1. Introduction

Pharmaceutical packaging, including glass, rubber, plastics, ceramics and metal, is an inescapable component of any drug [1]. It has been established that pharmaceutical packaging material is critical to the stability of a drug, because the packaging materials [2–4] can directly influence the clinical curative effect of the pharmaceuticals [5,6]. Furthermore, the more complex the packaging materials, the greater the risk to the patients, as there are more migrant compounds that may leach out of the packaging materials and into the drug during production and storage [7,8]. Therefore, the packaging-drug compatibility should be carefully evaluated by an extraction study and migration test to assess the effects on the quality and safety of the pharmaceuticals [9,10].

There have been many studies on packaging-drug compatibility [11–14]. It has been reported that the activity of heparin sodium was clearly decreased when it was put into a glass bottle for sodium chloride injection [15]. Turbidity also occurred for cefazolin sodium solution that was placed in a polyethylene plastic bottle [16]. Zhao [17] found that 2,6-di-tert-butyl-4-methylphenol (BHT) in the butyl stopper was the main factor that caused the issues with the clarity of ceftriaxone sodium, and Haverkamp et al. [18] identified clear variabilities in the property and amount of extractables among tubes from different vendors.

Rubber stopper is a type of medical packaging material with many superiorities, such as corrosion resistance, low moisture absorption rate, non-physiological toxicity, and excellent air tightness [19]. Lubricants, antioxidants, volatile additives, and vulcanizing agents are always added to rubber stoppers; hexadecanoic acid and stearic acid are often added as superior lubricants in the production of rubber stoppers. These components may leach into pharmaceuticals after long-term storage [20], which influences the stability and safety of pharmaceutical products. Therefore, the investigation of packaging-drug compatibility is a high priority. To evaluate the compatibility of the injection of recombinant antitumor-antivirus protein (RAAP) and the rubber stoppers, the contents of stearic acid and hexadecanoic acid in the rubber stoppers were first determined, and then a migration test was performed between the rubber stoppers and RAAP to assess the potential maximum risk of the injection.

The present investigation consisted of three parts: the extraction test of methyl hexadecanoate (MH) and methyl stearate (MS), the migration test for the two analytes, and the safety estimation of the injection. In this study, a simple, rapid, and sensitive gas chromatography-mass spectrometry (GC-MS) method with selective ion-monitoring (SIM) mode was developed and validated for the quantification of MH and MS owing to its high selectivity and sensitivity. Compared with other GC methods for the investigation
of packaging-drug compatibility [1], this method was time-efficient, simple, and convenient. The injection was stored for 3 months in accelerated aging conditions (25 °C ± 2 °C, RH 60% ± 5%), as recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines [21]. It was expected that the results of this work would provide a reference for the compatibility of rubber stoppers and injections after long-term storage to prevent unexpected toxicological problems.

2. Materials and methods

2.1. Chemicals and reagents

MH (99% purity) and MS (99% purity) were purchased from NuChek Prep. Inc (Minnesota, USA). Potassium hydroxide, sodium chloride, sodium sulfate and anhydrous analytical reagent were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shenyang, China). HPLC-grade n-heptane and methanol were purchased from Tianjin Kangkede Technology Co., Ltd. (Tianjin, China). Boron trifluoride-methanol solution for analytical reagent was obtained from Beijing Bailingwei Chemical Technology Co., Ltd. (Beijing, China). Super-pure water from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China) was used. The sample for extraction study was bromobutyl rubber (16111006) closures for injection. RAAP samples (lot numbers: 001, 002, 003, 004, 005, and 006) were used for the migration study.

2.2. Instruments and conditions

The analysis was performed by using an Agilent 6890N-5973 system equipped with an HP Hewlett 5973 autosampler and 5973 MS detector (Agilent Technology, USA). Chromatographic separation was performed on an HP-INNOWAX capillary column (30 m × 0.32 mm, 0.25 μm, Agilent Technology, USA) using helium as carrier gas at a constant flow rate of 25.1 mL/min. The oven temperature was initially held at 150 °C for 3 min. Subsequently, the temperature was increased to 210 °C at a heating rate of 50 °C/min and held for 4 min. Splitless mode was selected and 1 μL of the sample was injected. The temperatures of the ion source and injection port were held at 180 °C and 280 °C, respectively. Meanwhile, electron ionization was used for quantitative analytical detection, which was operated in SIM mode with the optimal target ion m/z 74.00 both for MH and MS.

