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Chemical stability of the lipid matrices of solid lipid nanoparticles (SLN) – development of an analytical method and determination of long-term stability

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Solid lipid nanoparticles (SLN) were produced with different lipids (cetylpalmitate, Dynasan 118, Softisan 154) by the hot homogenisation technique. A gas chromatography (GC) analysis in combination with a method for lipid extraction from aqueous SLN dispersions was established to investigate the chemical stability of the lipid excipient forming the particle matrix. A new procedure for transmethylation of liquid lipids was adopted to solid lipids. Storage of the aqueous SLN dispersions was performed at room temperature for 2 years. The particles sizes were determined by photon correlation spectroscopy and laser diffractometry. GC analysis was shown to be suitable for qualitative and quantitative determination of the chemical stability of the lipids used as excipients in the SLN matrix. Cetylpalmitate and Dynasan 118 showed the highest chemical stability (loss $<2\%$ within 2 years). Sufficient stability of the lipid excipients (content $>90\%$) – a prerequisite for a drug formulation – could be shown for these two lipids for 2 years.

1. Introduction

Solid lipid nanoparticles are a drug carrier system alternative to emulsions, liposomes and polymeric nanoparticles [1]. In contrast to emulsions and liposomes the particle matrix is composed of solid lipids. The solid state of the particle matrix protects incorporated active ingredients against chemical degradation [2] and allows to modify the drug release in a broad spectrum ranging from minutes [3] to days and weeks [4]. Various methods are described in the literature to prepare SLN. Preparation can be performed by the microemulsion technique [5], by high speed stirring or sonication [6, 7] and – preferentially with regard to scaling up aspects – by high pressure homogenization [8, 9]. Intensive data have been published about production [10], physico-chemical particle characterization [11, 12], drug incorporation and drug release [13] and also chemical stabilization of active ingredients by incorporation to SLN [14].
However, in contrast to stability data about incorporated active ingredients, no data have so far been published about the chemical stability of lipids in the SLN matrix. For successful formulation of drugs in SLN the chemical stability of the excipients used for particle production needs to be guaranteded. A prerequisite for analyzing the chemical stability of the lipids in the SLN is the development of a suitable method for extracting the lipids from the particles and subsequent analysis, e.g. gas chromatography. This paper describes the development of a suitable method and investigates the long-term stability of lipid excipients over a period of 24 months.

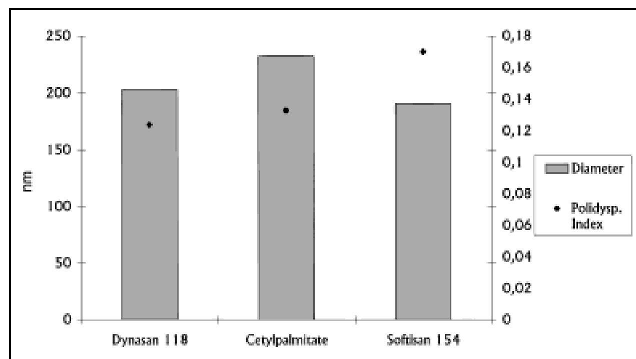

Fig. 1: Mean PCS diameters and polidyspersity indices (PI) of SLN particles produced with three different lipids

