols, whereas the available HPLC methods can only identify a few phytosterols and only under particular conditions\(^{12}\). We developed a simple HPLC analytical method for the routine determination of phytosterol content in parenteral LEs. The objective of this study was to determine differences in the phytosterol content of LEs available on the Spanish pharmaceutical market according to their formulation, brand, and batch.

Methods
We prospectively analysed intravenous LEs with different compositions available on the Spanish pharmaceutical market (Table 1) to determine daily exposure to phytosterols in patients with PN.

Results
The proposed analytical method allowed us to simplify sample preparation and conduct a single analysis, which led to the successful separation of 8 phytosterols, cholesterol, and squalene. The validation process showed that the method is suitable for routine analysis.

The analysis of LE brands (Table 2) showed that the fish-oil-based LE Omegaven\(^{2}\) did not contain phytosterols. This finding was in line with previously published results\(^{13}\), and therefore Omegaven\(^{2}\) was excluded from the statistical analysis. Intralipid is based completely on soybean oil. Its analysis showed that it contained the highest concentration of phytosterols (422.4 ± 130.5 µg/mL) and confirmed that soybean oil was the source of its high phytosterol content. The analysis showed that the other LE brands had variable phytosterol content ranging from 120 µg/mL to 210 µg/mL, depending on the percentage of soybean oil. Statistically significant differences were found between these brands (F = 42.97, p < 0.000). A weak correlation was found between phytosterol concentrations and greater plant-based lipid content, especially when the LE was based on soybeans.

The second part of the study analysed phytosterol content in various non-consecutive batches of LEs (Table 3). Statistically significant differences were also found between different batches. Clinoleic (F = 23.59, p < 0.000), Intralipid (F = 978.25, p < 0.000), Lipofundin LCT/MCT (F = 5.43, p < 0.045), Lipopuls (F = 123.53, p < 0.000), and Smolfiplid (F = 16.78, p < 0.000). Except in the case of Lipofundin LCT/MCT, the differences between batches were substantial.

Discussion
We developed an HPLC analytical method to simplify and reduce the cost of determining phytosterol content in LEs\(^{7}\). The validation process demonstrated its selectivity, linearity, precision, accuracy, and robustness, all of which support its routine use\(^8\). The sample treatment protocol for the commercially available LEs is an adapted version of published protocols\(^9\), and it took into account the properties of the samples and the requirements of the analytical method. We used this method to determine the phytoster-
Table 2. Differences in Total Phytosterol Content by Brand

<table>
<thead>
<tr>
<th>ID</th>
<th>Lipid emulsion</th>
<th>Mean total phytosterol concentration (µg/mL)</th>
<th>Statistically significant differences by ID (P&lt;0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clinoleic™ 20% (n = 12)</td>
<td>208.8 ± 39.4</td>
<td>2 y 5</td>
</tr>
<tr>
<td>2</td>
<td>Intralipid™ 20% (n = 9)</td>
<td>422.4 ± 130.5</td>
<td>1, 3, 4 y 5</td>
</tr>
<tr>
<td>3</td>
<td>Lipofundin™ LCT/MCT (n = 9)</td>
<td>187.9 ± 9.1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Lipoplus™ 20% (n = 9)</td>
<td>140.1 ± 20.9</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Smoflipid™ 20% (n = 15)</td>
<td>124.2 ± 15.3</td>
<td>1 y 2</td>
</tr>
</tbody>
</table>

F = 42.976; significance value = 0.000. Statistically significant differences with one-way ANOVA and non-parametric Kruskal-Wallis test.

Table 3. Differences in Total Phytosterol Content by Batch

<table>
<thead>
<tr>
<th>Snedecor’s F sig. (P value)</th>
<th>ID</th>
<th>Batch</th>
<th>Mean total phytosterol concentration (µg/mL)</th>
<th>Statistically significant differences between batches by ID (P&lt;0.05)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 3)</td>
<td>14H29N30</td>
<td>231.9 ± 15.7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2 (n = 6)</td>
<td>15F15N31</td>
<td>227.2 ± 21.0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3 (n = 3)</td>
<td>16F22N30</td>
<td>149.0 ± 3.9</td>
<td>1 and 2</td>
<td></td>
</tr>
<tr>
<td>F = 23.59; P = 0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (n = 3)</td>
<td>10H83671</td>
<td>451.3 ± 23.2</td>
<td>2 and 3</td>
<td></td>
</tr>
<tr>
<td>2 (n = 3)</td>
<td>10K7012</td>
<td>554.1 ± 36.5</td>
<td>1 and 3</td>
<td></td>
</tr>
<tr>
<td>3 (n = 3)</td>
<td>10KC3584</td>
<td>261.6 ± 12.8</td>
<td>1 and 2</td>
<td></td>
</tr>
<tr>
<td>F = 97.26; P = 0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (n = 3)</td>
<td>143638082</td>
<td>178.8 ± 3.7</td>
<td>3</td>
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</tr>
<tr>
<td>2 (n = 3)</td>
<td>14478082</td>
<td>189.7 ± 9.3</td>
<td>-</td>
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</tr>
<tr>
<td>3 (n = 3)</td>
<td>154818081</td>
<td>195.4 ± 3.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F = 5.43; P = 0.045</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1 (n = 3)</td>
<td>144538082</td>
<td>145.9 ± 6.1</td>
<td>2 and 3</td>
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<tr>
<td>2 (n = 3)</td>
<td>153938083</td>
<td>160.5 ± 1.5</td>
<td>1 and 3</td>
<td></td>
</tr>
<tr>
<td>3 (n = 3)</td>
<td>160128082</td>
<td>113.8 ± 1.6</td>
<td>1 and 2</td>
<td></td>
</tr>
<tr>
<td>F = 123.53; P = 0.000</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (n = 3)</td>
<td>16F1650</td>
<td>137.6 ± 2.9</td>
<td>3 and 4</td>
<td></td>
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<tr>
<td>2 (n = 3)</td>
<td>16H40273</td>
<td>138.9 ± 7.6</td>
<td>3 and 4</td>
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<tr>
<td>3 (n = 6)</td>
<td>1611719</td>
<td>121.1 ± 9.3</td>
<td>1, 2, and 4</td>
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</tr>
<tr>
<td>4 (n = 3)</td>
<td>16K65043</td>
<td>102.3 ± 1.9</td>
<td>1, 2, and 3</td>
<td></td>
</tr>
<tr>
<td>F = 16.79; P = 0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant differences with one-way ANOVA and non-parametric Kruskal-Wallis test.
** Post hoc Scheffe test: 1, Clinoleic™; 2, Intralipid™; 3, Lipofundin™ LCT/MCT; 4, Lipoplus™; 5, Smoflipid™.
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and, unlike the aforementioned studies, it confirms the great variability in phytosterol content by brand and batch with its consequent clinical implications. The results highlight the relevance of including the total phytosterol content by brand and batch with its consequent clinical implications, and, unlike the aforementioned studies, it confirms the great variability in phytosterol content by brand and batch with its consequent clinical implications.

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Bibliography

Conflicts of interests
No conflict of interest.

Contributions to the scientific literature
Recent studies have shown that long-term PN leads to liver function abnormalities, which have been attributed to the phytosterol content of LEs. This study determined the total phytosterol content of the LEs registered on the Spanish pharmaceutical market. The results confirm that there is significant variability between different brands of LEs and between different batches. The results provide a basis on which to design strategies to prevent their hepatotoxic effects.