Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry[1]; Service of Development of Medicines (SDM)[2], Faculty of Pharmacy and Food Sciences, University of Barcelona, Spain; Pharmacotherapy, Pharmacogenetics and Pharmaceutical Technology Research Group[3], Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Spain; Division "Pharmaceutical Analysis"[4], Faculty of Pharmaceutical Sciences, KU Leuven, Campus Gasthuisberg, Leuven, Belgium

Study of tocopherol content and its potential antioxidant activity in commercial lipid emulsions for parenteral nutrition

A. Novak[1,*], M. Gutiérrez-Zamora[2], P. Pérez-Lozano[1,2,3], J. M. Suñé-Negre[1,2,3], J. M. Llop[1,3], J. R. Ticó[1,2,3], M. Miñarro[1,2,3], E. García-Montoya[1,2,3], A. Van Schepdael[4]

Received March 22, 2020, accepted April 3, 2020

**Corresponding author: Ana Novak, Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain*
ana.novakova@gmail.com

Pharmazie 75: 240-241 (2020) *doi: 10.1691/ph.2020.0038*

The objective of this study was to determine the content and evaluate the potential antioxidant effect of tocopherols in commercially available lipid emulsions, using a simple validated method adequate for further routine use. During the study, variability between manufacturers as well as between three non-consecutive batches of the same emulsion was observed. Furthermore, addition of α-tocopherol to lipid emulsions as excipient yields more stable emulsions and potentially a beneficial clinical effect. It was concluded that the variation of the tocopherol content between batches implies the importance of control and specification of tocopherol content by the manufacturers.

1. Introduction

Lipid emulsions, used in parenteral nutrition of hospitalised patients, are being investigated for antioxidant activity, mainly attributed to α-tocopherol; however, the importance of other isomers, especially γ-tocopherol, is growing in recent studies (Seppanen et al. 2010; Xu et al. 2015; Saini and Keum 2016). The newer studies set optimal levels of tocopherols to inhibit *in vitro* oil oxidation from 350 to 550 ppm (Mäkinen et al. 2000).

In clinical studies, tocopherols have been investigated for their potential to prevent parenteral nutrition-associated liver dysfunction, a common complication in long term parenteral nutrition. Addition of vitamin E, up to 9.1 mg of α-tocopherol per day, is believed to have hepatoprotective effects due to its antioxidant activity as well as activation of enzymes and transporters in liver cells (Biesalski 2009; Burrin et al. 2014; Linseisen et al. 2000; Mitra and Ahn 2017; Ng et al. 2016; Vlaardingerbroek et al. 2014).

2. Investigations, results and discussion

Lipid emulsions available on the Spanish pharmaceutical market were systematically analysed for the first time for tocopherol content. The data, presented in the Table, show the content of the respective tocopherol isomers in lipid emulsions from various manufacturers and three non-consecutive batches per emulsion. Differences in tocopherol content between various manufacturers have been confirmed. The concentrations of total tocopherols varied from 40 µg/mL up to 250 µg/mL, as expected in view of each lipid emulsion composition. The highest concentration of α-tocopherol was found in Lipoplus 20%, which contained tocopherol as antioxidant to stabilise the emulsion. In Lipofundina MCT 20% and Omegaven 10%, the determined concentrations of α-tocopherol correspond to the declared concentrations. The lowest concentrations were found in Intralipid 20% and ClinOleic 20%, as both lacked α-tocopherol as an excipient. It was observed that vegetable oils, especially soybean oil, contribute low concentrations of α-tocopherol and the addition of α-tocopherol as an antioxidant is useful not only for emulsion stability, but also for clinical effects.

A correlation has been observed between the γ-tocopherol concentration and the soybean oil content. Intralipid 20%, based only on soybean oil, demonstrated the highest concentration of γ-tocopherol, meanwhile, in ClinOleic 20%, with lowest soybean oil content, and Omegaven 10%, based only on fish oil, the levels of γ-tocopherol were below the validated quantification limit (1.25 µg/mL). Determined concentrations of γ-tocopherol, from 10 to 40 µg/mL, were not found sufficient for noticeable clinical antioxidant activity (Linseisen et al. 2000; Biesalski 2009).

The isomer δ-tocopherol was identified only in Intralipid 20% and Lipoplus 20%, however the concentrations were below the validated quantification level, which was expected due to its low content in vegetable oils. Also, the antioxidant activity contribution of δ-tocopherol is minor compared to α-tocopherol and γ-tocopherol (Seppanen et al. 2010).

Analysis of three non-consecutive batches of each lipid emulsion revealed important differences in tocopherol content (Table 1). Furthermore, a comparison of the obtained results with previous studies (Xu et al. 2015) confirms less variability of tocopherol content in lipid emulsions with added α-tocopherol, compared to the ones that lack antioxidant stabilisation. Variation in the concentration is mainly attributed to the use of natural constituents. Lipid emulsions with a mixture of various vegetable oils in combination with fish oil, such as SMOFlipid and Lipoplus 20% demonstrated higher deviations in tocopherol content (176-206 µg/mL and 209-253 µg/mL, respectively). Omegaven 10% and Lipofundina MCT 20%, which had specified addition of α-tocopherol, resulted in relatively low variation (199-215 µg/mL and 181-197 µg/mL, respectively). In order to benefit from naturally occurring antioxidants in lipid emulsions for clinical effects, control and specification of the tocopherol content should be employed for each released batch.