2. Investigations, results and discussion

The SLN produced by the hot homogenization technique led to particles with a mean PCS diameter in the range of 200 to 250 nm for all three different lipid matrix materials (Fig. 1). The PCS polydispersity index (PI) is a measure for the width of the size distribution and ranges from 0 (monodisperse population) to 0.500 indicating a relatively broad distribution. For standard polystyrene latex particles considered as being practically monodisperse a PI typically in the range of about 0.020–0.050 is observed. Emulsions for parenteral nutrition possessing a very narrow size distribution have a PI of about 0.100, relatively broad distributed emulsions of about 0.250 (reference). The PI of all three SLN formulations is in the range of about 0.120 to 0.170 (Fig. 1), that means in the range of emulsions suitable for intravenous administration.
PCS is limited in the size range to particles below 3 μm. To assess if larger particles in the micrometer range are present, laser diffractometry (LD) was employed. Characterization parameters are the diameter 10% up to the diameter 95%, that means 10% up to respectively 95% of the particles are below the given size value (volume distribution). Especially the diameter 95% is sensitive to the presence of a few large particles, that means a high diameter 95% indicates the presence of micrometer particles and increases with increasing amount of these particles. The diameters 95% were in the range of 0.6 to 0.8 μm indicating a low amount of micrometer particles and a relatively narrow nanoparticle size distribution (Fig. 2). The diameters 50% of all three SLN formulations were about 300 nm and very close to the PCS diameters. This again proves the extremely low content of micrometer particles because the size distribution determined by LD is a volume distribution, where the presence of even a few large particles would shift the diameters to high sizes.
The SLN formulations were stored at room temperature for two years to assess their chemical stability under normal storage conditions. In addition, size analysis was performed after the storage period of two years. To avoid

interaction of preservatives with the particles, they were prepared preservative-free. Production by high pressure homogenization sterilizes the dispersion, as activation forces generated by the high production pressure disrupt bacteria. Therefore, the bacterial load after production is extremely low and bacterial growth can be excluded storing the samples at 4 °C in a fridge. The SLN samples for size analysis were stored in the fridge to exclude bacterial growth and possible interference with the size analysis. There was no (1 year) or little change (plus 20 nm after 2 years) in the mean PCS diameter of cetylpalmitate SLN over a storage period of two years, no significant change was observed in the PI. There was an increase in the diameter 95% determined by LD to 2.3 µm. Dynasan 118 and Softisan 154 SLN were stable for one year regarding the PCS diameter (no change) but showed an increase in the LD diameters indicating beginning aggregation. They showed increased viscosity and tendency of gel formation after 2 years as observed previously for some unstable formulations [15]. To assess the chemical stability of the lipid, the aggregation does not interfere.

The fatty acid composition of lipid samples is usually determined by GC of the corresponding methyl esters. This method describes well the composition and quantity of fatty acids. In this study a new transmethylation method of liquid lipids was used and adopted to the solid lipids of SLN. The procedure originally described by Garces and Mancha [16] is a simple and time-saving method. Lipid extraction from samples with a high water content and their transmethylation can be done in a single step. The resolution of GC could be improved by introducing a temperature program. Fig. 3 shows the GC chromatograms of Dynasan 118, cetylpalmitate and Softisan without temperature program indicating an insufficient resolution. In Figs. 4–6 the chromatograms of the pure lipids are obtained with temperature gradient indicating a high resolution. To ensure that the lipid extraction method had no effect on the chromatogram, the chromatograms of the pure lipids were compared with chromatograms obtained after extraction from the SLN dispersion (Figs. 4–6, upper versus lower part). It can clearly be seen that there is no distortion of the chromatogram caused by the sample preparation. Important for the obtained good resolution is the use of a silica capillary with high polarity. This is in contrast to the usually applied types of capillaries rather preferring less polar silicas as column materials.

The lipids were extracted from the SLN dispersion and the amount of lipid found was calculated as percentage of the nominal value. The percentages of lipids found ranged from about 92% to 99%, that means they were below the nominal 100% value. This can be explained by the production method using a small scale Micron LAB 40 homogenizer. This homogenizer is not fully temperature controlled, that means there is a certain loss of lipid crystallizing at colder parts of the homogenizer unit. Therefore the value found by GC after production needs to be set equivalent to 100% to assess chemical stability of the lipids (normalisation). The Table shows the amount of lipid found calculated in percent of the nominal value and the values calculated setting the analyzed amount at the day of production to be 100%.

In the SLN formulations with cetylpalmitate and Dynasan 118, the lipid matrix materials showed sufficient stability over a storage period of 24 months, the values were above 90% which is generally considered as being the critical limit. Even when extrapolating the data to 36 months full excipient stability can still be assumed.