2.3. Preparation of standard solution and calibration standards

Individual standard stock solutions of MH and MS were all prepared at 0.5 mg/mL in n-heptane. A mixed standard solution of the analytes was prepared by further dilution with n-heptane at the concentration of 0.05 mg/mL for MH and 0.1 mg/mL for MS. The mixed working solutions of MH and MS between 0.1 and 5 μg/mL and 0.2-10 μg/mL, respectively were prepared from the dilution of the mixed standard solution with n-heptane. All solutions were stored at 4 °C in a refrigerator before analysis.

2.4. Sample preparation

A powder sample of 0.1 g of rubber stoppers or 0.5 mL RAAP sample was transferred into a 50 mL conical flask, to which 2 mL potassium hydroxide methanol solution (0.5 mol/L) was added. The reflux process was conducted in a thermostatic water bath at 65 °C for 30 min. Subsequently, the sample was cooled to room temperature, and refluxed in a thermostatic water bath at 65 °C for 30 min, and 2 mL boron trifluoride-methanol solution (15%) was added. Then, the mixture was again cooled to room temperature and refluxed in a thermostatic water bath at 65 °C for 5 min after the addition of 4 mL n-heptane. Saturated sodium chloride solution (10 mL) was added to each sample to rinse; subsequently, the obtained solutions were well shaken and left to stand to separate. Finally, the supernatant liquid was transferred into another flask, desiccated by the addition of anhydrous sodium sulfate, and washed three times with 2 mL of super-pure water.

2.5. Method validation

The method was fully validated, including the selectivity, limits of detection (LOD) and quantification (LOQ), linearity, precision, recovery, and stability, with reference to the ICH guidelines for the validation of analytical methods. The selectivity of the methods was evaluated by the comparison of chromatograms of blank solvent with those of individual and mixed standard solutions of MH and MS, as well as sample solutions of rubber stoppers and RAAP that had been processed before analysis to confirm the absence of interfering peaks. The LOD and LOQ were determined by using the signal-to-noise (S/N) approach. LODs and LOQs were estimated for the concentration of each analyte that resulted in S/N ratios of 3:1 and 10:1, respectively. Linearity was verified in the concentration range of 0.1011–5.0570 μg/mL for MH and 0.2015–10.0740 μg/mL for MS. Precision was determined through the analysis of six identical standard solutions of MH and MS. Repeatability was verified through the analysis of six replicates of sample solutions of rubber stoppers and RAAP. The stability test was evaluated by the placement of the rubber stopper samples and injection samples, which were processed at room temperature for 23 h and sampled at the predetermined time points (0, 13, and 23 h) for analysis. The sample-added recoveries were determined through a comparison of the contents of MH and MS in samples at three concentrations (n = 3), which were spiked with the target analytes prior to extraction.

2.6. Migration studies

Packaging-drug compatibility is a major concern in the pharmaceutical industry and from a regulatory perspective; therefore, studies on the determination of migration from packaging materials into products are essential. In the present study, six batches of RAAP in penicillin bottles were stored for 3 months under accelerated aging conditions (25 °C ± 2 °C, RH 60% ± 5%) and migration studies were performed at the predetermined time points (0, 1, 2, and 3 months).

3. Results and discussion

3.1. Method development

3.1.1. Optimization of chromatographic and mass conditions

Linoleic acid, which is extremely common in rubber stoppers, was not detected in this study. The fatty acids of MH and MS were selected as the additive target analytes present in the rubber stoppers in this test. The chemical structures are shown in Fig. 1.

