The determination of cholesterol in edible oil by Gas Chromatography-Mass spectrometry

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Abstract

This To set up a simple, high-speed, and repeatable method used for determining cholesterol content in edible oil, to prevent the edible vegetable oil mixed with illegal cooking oil. The oil samples were saponified with 50% alkaline-ethanol mixture (volume ratio 40:12) solution, extracted mixed solution of petroleum ether-anhydrous ether mixture (volume ratio 1:1), dehydrated with anhydrous Na_2SO_4, and concentrated under N_2. The analyte was separated and quantitatively analyzed using GC-MS on a HP-5MS capillary column (30m × 0.25mm × 0.25μm). The linear range of the method was 1.62-243μg /mL with correlation coefficients of 0.9963. The recovery and the relative standard deviation of the method were 83.31% ~ 97.44% and 1.18%-2.89%, respectively. The minimum detection limit 0.51μg/mL, if the sample weight 5.0 g, the minimum detection limit 0.10 mg/kg. This method can be applied to determine the cholesterol content in edible vegetable oil.

Keywords

Cholesterol, Edible vegetable oil, GC-MS.

1. Introduction

Cholesterol is also called cholesterol alcohol, is a derivative of the cyclopentanoper hydrophenanthrene ring. It does not dissolve in water, soluble in ether, chloroform and other fat-soluble solvents. The cholesterol content in the human body is about 140 g, it is necessary to support the body's normal physiological function of material, too much cholesterol can form hyperlipidemia, cause atherosclerosis [1].

At present, Colorimetric method [2], high performance liquid chromatography (HPLC) method, the enzymatic[3], gas chromatography[4] and thin layer chromatography[5] are commonly used method for the determination of cholesterol of food. Colorimetric method simple, but the specificity is poor, more interference factors; Enzymatic is mainly used for determination of cholesterol in serum and plasma; HPLC method is more suitable for the analysis of cholesterol in biological products, application in food is relatively limited; At present, the GC-MS is a better method for the analysis on the cholesterol, has been recognized in the world. In this paper, using GC-MS for determination of cholesterol in cooking oil, ruled out the interference of oil in the natural sterol, qualitative and quantitative analysis of the results more accurate.

Illegal cooking oil composition is complicated, in the process of recycling inevitably mixed with animal oils and fats. The composition of illegal cooking oil is complicated, in the process of recycling inevitably mixed with animal oils and fats. Generally contains a lot of cholesterol in animal fats, but contains a tiny amount of cholesterol in the vegetable oil. Previous studies mainly focused on the determination of sitosterol in vegetable oil and other natural sterol. Therefore, establish a method for determination of the cholesterol content in vegetable oil, to infer whether oils mixed with animal oils, auxiliary differential cooking oil is of great significance [6].

2. Eexperiment

2.1 Instruments and reagents

Instrument: GC-MS instrument (agilent) : Equipped with Agilent 7890A gas chromatograph, 7683B automatic sampler, 5975C mass spectrometry detector and HP-5 MS chromatographic column(30 m×0.25 mm×0.25 μm); Turbo Vap(Ⅱ) N-EVAP(Caliper Life sciences).

Main reagents: Cholesterol (purity > 99%, Sigma reagent); methanol (chromatography); anhydrous ethanol; petroleum ether; ethyl ether; anhydrous sodium sulfate; potassium hydroxide. Other reagents are analytical pure.

2.2 Condition of Analysis and Test

Gas chromatographic conditions: Chromatographic column: HP-5MS chromatographic column (30m×0.25mm×0.25μm); vaporizing chamber temperature: 280℃; column temperature: using temperature programmed, initial temperature 220 ℃, keep 1 min, then at 30 ℃/min rate up to 280 ℃, keep for 10 min, the flow rate : 1 mL/min; detector: MSD; carrier gas: He, 2 mL/min (constant current mode); sample quantity: 1.0 μL.

Mass spectrometry conditions: EI ionization mode, electronic energy 70 ev, ion source temperature 250 ℃, electron multiplier voltage 1405 V, quality scanning range 50-650 u, transmission line temperature 265℃. The analysis parameters: mother ion m/z: 386, fragment ions m/z: 268, m/z: 301 and m/z: 231.