However, clear differences in stability could be seen between the two good lipids and Softisan. Cetylpalmitate and Dynasan proved to be the most stable, they showed only a non-significant reduction in the content by 0.1–0.2% after 12 months, a slightly higher reduction by 3–5% after 24 months (Table). The least stability of the three investigated lipids was observed for Softisan with a loss of 2% after 12 months and 11% after 24 months. In general, there was little degradation within the first 12 months, but a steeper decrease was observed within the second year of storage. At the moment it cnannot yet been foreseen how fast degradation continues, the study is still in progress.

Softisan 154 showed a distinctly reduced stability after 2 years being just below the value of 90%. As described above, some aggregation and gelling tendency occurred in the second year. It is known that the SLN gelling process is acompanied by internal changes of the liquid structure [15]. This can potentially accelerate the degradation process, e.g. by incorporation of water inside the lipid matrix. Cetylpalmitate SLN show no gelling, a stable structure on long-term [17] which is well in agreement with the observed high chemical stability of SLN made from this lipid.

Generally, the GC method showed a good reproducibility, the standard deviations were typically about 0.1–0.2% (Table). Two batches of SLN were produced using cetylpalmitate to study the in-batch variability. Both batches were relatively close on their data after one year, a small difference of 1.5% was found after 2 years (Table).

In conclusion, it can be stated that the method of lipid transmethylation and fatty acid methyl ester extraction is suitable for both quantitative and qualitative analysis of

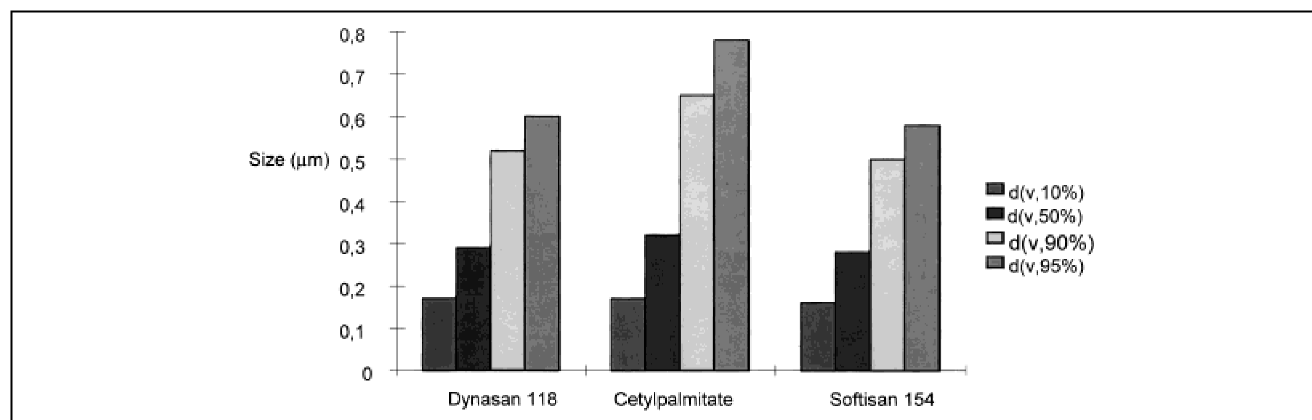

Fig. 2: Diameters 10%, 50%, 90% and 95% in µm determined by laser diffractometry (volume distribution data, measuring range 0.1 to 80 µm)

