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Development and Validation of Gas Chromatography-Mass Spectrometric Method for the Determination of Epoxidized Soybean Oil in Foods Stored in Glass Jars with Metal Lids

(Pembangunan dan Validasi Kaedah Kromatografi Gas-Spektrometri Jisim bagi Penentuan Minyak Kacang Soya Terepoksi dalam Makanan yang Disimpan di dalam Balang Kaca Berpenutup Logam)

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ABSTRACT

A method has been developed for the determination of epoxidized soybean oil (ESBO) in oily foods stored in glass jars with metal lids using gas chromatography-mass spectrometry (GC-MS). ESBO and its internal standard (cis,cis-11,12;14,15-diepoxyeicosanoate) were isolated from the matrix by transesterification process. The developed method showed good linear dynamic range between 0.7-20 µg mL^{-1} with coefficients of determination (R^2) > 0.9968 and acceptable limit of detection and limit of quantitation of 7 and 23 mg kg^{-1}, respectively, based on linearity calculations. Analyte recoveries were 90.84±27.24% for low concentration, 78.05±11.59% for medium concentration and 99.23±10.20% for high concentration. This first fully validated GC-MS method was successfully applied for the determination of ESBO in foods stored in glass jar with metal lid. Among the 31 food samples studied, 6 samples were found to exceed the specific migration limit of 60 mg kg^{-1} (based on EU Directive 2002/72/EC). The developed method is thus potentially useful for routine analysis for the determination of ESBO.

Keywords: Epoxidized soybean oil; foods stored in glass jars with metal lids; gas chromatography-mass spectrometry

ABSTRAK

Satu kaedah telah dibangunkan untuk menguji minyak kacang soya terepoksi (ESBO) dalam makanan berminyak di dalam balang kaca berpenutup logam menggunakan kromatografi gas-spektrometri jisim (GC-MS). ESBO dan piawai dalaman (cis,cis-11,12;14,15-diepoxyeicosanoate) telah dipisahkan daripada matrik menggunakan proses transesterifikasi. Kaedah yang dibangunkan ini menunjukkan dinamik linear yang baik antara 0.7-20 µg mL^{-1} dengan pekali penentuan (R^2) > 0.9968 dan had pengesanan serta had kuantifikasi yang boleh diterima iaitu 7 dan 23 mg kg^{-1}, masing-masing berdasarkan kepada pengiraan lineariti. Peratus pengembalian analit ialah 90.84±27.24%, bagi kepekatan rendah, 78.05±11.59% bagi kepekatan sederhana dan 99.23±10.20% bagi kepekatan tinggi. Kaedah GC-MS yang telah divalidasi sepenuhnya buat pertama kali telah berjaya diaplikasikan untuk penentuan ESBO dalam makanan yang disimpan di dalam balang kaca berpenutup logam. Daripada 31 sampel yang telah dikaji, 6 sampel didapati melebihi had 60 mg kg^{-1} mengikut undang-undang Eropah (EU Directive 2002/72/EC). Oleh itu, kaedah ini berpotensi untuk digunakan sebagai analisis rutin untuk penentuan ESBO.

Kata kunci: Kromatografi gas-spektrometri jisim; makanan disimpan di dalam balang kaca berpenutup logam; minyak kacang soya epoksi

INTRODUCTION

Plasticizers are widely used in the polymer industry due to its ability to change mechanical properties of plastics. Epoxidized soybean oil (ESBO) and other epoxidized vegetable and seed oils are primarily used in polyvinyl chloride (PVC) because they are highly effective in co-stabilizing PVC. The main functions of ESBO in PVC are to act as a thermal stabilizer, lubricant and also as a plasticizer. Percentage of ESBO in PVC can range between 0.1 and 27% (Castle et al. 1988a). It is used particularly in closure gaskets for the metal lids used to seal glass jars and bottles, forming the airtight seal needed to prevent microbiological contamination of foods. There is thus a potential for migration into food during both sterilization and storage.

Metal lids for glass containers come with a gasket sealing against the rim of the jar. A lid contains about 1 g of gasket material, of which typically 250 – 350 mg is in direct contact with food, i.e. on the food-oriented side of the seal (Fankhauser-Noti & Grob 2006). As the PVC usually contains 35 – 45% plasticizer, mostly more than 100 mg plasticizer is exposed to the food. A small amount of free oil (typically on top of the food) is sufficient to extract the plasticizers from the gasket to an extent approaching

completeness after a long storage time. However, there is negligible migration if the food is purely aqueous, like marmalade or pickles, or if the food is sufficiently firm not to get into contact with the lid (mustard) (Biedermann et al. 2008). ESBO became an issue when the migration into food far exceeded the legal limit of Europe as shown by Rapid Alert System of Food and Feed Week 2005/22 (RASFF 2005), Week 2007/40 (RASFF 2007) and Swiss June 2005 market survey (Fankhauser-Noti et al. 2006). In the Swiss survey, it was found that the highest violation could be up to 20-fold of European Union (EU) legal limit.

