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Short communication

Packed column supercritical fluid chromatography of sodium stearyl fumarate aqueous suspension

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Abstract

A method for the determination of sodium stearyl fumarate aqueous suspension is described. This straightforward method is based on homogenisation of the sample, dilution of a known aliquot with methanol to a suitable clear solution and mixing with an internal standard; (*S*)-naproxen. Separation and quantification is performed by packed column supercritical fluid chromatography on a commercial tartaric acid network polymeric column (*tert*butylbenzoyl) with UV-detection at 214 nm. The precision of the presented method upon repeated analysis of a 20 mg/ml suspension is 0.5% ($n = 8$), and the yield is near 100%. Less than 5 min is required for the chromatographic separation with a resolution of about 3 to the internal standard. With some modification of the chromatographic conditions water samples can also be analysed.

Keywords: Packed column supercritical fluid chromatography; Sodium stearyl fumarate; Pharmaceutical excipient; (*S*)-Naproxen

1. Introduction

Sodium stearyl fumarate is approved by the FDA for direct addition to food for human consumption as a conditioning or stabilizing agent in various bakery products, flour-thickened foods, dehydrated potatoes, and processed cereals. It is widely used in pharmaceutical processes as a lubricant in capsule and tablet formulations at 0.5–2.0% (w/w) concentration. The hydrophobicity is less than that of magnesium stearate or stearic acid, and it has a lower retardant effect on tablet dissolution than magnesium stearate [1–4].

For purity analysis of the substance we have developed a capillary GC-FID method based upon trimethylsilylation [5], which has been included in the European Pharmacopoeia after slight modifications [1]. A similar approach could perhaps be used for an aqueous suspension aimed for toxicological investigations. However, this would include both an acidification and extraction step followed by a derivatisation reaction. This should work, but the procedures required consume extra analytical laboratory skill and work and could be considered somewhat cumbersome even for a rather limited number of samples.

Reversed phase liquid chromatography is more suitable for less hydrophobic compounds than sodium stearyl fumarate and is consequently out of the question. Samples dissolved in water or methanol are not very compatible with traditional normal phase liquid chromatographic systems. However, we have found packed column SFC to be an interesting technique for the separation and determination of more or less lipophilic carboxylic acids [6,7]. The selectivity and retention behaviour is often similar to normal phase liquid chromatography systems but without the need to handle mobile phases based on volatile low molecular weight hydrocarbon solvents. For most basic and acidic analytes, it is common practise in SFC to have a basic or acidic additive in the mobile phase [8,9]. But with certain columns it is not necessary to have an acid additive present in the polar organic alcohol modifier [7,10,11]. This is partly due to the slightly acidic nature of the mobile phase bulk constituent carbon dioxide but also due to the well deactivated nature of the column such as Chiralpak AD or Chiralcel OD as well as those based on tartaric acid networks [7,12]. Another advantage of this is that detection can be performed at lower wavelengths and that system generated peaks are less prominent in the chromatograms. For an update of

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recent publications on SFC, the reader is referred to the latest bi-annual reviews by Chester and Pinkston published in Analytical Chemistry [13,14].

The aim of this study was to develop a method for the assay of sodium stearyl fumarate. The formulation was a stabilized suspension of the substance in water. The final method is based on homogenisation of the sample and dilution of an aliquot with methanol before mixing with an internal standard prior to SFC.

2. Experimental

2.1. Supercritical fluid chromatograph

The instrument was a Hewlett-Packard SFC Model G1205A (Little Falls Site, Wilmington, DE, USA). The samples were introduced using an auto-injector Berger Instrument ALS 3100 with a Valco valve (5 μl loop). Detection was accomplished at 214, 220, and 234 nm using a HP 1050 DA detector with a high-pressure flow cell. The instrument was controlled, and the chromatographic data were collected, by the Berger ChemStation version 3.3.6 software. Some experiments were performed with 20 and 50 μl loops.

Routine conditions were as follows. Flow-rate 1.5 ml/min of carbon dioxide with 15% of methanol at 30 °C with a back pressure of 150 bar.