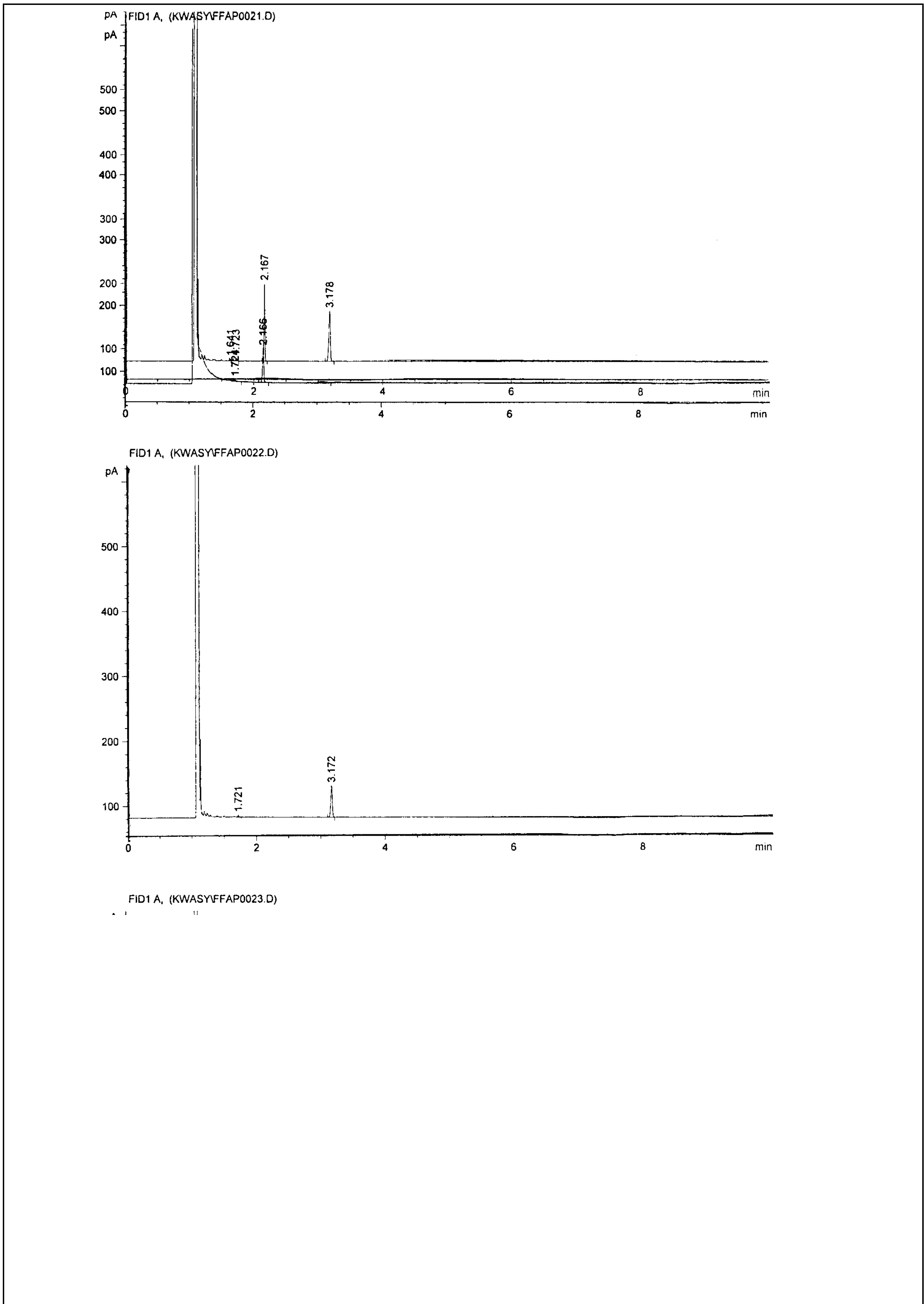

Fig. 3: GC of pure lipid analysed without applying a temperature gradient (from to top bottom: cetylpalmitate, Dynasan 118 and Softisan 154)