Several methods have been developed to determine ESBO in various food samples. A classic method have been developed by Castle et al. (1988b) and became a role model to almost all subsequent researchers. This method is based on the transmethylation of the triglycerides by sodium methoxide and derivatization of the fatty acid methyl esters (FAMEs) using boron trifluoridedietherate to form 1,3-dioxolanes. The target molecule is the diepoxy linoleic acid, which after the derivatization showed two well-separated peaks with almost identical mass spectra, as a result of the formation of two stereoisomers. The internal standard, cis,cis-11,14-diepoxyeicosanoate ethyl ester shows the same behavior. ESBO is quantified through the derivatives of the epoxidized linoleic acid by referring to the derivatives of the internal standard. Although the method is sensitive and specific, the preparation of the internal standards are tedious and the two-stage derivatization and double evaporation of sample to dryness made the method unattractive (Han & Szajer 1994). Furthermore, the method is also tedious for routine analysis of high number of samples because a method without a cleanup step, put a lot of strain on instrument and resulted in extensive downtimes caused by the need for cleaning (Ezerskis et al. 2007).

On the other hand, Fankhauser-Noti et al. (2005) analyzed ESBO migration from the gaskets of lids into food packed in glass jars using on-line liquid chromatography (LC)-gas chromatography (GC). The diepoxy components were isolated by normal-phase LC and then transferred on-line to GC with flame ionization detector (FID) using the on-column interface in the concurrent solvent evaporation mode. On-line LC-GC offers several advantages (Grob 2000) including sample clean up, sensitive and high reproducibility. Further details regarding the online LC-GC techniques were described by Grob and Schilling (1985). They reported a detection limit of 2 – 5 mg kg^{-1}, depending on food. Although this method is good for analyzing a large number of samples and for routine analysis, it was not used in this research due to lab capabilities constraint. The author prepared the internal standard, diepoxy methyl eicosanoate, according to Castle et al. (1988b). This is rather cumbersome as synthesizing internal standards is a lengthy process.

Weitzel et al. (2007) developed a miniaturized method based on, again, method devised by Castle et al. (1988b). The extraction method was basically similar to Castle et al. (1988b), but they added an additional cleanup process using gel permeation chromatography (GPC). The purpose of adding a GPC process was to reduce the strain on the analytical equipment as to make the method suitable for frequent routine usage. Since this method involved 2-steps derivatization, it was time consuming and tedious.

The method described by Biedermann-Brem et al. (2007) has a number of advantages. The sample preparation is simple where ESBO is transesterified with sodium methoxide directly in homogenated food without prior extraction. This is unlike methods proposed by other researchers where fats in foods need to be extracted first before performing transesterification. Secondly, instruments (GC-FID and GC-MS) used by the authors are common to analytical laboratories. This method employs direct transesterification in food. For analysis on a GC-FID, it requires a long column (100 m) to separate a complex food matrix. Polar column must be used because the difference in polarity is exploited to separate the methyl diepoxylinoleate (the analyte of interest) away from the bulk of the FAMEs. Using a 100 m column, this method reported a total retention time of 78 min. Overall, the method is simple and easy to be carried out although long analysis time has to be tolerated.

Suman et al. (2005) had analyzed ESBO using LC-electron ionization (EI)-Tandem mass spectrometer (MS). The major advantage of using LC as compared to GC is that the extraction step was extremely simple and fast. ESBO was extracted with dichloromethane and the extract was injected into LC without further cleanup. Separation was done using two C_{18} columns with an aqueous acetic acid-acetone-acetonitrile mixture as the mobile phase under gradient conditions. Limit of detection (LOD) was reported as 4 mg kg^{-1} with recoveries > 90%.

The aim of this research was to develop a new method based on transesterification followed by extraction and gas chromatography for the determination of residual quantity of ESBO in oily food migrated from gasket of metal lids. The developed method proved to be fast and simple as the transesterification was done directly in the food sample without the need of prior extraction.