2.2. Columns, reagents and chemicals

The enantioselective column investigated was a commercial Kromasil KR100-5CHI-TBB column (250 mm × 4.6 mm i.d.) from Eka Chemicals (Bohus, Sweden). The Chiralpak AD and Chiralcel OD columns were manufactured by Daicel (Tokyo, Japan) and were 250 mm × 4.6 mm i.d. The carbon dioxide used was grade 3.0 from AGA (Lidingö, Sweden) with a dipper tube in the 50 l cylinder. P.a. grade methanol, 2-propanol, citric acid monohydrate were obtained from E. Merck (Darmstadt, Germany). Sodium stearyl fumarate was obtained through AstraZeneca Bulk Productions Snäckviken (Södertälje, Sweden); manufactured by Moehs (Barcelona, Spain). The suspensions were prepared in-house with the aid of 0.1–0.5% (w/v) of Tween 80 and hydroxypropylcellulose 15000. (*S*)-Naproxen used as internal standard was from Sigma Chemical Co. (St. Louis, MO, USA).

3. Methods

The optimised method is as follows. The suspension of sodium stearyl fumarate in water is homogenised by stirring with a magnetic bar for 1 h (RCT basic, IKA Labortechnik, Staufen, Germany). Samples (1 ml) are withdrawn and weighed, diluted to 50 ml with methanol. Ultrasonication was often needed in order to get a clear solution. 1.00 ml of this dilution is mixed with 0.50 ml of a solution of the internal standard in methanol (typical 150 μg/ml) in an autosampler vial. Triplicate injections were normally done.

4. Results and discussion

4.1. Screening for a suitable column

From recent experience and results with lipophilic acids, preliminary experiments were performed with a Chiralpak AD column [7,11]. UV-detection was done at 214 nm since this wavelength was used for a pro-drug of naproxen [11]. Lower wavelengths did not improve the signal to noise ratio for stearyl fumarate to any significant extent down to 200 nm. Peaks were symmetric and column efficiency on Chiralpak AD was about 7000 at 25% of methanol at 30 °C. Retention with a flow-rate of 2 ml/min was typically about 5 min.

Much better column efficiency was obtained on a tartaric acid network column [12] with 15% of methanol and 1.5 ml/min total flow-rate. Plate numbers now were typically above 11,000 and the precision of the area ratio to the tentative internal standard (*S*)-naproxen after repeated injections was good. Over the period of this study, the column was in continuous heavy use. The plate number decreased some 10% but without significant effect on the quantitation performance. Reconditioning of the column will be discussed in a later section.

The tartaric acid TBB column was selected for the method since it was less retentive, with lower consumption of modifier, and gave decent precision for repeated injections at expected concentration levels of stearyl fumarate.

4.2. Selection of a suitable internal standard

Stearyl maleate was thought of as a possible internal standard, with closely related structure, in the quantification step. However, peaks were much more tailing on Chiralpak AD (longer retention) than for stearyl fumarate. Further, on the TBB column some asymmetry could be observed compared to the peak from stearyl fumarate though it now had shorter retention. The single enantiomer (*S*)-naproxen was investigated and gave a suitable peak immediately after stearyl fumarate in the chromatogram. It is contaminated with about 1% of its (*R*)-enantiomer which elutes just behind the (*S*)-enantiomer. The resolution is about 3, which gives room for speeding up the separation if needed.

4.3. Homogenisation of suspension and sample handling

The suspension of sodium stearyl fumarate sample is heterogeneous and upon standing a thick sediment of the substance is formed. Sodium stearyl fumarate is sparingly soluble in water at room temperature and practically insoluble in most organic solvents except methanol [2]. Suspension samples were fairly easily dissolved in this solvent though some ultrasonication was needed and stronger suspensions required more dilution in order to give clear solutions. Attempts with ethanol as dilution solvent were in vain.