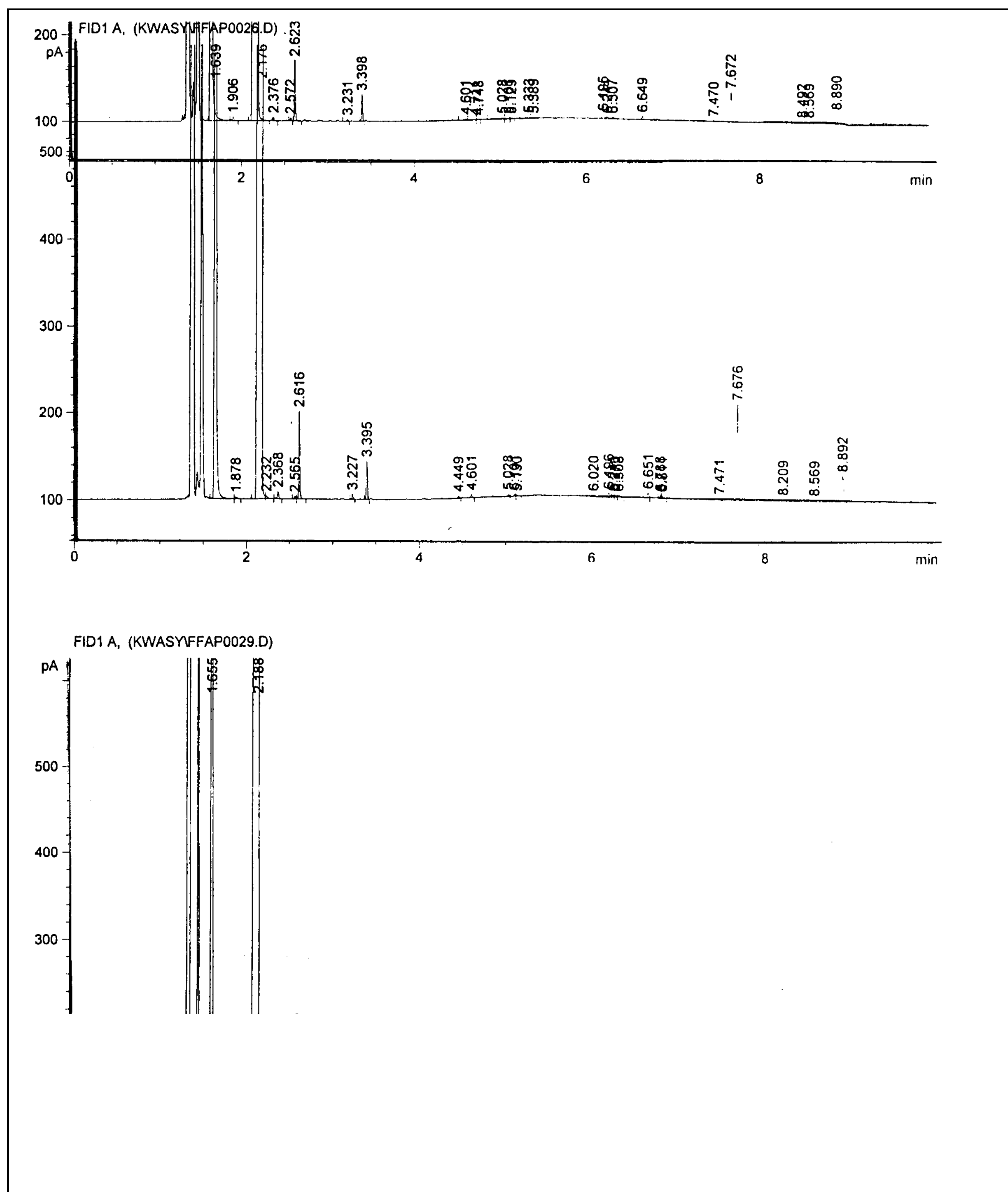

Fig. 4: GC of pure cetylpalmitate (top) and cetylpalmitate after extraction from the aqueous SLN mixture (bottom) analysed applying a temperature gradient

the lipids in SLN. In addition, this method can be considered as a convenient general method for fatty acid methyl ester preparation including solid lipids whereas a silica capillary column with high polarity gave the best separation of the analysed fatty acids.

In a drug formulation, not only the drugs but also the excipients need to comprise a sufficient chemical stability during long-term storage. For the first time it could be shown that this stability is given for lipids used as matrix forming excipients in SLN for at least 2 years.