2.3 Sample preparation

Choice of saponifier: Weigh sample oil 1 to 5 g (accurate to 0.1 mg) in the 100 mL triangle bottle with plug, with 50% KOH - methanol (volume ratio 40:12), 50% KOH - ethanol (volume ratio 40:12) two saponification liquid saponified, placed in a magnetic heating stirrer after blending, at 60 ℃ saponifing for 2 h.

Choice of extraction agent: Put all the saponification liquid in the 125 mL separatory funnel, with 10 mL of sodium chloride solution (50 g/L) washing tube three times, will wash together in a separatory funnel, gently shake, prevent emulsification, put into the 20 mL mixed solution of petroleum ether and ethyl ether (volume ratio 1:1) for extracting, shaking for 1 min, static layering. Transfer water phase in the second separating funnel, with 20 mL petroleum ether and ethyl ether mixture (volume ratio 1:1) repeat extraction twice, refuse the lower water phase, merge three organic phase, the organic phase was washed with distilled water to pH neutral (washing liquid was not red after joining the phenolphthalein), the anhydrous sodium sulfate dehydration, filtrate filter into 250 mL nitrogen blow for the bottle [7].

Concentration: Put above filtrate in the enrichment apparatus, 45℃ water bath, with nitrogen blow, concentrated to around 0.75 mL, with petroleum ether constant volume to 1.0 mL, through a straw into the sample bottle, gas chromatogram was analyzed.

2.4 Drawing standard curve

Put cholesterol standard stock solution and diluted with anhydrous ethanol mixture for cholesterol standard series: 1.62 μg/mL, 16.2 μg/mL, 48.6 μg/mL, 113.2g μ/mL, 16μg/mL, 243 μg/mL. According to the above chromatography conditions, respectively take 1.0 μL sample, measure retention time and peak area, with the mean peak area and concentration (μg /mL) drawing standard curve.

3. Results and discussion

3.1 Choice of saponifier

With 50% KOH - methanol (volume ratio 40:12) as a saponifier recovery was 79%, with 50% KOH - ethanol (volume ratio 40:12) the recovery was 98%, the former recovery rate was very low, this is because methanol boiling point (64.65℃) is lower than ethanol (78.4℃), not only in the

saponification process volatile reduces, cause the sample emulsification, lead to low recovery rate, but also toxic, so choose 50% KOH - ethanol (volume ratio 40:12) as saponification[1].

3.2 Choice of saponifier temperature

Because cholesterol allosteric easily at high temperature, oil saponification process should be controlled at lower temperature (40℃~80℃). Compared the saponification process respectively at 40℃, 60℃ and 80℃, found that higher temperature helps saponification speed, saponification time needed at three temperatures, respectively, 3 h, 2 h and 1 h. Considering the high temperature can cause the cholesterol oxidation allosteric, extraction solvent volatilization too fast, it was advisable to choose saponification temperature 60℃[8].

3.3 Choice of extraction agent

Petroleum ether, ethyl ether, petroleum ether-ethyl ether mixture (volume ratio 1:1) were selected as solvent extraction, recycling rate of cholesterol was investigated. The results showed that the recovery rate of petroleum ether and ethyl ether mixture was 98% that of petroleum ether was 95% that of ethyl ether was 92%, so petroleum ether-ether mixture (volume ratio 1:1) was choosed as extraction solvent.

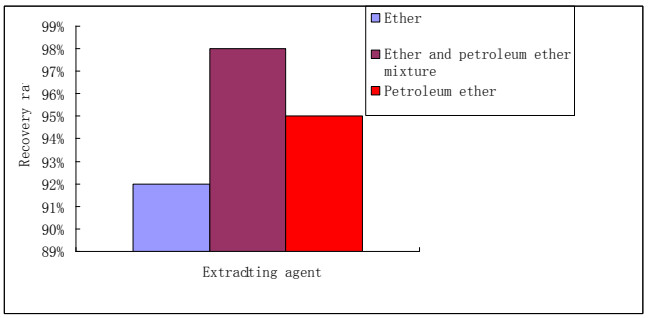

Fig.1 Recovery rate of cholestrol when using different

3.4 Choice of ion source and transmission line temperature

Transmission line temperature keeps the samples before entering the ion source from condensing, compared the influence of the temperature range(230℃~300℃), and the results showed that the transmission line temperature below 250 ℃, measured cholesterol peak area was lesser, at 300℃, cholesterol began to water loss , at the same time considering the highest use temperature of the chromatographic column, the service life of chromatographic column is reduced at high temperature, 270℃ as transmission line temperature in this experiment.