Homogenisation of the suspension before sampling was investigated using stirring with a magnetic bar and a speed that was high but did not form froth on the top of the surface. Fig. 1 illustrates the yield as a function of time for this process. Sam-

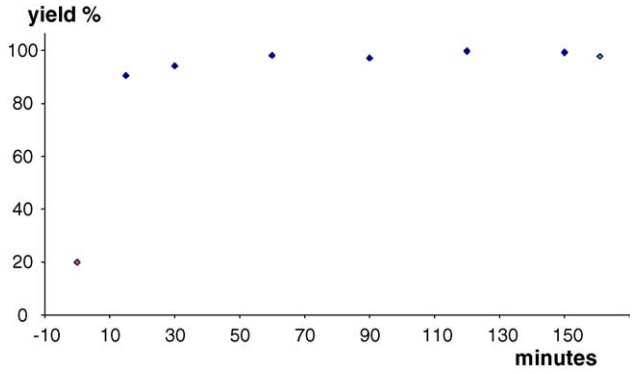

Fig. 1. Time course for the homogenisation of a 10 mg/ml sodium stearyl fumarate in water suspension. The first sample was taken before stirring was started and the last sample at 160 min was taken 10 min after the stirring had been stopped. For methods and details see Section 2. The yield was calculated with the aid of accurately weighed pure substance.

ples were withdrawn and weighed before dilution to volume with methanol followed by SFC analysis. After 60 min the yield is virtually constant. As shown from the first sample withdrawn, before the stirring was started, the supernatant has a low concentration of sodium stearyl fumarate; only some 20% of the full concentration. On the other hand, it is interesting to note that 10 min after stopping the bar the resulting value is only less than 2% lower than that of the average of the 120 and 150 min ones.

4.4. Calibration curve and linearity

A series of dilutions of sodium stearyl fumarate in methanol was prepared from two samples weighed of pure substance. A total of 12 samples were made in the range 640 down to 18 μg/ml plus blanks with and without internal standard in the vials. The graph of area ratio versus concentration was linear for this range with a regression coefficient of 0.9999. The slope was 0.0028 and the intercept calculated in the absence of blanks −0.0036. Representative chromatograms are given in Fig. 2 with the lowest level

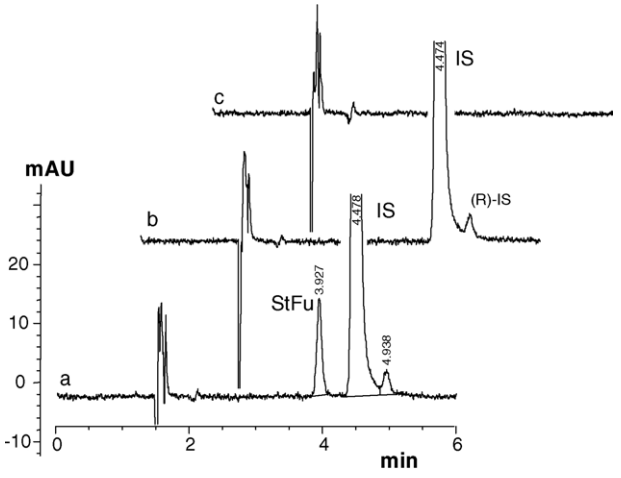

Fig. 2. Chromatograms from a standard curve: sodium stearyl fumarate 18 μg/ml and (*S*)-naproxen internal standard (a). Actual concentration of solution injected: 12 μg/ml. Blanks with internal standard (b) and pure methanol (c), respectively. UV-detection at 214 nm. Further details in Section 2.

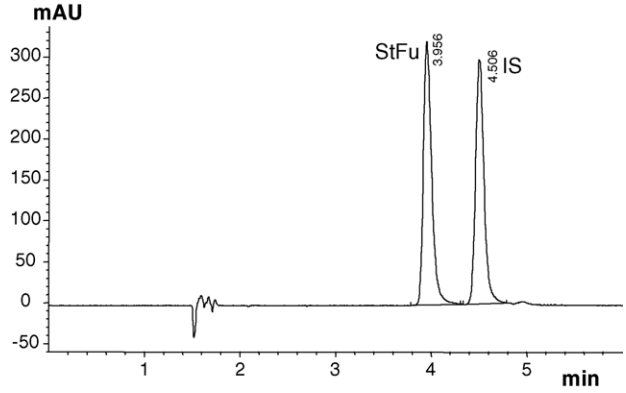

Fig. 3. Chromatogram from analysis of sodium stearyl fumarate aqueous suspension 20 mg/ml. Stearyl fumarate 266 μg/ml and (*S*)-naproxen internal standard 100 μg/ml. UV-detection at 214 nm. Further details in Section 2.