3. Experimental

3.1. Preparation of solid lipid nanoparticles

All SLN formulations consisted of 10% solid lipid, 1.2% surfactant and double destillated water added to 100%. Both the aqueous and lipid phase were heated to a temperature of 75 °C, then the aqueous phase containing the surfactant was added to the melted lipid and mixed by a stirrer at 8000 rpm for 1 min (Ultra Turrax type T25, Janke and Kunkel – IKA Labortechnik, Staufen, Germany). The obtained pre-emulsion was immediately fed to a high pressure homogeniser (Micron Lab 40, APV Homogeniser GmbH, Lübeck, Germany). Homogenisation was performed applying a pressure of 500 bar and 3 homogenisation cycles at the temperature of

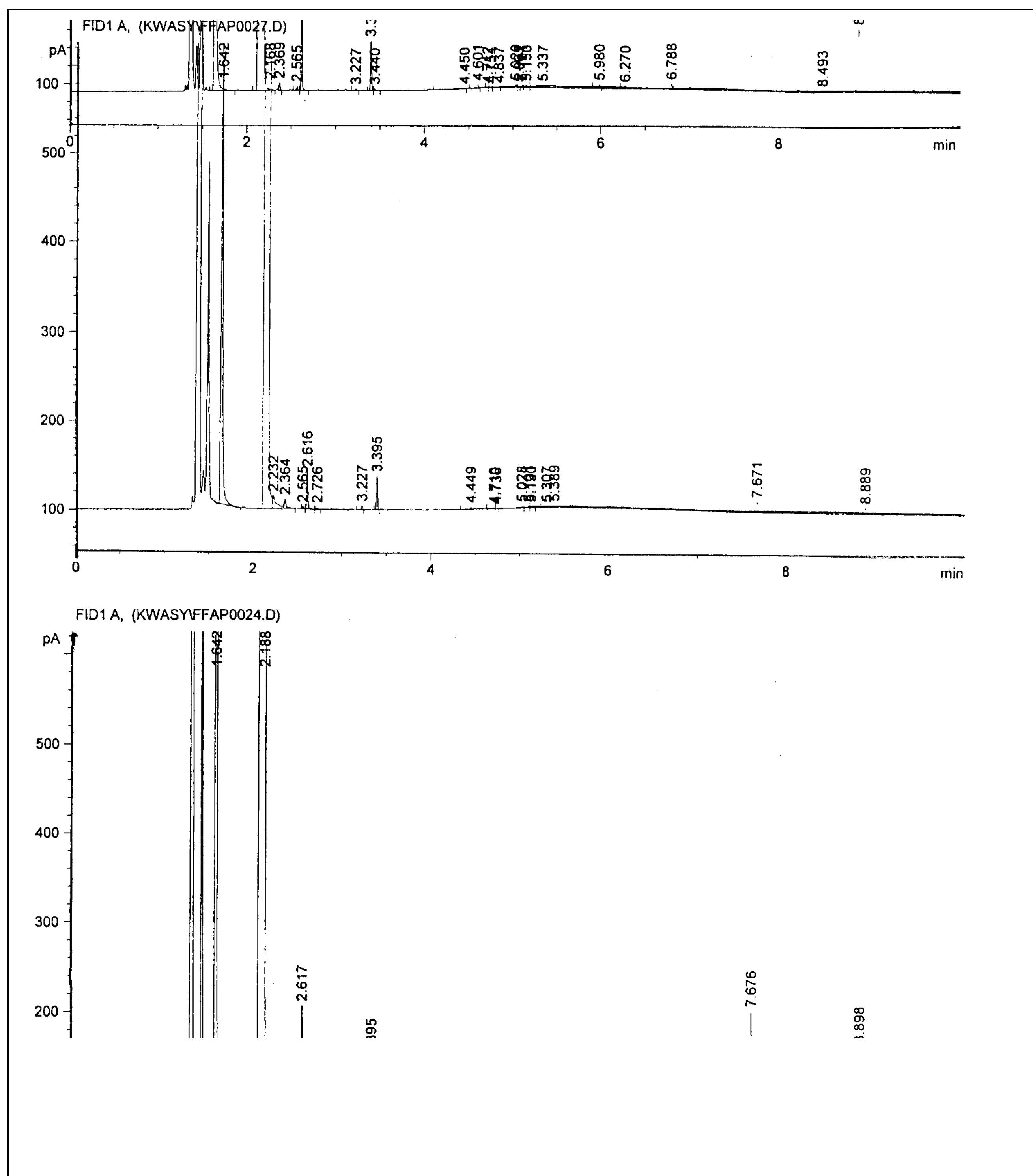

Fig. 5: GC of pure Dynasan 118 (top) and cetylpalmitate after extraction from the aqueous SLN mixture (bottom) analysed applying a temperature gradient