Experimental Details

Materials

The standard used in this study was epoxidized soybean oil from Fluka (Steinheim, Germany). Cis,cis-11,12;14,15-diepoxyeicosanoate from Fluka (Steinheim, Germany) was used as the internal standard. Other chemicals were 1,4-dioxane, sodium methoxide 25%, methanol anhydrous, hexane and disodium hydrogen citrate. All the chemicals were purchased from Sigma-Aldrich (Steinheim, Germany).

Instrumentations

Agilent Technologies 6890N GC (Santa Clara, California, USA) was used in this study. It was coupled with a 5975B

inert XL EI/CI mass spectrometer (Santa Clara, California, USA) and 7683 autosampler and autoinjector. The separation was performed on-column into a 30 m × 0.25 mm i.d. column coated with a 0.20 μm film of HP-88 and equipped with a 1 m × 0.53 mm i.d. uncoated, deactivated pre-column. The column flow (helium) was set at 1.2 mL min^{-1}. The oven temperature was programmed at 110°C (held for 1 min) and then programmed at 20°C min^{-1} to 200°C and at 8°C min^{-1} to 260°C (held for 2 min). The ion source was set at maximum temperature of 300°C to reduce contaminations and increase uptime.

METHODS

The method used was based on method by Biedermann-Brem et al. (2007). Samples were homogenized and mixed thoroughly before analysis. The upper layer of samples usually contained more ESBO and plasticizers than other part of samples as the upper layer is the nearest to the cap. After homogenization, samples were weighed into a 50 mL Erlenmeyer flask. Different sample weights were used depending on the oil percentage of the samples. One hundred mg for oil samples, 200 for sauces (moderately oily) and 500 mg for infant foods that are less oily.

Sample, dioxane (4 mL) and internal standard (methyl cis,cis-11,12;14,15-diepoxyeicosanoate) were mixed together by shaking the Erlenmeyer flask on a vortex for a short while (~ 5-10 s). Methanol 5% was added to start the transesterification. This process took about 1 – 1.5 min to complete. After that, 10 mL of hexane was added to extract out the fatty acid methyl esters (FAMEs) followed by 10 mL of disodium hydrogen citrate 15% to stop the reaction and separated into two phases. The upper phase (hexane layer) was carefully pipetted into a vial and 1 μL was injected into GC-MS. The hexane extract was preconcentrated up to 5 times when low ESBO concentrations were analyzed.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

A GC/MS method was developed for the determination of ESBO in foods with metal lid glass jars. A shorter column (30 m) was used for GC-MS as MS is very specific and the resolution power of a long column is not really necessary. The column flow was set at 1.2 mL min^{-1} (the optimum flow rate for MS applications) at constant flow mode. Ion source was set at 180ºC.

The oven was ramped at 20ºC min^{-1} for the first 6 min because the early eluting FAMEs were not the peaks of interest in this study. The oven ramp was slowed down from the sixth minute onwards to allow better separation of the peaks of interests (18:2E and 20:2E). The final ramp was only necessary for oil samples. It was found that if oil samples (olive oil) were analyzed, carry-over were observed if the run-time was only 15 min. Thus, it was further extended for another 2.50 min to burn out the remaining long chain of FAMEs. For normal samples, 15 min of run time proved to be sufficient. MS interface temperature was set at 250ºC. Ideally, the temperature should be about 20ºC higher than final oven temperature to prevent analyte loss at cold spots, but since the maximum allowable temperature for the column is only 250ºC (isothermal), it was set similar to the final oven temperature which is also the maximum temperature for the column to prevent column bleeding.

Solvent delay was set at 3 min. Although scan mode can be used, selected ion monitoring (SIM) mode was proven to be more sensitive. Two groups of ions were monitored. Each group consisted of two analytes. The first group was methyl diepoxylinoleate isomers. The target ion was m/z 155, qualifiers m/z 83, 109 and 187. Even though both analytes only differ in terms of geometric configuration, both analytes shared the same target as well as qualifier ions. The second group of ions monitored was the internal standard. The target ion was at m/z 183. Qualifiers were m/z 55, 69 and 81. Dwell time for each ion was at 80 m s. Low resolution was selected as it was more sensitive. High resolution is only for quantitation of ion at 0.5 to 1 amu apart.