Ion source temperature not too high [9], otherwise cholesterol before may into the ion trap water loss, that had an impact on the sensitivity analysis, This can be judged from abundance ratio of the molecular ion mass spectra peak m/z: 386 and its water loss peak m/z368, if cholesterol before not ionized water loss, the relative abundance of molecular ion peak m/z: 386 the weakened, but water loss peak m/z: 368 relative to strengthen. When ion source temperature 250℃ and transmission line temperature 270℃ the better results of the analysis was obtained.

3.5 Choice of mother ion and quantitative fragment ions

According to the molecular structure of cholesterol and scan mass spectrogram fragments of the abundance, in this experiment with molecular ion m/z: 386 for mother ion, with sub ion m/z: 368, m/z: 301, m/z: 231 as fragment ions for qualitative analysis. The scanning chart of total ion flow (48.6 μg/mL) was shown in figure 2, and NIST08 mass spectrum library retrieval rod mass spectrum diagram as shown in figure 3.

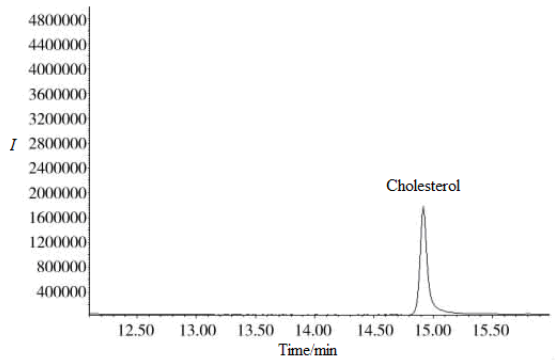

Fig.2 Total ion flow diagram of cholesterol standard solution

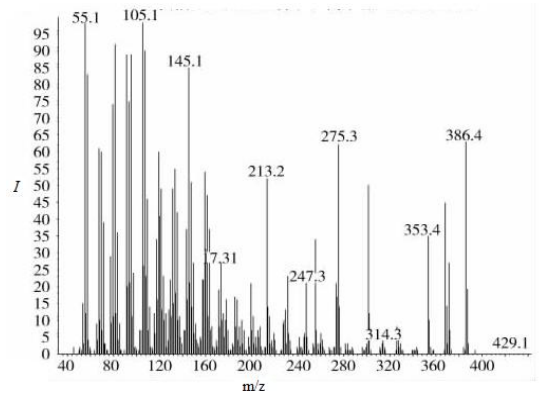

Fig.3 NIST08 mass spectrum library retrieval rod mass spectrum diagram of cholesterol standard solution

3.6 Linear range and linearity

In this experimental conditions, the standard curve was drawn with the mean peak area and concentration, cholesterol concentration within the scope of 1.62~243 μg/mL had good linear relationship, the regression equation was as follows: Y = 5.3×103 X-7.2×10-4 (r = 0.9994, n=6).

3.7 Method evaluation

Minimum detection limit: Under the condition of this study, with 1.62 μg/mL standard cholesterol solution as test fluid, signal-to-noise ratio (S/N) of cholesterol signal peak was measured, according to the lowest instrument detection limit (MDL) S/N = 3, the minimum quantitative limit (NQL) S/N = 10, the minimum detection limit of cholesterol in sample computed by GC-MS determination was 0.10 mg/kg.

Precision experiment: 16.2 μg/mL and 113.2 μg/mL standard solution were mad up respectively, on the above chromatography conditions, inject 1.0 μL sample, repeated six times, RSD between 1.22% ~ 3.72%.

Recovery experiments: Sampled from known content of edible vegetable oil, Accurately respectively to join low, medium and high concentrations of cholesterol standard liquid, each adding level repeat 6 times, according to item 2.3 to determine, calculate the average recovery, the results were shown in table 1. The average recovery of addition samples was 83.31% ~ 97.44%, relative standard deviation (RSD) was 1.28% ~ 2.89%.