Table: Percentage of lipid (detected as methyl esters) including standard deviation at the day of production and after 12 and 24 months of storage at room temperature

	Age of lipid related to nominal content/stand. dev. (%)			Age lipid set to 100% at 0 months (i.e. normalised) (%)		
Lipid	0 m	12 m	24 m	0 m	12 m	24 m
Cetylpalmitate batch 1	92.03 ± 0.17	91.81 ± 0.16	89.48 ± 0.26	100.00	99.87	97.23
Cetylpalmitate batch 2	91.98 ± 0.16	91.82 ± 0.21	88.02 ± 0.22	100.00	99.83	95.70
Dynasan 118	98.97 ± 0.16	97.55 ± 0.18	96.02 ± 0.16	100.00	98.57	97.02
Softisan 154	97.64 ± 0.26	95.81 ± 0.10	86.84 ± 0.23	100.00	98.13	88.94

The % ages are given related to the nominal content (left part of table) and normalized, i.e. by setting the lipid content detected at 0 months to be 100% (explanation cf. Text).

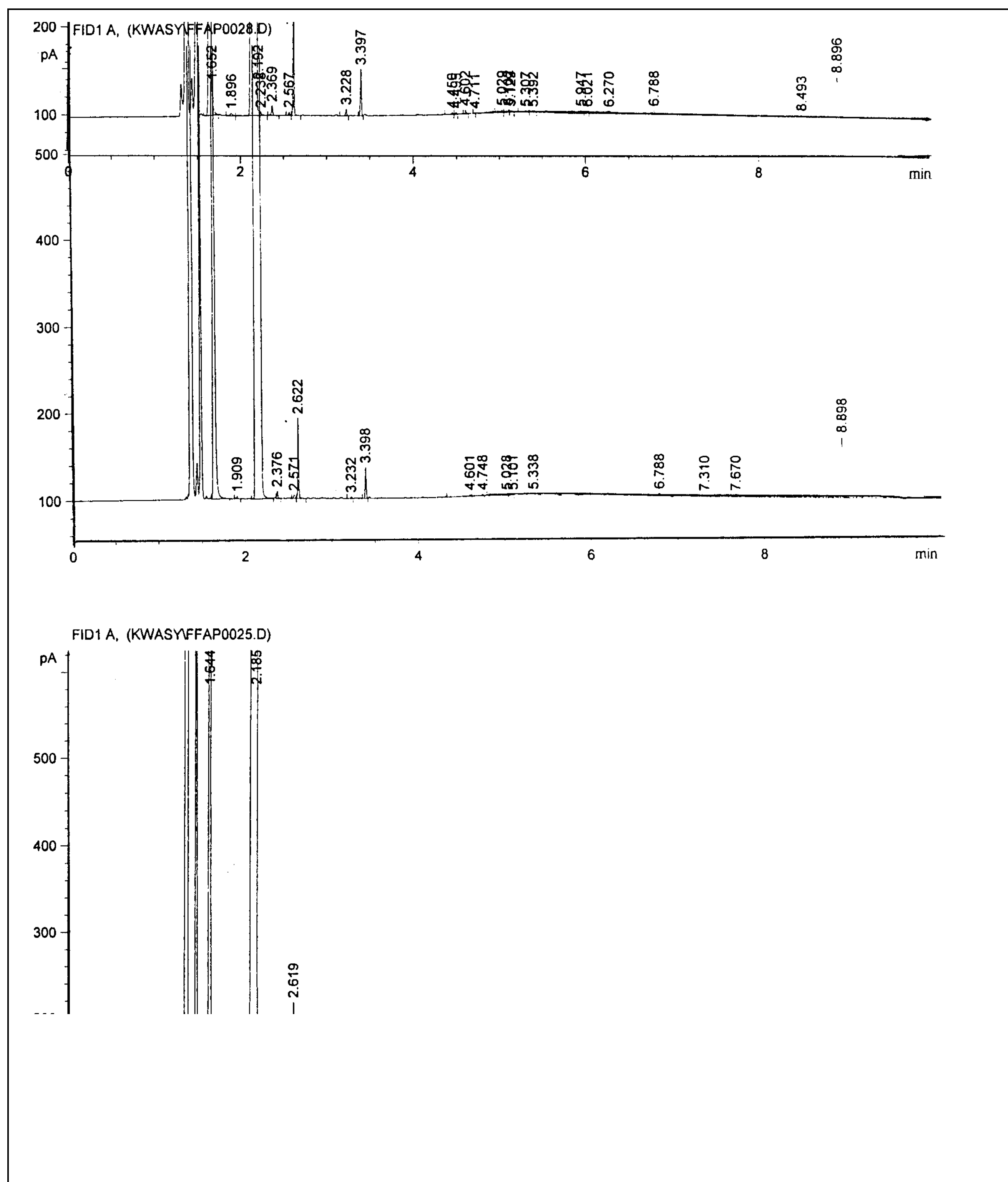

Fig. 6: GC of pure Softisan 154 (top) and cetylpalmitate after extraction from the aqueous SLN mixture (bottom) analysed applying a temperature gradient

75 °C. The obtained hot O/W nanoemulsion was cooled to room temperature and the lipid solidified forming SLN. About 40 g of SLN were produced for each formulation.

Three different SLN formulations were produced: A. 10% Dynasan 118 (glyceryl tristearate) + 1.2% Miranol (sodium cocoamphocetate); B. 10% cetylpalmitate + 1.2% Tego Care 450 (stearyl glucoside) and C. 10% Softisan 154 (glycerin ester) + 1.2% Miranol (sodium cocoamphocetate), each formulation contained water to 100%.

3.2. Particle size analysis

Particle size determination was performed by photon correlation spectroscopy (PCS) (N4, Coulter Electronics, Krefeld, Germany), measuring a range of 3 nm to 3 μm. Laser diffraction (MasterSizer, Malvern Instruments, Malvern, England) was applied in the size range of 100 nm to 80 μm.

3.3. Separation of SLN from the water phase