Quantification of ESBO was based on peak area ratio of ESBO standard with internal standard. The fatty acid composition of ESBO consists of 16:0, 18:0, 18:1, 18:2, $18:2E_1$ and $18:2E_2$. Since fatty acids such as 18:0, 18:1 and 18:2 are common to oils, only methyl diepoxylinoleate, 18:2E, is used for quantification of ESBO. Both methyl diepoxylinoleate and internal standard (diepoxy methyl eicosanoate, 20:2E) showed 2 peaks. The chromatograms and mass spectrum of diepoxylinoleate is shown in Figure 1. The main ion was m/z 155. Both $18:2E_1$ and $18:2E_2$ produced the same mass spectrum as they are diastereoisomers. Internal standards showed the intense peak at m/z 55. Nevertheless, m/z 183 was chosen as target ion as it is more selective. Both $20:2E_1$ and $20:2E_2$ also produced similar mass spectrum due to similar reason.

METHOD PERFORMANCE

The following criteria were used to evaluate the GC-MS method according to Resolution Oeno 10/2005 (2005), Analytical Laboratory Accreditation Criteria Committee (ALACC) Guide on method verification (2007) and IUPAC Guide (2002) guidelines: specificity, limit of detection (LOD), limit of quantification (LOQ), linearity and trueness.

SPECIFICITY

The specificity of a method is the ability to measure the analytes of interest in a sample. Specificity was determined by analyzing a standard mixture of ESBO in ten different matrices including mayonnaise, ketchup, pasta sauce and cooking sauce. All these samples are commonly consumed and stored in glass jars with metal lid. Each sample was spiked with one selected concentration within working range. Analysis of spiked and unspiked samples of each sample was performed. Figure 2 shows the graph of recovered concentration versus the added concentration