![Fig. 1. Chemical structures of methyl hexadecanoate (A) and methyl stearate (B).](image-url)
The optimal target ion of m/z 74.00 was selected for MH and MS. The ion mass spectra of MH and MS are shown in Fig. 2. GC-MS separation conditions were optimized through the comparison of inlet temperatures, injection mode, and programme temperatures. Among these factors, the inlet temperature and injection mode have few effects on the chromatographic resolution. Three injection temperatures (200 °C, 250 °C, and 280 °C) were tested during the optimization process. The results showed that peaks were completely distinct at all three inlet temperatures and the relative content of the major fatty acids was very similar. In general, inlet temperature is normally set to 50 °C above the boiling point of the analytes to ensure that all analytes are fully vaporized; therefore, 280 °C was selected as the final inlet temperature. Both split and splitless modes enabled complete separation. Studies have shown that the GC-MS programme temperature has a significant effect on the separation of fatty acids. In our preliminary experiment, heating rates of 20 °C/min and 50 °C/min were adopted to verify the effect on retention time and the shape of the chromatographic peak. Taking into account the column operating temperature range (40–260 °C), the maximum operating temperature was set to 210 °C to extend the life of the column. It has been shown that temperature increases to 210 °C at a heating rate of 50 °C/min offers advantages of high resolution and short analysis time. Under these conditions, volatile acid components could be completely separated with symmetrical peak shape.

3.1.2. Optimization of sample preparation

In general, the processing and determination of fatty acids are mostly performed on ester compounds. Therefore, fatty acids are usually derivatized to improve their stability prior to chromatographic analysis. Sample pretreatment strongly affects the accuracy, precision, and sensitivity of an analytical method; thus, the effects of reaction temperature and time on methyl ester yield were studied to improve the methyl esterification rate. The sample was pretreated in accordance with the extraction method of fatty acids in the Chinese Pharmacopoeia [22]. It was observed that a short methyl esterification time resulted in incomplete methyl esterification and that a low reaction temperature caused a lower methyl esterification rate. From these comprehensive optimization results, the optimal conditions for the derivatization of the target fatty acids were found to be an extraction performed by refluxing in a thermostatic water bath at 65 °C for more than 30 min. The
results showed that the polarity of the target fatty acid analytes decreased after methylation owing to the formation of hydrogen bonds. Improvements in the stability of analytes, a shorter analysis time and an expanded scope of analytical columns were found to result from the reduced polarity.

3.2. Method validation

The proposed method was validated in term of selectivity, LOD, LOQ, linearity, precision, reliability, stability and recovery.

3.2.1. Selectivity

The selectivity of the method was proved by the comparison of typical GC chromatograms of the blank solvent, individual solutions, and mixed reference standard solutions of MH and MS, and sample solutions of rubber stoppers and RAAP. The corresponding chromatograms are shown in Fig. 3. The retention time for MH and MS was 4.983 min and 6.159 min, respectively, which showed that the method had good selectivity and resolution.

3.2.2. LOD and LOQ

At an S/N ratio of 3, the LODs were 0.0030 and 0.0121 μg/mL for MH and MS, respectively. The LOQs for MH and MS were 0.0101 and 0.0403 μg/mL, respectively, with an S/N ratio of 10; these values were sufficient for this study.

3.2.3. Linearity

The calibration curves showed good linearity over the studied concentration range of 0.1011–5.0570 μg/mL for MH and 0.2015–10.0740 μg/mL for MS. The typical equations of the calibration curves

![Fig. 3. GC profiles of blank solvent (A), individual standard solutions of methyl stearate (B) and methyl hexadecanoate (C), and a mixed reference standard solution (D), as well as sample solutions of rubber stoppers (E), and recombinant antitumor-antivirus protein injection (F).](image-url)
are $y = 3.3 \times 10^3 x - 2.4 \times 10^4$ ($R^2 = 0.9994$) for MH and $y = 3.0 \times 10^3 x - 6.4 \times 10^4$ ($R^2 = 0.9991$) for MS, where $y$ and $x$ represent the peak area and the concentration of the analyte, respectively.

### 3.2.4. Precision of the instrument

The identical standard solutions of MH and MS were injected six times. The relative standard deviations (RSDs) of the retention time for MH and MS was 0.01% and 0.01% and the RSDs of the peak areas for MH and MS were 1.02% and 2.10%, respectively. The precision assay results were all within the acceptable range.

### 3.2.5. Repeatability

The RSDs of the peak area for MH and MS in six replicate analyses of rubber stopper samples were 4.13% and 0.98%, respectively, which confirmed the reproducibility and reliability of the method.

### 3.2.6. Stability

The stability of this method was evaluated by computation of the RSD of peak area for MH and MS stored at room temperature for 13 and 23 h. The RSDs for 23 h were 2.34% and 2.24% for MH and MS, respectively, and the two migrants were not detected in the injection. The results showed that both MH and MS had an excellent stability over 23 h.