Table: Tocopherol content in lipid emulsions

Lipid emulsion	Batch number	α-tocopherol (µg/mL) ± SD	γ-tocopherol (µg/mL) ± SD	δ-tocopherol (µg/mL) ± SD	Total tocopherols (µg/mL) ± SD
ClinOleic 20% (Baxter)	14H29N30	38 ± 1	ND*	ND*	38 ± 1
	15F15N31	45 ± 1	ND*	ND*	45 ± 1
	16F22N30	40 ± 1	ND*	ND*	40 ± 1
Intralipid 20% (Fresenius Kabi)	10HB3671	30 ± 1	40 ± 1	< 1.25	70 ± 1
	10IK7012	19 ± 1	37 ± 1	< 1.25	56 ± 1
	10KC3584	25 ± 2	36 ± 3	< 1.25	61 ± 3
Lipofundina MCT 20% (Braun)	143638082	169 ± 4	27 ± 1	ND*	181 ± 4
	144718082	169 ± 10	25 ± 3	ND*	194 ± 10
	154818081	171 ± 2	26 ± 1	ND*	197 ± 2
Lipoplus 20% (Braun)	144538082	228 ± 10	25 ± 1	< 1.25	253 ± 10
	153938083	223 ± 10	21 ± 1	< 1.25	244 ± 10
	160128082	196 ± 3	13 ± 1	< 1.25	209 ± 3
Omegaven 10% (Fresenius Kabi)	16H60131	209 ± 3	ND*	ND*	209 ± 3
	16IE1319	233 ±2	ND*	ND*	215 ± 2
	16KF4628	199 ± 2	ND*	ND*	199 ± 2
SMOFlipid 20% (Fresenius Kabi)	16HI0273	169 ± 17	7 ± 1	ND*	176 ± 17
	16IG1719	191 ± 2	9 ± 1	ND*	200 ± 2
	16K65043	197 ± 3	9 ± 1	ND*	206 ± 3

*ND - not detected

The proposed analytical method uses simple preparation procedures and equipment, which is common in every quality control laboratory and could be easily incorporated in lipid emulsion quality control. Variability between various brands and different batches has been confirmed, which suggests the importance of follow-up and specification of the tocopherol content by manufacturers. Determined concentrations of α- and γ-tocopherol in some lipid emulsions were below the recommended daily dose for vitamin E. Therefore, addition of α-tocopherol as an excipient is important for lipid emulsion stability as well as for antioxidant effects in patients on long term parenteral nutrition.

3. Experimental

A simple reverse phase high performance liquid chromatographic analytical method, using diode array detection (DAD), was primarily established for the determination of phytosterols, cholesterol and squalene (Novak et al. 2018). The method was extended for the analysis of tocopherols in order to ensure simultaneous quantification of various analytes for possible quality control of lipid emulsions. The solution stability, as well as method selectivity, linearity, precision, accuracy and robustness were validated, according to the official guidelines, in the range 5-500 µg/mL for α- and δ-tocopherol, whereas for γ-tocopherol, the range was set to 1.25-100 µg/mL.

Acknowledgements: This research was supported by the Investigation Agency of Spanish Society of Hospital Pharmacy [AISEFH 2014] and Institute of Health Carlos III [AES 2014, PI14/00706].

Conflicts of interest: None declared.

References

Biesalski HK (2009) Vitamin E requirements in parenteral nutrition. Gastroenterology 137: S92–S104.

Burrin DG, Ng K, Stoll B, Saenz De Pipaón M (2014) Impact of new-generation lipid emulsions on cellular mechanisms of parenteral nutrition-associated liver disease. Adv Nutr 5: 82–91.

Linseisen J, Hoffmann J, Lienhard S, Jauch K-W, Wolfram G (2000) Antioxidant status of surgical patients receiving TPN with an Ω-3-fatty acid-containing lipid emulsion supplemented with α-tocopherol. Clin Nutr 19: 177–184.

Mäkinen M, Kamal-Eldin A, Lampi A-M, Hopia A (2000) Effects of α- and γ-tocopherols on formation of hydroperoxides and two decomposition products from methyl linoleate. J Am Oil Chem Soc 77: 801–806.

Mitra A, Ahn J (2017) Liver disease in patients on total parenteral nutrition. Clin Liver Dis 21: 687–695.

Ng K, Stoll B, Chacko S, Saenz De Pipaón M, Lauridsen C, Gray M, Squires EJ, Marini J, Zamora IJ, Olutoye OO, Burrin DG (2016) Vitamin E in new-generation lipid emulsions protects against parenteral nutrition-associated liver disease in parenteral nutrition-fed preterm pigs. J Parenter Enter Nutr 40: 656–671.

Novak A, Gutiérrez-Zamora M, Domenech L, Suñé-Negre JM, Miñarro M, García-Montoya E, Llop JM, Ticó JR, Pérez-Lozano P (2018) Development and validation of a simple high-performance liquid chromatography analytical method for simultaneous determination of phytosterols, cholesterol and squalene in parenteral lipid emulsions. Biomed Chromatogr 32: 1–8.

Saini RK, Keum Y-S (2016) Tocopherols and tocotrienols in plants and their products: A review on methods of extraction, chromatographic separation, and detection. Food Res Int 82: 59–70.

Seppanen CM, Song Q, Saari Csallany A (2010) The antioxidant functions of tocopherol and tocotrienol homologues in oils, fats, and food systems. J Am Oil Chem Soc 87: 469–481.

Vlaardingerbroek H, Ng K, Stoll B, Benight N, Chacko S, Kluijtmans LAJ, Kulik W, Squires EJ, Olutoye O, Schady D, Finegold ML, van Goudoever JB, Burrin DG (2014) New generation lipid emulsions prevent PNALD in chronic parenterally fed preterm pigs. J Lipid Res 55: 466–77.

Xu Z, Harvey KA, Pavlina TM, Zaloga GP, Siddiqui RA (2015) Tocopherol and tocotrienol homologs in parenteral lipid emulsions. Eur J Lipid Sci Technol 117: 15–22.