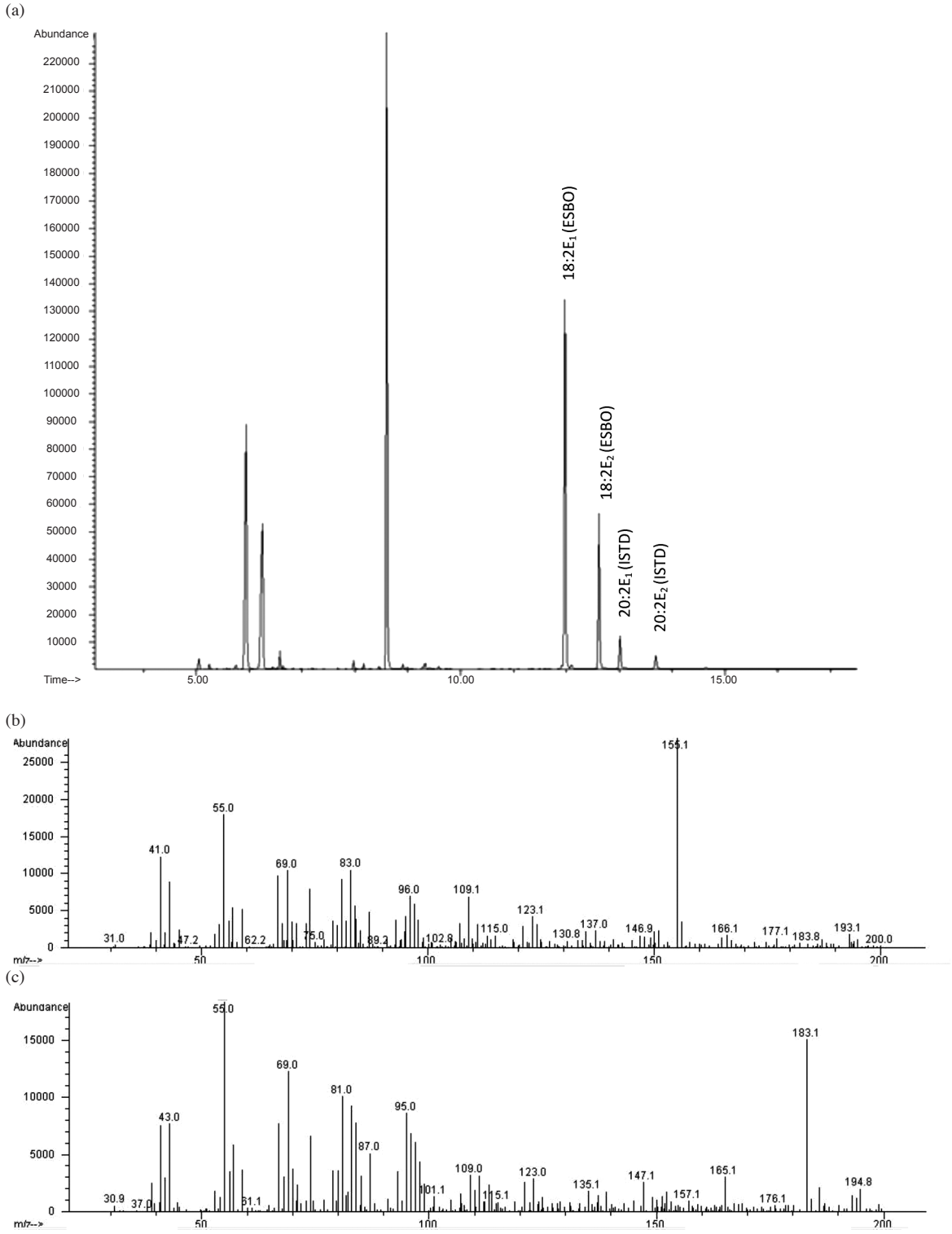

FIGURE 1. (a) Total ion chromatogram of ESBO with internal standard. Analyte of interest, methyl diepoxylinoleate, and internal standard, showed as two peaks due to diastereoisomerism. (b) Mass spectrum of methyl diepoxylinoleate – showing m/z 155 as the base peak and (c) Spectrum of methyl diepoxylinoleate – m/z 183 was chosen as the target ion

of ESBO. Recovery for pasta sauce 2 fell out of the graph. Possible reason was matrix interference.

Slope and intercept of the graph were tested using *t*-test to determine whether they are significantly different from 1 and 0. Since the critical values were higher than the calculated *t*-value, slope was not different from 1 and intercept was not different from 0 (Table 1). Specificity was deemed to be acceptable.

TABLE 1. Statistical calculation of specificity experiments using LINEST function in Microsoft® Excel to test the slope and intercept

Statistics	Value	Critical value	Conclusion
Slope, b	0.9445	1	
Slope std dev., S_b	0.2076		
Intercept, a	17.1160	0	
Intercept std. dev., S_a	24.7394		
Number of measurements, n	10		
Test for slope, t_1	0.2676	3.3554	Specificity accepted
Test for intercept, t_2	0.6919	3.3554	Specificity accepted

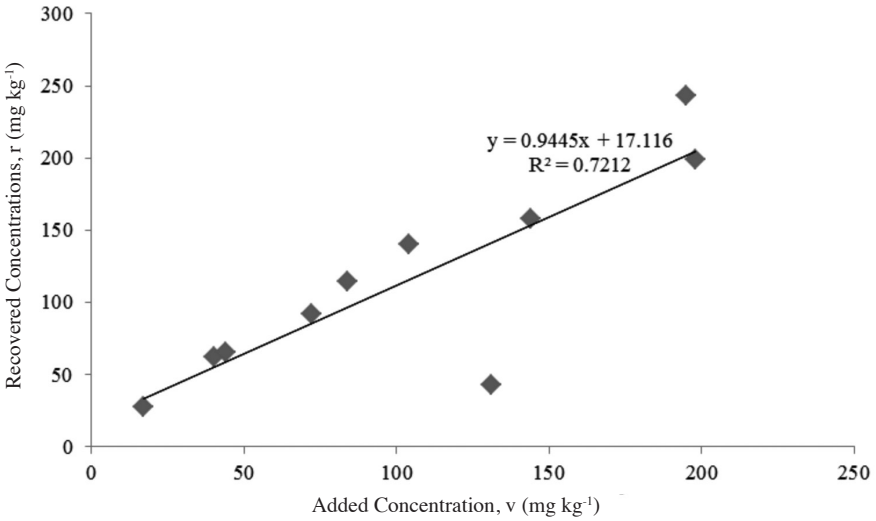

FIGURE 2. Correlation between recovered concentration, r, and known concentration added, v

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

In this study, two approaches have been used – based on signal to noise and based on linearity study. Determination of LOD and LOQ based on signal to noise is a very popular method as it is used by most researchers as it is fast and easy. Ten sets of independent sample blanks were spiked with 1 ppm of ESBO. Signals from both sample blanks and spiked samples were measured. Table 2 shows the data collected from experiments for determining of LOD and LOQ based on signal to noise. LOD and LOQ were 0.1 mg/L and 0.3 mg/L, respectively.