### 3.2.7. Sample-added recovery

The percentage recoveries were measured by the standard-addition method. Nine replicate samples of rubber stoppers (0.1 g each) and RAAP (0.5 mL each) were analyzed to determine the recoveries, to which three masses of MH (0.16, 0.20, and 0.24 mg) and MS (0.32, 0.40, and 0.48 mg) were added, respectively. The sample-added recoveries for MH and MS in samples are summarized in Table 1 and Table 2. The average recoveries of MH and MS in rubber stoppers were 109.09% and 111.30%, respectively, and the mean recoveries of MH and MS in RAAP were 103.14% and 101.50%, respectively, which indicated that this method was accurate and reliable.

### 3.3. Determination of migrates

The developed and validated method was successfully applied to determine MH and MS in RAAP. In the present study, samples from six batches of RAAP (lot number: 001, 002, 003, 004, 005 and

| Compound          | $m_{\text{sample}}$ (mg) | $m_{\text{added}}$ (mg) | $m_{\text{founded}}$ (mg) | Recovery (%) | Average recovery (%) | RSD (%) |
|-------------------|---------------------------|--------------------------|---------------------------|--------------|----------------------|---------|
| Methyl hexadecanoate | 0.114                     | 0.162                    | 0.282                     | 103.54       | 109.09               | 4.08    |
|                   | 0.115                     | 0.162                    | 0.281                     | 102.92       |                      |         |
|                   | 0.114                     | 0.162                    | 0.283                     | 104.95       |                      |         |
|                   | 0.114                     | 0.202                    | 0.335                     | 109.27       |                      |         |
|                   | 0.115                     | 0.202                    | 0.335                     | 108.91       |                      |         |
|                   | 0.112                     | 0.202                    | 0.335                     | 110.42       |                      |         |
|                   | 0.114                     | 0.243                    | 0.390                     | 113.61       |                      |         |
|                   | 0.114                     | 0.243                    | 0.391                     | 114.24       |                      |         |
| Methyl stearate   | 0.192                     | 0.326                    | 0.540                     | 106.29       | 111.30               | 4.16    |
|                   | 0.193                     | 0.326                    | 0.536                     | 104.74       |                      |         |
|                   | 0.191                     | 0.326                    | 0.539                     | 106.25       |                      |         |
|                   | 0.192                     | 0.407                    | 0.650                     | 112.14       |                      |         |
|                   | 0.193                     | 0.408                    | 0.647                     | 110.92       |                      |         |
|                   | 0.188                     | 0.407                    | 0.647                     | 112.45       |                      |         |
|                   | 0.192                     | 0.488                    | 0.763                     | 116.63       |                      |         |
|                   | 0.191                     | 0.488                    | 0.763                     | 116.69       |                      |         |

### Table 2

Sample-added recoveries of methyl hexadecanoate and methyl stearate from the injections of recombinant antitumor-antivirus protein.

| Compound          | $m_{\text{sample}}$ (mg) | $m_{\text{added}}$ (mg) | $m_{\text{founded}}$ (mg) | Recovery (%) | Average recovery (%) | RSD (%) |
|-------------------|---------------------------|--------------------------|---------------------------|--------------|----------------------|---------|
| Methyl hexadecanoate | 0                         | 0.162                    | 0.164                     | 101.02       | 103.14               | 1.41    |
|                   | 0.162                     | 0.166                    | 102.92                    |              |                      |         |
|                   | 0.162                     | 0.165                    | 102.16                    |              |                      |         |
|                   | 0.202                     | 0.206                    | 102.00                    |              |                      |         |
|                   | 0.202                     | 0.207                    | 102.65                    |              |                      |         |
|                   | 0.202                     | 0.208                    | 102.91                    |              |                      |         |
|                   | 0.243                     | 0.254                    | 104.69                    |              |                      |         |
|                   | 0.242                     | 0.253                    | 104.34                    |              |                      |         |
|                   | 0.243                     | 0.256                    | 105.59                    |              |                      |         |
| Methyl stearate   | 0                         | 0.326                    | 0.318                     | 97.59        | 101.50               | 2.37    |
|                   | 0.326                     | 0.324                    | 99.48                     |              |                      |         |
|                   | 0.326                     | 0.323                    | 99.26                     |              |                      |         |
|                   | 0.407                     | 0.410                    | 100.80                    |              |                      |         |
|                   | 0.408                     | 0.413                    | 101.41                    |              |                      |         |
|                   | 0.407                     | 0.418                    | 102.72                    |              |                      |         |
|                   | 0.488                     | 0.507                    | 103.82                    |              |                      |         |
|                   | 0.489                     | 0.508                    | 103.84                    |              |                      |         |
|                   | 0.488                     | 0.510                    | 104.54                    |              |                      |         |
006) were used for migration research after storage for 3 months under accelerated aging conditions. At the predetermined time points (0, 1, 2 and 3 months), 1 μL of the solution was subjected to the GC-MS system for analysis. The results showed that neither MH nor MS was detected in the samples from the six batches stored under accelerated conditions, indicating that the rubber stoppers in direct contact with the contained product did not influence the quality of RAAP.