Table 1 the test results of standard addition sample recovery rate (n=6, ×10-2)

Sample	Background values (μg)	Addition (μg)	Measured the amount (μg)	Average recovery rate (%)	RSD (%)
Vegetable oil	36.8	15.2	47.49	83.31	2.89
	36.8	47.6	84.38	95.51	1.66
	36.8	96.2	133.69	97.44	1.18

3.8 Samples determination

Samples from a city to sell edible vegetable oil, according to item 2.3 to deal with samples, under the condition of above chromatographic to determine, the result was shown in figure 4, with external standard method for calculating the content, the results were shown in table 2. Table 2 shows that the cholesterol content of most vegetable oil below 20 mg/kg. The cholesterol content of illegal cooking oil as high as 478.04 mg/kg, significantly higher than the normal vegetable oil, but campesterol, stigmasterol and sterol natural sterol content was obviously on the low side, such as shown in figure 5. The determination results consistent with Liu Bo'research results [10].

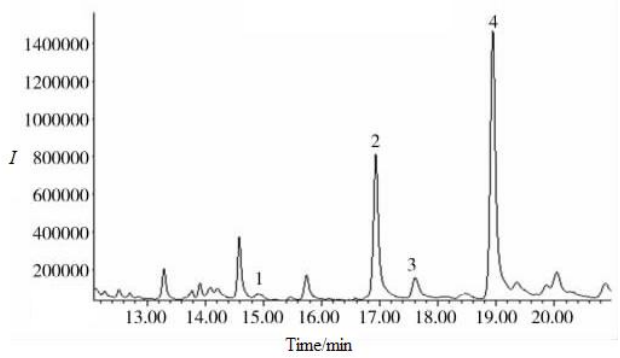

Fig.4 Total ion flow diagram of a vegetable oil saponification liquid
1. Cholesterol; 2. Campesterol; 3. Stigmasterol; 4. Sitosterol.

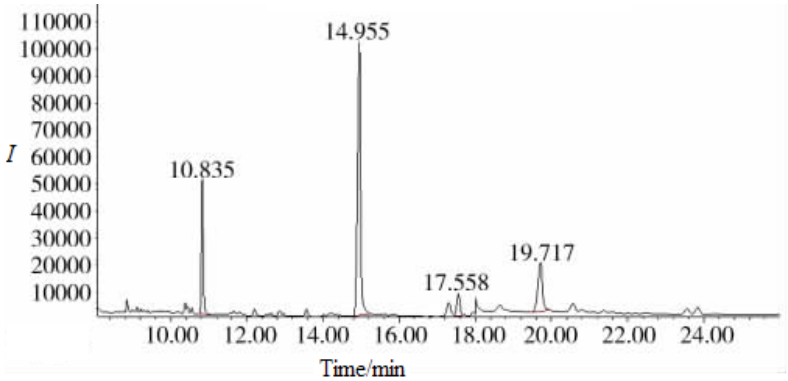

Fig.5 Total ion flow diagram of an illegal cooking oil saponification liquid

Table 2 Results of determination of cholesterol of vegetable oil and illegal cooking oil

Sample number	Sample types	Cholesterol content（mg/kg）
1	Sesame oil	5.48
2	Soybean oil	6.04
3	Peanut oil	4.38
4	Corn oil	5.16
5	Sunflower oil	3.06
6	Rapeseed oil	4.56
7	Olive oil	5.02
8	Salad oil	5.26
9	Cooking oil	478.04

4. Conclusion

In this study the high-throughput detection method by GC-MS was established for the determination of cholesterol content of edible oil, this method separation effect was good, high selectivity. Qualitative analysis by mass spectrometry, eliminate the effects of phytosterols on cholesterol detection can be applied to the detection of the cholesterol in edible oil. This paper compared the cholesterol levels of different oils, found that average cholesterol content of common vegetable oils below 20 mg/kg, so propose the limits of the cholesterol of vegetable oil below 50 mg/kg, provide reference for detecting vegetable oil mixed with illegal cooking oil.

Acknowledgements

The authors wish to thank the helpful comments and suggestions from my leaders and colleagues in college of chemistry and chemical engineering, zhoukou normal university. This work are supported by the laboratory open project of Zhoukou Normal University (Grant No. K201633), the Henan province education technology equipment and practice education research subject(Grant No. GZS370) and Henan Province Key Discipline of Applied Chemistry (Grant No. 201218692).

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