18.1 μg/ml of the standard curve plus the two blanks mentioned. One of them had the internal standard present. The small peak just after the internal standard is its (*R*)-enantiomer. Since for actual analytical work the expected concentration was known, calculation of the concentration of sodium stearyl fumarate was made by the aid of standard points near the expected concentration. These were prepared from at least two samples weighed and diluted followed by mixing volumes with the internal standard solution.

4.5. Precision of method upon repeated analysis and yield

The precision of the full method was evaluated after analysing eight samples from a 20 mg/ml suspension through the whole method. The R.S.D.% value obtained was 0.5. The absolute yield was better than 98% for a 20 mg/ml suspension. A representative chromatogram is shown in Fig. 3.

4.6. Future possibilities

In SFC with packed columns it is possible to inject samples dissolved in water when 2-propanol is used as modifier [15–18]. For acidic analytes it is necessary to have a small concentration of citric acid present in order to keep good column performance and peak shape since water appeared to activate the column [7]. Even with regular handling of Chiralpak AD columns, 2-propanol as modifier gives poor peak shape for profen acids compared to methanol as modifier [10,11]. Using 15% 2-propanol containing 1 mM of citric acid as polar modifier, the SFC system with the TBB column, water diluted samples of the suspension could be analysed. Further work was unattractive since pronounced system peaks surrounded the peaks. One advantage of the treatment of the TBB column with the 2-propanol/citric acid modifier was that the plate number improved, which had gradually decreased as mentioned above.

New experiments with the Ciralpak AD column and 2-propanol/citric acid were somewhat more successful. However, with Chiralcel OD it was possible to both minimize and separate system peaks using a relatively low concentration of modifier; 7.5%. The remaining problem is to prepare solutions of the

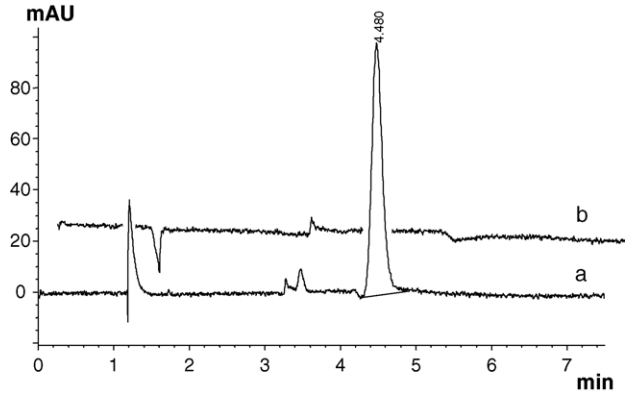

Fig. 4. Direct analysis of sodium stearyl fumarate aqueous solution 80 μg/ml with loop size 20 μl. Conditions: Chiralcel OD column with 2.5 ml/min of 7.5% 2-propanol containing 1 mM of citric acid in carbon dioxide at 30 °C. Sample identification: (a) solution containing 80 μg/ml in 50% water and methanol with 2-propanol and (b) neat water. Further details in Section 2.

sodium stearyl fumarate that do not precipitate before analysis. Thus, some alcohol was required in order to keep the analyte in solution.

Peak compression was also observed at certain higher concentrations of 2-propanol [17]. This beneficial effect could not be used since the system peaks could not be eliminated through shifting wavelength for detection. The detection limit could be improved by using larger injection loops. The system worked satisfactorily with both 20 and 50 μl loops though slight peak broadening and some tailing were observed with the latter. Fig. 4a and b show chromatograms obtained with the 20 μl loop. It is remarkable that so little disturbance is observed from the injection of water in this chromatographic system.

An interesting observation is that with 2-propanol stearyl fumarate is less retained than with methanol as modifier. One possible explanation is that comparatively less 2-propanol is adsorbed to the surface on the support.

5. Conclusions

A simple method for the determination of sodium stearyl fumarate suspensions in water has been developed. The method is based on dilution of the homogenised sample with methanol, and addition of an internal standard followed by packed column SFC. Each run takes about 5 min. The yield is near 100% and the precision upon repeated analysis of a 20 mg/ml suspension is 0.5% ($n = 8$). With minor modifications of the SFC conditions, water samples containing about 80 μg/ml can also be analysed.

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