3.4. Safety assessment

Although the result showed that neither MH nor MS was detected in the samples from six batches of RAAP, the potential concentration of migrants from the injection could be predicted from a theoretical perspective. The concentrations of MH and MS were 0.0030 μg/mL and 0.0121 μg/mL, respectively, on the basis of the LOD. From a report by the International Uniform Chemical Information Database (IUCID), the no-observed-effect level (NOEL) values were 5000 mg/kg/day (rat, oral, 150 days) and 5000 mg/kg/day (rabbit, oral, short-term) for MH and MS, respectively [23,24]. The permitted daily exposure (PDE) for humans is calculable from the following equation [25]:

\[
PDE = \frac{\text{NOEL} \times W}{(F_1 \times F_2 \times F_3 \times F_4)}
\]

where \(W\) represents the weight of an adult (50 kg); \(F_1\) is a factor to account for extrapolation between species, and \(F_1\) values were 5 and 2.5 for MH and MS, respectively; \(F_2\) is the variability between individuals (\(F_2 = 10\)); \(F_3\) is a factor to account for the short-term toxicity studies, with \(F_3\) values of 5 and 10 for MH and MS, respectively; \(F_4\) is a factor that may be applied in cases of severe toxicity (as severe toxicological information has not been reported to date, \(F_4 = 1\)); and \(F_5\) is a variable factor that may be applied if the NOEL is not established (the potential migrants were absent in the present study, \(F_5 = 1\)). \(F\) represents the conversion factor of the oral dosage form and injection (\(F = 100\)). Therefore, the PDE values for MH and MS were calculated from the following equations:

\[
PDE(MH) = \frac{5000 \times 5 \times 10 \times 5 \times 1 \times 1 \times 100}{5 \times 10 \times 5 \times 1 \times 100} = 10 \text{ mg/day}
\]

\[
PDE(MS) = \frac{5000 \times 2.5 \times 10 \times 10 \times 1 \times 1 \times 100}{2.5 \times 10 \times 10 \times 1 \times 1 \times 100} = 10 \text{ mg/day}
\]

Clinical data showed that the maximum clinical dosage of the RAAP was 1 mL. The possible maximum daily intakes were therefore 3.0 mg and 12.1 mg for MH and MS, respectively, based on the LODs. In the present study, the concentrations of the two migrants were both lower than the PDE, which indicated that the migrants were not considered to present a safety risk and did not influence the quality of the injection.

4. Conclusion

In the present study, a simple, rapid and sensitive GC-MS method for the quantification of the possible migrants in rubber stoppers has been developed and validated. This method was successfully applied for the study of packaging-drug compatibility and the safety estimation of the injection. The results of the safety estimation showed that the possible maximum daily intake for MH and MS was 3.0 mg and 12.1 mg, respectively; these values were both lower than the PDE, which indicated that the migrants were considered not to present a safety risk and did not influence the quality of the injection. The results in this study may offer a theoretical paradigm for the compatibility between rubber stoppers and RAAP and the long-term storage of the injection. Nevertheless, packaging-drug compatibility between the rubber stoppers and the injection should be thoroughly evaluated further in order to ensure quality and safety of pharmaceuticals.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