Sanagi et al. (2009) had shown that different approaches will give different values of LOD and LOQ and the values could vary by a factor of 5 to 6. Determination of LOD and LOQ based on linearity had been found to give a higher value than based on signal to noise, thus it is more convincing and can be confidently achieved. Six levels of standard solutions with low concentration values between 0.2 and 1.2 µg mL^{-1} were selected. The concentration selected should be regularly distributed over the lower range of values. The standard solutions were analyzed in 3 different batches. Each batch was prepared independently and analyzed on different days under reproducible conditions. By substituting the value of residual standard deviation and slope into the equation, LOD was determined to be 0.2 mg L^{-1} and LOQ was 0.7 mg L^{-1}.

By comparing the LOD and LOQ obtained from the above two methods, it was found out that LOD and LOQ obtained based on linearity study was two times higher than the LOD and LOQ obtained based on signal to noise. This finding was concurrent with the findings of Sanagi et al. (2009).

LINEARITY

The linearity of a method is its ability (within a specific range) to provide an informative value or results proportional to the amount of analyte to be determined in the sample. Ten levels of concentration were selected, starting from LOQ level – 0.7, 1, 5, 10, 15 and 20 µg mL^{-1}. Linearity was determined by the calculation of the regression line using the method of least-squares with a weighting factor of $1/x^2$ and it was expressed by the coefficient of determination (R^2). Calibration curves (based on the peak area ratio of each analyte to the internal standard) were plotted every day and were used for the

TABLE 2. Determination of LOD and LOQ based on signal to noise 3 and 10, respectively

Replicate	Peak height of spiked sample/reagent at 1 mg L^{-1}	Peak height of blank sample/blank reagent
1	589	19
2	943	18
3	587	19
4	771	20
5	373	18
6	727	20
7	437	19
8	633	14
9	162	17
10	120	15
mean	534	18
LOD = 3 × 17.9/534.2 × 1 ppm = 0.1005 mg L^{-1}		
LOQ = 10 × 17.9/534.2 × 1 ppm = 0.3351 mg L^{-1}		

determination of the concentration of analytes in the food samples. The linear dynamic range was 0.7-20 μg mL^{-1} with coefficients of determination (R^2) > 0.9968.

TRUENESS

Trueness of the method refers to the accuracy – the closeness of agreement between the values obtained compared with the true value, independent of the errors of precision of the two methods. Since there is no certified reference material (CRM) available for ESBO, estimation of trueness was done by using spiking/recovery study. Samples were spiked with low, medium and high concentration of ESBO. Recovery at 1 mg L^{-1} was 92.2 ± 29.0. Recovery for ESBO at 10 mg L^{-1} was 78.05 ± 11.59% and 99.23 ± 10.20% for 20 mg L^{-1}. The slightly lower recovery for medium concentration could be due to matrix effect.

APPLICATION TO REAL SAMPLES

Thirty one food samples were bought from local hypermarkets to be tested for their ESBO level in food. Samples that were found to violate the specific migration limit (SML) were tested in triplicate and the average readings of the triplicate were reported. Every batch of samples was run with two internal quality control (IQC) samples that were spiked with known concentration of ESBO. The results are only accepted if the recovery of IQC samples falls within acceptable range of 80–110%.

Out of the 31 samples, 6 samples were found to exceed the SML of 60 mg kg^{-1}. The sample that was found to contain the highest level of ESBO was anchovies in chili paste – 507 mg kg^{-1}. ESBO was well extracted by oil and hence the oilier the sample, the more ESBO will be extracted. Anchovies in chili paste are considered one of the oiliest samples among the 31 samples monitored in this work.

CONCLUSION

A GC/MS method was developed for the determination of ESBO in oily foods stored in glass jars using gas chromatography-mass spectrometry. The optimized method was validated to ensure that it is fit for purpose. The method was proven to be specific. Limit of detection and limit of quantitation were 7 mg kg^{-1} and 23 mg/kg, respectively. Working range was from 23– 667 mg kg^{-1}. No matrix effect was found and that means calibration curve can be prepared in aqueous solution. Repeatability was 0.8, 3.3 and 5.6 for low, medium and high concentration level, respectively. Trueness was 92.24 ± 28.99% for low concentration, 78.05 ± 11.59% for medium concentration and 99.23 ± 10.20% for high concentration. Out of 31 food samples purchased from hypermarkets in Malaysia were studied and 6 samples were found to exceed the specific migration limit of 60 mg kg^{-1} (based on EU Directive 2002/72/EC). The highest ESBO level detected was 507 mg kg^{-1} in an anchovies in chili paste sample. Nevertheless, tolerable daily intake of 1 mg kg^{-1} body weight, defined by Scientific Committee on Food of EU, is unlikely to be exceeded as consumption of foods in glass jars by Malaysian is not as much as Westerners.

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