In order to obtain the lipid phase, the aqueous SLN dispersions were centrifuged at 17000 rotations/min for 90 min. Two phases were obtained, one top phase with lipids and a second lower phase with water containing the water dissolved surfactants.

3.4. Preparation of fatty acids methyl esters (FAMES)

The fatty acid composition of lipids is usually determined by GC of the corresponding methyl esters. The preparation of fatty acid methyl esters

(FAMES) by transmethylation of lipids were made according the method described by Garces and Mancha [16] and adopted to solid lipids. Samples of 50 mg of SLN lipid phase with methylating mixtures containing methanol/toluene/dimethoxypropan/H_2SO_4 (39 : 20 : 5 : 2, by volume) were placed in tubes with teflon caps. The final quantity of methylating liquid was 3.3 ml of the above mixture plus heptan added to a total volume of 5 ml which was added to the lipid samples. The tubes were placed in a water bath at 80 °C for 90 min. Vigorous shaking after 3 min heating was necessary to mix all the components into a single phase. After heating, the tubes were cooled at room temperature and shaken. Two phases formed, the upper one containing the fatty acid methyl esters.

3.5. Gas chromatography: separation of fatty acids

The fatty acid composition was determined by GC in a silica capillary column with a high polarity. The capillary column 25 m× 0.25 mm (Macherey-Nagel) was operated at constant temperature (96 °C) and alternatively applying a temperature gradient from 96 °C to 196 °C with 25 °C/ 2 min rate. The temperatures of the injector and the detector (FID) were 200 °C and 220 °C, respectively. The carrier gas was helium. GC analysis was performed with a HP 5890 chromatograph (Hewlett-Packard, USA).

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