[1] L.G. Xie, X. Zhao, F. Wang, et al., Determination of fatty acids in rubber stoppers, Plast. Sci. Technol. 42 (2014) 102–104.
[2] K. Boven, S. Stryker, J. Knight, et al., The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes, Kidney Int. 67 (2010) 2346–2353.
[3] B. Sharma, F. Rader, T. Templeman, et al., Technical investigation into the cause of the increased incidence of anti-body-mediated pure red cell aplasia associated with Eprex®, Eur. J. Hosp. Pharm. 5 (2004) 86–91.
[4] D. Jenke, Evaluation of the chemical compatibility of plastic contact materials and pharmaceutical products; safety considerations related to extractables and leachables, J. Pharm. Sci. 95 (2006) 2566–2581.
[5] J. Feng, X.Y. Cai, Y. Liu, et al., Determination of antioxidant-BHT and vulcanizing agent extractable sulphur in medicinal butyl rubber, Chin. J. Pharm. Anal. 37 (2017) 702–706.
[6] B.M. Xiao, S.K. Gozln, L. Herz, et al., Development and validation of HPLC methods for the determination of potential extractables from elastomeric stoppers in the presence of a complex surfactant vehicle used in the preparation of parenteral drug products, J. Pharm. Biomed. Anal. 43 (2007) 558–565.
[7] F. Zhang, A. Chang, K. Karaiz, et al., Structural identification of extractables from rubber closures used for pre-filled semisolid drug applicator by chromatography, mass spectrometry, and organic synthesis, J. Pharm. Biomed. Anal. 34 (2004) 841–849.
[8] C.L. Stults, J.M. Ansell, A.J. Shaw, et al., Evaluation of extractables in processed and unprocessed polymer materials used for pharmaceutical applications, AAPS PharmSciTech 16 (2015) 150–164.
[9] Y.N. Ma, L. Ma, Y. Jiang, et al., Interpretation of the guideline of compatibility study of pharmaceutical products and packaging materials the assessment of experimental data, Chin. J. New Drugs 28 (2014) 940–943.
[10] Q.J. Qian, X.J. Zhao, Q.H. Ma, et al., Study on compatibility in between packaging materials and Haemophilus influenzae type b conjugate vaccine, Prog. Microbiol. Immunol. 40 (2012) 42–46.
[11] C.C. Corredor, T.A. Haby, J.D. Young, et al., Comprehensive determination of extractables from five different brands of stoppers used for injectable products, J. Pharm. Biomed. Anal. 58 (2012) 327–336.
[12] X.C. Pu, R. Chen, Y.J. You, et al., HPLC determination for migration of antioxidant 1076 from rubber stopper to micafungin sodium injection, Chin. J. Pharm. Anal. 37 (2017) 1298–1303.
[13] X.E. Pan, Y.F. Zhang, C.H. Zhang, et al., Evaluating compatibility between propofol injection and neutral borosilicate glass ampoule, China Med. 43 (2017) 45–48.
[14] G.X. Zhang, Compatibility Studies on Recombinant Human Erythropoietin Injection with Prefillable Syringes, South China University of Technology, Guangzhou, 2016.
[15] J.H. Zhang, The application of no-PVC coextruded film in the medical infusion packs, China Packag. Ind. 2 (2002) 23–24.
[16] X. Zhao, C.Q. Hu, S.H. Jin, et al., The analysis of volatile substances of butyl rubber closures, Chin. J. Pharm. Anal. 26 (2006) 315–318.
[17] X. Zhao, Study on the Compatibility between Butyl Rubber Clousures and Ceftriaxone Sodium, Chinese Peking Union Medical College, Beijing, 2006.
[18] J.B. Haverkamp, U. Lipke, T. Zapf, et al., Contamination of semi-solid drug applicator by chromatography, mass spectrometry, and organic synthesis, J. Pharm. Biomed. Anal. 34 (2004) 841–849.
[19] Z. Meng et al. / Journal of Pharmaceutical Analysis 9 (2019) 178–184
[23] G.A. Burdock, I.G. Carabin, Safety assessment of myristic acid as a food ingredient, Food Chem. Toxicol. 45 (2007) 517–529.

[24] S. O’Hagan, A. Menzel, A subchronic 90-day oral rat toxicity study and in vitro genotoxicity studies with a conjugated linoleic acid product, Food Chem. Toxicol. 41 (2003) 1749–1760.

[25] US Department of Health and Human Services Food and Drug Administration. Guidance for industry: Q3C Impurities: Residual Solvents, 1997. (https://www.fda.gov/downloads/drugs/guidanceregulatoryinformation/guidances/ucm073394.pdf).